REVEALING GENETIC BARRIERS AT A SMALL PROTECTED AREA FROM FINE-SCALE SPATIAL GENETIC STRUCTURE ANALYSIS OF *TAXUS CUSPIDATA* IN MT. SOBAEK, SOUTH KOREA

Yu-Jin Park,[1] Kyung-Nak Hong, Joon-Hyeok Kim, Yong-Pyo Hong, and Jin-Taek Kang

[1]Korea Forest Research Institute, South Korea

Introduction

Pacific yew (*Taxus cuspidata* L.) is an endangered species at subalpine mountains and a keystone species at a forest protected area in Mt. Sobaek, South Korea. Population genetic differentiation of the yew tree at species level (Gst =0.067, Lee *et al.* 2000) was moderate and relatively lower than those of other coniferous species. Also, some amount of differentiation among sub-subpopulations within a subpopulation (Fst = 0.042, Kwon 2003) was reported using isozyme markers. In this study, we analyzed fine-scale spatial genetic structure and found out genetic boundaries at a small forest protected area of *Taxus cuspidata* using microsatellite makers.

Material and Methods

Study site

To examine the spatial genetic structure and the distribution pattern of *Taxus cuspidata,* a study plot of 1.2ha (150m×80m) was set up at a protected area in Mt. Sobaek located in the middle of South Korea. The plot was composed of 20 subplots with the size of 30m x 20m. We investigated every yew tree in the plot.

Growth performance measurement

Every individual in the plot was estimated tree height and diameter in breast height (DBH). And their habit and vitality level were classified into the modified Grzegorz et al. (2005) categories. The categories of the habit were:

1. Shrub-like,
2. Monocormic (one main trunk without ramification), and
3. Polycormic (trunk bi- or trifurcate).

The categories of the vitality and health were:

1. Sound without cavity – no visible necroses and no reduction in number of needles,
2. Poor without cavity – small necroses and/or slightly reduced number of needles on tops of main branches,
3. Sound with cavity – no visible necroses and no reduction in number of needles with cavity,
4. Poor with cavity - small necroses and/or slightly reduced number of needles on tops of main branches with cavity, and

5. Very poor-large necroses and significantly reduced number of needles on most branches.

Microsatellite analysis

Needles from 111 individual trees in the plot were sampled for extracting total DNA. We screened 61 nSSR markers from congener species and selected six primer sets (TY24, TY16, TAX86, TB31, TB50, and TB56) that gave clear and polymorphic peak-patterns for the analysis. The PCR conditions were followed by modified Mahmoodi et al. (2010) and PCR products were electrophoresis using Prism 3130*xL* Genetic Analyzer (ABI) with GeneScan-500 size standard. All alleles were cropped using GeneMapper v4.0 and then manually double-checked.

Data analysis

Estimation of genetic diversity, spatial autocorrelation and genetic surface analysis were conducted with Arlequin (Excoffier et al. 2005), AIS (Miller, 2005), GenBMap (Cercueil et al. 2007), and Barrier (Guerard 2004) programs respectively.

Results and Discussion

Growth performance of yew trees in the plot showed that the trees were evenly categorized into three tree types as the habit and the tree vitality and health were classified into 84% of good condition. Average of tree height and diameter in breast height (DBH) were 4.9m (±3.1) and 32.9cm (±22.9) respectively. Genetic diversity indices as number of effective alleles (Ne), observed (Ho) and expected (He) heterozygosity were 3.72, 0.364 and 0.680, respectively (Table 1).

These values were little bit high taking restricted seed dispersal distance within a small stand of *Taxus cuspidata* into consideration. Spatial autocorrelation revealed that individuals in the plot were genetically homogeneous within approximately 20m of distance and randomly distributed from 20m to 50m of distance (Figure 2). Therefore, the results suggest that individual selection for *ex situ* conservation or the study on population genetic diversity in *Taxus cuspidata* should be made with at least 20 meters of spatial interval between samples.

Genetical bandwidth mapping (Cercueil *et al*. 2007) showed that the area of yew trees were separated by brown or white colored zones indicated intermediate or high values of genetic differences among individuals, respectively (Figure 3). Those colors represented the potential genetic barriers. Green colored zone in the map comes from less genetic information due to absence of yew trees. To verify more clearly the genetic barriers, Monmonier's algorithm was used and analyzed the individual genetic configuration. Red lines in the map were the genetic barriers following the algorithm. On the basis of the genetic barriers, the plot was divided into three genetic boundaries and 18% of total genetic variation from the AMOVA(Analysis of Molecular Variance) could be explained with the 'hidden' genetic structure. This study showed being of genetic barriers within a small forest protected area and it could help to make a decision for *in situ* conservation plan of *Taxus cuspidata* in this region.

Table 1. Genetic diversity indices of *Taxus cuspidata* at the study plot in Mt. Sobaek, Korea.

Locus	N	Na	Ne	I	Ho	He	F
TY24	100	12	5.2	1.896	0.130	0.807	0.839
TY16	99	5	3.6	1.411	0.747	0.720	-0.038
TaX86	95	8	3.1	1.404	0.305	0.673	0.546
ABRⅡ-TB31	87	3	1.9	0.768	0.218	0.485	0.549
ABRⅡ-TB50	82	13	6.4	2.048	0.634	0.843	0.248
ABRⅡ-TB56	96	3	2.2	0.885	0.146	0.552	0.736
Mean	93.2	7.3	3.72	1.402	0.364	0.680	0.480

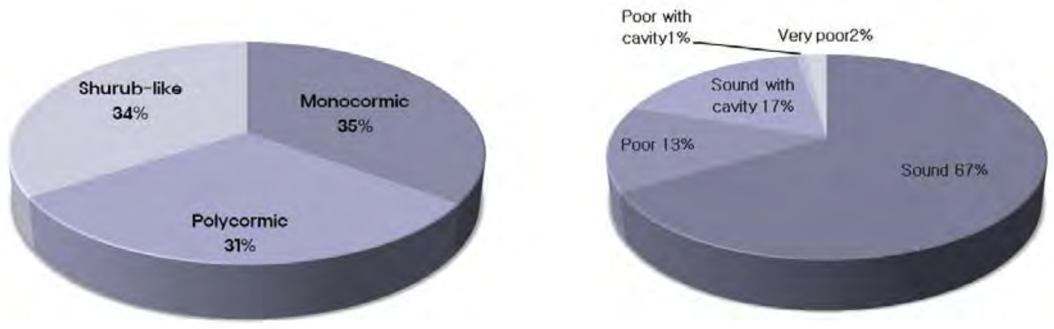

Figure 1. Proportion of tree form and growth performance of *Taxus cuspidata* at the study plot in Mt. Sobaek, Korea.

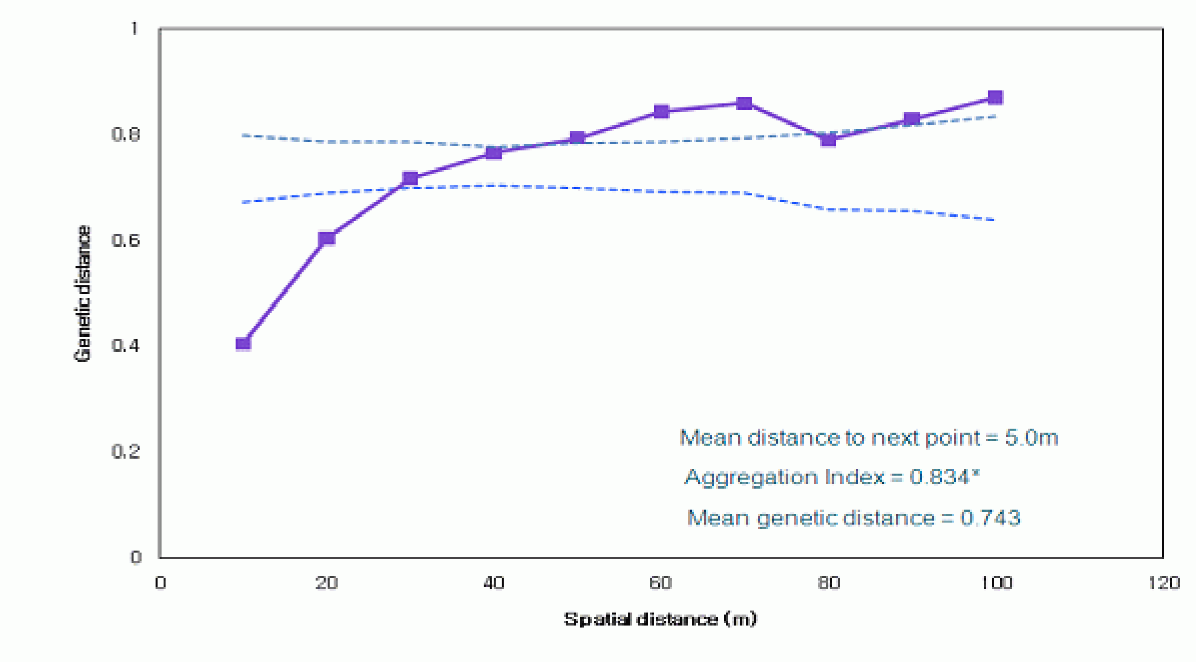

Figure 2. Correlogram of *Taxus cuspidata* at the study plot in Mt. Sobaek, Korea. The solid line and two spotted lines showed spatial autocorrelation and the upper and the lower confidence intervals as 95%, respectively.

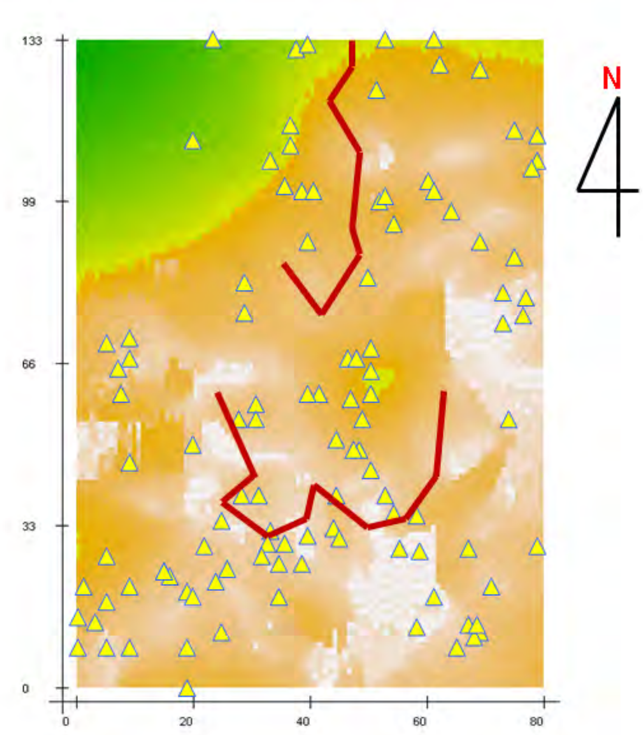

Figure 3. Genetical bandwidth map at the study plot of *Taxus cuspidata* in Mt Sobaek, Korea.

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