Genetic Differentiation of Damaged and Healthy Douglas-Fir Stands in Rheinland-Pfalz with Respect to Their Origin

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Summary

Sixteen provenances of the IUFRO-provenance trial from one site in Germany were sampled. According to their allele frequencies 2 subgroups could be distinguished (d0 close to 0.7). The differentiation pattern based on genetic structures at the gene locus 6-PGDH-A agreed strikingly with the presumed ranges of coastal and interior Douglas-fir.

Likewise clear differentiation showed allelic structures of Douglas-fir stands of unknown origin in Rheinland-Pfalz (Rheineland-Palatinate) in southwestern Germany. Damaged stands showed nearly the same genetic structures as the interior race, whereas healthy stands conformed closely to genetic structures of the coastal race.

Key words: Douglas-fir, damaged and healthy stands, genetic differentiation, origin, enzyme gene loci.

FDC: 232.11; 232.12; 165.5; 174.7 Pseudotsuga menziesii; (430.1–43.48).

Introduction

Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) has been cultivated in Germany for more than 100 years. Generally 3 varieties are distinguished in Germany (Flöhr, 1958; Schenck, 1939). First, coastal Douglas-fir (var. viridis) extends from Vancouver Island along the coastal mountains of British Columbia down through the coastal belt into California. Second, the interior type (var. glauca) extends along the Rocky Mountains from north British Columbia into the southwestern United States and Mexico. Third, the intermediate form (var. caesia) is located between the other 2 varieties.

In Germany, the first plantations of Douglas-fir were established by the Forest Research Institutes of Prussia, Baden-Wurttemberg and Braunschweig between 1880 and 1891. They resulted in well-growing, healthy stands of the coastal type. Later plantations presumably established with seeds from the interior variety showed less growing and susceptibility to disease of the needles by the fungus Rhabdocline pseudotsugae (Schöber, 1973). Therefore, further investigations should answer the question, of whether the differential performance of the 2 types of artificial Douglas-fir stands in Germany is caused by differences in the origin of the seeds from natural stands in North America.

The first 2 provenance trials in Germany were established in 1910 in Chorin by Schwappach and, with the same material, in 1912 in Kaiserslautern by Münch. During the following years, several provenance tests were established in Germany and other European countries. Generally, these tests yielded similar results (Schöber, 1988). On the basis of quantitative traits, such as growth rate and frost and disease resistance (Schöber et al., 1983, 1984; Stepghan, 1973), the coastal variety seemed to be more appropriate for transfer and planting in Europe than seeds from interior populations.

In German forestry, the origin of single Douglas-fir stands is often unknown. Thus, basic knowledge about interaction of genotype and environment in growth rate and disease resistance is lacking for these production stands.

For identification of the origin of stands, morphological markers are often poorly suited. In different environments, morphological characteristics such as needle length, needle colour and bark structure are extremely variable within the varieties (Schönbach, 1958). Thus, clear identification of the descent of stands is not possible.

In comparison with these morphological markers, genetic markers such as allozymes are better tools to analyse variation between and within the varieties of Douglas-fir. If individuals or populations differ on the basis of genetic markers, it is in principle possible to describe their differentiation independently of influences conditioned by different environments. According to Hoffmann (1994) solely rare alleles carry a high risk of getting lost during the transfer from North America to Europe. His results based on minor polymorphisms revealed similar genetic distances between artificial German stands and their known origin in North America as between natural stands of the coastal or interior race. Up to now, the most extensive work about genetic differentiation of Douglas-fir populations was published by Li and Adams (1989). These authors assayed 104 populations from the entire natural range of Douglas-fir. They distinguished 3 parts of the natural range; the coastal race and the interior race divided into a northern and a southern subgroup.

Several authors dealt with the identification of the origin of artificial Douglas-fir stands in Europe. Clear genetic differentiation between stands in the natural range and the corresponding artificial stands in Germany could not be ascertained yet. Klumpp (1995) constructed a qualitative model in order to distinguish seed or tree samples from 5 different parts of the Douglas-fir range. Stauffer and Adams (1993) reported that there are close similarities of genetic structures between 3 Douglas-fir stands in Switzerland and their probable seed source in North America. No significant deviation in genetic variation between 3 European stands and 1 stand in North America was observed by Prat and Arnal (1994). Allele frequencies were not significantly different in North American populations versus German cohorts (Hoffmann and Geburek, 1995).

Seriously ill stands of Douglas-fir (20 to 40 years of age) in Rheinland-Pfalz showed symptoms such as needle chlorosis, defoliation and bark necrosis. Such symptoms may have complex causes (Hartmann et al., 1988). Chemical analyses of needles revealed excessive amounts of manganese. Similar symptoms were reported by Schöne (1992).

The damaged stands comprised ill trees as well as healthy trees. In some cases there are healthy stands of the same age in the immediate neighbourhood (no environmental differences; same soils and climate) of those damaged stands.
In order to obtain information about the variation of gene markers between these stands they were assayed by means of starch gel electrophoresis.

Material and Methods

Together with the Forest Research Institute of Rheinland-Pfalz, pairs of damaged and healthy Douglas-fir stands were selected. The results from 3 stand pairs originating from the forest districts Daun and Salmtal (forest compartments Bruch and Dreis) located in the Eifel mountains are presented in this study. Buds from 50 trees per stand were sampled. Within the damaged stands healthy as well as ill trees were sampled. Each tree was classified according to the degree of visible damage.

Basic to investigations on the probable origin of the Rheinland-Pfalz stands is the knowledge of genetic variation in artificial German stands with known origin. Thus, buds from 16 populations of a IUFRO-provenance trial were sampled (Fig. 1). These populations represent 2 varieties of Douglas-fir, the coastal (var. viridis) and the interior variety (var. glauca). Buds were sampled on a site near Hann. Münden which is part of the IUFRO-provenance trial 1974 (for more information see JESTAEDT (1980)). Thinning has reduced the number of trees per provenance to an average of 20 trees.

Extracts from buds were analysed by standard methods of horizontal starch gel electrophoresis (BERGMANN, 1974; CHELIAK and PITEL, 1984).

The results for the isozyme 6-phophogluconate-dehydrogenase (E.C.-No. 1.1.1.44.) are presented in this study. There are 2 zones of activity for 6-PGDH. One zone shows only weak staining and was not scored.

To confirm the genetic control of the banding patterns of 6-PGDH-A, megagametophytes from single tree progenies of probably heterozygous trees were assayed. No deviation from the expected 1:1 ratio was observed.

For allele segregation in megagametophytes similar results were reported by ADAMS et al. (1990).

Data Analysis

The different allozymes at the gene locus 6-PGDH-A were named according to their migration distances during electrophoresis. The fastest variant is called $A_1$, the following $A_2$ and so on. In order to measure the degree of similarity among genetic structures of different populations, all pairwise genetic distances were computed. Allele frequencies and genetic distances were computed with the GSED program (GILLET, 1994). Allelic distance (GREGORIUS, 1974) is defined as:

\[
d_0 = \frac{1}{2} \sum_{i=1}^{n} |x_i - y_i|
\]

where $x_i$ is the frequency of the i-th allele in population x, $y_i$ is the frequency of the i-th allele in population y, and n is the number of alleles of the locus considered.
Minimum value for $d_0$ is zero in the case of identical allelic structures between populations. Maximum value for $d_0$ is 1 if 2 populations have no alleles in common. Cluster analysis based on genetic distance was performed by the UPGMA procedure (Sneath and Sokal, 1973, loc. cit. p. 573).

Genetic profiles were used for characterization of allele distributions. Genetic profiles are defined as distributions of allele frequencies, where the alleles are assorted to their frequencies. Main criteria is the frequency of alleles rather than the migration distance during electrophoresis (Finkeldy, 1993; Finkeldy and Gregorius, 1994).

Based on the allele frequencies at the gene locus 6-PGDH-A, genetic distances and genetic profiles were used to discriminate populations contained in the IUFRO-provenance trial. To assess the influence of selection and drift during the transfer from America to Europe, a comparison with allelic structures from natural stands was made. Finally, genetic structures of 3 pairs (damaged and healthy) of Douglas-fir stands were compared with the genetic structures from populations of the IUFRO-provenance trial as well as those from populations of the natural range.

**Results**

**IUFRO-provenance trial**

Eight alleles were observed at the gene locus 6-PGDH-A. Their frequencies were listed in Table 1.

The predominant alleles in all populations are $A_3$ and $A_6$. Rare alleles are $A_4$, $A_5$, $A_7$, and $A_8$; while alleles $A_1$ and $A_2$ exhibit intermediate frequencies. Allele $A_2$ reaches frequencies higher than 10% in samples No. 1105 and 1108. Over all populations, the frequencies of alleles $A_3$ and $A_6$ are the main determinants of the differences in allelic structures at the gene locus 6-PGDH-A.

The populations of the provenance trial clearly differed in their allele frequencies at the gene locus 6-PGDH-A. For 1 group, the allele $A_3$ is by far the most frequent, while allele $A_6$ and others are comparatively rare. Such frequency distributions are termed minor polymorphisms. On the contrary, $A_6$ is the most common allele in the samples belonging to the second group. These populations revealed a major polymorphism structure with more balanced frequencies of $A_3$ and $A_6$.

Cluster analysis was computed on the basis of genetic distances including all samples of the IUFRO-provenance trial (Fig. 3). In reference to the similarity of their genetic structure, the populations clustered into 2 subgroups. One group comprises the samples no. 1016, 1068, 1022, 1067, 1065, 1105, 1055 and 1105. The western-most population is 1067.

![Figure 3. – UPGMA-Cluster analysis based on genetic distances according to Gregorius (1974) at the gene locus 6-PGDH-A between the 16 populations sampled from the Douglas-fir provenance trial. Provenance-nos are listed on the right and genetic distance is shown on the x-axis above.](image)

A second group is formed by samples 1021, 1027, 1025, 1026, 1145, 1064, 1073 and 1143. The resulting genetic distance between these 2 groups after clustering is extremely high (close to 0.7).

The differentiation using genetic structures agree strikingly with the presumed range of the 2 races, coastal Douglas-fir and interior Douglas-fir, in the natural range of North America (Schenck, 1958).

Earlier studies on genetic structure showed similar patterns (Li and Adams, 1989; Klumpp, 1995). Because of the distinctive minor polymorphism of coastal provenances for the same allele $A_3$, genetic distances within this subgroup are lower than those within the interior subgroup. Only the 2 samples 1021 and 1027 are clearly differentiated here. The 2 samples share a comparatively low allele frequency for $A_3$ with respect to the remainder of this group. Heterogeneity among genetic structures of the interior subgroup is more extended. Less

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<td>1016</td>
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<tr>
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genetic distance for geographically adjacent populations could not be observed.

Average allele frequencies were calculated in both subgroups. The application of these mean values as references is striking only in the case of similar genetic structures between the populations of each subgroup. With regard to this, genetic profiles were used to characterise genetic structures for each population in the 2 subgroups.

All populations of the coastal race showed minor polymorphism structure with $A_3$ as the most common allele. If one assumes this to be characteristic of coastal Douglas-fir, a large similarity between those populations and their progenies can be expected. For all populations from the coastal range, genetic profiles were illustrated in figure 4. For each allele, mean values were calculated among the coastal populations. The resulting genetic profile for the coastal race is shown in the center of figure 4.

The mean frequency of allele $A_3$ is higher than 90%, and in some populations $A_3$ appears with frequencies of 100%. $A_3$ reached its lowest frequencies in the populations 1021 (82.5%) and 1027 (89.5%).

Genetic distances between populations from the interior range are comparatively larger than between populations of the coastal race. Despite this fact, similarity between genetic structures of single populations within the interior subgroup is obvious (Fig. 5). All samples (except 1055) showed major polymorphism structures with the most common allele $A_3$. The mean frequency value for $A_3$ is higher than 60% and for $A_6$ close to 30%.

Figure 6a shows the mean allele frequencies of both Douglas-fir races and the resulting allele structures side by side. The mean value of allele $A_3$ for the coastal race is higher than 90%, it is approximately 60% more frequent than in the interior race. On the contrary, allele $A_6$ is approximately 60% more frequent in the interior race with a mean value higher than 60%. Rare alleles showed higher frequencies in the interior race.

This result agrees with the study of Li and Adams (1989). For example, figure 6b shows allele frequencies in 6 populations of coastal and northern interior Douglas-fir (3 populations for each zone) selected from their basic data.

More alleles were observed in the IUFRO-provenance trial and the stands from Rheinland-Pfalz than were mentioned in the data of Li and Adams (1989). Therefore, the allele numbers used in this study differ from those which Li and Adams used for 6-PGDH. Based on the similarity of genetic structures between the 2 studies, it is assumed that the alleles $A_3$ and $A_6$ of the North American study correspond to the alleles $A_3$ and $A_6$ in this study. The resulting genetic structures for their natural populations from North America are shown in figure 6b. The results of the 2 studies are seen to be in agreement. Again, genetic structures of coastal Douglas-fir revealed

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I am much obliged to P. Li and W. T. Adams for their basic data.
minor polymorphism structures up to fixation of their most common allele $A_2$ ($A_3$ in this study). Most common allele in the interior populations is their allele $A_3$ ($A_6$ in this study).

Douglas-fir stands in Rheinland-Pfalz

Allele frequencies and the resulting genetic structures obtained from the artificial stands in Rheinland-Pfalz are shown in figure 7. Each pair of Douglas-fir stands represents one damaged stand marked by an $D$ and one healthy stand marked by a $H$ on the x-axis. Each pair showed 2 clearly distinct genetic structures. Obviously, damaged stands revealed allele structures as in populations from the interior range, and healthy stands showed genetic structures typical of the coastal race. Hence, genetic distances between the individual damaged stands and IUFRO samples from the interior range are always smaller than their genetic distance to coastal samples. Vice versa, healthy stands always revealed the smallest genetic distance to coastal provenances (cf. Table 2).

For all samples, from both the IUFRO-provenance trial and the stands in Rheinland-Pfalz, cluster analysis was computed and the result is shown in figure 8. All damaged stands clustered together as one subgroup of the northern interior race. This may indicate roughly the same seed source. The identification of their geographic origin is not possible because of comparably late clustering with other samples and stronger heterogeneity in this group as in the coastal group. This is reflected in the $\delta$-values$^2$ (GREGORIUS and ROBERDS, 1986) within each of the 2 subgroups. For the interior subgroup $\delta$ is 0.148 and for the coastal subgroup $\delta$ is 0.049. $\delta$ is the mean genetic distance of each population (deme) to its complement within a subgroup.

In the first cluster analysis both populations from southern British Columbia (1021 and 1027) are distinct from the remainder of the coastal group. Now, all healthy stands clustered closely with those populations to form one subgroup within the coastal race.

Discussion

Basically, there are 2 cases for differentiation among allele structures of populations or collectives. If alleles occur exclusively in one population or region, complete distinction seems to be clear. In contrast, differentiation of populations on the basis of quantitative differences in allele frequencies is less clear. In those cases, sample size is of great importance. Although sample sizes of the IUFRO-provenances are small, for this study it seems to be sufficient considering the
The differentiation between the coastal and the interior races is strong but the differentiation between the populations within each race is comparatively slight. Thus, only racial differentiation is possible for the Rheinland-Pfalz stands.

Damaged stands of Rheinland-Pfalz with no information about their exact origin reveal the same allelic structures as stands from the interior range. For the healthy stands, allelic structures were observed which are typical of the coastal race. Thus the racial differentiation between the stands from Rheinland-Pfalz seems to be responsible for damaged or healthy condition.


Close clustering of the healthy stands and samples No. 1021 and 1027 is supported by the fact that, up to the end of the 1960s and the beginning of the 1970s, many German stands were established with seeds from British Columbia (KLEINSCHMIT, 1973).

SCHOBER et al. (1984) mentioned that even in southern British Columbia, the 2 races are not completely separated. Thus, there is a higher risk of obtaining seed material from the interior race. The data of this study provided no evidence whether the damaged stands originate from this region.

Acknowledgements

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Literature

Coniferyl Alcohol Dehydrogenase, a Multifunctional Isozyme-Gene-System in Norway Spruce, Affects the Armillaria Resistance of Young Trees

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Summary

Coniferyl alcohol dehydrogenase (CADH) is a key enzyme in lignin biosynthesis of conifers. Because it functions differently in different ontogenetic stages, tissues and organs of trees, a more in-depth investigation of this isozyme-gene-system in Norway spruce (Picea abies L.) appeared worthwhile.

As a result of CADH analysis in somatic and gametic (megagametophytes of single trees) tissues of various spruce trees, one isozyme zone was detected in dormant buds, megagametophytes and embryos, which is controlled by one gene locus (CADH-A). Comparisons between a plus-tree and a random tree collection from the same Bavarian Norway spruce provenance showed a higher degree of heterozygosity at this gene locus among the plus-trees.

A study of isozymes from buds of young trees which suffer from Armillaria infections showed a reduced CADH activity in zymograms as compared to healthy trees of the same stand. PCR-based amplification experiments of the DNA encoding CADH were performed with needles from the same spruce material. Using a specific primer pair designed from a cDNA of this gene, only a single band of 520 bp could be detected in agarose gels. Whereas this band was strong in healthy trees, needles of infected trees showed only a very faint DNA band at this position in agarose gels. Since the DNA of other chromosomal regions in infected trees did not indicate similar reductions of the amplification process, it may be postulated that considerable mutations or modifications of the CADH open reading frame are correlated with the susceptibility of trees to Armillaria infections.

Key words: Picea abies, coniferyl alcohol dehydrogenase, isozymes, DNA amplification, Armillaria infections, susceptibility.

FDC: 165.3; 165.53; 443; 174.7 Picea abies

Introduction

The maintenance of isozyme major polymorphisms in tree populations is a process not yet satisfactorily understood, although a number of experimental and theoretical studies have dealt with this problem (for review and literature compilation, see MITTON, 1995). Based on a reanalysis of numerous isozyme data from 6 different conifer and broad-leaved species, it was recently suggested that major polymorphisms are the result of heterozygote advantage due to ontogenetic differences in the metabolic efficiency of the 2 allozymes (GREGORIUS and BERGMANN, 1995). A more convincing confirmation of this suggestion would be the finding of major polymorphisms for which the 2 frequent allozymes even exhibit different functions in different ontogenetic stages and/or different organs of a heterozygous tree.

One isozyme-gene-system that appears to have multifunctional properties during the ontogenetic development of trees is coniferyl alcohol dehydrogenase (CADH, also referred to as cinnamyl alcohol dehydrogenase, CAD, E.C. 1.1.1.195). This enzyme is primarily involved in lignin biosynthesis of conifers, where it catalyses the reduction of coniferyl aldehyde to the corresponding alcohol, which is the main lignin precursor in the xylem tissue of all conifers (GRISBACH, 1981).