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# Insignificant effect of management using irregular shelterwood system on the genetic diversity of European beech (*Fagus sylvatica* L.): A case study of managed stand and old growth forest in Slovenia

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ABSTRACT

In the presented case study, we aim to understand the impact of an irregular shelterwood system (ISS) on the genetic diversity of European beech (*Fagus sylvatica* L.) firstly by comparing managed stand to old growth beech forest and secondly by comparing two successive generations in both managed and old growth stands. Studies on European beech to date have not yet investigated the effect of ISS on its genetic diversity and have rarely addressed the effect of management on the genetic diversity of successive generations.

The study was conducted in two mixed beech stands in Slovenia; the unmanaged Rajhenavski Rog old-growth European beech forest reserve and beech forest in Osankarica, managed according to ISS. All 140 sampled adult trees and saplings were genotyped at 16 nuclear microsatellite loci.

ISS mimics genetic processes of the old growth rather well in the studied managed stand. The comparisons of diversity measures between managed and old growth stands did not reveal any significant differences between the two for any of the cohorts; the differences between the cohorts from the same stand were not significant. The observed significant shift in allele frequencies at four loci between successive generations could not be unambiguously attributed to management. Cohorts from the same stand had similar genetic structure, but six individuals from the managed stand formed a unique cluster.

No convincing evidence of the effect of ISS on genetic diversity of the studied managed beech stand was found.



## 1. Introduction

An elementary requirement for sustainable close-to-nature forest management that aims to maintain the adaptability of populations in future environments is an understanding and dynamic conservation of genetic variation. Contrasting evidence of the effect of management on genetic diversity in tree populations have been reported depending on the tree species and silvicultural methods used, ranging from negative (Buchert et al., 1997; Rajora et al., 2000; El-Kassaby et al., 2003; Paffetti et al., 2012) to weak or none (Adams et al., 1998; Aravanopoulos et al., 2001; Buiteveld et al., 2007; Fageria and Rajora, 2013; Rajendra et al., 2014). In this study, we present a case study of the evaluation of the effect of an irregular shelterwood system (also 'verfeinerte Femelschlag'; ISS) on the genetic diversity of European beech (*Fagus sylvatica* L.; hereafter beech) by (i) comparing a managed stand to old growth beech and (ii) comparing two successive generations in both managed and old growth stands.

Beech contributes almost 30% of the total growing stock in Slovenia and is one of the most ecologically and economically important tree species in the country. Since 1970 its area has been expanding by more than 1200 ha per year on average (Poljanec et al., 2010) and beech forests are found on 89% of the total forest area (Ficko et al., 2008). In Slovenia, beech is traditionally managed according to ISS (Diaci et al., 2012). ISS is defined as 'a system of successive regeneration fellings with a long and indefinite regeneration period, producing young crops of somewhat uneven-aged type' (Matthews, 1989) intended to create multispecies cohorts by adapting canopy openings to the light requirements of the

*Abbreviations:* ISS, irregular shelterwood system; PCR, polymerase chain reaction; ST, simulation test; WT, Waples test; $F_{ST}$, fixation index; $D_S$, standard genetic distance.

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**Table 1**
Attributes of regeneration centres, sampled beech saplings and the adult trees around the regeneration centres in the managed and old growth stands. *H* = height, *N* = number, $D_{0.5}$ = diameter at height 0.5 m, DBH = diameter at breast height.

| Description of regeneration centres | | | | Beech saplings[a] | | | Adult stand around regeneration centres[a] | |
|---|---|---|---|---|---|---|---|---|
| Location | Area ($m^2$) | Shape [1:5000] | Species composition[b] (%) | H interval (average) (m) | $D_{0.5}$ (cm) | N per $m^2$ | *H* (m) | DBH (cm) |
| *Managed stand* | | | | | | | | |
| I | | | | | | | | |
| 46,439085°N 15,381202°E | 1121 |  | *F. syl* 98<br>*A. pse* 1<br>*P. ab* 1<br>*A. alb* individually | 1.5–3.4 (2.4) | 1–3, individually up to 5 | 17 | 27 | 41–50 |
| II | | | | | | | | |
| 46,442405°N 15,378760°E | 3643 |  | *F. syl* 98<br>*P. ab* 2 | 1.0–3.1 (2.1) | 1–2 | 19 | 24 | 31–50 |
| III | | | | | | | | |
| 46,444322°N 15,373914°E | 1531 |  | *F. syl* 97<br>*A. pse* 1<br>*P. ab* 2 | 1.0–2.5 (1.8) | 1–3 | 22 | 26 | 36–55 |
| IV | | | | | | | | |
| 46,446159°N 15,374214°E | 368 |  | *F. syl* 97<br>*P. ab* 2<br>*A. alb* 1 | 0.5–2.6 (1.7) | 1–3 | 27 | 28 | 41–55 |
| *Old growth stand* | | | | | | | | |
| I | | | | | | | | |
| 45,658763°N 15,008832°E | 381 |  | *F. syl* 100 | 2.3–4.8 (3.2) | 1–4 | 4 | 30 | 51–60 |
| II | | | | | | | | |
| 45,658663°N 15,009943°E | 1337 |  | *F. syl* 100 | 1.4–6.5 (3.4) | 1–3, individually up to 6 | 2 | 27 | 41–50 |

[a] Data collected in 2014.
[b] *F. syl* = *Fagus sylvatica*, *A. pse* = *Acer pseudoplatanus*, *P. ab* = *Picea abies*, *A. alb* = *Abies alba*.

target tree species (Diaci, 2006; Raymond et al., 2009). ISS is a long-term oriented procedure with slow opening of the stand for which continuous and abundant regeneration is essential (Matthews, 1989). As a result, many parent trees can contribute over time to the next generation. Also, the tree species composition of the seedling layer may noticeably differ from that of the subsequent mature stand. In Europe, the most commonly used silvicultural system for beech is the shelterwood uniform system (Matthews, 1989) but lately a shift towards a more close-to-nature silvicultural systems has been observed (Wobst, 2006), adding importance to the research findings from silvicultural systems used on small scales such as ISS.

The territory of present-day Slovenia is one of the main sources for the post-glacial distribution of the beech and is supposedly the most important glacial refugia for its re-colonization in Europe (Magri et al., 2006; Brus, 2010). Studies on genetic structure of beech populations in central and southeastern Europe indicated a high level of genetic diversity in Slovenia (Brus et al., 1999; Gömöry et al., 1999) and the predominantly ecotypic character of genetic differentiation of populations (Brinar, 1971; Robson et al., 2010).

The effects of ISS on the genetic diversity of beech have not yet been studied. Other studies in beech have so far investigated the effect of the shelterwood uniform system, plantation, colonisation (Buiteveld et al., 2007; Piotti et al., 2013; Rajendra et al., 2014), conversion from coppice (Paffetti et al., 2012), selection forests (Rajendra et al., 2014) and patch cuttings (Konnert and Hosius, 2010) on genetic diversity or spatial genetic structure. While management has contrasting effects on the genetic diversity of beech, it significantly reduces the spatial genetic structure of beech stands (Paffetti et al., 2012; Piotti et al., 2013; Rajendra et al., 2014). This case study aims to answer the question of whether ISS affects genetic diversity of the studied beech stand by (i) comparing a managed stand with an old growth stand and (ii) comparing two successive generations in both managed and old growth stands.

## 2. Materials and methods

### 2.1. Study sites and field sampling

This study was conducted in the unmanaged Rajhenavski Rog old-growth European beech forest reserve and in the beech forest at Osankarica, managed using ISS.

Rajhenavski Rog is a 51.14 ha forest remnant located on a high karst plateau (850–920 m) in southeastern Slovenia (45.659°N, 15.009°E). The reserve is dominated by beech and silver fir (*Abies alba* Mill.). The total growing stock is 747 $m^3$ $ha^{-1}$ and dead wood residues in the forest remnant represent 247 $m^3$ $ha^{-1}$ (Hartman, 1999). The sampling area of 5 ha was located in the southern part of the old growth, 880 m above sea level with prevailing south exposition where beech is dominant. Management was banned in 1904 with revision of the Hufnagel's management plan from 1892 (Hartman, 2014: personal communication; Hartman, 1999); before that it was a virgin forest (Kraigher et al., 2002). Regeneration gaps where beech had formed the two studied regeneration centres were created during the last 10–20 years as a result of endogenous and external disturbances (i.e. death due to old age, wind, snow). Location, area and shape of the regeneration centres, species composition as well as sapling height, thickness and their abundance are presented in Table 1.

The research site at Osankarica is a 9.9 ha autochthonous forest stand overgrown by beech (89%), Norway spruce (*Picea abies* [L.] Karsten; 8%), sycamore maple (*Acer pseudoplatanus* L.; 2%) and silver fir (1%) with a total stand growing stock of 443 $m^3$ $ha^{-1}$ (Ahej et al., 2000) on the Pohorje Mountain in northern Slovenia (46.449°N, 15.376°E), 1200–1270 m above sea level with a prevailing northeast exposition. Adult beech trees are between 90 and 130 years old. The stand is managed according to ISS; smaller cohorts of regeneration are intermixed with larger cohorts of mature and rejuvenation stage resulting in a mixture of fine-grained and coarse-grained horizontal structures. According to forest management plans, before 1983, in the developmental phase of younger timber tree stage only thinnings were carried out in the stand at Osankarica. At that time, natural regeneration was absent from the stand. Between 1983 and 1994, the goal of management was based on ensuring the optimal growing stock by supporting beech. In 1987, a strong mast year was recorded, followed by three more in 1994, 1998 and 2004. When for the first time abundant regeneration was recorded under shelterwood, small regeneration gaps sized one to two tree heights were opened up in the stand. Regeneration centres subsequently extended into the stand and have been later released. Attributes of regeneration centres, measured on five 1 $m^2$ subplots situated in regeneration centres, are presented in Table 1. One of the subplots was always situated in the middle of the regeneration centre. The remaining four subplots were located in a cross; each subplot was situated halfway from the middle of the regeneration centre to its edge in the directions of north, south, east and west. The shape of the regeneration centres was plotted according to coordinates recorded during sampling.

Twigs with dormant buds from 35 adult trees and 35 saplings (>1.3 m tall and < 5 cm DBH) per site were collected in spring 2012. Trees >30 m apart from the entire sampling area were sampled and their geographical location was recorded using a Garmin GPSMAP 60CSx (Garmin International, Kansas, U.S.A.). For saplings, the midpoint of the regeneration centres and their borders were recorded. Differently sized regeneration centres at Osankarica were located in the prevailing horizontal structures (i.e. mature and rejuvenation stages), based on height and DBH of adult trees around the centres (Table 1). In the old growth, smaller regeneration centre was located in a gap while larger one was situated in the part of the old growth where different small gaps were already interconnected and regeneration was continuously present in the whole area. Regeneration centres where only seedlings were present (<0.5 m tall) were not considered for this analysis as initial phases of high mortality might not have come to an end. From the whole area of the regeneration centres, two (15–20 saplings per centre) and four (5–11 saplings per centre) regeneration centres in the old growth and managed stand, respectively, were randomly sampled.

### 2.2. Genotyping

Total DNA was isolated from dormant buds using a DNeasy plant kit (QIAGEN, Germany), as per the manufacturer's specifications. All adults and saplings were genotyped at 16 highly variable microsatellite loci using primers described by Lefevre et al. (2012). Primers were renamed with consecutive numbers to ease reporting; csolfagus_31 became Fs1 and DE576_A_0 became Fs16. Multiplex Kit 1 was split into two separate kits (kit 1a: primer pairs Fs1, Fs2, Fs4 and Fs5; kit 1b: primer pairs Fs3, Fs6, Fs7 and Fs8) to avoid the overlapping of alleles labelled with the same fluorescent dye. Polymerase chain reactions (PCRs) were performed as described by Lefevre et al. (2012) but primer pair concentrations required further optimisation and final concentrations differed from the published ones by a maximum of 0.9 for primer pair Fs16. The sizing of the PCR products was performed on an ABI PRISM 310 automatic sequencer with accompanying Gene Mapper 3.7 software.

### 2.3. Data analysis

Estimates of genetic diversity (mean number of alleles, rare, effective and private alleles and expected heterozygosity) were calculated using GenAlEx 6.5 (Peakall and Smouse, 2012). Deviations from the Hardy–Weinberg equilibrium and linkage disequilibrium were tested using 10,000 permutations with the Genepop 4.0 programme (Rousset, 2008). Inbreeding coefficient $F_{IS}$ was calculated and tested (10,000 permutations) with the SpaGeDi 1.3 programme (Hardy and Vekemans, 2002).

Temporal changes in allele frequencies were tested using the simulation test (ST) and $F_T$ test (Sandoval-Castellanos, 2010), and the Waples test (WT; Waples, 1989) using the TAFT 2.3 programme (Sandoval-Castellanos, 2010). ST is a statistical test based on the Bayesian theorem in which the distribution of the distances among sampling frequencies is simulated. Binominal sampling is used for generation changes and hypergeometric sampling for effective populations and samples. The simulation procedure has been described in detail by Sandoval-Castellanos (2010). The $F_T$ statistic corresponds to the fixation index ($F_{ST}$) minus the average $F_{ST}$ calculated among simulated samples and can be interpreted as the divergence which the population has undergone through time if the effect of gene drift is excluded. WT is a Chi-Square test adjusted to consider gene drift. The null hypothesis tested with all three tests was 'changes in observed allele frequencies between two samples taken from the same population at different times are the result of genetic drift and sampling error'. The following parameters were used for the above tests: full Bayesian algorithm, Plan I sampling strategy and one generation separated the two temporal samples. Population size was set at 10,000 and effective population size at 6000. The number of simulations was $10^6$. For comparison, pairwise $F_{ST}$ values according to Weir and Cockerham (1984) were calculated and significance was determined using 10,000 permutations with the SpaGeDi programme. Additionally, standard genetic distance ($D_S$) according to Nei (1978) was calculated in SpaGeDi.

Potential differences in the genetic structure between the cohorts were also investigated using a model-based clustering algorithm implemented in the Structure 2.3.4 programme (Pritchard et al., 2000; Falush et al., 2003; Hubisz et al., 2009). The best estimated number of distinct clusters was calculated according to Evanno et al. (2005) using Structure Harvester (Earl and von Holdt, 2012), whereas the 'Greedy algorithm' implemented in CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) was used to average the results of the replicated runs. The default model parameters using populations' priors were used for simulations, allowing number of populations $K$ to vary from 1 to 6. Each run, replicated 10 times, consisted of 150,000 burn-in iterations and 350,000 data collection iterations.

Bonferroni corrections were applied to adjust critical values in case of multiple comparisons. To test for significant difference in mean values of genetic diversities, a $t$-test with Welch modification for unequal variances between groups was calculated in R (R Development Core Team, 2008).

Though microsatellites are traditionally considered to be neutral markers, they were lately described to play a role in generating genetic variation underlying adaptive evolution (Kashi and King, 2006; Gemayel et al., 2010), possibly also in beech (Bilela et al., 2012). Therefore, we performed an outlier test using the Lositan outlier detection platform (Antao et al., 2008) to check for potential non-neutrality of the investigated loci.

Analysis of 10 families by Lefevre et al. (2012) revealed no null alleles at any of the 16 microsatellite loci used in our study; yet null alleles at a given locus may be present only in certain

populations (Heuertz et al., 2004; Westergren, 2010). Additionally, Oddou-Muratorio et al. (2008) found null alleles to be present at the only locus shared with our study, Fs4 (FS1_15). Therefore we tested the presence of null alleles in our dataset with Micro-Checker 2.2.0.3 (Van Oosterhout et al., 2004).

Data visualization was aided by Daniel's XL Toolbox Add-in for Excel, version 6.52, by Daniel Kraus, Würzburg, Germany.

## 3. Results

Significant deviations from the Hardy–Weinberg equilibrium were detected at locus Fs4 in the adult population of the managed forest ($p = 0.001$). At this locus null alleles were observed in the managed stand in both cohorts. Null alleles were also observed at loci Fs3 (old growth saplings), Fs10 and Fs15 (old growth adults). For locus Fs4, the null hypothesis of independent genotypes between two loci had to be rejected (in conjunction with loci Fs5, Fs8 and Fs12, $p = 0.000$ in all three comparisons). Therefore, locus Fs4 was omitted from further analysis.

The outlier test did not identify any outliers in the managed or old growth forests [managed forest: 0.171 (locus Fs11) $\leqslant p \geqslant$ 0.913 (locus Fs5)], old growth forest: [0.258 (locus Fs12) $\leqslant p \geqslant$ 0.971 (locus Fs6)].

### 3.1. Genetic diversity

The mean number of alleles, effective alleles, private alleles and expected heterozygosity across loci did not significantly differ between adult trees and their regeneration either in the managed or old growth stands (Fig. 1). In addition, the means of genetic diversity estimates between the managed and old growth stands did not significantly differ for either of the cohorts ($p$ values not reported but see vertical comparisons in Fig. 1). The mean number of rare alleles (frequency $\leqslant 0.05$) was lower in the managed stand (2.867) than in the old growth stand (4.133), but the means did not significantly differ from each other ($t = -1.589$, df = 27, $p = 0.124$). The mean number of rare alleles was also lower in saplings than in the adults for the managed (2.067 vs. 2.533; $t = 0.674$, df = 26, $p = 0.506$) and old growth stands (2.800 vs. 2.867; $t = 0.095$, df = 27, $p = 0.925$), but it was not significant. Genetic diversity estimates across loci indicated that ISS did not reduce mean allele indices either in the natural regeneration of the managed stand or in the managed stand itself when compared to the old growth forest.

Across loci, 12 out of 119 alleles were lost in the succeeding generation in the managed stand and 16 out of 123 in the old growth stand. In contrast, saplings from the old growth were more successful in recruiting new alleles into their population; they recruited 15 alleles not present in the sampled adult cohort in comparison to the managed stand where the saplings recruited 9 new alleles. All alleles lost in the next generation but one from the old growth were rare alleles. Majority of newly recruited alleles were also rare; 7 in the managed and 14 in the old growth stands.

The inbreeding coefficient $F_{IS}$ significantly departed from the expected value only in the sapling population in the old growth forest ($F_{IS} = 0.052$, $p = 0.017$) because of the departures from the expected value at locus Fs3 ($F_{IS} = 0.229$, $p = 0.021$). This was most likely caused by the presence of null alleles at this locus as identified with the Micro-Checker programme. Null alleles were also detected at loci Fs10 and Fs15 in the adult phase of the old growth stand but global $F_{IS}$ for this cohort did not significantly depart from the expected value under random mating ($F_{IS} = 0.016$, $p = 0.270$). The lack of inbreeding in the study was anticipated as inbreeding was not expected to occur in an outcrossing species like beech.

### 3.2. Temporal changes in allele frequencies

Temporal changes in allele frequencies that could not be attributed only to genetic drift and sampling error between the cohorts were detected in both the managed and old growth stands (Table 2, Fig. 2). In the managed stand significant temporal changes in allele frequencies were detected at loci Fs5, Fs6 and Fs8 while in the old growth temporal changes caused by factors other than genetic drift, sampling error and management were observed at loci Fs6 and Fs10. Repeating the simulations with frequencies adjusted for null alleles, according to Chakraborty et al. (1992) and Van Oosterhout et al. (2004), that were implemented in the Micro-Checker programme for loci exhibiting null alleles (Fs3, Fs10 and Fs15), changed the observed $F_{ST}$ values but did not alter the rejection of the null hypothesis for locus Fs10 and did not result in its rejection for the other two loci.

$F_{ST}$ values did not significantly differ from the expected values for any of the loci either in the managed or old growth stands after applying Bonferroni corrections for multiple comparisons. However, before the application of correction for multiple comparisons, $p$ values for loci Fs5 and Fs6 in the managed stand and loci Fs6 and Fs10 in the old growth stand were lower than 0.05, indicating a good fit with the results obtained with the $F_T$, ST and WT tests.

$F_{ST}$ value between adults and saplings in the managed stand (0.0042, $p = 0.069$) differed in order of magnitude from the unmanaged stand (0.0001, $p = 0.445$) while $D_S$ did not (0.0084 vs. 0.0011).

Apart from the drift and sampling effect, management alone did not explain temporal differences in allele frequencies because these were observed in both the managed and old growth stands, having locus Fs6 in common.

### 3.3. Genetic differentiation of the cohorts

Three genetic groups were identified for our data set. In the managed stand, the adult cohort and all but six saplings clustered together. The six individuals from regeneration centre I formed a genetically distinct group based on the analysis of 15 microsatellite loci using the Bayesian clustering implemented in the Structure 2.3.4 programme (membership proportions in the distinct group >0.6) The genetic structure of regeneration centres and adult cohort in the old growth forest was very similar yet differed from the genetic structure observed in the managed stand (Fig. 3).

## 4. Discussion

### 4.1. Influence of management on genetic diversity

In the presented case study, we examined the potential effects of ISS on the genetic diversity and structure of a European beech stand by (i) comparing a managed stand to an old growth beech stand and (ii) comparing two successive generations in both the managed and old growth stands. The pair-wise comparisons did not reveal significant differences in genetic diversity measures among the managed and old growth stands and among adult trees and saplings in either of the stands. In addition, the number of loci exhibiting significant temporal changes after the generation change was three in the managed stand and two in the old growth stand; one locus was shared between the two stands. With the exception of some individuals from one regeneration centre in the managed stand, the genetic structure of saplings was similar to the structure of adults in both studied stands. Based on the overall results, ISS is a suitable management method for sustaining genetic diversity in the studied beech stand.

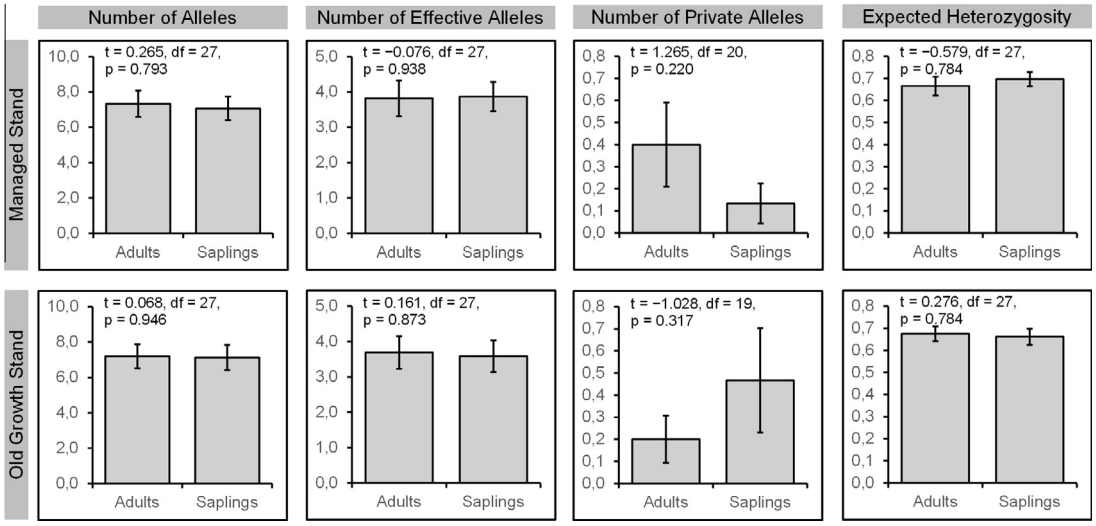


**Fig. 1.** Mean and standard errors across loci for each sample for number of alleles, number of effective alleles, number of private alleles and expected heterozygosity. Results of the *t*-test with Welch modification are given for each comparison.

**Table 2**
Loci with significant temporal changes in allele frequencies between adults and saplings. Observed Weir & Cockerham's $F_{ST}$ and test statistics for the simulation test ($p_{ST}$), $F_T$ test ($p_{FT}$) and Waples test ($p_{WT}$) for managed and old growth stands are presented. Significant test statistics and $F_{ST}$ values at $p < 0.05$ are marked in bold and those at $p < 0.10$ in italics.

| | Managed stand | | | Old growth stand | |
|---|---|---|---|---|---|
| Locus | Fs5 | Fs6 | Fs8 | Fs6 | Fs10 |
| $F_{ST}$ | 0.031 | 0.017 | 0.011 | 0.029 | 0.053 |
| Test statistic | | | | | |
| $p_{ST}$ | **0.007** | *0.060* | *0.057* | **0.004** | **0.016** |
| $p_{FT}$ | **0.027** | **0.040** | 0.170 | **0.009** | **0.015** |
| $p_{WT}$ | **0.026** | 0.132 | **0.029** | **0.012** | *0.083* |

This case study has a few drawbacks; two stand out in particular. Firstly, the sample size consisting of 35 individuals per cohort might be small for observations based on number of private or rare alleles. Theoretically 30 diploid individuals are necessary for a 95% probability of detecting an allele at a frequency of 0.05, which was also confirmed with an empirical dataset (Hale et al., 2012). Therefore, though we sampled 35 individuals, we probably did not sample all private or rare alleles, especially those with frequencies lower than 0.05, and their mean numbers deducted from the samples might differ from the actual ones in the cohorts. But for population-based studies, detecting all of the alleles present is not as important as ensuring that the frequencies of the alleles detected are representative of those in the total population, which can be achieved without sampling alleles present at very low frequencies (Hale et al., 2012). Ensuring representative allele frequencies for microsatellite loci can be achieved by sampling 25–30 individuals per population, particularly when sampling from relatively large populations, as was shown in a study based on a comparison of allele frequencies, expected heterozygosities and genetic distances between real and simulated populations by randomly subsampling 5–100 individuals from four empirical microsatellite genotype datasets belonging to different taxons (Hale et al., 2012). The result is also supported by recent assessment of the impact of sample size on genetic differentiation for highly polymorphic loci (Kalinowski, 2004) but in contrast to previous suggestions that large sample sizes are needed to accurately describe population structure (Nei, 1978; Ruzzante, 1998). Secondly, studied stands are not true pair populations as they are separated by 90 km and do not belong to the same ecological region (Kutnar et al., 2002) but nevertheless both belong to the same phytocenological alliance (*Aremonio-Fagion*) in the (alti)montane belt (Dakskobler, 2008). Also, the whole territory of Slovenia was one of the main source areas for the postglacial development of beech and the most important glacial refugia for its recolonization (Magri et al., 2006; Magri, 2008; Brus, 2010) though individual south facing microrefugia probably existed (Brus, 2010). In beech, most differentiation was found between regional populations originating from different glacial refugia and for different postglacial recolonization routes (Gömöry et al., 1999; Comps et al., 2001; Magri et al., 2006) therefore making the territory of Slovenia a relatively homogenous from the genetic perspective, apart from the Submediterranean (Brus et al., 1999). Yet due to a long development of beech forest in the same area ecological races might exist (Robson et al., 2010; Božič and Kutnar, 2012). In this study, highly polymorphic microsatellites were used and previously undiscovered genetic differences became clearly visible; also a beech stand belonging to the same ecological region and alliance as the studied old growth, 15 km away, differed significantly from the old growth (data not shown, only adults sampled). Despite the shortcomings, the results show the temporal dynamics of the shifts in genetic variability and structure of the cohorts in both managed and unmanaged stands as well as enable comparison of both studied stands.

Our observation that small scale management such as ISS did not affect genetic diversity of beech trees in this case study is supported by studies analysing the effect of the shelterwood uniform system (Buiteveld et al., 2007; Shanjani et al., 2011; Paffetti et al., 2012) and diverse silvicultural measures including stands managed according to group or individual tree selection (Konnert and

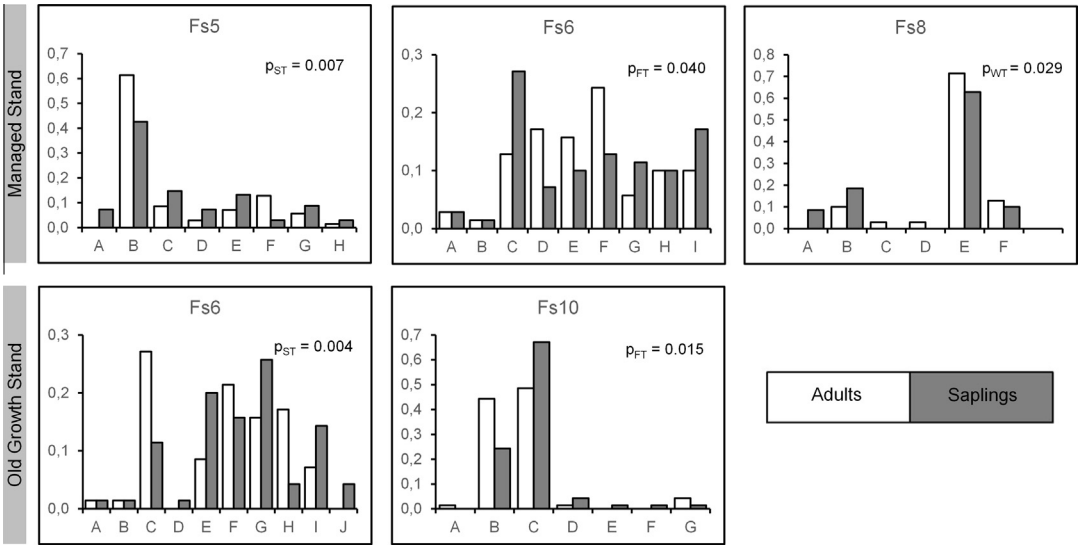


**Fig. 2.** Distribution of allele frequencies for loci exhibiting significant temporal changes in allele frequencies with at least one test [Simulation test (ST), $F_T$ test (FT), Waples test (WT)] in the managed and old growth stand. *p* values of the test with the lowest *p* value obtained after $10^6$ simulations with the TAFT 2.3 programme are reported.

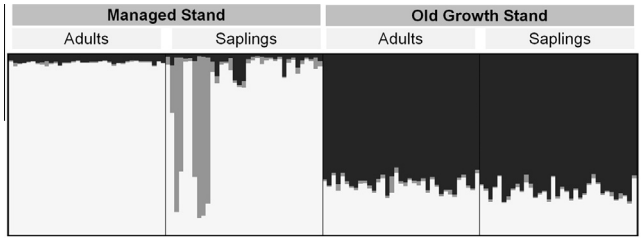


**Fig. 3.** Genetic structure of the four cohorts obtained with the Bayesian clustering using Structure 2.3.4 and printed using Distruct 1.1 (Rosenberg, 2004) software. Average membership proportion of each individual is printed.

Hosius, 2010; Rajendra et al., 2014) on the genetic diversity of beech. In the studied old growth, stand management activities were officially banned in 1904; prior to that it was a virgin forest. There was only a minor felling at the border zone of the reserve approximately 100 years ago and in 1948, when 7% of the growing stock was cut (Kraigher et al., 2002). This indicates that the natural development of the old growth stand was never directly disturbed, providing us with a true comparison of the managed stand. Results show that genetic diversity at microsatellite loci in the old growth strand was similar to the diversity levels observed in the managed stand. The biggest, although not significant, difference between the managed and old growth stands was in the number of observed rare alleles; fewer rare alleles were observed in the managed stand, an observation that could be a result of the different genetic composition of the two populations as discovered in the Structure analysis or influenced by our sample size. Still, sampling design should not be driven by the need to sample all the rare alleles present in a population, since they add very little information to population-based studies and on average the accuracy of their frequencies does not improve substantially with increasing sample size (Hale et al., 2012). The share of lost and gained alleles was slightly higher for the old growth than for the managed stand (0.13 and 0.10 for lost alleles and 0.12 and 0.08 for gained alleles) indicating that the old growth might be a more dynamic system than the managed stand. This observation could also be due to the reciprocal replacement of silver fir with beech, particularly in the Dinaric silver fir-beech forests (Boncina et al., 2003; Diaci et al., 2010). Still, proportion of beech in Slovenia has been increasing in its most optimal habitats belonging to forest category 'Beech forests' (Poljanec et al., 2010), into which forests of the alliance *Aremonio-Fagion* (i.e. both stands in our study) belong. Moreover, almost all alleles lost in the regeneration in both managed and unmanaged stands were replaced by new alleles, not observed in the adult cohort, indicating that ISS mimics the natural regeneration processes of the old growth rather well. While we compared the loss of alleles between two generations as our studied stands originate from different gene pools, loss of alleles in a coppice stand of beech compared to an old growth not managed for at least 400 years was reported by Paffetti et al. (2012). On the other hand, Rajendra et al. (2014) and Buiteveld et al. (2007) noted that where management of the unmanaged stands had recently ended (i.e. at most one to two generations ago with some exceptions) they did not observe any loss of rare alleles. As seen in an isoenzyme study for small scale patch regeneration of beech by Konnert and Hosius (2010) and suggested by Paffetti et al. (2012), small scale management systems such as ISS in our study did indeed successfully maintain genetic diversity in the next generation of the managed stand in this study as compared to the old growth strand, where slightly higher share of alleles was lost and gained than in the managed stand.

As expected for a highly outcrossing, wind pollinated species nil or weak inbreeding was observed in our study in both the managed and old growth stands. This was in contrast to studies using a low number of microsatellite markers with a high frequency of null alleles (Buiteveld et al., 2007; Paffetti et al., 2012), but in line with the results obtained by Rajendra et al. (2014). The low but significant value of the inbreeding coefficient in the sapling population of the old growth stand was explained by the presence of null alleles at locus Fs3.

### 4.2. Genetic differentiation of the cohorts

Both adult populations had genetically distinctive structures that were transferred to the offspring population. However, in the managed population six individuals from regeneration centre I differed in their genetic structure from the rest of the saplings and adults. A private allele at locus Fs5, possibly originating from the same unsampled mother tree (results not shown) found in five of this individuals, can partly explain their distinct genetic structure. As the centre was formed by natural regeneration,

two scenarios may explain the observed state. Firstly, the private allele could have originated from an unsampled adult tree in the vicinity of the regeneration centre. This is a very likely scenario as mean seed dispersal distance is approximately 10 m for beech (Oddou-Muratorio et al., 2010) and spatial genetic structure is reported to extend mainly up to 10 or 20, rarely to 40 m in beech (Piotti et al., 2013; Rajendra et al., 2014). The distance from the midpoint of regeneration centre I, where this six individual were sampled, to the closest sampled adult tree was 7 m; all other sampled trees were at least 30 m from the regeneration centre. Secondly, the distinct genetic structure may have been caused by pollen immigration. This regeneration centre is situated by a forest road, making long distance pollen immigration a convenient way to introduce new alleles. Beech has a high potential for pollen dispersal with mean within population pollen dispersal distances between 40 and 180 m (Oddou-Muratorio et al., 2010; Oddou-Muratorio et al., 2011; Piotti et al., 2012). In addition, high rates (approximately 75%) of pollen immigration into small to medium size plots were reported (Piotti et al., 2012). Additionally, saplings with the distinct structure could have originated from another mast year than the rest of the saplings; some saplings from this regeneration centre were by 0.5 m taller and up to 2 cm thicker than the rest of the saplings at Osankarica research site. Unfortunately, height and diameter measurements of saplings were not directly linked to the sampled individuals but rather represent averages for the regeneration centres and age of sampled seedlings was not recorded during sampling. As ISS is a long term oriented sylvicultural system with gradual opening of the canopy, seeds from more than one mast year coming from many parent trees will contribute to the new generation – the formation of the new stand. Numerous gene combinations will occur in the seedlings, on which natural selection will act and remove the maladapted genotypes.

There is no evidence to demonstrate that the distinct genetic structure of the six individuals was caused by management according to ISS. It is more likely a result of unsampled adult genotypes, no matter if the studied saplings originated from the same or different mast years.

### 4.3. Temporal changes in allele frequencies

Some studies of forest trees used $F_{ST}$ to evaluate differences between two temporally divergent populations, i.e. different developmental phases (Maghuly et al., 2006; Bilela et al., 2012). However, $F_{ST}$ indicates subpopulation differentiation because of restricted gene flow among subpopulations and is not meant to quantify temporal changes. In this study, we used a simulation procedure to test whether forces other than drift and sampling error contributed to differentiation of allele frequencies between two consecutive generations of beech. Although erroneous, we also used conventional $F_{ST}$ analysis for comparison.

Temporal changes in allele frequencies caused by forces other than genetic drift and sampling error between adult cohorts and saplings were detected in both the managed stand at three loci and in the old growth stand at two loci. Apart from the drift and sampling effect, management could have caused some changes in allele frequencies between the generations in the managed stand but could not explain all significant differences in allele frequencies because these were observed in both the managed and old growth stands, having locus Fs6 in common. Directional selection could have caused the observed changes but none of the loci were identifies to be outliers, making selection an unlikely cause of the temporal changes in allele frequencies in this study. Beech is currently expanding in Slovenia (Poljanec et al., 2010) and reciprocally replacing silver fir, particularly in the Dinaric silver fir-beech forests (Boncina et al., 2003; Diaci et al., 2010); both processes might contribute to the differences in allele frequencies between the adult and offspring generations in our study.

Only some of the individuals from the studied regeneration centres will be recruited into the canopy of the future stand; which ones will be greatly influenced by light conditions (Petritan et al., 2007) governed by gap size and canopy structure (Rozenbergar et al., 2007; Nagel et al., 2010), forest type, soil pH and basal area (Klopčič and Bončina, 2012). Yet our results show that genetic diversity and possibly structure of the recruited individuals will most likely be similar to that of the adults in both studied stands, at least according to neutral markers used in the study.

## 5. Conclusions

In the presented case study, we examined the potential effects of ISS on genetic diversity and structure of a European beech stand by (i) comparing managed stand to old growth beech stand and (ii) comparing two successive generations in both managed and old growth stands. No convincing evidence of the effect of ISS on genetic diversity of the managed beech stand was found despite the observed (but not significant) lower number of rare alleles in the managed stand and significant shift in allele frequencies between generations. The latter could not be unambiguously attributed to management.

Because this case study is exclusively based on neutral markers, the effect of ISS on the adaptive potential of the studied beech stand remains unknown.

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