GENETIC VARIATION AND MATING SYSTEM IN A NATIVE PROVENANCE AND THE DERIVED SEED ORCHARD OF DOUGLAS-FIR (*PSEUDOTSUGA MENZIESII* (MIRB.) FRANCO)

Daniel Prat[1, 3] **& Jarosław Burczyk**[2, 4]

[1)] Laboratoire INRA-ENGREF de Sciences Forestières, Unité de Génétique des Populations d'Arbres Forestiers, ENGREF, 14 rue Girardet, F–54042 Nancy Cedex, France
[2)] Institute of Dendrology, Polish Academy of Sciences, PL–62-035 Kórnik, Poland
[3)] Present address: INRA, Station d'Amelioration des Arbres Forestiers, F–45160 Ardon, France
[4)] Present address: Pedagogical University, Department of Biology and Environment Protection, ul. Chodkiewicza 30, PL–85064 Bydgoszcz, Poland

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ABSTRACT

Genetic diversity and mating system were assessed in Douglas-fir (*Pseudotsuga menziesii*) in two stands (Darrington IUFRO and Round Mountain) of the Darrington provenance area, and in the seed orchard established in France with clones derived from this provenance area. Genotypes of embryos and megagametophytes of about 10 seeds per mother tree were determined at 10 independent isozyme loci. This allowed genotyping of mother-tree. Fixation indices were higher in progenies than in mother-trees, however significant deviations from Hardy-Weinberg equilibrium were detected only at some loci in progenies with an excess of homozygosity, both in the natural stands and in the seed orchard. Genetic differentiation was very low ($F_{ST} < 0.03$) among natural stands, as well as among generations ($F_{ST} < 0.005$). All stands were predominantly allogamous (multilocus outcrossing rate > 0.90). The highest outcrossing rate (0.97) was noticed in the seed orchard. The difference between the expected fixation index based on this multilocus outcrossing rate and the observed values in mother-trees and progenies suggested a higher level of inbreeding in the Round Mountain stand than in the Darrington IUFRO stand. The seed orchard consisting of 70 phenotypically selected clones showed the same level of genetic variability than the original stands, but it exhibited a reduced genetic diversity at the progeny level.

Key words: *Pseudotsuga menziesii*, seed orchard, genetic variation, mating system, outcrossing, effective population size

INTRODUCTION

Genetic improvement of forest tree species consists of different steps. The first one is the evaluation of genetic diversity, usually through provenance and progeny trials. This leads to the identification and recommendation of the best natural seed sources for afforestation and to the estimation of genetic parameters (heritability of different traits) used in family and individual selection (NAMKOONG & KANG 1990). Plantations including the best provenances avoid use of maladapted material but they do not provide benefits of family or individual selection. The next step of tree improvement can be the establishment of seed orchards with material (families or clones) selected (phenotypically or genotypically) from the best seed sources (CHOLET 1986). The objective of this artificial population is for seed production for reforestation purposes. The seed crop collected in seed orchards should provide a genetic gain for one or more traits such as growth, adaptation, phenology, wood quality, stem straightness, etc. (MATHESON & LINDGREN 1985; COTTERILL & JACKSON 1989). Tree breeding can lead to a significant reduction in genetic diversity for traits under selection. However, it is desirable to maintain diversity for other traits, such as disease and stress resistance (NAMKOONG 1991). In this context, investigations on genetic diversity of seed crop from seed orchards is suitable to avoid the use of seedlots of excessively-reduced genetic diversity.

Most forest tree species exhibit little genetic differentiation among natural stands, when studied using neutral genetic markers (MERKLE & ADAMS 1987; LI & ADAMS 1989; ZANETTO & KREMER 1995), in spite of significant variation of quantitative traits detected from provenance trials (CHRISTOPHE & BIROT 1979; BASTIEN & ROMAN-AMAT 1990; LOO-DINKINS *et al.* 1991; LI & ADAMS 1993). Natural stands consist of numerous trees of the same species, and the population size can be large (YANG & YEH 1995). In fact, it is reduced by preferential crosses between neighbouring trees which could be moreover related genetically. This is generally taken into account when seeds are collected in a provenance by harvesting seeds from distant trees. In a seed orchard, seeds are collected from each seed-bearing tree, the potential population size is the number of different genotypes

growing in the seed orchard, which is quite a limited number in a clonal orchard when no or little contaminant pollen comes from surrounding stands. The effective population size is further decreased because of possible selfing (as well as in natural stands) and of several components of mating success limiting gene flow among trees (MUONA & HARJU 1989; PRAT 1995; BURCZYK & PRAT 1997). The expected genetic gain provided by a seed orchard have been assessed considering that the orchard is a panmictic population. Consequently, the mating system in a seed orchard plays a major role in the determination of quality of seed crop.

The present study compares the genetic diversity and mating system in a natural stand and in the seed orchard established with genetic material derived from this natural stand. It was carried out on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), a major species for reforestation in Northwest of North America and also for introduction in Europe (MAZE *et al.* 1989; BASTIEN & ROMAN-AMAT 1990; KLEINSCHMIT & BASTIEN 1992; ST. CLAIR & ADAMS 1993). Many studies have been conducted on genetic diversity and mating system in natural stands and seed orchards established within the natural range as well as in Europe (SHAW & ALLARD 1982; NEALE & ADAMS 1985; EL-KASSABY & RITLAND 1986; YEH & MORGAN 1987; LI & ADAMS 1989; STAUFFER & ADAMS 1993; EL-KASSABY & COOK 1994; PRAT & ARNAL 1994; PRAT & CAQUELARD 1995). Analysis of gene flow and gene diversity are easier in coniferous species than in broad-leaved species due to the haploid megagametophyte tissue which is identical to the maternal contribution to the embryo of the same seed. Thus comparison of megagametophyte and embryo genotypes allows the identification of pollen genotype. The original approach of this study is the analysis of a seed orchard established in the introduction range in Europe after phenotypic selection of trees originated from a single provenance, Darrington, in Washington State. This provenance has been recommended for reforestation in France following provenance trials (ROSSETTE unpublished; BASTIEN & ROMAN-AMAT 1990). Two stands of this provenance have been sampled. Seeds from the seed orchard and from separated trees in the natural Darrington provenance were available for comparison of genetic diversity and mating system. In this study we used isozymes as neutral genetic markers. They may reflect the potential effects of selection, like diversity loss, for agronomic traits on other characters.

MATERIAL AND METHODS

Plant material

Seed orchards for different conifer species were laid out at Lavercantière (near Cahors, France, 44°37'N, 1°20' E) in a broad-leaved forest area at an altitude of about 200 m. The annual rainfall (about 900 mm) is distributed throughout the year. A unique seed orchard was planted for each species. It is expected that no pollen contamination can occur between seed orchards or from surrounding stands. A clonal Douglas-fir seed orchard was planted in 1978 on 14 hectares. It consists of 70 grafted clones selected phenotypically by INRA (France) and Danish State Forestry (Denmark) for height growth, stem straightness and then wood density in three provenance plantations established in Denmark. All selected trees were originated from Darrington provenance in Washington State, the original seedlot was purchased at Darrington (48°15' N, 121°35' W). The 2863 ramets (about 40 per clone) of the seed orchard were planted at the density 208 trees per hectare (spacing: 8 × 6 m) and randomly distributed. At the time of analysis, only 1128 trees were still alive (mortality was due to graft rejection). Flowering began five years before the present study. Gibberellins $A_{4/7}$ treatments and girdling were practiced on some trees in order to enhance flowering and seed production (BONNET-MASIMBERT & WEBBER 1995). The seed orchard shows variation in the topography and sun exposure, it can be divided in two parts. These two parts differed in 1990 for the percentage of flowering trees (34% vs. 81% in the most sunny area). All ramets investigated in this study belong to the sunny area, and flowering ramets of each clone could be found there. Open-pollinated seeds were collected in 1990 from 132 ramets (generally two per clone) and maintained as separated seedlots in a cold room until analysis.

The natural provenance of Darrington (Washington State) was harvested in two different stands: Darrington IUFRO (48°16' N, 121°38' W, altitude 170 m) and Round Mountain (48°20' N, 121°36'W, altitude 200–450 m). These two stands were quite pure Douglas-fir stands. Trees were taller and older in the Round Mountain stand (about 100 year-old and 55 m in height) than in Darrington IUFRO (about 45 year-old and 35 m in height). Open-pollinated seedlots were collected in these two stands, spacing between trees was more than 50 m: 14 seedlots from the Darrington IUFRO stand (DAR) and 16 from the Round Mountain stand (RM) were available for the study.

Isozyme analysis

Seedlots from 30 adult trees of the natural range and from 70 clones (132 ramets) of seed orchard were available for comparison of gene diversity and mating system. Nine or 10 seeds from each seedlot (a total of 270 from natural stands and 1309 from the seed orchard) were sampled for genetic analyses. Pairs of megagametophyte and embryo tissues were analyzed electrophoretically using standard procedures similar to those presented in CONKLE *et al.*

(1982). The nine enzyme systems and 10 loci used (named according to LI & ADAMS 1989) were: (1) esterase (E.C. 3.1.1.1; *Est–1*), (2) formic dehydrogenase (E.C. 1.2.1.2; *Fdh*), (3) glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49; *G6pdh*), (4) glutamate oxaloacetate transaminase (E.C. 2.6.1.1; *Got–2*), (5) isocitrate dehydrogenase (E.C. 1.1.1.42; *Idh*), (6) leucine aminopeptidase (E.C. 3.4.11.1; *Lap–1*), (7) malate dehydrogenase (E.C. 1.1.1.37; *Mdh–1*, *Mdh–3*), (8) 6-phosphogluconic dehydrogenase (E.C. 1.1.1.44; *6-Pgd*), (9) superoxide dismutase (E.C. 1.15.1.1; *Sod*).

The enzyme systems were stained following various procedures (CONKLE *et al.* 1982, CHELIAK & PITEL 1984, NEALE *et al.* 1984, ADAMS *et al.* 1990). The staining, inheritance and linkage of the *Fdh* locus was described by LEWANDOWSKI & MEJNARTOWICZ (1992). No tight linkage was reported among the selected 10 loci (ADAMS *et al.* 1990, EL-KASSABY *et al.* 1982).

Genetic variation

Expected heterozygosity (H_e) was calculated in parental and filial populations in the natural stands and the seed orchard after NEI (1975). A contingency χ^2 test (WORKMAN & NISWANDER 1970) was used to test the homogeneity of allele frequency among populations. Deviations from Hardy-Weinberg proportions were determined using Wright's *F*-statistics (WRIGHT 1965). F_{ST} measures the amount of genetic variation in the whole population attributable at differentiation among subdivisions. F_{IS} measures the deviation from Hardy-Weinberg proportions within subdivisions and is often called the inbreeding coefficient. F_{IT} represents the level of deviation in the total population and is related to F_{IS} and F_{ST} by the following formula: $1-F_{IT}=(1-F_{IS})(1-F_{ST})$. The genotypic distribution observed within populations was compared with that expected under Hardy-Weinberg equilibrium by means of a *G*-test (SOKAL & ROHLF 1981).

Genetic variation was investigated at the progeny level as well as at the parental level. Parental genotypes were deduced from megagametophyte genotype segregations.

Mating system

The estimates of single- (t_s) and multilocus (t_m) outcrossing rates were calculated based on the maximum-likelihood procedures of RITLAND & EL-KASSABY (1985), that were developed for conifers, using MLTF computer program. Heterogeneity of single-locus estimates was investigated by Fisher's heterogeneity χ^2 test (RAO 1973), and the significance of the null hypothesis, that outcrossing rates do not differ from unity ($t = 1$) was determined by analyzing the limits of confidence intervals.

Equilibrium populations exhibit a relationship between Wright's fixation index (F_{IS}) and outcrossing rate (t) according to formula: $F_e = (1 - t) / (1 + t)$ (ALLARD *et al.* 1968). A substantial difference between F_{IS} and F_e indicates that inbreeding other than through selfing could occur in the population (SHAW & ALLARD 1982).

Effective numbers of pollen parents (exactly – variance effective population size of progeny population attributable to paternal contribution) were estimated for both natural and seed orchard populations. The method proposed by ROBERDS *et al.* (1991) was used, with slight modification allowing for the analyses of loci with multiple alleles (BURCZYK 1996). It is based on differences between the allele frequencies in parental and filial generations and is a reflection of temporal variation in allele frequencies (ROBERDS *et al.* 1991).

RESULTS

Genetic variation

The Round Mountain stand exhibited a slightly higher gene diversity of sampled reproductive trees ($H_e = 0.313$) and of progenies ($H_e = 0.308$), than the Darrington IUFRO stand ($H_e = 0.262$ and $H_e = 0.264$ respectively). Differentiation among these two stands was low ($F_{ST} = 0.030$ and $F_{ST} = 0.009$ at the reproductive and progeny levels respectively), thus only average data of both stands were shown. Heterozygosity of natural and seed orchard parental populations were similar, with the estimate slightly lower in the seed orchard (Table 1). When progeny populations were compared, the progeny of the natural stand was on average about 20 % more heterozygous than the progeny of the seed orchard. The seed orchard progeny maintained about 90 % of heterozygosity of the adult population.

Average number of alleles per locus was similar in adult populations from native stands and the seed orchard. However, among the progenies of natural stand six new alleles were detected, not present in parental population. They were provided by the pollen pool. In the filial population of the seed orchard there were no new alleles alien to the population of 70 clones. Ramets from the same clone produced a single multilocus isozyme genotype.

On average, Wright's fixation index (F_{IS}) was greater in natural stands (in fact only in Round Mountain stand) than in the seed orchard, in both parental and progeny populations. An excess of homozygotes as compared to theoretical population being in Hardy-Weinberg equilibrium was observed in all filial populations ($F_{IS} = +0.049$, $+0.087$ and $+0.028$ in Darrington IUFRO stand, Round Mountain stand and the orchard, respectively). The small excess of heterozygotes was observed only for the adults of seed orchard and Darrington IUFRO populations. The was observed among four loci in the progeny of natural

Table 1. Parameters of genetic diversity (H_e: expected heterozygosity; N_a: number of alleles per locus), and Wright's fixation index (F) for parental and progeny populations of the natural stand and seed orchard.

Locus	Natural population						Seed orchard					
	Parents			Progeny			Parents			Progeny		
	H_e	N_a	F	H_e	N_a	H_e	H_e	N_a	H_e	H_e	N_a	H_e
Est–1	0.623	4	–0.016	0.629	4	+0.317***	0.559	4	–0.176	0.541	4	+0.216***
Fdh	0.238	3	–0.121	0.186	3	+0.023	0.235	2	+0.087	0.248	2	–0.016
Got–2	0.316	3	+0.156	0.288	3	+0.241***	0.295	3	–0.113	0.190	3	+0.005
G6pd	0.491	2	+0.050	0.496	3	–0.016	0.474	3	–0.026	0.451	3	–0.014
Idh	0.231	2	–0.154	0.219	4	–0.016	0.247	3	+0.305	0.195	3	–0.048
Lap–1	0.491	2	+0.186	0.500	2	+0.259***	0.353	2	–0.053	0.303	2	+0.179***
Mdh–1	0.096	3	–0.040	0.100	3	+0.032	0.158	3	+0.005	0.112	3	+0.016
Mdh–3	0.255	2	+0.085	0.262	3	–0.033*	0.305	3	–0.218	0.325	3	–0.024
6Pgd	0.156	2	+0.359	0.143	3	+0.043	0.028	2	–0.014	0.026	2	–0.014
Sod	0.032	2	–0.017	0.072	3	+0.021	0.056	2	–0.029	0.037	2	–0.019
Mean	0.293	2.5	0.049	0.29	3.1	0.087	0.271	2.7	–0.23	0.243	2.7	0.028

*, **, ***: significant departure from Hardy-Weinberg equilibrium at 0.05, 0.01, 0.001 levels, respectively.

significant departure from expected genotypic distribution stand, while only in two loci in the progeny of the seed orchard.

The analysis of Wright's F-statistics revealed that there was no differentiation between parental and filial populations in the natural stands, as well as in the seed orchard ($F_{ST} < 0.002$). Small differences were observed between adult populations of natural stands and seed orchard ($F_{ST} = 0.025$), and consequently between their progenies ($F_{ST} = 0.026$). Thus less than 3 % of variation was attributable to variation between populations.

Similar results were obtained after the analyses of the homogeneity of allele frequencies among subpopulations. Generally, there was no heterogeneity detected between parental and filial generations within both native and seed orchard populations ($P > 0.6$). Only the *Got–2* locus exhibited some patterns of heterogeneity in the seed orchard ($P = 0.03$). However, when the two adult populations were compared, significant heterogeneity was found. It was mainly due to the effect of *Lap–1* ($P < 0.001$) and *6Pgd* ($P < 0.01$) loci. The heterogeneity was even strongly emphasized when the comparison was done between the progenies of both populations. Then, the heterogeneity was significant ($P < 0.001$) for most loci, *Mdh–3* ($P = 0.03$) and *Idh* excepted.

Mating system

Analyses of mating system revealed that only small, although significant, proportions of viable embryos resulted from self-fertilization (s, according to $s = 1 - t_m$) in Darrington IUFRO stand and especially in the seed orchard (Table 2). The respective selfing rates were 0.092 and 0.031. Single-locus estimates varied widely among loci and were significantly heterogeneous within each population (Table 2). Only a few t_s values were significantly different from t_s=1.0. The estimates obtained for *Est–1* locus were distinctly lower than calculated for other loci. While the estimates of single locus and multilocus outcrossing rates were quite similar in the seed orchard, they differed distinctly in natural stand, especially in Darrington IUFRO ($t_s = 0.881$ vs. $t_m = 0.970$). Inbreeding coefficients (F_e) calculated under mating system equilibrium (Table 2), based on the multilocus outcrossing rates were lower than fixation indices calculated directly from the progeny (F_{IS}). The difference was greater for the natural stands, particularly in Round Mountain stand.

Effective number of pollen parents

The variance effective number of progeny population attributable to the contribution of pollen parents in the natural stand was calculated to be 66.5 (confidence intervals (C.I.): 21.6–207.2), while the population-wide estimate for the effective number in the sampled trees male gamete pools across all analyzed trees (individual tree level) was equal to 9.8 (C.I.: 6.5–16.5). The respective effective numbers in the seed orchard were estimated to be 47.1 (C.I.: 20.1–88.5) and 28.1 (C.I.: 20.7–41.0). The estimates for the seed orchard were calculated, when the allele frequencies of parental population were determined based on the set of all 1128 ramets growing in the orchard. This included both the genetic variation among clones and the variation of number of ramets per clone, and reflected the real status of the mating population.

Table 2. Single- (t_s) and multilocus (t_m) estimates of outcrossing rate for the natural stand and seed orchard of Douglas-fir (standard deviation in parentheses)

Locus	Darrington IUFRO	Round Mountain	Seecd orchard
Est–1	0.513 (0.098) ***	0.741 (0.086) **	0.776 (0.032) ***
Fdh	0.901 (0.098)	0.971 (0.095)	1.034 (0.037)
Got–2	0.991 (0.059)	0.650 (0.110) **	0.984 (0.028)
G6pd	0.803 (0.129)	0.913 (0.111)	1.014 (0.022)
Idh	1.135 (0.097)	0.828 (0.143)	1.058 (0.025)
Lap–1	0.686 (0.118) **	0.777 (0.101) *	0.883 (0.036) **
Mdh–1	0.928 (0.109)	a	0.988 (0.034)
Mdh–3	1.149 (0.104)	1.227 (0.114) *	1.027 (0.047)
6Pgd	0.751 (0.262)	0.996 (0.085)	0.967 (0.051)
Sod	a	0.830 (0.206)	a
mean t_s	0.873	0.881	0.970
t_m	0.908 (0.046) *	0.952 (0.029)	0.969 (0.013) **
χ^2 for t_s heterogeneity	34.051***	20.249**	63.28 ***

*, **, ***: parameter significantly different from $t = 1.0$ or $\chi^2 = 0.0$ at 0.05, 0.01, 0.001 levels, respectively.
a: insufficient maternal genotype classes for estimating t_s

DISCUSSION

Mating system

Many studies provided outcrossing rates of Douglas-fir in natural stands and in seed orchard. The values observed for the present seed orchard ($t_m = 0.97$), planted out of the natural range are very similar to those observed in orchards in the natural range (OMI & ADAMS 1986; EL-KASSABY & RITLAND 1986; COPES & SNIEZKO 1991; EL-KASSABY & DAVIDSON 1991). However, the outcrossing rates noticed for the two natural stands of Douglas-fir were little lower ($t_m = 0.95$, $t_m = 0.91$). Such values have already been observed in some stands in natural range (NEALE & ADAMS 1985; YEH & MORGAN 1987), and also in introduction range in Europe (STAUFFER & ADAMS 1993; PRAT & ARNAL 1994). Our results confirm that Douglas-fir is largely allogamous.

Because single-locus estimators are more sensitive to the violations of the assumptions of mixed-mating model, the single-locus procedure tends to underestimate outcrossing rate, if there are matings among related individuals in the population (SHAW & ALLARD 1982). Although, in the seed orchard there was no difference between mean single-locus and multilocus estimates, the distinction was observed in the natural stand (Table 2). The later one may suggest matings among relatives. The presence of inbred mating (other than selfing) in the natural stand may be also observed by the comparison between inbreeding coefficients calculated directly from progeny data (F_{IS}) and those expected at the population equilibrium with the assessed selfing rate (F_e) (Tables 1 and 2). The most significant difference between t_m and t_s was noticed for Round Mountain stand for which F_e (0.025) was also the most different from fixation index calculated from adult trees ($F_{IS} = 0.078$) or from progenies ($F_{IS} = 0.083$). The inbreeding additional to selfing, detected in the natural population, may be attributed to family neighborhood structure, that is likely in natural stands (EPPERSON 1992; PRAT & ARNAL 1994; BOSHIER *et al.* 1995). Since most matings within a local population occur between near neighbors (ADAMS 1992), a family structure may cause the increase of biparental inbreeding of the progeny. As mentioned above, in the seed orchard where no family structure is expected, single- and multilocus estimates were nearly the same.

The phenomenon of lower outcrossing rates in natural stands than in seed orchards was often observed (MUONA & HARJU 1989; BARRETT *et al.* 1987; MORAN *et al.* 1989; FRIEDMAN & ADAMS 1985), and in general seed orchards have no more inbreeding than natural stands (SAVOLAINEN & KÄRKKÄINEN 1992). The major source of inbreeding in clonal seed orchard is selfing while in natural stands crosses between relatives also occur.

The effect of background pollination is considered to increase the proportion of outcrossing rates in seed orchards (EL-KASSABY *et al.* 1989; MUONA AND HARJU 1989; ADAMS & BIRKES 1991). However, the studied orchard was isolated from the natural stands of Douglas-fir and only a few signs of possible contaminant pollen gametes was detected (BURCZYK & PRAT in preparation), thus outcrossing rate was not altered by background pollination. In this case we should rather question, what is responsible for the relative decrease of outcrossing in the natural stand. The possible explanation may be mating

between relatives in natural stand. Although multilocus estimators are less sensitive to violations of the assumptions of mixed-mating model, considerable mating among related individuals could also affect multilocus estimates, however in smaller degree than the single-locus ones. The distance between collected trees in natural stands (about 50 m) might also bias outcrossing rate estimation because of variation in pollen pool from tree to tree (PRAT & MORINEAU in preparation) in spite of the observations of FU *et al.* (1992).

More attention should be also paid to the contrasts of patterns of pollination and pollen dispersal between natural stands and seed orchards. The differences in size of trees and spacing between individuals may cause large variation in pollination environment between seed orchards and natural stands. Effects of crosses between relatives appeared to be more important in Round Mountain than in Darrington IUFRO stand. Trees are older and bigger in Round Mountain than in Darrington. In these conditions pollen flow is more limited by surrounding trees and more crosses could occur between neighbor and related trees. EL-KASSABY *et al.* (1986) and OMI & ADAMS (1986) assessed outcrossing rate in the upper and lower part of crown in Douglas-fir and noticed a higher multilocus outcrossing rate in the upper part, where pollen flow was not limited by branches. In contrast, in the Lavercantière seed orchard, spacing between trees (6 × 8 m) was large enough to avoid contact between branches of trees, and pollen flow from tree to tree was not limited.

Esterase locus exhibited lower estimates of outcrossing than other loci. This was often found in other studies of mating system of Douglas-fir (EL-KASSABY *et al.* 1988; YEH & MORGAN 1987). This locus exhibited several major alleles, but it shows a higher heterozygosity in both natural stands and seed orchard studied here than in within populations on average (LI & ADAMS 1989). Advantage or disadvantage have been already recorded for some alleles at other loci in Douglas-fir (PRAT & CAQUELARD, 1995), such selection can provide unexpected results at a specific locus.

Effective population size

The effective number of pollen parents was distincly greater in the natural stand than seed orchard, especially when compared to the census population of the sampled progeny (*Ne/N*: 0.246 vs. 0.036 in the natural stand and seed orchard, respectively) (see BURCZYK 1996 for discussion). However, the difference between the population and the individual tree level estimates were greater in the natural stand, indicating larger variation among pollen gamete pools fertilizing sampled mother trees. This is probably due to different spacing of mother trees, which was greater in natural stand than in the seed orchard.

Reduced variance effective numbers in seed orchards may result from two reasons (ROBERDS *et al.* 1991): firstly, fertility variation among clones; and secondly, the influx of genes with immigrating pollen (contamination). In fact, in the case of background pollination the number of fathers (pollen donors) may be very large, including the trees located outside seed orchards. However, in such a situation, the effectiveness of a seed orchard, assumed as an isolated breeding unit, decreases with increasing contamination level. As mentioned above, no substantial background pollination occurred in the orchard, since the decrease of effective number may be attributed only to fertility variation among clones.

The variance effective number in another Douglas-fir seed orchard was found to be large (ROBERDS *et al.* 1991), however, individual tree estimates were much lower than those for the whole population. Obtained results confirm the hypothesis of large effective population sizes in natural stands as related by KOSKI (1970), YANG & YEH (1995).

Genetic variation

The seed orchard exhibited a higher average number of alleles (2.7) than natural populations at the parental level (2.5), but not at the progeny level (3.1). The small number of alleles of parental trees in the natural stand could result from the small sample size (30 individuals). The average number of alleles per locus was higher than that observed by LI & ADAMS (1989) in natural range of Douglas-fir (1.8). The loci analyzed in the present study were on average a little more polymorphic than those analyzed by LI & ADAMS (1989). The larger sample size at the progeny level (270 seeds) revealed a higher number of alleles per locus (3.1). No such variation was detected in the seed orchard since all potential pollinator trees were analyzed in the mother tree population. The intermediate number of alleles per locus in the seed orchard may result from the sample size (70 clones). These clones exhibited a larger genetic diversity (number of alleles) than 30 individuals of natural stands, but a less one was provided by potential father trees of 270 seeds of natural stands. If the seed orchard consisted of clones collected from a wide geographic area relatively to the size of a natural stand, this may increase variability in the orchard compared to wild populations. But in this study seed orchard originated from a single provenance, thus the reduction of diversity of the seed orchard may be rather expected. A sample size of 70 genotypes is sufficient to preserve from loss of an allele at the frequency larger than 0.06 with a risk of 0.01 (according to CROSSA 1989). Rarer genes have very few chances to be maintained in the seed orchard.

Gene diversity (expected heterozygosity under Hardy-Weinberg equilibrium) decreased a little from mother trees to progeny in natural stands. The presence of new alleles

provided by pollen increased the genetic diversity of progenies and compensated the decrease of heterozygosity due to selfing and matings between relatives. In the seed orchard, there was no pollen flow from surrounding stands, and gene diversity decreased because of selfing and matings within neighborhoods.

CONCLUSIONS

In the two natural stands as well as in the seed orchard, the genetic differentiation between generation was very low, however the heterozygosity level was different. A lower gene diversity at the progeny stage than at the reproductive stage have been already observed in forest trees (YAZDANI *et al.* 1985, PRAT & ARNAL 1994). Genetic differentiation between the two natural stands was also very low especially at the progeny level (F_{ST} = 0.01). At the adult level, a reduced number of sampled trees might influence measurements of differentiation. A higher differentiation was noticed between natural stands and the seed orchard (F_{ST} = 0.025), such values were already recorded for populations from the same region (MERKLE & ADAMS 1987; MORAN & ADAMS 1989), as well as from populations of introduction area (STAUFFER & ADAMS 1993; PRAT & ARNAL 1994). This revealed a small differentiation from the two stands of the same natural region. Thus, genetic composition of the progenies derived from the natural stands and from the seed orchard differed, despite that the seed orchard originated from the studied natural stand.

The slight loss of genetic diversity in the seed orchard might occur during the phenotypic selection carried out in the provenance trial in Denmark. The number of clones was nevertheless sufficient to maintain most of the genetic diversity. CHELIAK *et al.* (1988) have not observed any change due to selection on heterozygosity level, however some rare alleles were lost. The loss of genetic variation in selected material appeared more related to the sample size effect than to a peculiar effect of the selection.

The outcrossing rate assessed in the seed orchard was high enough to not affect the quality of seed crop. Although this seed orchard originated from one single provenance and might consist consequently of related trees, there were no evidence of crosses between relatives. Inbreeding of the seed orchard results then only from selfing, that was not the case in natural populations.

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