

Spatial Patterns of the Genetic Differentiation in European Beech (*Fagus sylvatica* L.) at Allozyme Loci in the Carpathians and the Adjacent Regions

By D. GÖMÖRY¹, L. PAULE¹, I. M. SHVADCHAK², F. POPESCU³, M. SULKOWSKA⁴, V. HYNEK⁵, R. LONGAUER⁶

(Received 18th November 2002)

Summary

Genetic differentiation and spatial patterns of the genetic diversity distribution in European beech were investigated in the Carpathian mountain range and surrounding regions using 12 isozyme loci. In total, 139 populations were included. No interperable trends of the genetic differentiation based on genetic distances were identified. However, genetic distogram indicates the existence of a stationary pattern: a significant similarity of populations separated by approx. 40 km and a significant dissimilarity in the 500 km distance class. Geostatistical analysis (kriging) revealed a clear geographical trend of the mean number of alleles per locus, with the highest levels observed in the southeastern edge of Carpathians and the lowest levels in the marginal regions. The trend of the genetic diversity was almost opposite, the highest levels were found in northern Poland.

Key words: *Fagus sylvatica* L., spatial pattern, kriging, genetic differentiation, allozymes.

Introduction

In the whole Carpathian region, common beech (*Fagus sylvatica* L.) is without ambiguity the most widespread and most important broadleaved tree species. The total area of beechwoods in the countries crossed or touched by the Carpathian arc is approx. 42,400 hectares, representing 13.3% of the total forest area (PHARE, 1998). This might appear not too much, but in the Czech republic, Poland or Ukraine, the occurrence of beech is concentrated just in the Carpathian mountain ranges, whereas it is much less represented or completely missing in the other parts of these countries. In Slovakia and Romania, beech is the most represented broadleaved species sharing more than 30% of the total forest area.

Owing to the economical and ecological significance of beechwoods, beech belongs to those tree species which have been an object of gene conservation programs and whose genetic variation has been intensively explored through provenance trials and genetic marker studies. In 1997, a European Network for Social Broadleaves was established within the EUFORGEN program, aimed at the coordination and promotion of gene conservation activities in beech and white oak species.

Except German provenances, the Carpathian ones are the most represented in beech provenance trials. The most recent and most extensive international beech provenance trial, established in 1995, includes 25 provenances of Carpathian origin

(mainly Slovak and Romanian) (VON WUEHLISCH *et al.*, 1997). Carpathian populations are also well represented in studies employing allozyme and molecular genetic markers on the rangewide scale (COMPS *et al.*, 1991, 2001; DEMESURE *et al.*, 1996). However, studies on local or regional scales, which are abundant for the other parts of the distribution range (COMPS *et al.*, 1990, 1998; GALLOIS *et al.*, 1998; GÖMÖRY *et al.*, 1999; KONNERT, 1995; VORNAM and HERZOG, 1996 and others) are almost missing for this region.

Beech is a widespread, wind-pollinated species. This group of tree species typically exhibits high levels of genetic variation (HAMRICK *et al.*, 1992). Most of this variation is harboured within population, the inter-population component rarely exceeds 5% (LARSEN, 1996; LEONARDI and MENOZZI, 1995; PAULE *et al.*, 1995). Yet, it does not mean that no patterns of differentiation can be observed. Both allelic frequencies and genetic multiplicity/diversity were found to be correlated with environmental variables and/or geographical coordinates. These patterns are explained by the adaptation to climate (COMPS *et al.*, 1990), or by the loss of alleles and changes in allelic frequencies due to the genetic drift during the postglacial recolonization (COMPS *et al.*, 2001; LEONARDI and MENOZZI, 1995). A similar pattern was observed in provenance trials. Although the geographic variation in morphological and phenological traits is rather ecotypic than clinal (VON WUEHLISCH *et al.*, 1997), some rangewide trends are evident mainly in adaptive traits (VON WUEHLISCH *et al.*, 1995). Nevertheless, the observed correlations between phenotypic traits and/or gene frequencies and geographical coordinates are generally quite weak. In many cases, there exists a geographical variation, but rather at local or regional scales than at the rangewide scale. In these cases, simple correlation and regression may become inefficient in revealing geographical trends and should be replaced by geostatistical analysis.

The aim of this paper is to fill the gap in regional genetic studies of beechwoods in Central and Eastern Europe by describing the patterns of genetic differentiation and spatial distribution of genetic diversity and multiplicity over the Carpathian arc and adjacent territories, employing both conventional approach and geostatistics.

Materials and Methods

In total, 139 indigenous beech stands were examined. The geographical locations of the investigated populations are shown in *Fig. 1*. In each beech stand twigs with dormant buds were sampled from at least 50 non-adjacent trees chosen at random. The populations were grouped into eight geographic regions: Hercynic (mainly Sudetic) ranges (17 populations), Polish lowlands (10), Western Carpathians (37), Eastern Carpathians (44), lowlands at the Eastern limit of the distribution range (Galicia, Podol'e – 6 populations), Moldova (former Soviet republic as well as the Romanian Moldova – 5 populations), Apuseni Mts. (9) and Southern Carpathians (10).

Enzymes were extracted from buds and cortical tissues of each individual, and were separated by means of starch elec-

¹ Technical University in Zvolen, Faculty of Forestry, T.G. Masaryka 24, SK-960 53 Zvolen, Slovakia

² University in L'viv, Faculty of Forestry, UA-290 052 L'viv, Ukraine

³ ICAS, Research Station and Arboretum Simeria, str. Biscaria 1, RO-2625 Simeria, Romania

⁴ Forestry Research Institute, ul. Bitwy Warszawskiej 1920 r. 3, PL-00 973 Warszawa, Poland

⁵ Ministry of Environment, Vršovická 65, CZ-100 10 Prague, Czech Republic

⁶ Forestry Research Institute, T.G. Masaryka 22, SK-960 92 Zvolen, Slovakia



Figure 1. – Distribution of the investigated populations.

trophoresis. Protein separation and staining procedures were described by MERZEAU *et al.* (1989) and MÜLLER-STARCK and STARKE (1993). Nine isozyme systems coded by twelve loci were scored: isocitrate dehydrogenase (*Idh-A*), malate dehydrogenase (*Mdh-A*, *Mdh-B*, *Mdh-C*), menadiene reductase (*Mnr-A*), phosphoglucumutase (*Pgm-A*), phosphoglucose isomerase (*Pgi-B*), peroxidase (*Px-A*, *Px-B*), glutamate-oxaloacetate transaminase (*Got-B*), leucine aminopeptidase (*Lap-A*), and shikimate dehydrogenase (*Skdh-A*).

Allelic frequencies at each locus were calculated on the basis of diploid genotypes. Genetic multiplicity was characterized by mean number of alleles per locus (n_e). Since this measure is very sensitive to the sample size, which varied considerably among regions, we used the measure of allelic richness developed by PETIT *et al.* (1998) based on the rarefaction technique. Allelic richness $A[g]$ corresponds to the number of different alleles expected to be found at a locus when g gene copies are sampled. The smallest regional sample size was 236 diploid individuals, so g was set to 400 (as the nearest lower round number). Expected Hardy-Weinberg heterozygosity (H_e) and effective number of alleles (n_e) were used to characterize the allelic diversity.

The geographical coordinates (latitude φ , longitude λ) of individual populations were converted to orthogonal coordinates $[x, y]$ using the general gnomonic projection (KUSKA, 1960):

$$x = R \frac{\sin \varphi \cos \varphi_Q - \cos \varphi \sin \varphi_Q \cos \lambda}{\sin \varphi \sin \varphi_Q + \cos \varphi \cos \varphi_Q \cos \lambda}$$

$$y = R \frac{\cos \varphi \sin \lambda}{\sin \varphi \sin \varphi_Q + \cos \varphi \cos \varphi_Q \cos \lambda}$$

(Q is the point with coordinates $[0, 0]$; $\varphi_Q = 48^\circ 27'$, $\lambda_Q = 21^\circ 20'$; to minimize the distortion, φ_Q and λ_Q were chosen as arithmetic averages of the latitudes and longitudes of the investigated populations, respectively. To avoid negative coordinates in the graphical presentations, the point $[0, 0]$ was shifted to the south by 600 km and to the west by 900 km.) Variogram models were derived and kriging estimates were calculated for the mean number of alleles per locus and the mean effective number of alleles. The exponential model ($\gamma(h) = C_0 + C \cdot [1 - e^{-h/a}]$) was used ($\gamma(h)$ is the semivariance, h is the lag distance, C is the sill, a is the range, and C_0 is the "nugget effect" – a term used in geostatistics to designate the variation

at the smallest distance classes which, in spite of the expectation, differs from zero due to the sampling error or unidentified sources of variation). Ordinary punctual kriging was performed using the Geo-EAS program (Geostatistical Environmental Exposure Assessment Software; U.S. Environmental Protection Agency, Las Vegas, Nevada, U.S.A.). The network of estimation points was a grid 33 km on a side. More details about the kriging procedure and its application in population genetics can be found in PIAZZA *et al.* (1981) or LE CORRE *et al.* (1998).

To quantify the degree of differentiation within and among populations, D_j and δ measures (GREGORIUS and ROBERDS, 1986) and F_{ST} (WEIR, 1990) were used. The heterogeneity of allele frequencies between pairs of regions was tested using exact probability tests (RAYMOND and ROUSSET, 1995). For the interpretation of genetic distances (GREGORIUS, 1974), principal coordinate analysis (PCoA) was used.

To identify the spatial scales of the genetic differentiation, distograms based on GREGORIUS (1974) genetic distances for progressively increasing distance classes (10, 20, 40, 80 and 160 km) were constructed employing the SGS program (DEGEN and SCHOLZ, 1998; DEGEN *et al.* 2001).

Results

Levels and distribution of genetic diversity

As shown in Table 1, genetic diversity is quite evenly distributed over the investigated area. The differences in both effective numbers of alleles and expected heterozygosities between regions are negligible. The lowest values were found in the southeastern edge of the distribution range (Romania and Moldova) (mean $n_e = 1.453$ – 1.462). However, diversity in the more northern located populations (Czech rep., Slovakia, Poland) is only slightly higher (mean $n_e = 1.478$ – 1.494). The geographical trends of genetic multiplicity are just opposite, whereby there are much greater differences among regions. Romanian Carpathians are unambiguously the centre of the allelic richness, both at the regional as well as population level. The excentrically located regions (Polish lowlands, Galicia/Podol'e) seem to be genetically depauperate. The discrepancy between the diversity and allelic richness, which is obvious at the regional level, exists also at the level of individual populations – the correlations between n_a and n_e and/or H_e are almost equal zero (-0.0502 and 0.0775 , respectively).

A more detailed information about the geographical distribution of genetic diversity and multiplicity than the averages over the defined regions was obtained using geostatistical methods. However, the observed trends confirm the conclusions derived from regional averages. There does not seem to exist any clear trend of genetic diversity (Fig. 2). The kriging estimates of n_e vary sometimes on a very small scale. Patches of sizes ranging from 30 to 400 km can be observed, whereby the values estimated at neighbouring points frequently differ considerably.

On the other hand, allelic richness exhibits very distinct trends (Fig. 3). Despite local variations, centres of high as well as low allelic multiplicity can easily be identified. Extremely low values occur at the northeastern limit of the distribution range – Baltic coast and centre of Poland. The highest values were found in Romanian Carpathians, mainly in the Apuseni Mts. and at the southeastern edge of Carpathians (regions Ploiesti and Brasov).

Genetic differentiation

In general, the differentiation within regions is much higher than the differentiation of individual regions from the comple-

Table 1. – Characteristics of the genetic multiplicity and diversity of the investigated regions.

Region	N_p	n_a		$A_{T(400)}$	n_e		H_e		δ	D_j
		mean	pooled		mean	pooled	mean	pooled		
Hercynic ranges	17	2.186	2.917	2.544	1.481	1.492	0.256	0.258	0.038	0.029
Polish lowlands	10	2.025	2.500	2.331	1.478	1.490	0.257	0.259	0.039	0.023
Western Carpathians	37	2.146	2.833	2.610	1.494	1.504	0.264	0.268	0.046	0.020
Eastern limit	6	2.139	2.667	2.583	1.482	1.496	0.260	0.266	0.052	0.034
Eastern Carpathians	44	2.235	3.167	2.712	1.462	1.476	0.255	0.261	0.043	0.014
Apuseni Mts.	9	2.315	2.750	2.633	1.462	1.471	0.267	0.262	0.041	0.021
Southern Carpathians	10	2.217	3.083	2.753	1.457	1.458	0.256	0.255	0.049	0.023
Moldova	6	2.167	2.667	2.592	1.453	1.480	0.251	0.264	0.078	0.034

N_p – number of populations, n_a – mean number of alleles per locus, $A_{T(400)}$ – allelic richness after rarefaction to a sample of 400 genes, n_e – mean effective number of alleles per locus, H_e – mean expected heterozygosity, δ – differentiation within region, D_j – differentiation of the j th region against the complement mean – average value of the respective parameter (average over the populations within a region), pooled – value of the respective parameter when the populations within a region are pooled.

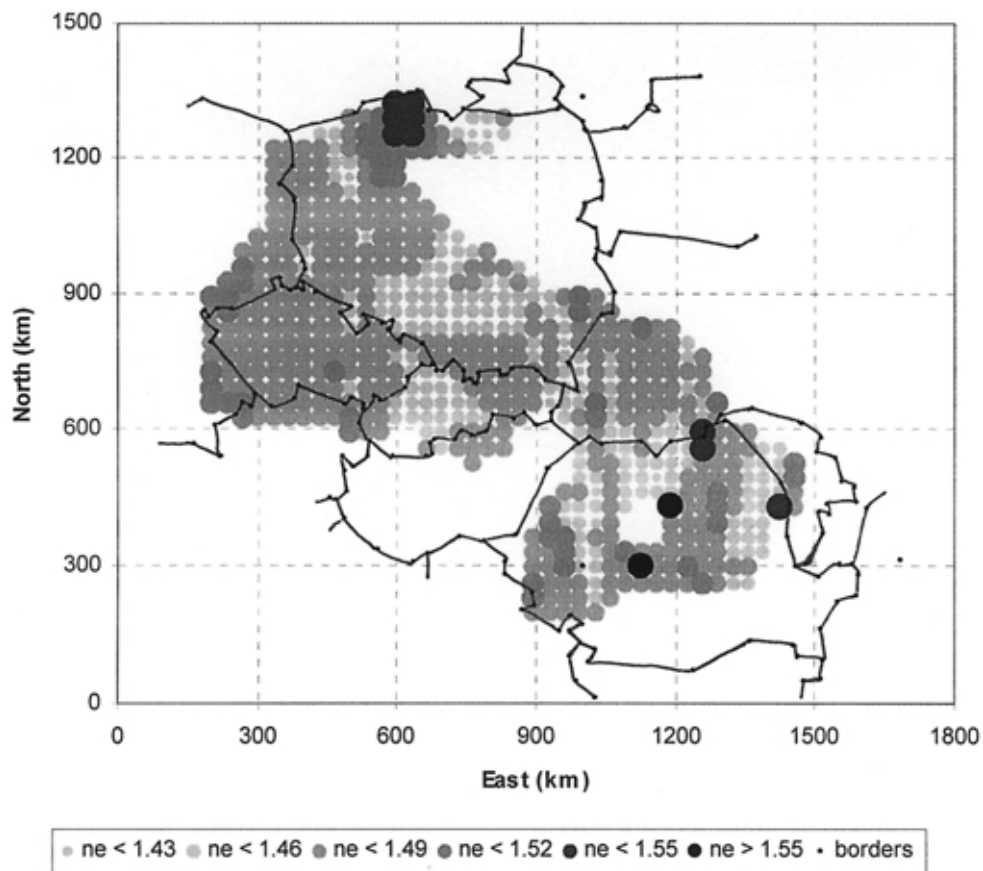


Figure 2. – Kriged estimates of the mean effective number of alleles (n_e) over the investigated territory.

ment (Table 1). It indicates that the differentiation pattern is mosaic-like. Yet, the distribution of D_j -values indicates that there exists a general differentiation trend: the centrally located regions (East and West Carpathians) exhibit the lowest differentiation from the rest, whereas most differentiated are the regions at the outer limit of the investigated area (Moldova, Galicia/Podol'e, Hercynic region). Despite the differences among regions, the overall level of differentiation is very low ($F_{ST} = 0.0297$).

Genetic distances do not reveal any interpretable geographical trend (Fig. 4). Even though the groups of point, represent-

ing individual regions do not overlap completely, neither any clinal trends nor any clear geographical pattern can be identified. The low percentages of the total variation, explained by the first two principal axes, resulting from the principal coordinate analysis of the genetic distance matrix, also indicate a complete absence of any area-wide trends, which could be identified using genetic distances. Cluster analysis (dendrogram not shown), neither revealed any pattern.

Pairwise comparison of regions is included in Table 2. The numbers of loci exhibiting significant differences in allelic frequencies between regions would be a good indicator of differen-

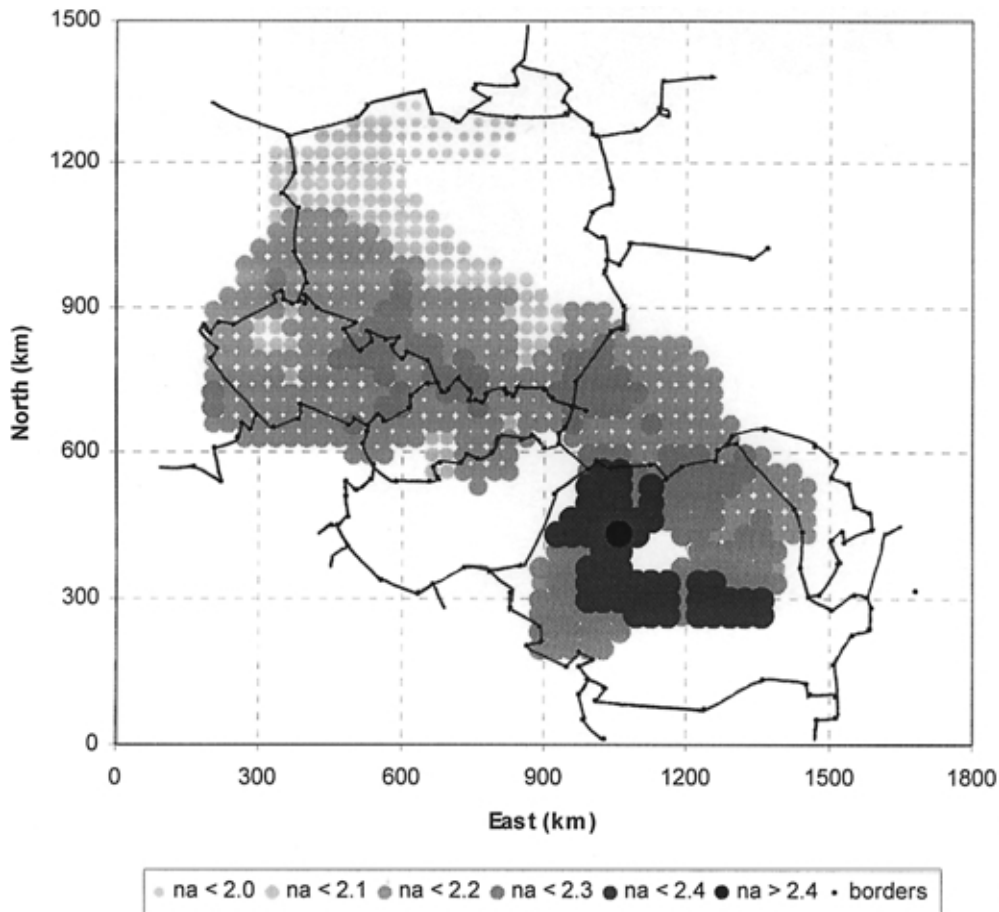


Figure 3. – Kriged estimates of the mean number of alleles per locus (n_a) over the investigated territory.

tiation if the sample sizes were equal. This is, however, not the case. Eastern and Western Carpathians, which are represented by the largest samples, appear then to differ significantly from the other regions at relatively many loci, although their genetic distances to neighbouring regions are quite small. Neverthe-

less, both genetic distances and heterogeneity tests of allelic frequencies indicate that among the investigated regions, the northernmost (Hercynic, Polish) and southernmost (Moldovian, South-Carpathian) regions are most differentiated from each other.

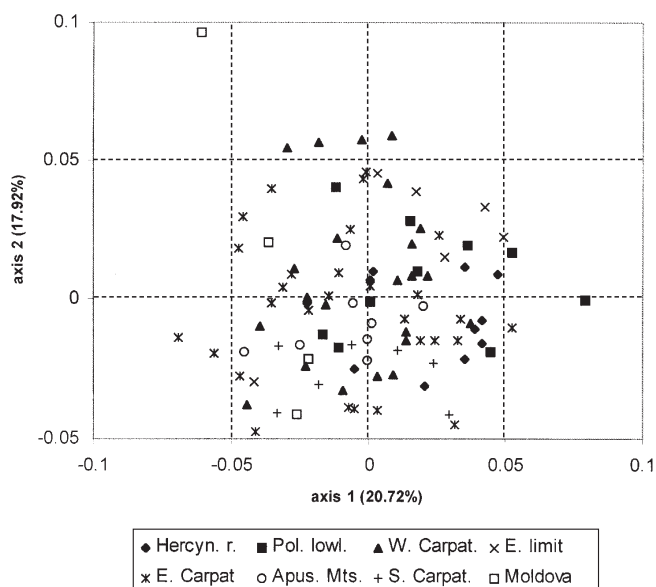


Figure 4. – Scattergram of the principal coordinate analysis of the matrix of genetic distances among populations.

Genetic distograms with distance classes of 10, 20, 40 and 80 km reveal essentially the same pattern (only the distograms for 10 km and 80 km distance classes are shown in Fig. 5). The course of the distogram polygon is more or less sinusoidal, with a "wavelength" of approx. 500 km and an amplitude decreasing from 0.056–0.0713 with 10 km classes to 0.060–0.068 with 80 km classes. Of course, oscillations occurring with small distance classes disappear and the course is smoother, when larger distance classes are used. However, in all distograms, a minimum at the distance of approx. 40 km and a maximum at 500 km, both exceeding the 95% confidence interval limits, was observed, what means that the pairs of populations separated by the distance of 40 km are genetically the most similar. Interestingly, the neighbouring populations (located up to 20 km from each other) are more differentiated than the populations separated by 30–60 km. At the smallest distance classes, mean genetic distances even do not differ significantly from the mean reference value expected in case of a random distribution, what indicates a strong "nugget effect".

Discussion

The analyzed beech populations are not regularly distributed over the beech distribution range in Central and Southeastern

Table 2. – Number of loci exhibiting significant differences (above diagonal) and genetic distances (below diagonal) between regional beech populations.

Region	Herc.	Pol.	W. Carp.	E. lim.	E. Carp.	Apus.	S. Carp.	Mold.
Hercynic ranges	-	5	7	6	9	9	9	10
Polish lowlands	0.025	-	9	3	8	6	10	8
West Carpathians	0.029	0.027	-	4	8	9	8	8
Eastern limit	0.037	0.028	0.035	-	4	5	8	6
East Carpathians	0.033	0.027	0.023	0.035	-	1	6	7
Apuseni Mts.	0.030	0.036	0.031	0.049	0.020	-	5	2
South Carpathians	0.043	0.035	0.032	0.043	0.015	0.026	-	7
Moldova	0.053	0.047	0.037	0.047	0.029	0.042	0.022	-

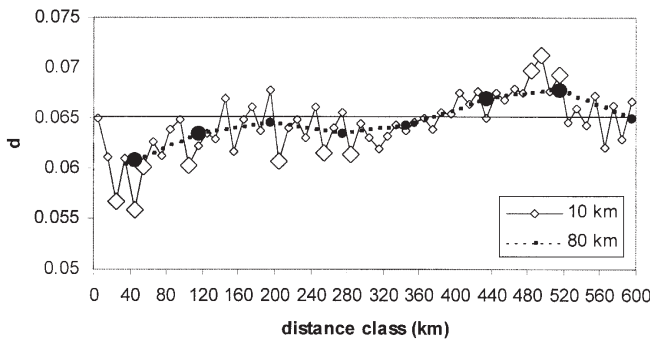


Figure 5. – Distogram based on GREGORIUS (1974) genetic distances for distance classes of 10 km (solid line) and 80 km (broken line). Large points represent values significantly ($\alpha < 0.05$) higher and/or lower than the mean reference value of 0.0651.

Europe. First, the range itself is irregular and beech is not uniformly represented everywhere. Second, the presented material does not originate from a single research project, but it is rather a result of a series of studies extended over 10 years, aimed at different topics in different regions. Nevertheless, all these studies were performed in a single laboratory (Technical University in Zvolen) using equal or very similar methodologies, so that their results are fully comparable. Despite the overrepresentation of some areas (e.g., biosphere reserves Pol'ana in Central Slovakia or Poloniny on the Slovak-Polish-Ukrainian border), the whole investigated area appears to be sufficiently covered without large gaps.

The observed levels of the genetic multiplicity and diversity are comparable with previous studies. Using the same set of loci, GÖMÖRY *et al.* (1999) found very similar values in the southern and southwestern parts of the Balkans. However, no substantial differences exist even compared to studies covering other parts of the distribution range of European beech (KONNERT, 1995; MÜLLER-STARCK and ZIEHE, 1991; HAZLER *et al.*, 1997 etc.), except those employing rather limited sets of polymorphic loci.

There is no concordance between the geographical trends of the genetic multiplicity (allelic richness) and genetic diversity. Whereas the populations containing most alleles are generally concentrated in the southeastern part of the investigated territory and a geographical trend is almost clinal, the trend of the effective number of alleles is much less obvious, with a tendency of increasing diversity in the western and northwestern populations. Such diverging and even opposite 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. Although the maps of the fossil pollen density by HUNTLEY and BIRKS (1981) allow to conclude, that a refugium may have been situated at the southeastern limit of the Carpathian arc, recent revisions of paleobotanical data indicate that the existence of such a refugium is a controversial issue, mainly due to a lack of reliably dated pollen records from the Romanian Carpathians (BREWER, 2002; DE BEAULIEU, MAGRI – unpublished data). The Slovenian and Croatian beech populations exhibit a close genetic similarity to the Romanian ones both at isozyme loci (GÖMÖRY *et al.*, 1999), and cpDNA and mtDNA haplotypes (DEMESURE *et al.*, 1996; VENDRAMIN *et al.*, 2001), although they are rather differentiated from the southern Balkans populations (Bulgaria, Greece, Serbia, Macedonia). No systematic differences between Romania and northern Balkans have been recorded in adaptive traits like bud flushing either (Von WUEHLISCH *et al.*, 1993). This allows to suppose a common post-glacial origin of populations on the whole investigated territory or even on a larger part of the range, but answering the question, whether the refugium was sheltered by the southern slopes of East Alps or by the southeastern edge of Carpathians, needs more palaeological evidence to be answered reliably.

The two approaches, used to identify the patterns of genetic differentiation, yielded contrasting interpretations. Neither principal coordinate analysis nor cluster analysis allowed to find any interpretable structure in the genetic distance matrix. On the other hand, the course of the distogram clearly indicates that populations located close to each other are genetically more similar than distant populations. Apparently, neither there is a clinal variation in allelic frequencies, nor the beechwoods in the investigated area can be subdivided into regional subpopulations strongly differentiated from each other, because both these patterns could have been identified by genetic distances. On the other hand, the presence of a more fine-grained structure of smaller regions which, despite a certain internal heterogeneity, are at least weakly differentiated, may become undiscovered using this approach, but it is reflected in the genetic distogram. In beech, most differentiation was found between regional populations originating from different glacial refugia and/or different postglacial recolonization routes (COMPS *et al.*, 2001; DEMESURE *et al.*, 1996; GÖMÖRY *et al.*, 1999). This is, however, not the case of the investigated area. On the other hand, differences among local or regional populations, which arose through the adaptation to local environments or through random processes and are maintained through isolation by distance, may explain the controversy between the distogram and the PCoA based on the same genetic distance matrix.

Stationary patterns of variation, described by a distogram or a variogram, have already been found in several broadleaved species with a continuous range, like oaks (LE CORRE *et al.*, 1998) and beech (DEGEN and SCHOLZ, 1998; GÖMÖRY *et al.*, 1998). In a set of German beech populations, DEGEN and SCHOLZ (1998) observed a similar sinusoidal course of the genetic distogram, with genetic distance smaller than expected with a random spatial distribution of allelic frequencies in low distance classes and bigger than expected in large distance classes. However, the "wavelength" was approximately a half of that observed within this study (250–300 km), indicating a smaller size of genetically homogeneous patches.

Absence of observable trends in the genetic distance matrix and a considerable "nugget effect" in the distogram indicate, that there is a substantial genetic differentiation even among populations separated by very small distances. This may be due to several reasons. First, the effects of human activities cannot be excluded. Although planting of beech in the area of interest has generally been limited and beechwoods have mostly been regenerated naturally, a part of the investigated populations may have originated from artificial regeneration. In this case, seed transfer, limited number of parental trees, nursery treatments and other factors may lead to a strong distortion of allelic frequencies (KONNERT and SCHMIDT, 1996). Second, Carpathians are a mountainous area where environmental conditions change at very small scales related mainly to altitude, but also slope aspect, parent rock etc. Gene flow between populations at various altitudes may be limited due to phenological isolation under such conditions, so that even populations separated by few kilometers may become strongly differentiated, whereas genetic structures of populations within the same altitudinal zone are more homogeneous (VYŠNÝ *et al.*, 1995). This may explain the observed pattern in the distograms: populations separated by approx. 40 km are genetically more similar than immediately neighbouring ones.

Acknowledgements

This study was supported by research grants 1/4036/97, 1/7056/20 and 1/0201/03 of the Slovak Grant Agency for Science and EVK2-1999-00244 of the EU. The technical assistance of Z. SLANČIKOVÁ and H. PAROKOVÁ is greatly appreciated.

References

BREWER, S.: Recolonisation postglaciaire de quelques taxons tempérés en Europe: une approche spatiale et temporelle. PhD Thesis, Université d'Aix-Marseille I., 179 pp. (2002). — COMPS, B., GÖMÖRY, D., LETOUZEY, J., THIÉBAUT, B. and PETIT, R. J.: Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. *Genetics* **157**(1): 389–397 (2001). — COMPS, B., MÁTYÁS, C., GEBUREK, T. and LETOUZEY, J.: Genetic variation in beech populations (*Fagus sylvatica* L.) along the Alp chain and in the Hungarian basin. *Forest Genetics* **5**(1): 1–10 (1998). — COMPS, B., THIÉBAUT, B., PAULE, L., MERZEAU, D. and LETOUZEY, J.: Allozymic variability in beechwoods (*Fagus sylvatica* L.) over central Europe: spatial differentiation among and within stands. *Heredity* **65**: 407–417 (1990). — COMPS, B., THIÉBAUT, B., SUGAR, I., TRINAJSTIĆ, I. and PLAZIBAT, M.: Genetic variation of the Croatian beech stands (*Fagus sylvatica* L.): spatial differentiation in connection with the environment. *Annales des Sciences Forestières* **48**: 15–28 (1991). — DEGEN, B., PETIT, R. J. and KREMER, A.: SGS – Spatial Genetic Software: A computer program for analysis of spatial genetic and phenotypic structures of individuals and populations. *Journal of Heredity* **92**: 447–448 (2001). — DEGEN, B. and SCHOLZ, F.: Spatial genetic differentiation among populations of European beech (*Fagus sylvatica* L.) in Western Germany as identified by geostatistical methods. *Forest Genetics* **5**(3): 191–199 (1998). — DEMESURE, B., COMPS, B. and PETIT, R. J.: Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. *Evolution* **50**: 2515–2520 (1996). — GALLOIS, A., AUDRAN, J. C. and BURRUS, M.: Assessment of genetic relationships and population

on discrimination among *Fagus sylvatica* L. by RAPD. *Theoretical and Applied Genetics* **97**(1-2): 211–219 (1998). — GÖMÖRY, D., HYNEK, V. and PAULE, L.: Delineation of seed zones for European beech (*Fagus sylvatica* L.) in the Czech republic based on isozyme gene markers. *Annales des Sciences Forestières* **55**(4): 425–436 (1998). — GÖMÖRY, D., PAULE, L., BRUS, R., ZHELEV, P., TOMOVIĆ, Z. and GRAČAN, J.: Genetic differentiation and phylogeny of beech on the Balkan Peninsula. *Journal of Evolutionary Biology* **12**(4): 746–754 (1999). — GREGORIUS, H.-R.: Genetischer Abstand zwischen Populationen. I. Zur Konzeption der genetischen Abstandsmessung. *Silvae Genetica* **23**: 22–27 (1974). — GREGORIUS, H.-R. and ROBERDS, J. H.: Measurement of genetical differentiation among subpopulations. *Theoretical and Applied Genetics* **71**: 826–834 (1986). — HAMRICK, J. L., GODT, J. W. and SHERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. *New Forests* **6**: 95–124 (1992). — HAZLER, K., COMPS, B., SUGAR, I., MELOVSKI, I., TASHEV, I. and GRAČAN, J.: Genetic structure of *Fagus sylvatica* L. populations in southeastern Europe. *Silvae Genetica* **46**: 229–236 (1997). — HUNTLEY, B. and BIRKS, H. J. B.: An Atlas of Past and Present Pollen Maps for Europe: 0-13000 Years Ago. Cambridge University Press, London – New York (1983). — KONNERT, M.: Investigations on the genetic variation of beech (*Fagus sylvatica* L.) in Bavaria. *Silvae Genetica* **44**(5-6): 346–351 (1995). — KONNERT, M. and SCHMIDT, S.: Genetische Konsequenzen der Grössensortierung in der Baumschule: Erste Ergebnisse. In: MÜLLER-STARCK, G. (Ed.) *Biodiversität und nachhaltige Forstwirtschaft*. Ecomed, Landsberg, p. 222–238 (1996). — KUSKA, F.: *Matematická kartografia [Mathematical Cartography]*. SVTL, Bratislava, 475 pp. (1960). — LARSEN, A. B.: Genetic structure of populations of beech (*Fagus sylvatica* L.) in Denmark. *Scandinavian Journal of Forest Research* **11**(3): 220–232 (1996). — LE CORRE, V., ROUSSEL, G., ZANETTO, A. and KREMER, A.: Geographical structure of gene diversity in *Quercus petraea* (Matt.) Liebl. III. Patterns of variation identified by geostatistical analyses. *Heredity* **80**: 464–473 (1998). — LEONARDI, S. and MENOZZI, P.: Genetic variability of *Fagus sylvatica* L. in Italy: the role of postglacial recolonization. *Heredity* **75**: 35–44 (1995). — MERZEAU, D., DI GIUSTO, F., COMPS, B., THIÉBAUT, B., LETOUZEY, J. and CUGUEN, J.: The allozyme variants of beech (*Fagus sylvatica* L.): inheritance and application to a study of the mating system. *Silvae Genetica* **38**: 195–201 (1989). — MÜLLER-STARCK, G. and STARKE, R.: Inheritance of isoenzymes in European beech (*Fagus sylvatica* L.). *Journal of Heredity* **84**: 291–296 (1993). — MÜLLER-STARCK, G. and ZIEHE, M.: Genetic variation in populations of *Fagus sylvatica* L., *Quercus robur* L., and *Quercus petraea* (Matt.) Liebl. in Germany. In: MÜLLER-STARCK, G. and ZIEHE, M. (Eds.) *Genetic Variation in European Populations of Forest Trees*. Sauerländer's Verlag, Frankfurt am Main. p. 125–139 (1991). — PAULE, L., GÖMÖRY, D. and VYŠNÝ, J.: Genetic diversity and differentiation of beech populations in Eastern Europe. In: S. F. MADSEN (Ed.), *Genetics and Silviculture of Beech*. *Forskningserien* **11**: 159–167 (1995). — PETIT, R. J., EL MOUSA-DIK, A. and PONS, O.: Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* **12**: 844–855 (1998). — PHARE: FOREST RESEARCH INSTITUTE ZVOLEN, PROSPECT CONSULTING AND SERVICES, JOINT RESEARCH CENTRE EC, SPACE APPLICATIONS INSTITUTE: Preparation of the Multi-country Forestry Programme PHARE. Multi-country Report. The European Commission Phare Programme, 450 pp. (1998) — PIAZZA, A., MENOZZI, P. and CAVALLI-SFORZA, L.: The making and testing of geographic gene-frequency maps. *Biometrics* **37**: 635–659 (1981). — RAYMOND, M. and ROUSSET, F.: An exact test of population differentiation. *Evolution* **49**: 1280–1283 (1995). — VENDRAMIN, G. G., ANZIDEI, M., PASTORELLI, R., CARNEVALE, S., FINESCHI, S., TAURCHINI, D. and SALVINI, D.: *Fagus sylvatica*. In: PETIT, R. J., LEGAY, M. and DEMESURE, B. (Eds.). *Phylogeography of European Forest Trees and Shrubs and Application for their Conservation and Management*. INRA – ONF, Fontainebleau (poster) (2001). — VON WUEHLISCH, G., JACQUES, D. and MUHS, H. J.: Phenological differences between beech provenances. In: VON WUEHLISCH, G. and MUHS, H. J. (Eds.): *Scientific Basis for the Evaluation of Genetic Resources of Beech*. BFH, Ahrensburg, p. 229–232 (1993). — VON WUEHLISCH, G., KRUSCHE, D. and MUHS, H. J.: Variation in temperature sum requirement for flushing of beech provenances. *Silvae Genetica* **44**(5-6): 343–346 (1995). — VON WUEHLISCH, G., LIESEBACH, M., MUHS, H. J. and STEPHAN, R.: A network of international beech provenance trials. In: TUROK, J., KREMER, A. and DE VRIES, S. (compilers). *First EUFORGEN Meeting on Social Broadleaves*. IPGRI, Rome, p. 164–172 (1997). — VORNAM, B. and HERZOG, S.: cpDNA-polymorphism of beech (*Fagus sylvatica*). *Allgemeine Forst- und Jagdzeitung* **167**(6): 122–126 (1996). — VYŠNÝ, J., SHVADCHAK, I. M., COMPS, B., GÖMÖRY, D. and PAULE, L.: Genetic diversity and differentiation of beech populations (*Fagus sylvatica* L.) in Western Ukraine. *Russian Journal of Genetics* **31**: 1309–1319 (1995). — WEIR, B. S.: *Genetic Data Analysis II*. Sinauer Associates, Sunderland, 445 pp. (1996).