

**“LANDSCAPE SHAPE INTERPOLATION” FOR DEFINING SPATIAL
PATTERN OF BEECH GENETIC DIVERSITY IN SERBIA**

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Knowledge about spatial distribution of genetic diversity is very important for foresters in order to make right decisions during establishing of management plans for conservation, afforestation or reforestation. Advanced molecular technologies provide us with efficiently measured genetic information's which can be analyzed powerful statistical tools. Landscape Shape Interpolation analysis was applied on protein based marker data sets from six natural populations of Balkan beech. Results indicate existence of three genetically distinct groups: 1) Djerdap, Kopaonik, Zubin potok and Ivanjica; 2) Bor and 3) Pirot. These results were compared with results of UPGMA clustering of sampled population based on NEI original distances, Principal Coordinate Analysis and Monmonier's Maximum Difference Algorithm Analysis. Landscape Shape

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Interpolation analysis represent a powerful tool in spatial genetic diversity research. Combining of Landscape Shape Interpolation analysis results with digitalized maps of investigated area in some of numerous GIS applications will for certainly improve visualization of results and provide better understanding of causal factors of genetic diversity.

Key word: beech, interpolation, spatial genetic diversity

INTRODUCTION

Knowledge about spatial distribution of genetic diversity of beech is very important for foresters in order to make right decisions during establishing of management plans for conservation, afforestation or reforestation (ISAJEV *et al.* 2003). Selection of appropriate seed source could be decisive for final success of forest tree plantations after long period of management cycle.

Spatial distribution of genetic diversity could be observed at different levels, from populations and subpopulations to neighboring individual trees. This diversity is result of different influences, like human activities, environment conditions and demographic history of species. At forest tree species, spatial genetic structure is consequence of genetic diversity distribution, mainly by pollen flow and seed dispersal. By time, this pattern could be modified by various causes, like natural or artificial selection. Because of that, information's about spatial genetic structure are very important in understanding of these causal factors (ESCUDERO *et al.* 2003).

Advanced molecular technologies provide us with efficiently measured genetic information's. On the other side, improvements in computer sciences provide us with powerful tools to analyze these information's by different statistical methods (MANEL *et al.* 2003). Different approaches could be implemented in investigations of spatial genetic diversity. In this paper, we will present Landscape Shape Interpolation for defining spatial pattern of beech genetic diversity in Serbia. This procedure is designed to facilitate visualizations of patterns of diversity across landscapes (MILLER, 2005).

MATERIAL AND METHODS

Six natural populations of Balkan beech (*Fagus moesiaca* /Domin, Maly/Czeczott.) were selected inside species natural range in Serbia (table 1). Seed was collected from ten individual trees from each population.

This seed material was used for genetic categorization of samples, analyzing protein complex by electrophoresis on PAA gel. Outputs of this analysis were binary sets of data, which was used as dominant markers for further investigation.

Table 1. Geographic location of sample populations

Locality	Coordinates		Altitude l (m)
	X (m)	Y (m)	
Djerdap	567958	4938651	540
Bor	575731	4888442	880
Stara Planina	643797	4796217	1050
Zubin Potok	475523	4760083	1160
Kopaonik	483926	4803881	620
Golija	442960	4803150	1250

The raw data was analyzed using software AIS (MILLER, 2005). The result of interpolation procedure is a 3-dimensional surface plot, with X and Y axes as geographical coordinates and surface heights (Z-axes) as genetic distance. The first step of analysis is constructing of connectivity network among all of the sampling locations in the data set, based on "all pairwise locations" connectivity network or connectivity networks based on Delaunay triangulations. Use of Delaunay triangulations is more preferred method with large number of sample data sets. For our research, because of relatively small number of samples, for calculation of surface we used pairwise connectivity matrix, combined with distance-corrected genetic distances (pseudoslopes) for calculating of surface heights. Second step is placing of genetic distances at the midpoints between samples connected in the network. Genetic distances were calculated as:

$$D_{ij} = \sum_{k=1}^n \frac{d_k}{n}$$

Where d_k is 1 if samples i and j contained different alleles at locus k and 0 if these samples contained same allele at locus k .

Next step is placing of grid over connectivity network. Testing showed that 50 x 50 grids as appropriate. Better resolution was shown with larger number of grids, but without significant influences on visualization of results. Final step is interpolation of genetic distances for all 2500 points of grid, by simple Inverse Distance Weighted interpolation procedure.

Given an evenly spaced grid defined around n actual data points (n X coordinates, Y coordinates, and Z values/surface heights), an inferred Z

value/surface height for a coordinate on the rectangular grid (x, y) can be obtained as:

$$Z = \frac{\sum_{i=1}^n w_i \times Z_i}{\sum_{i=1}^n w_i}$$

Where $w_i = \frac{1}{[(X_i - x)^2 + (Y_i - y)^2]^a}$; when $X_i = x, Y_i = y$, or $w_i = 1$ when $X_i = x, Y_i = y$,

and a is the distance weight value specified for the procedure. Greater values of a causes interpolated values to be more influenced by close points, and lower values of a create a tendency for all points to equally influence interpolated values. Overall, by calculating interpolated values of Z for all coordinates on the grid, we obtain new regularly spaced values of Z that can be plotted as a 3-dimensional surface. The result is 3-D surface plot (figure 2).

RESULTS AND DISCUSSION

Using pairwise connectivity matrix, provide us with 15 connections (geographic distances) between samples (figure 1). This network was used for further interpolation.

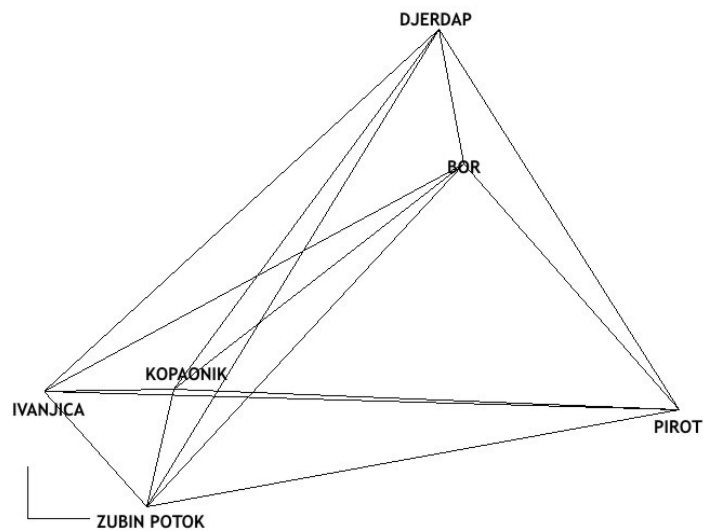


Figure 1. Connectivity network between samples

Calculated genetic distances (table 2) show us that biggest genetic distance is between locations Djerdap and Bor, even if their geographical distances is one of smallest. On the other side, samples from Djerdap showing smallest

genetic distances with samples from geographically distinct group: Ivanjica, Kopaonik, Zubin Potok.

Table 2. Genetic distances with coordinates of midpoints between sample locations

Connection	X coordinate	Y coordinate	'Height'*
DJER-BOR	571844,5	4913546,5	3,51469314970027E-06
DJER-PIR	605877,5	4867434	1,7705973880363E-06
DJER-ZUP	521740,5	4849367	7,10470431771559E-07
DJER-KOP	525942	4871266	6,74610978109652E-07
DJER-IVA	505459	4870900,5	7,74922353254704E-07
BOR-PIR	609764	4842329,5	2,80422415633288E-06
BOR-ZUP	525627	4824262,5	1,09658990066447E-06
BOR-KOP	529828,5	4846161,5	1,71683011547433E-06
BOR-IVA	509345,5	4845796	1,58421952978598E-06
PIR-ZUP	559660	4778150	1,24505191175327E-06
PIR-IVA	543378,5	4799683,5	1,06632816496679E-06
PIR-KOP	563861,5	4800049	1,56196702279567E-06
ZUP-KOP	479724,5	4781982	2,4024782008971E-06
ZUP-IVA	459241,5	4781616,5	2,64590398135864E-06
KOP-IVA	463443	4803515,5	2,61499300639668E-06

* reflection of the genetic distance between locations

This could be also noticed on 3D graphs (figure 2), which represent graphical visualization of interpolation results for 2500 grids. We can notice that highest peak is between localities Djerdap and Bor ($Z=2,658$). We can also notice that there is significant amount of genetic diversity between samples in southwestern group (Ivanjica, Kopaonik and Zubin potok).

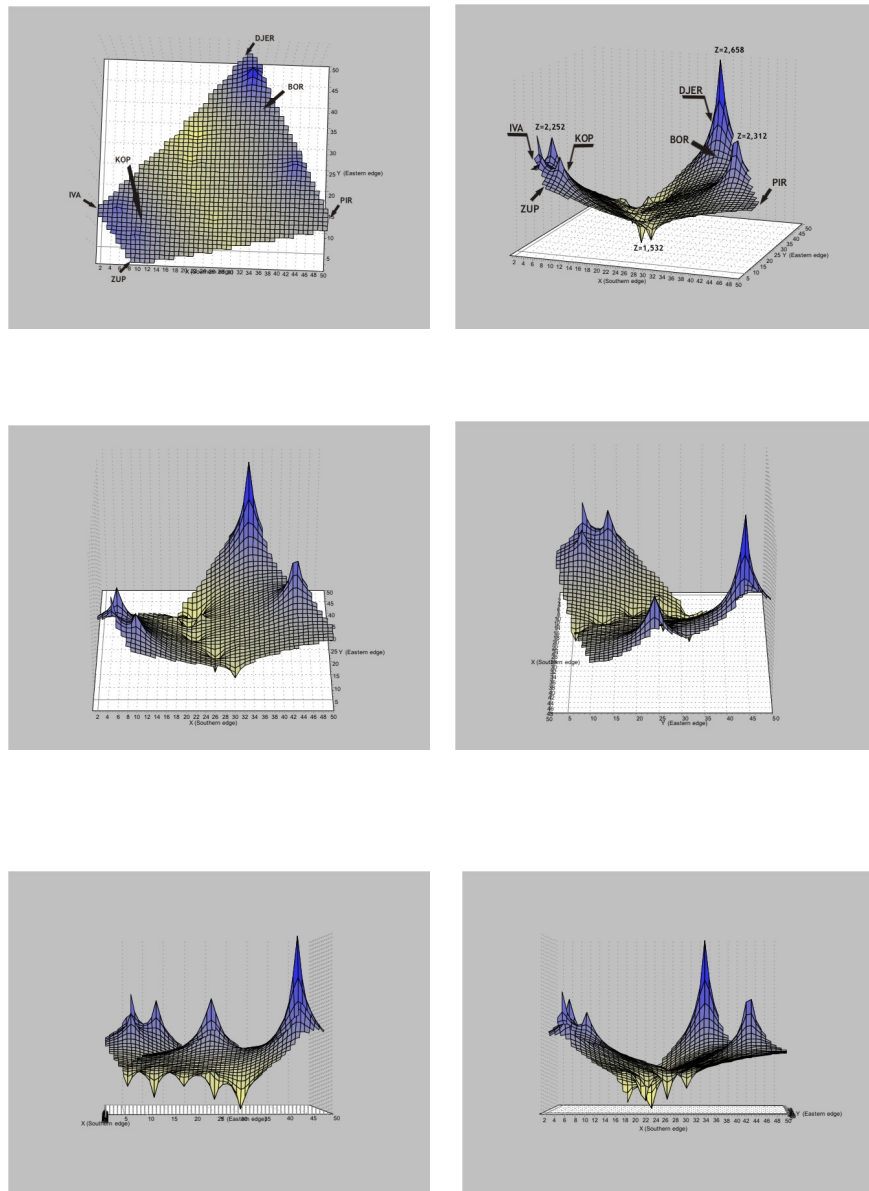


Figure 2. 3-D interpolation graph from different angles

Results of interpolation indicate that interpolated genetic distances between geographically closed populations are higher than between distinct groups of population. By geographical position of sampled population (figure 2a), we can form three groups: 1) northeastern group (Djerdap and Bor); 2) southwestern group (Kopaonik, Zubin potok and Ivanjica) and 3) southeastern group (Piot). Combining geographical position of sample populations and natural range of beech in Serbia with genetic distances, give us more information's. Populations from southwestern group are divided between themselves with peaks of calculated Z value (mountain complexes: Kopaonik, Rogozna – Zubin potok and Golija – Ivanjica), but without significant peaks between this group and northeastern group. The highest peak is between populations in northeastern group, Djerdap and Bor, which represent Homolje Mountain complex. But again, there are no significant peaks between northeastern and southwestern group. Southeastern group consisted is divided from northwestern group with one high peak (break in natural range), but without any peaks in direction of southwestern group.

These results are consistent with UPGMA clustering of sampled population, based on NEI (1972) original distances (figure 3), and with Principal Coordinate Analysis (figure 4).

Barriers between groups build by Monmonier's Maximum Difference Algorithm Analysis (MONMONIER, 1973), with geographical distance-corrected genetic distances (Pseudoslopes) used in computations (figure 5), additionally confirm existence of three genetically distinct groups: 1) Djerdap, Kopaonik, Zubin potok and Ivanjica; 2) Bor and 3) Piot (figure 5).

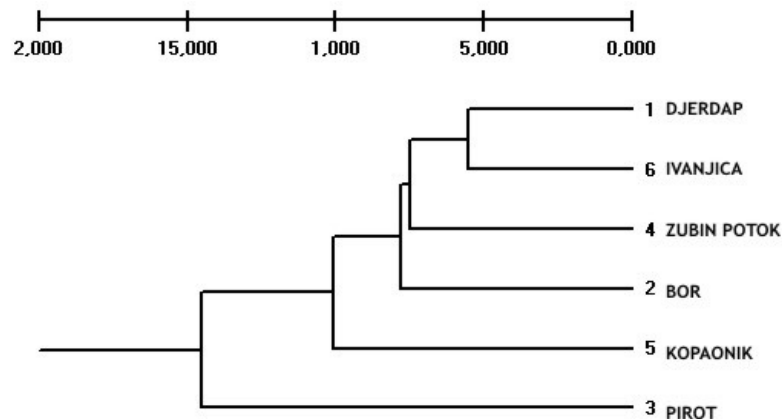


Figure 3. UPGMA clusters based on NEI (1972) original distances

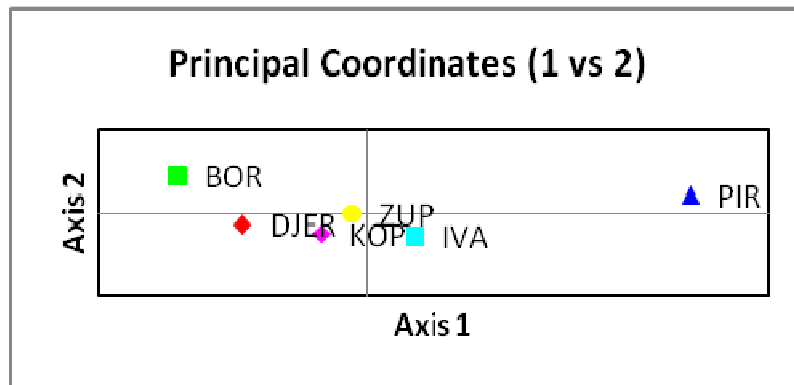


Figure 4. Principal Coordinate Analysis

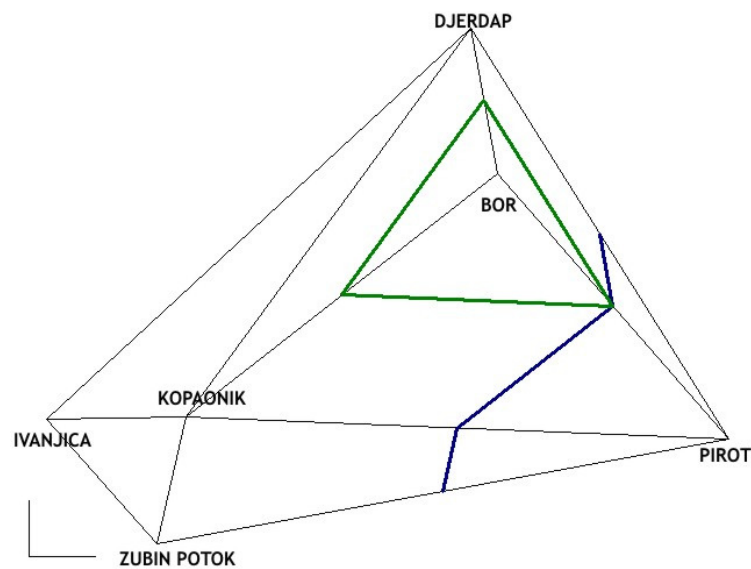


Figure 5. Barriers between sample populations build by Monmonier's Maximum Difference Algorithm

In this research we used pairwise connectivity matrix rather than Delaunay triangulations because relatively small number of samples. Further, we used geographic distance-corrected genetic distances (pseudoslopes) for calculating of surface heights, because results based on this parameter are more

consistent with other analysis results, compared with surface heights calculated by raw genetic distances or residual genetic distances. However, use of pseudoslopes has not been formally evaluated in any simulation based analyses. These "pseudoslopes" are derived by the AIS (MILLER, 2005) program as the quotient of congruent elements from the genetic and geographical distance matrices. Or more simply, if D_{gen} is the genetic distance between a pair of observations and D_{geo} is the geographical distance between the same pair of observations, then we may choose to perform analyses using the pseudoslope D_{gen}/D_{geo} to reflect the observation-specific change in genetic composition (genetic distance) of a pair of individuals relative to the change in geography (physical distance). Problems with the pseudoslope approach mainly seemed to appear when extremely large genetic distances are observed over very short geographical distances. In the case of Genetic Landscape Shape Interpolations, the resulting pseudoslope was large enough to overshadow any other feature of the genetic landscape shape (i.e., a single large peak was observed on what appeared to be an overall flat landscape).

Landscape Shape Interpolation analysis represent a powerful tool in spatial genetic diversity research. Selections of connectivity matrixes and parameters for surface height (Z) calculations have significant influence on results. Because of that, supporting of this analysis with other statistical tools are more than welcome. Combining of Landscape Shape Interpolation analysis results with digitalized maps of investigated area in some of numerous GIS applications will for certainly improve visualization of results and provide better understanding of causal factors of genetic diversity.

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**„LANDSCAPE SHAPE INTERPOLATION” ZA DEFINISANJE
PROSTORNOG OBRAZCA GENETIČKOG DIVERZITETA BUKVE U
SRBIJI**

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I z v o d

Poznavanje prostorne distribucije genetičkog diverziteta je veoma važno za šumare, u cilju donošenja odgovarajućih odluka prilikom praljenja planova gazdovanja u cilju konzervacije, pošumljavanja ili melioracije. Napredne molekularne tehnologije sa velikom efikasnošću obezbeđuju genetičke podatke koji se mogu analizirati moćnim statističkim alatima. “Landscape Shape Interpolation” je primenjena na podacima o proteinskim markerima dobijenim iz šest prirodnih populacija bukve u Srbiji. Rezultati ukazuju postojanje tri genetički udaljene grupe: 1) Đerdap, Kopaonik, Zubin potok and Ivanjica; 2) Bor and 3) Pirot. Rezultati pejzažne interpolacije su upoređeni sa rezultatima UPMGA klasterovanja ispitivanih populacija na osnovu NEI originalnih distanci, analize principijalnih koordinata (PCoA) i analize Monmonijerovog algoritma maksimalnih razlika. Analiza primenom analize “Landscape Shape Interpolation” predstavlja moćan alat u istraživanjima prostorne distribucije genetičkog diverziteta. Kombinovanje rezultata ove analize sa digitalizovanim kartama ispitivanog područja, u nekoj od brojnih GIS aplikacija, svakako će unaprediti vizualizaciju rezultata i omogućiti bolje razumevanje uzročnih faktora genetičkog diverziteta.

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