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Genetic Variability of Scots Pine Maternal Populations and Their Progenies

Joanna Kosinska, Andrzej Lewandowski and Wladyslaw Chalupka

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The genetic variability of Scots pine was investigated in six populations from Poland representing two maternal populations and their natural and artificial progenies. Thirteen enzyme systems controlled by 25 allozyme loci were analyzed using starch gel electrophoresis. Progeny populations maintained a high and similar level of genetic variation to that observed in the maternal populations. As expected, much closer genetic relationships were observed between maternal populations and their respective progeny than between the two maternal populations themselves. Progeny populations had the same major alleles, but differed in the number of rare alleles. Therefore, probably not all rare alleles were transferred from the maternal stands to the progenies. In addition, new rare alleles appeared in the progeny populations, possibly as a result of external pollen flow into the maternal populations.

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Authors' addresses *Kosinska*, Department of Human Medical Genetics, Medical University of Warsaw, Oczki 1, 02-007 Warsaw, Poland; *Lewandowski* (correspond.) and *Chalupka*, Polish Academy of Sciences, Institute of Dendrology, 62-035 Kórnik, Poland
E-mail alew@rose.man.poznan.pl
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1 Introduction

Scots pine (*Pinus sylvestris* L.) is the main economically and ecologically important forest tree species in Poland. It is distributed throughout Poland forming either pure or mixed stands on contrasting sites, varying from poor, sandy soils to highly productive and fertile sites. Stands of this species cover 4 885 000 hectares and represent 75.5% of the total forested land area (GUS – Leśnictwo 2005). Selection of Scots pine with the aim of genetic improvement of the species started in Poland in 1933 when the General Director of State Forests issued a special directive devoted

to Scots pine seed collection and use (Giertych 1999). However, the mass selection of phenotypically superior Polish stands of Scots pine began after the World War II. Approved seed stands, registered and protected by forest law, were chosen to preserve the best gene pool of the species. The seed stands, totaling 6896 hectares now (GUS – Leśnictwo 2005), are distributed throughout the country, mostly in regions where Scots pine is the dominant forest tree species. Over the years, the registry of approved seed stands has been updated: new stands are still being selected, with others being removed from the register due to damage or disease. Since the 1970s, progeny plantations have been established using seeds collected from standing trees in the approved seed stands in order to preserve the gene pool of the maternal populations (Giertych 1999). As some progeny plantations derived from approved seed stands have already started producing seed, an opportunity was created to examine the genetic structure and range of genetic variation in both the maternal and descendant populations. The aim of this study was to compare the genetic variation of maternal populations (approved seed stands) and its artificially and naturally regenerated progenies, using allozymes as genetic markers.

2 Materials and Methods

Six populations of Scots pine were analyzed, included two maternal populations of approved seed stands and their four progenies. Both maternal populations, i.e. Krotoszyn (K1) and Gubin (G1) are of artificial origin and the distance between them is about 190 km. Naturally regenerated progeny (G2) has developed under canopy of maternal population in Gubin. All planted progenies, i.e. K2, G3 and G4 were grown on new plots outside of maternal populations, but in the area of respective Forest District. Seeds for biochemical analyses were collected from randomly chosen trees of all mentioned populations. More information on the studied populations is shown in Table 1.

Horizontal starch gel electrophoresis was used to separate isozymes. Thirteen isoenzyme systems encoded by 25 loci were assayed. Individual trees were genotyped using seven macrogametophytes. The enzymes assayed, numbers of loci and alleles scored, and references for genetic interpretations are shown in Table 2. Alleles at each locus were numbered according to the electrophoretic migration of allozymes. The most anodally migrating band was named 1, the next 2, and so on. Alleles occurring in a population with a frequency of less than 5% are referred to as rare alleles. On the basis of the estimated allele frequencies, the following parameters of genetic variation were computed: average number of alleles per locus (N_A), effective number of alleles per locus (N_E, Kimura and Crow 1964), percentage of polymorphic loci (P, 99% criterion), expected heterozygosity (H_E), observed heterozygosity (H_O, Nei 1973), fixation index (F, Wright 1978). In order to test whether F estimates are significantly different from zero, as expected under Hardy-Weinberg equilibrium, the permutation test was employed (Weir 1996). Briefly, the test is based on the permutation of alleles over individuals that builds-up an array of genotypes under panmixia. Hence, the fixation index calculated for a permuted array of

Table 1. Characteristics of the Scots pine populations analyzed in this study.

Forest District (location)	Population	Age in 2006	Area, ha	Number of analysed trees
Krotoszyn (51°41´N 17°26´E)	Maternal (K1)	143	5.31	82
	Artificially regenerated progeny (K2)	38	4.14	98
Gubin (51°57´N 14°45´E)	Maternal (G1)	185	5.59	55
	Naturally regenerated progeny (G2)	20–50	5.59	60
	Planted progeny of 1975 (G3)	31	6.03	82
	Planted progeny of 1982 (G4)	24	5.33	71

Table 2. Enzymes assayed, numbers of loci and alleles (N) scored, and the references for their genetic interpretation.

Enzyme	Locus	N	Reference
Alcohol dehydrogenase (E.C. 1.1.1.1)	Adh1	3	Rudin and Ekberg 1978
	Adh2	4	
Fluorescent esterase (E.C. 3.1.1.2)	Fle	5	Yazdani and Rudin 1982
Glutamate dehydrogenase (E.C. 1.4.1.2)	Gdh	2	Goncharenko et al. 1994
Glutamate oxalo-acetate transaminase (E.C. 2.6.1.1)	Got1	4	Rudin 1975
	Got2	5	
	Got3	4	
Isocitrate dehydrogenase (E.C. 1.1.1.42)	Idh	2	Goncharenko et al. 1994
Leucine aminopeptidase (E.C. 3.4.11.1)	Lap1	4	Rudin 1977
	Lap2	5	
Malate dehydrogenase (E.C. 1.1.1.37)	Mdh1	2	Goncharenko et al. 1994
	Mdh2	3	
	Mdh3	7	
	Mdh4	5	
Menadione reductase (1.6.99.2)	Men1	5	Goncharenko et al. 1994
	Men2	2	
6-Phosphogluconate dehydrogenase (E.C. 1.1.44)	6-Pgd1	5	Szmidt and Yazdani 1984
	6-Pgd2	4	
Phosphoglucose isomerase (E.C. 5.3.1.9)	Pgi1	2	Szmidt 1984
	Pgi2	5	
Phosphoglucomutase (E.C. 2.7.5.1)	Pgm1	4	Goncharenko et al. 1994
	Pgm2	3	
Shikimate dehydrogenase (E.C. 1.1.1.25)	Shdh2	2	Szmidt and Yazdani 1984
Superoxide dismutase (E.C. 1.15.1.1)	Sod1	3	Lewandowski and Samoćko (unpublished results)
	Sod2	4	

genotypes is a variable drawn at random from the distribution of F under null hypothesis. Large number of permutations provides confidence limits for F=0, therefore the observed value of F beyond confidence intervals is considered significantly different from zero. In the present paper 10000 permutations were performed. Genetic differences between populations were measured by F_{ST} (Wright 1978) and the genetic distance index determined as D_N (Nei 1978). To characterize genetic relationships between populations, matrices of D_N values were clustered using the unweighted pair group method (UPGMA, Sneath and Sokal 1973).

3 Results

In general, a high and similar level of allozyme variation was observed in the six analyzed populations. A summary of genetic variability measures at 25 loci for the analyzed populations is shown in Table 3. The percentage of polymorphic loci (P_{99}) ranged from 80 to 96%. Mean (N_A) ranged from 2.56 to 3.12 and the effective (N_E) number of alleles per locus ranged from 1.43 to 1.48. Mean heterozygosity ranged from 0.231 for the planted progeny from Krotoszyn (K2) to 0.256 for the maternal population from Gubin (G1). Values of the fixation index were negative in the maternal

Table 3. Percentage of polymorphic loci (P_{99}), mean (N_A) , effective (N_E) number of alleles per locus, observed (H_O) and expected heterozygosity (H_E), and fixation index (F) for populations of Scots pine.

Population	P_{99}	N_A	N_E	H_O	H_E	F
Krotoszyn						
Maternal (K1)	92	3.08	1.46	0.254	0.254	–0.011
Planted progeny (K2)	92	3.12	1.43	0.231	0.242	0.053*
Gubin						
Maternal (G1)	80	2.76	1.48	0.256	0.257	0.035
Naturally regenerated progeny (G2)	80	2.72	1.45	0.237	0.240	0.006
Planted progeny of 1975 (G3)	96	2.96	1.43	0.233	0.234	–0.005
Planted progeny of 1982 (G4)	80	2.56	1.44	0.237	0.244	0.022

* Significant at $p \leq 0.05$

Table 4. Number of alleles in studied populations of Scots pine. Lost alleles – not found in the progeny populations; new alleles – not present in the maternal populations but found in the progeny populations.

Population	Total number of alleles	Rare alleles	New alleles	Lost alleles
Krotoszyn				
Maternal (K1)	77	33	-	-
Planted progeny (K2)	78	36	8	9
Gubin				
Maternal (G1)	69	23	-	-
Naturally regenerated progeny (G2)	68	29	6	7
Planted progeny of 1975 (G3)	74	32	11	6
Planted progeny of 1982 (G4)	65	22	6	11

population from Krotoszyn (K1) and in the older planted progeny from Gubin (G3). The fixation index was positive in the progeny population from Krotoszyn (K2) and three populations from Gubin (G1, G2 and G4). However, only in planted progeny from Krotoszyn (K2), the fixation index was significantly different from zero.

In the investigated material, 94 alleles were found and 50 of them were rare. The most common alleles were identical in all six populations at all loci. Several rare alleles that were present in the maternal populations were absent in the progeny populations. In contrast, a number of alleles were found in the progeny populations that were not present in their maternal populations (Table 4).

The results revealed much closer genetic relationships between maternal populations and their respective progeny than between the maternal populations themselves. The F_{ST} for all six populations was 0.036. The average F_{ST} calculated for the group of populations from Krotoszyn was 0.003 compared to 0.007 for the populations from Gubin. It should be noted that the average F_{ST} for the two maternal populations was 0.035. The maternal populations and their respective progenies formed two distinct groups (Fig. 1). In the group of populations from Gubin, the smallest genetic distance was observed between the maternal population and the younger planted progeny ($D_N = 0.0007$). In the same group of populations, the highest genetic distance was observed between the maternal population and

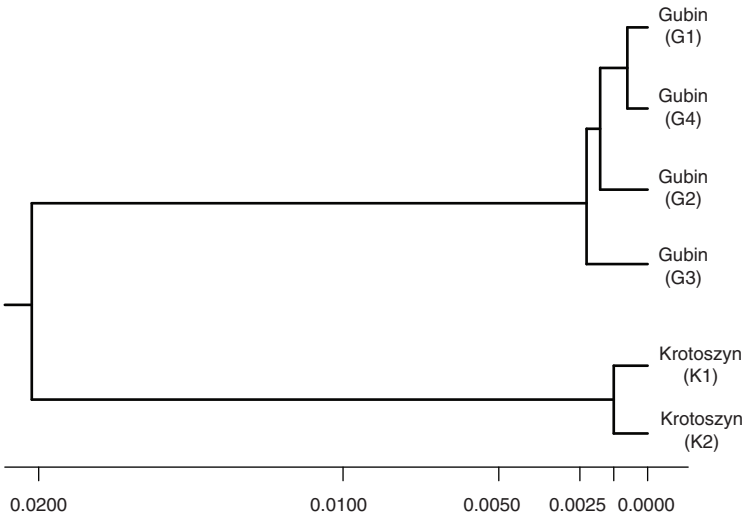

Fig. 1. Unweighted pair group method (UPGMA) dendrogram based on Nei's genetic distances among investigated populations of Scots pine.

the older planted progeny ($D_N = 0.0029$). In the group of populations from Krotoszyn, the genetic distance between the maternal population and the planted progeny was 0.0012.

4 Discussion

The high level of genetic variation in the studied populations is consistent with earlier reports for Scots pine (e.g. Szmidt 1984, Guldberg et al. 1985, Muona and Harju 1989, Wang et al. 1991, Goncharenko et al. 1994, Lewandowski et al. 2000). Except one, all analyzed populations were in Hardy-Weinberg equilibrium (statistically significant excess of homozygotes was detected in planted progeny (K2) from Krotoszyn). An excess of homozygotes in young populations is associated with the occurrence of selfing and mating between relatives. However, inbred individuals are progressively eliminated throughout the life span of the population. In naturally regenerating Scots pine stands, the elimination of homozygotic individuals has been observed at an age of 10–20 years (Yazdani et al. 1985). Muona et al. (1987) state that the elimination of inbred individuals starts at the age of 3 years in artificially regenerated Scots pine stands. An excess of homozygotes, although frequent in young populations, is rarely observed in mature populations (Tigersted et al. 1982, Muona and Harju 1989).

Our results indicate that the level of genetic variation in all progeny populations is similar to that found in maternal populations. As expected, genetic differentiation among the investigated populations (F_{ST}) was not high. About 96% of the genetic variation resides within the population and less then 4% among populations. The low level of interpopulation differentiation observed in the present study is consistent with other findings for conifer tree species with broad geographic ranges of distribution (Loveless and Hamrick 1984). The F_{ST} values were lower when comparing differences among groups of populations. F_{ST} for the Krotoszyn and Gubin population groups was 0.003 and 0.007, respectively. Nei's genetic distance values support close genetic relationships between maternal populations and their progenies. Each maternal population and its progeny populations were clustered together on the dendrogram. The genetic and life history characteristics of

forest trees result in populations, which are robust and resilient to genetic change. Consequently, many studies have found that artificial selection, breeding, and silviculture have had minor genetic effects on most tree species (Yazdani et al. 1985, Muona et al. 1987, El-Kassaby and Ritland 1996, Savolainen 2000, Wellman et al. 2003).

Despite similar levels of genetic diversity reported in this study, differences in the distribution of alleles between maternal and progeny populations were observed (Table 4), mainly among rare alleles with frequencies ranging from 0.005 to 0.036. Several alleles noted in the maternal populations were not found in any of the progeny populations and vice versa. In comparison with the maternal population from Gubin, seven rare alleles were absent in the naturally regenerated population, six alleles in the older progeny plantation, and up to 11 in the younger progeny plantation. New alleles, not found in the maternal population, were found in progeny populations. Six alleles were noted in the naturally regenerated population, 11 in the older progeny plantation, and six in the younger progeny plantation. Discrepancies with respect to rare allele composition were also noted between the maternal population from Krotoszyn and its progeny plantation. There were eight alleles absent in the progeny population in comparison with the maternal population. However, nine alleles from the plantation were absent in the maternal population. Moreover, the composition of rare alleles was different between two planted progenies from Gubin. Discrepancies with respect to composition of rare alleles may result from the low (less than 100) and different number of individuals analyzed in each population. However, it seems likely that such discrepancies may also result from the practice of seed collection in the maternal stands and its variation among years. This may be due to either collecting seeds from different parent trees and non-random sampling from individuals or sampling in a restricted part of the plantation, i.e. the center or edge. The appearance of new alleles in the progeny plantations was not surprising. Pollen contamination from background populations is one of the main factors accounting for the occurrence of new alleles. There are many studies indicating large effective pollen flow in trees (e.g. Lindgren et al. 1995, Harju and Nikkanen 1996, Adams and Burczyk 2000). Dzialuk (2003) in 3 years of observations found extensive pollen flow into *Pinus sylvestris* seed stands in Poland, attaining 86–88%. It is worth noting that a loss of some rare alleles in progenies was detected in present study. The significance of rare alleles in a population remains to be explained. According to some predictions, rare alleles may harbor genetic variation that may in the future enable a population to adapt to a changing environment, although their present influence on a population may be marginal (Müller-Starck 1995, Hu and Li 2001). The dynamics of rare alleles in planted populations needs further studies, using more extensive material.

In summary, our findings support management efforts aimed at gene-pool conservation of forest-tree species through the establishment of progeny plantations. Progeny plantations have the potential to ensure the continuation of seed stand selections, as the levels of genetic diversity present in the maternal populations are also reflected in their progeny populations. However, studies including populations of other tree species and DNA-based markers would shed more light on this issue.

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