DELINEATION OF BEECH PROVENANCE REGIONS IN SERBIA BY SPATIAL ANALYSIS OF GENETIC DIVERSITY

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The results of spatial analysis of genetic diversity have practical application in the definition and delineation of regional provenances of forest trees. Research in this paper, was based on the material from 27 natural populations of beech in Serbia. The genetic component of the research is based on the analysis of RAPD markers from bulk samples, using 28 primers. The spatial component of the research is based on the geographical position of the studied populations. Grouping of the studied populations in the regions, as well as their separation, was performed using the Monmonier's algorithm of maximum differences. To visualize the results and mapping the regions of beech provenances in Serbia, GIS was used, with database included the results of this study.

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INTRODUCTION

The theoretical basis for the application of molecular genetic markers in risk estimation for transfer of reproductive material between forest populations is in the assumption that the greater genetic differentiation between the two populations means the greater adaptive differentiation and therefore the higher the risk for transfer of seeds between them. Assumptions derived from this approach are: 1) that the genetic differences between populations is largely adaptive, 2) that the local population are the best adapted to specific sites, and 3) that the map of genetic variation is also the map of sites which have shaped the natural selection. Adaptability on the environment conditions is evident when the genetic and sites variability are correlated; for example, when dormant buds are established earlier at higher altitudes. However, testing at the level of molecular genetic markers does not assess all the risks. Transfer zones of forest reproductive material (regions of provenances) obtained in this manner must be considered as temporary, until they are proven in long-term provenance tests.

Given that the geographic and genetic variability typically are continuous variables, mathematical models can be developed for describing of their relationship. These models usually provide the best estimate of risk for the transfer of forest reproductive material. The greater genetic difference between the seed sources populations and natural populations near the planting site means the greater risk of reproductive material transfer. These models commonly use transfer distance; for example in degrees of latitude and longitude, and meters above sea level, in order to determine the risk level of failure of adaptation. Unfortunately, many of these models are very complex, and because of that they are often avoided. Also, as the site gradients and genetic cline are continuous value, in many cases, the boundaries between regions of provenances are questionable. In this paper, using spatial analysis of genetic diversity of beech in Serbia, we give a proposition of the method to delineate the regions of provenances, which includes two major components of populations distribution in nature: genetic and spatial. Spatial analysis in the study of spatial genetic structure of beech was previously applied by DEGEN and SCHOLZ 1998, VORNAM *et al*. 2004, IVETIĆ *et al*. 2008, 2010.

MATERIALS AND METHODS

In the area of the beech natural distribution, 27 populations that will represent the beech in Serbia on the most relevant way were selected. After georeferencing the center point of the site, at approximately equal distances from this point the parent trees were selected for collecting samples (seeds or buds). In doing so, attention was paid that the parent trees are apart at a sufficient distance, to avoid collecting samples which are the result of their mutual crossing.

In order for closer understanding of genetic diversity over short distances, within provenances Cer, Istočna Boranja, Veliki Jastrebac and Besna Kobila,

subpopulations have been separated. Also, in order to determine the impact of altitude on the genetic diversity, within provenances Kukavica and Kopaonik subpopulations have been separated in different height zones, namely: five populations at Kukavica in height zones of approximately 200 m and two populations at Kopaonik with altitude difference of 500 m.

The bulk samples were made of samples from each population. Bulk samples significantly reduce the resources required for testing of genetic variation and can be successfully used in the analysis using RAPD markers (GOTO *et al*. 2001). Analysis using RAPD markers was performed with 28 primers, 20 of which gave clear bands, while at the remaining eight there was no amplification or amplification was weak. Nineteen of the twenty primers that gave bands were polymorphic, and only one was monomorphic. Total of 82 bands were obtained, with a maximum of 6, and a minimum of 2 bands was obtained per primer.

Measures of multilocus variability which use genetic distance have proven to be very sensitive in detecting spatial genetic structure (SMOUSE and PEAKALL 1999). However, the statistical power of applied techniques depends on the actual structure of the population, the sample size and spatial aspects (number, orientation, location and layout of the total area covered by the studied population). In other words, the pattern and magnitude of sampling, in terms of population, are of decisive importance.

Certainly, determining the genetic boundaries (barriers) is one of the most obvious results of spatial analysis of genetic diversity. In geometric terms, the genetic boundaries (barriers) are the zone where the slope of the surface of different genetic variables (eg, allele frequency) is large. The four basic types of methods for finding these barriers are (FUZHONG *et al*. 2005): 1) spatial autocorrelation; 2) wombling, 3) Monmonier's algorithm and 4) contour area multifractal model (CAMM).

In contrast to spatial autocorrelation or regression models, which can examine the relationship between genetic and geographic distances and provide some clues to the form of genetic relief, but can not identify the location of genetic barriers, the Monmonier's algorithm of maximum differences is very easy and reliable method for the determination of such barriers.

The main objective of using the Monmonier's algorithm (MONMONIER 1973) in this paper is the visualization of data contained in the matrix of genetic data on the map, by drawing boundaries.

The technique consists of the following procedures:

1. Samples are located on the map in accordance with their geographical coordinates;
2. Delaunay triangulation is used to connect isolated geographic position of samples on the map, creating a network that connects all the samples;
3. Calculated the genetic distance between adjacent samples that are added to each edge (corresponding to the neighboring sample) on the network;

4. Monmonier's algorithm of maximum differences is used to identify boundaries:
- Selects the edge of the network with the greatest distance assigned, and is used to start drawing the border line perpendicular to the network;
- Edge which is directly linked to the growing border with the greatest genetic distance is selected to continue the border;
- The previous two steps are repeated until the growing boundary does not meet with other border or the border of researched area.

Delineation of the region using the Monmonier's algorithm was done in the AIS computer program (MILLER 2005). Using this program allows pre-determining the number of detected barriers by the user. Larger number of barriers provides greater accuracy, but adding new barriers do not affect the position of previous borders. In preliminary testing (IVETIĆ *et al*. 2008, 2010) was found that the best visualization for beech area of distribution in Serbia is achieved with five detected barriers.

Combining of GIS tools with molecular genetics technology increases the strength of results, by using the spatial dimension of information they provide and thus provides an alternative perspective that may lead to a better understanding of genomic functions. Visualization (spatial analysis research) and presentation (mapping) the spatial distribution of genetic data is likely to emphasize the patterns of diversity and thus further improve the interpretation of results. Furthermore, spatial analysis may allow the detection of relations between the regions of the genome, and habitat characteristics which surrounding the populations.

In this study, for visualization of genetic data and mapping of regions of provenances computer program DIVA-GIS Ver. 5.4 (HIJMANS *et al*. 2005), was used. DIVA-GIS is a free geographic information system for data analysis of biodiversity. It was developed by HIJMANS *et al*. 2001 with support from IPGRI and UC Berkeley Museum of Vertebrate Zoology.

As the first layer, the map of administrative border of Serbia, Srb0 (http://biogeo.berkeley.edu/bgm/gdata.php), which is converted to UTM Coordinate System - Zone 34 was taken. Then a new layer was added, with area of beech natural distribution, which was obtained based on the results of the National Forest Inventory (2009). The next layer contains the coordinates of the locations from which samples were collected for analysis (picture 1 – layer 3). These coordinates are connected by Delauny triangulation (picture 1 – layer 4). Then the coordinates of the barriers obtained using Monmonier's algorithm was added (picture 1 – layer 5). This layer is obtained by taking the results from the AIS program (MILLER 2005) and their introduction to GIS in the form shapefile, based on coordinates and the direction of barriers. The next layer is based on the map of Serbia (Military Encyclopedia, Second Edition, Volume 9, Sparta-Tirana, appendix in the offset - Serbia, p. 33), which is digitalized and georeferenced (picture 1 – layer 7). This map

was used to compare genetic to geographic barriers, as well as drawing maps of regions of provenances (picture 1 – layer 8).

RESULTS

The best visualization is achieved with five barriers. Also, depending on the method of processing raw genetic data, boundaries (borders) may be significantly different (IVETIĆ *et al.* 2010). The analysis can be performed based on raw genetic distances, genetic distances corrected on the basis of geographical distances and on residuals of genetic distances. Of the three methods of preparation of genetic distances (where a simple genetic distance - MILLER 2005 was used), using of genetic distances corrected on the basis of geographic distance (pseudoslopes) showed the best results. Using geographically corrected genetic distance is preferable in the case of samples collected in three dimensional space, or when the addition of latitude and longitude, altitude must be taken into account. In addition, this method of calculation should be used when dealing with a relatively small number of samples (IVETIĆ *et al.* 2008).

In this way, following regions are isolated (picture 1 – layer 8):
1. Northwestern Region – consists of Cer and Istočna Boranja;
2. Southwestern Region – Includes sites Bukovi, Tara, Murtenica, Jadovnik, Javor, Golija and Rogozna;
3. Kopaonik;
4. Eastern region – Including Rudnik, Goč, Veliki Jastrebac, Radan, Miroč, Žagubica, Rtanj and Stara Planina;
5. Kukavica;
6. Southeastern region – southeast of Besna Kobila.

Entering data and results of analysis of genetic diversity in geographic information system has enabled the creation of adequate maps of the regions of provenances, and visualization of beech genetic diversity in Serbia. The results of applying the computer program DIVA-GIS and developing geographic information system for beech in Serbia, it is certainly easier to introduce an interactive and graphic than in writing. In this paper we will gradually show all the layers which was used for creating map of beech regions of provenances.

DISCUSSION

Easy to apply and the fact that the input data consist only of spatial coordinates and the matrix of samples distance (regardless of the method of measurement), make the Monmonier's algorithm suitable for widely use. The results of the analysis to a large extent depend on the method of data processing, but perhaps even more depend on methods of sample collecting and patterns of spatial distribution in space. Ideally situation is the sample locations were evenly distributed on the investigated, with no differences in terms of altitude. Such a scenario is possible only in small areas and special purpose research fields. In a survey of populations of forest trees, which typically occur over large areas and high altitude.
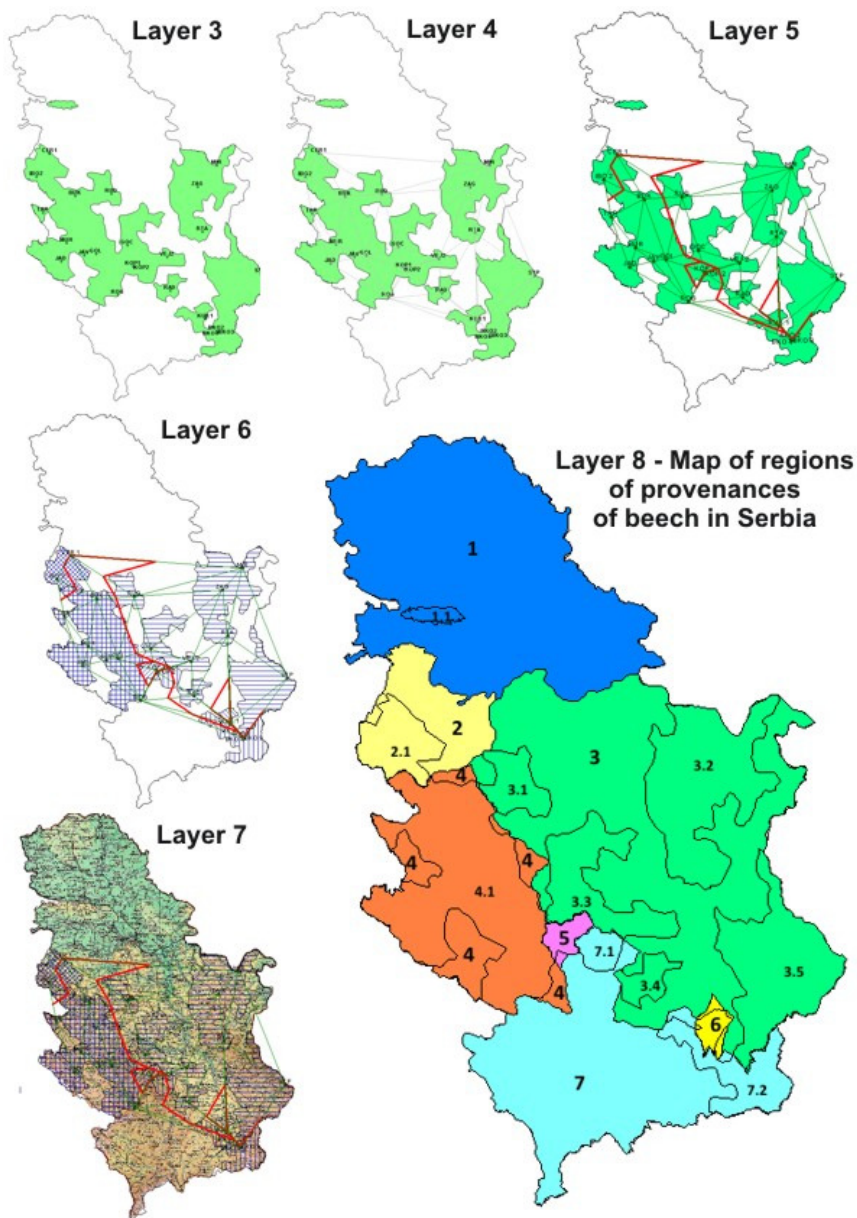
Picture 1: Procedure of delineation of regions of beech provenances in Serbia (based on Monmonier's geographical barriers obtained from corrected genetic distance)

amplitudes, primary attention should be paid to the method of data processing. In this regard, it is recommended to use geographically corrected genetic distance and the use of genetic distance residues, rather than using raw data. Due to the huge geographical distance between the variability of samples, it is recommended to use the remains of genetic distance obtained from linear regression of genetic vs. geographical distances (MANNI *et al*. 2004). During collecting samples, care should be taken to avoid large areas with no samples. Also, the precision of the method can be increased by adding virtual points in Delaunay triangulation, which increase the accuracy of the analysis at the border areas of the investigated surface. The analysis is simple and allows good visualization of genetic variation barriers, which combined with knowledge of the range and geographic barriers, can be successfully used for making recommendations for the transfer of reproductive material of forest trees.

Visualization can be further improved using the results of this analysis to draw maps at one of the widely used GIS programs, and their overlapping with the physical relief and other thematic maps. Database and digital georeferenced maps quickly and reliably determine membership of any location in Serbia, to a particular region or subregion of provenances. As the whole territory of a country must be covered by regions of provenances, parts of its beech natural range have been expanded with neighboring areas, based on the barrier obtained by using a Monmonier's algorithm of maximum differences on the basis of geographical barriers such as rivers or mountain peaks.

ACKNOWLEDGEMENTS

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IZDVAJANJE REGIONA PROVENIJENCIJA BUKVE U SRBIJI PROSTORNOM ANALIZOM GENETIČKOG DIVERZITETA

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I z v o d

Rezultati prostorne analize genetičkog diverziteta imaju praktičnu primenu u definisanju i razgraničenju regiona provenijencija šumskih vrsta drveća. Istraživanja u ovom radu, obuhvatila su materijal iz 27 prirodnih populacija bukve u Srbiji. Genetička komponenta istraživanja bazira se na analizi RAPD markera iz zbirnih uzoraka, korišćenjem 28 prajmera. Prostorna komponenta istraživanja, bazira se na geografskom položaju ispitivanih populacija. Grupisanje ispitivanih populacija u regione, kao i njihovo razgraničenje, izvršeno je primenom Monmonierovog algoritma najvećih razlika. Za vizualizaciju rezultata i izradu karata regiona provenijencija bukve u Srbiji, korišćen je GIS, čija je baza podataka obuhvatila rezultate navedenih istraživanja.