Genetic structure of spruce population and forest management

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Abstract

Genetic diversity and spatial structure of a young planted Norway spruce (*Picea abies* Karst.) stand, localized in the Giant Mts. near Špindlerův Mlýn, was investigated. Average tree height was 15.1 m, average diameter at breast height was 245 mm. The spatial distribution of trees was random. Spruce branches of all trees in the selected part of the stand were sampled in order to accomplish isozyme analysis. One–dimensional horizontal electrophoresis on starch gel was used to determine the alleles of isozyme loci G–6–PDH, GDH, SDH–A, SDH–B, PGM–A, IDH–B, AAT–B and AAT–C. Subsequently, allele numbers per loci, allelic frequency and heterozygosity were evaluated. Genotype combinations were a basis to calculate coefficients of genetic diversity: Shannon's index and Rao's quadratic entropy. Further, three variants of thinning with removal of 30% of the trees in each case were simulated: trees with the highest diameter were removed in the variant v1, 15 % of the trees with the highest and 15 % with the lowest diameter in the variant v2, and the trees with the lowest diameter in the variant v3. The genetic diversity is affected by the simulated thinning. Differences in heterozygosities and diversities between the whole population and subpopulations were statistically tested using the Monte–Carlo randomization test.

Keywords: genetic diversity, heterozygosity, isozyme, *Picea abies*, population structure, spatial structure

Introduction

The Norway spruce (*Picea abies* (L.) Karst.) dominated ecosystems represent a typical kind of forests near the alpine timberline in the Central Europe (SCHMIDT-VOGT 1977). These ecosystems grow in extreme environmental conditions. The spruce forests in the Czech Republic were often under high air-pollution impact, which has caused dieback of the stands. Such a situation was observed in a large area in the Giant Mountains (Krkonoše Mts.) (VACEK et al. 2007). Wind is another environmental factor that has caused the large-area disintegration of the tree stands during recent history in the Giant Mountains. These facts lead to existence of many ecosystems with young Norway spruce stands in sites near alpine timberline (8th forest altitudinal zone in sense of the Czech forest typological system; VIEWEGH et al. 2003). Under such circumstances, the studying of genetic properties of these young populations and possibilities of their further development is of outstanding importance.

Knowledge on the Norway spruce genetic features has the wide background in literature.

Many studies in the forest genetics have used isozymes belonging to the common types of molecular gene markers. The isozyme analysis represents a method useful to description basic genetic variation both within a population and between different populations.

Geographical isozyme variability

The large-area oriented research of the Norway spruce isozyme variation in Europe was carried out in several projects (KANNENBERG et GROSS 1999; PERRY et al. 1999; KRUTOVSKII et BERGMANN 1995). Isozyme polymorphism of the Norway spruce populations from 15 European provenances, which had been included into the IUFRO testing programme, showed that some alleles of the SDH–A, IDH–A, LAP–A and GDH loci exhibited a geographic pattern. The measures of genetic diversity showed lower values in Southern and Central Europe than in northern and north–eastern part of the continent, probably resulting from introgressive hybridization with other species (KRUTOVSKII et BERGMANN 1995). For most provenances, significant correlations were found between genetic dissimilarity and geographic distance (KANNENBERG et GROSS 1999). The genetic diversity and differentiation was also described in the Italian spruce populations by GIANNINI et al. (1991) who found that only a small part of the total genetic variation is due to interpopulational differentiation. On the other hand, no variation of allele frequency patterns along latitude or altitude in Norway spruce populations was found in the small geographical region – Black Forest (Schwarzwald) (KONNERT 1991b).

Regional studies of isozyme systems by Norway spruce are known from Italy (GIANNINI et al. 1991), Austria (GEBUREK 1998), Germany (KONNERT 1991a, b, KONNERT et FRANKE 1991), Poland (BURCZYK et al 2004; CHAŁUPKA et al. 2008), Latvia (GONCHARENKO et al. 1995) and Ukraine (KORSHIKOV et al. 2008). The study of spruce seed orchards (PAULE et al. 1993) belongs to the first isozyme studies of the Norway spruce populations, stands and seed orchards in region of the former Czechoslovakia. Norway spruce populations from the Ore Mts. and Giant Mts. were compared on the base of isozyme analyses in the framework of different environmental conditions (IVANEK 2000). MÁNEK (1999, 2001) was concerned in the Norway spruce stands in the Šumava Mts., having compared genetic characteristics of three autochthonous populations. Genetic study of the Norway spruce populations in the Czech Republic was accomplished for 10 ICP–Forests monitoring plots. The results suggested that the genetic diversity of the Norway spruce populations was determined both by reproductive material used and by genotype selection under different site factors (IVANEK 2006).

Population spatial pattern

Natural populations of plants can be spatially organized. This spatial pattern can be observable according to the genetic features, too. There are known statistical procedures, which are suitable to reveal these relations (e.g. HARDY et VEKEMANS 2002).

Using isozymes as gene markers and spatial autocorrelation of allozyme traits, random distribution of genotypes in space was found in most cases in the model spruce populations from the eastern Italian Alps. However, a few genotypes showed a significant clumped distribution over a small spatial scale (LEONARDI et al. 1996).

Weak spatial pattern and non-random association of genotypes were observed within autochthonous, high-elevation Norway spruce in Austria, which was explained as a result of microselection or statistical artefact (GEBUREK 1998).

Research in the Giant Mountains

The isozyme analysis of dormant buds of the Norway spruce clonal plantations from nurseries in the Giant Mts. was performed in order to certificate clonal homogeneity. Molecular genetic characteristics of the clones were compared with growth and phenology characteristics (IVANEK et MARTINCOVÁ 2005, 2006). Genetic differences between two Norway spruce tree subpopulations in the Giant Mountains (Krkonoše in Czech) National Park, the Sněžka Mt. region, differing markedly in the extent of damage after the Kyrill hurricane (2007), were investigated using isozyme analysis and biometric measurements including preliminary tree-ring analysis. Genetic distances of investigated spruce sets in framework of overall six spruce populations were calculated (IVANEK et al. 2009).

The main objectives of the present study were to:

- describe genetic structures in a Norway spruce population by using isozyme markers,
- study genetic variation within groups of young trees with different phenotypes and give evidence about possible genetic differentiation among them,
- assess the impact of different thinning models on the genetic variation of the population.

Methods

Stand description and sampling

The young planted Norway spruce stand was investigated (age of 39 years at 2008) at the locality near Špindlerův Mlýn in the Giant Mts., forest enterprise Vrchlabí, stand group $202F_4$, altitude 1080 m, inclination 25°, south–western orientation.

Actual vegetation can be described by the relevé (number 131/09, date 25.8.2009): total cover E_3 70 %, E_2 0 %, E_1 25 %, E_0 60 %. E_3: *Picea abies* 4, *Sorbus aucuparia* +; E_1: *Avenella flexuosa* +, *Blechnum spicant* r, *Calamagrostis villosa* 2, *Carex echinata* r, *Carex ovalis* +, *Dryopteris dilatata* 1–2, *Galium saxatile* +, *Gentiana asclepiadea* 1, *Hieracium lachenalii* +, *Homogyne alpina* +–1, *Juncus effusus* r–+, *Oxalis acetosella* 1, *Polygonatum verticillatum* r, *Rubus idaeus* +, *Rumex arifolius* r, *Senecio ovatus* 1–2, *Sorbus aucuparia* r, *Vaccinium myrtillus* +. This community corresponds to a transition between 7th and 8th forest altitudinal zone.

The investigated trees were selected in the field as continuous closed spatially-convex set of individuals. The research plot was determined as space occupied by these selected trees. All Norway spruce trees have been sampled in the plot. Spruce branches of all trees in the selected part of the stand were sampled (spring 2008) in order to accomplish isozyme analysis. Each tree was permanently marked at this time. All samples were transported into the laboratory immediately. The collected samples were stored in the freezer storage box at – 20 ± 3 °C by the time of analyze.

The position of each tree was measured using the Field–Map technology (www.fieldmap.cz) at May 2009. Tree perimeter at breast height (which was recalculated to tree diameter – DBH) and tree height (h) was recorded as the basic tree size features. The crown defoliation was assessed in per-cents. Occurrence of the trunk damage (breaks, forks, trunk curvature, visible fungus infection etc.) was recorded. The spruce individuals were separated in classes according to branching using known system (SAMEK 1964; SCHMIDT-VOGT 1972), which was simplified regarding the young population without sufficient manifestation of attributes to be used.

Isozyme analysis

Dormant buds from sampled branches were collected and homogenized with modified extraction buffer (pH 6.7) for tissues with high levels of interfering substances (WENDEL et WEEDEN 1990). The isozymes were separated by horizontal one-dimensional electrophoresis of the homogenate on starch gel at 3 – 5 °C in the Tris-citrate buffer (pH 7.5) using Multiphor II electrophoretic device. Each sample was randomly located at two gels. Each gel was completed by one comparative standard sample. Eight isozyme systems (Table 1) were stained according to PASTEUR et al. (1988) at 37 ± 0.5 °C. The gels were scanned and evaluated with ImageMaster software (Pharmacia Biotech).

Table 1. Assessed enzymes and scored loci.

Enzyme	E. C. No.	Scored loci
Glucose–6–phosphate dehydrogenase	1.1.1.49	G–6–PDH
Glutamate dehydrogenase	1.4.1.2	GDH
Shikimate dehydrogenase	1.1.1.25	SDH–A
Phosphoglucomutase	2.7.5.1	PGM–A
Malate dehydrogenase	1.6.99.3	MDH–B, MDH–C
Isocitrate dehydrogenase	1.1.1.42	IDH–A, IDH–B
Aspartate aminotransferase	2.6.1.1	AAT–B, AAT–C

Data processing

The spatial data was processed in GIS (TopoL software; www.topol.eu) and using special software (PlotOA; http://www.infodatasys.cz/software/hlp_PlotOA/PlotOA.htm). Spatial distribution of selected allele within the stand was evaluated using the L-function (derived from the Ripley's K-function). No correction on the border effect by the selected study plot was applied. Values L(h)-h against distances h were plotted (SCHABENBERGER et GOTWAY 2005: pp. 101-103).

Data exported from the ImageMaster programme was stored into the IsoEnz database (MATĚJKA 2009a). Each allele pair determination was carried out on the base of relative mobility using the SeqAn programme (MATĚJKA 2009b). The comparison within set of all gels was used in order to a unique identification of alleles over all trees analysed in a few gels. Alleles were numbered in increasing order from the slowest one.

Allele numbers per loci, allelic frequency and observed heterozygosity were evaluated as measures related to the single locus. Differences between observed heterozygosities h_1 and h_2 in the populations 1 and 2 of the population sizes n_1 and n_2 was statistically tested using test variable which has the Student t–distribution with n_1+n_2-2 degrees of freedom (e.g. ŠKRÁŠEK et TICHÝ 1990: pp. 259–262).

$$t = \frac{h_1 - h_2}{\sqrt{\left(h_1(1-h_1)/n_1 - 1\right) + \left(h_2(1-h_2)/n_2 - 1\right)}} \qquad (1)$$

Indices of diversity were evaluated using all processed enzymatic systems. Each individual tree was assigned in a genotype class using the combination of all studied allele pairs as a multilocus genotype. For instance, it is possible to use the sequence "222223222222222212" as an identification string of the genotype class identified on the base of the sequence of G6PDH (22), GDH (22), SDH–A (23), PGM–A (22), MDH–B (22), MDH–C (22), IDH–B (22), AAT–B (22) and AAT–C (12) enzyme loci. Genetic diversity of the population was evaluated using the following parameters:

- Number of classes (c) over the combinations of all-loci (number of different identification strings) is the simplest parameter.
- Potential number of classes (c_{pot}) – theoretical number of all possible identification strings combining all alleles presented in the investigated set of trees can be viewed as the indicator of diversity in a theoretical population of unlimited size.
- The Shannon's index *H which is given by*

$$H = -\sum_{i=1}^{c} p_i \ln_2 p_i \,, \tag{2}$$

where *c* is the number of the classes; p_i is relative proportion of trees within the *i*–th class; SHANNON et WEAVER 1949) was modified in the population genetics in several cases earlier (e.g. AFIF et al. 2008; LIA et al. 2008). Equitability $e = H / \ln_2 c_{pot}$ completes set of the indices.

- The last index is the Rao coefficient *R given by*

$$R = \sum_{i=1}^{c} \sum_{j=1}^{c} d_{ij}\, p_i\, p_j \,, \tag{3}$$

d_{ij} has been calculated according to the equation $d_{ij} = a_{ij} / 2N$, where a_{ij} is number alleles to be not contained in both identification strings and *N* is number of loci. This index is an adoption of the quadratic entropy measure (RAO 1982) in population genetics.

Because of any selection of the subset of individuals from the population would lead to changes in the population-genetic parameters (heterozygosities, indices of genetic diversity), the mere comparison between population and subpopulation parameters is not sufficient. Difference of these parameters is necessary to be statistically tested. But unfortunately, there is no easy connection between these numbers (diversity indices, heterozygosities) and a standard statistical distribution such as the Normal, Poisson or Chi–squared. Such classical statistical testing is problematic. The Monte–Carlo randomization test would be an effective manner of such testing (MCDONALD 2009). This test estimates the probability that a parameter in the random subpopulation (of the equal size as the comparable subpopulation) would be less or equal to the parameter, which has been calculated in the comparable subpopulation. Statistically important decrease of the parameter would be found if this probability is less or equal to the selected limit (obviously 5 %). Analogously, increase of the parameter would be found if this probability is great or equal to the value one minus selected limit (obviously probability 95 %). Similar simulation of the randomized sampling in the population study is known from the literature (e.g. WEGMANN et al. 2010).

All indices and Monte–Carlo tests were calculated using the IsoEnz software (MATĚJKA 2009a).

The population was divided into two or more subpopulations according to the selected feature. The subpopulations have been defined on the base of following features:

- Phenotype: plate-shape types × comb-shape types.
- Trunk damage (with breaking, doubled trunk, other damaged trunk): damaged trees × undamaged trees.
- Tree defoliation: all trees × less defoliated trees (up to the 30%-limit).
- Three subpopulations were defined using tree diameter (DBH) as a hypothetical result of thinning model.

Total 30 % of trees would be cut (removed) according to three simulated variants of thinning:

- 30 % trees with the highest diameter (the variant v1) should be removed, the subpopulation consists of individuals with low DBH;
- 15 % trees with the highest diameter and 15 % trees with the lowest one (v2) should be removed;
- 30 % trees of the lowest diameter (v3) should be removed.

Dissimilarity between subpopulations was calculated according to NEI (1978).

Results and discussion

The stand structure

The 112 trees were selected (108 individuals of Norway spruce and 4 rowan polycormons). Total 36 trees were indicated as spatially limit ones, used to determine the plot extent. Total plot area was 1391 m^2. The Norway spruce density was 777 individuals per 1 ha. Spatial distribution of trees in the stand in relation to the tree diameter at breast height is shown in the Fig. 1.

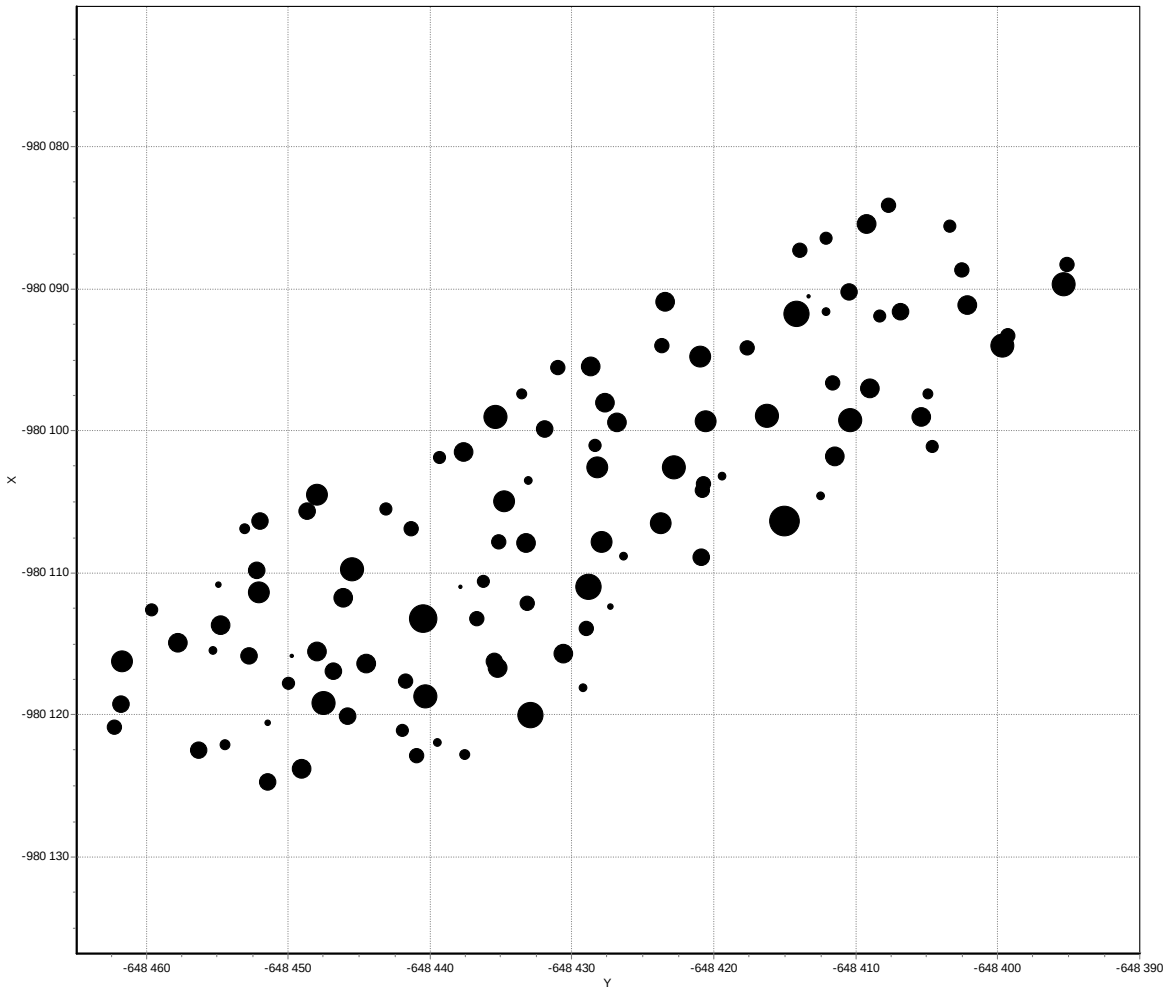

Fig. 1. Spatial distribution of trees in the stand. Point size is related to tree diameter at breast height (range of values is 114 – 388 mm).

Average tree height was 15.1 m (standard deviation ±2.3 m; total 106 individuals), average diameter at breast height was 245 ± 50 mm (2009). Mean tree defoliation was 29 ± 15 % with interval 5 – 90 %. Twenty six percent of trees were with damaged trunks. Average parameters of undamaged trees (n = 78) were slightly different: tree height 15.8 ± 1.3 m, DBH 258 ± 42 mm and defoliation 24 ± 9 %.

Although more branching types are known by Norway spruce (SAMEK 1964; SCHMIDT-VOGT 1972), only two classes were possible to be distinguished in the young population where phenotype signs have not been fully differentiated yet. Trees with the plate-shape branching prevail. The class of comb-shape types includes a wide variability from brush- to comb-shaped branching. It would be advisable to put the p-values in Table 2 for all two-factor ANOVA comparisons (A, B, C, D) and indicate significance with tree height, DBH or defoliation. Tree membership to the group according to branching type had no effect since average tree-size variables were slightly higher in the comb-shape type group and average defoliation was lower (Table 2).

Table 2. Differentiation of basic dendrometric features between selected groups of trees.

A. Number of individuals.

	Damaged	Undamaged
Plate-shape types	57	27
Comb-shape types	21	1

B. Tree height – mean ± standard error (in m).

	Damaged	Undamaged
Plate-shape types	15.5±0.3	13.1±0.4
Comb-shape types	16.6±0.4	12.9±2.0

C. DBH – mean ± standard error (in mm).

	Damaged	Undamaged
Plate-shape types	250±6	213±9
Comb-shape types	278±10	140±44

D. Defoliation – mean ± standard error (in %).

	Damaged	Undamaged
Plate-shape types	26±2	42±2
Comb-shape types	19±3	40±12

Genetic structure of the population

All loci except IDH–A were polymorphic whereas high allele numbers were observed by AAT–C and SDH–A. Rare allele of AAT–B and very rare allele of SDH–A were found (Table 3, number of alleles only shown). Polymorphic isozyme loci have previously been reported by KONNERT et MAURER (1995) in a study of spruce populations in Central Europe. In the same study, LAP-B (does not included in our study) was the most variable locus. The SDH-A and AAT-C loci were also highly variable. Observed polymorphism was only in half of the loci higher than in the work from IVANEK (2006). Comparing coefficients H and R in the studied population (Table 5) with several other spruce stands in the Czech Republic (IVANEK et al. 2009), moderate genetic diversity of the investigated spruce stand can be claimed. Slightly higher value of total allele number but lower number of classes, lower H coefficient and slightly higher R coefficient for the same set of isozyme loci were found in our study in comparison with the mature Norway spruce population growing near Sněžka Mt. (25 alleles, 37 classes, H= 4.45, R=0.097; IVANEK et al. 2009). Genotypes are not randomly distributed in the stand. The spatial distribution of some alleles in the stand was clumped. Size of such tree clusters was approximately 8 to 12 m. Such situation is visible by AAT-C locus, allele 0 (Fig. 2) and by SDH-A locus, allele 2 (Fig. 3). This fact can be viewed as a result of distribution of the planted trees. It is in accordance with clumped distribution over a small spatial scale reported by LEONARDI et al. (1996). Similar effects of family structures on the spatial genetic variation in young beech populations were concluded by DOUNAVI et al. (2010), whereby natural selection eliminated family structures in old beech stands.

Table 3. Number of alleles in the Norway spruce populations referred in some studies.

Data set	This study	Central Europe (KONNERT et MAURER 1995)	ICP Forests (IVANEK 2006)	Bohemian Forest (MÁNEK 1999)	Giant Mts. (IVANEK et al. 2009)
AAT–B	2	3	n.d.	2	1
AAT–C	4	5	n.d	3	3
G–6–PDH	2	4	2	n.d.	2
GDH	2	3	2	2	2
IDH–A	1	6	1	3	1
IDH–B	2	3	1	2	2
MDH–B	2	3	1	1	1
MDH–C	2	5	1	3	3
PGM–A	2	4	2	2	2
SDH–A	5	7	4	2	4
All	24	43	n.d.	n.d.	21

n.d. - not determined; A - total number of alleles.

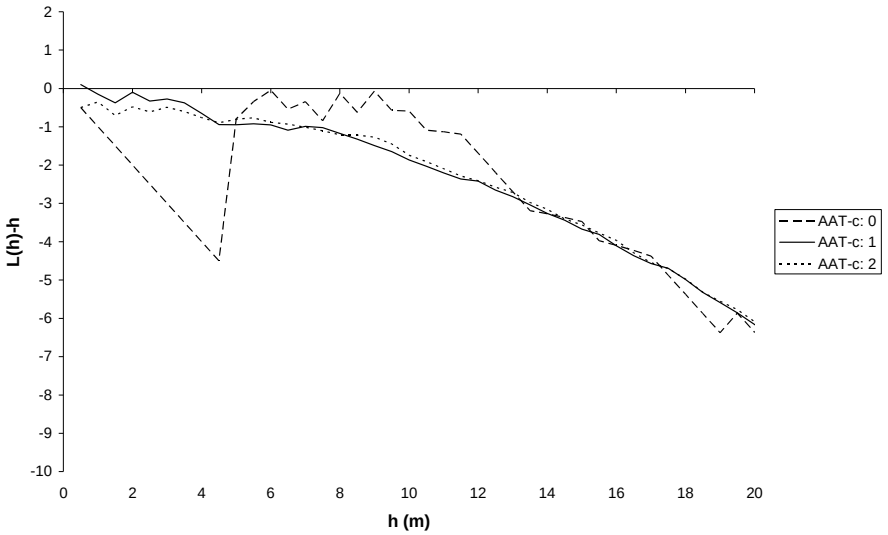

Fig. 2. Spatial distribution of trees with distinguished AAT–C alleles evaluated by the L function related to distance h.

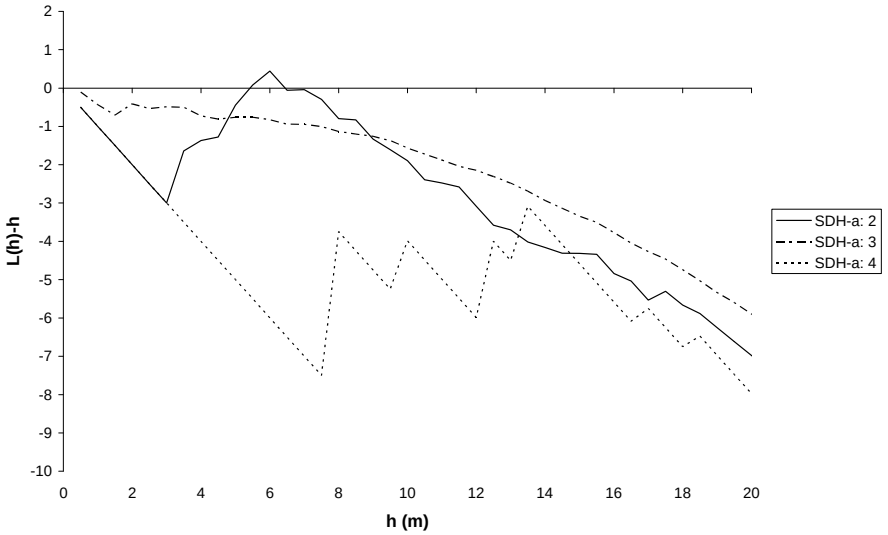

Fig. 3. Spatial distribution of trees with distinguished SDH–A alleles evaluated by the L function related to distance h.

Phenotype differentiation according to the branching type

Comparing genetic diversity of two phenotype groups, higher observed AAT–C heterozygosity was found in the group of comb-shape phenotypes. Difference between the G–6–PDH heterozygosities was statistically significant ($p<0.05$). On the other hand, Shannon's index of diversity (see eq. 2) do not differ between both subpopulations and Rao's index (eq. 3) would be higher in the plate-shape type subpopulation with considerable statistically importance (probabilities by the Monte–Carlo test was 87 % against 12 %; Table 4). The Nei–distance between two subpopulations of both phenotype groups is 0.0036 (data not shown) using all investigated loci – this value is comparable with the distance between populations growing in a region (Bohemian Forest, 0.004; MÁNEK 1999) and lower than the distance between populations of origin in different regions (Giant Mts. – Jeseníky Mts., 0.068; IVANEK et al. 2009).

Table 4. Comparing genetic diversity of two phenotype groups.

Phenotype	Plate-shape types	Comb-shape types	p
Tree count	85	22	
Observed heterozygosity:			
AAT–B	0.013 (75.0%)	0 (50.0%)	0.148
AAT–C	0.493 (13.3%)	0.650 (90.6%)	0.083
G–6–PDH	0.145 (85.8%)	0.046 (16.1%)	**0.049**
GDH	0.013 (29.6%)	0.048 (72.9%)	0.228
IDH–B	0.012 (38.5%)	0 (50.0%)	0.157
MDH–B	0.013 (75.0%)	0 (50.0%)	0.148
MDH–C	0.050 (62.5%)	0.046 (47.0%)	0.465
PGM–A	0.012 (38.0%)	0 (50.0%)	0.157
SDH–A	0.241 (34.4%)	0.318 (69.4%)	0.246
Diversity:			
Number of classes	28	13	
Potential classes no.	196830	2700	
H	4.0434 (59.4%)	3.4714 (75.4%)	
e	0.2299	0.3045	
R	0.1006 (87.3%)	0.0803 (11.6%)	

Probability of the Monte–Carlo test (for difference population – subpopulation) is in parenthesis. p – error probability by the t–test of heterozygosity difference.

Model thinning

Decrease of heterozygosity was observed in the variant v1 for the G–6–PDH, GDH and AAT–C loci. Considerable decrease of allele number per locus (indicated by the zero heterozygosity at the AAT-B, MDH-B and PGM-A loci) was observed in the variant v3. Heterozygosity loss was found for the MDH–B, MDH–C, and AAT–B loci in this variant, but none decrease is statistically significant. Commonly, the statistical significance of the heterozygosity differences is minor, changes in the total allele count are small (Table 5).

Number of genotype classes was reduced in all variants. Potential number of genotype classes was unchanged in the variant v1 and substantially reduced in the variant v3. The Shannon's index was the highest in case of the v2 variant (probability 95 %). Difference in the Rao's index between variants was highest comparing variants v2 and v3 (corresponding probabilities 94 % and 26 %; Table 6). While Shannon's index of genetic diversity is very sensitive to the subpopulation size (Fig. 4), Rao's index is stabile regarding the number of individuals (Fig. 5). Similar stability was observed by the observed heterozygosity (Fig. 6).

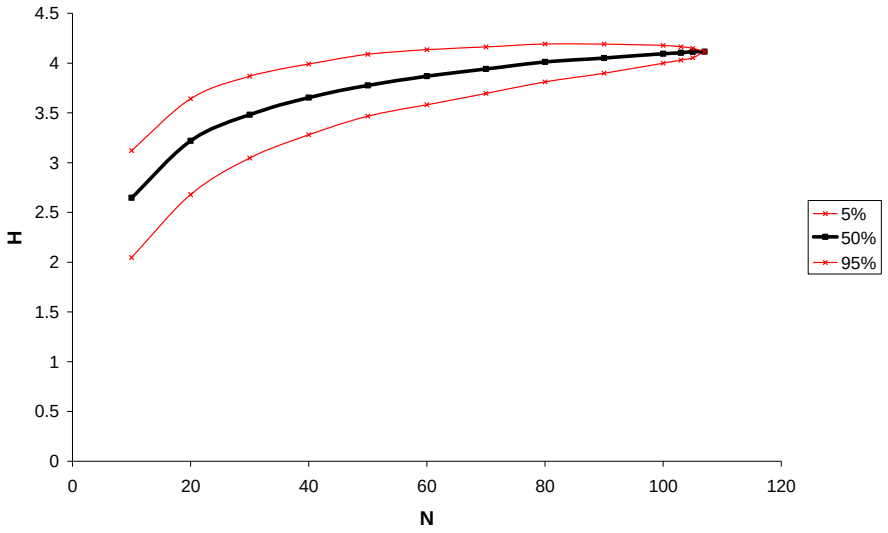

Fig. 4. Dependence of the Shannon's index of diversity (H) for the subpopulation of different size (N) – median and confidence interval.

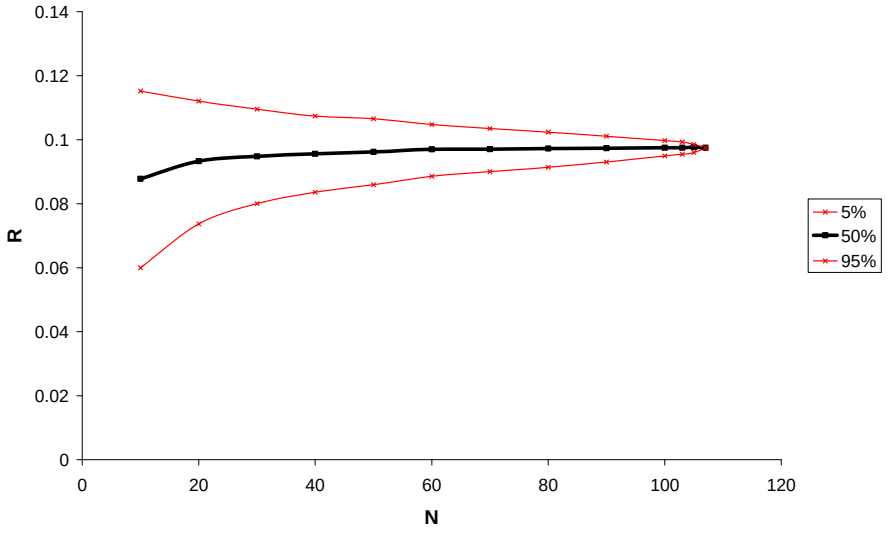

Fig. 5. Dependence of the Rao's index of diversity (H) for the subpopulation of different size (N) – median and confidence interval.

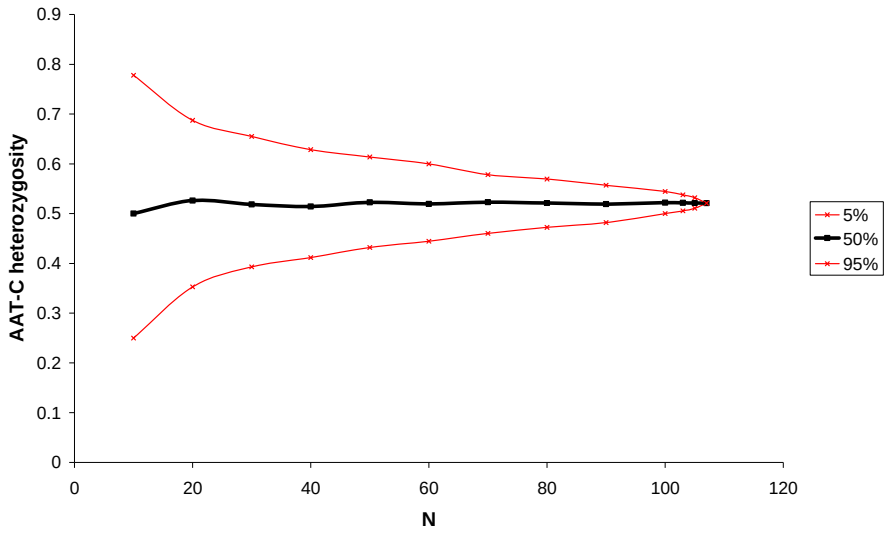

Fig. 6. Dependence of the observed heterozygosity (on example of AAT–C locus) for the subpopulation of different size (N) – median and confidence interval.

The Nei distance among three hypothetical populations and the original population without thinning were calculated (Fig. 7). The highest distance (0.00014) was observed between variants v1 and v3. This value is approximately four-time lower than dissimilarity between two mature Norway spruce subpopulations in the locality near Sněžka Mt. (IVANEK et MATĚJKA 2009. The combined thinning variant (v2) should be the best considering the genetic diversity preservation.

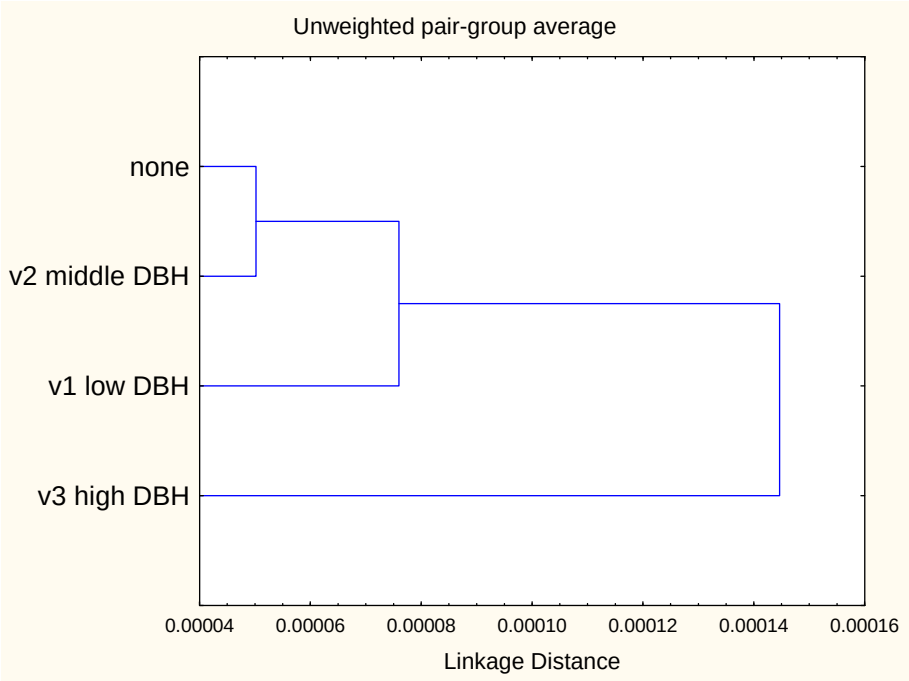

Fig. 7. Classification of the hypothetical Norway spruce populations formatted by different thinning (see Tables 5–6). The Nei distances were used as the base to calculate the linkage distance.

Loss of rare alleles and moderate changes of observed heterozygosity after thinning is known from literature. FINKELDEY et ZIEHE (2004) referred about such loss if the high proportion of trees is removed in small populations. According to HOSIUS (1993), strong selective thinning (50%) in the Norway spruce population resulted in the significantly

different genotypic structure for the LAP-B, 6-PGDH-B and G-6-PDH loci. These results are not directly comparable to ours due to different enzyme set and relatively low level of thinning in our variants.

Table 5. Effect of thinning according to tree size on observed heterozygosity and number of alleles of the polymorphic loci. Data without the monomorphic IDH-A locus.

Set of trees	all	with low DBH	with middle DBH	with high DBH	Maximal difference and their probability (p)
Thinning variant	none	v1	v2	v3	
	Alleles	Observed heterozygosity			
AAT–B	2	0.0097	0.0141 (55.6%)	0.0139 (50.0%)	0 (31.9%)	v1 – v3: 0.149
AAT–C	4	0.5208	0.4923 (16.9%)	0.5672 (87.9%)	0.5882 (94.0%)	v1 – v3: 0.111
G–6–PDH	2	0.1226	0.0959 (16.0%)	0.1233 (57.6%)	0.1507 (87.7%)	v1 – v3: 0.155
GDH	2	0.0196	0.0141 (15.6%)	0.0278 (56.5%)	0.0286 (94.2%)	v1 – v3: 0.267
IDH–B	2	0.0094	0.0137 (75.0%)	0.0137 (51.3%)	0.0137 (75.0%)	v1 – none: 0.397
MDH–B	2	0.0097	0.0141 (55.8%)	0.0139 (50.0%)	0 (29.5%)	v1 – v3: 0.149
MDH–C	2	0.0485	0.0563 (75.5%)	0.0694 (87.5%)	0.0423 (41.4%)	v2 – v3: 0.235
PGM–A	2	0.0094	0.0137 (75.0%)	0 (30.0%)	0 (28.4%)	v1 – v3: 0.156
SDH–A	5	0.2547	0.2603 (62.2%)	0.2740 (64.1%)	0.2740 (65.8%)	none – v3: 0.387
A	23		23	22	20	

Differences were evaluated by the t–test. Probability of the Monte–Carlo test is in parenthesis. A - total number of alleles.

Table 6. Effect of thinning according to tree size on selected features of genetic diversity.

Set of trees	all	with low DBH	with middle DBH	with high DBH
Thinning variant	none	v1	v2	v3
Classes	31	25	27	25
Potential classes no.	328050	328050	109350	12150
H	4.1162	4.0872 (81.3%)	4.2014 (**95.4%**)	3.9504 (39.2%)
e	0.2246	0.2231	0.2510	0.2911
R	0.0975	0.0997 (75.6%)	0.1031 (94.4%)	0.0949 (25.5%)

Probability of the Monte–Carlo test is in parenthesis.

Differentiation according to damage and defoliation

Trees with damaged trunks (including breaking, doubled trunk etc.) represent a group of a different genetic constitution comparing undamaged trees. The heterozygosity difference is significant by the AAT–C locus (Table 7). Similar but non-significant difference is found by locus SDH–A. While the subpopulation of damaged trees shows decreased diversity, the opposite trend has been recorded in the subpopulation of undamaged trees for both Shannon's and Rao's indices. Differentiation of both subpopulations is important – the corresponding Nei–distance was 0.0054.

There is only one important difference (AAT–C heterozygosity) between the whole set of trees and the subpopulation of trees with defoliation ≤ 30% (Table 8). Thinning the most defoliated trees is probably with minor effect on genetic diversity at the investigated loci, except for the mentioned AAT–C heterozygosity.

Table 7. Genetic differentiation according to trunk damage.

Set of trees	Damaged trees	Undamaged trees	p
Tree count	28	78	
Observed heterozygosity:			
AAT–B	0 (50.0%)	0.013 (50.1%)	0.151
AAT–C	0.333 (**1.6**%)	0.592 (**98.4**%)	**0.006**
G–6–PDH	0.107 (29.5%)	0.130 (72.7%)	0.374
GDH	0 (50.0%)	0.027 (72.5%)	0.068
IDH–B	0 (50.0%)	0.013	0.355
MDH–B	0 (50.0%)	0.013 (51.8%)	0.151
MDH–C	0.037 (31.8%)	0.053 (53.3%)	0.355
PGM–A	0.036 (74.7%)	0 (24.2%)	0.160
SDH–A	0.179 (18.3%)	0.286 (93.2%)	0.118
Diversity:			
Number of classes	12	29	
Potential classes no.	972	109350	
H	3.1887 (17.5%)	4.1478 (90.1%)	
e	0.3213	0.2478	
R	0.0853 (16.7%)	0.0985 (65.3%)	

Probability of the Monte–Carlo test (for difference population – subpopulation) is in parenthesis. p – error probability by the t–test of heterozygosity difference.

Table 8. Genetic differentiation according to defoliation.

Set of trees	All trees	Trees with defoliation ≤ 30%
Tree count	106	80
Observed heterozygosity:		
AAT–B	0.010	0.013 (28.4%)
AAT–C	0.521	0.587 (**98.2**%)
G–6–PDH	0.123	0.113 (27.8%)
GDH	0.020	0.026 (65.3%)
IDH–B	0.009	0.013 (45.8%)
MDH–B	0.010	0.013 (30.3%)
MDH–C	0.049	0.051 (48.8%)
PGM–A	0.009	0 (27.8%)
SDH–A	0.255	0.250 (43.3%)
Diversity:		
Classes	31	27
Potential classes no.	328050	109350
H	4.1162	3.9235 (27.5%)
e	0.2246	0.2344
R	0.0975	0.0933 (13.8%)

Probability of the Monte–Carlo test is in parenthesis.

Conclusions

Genetic structure of the planted Norway spruce population shows heterogeneous spatial pattern. The existence of spatial clumping of genotypes in a radius 10 m could be the result of planting and thinning schemas. Monte–Carlo randomization test is suggested for the partial study of populations as a method, which allows the generalization of results for the whole population.

The studied Norway spruce population allows to determine following conclusions comparing subpopulations constituted on the base of different phenotype features:

- Subpopulation of the comb-shaped types of trees has different genetic composition compared to the other subpopulation, but differences are mainly not statistically significant (except G–6–PDH heterozygosity) probably because of small sample size in the comb-shaped group.
- Thinning models based on size criteria suggest that the combined thinning (variant 2) is the most appropriate to preserve genetic diversity.
- Damaged trees (with breaking, doubled trunk, other damaged trunk) have different genetic composition than non-damaged trees.
- Thinning of young trees (about 40 years) with high defoliation does not reduce significantly the genetic variation.

Further investigations on the effects of different thinning variants on the genetic diversity of spruce populations are necessary to support the results of the present study.

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