Allozymic Variation in Some Norway Spruce Populations of the International IUFRO Provenance-testing Programme of 1964/1968

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Summary

Isoenzyme-polymorphisms of Norway spruce populations from the spruce regions of Central and Southeastern Europe were compared with those from Northern and Northeastern Europe. To do this, the electrophoretically detectable genetic variation was assayed at 19 coding gene loci (10 enzyme systems) for 15 provenances of the international IUFRO provenance-testing programme of 1964/1968. The results can be summarized as follows:

1. For the gene loci SKDH-A, IDH-A, LAP-A and GDH-A some alleles showed a marked geographic pattern.

2. The measures of genetic diversity as the mean number of alleles per locus (A/L), the proportion of polymorphic loci (P%), the expected heterozygosity (He) and the hypothetical gametic multilocus-diversity according to GREGORIUS (1978) all showed lower values in Southern and Central than in Northern and Northeastern Europe. Thereby, the genetic variation within the populations increased in going from the southwest to the north or northeast, although two provenances from the Balkans showed higher values as well. Hence, the conclusions of other authors could be confirmed.

3. For most provenances significant to highly significant correlations were found between the genetic distances and the geographic distances of the provenances.

4. The subpopulation differentiation (Dj), i.e. the genetic distance between each individual provenance and the complement of all other provenances, was highest for the most southerly provenances (Southwestern France and Northern Greece) and one Eastern Russian provenance.

In the discussion of the results it is pointed out that, along the altitude gradients and depending on the geographic latitude, numerous ecological adaptations advantageous for the species have arisen during the reinvasion into the Central European area after the last Ice Age. Thus, despite limited genetic variation at the experimentally accessible gene loci, the Norway spruce in Central Europe has demonstrated a good adaptive capacity.

Key words: Picea abies, genetic variability, electrophoresis, isozymes, provenance testing, geographic variation.

Zusammenfassung

Es wurden Isoenzym-Polymorphismen von Fichtenpopulationen aus den Fichtengebieten Mittel- bzw. Südosteuropas und Nordosteuropas verglichen. Dafür wurde die elektrophoretisch feststellbare genetische Variation an 19 kodierenden Genloci (10 Enzymsysteme) von 15 Provenienzen des internationalen Fichtenprovenienzversuchs der IUFRO von 1964/1968 ermittelt. Die wichtigsten Ergebnisse lassen sich folgendermaßen zusammenfassen:

1. An den Genloci SKDH-A, IDH-A, LAP-A und GDH-A zeigten manche Allele ein deutliches geographisches Muster (*Tab. 3*).

2. Die Maße der genetischen Variation innerhalb der Populationen wie mittlere Allelanzahl pro Locus (A/L), Anteil polymorpher Loci (P%), die erwartete Heterozygotie (He) und die hypothetische gametische Multilocus-Diversität nach GREGORIUS (1978) zeigten in Süd- und Mitteleuropa niedrigere Werte als in Nord- und Nordosteuropa. Die genetische Variation nimmt daher von Süd nach Nord bzw. Nordost zu, wobei zwei Provenienzen vom Balkan auch erhöhte Werte aufwiesen (*Tab. 4*). Aussagen aus Untersuchungen anderer Autoren konnten somit bestätigt werden.

3. Für die meisten Provenienzen wurden signifikante bis hoch signifikante Korrelationen zwischen den genetischen Abständen (D) und den geographischen Entfernungen der Herkunftsorte gefunden.

4. Die Subpopulationsdifferenzierung (D_j) als genetischer Abstand zwischen jeder einzelnen Provenienz und dem Komplement aller anderen Provenienzen war bei den südlichsten Provenienzen (Südwestfrankreich und Nordgriechenland) und einer ostrussischen Provenienz am höchsten (*Tab. 6, Abb. 3*).

In der Diskussion wird darauf hingewiesen, dass bei mittel- und südeuropäischen Fichten entlang von Höhengradienten und in Abhängigkeit von der geographischen Breite während der nacheiszeitlichen Wiederbesiedelung zahlreiche für die Art vorteilhafte sowohl morphologische als auch ökologische Differenzierungen entstanden sind. Somit erwies sich die Fichte in Mittel- und Südeuropa trotz eingeschränkter genetischer Variation an den methodisch zugänglichen Genloci als eine durchaus anpassungsfähige Baumart.

Schlagwörter: Picea abies, Elektrophorese, genetische Variation, geographische Variation, Isoenzyme, Provenienzversuche.

Introduction

Former provenance trials on the Norway spruce (*Picea abies* [L.] KARST.) have demonstrated for a number of characters a marked geographic variation in this tree species (KALELA, 1937). If one considers the natural range of Norway spruce, it seems likely that there would be a genetic isolation between populations on the range edges. The reasons for this assumption include not only the great distances separating such populations, but also the circumstance that they have migrated in since the last Ice Age from quite different isolated refuges (SCHMIDT-VOGT, 1977, 1986). A comprehensive knowledge of the characteristics of spruce populations from the whole distribution area was to be gained through a large-scale international inventory provenance-testing programme started 1964/1968 by the IUFRO, in which 1100 provenances were included. On the ca. 20 field trials scattered over Europe and Canada the individual provenances are, however, only represented by about 25 individuals each (KRUTZSCH, 1974, 1975). For a group of these provenances, investigations on allozymic variation could also be carried out later (LAGERCRANTZ and RYMAN, 1990; LIESEBACH, 1994). LAGERCRANTZ and RYMAN (1990) investigated 70 provenances primarily from West Germany, Austria, the former Czechoslovakia, Poland, the former Soviet Union and Sweden (none from the Balkans or the Carpathians). They were able to establish that the genetic variation of the spruce populations

increases on a large scale along a line from Southwestern France to Western Siberia. Both studies were based on relatively small numbers of individuals owing to the numerically limited material (41 or 29 trees per provenance, respectively).

A small group of 52 provenances of the IUFRO provenance-testing programme mentioned above is present on an experimental plot of the Institute of Silviculture of the University of Freiburg in Southern Germany, where each provenance is represented by ca. 20 trees. Since the danger existed that individual trees might die off with time, it seemed appropriate to investigate some of the provenances for isoenzyme-polymorphisms. Thus, the goal of the present investigation was to determine on the basis of the electrophoretically detectable isoenzyme-polymorphisms whether and how the populations from the outlying borders of the Central and Southern European spruce regions differ from those of the Northern European spruce region, which might have been colonized from different Ice-Age refuges. The disadvantage of numerically limited sample size (20 trees per provenance) was tolerated, as analogous investigations, such as those of LAGERCRANTZ and RYMAN (1990), were already available.

Methods

Provenances investigated

The provenances investigated (*Fig. 1, Tab. 1*) are located on an experimental plot of the Institute of Silviculture of the University of Freiburg in the vicinity of Stockach on Lake Constance (Lat.: 47° 51'N; Long.: 9° 01'E). Fifteen provenances were selected, having the places of origin as far apart as possible. These included provenances from the southern borders of the natural range (Southeastern France, Northern Greece, the Carpathians), as well as from Slovakia, from Eastern Poland, from Western Siberia and from Scandinavia. The alpine region was excluded, because information is already available from there (GIANNINI et al., 1991; MÜLLER-STARCK, 1995). In all, 293 trees were investigated (usually 20 trees per provenance) *(Tab. 1)*.

The IUFRO 1964/1968 provenance test with Norway spruce originally encompassed 1300 provenances, of which later only 1100 were ultimately included in the experiment, regardless of whether the seed sources were autochtonous or not. Provenances Nr. 8 and 15 given in *table 1* are two for which the material was not included in this experiment, so they have no experimental IUFRO number. Every provenance was characterized as belonging to one of six "seed classes".

1. Single tree progeny;
2. Multiple tree progeny from a single stand;
3. Seed stock from 10 to 20 trees of a single stand;
4. Seed stock from a single stand for practical purposes;
5. Seed stock from several neighboring stands;
6. Commercial seed stock from a forest district.

Two provenances (Nr. 4, Mpoukowaki, Northern Greece and Nr. 12, Hurdal, Norway) turned out to belong to seed class 1 and possibly should not be compared with the other groups. Because of the non-availability of other material from the regions involved these two provenances had to be accepted for this investigation. In any case the information on the seed class obtained was rated as not very reliable.

Electrophoresis

The trees of the provenances investigated were 29 years old at the time of the study (1993). Since they had not yet fructified, the investigation could not be performed on endosperm and was done on buds which had been removed from the trees in the winter of 1993. The genetic variation was determined and compared at a total of 19 coding gene loci from 10 enzyme systems, whose genetic control was already established *(Tab. 2)*. The enzyme crude extracts were prepared from 5 to 8 buds of a tree. The methodological details of the enzyme crude extraction, the starch-gel electrophoresis and the staining of the gels, as well as the composition of the gel buffer, starch gels, and electrode buffer, along with separation conditions, are based on those given by CHELIAK and PITEL (1984), and the newest instructions of KONNERT and MAURER (1995).

Table 1. – Investigated spruce provenances with their country of origin, the geographic location, the seed class, the IUFRO- and locality numbers, as well as the number of experimental trees per provenance in this study. Source: unpublished computer catalog of the Bundesforschungsanstalt für Forst- und Holzwirtschaft, Institut für Forstgenetik und Forstpflanzenzüchtung, D-22927 Grosshansdorf, Germany.

Sample Nr.	Provenance	Country	Latitude (°N)	Longitude (°E)	Elevation (m)	Seed-class	IUFRO Nr.	Locality Nr.	Number of Trees
1	Autrans	France	45,2	5,6	1250	3	0995	1112	20
2	Stirovaka	Croatia	44,8	15,0	1150	2	0790	5103	20
3	Marischka 2	Bulgaria	–	–	1360	3	0960	5308	20
4	Mpoukowaki	Greece	41,5	24,3	1490	1	0904	5508	20
5	Istebna	Poland	49,6	18,9	540	3	1045	6861	20
6	Lipt. Mikuláš	Slovakia	49,1	19,6	750	4	0923	4166	20
7	Bialowieza	Poland	52,8	23,7	150	3	1131	6231	20
8	Kamenec Pod.	Ukraine	48,4	26,3	224	2	–	7501	19
9	Molvotitsk	Russia	57,5	32,5	200	4	0834	7223	20
10	Udmurtsk	Russia	58,2	52,7	200	4	0166	7431	21
11	Tjumen	Russia	58,2	68,8	100	4	0200	7621	15
12	Hurdal	Norway	60,3	10,6	300	1	0589	8225	20
13	Mo Haerad	Sweden	57,6	13,8	250	4	1092	9213	20
14	Umeå Oestra	Sweden	63,9	19,9	149	6	0134	9701	18
15	Kuopio Ymp.	Finland	62,5	27,4	150	6	–	8723	20

Table 2. – Investigated enzyme systems and gene loci as well as Enzyme Commission Numbers.

Isozyme systems	Scored loci	E. C. – Nr.
Glutamate dehydrogenase (GDH)	A	1.4.1.3.
Glutamate – oxalacetate transaminase (GOT)	A,B,C	2.6.1.1.
Isocitrate dehydrogenase (IDH)	A,B	1.1.1.42.
Leucine aminopeptidase (LAP)	A,B	3.4.11.1.
Malate dehydrogenase (MDH)	A,B,C	1.1.1.37.
Nicotinamide – adenine – dinucleotide dehydrogenase (NADH)	A,B*	1.6.99.3.
6 – Phosphogluconate dehydrogenase (6PGD)	A,B,C	1.1.1.44.
Phosphoglucose isomerase (PGI)	A,B	5.3.1.9.
Phosphoglucomutase (PGM)	A,B	5.4.2.2.
Shikimate dehydrogenase (SKDH)	A,B	1.1.1.25.

*) In starch gels NADH-A stains only weakly or not at all. Nevertheless, it was possible in this study to score this locus by prolonging the time of staining. Therefore, the locus often referred to in the literature as A corresponds to the one indicated here with B.

Data analysis

For the quantification of the genetic variation two groups of parameters were determined.

1. Measures of the genetic variation *within* a population:

a) Number of alleles at all loci investigated (M);

b) Number of alleles per locus (A/L) calculated for all loci;

c) Proportion of polymorphic loci (P%) for all loci;

d) Genetic diversity [hypothetical gametic multi-locus diversity (v_p)] according to GREGORIUS (1978) and MÜLLER-STARCK and GREGORIUS (1986);

e) Population differentiation (δ_T) after GREGORIUS (1987) [δ_T corresponds to the diversity according to NEI (1973) for large sample sizes];

f) Heterozygosity [Ha: actual H., He: expected H. after NEI (1973), Hc: conditional H. after GREGORIUS et al. (1986)].

2. Measures of the genetic variation *between* populations:

a) Genetic distance (d_o) for each individual gene locus and the total distance (D) of populations (provenances) from each other after GREGORIUS (1974) and NEI (1972);

b) Subpopulation differentiation (D_j) after GREGORIUS and ROBERDS (1986), used to compare each provenance with the complement of the other provenances;

c) Cluster analysis using D;

d) Diversity analysis after NEI (1973). The total diversity (H_T) is the sum of the diversity within a population (H_e) plus the diversity between populations (D_{ST}).

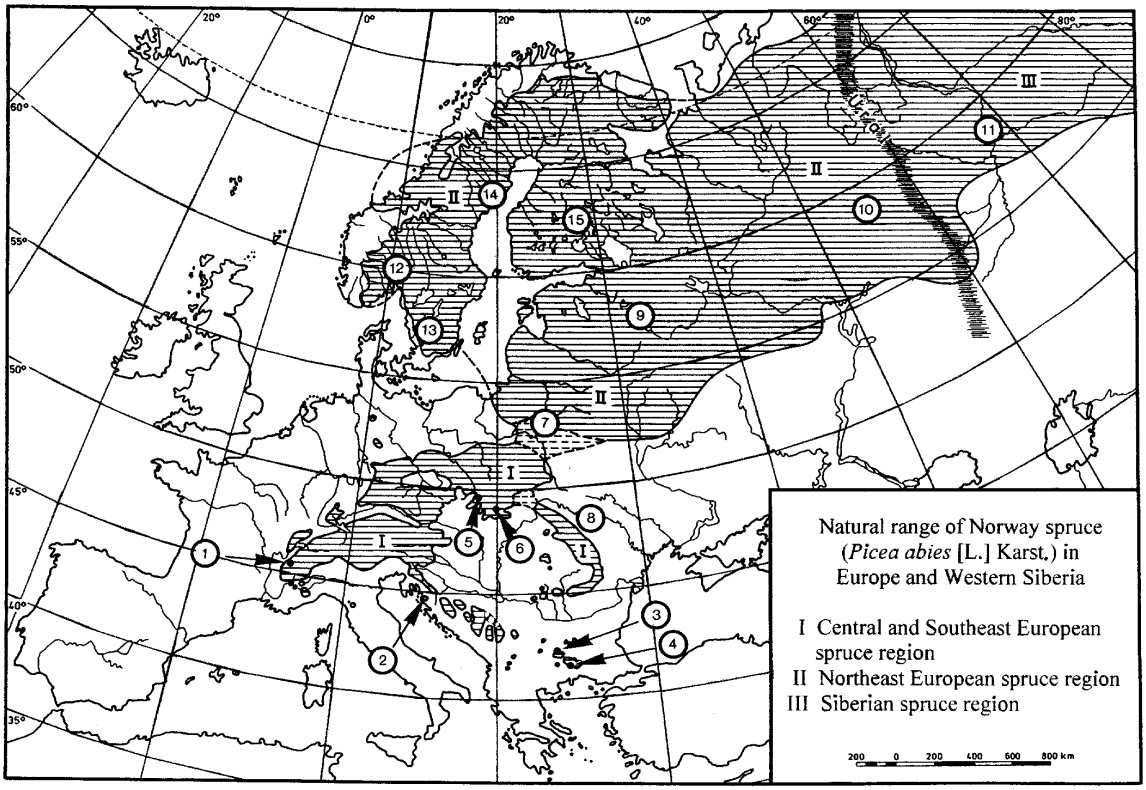

Figure 1. – The European natural range of Norway spruce [from SCHMIDT-VOGT (1977), slightly modified] and the places of origin of the 15 spruce provenances investigated.

Results

Allele frequencies

Of the 10 enzyme systems with altogether 19 coding gene loci investigated, there were 4 gene loci (GOT-A, MDH-B, PGM-A, SKDH-B) which were monomorphic in all 15 provenances. With one exception (Provenance Nr. 15, Kuopio) the locus GOT-B also appeared to be monomorphic. From the calculated results on the allele frequencies at the polymorphic gene loci *(Tab. 3)* the following can be concluded:

– The gene loci SKDH-A, IDH-A, LAP-A and GDH-A were found to be predominantly monomorphic for provenances from Southern and Central Europe.

– Some alleles (SKDH-A4, IDH-A1, NADH-B1*, PGM-B3, LAP-A1,-A3 and GDH-A3) were not found in the southern and southeastern reaches of the spruce region (Provenances Nr. 1 to 4). In contrast, the alleles LAP-B1,-B6 (with one exception) and -B7 were found only there.

– At the NADH-B locus the allele B3 occurred for the southern provenances with the numbers 1 to 4 noticably more frequently than for the remaining provenances. The allele B1* (see below) was particularly frequent for provenance Nr. 5 (Istebna, southern Poland) (22.5%).

– At 8 gene loci several rare alleles were found. There was no apparent relationship between the occurrence of these alleles and the geographic location of the places of origin.

A previously undescribed allele could be identified for two enzyme loci, NADH-B (for 4 provenances) and PGM-B (for 8 provenances). They were named B1* at the NADH-B locus and also B1* at PGM-B *(Tab. 3)*. Thereby the previous numbering for the B-alleles is changed, with each previous number now increased by one. Both alleles are now listed in KONNERT and MAURER (1995), as B1-alleles.

Genetic variation within the populations

For the genetic variation within the populations *(Tab. 4)* the individual parameters significantly increase in going from the south or southwest to the north or northeast (in the *Table* from top to bottom). For example, the *number of alleles* (M) increased from 27 to 42, the number of *alleles per locus* (A/L) from 1.4

Table 3. – Allele frequencies at 15 polymorphic gene loci of the 15 spruce provenances investigated.

Gene locus	Allele	Provenance														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
SKDH	A1	–	–	–	–	–	–	0,025	0,053	–	–	–	0,025	–	–	0,025
	A2	–	0,050	0,050	–	–	–	0,025	0,026	–	0,024	0,077	–	–	–	0,025
	A3	1,000	0,950	0,950	1,000	1,000	0,950	0,950	0,921	0,976	0,952	0,923	0,950	1,000	0,972	0,900
	A4	–	–	–	–	–	–	–	–	0,024	0,024	–	0,025	–	0,028	–
	A5	–	–	–	–	–	0,050	–	–	–	–	–	–	–	–	–
	A6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0,050
IDH	A1	–	–	–	–	0,050	0,025	–	0,053	0,024	0,095	0,033	0,075	–	0,139	0,025
	A2	0,950	1,000	1,000	1,000	0,950	0,975	1,000	0,947	0,976	0,905	0,967	0,925	1,000	0,861	0,950
	A3	0,050	–	–	–	–	–	–	–	–	–	–	–	–	–	0,025
IDH	B1	–	–	0,050	–	–	–	–	–	0,048	0,048	–	–	0,025	–	–
	B2	1,000	1,000	0,950	1,000	1,000	1,000	1,000	1,000	0,952	0,952	1,000	1,000	0,975	1,000	1,000
NADH	A1	–	0,050	0,025	0,194	0,025	0,050	0,050	–	0,048	0,095	0,033	0,111	0,100	0,200	0,075
	A2	1,000	0,950	0,975	0,806	0,975	0,950	0,950	1,000	0,952	0,905	0,967	0,889	0,900	0,800	0,925
NADH	B1	–	–	–	–	0,225	–	–	–	0,071	–	–	–	–	0,028	0,025
	B2	0,225	0,375	0,275	0,150	0,525	0,500	0,350	0,474	0,643	0,405	0,600	0,550	0,500	0,333	0,450
	B3	0,775	0,625	0,725	0,850	0,250	0,500	0,650	0,526	0,286	0,595	0,400	0,450	0,500	0,639	0,525
6PGD	B1	–	–	–	–	–	–	–	0,053	–	–	–	–	–	–	–
	B2	0,825	0,800	0,600	0,675	0,600	0,550	0,575	0,658	0,571	0,643	0,700	0,575	0,447	0,583	0,450
	B3	–	–	–	–	–	–	0,025	0,053	0,048	–	–	–	–	0,028	–
	B4	–	–	–	–	–	–	–	–	0,024	–	–	–	–	–	–
	B5	0,175	0,200	0,400	0,325	0,400	0,450	0,400	0,237	0,357	0,375	0,300	0,425	0,553	0,389	0,550
6PGD	C0	–	–	–	–	–	–	–	–	0,024	–	–	–	–	–	–
	C1	0,425	0,875	0,525	0,625	0,525	0,575	0,500	0,500	0,595	0,738	0,533	0,675	0,658	0,778	0,600
	C3	0,575	0,125	0,425	0,375	0,475	0,425	0,500	0,395	0,381	0,262	0,467	0,325	0,342	0,222	0,400
	C4	–	–	0,050	–	–	–	–	–	–	–	–	–	–	–	–
	C5	–	–	–	–	–	–	–	0,105	–	–	–	–	–	–	–
MDH	C1	–	–	–	–	–	–	0,025	–	–	–	–	–	–	–	–
	C2	–	0,025	0,025	–	–	0,025	0,100	0,105	0,024	–	–	0,025	0,050	–	0,050
	C3	1,000	0,975	0,975	1,000	1,000	0,975	0,875	0,895	0,976	1,000	1,000	0,975	0,950	1,000	0,950
GOT	B1	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0,925
	B2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0,075
GOT	C1	0,650	0,500	0,375	0,100	0,400	0,375	0,350	0,316	0,452	0,595	0,333	0,550	0,375	0,333	0,400
	C2	0,350	0,500	0,625	0,900	0,575	0,575	0,650	0,658	0,584	0,405	0,667	0,450	0,625	0,667	0,600
	C3	–	–	–	–	0,025	0,050	–	0,026	–	–	–	–	–	–	–
PGI	B1	0,400	0,300	0,325	0,475	0,200	0,300	0,200	0,263	0,381	0,500	0,267	0,250	0,225	0,444	0,375
	B2	0,600	0,675	0,675	0,525	0,800	0,675	0,800	0,737	0,619	0,500	0,733	0,600	0,750	0,500	0,600
	B3	–	0,025	–	–	–	0,025	–	–	–	–	–	0,150	0,025	0,056	0,025
PGM	B1	–	–	0,050	0,100	–	–	–	0,026	0,048	0,024	–	–	0,075	0,056	0,100
	B2	1,000	1,000	0,950	0,900	1,000	0,975	1,000	0,947	0,929	0,810	0,800	0,950	0,925	0,722	0,825
	B3	–	–	–	–	–	0,025	–	–	0,024	0,167	0,200	0,050	–	0,222	0,075
LAP	A1	–	–	–	–	–	–	0,050	0,053	0,167	0,262	0,077	0,200	0,075	0,111	0,125
	A2	1,000	1,000	1,000	1,000	1,000	1,000	0,925	0,947	0,810	0,667	0,923	0,725	0,925	0,889	0,850
	A3	–	–	–	–	–	–	0,025	–	0,024	0,071	–	0,075	–	–	0,075
LAP	B1	–	–	0,050	–	–	–	–	–	–	–	–	–	–	–	–
	B2	–	0,025	0,125	–	–	–	–	–	0,048	0,048	–	0,075	0,025	–	0,025
	B3	0,250	0,175	0,075	0,450	0,150	0,200	0,400	0,184	0,262	0,095	0,167	0,175	0,075	0,111	0,150
	B4	0,600	0,725	0,625	0,475	0,850	0,800	0,600	0,790	0,643	0,762	0,800	0,700	0,900	0,806	0,800
	B5	–	–	0,025	–	–	–	–	–	0,048	0,095	0,033	0,050	–	0,083	0,025
	B6	0,150	0,075	0,075	0,025	–	–	–	0,026	–	–	–	–	–	–	–
	B7	–	–	0,025	0,050	–	–	–	–	–	–	–	–	–	–	–
GDH	A2	1,000	1,000	1,000	1,000	0,975	1,000	0,975	0,921	0,929	0,643	0,833	0,725	0,925	0,722	0,750
	A3	–	–	–	–	0,025	–	0,025	0,079	0,071	0,357	0,167	0,275	0,075	0,278	0,250

to 2.2, and the proportion of *polymorphic loci* (P %) from 37% to 74%. For all three parameters, the highest values were found for the Russian provenance Molvotitsk (Nr. 9) and the Finnish provenance Kuopio (Nr. 15), and the lowest for the French alpine provenance Autrans (Nr. 1) and the Greek provenance Mpoukovaki (Nr. 4). The provenance Tjumen (Nr. 11) was only represented in the investigation by 15 experimental trees and for this reason seemed possibly genetically poorer.

The proportion of *observed heterozygosity* (Ha) lay between 19% and 31% (mean: 24%). The highest Ha-values were found for most of the Russian and Scandinavian provenances (Nr. 9, 10, 12, 14 and 15) and two Balkan provenances (Nr. 3 and 4). The *expected heterozygosity* (He) lay between 18% and 30% (mean: 23.5%). The deviations between Ha and He were minimal and could be neglected. The *conditional heterozygosity* (Hc), which gives the realized percentage of the maximal heterozygosity, was on average 68.5%. Above-average values of 72% to 82% were seen for the provenances of Southern and Western Europe (Nr. 1 to 4). Although the proportion of heterozygous genotypes (Ha) is higher in the northern area, here a noticeably lower proportion of the maximal heterozygosity is reached, with the exception of provenance Nr. 14 with 76%. The *hypothetical gametic multilocus-diversity* (v_p) reveals the potential for the creation of genetic variation in the next generation and thereby indicates the potential for adaptation. Here differences in the genetic diversity become particularly evident because the method of its calculation has a multiplicative effect. The Croatian provenance Stirovaka (Nr. 2), with 28.6, shows the lowest value, and the Russian-Scandinavian provenances (Nr. 9, 10, 12, 14 and 15) the highest (183 to 344), with the exceptions of Mo Haerad (Nr. 13) and Tjumen (Nr. 11). The same sequence found for v_p was also seen for *population differentiation* (δ_T), which gives, as a percentage, the differentness of the individuals within the populations.

Genetic variation between the populations

The differences between populations could be quantified with the use of the parameter *genetic distance* (D) after GREGORIUS (1974) and NEI (1972) *(Tab. 5)*. The genetic distances (x1000) lie between 47 and 166 according to GREGORIUS (1974) and between 6 and 72 according to NEI (1972). For 10 of the investigated provenances (Nr. 1 to 8, 14 and 15) a close correlation was found between D and the geographic distance of the places of origin. For the North Greek provenance Mpoukowaki (Nr. 4), coming from the most southerly reaches of the spruce range, this correlation was also significant, but the D-values were all higher relative to all other provenances. The D-values were, in contrast, lowest for the Siberian provenance Tjumen (Nr. 11), lying behind the Ural, and there was no correlation with the distances from the places of origin. For the provenances Nr. 2, 3, 5, 6, 7 and 8, the correlations between genetic distance and geographic separation of the places of origin were highly significant (r = 0.81*** to 0.93***); for the provenances with the numbers 1, 4, 14 and 15 they were significant (r = 0.69** to 0.78**). This shows that the differentiation between the populations can at least in part be attributed to genetic isolation. For the provenances Nr. 9 to 13, all from Northern and Northeast Europe, there were no unequivocal correlations. The calculations were carried out using the D-values according to GREGORIUS (1974). According to GRANNINI et al. (1991), this method appeared better suited for the geographic concept of the distance since these values represent metric data.

A dendrogram *(Fig. 2)* shows the results of the cluster analysis, which was performed on the basis of the distance-values (D) according to GREGORIUS (1974). These values are the measure of the relative similarity of the individual groups. There are four recognizable similarity groups. The first and last of these groups are each made up of two provenances, which also showed the largest D-values compared to all the

Table 4. – Genetic variation, heterozygosity, diversity and differentiation of the 15 spruce provenances investigated. M: number of alleles; A/L: mean number of alleles per locus; P(%): proportion of polymorphic gene loci based on the 99% criterion; Ha, He, Hc: mean observed, expected and conditional heterozygosities in %; vp: hypothetical gametic multilocus-diversity according to GREGORIUS (1978); δ_T(%): Population differentation according to GREGORIUS (1987). The first five columns are for all 19 gene loci investigated; the last two are for the 15 polymorphic loci.

Provenance	M	A/L	P(%)	Ha (%)	He (%)	Hc (%)	v_P	δ_T (%)
1 Autrans	27	1,42	36,8	20	18	73	36	19
2 Stirovaka	31	1,63	47,4	19	18	75	29	19
3 Marischka	36	1,89	57,9	25	22	76	82	23
4 Mpouwaki	29	1,53	42,1	24	19	82	44	20
5 Istebna	30	1,58	47,4	19	19	63	47	20
6 Lipt. Mikuláš	32	1,68	57,8	20	21	60	58	22
7 Bialowieza	34	1,79	57,8	22	22	65	74	23
8 Kamenec Pod.	37	1,95	63,2	23	23	67	98	25
9 Molvotitsk	41	2,16	73,7	26	26	68	183	27
10 Udmurtsk	37	1,95	68,4	27	30	61	344	32
11 Tjumen	32	1,68	63,1	23	24	69	91	26
12 Hurdal	37	1,95	68,4	26	29	61	313	31
13 Mo Haerad	34	1,79	63,2	22	23	66	77	24
14 Umeå	36	1,89	63,2	31	29	76	263	30
15 Kuopio	42	2,21	73,7	29	29	67	289	31

Table 5. – Total genetic distances D (x 1000) between the 15 provenances after GREGORIUS (1974) (above the diagonal) and after NEI (1972) (below the diagonal). P: Provenance

P	1	2	3	2	5	6	7	8	9	10	11	12	13	14	15
1		80	85	110	110	97	100	111	122	153	126	144	149	165	148
2	33		75	121	97	67	92	90	106	126	103	111	105	126	122
3	21	23		98	83	60	68	85	90	130	96	122	91	128	108
4	47	45	33		140	111	108	130	133	166	139	164	137	130	148
5	34	30	19	57		47	75	74	81	154	71	111	82	139	108
6	27	20	11	38	7		67	62	70	134	68	89	62	116	77
7	25	33	18	24	20	12		64	92	154	86	117	86	130	107
8	27	22	13	38	10	6	13		93	143	61	111	82	127	94
9	31	25	21	43	15	10	18	14		113	85	78	83	123	88
10	46	27	39	72	44	34	55	37	25		118	73	124	77	102
11	33	27	19	47	11	9	20	6	13	31		97	90	99	83
12	38	22	27	61	23	17	29	21	10	11	18		87	100	81
13	51	27	20	59	13	8	27	15	18	30	16	16		117	69
14	55	23	31	43	36	24	45	27	28	15	24	21	21		91
15	41	29	18	47	17	9	24	14	13	19	13	12	8	14	

other provenances (see *Tab. 5*). The provenances from the Central or Southern European spruce region and the Northeastern European spruce region (with the exception of Nr. 11) remain separated. Thus the dendrogram appears to reflect a special geographic pattern.

An additional measure of differentiation, the *subpopulation differentiation* (D_j) *(Tab. 6)*, is based on the comparison of allele and genotype frequencies. Each provenance represents a subpopulation, whose agreement with the complement made up of all the others is calculated. As regards the distribution of alleles, of all provenances Liptovský Mikuláš (Nr. 6) is the most "typical". Mpoukowaki (Nr. 4) and Autrans (Nr. 1) would appear to be most "atypical", since their allele structures most poorly match the overall picture.

The *subpopulation differentiation* (D_j) at the individual loci is graphically illustrated in the form of *differentiation spirals (Fig. 3)*. For example, for the GOT-C locus, provenance Nr. 4 had the highest D_j value (38%). At the individual loci the provenances are differentiated to different extents. The provenances Mpoukovaki (Nr. 4), Udmurtsk (Nr. 10) and Umeå (Nr. 14) frequently show high D_j-values. However, an unequivocal ranking order is not evident. The average of all D_j values at one locus is the *average differentiation* (δ) over all provenances (subpopulations). There is an especially strong differentiation of the allele variants at the loci NADH-B, LAP-B, and GDH-A, with δ-values of 15%, 14.7% and 11.4% for their alleles. The differentiation spirals of the loci SKDH-A, IDH-A/B, MDH-C and GOT-B are not shown, because their very low differentiation values (δ≤2%) cannot be satisfactorily represented visually as small-sized radial segments.

The diversity analysis according to NEI (1973), was introduced for comparison with other investigations. According to this analysis, the *total diversity* (H_T) was 0.252, the diversity within the provenances (H_e) 0.235, and the diversity between provenances (D_{ST}) 0.017 (G_{ST} = 0.067). Thus the overall genetic diversity between the provenances was 6.7%. G_{ST} is the relative proportion of D_{ST} compared to the total diversity H_T.

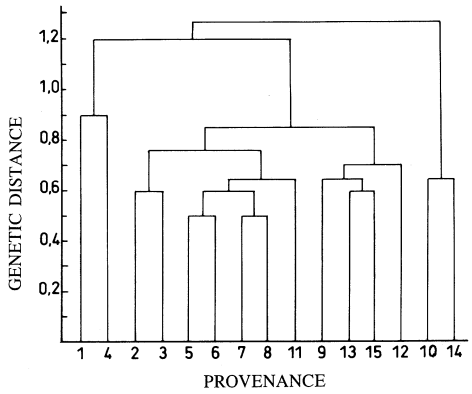

Figure 2. – Dendrogram of the 15 provenances based on the genetic distance (D) after GREGORIUS (1974).

Discussion

Before beginning the discussion it should be made clear that the experimental trees involved here, as well as those in the investigations of LAGERCRANTZ and RYMAN (1990) and LIESEBACH (1994), were derived from seed stock which had been planted in 1964 in the vicinity of Hamburg. There the prevailing selection pressures during germination and in the seedling stage were surely different from those in the original home of the trees. Based on the account of GREGORIUS et al. (1979), there is a certain probability that the genotypic compositions of the different provenances may not be precisely identical with

Table 6. – Subpopulation differentiation (D_j) in order of decreasing values. P: Provenance.

P	4	1	10	14	5	12	2	7	3	11	13	8	15	9	6
D_j%	11.8	11.5	10.6	9.9	8.1	8.0	7.8	7.6	7.1	7.0	6.9	6.4	6.2	5.9	5.1

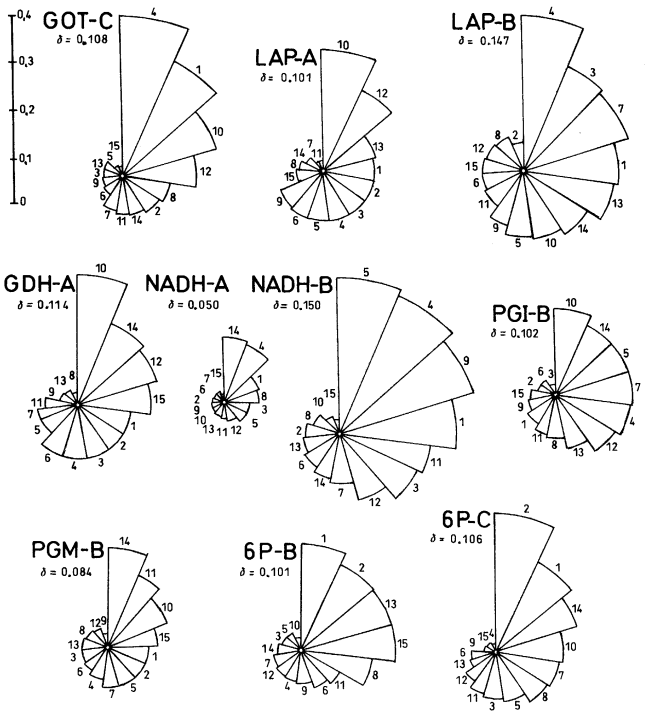

Figure 3. – Differentiation spirals for 10 gene loci for 15 provenances of Norway spruce (representation form after GREGORIUS, 1985). For a particular locus the sector radius represents the differentiation D_j for each numbered provenance (see scale at upper left; the value 1.0 equals 100%). δ gives the level of the average differentiation at each individual locus. The sector angle indicates the size of the sample.

those of the original populations. However, this difficult-to-estimate uncertainty had to be accepted, especially since it pertained equally well to all provenances.

Some of the results presented here as well as those of some similar investigations (LAGERCRANTZ and RYMAN, 1990; GIANNINI et al., 1991; KONNERT, 1991; LÖCHELT, 1993; LÖCHELT and FRANKE, 1993; LIESEBACH, 1994; MÜLLER-STARCK, 1995; KRUTOVSKIJ and BERGMANN, 1995), have been presented together in summary form for comparison (*Tab. 7* and *8*).

Most of the values given in *table 7* are quite comparable. But it is clear that the individual investigations are based on different sample sizes (20 to 100 experimental trees per population investigated) and were in part carried out with different enzyme systems. Hence, there are quite substantial variations in the values of the parameters A/L, P(%) and ML. For the investigations based on larger populations (LÖCHELT and FRANKE, 1993; MÜLLER-STARCK, 1995), more alleles per locus and more polymorphic loci were found than in the other investigations; moreover, no universally monomorphic loci were found. Hence, for small sample sizes, a particular gene locus may be observed to be monomorphic and all rare alleles in a population may not be detected (GREGORIUS, 1980). And even a respectable sample size of 100 trees per population, as in MÜLLER-STARCK (1995) does not necessarily guarantee a complete detection of all the genetic variation present. For example, in one of the stands investigated by MÜLLER-STARCK (1995) (*Le Brassus*) the loci GDH-A, MDH-B, GOT-B and IDH-A were found to be monomorphic. However, an extensive investigation of the youngest tree layer of this stand (THUMM, 1995) revealed two alleles for the GDH-A locus and three for MDH-B! In order to minimize such uncertainties, the current recommendation is to sample 400 trees per population (KÖHL et al., 1997).

The values for the intra- and interpopulation differentiation from the investigations *(Tab. 8)* have been obtained for the most part from populations of the Central and Southeastern European spruce region (spruce region I). Only the results of LAGERCRANTZ and RYMAN (1990) and those of this study are also derived from populations of the Northeastern European spruce region (spruce region II). In order to permit a comparison of the results from the last two studies mentioned, the items in *table 8* have been arranged according to the spruce regions mentioned above (see *Fig. 1*).

The comparison of the values in that table for the parameters *population differentiation* (δ_T), *heterozygosity* (Ha, He, Hc), *genetic distance* (D) and *subpopulation differentiation* (D_j) reveals relatively good agreement. Only the heterozygosity values of LAGERCRANTZ and RYMAN (1990) are of note upon comparison, being strikingly low. The values for the diversity between the populations (G_{ST}) lie considerably below 10% of the values for total diversity, as with other conifers (cf., MITTON, 1983; LEDIG, 1986).

Table 7. – Compilation of several parameters of genetic variation from various investigations. N: number of experimental trees per investigated population; ES: enzyme systems; L: gene loci; A/L: alleles per locus; P(%): proportion of polymorphic loci; EM: experimental material; ML: monomorphic loci in all populations. For better comparison, the data of some authors has been recalculated for all loci including the monomorphic ones.

Authors	N	ES	L	A/L	P(%)[1]		EM	ML
					95%	99%		
Lagercrantz & Ryman (1990)	41	12	22	1.4-1.8	27-54		Bud	6
Franke & Konnert (1990)	13-32	6	11	1.5-2.2		45-64	Endosp	2
Giannini et al. (1991)	≤40	11	21	1.7-2.0	45-52	52-71	Endosp	4
Löchelt & Franke (1993)	31-90	10	14	2.0-2.6		64-86	Bud	0
Liesebach (1994)	29	8	9	1.4-2.1		33-78	Bud	2
Müller-Starck (1995)	100	13	18	2.4-2.7		77-95	Bud	0
Krutovskij & Bergmann (1995)		14	26	2.3-2.9	53-62		Endosp	0
This study	20	10	19	1.4-2.2		37-74	Bud	4

[1]) A gene locus is considered polymorphic when the frequency of the most frequent allele does not exceed either 95% or 99%.

Table 8. – Summary of additional parameters of the genetic variation within and between populations from various investigations. δ_T: Population differentiation after GREGORIUS (1987) in %; Ha, He, Hc: actual, expected and conditional heterozygosities in %; D: genetic distance (x1000) after NEI (1972) (in parentheses) and after GREGORIUS (1974); D_j: subpopulation differentiation after GREGORIUS and ROBERDS (1986) in %; G_{ST}: Diversity between populations in % of the total diversity after NEI (1973); I.: Central and Southeastern European spruce region; II.: Northern and Northeastern European spruce region.

Authors	δ_T (%)		Ha(%)		He(%)		Hc(%)		D	D_j(%)	G_{ST}(%)
	I.	II.	I.	II.	I.	II.	I.	II.			
Lagercrantz & Ryman (1990)	–	–	7-12	8-19	7-10	10-17	–	–	(5-25) –	–	5,2
Franke & Konnert (1990)	–	–	17-36	–	19-31	–	–	–	– 24-158	3-9*	4,5
Giannini et al. (1991)	–	–	–	–	16	–	–	–	(2-42) 31-90	–	7,8
Löchelt & Franke (1993)	22-26	–	20-25	–	–	–	–	–	– 24-85	2-6	2,2
Liesebach (1994)	–	–	13-24	–	–	–	–	–	(3-53) 33-143	3-9	–
Müller-Starck (1995)	25-30	–	20-28	–	–	–	55-66	–	– –	3-7	–
Krutovskij & Bergmann (1995)	–	–	–	–	19	25-28	–	–	(5-49) –	5-13	–
This study	18-23	23-32	18-25	22-31	17-22	22-30	60-82	61-76	(2-72) 42-166	5-12	6,7

*) Values from KONNERT and FRANKE (1990)

Our own results presented here, as well as those of KRUTOVSKIJ and BERGMANN (1995) show that the genetic variation for populations from spruce region I is less than that of populations from spruce region II. LAGERCRANTZ and RYMAN (1990) have attempted to explain this phenomenon using a „bottleneck-hypothesis". According to this hypothesis, the spruce migrated into Central Europe from two Ice-Age refuges, one at the foot of the Carpathians, the other in the region of the Dinarian Mountains, There, according to FRENZEL (1968), spruce survived the last Ice Age in small, island-like forest occurrences, where the authmors propose that, due to the small population sizes, losses of genetic variation occurred. The time of ca. 10,000 years since the beginning of the reinvasion of the spruce into the Central European area was possibly insufficient for the spectrum of the allele frequencies of the populations, reduced in the "bottleneck", to return up to a steady-state or equilibrium level through mutation and/or migration. This formal genetic point of view corresponds approximately to the current interpretative possibilities. Put simply, the spruce populations from the Central and Southeastern European spruce region are seen as being, so to speak, "unfinished". According to these authors, this is also the reason why especially in the Central European area the spruce seems to be so sensitive to anthropogenically caused environmental changes.

Some of the results of the provenance research make this hypothesis, from a genetic point of view completely plausible, problematical.

– Thus the re-migration of the spruce back into Central Europe after the last Ice Age took place not only from east to west, but also from the south from a refuge on the Apennine Peninsula (SCHMIDT-VOGT, 1977; GIANNINI et al., 1991). Recently, it has even been speculated that the spruce migrated into western Switzerland from the southwest (MÜLLER-STARCK, 1995). Our previous views on the vegetation in Central Europe immediately after the last Ice Age may need to be revised, since KULLMAN (1996) has been able to demonstrate that the spruce was already present in Central Sweden 8000 years ago. Based on findings from pollen analysis, its extension limit at that time had been thought to be 1500 km further east.

– In evaluating the so-called spruce decline in Central Europe, it is sometimes overlooked that the spruce has largely been cultivated artificially for more than 150 years, often without considering the provenance problem and even outside its natural region. Moreover, in fumigation experiments, e.g. with realistic ozone concentrations, it has proven itself to be relatively robust (GROSS, 1994).

– In the light of previous provenance experiments, the spruce in Europe has already shown itself as a quite adaptable tree species (KALELA, 1937). If we limit our considerations to the Central European and Southeastern European spruce region (spruce region I), we find the climatically most favorable locations for spruce stands, with longer growing seasons at lower altitudes. In accordance with GRIME (1977), at most of these sites the plant species exist by using the strategy of competitive ability, which is characterized by a high growth capacity (high production of biomass) of the populations. Thus, one finds the most productive and adaptable spruce populations precisely in Central and Southeastern Europe (KRUTZSCH, 1974; SCHMIDT-VOGT, 1976), and there especially in the lower elevations of the Carpathians (GIERTYCH, 1976), i.e. in the vicinity of one of the Ice-Age refuges. With increasing altitude the emphasis shifts more to a strategy characterized by slower growth, namely that of stress tolerance. Correspondingly, the morphological and phenological properties of populations from low-lying locations and elevated sites are very different (ENGLER, 1913; NÄGELI, 1931). From an ecological viewpoint such populations behave in the extreme case like two different species. Neither is likely to survive in the long term when planted in the other one's habitat.

Such genetic adaptations of benefit for the species evidently must have arisen in the past 10,000 years for the Central European populations despite their observed reduced variation

at the gene loci which are methodologically accessible at present. The work of LIESEBACH (1994) may be regarded as an important contribution to the resolution of this contradiction. This author was able in his investigations to rule out unequivocally any relation between the isoenzyme polymorphisms and quantitative (often polygenically determined) characteristics such as growth rate and growth form. It may be concluded that while the isoenzyme analyses carried out previously permit deep insights into the genetic structure of populations, to answer the questions raised above they are evidently still not adequate.

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