Allozyme Variation and Mating System of Three Douglas-fir Stands in Switzerland[1])

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Summary

Ten enzyme systems representing 15 allozyme loci were assayed in seeds (megagametophyte [1N] and embryo [2N] tissues) from 3 Douglas-fir (*Pseudotsuga menziesii* [MIRB.] FRANCO) populations in Switzerland. The genetic affinity of the Swiss populations to those in the native range was assessed by the use of NEI's genetic distance measure and allozyme data available from a range-wide study of Douglas-fir. All 3 Swiss populations clustered clearly with populations of the coastal variety, which is consistent with their assumed origin. In addition, the Swiss populations had levels of genetic diversity consistent with observations reported for native populations of this variety. In all 3 Swiss populations, estimated proportions of outcrossed offspring ($\hat{t}_m$) were lower (mean $\hat{t}_m = 0.752$) than reported for native populations. Because of the high levels of selfing indicated by these low $\hat{t}_m$ values, special precautions should be taken in using seeds from these stands for reforestation purposes.

Key words: Genetic diversity, electrophoresis, mating system, inbreeding, land races.

Introduction

Douglas-fir (*Psudotsuga menziesii* [MIRB.] FRANCO) has been cultivated successfully as an exotic species in Switzerland for more than 100 years; it is customarily interplanted with other conifers and hardwood species. DIEZ and BUERGI (1991) described 39 stands where Douglas-fir is a major component, including stands in Wileroltigen and Boezingen that are part of the present study.

Little is known about the origin of European stands of Douglas-fir or their genetic structure. Nevertheless, seed collections are often made in these stands for reforestation purposes. Although low numbers of viable seed per cone are typically reported (KLEINSCHMIT, 1984), European populations may be better adapted to local climates than imported provenances; thus there is interest in propagating seed sources with proven performance under European conditions. In German tests, for example, higher frost-hardiness was observed for provenances from German populations than for American provenances with similar growth performance (KLEINSCHMIT *et al.*, 1974).

In the present study we were interested in confirming the native provenance of these first-generation Douglas-fir stands in Switzerland, which we suspected to be variety *menziesii* from coastal Oregon or Washington. We also used allozymes to compare the genetic structure and the mating system of the Swiss stands with native populations in North America.

Material and Methods

Seed collections

Wind-pollinated seeds from individual mother trees were collected in 3 stands in the Canton of Berne, Switzerland. The Lyss (3.5 ha) and Wileroltigen (4.5 ha) stands are in the Bernese Mittelland at an elevation of 500 m. Exact years of establishment are unknown, but both were planted around 1895 and belong to the same forest owner. The 15-ha Boezingen stand, at 670 m on the southeastern slope of the Jura mountains, was established in 1889 (MARCET, 1975).

The chosen stands grow in vegetation types where 18 out of the 39 stands investigated by DIEZ and BUERGI (1991) are located, and are representative of Douglas-fir populations in Switzerland. In all 3 stands, Douglas-fir is interplanted with Norway spruce (*Picea abies* [L.] KARST.), silver fir (Swiss pine) (*Abies alba* MILL.), and European beech (*Fagus silvatica* L.), but Douglas-fir predominates in the overstory. In Boezingen, Douglas-fir accounts for 90.6% (49.7 m^2/ha) of the total tree basal area, and in Wileroltigen it represents 76.7% (61.9 m^2/ha). No measurements are available for Lyss, but it has a stand structure similar to Wileroltigen. In all 3 stands, Douglas-fir trees are spaced about 7 m to 8 m apart (175 trees/ha to 200 trees/ha).

In 1989, 10 mother trees were sampled at Lyss and 14 at Wileroltigen. In 1990, 22 trees were sampled at Boezingen. Windpollinated seeds from 3 additional trees in Boezingen (collected in 1987) were already available from the Federal Institute for Forest Research, giving a total sample of 25 trees in this stand. Mother trees in each stand were chosen at random from trees with good cone crops. In all cases, seed collections were made in years with good cone crops and cones were sampled from upper crowns. Seeds of individual trees were kept separate during processing and storage. The seed lots were not screened to remove empty seeds.

Electrophoretic procedures

Seeds from each mother tree were soaked for 48 hours in 1% H_2O_2 and then germinated on moist filter paper in petri dishes. When radicles had emerged 3 mm to 5 mm beyond the seed coat, both the megagametophyte (1 N) and embryo (2N) of each seed were electrophoretically assayed by methods described in ADAMS *et al.* (1990). Different numbers of seeds per mother tree were analyzed in each population because of the different numbers of mother trees sampled (12 seeds per tree for Lyss, 9 for Wileroltigen, and 6 for Boezingen). Partly because of a seed chalcid (*Megastigmus spermatrophus* WACHTL) and the fact that empty seeds had not been removed during processing, germination rates were poor — 17% for Lyss, 34% for Wileroltigen, and 36% for Boezingen. Thus, in some cases, seed with radicles less than the indicated length had to be used. For Lyss, the requisite number of viable seeds could not be obtained from four of the trees, so the number of mother trees representing this stand was only 6. All

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seeds were assayed for 10 enzyme systems representing 15 loci: aconitase (*Aco1, Aco2*), phosphoglucomutase (*Pgm1*), leucine aminopeptidase (*Lap1, Lap2*), glutamate-oxalacetate transaminase (*Got1, Got2, Got3*), glucose-6-phosphate dehydrogenase (*G-6pd*), catalase (*Cat*), 6- phosphogluconate dehydrogenase (*6-Pgd*), isocitrate dehydrogenase (Idh), diaphorase (*Dia*), and malate dehydrogenase (*Mdh1, Mdh3*).

Data analysis

Genetic analyses of the populations were carried out at 3 lifecycle stages: adult trees pollen gametes, and embryos (diploid offspring). Genotypes of adult (mother) trees were inferred from allozymes in their megagametophytes. With the minimal sample of 6 megagametophytes, the probability of misidentifying the genotype of a mother tree at any one locus is less than 0.032 (Neale and Adams, (1985). Genotypes of pollen gametes were inferred by comparing the haploid genotypes of megagametophytes with the diploid genotypes of their embryos (Adams and Joly, 1980).

A subset of the data employed by Li and Adams (1989) to investigate range-wide patterns of allozyme variation in Douglas-fir was used to investigate the genetic affinity of the Swiss populations to populations in the native range. Unbiased genetic distances (Nei, 1978) were calculated for all population pairs based on the 15 loci in this study. Because of the small number of adult trees in each sample, allele frequencies of pollen gametes in the Swiss populations were utilized for the genetic distance calculations. Pollen gametes probably represent genes from a larger pool of parents, and thus better represent the genetic composition of the populations (Steinhoff *et al.*, 1983). Cluster analyses of the populations based on these genetic distances were performed by the UPGMA procedure (Sneath and Sokal, 1973). Genetic variation at all three life-cycle stages within the Swiss populations was quantified by calculating the mean number of alleles per locus (A), percentage of polymorphic loci (P; frequency of most common allele ≤ 0.95), observed heterozygosity (H_o; when possible), and expected heterozygosity (H_e; unbiased measure, Nei, 1978). All of the above population statistics were calculated with the BIOSYS-1 computer program (Swofford and Selander, 1989).

Single-locus ($\hat{t}_s$) and multi-locus ($\hat{t}_m$) estimates of the proportion of progeny due to random outcrossing (t; s = 1-t is the proportion due to selfing) were calculated for each population by the maximum likelihood procedures and computer programs described in Neale and Adams (1985). Several loci lacked the required minimum of 2 genotypic classes among the mother trees sampled in each population, which reduced the number of loci available for estimating $\hat{t}_s$ and $\hat{t}_m$ (6 to 10 actually used).

Results

Genetic affinity of Swiss populations to Douglas-fir in North America

Genetic distance and cluster analyses were performed in 2 steps. In the first analysis, in which the Swiss populations were compared to a subset of 37 populations from the entire species range (not shown), the Swiss populations clustered clearly with populations of the coastal variety *menziesii*, and were distinct from northern and southern populations of the interior variety *glauca* as well as from intermediate populations in transition zones in south-central Washington and central Oregon (see Li and Adams, 1989, for details on these taxonomic groupings). In the second analysis, which compared the Swiss populations to a sample of 36 coastal variety populations from British Columbia to northern California (*Figs. 1* and *2*), the 3 Swiss populations clustered very closely together, with a mean genetic distance of only 0.005. This suggests that these populations are of the same original provenance. In particular, Lyss and Wileroltigen, which have the same ownership, may have come from one seedlot. In agreement with the cluster analysis performed by Li and Adams (1989), populations of the coastal variety were weakly differentiated (mean genetic distance based on 15 loci = 0.025), and clustering was only roughly related to geographic location. The Swiss populations appear to be most closely associated with native populations in the coast ranges and on the west side of the Cascade range from northern California to southern British Columbia, and least associated with populations in northern British Columbia or east of the Cascades. It is unknown why population 35 is distinct from the remaining populations in this subsample. In the larger, range-wide analysis of Li and Adams (1989), which was based on 19 polymorphic loci, population 35 clustered closely with other west Cascade populations in Oregon and Washington.

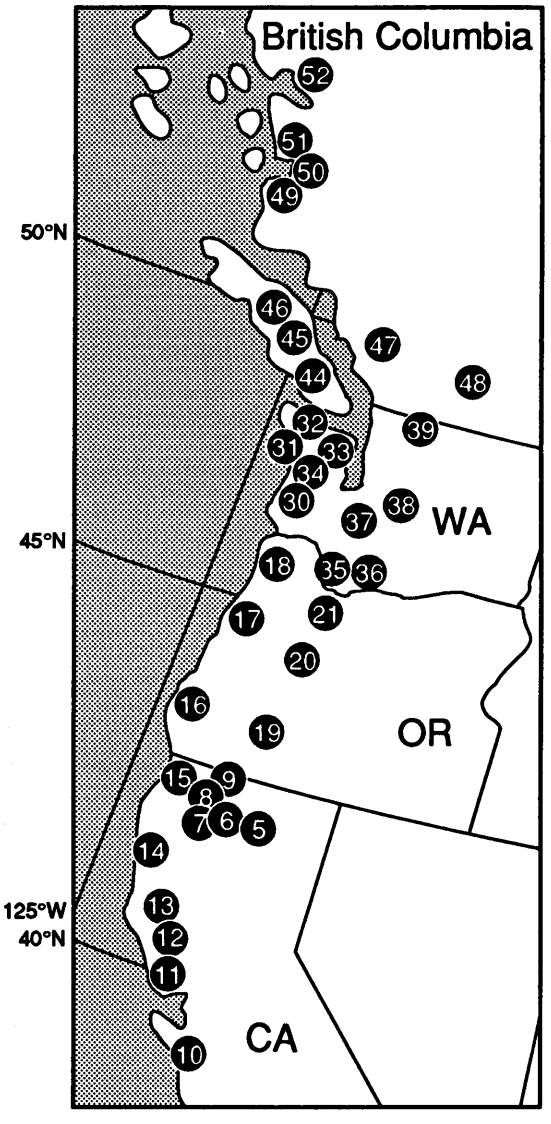

Figure 1. — Locations of 36 coastal variety populations of Douglas-fir included in the cluster analysis. Numbering of these populations is the same as in Li and Adams (1989).

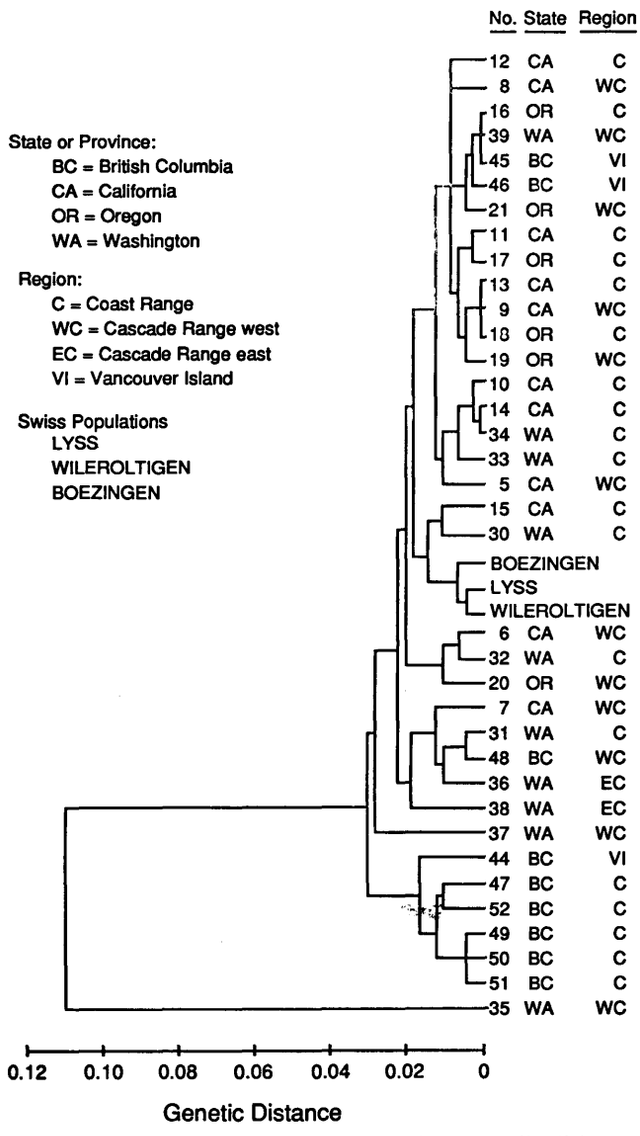

Figure 2. — Cluster diagram based on unbiased genetic distances (NEI, 1978) of 36 populations from the natural range of coastal Douglas-fir (see *Fig. 1*) and 3 exotic Swiss populations.

Levels of genetic variation

Measures of genetic diversity (i.e., A, P, and H_e) were similar for the 3 life-cycle stages, except that the number of alleles detected was always higher in pollen and embryos than in the adults; this difference is expected because of the larger sample sizes in the offspring (*Table 1*). The 3 Swiss populations had similar levels of genetic diversity, and the levels were consistent with observations reported for native populations of coastal Douglas-fir (NEALE, 1985; LI and ADAMS, 1989; MORAN and ADAMS, 1989). Mean H_e for the Swiss populations (0.209, based on pollen gametes), for example, falls well within the range of H_e calculated from the same 15 loci for the 36 native populations included in *Figure 1* (0.091—0.305, mean = 0.213).

Observed and expected heterozygosities in Swiss adults were very similar in magnitude in all 3 populations and were not significant different ($p > 0.05$). Differences between H_o and H_e were also nonsignificant at the embryo stage, but H_o was consistently about 27% less than H_e. Heterozygote deficiency relative to HARDY-WEINBERG expectation is frequently observed at the seed stage in conifer populations and is generally attributed to inbreeding (MUONA, 1989).

Mating system

Because of the small offspring sample sizes, single-locus estimates of outcrossed progeny varied widely over the loci used in each population (*Table 2*). Sampling error and violations of the mixed-mating model can lead to estimates of t greater than 1.0 (ADAMS and BIRKES, 1991), but in this case single-locus estimates as high as 2.167 were obtained. Because of the high standard errors of $\hat{t}_s$ and the greater susceptibility of single-locus estimates to violations of the mixed-mating model (NEALE and ADAMS, 1985), we feel that the multi-locus estimates more precisely reflect the true proportions of outcrossed progeny in these populations. The value of $\hat{t}_m$ for Wileroltigen is at the lower end of population estimates of t observed in native stands of Douglas-fir (SHAW and ALLARD, 1982; NEALE and ADAMS, 1985; YEH and MORGAN, 1987); $\hat{t}_m$ values for Lyss and Boezingen are considered low for conifers in general (MUONA, 1989; ADAMS and BIRKES, 1991).

Discussion

Affinity of the Swiss populations with the coastal variety of Douglas-fir was expected because of the appearance of the adult trees and offspring in the nursery. On the basis of DNA content and morphology of seeds, BERNEY (1972) concluded that the Boezingen population was derived from the coastal variety between 45° and 47° latitude. BERNEY's and our results agree nicely with the historical background of seed exported from the Pacific Northwest to Europe (D. GERDES, Silvaseed, Roy, WA, pers. comm.). The main seed exporter to Europe, Manning Seed Company, was founded in Tillamook, Oregon (latitude 45°25'; longitude 123°52'). After several years, the company moved to Chehalis, Washington (latitude 46°40'; longitude 122°58'). Seed collection was probably done in nearby natural stands in the Coast Range in northern Oregon and in the lower Columbia basin on both sides of the Columbia River. Afforestation with seed stocks from natural stands and subsequent growth under Swiss conditions has apparently had little, if any, influence on levels of genetic variation; the measures of genetic diversity in these small Swiss stands were not detectably different from those reported for native populations of the coastal variety. Similar results were observed for three artificial stands of Douglas-fir in France and Germany (D. PRAT and S. ARNAL, Laboratoire INRA-ENGREF de Recherche en Sciences Forestières, Nancy, France pers. comm.).

Although other explanations for low heterozygosity are possible (e.g., selective disadvantage of heterozygotes, population subdivision of the outcross pollen pool, chance sampling), the deficiency of heterozygosity observed in the embryos of all three populations (*Table 1*) is consistent with the low levels of outcrossed progeny found in these populations (*Table 2*). Proportions of selfed progeny in conifers appear to be negatively correlated with both tree density and overall pollen production within stands (FARRIS and MITTON, 1984; SHEA, 1987; SMITH *et al.*, 1988). When trees are widely spaced, self-pollen makes up a large proportion of the pollen cloud around each tree. Low seed set in our samples suggests that overall pollen production may have been low in the Swiss stands. This is not surprising, given the small size of the stands and their isolation from other sources of Douglas-fir pollen.

Table 1. — Estimates of mean number of alleles per locus (A), proportion of polymorphic loci (P, 0.95 criterion), and observed (H_o) and expected (H_e) heterozygosity for 3 Swiss populations of Douglas-fir[1)2)].

Population/ Lifestage	Mean sample size per locus	A	P	H_o	H_e
Lyss					
adult	6	1.7 (0.2)	66.7	0.256 (0.072)	0.227 (0.060)
pollen	72	2.3 (0.3)	46.7	–	0.189 (0.053)
embryo	72	2.5 (0.3)	40.0	0.135 (0.039)	0.188 (0.054)
Wileroltigen					
adult	14	2.0 (0.1)	60.0	0.200 (0.043)	0.207 (0.046)
pollen	126	2.3 (0.2)	73.3	–	0.227 (0.048)
embryo	126	2.4 (0.2)	66.7	0.154 (0.035)	0.208 (0.046)
Boezingen					
adult	25	2.5 (0.3)	66.7	0.211 (0.051)	0.224 (0.047)
pollen	150	2.9 (0.3)	60.0	–	0.211 (0.049)
embryo	150	2.9 (0.3)	66.7	0.158 (0.038)	0.213 (0.048)

[1)] Based on 15 loci.
[2)] Standard errors given in parentheses.

Table 2. — Single-locus ($\hat{t}_s$) and multi-locus ($\hat{t}_m$) estimates of proportions of outcrossed progeny in 3 Swiss populations of Douglas-fir.

Population	Number[1] of loci	$\hat{t}_s$ mean[2]	$\hat{t}_s$ S.D.	$\hat{t}_m$ mean[3]	$\hat{t}_m$ S.D.
Lyss	6	0.859 (0.562–2.167)	0.067	0.683	0.072
Wileroltigen	9	0.770 (0.610–1.100)	0.047	0.864	0.050
Boezingen	10	0.772 (0.375–1.341)	0.042	0.710	0.048
Mean		0.850		0.752	

[1)] Offspring (pollen gamets) sample size given in *table 1.*
[2)] Weighted mean based on inverse of variance. Range of $\hat{t}_s$ estimates over loci given in parentheses.
[3)] All 3 $\hat{t}_m$ estimates are significantly different ($p<0.05$) from $t=1.0$ based on chi-square likelihood ratio test (Brunk, 1975).

The spacing in these stands does not seem, by itself, to be large enough to limit good cross-pollination (Neale and Adams, 1985), but the effective distance between males was probably much greater because not all trees flowered and some asynchrony in flowering times between trees could be expected.

Higher frequencies of offspring resulting from self-fertilization may typify artificial Douglas-fir stands in other parts of Europe as well. In 2 of the 3 stands investigated by PRAT and ARNAL (pers. comm.), S was estimated to be 0.15 or greater. Because higher levels of selfed progeny lower the genetic quality of seed crops (MUONA, 1989; SORENSEN and MILES, 1982), special precautions should be taken in the collection and use of seed from these artificial stands. In order to minimize selfed offspring, seed collections should be limited to years with especially good flower crops. In addition, seedlings should be strongly culled in the nursery to help remove inbred individuals (SORENSEN and MILES, 1974). Tight spacing in plantations may also be employed to favor non-inbred individuals by encouraging competition (SORENSEN and MILES, 1982).

The results of this study illustrate a number of useful applications of isozymes in practical forestry. Isozymes may be employed to test the affinity of artificial stands from unknown seed sources, and, at least crudely, to identify their provenance of origin. In addition, isozyme analyses can help in evaluating the genetic quality and diversity of seed crops, and, subsequently, can help foresters make decisions regarding regeneration.

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Growth Differentiation in White Spruce Crop Tree Progenies

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1. Abstract

Stem analysis of 288 36-year-old dominant and codominant 'crop' trees in 18 white spruce open-pollinated families revealed a strong genetic control of height growth (heritabilities of 0.3 to 0.6). Heritability estimates for volume growth peaked around age 20 when they reached a level of 0.3 only to decline rapidly thereafter. Rank stability and the coefficient of genetic prediction between age 36 family means and means obtained at earlier ages were not sufficiently strong to warrant early selection for either height or volume. Height-age and volume-age relationships were successfully modelled with a quadratic log-linear model with three interpretable parameters. Heritabilities of the growth curve parameters varied from 0.14 to 0.72. The opportunity to improve the growth curves by 20% for all ages was explored by using a restricted selection index of the growth curve parameters. An improve-

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