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Three-way cross designs for animal breeding experiments

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ABSTRACT

Three-way crossbreeding has been a major tool for the development of present day commercial breeds. The hybrids thus produced are more stable and they exhibit individual as well as population buffering mechanism because of the broad genetic base. Here, 2 methods of constructing designs for breeding trials involving complete/ partial three-way crosses were developed. Among these, first method is based on mutually orthogonal Latin squares, which yield three-way crosses arranged in blocks. The other method is derived from two-associate class partially balanced incomplete block designs. The efficiency factor in terms of information per cross pertaining to general combining ability effects of half parents as well as full parents in comparison to a complete triallel cross plan, assuming the error variance to be same for both the plans, was computed. All these designs have a good efficiency. Parameters of the designs constructed are listed together with the efficiency factors.

Key words: Association scheme, Combining abilities, Partially balanced incomplete block designs, Triallel crosses, Triangular designs

Crossbreeding, a major tool for the development of present day commercial breeds, can be carried out as 2-way (diallel), 3-way (triallel) or 4-way (double) crosses, back crosses or rotational crosses. The crossbreeding approach normally involves a simple cross between an improved exotic and a local breed, to combine the better production capacity of the former with the later adaptability to harsh environment. This system also maximizes the expression of heterosis or hybrid vigour, in the cross, normally reflected in improved fitness characteristics (Hoffmann 2005). Two-way (diallel) cross is the most simple and easily manageable hybridization method. However, 3-way (triallel) and 4-way (double) cross hybrids are genetically more viable and consistent in performance than 2-way cross hybrids. There are many cases of animal species like swine and chicken for breeding purposes where 3-way and 4-way crosses are the commonly used techniques of producing commercial hybrids. These mentioned techniques help the animal breeders to improve economically and nutritionally important quantitative traits. Three-way and 4-way cross hybrids are more stable than pure lines as well as 2-way cross hybrids and they exhibit individual as well as population buffering mechanism because of the broad genetic base. At the same time more resources are needed in case of 4-way crosses. Thus, keeping in view of resource utilization, 3-way crosses are found to be most advisable.

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Three-way crossbred chickens show better egg traits than 2-way crossbred chickens with lower mortality (Khawaja *et al*. 2013). They also mentioned that 3-way cross breeding also improves traits like age at sexual maturity, annual egg number, feed intake and feed conversion.

Furthermore, commercial and backyard pig raisers prefer 3-way cross pigs' due to their impressive performance on traits like fast growth rate, good feed efficiency, and carcass quality (http: //www.pcaarrd.dost.gov.ph/home/momentum/ swine).

However, as the number of lines increases, the number of crosses in a complete 3-way cross (CTC) plan increases manifold and becomes unmanageably large for the investigator to handle. This situation lies in taking a sample of CTC, known as partial three-way crosses (PTC) or partial triallel crosses. The offsprings obtained from 3-way crosses can then be subjected to environmental conditions using a completely randomized design (CRD), randomized complete block (RCB) design or an incomplete block (IB) design. Many a times, crosses arranged in blocks can be obtained directly by using the block contents of an incomplete block design for making the systematic crosses.

Hinkelmann (1965) introduced the concept of partial triallel crosses and gave a method of construction using generalized partially balanced incomplete block (GPBIB) designs. A lot of methods of construction of the incomplete block designs were suggested by Ponnuswamy (1971) for triallel crosses, using Latin square and Graeco Latin square. Application of extended triangular design as the confounded triallel experiments was discussed by Arora and Aggarwal (1984). A method of construction of PTC using a special class of BIB and PBIB designs which preserves the property of triallel mating design was developed by Ponnuswamy and Srinivasan (1991). A systematic method of construction of PTC using Trojan square design was developed by Dharmalingam (2002). Mating designs were also obtained using generalized incomplete Trojan type designs by Varghese and Jaggi (2011). A method of construction of mating designs for partial triallel cross for $n > 3$ lines is proposed by using mutually orthogonal Latin squares of order n, where n is a prime or power of a prime (Sharma *et al.* 2012).

MATERIALS AND METHODS

Partial three-way cross [Hinkelmannn 1965]: Let there be n lines. A set of matings is said to be a PTC if it satisfies the following conditions:

(i) Each line occurs exactly r_H times as half-parent and r_F times as full-parent.

(ii) Each cross $(i\times j)\times k$ occurs either once or not at all.

Condition (ii) does not exclude the simultaneous occurrence of $(i\times j)\times k$, $(i\times k)\times j$ and $(j\times k)\times i$. To ensure the structural symmetric property (SSP) of the PTC, all the above mentioned three types of crosses are to be included in the plan. The total number of crosses is nr_F. Since each line is equally often represented as half-parent it follows immediately that $r_H = 2r_F$.

Three-way cross model: Let n be the number of inbred lines resulting in $N^* = n\ (n-1)\ (n-2)/2$ 3-way crosses. Consider 3-way crosses (ignoring reciprocal effects) of the form $i\times j\times k$ ($i, j, k = 1,2,\ldots,n$ and $i \neq j \neq k$) arranged in b blocks of size k^* and each cross replicated r times. The model for mating experiments can be expressed in the form

$$y_{lm} = \mu + \tau_{(ijk)l} + \beta_m + e_{lm} \tag{2.1}$$

where y_{lm}, response from the l^{th} cross ($l = 1,2,\ldots,N^*$) belonging to the m^{th} ($m = 1,2,\ldots,b$) block; μ, grand mean; $\tau_{(ijk)l}$, the effect of the l^{th} cross; e_{lm}, *i.i.d* following a normal distribution with 0 mean and constant variance σ^2.

The model in eqn. (2.1) can be rewritten in matrix notations

$$y = \mu 1 + \Delta' \tau + e \tag{2.2}$$

where $\boldsymbol{y}$, $Nr \times 1$ vector of responses; 1, a $Nr \times 1$ vector of ones; Δ', $Nr \times N$ incidence matrix of response versus crosses, τ, $N \times 1$ vector of cross effect, e, $Nr \times 1$ vector of errors.

Now, the cross effect τ itself is a mixture of various combining ability effects viz., gca effects of half as well as full parents. The reduced model for expressing the cross effects can be written as

$$\tau_{(ij)k}\ \bar{\tau} + h_i + h_j + g_k,\ (i, j, k = 1, 2, \ldots n,\ i \neq j \neq k) \tag{2.3}$$

where $\tau_{(ij)k}$, effect of 3-way cross of the type $(i\times j)\times k$; $\bar{\tau}$, mean effect of crosses; h_i, gca effect of i^{th} half parent involved in the 3-way cross; h_j, is the gca effect of j^{th} half parent involved in the 3-way cross; g_k, is the gca effect of k^{th} full parent involved in the 3-way cross.

Methods of construction

CTC/PTC plans with crosses arranged in blocks: Here, an easy and general method to obtain a series of CTC/PTC plan using mutually orthogonal latin squares (MOLS) has been explained. These are ready to lay out solutions to the breeders.

Let n, the number of lines be a prime. Construct a complete set of (n-1) MOLS for n symbols 1,2,…,n. Prime powers are not considered here. Retain first 3 rows of each array of size $n \times n$ corresponding to each of the Latin squares. Thus, n - 1 blocks each consisting of $3n$ crosses can be obtained easily by making all crosses within each column of each array. Further, in order to ensure the SSP of the plan, all 3 types of crosses are to be considered for each cross. The nature of resultant plans varies from complete to partial 3-way cross with increasing n. More precisely, the plan obtained is CTC for $n < 7$ and beyond that it leads to PTC plans. The method is advantageous in terms of its change-over from complete to partial 3-way cross plan as the number of lines increases since complete 3-way cross plans become difficult to handle due to large number of crosses. The parameters of this class of designs are: total number of crosses (N^*), $3n$ (n-1); number of blocks (b), (n-1); block size (k), $3n$ degree of fractionation (f), 6/(n-2) which is the ratio of number of crosses in a given plan to a CTC for the same number of lines.

The general form of information matrix ($\boldsymbol{C}$) for the joint gca effects is obtained as:

$$\begin{bmatrix} \frac{3(n-2)}{2n}(nI_n - J_n) & \frac{-3}{n}(nI_n - J_n) \\ \frac{-3}{n}(nI_n - J_n) & \frac{3(n-1)}{n}(nI_n - J_n) \end{bmatrix}$$

where, I_n and J_n are identity matrix and matrix of unities, respectively, of order $n\times n$.

Example 1: Let the number of lines be $n = 5$. A total of four MOLS are possible. All the 4 arrays obtained by retaining first 3 rows of MOLS areas all given below:

1	2	3	4	5
2	3	4	5	1
3	4	5	1	2

1	2	3	4	5
3	4	5	1	2
5	1	2	3	4

1	2	3	4	5
4	5	1	2	3
2	3	4	5	1

1	2	3	4	5
5	1	2	3	4
4	5	1	2	3

Thus, using the 4 arrays of 5 columns each and making crosses from each of the columns of the arrays we get a total of 60 crosses in 4 blocks of size 15 as given below:

Block 1	Block 2	Block 3	Block 4
(1×2)×3	(1×3)×5	(1×4)×2	(1×5)×4
(1×3)×2	(1×5)×3	(1×2)×4	(1×4)×5
(2×3)×1	(3×5)×1	(2×4)×1	(4×5)×1
(2×3)×4	(2×4)×1	(2×5)×3	(2×1)×5
(2×4)×3	(2×1)×4	(2×3)×5	(2×5)×1
(3×4)×2	(1×4)×2	(3×5)×2	(1×5)×2
(3×4)×5	(3×5)×2	(3×1)×4	(3×2)×1
(3×5)×4	(3×2)×5	(3×4)×1	(3×1)×2
(4×5)×3	(2×5)×3	(1×4)×3	(1×2)×3
(4×5)×1	(4×1)×3	(4×2)×5	(4×3)×2
(4×1)×5	(4×3)×1	(4×5)×2	(4×2)×3
(1×5)×4	(1×3)×4	(2×5)×4	(2×3)×4
(5×1)×2	(5×2)×4	(5×3)×1	(5×4)×3
(5×2)×1	(5×4)×2	(5×1)×3	(5×3)×4
(1×2)×5	(2×4)×5	(1×3)×5	(3×4)×5

The parameters of the designs are total number of crosses (N^*), 60; number of blocks (b), 4; block size (k), 15. The $\boldsymbol{C}$ matrix is given as follows:

$$\begin{bmatrix} (4.5I_5\text{-}0.9J_5) & (\text{-}3I_5+0.6J_5) \\ (\text{-}3I_5+0.6J_5) & (12I_5\text{-}2.4J_5) \end{bmatrix}$$

The variances of contrasts pertaining to estimated gca effects of half parents as well as full parents were computed using SAS codes and are given as $V_{hii'}$ σ^2= 0.5333σ^2 and $V_{gii'}$ σ^2= 0.2000σ^2 respectively, where i ≠ i ' = 1, 2,..., 5. Moreover, the design which we get for n = 5 is a complete 3-way cross plan.

In this example, it can be noticed that Block 1 and Block 4 have same contents; similarly, Block 2 and Block 3 have same contents and rest is randomization. Rather than repeating the same blocks, it would be better to use a design in less number of crosses and estimate gca effects with less precision many times. Thus, designs obtained using Method 3.1 will be helpful when the experimenter wants more precise comparisons among gca effects whereas the following particular case is advisable when the resources are limited:

A SAS code has been developed using PROCIML to compute variances pertaining to gca effects of half parents as well as full parents (available with the authors) for all classes of designs obtained under Section 3. Efficiency factor in terms of information per cross pertaining to gca effects of half parents as well as full parents in comparison to a complete triallel cross plan was also computed. These efficiency factors along with various parameters of the plans for number of lines < 25 have been listed in Table 1.

Table 1. CTC/PTC plans arranged in blocks using Method 1

Sl. No.	n	N	b	K	f	V_{hii}	V_{gii}	Eh	Eg
1	5*	60	4	15	6/6	0.5333	0.2000	1	1
2	7*	126	6	21	6/6	0.2857	0.1190	1	1
3	11	330	10	33	6/9	0.1515	0.0682	1	1
4	13	468	12	39	6/11	0.1231	0.0564	1	1
5	17	816	16	51	6/15	0.0896	0.0420	1	1
6	19	1026	18	57	6/17	0.0789	0.0373	1	1
7	23	1518	22	69	6/21	0.0638	0.0304	1	1

*These are CTC plans

Particular case

A special case of partial 3-way cross plans with crosses arranged in incomplete blocks having half degree of fractionation in comparison to the ones given under Method 3.1 can be obtained by considering MOLS as follows:

Let n, the number of lines be a prime. Construct any (n-1)/2 Latin squares of size n×n. Retain the first three rows of each Latin square. Thus (n-1)/2 blocks each consisting 3n crosses can be obtained easily by making all possible crosses within each column of each array. The parameters of this class of designs are: total number of crosses (N^*) = 3n(n-1)/2; number of blocks (b), (n-1)/2; block size (k), 3n crosses degree of fractionation (f), 3/(n-2). The general form of $\boldsymbol{C}$ matrix for joint gca effects is obtained as:

$$\begin{bmatrix} \frac{3(n-2)}{4n}(nI_n - J_n) & \frac{-3}{2n}(nI_n - J_n) \\ \frac{-3}{2n}(nI_n - J_n) & \frac{3(n-1)}{2n}(nI_n - J_n) \end{bmatrix}$$

where, I_n and J_n are identity matrix and matrix of unities, respectively, of order n × n.

Example 2: Let the number of lines n = 7. Construct first three rows of three orthogonal Latin squares as given below:

1	2	3	4	5	6	7
2	3	4	5	6	7	1
3	4	5	6	7	1	2

1	2	3	4	5	6	7
3	4	5	6	7	1	2
5	6	7	1	2	3	4

1	2	3	4	5	6	7
4	5	6	7	1	2	3
7	1	2	3	4	5	6

The crosses are obtained by making all the possible distinct 3-way crosses in 7 columns of each array. The partial 3-way cross plan so obtained satisfying the SSP is given below:

Block 1	Block 2	Block 3
(1×2)×3	(1×3)×5	(1×4)×7
(2×3)×4	(2×4)×6	(2×5)×1
(3×4)×5	(3×5)×7	(3×6)×2
(4×5)×6	(4×6)×1	(4×7)×3
(5×6)×7	(5×7)×2	(5×1)×4
(6×7)×1	(6×1)×3	(6×2)×5
(7×1)×2	(7×2)×4	(7×3)×6
(1×3)×2	(1×5)×3	(1×7)×4
(2×4)×3	(2×6)×4	(2×1)×5
(3×5)×4	(3×7)×5	(3×2)×6
(4×6)×5	(4×1)×6	(4×3)×7
(5×7)×6	(5×2)×7	(5×4)×1
(6×1)×7	(6×3)×1	(6×5)×2
(7×2)×1	(7×4)×2	(7×6)×3
(2×3)×1	(3×5)×1	(4×7)×1
(3×4)×2	(4×6)×2	(5×1)×2
(4×5)×3	(5×7)×3	(6×2)×3
(5×6)×4	(6×1)×4	(7×3)×4
(6×7)×5	(7×2)×5	(1×4)×5
(7×1)×6	(1×3)×6	(2×5)×6
(1×2)×7	(2×4)×7	(3×6)×7

The parameters of the designs are total number of crosses (N^*), 63; number of blocks (b), 3; block size (k), 21. The $\boldsymbol{C}$ matrix is:

$$\begin{bmatrix} (3.75I_7 - 0.54J_7) & (-1.5I_7 + 0.21J_7) \\ (-1.5I_7 + 0.21J_7) & (9I_7 - 1.29J_7) \end{bmatrix}$$

The average variances pertaining to the estimated gca effects of half parents and full parents is given as $V_{hij'}$ σ^2= 0.13σ^2 and $V_{gij'}$ σ^2= 0.2σ^2, respectively.

The efficiency factors along with various parameters of the designs obtained from this method for number of lines < 25 have been listed in Table 2.

Table 2. CTC/PTC plans arranged in blocks using particular case of Method 1

Sl. No.	n	N	b	K	f	V_{hii}	V_{gii}	Eh	Eg
1									
1	5*	30	2	15	3/3	0.5333	0.2000	1	1
2	7	63	3	21	3/5	0.5714	0.2381	1	1
3	11	165	5	33	3/3	0.3030	0.1364	1	1
4	13	234	6	39	3/11	0.2461	0.1128	1	1
5	17	408	8	51	3/15	0.1793	0.0840	1	1
6	19	513	9	57	3/17	0.1579	0.0746	1	1
7	23	759	11	69	3/21	0.1275	0.0609	1	1

*This is a CTC plan.

PTC plans using PBIB designs: Consider any partially balanced incomplete block (PBIB) design with parameters, v^*, b^*, r^*, k^*, ë*, having small block size ($k^* > 2$) and distinct block contents. When k > 3, 1 = 1 ensures that no two treatments are same in two different blocks of the design. A PBIB design [Bose and Nair 1939] based on m class ($m > 2$) association scheme is an arrangement of $v^* = n$ symbols in b^* blocks, such that (i) each block contains k^* ($<v^*$) distinct symbols, (ii) each symbols occurs in r^* blocks, (iii) if the symbols a and b are mutually i^{th} associates in the association scheme, then a and b occur together in l_2^* blocks, where the integer l_i^* does not depend on the pair (a, b) so long as they are mutually i^{th} associates, i = 1, 2, …, m. Further, not all l_i^*'s are equal. A large number of classes of PBIB designs are available in literature and most of them can be easily generated through the online software WS-PBIB developed by Sharma *et al.* (2013) available at http://nabg.iasri.res.in/pbibweb/.

Considering the symbols/ numerals of the block contents as lines and making all possible 3-way crosses within each block, one can obtain a partial 3-way cross plan. Total number of crosses in this class of designs are $N^* = b^*k^*(k^*-1)(k^*-2)/6$. These crosses are then to be laid out using an appropriate environmental design.

Example 3: Let the number of lines be 10. The block contents (blocks are given in rows) of a triangular PBIB design for n = 10 in blocks of size three is as follows:

1,	2,	5
1,	3,	6
1,	4,	7
2,	3,	8
2,	4,	9
3,	4,	10
5,	6,	8
5,	7,	9
6,	7,	10
8,	9,	10

A partial 3-way cross plan obtained by taking all possible distinct 3-way crosses within each block of the above design is given below:

(1×2)×5	(1×4)×7	(2×4)×9	(5×6)×8	(6×7)×10
(1×5)×2	(1×7)×4	(2×9)×4	(5×8)×6	(6×10)×7
(2×5)×1	(4×7)×1	(4×9)×2	(6×8)×5	(7×10)×6
(1×3)×6	(2×3)×8	(3×4)×10	(5×7)×9	(8×9)×10
(1×6)×3	(2×8)×3	(3×10)×4	(5×9)×7	(8×10)×9
(3×6)×1	(3×8)×2	(4×10)×3	(7×9)×5	(9×10)×8

Thus for n =10, the parameter of the design are N, 30; f, 1/12. The average variances pertaining to the estimated gca effects of half parents and full parents are $\bar{V}_{hii'}\sigma^2 = 0.8673\sigma^2$ and $\bar{V}_{gii'}\sigma^2 = 0.3218\sigma^2$ respectively, where $i \neq i' = 1,2,..., 10$.

Various parameters of the plans obtained using triangular designs for number of lines < 25 have been listed in Table 3.

Table 3. PTC plans using Method 2

Sl. No.	n	N	f	$\bar{V}_{hii}$	$\bar{V}_{gii}$	Eh	Eg
1	10	30	1/12	2.2000	0.8667	0.7013	0.7912
2	15	60	4/91	1.3460	0.5841	0.8090	0.8655
3	21	105	1/38	0.9921	0.4504	0.8533	0.8928

RESULTS AND DISCUSSION

Here, two methods of constructing designs for breeding trials involving complete/ partial 3-way crosses have been developed. The first method is based on a complete set of mutually orthogonal Latin squares (MOLS). This method yields PTC plans arranged in incomplete blocks for $n > 7$. For n = 5 and 7, the method yields CTC plans. The elementary contrasts pertaining to gca effects of full parents are estimated with same variance and of half parents are estimated with same variance, indicating that these designs are variance balanced.

Particular case of this method yields designs that are also based on MOLS, which yield PTC plans arranged in blocks except for n = 5 but are partially variance balanced. Degree of fractionation is reduced to half in this case. However, the variance of elementary contrasts due to estimated gca effects is double. For example, for n = 11 the degree of fractionation is 6/9 and 3/9 respectively in Tables 1 and 2 while the variance of elementary contrasts due to estimated gca effects of half parents is 0.1515 and 0.3030 and that of full parents is 0.0682 and 0.1364 respectively in Tables 1 and 2. So, Method 1 will be helpful when the experimenter wants more precise comparisons among gca effects whereas particular case is advisable when the resources are limited.

For constructing designs under Method 2, available two-associate class triangular designs with small block size have been used. The plans obtained are partially balanced and the number of variance groups for gca comparisons depend on the association scheme of the design used for construction. It can be seen from Table 3 that the degree of fractionation of these plans is very small and hence this type of designs can be advantageously used when the number of lines is large and resources are limited. A large number of two- and higher-associate class PBIB designs are available in literature that can yield designs involving 3-way crosses for almost all possible parametric combinations.

The variance of estimated gca effects due to full parents is less in all classes of designs in comparison to those of half parents. This efficiency is found to be 1 for all designs given in Tables 1 and 2 indicating that these designs are optimal in a competing class of designs. The efficiency factor for designs under Table 3 is seen to be fairly high.

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