Genetic Variability and Differentiation of Geographically Marginal Scots Pine Populations From Ukraine

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(Received 26th September 2000)

Abstract

Genetic variability of six geographically marginal Scots pine (*Pinus sylvestris* L.) populations from Ukraine situated mainly on a longitudinal gradient was studied using allozymes and vertical PAGE technique. The populations have average observed heterozygosity H_o=0.262, and expected heterozygosity H_e=0.281. The mean value of NEI's genetic distance was 0.016, and F_{ST} was 0.020, similar to the values reported for Scots pine populations from other parts of the range. The obtained values of NEI's genetic distances (0.004 to 0.026) suggest that southern geographically marginal populations of Scots pine are as much differentiated as the central populations and have probably slightly higher level of differentiation than the northernmost populations.

Key words: Scots pine, populations, genetic variability, Ukraine, allozymes.

Introduction

Scots pine (*Pinus sylvestris* L.) is a principal forest species of Eurasia covering a widest range of distribution among all pine species, with a great variability of ecological conditions (PRAVDIN, 1964).

During the last decades, many articles dealing with genetic variability of Scots pine populations from different areas of the range have appeared (e.g. GULLBERG et al., 1985; KINLOCH et al., 1986; MUONA et al., 1988; MUONA and HARJU, 1989; WANG et al., 1991; GONCHARENKO et al., 1994; PRUS-GŁOWACKI and BERNARD, 1994; ZHELEV et al., 1994; SHIGAPOV et al., 1995). However, only a few of them (GONCHARENKO et al., 1994; SANNIKOV et al., 1997) reported on southern marginal *P. sylvestris* populations from Ukraine. Meanwhile the Ukrainian populations are of particular interest for genetic study due to their mostly marginal status. It is generally accepted that the genetic structure of Scots pine populations in Europe was formed principally due to the post-glacial migration of the species from the refugia in its southwestern, southern and southeastern parts (GODWIN, 1956; LANGLET, 1959). Hence, geographically marginal Scots pine populations in the south are supposed to be the most ancient. Among them, the Ukrainian populations seem to be important as a subject for evolutionary study of *P. sylvestris* as a species in a whole.

In the intensive studies of Scandinavian Scots pine populations (see, for reference, SAVOLAINEN, 1996) it has been established that northernmost populations have a little higher rate of inbreeding and lower number of recessive lethals compared to southern ones (KÄRKKÄINEN et al., 1996). However, genetic differences at marker loci between these populations are small (GULLBERG et al., 1985; WANG et al., 1991).

Due to the discontinuous distribution of Scots pine in Ukraine (*Fig. 1*), gene migration between southern marginal *P. sylvestris* populations along the latitudinal transect is expected to be limited as compared to populations from the main segment of the range, and, also, gene flow from the south definitely does not occur. Thus, they are thought to have a higher level of differentiation and inbreeding than do the populations from the continuous part of the range. GONCHARENKO et al. (1994) reported on lower genetic variability of these populations as compared to central ones.

In this article I try to answer several questions. First, how different genetically are southern marginal Scots pine populations from those at the northern limits of the range? Second, is there significant genetic differentiation between geographically marginal Scots pine populations from Ukraine? And, finally, what factors can contribute to this differentiation?

Materials and Methods

Materials

Cones were collected from 140 trees 40 to 100 years old (22 to 24 trees per population, distances between the trees were 50 to 100 m) of six natural Scots pine populations of Ukraine situated principally on a latitudinal gradient (*Fig. 1*).

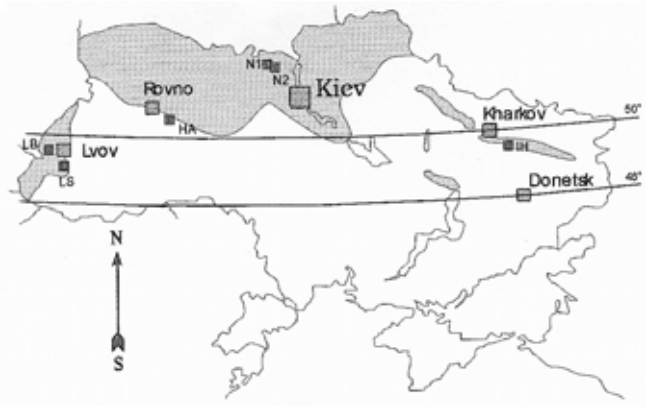

Figure 1. – The area of *Pinus sylvestris* distribution (shaded) in Ukraine and the locations of the studied populations. The populations designated: NR1 and NR2 – Nova Radcha 1 and Nova Radcha 2, LB – Bryukhovichi, LS - Stradch, HA – Neteshin, IH - Izum.

Isozyme electrophoretic analysis

Seeds were extracted from cones for each tree separately. For the isozyme study, 10 to 20 megagametophytes were analysed from each tree to determine a genotype of a maternal tree. The tissue was isolated from the seeds and homogenised with 0.025 ml of 0.2 M tris-glycine buffer, pH 7.5. The homogenates were subjected to vertical polyacrylamide gel electrophoresis (BREWER, 1970).

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Histochemical staining techniques described elsewhere (HARRIS and HOPKINSON, 1976; LOJDA et al., 1979; DVORNYK et al., 1996) were used. In total 21 loci of seven enzyme systems were scored. The enzymes assayed, and their abbreviations are as follows: glutamate dehydrogenase (*GDH*, EC 1.4.1.2, one locus), glutamic oxaloacetic transaminase (*GOT*, EC 2.6.1.1, 3 loci), diaphorase (*DIA,* EC 1.6.4.3, 3 loci), acid phosphatase (*ACP,* EC 3.1.3.2, one locus), malate dehydrogenase (*MDH,* EC 1.1.1.37, 4 loci), superoxide dismutase (*SOD,* 1.15.1.1, 4 loci), non-specific esterases (*EST,* EC 3.1.1-, 5 loci). Since the loci *Sod-1*, *Sod-2*, and *Sod-3* were monomorphic in all the populations studied, they were excluded from further analysis.

Alleles were designated such that the most anodally migrating isozyme was indicated as 1, the next as 2, and so on. Within each locus, the overall most frequent allele was assigned the value of 100. Other alleles of the locus were marked according to their relative mobility to the most frequent allele.

Due to the small number of the seeds analysed from each tree, MENDELian segregation was examined in single trees by c^2 test only for rare genotypes occurring in unique trees, while for the most common ones – pooled over all trees.

Statistical analysis

For each population basic characteristics of genetic variability were determined: allele frequencies, observed (H_o) and expected (H_e) heterozygosity, mean number of alleles per locus (A), mean effective number of alleles per locus (n_e), proportion of polymorphic loci at 95% criterion (P_{95}), and WRIGHT's F-statistics (WEIR and COCKERHAM, 1984). Genic differentiation between the populations was estimated using unbiased estimate of the P-value of the probability test (or FISHER's exact test), as described by RAYMOND and ROUSSET (1995*a*). NEI's genetic distance (D_N) (NEI, 1972) was calculated to estimate quantitatively the differentiation between populations. To determine confidence limits for F-statistics bootstrapping procedures were applied (WEIR, 1996).

The GENEPOP (RAYMOND and ROUSSET, 1995*b*) and PHYLIP (FELSENSTEIN, 1989) software was used for statistical analysis of the electrophoretic data.

Results

A total of 67 alleles were found at 21 loci in the six populations. 12 loci were polymorphic in all populations. Locus *Est-4* appeared to be most polymorphic with six alleles. Three loci, *Got-2*, *Got-3*, and *Dia-2*, had five alleles each. The frequencies of alleles at the 18 polymorphic loci are presented in *table 1.* The data of the table indicate that 19 alleles of the 64 ones listed occurred with frequencies less than 0.05, and 11 among them were "private", i.e. observed only in a particular population. The largest number of the "private" alleles were in populations HA and IH (4 in each). No such alleles have been found in population LS. Almost all polymorphic loci indicated no significant deviation from MENDELian segregation, except some combinations of rare alleles (*Table 2*).

Mean inbreeding coefficient over all the populations indicated a small deficiency of heterozygotes. This deficit was rather high in populations LS and HA. However, it was not significantly different from zero, as revealed by the bootstrap analysis (*Table 4*). The isolated populations HA and IH manifested the highest values of both expected and observed heterozygosity. Also, they possess highest values of some other measures of intrapopulation variability (*Table 3*).

The exact test for genic differentiation revealed significant differentiation between the studied populations at six loci at 0.05 level (*Table 4*). The most pronounced distinction was observed at loci of non-specific esterases: three of them (*Est-1*, *Est-2*, and *Est-5*) had highest level of genic differentiation.

Estimates of population subdivision using WRIGHT's F-statistics indicated rather low differentiation (*Table 4*). The mean F_{ST} value, which estimates the level of interpopulation differentiation, was 0.020. Thus about 98% of total genetic variation resides within each population. The magnitude of F_{ST} at some loci of the esterases (*Est-1* and *Est-2*) suggests that, among the loci tested, this group contributes most significantly to the total interpopulation variability. To assess contribution of isolation by distance to the interpopulation differentiation, MANTEL's tests (MANTEL, 1967) have been performed. They revealed no correlation between values of pair wise F_{ST} and geographic distances.

Discussion

The results of the investigation suggest that geographically marginal Scots pine populations from Ukraine are generally as highly differentiated as those from the other parts of the range. The value of F_{ST} is small (0.020, *Table 4*) and generally in agreement with F_{ST} or G_{ST} for Scots pine populations from the other segments of the range (GULLBERG et al., 1985; KINLOCH et al., 1986; WANG et al., 1991; GONCHARENKO et al., 1994; PRUS-GŁOWACKI and BERNARD, 1994; ZHELEV et al., 1994; SHIGAPOV et al., 1995). The mean values of WRIGHT's F_{IS} and F_{IT} indicated heterozygote deficiency at the respective levels of genetic structure of the Scots pine populations from Ukraine (*Table 4*). This deficiency estimated, on the average, about 6.7% at population level increases to more than 8.5% at level of the species. These values are a little higher than those reported by GONCHARENKO et al. (1994) (mean values of -0.014 and 0.015 for F_{IS} and F_{IT}, respectively). As the data of *table 4* suggest, loci *Dia-3*, *Mdh-4,* *Est*-2, *Est*-3, and *Est*-5 contribute most significantly to the value of inbreeding at both levels mentioned. However, on the other hand, bootstrap confidential intervals for both parameters overlap zero that suggests this deficiency is quite insignificant (*Table 4*).

Limited gene migration between the populations could be a reason for the higher level of F_{ST}. However, it is unlikely so. Thus, the gene flow calculated using both private alleles (BARTON and SLATKIN, 1986) and NEI's methods (NEI, 1987) was almost of the same value (5.11 and 6.86 migrants per generation, respectively). It is virtually the same as value of 8.68 reported by GONCHARENKO et al. (1994) and is high enough to maintain low interpopulation differentiation.

NEI's genetic distances between the populations were calculated for 18 polymorphic loci (*Table 5*). Their average value, 0.016, appeared to be about those for the populations from other segments of the species' distribution. Thus, GONCHARENKO et al. (1994) reported mean NEI's genetic distances of 0.025 for 18 Scots pine populations from various territories of Eurasia. Further, inferring phylogenetic relations between races and populations of *P. sylvestris*, the authors indicated the genetic distances between the populations of the same race within the range 0.005 to 0.012, between the isolated populations – 0.010 to 0.032, and between the races – 0.003 to 0.010. The geographically isolated populations from Spain manifested a slightly larger range of the D_N values (0.005 to 0.032 at 11 loci) (PRUS-GŁOWACKI and STEPHAN, 1994). Mean genetic distance between isolated populations of Caledonian Scots pine has been found to equal 0.013 (at 14 isozyme loci, KINLOCH et al., 1986). WANG et al. (1991) have reported D_N within 0.001 to 0.017 (at 14 loci) for *P. sylvestris* populations originating from Sweden and China. The genetic distances between three Swedish populations presented in that study were very low:

Table 1. – Frequencies of alleles in 18 polymorphic isozyme loci.

Loci	Alleles	Populations					
		N1	N2	LB	LS	HA	IH
1	2	3	4	5	6	7	8
Gdh-1	100	0.717	0.667	0.562	0.625	0.545	0.729
	111	0.283	0.333	0.438	0.375	0.455	0.271
Got-1	100	0.978	1.000	0.979	0.979	0.954	0.937
	111	0.022	0.000	0.021	0.021	0.046	0.063
Got-2	N	0.022	0.000	0.021	0.021	0.000	0.000
	100	0.652	0.521	0.687	0.750	0.704	0.646
	114	0.326	0.437	0.292	0.229	0.182	0.333
	128	0.000	0.042	0.000	0.000	0.068	0.021
	132	0.000	0.000	0.000	0.000	0.046	0.000
Got-3	N	0.000	0.000	0.000	0.000	0.023	0.000
	90	0.000	0.000	0.000	0.000	0.000	0.021
	100	0.609	0.646	0.667	0.771	0.659	0.729
	105	0.000	0.021	0.000	0.000	0.023	0.000
	112	0.391	0.333	0.333	0.229	0.295	0.250
Dia-2	85	0.000	0.000	0.000	0.000	0.000	0.062
	90	0.152	0.271	0.208	0.292	0.318	0.292
	95	0.000	0.000	0.021	0.000	0.000	0.000
	100	0.783	0.687	0.771	0.708	0.682	0.604
	105	0.065	0.042	0.000	0.000	0.000	0.042
Dia-3	95	0.022	0.000	0.042	0.000	0.000	0.000
	100	0.935	0.958	0.812	0.917	0.841	0.979
	105	0.043	0.042	0.146	0.083	0.159	0.021
Dia-4	80	0.022	0.021	0.083	0.021	0.068	0.063
	100	0.978	0.979	0.917	0.979	0.932	0.937
Acp-1	82	0.065	0.083	0.146	0.104	0.091	0.104
	92	0.196	0.188	0.208	0.208	0.205	0.229
	96	0.130	0.146	0.125	0.167	0.182	0.104
	100	0.609	0.583	0.521	0.521	0.522	0.563
Mdh-1	N	0.065	0.000	0.042	0.000	0.000	0.000
	100	0.935	1.000	0.958	1.000	0.954	1.000
	108	0.000	0.000	0.000	0.000	0.046	0.000
Mdh-2	N	0.000	0.000	0.000	0.000	0.000	0.021
	100	0.935	0.979	0.958	0.937	0.818	0.875
	105	0.022	0.000	0.000	0.000	0.000	0.000
	111	0.043	0.021	0.042	0.063	0.182	0.104
Mdh-3	N	0.022	0.021	0.021	0.000	0.023	0.021
	92	0.239	0.208	0.167	0.083	0.296	0.438
	100	0.739	0.750	0.812	0.896	0.681	0.541
	106	0.000	0.021	0.000	0.021	0.000	0.000

1	2	3	4	5	6	7	8
Mdh-4	100	0.630	0.583	0.687	0.542	0.477	0.625
	125	0.196	0.250	0.125	0.250	0.455	0.167
	250	0.174	0.167	0.188	0.208	0.068	0.208
Sod-4	N	0.022	0.021	0.021	0.000	0.000	0.063
	90	0.043	0.000	0.000	0.000	0.023	0.000
	95	0.000	0.021	0.000	0.000	0.000	0.000
	100	0.935	0.958	0.979	1.000	0.977	0.937
Est-1	N	0.913	0.958	0.979	1.000	0.636	0.854
	84	0.022	0.000	0.000	0.000	0.023	0.000
	100	0.065	0.042	0.021	0.000	0.341	0.146
Est-2	N	0.978	1.000	1.000	0.646	0.841	0.791
	100	0.022	0.000	0.000	0.333	0.159	0.188
	105	0.000	0.000	0.000	0.021	0.000	0.021
Est-3	N	0.000	0.000	0.000	0.000	0.046	0.021
	100	0.956	0.958	0.937	1.000	0.886	0.958
	100**	0.044	0.042	0.063	0.000	0.068	0.021
Est-4	N	0.000	0.021	0.021	0.000	0.046	0.083
	100	0.652	0.667	0.833	0.750	0.636	0.687
	112	0.304	0.229	0.125	0.146	0.204	0.188
	116	0.000	0.000	0.000	0.000	0.000	0.042
	125	0.022	0.083	0.021	0.083	0.068	0.000
	132	0.022	0.000	0.000	0.021	0.046	0.000
Est-5	N	0.065	0.125	0.188	0.042	0.000	0.104
	85	0.000	0.000	0.000	0.000	0.023	0.000
	100	0.935	0.875	0.812	0.958	0.977	0.896

N – null allele; **) – two-banded allele

Table 2. – Observed segregation of allozymes in endosperms of heterozygous trees and chi-square tests for goodness of fit to 1:1 ratio among the studied populations.

Locus	Tree	Allelic combination	Observed segregation	χ^2	P
1	2	3	4	5	6
Gdh-1	pooled	100 / 111	393:377	0.332	0.56
Got-1	pooled	100 / 111	41:29	2.057	0.15
Got-2	pooled	100 / 114	356:314	2.63	0.10
	pooled	100 / 128	10:10	0.000	1.00
	HA 15	100 / 132	4:6	0.400	0.53
	pooled	114 / 128	14:16	0.133	0.72
	HA 6	128 / 132	8:2	3.600	0.06
	LB 18	N / 100	5:5	0.000	1.00
	pooled	N / 114	6:14	3.200	0.07
Got-3	pooled	100 / 112	317:313	0.025	0.87
	HA 12	N / 100	6:4	0.400	0.53
	HA 6	100 / 105	6:4	0.400	0.53
	N2-41	105 / 112	6:4	0.400	0.53
Dia-2	pooled	90 / 100	256:274	0.611	0.43
	LB 19	90 / 95	15:5	5.000	0.03
	IH 16	85 / 100	8:12	0.800	0.36
	pooled	100 / 105	35:15	8.000	<0.01
	IH 15	90 / 105	7:13	1.800	0.16
Dia-3	pooled	95 / 100	14:26	3.600	0.06
	pooled	100 / 105	80:60	2.857	0.09
Dia-4	pooled	80 / 100	48:62	1.792	0.18
Acp-1	pooled	82 / 100	51:69	2.700	0.10
	pooled	92 / 100	104:126	1.409	0.15
	pooled	96 / 100	95:115	1.905	0.16
	pooled	82 / 92	51:49	0.040	0.84
	pooled	82 / 96	19:31	2.880	0.09
	pooled	92 / 96	23:27	0.320	0.57
Mdh-1	pooled	N / 100	13:17	0.533	0.47
	pooled	100 / 108	14:6	3.200	0.06
Mdh-2	N1-6	100 / 105	6:14	3.200	0.06
	IH 8	N / 100	3:7	1.600	0.21
	pooled	100 / 111	117:93	2.743	0.10
Mdh-3	pooled	92 / 100	262:298	2.314	0.13
	N1-7	N / 92	3:7	1.600	0.21
	pooled	N / 100	21:19	0.100	0.75
	LS 18	100 / 106	7:3	1.600	0.21
Mdh-4	pooled	100 / 125	79:81	0.025	0.87
	pooled	100 / 250	61:69	0.492	0.48
	pooled	125 / 250	82:68	1.307	0.25
Sod-4	pooled	N / 100	9:51	29.400	<0.01
	HA 5	90 / 100	5:5	0.000	1.00
	N2-44	95 / 100	6:4	0.400	0.53
Est-1	pooled	N / 100	93:87	0.200	0.66
	pooled	N / 84	9:11	0.200	0.66
Est-2	pooled	N / 100	32:28	0.533	0.61
	pooled	100 / 105	12:8	0.800	0.37
Est-3	pooled	100 / 100**	29:41	2.057	0.15
	IH 5	N / 100	4:6	0.400	0.53
Est-4	pooled	100 / 112	198:202	0.040	0.84
	N1-15	112 / 132	2:8	3.600	0.06
	pooled	100 / 116	10:10	0.000	1.00
	pooled	100 / 125	68:62	0.277	0.60
	pooled	100 / 132	19:11	2.133	0.14
	pooled	N / 100	29:21	1.280	0.26
	N2-18	N / 112	4:6	0.400	0.53
Est-5	pooled	N / 100	58:62	0.133	0.72
	HA 22	85 / 100	5:5	0.000	1.00

N – null allele; **) – two-banded allele

Table 3. – Basic genetic parameters of the studied Scots pine populations.

Population	Genetic parameters					
	H_e	H_o	P_{95}	A	n_e	F
N1	0.266	0.271	0.6	2.7	1.5	-0.019
N2	0.260	0.257	0.5	2.4	1.5	0.011
LB	0.263	0.236	0.7	2.4	1.5	0.105
LS	0.248	0.201	0.6	2.2	1.5	0.191
HA	0.340	0.301	0.7	2.7	1.6	0.118
IH	0.308	0.308	0.7	2.6	1.6	0.000
Mean	0.281	0.262	0.63	2.59	1.53	0.067

H_e – expected heterozygosity, H_o – observed heterozygosity, P_{95} – proportion of polymorphic loci at 95% criterion, A – mean number of alleles per locus, n_e – mean effective number of alleles per locus, F – WRIGHT's fixation index.

Table 4. – Estimates of F-statistics for 18 polymorphic loci (WEIR and COCKERHAM, 1984), their upper and lower bounds for 95% confidential interval (bootstrapping over loci, 1000 replications), and exact test for genic differentiation (P-values) (RAYMOND and ROUSSET, 1995*a*) in the studied populations.

Locus	F_{IS}	F_{IT}	F_{ST}	Genic differentiation
Gdh-1	-0.161	-0.152	0.008	0.314
Got-1	-0.023	-0.026	-0.003	0.519
Got-2	-0.211	-0.192	0.016	0.058
Got-3	-0.049	-0.053	-0.004	0.624
Dia-2	0.044	0.044	0.000	0.125
Dia-3	0.321	0.334	0.020	0.048
Dia-4	0.121	0.116	-0.006	0.564
Acp-1	0.105	0.088	-0.019	0.998
Mdh-1	0.266	0.276	0.014	0.007
Mdh-2	-0.103	-0.073	0.027	0.067
Mdh-3	-0.173	-0.106	0.057	0.014
Mdh-4	0.447	0.450	0.006	0.059
Sod-4	0.184	0.182	-0.002	0.233
Est-1	0.156	0.280	0.147	0.000
Est-2	0.521	0.593	0.150	0.000
Est-3	0.410	0.406	-0.006	0.317
Est-4	-0.100	-0.091	0.008	0.100
Est-5	0.403	0.414	0.019	0.016
Mean	0.067	0.085	0.020	—
Upper bound	0.201	0.223	0.043	—
Lower bound	-0.042	-0.021	0.003	—

Table 5. – Values of NEI's genetic distances (NEI, 1972) between the studied populations.

Populations	N2	LB	LS	HA	IH
N1	0.004	0.009	0.018	0.021	0.012
N2		0.009	0.017	0.022	0.012
LB			0.016	0.026	0.019
LS				0.022	0.018
HA					0.019

about 0.001, whereas the Chinese populations were much more differentiated. GULLBERG et al. (1985) found D_N ranging 0.001 to 0.015 for 9 populations from Sweden. Based on our results, one can conclude that the distances between the geographically marginal populations from Ukraine (0.004 to 0.026, *Table 5*) are of the same magnitude as those of the central and isolated populations from Spain and apparently slightly larger than those reported for the Scandinavian populations.

Comparing the northern and southern marginal populations of Scots pine one can conclude that the latter are slightly more differentiated, as inferred from NEI's genetic distances. This higher differentiation of the southern populations is apparently due to both the considerably longer period of their evolution after the last glaciation and the effect of somewhat isolated and semi-isolated character of the populations.

In fact, based on the results of allozyme analysis, it seems to be rather difficult to find any significant differences between central and geographically marginal (or isolated) Scots pine populations. The latter have been recognised having lower genetic variation (GONCHARENKO et al., 1994). However, this is probably not true for isozyme markers. Actually, as my data suggest (*Table 3*), two populations (LB and LS) among the six studied have indeed lower values of observed heterozygosity than the populations (N1 and N2) from the continuous part of *P. sylvestris* distribution, whereas other two isolated populations, HA and IH, possess the highest values of genetic variation. The only characteristic that maybe distinctive for the geographically marginal populations from Ukraine is apparently their slightly higher level of inbreeding as compared to central and northern populations. This can be due to the effect of some loci, such as *Dia-3, Mdh-4,* and non-specific esterases.

On the whole, the obtained data confirmed that Scots pine as a species maintains sufficient intrapopulation genetic variability and low interpopulation differentiation throughout its area of distribution. On the other hand, the southernmost populations appear to have slightly higher level of differentiation than the northernmost ones.

Acknowledgements

I wish to express my gratitude to Prof. OUTI SAVOLAINEN and Dr. CLAUS VOGL for helpful suggestions and fruitful discussion on early draft of the manuscript. The article would be impossible without invaluable help of my friends and colleagues, Dr. VALERY KOTOV (Donetsk State Medical University, Ukraine), Mrs. IRINA MIKHEENKO (University of Birmingham, UK), and Dr. SERGE YAROVOI (University of Massachusetts, USA). This work was partially supported by the Central European University within the Research Support Scheme, grant # 1304/93/1095.

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Genetic Control of Heartwood Content in Larch

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(Received 10th October 2000)

Abstract

Genetic variability for heartwood/sapwood extent in larch was examined at the population, progeny and clonal levels. For the study, increment cores were taken in 2 provenance trials (one of European larch at 34 years and one of Japanese larch at 36 years), and in 3 hybrid larch trials at around 15 years old (2 full-sib progeny trials and 1 clonal trial).

Heartwood appears early in larch and seems to progress quickly: at 15 years, it represents already more than 60% (along the radius) for hybrid larch and more than 70% for more mature (34 to 36 year old) European and Japanese larch. Genetic variability of about the same magnitude as that for radial growth was observed for heartwood/sapwood dimensions both at the provenance level (for European larch but not for Japanese larch) and at the progeny or clonal level (for hybrid larch). Broad-sense heritability levels for heartwood traits were high (0.75 to 0.92 for heartwood length, 0.63 to 0.99 for heartwood proportion). Heartwood and, to a lesser extent sapwood content, were positively linked to diameter growth in the different genetic entries studied (genetic correlations: 0.87 to 0.96 for heartwood length); heartwood extent was mostly independent of or positively correlated with sapwood content.

The proper choice of the species (Japanese versus European larch), of the origin (Central European larch populations rather than alpine ones), or of hybrid larch progenies or clones can result in significant combined genetic gains for both heartwood content and growth.

Key words: heartwood, sapwood, *Larix,* provenance, progeny, clone, hybrid, heritability, genetic variability.

Introduction

Among wood of coniferous species, European larch (*Larix decidua* MILL.) is much appreciated for its good mechanical properties but even more for the high natural durability of its heartwood (COLLARDET and BESSET, 1988).

Natural durability of wood is linked to its anatomical features (determining, for example, water permeability) and to its (qualitative and quantitative) content of extractives. Heartwood and sapwood are in many cases clearly distinguished by the amount of extractives; in European larch, KEITH and CHAURET (1988) have shown an increase of both water-soluble and alcohol-benzene extractives from the pith to the heartwood-sapwood boundary, and then a marked decrease in the sapwood. In this respect, the sapwood of larch is not durable and thus of low value as lumber but, in the native wood resource from the Alps, its extent is usually negligible: about 10% of a breast height (BH) diameter of 40 cm (RINGARD, 1980; COLLARDET and BESSET, 1988).

Wood from lowland plantations might not present the same characteristics in particular for the proportion of heartwood. Besides their establishment in more favourable ecological conditions, these stands are planted either with an exotic