Allozyme Variation of Indigenous Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] Populations and their Descendants in Germany[1])

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Abstract

Twenty-three populations of autochthonous Douglas-fir from British Columbia and Washington and from their descendants field-tested in the IUFRO-provenance trial at German sites were sampled to document early genetical impacts of species translocation. Tissue of vegetative buds was surveyed for allozyme variation at five loci. Coastal varieties revealed higher allozyme variation than interior populations. Allele frequencies were not significantly different in North American populations versus German cohorts. Unique allozymes were both detected in native and translocated populations. All loci studied were characterized by minor polymorphisms (frequency of the most common allele greater than 0.9) and therefore aggravate conclusions. Based on the comparably small number of marker genes that were used and the limited time during evolution could act there is no evidence that a translocation imposes different evolutionary forces detectable at the loci under study. Reasons for this lack of noticeable genetic differentiation between native and translocated Douglas-fir populations are discussed.

Key words: Pseudotsuga menziesii, allozymes, genetic differentiation, translocation.

FDC: 165.3; 165.5; 174.7 *Pseudotsuga menziesii;* (430).

Introduction

One of the greatest effects that humans have had on the genetics of forest tree populations is by bridging barriers to gene flow (LEDIG, 1992). During prehistoric and more recent human movement, many tree species have been translocated around the globe such as coconut (*Cocus nucifera*; OHLER, 1984) and black locust (*Robinia pseudoacacia*; KERESZTESI, 1980). In the genus *Pinus* for instance, natural and human-directed selection as well as bottlenecking have strongly reduced genetic diversity of semi-domesticated Afghan pine (*Pinus brutia* ssp. *eldarica*) compared to Calabrian pine (*P. brutia*) (CONKLE et al., 1988).

Populations transferred to a new environment adapt to it and often outperform its progenitors in fitness. In New Zealand, for instance, European larch (*Larix decidua*) seedlings originating from local seed orchards grow faster than those derived directly from European seed sources (MILLER, 1964). In an adventive population of common pear (*Pyrus communis*) in southern Ontario, natural selection is operating to eliminate poorly adapted genotypes as shown by growth analysis of trees phenotypically like and unlike the founding individuals (WALDRON et al., 1976). Also results of European provenance trials of Douglas-fir suggest that introduced provenances locally adapt (KLEINSCHMIT et al., 1974). Presumably new ecotypes have already evolved from German local populations, since progenies from certain isolated Douglas-fir stands in southwestern Germany, established at the turn of this century, grow exceptionally well. Often these trees even surpass the performance of Douglas-fir plants originating from Washington or British Columbia that are well adapted to German site conditions. Genetic comparisons of natural Douglas-fir populations with those translocated to Germany should provide information about evolutionary processes. Unfortunately the American sources of aforementioned Douglas-fir ecotypes in Germany are unknown and cannot be historically retraced.

Common garden experiments have shown that adaptive differentiation can stem from environmental heterogeneity attributable to elevation and/or exposition. In *Pseudotsuga menziesii* considerable adaptive variation on a macro- and microspatial scale has been reported (see literature cited in the review by KLEINSCHMIT and BASTIEN, 1992). Genetic effects by translocation are undoubted. Unfortunately, genetic structure at the allozyme level bears no or at best weak relation to geographical and environmental clines in Douglas-fir (MERKLE and ADAMS, 1987; MORAN and ADAMS, 1989). Allozymes have, therefore, some shortcomings to detect adaptation in this conifer.

Gene flow is pronounced in *Pseudotsuga menziesii* (e.g., FAST et al., 1986) and might counteract spatial differentiation caused by microhabitats in native stands. Since translocated populations are often isolated, mingling of topodemes will scarcely occur and consequently a counterbalance due to gene flow is not present or at least weakened. Besides spatial selection, translocated populations undergo strong SEWALL-WRIGHT effects. Those populations mostly descend from few open-pollinated families. Therefore, distinct genetic drift and marked selection might shape genetic differences between seed source populations and their descending cohorts that are detectable at the allozyme level in Douglas-fir. This paper reports about the genetic evaluation of 11 natural *Pseudotsuga menziesii* populations in British Columbia and Washington used as seed sources for the IUFRO-1970 Douglas-fir provenance test and their descendants in German field trials. To the best of the authors' knowledge, this is the first investigation of an allozyme comparison between tree seed sources and its progenies grown up on a different continent.

Materials and Methods

The IUFRO-1970 Douglas-fir provenance test comprises 124 seed sources. About one third of the natural stands in North America could still be identified. Timber of the remaining seed sources have been cut and forest stands have been reestablished partly with non-autochthonous plants. Since 50 individual trees were judged as the minimum sample size, number of available North American-German populations pairs further diminished to 23 samples [11 North American – German population pairs plus 1 German population (see below)]. *Figure 1* depicts North American locations where all 15 open-pollinated families were sampled. The seeds of these trees were used to establish the German field tests of the IUFRO-1970 Douglas-fir

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provenance trial. In spring 1990, at three German locations (forest office Katzenelnbogen, forest district Bärlach, Rhineland-Palatine, forest office Rauschenberg, forest district Wolferode, Hesse, forest office Bensheim, forest district Lorsch, Baden-Württemberg) buds from trees field tested in the IUFRO-provenance trial were collected. Bud samples in North American forest stands were collected in spring 1990. Fifty trees from each of North American populations and German cohorts were sampled. Populations were labelled according to the IUFRO-1970 trial (KLEINSCHMIT et al., 1974). Population #1003, a sample close to the northern limit of the interior distribution was included although resampling in North America was impossible. This population was only considered for comparisons between the coastal and interior variety. In addition to the IUFRO-provenance number, North American populations were designated with "*NA*" and German cohorts with "*G*". Age of *NA*-populations was approximately between 100 and 250 years. All *G*-cohorts were 23 years old when sampled. Since German cohorts have partly perished, viability selection is a conceivable evolutionary factor.

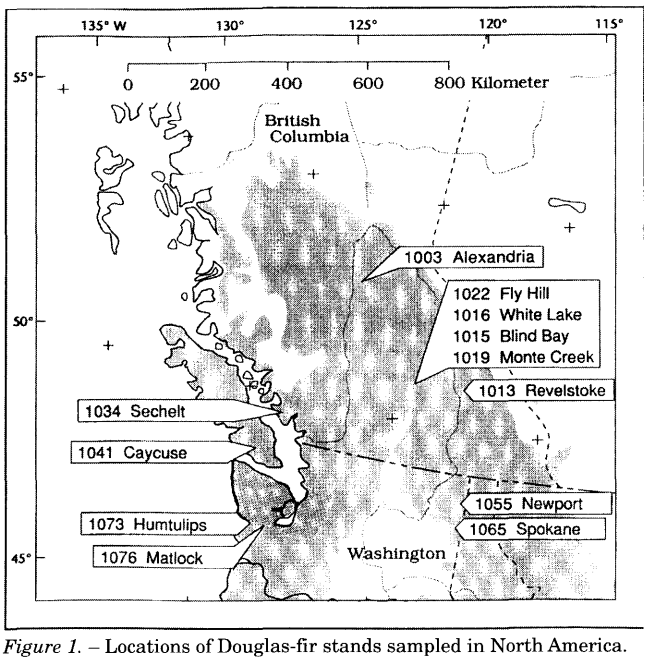

Figure 1. – Locations of Douglas-fir stands sampled in North America.

After scales were removed, enzymes of three dormant vegetative buds were extracted by using 30 µl to 50 µl of following buffer: 50 ml H_2O, 50 ml 0.3 m tris-HCl, pH 8.0, 12 g polyvinylpyrolidon 40, 150 mg DL-dithiothreitol (DDT). Horizontal electrophoresis was performed on 12% (w/v) Sigma Chemical Co. starch. Gel-electrode buffers, electrophoretic conditions, and staining solutions followed the methods described by CONKLE *et al.* (1982). Aspertate aminotransferase (AAT), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH), and malate dehydrogenase (MDH) were employed resulting in 5 gene loci (*Aat1, Aat2, Gdh, Idh, Mdh3*) that could be scored unambiguously. Genetic control of allozymes found at these loci is reported by ADAMS et al. (1990). *Aat2, Mdh3,* and *Idh* belong to the same linkage block. Linkage of these loci is moderate (*Idh* – 76 cM – *Aat2* – 42 cM – *Mdh3*)(ADAMS et al., 1990).

The BIOSYS computer program written by SWOFFORD and SELANDER (1989) was used to calculated allele frequencies, mean number of alleles per locus, observed and expected heterozygosities, and WRIGHT's *F*-statistics. Among populations differentiation was estimated using WRIGHT's F_{ST} that measures the amount of differentiation relative to the limiting amount under complete gene fixation as $F_{ST}=1-(1-F_{IT})(1-F_{IS})^{-1}$. Additionally, genetic distance (GREGORIUS, 1974), genic multiplicity, and genetic diversity (HATTEMER, 1991) were computed. Allele frequencies were checked using chi-square homogeneity test. Differences at genic multiplicity within population pairs was tested using the Wilcoxon matched pairs signed rank test (SOKAL and ROHLF, 1981, pp. 449 to 450). Probability of losing genic multiplicity in course of sampling was calculated according to HERNANDEZ and CROSSA (1993). Cluster analysis of population was conducted according to the UPGMA procedure (SNEATH and SOKAL, 1973) and based on genetic distances.

Results and Discussion

Of the 5 loci scored, 4 were polymorphic based on the 99% criterion. *Gdh* was monomorphic in all populations. Allozyme variation, measured as mean number of alleles per locus, percentage of polymorphic loci ($P_{.99}$), observed (H_o) and expected HARDY-WEINBERG heterozygosity (H_e), and genetic diversity (v) is shown by *table 1*. On average, mean number of alleles per locus was 1.9, 56.5% of the loci were polymorphic, heterozygosities (observed and expected) were each 0.07 and genetic diversity was 1.1. *Table 2* summarizes WRIGHT's *F*-statistics. The amount of genetic differentiation was very similar at all loci. F_{ST} averaged 0.04. Only 4% of the total allozyme variation was caused by genetic differentiation among all *NA*-populations and *G*-cohorts.

Table 1. – Mean estimate of number of allele per locus, percentage of polymorphic loci [99% criterion ($P_{0.99}$)], observed heterozygosity (H_0), expected heterozygosity (H_e), and genetic diversity [effective number of alleles (v)] in North American (*NA*) and German (*G*) populations.

Group	mean number of alleles	$P_{.99}$	H_o	H_e	v
coastal	2.1	62.5	0.108	0.104	1.17
interior	1.7	53.3	0.048	0.048	1.07
diff.:coastal - interior	+0.4	+3.2	+0.60	+0.56	+0.10
coastal					
NA	2.1	60.0	0.111	0.109	1.18
G	2.3	65.0	0.110	0.106	1.15
diff.: NA - G	-0.4	-5.0	+0.001	+0.003	+0.03
interior					
NA	1.8	57.1	0.050	0.051	1.07
G	1.7	50.0	0.047	0.045	1.06
diff.: NA -G	+0.1	+7.1	+0.003	+0.006	+0.01

Table 2. – WRIGHT'S *F*-statistics

Gene locus	F_{IS}	F_{IT}	F_{ST}
Aat1	-0.008	0.036	0.044
Aat2	-0.054	-0.019	0.034
Idh	-0.006	0.034	0.039
Mdh3	-0.050	-0.005	0.043
Mean	-0.029	0.012	0.040

In a range-wide Douglas-fir study, LI and ADAMS (1989) found higher measures of allozyme variation. For heterozygosity, for instance, an estimate of 0.137 was found. However, when only those loci of LI and ADAMS' study were considered which also have been employed in the present investigation H_e was 0.073, almost the same value found in the present paper. The coastal variety averaged higher measures of allozyme variation than the interior populations. Higher genetic diversity [= operating genetic potential (BERGMANN et al., 1990)] of the coastal seed sources coincides with better field performance irrespective of the chosen locality (KLEINSCHMIT and BASTIEN, 1992). Heterozygosities of the coastal variety were approximately twice as high compared to interior populations. In LI and ADAMS' study, H_e was 0.165 for the coastal and 0.151 for the interior variety (northern subgroup). Since allozyme variation in coastal populations decreases from Vancouver, Washington and Oregon to the southern and northern limits (LI and ADAMS, 1989), genetic variation of the coastal variety may be overestimated in this paper. In aforenamed study, allozyme variation among populations was estimated to be 0.071 and 0.043 for coastal and northern interior populations, respectively. GREGORIUS' (1974) genetic distance averaged 0.04 (range: 0.008 to 0.094). Cluster analysis revealed that the 2 varieties were genetically distinct (*Figure 2*). These results confirm previous findings (LI and ADAMS, 1989). The mean genetic distance in the interior variety was slightly smaller ($\bar{d}_o$=0.03, range 0.008–0.056) than in coastal populations ($\bar{d}_o$=0.04, range 0.020–0.072).

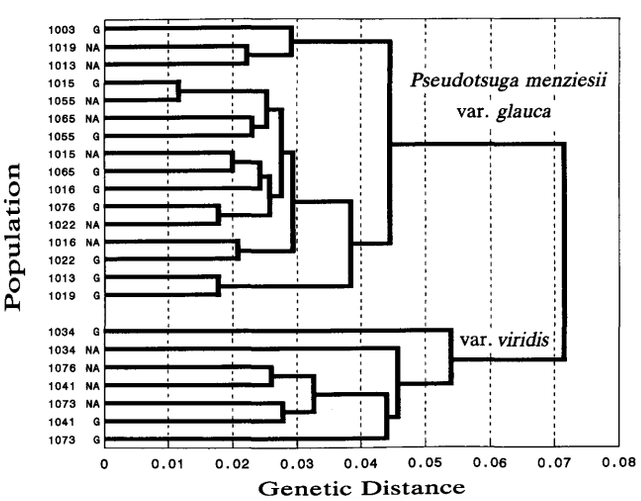

Figure 2. – Cluster diagram based on GREGORIUS' (1974) genetic distance.

Analysis of allele frequencies within population pairs resulted, with one exception, in no significant differences. However, not for all populations chi-square tests were conducted since expected number of alleles in certain classes was less than 5. Only at *Idh*, allele frequencies were significantly ($P \leq 0.01$) different within the *NA-G* population pair #1076. Mean genetic distance of populations pairs (*NA-G*) was $\bar{d}_o$=0.029. *Figure 3* illustrates a smaller genetic distance of a single pair (*NA-G*) (indicated by a black dot) than the mean genetic distance of the *NA* and *G* population of a certain sample # compared to all other populations (indicated by a horizontal bar). However, the genetic distance between *NA*-1076 and *G*-1076 was greater than the overall mean of average genetic distance of *NA*-1076 and *G*-1076 to all other provenances. Although, this population pair deviates from the general trend, genetic similarities between related *NA*- and *G*-populations are still noticeable.

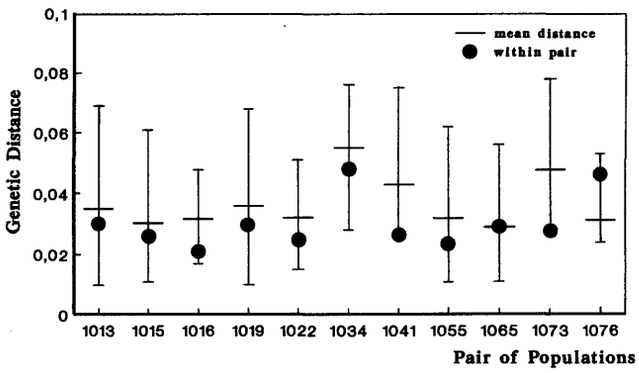

Figure 3. – GREGORIUS' (1974) genetic distances [mean (long horizontal bar), within the North American-German pair (black dot), range (small bars)] of 22 North American and German Douglas-fir populations.

Reduction of genic multiplicity depends on allele frequencies and sample size. In all *NA*-samples, 18 unique alleles were present, whereas 13 alleles were found exclusively in translocated populations. This difference was not significant. In 9 out of 11 cases, alleles from *NA*-populations were lost due to sampling drift and/or selection in German cohorts. If allele frequencies of *NA*-populations are unbiased expected probability of losing at least 1 allele was calculated. *Figure 4* shows the association of the actual allele loss and the probability of losing at least 1 allele. The point pattern indicates raising actual allele loss with higher expected probability of losing genic multiplicity. However, allele frequencies in *NA*-populations were biased due to limited sample size (N=50) and thus interfere with the point pattern of *figure 4*. Since all gene loci are characterized by minor polymorphism, i.e., the frequency of the most common allozyme is greater than 0.9, a sample size of N=50 is too small to detect differences of genic multiplicity between *NA*- and *G*-samples on a significant statistical level. If all alleles in *NA*-populations had to be sampled with a probability of 95%, sample sizes would range from 75 to 185 individuals depending on the *NA*-population concerned. Sampling variation imposes possible evolutionary factors acting at the loci under study and causes conclusions to be speculative.

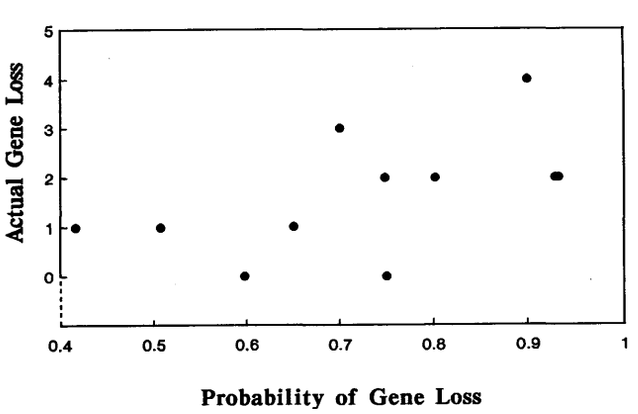

Figure 4. – Association between the Probability of losing genetic multiplicity and actual gene loss in Douglas-fir populations translocated to Germany.

Evolution surely modifies genetic structures in Douglas-fir and leads to differentiated topodemes (e.g., CAMPBELL, 1991). Morphological or physiological differentiation in space is by far more pronounced in Douglas-fir (e.g., ZHANG et al., 1993) than a spatial differentiation at the allozyme level (MERKLE and

ADAMS, 1987; MORAN and ADAMS, 1989). However, there is no evidence from this study that a translocation from native locations to Germany imposes different evolutionary forces detectable at the loci used in this study. Significant differences of allele frequencies at *Idh* within the population pair #1076 may be attributable to some form of selection and/or drift at this locus. Although all gene markers employed belong to the group-I enzymes that are probably adaptive (BERGMANN, 1991) the loci under study were neutral or evolutionary forces were too weak that they could be inferred by the sample size employed.

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Somatische Embryogenese und Sproßentwicklung in Abhängigkeit von 2,4-D- und BAP-Konzentrationen an zygotischen Embryonen der spätaustreibenden Stieleiche (*Quercus robur* L.)

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Zusammenfassung

Ein standardisiertes Verfahren zur in vitro-Vermehrung der spätaustreibenden Stieleiche konnte entwickelt werden. Durch gezielten Einsatz von Wachstumsregulatoren konnte sowohl somatische Embryogenese an den zygotischen Embryonen, als auch Sproßbildung bei zygotischen Embryonen stimuliert werden.

Sproßbildung bei zygotischen Embryonen konnte auf modifiziertem WPM mit 1 µM BAP + 1 µM 2,4-D, sowie auf modifiziertem WPM mit 10 µM BAP induziert werden. Auf modifiziertem WPM mit 10 µM BAP lag die Induktionsrate bei 87,9 %. In Kombination mit 5 µM 2,4-D fand bereits eine Hemmung der Sproßentwicklung statt.

Somatische Embryonen bildeten sich an den Keimblättern zygotischer Embryonen auf modifiziertem WPM (Woody plant medium) bei allen getesteten Kombinationen von 2,4-D und BAP, jedoch mit stark unterschiedlichen Induktionsraten. Die höchste Induktionsrate lag bei 34,8 % bei Verwendung von modifiziertem WPM mit 1 µM BAP + 1 µM 2,4-D.

Insgesamt wurden 36 Genotypen durch Sproßentwicklung aus zygotischen Embryonen und 17 Genotypen auf dem Wege der somatischen Embryogenese in vitro etabliert. Regeneration