Handbuch der Laubgehölze. Volume III. 416–423. Verlag Paul Parey, Hamburg and Berlin (1978). — LÖCHELT, S. and FRANKE, A.: Genetic structures of different levels of damage on permanent observation plots on forest decline in Baden-Württemberg. Research Report KfK–PEF 108. Kernforschungszentrum, Karlsruhe (1993). — MARKERT, C. L. and FAULHABER, L.: Lactate dehydrogenase isozyme patterns of fish. Journal of Experimental Zoology **159**: 319–332 (1965). — MAURER, W. D.: Isozymic and morphological investigations on linden. In: Proceedings of the 22nd Meeting of the Working Party of Forest Genetics and Tree Breeding. W. D. MAURER and U. TABEL (eds.). Mitteilungen der Forstlichen Versuchsanstalt Rheinland-Pfalz **34**, 234–243 (1995). — MAURER, W. D. and TABEL, U.: Erhaltung forstlicher Genressourcen in Rheinland-Pfalz. Allgemeine Forst Zeitschrift **2**: 102–105 (1995a). — MAURER, W. D. and TABEL, U.: Conservation of genetic resources of *Tilia* tree species in Rheinland-Pfalz. In: Population genetics and genetic conservation of forest trees. P. BARADAT (ed.). SPB Academic Publishing bv, Amsterdam, in press (1995b). — MEIZEL, S. and MARKERT, G. L.: Malate dehydrogenase isozymes in the marine snail *Ilyanassa obsoleta*. Archives of Biochemistry and Biophysics **122**: 753–765 (1967). — POULIK, M. D.: Starch gel electrophoresis in a discontinous system of buffers. Nature **180**, 1477–1478 (1957). — RHODES, M. J. C.: The extraction and purification of enzymes from plant tissue. Proceedings of the Phytochemical Society **14**, 245–268 (1977). — RIDGWAY, G., SHERBURNE, S. and LEWIS, R.: Polymorphisms in the esterases of Atlantic herring. Trans. American Fisheries Society **99**: 147–151 (1970). — ROTHE, G. M.: Electrophoresis of enzymes. p. 85. Springer Verlag, Berlin, Heidelberg, New York (1994). — ROTHE, G. M. and MAURER, W. D.: One-dimensional PAA-gel electrophoretic techniques to separate functional and denatured proteins. In: Gel electrophoresis of proteins. M. J. DUNN (ed.). 37–140. IOP Publishing Ltd., Bristol (1986). — SEITZ, F. W.: Chromosomenzahlenverhältnisse bei Holzpflanzen. Zeitschrift für Forstgenetik und Forstpflanzenzüchtung **1**: 22–32 (1951). — WEEDEN, N. F. and WENDEL, J. F.: Genetics of plant isozymes. In: Isozymes in plant biology. D. E. SOLTIS and P. S. SOLTIS (eds.). 46–72. Advances in Plant Sciences Series **4**. Dioscorides Press, Portland, USA (1989). — WEIMAR, M., MAURER, W. D. and ROTHE, G. M.: Erste Ergebnisse zum jahreszeitlichen Verlauf der Aktivität von Enzymen des Kohlenhydrat- und Stickstoffstoffwechsels in Fichtennadeln. In: Forschungszentrum Waldökosysteme/Waldsterben, Exkursionsführer Sommer 1985, 273–281 (1985).

Genetic Variation in High Elevated Populations of Norway Spruce (*Picea abies* (L.) KARST.) in Switzerland[1])

By G. MÜLLER-STARCK[2])

(Received 20th October 1995)

Abstract

20 autochthonous populations of Norway spruce (*Picea abies* (L.) KARST.) were studied which are located in mountainous and subalpine vegetation zones in Switzerland. For each of 2000 trees, multilocus genotypes were identified at 18 enzyme coding gene loci.

Genetic inventories revealed large genetic variation within populations and relatively small interpopulational variation. Compared to results from inventories in lower elevated regions northward and southward of the Alps, intrapopulational variation is not smaller in high elevated populations. Generally, frequency distributions of genetic types tend to deviate substantially from evenness.

The geographic variation of allele frequencies supports the hypothesis of postglacial re-immigration extra to the commonly accepted east-west routes. Results serve as criteria for gene conservation *in situ*. Preservation of genetic variability is required particularly under changing environmental conditions which challenge Alpine forest ecosystems.

Key words: Enzyme gene loci, genetic variation, heterozygosity, diversity, differentiation, gene conservation, Norway spruce (*Picea abies* (L.) KARST.).

FDC: 165.3; 165.5; 174.7 *Picea abies;* (494); (234.31).

Introduction

Norway spruce (*Picea abies* (L.) KARST.) is a predominant tree species which ranges from low elevations up to the subalpine vegetation zones. This species is subjected to a very large variety of site conditions. In particular, Norway spruce is an essential element of high elevated forest ecosystems which provide substantial benefits for the human society with respect to various protection and social functions. Generally, Norway spruce belongs to those tree species which are economically significant. Norway spruce is the most common tree species in Switzerland. It covers approximately 40% of the total forest area and makes up 49% of the total wood stock.

The postglacial re-immigration of spruce predominantly occurred from the eastern to the western part of the Alpes (e.g. KRAL, 1979; BURGA, 1988). Generally, re-immigration resulted in a competition between Norway spruce and pioneer species such as birches or pines which immigrated earlier. Silver fir (*Abies alba* MILL.) re-immigrated nearly at the same time as Norway spruce but predominantly the opposite way, i.e. from the western to the eastern parts of the Alps.

The development of the spruce habitat was severely affected by men. Inferences such as grazing or clearing by fire started already during the Neolithic Age around 3500 B.C. and became intensified during the Roman era and succeeding periods (e.g. exploitation following mining, salt works, charcoal-burning). These and other forms of ancient forest utilization modified substantial parts of high elevated forest ecosystems or replaced them. At least during the last two centuries, forestry interfered in the geographic distribution and the composition of tree species. Norway spruce was favoured by forest management because of its economic significance.

[1]) Dedicated to G. H. MELCHIOR

[2]) Lehrbereich Forstgenetik, Forstwissenschaftliche Fakultät der Universität München, Hohenbachernstraße 22, D-85354 Freising-Weihenstephan, Germany
(former address: Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft, Zürcherstrasse 111, CH-8903 Birmensdorf-ZH, Switzerland)

Because of these impacts, it is not possible to find populations which are autochthonous in a very strict sense. It cannot be excluded that the supposed autochthonous populations are partly or fully non-indigeneous in reality. In order to minimized the chance for misclassification, populations were carefully selected which were described by silviculturists and by documents of the Forest Service as autochthonous and show heterogeneous age and stand structures.

It is the aim of the present paper to describe intra- and interpopulational genetic variation in Norway spruce populations in Switzerland and to contribute to the characterization of genetic resources and to practical measures for preservation of genetic variation *in situ*. Genetic variation is considered to be a substantial determinant of adaptive abilities of populations (e.g. HAMRICK and GODT, 1989; SCHOLZ *et al.*, 1989; HATTEMER and GREGORIUS 1990; GREGORIUS, 1991; MÜLLER-STARCK and ZIEHE, 1991).

Characterization and preservation of genetic variation is essential in the case of tree populations which are exposed to a large variety of abiotic and biotic stress factors in time and space. Environmental heterogeneity cannot be controlled because prophylactic measures of control of environmental stress like in agricultural crops cannot be realized in long-lived tree populations. Future stress constellations following global warming will further challenge the adaptability of tree populations, which is determined by genetic variation in the present and future generations.

Material and Methods

Populations

Twenty populations were selected at different parts of the Swiss Alps and the Jura Mountains (*Figure 1*). According to studies of the Department of Silviculture, ETH Zürich (P. BONFILS, pers. comm.) and documents of the Forest Service, populations are recorded to be autochthonous. Apparently, populations reveal different age classes with natural regeneration in certain parts. The age of the studied trees approximately ranges between 50 years and a maximum of 200 years. The exact age is unknown. Population densities can vary considerably but none of the populations is recorded to reveal under average densities according to forest inventory standards.

Populations belong to the high mountaineous or sub-alpine vegetation zone. In each population, plot size is approximately 10 ha with a total of 100 studied trees per plot. Tree selection was based on a square grid system with an average distance of 33 m. Stands are enumerated in west-east direction. As can be seen from *table 1*, last column, Norway spruce is the predominant species in most populations. There are 3 exceptions: in population No. 4 (Gstaad) Norway spruce is mixed with Silver fir (*Abies alba*), in No. 8 (Aletschwald) predominantly with European larch (*Larix decidua*), and in No. 16 (Bondo) with *Abies alba* and *Larix decidua*. In the remaining populations, trees from other species are rare (mostly *Abies alba*).

Table 1. – Survey of site and stand characteristics of Norway spruce populations.

Pop. No.	Local designation	Sample size	Elevation (m)	Exposure	Inclination (°)	Spruce (%)
1	Le Brassus	100	1180–1200	S-O	0–5	85–90
2	Chaux du Milieu	100	1120–1160	N	0–5	80–90
3	Orsières	100	1300–1600	W	30–35	95
4	Gstaad	100	1260–1500	N-W	15–20	40–50
5	Adelboden	100	1460–1600	N	20	100
6	Saxeten	100	1400–1580	N-O	20–25	95–100
7	Grindelwald	100	1750–1810	N-O	15–20	95
8	Aletschwald	100	1650–1800	W	15–20	10–95
9	Simplon	100	1500–1680	S-W	25–30	85–90
10	Engstlenalp	100	1700–1800	W	15–20	90
11	Oberwald	100	1450–1680	N-W	20–25	85–95
12	Faido	100	1500–1640	S-W	5–15	100
13	Bödmeren	100	1500–1540	N	5–10	95–100
14	Scatlè	100	1580–1900	O	25–30	95–100
15	San Bernardino	100	1630–1650	S	0–5	100
16	Bondo	100	1350–1700 *	N-W	30–40	20–70
17	Rona	100	1580–1680	N-W	5–20	100
18	Conters	100	1700–1800	N-O	15–20	100
19	Poschiavo	100	1500–1750	N	25–35	95–100
20	Ardez	100	1500–1760	N	20–25	85–90

*)Two separate populations with 50 trees each (1350 m to 1400 m, 1630 m to 1700 m)

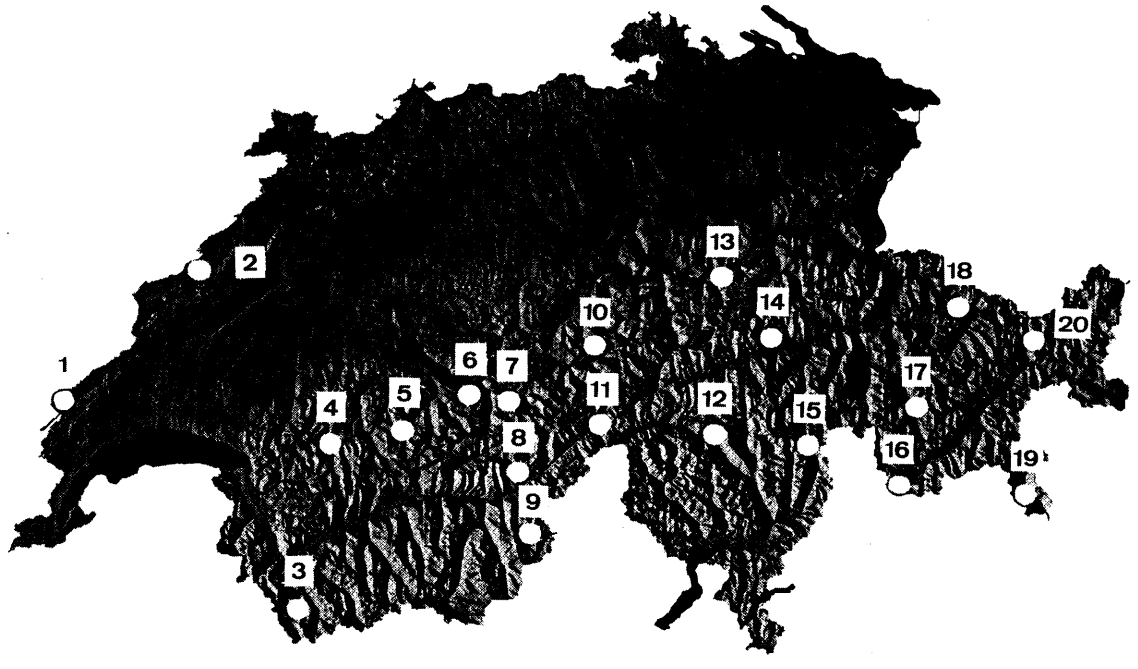

Figure 1. – Location and desination of studied populations of Norway spruce in Switzerland.

Electrophoretic methods and genotyping

Enzymes were separated by horizontal starch gel electrophoresis. For survey of electrophoretic methods see *table 2* and MÜLLER-STARCK (1995). The mode of inheritance of isoenzymes was verified by studying segregations among the offspring from controlled crosses at the seed stage (analysis of endosperm and corresponding embryo). The hypothesis of conformity with the expected Mendelian segregations was tested by utilizing full sib families (MÜLLER-STARCK, in prep.).

Table 2. – Survey of enzyme systems, E. C. No., electrophoretic methods and enzyme coding gene loci in buds and seeds of Norway spruce (MÜLLER-STARK, 1995, modified).

Enzyme system	E.C. No.	Buffer#	Gene locus
Alanin aminopeptidase (AAP)	3.4.11.1	1	AAP-B [1]
Aconitase (ACO)	4.2.1.3	1	ACO-A [1]
Diaphorase (DIA)	1.6.99.-	1, 2	DIA-A [3]
Glutamate dehydrogenase (GDH)	1.4.1.2	2	GDH-A [4]
Glutamate-oxaloacetate transaminase (GOT) *	2.6.1.1	1	GOT-A_2-B,-C [2]
Isocitrate dehydrogenase (IDH)	1.1.1.42	2	IDH-A [2]
Leucine aminopeptidase (LAP)	3.4.11.1	1	LAP-A,-B [1]
Malate dehydrogenase (MDH)	1.1.1.37	3.4	MDH-B,-C [2]
NADH-dehydrogenase (NDH)	1.6.99.3	1	NDH-A [4]
6-Phosphogluconate dehydrogenase (6PGDH)	1.1.1.44	3.4	6PGDH-B,-C [2]
Phosphoglucose isomerase (PGI)	5.3.1.9	1	PGI-B [2]
Phosphoglucomutase (PGM)	5.4.2.2	2	PGM-A [1]
Shikimate dehydrogenase (SKDH)	1.1.1.25	2	SKDH-A [1]

[1]) monomer, [2]) dimetric, [3]) tetrametric, [4]) polymetric
*) = aspartate aminotransferase (AAT)
#) Electrode and gel buffers:

No	Electrode buffer/pH	Gel buffer/pH
1	0.05 M LiOH-0.19 M boric acid/8.1	0.05 M tris-0.01 M citric acid/8.1
2	0.14 M tris-0.044 M citric acid/7.5	Electrode buffer: H_2O = 1 : 2.5
3	0.14 M tris-0.044 M citric acid/7.5	Electrode buffer: H_2O = 1 : 5
4	0.14 M tris-0.044 M citric acid/7.5	0.04 M tris-0.001 M EDTA-a 0.05 M histidinHCl/6.3

Quantification of genetic variation

Intrapopulational variation was measured by means of the number of alleles per locus (A_L), the actual (observed) heterozygosity (H_a), the conditional heterozygosity (H_C, GREGORIUS et al., 1986) and the genic diversity (GREGORIUS, 1978, 1987). In addition, the intrapopulation differentiation (δ_T, GREGORIUS, 1987) was calculated which is equal to NEI's "gene diversity" (H_e, NEI, 1973) in case of large sample sizes. The mode of frequency distribution of alleles was characterized by the evenness (e, GREGORIUS, 1990) which is based on genetic distances. In order to quantify the potential to create genetic variation, for each sample of 100 trees the hypothetical gametic multilocus diversity was calculated, i.e. the maximum number of genetically different 18-locus gametic types. Differences between frequencies of genetic types were tested statistically by employing the log likelihood ratio test (G-test) of homogeneity in contingency tables. Variation among populations is measured by differentiation among (sub-) populations (D_j, δ; GREGORIUS and ROBERDS, 1986).

Results and Discussion

(1) Intrapopulational genetic variation

In *table 3*, results are compiled which refer to the genotypes of 2000 trees at 18 polymorphic gene loci. Among these loci, GDH-A is close to fixation because polymorphism was observed in only 3 out of 20 populations (frequency of the major allele is 99% or more). For GOT-B, IDH-A, and MDH-B, polymorphism is indicated in at least nine populations, but analogously to GDH-A, the frequency of the major allele is very high (96% in one population (No. 4/IDH-A) and at least 98% in all other cases).

Table 3. – Intra- and interpopulational variation at 18 loci adult trees in subalpine populations of Norway spruce. Except heterozygosities, all measures refer to allele frequencies (for nomenclature see article).

Pop.	No. of trees	Heterozygosity H_a(%)	H_C(%)	A_L	Genic diversity $\bar{v}$	Intrapop. different. δ_T	Eveness (rel.) e	Pop. differ. (D_j, δ)
1	100	28.1	65.9	2.67	1.430	0.302	0.66	0.063
2	100	21.3	60.0	2.72	1.332	0.250	0.75	0.055
3	100	24.7	59.2	2.61	1.390	0.282	0.72	0.071
4	100	22.8	57.7	2.61	1.372	0.272	0.75	0.041
5	100	20.8	57.8	2.39	1.329	0.249	0.73	0.039
6	100	21.3	61.6	2.56	1.327	0.247	0.72	0.034
7	100	23.2	63.4	2.50	1.357	0.264	0.73	0.039
8	100	23.1	60.3	2.50	1.364	0.268	0.70	0.027
9	100	21.6	60.3	2.56	1.342	0.256	0.72	0.035
10	100	21.7	61.7	2.44	1.349	0.260	0.71	0.029
11	100	23.3	59.6	2.50	1.367	0.270	0.74	0.035
12	100	20.2	55.7	2.39	1.336	0.253	0.75	0.031
13	100	21.9	59.4	2.50	1.350	0.260	0.72	0.048
14	100	26.8	65.8	2.56	1.403	0.289	0.70	0.044
15	100	20.2	57.0	2.22	1.343	0.257	0.73	0.054
16	100	24.1	58.0	2.56	1.382	0.278	0.77	0.060
17	100	21.8	56.9	2.56	1.364	0.268	0.74	0.039
18	100	21.5	65.0	2.50	1.326	0.247	0.73	0.050
19	100	21.6	55.5	2.39	1.359	0.265	0.75	0.039
20	100	21.7	57.0	2.56	1.360	0.266	0.72	0.034
Grand mean		22.6	59.9	2.52	1.359	0.265	0.73	δ = 0.043

Grand means are arithmetic except for H_C (GREGORIUS et al., 1986) and for D_j according to GREGORIUS and ROBERDS (1986). In the case of the diversities, the *e*-values and δ_T, grand means are suggestive because of differences in the underlying allele frequencies.

Deviations among population samples are evident with respect to heterozygosities, the number of alleles per locus and particularly the hypothetical gametic multilocus diversities.

Heterozygosities (H_a, H_c)

The H_a-values range between 20.2% (Nos. 12, 15) and 28.1% (No. 1) which is equivalent to a ratio of 1 : 1.39. These results are confirmed if the underlying allele frequencies are taken into account: Sample No. 1 reveals the largest H_C-value, and Nos. 12 and 15 belong to those four samples which show the lowest values (see also Nos. 19, 20). The grand mean of H_a = 22.6% indicates slightly smaller average heterozygosities as reported for Norway spruce in adjacent but lower elevated regions in Southern Germany (H_a = 25.2% in 9-loci studies by KONNERT and FRANKE (1990) and KONNERT (1991), H_a = 22.2% in a 14-loci study by LÖCHELT and FRANKE (1993); *loc. cit.* MÜLLER-STARCK, 1995).

A study of genetic variation in South-West Switzerland (STUTZ, 1990) resulted in very large heterozygosities (H_a = 48.1%) but this value refers to 6 highly polymorphic gene loci which are not representative for the present study. This is evident in case of population No. 9 (Simplon) which was sampled in both studies (No. 16 in STUTZ, 1990; sample size: 40 trees). The value in the present study is 21.6%, the corresponding value in the previous one is 54%.

The grand mean of the H_C-values is 59.9%. This value and the average value for each of the 20 samples is based on 12 out of 18 loci, because 6 loci revealed zero values in at least one of the samples (fixation). Because H_C-values are independent of the underlying allele frequencies, the calculated grand mean clearly indicates a lack of heterozygotes as compared to the maximum attainable value which is equal to 100%. Because there are no data on previous life stages of the studied populations, it cannot be concluded whether the reproductive system and/or various forms of viability selection may account for this phenomenon.

Alleles per locus (A_L)

A_L-values range between 2.22 (No. 15) and 2.72 (No. 2) which is equivalent to a ratio of 1 : 1.23. The grand mean of 2.52 alleles per locus corresponds well with reference values from the above cited studies in Southern Germany which reveal $A_L = 2.6$ and $A_L = 2.4$ respectively (for survey of studies on Norway spruce in Europe see MÜLLER-STARCK, 1995). Compared to other tree species in Europe, Norway spruce reveals lower values than Scots pine (*Pinus sylvestris*) or oak species (*Quercus petraea, Q. robur*) but larger values than Silver fir (*Abies alba*) or Stone pine (*Pinus cembra*) in Central Europe (for survey see MÜLLER-STARCK *et al.*, 1992).

Genetic (genic) diversity (v)

Only small differences are indicated with respect to the genic (allelic) diversity v: values range between 1.326 (No. 18) and 1.430 (No. 1) which is equivalent to a ratio of 1 : 1.08. This ratio differs from the ratio between maximum and minimum A_L values (1 : 1.23). Both measures are related in that way, that the v is identical to A_L if alleles at any locus are observed in equal frequencies. In all other cases, v is smaller than A_L. The lowest possible value is $v = 1$ (fixation).

For each population, the comparison of A_L- and v-values allows to infer tentatively on the mode of frequency distribution of alleles. Samples with similar or identical A_L-values (e.g. $A_L = 2.56$ in case of Nos. 6, 14) can show distinct deviations with respect to the corresponding diversities ($v = 1.327$ and $v = 1.403$ resp.). This demonstrates the greater evenness of the allelic frequency distributions of sample No. 14 as compared to No. 6. Sample No. 2 reveals the largest A_L-value (2.72) but the corresponding low v-value (1.332) suggests a larger proportion of rare alleles due to greater deviations from even frequency distribution of alleles (see topic "Evenness").

Intrapopulational differentiation (δ_T)

The trends with respect to the genic diversities are evident also for the genic (allelic) differentiation δ_T: sample No. 1 shows the largest value ($\delta_T = 0.302$) and samples No. 6 and 18 the smallest ones ($\delta_T = 0.247$). These deviations are equivalent to a ratio of 1 : 1.22. This ratio exceeds the corresponding ratio of the genic diversities (1 : 1.08) and thus demonstrates the greater discriminative function of the genic differentiation as compared to the genic diversity. The reason is the applied transformation and normation of diversities on a scale between zero (genetic types at any locus are identical) and one (all types are different).

Evenness (e)

The mode of frequency distribution of alleles was quantified by means of the "eveness" (e) which measures the degree to which genetic types are equally represented (independent from the genetic diversity). It is defined as 1 minus the minimum genetic distance between the observed frequency distribution and the corresponding hypothetical uniform distribution (equally frequent types). In *table 3*, the "relative evenness" was calculated, which is one in the case of equally frequent types and approaches a lower bound of zero with increasing unevenness (further details see GREGORIUS, 1990).

The given e-values which are related to allele frequencies, show little variation among the samples. Maximum deviation from equal distribution of alleles is evident in case of sample No. 1 ($e = 0.66$), minimum deviation is indicated for sample No. 16 ($e = 0.77$). This is equivalent to a ratio of 1 : 1.17. Like the average value for each of the 20 samples, the grand mean was calculated with respect to 14 loci which were polymorphic in the majority of the samples. As indicated earlier, the remaining loci, i.e. GDH-A, GOT-B, IDH-A, MDH-B were excluded because of the obvious trends towards fixation. The value of $e = 0.73$ indicates distinct deviations from equal distribution of alleles.

If the e-values are compared with the previously discussed A_L-values in combination with the genic diversities v, it is evident that they do not necessarily correspond to each other. For instance, sample No. 1 reveals the maximum value for the genic diversity but not for A_L. In spite of this, the e-values suggest the largest deviations from equal distribution of alleles. The main reason for this phenomenon is the definition of diversity: one and the same v-value can be obtained by quite different frequency distributions. Even in the case of the same average number of alleles per locus, larger genetic diversities do not necessarily imply an increase of the evenness of frequency distributions.

Hypothetical gametic multilocus diversity (v_{gam})

This measure is suggested to quantify the ability of forest tree populations to create genetic variation and thus to facilitate adaptation to changing environmental conditions (e.g. GREGORIUS et al., 1986). As can be seen from *figure 2*, significant differences among samples are clearly indicated: the maximum number of genetically different 18-locus gametic types is 1592 (sample No. 1) the minimum is 329 (No. 6). This is equivalent to a ratio of 1 : 4.84. The calculated v_{gam}-values necessarily correspond with genic diversities v because they are derived from the single locus diversities.

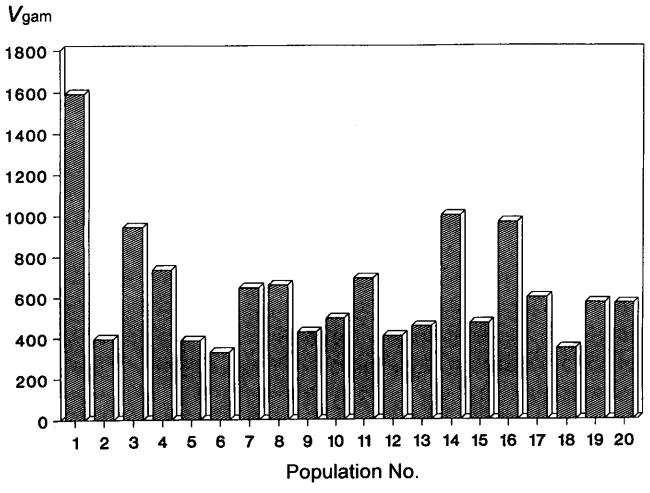

Figure 2. – Hypothetical gametic multilocus diversity (v_{gam}) as indicated from samples of 20 populations of Norway spruce (MÜLLER-STARCK *et al.*, 1995, modified; for population characteristics see *table 1*).

Sample Nos. 1, 14, 16 reveal the three highest values (the exact v_{gam}-values are 1592.7, 1000.5 and 967.5 resp.), sample Nos. 6, 18, 5 the lowest ones (329.1, 346.3 and 385.9 resp.). The arithmetic grand mean is 634.2. This value is suggestive because the multilocus mean of each sample refers to different constellations of frequency distributions at each locus.

In the present material, distinct geographic trends cannot be observed. The outstandingly great value of sample No. 1 (Le Brassus, Jura Mountains) and the relatively high value of No. 3 (Orsières, close to the valley of the river Rhône) suggest a greater adaptability of Norway spruce populations which may have immigrated from the South-West into Switzerland (see topics "Interpopulational variation" and "Conclusions").

Generally, the diversity measure v_{gam} strongly emphasizes differences among samples with respect to the genetic (genic) diversity because single locus diversities are multiplied. This measure appears to be highly specific but it clearly indicates the potential of a set of trees, to produce genetically different gametes and thus to submit genetic variation to the next generation (genetic variability). Particularly in the case of autochthonous tree populations in heterogeneous environments, genetic variability is a fundamental precondition for adaptation and survival.

(2) Interpopulational variation

Statistically significant deviations

Tests of homogeneity among allele frequencies of 20 population samples revealed statistically significant deviations for 16 out of 18 gene loci. For the majority of loci, deviations were highly significant (level of significance: 0.001): AAP-B, ACO-A, DIA-A, GOT-A, GOT-C, LAP-A, LAP-B, MDH-C, NDH-A, 6PGDH-B, 6PGDH-C, PGI-B, PGM-A and SKDH-A. At the level of 0.01, allele frequencies were statistically significant at the gene locus IDH-A, and at the level of 0.05 the frequencies at GOT-B. Deviations were not significant in case of the loci GDH-A and MDH-B.

Differentiation among populations (D_j, δ)

This measure of genetic differentiation among (sub-) populations is based on genetic distances. Frequencies of genetic types (alleles, genotypes) of one population are contrasted with the weighted averages of the frequencies of the remaining populations. Each population is considered as a subpopulation and differentiation is quantified by means of the genetic distances between one sample and the remaining ones which are pooled as the respective complement population. Consequently, genetic differentiation is quantified as a whole and not in pairs like in case of genetic distances.

In *figure 3*, the genetic differentiation is illustrated exemplarily for three out of 18 loci and for the entire gene pool. The graphs refer to the allele frequencies. In each graph, the radius of the circle is equal to the average level of differentiation (δ) at that particular locus. The given scale measures the average proportion of genes in which any sample differs from the remainder. For each graph, the radii of the population specific sectors are equal to the proportion of genes in which one population differs from the lumped remainder (D_j). The population with the largest radius and thus the greatest amount of differentiation between it and the remainder is placed on top of each graph. The more the sector radii approach the center, the more representative of the remainder is the genetic information of such a population.

As can be seen from the last column in *table 3*, D_j-values differ considerably from each other: the maximum value is evident for sample No. 3 ($D_3 = 0.071$), the minimum value for sample No. 8 ($D_8 = 0.027$). This is equivalent to a ratio of 1 : 2.63. In *figure 3*, the single locus graphs show that the average level of differentiation varies among loci and that the D_j-values of certain samples strongly deviate among gene loci. For instance, sample No. 1 differs substantially from the other samples in case of SKDH-A but is differentiated below average in case of LAP-A. Sample No. 3 reveals large D_j-values in all loci (see gene pool graph). This means that sample No. 3 (Orsières) shares the smallest proportion of the entire genetic information, i.e. this sample contains more specific genetic information than any other (see also topic "Conclusions").

The obvious deviations in the D_j-values among loci suggest locus-specific response to selective forces under the particular

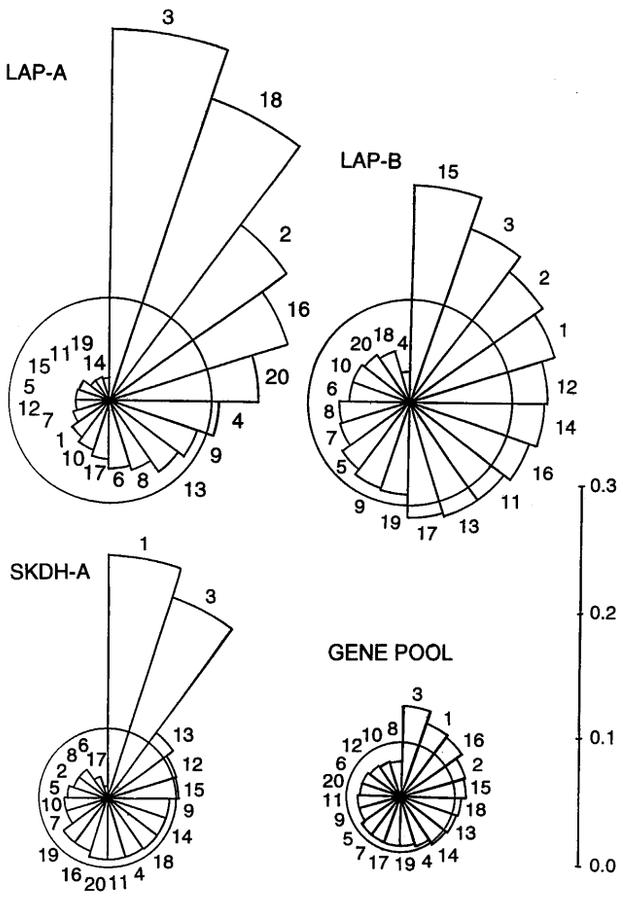

Figure 3. – Genetic differentiation (D_j, δ) among samples of 20 populations of Norway spruce for the gene loci LAP-A, LAP-B, SKDH-A and the gene pool with respect to 18 gene loci (for nomenclature see article).

environmental situations. If this interpretation is correct, the majority of the 18 loci appear as adaptive gene loci. The differentiation of single populations in contrast to the remainder can be studied in more detail if D_j-values are calculated for selected combinations of gene loci.

In case of the gene pool differentiation, diverging single-locus trends are compensated because all studied loci are adressed as a whole. Consequently, the given gene pool graph (*Figure 3*) reflects only general trends in differentiation. The gene pool differentiation is given by the radius of the circle of the gene pool graph, i.e. $\delta = 0.043$ (see scale in *Figure 3*). This means that any sample differs from the remainder on average by 4.3% of its alleles. The 20 samples are graphed in decreasing order of differentiation, i.e. sample No. 3 is the one which reveals the largest genetic differences to the remainder while sample No. 8 is the one that shares the largest proportion of common genetic information.

The gene pool graph suggests that the samples from the Jura Mountains and close to the Rhône valley in South-West Switzerland (Nos. 1, 2, 3) and also from regions in the South-East (Nos. 15, 16) tend to show a remarkably great differentiation. Adjacent regions in Central Switzerland (e.g. Nos. 6, 8, 9, 10, 11) reveal the opposite trend, i.e. these samples represent the entire gene pool to a larger extent and show only little specific information.

Conclusions

Genetic variation in Switzerland as compared to other regions in Europe

Surveys of genetic inventories in European tree populations (e.g. MÜLLER-STARCK *et al.*, 1992) clearly demonstrate problems which arise in the comparison of data. Studies refer to different sampling modes and different population and site characteristics. Furthermore, the utilized set of gene loci can differ substantially and concepts of quantification of genetic variation are not the same.

In the case of Norway spruce, the following studies correspond methodically to the present one and refer to adjacent regions northward or southward of the Swiss Alps: KONNERT and FRANKE, 1990; BERGMANN, 1991; KONNERT, 1991; MORGANTE and VENDRAMIN, 1991; LÖCHELT and FRANKE, 1993 (for survey see MÜLLER-STARCK, 1995).

With respect to these studies, the following main conclusions are suggested:

- The observed heterozygosities range within the values of reference studies in adjacent lower elevated areas northward of the Swiss Alps. The conditional heterozygosities appear to be at a low level (reference data are rare).
- The average number of alleles per locus is nearly the same as in Southern Germany and is substantially larger than in Italien spruce populations (2.5 vs. 1.8).
- The remaining intrapopulational measures suggest that variation within high elevated Swiss spruce populations is not smaller than in lower elevated reference populations (limited pool of reference data).
- Interpopulational variation (δ) in Swiss spruce stands is smaller than in Italien spruce populations (4.3 vs. 5.0) and appears to be within the range of reference populations northward the Swiss Alps (4.3 vs. 3.2, 4.9, 5.5).

Preliminary inferences on postglacial re-immigration

Peculiarities with respect to postglacial re-immigration may still considerably contribute to the present pattern of geographic variation among indigenous populations. For Norway spruce, such immigration is commonly accepted to predominantly follow east-west routes, although south-north routes such as for instance via the Simplon pass cannot be excluded (BURGA, 1988).

The present results support the hypothesis of re-immigration extra to the common east-west routes. As indicated in topic "Interpopulational variation", samples in the South-West of Switzerland tend to contain specific information and thus to share a relatively small portion of the common gene pool. Particularly the gene locus SKDH-A reveals a geographical pattern of genetic variation: The frequency of the allele SKDH-A_4 is 17.0% in sample No. 1 (Le Brassus), 17.5% in sample No. 3 (Orsières), but is equal or less than 2.5% in 11 samples (average is 0.8%) and is not represented in 7 samples.

If these findings are representative, a postglacial re-immigration from South-West into Switzerland is strongly suggested. This implies that Norway spruce re-immigrated earlier than expected and that populations in this area may genetically differ from the populations in Central or East Switzerland.

Preservation of genetic variability

Results clearly indicate substantial genetic variation within Norway spruce populations in contrast to relatively small interpopulational variation. In case of *in situ* preservation of genetic variation, results suggest a strategy in the declaration of gene conservation forests which should rather aim at the maintenance of fewer but larger units than of many small sized plots.

The main reason is the necessity to preserve the great number of rare alleles which are evident in forest tree populations and which represent a large potential to create genetic variation (genetic variability). The importance of rare alleles will be demonstrated briefly for sample No. 1 (Le Brassus). This population is the first out of the studied 20 populations which became part of the *in situ* preservation program of the Swiss Forest Service. Based on genetic criteria, further populations will be suggested for declaration as gene conservation forests (MÜLLER-STARCK *et al.*, in preparation). For sample No. 1 the present study monitored a total number of 48 genes (alleles) at 18 gene loci. If the loss of rare alleles, i.e. alleles with frequencies smaller than 5%, could not be avoided, the total number of genes would amount to 34 ($A_L = 1.89$) instead of 48 ($A_L = 2.67$).

In order to demonstrate possible consequences of such a reduction, the maximum number of genetically different 18-locus-genotypes is calculated which can be formed in case of the actual sample No. 1 and the hypothetical one with only 34 genes. The corresponding numbers are 49.601.160.000 and 12.754.584 individuals respectively (relation is 3889 : 1). It is self evident that the realization of such potentials depends on population sizes and the mode of distribution of alleles in populations.

Large gene conservation forests in heterogeneous environments will help to preserve rare alleles which cannot be expected to be maintained under forest management practices which favour relatively small population sizes. Rare alleles considerably increase the genetic variability and thus the ability of long-lived carrier species of complex forest ecosystems to adapt to and to survive under highly variable environmental conditions.

Acknowledgements

The technical assistance of ELIANE ESCHER and URS BÜCHLER is greatly appreciated. The choice of indigenous stands in the Swiss Alps was essentially facilitated by the help of PATRICK BONFILS (Eidgenössische Technische Hochschule, Zürich) and the Swiss Forest Service. The forest genetic group in Birmensdorf helped substantially in the collection of material. Also I would like to express my thanks particularly to ERWIN HUSSENDÖRFER, ERNST FÜRST and WALTER SCHÖNENBERGER, Birmensdorf, for stimulating discussions and very useful suggestions.

This study was supported financially by the Bundesamt für Umwelt, Wald und Landschaft, Eidgenössische Forstdirektion, Bern, Switzerland.

References

BERGMANN, F.: Causes and consequences of species-specific genetic variation patterns in European forest tree species: Examples with Norway spruce and Silver fir. In: MÜLLER-STARCK, G. and ZIEHE, M. (eds.): Genetic Variation in European Populations of Forest Trees. pp. 192–204. Sauerländer's Verlag, Frankfurt a. M. (1991). — BURGA, C. A.: Swiss vegetation history during the last 18 000 years. New Phytol. **110**, 581–602 (1988). — GREGORIUS, H.-R.: The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Math. Bioscience **41**, 253–271 (1978). — GREGORIUS, H.-R.: The relationship between the concepts of genetic diversity and differentiation. Theor. Appl. Genetics **74**, 397–401 (1987). — GREGORIUS, H.-R.: A diversity-independent measure of evenness. Amer. Natur. **136**, 701–711 (1990). — GREGORIUS, H.-R.: Gene conservation and the preservation of adaptability. P. 31–47. In: SEITZ, A. and LOESCHKE, V. (eds.): Species Conservation: A Population-Biological Approach. Basel, Birkhäuser Verlag (1991). — GREGORIUS, H.-R., KRAUHAUSEN, J. and MÜLLER-STARCK, G.: Spatial and temporal genetic differentiation among the seed in a stand of *Fagus sylvatica* L.. Heredity **57**, 255–262 (1986). — GREGORIUS, H.-R. and ROBERDS, J. H.: Measurement of genetical differentiation among subpopulations. Theor. Appl. Genet. **71**, 826–834 (1986). — HAMRICK, J. L. and GODT, M. J.: Allozyme diversity in plant

species. In: BROWN, H. D., CLEGG, M. T., KAHLER, A. L. and WEIR, B. S. (eds.): Plant Genetics, Breeding, and Genetic Resources. pp. 43–63. Sinauer Ass. Inc. Publ., Sunderland, Mass. (1989). — HATTEMER, H. H. and GREGORIUS, H.-R.: Is gene conservation under global climate change meaningful? P. 158–166. In: JACKSON, M. T., FORD-LLOYD, B. V. and PARRY, M. L. (eds.): Climatic change and Plant Genetic Resources. London and New York, Belhaven Press (1990). — KONNERT, M.: Die Fichte (*Picea abies* (L.) KARST.) im Schwarzwald: Genetische Variation und Korrelationen. Forstw. Cbl. **110**, 84–94 (1991). — KONNERT, M. und FRANKE, A.: Die Fichte (*Picea abies* (L.) KARST.) im Schwarzwald: Genetische Differenzierung von Beständen. Allg. Forst- u. J.-Ztg. **162**, 100–106 (1990). — KRAL, F.: Spät- und postglaziale Waldgeschichte der Alpen auf Grund der bisherigen Pollenanalysen. Veröff. Inst. f. Waldbau, Univ. f. Bodenkultur, Wien. Österreichischer Agrarverlag, Wien. pp. 175 (1979). — LÖCHELT, S. und FRANKE, A.: Bestimmung genetischer Merkmale von Fichten (*Picea abies* (L.) KARST.) mit unterschiedlich ausgeprägten Schadsymptomen auf baden-württembergischen Dauerbeobachtungsflächen. Allg. Forst- u. J.-Ztg. **165** (1993). — MORGANTE, M. and VENDRAMIN, G. G.: Genetic variation in Italian populations of *Picea abies* (L.) KARST. and *Pinus leucodermis* ANT. In: MÜLLER-STARCK, G. and ZIEHE, M. (eds.): Genetic Variation in European Populations of Forest Trees, pp. 205–227. Sauerländer's Verlag, Frankfurt a. M. (1991). — MÜLLER-STARCK, G.: Genetic variation under extreme environmental conditions. Proceedings of the International Symposium on Population Genetics and Gene Conservation of Forest Trees. August 1992. Carcans, France (1995, in press). — MÜLLER-STARCK, G., BARADAT, PH. and BERGMANN, F.: Genetic variation in European tree species. New Forests **6**, 23–47 (1992). — MÜLLER-STARCK, G., HUSSENDÖRFER, E. und SPERISEN, CH.: Genetische Diversität bei Waldbäumen – eine Voraussetzung für das Überleben unserer Wälder. S. 23–32. In: Erhaltung der Biodiversität – eine Aufgabe für Wissenschaft, Praxis und Politik. Forum f. Wissen, Eidgenöss. Forschungsanstalt f. Wald, Schnee u. Landschaft, Birmensdorf, Schweiz (1995). — MÜLLER-STARCK, G. and ZIEHE, M. (eds.): Genetic Variation in European Populations of Forest Trees. Sauerländer's Verlag, Frankfurt a. M.. pp. 271 (1991). — NEI, M.: Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA **70** (12), 3321–3323 (1973). — SCHOLZ, F., GREGORIUS, H.-R. and RUDIN, D. (eds.): Genetic Aspects of Air Pollutants in Forest Tree Populations. Springer Verlag Berlin, Heidelberg, New York, Tokyo. pp. 201 (1989). — STUTZ, H.-P.: Genetische Differenzierung und natürliche Selektionsfaktoren der Fichte (*Picea abies* (L.) KARST.) in einem zentralalpinen Gebiet der Schweiz. Dissertation Nr. 9101. pp. 128. Eidgenöss. Techn. Hochschule, Zürich (1990).

Multiple-Trait Combined Selection in Jack Pine Family-Test Plantations Using Best Linear Prediction

By J. I. KLEIN

Canadian Forest Service, Manitoba District Office, Winnipeg, Manitoba, Canada

(Received 2nd February 1995)

Summary

Best linear prediction (BLP) was applied to data from a jack pine open-pollinated family test in Manitoba to estimate trait breeding values for selection of parents for advanced generation breeding. The test includes 215 families and 1 control lot planted in 15 replications at 3 locations. Families in the test originated from 76 stands, with 1 to 5 families from each stand. Stands were grouped by proximity into 18 areas. Height and diameter at 20 years on individual trees were adjusted by subtraction of mean within-family deviations of neighboring trees. At 21 years on trees average or better for height growth and past stem quality (stage 2 trees), stem quality was scored, western gall rust galls were counted, and wood density was estimated from Pilodyn penetrations. The observation vector for each tree comprised deviations of its own measurement or score, its family mean, and its area mean from the test mean for all traits except stem quality, which had only tree value and family mean. For each stage 2 tree, the variance of the observation vector (V matrix) and the covariance of the observation vector with trait breeding values (C matrix) were calculated from variance and covariance components and family size values. Derivatives of V and C were used along with the observation vector to calculate BLP breeding values for 5 traits. Various weight vectors were applied to trait breeding values to produce total scores used for selection, until a satisfactory gain relationship among traits was found. Owing to relatively high heritabilities and low imbalance effect of family size differences, results from BLP differed little from results of index selection with these data.

Key words: Pinus banksiana, multiple-trait selection, combined selection, index selection, best linear prediction, sliding block adjustment, heritability, genetic gain, genetic correlation.

FDC: 232.11; 165.62; 165.3; 165.5; 174.7 *Pinus banksiana.*

Introduction

Although forest tree breeding programs are primarily concerned with increasing growth of planted trees, breeders often also wish to improve or maintain other traits, such as stem straightness (BROWN and MILLER, 1975; BRIDGWATER and STONECYPHER, 1979; COTTERILL and ZED, 1980; SHELBOURNE and LOW, 1980; DEAN et al., 1983; PARK et al., 1989; ADAMS and MORGENSTERN, 1991), wood quality (DEAN et al., 1983; ERNST et al., 1983; KING et al., 1988; PARK et al., 1989; MAGNUSSEN and KEITH, 1990; CORRIVEAU et al., 1991; YANCHUK and KISS, 1993), and rust resistance (WHITE and HODGE, 1988; HODGE et al., 1989; LAFARGE, 1989; SLUDER, 1994). Index selection is a method of assessing the genetic worth of genetic entities (individuals or families for example) as a linear function of multiple performance attributes (SMITH, 1936). Optimum weights for the attributes are calculated from assigned economic weights and from knowledge of genetic and phenotypic variances and covariances of the attributes (HAZEL, 1943). For improvement of multiple traits, index selection will always be equal or superior in efficiency to independent culling, which is always equal or superior to tandem selection (HAZEL and LUSH, 1942; BAKER, 1986). When trees grown in replicated genetic test plantations are being assessed for selection, genetic gain can be enhanced by combining information from observations on the individual trees being assessed with information from observations on their relatives. Simultaneous use of information from an individual being assessed along with information from the performance of relatives has been termed "combination selection" (LUSH, 1947) or "combined selection" (FALCONER, 1989, p. 236). Combined selection on a linear function of family and individual merit, weighted by within-family and family