

Pooling, Lattice Square, and Union Jack Designs

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Abstract

Simplified pooling designs employ rows, columns, and principal diagonals from square and rectangular plates. The requirement that every two samples be tested together in exactly one pool leads to a novel combinatorial configuration, the union jack design. Existence of union jack designs is settled affirmatively whenever the order n is a prime and $n \equiv 3 \pmod{4}$.

1 Introduction

Pooling experiments are used to screen large recombinant DNA libraries to isolate *clones* (sets of subintervals) containing a particular DNA sequence [?, ?]. Since it is not practical to screen each clone individually, subsets of clones, called *pools*, are used. The specification and collection of these pools is referred to as a “*pooling design*”. The primary objective is to determine, for each pool in the design, whether any of the clones in the pool contain a specified (small) subinterval. Given the results for each pool, we want to determine which clones contain the subinterval, and to do so unambiguously.

Clones are often stored and produced on square or rectangular plates, and hence, performing the pooling experiment requires the merging of material from a number of different positions on the plate. This operation is greatly simplified by defining the pools to be in a pattern, and in practice, the clones from an individual plate can be combined along lines such as rows, columns, and diagonals [?]. These combinations are sub-pools which form components of the final pools. Thus, the pooling design includes the specification of the templates used to obtain the sub-pools and the way that the sub-pools are combined to obtain the final pools. Since the physical limitations imposed by the templates and the presence of experimental error in the pooling outcomes must also be taken into account, it can be a challenge to construct pooling experiments [?].

When the rows and columns are considered, designs useful for constructing templates in pooling have been studied previously for the design of experiments [?]. A *lattice square design* of order n is a set of $\frac{n+1}{2}$ arrays, each $n \times n$ and each containing n^2 symbols once. Every pair of symbols occurs together once either in a row or a column, in exactly one of the arrays, and does not appear together in a row or column elsewhere. Note that the rows and columns of the arrays of a lattice square design of order n form the blocks of a resolvable $2-(n^2, n, 1)$ design [?, ?]. Here is a lattice square design of order three:

$$\left\{ \left[\begin{array}{ccc} 0 & 1 & 2 \\ 3 & 4 & 5 \\ 6 & 7 & 8 \end{array} \right], \left[\begin{array}{ccc} 0 & 5 & 7 \\ 4 & 6 & 2 \\ 8 & 1 & 3 \end{array} \right] \right\}.$$

Lattice square designs, known initially as *quasi-Latin square designs*, were one of the variants of Latin square designs examined by Yates [?]; see also [?, ?]. An explicit construction is given in [?]. Raghavarao [?, p. 171] remarks that such lattice square designs can always be obtained from an affine plane of odd order n ; partition the $n + 1$ parallel classes of blocks into pairs, and form an $n \times n$ array for each pair by interpreting blocks in one parallel class as rows and in the other as columns. This does not depend on the structure of the plane and so every affine plane of odd order gives rise to at least one lattice square design. Different pairings of the parallel classes can lead to different lattice square designs, and the enumeration of different lattice square designs appears to be more complex than the (already difficult) enumeration of the underlying planes. The affine plane can always be recovered from the lattice square design, since the $n + 1$ parallel classes from the rows and columns of the $\frac{n+1}{2}$ arrays form such a plane.

Naturally this approach requires the construction of an affine plane in order to obtain the lattice square design, and a direct construction might be preferred. However, whenever n is the order of a known affine plane, n is a power of a prime [?]. In this case, there is an affine plane (which we define subsequently) arising from the finite field of order n . It is a relatively simple matter to write a direct prescription for an affine plane using the field, and equally straightforward to write a prescription for the arrays of a lattice square design; we leave the details to the interested reader.

In this paper, we show that affine planes can also be used to construct analogous designs in which the rows, columns, front diagonals, and back diagonals of the arrays each form a parallel class of the plane. A

front diagonal is a set of array entries (i, j) satisfying $i - j \equiv b \pmod{n}$ for b a constant and $i, j \in \mathbb{Z}_n$. Similarly, a *back diagonal* satisfies $i + j \equiv b \pmod{n}$.

An *RCF design* of order n is a set of $\frac{n+1}{3}$ arrays, each $n \times n$ and containing n^2 symbols once. Every pair of symbols occurs together once in a row, column, or front diagonal in exactly one of the arrays, and does not appear together in a row, column, or front diagonal elsewhere. Here is an RCF design of order 5 defined on $\mathbb{Z}_5 \times \mathbb{Z}_5$:

$$\left\{ \begin{array}{l} \left[\begin{array}{ccccc} 00 & 10 & 20 & 30 & 40 \\ 01 & 11 & 21 & 31 & 41 \\ 02 & 12 & 22 & 32 & 42 \\ 03 & 13 & 23 & 33 & 43 \\ 04 & 14 & 24 & 34 & 44 \end{array} \right], \quad \left[\begin{array}{ccccc} 00 & 12 & 24 & 31 & 43 \\ 34 & 41 & 03 & 10 & 22 \\ 13 & 20 & 32 & 44 & 01 \\ 42 & 04 & 11 & 23 & 30 \\ 21 & 33 & 40 & 02 & 14 \end{array} \right] \end{array} \right\}.$$

RCF designs also arise from affine planes in the following manner. Form the affine plane from the finite field as follows (see [?] for relevant background). Let $X = \{(x, y) : x, y \in \text{GF}(n)\}$ be the n^2 points. Define for each $M, b \in \text{GF}(n)$ the line $L_{M,b} = \{(x, Mx + b) : x \in \text{GF}(n)\}$. Further define for each $b \in \text{GF}(n)$ the line $L_{\infty,b} = \{(b, y) : y \in \text{GF}(n)\}$. Let $\mathbb{P}_n = \text{GF}(n) \cup \{\infty\}$ denote the projective line. Then the $(n+1)n$ lines $L_{M,b}$ with $M \in \mathbb{P}_n$ and $b \in \text{GF}(n)$ are the blocks of the affine plane of order n . They form a resolvable 2 - $(n^2, n, 1)$ design. The parameter $M \in \mathbb{P}_n$ of the line $L_{M,b}$ is called the *slope* of $L_{M,b}$. For each $M \in \mathbb{P}_n$, the set $\mathcal{R}(M) = \{L_{M,b} : b \in \text{GF}(n)\}$ contains n lines which partition the n^2 points in X . We say that $\mathcal{R}(M)$ is the resolution class of lines with slope M . The $n+1$ resolution classes $\mathcal{R}(M)$, $M \in \mathbb{P}_n$, partition the $(n+1)n$ lines of the affine plane.

If n is a prime, then $n \equiv 5 \pmod{6}$, and it is easy to construct an RCF design from the affine plane. Partition \mathbb{P}_n into classes of size three, $X_1, \dots, X_{(n+1)/3}$ so that $X_1 = \{\infty, 0, 1\}$. The lines with slopes in X_1 lead directly to an array in which the parallel classes form rows, columns, and front diagonals. Now the group $\text{PGL}_2(n)$ acts 3-transitively on the set of slopes \mathbb{P}_n , and hence there is a group element mapping X_i to X_1 for each i , $2 \leq i \leq \frac{n+1}{3}$. Applying this automorphism places the parallel classes corresponding to the slopes in X_i in the roles of rows, columns, and front diagonals in an $n \times n$ array, and hence produces the arrays required. This establishes the following.

Theorem 1.1 *If $n \equiv 5 \pmod{6}$ is a prime, then there exists an RCF design of order n .*

Primality of n is employed here to ensure that the lines of slope one in the field are genuine front diagonals when interpreted in the $n \times n$ array. When n is a prime power but not a prime, this is not guaranteed; the prime power case is discussed in the last section.

2 Union Jack Designs of Prime Order

Simultaneous consideration of rows, columns, front diagonals, and back diagonals underlies the following combinatorial definition. A *union jack design* of order n is a collection of $n \times n$ arrays with distinct entries from a set X of n^2 points such that every pair of points appears exactly once among the rows, columns, front diagonals, and back diagonals of the arrays. Thus, the number of arrays in a union jack design is

$\binom{n^2}{2}/4n\binom{n}{2} = \frac{n+1}{4}$. For example, here is a union jack design of order 7 defined on $\mathbb{Z}_7 \times \mathbb{Z}_7$:

$$\left\{ \left[\begin{array}{cccccc} 00 & 01 & 02 & 03 & 04 & 05 & 06 \\ 10 & 11 & 12 & 13 & 14 & 15 & 16 \\ 20 & 21 & 22 & 23 & 24 & 25 & 26 \\ 30 & 31 & 32 & 33 & 34 & 35 & 36 \\ 40 & 41 & 42 & 43 & 44 & 45 & 46 \\ 50 & 51 & 52 & 53 & 54 & 55 & 56 \\ 60 & 61 & 62 & 63 & 64 & 65 & 66 \end{array} \right], \left[\begin{array}{cccccc} 00 & 15 & 23 & 31 & 46 & 54 & 62 \\ 56 & 64 & 02 & 10 & 25 & 33 & 41 \\ 35 & 43 & 51 & 66 & 04 & 12 & 20 \\ 14 & 22 & 30 & 45 & 53 & 61 & 06 \\ 63 & 01 & 16 & 24 & 32 & 40 & 55 \\ 42 & 50 & 65 & 03 & 11 & 26 & 34 \\ 21 & 36 & 44 & 52 & 60 & 05 & 13 \end{array} \right] \right\}.$$

When n is a prime and $n \equiv 3 \pmod{4}$, we give a construction using the blocks of the affine plane arising from the finite field on the set $X = \mathbb{Z}_n \times \mathbb{Z}_n$. Given $M, D \in \mathbb{P}_n$ with $M \neq D$, define $L_{M,D,i}$ to be the unique line in $\mathcal{R}(M)$ that intersects the line $L_{D,0}$ in the pair

$$\left\{ \begin{array}{ll} (i, Di) & \text{if } D \neq \infty \\ (0, i) & \text{if } D = \infty \end{array} \right\}$$

Let $R, C, D \in \mathbb{P}_n$ and define $M = M_{R,C,D}$ to be the n by n matrix whose $[i, j]$ -entry is

$$M[i, j] = L_{R,D,i} \cap L_{C,D,j} = (X_{ij}, Y_{ij})$$

where $i, j \in \mathbb{Z}_n$. Observe that

- (1) every element of $\mathbb{Z}_n \times \mathbb{Z}_n$ appears in some cell of M ,
- (2) the rows of M are the lines in $\mathcal{R}(R)$, and
- (3) the columns of M are the lines in $\mathcal{R}(C)$.

Theorem 2.1 *Let $R, C, D \in \mathbb{P}_n$, $R \neq C \neq D \neq R$. Then the front diagonals of $M = M_{R,C,D}$ contain the lines in $\mathcal{R}(D)$ and the back diagonals contain the lines in $\mathcal{R}(B)$, where*

$$B = B_{R,C,D} = \frac{RD + CD - 2RC}{2D - R - C}.$$

Proof: To simplify calculations, observe that

$$B_{\infty,C,D} = 2C - D$$

$$B_{R,\infty,D} = 2R - D$$

$$B_{R,C,\infty} = \frac{R + C}{2}$$

Case 1: $R \neq \infty, C \neq \infty$, and $D \neq \infty$. In this case,

$$L_{R,D,i} = \{(X, Y) : Y = RX + (D - R)i\}$$

and

$$L_{C,D,j} = \{(X, Y) : Y = CX + (D - C)j\}.$$

The intersection of these two lines is (X_{ij}, Y_{ij}) where

$$X_{ij} = \frac{(D - C)j - (D - R)i}{R - C}$$

$$Y_{ij} = RX_{ij} + (D - R)i = CX_{ij} + (D - C)j$$

Observe that

$$\begin{aligned} \frac{\Delta Y}{\Delta X} &= \frac{Y_{i+1,j+1} - Y_{i,j}}{X_{i+1,j+1} - X_{i,j}} = \frac{R\Delta X + (D - R)}{\Delta X} = R + \frac{(D - R)}{\Delta X} \\ &= R + \frac{(D - R)(R - C)}{(D - C) - (D - R)} = R + \frac{(D - R)(R - C)}{R - C} = D. \end{aligned}$$

Thus, the ordered pairs that appear on any front diagonal are on a line that has slope D . They account for all of the lines in $\mathcal{R}(D)$ since every ordered pair in $\mathbb{Z}_n \times \mathbb{Z}_n$ occurs exactly once among the cells of M . Observe that

$$\begin{aligned} \frac{\Delta Y}{\Delta X} &= \frac{Y_{i-1,j+1} - Y_{i,j}}{X_{i-1,j+1} - X_{i,j}} = \frac{R\Delta X - (D - R)}{\Delta X} = R - \frac{(D - R)}{\Delta X} \\ &= R - \frac{(D - R)(R - C)}{-(D - C) - (D - R)} = R + \frac{(R - D)(R - C)}{2D - R - C} \\ &= \frac{RD + CD - 2RC}{2D - R - C} = B. \end{aligned}$$

Thus the ordered pairs that appear on any back diagonal are on a line that has slope B . They account for all of the lines in $\mathcal{R}(B)$ since every ordered pair in $\mathbb{Z}_n \times \mathbb{Z}_n$ occurs exactly once among the cells of M .

Case 2: $R = \infty$. In this case,

$$L_{R,D,i} = \{(i, Y) : Y \in \mathbb{Z}_n\} \text{ and}$$

and

$$L_{C,D,j} = \{(X, Y) : Y = CX + (D - C)j\}.$$

The intersection of these two lines is (X_{ij}, Y_{ij}) where

$$X_{ij} = i$$

$$Y_{ij} = CX_{ij} + (D - C)j = Ci + (D - C)j$$

Observe that

$$\frac{\Delta Y}{\Delta X} = \frac{Y_{i+1,j+1} - Y_{i,j}}{X_{i+1,j+1} - X_{i,j}} = \frac{C\Delta X + (D - C)}{\Delta X} = C + (D - C) = D.$$

Thus the ordered pairs that appear on any front diagonal are on a line that has slope D . They account for all of the lines in $\mathcal{R}(D)$ since every ordered pair in $\mathbb{Z}_n \times \mathbb{Z}_n$ occurs exactly once among the cells of M . Observe that

$$\frac{\Delta Y}{\Delta X} = \frac{Y_{i-1,j+1} - Y_{i,j}}{X_{i-1,j+1} - X_{i,j}} = \frac{C\Delta X - (D - C)}{\Delta X} = C - \frac{D - C}{\Delta X} = 2C - D = B.$$

Thus the ordered pairs that appear on any back diagonal are on a line that has slope B. They account for all of the lines in $\mathcal{R}(B)$ because every ordered pair in $\mathbb{Z}_n \times \mathbb{Z}_n$ occurs exactly once among the cells of M .

Case 3: $C = \infty$. This case is the transpose of Case 2 (interchange R and C and repeat the argument in Case 2).

Case 4: $D = \infty$. In this case,

$$L_{R,D,i} = \{(X, Y) : Y = RX + i\}$$

and

$$L_{C,D,j} = \{(X, Y) : Y = CX + j\}.$$

The intersection of these two lines is (X_{ij}, Y_{ij}) where

$$\begin{aligned} X_{ij} &= \frac{j-i}{R-C} \\ Y_{ij} &= RX_{ij} + j = \frac{R(j-i)}{R-C} + j \end{aligned}$$

Observe that

$$\frac{\Delta Y}{\Delta X} = \frac{Y_{i+1,j+1} - Y_{i,j}}{X_{i+1,j+1} - X_{i,j}} = \frac{1}{\Delta X} = \frac{1}{0} = D.$$

Thus the ordered pairs that appear on any front diagonal are on a line that has slope D. They account for all lines in $\mathcal{R}(D)$ since every ordered pair in $\mathbb{Z}_n \times \mathbb{Z}_n$ occurs exactly once among the cells of M . Observe that

$$\begin{aligned} \frac{\Delta Y}{\Delta X} &= \frac{Y_{i-1,j+1} - Y_{i,j}}{X_{i-1,j+1} - X_{i,j}} = \frac{R\Delta X - 1}{\Delta X} = R - \frac{1}{\Delta X} \\ &= R - \frac{R-C}{2} = \frac{R+C}{2} = B. \end{aligned}$$

Thus the ordered pairs that appear on any back diagonal are on a line that has slope B. They account for all lines in $\mathcal{R}(B)$ because every ordered pair in $\mathbb{Z}_n \times \mathbb{Z}_n$ occurs exactly once among the cells of M . ■

To construct a union jack designs of prime order n , we need matrices $M_i = M_{R_i, C_i, D_i}$ for $i=1, 2, \dots, (n+1)/4$ so that $\{\{R_i, C_i, D_i, B_i\} : i = 1, 2, \dots, (n+1)/4\}$ partitions \mathbb{P}_n , where $B_i = B_{R_i, C_i, D_i}$. For each $A \in \mathbb{P}_n$, $B_{-A, \frac{1}{A}, \frac{A+1}{A-1}} = \frac{1-A}{1+A}$. Thus for each $A \in \mathbb{P}_n$, $M_A = M_{-A, \frac{1}{A}, \frac{A+1}{A-1}}$ handles the set of slopes $\mathcal{S}_A = \{-A, \frac{1}{A}, \frac{A+1}{A-1}, \frac{1-A}{1+A}\}$. The condition that $n \equiv 3 \pmod{4}$ implies that -1 is not a square modulo n . Thus, \mathcal{S}_A consists of four distinct elements. When $A = \infty$, this set is $\mathcal{S}_\infty = \{\infty, 0, 1, -1\}$. Consider the subgroup $H = \left\{ f_M(X) = \frac{MX+1}{-X+M} : M \in \mathbb{P}_n \right\}$ of $\text{PGL}_2(n)$. This subgroup is transitive on \mathbb{P}_n , because $f_{-A}(\infty) = A$. Furthermore,

$$\begin{aligned} f_A(\mathcal{S}_\infty) &= \{f_A(\infty), f_A(0), f_A(1), f_A(-1)\} \\ &= \left\{ -A, \frac{1}{A}, \frac{A+1}{A-1}, \frac{1-A}{1+A} \right\} = \mathcal{S}_A. \end{aligned}$$

Thus the orbit of \mathcal{S}_∞ under H is $\{\mathcal{S}_A : A \in \mathbb{P}_n\}$. The stabilizer of \mathcal{S}_∞ in H is

$$K = \{f_\infty, f_0, f_1, f_{-1}\} = \left\{ X \mapsto X, X \mapsto \frac{-1}{X}, X \mapsto \frac{1+X}{1-X}, X \mapsto \frac{X-1}{X+1} \right\}.$$

Hence the number of distinct items in the orbit $\{S_A : A \in \mathbb{P}_n\}$ is $|H|/|K| = (n+1)/4$. The transitivity of H shows that they partition \mathbb{P}_n . As a result, we may choose $(n+1)/4$ elements $A_1, A_2, \dots, A_{(n+1)/4} \in \mathbb{P}_n$ so that the $(n+1)/4$ matrices M_A form a union jack design of order n , when $n \equiv 3 \pmod{4}$. Consequently, we have:

Theorem 2.2 *If $n \equiv 3 \pmod{4}$ is a prime, then there exists a union jack design of order n .*

3 A Dual Formulation

Suppose a lattice square, RCF, or union jack design of order n exists. Each defines a set of $n+1$ resolution classes $\mathcal{R}_0, \dots, \mathcal{R}_n$ on n^2 points so that every pair of points occurs in exactly one block of exactly one class; hence the design is an affine plane. The association of resolution classes with the arrays from which they arose partitions the set of resolution classes $\mathcal{R}_0, \dots, \mathcal{R}_n$ into $\frac{n+1}{s}$ sets of s resolution classes each, where $s = 2, 3$, or 4 , depending on whether the underlying design is a lattice square, RCF, or union jack design.

Given the affine plane on elements X with lines \mathcal{B} , define a dual design (V, \mathcal{D}) where $V = \{v_B : B \in \mathcal{B}\}$ and $\mathcal{D} = \{D_x : x \in X\}$, by placing v_B in block D_x whenever $x \in B$. (Elements of the affine plane become lines of the dual and lines of the plane become elements of the dual.) So $|D_x| = n+1$ for all $x \in X$. Moreover, from each resolution class \mathcal{R}_i , we can define a *group* of points $G_i = \{v_B : B \in \mathcal{R}_i\}$. Two points from the same group do not appear together in a block of \mathcal{D} , but two points from different groups appear together in exactly one block of \mathcal{D} . Hence (V, \mathcal{D}) is a *transversal design* $TD(n+1, n)$ (see [?] for an extensive discussion of transversal designs).

The partition of resolution classes into sets of size s corresponds to a partition of the groups of a $TD(n+1, n)$, and hence to a collection of $TD(s, n)$ s. Each such $TD(s, n)$ must be obtained by applying the same dualization to a single array from the lattice square, RCF, or union jack design.

There is essentially only one $TD(2, n)$, and hence every $TD(n+1, n)$ admits a partition into appropriate $TD(2, n)$ s. However, when we turn to RCF and union jack designs, the picture is more complex. For RCF designs, a careful examination of the dual of a single array shows that it is a $TD(3, n)$ isomorphic to one with points $\mathbb{Z}_n \times \{0, 1, 2\}$; groups $\mathbb{Z}_n \times \{i\}$ for $i \in \{0, 1, 2\}$, and blocks $\{(i, 0), (j, 1), (i-j, 2)\} : i, j \in \mathbb{Z}_n$. Equivalently, this transversal design is the one obtained from the addition table of the cyclic group \mathbb{Z}_n . In the same manner, the dual of a single array in a union jack design is isomorphic to one with points $\mathbb{Z}_n \times \{0, 1, 2, 3\}$, groups $\mathbb{Z}_n \times \{i\}$ for $i \in \{0, 1, 2, 3\}$, and blocks $\{(i, 0), (j, 1), (i-j, 2), (i+j, 3)\} : i, j \in \mathbb{Z}_n$.

If an affine plane is to underlie an RCF or union jack design, a necessary condition is that the corresponding $TD(n+1, n)$ contain a $TD(3, n)$ isomorphic to the cyclic one. Immediately we see that the planes arising from the finite field cannot underlie such designs when their order is a prime power but not a prime. Indeed, in this case, every $TD(3, n)$ in the $TD(n+1, n)$ when $n = p^\alpha$ is isomorphic to that arising from $\mathbb{Z}_p \times \mathbb{Z}_p \times \dots \times \mathbb{Z}_p$, the elementary Abelian group, rather than from the cyclic group \mathbb{Z}_{p^α} .

There exist numerous planes of prime power order which do not arise from the finite field, and so it is possible that some other class of affine planes could be used to produce RCF or union jack designs. We do not know of any other examples of affine planes that can be used, but we cannot exclude the possibility since the classification of all such planes is not complete. Nevertheless, one natural class of planes to explore, the translation planes, can be eliminated from consideration using basic facts about the relation between translation planes and ‘quasifields’ (see [?]). All translation planes arise from quasifields, and all quasifields have an additive group which is elementary Abelian. It follows directly that the corresponding $TD(n+1, n)$ cannot contain a $TD(3, n)$ of the cyclic type, and hence that translation planes do not underlie RCF or union jack designs.

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