and stochastic dominance: a comparison. American Economic Review 64: 200—204 (1974). —— PORTER, R. B. and GAUMNITZ, J. E.: Stochastic dominance vs. mean-variance portfolio analysis: an empirical evaluation. American Economic Review 62: 348—446 (1972). —— QUIRK, P. and SAPOSNIK, R.: Admissibility and measurable utility functions. Review of Economic Studies 29: 140—146 (1962). —— RAY, A. A. (Editor): SAS User's Guide: Statistics, 1982 Edition. SAS Institute Inc., Cary, NC. (1982). —— VAN BUIJTENEN, J. P. and HANOVER, J. W.: Designing for yield. Journal of Forestry 84 (3): 28—30 (1986).

Allozyme Variation in Italian Populations of Picea abies (L.) Karst.

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Summary

Genetic diversity and genetic differentation of nine Italian populations of Norway spruce (*Picea abies* (L.) KARST.) were studied analyzing allozyme variation at 21 loci. On average, the expected heterozygosity was 0.165, 45.50% of loci were polymorphic, the number of alleles per locus was 1.831 and the effective number of alleles per locus was 1.198. Only 4.2% of the total genetic variation was due to interpopulational differentation. The mean value of NEI's genetic distance (0.019) confirmed that the variation among populations is low. The characteristics of the relic population of Campolino (the only natural stand located in Italy outside the Alps), such as a quite high gene diversity, the presence of some unique alleles and a peculiar genetic structure at the locus GOT-B, may provide some support to the hypothesis of a post-glacial recolonization of the Italian slope of the Alps in West-East direction, starting from refugial populations in Central Italy.

Key words: allozymes, *Picea abies*, genetic diversity, genetic differentiation, recolonization.

Zusammenfassung

Die genetische Diversität und die genetische Differenzierung von neun italienischen Fichtenpopulationen (*Picea abies* (L.) KARST.) wurden anhand von 21 Isoenzym-Genloci untersucht. Im Durchschnitt betrug der erwartete Heterozygotiegrad 0,165 und 45,50% der Loci waren polymorph. Die Anzahl der Allele pro Locus betrug 1,831 und die der effektiven Allelen pro Locus 1,198. Nur 4,2% der gesamten genetischen Variation basierte auf der Differenzierung zwischen Populationen. Der durchschnittliche Wert von NEI's genetischem Abstand (0,019) bestätigte, daß die Variation zwischen den Populationen gering war.

Auf Grund charakteristischer genetischer Eigenschaften der Reliktpopulation von Campolino, außerhalb des Alpengebietes der einzige autochtone Fichtenbestand Italiens, wie z. B. der relativ hohen genetischen Diversität, dem Vorkommen einiger einzigartiger Allele und einer besonderen genetischen Struktur am Locus GOT-B, wird vermutet, daß die Rückwanderung von Refugien in Mittelitalien in West-Ost-Richtung entlang der italienischen Alpenseite stattgefunden hat.

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Introduction

Up to now isoenzymatic analyses on Norway spruce have been restricted to populations of Middle-northern Europe and have had following goals: to estimate their level and distribution of genetic variability (BERGMANN, 1973a, 1974a; LUNDKVIST and RUDIN, 1977) and to study their genetic structure related to the spatial distribution of the trees (BRUNEL and RODOLPHE, 1985), to the distribution along altitudinal and latitudinal gradients (BERGMANN, 1978, 1988), and also to sensitivity to atmospheric pollution (BERGMANN and SCHOLZ, 1987). Moreover, some isoenzymatic variants have been useful to establish the processes of recolonization in Europe of Norway spruce in the post-glacial era (BERGMANN, 1984).

Nine Italian populations were electrophoretically investigated. The analysis of the relic population of Campolino seems to be particularly important. This narrow sized and isolated population may be useful in order to verify the hypothesis of a post-glacial migration in West-East direction, starting from refugial populations situated in the plains of Central Italy (FIRBAS, 1949 and 1952; GIACOMINI, 1958).

Materials and Methods

Populations sampled

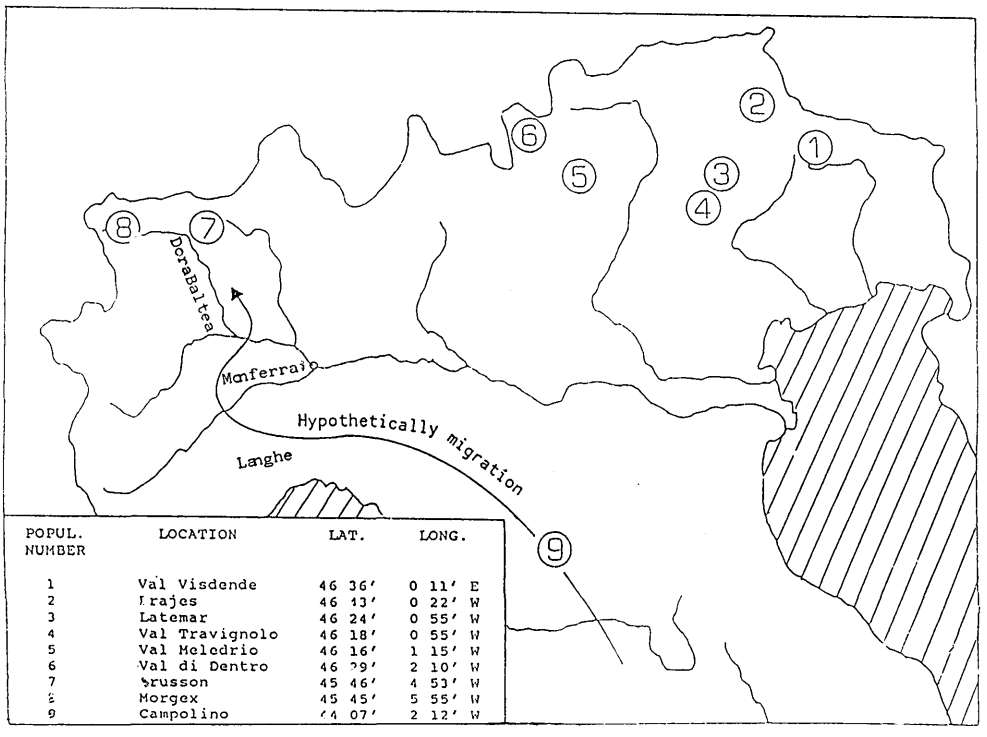
Nine native Italian populations were sampled: eight are located in the Alps, the ninth, a relic population, is situated in the natural reserve of Campolino nearby the Alpe delle Tre Potenze on the Tuscan Appennine (*Figure 1*). The population of Campolino is relic (MAGINI *et al.*, 1980; BORGHETTI et al., 1989), narrow sized and completely isolated.

Bulk provenance collections (populations No. 1, 2, 3, 5, 6) and seedlots from individual trees were analyzed to characterize the populations.

Electrophoresis

The allozyme analysis was carried out on the endosperm. About 140—160 endosperms were examined for the bulk provenance collections. For the populations with seeds from single trees the individual genotype was inferred by examining 6 endosperms for each tree. 20, 18, 33 and 40 trees were genotyped for populations No. 4, 7, 8, and 9, respectively.

Horizontal electrophoresis on starch gel was utilized to separate the isozymes at 11 enzyme systems. The systems assayed, their acronyms, the applied gel and electrode buffer systems, the number of gene loci scored and references for the inheritance of the allozymes are given



POPUL. NUMBER	LOCATION	LAT.	LONG.
1	Val Visdende	46 36'	0 11' E
2	Frajes	46 13'	0 22' W
3	Latemar	46 24'	0 55' W
4	Val Travignolo	46 18'	0 55' W
5	Val Moledrio	46 16'	1 15' W
6	Val di Dentro	46 29'	2 10' W
7	Brusson	45 46'	4 53' W
8	Morgex	45 45'	5 55' W
9	Campolino	44 07'	2 12' W

Figure 1. — Location of the nine populations studied; the arrow indicates the migration way between Valle d'Aosta and the Tuscan Appenine.

in *Table 1.* Staining was performed according to Müller-Starck (pers. comm.) and Cheliak and Pitel (1984).

Where several zones of activity were observed for a single enzyme, capital letters following the enzyme abbreviations were used; the most anodal zone was designated by the first letter. Within a zone of activity the fastest allele was designated by the lowest number. Silent alleles were designated with S. At ACP-B four alleles were found. B1 and B 2 coded for single bands and B3 and B4 coded for double bands. Because migration rates of both single- banded and both double-banded enzyme variants were not differentiated due to similar migration, "pooled" alleles B1-2 and B3-4 were used.

Statistical analysis

Measures of gene diversity

Four measures of gene diversity were used:

a) the expected proportion of heterozygotes H_e at each locus was calculated according to Nei (1975);
b) the average number of alleles per locus (all alleles found in the samples were counted);
c) the percentage of polymorphic loci. Following convention, loci were designated polymorphic if the most common allele had a frequency of less than 95%;
d) the effective number of alleles per locus (n_e), which was calculated following Crow and Kimura (1970). The average effective number of alleles per population is

Table 1. — Isozymes assayed, acronyms, applied gel and electrode buffer systems, number of scored loci, and reference of the genetic control.

ISOZYME SYSTEMS	E.C. No.	BUFFER SYSTEM	No. OF SCORED LOCI	REFERENCES
Acid phosphatase (ACP)	3.1.3.2	A	2	Bergmann (1974b)
Aconitase (ACO)	4.2.1.3	B	1	Muona et al. (1987)
Glutamate dehydrogenase (GDH)	1.4.1.2	A	1	Lundkvist (1979)
Glutamate-oxaloacetate-transaminase (GOT)	2.6.1.1	A	2	Muona et al. (1987)
Isocitrate dehydrogenase (IDH)	1.1.1.42	B	2	Muona et al. (1987)
Leucine aminopeptidase (LAP)	3.4.11.1	C	2	Bergmann (1973b)
Malate dehydrogenase (MDH)	1.1.1.37	B	3	Lundkvist (1979)
NADH dehydrogenase (NDH)	1.6.99.3	B	1	Morgante et al. (unpubl.)
Phosphoglucomutase (PGM)	2.7.5.1	B	2	Muona et al. (1987)
6-Phosphogluconate dehydrogenase (6PGD)	1.1.1.43	B	3	Morgante et al. (1989)
Shikimate dehydrogenase (SKDH)	1.1.1.25	B	2	Morgante et al. (1989)

A = Gel buffer: 0.08 M Tris — 0.01 M citric acid, ph 8.7/ electrode buffer: 0.03 M boric acid — 0.06 M NaOH, pH 8.2 (Poulik, 1957, modified)

B = Gel buffer: 0.14 M Tris — 0.04 M citric acid, pH 7.0/ electrode buffer: 0.035 M Tris — 0.01 M citric acid, pH 7.0 (Shaw and Prasad, 1970, modified)

C = Gel buffer: 0.05 M Tris — 0.008 M citric acid, pH 8.1/ electrode buffer: 0.19 M boric acid — 0.05 M LiOH, pH 8.1; gels were made using electrode and gel buffers in proportions 1:9 (Ashton and Braden, 1961)

Table 2. — Allele frequencies and expected heterozygosities.

LOCUS	ALLELE	POPULATIONS 1	2	3	4	5	6	7	8	9
ACP-A	A1	0.903	1.000	1.000	1.000	1.000	1.000	1.000	0.985	1.000
	A2	0.097	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000
Expected	het.	0.175	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000
ACP-B	B1-2	0.070	0.072	0.305	0.342	0.130	0.161	0.823	0.788	0.091
	B3-4	0.930	0.928	0.695	0.658	0.870	0.839	0.177	0.212	0.909
Expected	het.	0.130	0.134	0.424	0.450	0.227	0.271	0.291	0.334	0.165
ACO-A	A1	0.273	0.230	0.351	0.275	0.658	0.362	0.222	0.274	0.225
	A2	0.727	0.770	0.649	0.725	0.342	0.638	0.778	0.726	0.775
Expected	het.	0.397	0.354	0.456	0.399	0.450	0.462	0.345	0.398	0.349
GDH-A	A1	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000
	A2	1.000	1.000	1.000	1.000	1.000	0.994	1.000	1.000	1.000
Expected	het.	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000
GOT-A	A1	0.964	0.945	0.985	1.000	1.000	0.978	1.000	1.000	1.000
	A2	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000
	S	0.036	0.055	0.008	0.000	0.000	0.022	0.000	0.000	0.000
Expected	het.	0.069	0.011	0.029	0.000	0.000	0.044	0.000	0.000	0.000
GOT-B	B1	0.338	0.375	0.370	0.476	0.385	0.336	0.447	0.500	0.163
	B2	0.654	0.625	0.570	0.500	0.607	0.636	0.553	0.281	0.600
	B3	0.008	0.000	0.060	0.024	0.008	0.028	0.000	0.219	0.237
Expected	het.	0.458	0.469	0.534	0.523	0.483	0.482	0.494	0.623	0.557
IDH-A	A1	1.000	1.000	1.000	1.000	1.000	0.983	1.000	0.984	1.000
	A2	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.016	0.000
Expected	het.	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.031	0.000
LAP-A	A1	0.042	0.057	0.008	0.000	0.010	0.042	0.026	0.061	0.025
	A2	0.868	0.929	0.985	0.976	0.990	0.916	0.974	0.939	0.938
	A3	0.042	0.014	0.007	0.024	0.000	0.035	0.000	0.000	0.037
	S	0.049	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000
Expected	het.	0.241	0.133	0.030	0.046	0.020	0.157	0.051	0.114	0.119
LAP-B	B1	0.102	0.085	0.044	0.105	0.090	0.112	0.094	0.086	0.037
	B2	0.000	0.051	0.022	0.026	0.011	0.000	0.000	0.000	0.000
	B3	0.148	0.102	0.212	0.263	0.281	0.067	0.281	0.277	0.402
	B4	0.750	0.763	0.722	0.606	0.618	0.821	0.625	0.637	0.561
Expected	het.	0.405	0.398	0.431	0.552	0.531	0.309	0.522	0.510	0.522
MDH-B	B1	0.966	1.000	0.971	0.952	1.000	1.000	1.000	1.000	0.950
	B2	0.034	0.000	0.029	0.048	0.000	0.000	0.000	0.000	0.000
	B3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
Expected	het.	0.065	0.000	0.056	0.091	0.000	0.000	0.000	0.000	0.095
MDH-C	C1	0.008	0.076	0.029	0.024	0.000	0.072	0.132	0.061	0.000
	C2	0.992	0.924	0.935	0.976	1.000	0.928	0.868	0.939	0.950
	S	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.050
Expected	het.	0.017	0.141	0.124	0.046	0.000	0.134	0.229	0.114	0.095
NDH-A	A1	0.492	0.459	0.364	0.225	0.397	0.342	0.417	0.403	0.350
	A2	0.508	0.541	0.636	0.775	0.603	0.658	0.583	0.597	0.650
Expected	het.	0.500	0.497	0.463	0.349	0.479	0.450	0.486	0.481	0.455
PGM-A	A1	0.985	1.000	0.985	0.950	0.945	0.990	0.972	1.000	0.975
	A2	0.015	0.000	0.015	0.050	0.055	0.010	0.028	0.000	0.025
Expected	het.	0.030	0.000	0.030	0.095	0.104	0.020	0.054	0.000	0.049
6PGD-B	B1	0.695	0.695	0.878	0.850	0.749	0.637	0.750	0.661	0.712
	B2	0.305	0.305	0.122	0.150	0.251	0.363	0.250	0.339	0.288
Expected	het.	0.424	0.424	0.214	0.255	0.376	0.462	0.375	0.448	0.410
6PGD-C	C1	0.356	0.414	0.389	0.625	0.440	0.566	0.611	0.516	0.338
	C2	0.644	0.586	0.611	0.375	0.560	0.434	0.389	0.484	0.662
Expected	het.	0.459	0.485	0.475	0.469	0.493	0.491	0.475	0.499	0.447
SKDH-A	A1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
	A2	0.017	0.082	0.039	0.047	0.000	0.073	0.026	0.015	0.025
	A3	0.941	0.873	0.938	0.929	0.993	0.860	0.948	0.970	0.950
	A4	0.042	0.044	0.023	0.024	0.007	0.067	0.026	0.015	0.012
Expected	het.	0.111	0.228	0.118	0.135	0.015	0.251	0.101	0.059	0.097
SKDH-B	B1	0.958	0.975	0.961	0.976	0.993	0.933	0.974	0.985	0.988
	B2	0.042	0.025	0.023	0.024	0.007	0.067	0.026	0.000	0.012
	B3	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.015	0.000
Expected	het.	0.080	0.049	0.075	0.046	0.015	0.124	0.051	0.030	0.025

Table 3. — Genetic diversity analyses (Nei, 1973, 1975).

LOCUS	H_T	H_S	D_{ST}
ACP-A	0.0246	0.0228	0.0018
ACP-B	0.4272	0.2695	0.1577
ACO-A	0.4344	0.4011	0.0333
GDH-A	0.0014	0.0014	0.0000
GOT-A	0.0174	0.0171	0.0003
GOT-B	0.5420	0.5136	0.0283
IDH-A	0.0073	0.0072	0.0001
LAP-A	0.1037	0.1014	0.0023
LAP-B	0.4820	0.4645	0.0175
MDH-B	0.0351	0.0341	0.0010
MDH-C	0.1035	0.1000	0.0035
NDH-A	0.4727	0.4622	0.0106
PGM-A	0.0430	0.0423	0.0007
6PGD-B	0.3882	0.3765	0.0118
6PGD-C	0.4985	0.4771	0.0214
SKDH-A	0.1265	0.1239	0.0026
SKDH-B	0.0558	0.0551	0.0007
Mean	0.1792	0.1652	0.0140

Table 4. — Genetic distance (above the diagonal calculated following Gregorius (1974); below the diagonal following Nei (1972, 1975)).

POP.	1	2	3	4	5	6	7	8	9
1	-----	0.031	0.056	0.077	0.062	0.052	0.089	0.090	0.052
2	0.002	-----	0.051	0.071	0.055	0.039	0.072	0.079	0.050
3	0.008	0.008	-----	0.043	0.053	0.054	0.069	0.075	0.058
4	0.017	0.014	0.006	-----	0.065	0.065	0.055	0.070	0.072
5	0.012	0.014	0.009	0.016	-----	0.063	0.078	0.085	0.059
6	0.007	0.004	0.008	0.010	0.011	-----	0.079	0.081	0.068
7	0.041	0.038	0.022	0.017	0.042	0.032	-----	0.037	0.084
8	0.040	0.038	0.022	0.019	0.040	0.033	0.005	-----	0.083
9	0.008	0.009	0.010	0.016	0.016	0.013	0.042	0.038	-----

then the harmonic mean of the n values for individual loci (Gregorius, 1987).

Genetic differentiation

Several methods were used to quantify the degree of differentiation among populations:

a) gene diversity analyses as proposed by Nei (1973, 1975). Total gene diversity (H_T), gene diversity within populations (H_S), and among populations (D_{ST}) were used according following notation (n = number of populations):

$$H_T = 1 - \sum_{i=1}^{k} \bar{p}_i^2 \; ; \; H_S = \frac{\sum He}{n} \; ; \; D_{ST} = H_T - H_S$$

The ratio D_{ST}/H_T measures the relative degree of genetic differentiation (G_{ST}).

b) Genetic distance. Two measures of genetic distance were computed. Standard genetic distance D by Nei (1972, 1975) was calculated as

$$D = -\ln I$$

where I is the normalized identity of genes between two populations. We also used the genetic distance by Gregorius (1974)

$$d_o = 0.5 \sum_{i=1}^{k} |X_i - Y_i|$$

where X_i and Y_i are the frequencies of the i-th allele in the X and Y populations, respectively and k is the number of the alleles present at a locus. d_o is then averaged over all loci using the arithmetic mean.

Nei's genetic distance was used for clustering of populations by the unweighted pair-group method using arithmetic means (UPGMA; Sneath and Sokal, 1973).

Results

Four gene loci out of the 21 scored for 11 enzyme systems were monomorphic in all populations (MDH-A, IDH-B, PGM-B and 6PGD-A).

Genetic structure

Allele frequencies and expected heterozygosities for the 17 polymorphic loci are given in *table 2*. At ACP-B the frequency of B1-2 was very high in the two most western populations (No. 7 and 8); at ACO-A A2 was more frequent than A1 in Val Meledrio (No. 5); at GOT-B B1 was more frequent than B2 in Morgex (No. 8), whereas in all the others the frequence of B2 slightly exceeded that of B1; at 6PGD-C the most common allele was C2 in the populations of the eastern part of the Alps (No. 1, 2, 3, 5) as well as in Campolino (No. 9) and C1 was found most frequently in those of the central and western part (No. 4, 6, 7, 8).

With regard to the distribution of the rare alleles which are present in many loci, two of them, MDH-B3 and SKDH-A1, were found only in the relic population of Campolino (No. 9). On average at GOT-B B3 always had very low frequencies, in Morgex and Campolino, however, the frequencies of B3 exceeded 0.20.

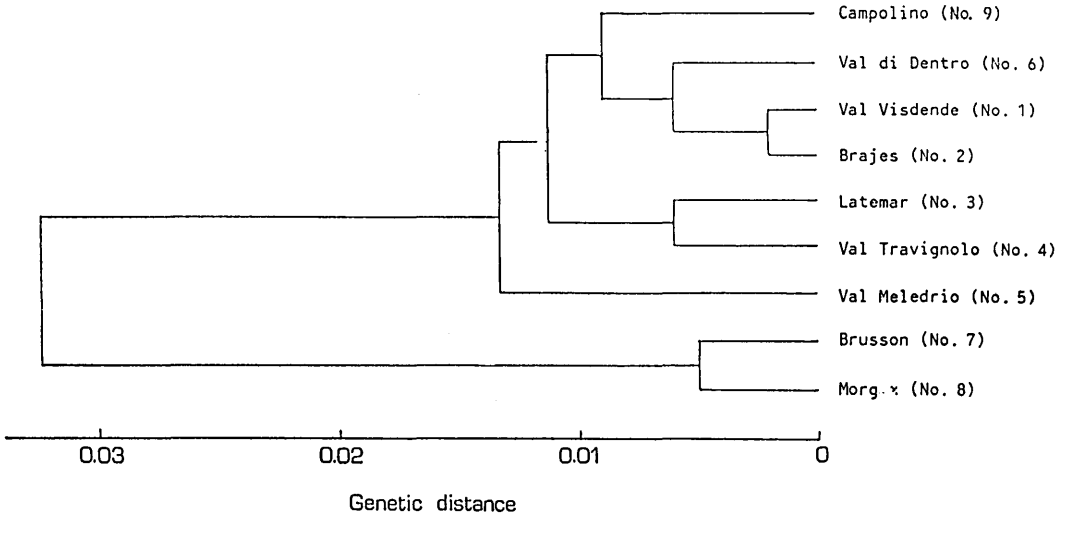


Figure 2. — Dendrogram of Nei's genetic distance obtained with UPGMA method

Genetic diversity

The mean expected heterozygosity per population ranged from 0.152 (Val Meledrio) to 0.176 (Val di Dentro) with a mean of 0.165. The average percentage of polymorphic loci was 45.50%, with a maximum of 52.38% (Val di Dentro, Campolino) and a minimum of 38.09% (Val Meledrio). The average number of alleles per locus was calculated as 1.831 and ranged from 1.667 (Val Meledrio, Brusson) to 2.048 (Latemar). The average effective number of alleles per locus was 1.198. Val di Dentro had the highest value (1.214) and Val Meledrio showed the lowest one (1.179).

Genetic differentiation

Total gene diversity (H_T) and gene diversity within populations (H_S) were very similar for single loci as well as for mean values (*Table 3*). Therefore total genetic variation among populations (G_{ST}) was low (4.2%).

Table 4 shows the matrix of the two measures of genetic distance. The mean Nei's standard genetic distance was 0.019, showing that the differentiation between populations is low and typical of the level of differentiation observed between local populations of conifers. The maximum value was found between Val Meledrio (No. 5) and Brusson (No. 7), and between Campolino (No. 9) and Brusson (No. 7); the minimum between the two most eastern populations, Val Visdende (No. 1) and Brajes (No. 2). The Gregorius genetic distance (mean value: 0.065) confirmed the results obtained with Nei's formula on the whole. A positive and significant correlation was found between both measures of genetic distance and the geographic distance ($r = 0.678$ for Nei's distance, $P<0.001$; $r=0.612$ for Gregorius distance, $P<0.001$).

The dendrogram obtained with the UPGMA method on the basis of Nei's genetic distance is shown in *Figure 2*.

Discussion

The value of the expected heterozygosity (H_e) pooled for nine populations is similar to those generally reported in conifers (Hamrick *et al.*, 1981). In previous studies on Norway spruce H_e values found were considerably higher than in the present study but were based on a low number of loci which were all highly polymorphic (Bergmann, 1973b; Bergmann and Gregorius, 1979; Lundkvist and Rudin, 1977). The differences among the studied populations are small. Also Lundkvist and Rudin (1977) and Bergmann and Gregorius (1979) found low H_e variation in Norway spruce.

The rankings of the populations provided by the average number of alleles per locus (N) and by the percentage of polymorphic loci (P) do not coincide with that provided by H_e. For N such discordance is foreseen by Crow and Kimura (1970). Low-frequency alleles contribute in fact to the expected heterozygosity to a small extent, but are significant for N. There is a smaller discordance between H_e and P than between H_e and N. Presumably the 5% criterion for P attenuates the effect of the low-frequency alleles. N and P are two genetic diversity parameters which strongly depend on the sample size. The effective number of alleles (n_e) was utilized, as it is a measure of genetic diversity which considers both the frequencies and the number of the alleles: thus it was independently regarded by Routledge (1979) and Gregorius (1978) as the best measure of genetic diversity. For population No. 5 (Val Meledrio), which was also the one with the minimum value of heterozygosity, we calculated the lowest values of P, N and n_e.

The greatest amount of the genetic diversity is localized within the populations: only the 4.2% of the total genetic diversity is interpopulational. An analogous scheme of distribution of the variation is found in most of the conifers: in *Pinus sylvestris* (Gullberg et al., 1985) and in *Pinus rigida* (Guries and Ledig, 1982) G_{ST} is equal to 0.02, in *Pinus contorta* (Yeh and Layton, 1979) to 0.04. Such small differentiation between populations is due to ecological characteristics of conifers, such as the density and large size of stands, the wide pollen- and seed-dispersal, the outcrossing rate and the high fecundity. Furthermore the actual range of all the forest tree species mentioned above largely occupies zones which, in the past, have been concerned with glaciations. We assume that since the end of the last glacial era there has not been sufficient time for a significant selection and/or genetic drift among the populations. Gullberg *et al.* (1985), who compared G_{ST} values in various studies on conifers, found that those populations which occupy areas not concerned with glaciations had a high genetic differentiation.

Among the studied loci ACP-B shows the highest G_{ST} value. In two populations, No. 7 and No. 8 (Brusson and Morgex), a clear prevalence of that allele which in all the others is the rarest (B1-2) can be observed. Bergmann (1978) presumed that the allelic frequencies of ACP-B show a clinal variation along altitudinal and latitudinal gradients; the frequencies of B1-2 would then decrease with a decreasing altitude and latitude, i. e. as the temperature increases, and B3-4 would change vice versa. We can therefore argue that the strong variation of the frequencies at this locus among the examined populations should be attributed more to the effects of the natural selection, as a consequence of the different climatic conditions, than to those of genetic drift.

The existence of a significant correlation between the genetic and the geographic distance ($r=0.678$, $P<0.001$ for Nei's distance; $r=0.612$, $P<0.001$ for Gregorius's distance) indicates that the differentiation among the populations is at least partially due to the isolation by distance (Nei, 1975): in our case the Alps, as orographic barriers, make gene flow more difficult. Between the two measures of genetic distance used, the one showing the lowest (but still significant) coefficient of correlation with the geographic distance is that of Gregorius, even if it is a distance of metric type which satisfies the triangular inequality condition and which comes closer to the geometric, therefore geographic, concept of distance.

Also the dendrogram shows the relation existing between genetic and geographic distance (*Figure 2*). Adjacent populations (with the exception of Val di Dentro population) are the first ones which group themselves, following the East-West direction. The separation of the two western populations which are located in Val d'Aosta (Brusson and Morgex) mainly depends on ACP-B. Val Meledrio (No. 5) is clearly distinguished from the remaining populations in the dendrogram and shows peculiar characteristics: low genetic diversity which is predominantly based on the absence of some low-frequency alleles at certain loci (MDH-B, MDH-C, SKDH-A, SKDH-B, LAP-A) and a very high frequency (0.658) of Al at the ACO-A locus. These characteristics of the Val Meledrio population may be due to its relatively marginal location in the Alpine range and/or to a bottle-neck effect. Also Bergmann and Gregorius (1979) observed that in geographi-

cally marginal populations the genetic variability of Norway spruce decreases.

The results of the allozyme analyses suggest a genetic peculiarity of the Campolino population. N and P values (1.857 and 52.38%, respectively) especially should be regarded elevated in the light of its characteristics. Moreover two alleles, SKDH-A1 and MDH-B3, were found in this population only. The high frequency (0.24) of allele B3 (GOT-B) is of particular interest. This locus is very polymorphic in Norway spruce and has two predominant alleles, B1 and B2, with similar frequencies. In most populations GOT-B3 is absent or present in very low frequencies (BERGMANN, 1988). The frequencies of GOT-B3 distinguish therefore the populations of Campolino and Morgex from Italian and also European populations. This fact may be of more importance than the observed genetic differences between these two populations. Based on morphometric traits also MAGINI *et al.* (1980) and BORGHETTI *et al.* (1989) suggested a strong relationship between Campolino and Morgex. MAGINI *et al.* (1990) supposed that a direct migration way between the Valle d'Aosta and the Tuscan Appennine, through the hills of the Langhe and of the Monferrato, existed in the past (*Figure 1*). The distribution of GOT-B3 found in Morgex and Campolino attests this hypothesis even if it must be emphasized that in the other population of the Valle d'Aosta (Brusson, No. 7), which is located further east than Morgex, GOT-B3 is completely absent. We should consequently think that this direct migration way did not involve all the Valle d'Aosta, but only the valley of the Dora Baltea.

The problem of the direction in which the migration occurred, remains unsolved: it is not known if it went from Campolino towards the Alps or vice versa. According to the most commonly accepted theory (BERTSCH, 1953; KRAL, 1977; SCHMIDT-VOGT, 1977) the Alpine range should have been recolonized in East-West direction starting from the refugial populations located in the Dinaric Alps. But according to other authors (FIRBAS, 1949 and 1952; GIACOMINI, 1958) the migration along the Italian slope of the Alps would have happened in the opposite direction, starting from refuges located in the plains of Central Italy. Therefore, on the ground of this second theory, Campolino should represent a starting point and should partly maintain those genetic characteristics peculiar to the refugial populations. As variability of this population is fairly high, especially if narrow size and isolation are considered, it may be assumed that the recolonization of the southern Alps started from central Italy in West-East direction.

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Literature Cited

ASHTON, G. C. and BRADEN, A. W. H.: Serum -globulin polymorphism in mice. Aust. J. Biol. Sci. **14**: 248—253 (1961). — BERGMANN, F.: Geographic pattern of genetic variation at 4 isozyme loci in the Finnish spruce population (*Picea abies*). Proc. IUFRO J. Workshop and Symp. Norway Spruce Prov., Biri, Norway, **35** (1973a). — BERGMANN, *F.: Gen*etische Untersuchungen bei *Picea abies* mit Hilfe der Isoenzym-Identifizierung. II. Genetische Kontrolle von Esterase- und Leucinaminopeptidase-Isoenzymen im haploiden Endosperm ruhender Samen. Theor. Appl. Genet. **43**: 222—225 (1973b). — BERGMANN, F.: Genetische Abstand zwischen Populationen. 2. Die Bestimmung des genetischen Abstandes zwischen europäischen Fichtenpopulationen (*Picea abies*) auf der Basis von Isoenzym-Genhäufigkeiten. Silvae Genetica **23**: 28—32 (1974a). — BERGMANN, F.: The genetics of some isoenzyme systems in spruce endosperm (*Picea abies*). Genetika **6**: 353—360 (1974b). — BERGMANN, F.: The allelic distribution at an acid phosphatase locus in Norway spruce (*Picea abies*) along similar climatic gradients. Theor. Appl. Genet. **52**: 57—64 (1978). — BERGMANN, F.: Ein besonderer Fall geographischer Variation an zwei Enzym-Genloci der Fichte (*Picea abies*). P. 8—14. In: Verh. Forum Genetik-Wald-Forstwirtschaft. 3. Arbeitstagung. Freiburg, 1983. Göttingen (1984). — BERGMANN, F.: Untersuchung der genetischen Differenzierung der Fichte in den Seetaler Alpen an Enzym-Genloci P. 163—172. In: Verh. Forum Genetik-Wald-Forstwirtschaft. 5. Arbeitstagung, Innsbruck, 1987. Schriftenr. Forstl. Bundesversuchsanst. Wien, Nr. 28 (1988). — BERGMANN, F. and GREGORIUS, H. R.: Comparison of the genetic diversities of various populations of Norway spruce (*Picea abies*). Proc. Conf. Biochem. Genet. Forest Trees. Umea, Sweden, 99—107 (1979). — BERGMANN, F. and SCHOLZ, F.: The impact of air pollution on the genetic structure of Norway spruce. Silvae Genetica **36**: 80—83 (1987). — BERTSCH, K.: Geschichte des deutschen Waldes. 4. Aufl. Jena (1953). — BORGHETTI, M., GIANNINI, R. and MENOZZI, P.: Geographic variation in cones of Norway spruce (*Picea abies* (L.) KARST.). Silvae Genetica **37**: 178—183 (1989). — BRUNEL, D. and RODOLPHE, F.: Genetic neighbourhood structure in a population of *Picea abies* L. Theor. Appl. Genet. **71**: 101—110 (1985). — CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Petawawa National For. Institute, Can. For. Serv., Information Rep. PI-X-42 (1984). — CROW, J. F. and KIMURA, M.: An Introduction to Population Genetics Theory. Harper and Row, New York, Evanston, London (1970). — FIRBAS, F.: Spät- und nacheiszeitliche Waldgeschichte Mitteleuropas nördlich der Alpen. I. Allgemeine Waldgeschichte. Jena (1949). — FIRBAS, F.: Spät- und nacheiszeitliche Waldgeschichte Mitteleuropas nördlich der Alpen. II. Waldgeschichte der einzelnen Landschaften. Jena (1952). — GIACOMINI, V.: La flora. In: "Conosci l'Italia". Vol. II. (Touring Club Italiano ed.). Milano (1958). — GREGORIUS, H. R.: Genetischer Abstand zwischen Populationen. I. Zur Konzeption der genetischen Abstandsmessung. Silvae Genetica **23**: 22—27 (1974). — GREGORIUS, H. R.: The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Mathemat. Biosci. **41**: 253—271 (1978). — GREGORIUS, H. R.: The relationship between the concepts of genetic diversity and differentiation. Theor. Appl. Genet. **74**: 397—401 (1987). — GULLBERG, U., YAZDANI, R., RUDIN, D. and RYMAN, N.: Allozyme variation in Scots pine (*Pinus sylvestris* L.) in Sweden. Silvae Genetica **34**: 193—201 (1985). — GURIES, R. P. and LEDIG, F. T.: Genetic diversity and population structure in pitch pine (*Pinus rigida* MILL.). Evolution **36**: 387—402 (1982). — HAMRICK, J. L., MITTON, J. B. and LINHART, Y. B. :Levels of genetic variation in forest trees: Influences of life history characteristics. Proc. Symp. on Isozymes of North American Forest Trees and Forest Insects. USDA For. Serv. Gen. Tech. Rep. PSW-46, 35—41 (1981). — KRAL, F.: Verbreitungsgeschichte der Fichte im Alpenraum. In: SCHMIDT-VOGT: Die Fichte. Band I. 180—186. Verlag Paul Parey, Hamburg, Berlin (1977). — LUNDKVIST, K.: Allozyme frequency distribution in four Swedish populations of Norway spruce (*Picea abies*). I. Estimation of genetic variation within and among populations, genetic linkage and a mating system parameter. Hereditas **90**: 127—143 (1979). — LUNDKVIST, K. and RUDIN, D.: Genetic variation in eleven populations of *Picea* abies as determined by isozyme analysis. Hereditas **83**: 67—74 (1977). — MAGINI, E., PELIZZO, A., PROIETTI PLACIDI, A. M. and TONARELLI, F.: La picea dell'Alpe delle Tre Potenze. Areale-Caratteristiche-Posizione sistematica. Ann. Acc. It. Sci. For. **XXIX**: 107—210 (1980). — MORGANTE, M., VENDRAMIN, G. G. and GIANNINI, R.: Genetics of 6PGD and SKDH in Norway spruce (*Picea abies* K.). J. Genet. and Breed. **43**: 67—72 (1989). — MUONA, O., YAZDANI, R. and LINDQVIST, G.: Analysis of linkage in *Picea abies*. Hereditas **106**: 31—36 (1987). — NEI, M.: Genetic distance between populations. Amer. Natur. **106**: 282—292 (1972). — NEI, M.: Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA **70**: 3321—3323 (1973). — NEI, M.: Molecular Population Genetics and Evolution. North Holland Publ., Amsterdam, Oxford (1975). — POULIK, M. D.: Starch gel electrophoresis in a discontinuous system of buffers. Nature **180**: 1477—1478 (1957). — ROUTLEDGE, R. D.: Diversity indices: Which ones are admissible? J. Theor. Biol. **76**: 503—515 (1979). — SCHMIDT-VOGT, H.: Die Fichte. Band I. Taxonomie, Verbreitung, Morphologie, Öko-

logie, Waldgesellschaften. Verlag Paul Parey, Hamburg, Berlin (1977). — Shaw, C. R. and Prasad, R.: Starch gel electrophoresis. A compilation of recipes. Biochem. Genet. 4: 297—320 (1970). — Sneath, P. H. A. and Sokal, R. R.: Numerical Taxonomy. W. H. Freeman and Co., San Francisco, CA (1973). — Yeh, F. C. and Layton, C.: The organization of genetic variability in central and marginal populations of lodgepole pine, *Pinus contorta* spp. *latifolia*. Can. J. Genet. Cytol. 21: 487—503 (1979).

Short Note: Genetic Control of Oak Shake; Some Preliminary Results

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Summary

The wood of ring porous oaks is frequently subject to a defect known as shake, which describes the development of extensive longitudinal fissures in the living tree. Previous work had suggested a relationship between the occurrence of shake and cross sectional area of earlywood vessels. This study investigated the degree of genetic control of vessel area in German *Quercus robur* and *Quercus petraea*, considered here as a single species. Ramets in a clonal seed orchard and open-pollinated progeny in an unreplicated trial were sampled. Heritability estimates for vessel area were high, ranging from 0.60 ± 0.25 (narrow sense, individual tree basis) to 0.93 ± 0.06 (broad sense, clonal mean basis). Interpretation of these results must acknowledge the limitations of the experimental material. However, the indication of strong additive genetic control of vessel area is consistent with information for other wood characteristics of other species, and suggests that simple selective breeding could be effective in reducing the frequency of shake in oaks.

Key words: Heritability, Quercus robur, Quercus petraea, shake, wood quality.

Introduction

The timber defect known as shake is described by Panshin and De Zeeuw (1980) as "... longitudinal separations of the wood which appear in the standing tree". Shake occurs in nearly all British oak plantations, frequently affecting more than 50% of the trees. The roadside value of shaken timber may be as little as 20% of that of sound timber (Brown, 1945; Henman, 1986).

An association betwen shake and soil has been proposed at least since 1679 when John Evelyn observed that oak "... which grows in gravel is subject to be frow [shaken] and be brittle". There is now little doubt that soils greatly influence the frequency of shake. Brown (1945) reported that sound oak is normally found on soils containing a reasonable proportion of clay; Henman (1986) reported that shake is most frequent on sites where water table levels are variable, particularly over drought-prone, light and sandy soils. Henman proposed that shake develops under the combined influences of "predisposing factors" and "triggers", suggesting that a predisposition results from weak or degraded wood which may be triggered to fracture by stresses such as those induced by droughts or gales.

The fact that even the most severely affected sites produce some sound stems implies that certain individuals are inherently less predisposed to shake than others. This encouraged Savill (1986) to examine wood samples for anatomical features that might be associated with shake. Of the many features investigated, only the mean cross sectional areas of the larger vessels in the earlywood, older than about 20 years from the pith, were found to be significantly correlated with shake: shaken trees possessed larger vessels than those in sound trees. This relationship between vessel size and shake conforms to findings by materials scientists, who have demonstrated that cracks are most easily propagated in cellular solids composed of large cells (Ashby and Gibson, 1983).

The working hypothesis upon which the studies described in this paper are based is that a predisposition to shake increases with vessel size. If this is so, then it would be of value to be able to recognise shake-prone trees in the field. This has been the subject of a separate investigation by Savill and Mather (1990), which has indicated that trees with large vessels flush later in the spring. It might therefore be possible, for example, to remove shake-prone trees in early thinning operations, or to select only early flushing trees in breeding programmes. The latter approach could be complicated by the greater sensitivity of early flushing trees to attack by *Tortrix viridana*.

The objective of this associated study was to investigate the extent to which vessel size in oak is heritable, and therefore amenable to genetic manipulation. Many anatomical properties of wood in many species are known to be under relatively strong genetic control (Burley, 1982; Zobel and van Buijtenen, 1989). In oaks characteristics associated with vessel qualities, such as the width of the earlywood zone and wood density, have been shown to be highly heritable (Nepveu, 1984a and b).

Materials and Methods

The material for which vessel size was examined is described in *Table 1*. Because of the limited availability of material, samples from both *Quercus robur* and *Quercus petraea* were pooled in subsequent analyses. This was regarded as legitimate because considerable hybridisation and introgression occurs between the two species and, as a result, there is an unresolved controversy about their status as two separate species (Cousens, 1962 and 1965; Gardiner, 1970; Valen, 1976).

The first available samples were radiographic images of 5 mm Pressler borer cores taken from a seed orchard of grafted clones near Hanover, Federal Republik of Germa-