Ann. Sci. For. 55 (1998) 425–436

Original article

Delineation of seed zones for European beech (*Fagus sylvatica* L.) in the Czech Republic based on isozyme gene markers

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(Received 21 March 1997; accepted 2 August 1997)

Abstract – Seed zones for European beech (*Fagus sylvatica* L.) in the Czech Republic were proposed on the basis of isozyme polymorphism. Twenty beech populations distributed over the natural range of beech in the target area were analyzed using 12 isozyme loci. Analysis of genetic distances revealed the existence of geographical differentiation patterns. Allelic frequencies were estimated for a square network of 300 points, covering the territory of the Czech Republic, employing kriging as an optimum spatial interpolation method. Cluster analysis based on allelic profiles of the kriging points made it possible to divide the investigated area into eight seed zones. (© Inra/Elsevier, Paris.)

***Fagus sylvatica* / seed zones / isozymes / kriging**

Résumé – Définition de régions de provenances pour le hêtre européen (*Fagus sylvatica* L.) en République Tchèque sur la base de marqueurs isoenzymatiques. La proposition de régions de provenances en République Tchèque pour le hêtre commun (*Fagus sylvatica* L.) a été basée sur l'étude de son polymorphisme isoenzymatique. Pour cela, vingt populations de hêtre, réparties sur l'aire d'extension naturelle dans le territoire examiné ont été analysées pour 12 loci isoenzymatiques. L'analyse des distances génétiques a montré l'existence d'une structuration géographique. Les fréquences alléliques ont été estimées par la méthode de krigeage, méthode d'interpolation spatiale, pour un réseau quadratique de 300 points recouvrant l'ensemble du territoire tchèque. L'analyse cladistique basée sur les profils alléliques en tout point du krigeage a permis de diviser la zone examinée en huit régions de provenances (© Inra/Elsevier, Paris.)

***Fagus sylvatica* / zone de provenance / isozymes / krigeage**

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1. INTRODUCTION

In most countries with a developed forestry, a concept of seed zones or provenance regions is used at least for economically important tree species. These terms are not equivalent, but both are based on the assumption that the intraspecific genetic variation is spatially structured due to adaptation to the environment or to other mechanisms. An uncontrolled transfer of seed or planting material can thus lead to a substantial reduction of survival and growth, and to economical losses.

Seed zones could therefore be defined as genetically more or less homogeneous regions [16]. However, genetic information was usually lacking at the moment when a need for regulation of transfer of propagation material was recognized; that is why seed zones were and are often based on some kind of ecological classification. Since the variation of soil properties is mostly too fine-grained to allow the delineation of reasonable regions, the classification is mostly confined to climatic data. When experimental data on morphological or physiological traits are available from provenance, ecophysiological or other studies, these preliminary seed zones are mostly revised and new zones based on ecological as well as experimental data are defined [1, 27]. At present, the Czech Republic is divided into 41 natural forest regions *(figure 1)* corresponding to the natural geomorphological division of the country and defined on the basis of environmental conditions, which, together with altitudinal vegetation zones, serve as the basis for seed transfer regulation. For European beech, a proposal of new seed zones is being prepared *(figure 1)*. The seed zones were defined on the basis of ecogeography and the introductory results of provenance tests. Within the proposed seed zones, 'core regions' were established, comprising the areas with the highest proportion of indigenous and valuable beech populations, to which no propagation material from other regions can be imported [17].

Allozymes have been considered unsuitable for the development of seed zones referring to the fact that a major part of the genetic variation in allozyme loci is allocated within, not among populations, and that there is no agreement between the allozyme loci differentiation and the distribution patterns of morphological and quantitative traits found in provenance experiments [11]. However, several studies have proven that there are clear geographical patterns in several tree species and/or loci [2, 9], indicating adaptational mechanisms operating on these loci. In some cases these mechanisms were described [3]. This indicates a potential usefulness of allozymes for the definition of the spatial structure of genetic variation.

Unless there is a special project aimed at the delineation of seed zones on the basis of allozyme gene markers, one of the problems of this approach is the density of the network of sample populations. Generally, only few populations (frequently selected and analyzed for completely different goals) have been included in countrywide studies of most tree species. Even in cases when the geographical pattern of gene frequencies is clear and the populations are clustered in well-defined groups, there may arise the problem of how to define the boundaries among individual zones.

Gene frequency can be considered a regionalized variable, i.e. its value depends on the geographical position of the sampling location. Regionalized variable theory assumes that the spatial variation of any variable can be expressed as the sum of three components: a structural component, associated with a constant mean value or a constant trend; a random, spatially correlated component; and a random

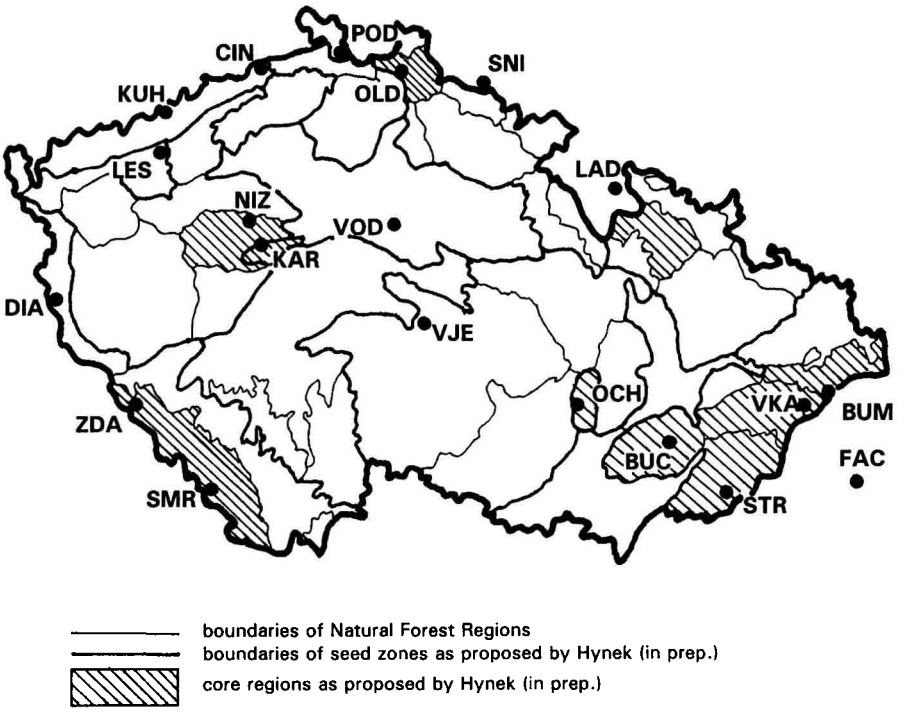

Figure 1. Location of the sampled populations within the natural forest regions of the Czech Republic. See *table I* for abbreviations.

noise [4]. Based on this assumption, Krige (1951 *ex* Clark [6]) and Matheron [18] developed a method of the optimum interpolation, providing a best linear unbiased estimate of a variable at a given point. The method is known under the name 'kriging.' Although the method was originally developed for use in the mining industry, it has recently found wide application in soil, groundwater and vegetation mapping, as well as in human and plant genetics. Piazza et al. [23] provide a detailed description of the principles of this method together with the application to mapping the gene frequencies in human populations.

In its simplest form, kriging is a method of weighted averaging of the observed values of a variable z within a neighbourhood V containing n points. In case of ordinary kriging, i.e. when no long-range trends are present, the average of differences of z between any two places $\mathbf{x}$ and $\mathbf{x} + \mathbf{h}$ separated by a distance vector h, is expected to be zero ($E[z(\mathbf{x}) - z(\mathbf{x} + \mathbf{h})] = 0$) and the variance of differences depends only on the distance between sites: ($E[\{z(\mathbf{x}) - z(\mathbf{x} + \mathbf{h})\}^2] = 2\gamma(\mathbf{h})$, where the function $\gamma(\mathbf{h})$ is known as semivariance. If the above-mentioned conditions are fulfilled, the semivariance can be estimated from sample data as

$$\hat{\gamma}(\mathbf{h}) = \frac{1}{2n} \sum_{i=1}^{n} \{z(\mathbf{x}_i) - z(\mathbf{x}_i + \mathbf{h})\}^2$$

where n is the number of pairs of sample points separated by distance h. The value of z at the point $\mathbf{x}$ can then be estimated as

$$\hat{z}(\mathbf{x}_0) = \sum_{i=1}^{n} \lambda_i \cdot z(\mathbf{x}_i)$$

where λ_i is the weight assigned to the i-th point, and

$$\sum_{i=1}^{n} \lambda_i = 1$$

The minimum variance of $\hat{z}(\mathbf{x})$ is

$$\sigma_e^2 = \sum_{i=1}^{n} \lambda_j \gamma(\mathbf{x}_j, \mathbf{x}_0) + \psi$$

and it is obtained when

$$\sum_{j=1}^{n} \lambda_j \gamma(\mathbf{x}_i, \mathbf{x}_j) + \psi = \gamma(\mathbf{x}_i, \mathbf{x}_0)$$

The solution of these equations provides the weights λ_i [4, 23].

We tried to apply this method for estimation of allozyme gene frequencies in a dense network of points by interpolation between analyzed populations and subsequently to propose seed zones as genetically homogeneous regions comprising points with similar allelic profiles.

2. MATERIALS AND METHODS

For this study, 17 European beech (*Fagus sylvatica* L.) populations, quite regularly distributed over the range of beech in the Czech Republic, were used. To complete the reference population network in areas where no Czech populations were sampled, one Slovak and two Polish populations from neighbouring regions were included. The location of the analyzed populations is given in *table I*. Only indigenous stands (mostly gene reserves) were sampled. Twigs with dormant buds were collected from 50 trees chosen at random in each population.

Proteins from buds and cambium were extracted using the 0.1 M Tris-HCl buffer pH

Table I. Geographical coordinates of the investigated beech populations.

Abbr.	Population	Geographical unit	Country	Longitude	Latitude	Altitude
DIA	Diana	Bohemian Forest	CZ	12°34'	49°09'	500
ZDA	Z6 ldánidla	S6 lumava	CZ	13°18'	49°07'	1 200
SMR	Smrc6 ina	S6 lumava	CZ	13°55'	48°43'	1 120
LES	Lestkov	Doupovské Mts.	CZ	13°15'	50°22'	550
KUH	Kühnhaide	Ore Mountains	CZ	13°12'	50°34'	800
CIN	Cínovec	Ore Mountains	CZ	14°35'	50°45'	800
POD	Podluz6 í	Luz6 ické Mts.	CZ	14°34'	50°52'	400
OLD	Oldr8 ichov	Jizerské Mts.	CZ	15°10'	50°48'	500
SNI	S8 nieżka	Giant Mountains	PL	15°40'	50°50'	750
NIZ	Niz6 bor	Kr6 ivoklát Forest	CZ	14°01'	50°01'	300
KAR	Karls6 lejn	Bohemian Karst	CZ	14°10'	49°56'	300
VOD	Vode6 ladské buc6 iny	Central Bohemian Hills	CZ	14°50'	49°55'	420
VJE	Ve6 lrny6 uJeníkov	Vysoc6 ina Mts.	CZ	15°30'	49°28'	650
LAD	LaJdek	Jeseníky Mts.	PL	16°50'	50°15'	970
OCH	Ochoz	Moravian Karst	CZ	16°11'	49°13'	450
BUC	Buchlov	Chr6 iby Mts.	CZ	17°20'	49°08'	400
STR	Strání	White Carpathians	CZ	17°42'	48°56'	400
VKA	V. Karlovice	Javorníky Mts.	CZ	18°18'	49°21'	600
BUM	Bumbálka	Javorníky Mts.	CZ	18°24'	49°23'	830
FAC	Fac6 kov	Stráz6 ovské Mts.	SK	18°38'	48°57'	820

CZ, Czech Republic; PL, Poland; SK, Slovakia.

7.0. The electrophoretic, staining procedures and zymogram interpretations followed Thiébaut et al. [25], Merzeau et al. [20] and Müller-Starck and Starke [21]. Eight enzyme systems coded by 12 loci were examined: glutamate-oxaloacetate transaminase (*Got-2*), isocitrate dehydrogenase (*Idh*), leucine aminopeptidase (*Lap-1*), malate dehydrogenase (*Mdh-1, Mdh-2, Mdh-3*), menadione reductase (*Mnr*), peroxidase (*Px-1, Px-2*), phosphoglucomutase (*Pgm*), phosphoglucose isomerase (*Pgi-2*) and shikimate dehydrogenase (*Skdh*). The allelic frequencies were calculated based on diploid genotypes. Heterogeneity of allelic frequencies among populations and between all pairs of populations was tested using the likelihood ratio test (*G*-test). To reveal the pattern of the genetic differentiation, genetic distances [15] between populations were calculated and the matrix of genetic distances was interpreted using the principal coordinate analysis [14].

The geographical coordinates (latitude, longitude) of individual populations were converted to orthogonal coordinates. The point 15°30' E / 50°00' N was chosen as the origin of the orthogonal coordinate system. Longitudinal distortion was rectified by multiplying the horizontal coordinate by the coefficient, corresponding to 0.97987 per latitudinal degree (Z6 Líhlavník, personal communication). Variogram models were derived and kriging estimates of gene frequencies were calculated for each allele separately (except for biallelic loci). The linear model

$$\left(\gamma(h) = C_0 + \frac{C}{a} \cdot h \text{ for } h \le a,\ \gamma(h) = C \text{ for } h > a\right)$$

was used most frequently – for 18 alleles, the exponential model

$$\left(\gamma(h) = C_0 + C \cdot \left[1 - e^{-h/a}\right]\right)$$

in 14 cases, and the spherical model

$$\left(\gamma(h) = C_0 + C \cdot \left(\frac{3h}{2a} - \frac{1h^3}{2a^3}\right)\right.$$

$$\left.\text{for } h \le a,\ \gamma(h) = C \text{ for } h > a\right)$$

in two cases (in the models, $\gamma(h)$ is the semivariance, h is the lag distance, C is the sill, a is the range and C_0 is the 'nugget effect'). Ordinary punctual kriging was performed using the Geo-EAS (Geostatistical Environmental Exposure Assessment Software U.S. Environmental Protection Agency, Las Vegas NV, U.S.A.) program. The network of estimation points was a grid 27.78 km on a side (15 latitudinal minutes and approximately 23 longitudinal minutes). For loci with more than two alleles, allelic frequencies were subsequently adjusted proportionately to the estimated values so that their sum was 1.0.

Genetic distances between estimation points were then calculated and the matrix of distances was subjected to cluster analysis using the UPGMA (Unweighted pair-group methode using averages) clustering procedure. The resulting dendrogram was subsequently divided on a level, providing a reasonable number of clusters (seed zones). The kriging standard deviations summed over all alleles were used for quantification of the precision of allele frequency estimates, and thus also for the precision of classification of kriging points to individual zones.

3. RESULTS

Allelic frequencies in the investigated populations are given in *table II*. The allelic frequencies within the whole population set proved to be heterogeneous in only one locus (*Lap-1*); however, significant heterogeneities were found between several pairs of populations in all loci exhibiting major polymorphisms (due to a large number of tests, they cannot be presented in a tabular form). Although a considerable variation of allelic frequencies can be observed, there are no clear latitudinal or longitudinal clines, nor any correlation with altitude. More likely, the character of the genetic variation appears to be mosaic in form.

The multilocus evaluation of the genetic differentiation using genetic distances provided quite similar results to the single locus patterns. However, it cannot be stated that there are no differentiation patterns observable. In *figure 2*, which is an

Table II. Allelic frequencies in the investigated beech populations.

Locus		Population									
		DIA	ZDA	SMR	LES	KUH	CIN	POD	OLD	SNI	NIZ
Px-1	*A*	.438	.255	.220	.278	.282	.345	.289	.264	.357	.198
	B	.563	.745	.780	.722	.718	.655	.711	.736	.643	.802
Px-2	*A*	.087	.138	.110	.148	.113	.167	.123	.094	.128	.112
	B	.762	.691	.710	.694	.585	.679	.763	.811	.723	.793
	C	.143	.170	.180	.157	.302	.155	.114	.094	.149	.095
	D	.008	.000	.000	.000	.000	.000	.000	.000	.000	.000
Lap-1	*A*	.026	.010	.020	.019	.032	.024	.000	.009	.021	.000
	B	.509	.469	.380	.426	.415	.476	.395	.330	.500	.403
	C	.298	.367	.310	.389	.277	.298	.491	.557	.323	.282
	D	.167	.153	.290	.167	.277	.202	.114	.104	.156	.315
Got-2	*A*	.349	.467	.408	.250	.218	.345	.360	.387	.378	.421
	B	.651	.533	.592	.750	.782	.655	.640	.613	.622	.579
Mnr	*A*	.000	.000	.000	.000	.000	.000	.009	.000	.010	.000
	B	.930	.878	.980	.972	.918	.976	.930	.943	.949	.857
	C	.008	.000	.000	.000	.000	.000	.000	.000	.000	.000
	D	.063	.122	.020	.028	.082	.024	.061	.057	.041	.143
Idh	*A*	.305	.337	.260	.147	.264	.274	.263	.212	.316	.230
	B	.672	.653	.740	.843	.709	.726	.737	.788	.684	.738
	C	.023	.010	.000	.010	.027	.000	.000	.000	.000	.033
Mdh-1	*C*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.981	.990	1.000
	D	.000	.000	.000	.000	.000	.000	.000	.019	.010	.000
Mdh-2	*A*	.031	.031	.080	.093	.073	.143	.026	.163	.051	.040
	B	.008	.000	.000	.000	.000	.000	.018	.000	.000	.000
	C	.961	.959	.920	.907	.927	.857	.956	.837	.949	.960
	D	.000	.010	.000	.000	.000	.000	.000	.000	.000	.000
Mdh-3	*A*	.250	.298	.213	.245	.409	.214	.272	.279	.296	.230
	B	.750	.702	.788	.755	.591	.786	.728	.721	.704	.770
Pgi-2	*A*	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	B	.930	1.000	.990	.970	.975	1.000	1.000	.990	1.000	.988
	C	.070	.000	.010	.030	.025	.000	.000	.010	.000	.013
Pgm	*A*	.000	.011	.000	.000	.013	.000	.000	.000	.000	.000
	B	1.000	.989	1.000	1.000	.988	1.000	1.000	1.000	.990	1.000
	C	.000	.000	.000	.000	.000	.000	.000	.000	.010	.000
Skdh	*A*	.000	.020	.000	.010	.000	.000	.000	.000	.000	.000
	B	.980	.980	.990	.960	1.000	1.000	.990	.990	.980	1.000
	C	.000	.000	.000	.010	.000	.000	.000	.000	.020	.000
	D	.020	.000	.010	.020	.000	.000	.010	.010	.000	.000

Locus		Population									
		KAR	VOD	VJE	LAD	OCH	STR	BUC	VKA	BUM	FAC
Px-1	*A*	.325	.260	.333	.415	.310	.443	.400	.420	.359	.350
	B	.675	.740	.667	.585	.690	.557	.600	.580	.641	.650
Px-2	*A*	.211	.183	.157	.160	.160	.189	.214	.278	.242	.265
	B	.614	.731	.787	.660	.690	.698	.704	.625	.645	.622
	C	.175	.087	.056	.181	.150	.113	.082	.097	.113	.112
	D	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
Lap-1	*A*	.009	.000	.010	.031	.000	.076	.011	.030	.067	.020
	B	.430	.461	.519	.354	.580	.511	.630	.330	.567	.530
	C	.254	.412	.356	.458	.310	.261	.207	.390	.217	.280
	D	.307	.127	.115	.156	.110	.152	.152	.250	.150	.170
Got-2	*A*	.421	.402	.283	.417	.400	.432	.409	.470	.382	.438
	B	.579	.598	.717	.583	.600	.568	.591	.530	.618	.563

Table II. Continued.

Locus		Population									
		KAR	VOD	VJE	LAD	OCH	STR	BUC	VKA	BUM	FAC
Mnr	*A*	.000	.000	.000	.000	.010	.000	.000	.000	.000	.000
	B	.991	.971	.917	.875	.890	.962	.970	.930	.941	.890
	C	.000	.000	.000	.010	.010	.000	.000	.000	.000	.010
	D	.009	.029	.083	.115	.090	.038	.030	.070	.059	.100
Idh	*A*	.237	.231	.269	.229	.300	.288	.223	.250	.364	.436
	B	.728	.769	.712	.771	.700	.692	.766	.750	.636	.564
	C	.035	.000	.019	.000	.000	.019	.011	.000	.000	.000
Mdh-1	*C*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	D	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
Mdh-2	*A*	.026	.067	.046	.052	.074	.071	.063	.040	.059	.063
	B	.044	.000	.009	.000	.000	.000	.000	.000	.000	.000
	C	.930	.933	.944	.948	.926	.929	.938	.960	.941	.938
	D	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
Mdh-3	*A*	.237	.279	.287	.362	.265	.188	.198	.250	.294	.347
	B	.763	.721	.713	.638	.735	.813	.802	.750	.706	.653
Pgi-2	*A*	.000	.000	.000	.000	.000	.000	.053	.000	.000	.000
	B	.990	1.000	.980	.990	.990	.981	.947	1.000	1.000	.940
	C	.010	.000	.020	.010	.010	.019	.000	.000	.000	.060
Pgm	*A*	.000	.000	.000	.000	.000	.009	.021	.000	.000	.000
	B	1.000	1.000	1.000	1.000	1.000	.991	.979	1.000	1.000	1.000
	C	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
Skdh	*A*	.000	.000	.000	.000	.010	.000	.000	.000	.015	.000
	B	1.000	1.000	.980	.990	.960	.969	.979	.990	.894	.990
	C	.000	.000	.000	.010	.000	.000	.000	.000	.000	.010
	D	.000	.000	.020	.000	.030	.031	.021	.010	.091	.000

See *table I* for abbreviations.

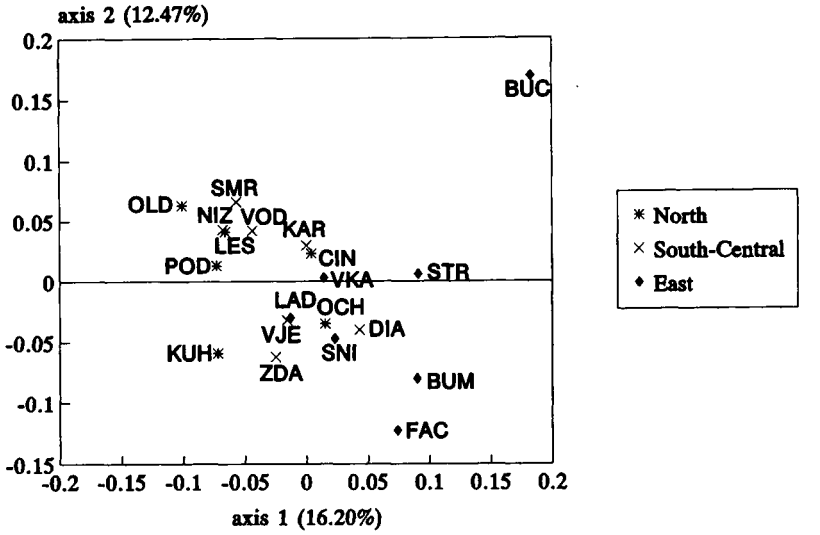

Figure 2. Principal coordinate analysis based on genetic distances between the investigated beech populations – projection into the first two principal axes. See *table I* for abbreviations.

interpretation of the genetic distance matrix, a concentration of points representing eastern Bohemian, Silesian and Moravian beech populations on the right side, those representing north-west and north Bohemia on the left side and those representing southern and central parts of Bohemia in the centre is recognizable. However, the groups overlap considerably. In addition, this figure presents only the projection into the first two principal axes, accounting together for only approximately 29 % of the total variation; a considerable portion of the variation is thus not displayed there. It also must be noted that the division of the territory into the eastern, northern and southern/central regions was arbitrary, demonstrating only that some patterns exist. No non-overlapping clusters of points corresponding to continuous regions could be identified in *figure 2*. The delineation of seed zones can thus hardly be based on the original samples. Firstly, the differentiation pattern is ambiguous (which, to a large extent, can be attributed to sampling error). Secondly, the sampling network is irregular, which does not allow any justifiable and objective method for drawing the boundaries between zones.

Therefore, our approach was based on estimation of allelic frequencies in a network of regularly distributed points using kriging as an optimum spatial interpolation method. As mentioned in the Methods section, kriging estimates were derived for each allele separately, except for the biallelic loci. Variogram equations were thus optimized for each allele (as an example, a variogram for the *Got-2/A* allele is presented in *figure 3*). The result was a matrix of allelic frequencies for 459 points (27 divisions in the longitudinal direction, 17 divisions in the direction of latitude). Before further treatment, 159 points lying outside the territory of the Czech Republic were excluded. For the remaining 300 points, genetic distances were calculated and subjected to cluster analysis. The resulting dendrogram *(figure 4)* was divided on a level, providing a reasonable number of eight clusters. The structure of the dendrogram, however, is not completely unequivocal, i.e. there are no really consistent clusters with tightly linked objects. Another number of clusters (six or three) could therefore be chosen as well. Decreasing the cutting level further would lead to a large number of excessively small clusters. Each kriging point was classified to a proposed seed zone corresponding to one cluster. The seed zones are continuous and do not overlap. Boundaries of seed zones divide the points classified to different clusters.

Figure 5 presents the seed zones defined on the basis of eight clusters. Choosing six clusters, the regions 1, 2 and 3 would be amalgamated. By choosing three clusters, the first zone would contain only cluster 6, i.e. Ore Mountains and the adjacent basin; the second zone would include clusters 7 and 8, i.e. Silesian and Moravian populations (except from the Českomoravská vrchovina Mountains); and the third zone would be comprised of the clusters 1 to 5, i.e. the rest of the territory. The grid density indicates the kriging standard deviation (summed over all loci), (a dense grid indicates high *KSD*, i.e. a low precision of allele frequency estimation and thus also a lower probability of a correct classification of kriging locations to individual seed regions).

4. DISCUSSION

The territory of the Czech Republic is ecophysiographically quite heterogeneous, but there are no clear and continuous ecological gradients like the north–south gradient in Scandinavia. This fact probably contributed considerably to the lack of clear patterns of the genetic differentiation observed in the presented material. A significant heterogeneity of allelic fre-

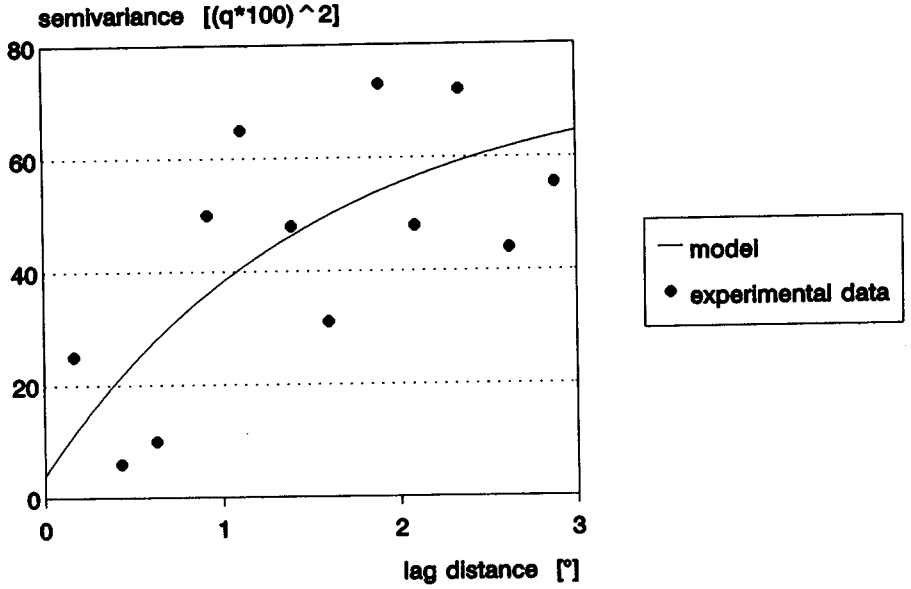

Figure 3. Variogram of the *Got-2/A* allele.

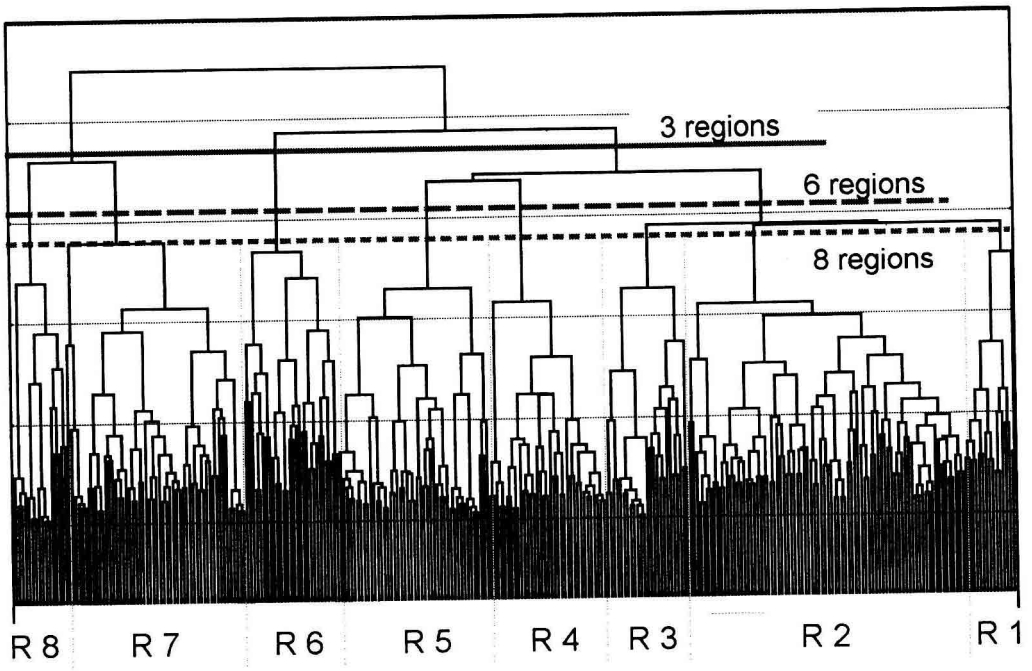

Figure 4. Dendrogram of the UPGMA cluster analysis based on genetic distances between kriging locations.

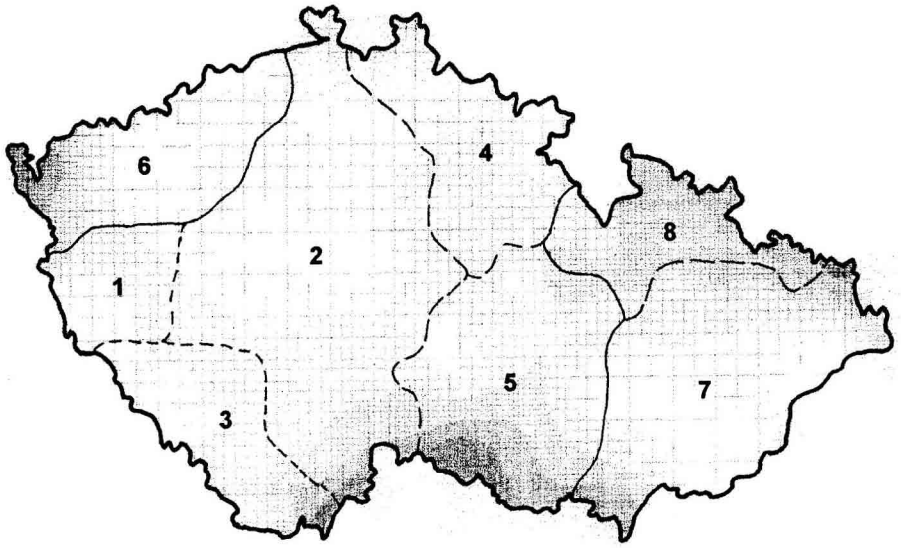

Figure 5. Delineation of the proposed seed zones.

quencies, but without unequivocal clines, probably results from random processes as well as the adaptation determined by a complex of environmental factors rather than by one predominating factor. The multilocus approach, however, indicated the existence of a spatial organization of the genetic variation in beech in the Czech Republic.

From the methodological point of view, the best solution for the delineation of genetically homogeneous zones would be to have a sufficiently dense network of populations with large sample sizes to reduce the sampling error and define the boundaries directly on the basis of the original samples. However, in addition to technical and financial demands of such an approach, even in this case the genetic differentiation pattern might not correspond enough to the geographical distribution of populations to allow an objective definition of zone boundaries. A clear clustering based on isozyme phenotypes, even corresponding with the morphological differentiation, as found in *Pinus rigida* [12], is more likely an exception than a rule. In European beech, an unequivocal spatial structure was found only in range-wide studies; the genetically homogeneous regions cover mostly the territory of several states [10, 21]. On a smaller scale, the groups of genetically similar populations always overlap considerably in the geographical context [7, 8, 9, 13, 26].

Westfall and Conkle [28] propose multivariate procedures for designing the breeding zones on the basis of allozyme markers. Their approach is based on sampling individual genotypes, transforming them to numerical scores using the procedure by Smouse and Williams [24] and subjecting the scores to multivariate analyses. Sampling individual trees makes a regular covering of the investigated territory technically feasible. A similar approach was applied by Cheliak et al. [5] for *Larix laricina*, Merkle et al. [19] for *Pseudotsuga menziesii* and Yeh et al. [29]

for *Pinus contorta*. Similar to the case of four conifers investigated by Westfall and Conkle [28], it led to overlapping groups and did not allow any clear territorial divisions.

Several objections can surely be raised against our procedure as well. We see the positive aspects of this approach in smoothing the random variation of allelic frequencies, which is due to sampling error, and in the fact that the delineation of zone boundaries is based on an objective interpolation method.

In Central Europe, including the Czech Republic, beech is an important commercial tree species, but primarily it is considered a stabilizing element of forest stands. Therefore, it is not an object of intensive breeding, but much more emphasis is given to the preservation of its adaptedness and ecological stability through the gene-pool conservation of the existing indigenous populations. Natural regeneration is generally considered the best tool for fulfilling these tasks. However, in several regions the share of beech in the tree species composition has been severely decreased in the last centuries, when the indigenous broad-leaved and mixed forests were replaced by coniferous monocultures. The reconstruction of a more natural tree species composition is hardly possible without extensive reforestation. The use of appropriate seed sources is thus a relevant topic for beech.

ACKNOWLEDGEMENTS

Thanks are due to Dr. Viliam Pichler, University of California, Riverside, Mr. Peter Jaloviar, Dr. Ján Tuček and Prof. Dr. Štefan Žíhlavník, Technical University in Zvolen, Slovakia for their assistance with the GIS procedures. The technical assistance of Mrs. Zuzana Slančíková is also heartily acknowledged.

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