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Genetic Characters and Diameter Growth of Provenances of Scots Pine (*Pinus sylvestris* L.)

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(Received 21st February 2001)

Abstract

Correlation between genetic characters and growth parameters was studied with respect to four provenance samples, which are part of a 47-year-old provenance trial of Scots pine. Two samples with superior growth were contrasted with two weakly growing samples. Based on a sample size of 100 trees per provenance, genotypes were monitored at 16 enzyme coding gene loci. Effects between genetic and growth traits were studied by means of two-factorial analysis of variance and linear regression models with respect to diameter classes and subsets of elite and non-elite trees within provenance samples as well as diameter subsets of pooled provenance samples.

Significant deviations are evident among the genetic structures of the four provenance samples. In each sample, deviations from HARDY-WEINBERG structures are indicated at single loci. Inbreeding does not primarily account for such deviations. The study of diameter classes reveals that an increase of stem volume tends to coincide with an increase of the observed heterozygosities in three out of four provenance samples. In the case of two-locus genotypes at 6-PGDH and MDH-C, significant effects of heterozygosity on diameter growth are observed. By focusing the two most frequent alleles at six differentiation effective gene loci, significant relations to diameter growth can be particularly verified for two locus combinations including AAT-A. The genetic comparison of the elite and non-elite trees, the first subset reveals larger values for diversities, differentiation and heterozygosities than the second does. The genetic comparison between the subset of the 50 thinnest and the 50 thickest trees among the 400 individuals indicates higher values for the genetic multiplicity and the gene pool and multilocus diversity for the subsets with superior growth. It is concluded for the present that isoenzyme gene markers reveal an indicative potential for quantitative traits.

Key words: Pinus sylvestris, provenances, isoenzyme gene markers, genetic variation, differentiation, diameter growth.

Introduction

Scots pine (*Pinus sylvestris* L.) covers a climatically divers continuous habitat, which ranges from Central and Northern Europe to Russia and Northern-Central Asia. Additional parts of the habitat are isolated, for instance the Pyrenees, Scotland and Northern Turkey. The vertical dimension of the habitat is extraordinary large: Scots pine is a significant carrier species of various ecosystems from low elevations up to the mountainous regions and in some cases also the sub-alpine vegetation zones.

A great variety with respect to morphological traits, especially habitus and growth, is evident throughout the habitat of Scots pine. The idea of a structuration of the Scots pine habitat into provenances goes back to the 16th century (HAUSRATH, 1982).

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The first ordinary provenance trials within the genus *Pinus* were established in France at the beginning of the 19th century (PRANGE, 1982). Since then, various provenance trials were initiated in Europe and Asia in order to verify and utilise the natural geographical differentiation of Scots pine populations. The present study addresses a provenance trial in Bavaria which was established as part of a network by the former Chair of Forest Seed Science and Plant Breeding of the University of Munich and is now part of the net of long term experimental areas of the Chair of Yield Science.

Genetic inventories in various parts of the habitat of Scots pine revealed large intrapopulational genetic variation and relatively little genetic differentiation among populations except in case of populations from different glacial refugia (MÜLLER-STARCK *et al.,* 1992). It appears that the great phenotypic variation does not coincide with a correspondingly great geographical genetic variation at the studied marker loci. This may be a consequence of the fact that until now the majority of genetic inventories were performed by nuclear markers such as isoenzyme gene markers, and not by extra-nuclear markers.

The great variation within populations is interpreted for the present to indicate a large potential for genetic adaptation to heterogeneous environmental conditions (e.g. GREGORIUS, 1996). In the majority of the studied Scots pine populations, genetic inventories revealed that the observed heterozygosities are higher than in other species. In the case of a natural regeneration of Scots pine in Northern Sweden, large heterozygosities were proven to correspond with survival (MUONA *et al.,* 1986). The genetic comparison between sensitive and tolerant sub-populations resulted in larger heterozygosities and multilocus diversities in the case of tolerant subsets (GEBUREK *et al.,* 1987). Similar trends were also verified in a study on the susceptibility of a Scots pine population against attacks by insects (MÄNNER, 1999). Very few studies addressed possible correlation between morphological and genetic characters. For instance, a sub-classification of morphological types revealed a statistically significant correspondence between homozygote genotypes and above average growth (KOHLSTOCK *et al.,* 1993). Other studies verified that the mode of thinning infers on the genotypic structures of respective populations and sub-populations (HERTEL and KOHLSTOCK, 1993; KOHLSTOCK *et al.,* 1993; HERTEL *et al.,* 1998; KÄTZEL, 2000, for Scots pine; HOSIUS, 1993, for Norway spruce; HUSSENDÖRFER and KONNERT, 2000, for Silver fir and European beech; KONNERT and SPIEKER, 1996, for European beech).

It is the aim of the present study to genetically characterise provenance samples with outstandingly low and high diameter growth, respectively. Because of possible inferences of inbreeding on growth characters, the quantification of inbreeding is part of this study. Furthermore, it is aimed at the verification of a correlation between genetic characters and quantitative traits in terms of growth parameters.

Material and Methods

Plant material

The study is based on a 47 year old Bavarian provenance trial of *Pinus sylvestris* L. in Bodenwöhr in the Northeast of Regensburg, Germany (*Fig. 1*) with 31 different provenance samples (KOLLER, 1981). According to a set of individual tree- and stand-based growth traits, measured in spring 1997, in each of two vegetation zones (FOERST and KREUTZER, 1978) a pair of provenance samples was selected, which revealed clear differences with respect to growth characters. The provenance samples Ebern and Schwabach originate from the "Fränkischer Keuper and Alpvorland", the samples Mitterteich and Waldsassen from the "Oberpfälzer Wald". Ebern and Mitterteich were classified as "superior in growth", because they reached maximum values in diameter growth whereas Schwabach and Waldsassen revealed lowest values and were characterised as "weak in growth" (see also BLUMENRÖTHER, 1999). In spring 1998, for each provenance sample 100 individual trees were randomly selected and probes taken for the genetic inventory.

Fig. 1. – Location of the provenance samples Ebern (9), Mitterteich (14), Schwabach-D. (28), Waldsassen (32) in the Northeast of Bavaria and of the provenance trial Bodenwöhr.

Identification of multilocus-genotypes

A total of 16 enzyme coding gene loci were utilised for multilocus genotyping. The enzymes were separated from crude homogenate by standard horizontal starch gel electrophoresis following methods that are compiled by HERTEL (1997) and MÜLLER-STARCK (1998). For verification of genetic control and mode of inheritance of the respective enzyme systems see MÜLLER-STARCK (1982a and b) and HERTEL (1997). Enzyme systems and coding gene loci are surveyed in *table 1.*

Statistical methods

Variation within populations was measured by means of genetic multiplicity and genetic diversity (GREGORIUS, 1978, 1987). In order to quantify the potential for creation of genetic variation, the hypothetical gametic multilocus diversity v_{gam} (GREGORIUS, 1987) was calculated i.e. the maximum number of genetically different multilocus gametic types. Heterozygosities were measured as observed proportions of heterozygotes (H_a-values) and calculated as conditional heterozygosities (H_c-values) which take into account the underlying allele frequencies (GREGORIUS *et al.,* 1986). Interpopulational differentiation was quantified by means of genetic distances D_0 (GREGORIUS, 1974) and the genetic differentiation among (sub-) populations (D_j, δ; GREGORIUS and ROBERDS, 1986). Differences among frequency distribution were tested statistically utilising the log likelihood ratio test (G-test) of homogeneity in contingency tables. Inbreeding was estimated by means of fixation coefficients F (WRIGHT, 1969) which are defined within the range of F = –1 (absence of homozogotes) and F = +1 (absence of heterozygotes) as compared to the corresponding HARDY-WEINBERG proportions. All parameters were computed with GSED (Genetic Structures from Electrophoresis Data; GILLET, 1994).

In addition to the four provenance samples (*Table 2a*), phenotypic subsets were formed (*Table 2b*) by using diameter

Table 1. – Enzyme systems, nomenclature, structure (mo = monomeric, di = dimeric, te = tetrameric, he = hexameric), metabolic category (I = primary, II = secondary) and enzyme coding gene loci.

Enzyme system	E. C. No.	Structure, Metabolic Category	Enzyme coding Polymorphic Gene loci
Aconitase	4.2.1.3	mo, I	ACO-A
Aspartate aminotransferase	2.6.1.1	di, I	AAT-A, -B, -C
Glutamate dehydrogenase	1.4.1.2	he, II	GDH-A
Isocitrate dehydrogenase	1.1.1.42	di, I	IDH-A
Leucine aminopeptidase	3.4.11.1	mo, II	LAP-A, -B
Malate dehydrogenase	1.1.1.37	di, I	MDH-A, -B, -C
Menadion reductase	1.9.992	te, II	MNR-A
6-Phosphogluconate dehydrogenase	1.1.1.44	di, I	6PGDH-B
Phosphoglucomutase	2.7.5.1	mo, I	PGM-A
Shikimate dehydrogenase	1.1.1.25	mo, II	SKDH-A, -B

Table 2. – Tripartite survey of methods (a, b, c) to analyse genetic variation and effects between genetic and yield-related characteristics of four provenance samples and subsets. For each provenance sample (Ebern, Mitterteich, Schwabach, Waldsassen) 100 individuals of Scots pine were selected randomly.

Focus	Genetic variation and differentiation Within and among (a) provenances	(b) phenotypic clusters	(c) Effects between genetic characters and growth of individual trees
Parameters	• Genotypes at 16 gene loci per tree • allelic / genotypic distance for cluster analysis	• genotypes at 16 gene loci per tree • provenance-independend classes of dbh: 50 weakest (thin) and 50 strongest (thick) • provenance-specific classes of dbh: lower third (dbh-1) and upper third (dbh-3) • provenance-specific affiliation to elite trees: elite tree (Z) and non-elite tree (R)	• genotypes including 15 (6) gene loci per tree • dbh [mm] • actual heterozygosity at 2 gene loci: homo-, heterozygotic [0;1] • genotypic rareness (< 10%) at 2 gene loci: rare, not rare [0;1] • representation of the 2 most frequent allels at 2 gene loci: n.a., allel 1 homozygous, heterozygous, allel 2 homozygous [0;1;2;3] • 0.1 classes of stem volume including heterozygosity
Methods of data-processing	• allelic / genotypic differentiation and diversity • allelic / genotypic distance • level of actual heterozygosity	• allelic / genotypic differentiation and diversity • allelic / genotypic distance • level of actual heterozygosity	• two-factorial analysis of variance • linear regression model

at breast height (dbh) or affiliation to elite tree classes, in the latter case selected by the diagnostics vitality, quality and distribution (KOHLSTOCK *et al.,* 1993; HERTEL and KOHLSTOCK, 1993).

In a second step, effects between genetic characters and growth traits of individual trees are tested using analytic statistics (*Table 2c*). The genetic impacts on the actual diameter growth as well as the stem volume are studied by using a linear regression respectively to a two-factorial analysis of variance. The genetic parameters were transformed taking into account heterozygosity (homo-, heterozygosity, transformed 0/1), allelic rareness (rare, not rare, transformed 0/1) as well as representation of the two most frequent alleles (n.a., allele 1 homozygous, heterozygosity, allele 2 homozygous, transformed 0/1/2/3). The analyses were performed for all 400 individuals of Scots pine or alternatively for each of the provenance sample.

Results

Genetic characterisation of provenance samples and subsets

For each parameter that is listed in t*able 2,* the results of the provenance samples are given first before presenting the data for the subsets which refer to different phenotypic characters within the provenance samples.

Genetic multiplicity and diversity

Provenance samples

The genetic inventory at 16 gene loci revealed a total of 70 different alleles (genes) among 400 individuals. The majority of

these alleles (43, i. e. 60.6%) were observed in frequencies below 10% (73.9% in the case of genotypes). Samples with similar geographic origin, i. e. Ebern and Schwabach of the vegetation zone "Fränkischer Keuper and Alpvorland" and Mitterteich and Waldsassen of "Oberpfälzer Wald" show nearly the same multiplicity. The sample Schwabach reveals the highest value of multiplicity with an amount of 3.75 alleles and 5.2 genotypes per locus (A_L) although there are only little differences among the populations. Values for gene pool diversity range from 1.39 in the case of sample Mitterteich and 1.46 in sample Ebern, those for hypothetical gametic multilocus diversity vary between 594.1 in sample Waldsassen and 803.3 in sample Ebern (*Table 3*).

Subsets within provenance samples

The different diameter classes within the provenance samples show quite similar values for multiplicity but refer to deviating sample sizes. Values for allelic and genotypic multiplicity vary from 2.6 to 3.0 alleles per locus and 3.3 to 3.8 genotypes per locus in the case of dbh-1 collectives and from 2.5 to 3.2 alleles and 3.0 to 4.2 genotypes per/locus in the case of dbh-3 collectives (*Table 3*). The dbh-3 subset of Ebern shows an exceptional high multiplicity within a sample of only 33 individuals. Generally the values for multiplicity of the two different dbh-classes within the provenance samples are quite homogenous. Analogously to the gene pool-diversity, there are only small differences between samples. The hypothetical gametic multilocus diversity is much higher in subsets dbh-1 of Ebern with 1.48 and dbh-3 of Schwabach with 1.47 than in the others, although sample sizes are smaller than those of the remaining samples (*Table 3*).

Between the categories of the elite (Z) and non-elite (R) tree samples, different values in the allelic and genetic multiplicity are evident. The multiplicity of the elite trees varies between 2.4 and 2.6 alleles/locus and between 2.6 to 3.6 genotypes/locus, while non-elite trees show an amount of 3.4 to 3.7 alleles and 4.8 to 5.1 genotypes per locus (*Table 3*). There are no great differences in gene pool diversity and hypothetical gametic multilocus diversity between elite and non-elite trees of the same provenance with the exception of Mitterteich: subset Mitterteich Z reveals values of 1.46 for the gene pool diversity and of 647.2 for the hypothetical gametic multilocus diversity while the corresponding values are 1.39 and 287.0 in the subset Mitterteich R (*Table 3*). When grouping all elite tree subsets versus all non-elite tree subsets, at 11 out of 15 gene loci the group of the elite trees reveal a higher allelic diversity than the group of the non-elite trees (data not shown).

In the case of the 50 thinnest and 50 thickest trees among the 400 individuals, there are obvious distinctions in multiplicity and diversity between these collectives. As for genetic multiplicity, gene pool diversity and particularly multilocus diversity values are higher in the sample of thick trees as compared to the thin trees: 3.5 vs. 3.3 alleles per locus, 4.8 vs. 4.3 genotypes per locus, gene pool diversity of 1.47 vs. 1.43 and a multilocus diversity of 860.1 vs. 474.9.

Heterozygosity

Provenance samples

Proportions of heterozygotes differ very little between the samples, i.e. between 27.6% (Waldsassen) and 28.5% (Ebern), while sample Mitterteich reveals 28.4% heterozygotes, and Schwabach 28.0% (data not shown). The average observed heterozygosity of all provenance samples is 28.1%. Sample Mitterteich realised the highest value for the conditional heterozygosity with 77.5%, followed by Schwabach with 67.2%, Ebern with 66.8% and Waldsassen with 66.3%.

Subsets within provenance samples

In the diameter subsets of the provenance samples Ebern and Mitterteich, the mean values of actual heterozygosity are lower in the dbh-3 collectives (28.2% for Ebern, 27.8% for Mitterteich) than in the dbh-1 collectives (30.9% for Ebern and 29.1% for Mitterteich). Higher levels of heterozygosity in the dbh-3 collectives are indicated in the case of Schwabach (34.6%) and Waldsassen (28.6%) as compared to the dbh-1 subsets for these provenance samples (24.9% for Schwabach and 26.9% for Waldsassen). When grouping all dbh-3 and all dbh-1 individuals the group of all upper third trees reveal a higher mean heterozygosity at 9 out of 15 loci (*Fig. 2*).

The elite and non-elite trees reveal an average actual heterozygosity of 31.4%. Sample Mitterteich Z shows the highest average heterozygosity (37.5%), the samples Schwabach R and

Table 3. – Number of trees (N), allelic and genetic multiplicity (A_L, G_L), genetic diversity ($v_{gene\ pool}$) and hypothetical gametic multilocus diversity (v_{gam}) of the provenance samples themselves (all), the dbh-1 and dbh-3 subsets, the elite (Z) and non-elite (R) trees of the provenance samples Ebern, Mitterteich Schwabach, Waldsassen and of the subsets of thin and thick trees.

Parameter	Ebern					Mitterteich					Thin
	All	dbh-1	Dbh-3	Z	R	all	dbh-1	dbh-3	Z	R	
N	100	16	33	21	79	100	40	18	8	92	50
A_L	3.6	2.6	3.2	2.8	3.5	3.5	3.0	2.5	2.4	3.5	3.3
G_L	5.2	3.3	4.2	3.6	4.8	4.8	3.8	3.0	2.6	4.8	4.3
$v_{gene\ pool}$	1.46	1.48	1.45	1.46	1.45	1.39	1.40	1.37	1.45	1.39	1.43
v_{gam}	803.3	1130.8	744.4	851.6	768.9	314.2	326.7	236.7	647.2	287.0	474.9
Parameter	Schwabach					Waldsassen					Thick
	All	Dbh-1	dbh-3	Z	R	all	dbh-1	dbh-3	Z	R	
N	100	41	13	12	88	100	36	21	19	81	50
A_L	3.8	2.9	2.6	2.4	3.7	3.5	2.9	2.8	2.6	3.4	3.5
G_L	5.2	3.8	3.0	2.8	5.1	4.8	3.8	3.2	3.3	4.8	4.8
$v_{gene\ pool}$	1.44	1.40	1.47	1.42	1.44	1.43	1.42	1.43	1.43	1.43	1.47
v_{gam}	663.1	408.5	907.1	608.4	643.2	594.1	494.1	554.9	633.7	578.5	860.1

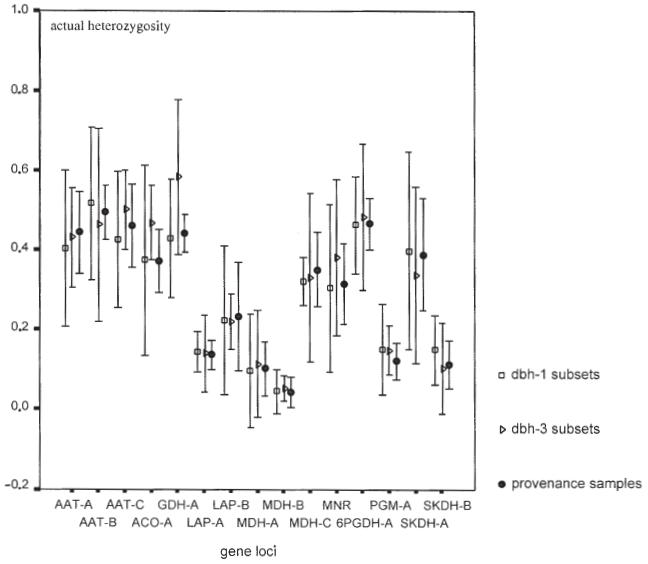

Fig. 2. – Error bar diagram for the actual heterozygosity with mean values and intervals of confidence of the pooled provenance samples, the dbh-1 and the dbh-3 subsets within the provenance samples.

Waldsassen R the lowest ones (29.3%). Proportions of heterozygotes in the other samples range between these values, i.e. 31.1% (Ebern Z), 30.2% (Ebern R), 29.6% (Mitterteich R), 34.4% (Schwabach Z) and 29.5% (Waldsassen Z). All samples of the elite trees assemble more heterozygote individuals than those of the non-elite trees. At 11 gene loci, the mean values of heterozygosity are higher in the pooled samples of the elite trees than in the grouped samples of non-elite trees (data not shown).

As far as the subsets thin and thick of the provenance samples are concerned, the proportion of heterozygous genotypes is lower in the sample of thin trees as compared to the thick ones (27.8% vs. 29.5%). In 9 out of 15 loci, more heterozygotes are observed in sample thick than in sample thin (data not shown), though only 67.2% of the possible heterozygosity is realised as compared to 70.7% in the sample of thin trees.

HARDY-WEINBERG proportions and fixation index F

Tests refer to the four provenance samples Ebern, Mitterteich, Schwabach and Waldsassen. As listed in *table 4*, only a few loci, i. e. AAT-A, AAT-B, LAP-A, MNR and 6-PGDH-B, reveal remarkable deviations between the observed genotypic structures and corresponding HARDY-WEINBERG proportions. The fixation indices verify a deficiency of heterozygots in the majority of the studied cases. The F-values range from 0.500 for the sample Ebern to -0.041 for Mitterteich, both at the locus MNR. The great heterogeneity with respect to the F-values clearly indicates that inbreeding alone cannot account for the existing deviations from HARDY-WEINBERG proportions.

Genetic distance d_0

Provenance samples

Focusing the genotypic distances, the highest values are indicated for loci AAT-A and MNR (*Table 5*). At locus AAT-A, the largest genotypic distances are observed in the pairs Ebern/Waldsassen (d_0=0.32) and Mitterteich/Waldsassen (d_0=0.29). At locus MNR, the samples Ebern and Mitterteich reveal the largest genotypic distance (d_0=0.35) (*Table 5*). The values for the genetic distances of their gene pool vary between 0.06 and 0.08.

Subsets within provenance samples

The allelic distances between lower third and upper third of the dbh-samples of the same provenance are low at most loci, except locus AAT-A with an allelic distance of 0.18 between the dbh-1 and dbh-3 subsets of Waldsassen and locus MNR with an allelic distance of 0.24 between samples dbh-1 and dbh-3 of Ebern. Remarkable though are the distance values at locus AAT-A in pairs of samples combined with one of the dbh-subsets of Waldsassen: values vary between 0.20 (dbh-3 Ebern/dbh-1 Waldsassen) and 0.43 (dbh-1 Mitterteich/dbh-3 Waldsassen).

Subsets of elite and non-elite trees of the same site reveal maximum allelic distances of 0.10 between Waldsassen Z and R at locus SKDH-A up to 0.26 between Schwabach Z and R at AAT-C. Again, at locus AAT-A samples paired with subsets of

Table 4. – Results of G- and χ^2 -test and fixation index F for the samples Ebern, Mitterteich, Schwabach and Waldsassen at the loci AAT-A, AAT-B, LAP-A, MNR and 6-PGDH-B. Levels of probability: * (p = 0.05), ** (p = 0.01), *** (p = 0.001).

Locus	Ebern		Mitterteich		Schwabach		Waldsassen	
	G/χ^2	F	G/χ^2	F	G/χ^2	F	G/χ^2	F
AAT-A	***/***	0.263	n.a./***	-0.026	n.a./***	-0.040	***/***	0.064
AAT-B	**/**	0.050	n.a.	0.021	**/*	0.172	***/***	0.091
LAP-A	n.a.	0.072	n.a.	0.097	n.a./***	0.214	n.a.	0.218
MNR	***/***	0.500	n.a.	-0.041	***/***	0.450	***/***	0.499
6-PGDH-B	n.a.	0.005	**/***	0.165	n.a./**	0.051	n.a.	0.072

Table 5. – Genotypic distances at the loci AAT-A and MNR (upper and lower diagonal half, respectively) for the samples Ebern, Mitterteich, Schwabach and Waldsassen.

		AAT-A			
	Sample	Ebern	Mitterteich	Schwabach	Waldsassen
M	Ebern	–	0.13	0.15	0.32
N	Mitterteich	0.35	–	0.06	0.29
R	Schwabach	0.22	0.19	–	0.26
	Waldsassen	0.25	0.19	0.22	–

Waldsassen show high distance values ranging from 0.20 (Mitterteich Z/ Waldsassen Z) to 0.32 (Ebern Z/ Waldsassen Z).

The loci AAT-A and MNR again appear to show largest values if samples of thin and thick trees are paired. Maximum allelic distance values appear at AAT-A (d0=0.16) and at locus MNR (d0=0.13); the distance of the gene pool is 0.05.

Genetic differentiation

The genetic (allelic) differentiation is demonstrated in *figures 3, 4,* and *5* for the same categories of samples at each of the loci AAT-A, LAP-A and MNR. These loci reveal larger deviations among allele frequencies than the remaining ones. In contrast to the selected three gene loci, the differentiation with respect to all studied loci (gene pool, *Fig. 6*) is very low. The radii of the dotted circles are equal to the average level of differentiation at the particular locus. The arc of each sector represents the size of the respective sample. The given scale measures the average proportion of alleles in which any sample differs from the remainder: the larger the radius of the dotted circle, the more effective the respective locus is in reflecting genetic differentiation. The sample with the largest radius assembles the greatest amount of differentiation compared to the remaining collectives. The more the sector radii approach to the centre, the more representative is the genetic information of the respective sample.

Provenance samples

In case of locus AAT-A, Waldsassen represents a more specific allelic differentiation than the other samples. The same holds for Schwabach at locus LAP-A, whereas at locus MNR the provenance samples Ebern and Mitterteich are more differentiated than Schwabach.

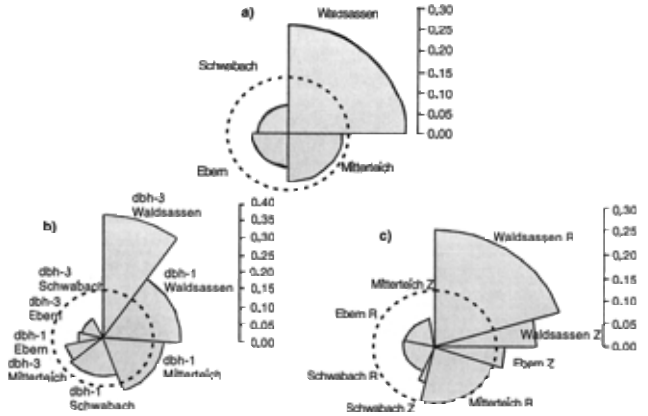

Fig. 3. – Genetic differentiation of provenance samples (a), dbh-classes (b) and elite and non-elite tree subsets (c) at gene locus AAT-A.

Subsets within provenance samples

Evidently, certain collectives assemble most of the differentiation of each provenance sample. At AAT-A, all subsets derived from sample Waldsassen show a greater differentiation than the others. In the case of Schwabach at locus LAP-A, the samples of the thicker trees, i. e. collectives dbh-3, Schwabach and Schwabach Z, substantially contribute to the given differentiation. In contrast to this, the specific information at locus MNR of Ebern is caused by the subsets of trees with lower dbh, i. e. dbh-1 of Ebern and Ebern R. Remarkable though are the small radii of dbh-3 of Schwabach and Mitterteich Z at AAT-A and Waldsassen Z at locus MNR. These samples represent the

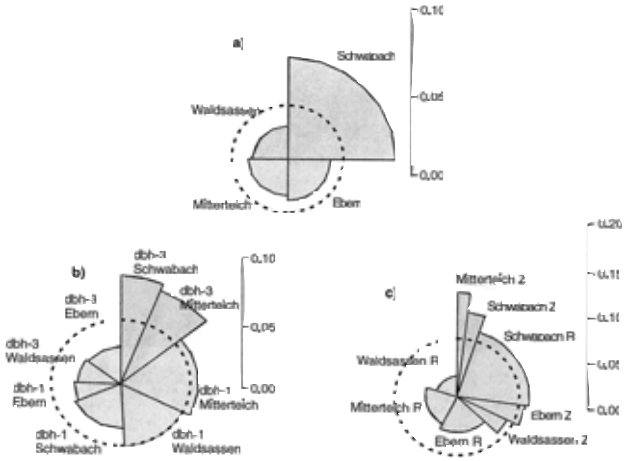

Fig. 4. – Genetic differentiation of provenance samples (a), dbh-classes (b) and elite and non-elite tree subsets (c) at gene locus LAP-A.

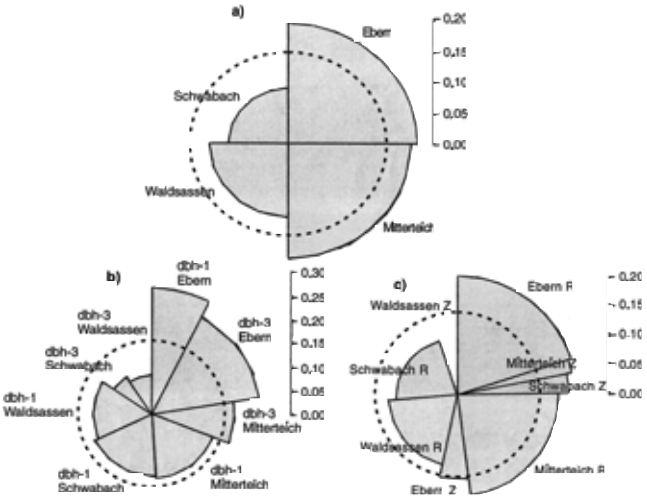

Fig. 5. – Genetic differentiation of provenance samples (a), dbh-classes (b) and elite and non-elite tree subsets (c) at gene locus MNR.

best average differentiation at these loci. In case of the gene pool (*Fig. 6b* and *6c*), only the subsets dbh-1 of Ebern, Ebern Z, Mitterteich Z and Schwabach Z reveal a considerable higher gene pool differentiation as compared to the remaining subsets.

Log likelihood ratio test (G-test) of homogeneity among frequency distributions

Over all provenance samples, dbh-classes and subsets of elite and non-elite trees, particularly the loci AAT-A, LAP-A and MNR reveal a significant heterogeneity among allelic frequency distributions. Within the provenance samples, the frequency distributions at the loci AAT-A, MNR and SKDH-A deviate statistically significant from each other. The samples of thin and thick trees reveal statistically significant deviations among the allelic structures at the locus AAT-A.

Genetic characters and individual tree growth

Effects of actual heterozygosity at the gene pool level and at single locus combinations

Results of the linear regression analyses point out, that with increasing mean heterozygosity at 15 gene loci (IDH excluded because of lacking polymorphism), provenance samples Mitter-

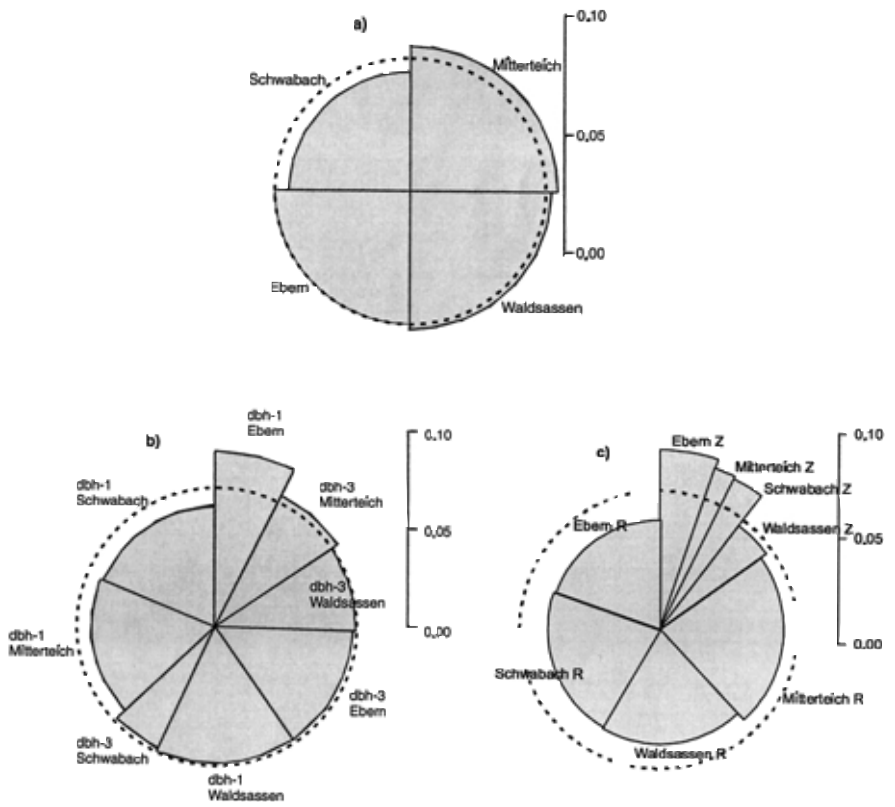

Fig. 6. – Genetic differentiation of the gene pool of the provenance samples (a), dbh-classes (b) and elite and non-elite tree subsets (c).

teich, Schwabach-D. and Waldsassen show a slightly positive tendency towards increasing stem volume (wood above 7 cm dbh). Except Ebern, especially rare trees with a volume above 0.4 m^3 share higher portions of heterozygosity (*Fig. 7*).

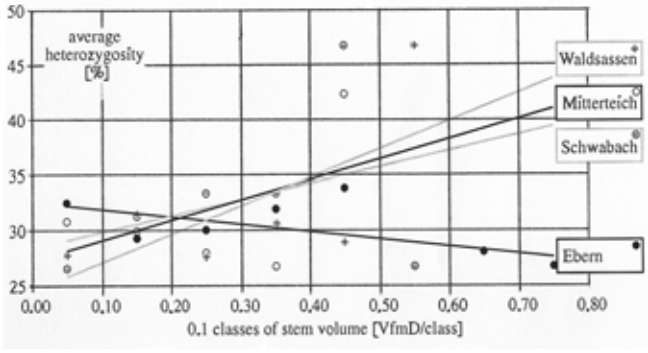

Fig. 7. – Average heterozygosity – including 15 gene loci – stratified by 0.1 classes of stem volume (above 7 cm dbh) and provenance samples. The adjustment was determined via linear regression (R2 = 0.1342…04354).

The test of the effect of actual heterozygosity at single locus combinations results in significant mean effects on diameter growth of all provenance samples, i.e. three combinations of gene loci for Schwabach, two for Ebern and for Waldsassen and one for Mitterteich (*Table 6*). In most cases, these mean effects are accompanied by significant interactions between the two gene loci.

Based on the pooled provenance data, the combination of MDH-C and 6PGDH-B reveals interactions which are indicated by the crossing lines in *figure 8* (Bortz, 1993). The factor loads of the two gene loci can be combined in four ways, two of them corresponding with lower values of dbh, i.e. both gene loci are either homozygous or heterozygous. On the other hand, higher values of dbh are connected with combinations of homozygous and heterozygous gene loci.

Effects of genotypic rareness

With the exception of provenance Ebern, the two-factorial analysis of variance including genotypic rareness (<10%) entails significant mean effects on diameter growth at two of

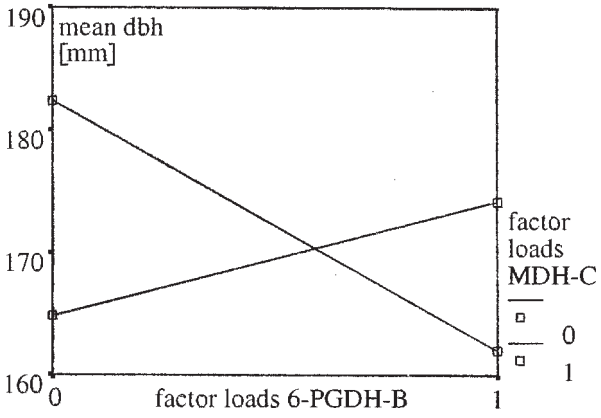

Fig. 8. – Two-factorial analysis of variance with respect to the gene loci 6-PGDH-B and MDH-C: Mean diameters at breast height (dbh) are given for different combinations of factor loads (load 0 „homozygosity“, load 1 „heterozygosity“). In case of two-locus heterozygosity, interactions lead to significant effects on diameter growth.

Table 6. – Significant effects (related to mean effects) following the two-factorial analysis of variance. The database is sub-structured into four provenance samples (4x100 trees) and the entire data pool (400 trees). Dependent variable is diameter at breast height. Alternating factors are actual heterozygosities per locus (transformed 0/1) for combinations of two loci out of 15. Beside f-values, the following significance is given: for two factors, for interactions (two-way-sig.) and for mean effects (M. effect).

Provenance	Factor 1			Factor 2			Interaction	M. effect
	gene locus	f-value	Sig.	Gene locus	f-value	sig.	two-way sig.	Exp-sig
Ebern	MDH-C	0.173	0.678	6PGDH-B	0.425	0.516	0.001	0.01
	6PGDH-B	6.091	0.015	PGM-A	0.326	0.569	0.000	0.002
Mitterteich	LAP-A	3.717	0.057	PGM-A	4.219	0.043	0.003	0.031
Schwabach	AAT-B	0.993	0.322	6PGDH-B	0.001	0.980	0.003	0.017
	GDH-A	2.598	0.110	MNR	5.871	0.017	0.999	0.047
	MDH-A	1.977	0.163	MNR	0.536	0.466	0.035	0.01
Waldsassen	AAT-B	5.876	0.017	SKDH-A	0.295	0.589	0.004	0.004
	LAP-B	5.992	0.016	MDH-A	1.579	0.212	0.114	0.044
All data	MDH-C	0.748	0.372	6PGDH-B	1.120	0.291	0.009	0.046

Table 7. – Significant effects (related to mean effects) following the two-factorial analysis of variance with respect to four provenance samples. Alternating factors are genotypic rarenesses (<10%) per locus (transformed 0/1) for combinations of two loci out of 15. For further explanation see table 6.

Provenance	Factor 1			Factor 2			Interaction	M. effect
	Gene locus	f-value	Sig.	gene locus	f-value	sig.	two-way sig.	Exp-sig
Mitterteich	AAT-C	0.019	0.892	LAP-A	6.234	0.014	n.a.	0.049
	ACO-A	0.350	0.555	LAP-A	6.009	0.016	n.a.	0.041
	GDH-A	1.129	0.291	LAP-A	5.901	0.017	n.a.	0.028
	LAP-A	5.782	0.018	LAP-B	1.410	0.238	n.a.	0.024
	LAP-A	6.405	0.013	MDH-A	1.233	0.270	n.a.	0.027
	LAP-A	8.187	0.005	MDH-B	0.116	0.735	0.152	0.042
	LAP-A	6.574	0.012	MDH-C	1.416	0.237	n.a.	0.024
	LAP-A	7.464	0.007	MNR	4.391	0.039	0.162	0.014
	LAP-A	6.150	0.015	6PGDH-B	0.003	0.957	n.a.	0.049
	LAP-A	5.970	0.016	SKDH-A	1.805	0.182	n.a.	0.020
	LAP-A	6.059	0.016	SKDH-B	0.418	0.520	n.a.	0.040
Schwabach	MDH-A	4.913	0.029	SKDH-A	5.434	0.022	0.032	0.035
Waldsassen	LAP-B	6.126	0.015	MDH-A	1.597	0.209	0.114	0.041
	MDH-A	2.815	0.097	MDH-C	3.890	0.051	n.a.	0.048

15 gene loci (*Table 7*). Regarding provenance Mitterteich, especially combinations with LAP-A are indicative. Only for MDH-A in combination with SKDH-A, provenance Schwabach reveals additional significant interactions. In the case of pooled provenance data, significant effects are not evident.

Effects of most frequent alleles

For six polymorphic gene loci (AAT-A, GDH-A, LAP-A, LAP-B, MNR, SKDH-A) the two most frequent alleles per locus were studied in detail. Within provenance samples Mitterteich and Schwabach combinations including LAP-A respectively to SKDH-A show significant mean effects on dbh growth, whereas significant interactions are not evident (*Table 8*).

In the case of the pooled data, AAT-A is particularly indicative (*Fig. 9*). Heterozygosity at this gene locus (factor load 2, representing A2A4) corresponds with a remarkable reduction in diameter dimension.

Table 8. – Significant effects (related to mean effects) following the two-factorial analysis of variance with respect to four provenance samples and the entire data pool. Alternating factors are representations of the two most frequent alleles per locus (transformed 0/1/2/3) for combinations of two loci out of 15. For further explanation see table 6.

Provenance	Factor 1			Factor 2			Interaction	M. effect
	gene locus	f-value	Sig.	gene locus	f-value	sig.	two-way sig.	Exp-sig
Mitterteich	AAT-A	1.222	0.306	LAP-A	6.160	0.001	n.a.	0.003
	GDH-A	0.214	0.886	LAP-A	5.031	0.003	n.a.	0.011
	LAP-A	5.650	0.001	LAP-B	0.770	0.466	n.a.	0.003
	LAP-A	5.985	0.001	MNR	0.819	0.444	n.a.	0.003
	LAP-A	6.015	0.001	SKDH-A	1.347	0.264	n.a.	0.003
Schwabach	AAT-A	0.863	0.463	SKDH-A	3.733	0.014	n.a.	0.019
	GDH-A	1.682	0.176	SKDH-A	4.579	0.005	n.a.	0.007
	LAP-A	0.263	0.852	SKDH-A	4.103	0.009	n.a.	0.037
	LAP-B	0.594	0.554	SKDH-A	4.188	0.008	0.154	0.022
All data	AAT-A	3.144	0.025	GDH-A	1.206	0.307	n.a.	0.046
	AAT-A	3.536	0.015	LAP-A	1.262	0.287	n.a.	0.043

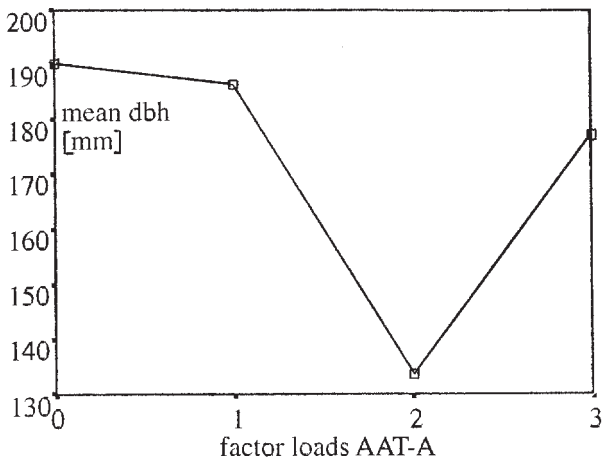

Fig. 9. – Two-factorial analysis of variance with respect to the two most frequent alleles at two out of six gene loci: Mean diameters at breast height (dbh) are given for factor loads 0 (n.a), 1 (allel 1 homozygous), 2 (heterozygosity) and 3 (allel 2 homozygous). Combinations with locus AAT-A revealed significant effects on diameter growth.

Discussion

Sustainable forestry connected with genetic resources has been acknowledged politically as a significant topic (e.g. MERKER and SPELLMANN, 2000; STEPHAN, 2000). Provenance samples originating from a broad spectrum of topographical characteristics and site factors were commonly accepted to ensure high levels of genetic variation (e.g. ROTACH, 1994). Gene markers efficiently help to quantify genetic variation within and among populations.

Genetic variation in provenance samples and subsets

Provenance samples

In terms of diversities, sample Ebern distinctively possesses the highest values of gene pool-diversity (1.46) and gametic multilocus diversity (803.3) in particular. This sample reflects the largest potential to create genetic variation in the following generation in terms of producing different gametes.

The observed relatively low values for the allelic multiplicity and diversity compared to the results of other studies (BERGMANN and HOSIUS, 1996; HERTEL *et al.,* 1998) may result from a reduction of genetic variation of the provenance samples following the collection of the respective reproductive material from only few seed trees and/or genetic selection during the growing up of the populations.

The actual heterozygosities range within the results of other studies on Scots pine (HERTEL *et al.,* 1998; HERTEL and KOHLSTOCK, 1993; BERGMANN and HOSIUS, 1996; MEJNARTOWICZ and PALOWSKI, 1989; MÜLLER-STARCK, 1987; GULLBERG et al., 1982). Investigating different growth types (a- and b-type) of Scots pine, HERTEL *et al.,* (1998) reported lower values of actual heterozygosity in trees of higher mean dbh (b-type) than in those of lower dbh. With the exception of provenance sample Ebern, higher volume growth tends to be positively correlated with increasing heterozygosity.

In contrast to a study on Norway spruce by ERIKSSON *et al.,* (1973) who proved inbreeding to be responsible for a depression in volume growth of trees, the underlying study does not indicate such a correlation between fixation indices and growth within the provenance samples. There are only a few loci within the collectives that do not correspond to HARDY-WEINBERG proportions because of surplus homozygotes. It is concluded for the present that those individuals, which are homozygous at the respective loci, were favoured by viability selection and/or that inbred individuals were already eliminated by selective processes through the 47 years of existence of the provenance trial.

Though genetic distance values among the four provenance samples seem to be quite low, the gene pools differ more from each other than those of samples of two Central European and one Polish provenance in a study by HERTEL and KOHLSTOCK (1993). In particular, the provenance sample Waldsassen is characterised by a specific genetic structure at locus AAT-A, while locus MNR revealed to have a great genetic variation in each provenance sample.

Despite the low differentiation of the gene pool of all sample categories at the loci AAT-A, LAP-A and MNR, the genetic differentiation at these loci obtained some remarkable aspects. The differentiation at locus AAT-A is dominated by sample Waldsassen, each subset of which represent a specific amount of differentiation as compared to all the remaining subsets. At LAP-A, the great differentiation of sample Schwabach is particularly due to the sample of thicker trees represented by the subsets dbh-3 of sample Schwabach and Schwabach Z. Remarkable though is the pronounced differentiation of Ebern following the subsets of the thinner trees in sample dbh-1 of Ebern and Ebern-R.

KOHLSTOCK *et al.,* (1993) found statistically significant differences in the allelic frequencies at loci SKDH-A after examining two morphological different types of Scots pine. Beside loci AAT-A, LAP-A and MNR, locus SKDH-A also revealed statistically significant differences in the allelic structures in a log likelihood ratio test (G-test) over all samples.

Subsets of provenance samples

For quantification of phenotypic traits, classes of diameter growth and of vitality (both integrated in silvicultural decisions) were employed and associations between these traits and genetic characters studied. At 9 out of 15 gene loci, the mean actual heterozygosity was verified to be larger in the pooled samples of dbh-3 trees than in the grouped samples of dbh-1 trees with lower diameters. Pairs of subsets of different geographic origin and different dbh- or elite tree-categories, e. g. dbh-1 of Mitterteich and dbh-3 of Waldsassen (d_0=0.43) are genetically most distant from each other as could be expected according to the strongly deviating metric characters of both provenance samples. With the exception of provenance sample Schwabach, the subsets of the elite trees show a tendency to a more homogenous allelic distribution, which results in higher values of the hypothetical gametic multilocus diversity but a lower allelic multiplicity. All subsets of elite trees within provenance samples show a higher actual heterozygosity and the pooled subsets of the elite trees reveal a higher proportion of actual heterozygosity at 11 out of 15 gene loci than the pooled samples of non-elite trees. Values for genetic multiplicity and gene pool diversity are greater in the sample of thick trees and the hypothetical gametic diversity is twice as high in the sample of thick trees compared to the thin ones. The potential of trees of higher diameter to create genetic variation is greater than of trees with lower dbh. Furthermore, the mean actual heterozygosity is higher in the sample of thick trees as compared to the thin ones; in 9 out of 15 loci the sample of thick trees reveals a larger proportion of heterozygotes than the thin trees subset.

Single tree growth in relation to genetic parameters

Diameter at breast height – available for every tree – was used as dependent variable, although this parameter is expect-

ed to be mainly influenced by environmental conditions (EBERT, 1997). While site conditions are more or less equal within the trial area, the effects of thinning from above cannot be denied. Nevertheless, also other authors detected genetic effects on diameter growth e.g. of the order of 10% (e.g. LIESEBACH *et al.,* 2001). To verify genetic impacts on diameter growth, three different strategies were applied and all of them indicate effects.

The first approach focuses heterozygosity. This contradictory discussed parameter is an important buffer for rare alleles (HUSSENDÖRFER and MÜLLER-STARCK, 1997). Analysis of variance based on the pooled provenance data emphasises factors MDH and 6PGDH-B as well as their interactions (both metabolic category I). Under complex environmental stress, the response of the enzyme system MDH is well known for beech and silver fir (KONNERT, 1992; MÜLLER-STARCK, 1993). HUSSENDÖRFER and MÜLLER-STARCK (1997) describe positive physiological reactions for silver fir resulting from heterozygosity at 6PGDH gene loci.

In a second approach, significant response with respect to rare alleles (frequency < 10%) is observed. All provenance samples except Ebern show significant mean effects on diameter growth. The meaning of these rare alleles for the preservation of adaptability is also discussed controversial (KÄTZEL, 2000), although they represent a latent potential of adjustment to drastically changing environmental conditions (BERGMANN *et al.,* 1990). Furthermore, an efficient combination of rareness and heterozygosity was observed by HERTEL *et al.,* (1998).

In the last step, the representation of the two most frequent alleles and their importance for the dimension of diameter was studied. In particular the presence of AAT-A in connection with an analysis of pooled data was obvious (especially the heterozygous combination A2A4). Holding a key position within amino acid synthesis, AAT-A belongs to metabolic category I (RICHTER, 1988). KONNERT (1992) points out, that according to this enzyme system, in air-polluted stands of silver fir significant differences between tolerant and sensitive individuals occurred.

Concluding this attempt to detect genetic impacts on diameter growth, two major restrictions must be pointed out. Firstly, the frequencies of the factor loads and their combinations within the analysis of effects of rare and most frequent alleles are often small. Secondly, only provenance sample Ebern (with the best growth performance) shows exceptionally significant effects. This measured growth efficiency may be caused by combinations of more than two of the studied gene loci and/or there are inferences by various gene loci that have not been taken into account in this analysis.

Nevertheless, this study revealed evidence for relation between genetic parameters and diameter growth. In a further step, other environmental influences should be considered in more detail. By the time growth simulators for single trees are able to integrate external factors, but do not yet take into account provenance-specific information, especially genetic structures.

Acknowledgements

The authors greatly appreciate valuable suggestions and comments of Prof. Dr. H. PRETZSCH, Freising and Prof. Dr. G. ROTHE, Mainz. This study was supported financially by the State Forest Administration of Bavaria, Munich.

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Balancing Genetic Gain and Relatedness in Seed Orchards

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(Received 26th February 2001)

Summary

The traditional way to avoid related mating and subsequent inbreeding depression in seed orchards is to use only unrelated clones for orchard establishment. As tree-breeding programs move to advanced generations, relatedness (coancestry) among candidates for seed orchard selections becomes more common, especially for high breeding value candidates. The traditional way of selecting the ones with the highest breeding value, provided they are unrelated, is referred to Restricted Selection (RS). In order to consider breeding value as well as relatedness, an alternative selection method, based on a value criterion for the whole group of selected clones, is presented in this paper. The method, here called Group Merit Selection (GMS), is based on a suggestion by LINDGREN and MULLIN (1997), but modified for seed orchard selection by neglecting selfing and self-coancestry. The method can be regarded as the selection of a group of clones that maximizes expected genetic value (predicted genetic gain minus inbreeding depression).

A case study was conducted in which twenty clones for a seed orchard were selected among second-generation loblolly pine (*Pinus teada* L.) selections from the NCSU-Industry cooperative breeding program. Assuming an observed inbreeding depression of 40% for one unit coefficient of inbreeding, penalty constants based on estimated breeding values at age 8 was corresponding with inbreeding depression. That gave 12% more genetic value for GMS than Restricted Selection. Predictions of the penalty constant considering additional relevant factors (such as pollen contamination, breeding values based on immature trials, and unrepresentative experimental sites) resulted in selection of the same clones. Changes among the selected clones did not occur until relatedness reached twice the penalty constant, suggesting that GMS solutions are rather robust.

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Key words: seed orchard, group merit selection, restricted selection, inbreeding, relatedness, status number, genetic gain, loblolly pine.

Introduction

The objective of a seed orchard is to produce seeds with high genetic quality. The implication may be that a seed orchard should give seeds with high breeding value and low inbreeding. To avoid inbreeding depression only unrelated clones are traditionally used for seed orchard establishment. When a tree-breeding program moves to advanced generations, the number of unrelated families is reduced. Breeders are thus faced with a dilemma. Would the acceptance of related selections in a seed orchard result in substantial genetic gain or would it result in an intolerable inbreeding depression? LINDGREN and MULLIN (1997) suggested a selection algorithm to select a group of genotypes, where both breeding value and relatedness are considered in an optimal way. It seems possible to develop this algorithm as a means for seed orchard composition, where related clones can be considered.

The loblolly pine (*Pinus teada* L.) breeding program at the N.C. State University has completed two cycles of breeding with 45 years of genetic improvement operations. Substantial genetic gains have been achieved in forest tree species by establishment of seed orchards with phenotypically and genetically selected parents (ZOBEL and TALBERT, 1984). The secondgeneration breeding program began in the 1970's and 2nd generation seed orchards are now producing more than 50% of the total seed harvest in the program with an average gain of 10% in rotation volume compared to the 1st generation seed orchards (LI *et al.*, 1999). If only the best 30% of the parents were to be used to establish new seed orchards, i.e. 2.5 generation seed orchards (ZOBEL and TALBERT, 1984), an additional 13.5% gain increase compared to unrogued 2nd generation seed orchards could be expected (LI *et al.,* 1999). This prediction assumes that no inbreeding depression would occur. However, in reality, there is a high relatedness among the top-ranked clones (based on progeny tests) in an advanced tree-breeding program.

It is generally well known that mating of related individuals can result in inbreeding depression of growth and adaptation