GENETIC VARIATION AMONG PROVENANCES OF *ABIES GRANDIS* FROM THE PACIFIC NORTHWEST

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ABSTRACT

Isoenzyme analysis was used to investigate the genetic structure of eleven *Abies grandis* provenances on eleven gene loci. Bud tissue was taken from nine provenances in a 17 year old field trial and two samples were taken from a commercial seed collection.

Clear differences in the genetic structure were found at the polymorphic gene loci *Idh–B* and *Pgm–A*. Under consideration of all gene loci a clear regional differentiation between provenances from Washington/British Columbia and western Oregon was observed. The northern provenances had a higher genetic diversity and heterozygosity. The provenance Post (1450 m) from central Oregon (Interior) had by far the highest genetic variability and a very specific genetic structure and could not be assigned to any of the two regional groups. The results of the isozyme analysis is discussed in relation to the field trials and ecophysiological studies.

Key words: *Abies grandis*, provenances, isozyme analysis, genetic variation

INTRODUCTION

Among the true firs of the western United States, grand-fir is the species able to grow under the most diverse site conditions. It is found growing in the mild, humid coastal zone along the Pacific as well as on the dry high elevation sites in central Oregon, where frosts can occur during all months of the year. The sites where grand fir is found, were described and documented in great detail by MÜLLER (1935/36). Furthermore grand fir is the fastest growing and largest of the true firs.

Abies grandis has been planted in Europe since seed from the coastal areas of the Pacific Northwest was first brought to Europe around 1830. In Germany plantations with grand fir were established in various locations. An American forester visiting a species trial in Weinheim once said "no one plants grand-fir except you Germans!". Thus it is not surprising that some of the first provenance trials with grand fir were initiated in Germany by Fabricius in 1938. The results of these trials have been reported by ROHMEDER (1953), ROHMEDER & DIMPFLMEIER (1960), BEUSCHEL (1968) and WOLF & RUETZ (1988).

The provenance trials demonstrate that *Abies grandis* can adapt to a variety of environmental and site conditions without losing its growth potential and that there are large differences between provenances (RAU *et al.* 1991). The provenances Elwha (from the Olympic Peninsula) and Darrington (from the Western Cascades of Washington) performed very well in numerous provenance studies in Europe (KÖNIG 1995, KLEINSCHMIT *et al.* 1995, VANČURA & BERAN 1995).

On the basis of the adaptability and the differential behaviour of the provenances one would expect, that grand fir would also have a high genetic variability. Since data on the genetic variation of grand fir is lacking, it was our goal to obtain information on the genetic structure of *Abies grandis* and the genetic variation among Pacific Northwest provenances.

Isozyme analysis has been used succesfully in determining the genetic variation of numerous abies species *e.g. Abies alba* (BERGMANN & KOWNATZKI 1988, SCHROEDER 1989, BREITENBACH-DORFER *et al.* 1992, KONNERT 1992, LONGAUER 1994, KONNERT & BERGMANN 1995, HUSSENDÖRFER 1995), *Abies amabilis* (DAVIDSON 1990), *Abies balsamea* (NEALE & ADAMS, 1986), *Abies borisii-regis* (FADY & CONKLE 1993), *Abies concolor* (WESTFALL & CONKLE 1992), *Abies fraseri* (DIEBEL & FERET 1990).

MATERIAL AND METHODS

From the IUFRO and our own seed collection four provenance field trials were established in Bavaria in 1980 and 1981 (RAU *et al.* 1991). In order to investigate the genetic variation, we collected bud tissues from ten provenances in the field trial "Buchenau" (age 17) in March 1992.

The provenances which were investigated, are listed in Table 1 whereby the provenance number refers to the number in the provenance trial. For provenance

Table 1. Investigated provenances of *Abies grandis*.

Prov. Nr.	Name	Seed zone	Altitude (m)	Latitude	Longitude	Nr. of analyzed individuals
1	Santiam, Oregon	461	380	44°25'	122°30'	59
2	Santiam, Oregon	461	820	44°25'	122°30'	52
3	Mohawk R., Oregon	471	300–600	44°16'	122°42'	43
6	Philomath, Oregon	252	300	44°30'	123°26'	52
7	Darrington, Washington (IUFRO 12001)	403	450	48°16'	121°21'	62
8	Elwha, Washington (IUFRO 12003)	221	140	48°04'	123°38'	62
9	P. Alberni, British Columbia (IUFRO 12043)	–	25	49°18'	124°58'	65
10	Post, Oregon	673	1425	44°30'	120°40'	50
11	Lk Keechelus, Washington	631	760	47°18'	121°20'	64
12	Sears Cr. Idaho (commercial seed collection)	–	~900	~47°	~116°	111
13	Elwha, Washington (commercial seed collect.)	221	~150	~48°	~123°	144

*) Prov. 3 + 4 from the provenance trial "Buchenau"

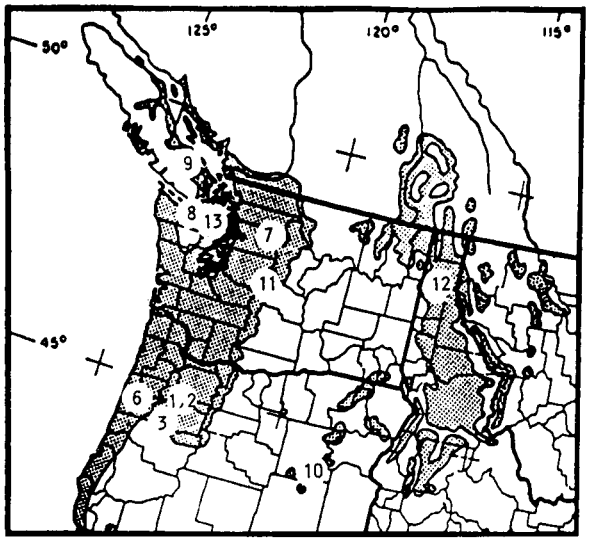

Figure 1. Location of *Abies grandis* provenances (original stands).

listed in Table 1 whereby the provenance number refers to the number in the provenance trial. For provenance number 3 two provenances from Mohawk R., Oregon were combined since they come from the same area but different elevations (300 m and 600 m). During the analysis the two provenances showed no difference in their genetic structure so they were lumped together. Furthermore we had seed available from two commercial seed collections (Elwha, Washington and Sears Cr., Idaho), which are noted as numbers 12 and 13 in Table 1. The location of provenances (original stands) is illustrated in Fig. 1.

Electrophoretic procedure

Isozyme analysis was carried out on bud tissue (buds from 43 to 65 individual trees per provenance, see Tab. 1) and endosperm (111 resp. 144 seeds from the two commercial seed collections). The vegetative material was homogenized in a 0.1 M TRIS-HCl extraction buffer, pH = 7.0 with 7-mM-mercaptoethanol and 3 % w/v PVP-40. After extraction the isozymes were separated by horizontal starch gel (12 %) electrophoresis.

Seven enzyme systems were investigated (Tab. 2), by utilizing three electrode and gel buffer systems: a Tris-citric acid buffer pH = 8.0 (to separate IDH, 6-PGDH and DIA), a Tris-citric acid/lithium hydroxide-boric acid buffer pH = 8.1 (to separate LAP and PGI) and a Tris-citric acid pH = 8.7/Sodium hydroxide-boric acid pH = 8.2 (to separate GOT and PGM). The method of electrophoretic separation used in this study was identical to that used for *Abies alba* (KONNERT 1992, HUSSENDÖRFER *et al.* 1995). The isozyme patterns found for *Abies grandis* are very similar to those found for other *Abies* species *e.g. Abies balsamea* (NEALE & ADAMS 1986), *Abies amabilis* (DAVIDSON 1990), *Abies lasiocarpa* (SHEA 1987).

Isozyme Data Analysis

For each population the genotype and gene frequencies at eleven gene loci were calculated.

As measures of genetic variability the following parameters were used: G_M = maximum genotypic multiplicity; P = proportion of polymorphic loci; M/L = mean number of alleles per locus (HATTEMER *et al.* 1993).

Table 2. Enzyme systems, E.C. reference number, designation of scored loci and number of alleles.

Enzyme system	E.C. Number	Scored loci	No. of alleles
Isocitrate dehydrogenase	1.1.1.42	*Idh–A, –B*	1, 4
Glutamate oxaloacetate transaminase	2.6.1.1	*Got–A, –B, –C*	3, 1, 3
Leucine aminopeptidase	3.4.11.1	*Lap–A, –B*	3, 3
Phosphoglucose isomerase	5.3.1.9	*Pgi–A, –B*	1, 2
Phosphoglucomutase	2.7.5.1	*Pgm–A*	3
Diaphorase	1.6.4.3	*Dia–A*	2

The genetic diversity of the analyzed provenances was quantified using the parameters: n_e = effective number of alleles per locus (CROW & KIMURA 1970), v_{gam} = hypothetical gametic multilocus diversity (GREGORIUS 1978), and H_e = expected level of heterozygosity (NEI 1978).

The genetic distance, a measure of differentiation between populations, was calculated according to GREGORIUS(1974). On the matrix of the calculated distance, a cluster analysis (average linkage procedure) was performed using the computer program SAS.

The apportionement of variation among and within populations was estimated using a population differentiation measure, developed by GREGORIUS & ROBERDS (1986) and the diversity statistics devised bei NEI (1973, 1977). The measure D_j (GREGORIUS & ROBERDS 1986) can be interpreted as the proportion of the effective number of genetic elements (in our case alleles) by which the j^{th} population differs from all other populations pooled. According to NEI (1973, 1977) the gene diversity in the total population (H_T) can be subdivided into a within provenances component (H_S) and an among provenances component (D_{ST}). The relative amount of gene diversity due to differences among provenances is then:

$$G_{ST} = D_{ST} / H_T$$

For the computation of the genetic diversity, genetic distance and population differentiation, gene loci which were monomorphic in all provenances were not considered.

RESULTS

Allele frequencies

Of the eleven investigated gene loci, *Idh–A, Got–B* and *Pgi–A* showed no variation in the 11 provenances. Allele frequencies for the eight variable loci (*Idh–B, Got–A, Got–C, Lap–A, Lap–B, Pgi–B* and *Pgm–A*) are listed in Table 3.

Only the loci *Idh–B* and *Pgm–A* were variable in all surveyed populations. *Idh–B* displays a high degree of polymorphism, with two codominant alleles (B_1 and B_3) in all provenances and two rare alleles (B_2 and B_4) in only a few provenances. B_4 is found only in the three interior provenances east of the Cascades (10 – Post, 11 – Keechelus and 12 – Sears Cr.). The high elevation provenances 10 – Post (1450 m) differs significantly in its genetic structure at this gene locus from the other provenances. This provenance has the lowest frequency for *Idh–*B_1 with 18 % and the highest frequency for *Idh–*B_4 with 10 %. It should be noted that the two other high elevation provenances 2 – Santiam (830 m) and 12 – Sears Cr. (900 m), with 25 % and 23.4 % respectively, also have a lower frequency of *Idh–*B_1 than the other provenances.

In a study on *Abies alba* the gene loci *Idh–B* was found to show clinal variation; BERGMANN (1993) suggested, that this might be due to varying thermostability of the isoenzyme coded by this gene loci. The variation we found in our study indicates, that this might also be true for *Abies grandis*. Further investigations involving more provenances are suggested to determine if such a clinal trend can also be found in *Abies grandis*.

The *Pgm–A* locus is less polymorphic, whereby in all provenances the allele A_1 is dominant. The variant A_3 is missing in the population 10 – Post and 12 – Sears Cr. On this gene locus the more northern provenances from Washington (7, 8, 11) and British Columbia (9) clearly differentiate from the more southern provenances from Oregon (1, 2, 3, 6 and 10). The northern provenances have a lower frequency of the allele A_1 (62–70 % in the north, vs. 78 –97 % in the south) and a higher frequency of the allele A_3 (8–16 % in the north vs. 0–6 % in the south).

The other five investigated loci *(Got–A, Got–C, Lap–A, Lap–B* and *Pgi–B*) are variable in only some of the populations with generally the same allele being the most frequent (frequency greater than 90 %). Notable exceptions are the provenances 10, 11 and 12 which have a high proportion of rare alleles.

Provenance 10 – Post can also be differentiated from the others by the allelic distribution at the locus *Pgi–B*, where B_1 has a frequency of 18 %, and at the locus *Lap–B*, where the allele B_3 appeared (3 %).

Table 3. Allelic frequencies at eight enzyme gene loci for eleven provenances of *Abies grandis*.

Gene locus	Allele	Provenances										
		1	2	3	6	7	8	9	10	11	12	13
Idh–B	B_1	0.456	0.250	0.349	0.298	0.476	0.484	0.492	0.180	0.531	0.234	0.333
	B_2	0.017	–	0.012	0.019	0.145	–	–	–	–	–	0.042
	B_3	0.517	0.750	0.640	0.683	0.379	0.516	0.508	0.720	0.438	0.703	0.625
	B_4	–	–	–	–	–	–	–	0.100	0.031	0.063	–
Got–A	A_1	–	–	–	–	–	–	–	0.010	0.016	–	–
	A_2	1.000	1.000	1.000	1.000	0.984	0.960	0.985	0.970	0.984	0.964	0.931
	A_3	–	–	–	–	0.016	0.040	0.015	0.020	–	0.036	0.069
Got–C	C_1	0.008	0.019	0.023	0.029	–	–	–	0.050	0.016	0.036	–
	C_2	0.992	0.981	0.977	0.971	1.000	1.000	1.000	0.930	0.937	0.964	1.000
	C_3	–	–	–	–	–	–	–	0.020	0.047	–	–
Lap–A	A_1	–	–	–	–	–	–	–	0.010	–	0.010	–
	A_2	1.000	1.000	0.977	1.000	1.000	1.000	0.985	0.960	0.969	0.847	1.000
	A_3	–	–	0.023	–	–	–	0.015	0.030	0.031	0.144	–
Lap–B	B_1	–	–	–	0.019	–	–	0.038	0.030	–	0.036	–
	B_2	1.000	1.000	1.000	0.981	1.000	1.000	0.962	0.940	1.000	0.964	1.000
	B_3	–	–	–	–	–	–	–	0.030	–	–	–
Pgi–B	B_1	0.034	0.057	–	–	0.008	0.016	–	0.180	0.062	–	0.028
	B_2	0.966	0.943	1.000	1.000	0.992	0.984	1.000	0.820	0.938	1.000	0.972
Pgm–A	A_1	0.924	0.827	0.779	0.885	0.636	0.619	0.658	0.970	0.703	0.811	0.653
	A_2	0.68	0.115	0.163	0.105	0.205	0.238	0.263	0.030	0.204	0.189	0.236
	A_3	0.008	0.058	0.058	0.010	0.159	0.143	0.079	–	0.093	–	0.111
Dia–A	A_1	1.000	1.000	0.953	1.000	0.978	1.000	1.000	1.000	1.000	1.000	1.000
	A_2	–	–	0.047	–	0.022	–	–	–	–	–	–

Table 4. Measures of genetic multiplicity and diversity in eleven provenances of *Abies grandis*.

Provenance		Genetic multiplicity			Genetic diversity		Heterozygosity
		P %	M/L	GM	n_e	υ_{gam}	H_e
1	Santiam	36	1.54	324	1.165	2.62	0.092
2	Santiam	36	1.45	162	1.148	2.66	0.084
3	Mohawk	45	1.64	972	1.206	3.57	0.126
6	Philomath	36	1.54	324	1.150	2.56	0.093
7	Darrington	45	1.64	972	1.346	5.93	0.153
8	Elwha	36	1.45	162	1.286	4.86	0.143
9	P. Alberni	45	1.54	486	1.205	3.72	0.140
10	Post	63	2.09	69984	1.211	4.02	0.147
11	Lk Keechelus	54	1.82	5832	1.333	6.52	0.174
12	Sears Cr.	54	1.73	2916	1.228	4.38	0.153
13	Elwha	36	1.54	324	1.277	4.87	0.148

Table 5. Estimates of genetic distances between pairs of provenances of *Abies grandis*.

Population	2	3	6	7	8	9	10	11	12	13
1	0.046	0.049	0.035	0.063	0.048	0.050	0.085	0.055	0.082	0.061
2	–	0.031	0.021	0.076	0.062	0.060	0.078	0.076	0.050	0.050
3		–	0.034	0.062	0.056	0.049	0.094	0.058	0.059	0.043
6			–	0.080	0.069	0.063	0.070	0.077	0.048	0.054
7				–	0.029	0.039	0.144	0.050	0.105	0.049
8					–	0.021	0.126	0.043	0.086	0.028
9						–	0.118	0.040	0.076	0.041
10							–	0.108	0.073	0.110
11								–	0.088	0.059
12									–	0.073

Genetic multiplicity and diversity

The values calculated for the genetic multiplicity and diversity measures are listed in Table 4. They show large fluctuation between the provenances. By far the highest variability is found in provenance 10 – Post where 63 % of the gene loci are polymorphic. It is the only provenance with more than 2 alleles per gene locus ($M/L = 2.09$) and has, in comparison to the other provenances, an extremely high potential genotypic multiplicity ($G_M = 69{,}984$). Among the other provenances G_M only reaches 0.2 % to 8 % of this value.

Among the values of genetic diversity however, the provenance 10 – Post does not rank first. The four provenances from Washington (7 – Darrington, 8 and 13 – Elwha and 11 – Keechelus) as well as provenance 12 – Sears Cr. from Idaho are genetically more diverse than the more southern provenances from Oregon and the northwest provenance 9 – Port Alberni from British Columbia. These higher values are primarily due to the allele structure at the *Pgm–A* gene locus (see also 2.1). Noticeably less diverse are the provenances 1 and 2 – Santiam as well as 6 – Philomath.

These results are also valid for the mean heterozygosity with the noticeably low heterozygosity ($H_e <$ 10 %) of the low elevation provenances from Oregon (1, 2, 3 and 6).

Differentiation between provenances

Estimates of genetic distances (D) (GREGORIUS 1974) between pairs of *Abies grandis* provenances are presented in Table 5. The values of D varied between 0.021 (2 – Santiam to 6 – Philomath) and 0.144 (7 – Darrington to 10 – Post). The high elevation provenance 10 – Post had a large genetic distance in respect to all other provenances.

The genetic distances between the Oregon provenances (1, 2, 3, 6) as well as between the Washington (7, 8, 9, 11, 13) and British Columbia provenances are much less than between the Oregon and Washington, British Columbia collective. The provenance 12 – Sears Cr. also has a relatively high genetic distance to all other provenances (D between 0.048 and 0.105).

The indicated similarities and differences listed in the genetic distance matrix become clearly visible in the cluster analysis (Figure 2). The dendrogram shows two distinct groups: one group encompasses the northern provenances from Washington and British Columbia (7, 8, 9, 11, 13) and the other group the provenances from Western Oregon (1, 2, 3, 6) as well as 12 – Sears Cr. from Idaho; whereby 10 – Post is clearly distinguishable from the rest of the stands by being the most remote genetically.

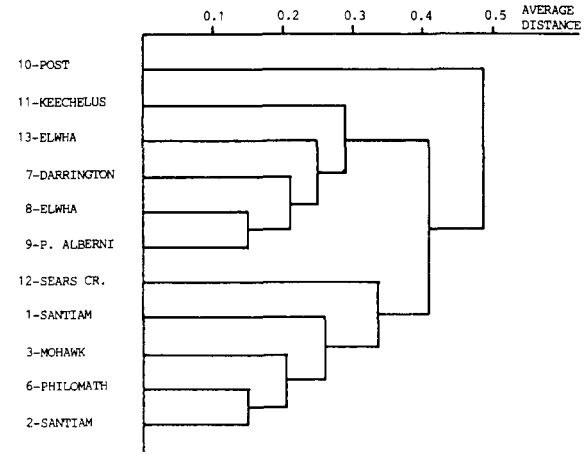

Figure 2. Dendrogram from cluster analysis based on GREGORIUS (1974) genetic distances between eleven provenances of *Abies grandis*.

The values calculated for the parameter D_j (D_j = differentiation among provenances) indicate that the provenances are clearly differentiated. Substantial variation exists among loci: For the gene loci *Idh–B*

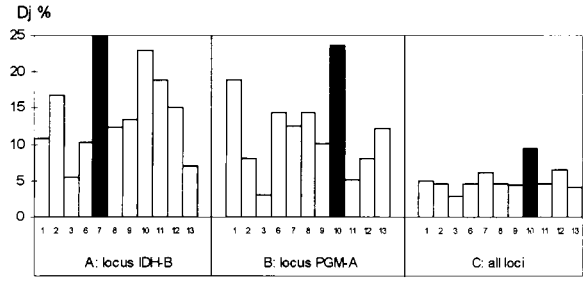

Figure 3. Genetic differentiation between populations of *Abies grandis*.

and *Pgm–A*, which discriminate better than others, the D_j values are presented in Figure 3. The average D_j computed over all polymorphic loci are also listed. One can clearly see that the sequence of differentiation among provenances can be quite varied (populations with the highest differentiation are indicated by the shaded bar). The population 10 – Post is clearly the provenance with the highest degree of differentiation over all 10 loci. On the average 9,4 % of the alleles differ from the other provenances, a value which can be considered as being quite high. The least differentiated provenance was the population 3 – Mohawk with $D_j = 2{,}9$ %.

The average D_j value of 5,2 % was almost identical to the value $G_{ST} = 5{,}72$ %, (G_{ST} = coefficient of gene differentiation among provenances) calculated with the diversity statistics of NEI. This means that approximately 6 % of the total diversity detected in all the samples can be attributed to genetic differences among provenances and 94 % of the allelic variation resides within individual provenances.

DISCUSSION

In field trials (RAU *et al.* 1991, KÖNIG 1995, KLEINSCHMIT *et al.* 1995) as well as in ecophysiological and morphological studies (LARSEN 1978, LARSEN & RUETZ 1980, ZOBEL 1973, 1974) large differences were found between *Abies grandis* provenances in respect to growth, frost hardiness, drought tolerance etc. The expected high degree of genetic variation between provenances was only partially verified by the presented isoenzyme analysis. In some cases the response of specific provenances was associated with specific genetic structure.

Thus, for example, the provenance 10 – Post, Oregon from a region of diverse and extreme site conditions (frost, drought), can be clearly differentiated from other provenances. In the field trials it is the slowest growing provenance. Studies on frost resistance (LARSEN & RUETZ 1980) have also shown it to have a extremely high resistance against late and early frost. Similar traits (slower growth than coastal provenances and high frost resistance) are also found among the other two inland provenances 11 – Keechelus from the east slope of the Cascades in Washington and 12 – Sears Cr., Idaho. Both provenances show a high interpopulational genetic variation. This can be the result of the more variable site conditions where these provenances are found. On the other hand, the possibility that it is also an expression of possible hybridization with *Abies concolor* cannot be excluded since according to STEINHOFF (1978) and ZOBEL (1973, 1974) the provenance 10 – Post lies in the introgression area. KONNERT & BERGMANN (1995) also found a higher genetic variability in *Abies alba* in its introgression zone with *Abies borisii-regis*.

A direct correlation between the genetic structure at specific gene loci and phenotypical parameters was not found. This is in agreement with the results found by numerous authors who state: "we still know little about the phenotypic effects associated with particular allozyme alleles in forest trees" (BUSH & SMOUSE 1992).

In order to draw conclusions from these results in regards to the genetic variation of *Abies grandis* in its natural range one must assume that there was no loss of genetic variation in the nursery (treatment, sorting etc.) nor on the test site (selective effects due to the environment, drift effects due to limited number of plants). In other words we must base our assumptions on the basis that the investigated provenances from the trial are genetically identical to the originals stands. That this can be assumed is shown by a comparison of the provenance 8 – Elwha – bud tissue from the provenance trial – and 13 – Elwha – commercial seed collection in the same area. Both "provenances" are found to be largely genetically similar, only the allele B_2 from the *Idh–B* locus is missing in the sample from the provenance trial. There are two possible explanations for this. On one hand the number of trees sampled in the provenance trial was relatively small ($N = 62$) so that a rare allele can be missing or was not found in the small sample. On the other hand we know that the IUFRO seed collection for the provenance 8 – Elwha was done in a relatively restricted area near the Indian Cr. Motel, so that the allele $Idh\text{–}B_2$ could have been missing in the original seed sample.

Our studies indicate that there are definite genetic differences between *Abies grandis* stands from the northern limits of its range (Washington, British Columbia) and the more southern provenances from Oregon. Within each group the genetic differences are low, especially for provenances from the same region but from slightly differing elevations (*e.g.* Mohawk, R., O.).

For *Abies grandis* some of the variation which was found can be explained by population history. The migration northward following the ice age could have occured along the eastern slope of the Cascades northward or perhaps even westward from Idaho across the mountains along the Canadian/US border. The occurence of certain allele variants from both the Oregon populations and the interior provenances among the Washington and British Columbia population indicate that there must have been contact with both groups. This was also suggested by STEINHOFF (1978) who mentioned that ZAVARIN *et al.* (1977) found "concolor type terpene composition" among grand fir from the eastern slope of the Washington Cascades, as well as DANIELS (1969) who found a low frequency of trees with yellow bark (a characteristic of *Abies concolor*) as far north as the central Washington Cascades. This could only be verified by a more intensive study of the allele distribution of provenances along the eastern Cascades northward from Oregon and among provenances in northern Washington between northern Idaho and the northern Cascades.

The lack of microgeographic differentiation was also found among other North American Abies species. DAVIDSON (1990) reported on a very low genetic differentiation among *Abies amabilis* provenances from Vancouver Island. The value for G_{ST} found by DAVIDSON(1990) was much lower (1,8 %) than the value we found for *Abies grandis* (G_{ST} = 5,7 %) whereby this is largely due to the large regional differences. A lack of differentiation among smaller, regional population was also found By DIEBEL & FERET (1991) for *Abies fraseri* and By NEALE & ADAMS (1985) for *Abies balsamea.*

A similar pattern of variation as found by *Abies grandis* – clear differentiation among larger regions, small differentiation within the region – was also found among the European *Abies alba* (KONNERT 1995). By analysis of over 100 populations from the entire natural range of *Abies alba* it was possible to find an interrelation between the genetic structure and the northward migration following the ice age (KONNERT & BERGMANN, 1995).

Under consideration of all the mentioned limitations of the material used in this study the results are nevertheless an indication that isoenzymatic investigations can be used to separate larger regions of provenance such as those from the coastal region and Cascades of Oregon from the Interior provenances as well as from provenances in the Cascades and Olympic Peninsula of Washington – including those from Vancouver Island, British Columbia. Such results can potentially be used in seed control as well as in helping identify the origin of older grand fir plantations.

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