

ANALYSES OF VARIANCE OF DIALLEL CROSSES IN SPLIT-PLOT EXPERIMENTS*

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Summary

The analysis and interpretation of four split-plot type designs involving diallel crosses are presented. In two designs all crosses in a given whole plot have one parent in common; the distinction between the two designs arises from the inclusion or non-inclusion of parents. In the other two designs (which are split-split-plot designs) reciprocal crosses appear together in split plots while whole plots are composed of all split plots with one common parent. Mean square expectations are given for all four designs for both random and mixed (all effects except genetic ones are considered random) models. The estimation and interpretation of genetic variance components under random models is discussed and a numerical example is presented.

I. INTRODUCTION

Griffing (1956*b*) summarized the analyses and interpretation of a variety of diallel crossing systems. The procedures presented for computing the analysis of variance, estimation of combining ability, and population parameters are general for any design where all genotypes are measured with equal precision. For convenience or in order to obtain more precision on either the differences among crosses arising from a common parent or the reciprocal cross differences, crosses may be blocked by some characteristic of genotype such as common maternal or paternal parent. By such blocking, however, precision is lost on the comparison of genotypes from different parents and on the comparison of non-reciprocal genotypes, respectively. In those designs where such blocking is employed, the customary diallel analyses are not usually appropriate.

All four of Griffing's methods [1, crosses and reciprocals with parents; 2, crosses and parents, no reciprocals; 3, crosses and reciprocals without parents; and 4, crosses, no reciprocals or parents] can be blocked by maternal or paternal parent or both. The analyses of two of these blocked experiments (*viz.* methods 1 and 3) have been presented in detail by John (1963). In addition to these split-plot designs, it is possible to form split split-plot designs for methods 1 and 3. In the latter designs whole plots are blocked by one common parent and subplots by the second common parent; i.e. subplots consist of two sub-subplots, the cross $i \times j$ and its reciprocal $j \times i$. In this paper the split-plot designs for methods 2 and 4

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and split-split-plot designs for methods 1 and 3 are considered. Common to all four designs is the following notation: n inbred lines as the source of the crosses which are grown in each of r replicates.

II. SPLIT-PLOT DESIGNS

(a) *Split-plot Designs for Method 4: Crosses, No Reciprocals or Parents*

In these split-plot designs the cross 1×2 (or 2×1) appears in both blocks 1 and 2; however, for convenience in notation the cross appears as 1×2 and 2×1 in blocks 1 and 2, respectively. In general, the $i \times j$ th cross is randomly assigned to the i th and j th blocks; in effect the number of replicates is therefore $2r$. The tabulated data for the first replication will take the following form:

		Split Plot			
Whole Plot		1	2	3	... n
	1		X_{112}	X_{113}	... X_{11n}
	2	X_{121}		X_{123}	... X_{12n}
	3	X_{131}	X_{132}		... X_{13n}
	.	.	.	.	.
	.	.	.	.	.
	n	X_{1n1}	X_{1n2}	X_{1n3}	...

There are, therefore, $n-1$ experimental units (split-plots) within each whole plot and n whole plots in each of r replications, giving a total of $rn(n-1)$ experimental units.

The model appropriate to these data is

$$X_{gij} = \mu + \rho_g + \alpha_i + \alpha_j + \delta_{gi} + \gamma_{ij} + \epsilon_{gij},$$

where X_{gij} is the observed phenotypic value, $g = 1, 2, \dots, r$; $i \neq j = 1, 2, \dots, n$; μ = overall mean, ρ_g = replication effect, α_i = general combining ability effect of the i th parental line; δ_{gi} = whole plot effect; γ_{ij} = specific combining ability effect; and ϵ_{gij} = residual effect. The analysis of variance corresponding to this model is shown in Table 1. In the Appendix the derivation of the least squares estimates of whole-plot effects and corresponding sums of squares is given. This shows, incidentally, that John's (1963) two alternative analyses are really identical.

Table 1 gives the computing formulae for all sums of squares as well as mean square expectations under the completely random model and under a mixed model where α_i and γ_{ij} are considered to be fixed effects. The variance components for the former model can be estimated as indicated in the table. To make an approximate test of significance for σ_δ^2 , σ_γ^2 , and σ_α^2 , which cannot be estimated directly,

TABLE 1
SPLIT-PLOT DIALLEL ANALYSIS OF METHOD 4: ONE SET OF CROSSES, NO RECIPROALS, NO PARENTS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Mean Square Expectation	
				Random Model	Mixed Model
Replications	$r-1$	S_R	M_R	$\sigma_e^2 + (n-1)\sigma_g^2 + n(n-1)\sigma_p^2$	$\sigma_e^2 + (n-1)\sigma_g^2 + n(n-1)\sigma_p^2$
General combining ability (GCA)	$n-1$	S_g	M_g	$\sigma_e^2 + 2r\sigma_y^2 + \frac{1}{2}(n-2)\sigma_g^2 + 2r(n-2)\sigma_a^2$	$\sigma_e^2 + \frac{1}{2}(n-2)\sigma_g^2 + 2r(n-1)\Sigma\alpha_i^2/(n-2)$
Whole-plot error	$(r-1)(n-1)$	S_{Rg}	M_{Rg}	$\sigma_e^2 + (n-1)\sigma_g^2$	$\sigma_e^2 + (n-1)\sigma_g^2$
Specific combining ability (SCA)	$\frac{1}{2}n(n-3)$	S_s	M_s	$\sigma_e^2 + 2r\sigma_y^2$	$\sigma_e^2 + 4r\Sigma\gamma_{ij}^2/n(n-3)$
Error	$rn(n-2) - \frac{1}{2}n(n-3)$	S_e	M_e	$\sigma_e^2 + (n-1)\sigma_g^2/K_1$	$\sigma_e^2 + (n-1)\sigma_g^2/K_1$
Total	$rn(n-1) - 1$	S_t			

Formulae:	Variance component estimation:
$S_t = \Sigma\Sigma X_{ij}^2 - X^2/rn(n-1)$	$s_e^2 = (K_1M_e - M_{Rg})/(K_1-1)$
$S_R = \Sigma X_{g..}^2/n(n-1) - X^2/rn(n-1)$	$s_g^2 = [(K_1-1)M_g - K_1(M_e - M_{Rg})]/2r(K_1-1)$
$S_g = \Sigma (X_{.i.} + X_{.i.})^2/2r(n-2) - 2X^2/rn(n-2)$	$s_y^2 = K_1(M_{Rg} - M_e)/(K_1-1)(n-1)$
$S_{Rg} = \Sigma [\Sigma X_{gi.}^2/(n-1) - X_{.i.}^2/r(n-1)] - S_R$	$s_a^2 = (M_g - M_e - K_2M_{Rg} - K_3M_e)/2r(n-2)$
$S_s = \Sigma\Sigma (X_{ij} + X_{ji})^2/2r - X^2/rn(n-1) - S_g$	where $K_1 = 2r(n-2) - n + 3$
$S_e = S_t - S_R - S_g - S_{Rg} - S_s$	$K_2 = \{K_1(n-2) - 2(n-1)\}/2(K_1-1)(n-1)$
	$K_3 = \{nK_1 - 2(n-1)\}/2(K_1-1)(n-1)$

Satterthwaite's (1946) approximation for degrees of freedom may be used. The variance component σ_ρ^2 , if of interest, can be tested directly by M_R/M_{Rg} . Under either the random or mixed effects model σ_δ^2 may be tested by

$$F = (K_1 - 1)M_{Rg}/(K_1M_e - M_{Rg})$$

or

$$F = M_{Rg}/s_\epsilon^2.$$

σ_γ^2 and σ_a^2 may be tested by

$$F = M_s/s_\epsilon^2,$$

and

$$F = M_g/[s_\epsilon^2 + 2rs_\gamma^2 + (n-2)s_\delta^2/2],$$

respectively under the random model. One may test for zero specific combining ability (SCA) and general combining ability (GCA) effects under the mixed model as

$$F = M_s/s_\epsilon^2,$$

and

$$F = M_g/[s_\epsilon^2 + (n-2)s_\delta^2/2],$$

respectively, where the K 's and s_i^2 are defined in Table 1. An alternative method of estimation of the variance components is one proposed for such non-orthogonal data by Henderson (1953).

(b) *Split-plot Designs for Method 2: Crosses and Parents, No Reciprocals*

The crosses are blocked as in the previous design; there is, however, an additional subblock in each block, consisting of the parental line associated with the block. Hence there are rn^2 observations. The tabulated data will take similar form to that design shown previously.

The model appropriate to these data is

$$X_{gij} = \mu + \rho_g + \alpha_i + \alpha_j + \delta_{gi} + \gamma_{ij} + \epsilon_{gij},$$

where $g = 1, 2, \dots, r$, and $i, j = 1, 2, \dots, n$. Definitions of the terms are similar to the previous model.

The corresponding analysis of variance is shown in Table 2. Included in this table are the computing formulae as well as the mean square expectations for both the random and mixed models. Variance components may be estimated as shown in Table 2 or by the Henderson procedure. Tests of significance of these variance components or effects or both can be constructed similarly to those tests given for the previous model.

III. SPLIT-SPLIT-PLOT DESIGNS

(a) *Split-split-plot Designs for Method 3: Crosses and Reciprocals, No Parents*

In these split-split-plot designs, each split-plot is comprised of two crosses, $i \times j$ and its reciprocal $j \times i$. Whole plots in turn are composed of all split plots with one common parent, say the i th. Therefore every cross must appear twice in every replication giving again effectively $2r$ replications. In the field layout,

randomization is employed in identifying whole plots with parental lines and in assigning particular crosses (ignoring reciprocals) to the subplots within whole plots and subsequently the two reciprocal crosses to sub-subplots within subplots. The tabulated data of the first replication will take the following form:

Split Plot					
Whole Plot		1	2	3	n
	1		$X_{1112}X_{1121}$	$X_{1113}X_{1131}$	$\dots X_{111n}X_{11n1}$
	2	$X_{1221}X_{1212}$		$X_{1223}X_{1232}$	$\dots X_{122n}X_{12n2}$
	3	$X_{1331}X_{1313}$	$X_{1323}X_{1332}$		$\dots X_{133n}X_{13n3}$
	.	.	.	.	.
	.	.	.	.	.
	.	.	.	.	.
	n	$X_{1nn1}X_{1n1n}$	$X_{1nn2}X_{1n2n}$	$X_{1nn3}X_{1n3n}$	$\dots$

There are, therefore, $n-1$ split plots within each of n whole plots for each of r replications. Since two split-split-plots comprise a split plot, the total number of experimental units is $2rn(n-1)$.

The model corresponding to these data is

$$X_{ghij} = \mu + \rho_g + \alpha_i + \alpha_j + \delta_{gi} + \gamma_{ij} + \beta_{gij} + \Delta_{ghi} + \pi_{ij} + \epsilon_{ghij},$$

where $g = 1, 2, \dots, r$; $i \neq j = 1, 2, \dots, n$; and $h = i, j$. Additional components not previously identified include: β_{gij} , split-plot effect; Δ_{ghi} , duplicate split-plot effect; and π_{ij} , reciprocal cross effect. The analysis of variance derived from this model is indicated in Table 3, including mean square expectations for both random and mixed models and variance component estimation. There are two checks on computations in the two split-split-plot designs: (1) $S_g + S_s + S_r$ must equal the genotype sum of squares; and (2) the following relationship of independently computed sums of squares must hold:

$$S_t = S_R + S_g + S_{Rg} + S_s + S_{Rs} + S_d + S_r + S_e.$$

Table 3 also indicates how the variance components may be estimated under the random model. Only the test of significance of the hypothesis $\sigma_\pi^2 = 0$ is straightforward; other variance components or effects or both may be tested as previously indicated with approximate degrees of freedom as given by Satterthwaite's approximation.

(b) *Split-split-plot Design for Method 1: Crosses, Reciprocals, and Parents*

Crosses in this design are blocked in analogous fashion to the split-split-plot design previously indicated. However, the i th whole plot now contains an additional split plot composed of two identical split-split-plots of the i th parent; the total number of experimental units is therefore $2rn^2$.

TABLE 2
SPLIT-PLOT DIALLEL ANALYSIS OF METHOD 2: ONE SET OF CROSSES, NO RECIPROCALLS, PARENTS INCLUDED

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Mean Square Expectation	
				Random Model	Mixed Model
Replications	$r-1$	S_R	M_R	$\sigma_e^2 + n\sigma_g^2 + n^2\sigma_p^2$	$\sigma_e^2 + n\sigma_g^2 + n^2\sigma_p^2$
GCA	$n-1$	S_g	M_g	$\sigma_e^2 + 2r(n-1)\sigma_y^2/n + \frac{1}{2}n\sigma_g^2 + 2nr\sigma_a^2$	$\sigma_e^2 + \frac{1}{2}n\sigma_g^2 + 2rn\Sigma\sigma_i^2/(n-1)$
Whole-plot error	$(r-1)(n-1)$	S_{Rg}	M_{Rg}	$\sigma_e^2 + n\sigma_g^2$	$\sigma_e^2 + n\sigma_g^2$
SCA	$\frac{1}{2}n(n-1)$	S_s	M_s	$\sigma_e^2 + 2r(n^2 - n + 1)(\sigma_y^2/n^2)$	$\sigma_e^2 + 2r\Sigma\gamma_{ij}^2/n(n-1)$
Error	$\frac{1}{2}n(n-1)(2r-1)$	S_e	M_e	$\sigma_e^2 + \sigma_g^2/(2r-1)$	$\sigma_e^2 + \sigma_g^2/(2r-1)$
Total	rn^2-1	S_t			
Computing formulae:				Variance component estimation:	
$S_t = \Sigma\Sigma\Sigma X_{gij}^2 - X^2.../rn^2$				$\sigma_e^2 = K_1[nM_e - M_{Rg}/K_1]/K_2$	
$S_R = \Sigma X_g^2/n^2 - X^2.../rn^2$				$\sigma_y^2 = n^2(M_s - \sigma_e^2)/2rK_3$	
$S_g = \Sigma(X_{..i} + X_{.i.})^2/2nr - 2X^2.../rn^2$				$\sigma_g^2 = K_1(M_{Rg} - M_e)/K_2$	
$S_{Rg} = \Sigma[\Sigma X_{gi}^2/n - X_{.i.}^2/rn] - S_R$				$\sigma_a^2 = [M_g - n(n-1)M_s/K_3 - (K_2-1)M_{Rg}/2K_2 - \{nK_1/2K_2 - n(n-1)/K_3\}M_e]/2nr$	
$S_s = \Sigma\Sigma X_{.ij}(X_{.ij} + X_{.ji})/2r - X^2.../rn^2 - S_g$				where $K_1 = 2r-1$	
$S_e = S_t - S_R - S_g - S_{Rg} - S_s$				$K_2 = nK_1 - 1$	
				$K_3 = n^2 - n + 1$	

TABLE 3
SPLIT-SPLIT-PLOT DIALLEL ANALYSIS OF METHOD 3: ONE SET OF CROSSES, RECIPROCALLS, NO PARENTS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Mean Square Expectation	
				Random Model	Mixed Model
Replications	$r-1$	S_R	M_R	$\sigma_e^2 + 4\sigma_\beta^2 + 2(n-1)\sigma_\delta^2 + 2n(n-1)\sigma_\rho^2$	$\sigma_e^2 + 4\sigma_\beta^2 + 2(n-1)\sigma_\delta^2 + 2n(n-1)\sigma_\rho^2$
GCA	$n-1$	S_g	M_g	$\sigma_e^2 + 4\sigma_\beta^2 + 4r\sigma_\gamma^2 + (n-2)\sigma_\delta^2 + 4r(n-2)\sigma_a^2$	$\sigma_e^2 + (n-2)\sigma_\delta^2 + 4r(n-1)\Sigma\sigma_{ij}^2/(n-2)$
Whole-plot error	$(r-1)(n-1)$	S_{Rg}	M_{Rg}	$\sigma_e^2 + 2(n-2)\sigma_\beta^2/(n-1) + 2(n-1)\sigma_\delta^2$	$\sigma_e^2 + 2(n-2)\sigma_\beta^2/(n-1) + 2(n-1)\sigma_\delta^2$
SCA	$\frac{1}{2}n(n-3)$	S_s	M_s	$\sigma_e^2 + 4\sigma_\beta^2 + 4r\sigma_\gamma^2$	$\sigma_e^2 + 4\sigma_\beta^2 + 8r\Sigma\sigma_{ij}^2/n(n-3)$
Split-plot error	$\frac{1}{2}(r-1)n(n-3)$	S_{Rs}	M_{Rs}	$\sigma_e^2 + 4(n-2)\sigma_\beta^2/(n-3) - 2(n-1)\sigma_\delta^2/(n-3)$	$\sigma_e^2 + 4(n-2)\sigma_\beta^2/(n-3) - 2(n-1)\sigma_\delta^2/(n-3)$
Duplicate subplots in replications	$\frac{1}{2}rn(n-1)$	S_d	M_d	$\sigma_e^2 + 4r\sigma_d^2 + 2\sigma_\delta^2$	$\sigma_e^2 + 4r\sigma_d^2 + 2\sigma_\delta^2$
Reciprocal crosses	$\frac{1}{2}n(n-1)$	S_r	M_r	$\sigma_e^2 + 4r\sigma_\pi^2$	$\sigma_e^2 + 8r\Sigma\sigma_{ij}^2/n(n-1)$
Duplicate reciprocal crosses	$\frac{1}{2}(2r-1)n(n-1)$	S_e	M_e	σ_e^2	σ_e^2
Total	$2rn(n-1)$	S_t			
Computing formulae:					
C.F. = $X^2/2rn(n-1)$					
$S_t = \Sigma\Sigma\Sigma X_{ghij}^2 - \text{C.F.}$					
$S_R = \Sigma X_g^2/2n(n-1) - \text{C.F.}$					
$S_g = \Sigma(X_{..i} + X_{..j})^2/4r(n-2) - X^2/2rn(n-2)$					
$S_{Rg} = \Sigma[\Sigma X_{gi}^2/2(n-1) - X_{..i}^2/2r(n-1)] - S_R$					
$S_s = \Sigma\Sigma(X_{..ij} + X_{..ji})^2/4r - \text{C.F.} - S_g$					
$S_{Rs} = \Sigma\Sigma[\Sigma(X_{gij} + X_{gji})^2/4 - (X_{..ij} + X_{..ji})^2/4r] - S_{Rg} - S_R$					
$S_d = \Sigma\Sigma\Sigma(X_{gij} - X_{gji})^2/4$					
$S_r = \Sigma\Sigma(X_{..ij} - X_{..ji})^2/4r$					
$S_e = \Sigma\Sigma\Sigma(X_{ghij} - X_{ghji})^2/2 - S_r$					
Variance component estimation:					
$\sigma_e^2 = M_e$					
$\sigma_\pi^2 = (M_r - M_e)/4r$					
$\sigma_\delta^2 = [2(n-1)M_{Rg} - (n-3)M_{Rs} - (n+1)M_e]/2(n-1)(2n-1)$					
$\sigma_\beta^2 = (n-1)[(n-3)M_{Rg} + M_{Rs} - (n-2)M_e]/2(n-2)(2n-1)$					
$\sigma_d^2 = (M_d - M_e - 2\sigma_\delta^2)/4$					
$\sigma_\gamma^2 = (M_s - M_e - 4\sigma_\beta^2)/4r$					
$\sigma_a^2 = [M_g - M_e - (n-2)\sigma_\delta^2 - 4r\sigma_\gamma^2 - 4\sigma_\beta^2]/4r(n-2)$					

The model pertinent to data of this nature is

$$X_{ghij} = \mu + \rho_g + \alpha_i + \alpha_j + \delta_{gi} + \gamma_{ij} + \beta_{gij} + \Delta_{ghi} + \pi_{ij} + \epsilon_{ghij},$$

where $g = 1, 2, \dots, r$; $i, j = 1, 2, \dots, n$; and $h = i, j$. The terms in this model are defined similarly to the previous model. The analysis of variance derivable from the model is indicated in Table 4, including mean square expectations under both the random and mixed models and variance component estimation. Tests of hypotheses concerning variance components are similar to the previous design in execution. The same computational checks are available for this design as for the previous design.

IV. ALTERNATIVE MODELS

Although it appears logical to provide two and even three "error terms" in the split-plot and split-split-plot designs, respectively, it is apparent that a number of alternative models could have been considered. For example, if one is justified in assuming there are no whole-plot effects ($\delta_{gi} = 0$) in the split-plot designs, the analyses reduce to Griffing's methods 2 and 4 with the residual terms containing $r(n^2-1)-(n-1)(n+2)/2$ and $r(n^2-n-1)-(n^2-n+2)/2$ degrees of freedom, respectively. The coefficients of the variance components in the mean square expectations, with the exception of σ_e^2 , will be doubled, however. The justification for assuming this absence of whole-plot effects may stem either from previous or current empirical evidence. In the latter case Paull (1950) presents a general exposition of such pooling procedures.

The split-split-plot analyses could have been analysed without the β_{gij} term, in which case the σ_β^2 component would disappear from all mean square expectations. The two sources of variation, "split-plot error" and "duplicate reciprocal crosses" sources of variability would coalesce into a single source of variation, say "residual" with $n[2r(n-2)-(n-3)]/2$ degrees of freedom for method 3. The split-split-plot analysis for method 1 could also have the β_{gij} term deleted with analogous changes.

In the special circumstance where σ_A^2 , σ_B^2 , and σ_g^2 are all of no consequence in the split-split-plot designs, the analyses reduce to the diallel analyses of Griffing's methods 1 and 3 with the pooled error terms containing the pooled degrees of freedom from the three respective sources of variability. Similar to the split-plot analyses, the coefficients of the variance components in the mean expectations will be doubled as contrasted to the customary expectations.

V. INTERPRETATION OF VARIANCE COMPONENTS FOR ALL DESIGNS

Matzinger and Kempthorne (1956) show that if each of the inbred lines used in the diallel cross had an arbitrary but equivalent amount of inbreeding, F , then σ_a^2 , and σ_v^2 would be related to additive genetic, dominance, and epistatic variances as follows:

$$\begin{aligned}\sigma_a^2 &= \frac{1}{4}(1+F)\sigma_A^2 + \frac{1}{16}(1+F)^2\sigma_{AA}^2 + \dots = \sum_i \frac{1}{4i}(1+F)^i\sigma_{A^i}^2, \\ \sigma_v^2 &= \frac{1}{4}(1+F)^2\sigma_D^2 + \frac{1}{8}(1+F)^2\sigma_{AD}^2 + \frac{1}{8}(1+F)^3\sigma_{AD}^2 + \frac{1}{16}(1+F)^4\sigma_{DD}^2 + \dots \\ &= \sum_{i=2} (1+F)^i \{2^{i-1} - 1\} / 2^{2i-1} \sigma_{A^i}^2 + \sum_{i=0} \sum_{j=1} (1+F)^{i+2j} / 2^{i+2j} \sigma_{A^i D^j}^2,\end{aligned}$$

TABLE 4
SPLIT-SPLIT-PLOT DIALLEL ANALYSIS OF METHOD 1: CROSSES, RECIPROALS, AND PARENTS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Mean Square Expectation	
				Random Model	Mixed Model
Replication	$r-1$	S_R	M_R	$\sigma_e^2 + 2(2n-1)\sigma_\beta^2/n + 2n^2\sigma_p^2 + 2n\sigma_d^2$	$\sigma_e^2 + 2(2n-1)\sigma_\beta^2/n + 2n\sigma_d^2 + 2n^2\sigma_p^2$
GCA	$n-1$	S_o	M_o	$\sigma_e^2 + 4(n-1)\sigma_\beta^2/n + 4r(n-1)\sigma_\gamma^2/n + n\sigma_d^2 + 4rn\sigma_a^2$	$\sigma_e^2 + 4(n-1)\sigma_\beta^2/n + n\sigma_d^2 + 4rn\sigma_a^2/(n-1)$
Whole-plot error	$(r-1)(n-1)$	S_{Ro}	M_{Ro}	$\sigma_e^2 + 2(n-1)\sigma_\beta^2/n + 2n\sigma_d^2$	$\sigma_e^2 + 2(n-1)\sigma_\beta^2/n + 2n\sigma_d^2$
SCA	$\frac{1}{2}n(n-1)$	S_s	M_s	$\sigma_e^2 + 4(n^2-n+1)\sigma_\beta^2/n^2 + 4r(n^2-n+1)\sigma_\gamma^2/n^2$	$\sigma_e^2 + 4(n^2-n+1)\sigma_\beta^2/n^2 + 4r\sum \sigma_{ij}^2/n(n-1)$
Split-plot error	$\frac{1}{2}(r-1)n(n-1)$	S_{Rs}	M_{Rs}	$\sigma_e^2 + 4\sigma_\beta^2 - 2\sigma_d^2$	$\sigma_e^2 + 4\sigma_\beta^2 - 2\sigma_d^2$
Duplicate subplots in replications	$\frac{1}{2}rn(n-1)$	S_d	M_d	$\sigma_e^2 + 4\sigma_d^2 + 2\sigma_d^2$	$\sigma_e^2 + 4\sigma_d^2 + 2\sigma_d^2$
Reciprocal crosses	$\frac{1}{2}n(n-1)$	S_r	M_r	$\sigma_e^2 + 4r\sigma_\pi^2$	$\sigma_e^2 + 8r\sum \sigma_{ij}^2/n(n-1)$
Duplicate reciprocal crosses	$rn^2 - \frac{1}{2}n(n-1)$	S_o	M_o	σ_e^2	σ_e^2
Total	$2rn^2 - 1$	S_t			

Computing formulae:	Variance component estimation:
C.F. = $X^2.../2rn^2$	$s_e^2 = M_e$
$S_t = \sum \sum \sum X_{ohij}^2$ - C.F.	$s_r^2 = (M_r - M_o)/4r$
$S_R = \sum X_{o..i}^2/2n^2$ - C.F.	$s_o^2 = [M_{Ro} + nM_{Rs} - (n+1)M_d]/K_1$
$S_o = \sum (X_{o..i} + X_{o..i})^2/4nr - X^2.../rn^2$	$s_\beta^2 = [2nM_{Ro} - (n-1)M_{Rs} - (n+1)M_o]/K_1$
$S_{Ro} = \sum [\sum X_{oi..}^2/2n - X_{o..i}^2/2rn] - S_R$	$s_\gamma^2 = (M_d - M_o - 2s_\beta^2)/4$
$S_s = \sum \sum X_{o..ij}(X_{o..ij} + X_{o..ji})/4r$ - C.F. - S_o	$s_d^2 = (M_o - M_o - 4s_\beta^2)/4r$
$S_{Rs} = \sum \sum [\sum X_{oi..j}(X_{oi..j} + X_{oi..ji})/4 - X_{o..i}(X_{o..i} + X_{o..ji})/4r] - S_{Ro} - S_R$	$s_\pi^2 = [M_o - M_o - (n-2)s_\beta^2 - 4rs_\gamma^2 - 4s_\beta^2]/4r(n-2)$
$S_d = \sum \sum (X_{oi..j} - X_{oi..i})^2/4$	
$S_r = \sum \sum (X_{o..ij} - X_{o..ji})^2/4r$	
$S_e = \sum \sum \sum (X_{ohij} - X_{ohji})^2/2 - S_r$	
$a, h, i \leq j$	

where

- σ_A^2 = additive genetic variance,
- σ_D^2 = dominance variance,
- σ_{AA}^2 = additive $\times$ additive epistatic variance,
- σ_{AD}^2 = additive $\times$ dominance epistatic variance,
- σ_{DD}^2 = dominance $\times$ dominance epistatic variance, etc.

TABLE 5
SIMULATED SPLIT-SPLIT-PLOT DIALLEL DATA OF METHOD 3: CROSSES AND RECIPROALS, NO PARENTS (4 PARENTAL LINES AND 2 REPLICATIONS)
Tabulated data*

		Split Plot							
		1		2		3		4	
Whole Plot	1			133 127	131 122	116 102	110 99	102 90	106 84
	2	133 126	136 127			115 113	117 112	108 97	105 91
	3	110 102	114 100	121 113	117 115			81 76	85 70
	4	105 92	105 89	100 90	101 90	83 79	86 79		

Analysis of Variance

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Replications	1	1,150.5	1,150.5
GCA	3	11,522.4	3,840.8**
Whole-plot error	3	33.2	11.1
SCA	2	119.2	59.6
Split-plot error	2	129.4	64.7
Duplicate split plots in replicates	12	126.2	10.5
Reciprocal crosses	6	47.1	7.8
Duplicate reciprocal crosses	18	101.5	5.6
Total	47	13,229.5	

* Upper and lower values for each vertical pair are from replications 1 and 2, respectively.
** Significant at 0.01 level.

However, both Matzinger and Kempthorne (1956) and Griffing (1958) indicate that estimates of population variance components are biased if the parents are included except in the special circumstance of the absence of epistasis. Griffing (1956*a*, 1958) further shows that estimates of the population variance components are unbiased when the parents are not included in the analysis.

VI. NUMERICAL EXAMPLE OF SPLIT-SPLIT-PLOT DESIGN

Assume that four barley lines have been crossed in all possible combinations, excluding parents, and the resultant seed planted in the split-split-plot design without parents included. The data in Table 5 simulate seed yield in grams per 8-ft plot. These data demonstrate computing formulae and make no attempt to demonstrate biological phenomena. Using the necessary totals calculated from these data, one obtains:

$$\text{C.F.} = (5005)^2/48 = 521,875.5;$$

$$S_t = (133)^2 + (127)^2 + \dots + (79)^2 - \text{C.F.} = 13,229.5;$$

$$S_R = \{(2620)^2 + (2385)^2\}/24 - \text{C.F.} = 1,150.5;$$

$$S_g = \frac{1}{16}\{(2661)^2 + (2740)^2 + \dots + (2194)^2\} - \frac{1}{16}(5005)^2 = 11,522.4;$$

$$S_{Rg} = \frac{1}{6}\{(698)^2 + (624)^2 + \dots + (519)^2\} - \frac{1}{12}\{(1322)^2 + \dots + (1099)^2\} - S_R = 33.2;$$

$$S_s = \frac{1}{8}\{(1035)^2 + (853)^2 + \dots + (639)^2\} - \text{C.F.} - S_g = 119.2;$$

$$S_{Rs} = \frac{1}{4}\{(533)^2 + (502)^2 + \dots + (304)^2\} - \frac{1}{8}\{(1035)^2 + (923)^2 + \dots + (639)^2\} \\ - S_{Rg} - S_R = 129.4;$$

$$S_d = \frac{1}{4}\{(269-264)^2 + (224-226)^2 + \dots + (158-146)^2\} = 126.2;$$

$$S_r = \frac{1}{8}\{(523-512)^2 + (432-421)^2 + \dots + (322-317)^2\} = 47.1;$$

$$S_e = \frac{1}{2}\{(133-136)^2 + (126-127)^2 + \dots + (76-70)^2\} - S_r = 101.5.$$

Two computational checks are as follows

$$(1) S_e = 13,229.5 - 1,150.5 - \dots - 47.1 = 101.5, \text{ and}$$

$$(2) \text{ The sum of squares for genotypes is } \frac{1}{4}\{(523)^2 + (512)^2 + \dots + (317)^2\} - \\ \text{C.F.} = 11,688.7, \text{ which is also } S_g + S_s + S_r (= 11,522.4 + 119.2 + 47.1 = \\ 11,688.7).$$

From the above computations, an analysis of variance is derived as shown also in Table 5. From the mean squares, one obtains

$$s_e^2 = 5.6;$$

$$s_\pi^2 = \frac{1}{8}(7.8 - 5.6) = 0.28;$$

$$s_\delta^2 = \{2(3)(11.1) - (1)(64.7) - 5(5.6)\}/\{2(2)(7)\} = -0.93 \text{ or } 0;$$

$$s_\beta^2 = \{3[1(64.7) + 11.1 - (2)(5.6)]\}/\{2(2)(7)\} = 2.31;$$

$$s_d^2 = \frac{1}{4}\{10.5 - 5.6 - 2(0)\} = 1.22;$$

$$s_\gamma^2 = \frac{1}{8}\{59.6 - 5.6 - 4(2.31)\} = 5.60;$$

$$s_\alpha^2 = \{3840.8 - 5.6 - (2)(0) - 8(5.60) - 4(2.31)\}/\{4(2)(2)\} = 236.3.$$

In the absence of epistasis, additive genetic variance is estimated from

$$\sigma_a^2 = \frac{1}{4}(1+F)\sigma_A^2.$$

Hence

$$s_A^2 = \hat{\sigma}_A^2 = \{4/(1+F)\} s_a^2.$$

Substituting the estimate of σ_a^2 yields

$$s_A^2 = \{4/(1+F)\}(236).$$

If the parental lines are homozygous, $F = 1$, and

$$s_A^2 = 2(236) = 472.$$

Similarly,

$$s_D^2 = \{2/(1+F)\}^2 s_v^2,$$

which yields the estimate

$$s_D^2 = 5.60.$$

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APPENDIX

Derivation of Least Squares Estimates of Whole-plot Effects for the Split-plot Designs for Method 4: Crosses, No Reciprocals or Parents

Consider the portion of the model:

$$X_{gij} = \mu + \rho_g + \alpha_i + \alpha_j + \delta_{gi}.$$

Introducing the customary dummy variables, e.g. Y_g ($= 1$ for the g th replicate, 0 otherwise), the matrix of uncorrected sums of squares and cross-products is:

$$\mathbf{Y}'\mathbf{Y} = \begin{bmatrix} a & \mathbf{b} & \mathbf{c} & \mathbf{d} \\ \mathbf{b}' & E & F & G \\ \mathbf{c}' & F' & H & K \\ \mathbf{d}' & G' & K' & L \end{bmatrix}$$

where

$$\begin{array}{llll} a = rn(n-1) & & E = n(n-1)\mathbf{I} & (r \times r) \\ \mathbf{b} = n(n-1)\mathbf{1} & (1 \times r) & F = 2(n-1)\mathbf{1}'\mathbf{1} & (r \times n) \\ \mathbf{c} = 2r(n-1)\mathbf{1} & (1 \times n) & H = 2r[(n-2)\mathbf{I} + \mathbf{1}'\mathbf{1}] & (n \times n) \\ \mathbf{d} = (n-1)\mathbf{1} & (1 \times nr) & K = [PP \dots P] & (n \times rn) \\ \mathbf{1} = 1 \cdot 1 \dots 1 & & L = (n-1)\mathbf{I} & (rn \times rn) \\ & & P = (n-2)\mathbf{I} + \mathbf{1}'\mathbf{1} & (n \times n) \end{array}$$

$$\mathbf{G} = \begin{bmatrix} n-1 & \dots & n-1 & 0 & \dots & 0 & \dots & 0 & \dots & 0 \\ 0 & \dots & 0 & n-1 & \dots & n-1 & \dots & 0 & \dots & 0 \\ \cdot & & \cdot & & & & & \cdot & & \cdot \\ \cdot & & \cdot & & & & & \cdot & & \cdot \\ \cdot & & \cdot & & & & & \cdot & & \cdot \\ 0 & \dots & 0 & 0 & \dots & 0 & \dots & n-1 & \dots & n-1 \end{bmatrix}. \quad (r \times rn)$$

Also the matrix of uncorrected sum of cross-products with the observed X values gives

$$(\mathbf{Y}'\mathbf{X}) = \begin{bmatrix} X \dots \\ \{X_{g..}\} \\ \{X_{.i.} + X_{..i}\} \\ \{X_{gi.}\} \end{bmatrix};$$

After introducing the usual restrictions that

$$\Sigma \rho_g = \Sigma \alpha_i = \Sigma_g \delta_{gi} = \Sigma_i \delta_{gi} = 0,$$

the reduced matrix is

$$(\mathbf{Y}'\mathbf{Y})_r = \begin{bmatrix} a & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0}' & E_r & 0 & 0 \\ \mathbf{0}' & 0 & H_r & 0 \\ \mathbf{0}' & 0 & 0 & L_r \end{bmatrix}$$

where

$$\begin{aligned} E_r &= n(n-1)M, & (r-1) \times (r-1) \\ H_r &= 2r(n-2)M, & (n-1) \times (n-1) \\ L_r &= (n-1) \begin{bmatrix} 2M & M & \dots & M \\ M & 2M & \dots & M \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ M & M & \dots & 2M \end{bmatrix}, & (r-1)(n-1) \times (r-1)(n-1) \end{aligned}$$

and

$$M = \mathbf{I} + \mathbf{1}\mathbf{1}'.$$

It can be shown that

$$L_r^{-1} = \frac{1}{rn(n-1)} \begin{bmatrix} (r-1)N & -N & \dots & -N \\ -N & (r-1)N & \dots & -N \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ -N & -N & \dots & (r-1)N \end{bmatrix}$$

where

$$N = n\mathbf{I} - \mathbf{1}\mathbf{1}'.$$

It therefore follows from the form of $(\mathbf{Y}'\mathbf{Y})_r$ that

$$\{\hat{\delta}_{gi}\} = L_r^{-1}(\mathbf{Y}'\mathbf{X})_r$$

where

$$(\mathbf{Y}'\mathbf{X})_r = \{X_{gi.} - X_{gn.} - X_{ri.} + X_{rn.}\}.$$

After algebraic reduction, it follows that

$$\{\hat{\delta}_{gi}\} = \{rnX_{gi.} - nX_{.i.} - rX_{g..} + X_{...}\}/rn(n-1).$$

The sum of squares associated with these effects is

$$S_{Rg} = \{\hat{\delta}_{gi}\}'(\mathbf{X}'\mathbf{Y})_r.$$

After a considerable amount of reduction, it can be shown that

$$\begin{aligned} S_{Rg} &= \frac{\Sigma \Sigma X_{gi.}^2}{n-1} - \frac{\Sigma X_{.i.}^2}{r(n-1)} - \frac{\Sigma X_{g..}^2}{n(n-1)} + \frac{X_{...}^2}{rn(n-1)} \\ &= \sum_i \left[\frac{\Sigma_g X_{gi.}^2}{n-1} - \frac{X_{.i.}^2}{r(n-1)} \right] - S_R. \end{aligned}$$