GENETIC VARIATION OF NORWAY SPRUCE (*PICEA ABIES* [L.] KARST.) POPULATIONS IN AUSTRIA

III. MACROSPATIAL ALLOZYME PATTERNS OF HIGH ELEVATION POPULATIONS

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ABSTRACT

In the Austrian Alps and Bohemian Massif, 29 autochthonous, high elevation Norway spruce (*Picea abies*) populations were studied using 17 isozyme loci. For each population, 100 adult trees were genotyped. Intraspecific genetic variation was mediocre. Average observed heterozygosity was approximately 15%, mean number of alleles per locus and effective number of alleles was 2.2 and 1.2, respectively. On average 44% of the loci were polymorphic based on the 0.05 criterion. The measure of genetic similarity or the relative evenness *e* (GREGORIUS 1990) reached 0.88 indicating relatively high similarity. Interpopulational genetic variation was small. Population genetic differentiation (D_j, GREGORIUS & ROBERDS 1996) of one population to the remaining 28 populations varied from 0.019 to 0.036. On average, genetic differentiation δ was 0.026 and F_{ST} was 0.012. Overall relationship between Gregorius' allelic distance and the geographic distance was weak ($r = 0.178$) but significant as indicated by MANTEL (1967) test. Mantel correlogram indicated a significant positive *r* value in the first distance class (0–75 km) and a significant negative value in the distance class of 150–225 km. Mantel *r* values for the other distance classes up to 450 km were not significant at the < 5% level. Multivariate analysis showed that 4.8% of the total variation, which could be assigned to the first three canonical variables, was due to population differences. The first two canonical variables seemed to separate the population in an eastwestern fashion. The Mantel test was also used to check the goodness-of-fit of allelic data (matrix ***Y***) to the basic rock type (matrix ***X***). Expectedly influence on basic rock type on the gene marker under study was negligible although significant for *Fest2*. Data presented are also discussed in the light of *in situ* conservation of forest genetic resources.

Key words: *Picea abies*, Alps, Bohemian Massif, genetic conservation, geographic differentiation, multivariate analysis, Mantel correlogram, Mantel test.

INTRODUCTION

Norway spruce [*Picea abies* (L.) Karst.] is the most common forest tree species in Austria. Today it is found on 61.8 % of the wooded area. This species can cope successfully with varying forest sites conditions and occupies all soil types, but is preferably found on podsolic and rendzina types (SCHADAUER 1994). A meticulous depiction of its range in Austria was given by TSCHERMAK (1949). In short, the Alps, mountains of the Bohemian Massif [Mühlviertel (Upper Austria) and Waldviertel (Lower Austria)] and higher elevations of the Alpine foothills (Hausruck, Kobernaußerwald) are naturally covered by *Picea abies*. The Danube basin separates the range into the Alpine part and the Bohemian Massif with a sparsely covered link in the Strudengau between the cities Grein and Ybbs. Pure stands are found in the *Piceetum subalpinum*. In this forest community the range varies in the Central Alps between 1400 and 2100 m and between 1000 and 1400 m in the Bohemian massif. In the northern Alpine transitional zone, an elevational range of this forest community is typical at 1400 to 1900 m and in the southern Alpine transitional zone at 1500 to 2100 m. In the *Piceetum montanum*, Norway spruce is also the dominant species. Elevational zones vary strongly from ecoregion to ecoregion, for instance in the Central Alps from 650 to 1700 m and in the southern Limestone Alps from 1000 to 1700 m. Timber production of Norway spruce amounts to approximately 12 million m^3/a and plays both for forestry and wood industry a major role in Austria. Thus, this conifer is of utmost importance for forestry and nature protection including torrent and avalanche control.

Since the early allozyme studies on the geographic variation in Norway spruce (*e.g.*, TIGERSTEDT 1973, BERGMANN 1974, LUNDKVIST & RUDIN 1977) a much better knowledge of the macrospatial pattern have been

gained. It looks like that in certain areas, such as the Carpathians (GONCHARENKO & POTENKO 1990, GÖMÖRY & PAULE 1993) and the Baltic region (GONCHARENKO & POTENKO 1990, LAGERCRANTZ & RYMAN 1990) genetic centers are present. Values of population genetic differentiation, estimated as G_{ST} parameter, typically are about 5 % (for review see MÜLLER-STARCK 1992) indicating that much of the allozyme variation of this conifer resides within single populations. Marginal populations are genetically less variable than central ones (TIGERSTEDT 1979, BERGMANN & GREGORIUS 1979) but data are still scanty. In the Alps, Norway spruce populations have been studied on a macrogeographical scale using allozyme loci in Italy (GIANNINI *et al.* 1991, MORGANTE & VENDRAMIN 1991), Switzerland (MÜLLER-STARCK 1995) and Germany (BERGMANN 1991). Data on Austrian populations are still missing.

Objective of this study was to study allozyme variation within and among 29 high elevation and putatively autochthonous *Picea abies* populations in the Austrian Alpine range and Bohemian Massif. While Italian spruce populations probably share different ancestral gene pools due to different glacial refugia on the Balkan peninsula and in the plains of Central Italy (GIANNINI *et al.* 1991, MORGANTE & VENDRAMIN 1991), and a postglacial immigration of *Picea abies* into the western Swiss Alps from southern locations is not unlikely (MÜLLER-STARCK 1995), this study should also elucidate the immigration history of this species in the eastern Alps and should examine whether spruce immigrated into the Austrian Alps besides the presumptive main eastwest path via additional routes. Furthermore, the hypothesis of a genetic difference of spruce populations growing on soils originating from calcareous and silicate basis rock is addressed. Data presented are also discussed in the light of *in situ* conservation of forest genetic resources. This study continues a series of studies on the genetics of this conifer in Austria and the reader is respectfully referred to previous papers (GEBUREK 1998, GEBUREK *et al.* 1998).

MATERIAL AND METHODS

Study sites

In total, 29 *Picea abies* populations, all are located in the montane and subalpine vegetation zone, were studied (Fig. 1). The origin of stands selected is putatively autochthonous. Stand selection was based on information derived from local forest district offices. Additionally local inspection proved that stands were heterogenous both in their age classes (juvenile to adult trees) and their stand structure. However, it cannot be surely excluded that also non-autochthonous plant material was used. For all populations 100 trees each were selected and buds from adult trees were sampled. It was tried to obey a minimum distance from tree to tree of approximately 30 m. Usually this was possible, however, sometimes this distance fell short. Population number, local designation, elevation, and tree mixture are arranged in Table 1. Populations were consecutively numbered, starting in the far west, *i.e.*, the province of Vorarlberg, and then following in west-eastern direction.

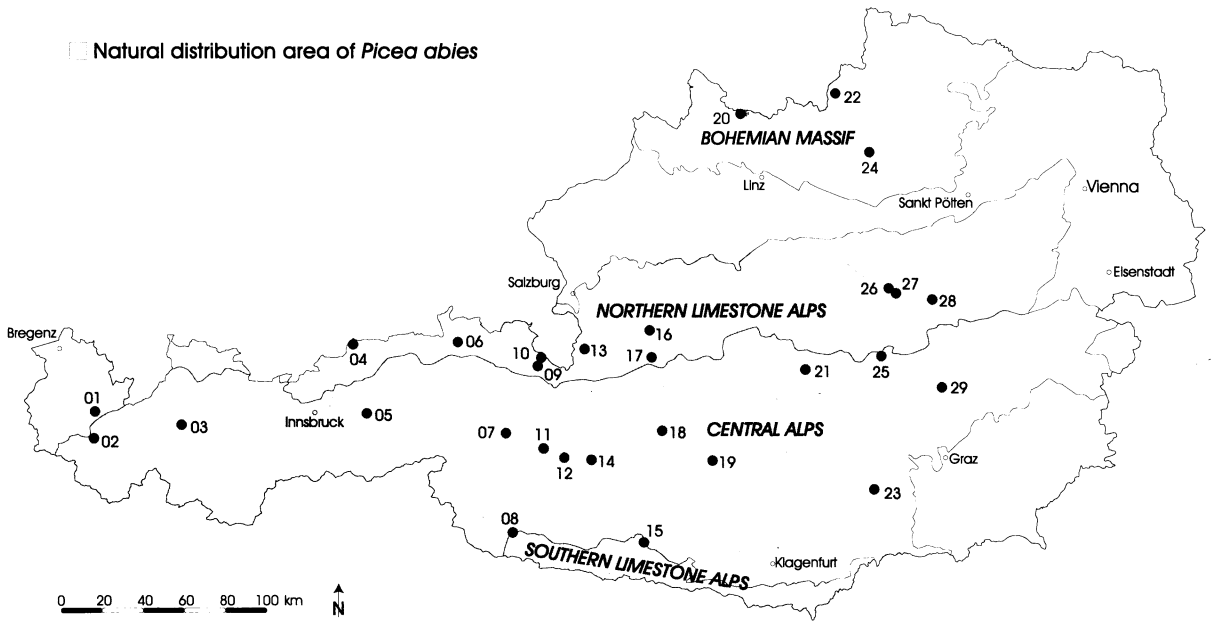

Figure 1. Location of Norway spruce (*Picea abies*) populations sampled in Austria. Dark area shows the natural range.

Table 1. List of Norway spruce (*Picea abies*) population characteristics in Austria.

Pop	Local Name/Province	Elevation [m]	Proportion of spruce	Basic Rock
1	Gadental / Vorarlberg	1100–1350	1.0	calcareous
2	Schruns / Vorarlberg	1500–1580	1.0	silicate
3	Lochalm / Tyrol	1500	0.8	calcareous
4	Schulterberg / Tyrol	1400–1500	0.9	calcareous
5	Weerberg / Tyrol	1500–1700	0.7	calcareous
6	Hoher Winkel / Tyrol	1300–1400	1.0	calcareous
7	Wiegenwald/Salzburg	1650	0.7	silicate
8	Thaler Alpl/Tyrol	1440–1500	1.0	calcareous
9	Stoißen/Salzburg	850–1150	0.3	calcareous
10	Mitterkaser/Salzburg	1400–1500	1.0	calcareous
11	Seidlwinkl/Salzburg	1700	0.4	silicate
12	Durchgangswald/Salzburg	1650–1700	0.9	silicate
13	Hagengebirge/Salzburg	1500–1550	0.7	calcareous
14	Anlauftal/Salzburg	1600–1800	0.8	silicate
15	Paternion/Carinthia	1400	1.0	calcareous
16	Hinterer Wieswald/Upper Austria	1500	1.0	calcareous
17	Koglgassenwald/Upper Austria	1400–1500	0.4	calcareous
18	Ulnwald/Salzburg	1540–1720	0.8	silicate
19	Turrach/Styria	1700–1800	0.9	silicate
20	Sternstein/Lower Austria	900	0.9	silicate
21	Trieben/Styria	1500	0.9	silicate
22	Riesenkopf/Lower Austria	850	0.4	silicate
23	Pomseben/Carinthia	1650	1.0	silicate
24	Bärnkopf/Lower Austria	900	1.0	silicate
25	Lahnhube/Styria	1300–1400	?	calcareous
26	Rothwald/Lower Austria	1100	0.3	calcareous
27	Greith/Styria	1150–1250	0.5	calcareous
28	Hoher Student/Styria	1350	1.0	calcareous
29	Hochlantsch/Styria	1200–1300	1.0	silicate

Tissues, electrophoretic methods, and genotyping

Buds were collected in the field and were stored at –80° C until usage. Enzyme extraction, electrophoresis, staining, and genotyping followed KONNERT (1995) unless otherwise stated. Following enzyme systems were used (in brackets acronym, EC number, and locus designation are given): aconitase (ACO, 4.2.1.3, *Aco*), aspartate aminotransferase (AAT; 2.6.1.1, *Aat1,Aat2, Aat3*), fluorescent esterase (FEST, 3.1.1.1, *Fest1,Fest2,* enzyme staining according to CHELIAK & PITEL (1984), putative genetic control), diaphorase (DIA, 1.6.4.3, *Dia1*), glutamate dehydrogenase (GDH, 1.4.1.3, *Gdh*), hexokinase (HEX, 2.7.1.1, *Hex,* enzyme staining according to CHELIAK & PITEL (1984), putative genetic control), isocitrate dehydrogenase (IDH, 1.1.1.42, *Idh1, Idh2*), NADH-dehydrogenase (NDH, 1.6.99.3, *Ndh*), phosphoenolpyruvate carboxylase (PEPCA, 4.1.1.31, *Pepca*), phosphogluconate isomerase (PGI, 5.3.1.9, *Pgi*), phosphoglucomutase (PGM, 2.7.5.1, *Pgm1, Pgm2*), superoxide dismutase (SOD,1.15.1.1., *Sod,* enzyme staining according to CHELIAK & PITEL (1984), putative genetic control).

Measures of genetic variation

To assess genetic variation within and among populations the following measures were used: gene pool diversity v, which is the mean effective number of alleles (GREGORIUS 1978), total population differentiation δ_T (GREGORIUS 1987), and genetic (allelic) distance (GREGORIUS 1984). Hypothetical gametic diversity v_{gam} was calculated according to (GREGORIUS 1978). This parameter is the product of single allelic diversities and hence reveals potential differences in gametic frequencies among populations. Similarity of allele frequencies were investigated by the relative evenness (e) (GREGORIUS 1990). This measure varies from 0 (not similar) to 1 (similar). As measures of genetic variation between populations, population differentiation parameters Dj and δ were employed (GREGORIUS & ROBERDS 1986). The calculations were performed using the PC programme GSED (GILLET 1994). F-statistics were calculated including the 95%

confidence interval (CI) by bootstrapping over loci (GOUDET 1994). Comparisons of distance matrices (allelic and geographic) among the same sampling populations were performed according to MANTEL (1967) in order to test the null hypothesis H_o: Allelic distances among populations in matrix $\boldsymbol{X}$ are not linearly related to the corresponding geographic distances of matrix $\boldsymbol{Y}$, *i.e.*, the allelic distances are not autocorrelated as a gradient. MANTEL (1967) statistic was

$$z = \sum_i \sum_j x_{ij} y_{ij}$$

for $i \neq j$ where i and j were row and column indices of the matrices. Mantel z was normalized according to

$$r = [1/(n-1)] \sum_i \sum_j [(x_{ij} - \bar{x})/s_x][(y_{ij} - \bar{y})/s_x]$$

for $i \neq j$ where i and j were row and column indices, and n was the number of pairs of distances in the two matrices (diagonal excluded). This statistic varies between −1 and +1. As in any other statistical test, the decision is made by comparing the actual value of Mantel r to the reference distribution obtained under H_o. The reference distribution was achieved by permuting at random one of the matrices. By repeating this operation (1000 times), different permutations produced a set of values of Mantel r obtained under H_o and these values represented the sampling distribution. *P*robabilities (p) were computed following HOPE (1968). According to EDGINGTON (1987) HOPE's probabilities have to be interpreted in terms of being 'strictly smaller' or 'strictly greater' than the stated value. For instance, if the permutation test results in 0.05, then the probability of the null hypothesis to be true is strictly smaller than 0.05 (one-tailed test). Mantel r was additionally computed for six geographic distance classes (Mantel correlogram) (0 – 75 km, >75 – 150 km, >150 – 225 km, >225 – 300 km, > 300 – 375 km, >375 – 450 km). Comparisons between the allelic distance matrix and the model matrix derived by the ecological hypothesis (populations originating from calcareous and silicate rock types differ in the allelic distances at the isozyme loci under study) were also performed according to MANTEL (1967). While the allelic distance matrix comprised the allelic distances between the respective populations, the basic rock type matrix contained values of '0' if the two populations differed in their basic rock types or '1' if the two populations shared identical basic rock types. Calculations were performed with the R-computer package (LEGENDRE & VAUDOR 1991).

Besides the univariate approach a canonical discriminant analysis was performed. Individual tree genotypes were coded following the method described by SMOUSE & NEEL (1977) resulting in $n-1$ variables for each locus, where n equals the number of allozymes per locus. Data were subjected to the MDA routine of the BIOΣTAT programme package (PIMENTEL 1993). Allozymes with an overall frequency less than 1% were not considered for the analysis were pooled into a synthetic allele class for proper MDA function (cf. PERRY & KNOWLES 1989).

RESULTS

Different measures to quantify genetic variation are presented in Table 2. Observed heterozygosities (H_{obs}) ranged from 12.9% (POP–28, 'Hoher Student', Styria) to 16.9% (POP–25, 'Lahnhube', Styria) and averaged over all 29 populations to 14.7%. Mean number of alleles per locus (A_L) was very similar among the populations and was 2.2. Due to unequal allele frequencies per locus, allelic or genic diversity (v) was smaller than the mean number of alleles per locus. Hypothetical gametic multilocus diversity (v_{gam}) varied from 18.01 for POP–28 ('Hoher Student', Styria) to the maximum value of 45.82 estimated for POP–7 ('Wiegenwald', Salzburg). Due to the high number of sampled trees per population intrapopulational differentiation δ_T almost equaled expected Hardy-Weinberg heterozygosities (H_e), which varied from 0.137 (POP–28, 'Hoher Student',Styria) to 0.175 (POP–4, 'Schulterberg', Tyrol, and POP–7, 'Wiegenwald', Salzburg). The locus *Gdh* was close to fixation. Relative evenness (e) showed that allele frequencies were very similar. Population differ-

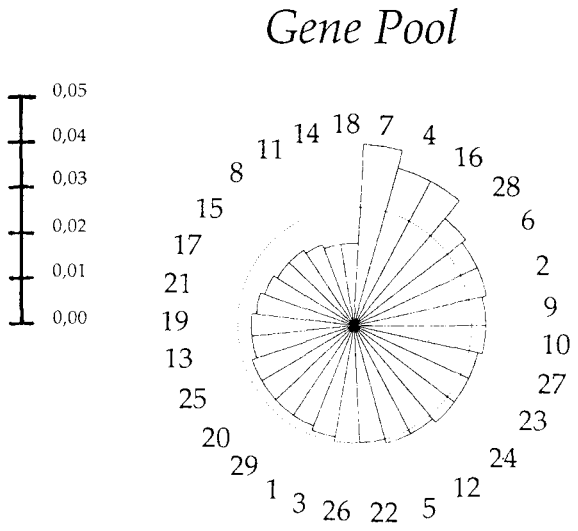

Figure 2. Gregorius' gene pool differentiation 'snail' based on 17 isozyme loci for 29 high elevation, autochthonous *Picea abies* populations in Austria. Numbers given refer to population designation.

Table 2. Different measures of allozyme variation within and among adult Norway spruce (*Picea abies*) population in Austria.

Population	N	H_{obs}	A_L	v	v_{gam}	$P_{0.05}$	δ_T	e	D_j
POP- 1	100	14.3	2.2	1.17	24.99	41.2	0.149	0.898	0.024
POP- 2	100	15.9	2.1	1.19	29.47	52.9	0.160	0.869	0.030
POP- 3	100	13.3	2.3	1.17	25.39	47.1	0.149	0.888	0.025
POP- 4	100	16.2	2.5	1.21	39.46	58.8	0.175	0.842	0.036
POP- 5	100	14.6	2.2	1.17	22.85	35.3	0.145	0.889	0.027
POP- 6	100	15.1	2.1	1.19	31.09	41.2	0.159	0.872	0.030
POP- 7	100	16.8	2.3	1.21	45.82	47.1	0.175	0.870	0.040
POP- 8	100	13.8	2.1	1.17	22.84	35.3	0.146	0.889	0.020
POP- 9	100	14.1	1.9	1.18	25.58	47.1	0.153	0.852	0.029
POP-10	100	14.2	2.2	1.19	30.68	47.1	0.158	0.879	0.029
POP-11	100	14.6	2.1	1.18	25.93	41.2	0.151	0.886	0.019
POP-12	100	15.4	2.2	1.19	33.29	47.1	0.161	0.892	0.027
POP-13	100	15.0	2.4	1.20	33.32	47.1	0.165	0.859	0.023
POP-14	100	14.2	2.0	1.18	25.85	47.1	0.152	0.882	0.018
POP-15	100	14.8	2.6	1.18	25.08	47.1	0.153	0.866	0.020
POP-16	100	16.4	2.4	1.21	43.18	47.1	0.174	0.886	0.036
POP-17	100	14.6	2.3	1.17	21.40	41.2	0.144	0.872	0.021
POP-18	100	14.3	1.8	1.17	22.35	41.2	0.145	0.879	0.018
POP-19	100	13.3	2.2	1.17	21.49	47.1	0.144	0.877	0.023
POP-20	100	14.2	2.4	1.18	27.39	47.1	0.154	0.888	0.024
POP-21	100	13.9	2.2	1.17	24.46	35.3	0.146	0.900	0.022
POP-22	100	14.9	2.3	1.19	27.54	52.9	0.157	0.859	0.026
POP-23	100	13.3	1.9	1.17	22.88	29.4	0.144	0.884	0.028
POP-24	100	15.2	2.1	1.17	23.90	41.2	0.148	0.886	0.028
POP-25	100	16.9	2.1	1.19	33.29	41.2	0.161	0.886	0.024
POP-26	100	15.2	2.4	1.18	28.29	47.1	0.156	0.876	0.026
POP-27	100	15.4	2.2	1.19	29.89	47.1	0.161	0.864	0.028
POP-28	100	12.9	2.0	1.16	18.01	35.3	0.137	0.865	0.031
POP-29	100	14.9	2.2	1.17	24.22	41.2	0.146	0.895	0.024
		14.7	2.2	1.18	27.93	44	0.154	0.879	$\delta = 0.026$

N – number of trees, H_{obs} – observed heterozygosity, A_L – mean number of alleles per locus, v – genetic diversity, v_{gam} – hypothetical gametic multilocus diversity, $P_{0.05}$ – percentage of polymorphic loci, δ_T – interpopulation differentiation, e – relative evennes, D_j – population differentiation.

entiation (D_j) was small for all populations (Table 2). D_j values and gene pool differentiation are shown using Gregorius' differentiation 'snail' (Fig. 2). Additionally, hypothecial gametic multilocus diversity (v_{gam}), intra-populational differentiation (δ_T), relative evenness (e) and genetic differentiation (D_j) were each standardized by their respective maximum value among the samples. Thus, all these relative values potentially could vary between 0 and 1. These standardized values are depicted in a radar chart (Fig. 3). Wright's F-statistics was: F_{IT} =0.057 (95% *CI*: 0.014; 0.119), F_{ST} =0.012 (95% *CI*: 0.007; 0.019), F_{IS} = 0.043 (95% *CI*: 0.005; 0.106). Overall relationship between Gregorius' allelic distance and the geographic distance was weak (Mantel $r = 0.178$) but significant ($p < 0.05$). Mantel correlogram indicated a significant positive r value in the first distance class (0–75 km) and a significant negative value in the distance class of 150 – 225 km. Mantel r values for the other distance classes up to 450 km were not significant at $p < 0.05$ (Fig. 4). The hypothesis that basic rock type and allelic distance is autocorrelated was rejected in most cases. Only for *Fest2*, Mantel r was significant ($p < 0.03$), however relationship was very weak as indicated by $r = 0.053$.

Canonical discriminant analysis showed differences among populations (Table 3, Fig. 5). The first canonical variable accounted for 21.4% of the total variance, 11.1% (*i.e.*, squared canonical correlation $R = 0.334$) of which was explained by population differences. In this canonical variable alone, ($0.111 \times 0.214 = 0.024$) 2.4% of the total genetic variation could be assigned to population effects. While relatively high correlations (loadings) for the first canonical variable were found only for alleles at *Aat1*, *Fest1* and *Fest2*, the second

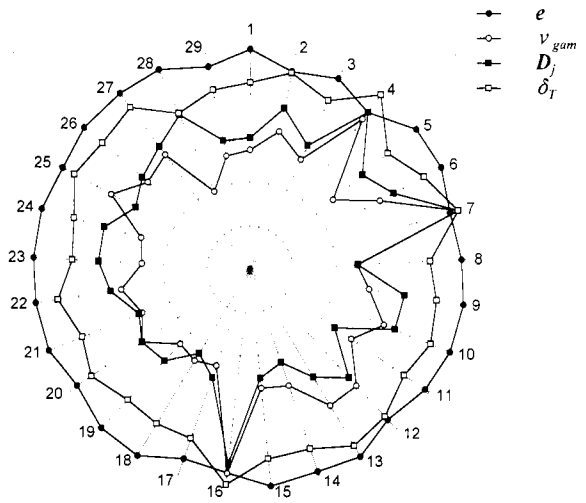

Figure 3. Radar chart showing relative measures of genetic variation. Numbers given refer to population designations. For details see text.

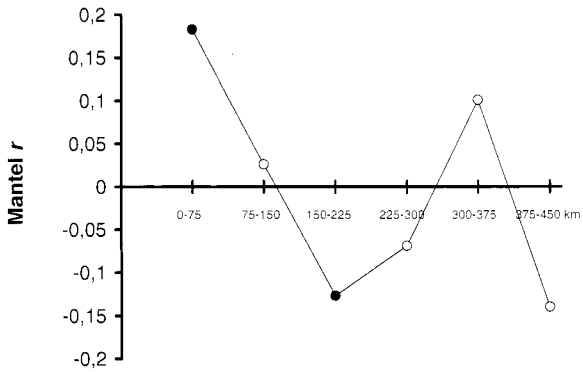

Figure 4. Mantel correlogram for the relationship between the matrix Gregorius' gene pool distance and the geographic distance. Ordinate shows standardized Mantel statistic (r) calculated for six distance classes. Black dots represent significant values of the Mantel statistic ($p < 0.05$).

canonical variable was essentially loaded by additional alleles (Table 3). The second variable accounted for 17.8 % and the third 11.7 % of the total variation. Hence, 1.7 % and 0.7 % could be assigned to population effects, respectively. Thus in the first three variables 4.8 % of the total variation was caused by populations effects. The first canonical variable (vertical axis) separated the population in an east-western fashion. Western populations (such as POP–1, POP–2, POP–3, POP–4) seemed to have centroids which were negative for the first (F1) and positive for the second canonical variable (F2), while centroids of eastern population (such as POP–9, POP–22, POP –27) tended to have positive values for the first and negative for the second variable (Fig. 5). Both axis separated POP–4

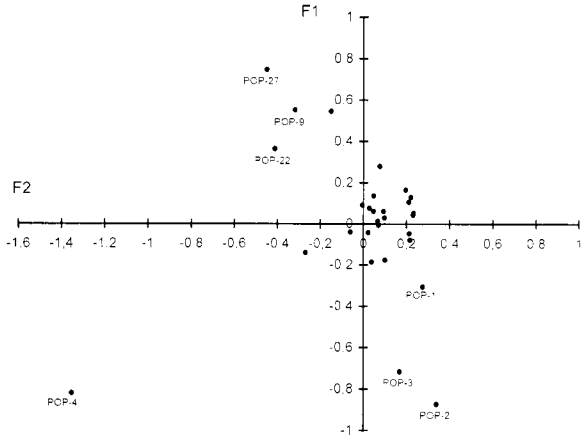

Figure 5. Mean canonical variable scores of 29 *Picea abies* populations plotted for the first two canonical variables (not all centroids were labeled, see Tab. 1 for population abbreviations).

('Schulterberg', Tyrol) from the pool.

DISCUSSION

Intrapopulational genetic variation was mediocre and genetic differentiation among populations was very small at the loci under study. While Austrian silver fir (*Abies alba*) populations can be traced back to two immigration routes revealed by isozyme markers (BREITENBACH-DORFER *et al.* 1997), this study does not furnish evidence that the *Picea abies* populations are derived from two or more ancestral sources in Austria. However, strictly speaking it must be taken for granted that the refugial populations differ in their genetic structures which – of course – must not be necessarily the case. With this limitation in mind, results of this study do not reject the hypothesis based on paleoecological data that *Picea abies* populations are derived from a single ancestral population in the southeastern foreland of the Alps (KRAL 1979). Although Norway spruce could persist at the northern Alpine slopes (BORTENSCHLAGER 1970), scientific support of such additional refugium is still missing. It is very likely that Norway spruce immigrated from the southeastern Alpine edge and maybe also from the Dinaric Alps into central parts of the eastern European Alps (LANG 1994, *l.c.* pp.138–145). Previous results indicate that *Picea abies* in the Italian Alps resulted from mingling of immigrating population(s) along the main immigration route in eastwestern direction with population(s) originating in the Italian plains during glacial periods (GIANNINI *et al.*1991, MORGANTE & VENDRAMIN 1991), however paleoecological evidence (*e.g.*, pollen findings) is still missing. In Switzerland southnorth routes have not been excluded (BURGA

Table 3. Correlation coefficients of four canonical variables for 29 Norway spruce (*Picea abies*) populations in Austria.

Allozyme	1	2	3	4
Aat1–2	0.733	0.257	0.397	–0.187
Aat1–5	–0.622	0.069	–0.539	0.091
Aat2–1	0.094	–0.130	0.310	–0.186
Aat3–1	0.008	–0.143	–0.207	–0.376
Aat3–2	0.020	0.169	0.220	0.387
Aat3–3	–0.076	–0.090	–0.031	–0.039
Aco–1	–0.111	0.102	0.205	0.293
Aco–2	0.105	–0.100	–0.197	–0.299
Dia–2	0.113	–0.077	0.179	–0.214
Fest1–2	–0.273	0.285	0.015	–0.257
Fest1–3	0.308	–0.108	–0.159	0.126
Fest1–4	0.125	–0.317	–0.029	0.010
Fest2–1	–0.194	0.116	–0.127	–0.191
Fest2–2	–0.020	0.037	0.353	0.117
Fest2–3	0.482	–0.339	–0.567	0.142
Gdh–2	–0.069	–0.059	–0.003	–0.186
Hex–2	0.193	0.128	0.019	0.138
Hex–3	–0.208	–0.115	–0.006	–0.174
Idh1–2	–0.033	0.047	0.101	0.034
Idh2–2	–0.059	–0.067	–0.117	–0.002
Ndh–1	–0.104	0.315	–0.099	0.453
Ndh–2	0.106	–0.342	0.081	–0.370
Ndh–3	–0.001	0.103	0.081	–0.273
Eigenvalue	0.125	0.104	0.069	0.05
Cummulative percentage of variation	21.39	39.24	50.97	59.47
Canonical correlation	0.334	0.307	0.253	0.218
p	<0.001	>0.001	<0.001	<0.001

1988) and MÜLLER-STARCK's (1995) allozymic data suggest additional immigration routes from a south-western direction. It is interesting that populations from the Bohemian Massif (POP–20, POP–22, POP–24) could not be distinguished from Alpine populations based on isozyme marker, although provenance data have shown that growth performance of *Picea abies* from this region is likely to be different to Alpine sources. Provenances of this region exhibit a growth pattern which is similar to provenances originating from the Carpathians (GÜNZL 1979) which supports KRAL's (1979) hypothesis that this region is influenced by populations originating from the Caparthian refugium. But neither the univariate analysis, especially based on D_j-values [multilocus and single-locus (data not shown)], nor the multivariate analysis differentiated this region from the Alpine range. When allelic richness (data not shown) of *Aat1, Aat2, Aco, Gdh* and *Pgi* of Austrian populations (POP–20, –22, –24, Fig. 1) originating from the Bohemian Massif were compared with data derived from virgin *Picea abies* forests in the Carpathians (GÖMÖRY 1992) it was found that these Austrian populations harbor less alleles at *Aco, Gdh,* and *Pgi,* while no difference was found at *Aat1* and *Aat2*. Since the detection of rare alleles is very sensitive to the sample size it is noteworthy that in GÖMÖRY's study between 30 and 60 mature trees were genoytped, a sample size which is considerably smaller than that used in the present study. The lack of rare alleles which were found in the Carpathians but were not detected in populations in the Bohemian Massif does not support the hypothesis that *Picea abies* in the Bohemian Massif can be traced back to Carpathian sources, at least this holds true for POP–20, –22, and –24.

The locus *Gdh*, which was close to fixation, supports former findings (BERGMANN 1983, MÜLLER-STARCK 1995) that in the Alps no or – at most – very small genetic variation can be detected. In Norway spruce, *Gdh* is highly polymorphic outside in northeastern Europe. Thus this study supports earlier reports (BERGMANN 1983, GÖMÖRY 1992) on the diagnostic uselfulness of this marker and it can serve as a rough indicator that populations are of Alpine or Carpathian origin.

Relationship between genetic and geographic distance in forest tree species is generally weak when relative small regions are considered. Distinct gene flow through intensive pollen and seed distribution is typical for forest trees and counteracts a genetic differentiation (GOVINDARAJU 1988). Present data show that isolation-by-distance contributed to a genetic differentiation of *Picea abies* in Austria. However, its effect is small as shown by a significant, but small Mantel's *r* based on the matrices of allelic and geographical distance. Lack of a pronounced correlation has been also observed in *Fagus sylvatica*, when a small part of the natural range was considered (BELLETTI & LANTERI 1996). Conversely, in the tropical tree *Pterocarpus* a correlation coefficient exceeding 0.5 between genetic and geographic distance was obtained, when 11 Thai populations were studied (LIENGSIRI *et al.* 1995). However, a comparison of these studies with the present one is very limited since in above-mentioned studies mistakenly product-moment correlations were used to assess the relationship between genetic and geographic distance.

The inability to detect differences at the single-locus level among forest tree populations has prompted the application of multivariate analyses (*e.g.*, MERKLE *et al.* 1988) and this study shows that a greater part of the genetic variation could be assigned to population differences [1.2 % (F_{ST}) vs. 4.8% populational effects

based on the first three variables]. Although it was possible to assign a larger proportion of the genetic variation to differences among populations, the geographical pattern of variation was still weak since many populations could not be clearly differentiated (Fig. 5). Direct comparisons of present data to other investigations in *Picea abies* are troublesome. It has been already mentioned that, for instance, different sample sizes detect rare alleles with different probabilities and in turn certain genetic measure such as v_{gam} or A_L will be strongly affected. Furthermore, different markers provide different results. This is not only true when identical Norway spruce populations were studied by different types of markers [see for instance allozyme *vs.* DNA markers (GEBUREK *et al.* 1998)] but this is also true for different sets of loci of a certain type of marker. Thus, it is known that the genetic variability of gene loci coding for 6-phosphogluconate dehydrogenase (6-PGDH) is high in Norway spruce, but since diploid material was used for the present study this enzyme was excluded due to overlapping allozyme banding. In a strict sense only results from other studies can be used for comparisons which are based on identical methods. Norway spruce has been studied by using allozymes in the Alps and their foreland by different authors. In the Black Forests (Germany) (KONNERT & FRANKE 1990, KONNERT 1991), Bavarian Alpine foreland (BERGMANN 1991), Italian Alps (GIANNINI *et al.* 1991, MORGANTE & VENDRAMIN 1991), and Swiss Alps (MÜLLER-STARCK 1995), this conifer was studied using similar but not identical methods. Samples size, material (bulked seed lots, buds from single trees, single tree seed lots), enzyme systems, loci per enzyme system, etc. were partly different and often different genetic parameters were estimated. Compared with this study the most congruent method was used in MÜLLER-STARCK's (1995) paper and thus a comparison of Austrian to Swiss *Picea abies* populations is the most meaningful. To a slightly lesser extant, data can be purposefully compared with the Italian study by MORGANTE & VENDRAMIN (1991). Grand mean number of alleles found in Austrian populations was very close to the slightly smaller value in Italian (A_L = 1.8) and to the slightly higher value in Swiss populations (A_L = 2.52) and percentage of polymorphic loci ($P_{0.05}$) of the present study was nearly identical to the value in Italy ($P_{0.05}$ = 43.2%). Expected Hardy-Weinberg heterozygosities (H_e) amounted to 0.162 in the Italian study while δ_T averaged 0.154 for Austrian populations. δ_T is nearly identical to H_e since a large sample size was used in the present study. Compared with Swiss and Italian populations this estimate is smaller, but this may stem from the use of more gene loci including highly polymorphic ones, *e.g.* 6PGDH coding genes, both in the Italian and Swiss investigations. While sample size (100 trees sampled per population) was identical in the Swiss study, sample size in the Italian study was at least twice as small. Hence, a pronounced difference regarding A_L between Austrian and Italian data was not to be expected, because the probability to detect alleles depends strongly on sample size. All genes were considered for the relative evenness in the present study, whereas those gene loci close to fixation (such as *Gdh*) were excluded in the Swiss investigation. This probably explains why estimates of the relative evenness were higher in Austrian populations compared with the Swiss ones. Gene pool differentiation (δ) was estimated as 0.043 for 20 Swiss *Picea abies* populations, but when populations presumably influenced by a southwest immigration were excluded δ decreases to 0.037. This estimate is still higher than δ in the present study but differences are small. Based on the limited comparability among studies, a careful conclusion would be that Austrian spruce populations are genetically very similar to those spruce populations in Switzerland which are not affected by southern refugial populations. Summing up, it looks like that Norway spruce in the Alpine range is genetically very similar when populations of the same altitudinal band are compared.

Allozyme differences between populations grown on calcareous and silicate soils are conjectural. Although a significant relationship between allelic distances at one locus (*Fest2*) and the 'pedogenetic distances' cannot be denied, a very low Mantel r does not bespeak a strong selection at this isozyme locus. In *Picea abies,* allozymes or closely linked genes may be adaptive under heavy pollution, for instance by heavy metals (BERGMANN & HOSIUS 1996), however, it is not very likely that 'normal' environmental differences such as different basic rock types can affect allozyme structure as shown for instance in Douglas-fir (*Pseudotsuga menziesii*) by a multivariate analysis (MERKLE *et al.* 1988).

How can the results of the present paper be used for conservation of genetic resources of *Picea abies* in Austria? While substantial allozyme variation can be found at the intraspecific level, small genetic differences among populations favor maintaining a few populations for *in situ* conservation. It can be concluded from Fig. 3 (*relative Dj*) that for instance POP–4, POP–7, POP–16 are genetically more variable than others. However, relative evenness and other important measure such as multilocus hypothetical diversity are higher in POP–16. Thus if a single population has to be selected based on these data the latter one is to be selected. However, gene conservation should not – as several authors have already stated (*e.g.*, EL-

KASSABY & RITLAND 1996, GEBUREK 1997) – be based exclusively on allozymes but also on other valuable genetic information derived from other nuclear and extrachromosomal gene markers and from other genetical studies (GEBUREK in press). Since these data are partly missing for Austrian Norway spruce stands, it can only be concluded *sensu stricto* that few *in situ* populations would sufficiently harbor allozyme variation. However, it is tempting to speculate that a prominent geographic variation among high elevation Alpine populations does not exist, because (1) the Alpine populations are likely descendants from a single glacial refugial population, (2) phenotypic variation in high elevations is not conspicuous, and (3) results from field trials do not suggest prominent geographic variation among high elevation Alpine populations (GÜNZL 1979), and, hence, other than allozyme variation is likely to be preserved in a few *in situ* populations within a certain elevational band. As already proposed by MÜLLER-STARCK (1995) for Swiss *Picea abies* conservation, single, but large *in situ* populations are preferable than several small conservation units. Since neither pronounced phenotypic nor quantitative genetic variation can be found in high elevation Austrian populations, it is proposed to select huge (>100 ha) areas in the Central Alps and in the northern and southern Limestone Alps for gene conservation purposes (cf. KOSKI *et al.* 1997). Populations from the Bohemian massif may be treated separately. It is not absolutely clear whether biologically significant differences between silicate and calcareous basic rock types exist and unless better knowledge is acquired both types should be considered adequately for gene conservation.

In this context it may be added that in Austrian forest gene reserves forest management is not significantly restricted (GEBUREK & MÜLLER 1995). While knowledge whether forest management in the genus *Picea* will alter allozyme population structure is still insufficient (BERGMANN & RUETZ 1991, CHAISURI & EL-KASSABY 1994), more recent results in eastern white pine (*Pinus strobus*) furnished strong evidence that logging results in a real and repeatable genetic erosion (BUCHERT *et al.* 1997) and alternative regeneration methods (group selection *vs.* shelterwood) have effects on rare, presumably deleterious alleles in Douglas-fir (Pseudotsuga menziesii) (ADAMS *et al.* 1998). Unless a better knowledge is acquired genetic resources in *Picea abies* should be also conserved in so called strict nature reserves in which conventional wood harvests are restricted.

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