GENETIC VARIATION OF SILVER FIR POPULATIONS (*ABIES ALBA* MILL.) IN SWITZERLAND

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ABSTRACT

Levels of genetic variation of 18 indigenous populations of silver fir (*Abies alba* Mill.) were studied in Switzerland. For each of 100 trees per population, multilocus genotypes were identified using isoenzyme gene markers at 14 enzyme coding gene loci.

The observed numbers of alleles per locus deviate only little between populations and are comparable to those found in other parts of the Central European range. The occurrences of area-specific alleles indicate that in Switzerland introgression zones between various refugia exist. According to actual pollenanalytical studies it can be concluded that Swiss silver fir mainly re-imigrated from the northern and central Italy and that genetic structures are additionally influenced by an eastern gene pool.

Levels of actual heterozygosity and genetic diversity are similar to those observed for other silver fir populations. In general, both parameters deviate only slightly between populations, but deviations are obvious between populations from outside and within the Alpine region. Especially, higher values of genetic diversity were observed for populations within the Alpine region. This finding may suggest that genetic variability is crucial to adapt to and to survive under the heterogeneous environmental conditions within the Alpine region.

Levels of interpopulational genetic variation (genetic distance, genetic differentiation) revealed distinct deviations between populations within and outside the Alpine region as well as between neighbouring populations growing under different environmental conditions. Both results indicate to be primarily dependent on processes of adaptation to different or specific environmental conditions.

Results clearly indicate that for the preservation of genetic variation a larger number of gene reserve areas should be established to enable preserving many genetically differentiated populations and ecotypes. Overall, in order to extensively preserve genetic resources, the results indicate the necessity to focus on the preservation of genetic variation as an essential goal in sustainable management of forest ecosystems.

Key words: *Abies alba*, isoenzymes, geographic variation, gene preservation, vegetation history

INTRODUCTION

Natural range of silver fir in Switzerland

In Switzerland, silver fir is an important ecological and economical tree species of mixed forests of the mountainous and the lower Alpine region. Silver firs stock on approximately 180.000 hectares and cover about 15 % of the total forest area (ANONYMUS 1988a). This is significantly higher when compared to other countries of Central Europe (SCHÜTT 1991). Within Swiss forest regions, most of the silver firs occur in the Prealps (22 %), in the Jura (21.4 %), and in the Plateau (15.4 %). Lower occurrence is reported for the Alps (6.4 %), and the South Alpine region (6 %). The natural range of silver fir is characterized by distinct heterogeneity of soil types (BACH *et al.* 1954), vegetation communities (KUOCH 1954), climatic conditions (LEIBUNDGUT 1978, LINGG 1986), and elevation zones (ELLENBERG & KLÖTZLI 1972). For example, silver firs range from 400 m above sea level (a.s.l.) in the Plateau up to 1900 m a.s.l. in the central Alps, close to the timberline, and they grow under moist climatic conditions in the Prealps (over 2000 mm rainfall per year) as well as under rather dry and continental climatic conditions in the interior Valais (approximately 600 mm rainfall per year).

Vegetation history of Swiss silver fir

During the last glacial period, the main refugia for Swiss silver fir are thought to be in the central and northern part of the Apennine, with a mosaic of varied steppe vegetation and local forests (BURGA 1988, LANG 1992). SCHNEIDER (1978, 1985) assumed that additional refugia existed close to the southern Alpine region because silver firs occurred immediately after the glacial period in the western Po region and in the

southern Alps. A western refugial influence, especially for the Jura region, is assumed by KONNERT & BERGMANN (1995), who found an area-specific isozyme allele ($Mnr\text{-}B_1$) in populations of the western European range.

After the glacial period silver fir re-immigrated generally from the south and the southwest to Switzerland (BURGA 1988, BURGA & PERRET 1998). The re-immigration is predominantly influenced by the Alpine chain extending from the South-west to the North-east of Switzerland and by only a few passes facilitating the re-immigration in south-north direction. According to BURGA (1988), at least five re-immigration routes can be distinguished (Fig. 1): (a) from the French Jura into the Swiss Jura, (b) from the Savoie region (France) over the Forclaz pass into the western Prealps and parts of the Plateau, (c) from northern Italy over the Simplon pass into the upper and middle Valais, (d) from Northern Italy and the Tessin valleys over the Lukmanier pass and via the upper Rhine valley into the eastern Prealps, and (e) from northern Italy via the Etsch valley and over the Reschen pass into the Lower Inn valley.

In Switzerland, the earliest occurrences of silver firs are reported in the south Alpine region at about 12.000 yr. B.P. (years before present) and in the Tessin valleys at about 10.000 yr. B.P. (ZOLLER & KLEIBER 1971). The main development of today's silver fir range began with re-immigration and expansion during the early Preboreal (10200–8800 yr. B.P.). Silver fir became well established in the Swiss South Alps (Tessin valleys) during the Boreal (8800–7300 yr. B.P.) and immigrated into the upper Valais over the Simplon pass (WELTEN 1982), which is about 2005 m a.s.l. high. At the beginning of the Older Atlantic (7300–6000 yr. B.P.), silver fir immigrated into the Upper Rhine valley and the eastern part of the Swiss Alps. During the Younger Atlanticum (6000–4800 yr. B.P.), silver fir dominated in the mountainous and subalpine belts both south and north of the Alps, and became established in the Jura and the Plateau. Beginning from the Subboreal (4800–2800 yr. B.P.), the range of silver fir decreased due to changing climatic conditions, re-immigration and expansion of Norway spruce and European beech, and human activities (*e.g.* exploitation of silver fir forests, reforestation with Norway spruce). The area reduction caused, on the one hand, a fragmentation of silver fir populations into many subpopulations; for example, separated within disjunct Alpine valleys. On the other hand, population sizes were strongly reduced. For example, within the region of the Lower Inn valley only one small relic population of about 400 individuals exists today.

Interestingly enough, the chronological differences of re-immigration into different regions of Switzerland caused a distinct deviation in development of silver fir ecotypes. Silver fir re-immigrated into the Swiss South Alps and the south-western parts of the Swiss Alps before the other climax tree species, spruce and beech. In contrast, the re-immigration into the Jura, the Plateau, the eastern parts of the Alps, and the Prealps resulted in a competition between silver fir and spruce or beech. Due to these different evolutionary processes, silver firs of the Tessin valleys and of the south-western part of the Swiss Alpine region show a different eco-physiological behaviour when compared to other silver firs, *e.g.* distinct pioneer characteristics, lower shade requirement, and higher drought resistance (MAYER 1962, ZOLLER 1964, LINGG 1986).

Overall, one can assume, that genetic structures of silver fir populations of Switzerland are influenced by refugial behaviour, postglacial re-immigration history, adaptation to different environmental conditions, and natural and human impacts during the development of today's range. However, up to now only little information about genetic variation of silver fir in Switzerland exists, measured by either quantitative traits (*e.g.* ENGLER 1905, LEIBUNDGUT 1978, COMMARMOT 1997) or genetic markers (*e.g.* BERGMANN *et al.* 1990, WOLF 1992, HUSSENDÖRFER 1995a,b, HUSSENDÖRFER & MÜLLER-STARCK 1994). Meanwhile, further genetic inventories using izoenzyme gene markers were performed which focused mainly on the designation of gene reserve areas (HUSSENDÖRFER 1997). This report gives a survey of the actual results of genetic variation patterns and conclusions for vegetation history and gene preservation of Swiss silver fir.

MATERIAL AND METHODS

Within the natural range of silver fir in Switzerland, samples of eighteen indigenous populations were chosen (Table 1, Figure 1). For each population, 100 adult trees were selected on an area of approximately 10 hectares. Tree selection was based on a square grid system with an average distance of 33 m. The age of the studied trees ranges from 60 to a maximum of 350 years.

Using bud tissue, the genotype of each of 1800 trees was determined by isoenzyme gene markers at 14 polymorphic gene loci (Table 2). For the survey of electrophoretic methods see HUSSENDÖRFER *et al.* (1995). Intrapopulational variation was measured by number of alleles per locus A_L, actual (observed) heterozygosity H_A, genetic diversity v (GREGORIUS 1978, 1987), and hypothetical gametic multilocus diversity v_{gam} (GREGORIUS *et al.* 1985). Variation among populations is measured by genetic distance d_0 (GREGORIUS 1974) and by differentiation among (sub-)

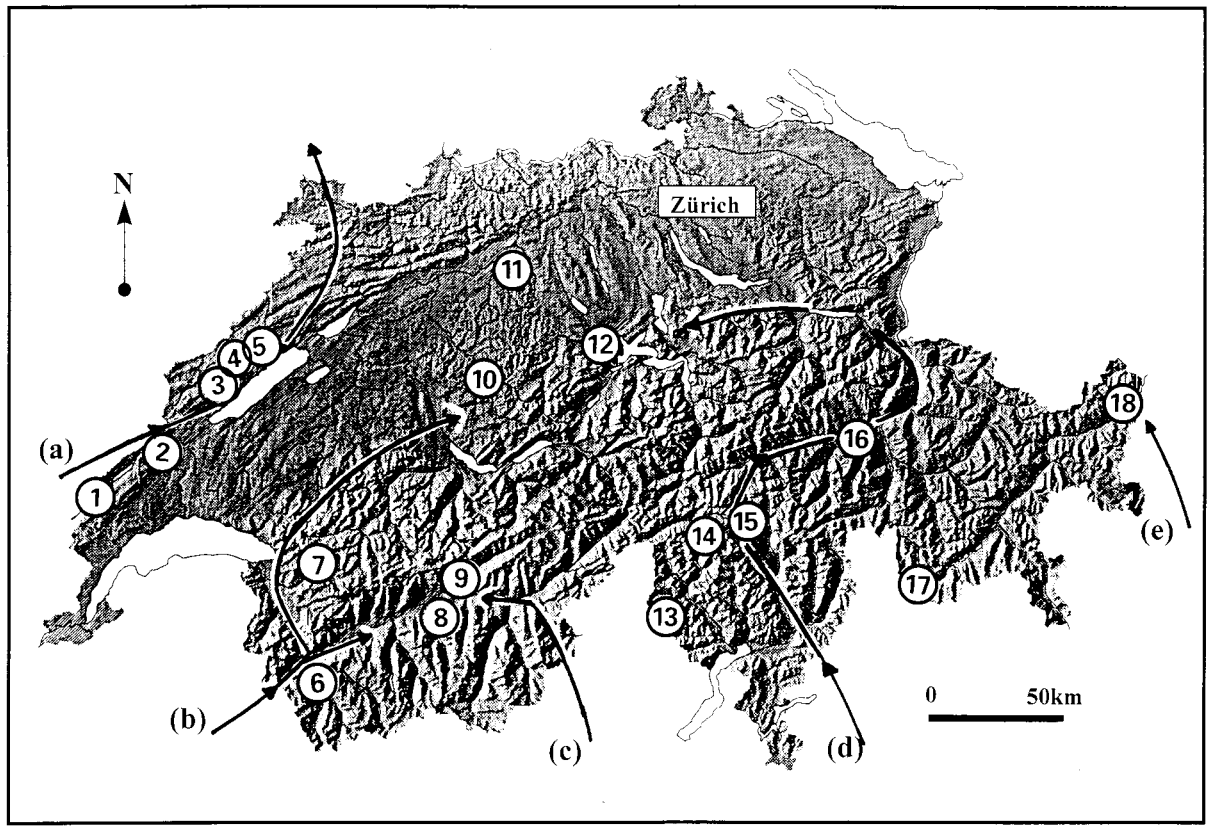

Figure 1. Location and designation of investigated populations of silver fir in Switzerland. Suggested Holocene re-immigration routes (Nos. a, b, c, d, e) are given according to BURGA (1988); for detailed information see text.

Table 1. Survey of site and stand characteristics of Silver fir populations.

Pop. No.	Local designation	Sample size	Forest region	Altitude (m)	Exposition
1	Le Brassus	100	Jura	1190–1240	–
2	Bretonnières	100	Jura	810–830	–
3	Couvet-South	100	Jura	820–860	S
4	Couvet-North	100	Jura	840–900	NW
5	Neuenburg	100	Jura	780–860	NW
6	Martigny	100	Swiss Alps	1080–1160	NW
7	Gstaad	100	Prealps	1280–1390	NW
8	Ochsenboden	100	Swiss Alps	1070–1220	NO
9	Leuk	100	Swiss Alps	1200–1320	SW
10	Signau	100	Prealps	900–980	NO
11	Zofingen	100	Middle Land	480–490	–
12	Schwarzenberg	100	Prealps	900–940	NO
13	Vergeletto	100	South Alps	1100–1280	N
14	Prato	100	South Alps	1250–1480	NO
15	Cavagnago	100	South Alps	1300–1490	SW
16	Präz	100	Swiss Alps	1350–1450	NO
17	Bondo	100	Swiss Alps	1290–1410	NW
18	Tschlin	100	Swiss Alps	1200–1310	NO

populations D_j, δ (GREGORIUS & ROBERDS 1986). Differences between frequencies of genetic types were tested statistically by employing the log likelihood ratio test (G-test) of homogeneity in contingency tables;

Table 2. Survey of enzyme systems, standard abbreviation, E. C. No., electrophoretic buffer systems, and enzyme coding gene loci in buds of silver fir.

Enzyme system	Abbreviation	E.C. Nr.	Buffer system*	Gene locus
Alanin aminopeptidase	AAP	3.4.11.1	A	*Ap*–D
Aspartat aminotransferase	AAT	2.6.1.1	B	*Aat*–A, –B, –C
Isocitrate dehydrogenase	IDH	1.1.1.42	C	*Idh*–A, –B
Leucin aminopeptidase	LAP	3.4.11.1	A	*Ap*–A, –C
Malate dehydrogenase	MDH	1.1.1.37	C	*Mdh*–A
Menadione reductase	MNR	1.6.99.2	C	*Mnr*–B
NADH-dehydrogenase	NDH	1.6.99.3	C	*Ndh*–A
6-Phosphogluconate dehydrogenase	6PGDH	1.1.1.44	C	*6Pgdh*–A, –B
Phosphoglucomutase	PGM	2.7.5.1	C	*Pgm*–A

* A = Ashton-system, B = Tris-citric acid pH 8.5, C = Tris-histidin pH 7.5 (see HUSSENDÖRFER *et al.* 1995).

levels of significance are given by $\alpha = 0.05$ (*), $\alpha = 0.01$ (**) and $\alpha = 0.001$ (***).

RESULTS AND DISCUSSION

(1) Genetic variation of the enzyme systems (see Appendix 1)

Among the investigated loci, *Pgm–A* is close to fixation since the allele $Pgm–A_2$ was observed in only 5 out of 18 populations. For *Ndh–A*, polymorphism was found in 10 populations, for *Aat–C*, *Mnr–B*, *6Pgdh–B* in 14 populations, and for *Ap–D* and *Aat–A* in 16 populations. At some loci rare alleles were observed which are considered to be area-specific for silver fir (for an overview see KONNERT & BERGMANN 1995): $Ap–A_2$ (named $Ap–A_1$ in KONNERT & BERGMANN 1995), $Mnr–B_1$, $6Pgdh–B_3$.

For the $Mnr–B_1$ allele, decreasing frequencies were observed from west to east and missing entirely in the eastern populations Nos. 17 and 18. This finding corresponds partly to studies of KONNERT & BERGMANN (1995) who observed a limited geographical distribution of the $Mnr–B_1$ allele. They postulated an area-specificity for the $Mnr–B_1$ allele characterizing silver fir populations originating from a western refugium (located probably in the Massif Central, France). According to the hypothesis of KONNERT & BERGMANN (1995), the geogrophical distribution of the $Mnr–B_1$ allele should be limited to the western part of Switzerland (Jura region, western Prealps). In the present study, however, the $Mnr–B_1$ allele was not exclusively found in populations of the Jura region or the western Prealps but also in one population of the Southern Alps (samples No. 13), in populations of the Valais (samples Nos. 6, 8, 9), in populations of the Plateau (samples Nos. 10, 11, 12) and even in one population of the eastern Swiss Alps (sample No. 16). In other studies this allele was observed in populations of northern Italy (HUSSENDÖRFER 1997) and in populations of Austria (BREITENBACH-DORFER *et al.* 1997). Thus, it seems doubtful whether the $Mnr–B_1$ allele characterizes silver fir populations originating from a western refugium as was assumed by KONNERT & BERGMANN (1995). In contrast, due to the actual geographical distribution it can not be excluded that the $Mnr–B_1$ allele originated from an Italian gene pool and spread to todays western and eastern range of silver fir. This assumption would be in accordance with pollenanalytical studies. LANG (1992) concluded that silver fir re-immigrated into the western range of Central Europe (French and Swiss Jura, Vosges Mts., Black Forest) and further into the Massif Central via a western Alpine route originating from northern and central Italy (see also BURGA & PERRET 1998).

In some samples of the western and eastern range, the allele $Ap–A_2$ (sample Nos. 2, 10, 15) was observed and the allele $6Pgdh–B_3$ was found in one sample of the Jura (sample No. 2). According to SCHROEDER (1989b), KONNERT & BERGMANN (1995) and LONGAUER (pers. comm.) these alleles are considered to be area-specific in particular for silver fir populations of the eastern European range (Austria, Balkan). In Austria, BREITENBACH-DORFER *et al.* (1997) found the $6Pgdh–B_3$ allele in silver fir populations as well and assumed a minor gene flow from the Balkan refugia into Austria. By means of isoenzyme studies, PARDUCCI *et al.* (1996) also assumed that silver fir populations of the southeastern Alps and northern Italy could have been originated from a Balkan refugium. Considering the main re-immigration from northern and central Italy into Switzerland, and the possibility of an influence into Switzerland from an eastern refugia which was already assumed by pollenanalytical studies (LANG 1992), it seems to be very likely that the area-specific alleles are dependend on migration and/or gene flow

Table 3. Intra- and interpopulational variation at 14 polymorphic loci for Silver fir populations of Switzerland. Except heterozygosities, all measures refer to allele frequencies (for nomenclatur see text).

Pop. No.	Alleles per locus A_L	Heterozygosity (%) H_A	Genic diversity v	Hypothetical multilocus diversity v_{gam}	Differentiation among populations D_j, δ
1	2.21	17.2	1.22	22.13	3.9
2	2.21	18.8	1.25	33.39	3.3
3	2.21	18.8	1.26	35.74	3.3
4	2.29	19.6	1.26	34.80	3.2
5	2.14	22.2	1.27	45.34	5.1
6	2.07	19.6	1.27	41.49	4.1
7	2.00	19.9	1.26	40.91	3.9
8	2.29	21.4	1.29	56.79	5.5
9	2.14	18.2	1.24	30.29	4.6
10	2.21	16.3	1.22	23.98	3.0
11	2.00	19.7	1.26	37.58	3.6
12	2.14	18.6	1.24	28.43	3.1
13	2.14	16.7	1.23	28.90	4.1
14	2.14	21.8	1.29	65.96	6.6
15	2.07	21.7	1.28	63.48	6.7
16	2.36	18.6	1.28	55.67	3.5
17	2.21	19.9	1.29	59.05	5.0
18	2.00	18.6	1.25	48.14	7.7
Mean	**2.16**	**19.3**	**1.26**	**41.78**	**δ = 4.4**

from an eastern gene pool on Swiss silver fir populations.

For the loci *Ap–A*, *Ap–C*, *Aat–B*, *Idh–A*, *Idh–B*, *Mdh–A*, *6Pgdh–A* a distinct major-polymorphism (according to FINKELDEY (1993): two or more alleles showing frequencies greater than 20 %) was observed in each of the investigated populations. Overall, no geographic trend was obvious by the frequency distribution at any of the investigated gene loci. This is in contrast to findings of other studies which revealed clinal geographic variation pattern's for silver fir, especially for allelic distribution at the *Idh–B* gene locus. At this gene locus some studies showed a temperature dependent cline from southern Italy to southern Germany was observed (MOLLER 1986, BERGMANN & KOWNATZKI 1988, BERGMANN & GREGORIUS 1993), while others showed a southwest to northeast cline which was regarded to be mainly associated to migration history (SCHROEDER 1989a, BREITENBACH *et al.* 1992). The lack of geographic variation patterns in the present study might be explained by overlapping effects due to the various postglacial re-immigration routes and selectional processes under different environmental conditions, for example dependent on exposition.

(2) Intrapopulational genetic variation

Alleles per locus

As can be seen from Table 3, average A_L-values per population range from 2.00 (samples Nos. 7, 11, 18) to 2.36 (sample No. 16) and the grand mean is 2.16. Similar values were found in silver fir populations of Baden-Württemberg (KONNERT 1992), Bavaria (KONNERT 1993) and Italy (PARDUCCI *et al.* 1996). In the present study, among the investigated populations no trend corresponding either to migration history or to geographic aspects is evident. That means, that a reduced level of genetic variation, which is assumed especially for populations located north of the Alps due to genetic drift within small refuge populations as well as due to bottleneck effects during re-immigration over small and high elevated passes, is not obvious at the investigated gene loci.

Heterozygosity

The H_A-values (Table 3) range from 16.3 % (sample No. 10) to 22.2 % (sample No. 5), which is equivalent to a ratio of 1 : 1.36. For most of the populations H_A-values range between 18 % and 20 %, and the grand mean is 19.3 %. These results indicate slightly lower values of heterozygosity than reported for silver fir in Baden-Württemberg (21.6 % in 10-loci studies by

KONNERT 1992) or in Bavaria (23.3 % in a 9-loci studies by KONNERT 1993), but significantly higher than observed in populations of Italy (13.3 % in a 14-loci studies by PARDUCCI *et al.* 1996). However, it must be acknowledged that the latter result depends on a different choice of enzyme systems, and thus methodical effects on the results can not be excluded (see BERGMANN 1991b, MÜLLER-STARCK *et al.* 1992).

According to BERGMANN (1993) and MEJNARTOWICZ *et al.* (1995) one can assume that especially for silver fir low H_A-values are mainly dependent on increased selfing due to low tree density in the parental population. Unfortunately, in most cases no data exists about stand structures of the parental populations of today's adult silver firs to confirm this hypothesis. However, for example in case of the population Vergeletto (sample No. 13), a low population density as well as distinct distances between single-trees of the parental population are very likely because of the specific site conditions (rock sites). This could possibly explain the outstandingly low H_A-value observed for this population (H_A = 16.7 %).

Genetic diversity

Levels of genetic (genic) diversity *v* varied very little between populations (Table 3): *v*-values range from 1.22 (samples No. 1, 10) to 1.29 (samples No. 8, 14, 17) and the grand mean is 1.26. These values correspond well with values observed in Baden-Württemberg (KONNERT 1992), and Bavaria (KONNERT 1993). The values of hypothetical multilocus diversity v_{gam} clearly distinguished samples: the minimum number of genetically different 14-locus gametic types is 23.98 (sample No. 10), and the maximum is 65.96 (sample No. 14). This is equivalent to a ratio of 1 : 2.75. The grand mean of v_{gam} is 41.78. In contrast to *v*-values, the v_{gam}-values of other studies are not comparable because single locus diversities are multiplied (see GREGORIUS *et al.* 1986), and thus significantly affect values due to the number of investigated gene loci.

In general, the comparison of A_L- and v-values allows the tentative inference about the mode of allelic frequency distribution for each population. Both measures are related in that diversity v is identical to A_L if alleles at any locus are in equal frequencies and v = 1 in case of fixation at all gene loci. In all other cases, v lies between the A_L-value and "1". As can be seen from Table 3, some populations with identical A_L-values show identical or similar v-values (*e.g.* samples Nos. 9, 12). However, for some other populations possessing identical A_L-values deviations of the corresponding diversities are obvious. For example, A_L-values are identical for populations Nos. 1 and 17 (A_L = 2.21), whereas the diversities range from the lowest value in case of population No. 1 (v = 1.22) to the highest value in case of population No. 17 (v = 1.29). This is explained by the greater evenness of the frequency distribution of the alleles of population No. 17 as compared to No. 1.

Among the investigated populations a distinct geographical trend can be observed for v-values, and in particular for v_{gam}-values (Figure 2). Both measures of diversity are significantly higher for most populations inside the Alpine region (samples Nos. 6, 8, 9, 13, 14, 15, 16, 17, 18) when compared to most populations outside the Alpine region (samples Nos. 1, 2, 3, 4, 5, 7, 10, 11, 12). The grand mean of v_{gam} for populations outside the Alpine region is 33.59 and for populations within the Alpine region 49.97, which is equivalent to a ratio of 1 : 1.48. In general, the v_{gam}-value is suggested to quantify the ability of populations to create genetic variation (GREGORIUS *et al.* 1986). According to this hypothesis, the significantly higher v_{gam}-values for populations of the Alpine region may suggest the necessity of high genic diversity in order to maintain the adaptability of silver fir populations, especially under the heterogeneous environmental conditions within the Alpine region.

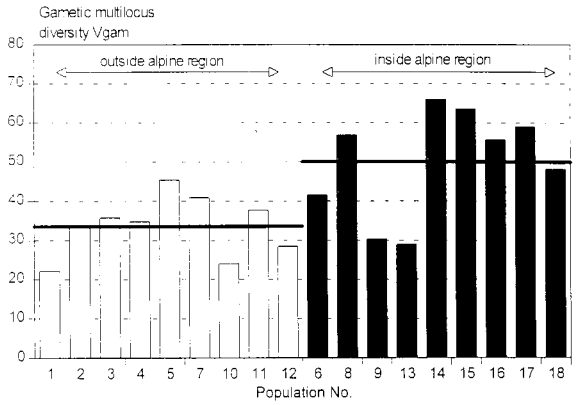

Figure 2. Levels of hypothetical multilocus diversity (v_{gam}) as indicated for populations outside and inside alpine region. The lines mark the grand mean for each region.

(3) Interpopulational variation

Genetic distance

Genetic distances d_0 of the gene pool were compiled to quantify differences of allele frequencies between each pair of population. As can be seen from Table 4, the largest genetic distance is obvious between samples No. 5 and No. 18 (d_0 = 10.8 %), and the lowest genetic distance between samples No. 10 and No. 12 (d_0 = 2.4 %). Overall, a slight trend is obvious, indicating larger gene pool distances between most populations of the western range (*i.e.* samples Nos. 1, 2, 3, 4, 5, 6, 7) and

most populations of the eastern range (*i.e.* samples Nos. 14, 15, 16, 17, 18). In particular, sample No. 18 shows large genetic distances when compared to the majority of the other samples. The latter finding supports the hypothesis of a separate re-immigration into the lower Inn valley (ZOLLER 1964).

Between some neighbouring populations an outstandingly high gene pool distance, as well as significant differences of allele frequencies at some gene loci are obvious. In Table 5, examples are given for the pairwise comparison between population No. 8 (Ochsenboden) and No. 9 (Leuk), as well as between population No. 14 (Prato) and No. 15 (Cavagnago). As can be seen from Table 5, between populations Nos. 8 and 9, d_0-values and G-values reveal highly significant deviations for gene loci *Ap–C*, *Idh–A* and *6Pgdh–A*, and between population Nos. 14 and 15 for gene loci *Ap–A*, *Ap–C*, *Ap–D* and *Idh–A*. The average geographical distance between each of the pairs is approximately 15 km only, but populations of each pair grow under various expositions (see Table 1), and populations Nos. 14 and 15 additionally grow on different soil substrate (No. 14: limestone, No. 15: granit and gneis). Thus, the distinct deviations in genetic structures may suggest locus-specific response to selective forces under the particular microsite conditions and indicate a direct or linked adaptive significance of these enzyme systems (see BERGMANN & GREGORIUS 1993, LONGAUER 1995).

Overall, the observed variation patterns by means of genetic distances suggest that mainly are dependent on adaptional processes under both, the particular macro- site and microsite conditions. However, it can not be excluded that the observed trend between populations of the western range and the eastern range is additionally influenced by re-immigration via different routes into the western and eastern part of Switzerland.

Genetic differentiation

Genetic differentiation (D_j, δ) contrasts the frequencies of genetic types (alleles, genotypes) either at any of the gene loci or the entire gene pool of one population with the average frequencies of the remaining populations, which are pooled as the respective complement population (GREGORIUS & ROBERDS 1986).

As can be seen from Table 3 (last column), the grand mean of genetic differentiation is δ = 4.4 %, and the D_j-values for the allelic gene pools range between 3.0 % (sample No. 10) and 7.7 % (sample No. 18). This means, that population No. 10 shares the largest proportion of common genetic information while population No. 18 reveals the largest genetic differences compared to the remainder populations. Overall, the following trend is obvious: for the majority of populations inside the Alpine region D_j-values are above average (samples Nos. 18, 14, 15, 8, 17, 9), whereas for the majority of populations outside the Alpine region D_j-values are under average (samples Nos. 10, 12, 3, 4, 1, 2, 11).

For single locus differentiation, the following results are evident (Table 6): the maximum δ-value is δ = 11.0 % (gene locus *Ap–C*), the minimum value δ = 0.5 % (gene locus *Pgm–A*). High δ-values are additionally obvious for gene loci *Mdh–A* (δ = 10.2 %), *Ap–A*

Table 4. Genetic distances (d_0) of the gene pool between each pair of populations.

Pop. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2	4.6																
3	3.8	4.2															
4	2.6	3.7	3.4														
5	4.9	3.6	3.5	4.0													
6	4.9	3.0	4.7	4.1	4.5												
7	6.7	4.0	5.3	4.9	5.0	4.0											
8	5.6	5.6	5.0	4.8	3.9	5.7	5.3										
9	3.5	4.8	4.5	3.6	5.7	5.5	6.4	6.3									
10	3.5	4.6	4.3	4.0	6.4	5.2	4.4	6.8	4.8								
11	5.3	4.2	3.9	3.7	4.4	4.9	4.2	5.4	5.5	4.3							
12	4.1	5.3	4.3	4.0	5.9	5.7	3.8	6.1	5.7	2.4	4.6						
13	5.4	5.2	5.9	6.1	6.5	6.4	4.5	8.2	6.0	4.1	6.5	3.6					
14	5.5	8.5	7.2	5.9	7.9	8.4	7.6	6.4	6.5	6.5	7.9	5.7	7.7				
15	6.1	8.8	8.0	7.4	9.3	9.0	9.0	8.9	5.6	6.3	7.9	6.9	6.8	6.1			
16	4.5	4.8	5.2	4.9	6.0	4.6	5.7	7.4	4.4	5.2	5.5	5.8	5.3	6.9	5.0		
17	7.5	7.0	7.0	6.9	7.5	6.6	5.0	7.0	7.4	5.2	5.5	4.6	4.3	8.6	7.3	5.9	
18	8.7	9.7	9.5	8.9	10.8	9.2	7.6	9.9	9.1	7.1	7.5	7.1	5.9	10.0	8.0	7.6	5.0

Table 5. Genetic distances (d_0) and *G*-values of the frequency distributuion of alleles between population pairs (for nomenclatur see table 1). In case of *G*-values, levels of significance are marked: α = 0.001 (***); n.t. means not testable.

Gene locus	Population pairs			
	Ochsenboden – Leuk		Prato – Cavagnago	
	d_0	*G*-values	d_0	*G*-values
Ap–A	5.0	4.73	17.5	23.48***
Ap–C	20.0	43.22***	21.0	45.19***
Ap–D	1.5	2.00	13.5	28.06***
Aat–A	1.5	1.04	2.0	5.58
Aat–B	3.5	n.t.	1.5	n.t.
Aat–C	0.5	n.t.	–	–
Idh–A	19.5	27.38***	16.5	16.93***
Idh–B	9.0	4.54	2.5	0.25
Mdh–A	5.5	1.26	2.5	0.26
Mnr–B	0.5	n.t.	0.1	n.t.
Ndh–A	–	–	5.5	n.t.
6Pgdh–A	19.5	19.28***	1.5	4.29
6Pgdh–B	0.5	n.t.	0.5	n.t.
Pgm–A	0.1	n.t.	–	–
Gene pool	63		61	

Table 6. Minimum and maximum values of genetic differentiation *Dj*, δ-values, and *G*-values of the allelic frequency distribution of the gene pool among samples of 18 silver fir populations in Switzerland (for nomenclature see text). In case of *G*-values, levels of significance are marked: α = 0.05 (*), α = 0.01 (**) and α = 0.001 (***).

Gene locus	D_{jmin}		D_{jmax}		δ	*G*-values
	D_j	Pop. No.	D_j	Pop. No.		
Ap–A	2.0	12	24.2	18	8.0	219.986***
Ap–C	3.6	7	21.6	14	11.0	328.057***
Ap–D	1.0	13	15.3	14	4.2	226.704***
Aat–A	0.2	1, 6, 11, 14	2.5	3	1.3	60.901**
Aat–B	1.1	3, 16	12.2	18	5.9	161.802***
Aat–C	0.4	1	3.8	5	1.4	62.136***
Idh–A	0.8	4	12.1	8	5.3	137.143***
Idh–B	0.2	15	9.5	2	4.4	54.784
Mdh–A	0.1	11	23.4	18	10.2	264.034***
Mnr–B	0.5	4, 8, 10	6.8	6	1.7	86.504***
Ndh–A	0.1	4, 12, 16	2.2	13	1.0	63.414***
6pgdh–A	0.9	3	12.5	18	6.3	104.388***
6pgdh–B	0.2	1, 8, 17	3.9	5	1.1	49.656*
Pgm–A	0.2	5	1.3	18	0.5	28.609*

(δ = 8.0 %), and *6Pgdh–A* (δ = 6.3 %). At most of gene loci distinct deviations between the minimum and the maximum D_j-value can be observed. For example at gene locus *Mdh–A* the minimum D_j-value is 0.1 %, the maximum D_j-value 23.4 % (see Table 6).

Statistical tests

Tests of homogeneity among allele frequencies of the 18 samples revealed statistically significant deviations for 13 gene loci (see Table 6). For the majority of loci, deviations were highly significant (levels of significance 0.001): *Ap–A*, *Ap–C*, *Ap–D*, *Aat–B*, *Aat–C*,

Idh–A, *Mdh–A*, *Mnr–A*, *Ndh–A*, *6Pgdh–A*. For the locus *Aat–A* deviations were significant at the level of 0.01, and for the loci *6Pgdh–B* and *Pgm–A* at the level of 0.05. For the gene locus *Idh–B* the observed frequency distributions of alleles deviated not significantly.

Overall, the observed significant deviations both for gene pool as well as for gene loci, correspond well with findings among silver fir populations of other ranges (*e.g.* SCHROEDER 1989a,b; BERGMANN 1991a; KONNERT 1992, 1993; BREITENBACH *et al.* 1995; LONGAUER 1995; PARDUCCI *et al.* 1996). In general, for these remarkable genetic deviations among silver fir populations the background can be mainly explained by either historical events (refugial behaviour, re-immigration history, human impact) or by adaptation processes. In the present study, the opposite trend in D_j-values which was found between populations within and outside the Alpine region, the distinct deviations within and among gene loci, and the statistically significant deviations among allele frequencies at most of the gene loci suggests to be mainly dependent on adaptation processes. However, an influence on genetic differentiation between western and eastern populations due to different re-immigration can not be completely excluded. Especially, the outstandingly high D_j-value of sample No. 18 indeed suggests a good accordance with historical processes depending on the separate re-immigration into the lower Inn valley (ZOLLER 1964). In addition, the low D_j-values which were found for population Nos. 10 and 12 could be dependent on historical events. The prealpine region, where both populations are located, is assumed to be the introgression zone between the western and eastern re-immigration routes around the Swiss Alps (see Figure 1). Thus, it is very likely that these both populations share more common genetic information when compared to other populations.

CONCLUSIONS

Vegetation history of the Swiss silver fir

The results of the present study indicate some new aspects of the vegetation history of silver fir. The observed geographical distribution of the *Mnr–B*$_1$ contrasts with the assumed area-specificity of this allele characterizing silver firs originating from a western refugium supposed by KONNERT & BERGMANN (1995). The results of the present study, however, confirm the pollenanalytical hypothesis that silver fir re-immigrated into Switzerland (including the western (Swiss) Alps and the Jura region) mainly from refugia located in the northern and central Italy (LANG 1992).

Furthermore, the occurrence of other area-specific alleles indicates an additional influence from eastern refugia on the Swiss gene pool. These findings seem to suggest that an introgression zone between various refugia exists in Switzerland which extended from the southern Alpine region to the Swiss Jura in the west, and to the Swiss Alps in the east. The geographical distribution of area-specific alleles observed in recent investigations of populations of Austria and Baden-Württemberg (Germany) indicate that such an introgression zone might even extend further to the east and to the north (see BREITENBACH-DORFER *et al.* 1997, HUSSENDÖRFER & KONNERT 1998).

Gene preservation

The Swiss gene preservation programme is focused on the designation of gene reserve areas for *in situ* conservation of genetic variation of significant forest tree species (ANONYMUS 1988b). For this purpose, the study of genetic variation of spruce in Switzerland revealed that genetic investigations supply essential criteria for the strategy in designation of gene reserve areas (MÜLLER-STARCK 1995). For spruce, the number of alleles was regarded to be most suitable criteria for the selection of gene conservation populations. To preserve great numbers of alleles, MÜLLER-STARCK (1995) concluded that designation of gene conservation areas should rather aim at the selection of fewer but larger populations.

In general, when compared to spruce, silver fir populations usually are characterized by lower levels of intrapopulational genetic variation (number of alleles, diversity, heterozygosity), but reveal distinct deviations between populations (*e.g.* BERGMANN 1991a; MÜLLER-STARCK *et al.* 1992). This trend can be confirmed by the results of this study.

Furthermore, the present results indicate that genetic structures are influenced by adaptation processes which are asumed to be dependent on selection pressure resulting from different or specific environmental conditions. This refers to macrosites as well as to microsites. In particular, the observed higher levels of hypothetical multilocus diversity v_{gam} for most populations of the alpine regions suggest that genetic variation is crucial to adapt and to survive under heterogeneous environmental conditions. In addition, one can assume that the outstandingly high levels of genetic distance and genetic differentiation between populations of the alpine regions may reflect evolutionary processes of development of silver fir's ecotypes.

Overall, these results suggest following recommendations for the designation of forest gene reserve areas for the silver fir in Switzerland:

- a greater number of gene reserves must be established to enable preserving a lot of genetically differentiated populations and ecotypes;
- the observed peculiarities of silver fir populations within the Alpine region suggest that a larger number of units should be established within the Alpine region, whereas less units would suffice outside the Alpine region;
- populations showing a high level of gametic multilocus diversity should be preferably selected to preserve the ability of silver firs to adapt to heterogeneous environmental conditions;
- furthermore, populations possessing rare or unique alleles should be selected to maintain genetic variability of silver fir.

In general, it must be acknowledged that the *in situ* preservation of genetic resources of silver fir in Switzerland within designated areas (*e.g.* gene reserve areas, strict forest reserve areas) will extend hardly more than 1 % of the total forest area of this species. Thus, to enable the extensive preservation of silver fir's genetic resources within and among genetically differentiated populations and ecotypes, it seems to be necessary to understand the maintenance of genetic variation as an essential goal in sustainable management of forest ecosystems.

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Appendix. Alleic frequencies of investigated silver fir populations

Enzyme gene locus	Allele	Population								
		1	2	3	4	5	6	7	8	9
Ap–A	2	–	.015	–	–	–	–	–	–	–
	3	.870	.840	.850	.825	.880	.825	.805	.880	.845
	4	.055	.060	.075	.030	.040	.045	.030	.035	.085
	5	.075	.085	.075	.145	.080	.130	.065	.085	.070
	6	–	–	–	–	–	–	–	–	–
Ap–C	1	.050	.040	.030	.045	.050	.045	.120	.135	.035
	2	.740	.711	.770	.735	.760	.670	.630	.720	.620
	3	.025	.050	.085	.030	.045	.030	.085	.015	.155
	4	.185	.200	.115	.190	.145	.255	.165	.125	.185
Ap–D	1	.050	.010	.015	.050	.010	.025	–	.045	.030
	2	.920	.990	.975	.935	.960	.975	1.000	.955	.965
	3	.030	–	.010	.015	.030	–	–	–	.005
Aat–A	1	.020	–	.045	.040	.040	.020	.010	.030	.015
	2	.980	1.000	.955	.960	.960	.980	.990	.970	.985
Aat–B	2	.940	.810	.895	.830	.795	.825	.825	.815	.850
	3	.060	.190	.105	.170	.205	.175	.175	.185	.150
Aat–C	1	.010	.025	.040	.020	.050	.040	.020	.010	.005
	2	.990	.975	.960	.980	.950	.960	.980	.990	.995
Idh–A	1	.130	.165	.220	.190	.225	.135	.270	.280	.115
	2	–	.010	–	–	–	–	–	.030	–
	3	.870	.825	.780	.810	.775	.865	.730	.690	.885
	4	–	–	–	–	–	–	–	–	–
Idh–B	1	–	–	–	–	–	–	–	.005	–
	2	–	–	–	–	–	–	–	–	–
	3	.420	.540	.430	.400	.515	.500	.495	.450	.365
	4	.580	.460	.570	.595	.485	.500	.505	.545	.635
	5	–	–	–	.005	–	–	–	–	–
Mdh–A	1	.680	.740	.670	.680	.635	.745	.845	.575	.630
	2	.320	.260	.330	.320	.365	.255	.155	.425	.370
Mnr–A	1	.010	.030	.065	.025	.010	.085	.035	.025	.020
	3	.990	.970	.935	.975	.990	.915	.965	.975	.980
Ndh–A	1	1.000	.990	.985	1.000	1.000	1.000	1.000	1.000	1.000
	2	–	.010	.015	–	–	–	–	–	–
6Pgdh–A	1	–	–	–	–	–	–	–	–	–
	2	.200	.255	.265	.210	.300	.320	.285	.375	.180
	3	.800	.745	.735	.790	.700	.680	.715	.625	.820
6Pgdh–B	1	.015	.025	.010	.005	.050	–	.010	.015	.010
	2	.985	.975	.990	.995	.950	1.000	.990	.985	.990
	3	–	.005	–	–	–	–	–	–	–
Pgm–A	1	.990	1.000	1.000	1.000	1.000	.990	1.000	.990	1.000
	2	.010	–	–	–	–	.010	–	.010	–

Appendix (continued).

Enzyme gene locus	Allele	Population								
		10	11	12	13	14	15	16	17	18
Ap–A	2	.005	–	–	–	–	.010	–	–	–
	3	.780	.715	.805	.815	.825	.650	.670	.711	.560
	4	.085	.135	.065	.090	.035	.130	.135	.145	.165
	5	.130	.150	.130	.095	.145	.200	.195	.150	.275
	6	–	–	–	–	–	.010	–	–	–
Ap–C	1	.095	.075	.150	.070	.165	.120	.060	.170	.095
	2	.690	.775	.675	.605	.495	.485	.580	.580	.555
	3	.075	.055	.055	.160	.045	.255	.095	.145	.145
	4	.140	.095	.120	.165	.295	.140	.265	.105	.205
Ap–D	1	–	–	.035	.045	.050	.075	.040	.080	.085
	2	.995	1.000	.935	.935	.800	.910	.960	.880	.915
	3	.005	–	.030	.020	.150	.015	.040	–	.105
Aat–A	1	.035	.020	.035	.010	.020	–	.015	.030	–
	2	.965	.980	.965	.990	.980	1.000	.985	.970	1.000
Aat–B	2	.955	.810	.925	.955	.945	.960	.895	.865	1.000
	3	.045	.190	.075	.045	.055	.040	.105	.135	–
Aat–C	1	–	–	.005	.005	–	–	.020	.005	–
	2	1.000	1.000	.995	.995	1.000	1.000	.980	.995	1.000
Idh–A	1	.170	.225	.230	.205	.290	.125	.120	.185	.195
	2	–	–	–	–	–	–	–	–	.015
	3	.830	.775	.770	.795	.710	.875	.865	.815	.775
	4	–	–	–	–	–	–	.015	–	.015
Idh–B	1	–	–	–	–	–	–	.005	–	–
	2	–	–	–	–	–	–	–	.005	–
	3	.430	.395	.440	.500	.425	.450	.500	.470	.385
	4	.570	.605	.560	.500	.575	.550	.494	.525	.615
	5	–	–	–	–	–	–	–	–	–
Mdh–A	1	.800	.730	.840	.905	.566	.590	.665	.885	.955
	2	.200	.270	.160	.095	.435	.410	.335	.115	.045
Mnr–A	1	.025	.010	.015	–	.010	–	.015	–	–
	3	.975	.990	.985	1.000	.990	1.000	.985	1.000	1.000
Ndh–A	1	.995	.990	.970	.995	.940	.990	.985	1.000	.985
	2	.005	.010	.030	.005	.060	.010	.015	–	.015
6Pgdh–A	1	–	–	–	–	–	.015	–	–	–
	2	.200	.285	.210	.240	.175	.160	.235	.365	.375
	3	.800	.715	.790	.760	.825	.825	.765	.635	.625
6Pgdh–B	1	.010	.005	–	.040	.005	–	.020	.015	.005
	2	.990	.995	1.000	.960	.995	1.000	.980	.985	.995
	3	–	–	–	–	–	–	–	–	-
Pgm–A	1	.990	1.000	1.000	1.000	1.000	1.000	.995	1.000	.985
	2	.010	–	–	–	–	–	.005	–	.015