Optimal diallel cross designs for estimation of heritability

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Abstract

Diallel crosses as mating designs are used to study the genetic properties of inbred lines in plant breeding experiments. Most of the theory of optimal diallel cross designs is based on standard linear model assumptions where the general combining ability effects are taken as fixed. In many practical situations, this assumption may not be tenable since we are studying only a sample, of inbred lines, from a possibly large hypothetical population. A random effects model is proposed that allows us to first estimate the variance components and then obtain the variances of the estimates. We address the issue of optimal designs in this context by considering the A-optimality criteria. We obtain designs that are A-optimal for the estimation of heritability in the sense that the designs minimize the sum of the variances of the estimates of the variance components. The approach leads to certain connections with the optimaztion problem under the fixed effects model. Some numerical illustrations are given.

Keywords: A-optimality, Variance components, Heritability.

1. Introduction

Diallel crosses as mating designs are used to study the genetic properties of inbred lines in plant breeding experiments. Plant breeders frequently need overall information on average performance of individual inbred lines in crosses- known as general combining ability, for subsequent choosing the best amongst them for further breeding. For this purpose diallel crossing techniques are employed.

Consider a hypothetical population involving large number of lines and crosses so that all means are estimated without error. Crossing a line to several others provides the mean performance of the line in all its crosses. This mean performance, when expressed as a deviation from the mean of all crosses, is called the general combining ability of the line. Any particular cross, then, has an expected value which is the sum of the general combining abilities of its two parental lines. The cross may, however, deviate from this expected value to a greater or lesser extent. This deviation is called the specific combining ability of the two lines in combination. In statistical terms, the general combining abilities are main effects and the specific combining ability is an interaction. Griffing (1956) defines diallel crosses in terms of genotypic values where the sum of general combining abilities for the two gametes is the breeding value of the cross (i, j). Similarly, specific combining ability represents the dominance deviation value in the simplest case ignoring epistatic deviation; see Kempthorne (1969) and Mayo (1980) for details.

From the population consisting of a large number of lines the plant breeder carries out a diallel cross experiment after drawing a random sample of p lines. Since we are observing a sample

from a large hypothetical population of lines and crosses, the expected value of an observation Y_{ij} (conditional on the realized value of the general combining ability and specific combining ability) arising out of cross (i, j) involving lines i and j, i < j; i, j = 1, ..., p is modeled as

$$E(Y_{ij}) = \mu + g_i^* + g_j^* + s_{ij}^* , \qquad (1.1)$$

where μ is the general mean, g_i^* (g_j^*) is the realized value of g_i (g_j), the general combining ability effect of sampled *i*-th (*j*-th) line and s_{ij}^* is the realized value of s_{ij} , the specific combining ability effect of cross (i, j).

Accordingly, in experimental mating design, the analysis of the observations arising out of n crosses involving p lines will be carried out based on a model

$$Y_{iil} = \mu + g_i + g_j + e_{iil} \; ; \; i < j, \tag{1.2}$$

where Y_{ijl} is the observation out of the l-th replication of the cross (i,j), g_i is the i-th line effect with $E(g_i) = 0$, $Var(g_i) = \sigma_g^2$, $Cov(g_i, g_j) = 0$, μ is the general mean and e_{ijl} is the random error component with expectation zero and variance σ_e^2 , $i < j; i, j = 1, \ldots, p$. Here the specific combining ability effects are assumed to be negligible and have been absorbed in the error component. In the model, as given in (1.2), μ is the fixed effect while g_i , g_j (i < j) and e_{ijl} are random effects. This is the characteristic of what is called a random effects model, named Model II by Eisenhart (1947) and Griffing(1956).

The basic idea in the study of variation among observations arising out of crosses is its partitioning into components attributed to different causes like additive value, dominance deviation and epistatic deviation; see Falconer (1991). The relative magnitude of these components determines the genetic properties of the population. One of such properties is heritability which is of paramount interest to plant breeders to understand the gene action on which depends the breeding policies. The relative importance of heredity in determining phenotypic values is called the heritability of a character in broad sense. Thus the ratio σ_g^2/σ_p^2 gives a measure of heritability, where $\sigma_p^2 = \sigma_g^2 + \sigma_e^2$ is the phenotypic variance and σ_g^2 is the genotypic variance. Such a measure expresses the extent to which individual's phenotypes are determined by the genotypes.

Our primary interest is thus in $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$. It may also be mentioned here that the best linear unbiased predictor (BLUP) of the unobserved line effects $\mu + g_i$ depends on good estimates of σ_g^2 and σ_e^2 . The BLUP(g) of g is used to rank the values of inbred lines, which are unobservable, so that the predictors of g_i and g_j have the same pairwise ranking as g_i and g_j with maximum probability. Thus in order to get a good estimate of h^2 and an efficient BLUP, we propose optimal designs for estimation of σ_g^2 and σ_e^2 . To find the estimates of the variance components σ_g^2 and σ_e^2 , we adopt the Henderson Method III (See, Searle, Casella and McCulloch (1992, pg. 202). Such a method has been used here since it gives an unbiased estimate of the variance components.

An experiment is carried out using a diallel cross design with p lines and n crosses. A diallel cross design is said to be complete if each of the $\binom{p}{2}$ crosses appear equally often in the design otherwise it is said to be a partial diallel cross design. Customarily, diallel cross experiments have been carried out using a completely randomised design or a randomised complete block design. Several methods of obtaining such diallel cross designs have been given, together with their efficiency factors, by Kempthorne and Curnow (1961), Curnow (1963), Hinkelmann and Kempthorne (1963) and Singh and Hinkelmann (1988, 1990). However, with the increase in the number of lines p, the number of crosses in the experiment increases rapidly, and in such a situation, adoption of a complete block design is not appropriate. Singh and Hinkelmann (1995) used conventional partially

balanced incomplete block designs both to select the diallel crosses to be observed and to arrange them into blocks. Some orthogonal blocking schemes had also been advocated by Gupta, Das and Kageyama (1995). Most of the theory of optimal diallel cross designs is based on standard linear model assumptions where the general combining ability effects are taken as fixed and the primary interest lies in comparing the lines with respect to their general combining ability effects. Under such a model, Gupta and Kageyama (1994), Dey and Midha (1996), Mukerjee (1997), Das, Dey and Dean (1998), Das, Dean and Gupta (1998), Chai and Mukerjee (1999), Parsad, Gupta and Srivastava (1999) and Das and Ghosh (1999) have characterised and obtained optimal completely randomised designs and incomplete block designs for diallel crosses. In many practical situations, the fixed effects assumption may not be tenable since we are studying only a sample, of inbred lines, from a possibly large hypothetical population. A random effects model is proposed that allows us to first estimate the variance components and then obtain the variances of the estimates. We address the issue of optimal designs in this context by considering the A-optimality criteria. We obtain designs that are A-optimal for the estimation of heritability in the sense that the designs minimize the sum of the variances of the estimates of the variance components.

In Section 2 we first obtain the estimate of σ_g^2 and σ_e^2 , under an unblocked model, and then obtain the variances of these estimates. In Section 3 similar thing is done under a block model. Finally, in Section 4 we characterize A-optimal designs.

2. Unbiased Estimates of Variance Components and their Variances

In this section we consider the unblocked model. An experiment is carried out using unblocked diallel cross design with p lines and n crosses. From (1.2) we can represent our model in matrix notation as

$$Y = \mu 1 + D_1' g + e, (2.1)$$

where Y is the vector of n observations, g is the $p \times 1$ vector of general combining ability effects with E(g) = 0 and $Var(g) = \sigma_g^2 I$, e is the error vector with E(e) = 0 and $Var(e) = \sigma_e^2 I$, and $D_1 = (d_{uv}^{(1)})$ is the $p \times n$ line versus observation matrix with $d_{uv}^{(1)} = 1$ if v-th observation is out of a cross involving the u-th line and $d_{uv}^{(1)} = 0$ otherwise. Here 1 represents a column vector of all ones and I denotes an identity matrix. We assume that D_1 has full row rank. Equivalently,

$$Y = X \binom{\mu}{g} + e,$$

where $X = (1 \ D'_{1}).$

In general, for a matrix $X = (X_1 \ X_2)$, we have an identity among the matrices of quadratic forms given by,

$$X(X'X)^{-}X' = X_1(X_1'X_1)^{-}X_1' + M_1X_2(X_2'M_1X_2)^{-}X_2'M_1,$$
(2.2)

where T^- is a g-inverse of a matrix T and $M_1 = I - X_1(X_1'X_1)^-X_1'$ is idempotent. From this identity we can obtain three quadratic forms, that is, the total corrected sum of squares (SST), the sum of squares due to lines (SSL) and the sum of squares due to error (SSE). Partitioning SST into SSL and SSE, based on Henderson's Method III, we have

$$SST = SSL + SSE$$
.

Now,

$$SST = Y'MY, (2.3)$$

$$SSL = Y' \left[MD_1' \left(D_1 M D_1' \right)^- D_1 M \right] Y, \tag{2.4}$$

$$SSE = Y'M_0Y, (2.5)$$

where $M = I - \frac{1}{n}11'$ and $M_0 = I - (1 D_1') \left[(1 D_1')' (1 D_1') \right]^- (1 D_1')'$. We now obtain the expected values of SSL and SSE using results given in Searle, Casella and McCulloch (1992, pages 204 and 466). Let $G = D_1 D_1' = (g_{ij})$ and $s = D_1 1$. Using the definition of D_1 it can be verified that for $i \neq j$, g_{ij} gives the number of times cross (i,j) appears in the design, $g_{ii} = s_i$ where $s = (s_1, s_2, \dots, s_p)'$ and s_i is the replication of the *i*-th line. Also, since we assume $Rank(D_1) = p$, G is symmetric with Rank(G) = p and tr(G) = 2n where for a square matrix A, tr (A) stands for the trace.

$$\begin{split} E\left[SSL\right] &= \sigma_g^2 \; \mathrm{tr} \; [MD_1'D_1] + \sigma_e^2 \left(Rank \left(1 \; D_1'\right) - Rank \left(1\right)\right) \\ &= \sigma_g^2 \; \mathrm{tr} \; \left[\left(I - \frac{1}{n}11'\right)D_1'D_1\right] + \sigma_e^2(p-1) \\ &= \sigma_g^2 \; \mathrm{tr} \; \left[D_1D_1' - \frac{1}{n} \left(D_11\right) \left(D_11\right)'\right] + \sigma_e^2(p-1) \\ &= \sigma_g^2 \; \mathrm{tr} \; \left[G - \frac{1}{n}ss'\right] + \sigma_e^2(p-1), \end{split}$$

and

$$E[SSE] = (n-p)\sigma_e^2$$

Thus,

$$E \begin{bmatrix} SSL \\ SSE \end{bmatrix} = L \begin{pmatrix} \sigma_g^2 \\ \sigma_e^2 \end{pmatrix} = L \sigma^2, \tag{2.6}$$

where $L = \begin{pmatrix} \operatorname{tr} \left[G - \frac{1}{n} s s' \right] & (p-1) \\ 0 & (n-p) \end{pmatrix}$, and $\sigma^2 = \begin{pmatrix} \sigma_g^2 \\ \sigma_e^2 \end{pmatrix}$.

From (2.6) it follows that an unbiased estimator of σ^2 is

$$\hat{\sigma}^2 = \begin{pmatrix} \hat{\sigma}_g^2 \\ \hat{\sigma}_e^2 \end{pmatrix} = L^{-1} \begin{pmatrix} SSL \\ SSE \end{pmatrix}, \tag{2.7}$$

where $L^{-1} = \frac{1}{(n-p)\operatorname{tr}\left[G - \frac{1}{n}ss'\right]} \begin{pmatrix} n-p & -(p-1) \\ 0 & \operatorname{tr}\left[G - \frac{1}{n}ss'\right] \end{pmatrix}$.

Let $C_0 = G - \frac{1}{n}ss'$ and $W = D_1' - \frac{1}{n}1s'$. Then $L^{-1} = \frac{1}{(n-p)\operatorname{tr} C_0} \begin{pmatrix} n-p & -(p-1) \\ 0 & \operatorname{tr} C_0 \end{pmatrix}$. Also, we can write

$$SSL = Y' \left(MD_1' \left(D_1 M D_1' \right)^- D_1 M \right) Y = Y' W C_0^- W' Y, \tag{2.8}$$

since

$$MD_1' = D_1' - \frac{1}{n}1s' = W,$$
 (2.9)

and

$$D_1 M D_1' = D_1 \left(I - \frac{1}{n} 11' \right) D_1' = D_1 D_1' - \frac{1}{n} ss' = G - \frac{1}{n} ss' = C_0.$$
 (2.10)

Note that,

$$D_1W = D_1 \left(D_1' - \frac{1}{n} 1s' \right)$$

= $G - \frac{1}{n} ss' = C_0,$

and

$$W'W = (D_1 - \frac{1}{n}s1')(D_1' - \frac{1}{n}1s')$$
$$= G - \frac{1}{n}ss' = C_0.$$

Also, since $C_0 1 = 0$, $Rank(C_0) \le p-1$. However, since $Rank(D_1) = p$, it follows that $Rank(C_0) = p-1$. To study the sampling distribution of $\hat{\sigma}^2$ in terms of its sampling variance-covariance matrix, we first derive the dispersion matrix of $\binom{SSL}{SSE}$.

Let $A_1 = WC_0^-W'$. Then using the results given in Searle, Casella and McCulloch (1992, pg. 467) on variance and covariance of quadratic forms, under normality, we obtain

$$\begin{split} Var(SSL) &= Var(Y'WC_0^-W'Y) \\ &= Var(Y'A_1Y) \\ &= 2 \text{ tr } (A_1V)^2 \text{ where } V = Disp(Y) = \sigma_g^2 D_1' D_1 + \sigma_e^2 I \\ &= 2 \text{ tr } \left[WC_0^-W'\left(\sigma_g^2 D_1' D_1 + \sigma_e^2 I\right)\right]^2 \\ &= 2 \text{ tr } \left[\sigma_g^4 WC_0^-W'D_1' D_1 WC_0^-W'D_1' D_1 + \sigma_g^2 \sigma_e^2 WC_0^-W'D_1' D_1 WC_0^-W' + \sigma_g^2 \sigma_e^2 WC_0^-W'WC_0^-W'D_1' D_1 + \sigma_e^4 WC_0^-W'WC_0^-W'\right]. \end{split}$$

Now evaluating each of the above four terms, we have

$$\operatorname{tr} \left[\sigma_g^4 W C_0^- W' D_1' D_1 W C_0^- W' D_1' D_1 \right]$$

$$= \sigma_g^4 \operatorname{tr} \left[(D_1 W) C_0^- (D_1 W)' (D_1 W) C_0^- (D_1 W)' \right]$$

$$= \sigma_g^4 \operatorname{tr} \left[C_0 C_0^- C_0 C_0 C_0^- C_0 \right]$$

$$= \sigma_g^4 \operatorname{tr} C_0^2,$$

$$\operatorname{tr} \left[\sigma_g^2 \sigma_e^2 W C_0^- W' D_1' D_1 W C_0^- W' \right]$$

$$= \sigma_g^2 \sigma_e^2 \operatorname{tr} \left[(W' W) C_0^- (D_1 W)' (D_1 W) C_0^- \right]$$

$$= \sigma_g^2 \sigma_e^2 \operatorname{tr} \left[C_0 C_0^- C_0 C_0 C_0^- \right]$$

$$= \sigma_g^2 \sigma_e^2 \operatorname{tr} \left[C_0 C_0^- C_0 C_0^- \right]$$

$$= \sigma_g^2 \sigma_e^2 \operatorname{tr} \left[C_0 C_0^- C_0 \right]$$

$$= \sigma_g^2 \sigma_e^2 \operatorname{tr} C_0,$$

$$\operatorname{tr} \left[\sigma_g^2 \sigma_e^2 W C_0^- W' W C_0^- W D_1' D_1 \right]$$

$$= \sigma_g^2 \sigma_e^2 \operatorname{tr} \left[(D_1 W) C_0^- C_0 C_0^- (D_1 W)' \right]$$

$$= \sigma_g^2 \sigma_e^2 \operatorname{tr} \left[C_0 C_0^- C_0 C_0^- C_0 \right]$$

$$= \sigma_g^2 \sigma_e^2 \operatorname{tr} \left[C_0 C_0^- C_0 \right]$$

$$= \sigma_g^2 \sigma_e^2 \operatorname{tr} C_0,$$

$$\operatorname{tr} \left[\sigma_e^4 W C_0^- W' W C_0^- W' \right]$$

$$= \sigma_e^4 \operatorname{tr} \left[(W' W) C_0^- (W' W) C_0^- \right]$$

$$= \sigma_e^4 \operatorname{tr} \left[C_0 C_0^- C_0 C_0^- \right]$$

$$= \sigma_e^4 \operatorname{tr} \left[C_0 C_0^- C_0 C_0^- \right]$$

$$= \sigma_e^4 \operatorname{Rank}(C_0)$$

$$= (p-1) \sigma_e^4.$$

Therefore,

$$Var(SSL) = 2\{\sigma_q^4 \operatorname{tr} C_0^2 + 2\sigma_e^2 \sigma_q^2 \operatorname{tr} C_0 + (p-1)\sigma_e^4\}.$$

Let $A_2 = M_0$. Now using the fact $D_1 M_0 = 0$ and $A_1 M_0 = 0$, we have

$$A_1 V A_2 = A_1 \left(\sigma_q^2 D_1' D_1 + \sigma_e^2 I \right) M_0 = A_1 \left(\sigma_q^2 D_1' (D_1')' M_0 + \sigma_e^2 M_0 \right) = 0.$$

Therefore,

$$Cov (SSL, SSE)$$

$$= Cov (Y'A_1 Y, Y'A_2 Y)$$

$$= 2 \operatorname{tr} [A_1 V A_2 V]$$

$$= 0.$$

Finally,

$$V(SSE) = V(Y'A_2Y)$$

$$= 2 \operatorname{tr} \left[M_0 \left(\sigma_g^2 D_1' D_1 + \sigma_e^2 I \right) M_0 \left(\sigma_g^2 D_1' D_1 + \sigma_e^2 I \right) \right]$$

$$= 2 \operatorname{tr} \left[\sigma_e^4 M_0 M_0 \right]$$

$$= 2 \sigma_e^4 \operatorname{tr} M_0$$

$$= 2(n-p) \sigma_e^4.$$

Thus, we now have

$$Disp\binom{SSL}{SSE} = \begin{pmatrix} 2\{\sigma_g^4 \text{ tr } C_0^2 + 2\sigma_e^2 \sigma_g^2 \text{ tr } C_0 + (p-1)\sigma_e^4\} & 0\\ 0 & 2(n-p)\sigma_e^4 \end{pmatrix}.$$
(2.11)

From (2.7) and (2.11) we finally have

$$Disp\begin{pmatrix} \hat{\sigma}_g^2 \\ \hat{\sigma}_e^2 \end{pmatrix} = L^{-1}Disp\begin{pmatrix} SSL \\ SSE \end{pmatrix}(L^{-1})' = 2\begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix},$$
 (2.12)

where

where
$$a_{11} = \{(n-p)(\sigma_g^4 \operatorname{tr} C_0^2 + 2\sigma_e^2 \sigma_g^2 \operatorname{tr} C_0 + \sigma_e^4) + \sigma_e^4 (p-1)^2\} / \{(n-p)(\operatorname{tr} C_0)^2\},$$

$$a_{12} = a_{21} = -\sigma_e^4 (p-1) / \{(n-p) \operatorname{tr} C_0\},$$
and
$$a_{22} = \sigma_e^4 / (n-p).$$

3. Unbiased Estimates and their Variances under a Block Model

An experiment is carried out using a diallel cross design with p lines and b blocks each having k crosses. Here, n = bk.

We represent our model in matrix notation as

$$Y = \mu 1 + D_2'\beta + D_1'g + e, (3.1)$$

where as before, Y is the vector of n observations, g is the $p \times 1$ vector of general combining ability effects with E(g) = 0 and $Var(g) = \sigma_g^2 I$, β is the fixed effect due to blocks and e is the error vector with E(e) = 0 and $Var(e) = \sigma_e^2 I$. Also, $D_1 = (d_{uv}^{(1)})$ is the $p \times n$ line verses observation matrix, as mentioned earlier, and $D_2 = (d_{uv}^{(2)})$ is the $b \times n$ block versus observation matrix with $d_{uv}^{(2)} = 1$ if the v-th observation arise from the u-th block and $d_{uv}^{(2)} = 0$ otherwise. Equivalently,

$$Y = X \begin{pmatrix} \mu \\ \beta \\ g \end{pmatrix} + e,$$

where $X = \begin{pmatrix} 1 & D_2' & D_1' \end{pmatrix}$. Then,

$$X'X = \begin{pmatrix} 1'1 & 1'D_2' & 1'D_1' \\ D_21 & D_2D_2' & D_2D_1' \\ D_11 & D_1D_2' & D_1D_1' \end{pmatrix}, \tag{3.2}$$

where $D_1 1 = s$, $D_2 1 = k1$, $D_1 D'_1 = G$, $D_2 D'_2 = kI$, $D_1 D'_2 = N$ and $N = (n_{ij})$ is the incidence matrix with n_{ij} indicating the number of times the *i*-th line occurs in the *j*-th block. In our model (3.1) we may consider β to be a random effects block parameter. Such a consideration do not alter the results obtained here.

Now, using the identity given in (2.2), we can obtain four quadratic forms, that is, the total corrected sum of squares (SST), the sum of squares due to lines (SSL), the sum of squares due to blocks (SSB) and the sum of squares due to error (SSE). Partitioning SST into SSL, SSB and SSE, based on Henderson's Method III, we have

$$SST = SSL + SSB + SSE.$$

Now, as in previous section, SST = Y'MY,

$$SSL = Y' \left[M_2 D_1' \left(D_1 M_2 D_1' \right)^- D_1 M_2 \right] Y, \tag{3.3}$$

$$SSB + SSL = Y' \left[M(D_2' \ D_1') \{ (D_2' \ D_1')' M(D_2' \ D_1') \}^{-} (D_2' \ D_1')' M \right] Y, \tag{3.4}$$

$$SSE = Y'M_3Y, (3.5)$$

where

$$M = I - \frac{1}{n} 11',$$

$$M_2 = I - (1 \ D_2') \left[(1 \ D_2')' (1 \ D_2') \right]^- (1 \ D_2')',$$

$$M_3 = I - (1 \ D_2' \ D_1') \left[(1 \ D_2' \ D_1')' (1 \ D_2' \ D_1') \right]^- (1 \ D_2' \ D_1')'.$$

Let $C = G - k^{-1}NN'$. It is easy to see that $Rank(C) \leq p-1$. We now obtain the expected values of SSL, SSB and SSE. Here we shall use the fact that Rank(X) = b + Rank(C) and also use results given in Searle, Casella and McCulloch (1992, pages 204 and 466).

$$E[SSL] = \sigma_g^2 \operatorname{tr} [M_2 D_1' D_1] + \sigma_e^2 (Rank(X) - Rank(1 D_2'))$$

$$= \sigma_g^2 \operatorname{tr} \left[D_1 D_1' - D_1 (1 D_2') \begin{pmatrix} 0 & 0' \\ 0 & k^{-1/2} I \end{pmatrix} \begin{pmatrix} 0 & 0' \\ 0 & k^{-1/2} I \end{pmatrix}' (1 D_2')' D_1' \right]$$

$$+ \sigma_e^2 ((b + Rank(C)) - b)$$

$$= \sigma_g^2 \operatorname{tr} \left[G - k^{-1} (D_1 D_2') (D_1 D_2')' \right] + \sigma_e^2 Rank(C)$$

$$= \sigma_g^2 \operatorname{tr} \left[G - k^{-1} NN' \right] + \sigma_e^2 Rank(C)$$
and
$$E[SSE] = (n - b - Rank(C)) \sigma_e^2.$$

Therefore,

$$E\begin{bmatrix} SSL \\ SSE \end{bmatrix} = L \begin{pmatrix} \sigma_g^2 \\ \sigma_e^2 \end{pmatrix} = L \sigma^2, \tag{3.6}$$

where $L = \begin{pmatrix} \operatorname{tr} C & Rank(C) \\ 0 & n - b - Rank(C) \end{pmatrix}$.

From (3.6) it follows that an unbiased estimator of σ^2 is

$$\hat{\sigma}^2 = \begin{pmatrix} \hat{\sigma}_g^2 \\ \hat{\sigma}_e^2 \end{pmatrix} = L^{-1} \begin{pmatrix} SSL \\ SSE \end{pmatrix},$$
 where $L^{-1} = \frac{1}{(n-b-Rank(C)) \text{ tr } C} \begin{pmatrix} n-b-Rank(C) & -Rank(C) \\ 0 & \text{ tr } C \end{pmatrix}$. Note that since $D_1 M_2 D_1' = G - \frac{1}{k} NN' = C$ we can write,

$$SSL = Y' (M_2 D'_1 (D_1 M_2 D'_1)^- D_1 M_2) Y$$

= Y'HC^-H'Y

where $H = M_2 D_1' = D_1' - k^{-1} D_2' N'$.

The following results are useful and would be used subsequently.

$$D_{1}H = D_{1}D'_{1} - k^{-1}D_{1}D'_{2}N'$$

$$= G - \frac{1}{k}NN' = C,$$

$$H'H = \left(D_{1} - k^{-1}ND_{2}\right)\left(D'_{1} - k^{-1}D'_{2}N'\right)$$

$$= D_{1}D'_{1} - k^{-1}NN' = C,$$
and
$$D_{2}H = D_{2}\left(D'_{1} - k^{-1}D'_{2}N'\right)$$

$$= N' - k^{-1}kN' = 0.$$

To study the sampling distribution of $\hat{\sigma}^2$ in terms of its sampling variance-covariance matrix we now, working on lines similar to that in Section 2, obtain the dispersion matrix of $\binom{SSL}{SSE}$.

Let $B_1 = HC^-H'$. Then,

$$\begin{split} Var(SSL) &= Var(Y'HC^-H'Y) \\ &= Var(Y'B_1Y) \\ &= 2 \text{ tr } (B_1V)^2 \text{ where } V = Disp(Y) = \sigma_g^2 D_1' D_1 + \sigma_e^2 I \\ &= 2 \text{ tr } \left[HC^-H' \left(\sigma_g^2 D_1' D_1 + \sigma_e^2 I \right) \right]^2 \\ &= 2 \text{ tr } \left[\sigma_g^4 HC^-H'D_1' D_1 HC^-H'D_1' D_1 + \sigma_g^2 \sigma_e^2 HC^-H'D_1' D_1 HC^-H' + \sigma_g^2 \sigma_e^2 HC^-H'HC^-H'D_1' D_1 + \sigma_e^4 HC^-H'HC^-H' \right]. \end{split}$$

Now evaluating each of the above four terms, as in unblocked case, we have

$$\operatorname{tr}\left[\sigma_g^4 H C^- H' D_1' D_1 H C^- H' D_1' D_1\right] = \sigma_g^4 \operatorname{tr} C^2,$$

$$\operatorname{tr}\left[\sigma_g^2 \sigma_e^2 H C^- H' D_1' D_1 H C^- H'\right] = \sigma_g^2 \sigma_e^2 \operatorname{tr} C,$$

$$\operatorname{tr}\left[\sigma_g^2 \sigma_e^2 H C^- H' H C^- H D_1' D_1\right] = \sigma_g^2 \sigma_e^2 \operatorname{tr} C,$$
and
$$\operatorname{tr}\left[\sigma_e^4 H C^- H' H C^- H'\right] = \sigma_e^4 \operatorname{Rank}(C).$$

Therefore,

$$Var(SSL) = 2\{\sigma_q^4 \operatorname{tr} C^2 + 2\sigma_e^2 \sigma_q^2 \operatorname{tr} C + \sigma_e^4 \operatorname{Rank}(C)\}.$$

Let $B_2 = M_3$. Now using the fact $D_1 M_3 = 0$ and $B_1 M_3 = 0$, we have

$$B_1 V B_2 = B_1 \left(\sigma_g^2 D_1' D_1 + \sigma_e^2 I \right) M_3 = B_1 \left(\sigma_g^2 D_1' (D_1')' M_3 + \sigma_e^2 M_3 \right) = 0.$$

Therefore,

$$Cov (SSL, SSE)$$

$$= Cov (Y'B_1 Y, Y'B_2 Y)$$

$$= 2 \operatorname{tr} [A_1 V A_2 V]$$

$$= 0.$$

Finally,

$$V(SSE) = V(Y'B_2Y)$$

$$= 2 \operatorname{tr} \left[M_3 \left(\sigma_g^2 D_1' D_1 + \sigma_e^2 I \right) M_3 \left(\sigma_g^2 D_1' D_1 + \sigma_e^2 I \right) \right]$$

$$= 2 \operatorname{tr} \left[\sigma_e^4 M_3 M_3 \right]$$

$$= 2\sigma_e^4 \operatorname{tr} M_3$$

$$= 2(n - b - Rank(C)) \sigma_e^4.$$

Thus, we now have

$$Disp\binom{SSL}{SSE} = \begin{pmatrix} 2\{\sigma_g^4 \operatorname{tr} C^2 + 2\sigma_e^2 \sigma_g^2 \operatorname{tr} C + \sigma_e^4 \operatorname{Rank}(C)\} & 0 \\ 0 & 2(n - b - \operatorname{Rank}(C)) \sigma_e^4 \end{pmatrix}. \quad (3.8)$$

From (3.7) and (3.8) we finally have

$$Disp\begin{pmatrix} \hat{\sigma}_g^2 \\ \hat{\sigma}_e^2 \end{pmatrix} = L^{-1}Disp\begin{pmatrix} SSL \\ SSE \end{pmatrix}(L^{-1})' = 2\begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix}, \tag{3.9}$$

where

 $a_{11} = \{(n-b-Rank(C))(\sigma_g^4 \text{ tr } C^2 + 2\sigma_e^2\sigma_g^2 \text{ tr } C + \sigma_e^4) + \sigma_e^4 (Rank(C))^2\} / \{(n-b-Rank(C))(\text{tr } C)^2\},$ $a_{12} = a_{21} = -\sigma_e^4 Rank(C) / \{(n-b-Rank(C)) \text{ tr } C\},$ and

 $a_{22} = \sigma_e^4 / (n - b - Rank(C)).$

In the remaining paper we take Rank(C) = p - 1 since we assume that $D'_2 1 = \frac{1}{2}D'_1 1 = 1$ are the only two independent restrictions among the columns of the design matrix.

4. A-Optimal Designs

In Section 2 and Section 3 we have explicitly obtained the variance of $\hat{\sigma}_g^2$ and $\hat{\sigma}_e^2$ under an unblocked and a blocked model. Let $\mathcal{D}(p,n)$ be the class of diallel cross unblocked designs involving p lines and n crosses and $\mathcal{D}(p,b,k)$ the class of diallel cross designs with p lines arranged in b blocks of k crosses each. Also, we use $\mathcal{D}_0(p,n)$ to denote the subclass of designs in $\mathcal{D}(p,n)$ having designs with $s_i = s = 2n/p$; $i = 1, \ldots, p$. In fact, among designs in $\mathcal{D}(p,n)$, only designs in the subclass $\mathcal{D}_0(p,n)$ has maximal tr C_0 . Finally, $\mathcal{D}_0(p,b,k)$ is the subclass of designs in $\mathcal{D}(p,b,k)$ for which tr C is maximum. A design d is said to be A-optimal if, among all designs in \mathcal{D} , d minimizes $Var(\hat{\sigma}_g^2) + Var(\hat{\sigma}_e^2)$. We need the following wellknown result, see, for example, Cheng (1978, page 1246).

Lemma 4.1 For given positive integers v and t, the minimum of $n_1^2 + n_2^2 + \cdots + n_v^2$ subject to $n_1 + n_2 + \cdots + n_v = t$, where n_i 's are non-negative integers, is obtained when t - v[t/v] of the n_i 's are equal to [t/v] + 1 and v - t + v[t/v] are equal to [t/v], where [z] denotes the largest integer not exceeding z. The corresponding minimum of $n_1^2 + n_2^2 + \cdots + n_v^2$ is t(2[t/v] + 1) - v[t/v]([t/v] + 1). We now give a Lemma that would be used subsequently.

Lemma 4.2 Consider a real symmetric square matrix A of order m having rank r. Then

$$\frac{tr\,A^2}{(tr\,A)^2} \ge \frac{1}{r}$$

and the equality is attained when the non-zero eigenvalues of A are equal.

Proof. Let $\lambda_1, \lambda_2, \ldots, \lambda_r$ be the non-zero eigenvalues of A. Then

$$\frac{\operatorname{tr} A^2}{(\operatorname{tr} A)^2} = \frac{\sum_{i=1}^r \lambda_i^2}{(\sum_{i=1}^r \lambda_i)^2}$$

But,

$$\sum_{i=1}^{r} (\lambda_i - \frac{1}{r} \sum_{i=1}^{r} \lambda_i)^2 \geq 0$$
or,
$$\sum_{i=1}^{r} \lambda_i^2 - \frac{(\sum_{i=1}^{r} \lambda_i)^2}{r} \geq 0$$
or,
$$\frac{\sum_{i=1}^{r} \lambda_i^2}{(\sum_{i=1}^{r} \lambda_i)^2} \geq \frac{1}{r}$$

and the result follows.

In the unblocked situation, rewriting the variance expressions as given in (2.12), we have

$$Var(\hat{\sigma}_g^2) = 2 \, \sigma_g^4 \, \left[\frac{\operatorname{tr} \, C_0^2}{(\operatorname{tr} \, C_0)^2} \right] + 4 \, \sigma_e^2 \sigma_g^2 \, \left[\frac{1}{\operatorname{tr} \, C_0} \right] + 2 \, \sigma_e^4 \, \left[\frac{(p(p-3)+n+1)}{(n-p) \, (\operatorname{tr} \, C_0)^2} \right]$$

and

$$Var(\hat{\sigma}_e^2) = 2 \sigma_e^4/(n-p).$$

In order to minimize $Var(\hat{\sigma}_g^2) + Var(\hat{\sigma}_e^2)$, within the class of designs $\mathcal{D}(p, n)$, it is sufficient to minimize $\frac{\operatorname{tr} C_0^2}{(\operatorname{tr} C_0)^2}$ and $\frac{1}{\operatorname{tr} C_0}$.

Similarly from (3.9) it follows that an A-optimal design in $\mathcal{D}(p, b, k)$ minimizes $\frac{\operatorname{tr} C^2}{(\operatorname{tr} C)^2}$ and $\frac{1}{\operatorname{tr} C}$. Next we have the following two Lemmas

Lemma 4.3 For any design $d \in \mathcal{D}(p, n)$,

$$\operatorname{tr}(C_{0d}) \le 2n(p-2)/p.$$

Equality holds if and only if $s_{d_i} = 2n/p = s$ for i = 1, ..., p.

Proof. For any design $d \in \mathcal{D}(p, n)$,

$$\operatorname{tr}(C_{0d}) = \sum_{i=1}^{p} s_{di} - \frac{1}{n} \sum_{i=1}^{p} s_{di}^{2}.$$

Now, since $\sum_{i=1}^{p} s_{di} = 2n$ and 2n/p = s, using Lemma 4.1,

$$\sum_{i=1}^{p} s_{di}^2 \ge 4n^2/p.$$

Hence,

$$\operatorname{tr}(C_{0d}) \le 2n - 4n/p = 2n(p-2)/p.$$

By Lemma 4.1, equality above is attained if and only if $s_{di} = 2n/p = s$ for i = 1, ..., p.

Lemma 4.4 For any design $d \in \mathcal{D}(p, b, k)$,

tr
$$(C_d) \le k^{-1}b\{2k(k-1-2x) + px(x+1)\},\$$

where x = [2k/p]. Equality holds if and only if $n_{dij} = x$ or x+1 for all i = 1, 2, ..., p, j = 1, 2, ..., b.

Proof. For any $d \in \mathcal{D}(p, b, k)$, we have

$$\operatorname{tr}(C_d) = \sum_{i=1}^{p} s_{di} - k^{-1} \sum_{i=1}^{p} \sum_{j=1}^{b} n_{dij}^2$$
$$= 2bk - k^{-1} \sum_{i=1}^{p} \sum_{j=1}^{b} n_{dij}^2.$$

Now, $\sum_{i=1}^{p} \sum_{j=1}^{b} n_{dij} = 2bk$. Therefore, using Lemma 4.1,

$$\sum_{i=1}^{p} \sum_{j=1}^{b} n_{dij}^{2} \ge b\{2k(2x+1) - px(x+1)\},\,$$

where x = [2k/p]. Hence,

tr
$$(C_d) \le 2bk - k^{-1}b\{2k(2x+1) - px(x+1)\}\$$

= $k^{-1}b\{2k(k-1-2x) + px(x+1)\}.$

By Lemma 4.1, equality above is attained if and only if $n_{dij} = x$ or x + 1, for i = 1, 2, ..., p; j = 1, 2, ..., b.

Note that if 2k < p then x = 0 and in that case we have

$$\operatorname{tr}(C_d) \le 2b(k-1), \quad d \in \mathcal{D}(p, b, k). \tag{4.1}$$

Making an appeal to the results of Lemmas 4.2 and 4.3, we have the following result.

Theorem 4.1 Let $d_0^* \in \mathcal{D}(p, n)$ be a diallel cross design, and suppose $C_{0d_0^*}$ satisfies

- (i) $tr(C_{0d_0^*}) = 2n(p-1)/p$, and
- (ii) $C_{0d_0^*}$ is completely symmetric in the sense that $C_{0d_0^*}$ has all its diagonal elements equal and all its off-diagonal elements equal.

Then d_0^* is A-optimal in $\mathcal{D}(p, n)$.

Theorem 4.1 establishes the A- optimality of complete diallel cross designs in $\mathcal{D}(p, n)$. Again, making an appeal to the results of Lemmas 4.2 and 4.4, we have

Theorem 4.2 Let $d^* \in \mathcal{D}(p, b, k)$ be a block design for diallel crosses, and suppose C_{d^*} satisfies

- (i) $tr(C_{d^*}) = k^{-1}b\{2k(k-1-2x) + px(x+1)\}$, and
- (ii) C_{d^*} is completely symmetric.

Then d^* is A-optimal in $\mathcal{D}(p, b, k)$.

We now show a connection between nested balanced incomplete block design of Preece (1967) and optimal designs for diallel crosses. For completeness, we recall the definition of a nested balanced incomplete block design.

Definition 4.1 A nested balanced incomplete block design with parameters $(v, b_1, k_1, r^*, \lambda_1, b_2, k_2, \lambda_2, m)$ is a design for v treatments, each replicated r^* times with two systems of blocks such that:

- (a) the second system is nested within the first, with each block from the first system, called henceforth as 'block' containing exactly m blocks from the second system, called hereafter as 'sub-blocks':
- (b) ignoring the second system leaves a balanced incomplete block design with usual parameters $v, b_1, k_1, r^*, \lambda_1$;
- (c) ignoring the first system leaves a balanced incomplete block design with parameters $v, b_2, k_2, r^*, \lambda_2$.

From the well-known parametric relations for a balanced incomplete block design, it is easy to see that the following parametric relations hold for a nested balanced incomplete block design:

$$vr^* = b_1k_1 = mb_1k_2 = b_2k_2, (v-1)\lambda_1 = (k_1-1)r^*, (v-1)\lambda_2 = (k_2-1)r^*.$$

Consider now a nested balanced incomplete block design d with parameters $v = p, b_1, k_1, k_2 = 2, r^*$. If we identify the treatments of d as lines of a diallel experiment and perform crosses among the lines appearing in the same sub-block of d, we get a block design d^* for a diallel experiment involving p lines with $v_c = p(p-1)/2$ crosses, each replicated $r = 2b_2/\{p(p-1)\}$ times, and $b = b_1$ blocks, each of size $k = k_1/2$. Such a design $d^* \in \mathcal{D}(p, b, k)$; also, for such a design, $n_{d^*ij} = 0$ or 1 for $i = 1, 2, \ldots, p, j = 1, 2, \ldots, b$ and $C_{d^*} = (p-1)^{-1}2b(k-1)(I-p^{-1}11')$. Clearly, C_{d^*} given above is completely symmetric and tr $(C_{d^*}) = 2b(k-1)$ which equals the upper bound for tr (C_d) given by (4.1). Thus, from Theorem 4.1, the design d^* is A-optimal in $\mathcal{D}(p, b, k)$. Summarizing, therefore, we have

Theorem 4.3 The existence of a nested balanced incomplete block design d with parameters $v = p, b_1 = b, b_2 = bk, k_1 = 2k, k_2 = 2$ implies the existence of an A-optimal incomplete block design d^* for diallel crosses.

The construction methods and elaborate tables of nested balanced incomplete block designs are available in a recent review paper by Morgan, Preece and Rees (2000). The tables in their paper provide solutions to our A-optimal diallel cross designs within the parametric range $2k , <math>s \leq 30$. The case 2k = p is dealt in Gupta and Kageyama (1994). The nested balanced incomplete block designs have been extended to nested balanced block designs and a series of designs, A-optimal under our setup, is given in Das, Dey and Dean (1998).

We now have the following

Theorem 4.4 A design d_0^* with p lines is A-optimal in $\mathcal{D}_0(p,n)$ with s = 2n/p if and only if the number of times, $g_{d_0^*ii'}$, that cross(i,i') occurs in d_0^* satisfies

$$|g_{d_0^*ii'} - s/(p-1)| < 1 \text{ for } i \neq i', i, i' = 1, \dots, p.$$

Proof. For any design $d_0 \in \mathcal{D}_0(p,n)$, $C_{0d_0} = G_{d_0} - \frac{2s}{p}11'$ and

$$\operatorname{tr}(C_{0d_0}) = 2n - \frac{4n}{p} = \frac{2n(p-2)}{p},$$

which is fixed for the class of competing designs. Now, using the fact that $\sum \sum_{i < i'} g_{d_0 i i'} = n$,

$$\operatorname{tr}(C_{0d_0}^2) = \sum \sum g_{d_0ii'}^2 - \frac{4s}{p} \sum \sum g_{d_0ii'} + 4s^2$$

$$= 2 \sum_{i < i'} \sum_{i'} g_{d_0ii'}^2 + s^2 p - \frac{8s}{p} \sum_{i < i'} \sum_{i'} g_{d_0ii'}$$

$$= s^2 p - 8sn/p + 2 \sum_{i < i'} \sum_{i'} g_{d_0ii'}^2$$

$$= s^2 (p - 4) + 2 \sum_{i < i'} \sum_{i'} g_{d_0ii'}^2.$$

But, from Lemma 4.1, with v = p(p-1)/2 and t = n, we have

$$\sum_{i < j} \sum_{i'} g_{d_0 i i'}^2 \ge n(2[s/(p-1)] + 1) - \frac{(p(p-1))}{2}[s/(p-1)]([s/(p-1)] + 1).$$

Hence,

tr
$$(C_{0d_0}^2) \ge s^2(p-4) + n(2[s/(p-1)]) + 1) - \frac{p(p-1)}{2}[s/(p-1)]([s/(p-1)] + 1).$$

By Lemma 4.1, equality above is attained if and only if $g_{d_0ii'} = [s/(p-1)]$ or [s/(p-1)] + 1, for $i \neq i'$.

From Theorem 4.4, partial diallel cross designs in which every line appears the same number s=2n/p of times and in which each cross appears either $\lambda=[s/(p-1)]$ or $\lambda+1$ times are A-optimal. A common way to construct a partial diallel cross design is to take a conventional binary incomplete block design with p treatments each occurring s times, n distinct blocks of size 2 and treatment concurrences λ and $\lambda+1$ (called the auxiliary design by Singh and Hinkelmann, 1995) and to form crosses between the two treatments in each block. Any such partial diallel cross design satisfies the conditions of Theorem 4.4 and is A-optimal. Among others, this includes the M-designs of Singh and Hinkelmann (1995), the first series of designs of Mukerjee (1997), and the designs formed from the basic plans listed by Gupta, Das and Kageyama (1995).

Finally, we have

Theorem 4.5 A design d^* in $\mathcal{D}_0(p, b, k)$ with 2k/p an integer is A-optimal in $\mathcal{D}_0(p, b, k)$ if and only if the number of times, $g_{d^*ii'}$, that cross (i, i') occurs in d^* satisfies

$$|g_{d^*ii'} - s/(p-1)| < 1 \text{ for } i \neq i', i, i' = 1, \dots, p.$$

Proof. It follows from Lemma 4.4 that a design d in $\mathcal{D}_0(p,b,k)$ with 2k/p integer has $n_{dij}=2k/p$ for all $i=1,2,\ldots,p, j=1,2,\ldots,b$. Thus, each line occurs s=2kb/p times and $C_d=G_d-\frac{4bk}{p^2}11'=G_d-\frac{2s}{p}11'$ with tr (C_d) fixed for the class of competing designs.

Now, using the fact that n = bk and arguing on lines similar to the proof of Theorem 4.4 the result follows.

Das, Dean and Gupta (1998) gave two general methods of construction of Partial diallel cross designs. Their designs belong to $\mathcal{D}_0(p, b, k)$ with 2k/p an integer. Moreover the designs satisfy the conditions of Theorem 4.5 and are thus A-optimal in $\mathcal{D}_0(p, b, k)$.

Example 4.1 Suppose we have p = 8 lines and n = 16 crosses. We recommend the following design: $\{(1,6);(2,5);(3,4);(0,7);(2,0);(3,6);(4,5);(1,7);(3,1);(4,0);(5,6);(2,7);(5,3);(6,2);(0,1);(4,7)\}$. This design is A-optimal in $\mathcal{D}_0(8,16)$. The condition of Theorem 4.4 is satisfied since every cross (i,i') appears 0 or 1 time in the design.

Example 4.2 Consider the following design (rows are blocks) with parameters p = 8, b = 4 and k = 4.

 $\begin{array}{ccccc} (1,6) & (2,5) & (3,4) & (0,7) \\ (2,0) & (3,6) & (4,5) & (1,7) \\ (3,1) & (4,0) & (5,6) & (2,7) \\ (5,3) & (6,2) & (0,1) & (4,7) \end{array}$

This design is A-optimal in $\mathcal{D}_0(8, 4, 4)$. An A-optimal design in $\mathcal{D}_0(8, 11, 4)$ with b = 11 blocks of size k = 4 can be obtained by appending the full set of p - 1 = 7 blocks as indicated in Das, Dey and Dean (1998).

Remark 4.1 The A-optimal design in \mathcal{D} also minimizes the $Var(\hat{\sigma}_g^2)$ in \mathcal{D} since $Var(\hat{\sigma}_e^2)$ is independent of the design.

Remark 4.2 The results on the characterization of A-optimal designs hold for D-optimality as well. This is so because, for unblocked case, the minimization of determinant of $Disp\begin{pmatrix} \hat{\sigma}_g^2 \\ \hat{\sigma}_e^2 \end{pmatrix}$ is equivalent to the minimization of $\frac{\operatorname{tr} C_0^2}{(\operatorname{tr} C_0)^2}$ and $\frac{1}{\operatorname{tr} C_0}$ as is required for A-optimality. The same argument hold for designs under block model.

Remark 4.3 As a result of the very nature of the derived objective function under the random effects model that we are minimizing, every result on previously known MS-optimal designs under the fixed effects model remains valid under A- and D-optimality of designs in \mathcal{D}_0 for random effects model.

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