

**IMPLEMENTATION OF MONMONIER'S ALGORITHM OF MAXIMUM DIFFERENCES FOR  
THE REGIONALIZATION OF FOREST TREE POPULATIONS  
AS A BASIS FOR THE SELECTION OF SEED SOURCES**

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*Abstract* - The regionalization of forest tree populations was researched on an example of beech, as the species with the largest range and the widest ecological amplitude in Serbia. The implementation of Monmonier's algorithm of maximum differences to analyze the spatial distances and the matrix of genetic distances generated by RAPD markers produced different results, depending on the method of addressing the genetic distances, so that data processing should be planned in accordance with the number of samples and their geographic location. The analysis is simple and enables a good visualization of genetic variability barriers which, in combination with the data on the distribution and the geographic barriers, can be utilized for recommending the transfer of forest tree reproductive material.

*Key words:* Monmonier's algorithm, spatial analysis, beech, genetic diversity

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## INTRODUCTION

With the aim of recommending the transfer of forest reproductive material, we studied how the barriers of regions of forest tree populations can be detected. To this end, we selected beech as the species with the largest range and the widest ecological amplitude in Serbia. Although beech is characterized by a high degree of genetic variability, most of it is intra-population variability while inter-population variability rarely exceeds 5% (Paule et al., 1995; Leonardi and Menozzi, 1995; Larsen, 1996). However, this does not mean that spatial forms of this variability cannot be detected. The most reliable recommendations for seed transfer can be obtained based on long-term provenance tests established from seeds collected from all parts of the species' range and from diverse ecological conditions. However, the tests usually last for several decades. In situations where there are no data from long-term provenance tests, or where there is no time to perform the tests, some efficient approaches are the mapping of genetic variability conditioned by geographic variability,

the testing the seedlings in the nursery, and the use of molecular markers as the fastest method. The theoretical base of this approach is the hypothesis that the greater the genetic differentiation between two populations, the greater the adaptive genetic differentiation, and consequently the higher the risk for the transfer of seeds between them. As geographical and genetic variabilities are usually continuous variables, mathematical models can be developed to describe their interrelationship. When the locations of the individuals or populations under examination are known, the association between the genetic and geographic distances can be studied by spatial autocorrelation or regression methods. However, the results of these analyses, even if they can suggest the shape of the genetic landscape, cannot identify the locations of genetic barriers, i.e. the areas where the observed variables show great changes. The genetic barriers can be defined by Monmonier's maximum difference algorithm (Monmonier, 1973), which can be applied not only to genetic data, but also to any other type of data forming a distance matrix, when the locations are available.

## MATERIALS AND METHODS

The data on beech distribution in Serbia were taken from the National Forest Inventory, with a resolution of 4 x 4 km. The map of beech distribution in Serbia was constructed using computer program DIVA-GIS Version 5.4 (Hijmans et al., 2005). Based on the map of beech distribution, we selected the most relevant regions which represent beech in Serbia, and within them the sampling populations were selected in the field. The geographic position of the samples (latitude, longitude and altitude) were recorded by GPS navigator GARMIN eTrex Vista in the geographical coordinate system, in format (hh°hh'hh"). The data were then converted using the GEOTRANS V2.4.1 programme in format (X m, Y m), UTM coordinate system – zone 34. Altogether 27 populations that were selected covered the spatial and ecological distribution of beech populations relatively uniformly (Maps 1, 2 and 3). Ten parent trees were selected in each of the populations, and dormant buds were collected in 2007. Because of the great number of samples (270), the samples from each population were bulked. The bulking procedure significantly reduces the necessary resources for the testing of genetic variability and can be effectively applied in the RAPD analysis (Goto et al., 2001). The analysis using RAPD markers was performed with 28 primers, twenty of which produced bright bands, while the remaining 8 failed to be amplified, or the amplification was weak. Altogether 82 bands were produced, i.e. a maximum of 6, and a minimum of 2 bands per primer.

The simple genetic distances (Miller, 2005) were then computed by the software package AIS to quantify the percentage of non-overlapping of the samples  $i$  and  $j$  in the loci, expressed as:

$$D_{i,j} = \frac{\sum_{k=1}^n d_k}{n} \quad (\text{Miller, 2005})$$

$d_k = 1$  if individuals  $i$  and  $j$  have different alleles in locus  $k$ , and  $d_k = 0$  if individuals  $i$  and  $j$  have identical alleles in locus  $k$ .

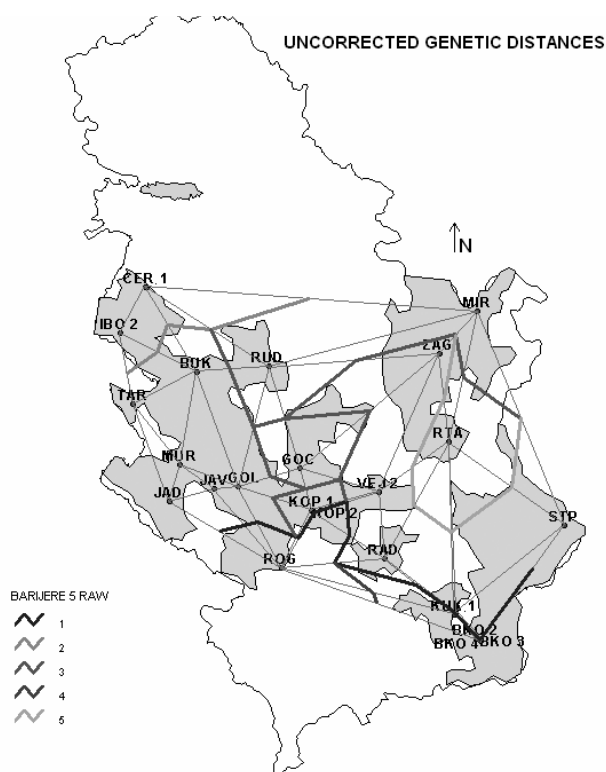
The visualization of the data which were not in the matrix of genetic data, by constructing the boundaries on the geographic map, was performed by Monmonier's algorithm in the AIS software package. The method consists of the following steps:

1. The samples are located on the map according to geographical coordinates.
2. A Delaunay triangulation is used to connect the geographic locations of the samples on the map, to generate the connectivity network among the samples.
3. The genetic distances are calculated between the adjacent samples and they are added to each edge (corresponding to the adjacent sample) on the grid.
4. Monmonier's maximum difference algorithm is used for the identification of barriers as follows: a) by selecting the edge of the network with the largest allocated distance and using it as the starting point of the barrier perpendicular to the network boundary; b) selecting the edge which is directly connected with the growing barrier with the largest genetic distance for the continuation of the barrier. The first two steps are repeated until the growing barrier meets the other barrier or the barrier of the investigated region.

## RESULTS AND DISCUSSION

The number of barriers detected in the analysis can vary. In our research, the best visualization was achieved with five barriers. Also, barriers can differ significantly depending on the method of genetic data processing. For this reason, we present the results of three methods of preparation of genetic distances.

- A. The results of applying the raw genetic distances (Map 1) show the greatest number of regions (9). They are: 1) North-western region – covering the localities Cer and Eastern Boranja; 2) Western region – Tara, Bukovi, Jadovnik, Murtenica, Javor and Golija; 3) Central-North-

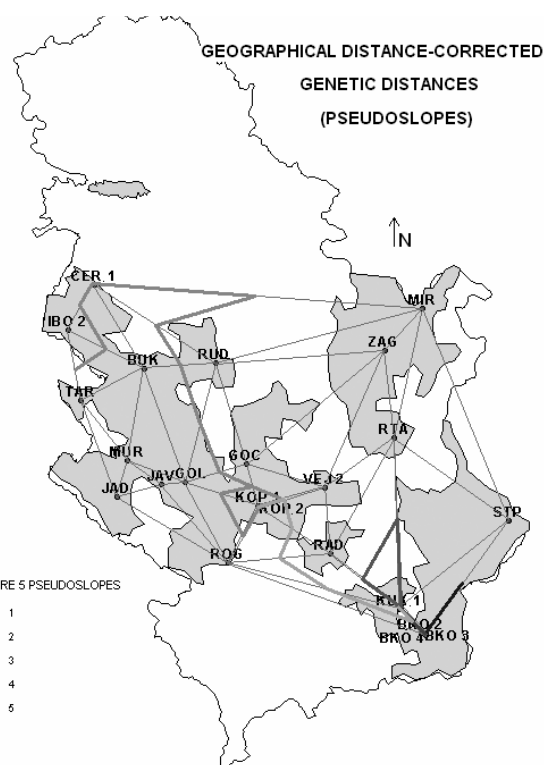


**Map 1.** Barriers generated by Monmonier's algorithm, based on raw genetic distances

Western region – from Rudnik, Northeast, to Miroč; 4) Central region – Goč and the surrounding mountains; 5) Kopaonik; 6) South-Western region – Rogozna and in the North, to the Southern slopes of Kopaonik; 7) Central-Eastern region – beech forest complexes from Žagubica, Veliki Jastrebac, Radan Planina to Stara Planina in the far East; 8) Eastern region – a small complex around Rtanj; 9) South-Eastern region – ranging South-East of Kukavica and Besna Kobila.

The above representation suggests a small spatial autocorrelation, because it can be taken as a rule that the more numerous the barriers and segments resulting from them, the weaker the interrelationship between the genetic and geographic distances.

B. The results of applying the geographical distance-corrected genetic distances (Map 2) show a somewhat smaller number of regions (6). They are:

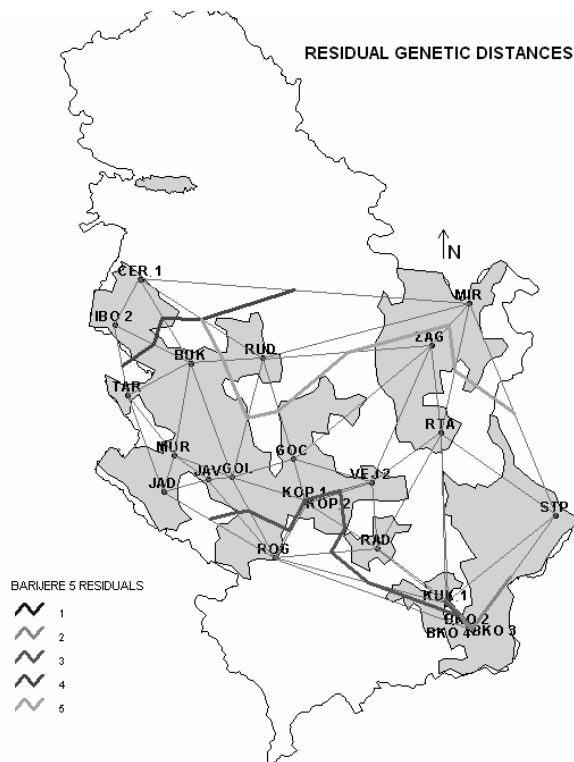


**Map 2.** Barriers generated by Monmonier's algorithm, using the geographical distance-corrected genetic distances

1) North-Western region – Cer and Eastern Boranja; 2) South-Western region – Bukovi, Tara, Murtenica, Jadovnik, Javor, Golija and Rogozna; 3) Kopaonik; 4) Eastern region – Rudnik, Goč, Veliki Jastrebac, Radan, Miroč, Žagubica, Rtanj and Stara Planina; 5) Kukavica; 6) South-Eastern region – South-East of Besna Kobila.

The application of the geographical distance-corrected genetic distances is desirable when the samples are collected in a three-dimensional space, i.e. when the altitude of the sampled location must be taken into account in addition to longitude and latitude. Also, this calculation method should be used in the cases of a relatively low number of samples (Ivetić et al., 2008).

C. The results of applying the residual genetic distances (Map 4) show the lowest number of segments (regions). They are: 1) North-Western region – Cer and Eastern Boranja; 2) Northern region



**Map 3.** Barriers generated by Monmonier's algorithm using residual genetic distances

- the stretch from Rudnik to Miroč; 3) Central region – covering the greatest part of beech range;
- 4) Southern region – Rogozna and Besna Kobila.

The use of residual genetic distances, i.e. the calculation of the matrix of expected genetic distances based on the previously computed regression, and their removal from the matrix of original genetic distances is efficient in cases of weak spatial autocorrelation. Presumably, this procedure also enables the most accurate visualization of genetic diversity because it reflects a variability which is not linked to migration (Manni et al., 2004). Only four segments (regions) are obtained. Also, it is interesting that, in this case, barriers are predominantly oriented in the East-West direction.

The simple application and the fact that input data consist only of the spatial coordinates and the matrix of the sample distances (regardless of the measuring method), make Monmonier's algorithm

suitable for wide implementation. Certainly, the results of the analysis significantly depend on the data processing method, and perhaps even more on the method of collection, i.e. on the spatial distribution of the samples. It would be ideal if the sample locations were uniformly distributed throughout the sample plot, without deviations in elevation. This scenario is possible only in small sample plots and specific-purpose sample plots. In the study of forest tree populations, which generally occur over large areas and high altitudinal amplitudes, primary attention should be focused on data processing methods. In this sense, the application of the geographical distance-corrected genetic distances and residual genetic distances can be recommended, rather than the application of raw data. In sample collection, care should be taken to avoid large areas without any samples. Also, the precision of the method can be increased by adding the virtual points in the Delaunay triangulation, which increases the precision of the analysis in the sample plot fringe areas.

The analysis is simple and it enables a good visualization of genetic variability barriers, which, in combination with the data on the distribution and the geographic barriers can be effective in the recommendations for the transfer of forest tree reproductive material. Using the results of this analysis, the visualization can be additionally improved for the construction of maps in some of the widely used GIS programs, and by their overlying with physical relief maps and other thematic maps.

## REFERENCES

- Goto, S., Miyahara, F., and Y. Ide, (2001). A Fast Method for Checking the Genetic Identity of Ramets in a Clonal Seed Orchard by RAPD Analysis with a Bulking Procedure. *Silvae Genetica* 50, 5–6. Pp. 271–775
- Hijmans, R. J., Guarino, L., Jarvis, A., O'Brien, R., Mathur, P., Bussink, C., Cruz, M., Barrantes, I., and E. Rojas, (2005). *DIVA-GIS Version 5.2 – Manual*. 79 p.
- Ivetić, V., Isajev, V., and S. Mladenović-Drinić, (2008). "Landscape Shape Interpolation" for defining spatial pattern of beech genetic diversity in Serbia. *Genetika*. Vol. 40, No. 3. Pp. 283–292

- Larsen, A.B. (1996). Genetic structure of populations of beech (*Fagus sylvatica* L.) in Denmark. *Scandinavian Journal of Forest Research* **11**(3): pp. 220-232
- Leonardi, S. and P. Menozzi, (1995). Genetic variability of *Fagus sylvatica* L. in Italy: the role of postglacial recolonization. *Heredity* **75**: pp. 35-44
- Manni, F., Guérard, E., and E. Heyer, (2004). Geographic patterns of (Genetic, Morphologic, Linguistic) Variation: How Barriers Can Be Detected by Using Monmonier's Algorithm. *Human Biology*. Vol. **76**, No. 2. Pp. 173-190
- Miller, M.P. (2005). Alleles in Space: Computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity* **96**, p. 722-724
- Monmonier, M.S. (1973). Maximum-Difference Barriers: An Alternative Numerical Regionalization Method. *Geographical Analysis*. Volume V, Nr. 3, pp. 254-261
- Paule, L., Gömöry, D. and J. Vyšný, (1995). Genetic diversity and differentiation of beech populations in Eastern Europe. In: *Genetic and silviculture of beech*. (Ed. Madsen, S.F.), *Forskningserien* **11**, 159-167

## ПРИМЕНА МОНМОНИЈЕОВОГ АЛГОРИТМА МАКСИМАЛНИХ РАЗЛИКА ЗА РАЗГРАНИЧЕЊЕ РЕГИОНА ПОПУЛАЦИЈА ШУМСКОГ ДРВЕЋА КАО ОСНОВЕ ЗА ИЗБОР ИЗВОРА СЕМЕНА

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У раду је истражена могућност одређивања граница региона популација шумског дрвећа, у циљу давања препорука за трансфер репродуктивног материјала, на примеру букве. На основу података из Националне инвентуре шума добијена је распрострањеност букве у Србији са резолуцијом 4x4 km. На основу овако добијене карте ареала, одабране су области које ће на најрелевантнији начин представљати букву у Србији. У оквиру овог истраживања, испитано је 27 популација, које релативно равномерно покривају просторну и еколошку дистрибуцију популација букве. Анализа применом RAPD маркера, урађена је са 28 прајмера. Укупно су добијене 82 траке. За визуализацију оних података који су садржани у матрици генетичких података, путем цртања граница на географској карти, коришћен је Монмонијеов алгоритам у програму AIS. Приказани су резултати три начина припреме генетичких дистанци за анализу, са задатим бројем од 5 баријера за детекцију. Резултати коришћења сирових генетичких дистанци показују највећи број сегмената (региона), што наговештава малу просторну ауто-

корелацију. Резултати коришћења генетичких дистанци исправљених на основу географских дистанци показују нешто мањи број сегмената (региона), што указује на већу прецизност добијену оваквом припремом генетичких дистанци за релативно мали број узорака који су просторно удаљени. Резултати коришћења остатака генетичких дистанци показују најмањи број сегмената (региона), што указује на велику снагу овакве припреме генетичких дистанци у случајевима слабо изражене просторне аутокорелације. Овим приступом, највероватније је омогућена и најтачнија визуализација генетичког диверзитета. Анализа је једноставна и омогућује добру визуализацију баријера генетичке варијабилности, што се у комбинацији са познавањем ареала и географских баријера, може успешно искористити за давање препорука за трансфер репродуктивног материјала шумског дрвећа. Визуализација се може додатно побољшати коришћењем резултата ове анализе за цртање карата у неком од широко коришћених програма ГИС-а, и њиховим преклапањем са картама физичког рељефа и другим тематским картама.