Trees grown from cuttings compared with trees grown from seed (*Pinus radiata*). Silvae Genetica **19**, 54—63 (1970). —— HOOD, J. V. and LIBBY, W. J.: Continuing effects of maturation state in radiata pine and a general maturation model. In: Propagation of Higher Plants Through Tissue Culture. K. W. (1978). —— HUSHES, R. H. and CONSTANTIN, M. (Eds.): U.S. Department of Energy, Washington, DC. Pp: 220—232 (1978). —— LIBBY, W. J., BROWN, A. G. and FIELDING, J. M.: Effects of hedging radiata pine on production, rooting and early growth of cuttings. New Zealand Journal of Forest Science **2**: 263—283 (1972). —— LIBBY, W. J. and HODD, J. V.: Juvenility in hedged radiata pine. Acta Hortic. **56**: 91—98 (1976). —— OLD, K. M., LIBBY, W. J., RUSSELL, J. H. and ELDRIDGE, K. G.: Genetic variability in susceptibility of *Pinus radiata* to western gall rust. Silvae Genetica **35**: 145—149 (1986). —— POWER, A. B. and DODD, R. S.: Early differential susceptibility of juvenile seedlings and more mature rooted cuttings of *Pinus radiata* to *Dothistroma pini*. New Zealand Forestry Science **14**: 223—228 (1984). —— TUFOUR, K. and LIBBY, W. J.: First-lift pruning times of radiata pine seedlings and rooted cuttings in a small California experiment. New Zealand Journal of Forestry **18**: 124—132 (1973). —— ZAGORY, D. and LIBBY, W. J.: Maturation related Resistance of *Pinus radiata* to Western Gall Rust. Phytopathology **75**: 1443—1447 (1985).

Genetic Variation of Pinus sylvestris from Spain in Relation to Other European Populations

By W. PRUS-GLOWACKI[1]) and B. R. STEPHAN[2])

(Received 10th February 1993)

Summary

Seven populations of Scots pine (*Pinus sylvestris* L.) from the natural distribution of the species in Spain were investigated isoenzymatically at 11 enzyme loci, and compared with 16 populations from northern and eastern Europe at seven loci. Populations from Spain are genetically differentiated in relation to the frequencies of genes and genotypes. The similarity between the 7 populations was studied and agreed to a significant extent with taxonomical subdivisions of Scots pine on the Iberian peninsula. The Spanish and the northern and eastern European populations form 2 very distinct and heterogeneous groups according to the genetic similarity. In comparison with the other European populations the Spanish material showed a slightly higher number of alleles per gene locus, a higher number of genotypes, and a slightly lower degree of heterozygosity. In the Spanish populations five alleles were detected, which are not present in the other European populations, whereas in the northern and eastern European populations only 2 alleles were found, which were not present in the Spanish material.

Key words: Pinus sylvestris, genetic variation, allozymes, heterozygosity.

Introduction

The genetic structure and the geographical variation of populations of *Pinus sylvestris* L. in Europe were formed by many different factors. In central and eastern Europe besides the climatic and environmental conditions the post-glacial migration of the species from refugia in south-western, southern and south-eastern Europe have had the most important influence (WULF, 1943; GODWIN, 1956; LANGLET, 1959). Also man's activity changed the "original" gene pool of *Pinus sylvestris* populations by cutting, reforestation and more recently by effects of air pollution, which is especially dramatic in central and eastern Europe.

Populations of *P. sylvestris* from the Iberian peninsula have had their own and different history compared to the rest of Europe. They are considered as relics of the tertiary period and represent "old" gene pools. Today, *P. sylvestris* inhabits disjunct, isolated areas separated by long distances, as a consequence of edaphic and climatic factors (PRAVDIN, 1969). The Spanish Scots pine stands are marginal populations of the natural range of the species in south-western Europe. Therefore, *P. sylvestris* populations from Spain represent a unique material highly valuable for genetic studies, also in connection with gene conservation programs. But they are also very interesting from the taxonomical point of view, because they represent different ecotypes or varieties of the species (SVOBODA, 1953; GAUSSEN, 1960; NICOLAS and GANDULLO, 1969).

Unfortunately, data concerning isoenzymatic differentiation of Scots pine as related to geographic and racial aspects and also our knowledge about the genetic variation of *P. sylvestris* populations and especially of populations from the Iberian Peninsula remain still largely incomplete (for review see PRUS-GLOWACKI, 1991; MÜLLER-STARCK et al., 1992). Therefore, we investigated the genetic variation and geographic differentiation of indigenous, mountainous populations of 7 isolated provenance regions in Spain and compared several genetic parameters of these populations with 16 populations from eastern and northern Europe.

Materials and Methods

For the isoenzyme study open pollinated cones of seven populations of *P. sylvestris* from Spain were collected. The seeds of each of the populations (21 to 25 trees from each population) were put together and used as random samples. From each population 40 to 112 embryos from germinated seeds were isolated and analysed.

As reference, 16 populations from eastern and northern Europe were chosen from provenance trials (Lubien IUFRO 1937, Kórnik 1968) and from four natural stands in Poland. For these isoenzyme analyses winter buds of the trees (29 to 51 from each population, mostly 30) were used.

The data for the origin of the populations are given in *table 1* and *figure 1*. Edaphic and climatic conditions for the main Scots pine areas in Spain were described by ALIA et al. (1991) and PARDOS and STEPHAN (1988).

In the Spanish material the variability of 11 enzyme loci were analysed: Alcohol dehydrogenase (ADH) E.C. 1.1.1.1, Diaphorase (DIA) E.C. 1.6.99, Fluorescent esterase (FEST) E.C. 3.1.1.1, Glutamate dehydrogenase (GDH) E.C. 1.4.1.2, Glutamate oxaloacetate transaminase (GOT), 2 loci, E.C. 2.6.1.1, Malate dehydrogenase (MDH), 2 loci, E.C. 1.1.1.37,

[1]) A. Mickiewicz University, Genetics Department, Faculty of Biology, ul. Dabrowskiego 165, PL-60-594 Poznan, Poland

[2]) Federal Research Centre for Forestry and Forest Products, Institute of Forest Genetics, Sieker Landstr. 2, D-22927 Grosshansdorf, Germany

Table 1. — Data of the *Pinus sylvestris* stands studied.

Locality	Country	Lat. (N)	Long.	Alt. (m)	Denotation	Embryos (n)
Valsain	Spain	40°49'	4°01'W	1550	H1	78
Gudar	Spain	40°25'	0°41'W	1700	H2	40
Baza	Spain	37°22'	2°51'W	2050	H3	40
Covaleda	Spain	41°56'	2°48'W	1550	H4	66
Borau	Spain	42°42'	0°35'W	1550	H5	102
Orihuela	Spain	40°31'	1°38'W	1750	H6	112
Refalgueri	Spain	40°45'	0°12'E	1150	H7	80
						Trees (n)
Inari	Finland	68°40'	27°37'E	140	FI	30
Rovaniemi	Finland	66°25'	26°26'E	250	FR	30
Saaminki	Finland	61°40'	28°55'E	85	FS	30
Tonset	Norway	62°22'	10°48'E	550	NT	31
Asnes, Hamar	Norway	60°32'	12°11'E	230	NA	30
Svanoy	Norway	61°30'	5°07'E	50	NS	30
Vecmokas	Latvia	57°03'	23°10'E	80	LT	30
Glen Garry	Scotland	57°04'	4°55'W	150	SK	30
Ruciane	Poland	53°41'	21°26'E	120	PU	30
Szczeliniec	Poland	50°17'	16°36'E	900	SZ	51
Kampinos	Poland	52°19'	20°41'E	95	KP	30
Pieniny	Poland	49°20'	19°20'E	770	PP	30
Lubmol	Ukraine	51°15'	24°05'E	195	UK	30
Mustejki	Lithuania	54°08'	24°25'E	130	LI	30
Värmland	Sweden	59°30'	--	1-200	V1	29
Värmland	Sweden	60°	--	1-200	V3	31

6-Phosphogluconate dehydrogenase (6-PGD) E.C. 1.1.1.44, and Shikimic acid dehydrogenase (SKDH), 2 loci, E.C. 1.1.1.14.

In the material from eastern and northern Europe the following 7 loci were analysed: *Adh*, *Dia*, *Fest*, *Gdh*, *Got-A*, *Got-B*, and *Skdh-A*.

In both the embryos and the winter buds the expression of the same alleles was verified. Isozyme electrophoresis, staining procedure and genetic interpretation of the results correspond to those described by RUDIN and EKBERG (1978), YAZDANI and RUDIN (1982), GULLBERG et al. (1985), SZMIDT and YAZDANI (1984). Calculations of genetic parameters such as heterozygosity observed, expected and total (H_o, H_e, H_t), fixation indices (F), relative measure of genetic differentiation between populations (G_{ST}, D_{ST}), polymorphism indices of genotypes (Pg), actual (n) and effective number of alleles (n_e) per locus, number of genotypes, and genetic similarities of populations, based on the frequency of genes (S_N), genotypes (S_H) and genetic distances (D_N, D_H), were conducted as described by JAIN and WORKMAN (1967) NEI and ROYCHOUDHRY (1974), HEDRICK (1974), NEI (1987) and EL-KASSABY (1991) using the GEN computer program (NOWAK-BZOWY and BZOWY, unpublished). Statistical significance of differences in allele and genotype frequencies was evaluated using a chi-square test with hypothesis of equal frequency of alleles and genotypes in the populations studied.

Results

I. Populations of Pinus sylvestris from Spain

Alleles and genotypes

The number of detected alleles showing the genetic "richness" of the populations varies from 25 for the population from Baza (H3) and Gudar (H2) to 32 for the Valsain population (H1) with a mean number for all Spanish populations of 28.2 (*Table 2*). The most striking differences in the number of alleles are recorded for the population from Baza (H3), compared to other populations, for the loci *Adh*, *Dia* and *Skdh-A*.

The number of multilocus genotypes in the populations studied and the average number of genotypes per locus corresponded with the number of alleles: the highest number of genotypes are noted in the population from Valsain (H1), the lowest numbers in the populations from Baza (H3) and Gudar (H2).

Allele frequencies are presented in *table 3*. For several alleles statistically significant differences in the frequency are observed, e.g. for *Fest 1* and *3*, *Gdh 2*, locus *Got-B* alleles *1* and *2*, *Mdh-C 2*, *6-Pgd 2*, and *Skdh-A 1* and *2*, and mostly attributed to deviations of populations from Baza (H3) and Gudar (H2).

Heterozygosity and genetic diversity

The highest heterozygosity H_o is noted for the population from Orihuela (H6), the lowest one for Gudar (H2). It should be mentioned that the values of heterozygosity for all populations studied are similar. The extreme dif-

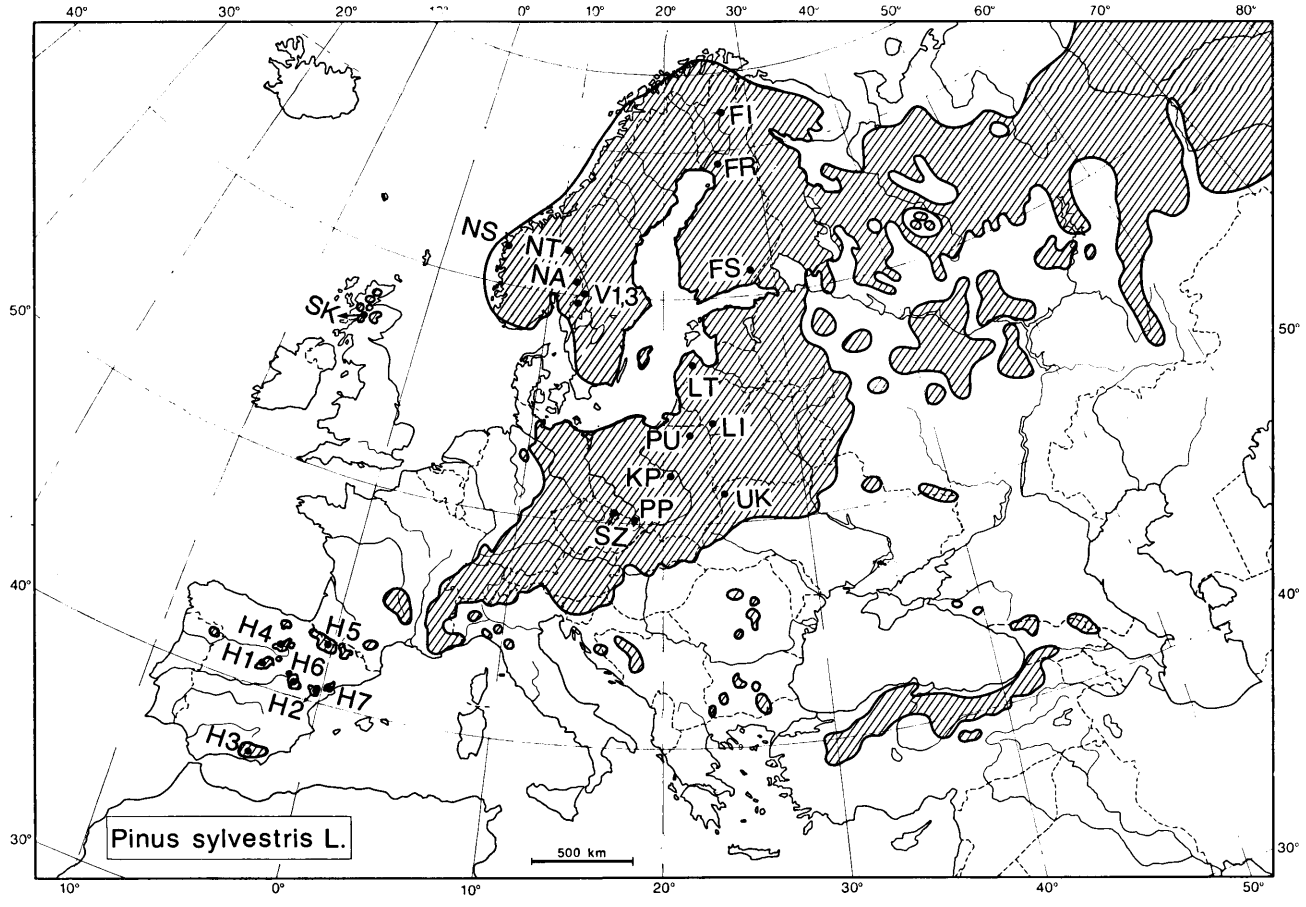

Figure 1. — Location of 23 populations of *Pinus sylvestris* from Spain, eastern and northern Europe used in this study. (Range of the natural distribution area after MEUSEL et al., 1965).

Table 2. — Number of alleles at a single locus in Spanish populations.

Population ⇒ / Locus ⇓	H1	H2	H3	H4	H5	H6	H7	Average
Adh	3	2	4	3	2	2	3	2.7
Dia	3	3	2	3	4	3	2	2.9
Fest	4	4	3	3	3	3	3	3.3
Gdh	2	2	2	2	3	2	2	2.1
Got-A	3	1	1	1	1	2	1	1.4
Got-B	4	3	3	4	3	4	4	3.6
Mdh-A	1	1	2	1	2	1	2	1.4
Mdh-C	2	2	2	2	2	2	2	2.0
6-Pgd	3	2	2	3	2	3	2	2.4
Skdh-A	5	3	2	4	5	5	4	4.0
Skdh-B	2	2	2	3	3	2	3	2.4
A	32	25	25	29	30	29	28	28.2
Al/Loc	2.9	2.3	2.3	2.6	2.7	2.6	2.5	2.6

A = number of alleles found in the particular population
Al/Loc = average number of alleles per locus

ference of this index was about 18% for the populations from Orihuela (H6) and Gudar (H2). This is the case also for the polymorphism indices of genotypes (Pg), where again the most contrasting population is from Gudar (H2).

The fixation indices in all populations are close to zero indicating that the populations are in a HARDY-WEINBERG equilibrium.

The relative measures of genetic differentiation between populations (G_{ST}) and within populations (%) show that between the populations the average difference for 11 analyzed loci is ca. 0.033. It means that the "average" population has in common 96.7% genetic diversity of the group of Spanish populations. The highest value of G_{ST} is observed for the locus *Got-A* (0.100) and *Fest* (0.055), the lowest one for *Skdh-B* (0.011).

Table 3. — Frequencies of the alleles in the populations of *Pinus sylvestris* from Spain (H1 to H7) and the range of frequencies (min-max) of the alleles in 16 populations from eastern and northern Europe.

Locus	Allele	Spanish Populations								E- and N-European Populations	
		H1	H2	H3	H4	H5	H6	H7	Mean	Range min-max	Mean
Adh	1	0.62	0.60	0.50	0.68	0.49	0.54	0.58	0.573	0.43-0.75	0.611
	2	0.36	0.40	0.47	0.30	0.51	0.46	0.41	0.416	0.25-0.50	0.389
	3	0.02	0.00	0.01	0.01	0.00	0.00	0.01	0.007	0.00	0.000
	4	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.001	0.00	0.000
Dia	1	0.91	0.94	0.96	0.92	0.89	0.92	0.92	0.923	0.72-0.93	0.842
	2	0.07	0.04	0.04	0.05	0.10	0.05	0.08	0.061	0.07-0.26	0.136
	4	0.01	0.03	0.00	0.03	0.01	0.03	0.00	0.016	0.00-0.10	0.018
	5	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.001	0.00	0.000
Fest	*1	0.68	0.84	0.48	0.67	0.73	0.84	0.81	0.721	0.65-0.79	0.707
	2	0.06	0.04	0.24	0.05	0.07	0.07	0.03	0.079	0.02-0.18	0.102
	*3	0.25	0.11	0.29	0.28	0.20	0.09	0.16	0.197	0.02-0.27	0.158
	5	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.003	0.00-0.05	0.020
Gdh	1	0.95	0.91	0.78	0.94	0.93	0.83	0.84	0.883	0.50-0.76	0.657
	*2	0.05	0.09	0.22	0.06	0.06	0.17	0.16	0.116	0.22-0.50	0.341
	3	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.001	0.00-0.02	0.001
Got-A	1	0.98	1.00	1.00	1.00	1.00	0.99	1.00	0.996	0.86-1.00	0.933
	2	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.001	0.00-0.05	0.006
	3	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.003	0.00-0.15	0.054
Got-B	*1	0.48	0.60	0.25	0.50	0.41	0.48	0.48	0.457	0.36-0.67	0.646
	*2	0.47	0.39	0.70	0.47	0.58	0.51	0.48	0.514	0.28-0.60	0.374
	3	0.03	0.00	0.05	0.02	0.01	0.01	0.00	0.017	0.00-0.12	0.060
	4	0.03	0.01	0.00	0.02	0.00	0.00	0.04	0.014	0.00-0.03	0.003
	5	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.003	0.00	0.000
Mdh-A	1	1.00	1.00	0.91	1.00	0.94	1.00	0.98	0.976		
	2	0.00	0.00	0.09	0.00	0.06	0.00	0.02	0.024		
Mdh-C	1	0.76	0.63	0.84	0.68	0.82	0.63	0.70	0.723		
	*2	0.24	0.37	0.16	0.23	0.18	0.37	0.30	0.264		
6-Pgd	1	0.76	0.80	0.84	0.81	0.65	0.75	0.80	0.773		
	*2	0.23	0.20	0.16	0.18	0.35	0.24	0.21	0.224		
	3	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.003		
	4	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.003		
Skdh-A	*1	0.46	0.69	0.66	0.45	0.63	0.61	0.51	0.573	0.75-0.92	0.833
	*2	0.42	0.28	0.34	0.53	0.30	0.32	0.42	0.373	0.03-0.17	0.104
	3	0.02	0.00	0.00	0.01	0.02	0.02	0.05	0.017	0.00-0.08	0.024
	4	0.06	0.00	0.00	0.00	0.01	0.03	0.03	0.019	0.00	0.000
	5	0.05	0.01	0.00	0.01	0.04	0.02	0.00	0.019	0.00-0.05	0.014
	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.02-0.07	0.026
	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.02-0.03	0.006
Skdh-B	1	0.97	0.96	0.99	0.94	0.92	0.92	0.96	0.951		
	2	0.03	0.04	0.01	0.05	0.08	0.08	0.04	0.047		
	3	0.00	0.00	0.00	0.02	0.01	0.00	0.01	0.006		

Mean = arithmetic mean of frequencies
* = statistically significant differences (0.05)

Genetic similarities

The genetic similarities of the populations based on allele (NEI) and genotype (HENDRICK) frequencies in form of a dendrogram and dendrite are shown in *figure 2*. The populations are forming 2 clusters. The first cluster consists of populations from Valsain (H1) and Covaleda (H4). In the second group there are geographically close populations from Orihuela (H6), Refalgueri (H7) and from Gudar (H2). The population from Borau (H5) is on the basis of NEI's similarity indices loosely connected to these two groups and on basis of HEDRICK's similarity indices to populations from Covaleda (H4) and Valsain (H1). The most southern pop-

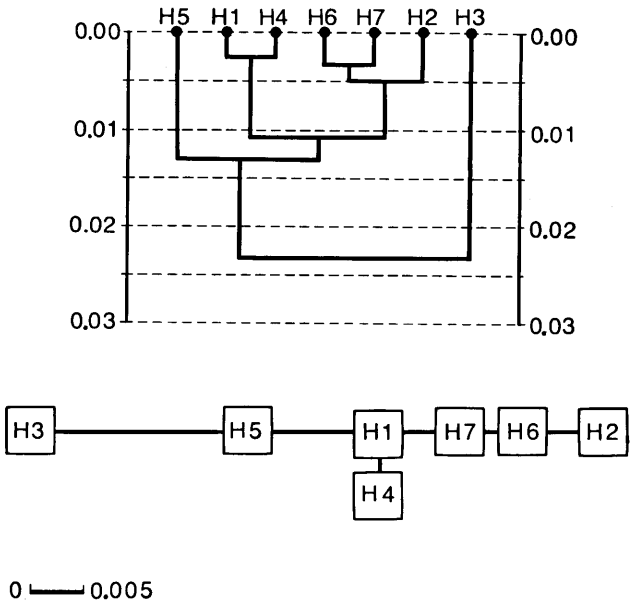

Figure 2. — Dendrogram (top) and dendrite (bottom) showing the genetic similarities of *Pinus sylvestris* populations from Spain based on frequency of alleles for 11 loci.

ulation from Baza (Sierra Nevada, H3) exhibited the most pronounced differences in relation to the other populations studied. Dendrites are showing that this population (Baza, H3) is connected to populations from Borau (western Pyrenees, H5) (NEI's similarity indices) and from Refalgueri (H7) (HEDRICK's similarity indices). In both cases (S_N and S_H) the population from Borau (H5) is loosely related to the populations from Covaleda (H4) and Valsain (H1). Three populations from eastern Spain (H2, H7, and H6) are clustering together.

II. Relation of Spanish Pinus sylvestris populations to populations from eastern and northern Europe

Genetic structure and genetic similarites of the 2 groups of populations were compared on basis of 7 enzyme loci.

In *table 4* several genetic parameters for both groups of populations are given. The average number of alleles per locus (n) is slightly higher in populations from Spain, but the effective number of alleles (n_e) is higher in the second group. The number of genotypes (G) is also higher in Spanish populations. Regarding the multilocus heterozygosity observed (H_0), the mean value for 7 loci and the populations is ca. 15% lower for the Spanish material. Both groups are in a HARDY-WEINBERG equilibrium

Table 4. — Mean number of alleles (n) and genotypes (G) per locus and effective number of alleles (n_e), heterozygosity observed (H_0) and heterozygosity expected (H_e), fixation indices (F) and polymorphism indices of genotypes (Pg) in 7 Scots pine populations from Spain and 16 populations from northern and eastern Europe (for 7 loci).

Population	n	n_e	G	H_0	H_e	F	Pg
H1	3.429	1.705	5.143	0.292	0.343	0.147	0.475
H2	2.571	1.491	3.000	0.265	0.284	0.069	0.403
H3	2.429	1.721	3.571	0.342	0.352	0.027	0.467
H4	2.857	1.597	3.857	0.304	0.318	0.043	0.438
H5	3.000	1.592	4.571	0.316	0.321	0.018	0.448
H6	3.000	1.587	4.286	0.337	0.324	-0.039	0.446
H7	2.714	1.642	3.857	0.316	0.334	0.056	0.466
Mean 1	2.857	1.619	4.041	0.310	0.325	0.046*	0.449
FI	2.714	1.715	3.857	0.357	0.390	0.083	0.543
FR	3.000	1.685	4.143	0.386	0.386	0.000	0.536
FS	2.714	1.616	3.571	0.348	0.353	0.015	0.508
NT	2.714	1.725	3.857	0.410	0.401	-0.022	0.552
NA	2.857	1.688	3.857	0.333	0.375	0.112	0.519
NS	3.143	1.604	3.714	0.338	0.347	0.025	0.511
PU	2.857	1.592	3.714	0.329	0.332	0.010	0.463
LT	2.714	1.698	3.857	0.357	0.379	0.059	0.527
LI	2.714	1.695	3.571	0.371	0.368	-0.008	0.510
UK	2.571	1.697	3.714	0.357	0.379	0.058	0.535
SK	2.857	1.597	3.857	0.314	0.340	0.076	0.486
PP	2.714	1.625	3.286	0.414	0.347	-0.195	0.466
KP	2.571	1.561	3.286	0.357	0.328	-0.088	0.447
V1	2.429	1.663	3.429	0.379	0.364	-0.041	0.500
V3	2.857	1.650	3.429	0.357	0.378	0.054	0.533
SZ	2.429	1.590	3.143	0.362	0.335	-0.081	0.482
Mean 2	2.741	1.650	3.643	0.361	0.363	0.006*	0.507
Mean 1+2	2.776	1.641	3.764	0.335	0.351	0.017*	0.489

F* — value calculated on basis of the arithmetic mean H_0 and H_e (mean 1 and mean 2).

Table 5. — Relative measure of genetic differentiation (G_{ST}) for 7 loci between 7 populations of *Pinus sylvestris* from Spain (1) and for 16 populations from northern and eastern Europe (2).

Locus	H_T		H_S		D_{ST} (H_T-H_S)		G_{ST}		Within Population %	
	1	2	1	2	1	2	1	2	1	2
Adh	0.499	0.475	0.490	0.464	0.009	0.011	0.018	0.022	98.2	97.7
Dia	0.144	0.267	0.142	0.257	0.002	0.010	0.014	0.037	98.6	96.2
Fest	0.435	0.460	0.411	0.451	0.024	0.009	0.055	0.019	94.5	98.1
Gdh	0.205	0.451	0.198	0.436	0.007	0.015	0.034	0.033	97.6	96.6
Got-A	0.010	0.124	0.009	0.118	0.001	0.005	0.100	0.041	90.0	95.8
Got-B	0.528	0.538	0.511	0.527	0.017	0.011	0.032	0.019	96.8	98.0
Skdh-A	0.532	0.289	0.517	0.282	0.015	0.007	0.028	0.022	97.2	97.7
Average	0.336	0.372	0.325	0.362	0.010	0.010	0.040	0.025	96.0	97.5

% = the amount of genetic differentiation within populations

as indicated by the mean fixation indices (F). However, there are some exceptions at the population level. One population from Spain (H1) and another one from Norway (NA) are showing an excess of homozygosity. A population from Pieniny National Park (PP) on the other hand is exhibiting an excess of heterozygosity.

The group of populations from eastern and northern Europe is more polymorphic regarding the genotypes concerned (Pg).

Relative measure of genetic differentiation (*Table 5*) indicates that the populations from Spain are more differentiated than the populations from the rest of Europe. The index of G_{ST} varies for the Spanish populations from 0.014 to 0.100 with an average of 0.040, and for the eastern and northern European populations from 0.019 to 0.041 with an average of 0.025 (*Table 5*). For 5 of the 7 loci the G_{ST}-values were higher in the Spanish than in the other populations.

Concerning the frequency of alleles and genotypes there are distinct differences between both groups, particularly in the loci *Dia*, *Gdh*, *Got-A*, and *Got-B* (*Table 3*). The differences between the group of Spanish populations and the group from eastern and northern Europe are also expressed by the presence of unique alleles. In Spanish populations 5 alleles (*Adh 3* and *Adh 4*, *Dia 5*, *Got-B 5* and *Skdh-A 4*) were detected which are not present in 16 populations from eastern and northern Europe and vice versa 2 alleles (*Skdh-A 6* and *Skdh-A 7*) are not noted in Spain (*Table 3*). The alleles *Adh 4* and *Dia 5* were found in only 1, *Got-B 5* in 2, and *Skdh-A 4* and *Adh 3* in 4 Spanish populations. Some of them were infrequent in the respective population (*Table 3*). The Scots pine population from Refalgueri (H7) has 3 of the 5 rare alleles.

Genetic similarity between populations, based on 7 enzyme loci, is presented in *figure 3*. According to the genetic similarity based on gene frequency (Nei) the populations form 2 very distinct, but heterogenous groups. One of them consists exclusively of populations from Spain, the second one of populations from eastern and northern Europe. The mutual position of the populations from Spain are the same as in the case of 11 loci investigated, except the population from Borau (H5), which is loosely connected to 3 populations from eastern Spain (H2, H6, H7). In the case of 11 loci this population is showing more pronounced specificity compared to 5 populations from eastern and central Spain. In the populations from eastern and northern Europe some geographical grouping can be observed. Populations from Finland and Norway form 1 group, populations from Scotland and 2 from Poland another one. The geographically close populations from Latvia, Lithuania and Ukraine are forming a third cluster, and 2 populations from south Sweden the last group. A relic mountain population from Pieniny National Park from Poland shows the most distinct character of the whole group.

As could be shown the hiatus between both groups of populations from Spain and from northern and eastern Europe is almost twice as much as the distances between the populations in each separate group (D_N = 0.062). The special position is characteristic for the southernmost population from the Spanish Sierra Nevada (Baza, H3) exhibiting the most significant specificity in relation to the other populations studied.

Discussion and Conclusions

The investigation revealed that the populations of *P. sylvestris* from Spain are genetically significantly different. These differences are caused by divergent gene and genotype frequencies in certain populations and are especially pronounced in the *Fest*, *Gdh*, *Got-A* and *Mdh-C* loci. In spite of this, the level of heterozygosity in the populations studied are similar. The population from Orihuela (H6) is the most heterozygous one. Less heterozygotic is the geographically neighbouring population from Gudar (H2). The differences between the values for heterozygosity reach only 18%. In the population from the southernmost limit of the species in Europe (Baza, H3) the heterozygosity attains one of the highest value of all populations studied in contrast to the hypothesis of low genetic variability in marginal populations (Mayr, 1963). It could be possible that adaptive strategy of coniferous species in marginal populations is connected with a high genetic variability as already reported by Szmidt and Muona (1985) for northern Swedish populations of *P. sylvestris*, by Kinloch et al. (1986) for Scotish populations of *P. sylvestris*, and by Tigerstedt (1973) and Bergmann (1978) for populations of *Picea abies*. On the other hand genetic "richness" as measure by the number of alleles indicates that the genetic pool of the population from Valsain (H1), central Spain, is richer than the southernmost population from Baza (H3) (*Table 2*). But this phenomenon could also have evolved by local environmental conditions, e.g. selection, eliminating some

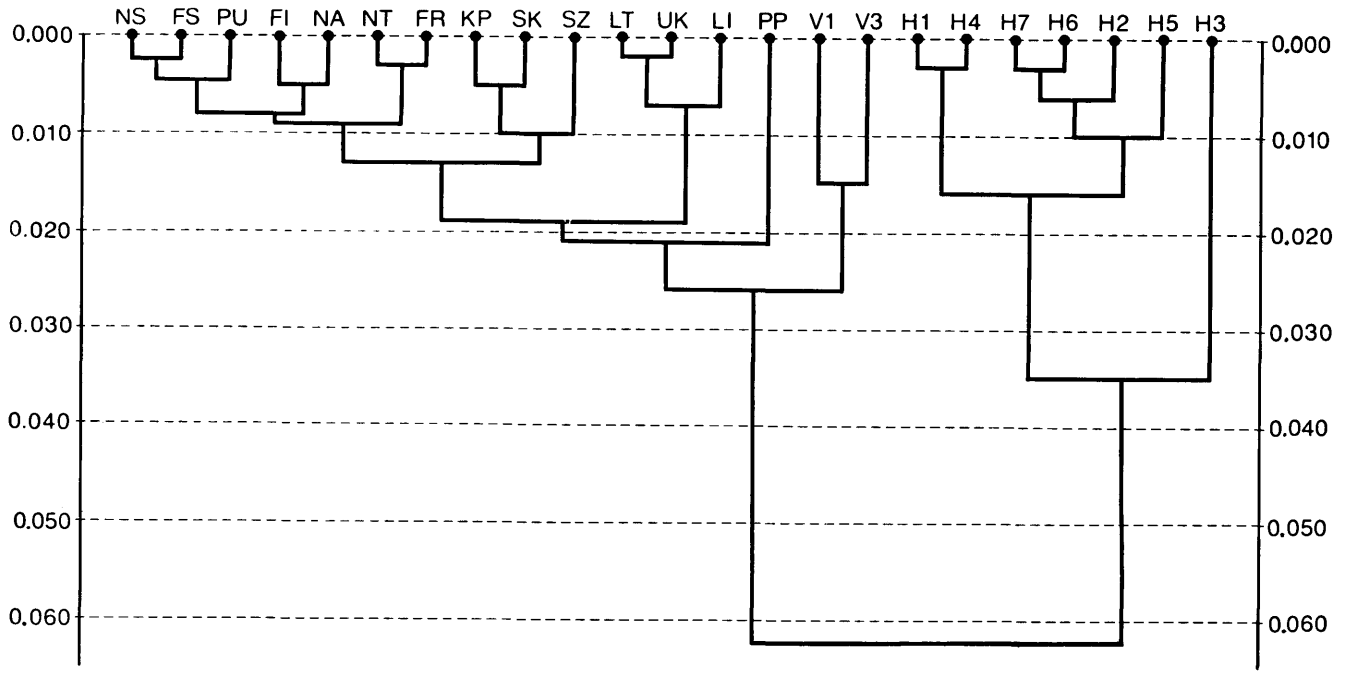

Figure 3. — Dendrogram illustrating the genetic similarities of *Pinus sylvestris* populations from Spain and eastern and northern Europe based on frequency of alleles for 7 enzyme loci.

not adaptive alleles from the southernmost population. But also gene flow by pollen input from adjacent populations is probable for the higher number of alleles in the central population of Valsain (H1).

In all populations studied, also those from Spain, the fixation index (F) is close to 0. This indicates that the genetic structure of the populations is in an equilibrium.

In relation to the genetic similarities, our data confirm to a great extent the suggestions of some authors. They distinguished on the basis of morphological characters the existence of several varieties or climatic ecotypes of *Pinus sylvestris* in Spain (Svoboda, 1953; Gaussen, 1960; Nicolas and Gandullo, 1969; Staszkiewicz, 1970). From our point of view, genetically similar populations from Covaleda (H4) and Valsain (H1) and also populations from Orihuela (H6), Gudar (H2) and Refalgueri (H7) belong to the Iberian variety *iberica* Svob. of Svoboda (1953), Gaussen (1960) and Nicolas and Gandullo (1969). At the same time we suggest that the Iberian variety is composed by two genetically different groups of populations: north-western (Covaleda and Valsain) and east-central (Orihuela, Refalgueri and Gudar). One of the groups occurs in the Central Mountains, the second one in the Iberian chain (*Figure 1*). The population from Borau (H5) represents the Pyrenean variety *pyrenaica* Svob. and is related to some extend to both groups of populations. The southernmost population from Baza (H3) is genetically most distinct from the rest of the populations from Spain and in this regard our results support the opinion of the existence of the variety *nevadensis* Christ. Also Pardos et al. (1990) indicated on the basis of the monoterpene composition of *P. sylvestris* from the Sierra Nevada the special position of this population.

The comparison of several genetic parameters and systematic relations of the populations of *P. sylvestris* from Spain (embryos) to populations from eastern and northern Europe (mature trees) indicates the exceptional position of the Spanish populations (*Fig. 3, Table 4* and *5*). We are fully aware of the fact that the genetic structure of embryo populations and mature trees derivated from them could be different due to selection processes during the life cycle, but these changes are probably not as significant as that they could have influenced the general picture of the variation. This is supported by fixation indices near 0, which indicate that effects by selection acting during the life cycle from the embryos to the mature trees are negligible. The special taxonomical position of Scots pine from Spain was also stressed by Lange and Weissmann (1988), Pardos et al. (1990), and Weissmann and Lange (1990), who found that populations from Spain are completely free from Δ3-carene. About the special taxonomical position and the unique genetic structure of Scots pine from the Iberian peninsula one can reflect also on the basis of the work of Wang et al. (1991), who compared several genetic parameters of 3 populations of *P. sylvestris* var. *lapponica* Fries from Sweden and 3 populations of var. *mongolica* Fomin from China. In spite of a very large geographical distance between these populations the genetic divergence of these two groups of Scots pine populations were much smaller than we found for the material from Spain and north-eastern Europe. The number of alleles and genotypes are higher in Spain indicating that these populations are genetically richer in spite of relatively small areas they occupied, in comparison to the area from which the other populations originated. In the Spanish material we detected 5 unique alleles, not present in the populations studied from the rest of Europe and 2 existing in these populations, but not found in Spain (*Table 3*). From this point of view the Spanish *P. sylvestris* should be considered for a gene conservation program. The Spanish populations are also more differentiated than all eastern and northern populations from the much larger area (*Table 5* — G_{ST} indices). Presumably the reason for this pattern of variation is that these populations are geographically isolated without any chance for effective gene exchange with the other European populations. Secondly, there is the opinion that they originated from the

tertiary period (BERTSCH, 1940; MIROV, 1967) and they could gain and maintain the unique alleles in particular populations, in contrast to other populations in Europe, where the relatively recent reforestation is a result of the migration of Scots pine from several refugia after the last glaciation. An intensive genes exchange from different sources was possible.

As the pattern of geographic variability reflects the migration of Scots pine after the glaciation one can presume that the populations from the Iberian peninsula are relics and did not take part in reforestation of Europe after the last glaciation. This hypothesis is supported by our findings of the unique alleles not present in populations from eastern and northern Europe. Also data of PARDOS et al. (1990) from monoterpene analysis confirm this opinion.

Acknowledgements

We are grateful to JOLANTA LUKASZEWICZ, M. Sc., and DOROTA WYSOCKA, M. Sc., for their technical help. We have to thank also Prof. JOSÉ PARDOS and Dr. RICARDO ALIA, both Madrid, for supplying the seed samples from Spain. The study was conducted in the frame of the German-Spanish cooperation program and financially supported by the Ministry of Food, Agriculture and Forestry (BML), Bonn, and the Instituto Nacionale de Investigaciones Agrarias (INIA), Madrid. The work was also partly financed by the grant CPBP 09.02.04 from the Polish Academy of Sciences. We are grateful to Mr. H. WOHLTORF for preparing the drawings.

Literature

ALIA MIRANDA, R., MARTIN ALBERTOS, S. and GIL MUNOZ, P.: Delineation of pine provenance regions and select stands selection in Spain. In: NATHER, J. (ed.): Actual problems of the legislation of forest reproductive material and the need for harmonization of rules at an international level. Proc. of the meeting of IUFRO WP S2.02-21, Gmunden/Vienna, Austria, June, 1991. 155—162 (1992). BERGMANN, F.: The allelic distribution at an acid phosphatase locus in Norway spruce (*Picea abies*) along climatic gradients. Theor. Appl. Gen. **52**: 57—64 (1978). — BERTSCH, K.: Geschichte des deutschen Waldes. Verlag G. Fischer, Jena (1940). — EL-KASSABY, Y. A.: Genetic variation within and among conifer populations: review and evaluation of methods. In: FINESCHI, S., MALVOTTI, M. E.: CANNATA, F. and HATTEMER, H. H. (eds): Biochemical markers in forest genetics. Academic Publ. BV, The Hague. 61—76 (1991). — GAUSSEN, H.: Les gymnospermes actuelles et fossiles. Travaux du Laboratoire Forestal de Toulouse. Vol. **6**, 272 pp. (1960). — GODWIN, H.: The history of the British flora. Cambridge Univ. Press, London (1956). — GULLBERG, U., YAZDANI, R., RUDIN, D. and RYMAN, N.: Allozyme variation in Scots pine (*Pinus sylvestris* L.) in Sweden. Silvae Genetica **34**: 193—200 (1985). — HEDRICK, P. W.: Genetic similarity and distance; comments and comparisons. Evolution **29**: 362—366 (1974). — JAIN, S. K. and WORKMAN, P. L.: Generalized F-statistics and the theory of inbreeding and selection. Nature **214**: 674—678 (1967). — KINLOCH, B. B., WESTFALL, R. D.: and FORREST, G. I.: Caledonian Scots pine: Origins and genetic structure. New Phytol. **104**: 703—729 (1986). — LANGE, W. and WEISSMANN, G: Die Zusammensetzung der Harzbalsame von *Pinus sylvestris* L. verschiedener Herkünfte. Holz Roh-Werkstoff **46**: 157—161 (1988). — LANGLET, O.: A cline or not a cline, a question of Scots pine. Silvae Genetica **8**: 13—22 (1959). — MAYR, E.: Animal Species and Evolution. The Belknap Press of Harvard Univ. Press, Cambridge, Mass. 797 pp. (1963). — MEUSEL, H., JÄGER, E. and WEINERT, T.: Vergleichende Chorologie der zentraleuropäischen Flora. Verlag G. Fischer, Jena (1965). — MIROV, N. T.: The Genus *Pinus*. Ronald Press, New York (1967). — MÜLLER-STARCK, G., BARADAT, PH. and BERGMANN, F.: Genetic variation within European tree species. New Forests **6**: 23—47 (1992). — NEI, M.: Molecular Evolutionary Genetics. Columbia University Press, New York. 512 pp. (1987). — NEI, M. and ROYCHOUDHRY, A. K.: Sampling variances of heterozygosity and genetic distance. Genetics **76**: 379—390 (1974). — NICOLAS, A. and GANDULLO, J. M.: Ecologia de los pinares espanoles. II. *Pinus sylvestris*. Ministerio de Agricultura, IFIE Madrid. 303 pp. (1969). — PARDOS, J. A., LANGE, W. and WEISSMANN, G.: Morphological and chemical aspects of *Pinus sylvestris* L. from Spain. Holzforschung **44**: 143—146 (1990). — PARDOS, J. A. and STEPHAN, B. R.: Die Verbreitung der Kiefer (*Pinus sylvestris* L.) in Spanien und in der Bundesrepublik Deutschland und Vorbereitung eines deutsch-spanischen Herkunftsversuchs. In: MBL: (ed.): Deutsch-spanische Zusammenarbeit in der Agrarforschung 1979 bis 1988, pp. 35—50 (1988). — PRAVDIN, L. F.: Scots Pine. Variation, intraspecific taxonomy and selection. Israel Program for Scientific Translation, Jerusalem. 208 pp. (1969). — PRUS-GLOWACKI, W.: Biochemical polymorphism. In: GIERTYCH, M. and MATYAS, C. (eds.): Genetics of Scots Pine. Elsevier Sc. Publ., Amsterdam. 73—86 (1991). — RUDIN, D. and EKBERG, T.: Linkage studies in *Pinus sylvestris* using macrogametophyte allozymes. Silvae Genetica **27**: 1—11 (1987) — STASZKIEWICZ, J.: Systematics and variation in Scots pine. In: BIALOBOK, S. (ed.): Scots Pine — *Pinus sylvestris*. Translation of the Popular Scientific Monograph "Our Forest Trees". Institute of Dendrology and Kórnik Arboretum, Kórnik, Poland. 52—71 (1970). — SVOBODA, P.: Forest trees and their communities. Statni. Zem. Nakl., Prague. Vol. 1, 411 pp. (1953). — SZMIDT, A. E. and MUONA, O.: Genetic effects of Scots pine (*Pinus sylvestris* L.) domestication. Lect. Notes Biomath. **60**: 241—252 (1985). — SZMIDT, A. E. and YAZDANI, R.: Electrophoretic studies of genetic polymorphism of shikimate and 6-phospho-gluconate dehydrogenases in Scots pine (*Pinus sylvestris* L). Arboretum Kornickie **29**: 63—72 (1984). — TIGERSTEDT, P. M. A.: Studies on isozyme variation in marginal and central populations of *Picea abies*. Hereditas **75**: 47—60 (1973). — WANG, X.-R., SZMIDT, A. E. and LINDGREN, D.: Allozyme differentiation among populations of *Pinus sylvestris* L. from Sweden and China. Hereditas **114**: 219—226 (1991). — WEISSMANN, G. and LANGE, W.: A carene-free chemotype of *Pinus sylvestris*. Phytochemistry **29**: 2897—2898 (1990). — WULF, E. V.: An introduction to historical plant geography. Chronica Botanica, Waltham, Mass. (1943). — YAZDANI, R. and RUDIN, D.: Inheritance of fluorescence esterase and β-galactosidase in haploid and diploid tissues of *Pinus sylvestris* L. Hereditas **96**: 191—194 (1982).

Interspecific Hybridization of Swiss Stone Pine (Pinus cembra L.)

By I. BLADA

Forest Research Institute, Sos. Stefanesti 128,
Bucharest 11, Romania

(Received 22nd February 1993)

Summary

Many crosses between *P. cembra* and other white pines have been attempted but only *P. cembra* x *P. wallichiana* A. B. JACKS. and *P. cembra* x *P. monticola* DOUGL. were successful. After 11 years of testing, the following important results were observed: (1) Significant genetic differences were found between hybrids and their parents for most traits; (2) Both hybrid crosses resulted in individuals that have some important traits outside the range of the parents; (3) *P. cembra* x *P. wallichiana* was intermediate between parents in both blister rust resistance and growth traits; this hybrid incorporated blister rust resistance genes from the female parent and fast grvwth from the male parent; (4) *P. cembra* x *P. monticola* displayed heterosis in 6 of the 9 tested traits, including all growth traits; it inherited outstanding form and growth from the male parent; (5) Both hybrids represent a potentially important planting stock for middle to high altitude sites.

Key words: Pinus cembra, P. wallichiana, P. monticola, hybrid, heterosis, blister rust resistance, growth traits.