THE GENETIC STRUCTURE OF SCOTS PINE (*PINUS SYLVESTRIS* L.) POPULATION FROM THE TOP OF SZCZELINIEC WIELKI MT., CENTRAL SUDETES

JERZY SZWEYKOWSKI¹, WIESŁAW PRUS-GŁOWACKI¹, JERZY HRYNKIEWICZ²

¹Department of Genetics, Faculty of Biology, Adam Mickiewicz University,
Międzychodzka 5, 60-371 Poznań, Poland.

²Museum of Natural History, Wrocław University,
Sienkiewicza 4, 50-335 Wrocław, Poland

(Received: November 3, 1994. Accepted: November 25, 1994)

ABSTRACT

The comparison of the genetic structure of the population of Scots pine from Szczeliniec Wielki Mt. with the population of its progeny and three other populations from the same geographic region shows great similarity of both populations as far as the frequencies of genes and genotypes are concerned. Also other genetic parameters, such as heterozygosity, genotypic polymorphism indices and a number of alleles per locus were in both populations very similar. It seems that our data proved that the provenance trial experiments reflect rather closely the genetic structure of maternal populations. The fact that the population from Szczeliniec Wielki and its "replica" are genetically most similar to the geographically closest population from Bystrzyca Kłodzka leads to the conclusion, that these populations are of common origin. Though the relic character of the population from Szczeliniec can not be excluded. The morphological distinctness of the population from Szczeliniec is caused by a modifying influence of environmental conditions.

KEY WORDS: *Pinus sylvestris*, genetic structure, maternal and progeny populations, provenance trials.

INTRODUCTION

Single trees and small groves of Scots pine, found on isolated rock complexes of the Góry Stołowe Mts. (Central Sudetes) catch the eye by their characteristic form. With few exceptions, the height of the trees does not exceed 3-4 meters, and these growing on rocks are even dwarfish (1.5-2 m high), with relatively thick and quickly tapering trunks and crowns, formed by few twisted branches. The environmental conditions on the rocky peaks are rather harsh. The cretaceous sandstone weathers to a poor, sterile sandy soil, which supports only a poor plant association, such as *Leucobryo-Pinetum* (Matuszkiewicz and Matuszkiewicz 1973). Strong, cold winds are a characteristic feature of the climate, in which the growing period is short. On average there are 126 days in a year, during which the temperature drops below zero (Walczak 1968).

One of the most spectacular pine groves is situated on the Szczeliniec Wielki Mountain at an elevation of 900-919 m, with about 500 trees located on the top plateau. The trees are loosely spaced, but, in spite of their frequently bizarre habitus, they are healthy and in some years produce cones with viable seeds.

In 1966 the seeds were collected from this population and sown in a tree-nursery in the forest region of Borowina, situated 17 km north of the original population at an elevation of about 500 m. After two years, seedlings were transplanted to an experimental plot in the same region. In 1987 a comparison

between the original population and its progeny growing in Borowina was carried out. In the present paper, the results concerning the genetic structure are published.

Regardless of some reservations, which will be discussed below, there is the possibility to address two important questions.

(i) A comparison between the genetic structures of the original population and its progeny, established exactly in the way the provenance trials are constructed, gives a unique opportunity to evaluate the influence of the procedure of setting up a provenance trial on the genetic structure of the derivative population. In other words, we tried to answer the basic question: whether these "replicate" populations can be treated as representative samples of the natural population. If so, population geneticists could with much less effort use these samples for studying the variability and the genetic structures of populations, as in the case of sampling from natural stands. Moreover, the results of a study of trees growing on provenance trials could provide us with valuable data on the geographical variability of several kinds of morphological, anatomical, chemical and other characters.

Provenance trials are widely used to obtain valuable data for forestry. But one has to realize that the sample on the provenance plot need not necessarily be representative of the whole original population: it frequently happens that it is only a representative of a group of chosen individual trees, e.g. plus trees (see discussions in Wright 1976, Giertych 1993).

TABLE 1. Allele frequency in population samples (number of trees assayed in brackets).

Gene	Populations					Mean [weighted]
	SZ	PL	BK	PR	KB	
FEst.	(51)	(137)	(29)	(30)	(30)	
1.	0.726	0.697	0.879	0.767	0.633	0.7220
2.	0.020	0.047	0.069	0.050	0.250	0.0667
3.	0.216	0.208	0.017	0.150	0.117	0.1751
5.	0.029	0.047	0.035	0.033	0.000	0.0361
GOT A	(51)	(137)	(29)	(30)	(30)	
1.	1.000	0.993	0.948	0.967	0.967	0.9839
3.	0.000	0.007	0.052	0.033	0.033	0.0162
GOT B	(51)	(137)	(29)	(30)	(30)	
1.	0.520	0.547	0.552	0.467	0.467	0.5253
2.	0.373	0.394	0.431	0.517	0.483	0.4170
3.	0.108	0.051	0.017	0.016	0.050	0.0542
4.	0.000	0.007	0.000	0.000	0.000	0.0036
Diaf.	(51)	(137)	(29)	(30)	(30)	
1.	0.824	0.799	0.948	0.833	0.950	0.8394
2.	0.118	0.128	0.052	0.100	0.033	0.1047
3.	0.059	0.073	0.000	0.050	0.017	0.0542
4.	0.000	0.000	0.000	0.017	0.000	0.0018
GDH	(51)	(137)	(29)	(30)	(30)	
1.	0.755	0.745	0.707	0.683	0.650	0.7296
2.	0.245	0.256	0.293	0.317	0.350	0.2794
ADH	(48)	(133)	(27)	(29)	(28)	
1.	0.750	0.696	0.630	0.552	0.554	0.6679
2.	0.250	0.305	0.370	0.448	0.446	0.3321
ShDH A	(51)	(137)	(29)	(30)	(30)	
1.	0.824	0.850	0.828	0.800	0.700	0.8213
2.	0.118	0.124	0.103	0.067	0.283	0.1318
3.	0.059	0.011	0.052	0.000	0.000	0.0217
5.	0.000	0.015	0.017	0.133	0.017	0.0253
ShDH B	(51)	(137)	(28)	—	—	
1.	0.755	0.839	0.946	—	—	0.8378
2.	0.266	0.102	0.054	—	—	0.1251
3.	0.000	0.058	0.000	—	—	0.0003
MDH A	(51)	(137)	(29)	—	—	
1.	0.990	0.978	1.000	—	—	0.9838
2.	0.010	0.022	0.000	—	—	0.0162
MDH C	(51)	(137)	(28)	—	—	
1.	0.745	0.800	0.643	—	—	0.7294
2.	0.255	0.200	0.357	—	—	0.2706

(ii). Isolated Scots pine populations growing in the south Polish mountains, living usually in inhospitable places (rocky outcrops, rocky, isolated summits) are considered as relic stands (e.g. Pancer-Kotejowa et al. 1982). In this respect, the isolated pine population in the Góry Stołowe Mts are very similar to those of Pieniny Mts. Thus the second issue we are going to raise is, whether the genetic structure can possibly be

of some help in resolving the problem of the relic character of these pine groves.

There are some reservations as to the question whether the population growing in Borowina is representative of its maternal stand, as no protocols of the seed collecting procedure are available. We believe, however, that since the population in Borowina was planted exactly in the same manner as the

provenance trials, the present study can answer the first question in a proper way. An additional advantage is that the maternal population still exists; seeds for provenance trials are collected usually from cut trees. Thus, we may also attempt to resolve the second question.

MATERIAL AND METHODS

Winter buds collected in late autumn of 1989 from the trees of the original population (Szczeliniec Wielki Mt. – SZ) and from its progeny growing in Borowina (PL) were used for electrophoretic analysis of 8 enzyme systems governed by 11 loci. These loci were: one locus of the Fluorescent Esterase (E.C.3.1.1.1. – FEst); two loci of the Glutamate oxalo-acetate Transaminase (E.C.2.6.1.1 – GOT); one locus of Diaforase (E.C.1.6.4.3. – Diaf.); one locus of Glutamate Dehydrogenase (E.C.1.4.1.3. – GDH); one locus of the Alcohol Dehydrogenase (E.C.1.1.1.1. – ADH); two loci of the Shikimic Acid Dehydrogenase (E.C.1.1.1.25 – ShDH); two loci of the Malate Dehydrogenase (E.C. 1.1.1.37. – MDH) and one locus of Isocitric Dehydrogenase (E.C.1.1.1.42 – IDH).

A 12% starch gel was used for electrophoresis. The methods of performing analysis, staining procedures and genetic interpretation described by Rudin and Ekberg (1978), Szmidi and Yazdani (1984), Yazdani and Rudin (1982), Gulberg et al. (1982), were followed.

Genetic structures of both populations were then compared with some other pine populations assayed previously. There were three of them: Bystrzyca Kłodzka (BK, 20 km SE of Szczeliniec Wielki Mt), Kubryk (KB, ca 60 km N of Szczeliniec Wielki Mt) and Pruszków (PR, about 65 km E from Szczeliniec Wielki Mt). Buds from these populations were taken from a provenance trial in Kórnik, near Poznań. Two of these populations, KB and PR, were assayed in respect of 7 loci, ShDH B and both loci of Malate Dehydrogenase (MDH A and MDH C) were not studied.

The genetic structure of the studied populations was characterised by gene and genotype frequencies and also the F-statistics (Wright 1943, 1951, 1965, 1969, cited in Nei 1987) and D_{ST} coefficient (Nei 1987) and genotypic polymorphism indices P_g (Jain and Marshall 1967) were calculated.

Genetic distances were calculated for gene and genotype frequencies, and constructed on the basis of distance matrices dendrograms by the UPGMA technique (Nei 1973, Hedrick 1974, Sneath and Sokal 1973). As the genetic structures of two populations (PR and KB) were based on assaying 7 loci, we compared the studied populations in two ways. First, these populations of which the genetic structures were analysed on the basis of 10 loci were treated together; second: to make all five populations comparable, we omitted three loci (ShDH B, MDH A, and MDH C), which were not assayed for the two populations mentioned above. In this way, in the second variant we compared all five of the studied populations.

Once again, it should be remembered that only one population – that from Szczeliniec Wielki Mt. (SZ) – was sampled from the natural stand. The material from the four remaining populations came from plants growing in provenance plots located either in Borowina (PL) or in Kórnik (BK, KB, PR).

RESULTS

As can be seen from Table 1, the analysed loci were polymorphic. Only a locus of isocitric dehydrogenase turned out

to be monomorphic and was omitted in the discussion that follows. As far as allele frequencies are concerned (Table 1) in three cases the sample taken from Szczeliniec Wielki Mt differed markedly from the rest; the allele of FEst3 and the allele ShDH B2 show much higher frequencies than in the three samples from the provenance trial in Kórnik. The sample of Borowina origin resembles the one of the parental population, although the frequencies of the mentioned alleles are here a little lower, approximating appropriately the remaining samples from the provenance trial in Kórnik. The frequency of the allele of the GOT B2 is on the contrary much lower; again, the derivative population demonstrates a slightly higher frequency, similarly to the previous case.

There is a visible similarity in gene frequencies of the natural population from Szczeliniec Wielki Mt and its progenial population growing in Borowina. This is evident from the results of the clustering methods. Dendrograms based on mean allele frequencies show very high similarity between the sample from Szczeliniec Wielki Mt. and its descent population (PL) (Fig. 1).

It is worth stressing, however, that in all instances (similarity based on single locus or on mean values for all studied loci) the population which shows the greatest similarity to the population from Szczeliniec (SZ) or to its "replicate" from Borowina (PL) is the one coming from Bystrzyca Kłodzka which is in the closest neighbourhood to the Szczeliniec Wielki population.

Only in two cases our samples were monomorphic: the first (case) was the sample from Szczeliniec Wielki Mt., which is fixed for the allele GOT A1, the second monomorphic locus MDH A was fixed for the allele MHD A1 in the sample from Bystrzyca Kłodzka. Allele MHD A2 had also a low frequency in the two remaining populations.

A quite different variation occurs as far as the number of alleles per locus is concerned. Five loci show only two alleles in our material. These are: GOT A, GDH, ADH, MDH A, and MDH C. The remaining 5 loci are characterised by showing more than two alleles: most frequently there are four of them (FEst, GOT B, Diaf, ShDH A). The locus ShDH B shows – in our material – three different alleles. The effective number of alleles per locus (Table 2) amounts from 1.0 in MDH A in the population BK sample and in the locus GOT A in the sample coming from Szczeliniec Wielki Mt., to 2.37 in the locus GOT B in the same sample.

The distribution of the effective number of alleles (n_e) is bimodal (data not shown). The first maximum lies between values 1.000 and 1.20; the loci showing such low values of the effective number of alleles per locus are nearly monomorphic, i.e. only one allele shows a very high frequency (over 0.9) – examples are GOT A and MDH A. Most of the studied loci, however, show distinctly higher values of n_e (over 1.50). Here at least two different alleles reach high frequencies; the locus GOT B showing 3, or even (in PL sample) 4 alleles, reaches the highest values of n_e of all the studied samples. The second locus with a high n_e values is ADH. It shows only two alleles, both of high frequency. All five populations assayed are rather similar in respect of the effective number of alleles per locus and in respect of values of the polymorphic index.

The genotypic frequencies were used for calculation of heterozygosities, F-statistics, and polymorphic indices (Hedrick 1974). The last parameter, i.e. P_g (Table 2) reaches the highest values (between 0.6555 and 0.7426) in locus GOT B, which shows, at the same time, the highest effective number of alleles. Similarly, the lowest values of the polymorphic

index (between 0.0000 and 0.1854) are found in the locus GOT A, showing the lowest effective number of alleles. The correlation, however, is far from being complete. As these two indices (n_e and P_g) estimate different parameters, we can find population samples with high effective number of alleles showing relatively low polymorphic index values (e.g. locus ADH in the BK sample) and vice versa (e.g. locus ShDH B in the sample from Borowina (PL): having $n_e = 1.39$ it shows the P_g value of 0.4627, which is only a little lower than in the ADH locus in PR sample (0.4708) where the effective number of alleles amounts to 1.98). Taking into account the polymorphic index value the most polymorphic population is the only natural population from Szczeliniec Wielki Mt.

Three kinds of heterozygosity coefficients were calculated. H_I – which is the observed fraction of heterozygotes in the sample assayed, H_S – expected heterozygosity under assumption of Hardy-Weinberg equilibrium (HWE) in each sample treated as subpopulation of the total population, which was formed by pooling all the samples assayed together. H_T is the expected fraction of heterozygotes in such a "total" population (Table 3).

Two loci (GOT A and MDH A) show the observed (H_I) heterozygosity values, one order of magnitude lower than the remaining ones. The rest of loci and populations show rather high values of H_I between 0.25 and 0.55, which is not unusual for gymnospermous trees (Kinloch et al. 1986, Mitton 1983, El-Kassaby 1991, Prus-Głowacki 1991, Müller-Starck et al. 1992).

The departure from the HWE was measured in two different ways: (i) as the f statistics ($= F_{IS}$ in Wright's notation), and (ii) by means of the χ^2 test (Weir 1990). Only in the natural population (SZ) the f values are frequently lower than 0 (Table 4). Only in one locus ShDH B the fixation index is positive and significantly different from zero. It is interesting that the population from Borowina shows also a positive f value in this case, however, not significantly different from zero. The samples from Kórnik provenance trial in which the ShDH B locus was assayed, show a negative value of that index. The population from Szczeliniec Wielki Mt. shows $f=0$ in two loci. The first case is the GOT A locus, in which only homozygotes of the allele were discovered. The heterozygotes were, however, present in excess in the population from Bo-

Fig. 1. Dendrograms and dendrites showing the genetic similarities of studied *P. sylvestris* populations (UPGMA), based on genes (DN) and genotypes (DH) frequencies. SZ – population from Szczeliniec Wielki Mt, PL – "replica" of this population from Borowina. PR, BK and KB populations from Pruszków, Byszyca Kłodzka and Kubryk respectively.

TABLE 2. Effective number of alleles (n_e , first row) and polymorphic index (P_g , second row).

Locus (no. of alleles)	Populations					Mean
	SZ	PL	BK	PR	KB	
FEst [4]	1.73 0.5890	1.87 0.6579	1.28 0.3591	1.63 0.5667	2.10 0.6622	1.72 0.5670
GOT A [2]	1.0 0.0000	1.01 0.0288	0.11 0.1854	1.07 0.1245	1.07 0.1245	1.05 0.0926
GOT B [4]	2.37 0.7436	2.18 0.6683	2.04 0.6825	2.05 0.6555	2.20 0.7177	2.17 0.6935
Diaf. [4]	1.44 0.5121	1.51 0.5339	1.11 0.1854	1.42 0.4667	1.11 0.1844	1.32 0.3765
GDH [2]	1.59 0.5368	1.61 0.5471	1.71 0.5779	1.76 0.5844	1.83 0.6067	1.70 0.5707
ADH [2]	1.60 0.5391	1.73 0.5667	1.87 0.3841	1.98 0.4708	1.98 0.5485	1.83 0.5018
ShDH A [4]	1.44 0.5121	1.35 0.4082	1.43 0.4922	1.51 0.4844	1.75 0.5844	1.50 0.4963
SdDH B [3]	1.53 0.5136	1.39 0.4627	1.11 0.1993	— —	— —	1.34 0.3892
MDH A [2]	1.02 0.0384	1.04 0.0838	1.00 0.0000	— —	— —	1.02 0.0407
MDH C [2]	1.65 0.5321	1.47 0.4845	1.85 0.6250	— —	— —	1.66 0.5472
Mean over 7 loci	1.60 0.4904	1.61 0.4873	1.51 0.4095	1.63 0.4791	1.72 0.4898	1.61 0.4712
Mean over 10 loci	1.51 0.4517	1.52 0.4442	1.45 0.3683	— —	— —	1.49 0.4214
Average number of alleles per locus	2.57	3.00	2.71	2.85	2.57	2.74

rowina (PL) and in all samples from the Kórnik provenance trial (BK, PR and KB). The second locus in which the fixation index value equals zero is that of ADH. In this case all three categories of genotypes are present and they are exactly in HWE proportions. Again, in both populations from Borowina (PL) and in the three samples from the Kórnik provenance trial, we found an excess of heterozygotes.

Chi-square values are generally low, and are insignificant in most of cases as far as the departures from Hardy-Weinberg equilibrium are concerned. There are, however, two notable exceptions. The first concerns the locus FEst. The χ^2 value is well over the significance level in the natural population on Szczeliniec Wielki Mt. (SZ) and it is still higher in the population from Borowina (PL). The remaining samples show much

lower χ^2 values, however they are still higher than in most loci of these populations. The second case is that of ADH locus. The three genotypes are in perfect agreement with HWE expectations in the natural population from Szczeliniec Wielki Mt. (SZ) only. The population from Borowina (PL) and all three samples from the Kórnik provenance trial show much higher χ^2 values, and in BK that value is significantly higher than the critical value. In the PR population it is only a little lower than that.

Pooling particular samples together and treating them thereafter as one "total" population gives an opportunity to check the goodness-of-fit for the HWE expectations. In the present paper we combined the samples in three different ways, thus obtaining three different "total" populations. First, we put

TABLE 3. Heterozygosities (H_I , H_S and H_T) in the samples assayed (number of studied trees in brackets).

Locus		Populations					Total population
		BK [29]	PR [30]	KB [30]	SZ [51]	PL [137]	
FEst	H_I	0.1724	0.3000	0.6000	0.5294	0.5328	0.4765
	H_S	0.2206	0.3861	0.5228	0.4216	0.4663	0.4298
	H_T						0.4423
GOT A	H_I	0.1034	0.0667	0.0667	0.0000	0.0146	0.0325
	H_S	0.0981	0.0644	0.0644	0.0000	0.0145	0.0314
	H_T						0.0320
GOT B	H_I	0.3448	0.5000	0.4667	0.5882	0.5839	0.5379
	H_S	0.5096	0.5149	0.5461	0.5796	0.5423	0.5432
	H_T						0.5473
Diaf	H_I	0.1034	0.2667	0.1000	0.3529	0.3139	0.2708
	H_S	0.0981	0.2928	0.0961	0.3046	0.3395	0.2764
	H_T						0.2816
GDH	H_I	0.3793	0.4333	0.4333	0.3333	0.3358	0.3610
	H_S	0.4144	0.4328	0.4550	0.3701	0.3804	0.3958
	H_T						0.3982
ADH	H_I	0.7407	0.6897	0.6071	0.3750	0.4586	0.5132
	H_S	0.4664	0.4947	0.4943	0.3750	0.4238	0.4344
	H_T						0.4436
ShDH A	H_I	0.2759	0.2333	0.4333	0.3529	0.2117	0.2708
	H_S	0.3014	0.3378	0.4295	0.3046	0.2611	0.2999
	H_T						0.3070
ShDH B	H_I	0.1071	–	–	0.2546	0.2941	0.2546
	H_S	0.1015	–	–	0.3493	0.2816	0.2742
	H_T						0.2808
MDH A	H_I	0.0000	–	–	0.0196	0.0438	0.0323
	H_S	0.0000	–	–	0.0194	0.0428	0.0317
	H_T						0.0317
MDH C	H_I	0.2584	–	–	0.3277	0.3062	0.3049
	H_S	0.2669	–	–	0.3104	0.3072	0.3026
	H_T						0.3056
Mean over 7 loci	H_I	0.3029	0.3557	0.3867	0.3617	0.3502	0.3519
	H_S	0.3012	0.3605	0.3726	0.3365	0.3468	0.3444
	H_T						0.3503
Mean over 10 loci	H_I	0.2584	–	–	0.3277	0.3062	0.3049
	H_S	0.2669	–	–	0.3104	0.3072	0.3026
	H_T						0.3056

together the samples taken from the natural population on Szczeliniec Wielki Mt. (SZ) and its progeny growing in Boro-wina (PL). Secondly, we added to the former two, the sample from Bystrzyca Kłodzka (BK, taken from the provenance trial in Kórnik). The reason for doing this was that the population from BK is geographically closest to the Szczeliniec Wielki

Mt. Thirdly, we pooled all five samples together; as the two remaining samples (KB and PR) were analysed according to 7 loci, the "total" population formed in this way could be char-acterised by seven loci only.
Table 5 contains F statistics characterising the three de-scribed "total" populations. The F_{IT} values measuring the de-

TABLE 4 Departure from HWE expectations measured by the f coefficient ($=F_{IS}$) (first row) and chi-square values; number of trees assayed in brackets.

Locus	Populations					Mean
	SZ	PL	BK	PR	KB	
FEst	[51]	[137]	[29]	[30]	[30]	
	-0.2557	-0.1426	+0.2185	+0.2230	-0.1477	-0.0209
	5.4065	8.9431	0.1553	0.4684	2.4583	
GOT A	[51]	[137]	[29]	[30]	[30]	
	0.0000	-0.0069	-0.0540	-0.0357	-0.0357	-0.0331
	0.0000	0.2518	0.0000	0.0000	0.0000	
GOT B	[51]	[137]	[29]	[30]	[30]	
	-0.0148	-0.0767	+0.3234	+0.0289	+0.1454	+0.0812
	2.0846	4.6777	1.5000	0.0000	0.0524	
Diaf	[51]	[137]	[29]	[30]	[30]	
	-0.1586	+0.0754	-0.0540	+0.0891	-0.0406	-0.0177
	0.7310	0.0086	0.0000	0.0000	0.0000	
GDH	[51]	[137]	[29]	[30]	[30]	
	+0.0994	+0.01172	+0.0847	-0.0012	+0.0477	+0.0696
	0.2108	1.3579	0.0052	0.0000	0.0000	
ADH	[48]	[133]	[27]	[29]	[28]	
	0.0000	-0.0826	-0.5881	-0.3942	-0.2282	-0.2586
	0.0000	0.6453	7.5032	3.3195	0.8851	
ShDH A	[51]	[137]	[29]	[30]	[30]	
	-0.1586	+0.1892	+0.0846	+0.3094	-0.0089	+0.0831
	0.7310	3.0107	0.0163	0.6224	0.0336	
ShDH B	[51]	[137]	[28]	—	—	
	+0.1604	+0.0364	-0.0552	—	—	+0.0479
	0.1976	0.0651	0.0000	—	—	
MDH A	[51]	[137]	[29]	—	—	
	+0.0101	-0.0186	0.0000	—	—	-0.0169
	0.0000	0.0000	0.0000	—	—	
MDH C	[52]	[137]	[29]	—	—	
	-0.1356	+0.0741	+0.2223	—	—	+0.0536
	0.2047	1.7933	0.7262	—	—	
Mean over 10 loci	-0.0453	+0.0165	+0.0318	—	—	+0.0040
	0.9566	2.6993	0.9906	—	—	
Mean over 7 loci	-0.0749	-0.0098	-0.0056	+0.0133	-0.0378	-0.0219
	1.3091	2.0754	1.3114	0.6300	0.4899	

viations from the HWE expectations, show in particular variants quite different values. For instance, F_{IT} for FEst locus shows a clear-cut excess of heterozygotes in the first and second "variants" but a deficiency in the third one. Thus the samples combined in the first two variants are similar in respect of their genetic structures, which are determined by mating system. On the contrary, the ShDH A locus shows a

deficiency of heterozygotes in all three variants, although the values of F_{IT} in the first two variants are identical. Thus, also in this case, the three populations (SZ, PL, BK) coming from one small geographical area are similar and differ from the remaining two populations which grow much further away.

The indices of population subdivision (F_{ST} and D_{ST}) show in all cases a similar trend: their values are becoming higher

as the number of samples forming a particular "total" population increases. Evidently the two populations (SZ and PL) are most similar as far as their genetic structure is concerned.

DISCUSSION

The first problem we would like to discuss here is, whether the samples grown on plots of a provenance trial are representative of their original populations, as regards their population genetic parameters. The material studied in our work allows for a comparison between such parameters in the case of the original population situated on the top of Szczeliniec Wielki Mt. (SZ) and a derivative population growing in Borowina (PL).

It is evident from our results (Fig. 1, Tables 1, 2, 3, 4, 5) that the most similar samples in the examined material are those taken from the original population (SZ) and its "replicate" (PL). But similar does not mean identical. There are some differences in gene (Table 1) and genotype frequencies. The differences in question will be discussed later; now, we would like to focus on the discrepancies and similarities of more complex parameters, namely on the characteristics of such important population attributes as their mating system. One of such parameters is the goodness of fit with the HWE expectations.

The departures from these expectations can be caused by various factors: (i) by deviation from random mating, thus reflecting the characteristics of the actual mating system of the population in question, (ii) by population subdivision, and (iii) by selection (Nei 1987). In our case we could be reasonably sure that because of the small size of the populations sampled and the little space they occupy, the effects of possible population subdivision (ii) can be neglected.

Thus, the replicates growing in the provenance trials should reflect the properties of the mating systems of the original populations, and the selective forces operating in their places of living. It follows that the genetic structures of both the

original populations and their "replicates" should be very similar if additional sampling effects or artificial and natural selection are not acting. However, the artificial selection is performed, frequently quite unconsciously, by foresters during seeds collection and on the occasion of transplanting seedlings from the nursery to the experimental plot. The natural selection is caused by ecological conditions, which both in the nursery and in the experimental plot can be quite different from those operating in places where the original populations live.

Because of their importance to our problem, we shall discuss these matters at length. First, when a seed sample is taken from the original population, the sampling error is frequently caused by a tendency to collect seed from healthy trees producing large amounts of seed. Even if the sampling error is negligible, e.g., when the seed sample is taken at random from many trees, the seedlings from the seed sample grow for a period of two years in a nursery, where the ecological conditions are usually quite different from those prevailing in the original stand. They will be therefore affected by quite different factors of selection processes than acting in native population. Moreover, when seedlings are transplanted from the nursery to the experimental plot, they are additionally selected by man, who prefers usually vigorous and healthy looking plants for transplantation. After the planting into the experimental plot, new selective factors can operate on the genetic structure of the sample. Thus, there are numerous factors that can change the genetic structure of the experimental population growing in a provenance trial.

Thus, the genetic structure of the "replicate" populations growing in a provenance plot can reflect additional effects of the sampling. Comparison of such complex parameters as the departure from the HWE expectations or of fixation indices, in both the original population and its "replicate" from the plots of a provenance trial, can allow us to evaluate the impact of the additional sampling effects, in other words, we can possibly evaluate the influence of the procedure of forming a provenance trial on the genetic structure of the "replicates".

TABLE 5. Values of F_{ST} , F_{IT} and D_{ST} in three different combinations of the studied populations.

Locus	Variant I: 2 pop. [SZ, PL] 10 loci			Variant II: 3 pop. [SZ, PL, BK] 10 loci			Variant III: 5 pop. [SZ, PL, BK, PR, KB] 7 loci		
	F_{ST}	F_{IT}	D_{ST}	F_{ST}	F_{IT}	D_{ST}	F_{ST}	F_{IT}	D_{ST}
FEst	0.0009	-0.1700	0.0004	0.0196	-0.1215	0.0084	0.0238	+0.0773	0.0123
GOT A	0.0094	0.0000	0.0001	0.0219	-0.0088	0.0005	0.0188	-0.0156	0.0006
GOT B	0.0016	-0.0574	0.0009	0.0024	-0.0090	0.0013	0.0075	+0.0172	0.0041
Diaf.	0.0006	+0.0174	0.0002	0.0121	+0.0257	0.0037	0.0185	+0.0385	0.0052
GDH	0.0000	+0.1125	0.0000	0.0010	+0.1093	0.0004	0.0060	+0.0934	0.0024
ADH	0.0026	-0.0597	0.0011	0.0059	-0.1321	0.0025	0.0207	-0.1569	0.0092
ShDHA	0.0026	+0.0863	0.0007	0.0025	+0.0862	0.0007	0.0232	+0.1180	0.0071
Mean over 7 loci	0.0025	-0.0101	0.0005	0.0093	-0.0072	0.0025	0.0168	-0.0046	0.0059
ShDH B	0.0148	+0.0895	0.0045	0.0234	+0.0932	0.0066	—	—	—
MDH A	0.0000	-0.0136	0.0000	0.0032	-0.0189	0.0001	—	—	—
MDH C	0.0038	+0.0130	0.0013	0.0157	+0.0605	0.0056	—	—	—
Mean over 10 loci	0.0036	+0.0018	0.0009	0.0098	+0.0022	0.0030	—	—	—

As can be seen from Table 4, there exist differences in the values of parameters denoting the mating system the original population (SZ) and its "replicate" (PL). The f (F_{IS}) values (Table 4) show that in nearly all particular loci the original population differs from its replicate. And the χ^2 values (Table 4) with only two exceptions are distinctly higher in the "replicate" than in the original population, showing that the extent of departure from the HWE expectations is more pronounced here. It seems then, that those aspects of the genetic structure of populations that are dependent on the mating system are also more vulnerable to the effects of activities connected with establishing of provenance trial experiments.

The discussion on the problem of influence of factors operating in provenance trial experiments, allows some important conclusions to be drawn.

(i) It can be stated that in spite of many opportunities for change of the genetic structure of population samples used in provenance trials, our results show that the original population (SZ) and its "replicate" (PL) are genetically the most similar among all populations studied. This similarity concerns both multivariate measures and frequencies of alleles and/or genotypes in particular loci. It seems that this striking similarity can be explained by the neutral (or nearly neutral) character in these particular ecological conditions, of most the genes we studied. One has to realize, however, that the factors operating during the formation of a provenance trial do change the genetic structure of population samples, although in most instances to a rather small extent.

There are two reasons for this opinion. First, in some instances (e.g. the allele of *Fest 3* and the allele of the *Shdh B2*) we observed rather obvious differences in gene frequencies between the natural population (SZ) and the population samples cultivated in Kórnik (BK, KB, PR). In all these instances, the "replicate" from Borowina (PL) deviates from the original population (SZ) – although very little – in the same direction; it seems, then, that the milder conditions of living in both the nursery and in provenance plots change the genetic structures in one direction, thus causing them to get more similar than are the original populations.

(ii) Significant deviations from the genetic structure of the original population can occur, when the genes in question are under a strong pressure of selection. We met two cases of that kind in our study: the loci *Adh* and *Shdh B*.

(iii) As we have shown, the parameters characterising more complex aspects of the genetics of populations as, e.g., of the mating system, are more vulnerable to the factors operating during the formation of a provenance trial.

Thus, when studying the genetic parameters of samples growing in provenance plots, one has to be cautious when drawing more general conclusions. In some instances the values of genetic parameters of the sample from a provenance trial plot can be different from those of the original population.

We can come also to another important conclusion from our data. The very unusual morphological appearance of the trees from the Szczeliniec Wielki is the result of the modifying influence of environmental conditions, because the genetic structure of SZ and PL ("replicate") populations are genetically most similar among populations we studied. The progeny of these trees (SZ – population) growing in Borowina are showing "normal" growth and the appearance of regular Scots pine and do not repeat the characteristics of maternal trees.

The second problem we were going to investigate was the supposed relic character of the natural population situated at the top of Szczeliniec Wielki Mt. (SZ). This population is

quite isolated from other pine forests except for similar pine groves on various rock complexes of the Góry Stołowe ridge, with a genetic structure virtually unknown. The closest "regular" pine forest, most probably planted by man, is situated about 10 km NE of Szczeliniec Wielki Mt. This forest occurs at a significantly lower elevation – at least 400 meters below the top plateau of Szczeliniec Wielki Mt.

The comparison of genetic parameters of the natural population (SZ) and its "replicate" from Borowina (PL) with the samples growing in the Kórnik provenance trial (BK, KB, PR) and originated from a geographic region close to Szczeliniec Mt., shows that in few instances (loci *Shdh A*, *Got B*, *Mdh A* as far as gene frequencies are concerned and also locus *Shdh A* in respect to genotypic structure) these two samples (SZ and PL) do not show the greatest similarity to each other, but to the sample coming from the vicinity of the town of Bystrzyca Kłodzka (BK). This population does not exist any more, but it was probably a seminatare pine population formed from the seed produced in situ and situated relatively close (about 20 km SE) to the natural population on the top of Szczeliniec Wielki Mt. Thus, the possibility cannot be excluded that both populations (SZ and BK) are derivatives from one and the same ancestral population, which inhabited the Kotlina Kłodzka valley at the dawn of the glacial epoch.

However, the possibility cannot be denied that the similarity of the genetic structures of SZ and BK has been caused by a relatively recent gene flow via long distance pollen transport. However the significant difference in elevation (BK was situated about 500 m lower) causes the time of flowering at the top of Szczeliniec Wielki Mt. to be at least two weeks later than in the site of BK. Thus, the gene flow via pollen seems to be negligibly small, if any.

Thus, the problem of a possible relic character of the isolated pine stand inhabiting the top of the Szczeliniec Wielki Mt. cannot be answered unequivocally on the basis of the genetic data at hand.

ACKNOWLEDGEMENTS

We are grateful to Mrs Romana Nowak-Bzowy for the technical help and computer processing data.

This work was supported by grants CPBP no. 09.02.04 and 04.03.II/1 from the Polish Academy of Sciences.

LITERATURE CITED

- EL-KASSABY Y.A., 1991. Genetic variation within and among Conifer populations: review and evaluation of methods in: Biochemical markers in the population genetics of forest trees, SPB Academic Publishing, The Hague: 61-76, FINESCHI S., MALVOLTI M.E., CANNATA F., HATTEMER H.H. (eds.).
- GIERTYCH M., 1993. Zmienność proveniencyjna (Provenance variability, in Polish), in: Biologia sosny zwyczajnej (The Biology of Scotch Pine, in Polish), Białobok S., Boratyński A., Bugała W. Poznań, Sorus Editions: 325-339, (eds.).
- GULBERG U., YAZDANI K., RUDIN D., 1982. Genetic differentiation between adjacent populations of *Pinus sylvestris*, Silva Fennica 6 (2): 205-214.
- HEDRICK P.W., 1974. Genetic similarity and distance; comments and comparisons, Evolution 29 (2): 362-366.
- JAIN S.K., MARSHALL D.R., 1967. Population studies in predominantly self-pollinating species X. Variation in natural populations of *Avena fatua* and *A. barbata*, Amer. Natur. 101: 19-33.

- KINLOCH B.B., WESTFALL R.D., FORREST G.I., 1986. Caledonian Scots Pine: origins and genetic structure, *New Phytol.* 104: 703-729.
- MATUSZKIEWICZ W., MATUSZKIEWICZ J., 1973. Przegląd fitosocjologicznych zbiorowisk leśnych Polski, cz. 2. Bory sosnowe, *Phytocoenosis* 4(2): 273-356.
- MITTON J.B., 1983. Conifers, in: *Isozymes in Plant Genetics and Breeding*, Elsevier: 443-472, TANKSLEY S.D., ORTON T.J. (eds.).
- MÜLLER-STARCK G., BARADAT Ph., BERGMANN F., 1992. Genetic variation within European tree species. *New Forest* 6: 23-47.
- NEI M., 1973. Analysis of gene diversity in subdivided populations, *Proc. Natl. Acad. Sci. USA* 70: 3321-3323.
- NEI M., 1987: *Molecular Evolutionary Genetics*, Columbia University Press, N. York, 512 pp.
- PANCER-KOTEJOWA E., BAZYLUK W., LIANA A., 1982. Forest Biocenoses (Biocenozy leśne, in Polish) in: *Biota of Pieniny Mts. in the changing world (Przyroda Pienin w obliczu zmian, in Polish)* Zarzycki K. (ed.): 315-328.
- PRUS-GŁOWACKI W., 1991. Biochemical polymorphism. in: *Genetics of Scots pine*, Elsevier Sci. Publ. ed. M. Giertych, C. Matyas, 73-86.
- RUDIN D., EKBERG J., 1978. Linkage studies in *Pinus sylvestris* L. using macrogametophyte allozymes, *Silvae Genetica* 27: 1-12.
- SNEATH P.H.A., SOKAL R.R., 1973. *Numerical Taxonomy*, W.H. Freeman & Co., San Francisco, 573 pp.
- SZMIDT A.E., YAZDANI K., 1984. Electrophoretic studies of genetic polymorphism of shikimate and 6-phosphogluconate dehydrogenases in Scots pine (*Pinus sylvestris* L.), *Arboretum Kórnickie* 29: 63-72.
- WALCZAK K., 1968. *Sudety (Sudetes, in Polish)*, PWN Warszawa.
- WEIR B.S., 1990. *Genetic Data Analysis*, Sinauer Ass., Sunderland, 377 pp.
- WRIGHT J.W., 1976. *Introduction to forest genetics*, Academic Press, 461 pp.
- WRIGHT S., 1943. Isolation by distance, *Genetics* 28: 114-138.
- WRIGHT S., 1951. The genetical structure of populations, *Ann. Eugen.* 15: 323-354.
- WRIGHT S., 1965. The interpretation of population structure by F-statistics with special regard to systems of mating, *Evolution* 19: 395-420.
- WRIGHT S., 1969. *Evolution and the Genetics of Populations. Vol. 2. The Theory of Gene Frequencies*, University of Chicago Press, 511 pp.
- YAZDANI K., RUDIN D., 1982. Inheritance of fluorescence esterase and β -galactosidase in haploid and diploid tissues of *Pinus sylvestris* L., *Hereditas* 96: 191-194.

STRUKTURA GENETYCZNA POPULACJI SOSNY ZWYCZAJNEJ (*PINUS SYLVESTRIS* L.) ZE SZCZYTU SZCZELIŃCA WIELKIEGO W ŚRODKOWYCH SUDETACH

STRESZCZENIE

Porównanie struktury genetycznej populacji sosny ze Szczelińca Wielkiego i populacji potomnej oraz trzech innych populacji z tego regionu geograficznego wykazało bardzo duże podobieństwo tych dwóch populacji pod względem częstości genów i genotypów. Także inne parametry genetyczne takie jak heterozygotyczność, współczynnik polimorfizmu genotypowego oraz liczba alleli na locus były w obu populacjach bardzo zbliżone. Uzyskany wynik daje podstawy do tego, aby sądzić, że doświadczenia proveniencyjne dość wiernie odzwierciedlają strukturę genetyczną populacji macierzystych. Populacja ze Szczelińca Wielkiego i jej "replika" są genetycznie najbardziej podobne do najbliższej im geograficznie populacji z Bystrzycy Kłodzkiej, co sugerować może wspólne pochodzenie tych populacji. Nie wyklucza się więc relikтового charakteru badanej populacji. Obserwowana odrębność morfologiczna populacji sosny ze Szczelińca jest, w świetle uzyskanych wyników, zmiennością modyfikacyjną.

SŁOWA KLUCZOWE: *Pinus sylvestris*, struktura genetyczna, populacja macierzysta i potomna, doświadczenie proveniencyjne.