

high level of variation to the geographic range and distribution of the species and other life-history features (EL-KASSABY, 1991). For example, "La Losilla" and "Cerro Gordo" have not undergone an strong silvicultural management (Personal comm. J. A. GÓMEZ LORANCA).

Summarizing, the genetic difference between the 2 locations could be attributable to natural selection. Morphological features are more sensitive to selective pressures from the local environment, so the seed-size-difference among the studied populations could be induced environmentally.

The genetic identity (NEI, 1972), calculated on the basis of the allele frequencies obtained, gave a value of $I = 0.967$. This high rating is yet another index of the proximity of the 2 populations, but it suggests that the similarity would be lower if the study were widened to include populations of the same subspecies geographically further afield, and naturally to include other subspecies of *Pinus nigra* in the Mediterranean area.

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Allozyme Variation and Mating System in Three Artificial Stands of Douglas-Fir (*Pseudotsuga menziesii* (Mirb.) Franco) Planted in Europe

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Summary

Seed collected from some artificial stands of Douglas-fir produce trees having good adaptation to the local environment and substantial growth. The origin of these artificial stands is completely unknown and might be assessed by studying genetic variability and mating system, using isozymes as genetic markers.

Three European artificial stands (Au Charnay, Barlohe, En Argaud) and 1 natural stand (Bacon Creek) of Washington State were analysed in the present study. No significant variation of allelic frequency was observed among stands or between generations (mother trees and offsprings); nevertheless the gene diversity in the progenies was reduced. According to a multivariate analysis, the genetic variation, was mainly within open progenies, and weakly among provenances (about 2%); artificial provenances could not be differentiated.

The increase of homozygosity from mother trees to their progenies was general; it was higher than that expected from the observed outcrossing rate. This suggested pollination and crossing between related trees and neigh-

bourhood effect, that was also supported by the difference between multilocus and single locus outcrossing rate. Different levels of crossing between related trees were assessed (more than 45%) considering that fixation index resulted only from selfing and related mating. In 1 artificial provenance, the mother trees showed a weak and positive fixation index, and the level or related mating was relatively low. This artificial provenance (Au Charnay) may have resulted from a mixture of several progenies collected in different natural provenances, inducing a deficit of heterozygosity by structuring into subpopulations. Trees from seed collected in this stand produced a higher growth than those from En Argaud stand, which showed a presumed higher frequency of inbred mating.

Key words: allozyme variation, artificial provenance, genetic variation, genetic structure, mating system, provenance, *Pseudotsuga menziesii*.

FDC: 165.3; 165.53; 232.1; 174.7 *Pseudotsuga menziesii*; (4).

Introduction

Plantations of Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO) have been developed in Europe and especially in France since its introduction during the last century. The

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first introductions were of unknown origins. Douglas-fir have now become the main planted (ca. 8000 ha per year) forest tree species in France (BASTIEN et al., 1986). The need for seeds is great. Thus, in order to meet the demand, selection of the best seed sources in native and introduction area have been carried out, using adaptation growth traits, and wood quality (CHRISTOPHE and BIROT, 1983; BASTIEN et al., 1986; ROSETTE, unpublished; BASTIEN and ROMANT-AMAT, 1990; KLEINSCHMIT and BASTIEN, 1992). These controlled provenances provide seed of a good genetic quality for afforestation.

Native and artificial provenances consisting generally of open pollinated progenies were tested in various provenance trials in France. In terms of adaptation (vegetative phenology, low G x E interaction, ...) and growth in height, certain artificial provenances were at least as good as the best natural ones (ROSETTE, unpublished; ARBEZ, 1987). Four hypotheses are usually put forward to explain this good behaviour of artificial provenances. (1) They originate from the best zones of the natural range, which either disappeared or were not represented in the French provenance trials. (2) Selection during the first generation of introduction (traditional forestry with thinning) was efficient enough to improve the genetic value of the stand. (3) Artificial stands originate from provenance mixture. (4) Planting suppresses the so called "inbreeding circles" and subsequently results in a maximum reduction of the mating of related trees, leading to no (or low) inbreeding and to a substantial growth.

Hypothesis (1) can be tested with the analysis of genetic differentiation among native and artificial provenances. Hypothesis (2) can be tested by comparing genetic variation of mother-tree and progeny generations. Analysis of mating systems with the respect of possible crossing between related trees can be used to test hypotheses (3) and (4), hypothesis (3) supports an increase of gene diversity of artificial populations. As hypotheses are not exclusive and as some deviations are possible by random, hypotheses can not be strictly tested. Analysis of artificial populations requires native population study for comparing genetic variation and differentiation, and mating systems.

Genetic markers are so far the best tool to analyse genetic variation and mating systems. Genetic diversity of Douglas-fir in its natural range based on isozymes have been studied by YEH and O'MALLEY (1980), MERKLE and ADAMS (1987) and by LI and ADAMS (1989); their results can be used to compare natural and artificial provenances. In the present study, an attempt is made to explain the high quality of seed collected in certain artificial provenances. Three different artificial provenances and 1 natural provenance were included in the study.

Materials and Methods

Two French artificial provenances (Au Charnay, and En Argaud) included in multiside provenance trials, 1 German artificial provenance (Barlohe) and 1 natural provenance (Bacon Creek, Washington State) were analysed (Table 1). Open-pollinated families were individually collected in Bacon Creek, and in 1988 in the European stands.

Several seeds (8 to 10) per single tree progeny were analysed by isozyme electrophoresis. Genotypes of embryo and megagametophyte of each seed were then scored, the genotype of each mother tree was assessed from segregations observed in related megagametophytes. Genotypes of male gametes were inferred by comparing embryo and megagametophyte (female gamete) genotypes of each seed. Genotypes of the mother trees and of only one randomly selected embryo per progeny were considered in genetic variation analysis to avoid differential contributions of male and female alleles and consequently biased estimates of expected heterozygosity and fixation index. In multivariate analyses and mating system study, each embryo (8 to 10 per open-pollinated family) was considered.

Megagametophytes and embryos of every seed (soaked in water for 2 days) were dissected and separately crushed in extraction buffer (Tris-HCl 10 mM pH 7.4, KCl 25 mM, sucrose 29 mM, 30 μ l per extract). Electrophoreses were carried out in vertical polyacrylamide gels (continuous buffer system: Tris 90 mM, H_3BO_3 90 mM, ethylenediaminetetraacetic acid disodium salt 2.5 mM, pH 8.4). Four polymorphic enzymatic systems corresponding to 7 loci were revealed according to CONKLE et al. (1982): α -esterase (α -Est, E.C. 3.1.1.1), glucose-6-phosphate dehydrogenase (G6pdh, E.C. 1.1.1.49), leucine-amino peptidase (Lap-1 and Lap-2, E.C. 3.4.11.1), and malate dehydrogenase (Mdh-1, Mdh-2 and Mdh-3, E.C. 1.1.1.37). G6pdh, Mdh-1, Lap-2 and Mdh-3 are linked on the same chromosome but not closely (recombination > 0.30) according to ADAMS et al. (1990). Loci analysed were chosen in order to avoid strong linkage. Genetic patterns and inheritance of isozymes were identical to those described by ADAMS et al. (1990). Alleles are designated from the fastest to the slowest observed in our experiment.

Study on genetic diversity included the number of polymorphic loci (frequency of the main allele less than 0.95), the mean number of alleles (observed and effective) per locus and the expected rate of heterozygosity. The gene diversity, expected heterozygosity in the total population (H_T), average expected heterozygosity in the subpopulations (H_S) and among subpopulations ($D_{ST} = H_T - H_S$, $G_{ST} = D_{ST} / H_T$, NEI, 1973) already used by LI and ADAMS

Table 1. — Characteristics of the stands studied.

Stand	Bacon Creek	Au Charnay	Barlohe	En Argaud
State	Washington State	France	Germany	France
Latitude	48°36'N	46°18'N	54°09'N	46°18'N
Longitude	121°23'W	4°25'E	9°38'E	4°30'E
Elevation	—	560 m	50 m	550 m
Number of open-pollinated families	25	24	39	20

Table 2. — Gene diversity at allozyme loci over 1 natural and 3 artificial populations of Douglas-fir.

Locus	Number of alleles		Frequency of most common allele minimum-maximum	Gene diversity	
	Observed	Effective		H_T	G_{ST}
Mother trees (108^a)					
α -EST	5	2.1	0.550-0.625	0.592	0.010
G6PDH	4	2.1	0.475-0.603	0.544	0.031
LAP-1	5	3.1	0.346-0.500	0.689	0.017
LAP-2	5	1.5	0.740-0.854	0.335	0.023
MDH-1	2	1.2	0.750-0.938	0.197	0.049
MDH-2	3	1.1	0.923-1.000	0.063	0.029
MDH-3	3	1.4	0.775-0.900	0.293	0.018
Mean	3.9	1.8		0.387	0.025
Progenies (108^a)					
α -EST	5	2.8	0.350-0.615	0.662	0.029
G6PDH	4	2.1	0.438-0.680	0.544	0.030
LAP-1	5	3.0	0.372-0.458	0.687	0.023
LAP-2	4	1.5	0.680-0.875	0.358	0.027
MDH-1	2	1.2	0.775-0.958	0.183	0.042
MDH-2	3	1.0	0.936-1.000	0.045	0.036
MDH-3	2	1.4	0.825-0.880	0.266	0.004
Mean	3.6	1.9		0.392	0.027
Pollen (900^a)					
α -EST	5	3.2	0.412-0.541	0.697	0.011
G6PDH	5	2.4	0.455-0.570	0.586	0.013
LAP-1	5	3.2	0.349-0.457	0.697	0.017
LAP-2	5	1.6	0.749-0.828	0.367	0.005
MDH-1	3	1.2	0.857-0.939	0.201	0.008
MDH-2	3	1.0	0.991-1.000	0.008	0.003
MDH-3	3	1.3	0.783-0.911	0.256	0.013
Mean	4.1	2.0		0.402	0.010

^a) sample size

(1989) in their study of natural populations were also assessed.

Genetic variation was analysed by F-statistics (WRIGHT, 1965; NEI, 1977: F_{IT} structure of the total population; F_{IS} structure within subpopulation; F_{ST} structure among subpopulations and usually similar to G_{ST}) using the Biosys-1 computer programme (SWOFFORD and SELANDER, 1981).

A multivariate analysis (principal component analysis) was carried out as by MERKLE et al. (1988) on the alleles observed in each embryo (8 to 10 per family) scoring 2 if homozygous, 1 if heterozygous and 0 if not present in the embryo; or on the genotypes of each embryo scored 1 or 0.

Hierarchical decomposition of the variance was carried out on the individual values on the 5 main axes of the multivariate analysis. The variability was decomposed into between-stand variability, within-stand and between-progeny variability, and within-progeny variability according to the ratios of the sums of squares.

The outcrossing rates were determined in the stands according to RITLAND (1986) from single locus and multi-locus observations. Maternal genotypes assessed from megagametophyte segregations were taken into account. The confidence intervals of the outcrossing rates were estimated after 100 boot-straps. Selfing rate was assessed

Table 3. — Genetic characteristics of mother trees (MT) and their progenies (P, 1 seed per progeny) in 4 Douglas-fir stands.

Stand	Bacon Creek		Au Charnay		Barlohe		En Argaud	
	MT	P	MT	P	MT	P	MT	P
Sample size	25	25	24	24	39	39	20	20
Polymorphic loci ($P_{alleles} > 5\%$)	6/7	6/7	6/7	5/7	7/7	7/7	6/7	6/7
Mean number of alleles per locus	3.00	2.86	3.00	2.86	3.43	3.14	3.29	3.14
Mean expected heterozygosity	0.365	0.380	0.345	0.360	0.386	0.374	0.426	0.426
Mean observed heterozygosity	0.371	0.320	0.339	0.274	0.396	0.322	0.471	0.350
Mean fixation index *	-0.040	0.159	0.014	0.170	-0.021	0.130	-0.087	0.141

*1) locus MDH-2 excluded

from multilocus outcrossing rate as its complement to 1. Multilocus outcrossing rate should include each kind of allogamy, even crosses between related trees. Single locus outcrossing rate allows an estimation of selfing rate but is affected by other consanguineous mating (SHAW and ALLARD, 1982).

Results

Genetic variation within populations

Only the Mdh-2 locus was not polymorphic in each population analysed. The major allele at each locus was the same in the 4 populations, except at the locus Lap-1. Mdh loci showed the lowest allelic polymorphism: 1 allele only was observed in all the embryos from the Au Charnay at the locus Mdh-2, while the same allele showed its lowest frequency (0.923) in the Barlohe stand; the frequency of other Mdh major alleles ranged from 0.750 to 0.936 according to the locus and to the stand (Table 2). Lap-1 was the most polymorphic locus.

Minor alleles, including the inactive (null) alleles observed (loci Mdh-2, Mdh-3, Lap-1 and Lap-2), were noticed in the 4 populations; they did not exhibit a significant variation of frequency among populations. The variation of frequency was observed essentially between male and female and female contributions and remained low. The pollen frequencies were similar to the allelic frequencies of mother trees, except at the locus α -Est in Au Charnay and the locus Lap-1 in Bacon Creek stands. The total number of alleles observed was higher in the pollen than in the mother tree sample (Table 2), additional alleles had a low frequency (less than 0.05). This is probably due to the sample size.

The expected heterozygosity was high and ranged from 0.345 to 0.426 in mother trees, and from 0.274 to 0.350 in progenies (Table 3). This showed a great allelic diversity at the analysed loci. Mother trees and their progenies exhibited a similar genetic diversity. Each mother tree had a unique genotype in its respective stand, excepted in Barlohe stand where 5 different genotypes (at the 7 loci) were presented each by 2 trees.

The mother trees exhibited a negative fixation index, particularly in En Argaud stand, but not in Au Charnay stand where there was a little excess of homozygosity (Table 3). Most loci showed the same tendency resulting in a negative mean F_{IS} (Table 4) and the total population of mother trees also showed a small excess of heterozygosity (negative F_{IT}).

The progenies (1 seed considered per progeny) showed an excess of homozygosity in the 4 stands. Although most loci exhibited this tendency only 2 loci significantly contributed to this excess in most populations: α -Est and Lap-1. Average F_{IS} and F_{IT} were positive. This excess of homozygosity of the progenies was general in the total population and subpopulations.

Genetic variation among populations

The similarity of F_{IS} and F_{IT} in the mother tree generation and in their progenies resulted in a small value of F_{ST} showing little differentiation between stands. The same value of F_{ST} (0.026) was observed in both generations (Table 4). This confirmed the little genetic diversity between stands ($G_{ST} = 0.026$) observed in average single locus studies (Table 2). Gene diversity of pollen exhibited a reduced differentiation among stands ($G_{ST} = 0.010$).

Principal component analysis was carried out on the 27 (total of 7 loci) alleles, each axis being mainly determined by 1 or 2 loci (Table 5). The 5 major axes accounted for 62 % of the variance. The 3 most polymorphic loci (G6pdh, α -Est and Lap-1) exhibited the major contribution to the main axes. The main source of variation was within progenies and was about 57 % according to the hierarchical analysis. The variability between stands remained

Table 4. — Genetic variation among 4 Douglas-fir stands.

Locus	Mother trees			Progenies*		
	F_{IT}	F_{IS}	F_{ST}	F_{IT}	F_{IS}	F_{ST}
α -EST	0.021	0.009	0.011	0.278	0.258	0.027
G6PDH	-0.030	-0.063	0.031	-0.057	-0.093	0.033
LAP-1	-0.065	-0.084	0.018	0.393	0.379	0.023
LAP-2	0.042	0.021	0.021	0.085	0.058	0.029
MDH-1	-0.027	-0.085	0.053	0.002	-0.049	0.048
MDH-3	0.014	-0.004	0.017	0.362	0.359	0.004
Mean	-0.014	-0.037	0.022	0.196	0.173	0.027

*) 1 seed per progeny

low at about 2.1 %. Bacon Creek and Au Charnay stands differed significantly according to the first axis (G6pdh₂ and G6pdh₃ alleles had major contribution) showing most genetic differentiation among stands. Variances of coordinates on major axes, within or between progenies of the same stand, showed no significant variation according to the considered stand. However Barlohe stand exhibited the largest variance between progenies and En Argaud the lowest.

Multivariate analysis was also carried out on the most frequent genotypes (frequency > 0.02) at each locus; 2 to 10 genotypes per locus were considered, giving in a total of 36 genotypes analysed. The 5 main axes accounted for only 22 % of the variance. Various genotypes defined axes. The genetic diversity between stands was still low (1.9%) and remained mainly within progenies (64 %). The genetic differentiation between stands was about 2 % whatever the method of estimation.

Mating system

The lowest outcrossing (single locus or multilocus) rate was observed in Au Charnay stand, while En Argaud

stand, the nearest stand, exhibited 1 of the highest (Table 6). Allogamy was predominant in every stand.

According to the considered locus, the heterogeneity of the single locus estimation of outcrossing rate was largest (0.29 to 1.16) in Bacon Creek, the natural provenance, and lowest (0.75 to 1.16) in En Argaud. Within-stand single-locus estimates of outcrossing rate differed significantly according to the locus, except in En Argaud.

In Bacon Creek, the multilocus selfing rate was about 0.069. According to this value and that of the fixation index of mother trees (-0.040, Table 3), the fixation index of progenies should be 0.033 (Table 7), and not 0.159 (Table 3). A part of the fixation index (0.131) of progenies did not result from selfing or fixation index of mother trees. The same situation was observed in every analysed stand. In Barlohe and Au Charnay the part of fixation index of progenies not linked to selfing or fixation index of mother trees was smaller (Table 7).

Bacon Creek showed the highest difference between multilocus and single locus outcrossing rates and the highest part of progeny fixation index not resulting from

Table 5. — Hierarchical genetic variation according to principal component analysis on the alleles, based on the ratios of the sums of squares.

	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Weighted average
Total variability (%)	15.5	13.7	11.9	11.3	9.3	61.7 ^{a)}
Between stand variability (%)	3.8 *	0.7	2.3	0.4	3.2	2.1
Within stand and between progeny variability (%)	36.9	46.3	38.2	39.7	44.7	40.9
Within progeny variability (%)	59.3	53.0	59.5	59.9	52.1	57.0
Major alleles contributing to the axis	G6PDH ₂ G6PDH ₃	α -EST ₃ α -EST ₄	LAP-1 ₁ LAP-1 ₃	LAP-1 ₁ LAP-1 ₄	LAP-2 ₁ LAP-2 ₂ MDH-3 ₂ MDH-3 ₃	

^{a)} sum of the 5 main axes

^{b)} variability between stands significant at the 5% level

Table 6. — Outcrossing rates of mother trees in each of 4 Douglas-fir stands.

Stand	Bacon Creek	Au Charnay	Barlohe	En Argaud
Mean single locus outcrossing rate	0.781 ± 0.074	0.684 ± 0.102	0.745 ± 0.086	0.823 ± 0.119
Multilocus outcrossing rate	0.931 ± 0.050	0.797 ± 0.092	0.850 ± 0.082	0.924 ± 0.094

Confidence interval at 5%

selfing. On the contrary, Barlohe and Au Charnay stands exhibited the lowest values.

Discussion

Genetic variation in artificial Douglas-fir populations

Except for the pollen Mdh-2 locus, the gene diversity found in the present study was higher than that observed by LI and ADAMS (1989) at the same loci in populations of

the entire natural range. Coastal variety of Douglas-fir showed a higher gene diversity than interior variety (LI and ADAMS, 1989). Most populations introduced in Europe, as the studied stands, came from coastal variety. Thus a higher gene diversity is expected in artificial stands than in average range of Douglas-fir. There is no obvious differentiation of artificial stands according to their polymorphisms.

The greatest part of the gene diversity was within provenances and particularly within progenies, whatever the analysed stand, native or artificial. A large gene diversity, based on isozymes, has been already shown at stand level in Douglas-fir by YEH and O'MALLEY (1980) and MORAN and ADAMS (1989). The same situation was now observed within Douglas-fir artificial stands. According to our multivariate analysis, the within progeny variation accounted for about 57% of total variation. This value reached 66 % to 73 % for some quantitative traits like girth and height (BASTIEN and ROMAN-AMAT; 1990). The genetic variation was mainly within progenies.

The genetic variation (based on isozymes) among provenances of Douglas-fir is low in the natural range: 2.6 % among 11 populations of British Columbia (YEH and O'MALLEY, 1980), 1.3 % in 12 populations of Southwest Oregon (MORAN and ADAMS, 1989), less than 1 % in 22 breeding zones of South Oregon (MERKLE and ADAMS, 1987) in spite of the analysis of numerous loci. It amounts to only 7.1 % among 43 populations in the whole coastal natural range (LI and ADAMS, 1989). The genetic differentiation among the artificial populations we analysed ranged within the same limits than those observed inside a State like Oregon. Pollen represented the best sample of genetic variation, because of the numerous and independent scored alleles. It also exhibited a low gene differentiation among 3 artificial and 1 natural provenance ($G_{ST} = 1\%$).

Artificial populations of Douglas-fir appeared not particularly different from the natural ones. In *Cryptomeria* the gene diversity was larger in artificial stands, and the artificial stands appeared quite different from the natural ones (TSUMARA et al., 1989). But in Japan, plantation of *Cryptomeria* has been carried out since a long time; after several regenerations and vegetative multiplication cycles, the genetic structure may have been changed. This is not yet the case for Douglas-fir in Europe. In Slovakia, plantations of *Picea abies* exhibited a reduced genetic variation and a high genetic distance with native populations. Genetic differentiation among planted stands remained also great (GÖMÖRY, 1992). This author considered that the loss of genetic variation and the genetic drift resulted from a reduced number of trees collected in close native stands for establishing planted stands. Most planted stands of *Pinus halepensis* in Israel can be recognized by the presence of alleles not found in native populations. These alleles were introduced by introgression (GRUNWALD et al., 1986). In these 3 coniferous species, planted stands exhibited a differentiation from native stands. This situation was not observed in the present study on Douglas-fir probably because of the lack of pollination with native species in Europe (no introgression). One generation is not sufficient to allow a differentiation by genetic drift (the mother trees analysed grew from seeds collected in the natural range). Finally, it is very likely that a sufficient number of trees were involved in the original collection. They thus maintain genetic variation. Moreover, no

Table 7. — Effects not due to selfing on fixation index and outcrossing rate.

Stand	Bacon Creek	Au Charnay	Barlohe	En Argaud
Progeny fixation index expected after selfing rate	0.033	0.103	0.073	0.035
Part of progeny fixation index not due to selfing rate	0.131	0.066	0.058	0.116
Difference between multilocus and single locus outcrossing rate	0.150	0.113	0.105	0.127

allele showed a deviation of allelic frequency and appeared favoured in our study.

Mating system in natural and artificial Douglas-fir stands

A high outcrossing rate of natural population is observed in our study (0.93), this confirm other studies in natural populations of Douglas-fir (NEALE and ADAMS, 1985; YEH and MORGAN, 1987). The lower rate in two artificial populations is not explained. This is not typical of French conditions: selfing rate in a French seed orchard was only 5 % (PRAT and CAQUELARD, 1991).

In a natural stand, like Bacon Creek crosses between related trees may occur, leading to an underestimation of outcrossing rate by single locus method and to an increase of the fixation index of progenies. The fixation index of progenies was higher than that expected after the level of the selfing rate and of the fixation index of mother trees. The significant difference observed between multilocus and average single locus estimations of outcrossing rate of Bacon Creek pointed out the importance of assortative mating (RITLAND and JAIN, 1981), and suggested pollination between related trees. Supposing that the part of fixation index of progenies not due to selfing resulted only from crossing between related trees, frequency of related mating might be assessed when type of relatedness was known. In natural stands related trees are mainly half-sib (PARK et al., 1984; BRUNEL and RODOLPHE, 1985). Thus the fixation index in progenies due to crossing between related trees considered as half-sib was 1/8 of their frequency. This frequency could be assessed to 1.05 in Bacon Creek stand, it was too much important because of possible occurrence of crossing between full sib also, and of other causes (crossing between neighbour trees, time of flowering ...) influencing fixation index in progenies. The reproductive phenology affects significantly the mating system (EL-KASSABY and RITLAND, 1986) and induces a larger part of crossing within the same phenological class. Since this is a heritable parameter (ERICKSON and ADAMS, 1989) it can also be partly included in related mating. Therefore crossing between related trees should represent a major part of mating system in natural populations of Douglas-fir.

The same approach can be applied in artificial provenances. The frequency of crossing between related trees, considered as half-sibs, was estimated to 0.53 in Au Charnay, 0.46 in Barlohe, and 0.93 in En Argaud. These values might be overestimated because of other factors influencing fixation index. Artificial stands exhibit a lower level of related mating than native one. Frequencies of crossing between related trees were proportional (about 4 times) of the difference between multilocus and single locus estimations of outcrossing rate in the artificial stands.

In introduced and planted stands, crosses between related trees should not take place frequently, the effects of inbreeding (selfing excluded) were less marked. The higher homozygosity due to selfing in progenies compared to the parent tree generation did not necessarily influence performance of progenies, because of a selection in favour of heterozygous as observed for instance in *Picea abies* (SZMIDT and MUONA, 1985; YAZDANI et al., 1985).

Origin of artificial stands and foundation effects

The low genetic variation among populations does not allow the identification of the European provenance origin, even with a rough estimation. In regard to our results, it can not be assumed that artificial provenance originated from a particular provenance, differing from those collected recently.

The assessed frequency of crossing between related trees in En Argaud stand according to the fixation index and to the difference between multilocus and single locus outcrossing rates was similar to that observed in natural stand. This stand was probably largely established with related plants as a native one. This suggests that it was constituted of trees sampled in a single provenance or of close provenances.

Au Charnay and Barlohe stands showed a reduced frequency of related mating. These provenances very

likely consist of the sampling of unrelated trees of a larger area such as a provenance region, considering the low genetic structure observed by LI and ADAMS (1989) in Douglas-fir. The positive fixation index of the mother tree generation of Au Charnay might result from a mixture of progenies of various origins. The precise origin of artificial provenances can not be determined with our analysis.

Conclusion

Artificial provenances, under study here, showed a similar genetic variation to that of native stands. No noticeable variation of the allelic frequencies was observed from one generation (mother trees) to another (progenies); and no disadvantage of an allele was detected in the artificial provenances. Genetic markers used did not reveal any evidence of selection that can explain performance of progenies from artificial stands.

The mating system appeared important in artificial stands. Three main components determine the mating system: selfing, crossing between related trees, and outcrossing (between unrelated trees). Crossing between related trees (considered as half-sib), estimated after selfing rate and fixation indices under restrictive hypotheses (no other factor influencing fixation index), might represent the major component of mating system in natural stand and a variable component in artificial stands according to their genetic constitution (presence of related trees).

The good genetic quality of some artificial stands should result from their genetic structure. Their genetic structure is the consequence of the genetic variation introduced into the stand and of mating system. Progenies from Au Charnay and En Argaud were tested in French provenance trials. The best growth and adaptation were observed in Au Charnay progenies (ROSETTE, unpublished). The artificial provenance with the presumed lowest rate of inbreeding produced seed of the genetic best quality because of reduction of inbreeding. This can be considered in tree breeding program.

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