PATTERNS OF GENETIC DIVERSITY DISTRIBUTION IN THREE MAIN CENTRAL EUROPEAN MONTANE TREE SPECIES: PICEA ABIES KARST., ABIES ALBA MILL. AND FAGUS SYLVATICA L.

Ladislav PAULE1, Dušan GÖMÖRY1, Roman LONGAUER2 and Diana KRAJMEROVÁ2

1Technical University in Zvolen, Faculty of Forestry, T. G. Masaryka 24, SK – 960 53 Zvolen
2Forest Research Institute Zvolen, T. G. Masaryka 22, SK – 960 92 Zvolen


The contribution summarizes the research on the genetic differentiation and diversity of populations of three forest tree species, which are principal constituents of the montane and subalpine forest belts in Europe: Norway spruce (Picea abies Karst.), silver fir (Abies alba Mill.), and common beech (Fagus sylvatica L.). The footprints of the postglacial recolonization process in the pattern of the allelic differentiation and diversity distribution are shortly discussed.

In beech, a relatively homogeneous allelic structure was found. The differentiation among regional population is not strong, there is, however, a considerable within–region differentiation. There does not seem to be any concordance between the trends of allelic richness (genetic multiplicity) and genetic diversity in this species.

In silver fir, a strong differentiation among regions and a much more clear geographic pattern was found. There exists an East–West clinal variation. Western Carpathians exhibit the lowest levels of genetic diversity, which increases subsequently both in the West (Herzynic and Pre–Alpine region) and East (Eastern and Southern Carpathians, Balkans). On the other hand, allelic richness is quite regularly distributed over regional populations.

In Norway spruce, two different kinds of studies on smaller scales were performed. In a primeval forest, a weak spatial pattern of the distribution of alleles was found. Within a regional study, different patterns of the genetic variation between Herzynin and Carpathian populations were observed.

Keywords: Picea abies, Abies alba, Fagus sylvatica, genetic diversity, genetic multiplicity, Carpathians, spatial autocorrelation

1. Introduction

Mountain forests complex represents undoubtedly the most important component of forest resources in the Central and Eastern Europe. In contrast to lowlands, where forests have been extensively converted to agricultural land since the neolithic, the mountain forest belt has been mostly preserved. Partially, in the higher elevations, alpine meadows have been extended on account of the mountain dwarf pine and subalpine spruce forests and/or forests on the rounded summits and ridges were converted to pastures. It happened mainly since the 15th century in association with the so–called Wallachian colonization (shepherds). However, large and continuous forest complexes in the mountains have largely been preserved.

Due to their nearly natural character, mountain forests harbour an incomparably greater biodiversity than the other ecosystems except wetlands. However, it is not comparable with
the species and genetic diversity in the West Asian mountain ranges like Caucasus or Alborz. The genetic resources as well as species richness were largely depleted by Pleistocene glaciations. The unfortunate East–West orientation of the main European mountain ranges (Alps, Pyrenees, Carpathians) hampered the southward retreat of vegetation during the glacials. At the same time, Europe is bordered by Mediterranean Sea from the South, so that there was not enough space where forest plant populations could withdraw. The survival of species became a matter of chance. Paleontological investigations indicate, that the number of tree species in Europe in Tertiary was comparable with temperate regions of West Asia or North America, but several genera disappeared completely, or were impoverished of most species (*Pseudotsuga, Taxodium, Zelkova, Carya* etc.). Gene pools were depleted in a similar way. The reproduction–effective sizes of forest tree populations in glacial refugia were strongly limited, so that many genes disappeared through genetic drift.

Another factor which contributed to a poorer genetic and species structure is the human influence, mainly forest management. In several regions of Central Europe, natural forests were converted to spruce or pine monocultures. At the same time, unindigenous reproduction material was used. There are species, which have mostly been regenerated naturally (common beech, silver fir), so that most of their present stands possess unaltered or slightly altered genetic structures. However, in case of Norway spruce or white oaks, extensive planting has caused that the indigeneity of almost all stands (except those in natural reserves) is dubious. Recently, forest ecosystems have been strongly affected by industrial pollution.

The most productive forest ecosystem in Central Europe is the so–called “carpatho–herzynic mixture”: natural mixed stands of European beech, silver fir and Norway spruce (Korpeľ, 1995). In this contribution, we summarize the results of our investigations of the genetic structures of these three species (which have their analogy in the Caucasus, where Eastern beech (*Fagus orientalis*), Caucasian fir (*Abies nordmanniana*) and oriental spruce (*Picea orientalis*) form extensive stands (Grossgejm, 1952; Mayer & Aksoy, 1986)) in the Carpathians and the Herzynic range.

### 2. Material and Methods

European beech and silver fir in the Central and Eastern Europe and West Asia have been object of intensive genetic investigations at the Faculty of Forestry, Technical University in Zvolen and Forest Research Institute, Zvolen, since the late 80's. The regional Carpathian and Herzynic beech and fir population ranges have been quite densely sampled. In total 89 beech and 68 fir local populations were analyzed.

In Norway spruce, a study of the interpopulation differentiation on a smaller scale was performed. It comprised 11 Carpathian (Slovak) and 3 Herzynic (Czech) spruce populations.

During the dormancy seasons, twigs with buds were collected from randomly selected trees and subjected to isozyme analysis. Buds without scales were homogenized in a Tris–HCl extraction buffer pH 7.3. Enzyme separation was performed electrophoretically in 12% (w/v) starch gels using four buffer systems (lithium–borate – Tris–citrate pH 8.1, sodium–borate pH 8.0 – Tris–citrate pH 8.7, continuous Tris–citrate pH 7.8, and Tris–histidine – Tris–citrate buffer pH 7.0). The following enzyme systems were assayed in individual tree species (NS – Norway spruce, SF – silver fir, EB – European beech): aconitase (SF), unspecific aminopeptidase (NS), diaphorase (SF), fluorescent esterase (NS), glucose–6–phosphate dehydrogenase (NS), glutamate dehydrogenase (NS, SF), glutamate–oxaloacetate transaminase (all three species), isocitrate dehydrogenase (all), leucine aminopeptidase (all), malate dehydrogenase (all), menadione reductase (EB), NADH dehydrogenase (NS, SF), phosphoenolpyruvate carboxylase (NS), peroxidase (all), 6–phosphogluconate dehydrogenase (SF), phosphoglucose isomerase (all), phosphoglcomutase (NS, EB), and
shikimate dehydrogenase (NS, EB). In Norway spruce, zymogram interpretation followed Muona et al. (1987) and Lagercrantz et al. (1988). In common beech, genetic interpretation was completed as per Merzeau et al. (1989) and Müller–Starck & Starke (1993). In silver fir, Mendelian inheritance was proven by Hussendörfer et al. (1995) and Fady & Conkle (1992).

Allelic frequencies at each locus were calculated based on diploid genotypes. Genetic diversity was characterized by the mean number of alleles per locus, effective number of alleles \( n_e = 1/\Sigma p(A_i)^2 \), and observed \( H_o = \Sigma \Sigma_j p(A_i,A_j) / \Sigma \Sigma_j p(A_i,A_j) \) and expected heterozygosities \( H_e = 1 - \Sigma p(A_i)^2 \) \( p(A_i) \): frequency of the allele \( A_i \), \( P(A_i,A_j) \): frequency of the genotype \( A_i,A_j \). Genetic differentiation was quantified using genetic \( ne \) distances following Nei (1978). The matrix of genetic was interpreted employing principal coordinate analysis.

Within the study of the intrapopulation spatial genetic structure, a transect of 40 × 150 m was established near the summit of the mountain Zadná Poľana (1,458 m a.s.l.) in a primeval Norway spruce forest (nature reserve). Twigs with dormant winter buds were sampled from all adult individuals and those young spruces on the transect, whose size allowed to take enough material for the analysis without destroying the tree (200 samples in total). The position (coordinates \( x, y \) and breast–height–diameter were recorded for each individual. Isozyme analysis was performed as given above.

The patterns of the spatial distributions of individual alleles were identified using the spatial autocorrelation analysis. The alleles with a frequency less than 0.05 or more than 0.95 were excluded from the analysis, in biallelic loci (or when the sum of the two most frequent alleles was more than 0.95), only the most frequent allele was used. Moran's \( I \)–statistics (Sokal & Oden, 1978) was estimated for 15 distance classes with approximately equal sample sizes.

3. Results
3.1. Norway spruce
3.1.1 Spatial genetic structure of a primeval forest

Within the investigated transect, 21 groups of identical genotypes containing 2 to 6 individuals occurred. Most of them covered an area corresponding to a crown or a root system of an adult tree, but two groups were larger and seven groups of identical genotypes contained individuals distributed over different parts of the transect. In 8 cases, compact groups contained individuals of different ages, in one case even different generations.

Spatial autocorrelation analysis was performed for 8 frequent and independent alleles. Since this analysis was aimed at discovering the patterns arising through the generative regeneration, the genotypes belonging to the same putative clonal group were pooled and the center was taken as the position of the group. There is no space for presenting the single–allele correlograms, but some generalization based on the average \( I \) indices and the occurrence of significant values is possible. Out of 120 values, ten Moran's indices were significant (Fig. 1). Surprisingly, only one positive Moran's \( I \) was found in the first distance class. The autocorrelation is generally positive in the first distance class (0–10.8 m). Then, it varies around the expected value, and for distance classes of 31.4–36.6 m, 36.6–42.1 m, and 42.1–48.1 m it becomes negative. Significant positive values occur again in the distance class of 71.5–81.6 m.
1. Correlogram (average Moran’s I over 8 alleles) over distance classes with equal frequencies, including the numbers of significant I-values.

3.1.2 Regional patterns of diversity distribution

Geographical distribution of genetic structure characteristics is shown in Fig. 2. Generally higher levels of both genetic multiplicity (as measured by the mean number of alleles per locus $n_a$) as well as diversity (as measured by effective number of alleles $n_e$) were observed in the Herzynic (Czech) populations. Despite the fact that the trends of diversity and multiplicity are not perfectly correlated, within Western Carpathians, more southern populations (Low Tatra range, Slovak Ore Mts.) exhibited a greater allelic richness and diversity than more northern populations from the High Tatra Mts. Clearly the lowest levels of both genetic multiplicity and diversity and a considerable proportion of monomorphic loci were observed in the Babia hora population, which is the northernmost one.

2. Distributions of the genetic multiplicity ($n_a$) and genetic diversity ($n_e$) over the sampled Norway spruce populations in Czecho-Slovakia. Size of the circle indicates deviation from the overall mean, black circles indicate positive deviations (greater than mean), empty circles indicate negative deviation (lower than mean).

3.2. Silver fir

In contrast to Norway spruce, where we focused on a smaller territory and/or a single population, patterns of the genetic differentiation and diversity over larger regions were investigated in silver fir. Allelic richness seems to be quite regularly distributed over Carpathians and Herzynic range. The values range from 2.0 in the Ukrainian Carpathians to 2.2 in the Northern and Central Slovakia. On the other hand, there are bigger differences in the diversity, which follows a clear geographical trend: low levels of diversity were found in the Czech, Slovak and Polish populations, whereas diversity increases towards Ukraine and Romanian Eastern and mainly Southern Carpathians (Fig. 3).
3. Distributions of the genetic multiplicity ($n_a$) and genetic diversity ($n_e$) over the regional silver fir populations. Size of the circle indicates deviation from the overall mean, black circles indicate positive deviations (greater than mean), empty circles indicate negative deviation (lower than mean).

A similarly unambiguous trend as in diversity distribution was found in the genetic differentiation. Genetic distances among regional populations follow the Herzynic mountain range and the Carpathian arc with an almost absolute perfection (Fig. 4). Three separate groups can be distinguished: populations from the Herzynic massive, Western Carpathians together with Ukrainian Eastern Carpathians, and Romanian Eastern and Southern Carpathians.


3.3. European beech

Like in silver fir, large-scale distribution of the genetic diversity and multiplicity levels over the Carpathians and Herzynic range was investigated in European beech. Considerable differences in allelic richness as well as genetic diversity were revealed (Fig. 5). Mean number of alleles per locus ranged from 2.1 in the Beskid Nisky Mts. (South Poland) to 3.1 in the Banat Mts. There is no clear trend in the genetic multiplicity, but in general, populations in the South (Southern Carpathians, Banat Mts.) are most rich in alleles, whereas those at the
edges of the distribution range or localized more apart from the former glacial refugia, possess generally less allelic variants. However, the trend of the genetic diversity does not conform with this pattern. Like allelic richness, effective number of alleles varies – from 1.332 in the Apuseni Mts. (western Romania) to 1.376 in the Central Slovakia (Tatra and Fatra ranges, Central Slovakian volcanic range). Frequently, regional population which are rather poor in alleles, show the highest diversity (Podol’e, Vihorlat volcanic range) and, on the other hand, Banat Mts. with the highest allelic richness exhibit only a moderate diversity.

5. Distributions of the genetic multiplicity ($n_a$) and genetic diversity ($n_e$) over the regional common beech populations. Size of the circle indicates deviation from the overall mean, black circles indicate positive deviations (greater than mean), empty circles indicate negative deviation (lower than mean).

Genetic differentiation does not seem to follow a clear geographical pattern. In Fig. 6, presenting the principal coordinate analysis based on Nei’s genetic distances among regional populations, no geographical grouping can be identified. Differentiation pattern is rather a mosaic one.

4. Discussion

As mentioned in the introduction, this contribution summarizes research results of several independent projects with different focuses.

In Norway spruce, the studies were aimed at the assessment on small–scale patterns. Although in the study of the intrapopulation genetic patterns, we repeated the procedure of BRUNEL & RODOLPHE (1985) step–by–step, we received quite different results: an important share of full sibs and a lower share of non–related trees. This can be due to a different life history and character of investigated populations. Brunel & Rodolphe examined an almost even–aged, semi–natural forest, regenerated from regularly distributed seed–trees, growing in optimum conditions. The family structure, which might have existed at early stages of the stand ontogeny due to a limited seed dispersal, is gradually destroyed by the stochastic process of reduction of the number of trees. On the other hand, our object was a plot within a virgin forest, uneven–aged, growing in adverse climatic conditions of a high–elevated mountain summit. The regeneration is spatially and temporally irregular, mostly limited to decaying stems, rocks and other elevated places. Seed crops are small and scarce due to unfavourable climate. Since the spatial distribution of trees is incomparably more irregular than in a managed forest and there is no human intervention aimed at a more regular spacing, the chance for a continuous preservation of a family structure is much higher. Different sizes of the investigated plots maybe also contributed to different results. Finally, we cannot exclude that in some cases, parent–offspring relationships between trees occurred in our material, although we excluded younger trees from the analysis. MITTON (1983) provides many examples of the existence of family structure in conifers. Although the Norway spruce pollen may be transported by the wind over tens of kilometers without the loss of viability, most matings occur between the closest neighbours (LANGLET ex SCHMIDT–VOGT, 1978). Similarly, the reach of seeds (defined as the sedimentation distance of 96% of grains) amounts to 66 m, (MÜLLER ex SCHMIDT–VOGT, 1978), but most seeds fall beneath the tree. At high elevations, vegetative regeneration becomes rather common. Based on these considerations, a natural Norway spruce stand may be expected to be composed of overlapping, loosely aggregated families and even clones.

A spatial structure can be identified, although the patterning is very weak. The sinusoidal course of the average correlogram indicates a patchy structure, with a patch size of approx. 20–25 m. A similar patchy structure has been found in other forest trees as well (e.g. KNOWLES, 1991). On the other hand, EPPERSON & ALLARD (1989) found practically no spatial pattern of allele distribution in lodgepole pine. There are several mechanisms of the formation of patches, among them the restricted pollen and seed dispersal and spatially varying microenvironmental selection seem to be the most important (EPPERSON, 1992). In the conditions of the Poiana virgin forest, where the intraspecific competition is weak due to a low density of regeneration and due to the fact that most seedlings survive on decaying stems, selection does not seem to play an important role.

In silver fir, a rather homogeneous distribution of the allelic richness over the investigated area was observed, contrasting with a clear trend of the genetic diversity distribution. The pattern of the genetic differentiation is similarly unambiguous, and fully corresponds to morphological variation. In contrast to morphologically uniform northern populations, southern populations are much more variable in several traits (more frequent occurrence of adaxial stomata, fewer needles with incised apex, variation in needle thickness, needle width and width of stomatal bands) (AAS et al., 1994; DEACONU, 1995). Increasing phenotypic variation in the habitus and growth towards the South was described in provenance trials by HORVAT–MAROLT & KRAMER (1982) and KRAMER (1985). Romanian provenances proved also to be superior in growth, vigour and adaptability as compared with the West–Carpathian and Herzynic ones (RUETZ & STIMM, 1995; SVOLBA, 1995). In monoterpenes studies, the
lowest variation in the occurrence of monoterpene types and their proportions was found in the Herzynic region and Western Carpathians (LANG, 1994), i.e. exactly in those populations, which exhibit the lowest levels of diversity in our study.

In beech, the observed levels of the genetic multiplicity and diversity are comparable with previous studies (GÖMÖRY et al., 1999; HAZLER et al., 1997; KONNERT, 1995; MÜLLER–STARCK & ZIEHE, 1991), except those using few polymorphic loci. There is no concordance between the geographical trends of the genetic multiplicity and genetic diversity. The populations most rich in alleles are more frequently occurring at the southeastern edge of Carpathians, whereas the populations near the distribution range limits exhibit generally rather low levels of the genetic multiplicity. The trend of diversity, as measured by the effective number of alleles, is much less obvious, with the highest levels in the Western Carpathians. Such diverging trends of diversity and multiplicity have already been observed in European beech, both on the regional scale (GÖMÖRY et al., 1999) and on the rangewide scale (COMPS et al., 2001). A high allelic richness in southern populations is generally explained by the proximity of glacial refugia. The existence of a beech refugium at the southeastern limit of Carpathians is a controversial issue, since reliably dated pollen records from this area have not been available. However, a close genetic similarity of Slovenian and Croatian beech populations to the Romanian ones both at isozyme loci (GÖMÖRY et al., 1999), and cpDNA haplotypes (DEMESURE et al., 1996) along with a strong differentiation from beech in the southern Balkans indicates a common postglacial origin of beech in a large part of its range, whereby the question, whether the refugium was sheltered by the southern slopes of East Alps or by the southeastern edge of Carpathians, remains to be answered. The non–concordance of the geographical patterns of genetic diversity and multiplicity may be related to the postglacial colonization process. As shown by MARUYAMA & FUERST (1985), loss of alleles after a restriction of the population size is more rapid than the loss in gene diversity. The traces of population bottlenecks, which are unavoidable in colonization, could have been preserved in more northern populations which established themselves more recently.

The question which remains unanswered is why two species with a similar postglacial history, which occur in the Carpathian forests largely together and have very similar ecological requirements, exhibit completely different geographical trends of genetic diversity and multiplicity. Several hypotheses could be formulated about this, but leaving this question for further research seems more reasonable at this stage.

References