VARIATION AND INHERITANCE OF SOME PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS IN DOUGLAS-FIR

by Oscar Sziklai1

Forest genetics is the study of variation and heritability in forest trees. It is concerned with similarities and differences of various traits between related trees and their transmittance to the next generation.

Variation itself is a product of differences between individuals and the effects of environmental modifications, genetic recombinations and mutations (Stebbins 1957). An understanding of the role of the above factors is essential in any genetic studies. The variation pattern from tree to tree and from stand to stand as well as the population composition throughout the range of the species must be known before any improvement work can be planned on a logical basis. The calculation of genetic gain requires an estimate of heritability which will express the probability that certain characteristics will appear in future generations. Only limited information is available at present on the inheritance of various traits of forest tree species.

1Associate Professor, University of British Columbia, Faculty of Forestry, Vancouver. *Financial support was provided by the National Research Council of Ottawa (67-1375) and the Committee on Research, University of British Columbia (68-0316).*

Materials and Methods

Four Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii)* trees were selected on the U.B.C. Campus and designated A, B, E, and 11. Trees A, B, and E originated from natural regeneration and represent the local population of Douglas-

fir, while tree 11 was selected from a plantation established in 1934, from an unknown coastal provenance.2

Phenological observations on the flushing date of the vegetative bud were carried out for 4 years, commencing in 1959.

Isolation of the megasporangiate strobili was carried out 2 to 3 weeks prior to pollination. For isolation, viscose casings were used as recommended by Duffield (1950). Pollen was extracted by a modification of the method described by Orr-Ewing 1954) and was stored at 0° C. until required.

Three different pollination methods were used: dry, wet, and dry-wet as described by Allen and Sziklai (1962), in all, 16 combinations. Isolation bags were replaced by fiber-glass screens 6 to 8 weeks after pollination to protect the developing conelets from insect damage.

After collecting the cones during the first half of September, the seeds were extracted, counted, and separated by weight into two classes—filled seed and empty seed. Seven milligrams were used as the lower limit for filled seed. Germination tests of filled seed, using the method described by Allen and Bientjes (1954), were run for 40 days at a constant temperature of 25° C. Light was not applied, except for a short period every day when the germinants were removed.

Later, 6 average germinants were transplanted into a plastic container (100 x 100 x 135 mm) filled with vermiculite to a depth of 130 mm. Irrigation with Hoagland's solution was provided daily. Long-day environmental condition was established in a Percival PGC-78 growth chamber with 15 hours of illumination and with a light intensity of 3500 foot-candles. The temperature during the illumination period was 25° C., while 15° C. was maintained during the dark period. Although the relative humidity was not controlled, it correlated closely with temperature changes; 50-60 percent relative humidity occurred during the illumination period and 80-100 percent during the dark period.

Each cross was represented by 6 seedlings grown in one container in the chamber. The containers were placed randomly on the shelves of the growth chamber and were rearranged randomly every 10-14 days to further reduce biases that might have been caused by variations in the distance from lights, observation windows, or circulation fans.

Although the repeated moving of the containers reduced environmental variation and provided some randomization, the experimental design was not randomized and the results and analyses must be considered with some reservation. However, it is felt that they are of sufficient interest to warrant publication. Furthermore, other tests (which space does not permit publishing at this time) have verified the results in most respects.

2Personal communications from F. M. Knapp, 1963.

The progeny test in the growth chamber ran from February 25, 1963, to July 6, 1963, a period of 132 days. The seedlings were then examined for: length of roots to 1.0 mm, length of hypocotyl to 1.0 mm, length of epicotyl to 1.0 mm, length of branches to 1.0 mm, number of branches, number of cotyledons, diameter of root collar to 1.0 mm, green weight of shoots to 0.1 g, dry weight of roots to 0.001 g, dry weight of shoots to 0.001 g, light transmittance of chlorophyll.

The transmittance of chlorophyll was measured, using the method described by Madison and Anderson (1963). One gram sample of needles was extracted in a 20 ml. methanol. The needles were collected from the middle portion of the shoot and were cut into approximately one-mm lengths to provide better penetration of methanol. A Beckman colorimeter was used, with a filter to pass wave lengths of about 440 millimicrons. An extract of needles of open-pollinated progeny from tree E was used as the standard. The readings were obtained in percent and later on were transformed to arcsine values for analysis.

Methods of Statistical Analysis

The individual seedling records were used in the calculation, which is based on the following mathematical model:

$$x_{ijk} = m + a_{s_i} + b_{p_j} + c_{sp_{ij}} + e_{ijk}$$

where: x_{ijk} = the observation of the k-th offspring of a cross, in which the seed (female) parent belonged to the i-th tree, and the pollen (male) parent belonged to the j-th tree.

i = the number of trees used as seed parent,

j = the number of trees used as pollen parent,

k = the number of seedlings from each cross,

m = the sample mean,

a_{s_i} = the effect of the i-th seed parent,

b_{p_j} = the effect of the j-th pollen parent,

$c_{sp_{ij}}$ = the interaction of the i-th seed and the j-th pollen parent,

e_{ijk} = the error term

The expected mean squares were derived from analysis of variance (table 1) and the component of variance was calculated, using the following forms for each analysis.

When the mean square of the seed-pollen (S x P) interaction was less than the mean square of the residual variance, its component of variance was considered as non-existent. To obtain more accurate estimates of the residual variance, the two sums of squares and degrees of freedom were pooled and

Table 1.--Analysis of variance and component of variance using individual seedlings from a polyallel cross of Douglas-fir

Source of variation	Degree of freedom 1/	Components of variance 1/
Seed (S)	i-1	$MS_s = S_e^2 + kS_{sp}^2 + kj\ S_s^2$
Pollen (P)	j-1	$MS_p = S_e^2 + kS_{sp}^2 + ki\ S_p^2$
S x P	(i-1)(j-1)-1'	$MS_{sp} = S_e^2 + kS_{sp}^2$
Residual	ijk-ij-(k-1)1'	$MS_e = S_e^2$

1/ Explanation of symbols is as follows:

S_s^2 = the genetic component of the seed parents

S_p^2 = the genetic component of the pollen parents

S_{sp}^2 = the genetic component from interaction of seed and pollen parents

S_e^2 = the residual variance, which cannot be allocated to the other three terms

1' = the number of selfings from polyallel cross

a new residual mean square was calculated. The heritability coefficient was calculated for the seed and pollen parents separately by dividing the genetic-variance components of each parent with the phenotypic variance:

$$\text{for seed parents: } h_s^2 = \frac{S_s^2}{S_s^2 + \frac{S_{sp}^2}{i} + \frac{S_e^2}{ik}}$$

$$\text{for pollen parents: } h_p^2 = \frac{S_p^2}{S_p^2 + \frac{S_{sp}^2}{j} + \frac{S_e^2}{jk}}$$

Phenological Differences

Vegetative bud flushing was consistent, and significant tree-to-tree differences were demonstrated. Tree B was consistently earliest and tree 11 latest in flushing during 4 years (table 2). The difference was 16 days on the average. Griffith noted a maximum difference of 5 weeks between early- and late-flushing trees among the 154 trees observed at the University Research Forest, near Haney, B.C.[3] Early-flushing tree B appears to be subject

Table 2.--The average date of vegetative bud-flushing in 1959, 1960, 1961, and 1963

Year	Tree number				Average
	A	B	E	11	
	Number of days since January 1				
1959	150	143	148	157	149.5
1960	140	132	136	154	140.5
1961	148	141	144	155	147.0
1963	146	139	139	153	144.3
Average	146.0	138.7	141.7	154.7	

to greater variation (SD = ±4.79 days) than they late-flushing tree 11 (SD = ±1.73 days).

Although flowering characteristics were not systematically observed, notes on pollination reveal. that the times of flowering and flushing were not necessarily correlated (r = .24) :

Tree number	*Pollination: Number of days since January 1*
A	108
B	99
E	103
11	102

Ripening of pollen flowers started a few days before the first ovulate flowers reached the fully receptive stage. This slight protandry was also

[3] *Griffith, B. G. 1963. Phenology, growth,, and flower and cone production of open-grown Douglas-fir trees. In manuscript, University of British Columbia.*

observed by Orr-Ewing (1956) and Stoate *et al.* 1961). Simultaneous maturation of the male and female flowers (synacme) dominated the large part of the flowering period, and would allow self-pollination.

Results of Controlled Pollination

Of the pollinated ovulate conelets, 201 out of 290 developed into cones in 1962, and 193 out of 413, in 1964. In both years, the loss was largest, 58 and 52 percent respectively, on the earliest (B) and latest (A) flowering trees. The loss on tree 11 (average of 17 percent) was the least in both years.

The different pollination methods used in cross-pollination (table 3) gave cone mortalities of 49 percent for the dry method, 34 percent for the dry-wet method, and only 19 percent for the wet method. When selfing, only the wet pollination method was used and the loss of conelets was 24 percent at the end of the growing season.

Specific gravity of the pollen grains in Douglas-fir is larger than one, and the pollen could sink close to the integument of the ovule when wet pollination is applied and water is accumulated between the ovuliferous scales and the bracts. That water also does not interfere with the stigmatic inner surface of the integument during the pollination process was found by Allen and Sziklai (1962). Silen and Krueger (1962) indicated that rain during the pollination period could not cause a major reduction in Douglas-fir seed set. The number of filled seed per cone on tree B in 1962 was 19.00 when dry, 19.38 when wet, and 26.71 when dry-wet pollination methods were used.

To further investigate seed production, artificial pollination was separated into cross- and self-pollination groups, and wind-pollination was also included (table 4).

Since the development of seed does not depend on pollination, the total number of seeds per cone should not differ greatly on the same tree as a result of different pollination methods. Although the different average values among the total number of seeds did not differ significantly, wind-pollination resulted in only 30.87 seeds per cone, compared to 37.09 for self- and 42.42 for cross-pollination. The low number of seeds per cone in wind-pollination may be attributed to the damage caused by *Contarinia oregonensis* Foote. The seeds become fused with the scale and are not released from the cone (Hedlin 1958).

The number of filled seeds per cone from cross-pollination (13.81) is significantly different from

Table 3.-- Number of ovulate conelets isolated and cones collected, using the various pollination methods in 1962

Method of pollination	Tree number A Poll.	A Coll.	B Poll.	B Coll.	E Poll.	E Coll.	11 Poll.	11 Coll.	Totals Poll.	Totals Coll.	Conelets lost (percent)
Dry	15	2	17	7	12	6	20	18	65	33	49
Wet	22	20	19	16	11	9	21	14	73	59	19
Dry-wet	18	7	15	7	19	17	15	13	67	44	34
Self-wet	23	18	18	10	17	15	27	22	85	65	24
Total	79	47	69	40	59	47	83	67	290	201	31

Table 4.-- Average total and filled seeds per cone from cross-, self-, and wind-pollination in 1962

Tree no.	Cross Total	Cross Filled	Self Total	Self Filled	Wind Total	Wind Filled
A	38.8	9.6	38.8	.4	37.1	2.0
B	42.2	21.0	39.5	.0	23.0	1.8
E	53.4	2.7	45.5	.9	32.7	4.1
11	35.3	21.9	24.5	6.3	30.6	4.3
Average	42.42	13.81	37.09	1.91	30.87	3.05

self- (1.91) and from wind-pollination (3.05) (table 4). Compared with wind-pollination, cross-pollination increased the number of filled seeds 4.8 times on tree A, 5.1 times on tree 11, and 11.4 times on tree B. However, the wind-pollination method produced more filled seeds than did cross-pollination on tree E, probably because the controlled pollination took place too late in 1962.

Application of artificial pollination in seed production areas, and especially in seed orchards, has obvious potential value in tree improvement programs.

Self-pollination gave the lowest average number (1.91) of filled seed per cone (range from 0 to 6.27). Orr-Ewing (1956) indicated a range between 0.37 and 13.82, and pointed out that self-pollination might result in the same number of filled seeds as in cross-pollination. On the other hand, certain self combinations might produce only empty seeds, as on tree B. Self-pollinations were attempted in previous years on tree B, but filled seeds were never obtained. It is logical to assume that deleterious mutant recessive genes have accumulated in *Pseudotsuga*. Since this is an outcrossing species, the appearance of these genes is hidden by the corresponding dominant alleles. In self-pollination (inbreeding), these genes could arrive at a homozygous condition, and their effect could be clearly expressed in the seeds or progeny. It is also possible to assume that some of these recessive genes are lethal and are able to block fertilization or destroy the embryo shortly after fertilization takes place.

Germination Tests

In the germination tests on seeds from controlled crosses all of the above-mentioned variations in environmental factors were eliminated or reduced. This helped to bring to light the heritability variances.

The seed germination percent varied by mother tree as follows: E, 91.3; A, 76.2; D, 74.1; and 11, 42.0. When tree E pollen was applied, a high germination value was observed in every case. On tree E, no change in germination percent was evident in any cross when a different pollen source was used, indicating a high combining ability for this tree.

Progeny Test in the Growth Chambers

Data available from the polyallel crosses permitted an assessment of the combining ability of the four Douglas-fir trees, both as seed and as pollen parents. Tree 11 appears to be the best seed parent, followed by tree E, B, and A. As a pollen partner, tree B proved to be the best, followed by tree 11, A, and E. Tree 11 as a pollen parent was not far behind tree B. In a few important characteristics, such as length of epicotyl, diameter of root collar, and green and dry weight of shoot, the crosses with pollen from tree 11 surpassed the crosses with pollen from tree B.

The combining ability of the parents provides evidence that intraspecific crosses can yield significantly different positive results in 132-day-old progenies of Douglas-fir. The polyallel cross in connection with a short testing method, using growth chambers, seems very useful, but will have to be modified to best fulfill the requirements of forest tree improvement.

The correlation coefficients between total height of 4-year-old and the epicotyl lengths of 132-day-old progenies were calculated, using the four available 1958 crosses—B x A, B x E, E x A, and E x B. A significant correlation was found with a value of 0.895 (r_{05} =0.879 and r_{01} =0.959, df = 3). The first year's total height growth also showed significant or highly significant correlation between total heights after 1 year and after 4 years of the same crosses. This may mean that the effect of the seed is not well pronounced in these crosses, or diminishes at a very early stage of development. On the other hand, the correlation between shoot (epicotyl) length at 132 days and the total height after 4 years is a useful one, and promises a certain time reduction in progeny testing in forest tree breeding, where time is one of the greatest obstacles.

It was possible to calculate narrow-sense heritability values only for length of epicotyl (seed parent; 0.36), length of branches (seed parent; 0.72, pollen parent; 0.58), number of cotyledons (pollen parent; 0.42), green weight of shoots (pollen parent; 0.22) and for light transmittance of chlorophyll (seed parent; 0.98, pollen parent; 0.97).

The moderate inheritance in epicotyl length attributed to seed parents (0.36) is important in forestry practice. By using such seedlings, rapid juvenile growth may overcome brush competition earlier and the new forest stand could be established more quickly and surely. At the end of the 132-day experimental period, for instance, seedlings had grown to average heights, by crosses, of 224.3 mm for 11 x A, 129.3 mm for B x A, and 126.0 mm for: E x A. In all these crosses, the pollen parent was the same; only the seed parents were different. By selecting seedlings from 11 x A crosses, the increase could be between 73 and 78 percent in comparison to seedlings from B x A or E x A crosses respectively. Such trees could be propagated in seed orchards to give a quick increase in seedling height. This might be worthwhile even if we are taking a calculated risk in not knowing the correlation between seedling and mature tree height.

The length of branch was under strong genetic control, and the seed parent effect was larger than that of the pollen parent.

Generally, except for the light transmittance of chlorophyll, the heritability estimates are moderate, which confirms the assumption of Toda *et al.* (1959) that in many characteristics the additive

genetic variance is small compared to other variances. The moderate heritability values also indicate that selection based on phenotypic values may not be a reliable indication of the success of such a breeding program. The writer wholeheartedly agrees with the statement of Wright *et al.* (1958): "Most significant improvement will come from programs in which every parent is carefully selected and carefully progeny-tested and in which controlled pollination plays a major part."

Literature Cited

Allen, G. S. 1963. Origin and development of the ovule in Douglas-fir. Forest Sci. 9: 386-393.

Allen, G. S. and W. Bientjes. 1954. Studies on coniferous tree seed at the University of British Columbia. Forest. Chron. 30: 184-196.

Allen, G. S. and 0. Sziklai. 1962. Pollination of Douglas-fir with water suspensions of pollen. Forest Sci. 8:64-65.

Duffield, J. W. 1950. Techniques and possibilities for Douglas-fir breeding. J. Forest. 48: 41-45.

Hedlin, A. F. 1958. Insects causing seed losses in Douglas-fir on Vancouver Island in 1957. Entomol. Soc. B.C. 53: 37-39.

Madison, J. H. and A. H. Anderson. 1963. Chlorophyll index to measure turf grass response. Agron. J. 55: 461-464.

Orr-Ewing, A. L. 1954. Inbreeding experiments with Douglas-fir. Forest. Chron. 30: 7-16.

Orr-Ewing, A. L. 1956. An investigation into the effect of self-pollination on *Pseudotsuga menziesii* (Mirb.) Franco. U.B.C. Dep. Biol. Bot. Ph.D. thesis. 110 pp.

Silen, R. R. and K. W. Krueger. 1962. Does rainy weather influence seed set of Douglas-fir? J. Forest. 60: 242-244.

Stebbins, G. Jr. 1957. Variation and evolution in plants. New York: Columbia Univ. Press. 643 pp.

Stoate, T. N.; I. Mahood; and E. C. Crossin. 1961. Cone production in Douglas-fir. Empire Forest. Rev. 40: 104-110.

Toda, R.; K. Nakamura; and T. Satoo. 1959. The heritability of tree height and stem girth in *Cryptomeria* through sexual reproduction. Silvae Genet. 8: 43-49.

Wright, J. W.; R. T. Bingham; and K. W. Dorman. 1958. Genetic variation within geographic ecotypes of forest trees and its role in tree improvement. J. Forest. 56: 803-808.