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Analysis of Genetic Variation in 1-, 2-, and 3-year old Eastern White Pine in Incomplete Diallel Cross Experiments

By H. B. KRIEBEL, G. NAMKOONG and R. A. USANIS[1])

This paper reports the results of diallel analyses of juvenile height growth in eastern white pine (Pinus strobus L.), and compares the results with estimates of heritability and the components of variance of a l-parent progeny test.

The questions discussed are: (1) How much variation in parameter estimates is caused by crossing two randomly selected sets of parent trees from the same breeding population, one set in one year and the other set the next year?; (2) How do variance components and heritabilities obtained at ages 1, 2 and 3 compare for the families resulting from each year's diallel crosses and how high is the genetic correlation between responses in different years?; (3) How can a diallel analysis be made on a large number of families when there are missing cells and family size varies?; (4) What is the effect of a high level of environmental uniformity in the progeny test on the heritabilities obtained?

Materials and Methods

All crosses were made in a young stand of native white pine in central Ohio. The parent trees could be considered a random selection with respect to vigor. Actually, reproductive fertility was the most important consideration in tree selection, because female cone production was necessary for the wind pollination study and a moderate or greater level of bisexuality was required for the diallel cross experiments.

The inclusion of reciprocals and unpollinated controls in the diallels, along with a need for replication on each tree, limited the number of suitable parents to 10 in 1962 and 9 in 1963. From 5 to 7 bags of each cross combination were pollinated on each tree. The reciprocals were included in both experiments to estimate a reciprocal-maternal component of variance for the three ages. Though seed weight is known to influence early growth rate (SPURR 1944), the significance of this component of variance has not been investigated previously in eastern white pine.

Fresh pollen was used for all crossing. The flowers were isolated in synthetic sausage-casing bags prior to local pollen dissemination. The conelets were subsequently protected from insects during both growing seasons by white cloth bags. There were, nevertheless, some cone losses from wind- and man-caused branch breakage before maturity. Such losses are difficult to avoid in P. *strobus* because female cone production is restricted to the upper part of the crown. As a result, some cross combinations yielded too small a quantity of seed for inclusion in the experiments and family size varied. Thus, the diallels were irregular and incomplete.

Seed from the 1962 diallel crosses was collected in late August, 1963 and that from the 1963 crosses in late August, 1964. Wind-pollinated seed was collected from 20 trees in the same breeding population in August, 1962.

After cone collection, the seeds were air-separated and stratified for 90 days at 5° C on Perlite in petri dishes. We then placed them in trays in a specially constructed germinator and put them in a warm greenhouse. As the seeds germinated, we sowed them in individual small clay pots in a sterilized potting mixture consisting of 2 parts Wooster silt loam, 1 part sand, and 1 part German peat moss.

In the 1962 diallel experiment (1963 seed), the pots were placed in a greenhouse during the early spring of 1964 in 2 randomized blocks with families as plots. They were moved to a lath house in June and kept there during the first summer. In the autumn, each family in each block was divided in two, and the 4 resulting replications were planted in a nursery, where they remained during the next 2 growing seasons. In the 1963 diallel experiment (1964 seed), the trees were grouped in 4 replications from the start. They were also started in the greenhouse, but were subsequently kept in pots in the lath house until the end of the third growing season. During the intervening winters, the trees, still in the 4 replications, were kept in a hotbed at temperatures slightly above freezing. The seedlings were repotted at the end of the first growing season without major disturbance to the original soil around the roots.

In the test of wind-pollinated progenies, the seedlings were also kept in pots throughout the first three years. The family groups were unreplicated during the first and second years, then divided into 4 blocks prior to the third growing season. The pots were changed to a larger size at the end of the first year as in the 1963 diallel experiment.

The basic measurement used for analysis of variance was total plant height, measured for each tree at the end of each of the first 3 growing seasons. In the diallel experiments, the group of trees selected for crossing each year was considered to be a sample from a general breeding population. The progenies were obtained by using each

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tree as a male and a female parent. With the assumption that success in crossing and seedling establishment was independent of height growth, the resulting incomplete set of families was considered to be a random sample of all possible crosses in a diallel table, without selfs but with reciprocals.

The analysis of each experiment, for each age, was performed in the manner of GRIFFING (1956). Each progeny's height was considered to be a linear function of:

(1) A general mean level of growth.
(2) A deviation from the mean due to block effects.
(3) A deviation from the mean due to the general combining ability of each parent.
(4) A deviation from the mean of each parent due to the specific combining ability of the particular parental genotypes.
(5) A deviation from the specific combining ability due to reciprocal differences.
(6) A random error.

The statistical model is:

$$Z_{hij} = \mu + b_h + g_i + g_j + s_{ij} + r_{ij} + e_{hij}$$

where Z_{hij} is the mean performance in the h^{th} block of the i^{th} parental line mated to the j^{th} parental line; μ is the general mean, b_h is the block deviation from the mean, g_i (g_j) is the general combining ability of the i^{th} (j^{th}) parental line, s_{ij} is the interaction of the i^{th} and j^{th} parents, r_{ij} is the difference caused by the direction of the cross ($r_{ij} = -r_{ji}$) and e_{hij} is the random error.

A general least squares analysis (SCHAFFER and USANIS 1969) was used because the diallel table was incomplete and because of the imbalance caused by missing block-family cells. This computation method uses an abbreviated forward DOOLITTLE solution rather than direct matrix inversion, because of the number of equations involved. The Expected Mean Squares for this analysis are listed in *Table 1*. The analysis of covariance was performed in an exactly analogous form, with the exception that mean cross products between ages were computed instead of mean squares for each age.

The cross combinations from which we obtained families large enough to include in the analyses are shown in *Table 2*. The analyses were based on plot means. On this basis and assuming that mortality or missing crosses are random with respect to height, $\sigma^2_e = \frac{\sigma^2_w}{n} + \sigma^2_p$, where:

σ^2_w = within plot variance
n = harmonic mean of the number of trees per plot
σ^2_p = plot error

Since the data on individual trees were available, we estimated σ^2_w by pooling the "among trees within plots" variances. Then, given our estimate of σ^2, σ^2_w and n, we obtained σ^2_p.

The K values in the expected mean squares were obtained from a complete least squares solution for all effects and variances in the analysis (SCHAFFER and USANIS 1969).

Table 1. — Model for the diallel analysis with reciprocals.

Source	DF	MS	Expectation of mean squares
Replications	b	MS_{reps}	$\sigma^2_e + K_0\theta_b$
GCA	g	MS_{gca}	$\sigma^2_e + K_1\sigma^2_{rec} + K_2\sigma^2_{sca} + K_3\sigma^2_{gca}$
SCA	s	MS_{sca}	$\sigma^2_e + K_4\sigma^2_{rec} + K_5\sigma^2_{sca}$
Reciprocal	r	MS_{rec}	$\sigma^2_e + K_6\sigma^2_{rec}$
Pooled error		MS_e	σ^2_e

Table 2. — Mating designs of 1962 and 1963 breeding experiments, showing combinations included in the analyses.

1962

	A	B	C	D	E	F	G	H	I	J
A		X	X	X	X	X	X	X	X	X
B										
C										
D					X	X				
E		X		X		X	X	X		
F		X	X		X					X
G		X	X	X	X			X	X	X
H			X		X				X	X
I				X		X		X		
J										

1963

	A	B	C	D	E	F	G	H	I
A		X		X			X		
B					X	X		X	X
C									
D					X				
E							X	X	
F	X			X	X				
G	X	X	X	X	X	X		X	X
H	X	X	X	X	X	X	X		X
I	X		X	X	X		X	X	

Standard errors of variance components were computed as:

$$\text{S.E.}(\sigma^2_j) = \sqrt{\sum_i \frac{2a^2_i (MS_i)^2}{d.f._i + 2}}$$

where the a_i are the coefficients in the inverse of the matrix of expected mean squares used to estimate the j^{th} variance component (ANDERSON and BANCROFT 1952).

The component of variance σ^2_{gca} was used to estimate the variance in general combining ability among all of the parents in these experiments and is used as an estimator of $\frac{1}{4}\ \sigma^2_A$. We assumed that all epistatic components of genetic variance were insignificantly small. The component σ^2_{sca}, the estimated variance in specific combining ability, is an estimator of $\frac{1}{4}\ \sigma^2_D$ (with the same assumptions). Therefore, an estimator of the additive genetic variance is $4\ \sigma^2_{gca}$, and an estimate of the dominance genetic variance is $4\ \sigma^2_{sca}$.

Since the 1963 cross material had been grown under more uniform conditions than the 1962 material, we expected a reduction in environmental error in the second year's analysis compared to that of the first year. Any such reduction should be reflected in a lower variation among replicates (Θ_b) and in the plot error variances (σ^2_e). As long as the extra care did not bias family comparisons, a significantly reduced environmental error could be useful in selection. Therefore, comparisons were made of Θ_b and $\sigma^2_p + \frac{\sigma^2_w}{n}$ for both years' progenies.

To estimate the effectiveness of selection for early height growth, two heritabilities for each age and each experiment were calculated. The first heritability is the one commonly used for estimating the ratio of additive genetic to total variance, which is also appropriate for estimating

gain from mass selection among randomly placed seedlings in small test plantings. This heritability is estimated by:

$$h_1 = \frac{4\,\sigma^2_{gca}}{\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{rec} + \sigma^2_p + \sigma^2_w}$$

Mass selection gain is estimated by:

$$\Delta G_1 = i_1 h^2_1$$

where i_1 is the selection differential for individual seedling selection. The second heritability is appropriate for estimating gain from selection among half-sib families if 10 seedlings per plot and 4 replicates of each family plot are used to judge the height growth potential of each family. This heritability is estimated by:

$$h^2_2 = \frac{\sigma^2_{gca}}{\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{rec} + \frac{\sigma^2_p}{4} + \frac{\sigma^2_w}{40}}$$

and gain from half-sib family selection is estimated by:

$$\Delta G_2 = i_2 h^2_2$$

where i_2 is the selection differential for half-sib families. If the parents of the best families are to be selected and intermated, i_2 should be doubled to give the expected gain. Generally, i_2 would be much smaller than i_1.

While juvenile height growth may be of direct value as an indicator of early competitive ability, it is also useful in selection for later growth if the juvenile-mature genetic correlation is high. Environmentally caused correlations cannot usually be used in a selection program. Therefore, correlations of genetic effects as well as total phenotypic correlations were estimated for the 1963 diallel but not for the less-uniformly treated 1962 trees. Though the analysis only covered a 3-year period for one year's progeny, the results may be useful as an indication of the correlation over the early years in the population. All of the covariance components for each source of variance for each pair of ages were computed by the same least squares diallel computer program used to compute the variance components. The correlations of plot error deviations and general combining abilities were computed by dividing the appropriate covariance components by the square root of the product of the appropriate pair of variances components, i. e.

Table 3. — Variance component estimates and standard errors for the 1962 and 1963 diallel crosses at ages 1, 2, and 3, years.

Source	Age 1	Age 2	Age 3
1962 Diallel Cross		Variance Component	
Replication (θ_b)	.0079 ± .0105	2.2510 ± 1.4566	.8996 ± .7484
General Combining Ability (σ^2_{gca})	.1192 ± .0571	.3744 ± .4566	.7615 ± .4268
Specific Combining Ability (σ^2_{sca})	-.0478 ± .0531	-.6010 ± .3848	-1.6999 ± 1.0444
Reciprocal-Maternal Effects (σ^2_{rec})	.0734 ± .0623	.7352 ± .5071	1.2879 ± 1.3152
Error (σ^2_e)	.1666 ± .0398	1.6445 ± .2399	8.9482 ± 1.3052
Plot error (σ^2_p)	.1391	1.0687	6.1917
Within plot error (σ^2_w)	.5230	4.7170	12.0254
Σ(plot mean) = $\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{rec} + \frac{\sigma^2_p}{4} + \frac{\sigma^2_w}{40}$	.1927	.8937	2.1981
h^2(plot) = σ^2_{gca}/Σ(plot mean)	.62	.42	.35
Σ(individual) = $\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{rec} + \sigma^2_p + \sigma^2_w$	.8070	6.2942	18.5666
h^2(indiv.) = 4 σ^2_{gca}/Σ(indiv.)	.59	.24	.16
1963 Diallel Cross		Variance Component	
Replication (θ_b)	.0428 ± .0285	.0623 ± .0440	.7758 ± .5403
General Combining Ability (σ^2_{gca})	.0456 ± .0243	.1495 ± .0823	.3968 ± .2450
Specific Combining Ability (σ^2_{sca})	.0152 ± .0174	.0701 ± .0672	-.1198 ± .3868
Reciprocal Maternal Effects (σ^2_{rec})	.0181 ± .0145	.0732 ± .0544	.4672 ± .4177
Error (σ^2_e)	.0712 ± .0104	.2263 ± .0330	2.4768 ± .3613
Plot error (σ^2_p)	.0282	.0429	1.8392
Within-plot error (σ^2_w)	.4006	1.6250	5.1726
Σ(plot mean) = $\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{rec} + \frac{\sigma^2_p}{4} + \frac{\sigma^2_w}{40}$	.0960	.3441	1.334
h^2(plot) = σ^2_{gca}/Σ(plot mean)	.47	.43	.30
Σ(individual) = $\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{rec} + \sigma^2_p + \sigma^2_w$	.5077	1.9607	7.7561
h^2(indiv.) = 4 σ^2_{gca}/Σ(indiv.)	.36	.30	.20

$$\varrho \text{ age 1, age 2} = \frac{\text{Cov [gca (age 1), gca (age 2)]}}{\sqrt{\sigma^2_{\text{gca (age 1)}} \cdot \sigma^2_{\text{gca (age 2)}}}}$$

A total plot mean phenotypic correlation was computed from the sum of covariance components in the numerator and the sum of variance components in the denominator for gca, sca, reciprocal and plot mean error effects.

Results

Variance component estimates and their standard errors for the six incomplete diallels are listed in *table 3* with their standard errors. The table also shows estimates of heritabilities. Correlation coefficients for ages 1, 2, and 3 are shown in *table 4*. Points of interest include the following:

1) Careful handling clearly reduced the replication differences and all error terms after the first year. In the 1962 diallel cross material, the ratio of all of the "genetic" components to the sum of components, i. e.

$(\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{rec}) / (\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{rec} + \sigma^2_p + \sigma^2_w)$

dropped from 18% at age 1 to 8% at age 2 and 2% at age 3. But in the 1963 cross families, the ratio was 16%, 15% and 10% for the three successive years of measurement.

2) The reciprocal-maternal efects were moderately large, accounting for about the same percentage of the total variance as did the gca effects.

3) The dominance genetic variance was low and was often estimated by a negative component. The irregular behavior of the more roughly handled 1962 material resulted in negative estimates in all 3 years. In the 1963 material the estimate was negative only at age 3 but was less than its standard error at age 1, and at age 2 was barely larger than its standard error.

4) The estimates of heritability fell as the material aged. On the basis of individual trees, heritability fell sharply from .59 to .24 to .16 in the 1962 material. This may reflect the accumulation of relatively large environmental sources of variation or a true loss of genetic variation in accumulated height. In the more carefully handled 1963 materials, heritability did clearly decline but at a more moderate rate from .36 to .30 to .20.

5) The analysis of 3 year old open-pollinated material provided additional estimates of σ^2_A and of a plot error term. In this analysis, the family variance includes both the gca and reciprocal effects. The estimates were: $\Theta_b = .03$, $\sigma^2_{(family)} = 2.05$, $\sigma^2_p = .36$, and $\sigma^2_w = 11.71$. If the diallel results are accurate, we may assume that σ^2_{rec} would be as large as σ^2_{gca}. On this assumption, heritability on an individual tree basis for age 3 was .28. This is slightly larger than the .16 and .20 estimates derived from the diallel crosses and may be due to the even more strictly controlled environment of this experiment.

6) Estimates of heritability based on plot means followed the same trends as the estimates based on individual trees. Since most of the error variance was in the σ^2_w component, samples consisting of 10 trees per plot were useful in reducing the error of estimating family means. In this material, the reduction in error variance more than compensated for the use of only ¼ σ^2_A in the numerator of heritability; h^2 on a plot mean basis was consistently higher than h^2 (individual). These results indicate that selection on the basis of family comparisons can be effective and may be economically acceptable if the loss in selection differential and testing time can be made small.

7) The decline in the phenotypic correlation of the 1963 trees as shown in *table 4* from .74 (age 1 vs. age 2) to .49 (age 2 vs. age 3) to .36 (age 1 vs. age 3) is a reflection of the balance provided by the consistently high correlation of genetic effects and the decline of correlation caused by environmental effects. Even in this more carefully handled material, environmental sources of variation apparently changed enough in 3 years so that only 24% of the plot error variance in third year height was accounted for by first year height. In contrast, the genetic correlation only declined from .99 to .94 in the same period.

Discussion

There was a similarity in the results obtained from the two incomplete diallels. The generally high additive variance and low dominance variance indicate that the methods used provided an environment in which we could effectively select for early height growth. This environment is quite different from that occurring in plantations or natural stands; therefore, heritability estimates are higher than would be expected under field conditions. The technique provides a sensitive method for juvenile selection of fast-growing families as long as selection is unbiased by any genotype-by-handling interaction. We have no tests which indicate the size of any such genotype × environment interaction. The value of the technique in a breeding problem depends on the strength of the correlation between juvenile growth rate and later performance in the field, as it does in any juvenile selection program. This information is not yet available, but the early correlations suggest that the genetic correlations can be expected to fall off much more slowly than the total phenotypic correlations.

The decline in heritability of height growth estimated in these experiments may have been caused by many factors. Two possible factors are (1) a relative increase in environmental sources of error and (2) an actual reduction of genetic variance in growth caused by a convergence of growth curves implying continued genetic control but with population uniformity. Since all variance components except σ^2_{sca} increased over the years, there was little apparent reduction in the gross amount of variation caused by additive genetic differences. Further, the strong positive genetic correlations indicate consistent relative performance for 3 years and hence no strong tendency towards growth curve convergence. It therefore seems most reasonable to suppose that our trees met new environmental

Table 4. — Correlation coefficients among ages 1, 2, and 3 for genetic and error effects.

1963 Diallel Cross								
Correlation of General Combining Ability			Correlation of Error			Total Phenotypic Correlation		
	Age 2	Age 3		Age 2	Age 3		Age 2	Age 3
Age 1	.99	.94	Age 1	.38	.14	Age 1	.74	.36
Age 2		.98	Age 2		.32	Age 2		.49

sources of variation each year which contributed heavily to among-plot and within-plot errors. The decrease in the plot error correlation indicates that such sources of variation are more or less random. In addition, the much slower decline in heritability experienced by the more carefully handled 1963 cross material indicates that these sources of error can be controlled by experimental procedures. Thus we conclude that selection can be carried out more effectively under controlled rather than common handling methods if genotype × handling interaction does not exist.

The breeding population from which the parent trees were drawn was limited in size and was itself derived from a very limited number of parent trees. Consequently, the parents are probably partly related and could be all half-sibs or even all full-sibs. If so, the additive genetic variance obtained in these analyses probably underestimates the actual parameter for the population (DICKERSON 1942). Within the conditions of the experiments, our heritability estimates are, therefore, probably conservative for the population sampled.

Summary

Diallel crosses with reciprocals were made in a native stand of *Pinus strobus* L. in Ohio, in two successive years. An analysis was also made of open-pollinated families from the same breeding population. Analyses of tree heights in each of the resulting incomplete sets of families were made at ages 1, 2 and 3, prior to field planting. A general least squares analysis was made for each set of crosses at each age.

Dominance genetic variance was small but reciprocal-maternal effects were moderately large. Heritability of height growth decreased with age. Heritability based on plot means was consistently higher than that based on individual trees.

Results indicated that the decline in heritability could be attributed to an increase in environmental variance rather than an actual reduction in genetic control of growth. Genetic correlation measured in trees from the 1963 crossing remained fairly constant with increasing age.

The careful experimental technique reduced environmental variation as compared to that expected in field tests. If no genotype × handling interaction existed, and if later analysis shows that strong juvenile-mature correlations exist in growth rate, the technique should be useful for juvenile selection of fast-growing families.

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Germination of Douglas-fir Pollen

By RONGHUI HO and OSCAR SZIKLAI[1])

Introduction

ALLEN and SZIKLAI (1962) reported that water suspensions of *Pseudotsuga menziesii* (Douglas-fir) pollen offered possibilities for obtaining satisfactory seed yields. This opens up the potentiality of stimulating the rate of pollen germination and tube elongation by adding nutrients to the suspensions. However, evidence is scanty on the beneficial effects of nutrients supplied to water suspensions to give high rates of pollination and fertilization of Douglas-fir. Therefore, the types of substances that stimulate pollen germination and elongation of the pollen tube should be studied, *in vitro*, for the practical value of recovering filled seed for Douglas-fir tree improvement programs.

LARUE (1953) put Douglas-fir pollen into a liquid form of WHITE's solution, but found no germination. ORR-EWING (1956) was the first to determine the viability of Douglas-fir pollen by incubating it on an agar medium. CHING and CHING (1959) introduced 10% sucrose and a series of gibberellic acid concentrations into the agar medium to culture Douglas-fir pollen and obtained a partial development of the male gametophyte to the three-celled stage. The complete development of the male gametophyte of Douglas-fir *in vitro* has not as yet been reported. BARNER and CHRISTIANSEN (1962) stated, "The actual germination of Douglas-fir pollen *in vitro* proved impossible." CHRISTIANSEN (1969) reported, "Pollen of *Larix* and *Pseudotsuga* cannot be germinated *in vitro*."

This study utilized a mineral ion solution, a long-term cultivation period, and methods different from those previously reported, and obtained the complete development of the male gametophyte of Douglas-fir.

Materials and Methods

Microsporangiate strobili of Douglas-fir were collected from 4-year-old grafted clones at Caycuse, Vancouver Island on May 2, 1967, and in 1968 from five trees on the University of British Columbia campus. Pollen was extracted from the strobili at room temperature (about 23° C) and stored in a closed vial at 0—2° C in the refrigerator.

Instruments were sterilized either with alcohol, then flamed, or by autoclaving at 15 psi for 20 minutes. In experiments in which boron was studied, all glassware was first soaked for 12 hours in *3N* HCl, rinsed 5 times in double-distilled water and covered.

A modified cotton blue staining technique (COLE, 1958), followed by destaining in distilled water, was used to test for pollen viability. Nuclei were stained by BELLING's iron-acetocarmin method (JOHANSEN, 1940). Identification of the separated cells of the male gametophyte was facilitated by smears and dehydration.

Stock solutions A and B *(Table 2)* were adopted from BREWBAKER and KWACK (1963) and used for viability tests

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