The Genetic Structure of a Population of Douglas-fir (Pseudotsuga menziesii var. glauca) as Reflected by Its Wind-Pollinaded Progenies

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Summary

The genetic structure of a single population of Douglas-fir was studied. Families derived from wind, self, and local cross pollinations of 10 trees were compared in two nursery environments.

Results indicated: (1) equivalence of mean performance of families derived from local cross or wind pollinations; (2) essentially equal variances within families derived from wind and local cross pollinations; (3) three of the 10 trees used as maternal parents carried a gene for albinism; (4) of an additional 32 trees tested, no other carriers of albinism were identified; (5) reduced growth of families within which the albino trait was expressed; and (6) large inbreeding effects on seed yield and seedling growth.

Results are discussed in terms of genetic structure of the populatian, the genetic system of the variety, and tree improvement.

Key words: genetic structure, inbreeding depression, Pseudotsuga menziesii.

Zusammenfassung

In einem ca. 1,6 ha großen, etwa 60 Jahre alten Bestand von Pseudotsuga rnenziesii in 1150 m Seehöhe in der Nähe von Clarkia (Idaho, USA), der vermutlich aus Naturverjüngung nach einem Waldbrand hervorging, wurden an zehn Baumen kontrollierte Kreuzungen mit Pollen anderer Bäume desselben Bestandes, mit Pollengemischen von mehreren Bäumen sowie Selbstungen durchgeführt. Gleichzeitig unterlagen die Bäume der freien Windbestäubung. Aus der Untersuchung der Sämlingsnachkommenschaften geht hervor, daß die mittlere Leistung der aus Kreuzung hervorgegangenen Familien derjenigen der aus Windbestäubung hervorgegangenen Familien gleichwertig war. Die Familienleistungen hatten eine ähnliche Streuung. Drei der zehn Mutterbäume vererbten Albinismus, der mit eingeschränktem Wachstum verbunden war.

Introduction

Population differentiation of forest trees is commonly assessed by comparing wind-pollinated progenies. But proximal populations exchange genes. Thus, wind pollinations not only reflect the genetic structure of a population, but also reflect migration rates. Wind-pollinated progenies represent descendants of selected ancestors, but they have not undergone selection themselves (HESLOP-HARRISON 1964). Consequently, differentiation among selected populations is assessed from the performance of their unselected progenies.

The degree to which wind-pollinated seeds accurately represent particular populations depends on rates of migration and selection, the amount of genetic variation, the manner by which variation is stored, and recombination indices; it depends on the genetic structure*) of populations within the genetic system.

The primary objective of the present study was to compare the genetic structure of a single population of Douglas-fir to that of the wind-pollinated progenies produced by that population. Accidental selection of a population containing a relatively high frequency of a gene for albinism allowed additional elucidation of the genetic structure of the population as a component of the genetic system of Douglas-fir in the Northern Rocky Mountains.

Materials and Methods

A single population, located near Clarkia, Idaho, was selected for study. This population occupies about 1.6 ha on a Tsuga *heterophylla/Pachistima* rnyrsinites habitat type (DAUBENMIRE and DAUBENMIRE 1968) at an elevation of 1150 m. Sixty-year-old Douglas-fir trees accaunt for approximately 30% of the forest composition on a site that approaches full stocking (875 stems/ha). The population apparently originated after a fire that covered much of the area (50 km^2) drained by the Middle Fork of the St. Maries River. This particular population represents one of the few on the northern aspects of the drainage in which composition is dominated by Douglas-fir. The species is represented by scattered individuals in surrounding areas.

Controlled pollinations were performed on 10 trees located within the central hectare occupied by the population. Self, wind, and local cross pollinations were performed on five trees in two consecutive years. For local crosses performed in a particular year, pollen from the 5 trees being used as maternal-parents and pollen from 6 additional trees within the population were combined such that: (1) all local crosses involved pollen mixtures of 10 trees, and (2) no self pollinations were represented in local crosses. Thus, slightly different pollen mixtures were used for the trees pollinated in a given year, and much different pollen mixtures were used between years.

Seeds were sown in unreplicated nursery beds on the Priest River Experimental Forest (PREF). After one growing season, seedlings were transplanted at a spacing of 15 cm into randomized complete block designs consisting of three replications at the Coeur d'Alene Forest Nursery (Cd'A) and at PREF. Although all families except those derived from self pollinations were represented originally by 14 seedlings in each replication, high mortality during the first winter reduced subclass size to an average of 11 for wind- and cross-pollinated families but only 2 for self-pollinated families.

The following data were obtained from each seedling: (1) tree height after three and four years of growth, (2) top dry weight after four years of growth, and (3) the number of leaves on a central 10 cm segment of the shoot produced during the fourth grawing season. In addition, the frequency of albino seedlings within each family was scored

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[2]) *In accordance with* STERN *and* ROCHE (1974), *genetic structure reflects the distribution of genes to individuals; structure develops from interactions of the breeding system and natural selection. The genetic system describes the intraspecific organization, storage and release of genetic variability to account for the adaptation of populations and maintenance of variability.*

weekly during germination. The albino phenotype was characterized by pure white cotyledons and a pink hypocotyl.

Because variances were proportional to means, logarithmic transformations (Steel and Torrie 1960) were applied to data on height and weight before statistical analyses were made on four variables: 4-year height, dry weight, number of leaves, and deviation from regression of 4-year height on 3-year height. Deviations from the height regression represent an assessment of relative rate of growth from a constant height at age 3; thereby, differential environmental effects such as seed weight and transplanting shock on seedling height were minimized.

Analyses of variance were made for assessing mean performance according to a factorial design that included main effects of nurseries, replications, pollen types, and maternal trees. Secondly, variation in performance was assessed by analyses of variance for which basic data included variance estimates within each family at each nursery; replication was ignored in order to maximize the sample size on which variance estimates were based. For both types of analyses, all variates except pollen types were assumed to be random. Because of missing data and low subclass size, mean values for families from self pollinations are presented but were excluded from statistical analyses.

Table 1. — Intraclass correlations derived from analyses of variance of mean performance.

Source of variance	Degrees of freedom	Variable 4-year height	 No. of leaves	 Dry weight	 Height deviation[1]
Nurseries	1	0.15**	0.07**	0.10**	0.22**
Replication	2	.02**	0	.01*	.02**
Maternal trees	9	.23**	.17**	.19**	.09**
Pollens	1	0	.01	0	.01
NxT	9	.08**	.03*	.06**	0
NxP	1	0	0	0	.01
TxP	9	.04*	.07**	.06**	0
NxTxP	9	0	0	.02	0
Experimental error[2]	78	.14**	.05**	.10**	.09**
Within	1142	.33 (0.0844)[3]	.60 (0.0425)[3]	.45 (0.0838)[3]	.56 (0.0295)[3]

[1]Deviation from regression of 4-year height on 3-year height.
[2]Includes all sources of variance involving replication interactions.
[3]Mean square within cells.
** Significance of F value at the 0.99 level of probability.
* Significance of F value at the 0.95 level of probability.

To assess the frequency of trees that carried an albino trait, wind-pollinated cones were collected from an additional 32 trees dispersed throughout the 1.6 ha occupied by the population. Between 100 and 2,000 seeds from each tree were sown in pots in a glasshouse. The occurrence of albino seedlings was scored periodically during germination.

Results

Albino seedlings occurred at the following percentages in families of three maternal trees:

Tree no.	Pollen type Self	 Local cross	 Wind
8	8.3	5.6	4.9
9	14.0	7.1	2.9
10	17.9	4.6	0.4

No additional carriers of genes for albinism were detected in germination trials of seeds from 32 additional trees.

Analyse of variance for assessing mean performance *(Table 1)* detected no differences associated with the two types of pollen; growth responses for families derived from local crosses were similar to those for wind-pollinated families. Main effects of nursery environments and maternal trees dominate results of these analyses. The latter effects result primarily from the poor growth of families carrying the albino trait *(Table 2)*. Wind- and cross-pollinated families of the three carrier trees were characterized by growth responses that deviated from overall means in the same direction but to a lesser extent than selfed lines.

Table 2. — Mean values of four variables according to pollen types (W = wind, L = local cross, S = self) and maternal trees.

Maternal tree	Variable 4-year height (cm) L	 W	 No. of leaves L	 W	 Dry weight (g) L	 W	 Height deviation[1] L	 W
1	51.4	41.7	83.1	85.4	59.3	44.5	+0.10	+0.08
2	41.2	46.5	91.6	90.2	45.3	49.1	+ .10	+ .11
3	35.9	37.3	87.9	87.7	42.4	43.3	+ .02	+ .04
4	42.1	38.5	87.9	86.8	48.0	43.2	+ .03	+ .04
5	30.0	34.5	101.1	90.2	35.6	37.5	+ .04	+ .12
6	35.5	33.1	95.6	95.9	41.2	41.4	+ .04	- .02
7	26.0	27.7	104.5	103.9	32.4	31.7	+ .05	+ .09
8[2]	26.6	34.8	106.1	100.1	32.7	42.0	- .07	+ .02
9[2]	23.3	24.0	117.7	102.7	29.9	31.2	- .01	- .02
10[2]	22.4	31.2	114.7	96.5	30.9	39.9	- .11	- .05
Mean	33.4	35.5	99.0	93.9	39.8	40.4	+ .02	+ .04
	(S = 22.8)		(S = 120.5)		(S = 33.1)		(S = -0.21)	

[1]Deviation from regression of 4-year height on 3-year height. Since deviations were calculated from the difference of two logarithms, mean values are without units.
[2]Carriers of albinism.

Table 3. — Intraclass correlations derived from analyses of variance of variation in performance.

Source of variance	Degrees of freedom	Variable 4-year height	 No. of leaves	 Dry weight	 Height deviation[1]
Nurseries	1	0.05	0	0.18**	0.03
Maternal trees	9	.09	0.03	.23***	.07
Pollens	1	.13*	0	.03	.14*
NxT	9	.08	.14	.15**	.19
NxP	1	0	0	0	0
TxP	9	.46***	0	.30***	.34**
NxTxP	9	.20 (0.0624)[2]	.82 (0.0222)[2]	.12 (0.0358)[2]	.23 (0.1280)[2]

[1]Deviation from regression of 4-year height on 3-year height.
[2]Mean square x 10^{-2} for NxTxP.
***Significance of F value at 0.99 level of probability.
**Significance of F value at 0.95 level of probability.
*Significance of F value at 0.90 level of probability.

Maternal trees significantly affected deviations from the regression of 4-year height on 3-year height *(Table 1)*. Families were characterized by contrasting growth rates. Even if all seedlings had been of similar height at age 3, differences in height would have been evident at age 4. Again, families that carry the albino trait are characterized by the lowest mean growth rates (negative deviations from regression).

Analyses designed for testing variation in performance *(Table 3)* indicate that pollen types significantly affected only two variables at a low level of probability. Yet, for all variables, mean values for families derived from wind pollinations were slightly more variable than those derived from local crosses *(Table 2)*. Also, for three of the variables, larger variances were associated with wind pollinations than local pollinations for 7 of the 10 trees. And because interactions between pollen types and maternal trees were relatively large, it is possible that real effects of pollens for all variables except number of leaves were not detected because of low statistical power. However, even if pollen type had a real effect, small mean differences probably reflect a larger number of trees represented in pollen borne by the wind than the 10 trees represented in local crosses. Thus, essentially no differences in variance characterize families from wind or local cross pollinations.

Effects of selfing were pronounced. Self-pollinated cones averaged only 1.3 filled seeds; local cross-pollinated cones averaged 16.0 filled seeds. A large inbreeding depression in selfed lines was expressed on the average by a 32% reduction in 4-year height, 17% reduction in dry weight, and a 25% increase in the number of leaves on a 10 cm segment of shoot *(Table 2)*. The large reduction in growth rate (deviation from regression) shows that the difference in performance of selfed families and outcrossed families continues to increase at age 4. For selfed families, a reduction in shoot elongation accompanied by an increase in leaves per unit length reflects greatly reduced internodal elongation.

Similar effects of inbreeding on seed yield (Sorensen 1971) and growth depressions (Orr-Ewing 1965; Sorensen and Miles 1974) have been observed in the coastal variety of Douglas-fir *(Pseudotsuga menziesii* var. *menziesii)*. Also, the two varieties of Douglas-fir seem to have similar genetic loads. That values of relative self fertility correspond to those estimated for the coastal variety (Sorensen 1969) suggests that the interior variety also carries a load of about 10 lethal equivalents per zygote.

Discussion

Analyses of progenies from a single population have shown: (1) that three of the 42 trees tested carried an albino trait, (2) reduced growth for families expressing the albino character, (3) equivalence in mean performance of families derived from local cross or wind pollinations, (4) essentially equal variances within families derived from wind and local cross pollinations, and (5) large inbreeding effects on seed yield and seedling growth after self pollinations. These results provide an opportunity to assess the genetic structure of the population as a component of the genetic system of Douglas-fir in the Northern Rocky Mountains.

Because genetic structure reflects the distribution of genes among individuals, high frequency of an albino marker within the population provides opportunity to assess genetic structure. The albino phenotype was evident in progenies of local crosses on three trees; therefore, the character is probably controlled by a common allelic series. Also, albino seedlings occurred in self-pollinated families of three trees at ratios that deviated considerably from expected ratios for a trait governed by a recessive single gene. However, as noted by Sorensen (1973), deviations can occur for a variety of reasons, particularly in a species such as Douglas-fir that is characterized by a large inbreeding depression. Therefore, it is assumed that the albino phenotype is controlled by a single recessive gene that is present at a common locus in three maternal trees.

Although the frequency of the albino allele is much higher in this population than expected for lethal alleles, the actual frequency is low. Observed frequencies of albino seedlings after wind and local cross pollinations approach the frequencies expected from the assumption that only three trees carry the allele. After cross pollinations, albinism averaged 5.8%. The three heterozygous trees were pollinated during the same year, and therefore the pollen mixture used for local crosses on each of these trees contained pollen from the other two heterozygous trees. Thus, albinism should average 5% in local crosses of heterozygous trees. Because 8% albino seedlings would have been expected if an additional heterozygous tree had been represented in the pollen mixture, it is assumed that none of the six additional trees represented in the pollen were heterozygous.

If self pollinations account for 7% of wind-pollinated seedlings (Sorensen 1974), 1.75% of wind-pollinated seedlings of heterozygous trees should be albino; 2.7% were observed. The discrepancy could arise from (1) unequal rates of self fertility as shown by Sorensen (1971), (2) 2% (4 heterozygous trees per 100) albino alleles in the pollen cloud borne by the wind from the population as a whole, or (3) the presence of albino alleles in the pollen cloud at higher frequencies near the heterozygous trees than in the remainder of the population. The last alternative arises because the three heterozygous trees were growing in the same general area (0.02 ha).

Three trees are heterozygous. Ten maternal trees and six paternal trees were involved in original pollinations. Germination trials involved 32 additional trees. Thus, a maximum of 6% of the trees are heterozygous.

Evidence suggests that the three heterozygous trees have a common ancestor. First, the character seems to be controlled by alleles at a single locus. Second, the three heterozygous trees occur within the same 0.02 ha. Also, progeny tests indicate that additive genetic variance *(Table 1)* for this population is nearly twice as large as for other populations (Rehfeldt 1974 a, 1974 b). (Inbreeding inflates estimates of additive genetic variance in proportion to the inbreeding coefficient (Falconer 1960)).

Finally, growth depression is evident in families carrying the albino allele. Growth depression could have resulted from either pleiotropism or linkage disequilibrium between the albino allele and alleles deleterious to growth. A pleiotropic effect requires a small height for all seedlings heterozygous for the albino allele. The three heterozygous trees should produce about 50% heterozygous progenies regardless of the type of pollination. Pleiotropic effects should produce bimodal frequency distributions, but bimodal distributions were not apparent.

If linkage disequilibrium exists between the albino allele and other deleterious genes, the relationship between poor growth and frequency of albinism seems to represent an inbreeding depression. The genetic system of Douglas-fir contains many lethal equivalents (Sorensen 1969); even silght inbreeding increases the probability of homozygosis of several semilethal alleles. Also, dwarfing of Douglas-fir is common in the S_2 generation (Orr-Ewing 1974). Thus, albinism and dwarfism seem to indicate an inbreeding depression in progenies from three trees.

A maximum of 6% of the trees in the population carry the albino trait. Yet, if the relatively high frequency of the albino allele results from inbreeding, occurrence of the albino phenotype marks only half of the inbred trees. This may be evidenced by tree 7 *(Table 2)* which was not heterozygous but produced progenies characterized by poor growth. Therefore, as much as 12% of the trees in the population could be offspring of a single tree. In addition, Douglas-fir is at a high density in this small population but is at a low density in surrounding areas. Much of the current population may be descendants of only a few trees that survived the original fire at this particular site. It is even possible that more than one inbred line may be represented in the population.

On the other hand, additional evidence does not support high levels of inbreeding. Trees found to be heterozygous for the albino allele were growing in close proximity. Wind-pollinated progenies from most trees did not show obvious inbred characteristics. Performance of progenies of this population in two other tests (Rehfeldt 1974 a, 1974 b) was normal. And the performance of progenies developed from interpopulational crosses that included the present population did not differ from the mean performance of intrapopulational crosses of parental populations (G. E. Rehfeldt, unpublished data). Therefore, either the percentage of inbred trees (12%) within the present population is overestimated, or a similar level of inbreeding is characteristic of the genetic structure of all populations. The latter alternative may be of particular importance. Douglas-fir is a seral species on the *Tsuga heterophylla* series of habitats of northern Idaho, a region in which wild fire has been the primary agent of forest disturbance (Daubenmire and Daubenmire 1968).

The level of inbreeding within the population should be expressed in the frequency of numerous lethal or semi-lethal traits. Unfortunately, the objectives for which this study was designed did not include an assessment of inbreeding within the species' mating system. Consequently, data on seed development and the occurrence of other mutant phenotypes were not obtained.

Still, results of this study have implications for studies in ecological genetics and for tree improvement of Rocky Mountain Douglas-fir. No differences in mean performance or variation of performance could be associated with wind- or local cross-pollinated seeds. For species of similar genetic systems, wind-pollinated seeds seem to adequately reflect population structure. Secondly, even for Douglas-fir, a species characterized by high inbreeding depressions and relatively large genetic loads, inbreeding may be a constituent of the mating system. Even though inbreeding could account for random patterns of local differentiation (Rehfeldt 1974 a), the importance of inbreeding on the evolution of the genetic system requires further assessment.

That inbreeding inflates additive genetic variances is well documented. For Rocky Mountain Douglas-fir, genetic components of variance and genetic gains can be estimated only after assessing the importance of inbreeding on the genetic structure of populations.

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Efficacy of supplemental mass-pollination in a Douglas-fir seed orchard

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Summary

A study of eight different pollination regimes, encompassing a common ten-day period in a 16-year-old Douglas-fir orchard, quantified day-to-day effects of wind pollination, controlled pollination, and supplemental mass-pollination on filled seed production of a single clone. Wind pollination had a marked cumulative effect, with each additional day of open pollination resulting in an average increase in filled seed percent (FS%) of eight percentage points. Wind-pollinated controls averaged 59.5% filled seed with 22.5 filled seeds per cone.

Control-pollinated treatments exhibited a temporal pattern of FS%, reflecting female strobilus receptivity, that peaked at the level of the wind-pollinated control and declined sharply between the seventh and eighth days. This rapid loss of receptivity was not signalled by any gross change in outward appearance of female strobili. Onset of female receptivity was not clearly defined due to a putative "carryover pollination" effect.

Supplemental mass-pollination treatments (i.e., a single application of pollen to unbagged strobili) did not appreciably increase FS% above the mean of the wind-pollinated control, in this particular orchard clone. However, analysis of other pollination regimes indicated that supplemental mass-pollination could significantly increase filled seed production in very early or late-flowering clones or in young orchards where wind pollination is more inhibited than in this study.

Proper timing of pollen application is the key to maximizing effects of supplemental mass-pollination. Special equipment and procedures are needed to facilitate proper timing and repeated applications, while conserving the