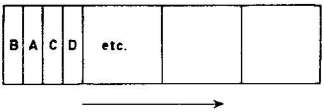
CHAPTER 14

THE LATIN SQUARE DESIGN

14.1 Two systems of blocks in a single design

14.1.1 Consider the following randomized blocks lay-out in which as a special case there is the same number of replications as treatments, the fertility gradient being in the direction of the arrow:



We saw how the blocks eliminate a considerable portion of the error due to soil heterogeneity as compared with a simple random design, but that errors due to soil heterogeneity within blocks cannot be reduced except by an arrangement of plots with length parallel to the arrow, a state of affairs which can come about only by chance or good guesswork. We also saw that the following lay-out, in which the plots form a 4 × 4 square on the ground, is equally efficient:

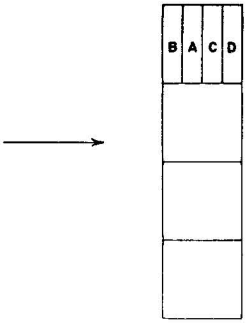


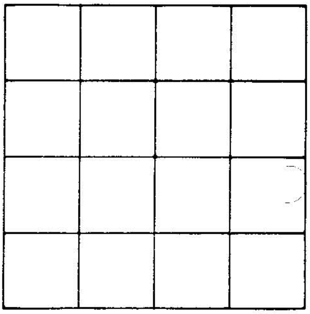
Figure 14.1: Randomized blocks design with $r = t = 4$ arranged in a 4 × 4 square.

14.1.2 If now we arrange the randomization within each block of the lay-out of Figure 14.1 so that each treatment occupies the four possible positions in a block once and once only, we obtain an arrangement such as the following:

B	A	C	D
D	C	A	B
A	D	B	C
C	B	D	A ,

and not only may each row be treated as a block but also each column, since each row and each column contains each treatment once and once only. We are thus in a position to eliminate from experimental error fertility differences in two directions at right angles simultaneously, and it is clear that in the lay-out of Figure 14.1 the introduction of the column-blocks would be very effective in reducing the error below that of the randomized blocks lay-out.

14.1.3 Looking at this in another way: if we have a square array of 16 plots for the testing of 4 treatments, a randomized blocks design using either the rows or columns as blocks would be more efficient than a simple random design, except when the fertility gradient was parallel to the length of the blocks, as in Figure 14.1. The relative efficiency of such a randomized blocks design in removing the effects of soil heterogeneity varies from equal efficiency to very much greater efficiency, since the design eliminates effects of fertility or positional differences in a single direction only. If now we arrange matters so that we can treat both rows and columns as blocks, we can see that when the fertility gradient is such that the blocking effect of the rows is negligible then that of the columns is at a maximum, and *vice versa*. Should the fertility gradient run at an angle, it resolves itself into two components at right angles, one of which is countered by the rows and the other by the columns. It is clear therefore that a design using rows and columns as blocks is in general more efficient than a randomized blocks design using only the rows or the columns as blocks. The worst that can happen (when the gradient is perpendicular to the rows or the columns) is that it should be of equal efficiency. Relative to the simple random design, however, this new design will always be more efficient unless there is no fertility gradient at all.



14.1.4 A design laid out in rows and columns in this way is called a **Latin square**. Each treatment occurs once in each row and each column, and the number of replications must equal the number of treatments. The two sets of blocks are usually called **rows** and **columns**; which is called which is immaterial. The efficiency of a Latin square design is virtually independent of the direction of any major fertility changes across the experimental area, so that low experimental errors are assured without having to make a successful guess at the direction of the fertility slope. The possibility of choosing an unfortunate orientation of plots within the blocks of a randomized blocks design in relation to the fertility gradient, and hence the possibility of a rather high error, is avoided.

14.1.5 What we are virtually doing is to make a two-way mapping of the fertility of the experimental area, the fertility of each plot being regarded as the sum of two yield components arising from the two component fertility slopes at right angles into which we may regard any fertility gradient as being resolved. With blocks only, the allowance for systematic fertility differences is analogous to knowing only the "latitude" or the "longitude" of the plot with respect to its fertility; with rows and columns we know both the "latitude" and the "longitude" and can pinpoint the fertility much more accurately. The analogy is, however, not quite exact since there is still room for the fertility of individual plots to deviate from the sum of its row and column components, and these deviations contribute to experimental error. As usual a process of randomization enables these contributions to error to be regarded as random.

14.1.6 The arrangement of the randomization mentioned in § 14.1.2 amounts to a further restriction on the randomization of the randomized blocks design in addition to the restriction imposed by the blocks and by the orthogonality of blocks and treatments. With two sets of blocks, additional parameters have to be brought into the model and more D.F. (i.e. more constants) devoted to the elimination of the effects of soil heterogeneity. These D.F. can only be used in this way at the expense of the number of D.F. available for estimation of error. In discussing the Latin square design relative to randomized blocks the remarks of § 13.10.3 are of equal application here. In the smaller designs, however, the loss of D.F. can be serious.

14.2 Partitioning of the Total S.S. in the Latin square design

14.2.1 Suppose we have a 5 × 5 square array of plot yields:

						Row means
	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{10}
	y_{21}	y_{22}	y_{23}	y_{24}	y_{25}	y_{20}
	y_{31}	y_{32}	y_{33}	y_{34}	y_{35}	y_{30}
	y_{41}	y_{42}	y_{43}	y_{44}	y_{45}	y_{40}
	y_{51}	y_{52}	y_{53}	y_{54}	y_{55}	y_{50}
Column means	y_{01}	y_{02}	y_{03}	y_{04}	y_{05}	$\bar{y}$

Here the first suffix represents row and the second, column, so that y_{ij} is the treatment in the i^{th} row and the j^{th} column, and the above array represents actual field positions of the plots. The Total S.S. may first be partitioned according to [13.2] as

$$\sum_i \sum_j (y_{ij} - \bar{y})^2 = 5\sum_i (y_{i0} - \bar{y})^2 + 5\sum_j (y_{0j} - \bar{y})^2 + \sum_i \sum_j (y_{ij} - y_{i0} - y_{0j} + \bar{y})^2, \qquad [14.1]$$

but the S.S.'s on the R.H.S. now represent rows, columns, and residuals respectively.

In a Latin square design each y_{ij} has a third suffix k, representing the k^{th} treatment allotted at random to each plot, so that each value of k occurs once in each row and column. Thus y_{i0} can be written y_{i00}, since the mean is also over all values of k, and similarly we may write y_{0j0} for y_{0j}. The residual S.S. must be a mixture of treatment and error effects which it is required to separate.

14.2.2 Suppose we consider an actual arrangement:

$$\begin{matrix} y_{111} & y_{122} & y_{133} & y_{144} & y_{155} \\ y_{212} & y_{224} & y_{235} & y_{241} & y_{253} \\ y_{313} & y_{325} & y_{331} & y_{342} & y_{354} \\ y_{414} & y_{423} & y_{432} & y_{445} & y_{451} \\ y_{515} & y_{521} & y_{534} & y_{543} & y_{552} \end{matrix},$$

and write down the 25 residuals of [14.1] in 5 columns according to the suffix k. Let the mean of the k^{th} treatment be y_{00k}. The residuals are:

$$\begin{array}{lll} & \underline{k=1} & \underline{k=2} \\ & y_{111}-y_{100}-y_{010}+\bar{y} & y_{122}-y_{100}-y_{020}+\bar{y} \\ & y_{241}-y_{200}-y_{040}+\bar{y} & y_{212}-y_{200}-y_{010}+\bar{y} \\ & y_{331}-y_{300}-y_{030}+\bar{y} & y_{342}-y_{300}-y_{040}+\bar{y} \quad \text{etc.} \\ & y_{451}-y_{400}-y_{050}+\bar{y} & y_{432}-y_{400}-y_{030}+\bar{y} \\ & y_{521}-y_{500}-y_{020}+\bar{y} & y_{552}-y_{500}-y_{050}+\bar{y} \\ \hline \text{Means:} & y_{001}-\bar{y}-\bar{y}+\bar{y} & y_{002}-\bar{y} \\ & =y_{001}-\bar{y} & \end{array}$$

Applying [10.15], viz. $\Sigma x^2 = n\bar{x}^2 + \Sigma(x - \bar{x})^2$, to the first column, we have

$$\sum_i\sum_j(y_{ij1}-y_{i00}-y_{0j0}+\bar{y})^2=5(y_{001}-\bar{y})^2+\sum_i\sum_j(y_{ij1}-y_{i00}-y_{0j0}-y_{001}+2\bar{y})^2,$$

where i and j may have the pairs of values 1, 1; 2, 4; 3, 3; 4, 5; and 5, 2. Summing over all values of k, we get

$$\sum_i\sum_j\sum_k(y_{ijk}-y_{i00}-y_{0j0}+\bar{y})^2=5\sum_k(y_{00k}-\bar{y})^2+\sum_i\sum_j\sum_k(y_{ijk}-y_{i00}-y_{0j0}-y_{00k}+2\bar{y})^2,$$

and substituting in [14.1], we have

$$\sum_i\sum_j\sum_k(y_{ijk}-\bar{y})^2 = 5\sum_i(y_{i00}-\bar{y})^2+5\sum_j(y_{0j0}-\bar{y})^2+5\sum_k(y_{00k}-\bar{y})^2 + \sum_i\sum_j\sum_k(y_{ijk}-y_{i00}-y_{0j0}-y_{00k}+2\bar{y})^2, \quad [14.2]$$

illustrating for the Latin square design how the Total S.S. is partitioned into S.S.'s for rows, columns, treatments, and error. Actually, the triple sigma sign in the Total S.S. and the Error S.S. contains one superfluous sigma, because summation over i and j alone covers all possible values of y.

14.2.3 As always, the above partitioning is purely algebraic and the y_{ijk} need not be statistical variates. If they are, then we have an orthogonal subdivision of the Total S.S. The concomitant partitioning of D.F. follows similarly to before. If the square is **of order r** (i.e. has r rows, r columns, and r treatments), there are $r - 1$ D.F. each for rows, columns, and treatments, leaving $r^2 - 1 - 3(r - 1) = (r - 1)(r - 2)$ D.F. for error. The above partitioning into component S.S.'s and D.F. may be displayed in a symbolic analysis of variance table as follows:

Table 14.1: Symbolic analysis of variance for a Latin square design

Source	D.F.	S.S.	M.S.
Rows	$r-1$	$r\sum_i(y_{i00}-\bar{y})^2$	
Columns	$r-1$	$r\sum_j(y_{0j0}-\bar{y})^2$	
Treatments	$r-1$	$r\sum_k(y_{00k}-\bar{y})^2 = A$	$\frac{A}{r-1}$
Error	$(r-1)(r-2)$	$\sum_i\sum_j\sum_k(y_{ijk}-y_{i00}-y_{0j0}-y_{00k}+2\bar{y})^2 = B$	$\frac{B}{(r-1)(r-2)}$
Total	r^2-1	$\sum_i\sum_j\sum_k(y_{ijk}-\bar{y})^2$	

On the usual assumption of N.I.D. errors the F-test of $\frac{\text{Treatments M.S.}}{\text{Error M.S.}}$ may be made to test whether significant differences exist between treatments.

14.3 Statistical analysis of the Latin square design

The calculation of S.S.'s for rows, columns, and treatments follows the usual rules (e.g. Formula [13.4]), and is, in fact, a little easier since the divisors are all the same.

Example 14.1 The following are the plan and yields of grain (in lb.) from a Latin square fertilizer experiment on wheat conducted at Rothamsted Experimental Station (*Rothamsted Report*, 1932, page 147):

D 72·2	*SS* 55·4	*O* 36·6	*C* 67·9	*S* 73·0
O 36·4	*C* 46·9	*SS* 46·8	*S* 54·9	*D* 68·5
SS 71·5	*S* 55·6	*D* 71·6	*O* 67·5	*C* 78·4
S 68·9	*O* 53·2	*C* 69·8	*D* 79·6	*SS* 77·2
C 82·0	*D* 81·0	*S* 76·0	*SS* 87·9	*O* 70·9

The treatments are:

O = no fertilizer
S = single dressing of nitrogen (sulphate of ammonia) in March
SS = same as S, but applied over 6 monthly dressings (Nov.–April)
C = equivalent quantity of cyanamide in October (just before planting)
D = 50–50 mixture of cyanamide and dicyanadiomide in October.

Analyse the data, presenting results in bags per acre. The plot size = $\frac{1}{40}$ acre, and 1 bag = 200lb.

Computation sheet (basic analysis)

(A)

						Row totals
	D 72·2	*SS* 55·4	*O* 36·6	*C* 67·9	*S* 73·0	305·1
	O 36·4	*C* 46·9	*SS* 46·8	*S* 54·9	*D* 68·5	253·5
	SS 71·5	*S* 55·6	*D* 71·6	*O* 67·5	*C* 78·4	344·6
	S 68·9	*O* 53·2	*C* 69·8	*D* 79·6	*SS* 77·2	348·7
	C 82·0	*D* 81·0	*S* 76·0	*SS* 87·9	*O* 70·9	397·8
Column totals	331·0	292·1	300·8	357·8	368·0	1649·7 (B)

Treatment totals (C)

O	264·6
S	328·4
SS	338·8
C	345·0
D	372·9
	1649·7

Skeleton analysis of variance (D)

	D.F.
Rows	4
Columns	4
Treatments	4
Error	12
Total	24

$$\text{C.F.} = \frac{(1649\cdot7)^2}{25} = 108{,}860\cdot4036$$

Total S.S. = 113,574·73
108,860·40
4,714·33

(E) Rows S.S. = 111,186·79
108,860·40
2,326·39

Columns S.S. = 109,761·78
108,860·40
901·38

Treatments S.S. = 110,144·91
108,860·40
1,284·51

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	*F*
Rows	4	2,326·39		
Columns	4	901·38		
Treatments	4	1,284·51	321·13 (F)	19·1**
Error	12	202·05	16·84	
Total	24	4,714·33		

S.E. of a single yield = 4·104 (G)

$$\text{C.V.} = \frac{4\cdot104}{1649\cdot7} \times 25 \times 100 = 6\cdot2\%$$

S.E. of a single treatment total = $\sqrt{5 \times 16\cdot84} = 9\cdot18$.

Least significant differences (2 treatment totals) = $\sqrt{10 \times 16\cdot84} \times t$ (12 D.F.)

$$= 12\cdot977 \times \begin{cases} 2\cdot179 \\ 3\cdot055 \end{cases}$$

= 28·28 (5%)
39·64 (1%)

Conversion factor, treatment totals to means in bags per acre = $\frac{40}{5} \times \frac{1}{200} = 0\cdot04$ (H)

Notes on basic analysis

(A) Usually Latin square data are given in the form of a field plan which already consists of a rectangular array so far as rows and columns are concerned.

(B) Totals for rows and columns are obtained and *the G.T. checked as the sum of the row totals as well as the sum of the column totals.*

(C) Treatment totals are calculated *and their sum checked to the G.T.* There is no need to write the yields of the separate treatments out afresh. They can be picked out from the data and added on a machine.

(D) The analyses we are describing are gradually becoming more complex as we go along. It may be helpful to set out at the start of the computations, therefore, a "skeleton analysis of variance table" which contains the various sources of variation and the D.F. This then provides the "plan of campaign"—the S.S.'s which have to be calculated, etc.

(E) The Rows S.S. is calculated from $\frac{1}{5}\Sigma T^2 - C.F.$, and the same divisor applies for the Columns and Treatments S.S.'s also.

(F) In this particular example the treatments comprise a classified set and so the calculation of the Treatments M.S. and F is really superfluous. The Rows and Columns M.S.'s are of little interest and are not calculated in a routine analysis. We may notice, however, that both M.S.'s are much larger than the Error M.S., indicating that, if a randomized blocks design had been used with either rows or columns as blocks, the error would have been considerably higher.

(G) $4\cdot104 = \sqrt{16\cdot84}$.

(H) The decimal happens to be exact here, otherwise many more decimal places would have had to be retained.

Computation sheet (continued)

S.S. Control *v.* Remainder: (I)

Linear function = 5 × 264·6 − G.T. (J) = −326·7

Divisor = {4² + (4 × 1)} × 5 = 100 (K)

S.S. Remainder among themselves = 96,142·28 (L)

$$C.F. = \frac{(1649\cdot7 - 264\cdot6)^2}{20} \qquad \frac{95{,}925\cdot10}{217\cdot18}$$

	D.F.	S.S.	M.S.	F
Control *v.* Average of nitrogen treatments	1	1,067·33	1067·33	63·4** (N)
Nitrogen treatments	3	217·18	72·39	4·3* (N)
Treatments	4	1,284·51 (M)		
Error	12		16·84	

Subdivision of S.S. between nitrogen treatments:

	Comparison (O)	Linear function		Divisor	S.S.	D.F.	F
1.	*S v. SS*	338·8 (P) 328·4 10·4		10	10·82	1	<1 (S)
2.	*C v. D*	372·9 (P) 345·0 27·9		10	77·84	1	4·62 (nearly*)
3.	Average of sulphate of (Q) ammonia treatments *v.* Average of cyanamide treatments = $(S + SS)$ *v.* $(C + D)$	345·0 372·9 717·9 667·2 50·7	338·8 328·4 667·2	20	128·52 217·18 (R)	1	7·63*

Presentation of results

TREATMENT MEANS IN BAGS PER ACRE

O	*S*	*SS*	*C*	*D*	Mean
10·58	13·14	13·15	13·80	14·92 (T)	13·20

S.E. = ±0·367 (U)

L.S.D's: 1·13 (5%) and 1·58 (1%) (U)

S.E. of a single plot as % of mean yield = 6·2%
(coefficient of variation)

All the nitrogen treatments are superior to the control at the 1% level, the responses ranging from 2·56 bags per acre to 4·34 bags per acre, the largest response being recorded for the cyanamide-dicyanodiomide mixture. The average of the cyanamide treatments applied before sowing is significantly higher than the average of the sulphate of ammonia treatments. The cyanamide-dicyanodiomide mixture in October has yielded 1·12 bags per acre more than cyanamide applied at the same time (the difference being very nearly significant at the 5% level), 1·37 bags per acre more than sulphate of ammonia applied as 6 monthly dressings (significant at 5% level), and 1·78 bags per acre more than sulphate of ammonia applied in March (significant at 1% level). A difference of 0·41 bags per acre in favour of sulphate of ammonia applied in 6 monthly dressings starting just after planting as compared with the same quantity applied in March is not significant. (V)

Notes on additional analysis

(I) The subdivision of the Treatments S.S. into Control *v.* remainder and Remainder among themselves (§ 11·8) is appropriate here. Although the fertilizer treatments are not an unclassified set, they are a set of treatments which are on exactly the same footing, i.e. they are potential nitrogen treatments. In Example 13.1 the treatments other than control consisted of a set in which various additions to a basic nitrogen treatment were under test, and it would have been unnatural to regard them as being on an equal footing.

(J) $4 \times \text{Control} - \text{Remainder}$ [14.3]
$= 5 \times \text{Control} - \text{G.T.}$ [14.4]

(K) The divisor is obtained as in § 11.8.3. *The coefficients squared must be those of the linear function [14.3]* (*i.e.* $4 - 1 - 1 - 1 - 1$), not those of the more convenient computational form [14.4]. In the latter the Control has two separate coefficients 5 and -1; these must be reduced to a single coefficient. The S.S. obtained as $\frac{(326\cdot 7)^2}{100}$ is entered directly in the table below.

(L) The usual rules apply. The divisor is 5. The total of the remainder is G.T. − Control = 1649·7 − 264·6, made up of 20 plots, and this determines the C.F.

(M) Because this is an orthogonal subdivision, the two components add to the Treatments S.S.

(N) Most of the Treatments S.S. is accounted for by the difference between the control and the remainder. Significant differences do exist, however, between the nitrogen treatments, and we are therefore permitted to proceed to individual comparisons.

(O) The nitrogen treatments are, nevertheless, not an unclassified set and the individual comparisons we make should be capable of being, and should be, determined beforehand. The set is, however, a rather miscellaneous one, a number of different points (sulphate of ammonia *versus* cyanamide, type of cyanamide treatment, and time of application) being under simultaneous study. Even the comparison of sulphate of ammonia and cyanamide appears intermingled with a time of application effect, but apparently there is really no question of wishing to study sulphate of ammonia applied before sowing or cyanamide after sowing. In this case, therefore, the time of application cannot be separated from the type of fertilizer.

The only clear-cut comparisons are S *v.* SS (time of application of sulphate of ammonia) and C *v.* D (cyanamide alone *versus* the mixture, the time of application being the same for both). The analysis made includes these two comparisons; the third has been chosen so as to make up a complete orthogonal set. The third comparison does not really convey very much since there is no single point being decided. Perhaps the non-orthogonal comparison, S *v.* C, sulphate of ammonia in March *versus* cyanamide in October (regarding the two times of application as being inseparable from the two types of fertilizer), might have been preferable.

(P) The linear functions here are simple differences of two treatment totals each of 5 plots. The divisor is therefore 10, and $10\cdot 82 = \frac{(10\cdot 4)^2}{10}$, etc.

(Q) As explained in § 11.3.2 we get rid of fractions by considering the linear function

$$S + SS - C - D,$$

which is obviously orthogonal to $S - SS$ and $C - D$. This partitioning is a special case of the type of subdivision discussed in § 11.7.1; the two "within" categories (within sulphate of ammonia treatments and within cyanamide treatments) have only 1 D.F. here.

The divisor for this S.S. is $(1^2 + 1^2 + 1^2 + 1^2) \times 5 = 20$, and $128\cdot 52 = \frac{(50\cdot 7)^2}{20}$.

(R) This being an orthogonal subdivision, the component S.S.'s must sum to the S.S. for between nitrogen treatments.

It is possible to present a combined analysis of variance table of the following type:

		D.F.
Rows		4
Columns		4
Treatments:		
Control *v.* nitrogen treatments	1	
Time of application of sulphate of ammonia	1	
Cyanamide *v.* mixture	1	
Sulphate of ammonia treatments *v.* cyanamide treatments	1	4
Error		12
Total		24

but this is not really necessary.

(S) These F-tests are, of course, made against the Error M.S. (16.84).

(T) $10 \cdot 58 = 264 \cdot 6 \times 0 \cdot 04$, etc.

(U) $0 \cdot 367 = 9 \cdot 18 \times 0 \cdot 04$; $1 \cdot 13 = 28 \cdot 28 \times 0 \cdot 04$, etc.

(V) The original report of this experiment made a point of stating the non-significance of the comparison S v. C, which was apparently of interest. The above summary is based more on regarding the four nitrogen treatments as equivalent possibilities than on the subdivision of the S.S. between nitrogen treatments, which has not helped very much in this example.

14.4 Statistical model for the Latin square

14.4.1 A statistical model for the Latin square design may be proposed on the same lines as hitherto. We may assume for any plot yield the equation

$$y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + \epsilon_{ijk}, \qquad [14.5]$$

where μ is a component common to all plots, α_i is a component common to all plots in the i^{th} row, β_j is a component common to all plots in the j^{th} column, τ_k is a component common to all plots receiving the k^{th} treatment, and ϵ_{ijk} is a random component N.I.D.$(0, \sigma^2)$. If we regard the three sets of parameters α_i, β_j, and τ_k as representing fixed effects, then we must impose one linear restriction on each set, and these are conveniently taken as $\Sigma\alpha_i = 0$, $\Sigma\beta_j = 0$, and $\Sigma\tau_k = 0$.

14.4.2 Examining this model, we see that the mean or expected value of any plot on the null hypothesis $(\mu + \alpha_i + \beta_j)$ is made up of three additive components. In general no two plots have the same expected value, the fertility of each individual plot being "pinpointed" by the fact that it receives a component from the row it is in as well as one from the column it is in. The treatment effect is again a purely additive component and is assumed not to "interact" with rows or columns; that is to say, it is assumed to be quite unaffected by the differences in fertility between rows, columns, or plots.

14.4.3 In estimating the parameters of Model [14.5] we equate each plot yield to its expected value in terms of the estimates thus:

$$y_{ijk} = m + a_i + b_j + t_k \qquad [14.6]$$

Sometimes r_i and c_j are used for row and column constants, but these do not give rise to convenient Greek letters for Model [14.5].

Normal equations involving the G.T., row totals, column totals, and treatment totals are formed similar to Equations [13.14] in accordance with the principle of least squares, and the parameter estimates

$$m = \hat{\mu} = \bar{y}, \qquad a_i = \hat{\alpha}_i = y_{i00} - \bar{y},$$
$$b_j = \hat{\beta}_j = y_{0j0} - \bar{y}, \qquad t_k = \hat{\tau}_k = y_{00k} - \bar{y},$$

are obtained with similar ease upon adopting the linear restrictions $\Sigma a_i = \Sigma b_j = \Sigma t_k = 0$. The fitting of constants is such that the S.S. of error residuals, $\sum_i \sum_j \sum_k (y_{ijk} - m - a_i - b_j - t_k)^2$, is a minimum.

This minimum value, viz.

$$\sum_i \sum_j \sum_k \{y_{ijk} - \bar{y} - (y_{i00} - \bar{y}) - (y_{0j0} - \bar{y}) - (y_{00k} - \bar{y})\}^2 \qquad [14.7]$$
$$= \sum_i \sum_j \sum_k (y_{ijk} - y_{i00} - y_{0j0} - y_{00k} + 2\bar{y})^2,$$

is the same as the Error S.S. of the analysis of variance. The numbers of independent constants fitted, viz. $r - 1$ for rows, $r - 1$ for columns, and $r - 1$ for treatments, correspond to the D.F. in the analysis of variance. The single D.F. for m is already deducted from r^2 in the D.F. for the Total S.S.

14.5 Orthogonalityof the Latin square design

14.5.1 The simple solution to the normal equations presented above is possible only because of the orthogonality of the design. An inspection shows that each row total in terms of [14.6] contains all the b_j and all the t_k. Simple subtraction therefore enables differences among the a_i to be evaluated free from column or treatment effects, i.e. rows are orthogonal to columns and treatments. Similarly each column total in terms of [14.6] contains all the a_i and all the t_k, and each treatment total contains all the a_i and all the b_j, since each treatment appears once in each row and column. Evidently rows, columns, and treatments are all mutually orthogonal. It is also apparent from the exact partitioning of the Total S.S. that all four categories (rows, columns, treatments, and error) are mutually orthogonal, but the above discussion of the orthogonality of rows, columns, and treatments enables the idea to be more firmly grasped.

14.5.2 Because of the orthogonality, each of the S.S.'s for rows, columns, and treatments in the analysis of variance represents deviations due to the indicated source only, being directly related to the estimates of these respective effects given in § 14.4.3. It is also apparent from [14.7] that the Error S.S. represents solely error deviations. Should the orthogonality of the design be upset, S.S.'s calculated from row, column, or treatment totals in the usual way will not represent purely row, column, and treatment effects respectively, but each will be affected by the others.

14.6 Randomization of the Latin square design

14.6.1 Examples of Latin squares of order up to 12 are given in Fisher and Yates's tables. The easiest way to draw up a design is to choose one of these squares of the required order at random and to randomize the rows and columns. Then allot the treatments to the letters at random. Each step can be carried out with random numbers in the usual way. For the smaller squares not all these steps are really necessary, but it is easiest to prescribe a general method to be followed in all cases.

14.6.2 To illustrate, let us take a square of the 5th order from Fisher and Yates:

A	B	C	D	E	(1)
B	A	D	E	C	(2)
C	E	B	A	D	(3)
D	C	E	B	A	(4)
E	D	A	C	B	(5),

which has its first row and column in set order. Let us say that the random numbers drawn for rows were 2, 4, 1, 3, 5. We may interpret this either as 2nd row in 1st position, etc., or row 1 in 2nd position, etc. Adopting the former, we get:

B	A	D	E	C
D	C	E	B	A
A	B	C	D	E
C	E	B	A	D
E	D	A	C	B
(1)	(2)	(3)	(4)	(5)

We now randomize the columns, and let us say the random numbers drawn are 3, 5, 2, 4, 1. The square then becomes:

D	C	A	E	B
E	A	C	B	D
C	E	B	D	A
B	D	E	A	C
A	B	D	C	E

A further set of random numbers, e.g. 4, 1, 2, 5, 3, allows us to allot the treatments to letters thus:

A = Treatment 4
B = Treatment 1
C = Treatment 2
D = Treatment 5
E = Treatment 3,

and the square in its final form is:

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

14.7 Field lay-out of the Latin square design

14.7.1 Normally the plots of a Latin square design form a square array on the ground, though, of course, the experimental area need not be an exact square—for example, if the plots are oblong. Remembering, however, our introduction to the design in § 14.1.1, we can see that what the second set of blocks in a Latin square really does is to eliminate the effects of consistent differences in fertility due to plot position within the first set of blocks. Thus in Figure 14.1, if all the first plots in each block are higher in fertility on the average than all the second plots in each block, then this difference is eliminated by considering all the first plots as comprising one column, all the second plots another column, and so on.

14.7.2 It is therefore apparent that the type of field plan shown in Figure 14.2 could also function as a Latin square, and could be very convenient if the field happened to be shaped like a parallelogram. All that has been done

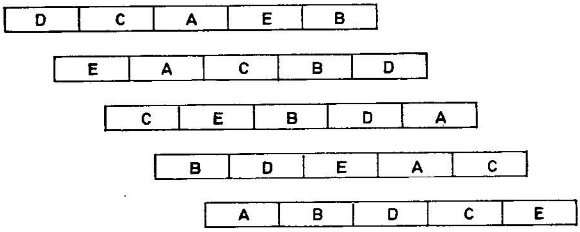


Figure 14.2: Latin square design with columns on an angle.

is to rotate the columns through an angle, and this in no way prevents them from performing their function of eliminating effects of plot position within blocks.

14.7.3 Continuing the process further, we can end up with the plots all in one long line as in Figure 13.1 but with the randomization within blocks restricted in the Latin square manner, so that each treatment appears once in the first position in each block, once in the second position, and so on. This is actually a Latin square design, of course, and must be analysed accordingly, but it departs rather radically from a "square" in form and is not a common type of lay-out.

14.7.4 In the ordinary type of square lay-out it is not desirable to have plots which depart too greatly from a square shape. Otherwise either the rows or the columns will become unduly elongated and will tend to lose their efficiency in controlling error. This is because fertility gradients are not perfectly regular; consequently, long narrow rows or columns will tend to be alike in yield for the same general reason as long narrow plots will tend to be alike (§ 12.11.8), and this will militate against the removal of fertility effects which are definitely present. Wishart has mentioned a theoretical fertility situation in which the Latin square design would be useless. This is illustrated in Figure 14.3, where it is easily seen that the rows and columns would all be of more or less equal fertility:

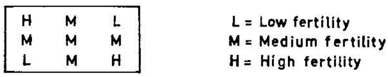


Figure 14.3: An experimental area in which the Latin square design would be ineffective in controlling error.

The longer the rows or columns the more likely is this situation to be reproduced in respect of the long rows or columns, and the more likely it is that a randomized blocks design with the set of elongated blocks excluded would be just as efficient. Since (as we shall see in § 14.8) the decision to use a Latin square in preference to a randomized blocks design is by no means an automatic one in the same way as the decision to use a randomized blocks design in preference to a simple random design, the randomized blocks design would probably be preferred if the retention of long narrow plots is considered essential.

14.8 Limitations of the Latin square design and comparison with the randomized blocks design in field experiments

14.8.1 By reason of its two-way elimination of soil heterogeneity effects, the Latin square design is more efficient than the randomized blocks design in nearly all fertility situations, since the reduction in experimental error usually more than offsets the loss of error D.F. Nevertheless, the number of D.F. being devoted to control of error as opposed to estimation of error is becoming appreciable, especially with small designs. In fact, it is necessary to rule out 3×3 and 4×4 squares altogether so far as field experiments are concerned, since these designs provide only 2 D.F. and 6 D.F. respectively for error (see § 12.8.4). The 2×2 square does not permit any estimation of error at all. Furthermore, since the randomized blocks design already gives a reasonable control of error, it is necessary to balance the desirability for still greater efficiency against certain disadvantages of the Latin square.

14.8.2 The chief disadvantage of the Latin square as a general design is the fact that the number of replications must always equal the number of treatments. This virtually limits its use to experiments with 5 to 9 treatments. Smaller squares are ruled out, as we have just seen. With large numbers of treatments we are committed to a number of replications which is probably excessive, whereas with the randomized blocks design any desired number of replications is permissible and a number of treatments up to about 16 can be considered. A Latin square of order greater than 9 would tend to become unwieldy and to strain the resources of the experimenter (cf. § 12.11.7). Furthermore, the efficiency tends to become impaired when the number of treatments is large, due to long rows and columns, but this limitation applies equally to the randomized blocks design, the blocks of which may not operate efficiently if they are too large.

Nevertheless, for an experiment in which the desired number of treatments ranges from 6 to 8 the Latin square provides just about the right number of replications and is excellent from the point of view of efficiency.

14.8.3 The difficulty with the Latin square in respect of the number of replications is only one aspect of the greater *flexibility* of the randomized blocks design as compared with the Latin square. Just as the randomized blocks design is a more complex design than the simple random design, so the Latin square represents a further step up in complexity. The Latin square design is therefore more susceptible to mishaps with the data, in the sense that there is now no kind of mishap which does not upset the orthogonality of the design and hence cause the simple analysis of variance to break down. For example, if all the plots of one or more treatments have to be discarded in a randomized blocks design, the yields of the remaining treatments may be analysed in a straightforward manner, as if the missing treatments had never existed; in the case of a Latin square, the analysis is more complex. Then, again, whereas in the randomized blocks design it is possible to drop one or more blocks from the analysis, it is impossible to drop a row or a column from a Latin square design without fairly severe complications.

In general, too, it is not so easy to fit the Latin square design into an irregular area.

14.8.4 In its usual square form the Latin square is more suitable for crops such as potatoes, which are sown and cultivated by hand, than for crops requiring machine operations, such as small-grain cereals. This is because the square lay-out does not make for ease of access to plots and excessive space would be wasted in the way of pathways if room had to be left for machinery to turn, etc. Also, as seen above, it is usually best not to have long narrow plots.

There will be no difficulties of access, however, if the design is laid out in a long line as in Figure 12.3, a possibility explained in § 14.7.3. This type of lay-out is, as we know, exceptionally convenient for machine work. In view of the fact that, if we use a randomized blocks design in this type of lay-out, errors due to soil heterogeneity are limited to variations in fertility within blocks, it would seem a logical step to use a Latin square design in the same type of lay-out to guard against the possibility that the lengths of the plots do not lie parallel to the fertility gradient. However, there is still the point that the plots belonging to any one of the additional set of blocks extend over almost the whole length of the experimental area, so that these blocks will tend to be inefficient. It follows that this type of lay-out should be reserved for circumstances where a strong fertility gradient is known or suspected to run parallel to the length of the area and it is impossible or inconvenient to have the lengths of the plots parallel to the gradient.

14.8.5 From one point of view we may have been too hard on the Latin square as a design for an experiment with fewer than 5 treatments, for it is always possible to gain error D.F. by repeating the design in two or more squares alongside. Even the 2 × 2 square could be used in this way.

*The analysis of repeated squares is not difficult. Suppose we have a number of squares separately randomized. S.S.'s for rows are computed for each square separately and added; similarly for columns. The Treatments S.S. is, however, not derived from each square separately, but from treatment totals added over all squares. In addition a S.S. between squares is calculated from the totals of the squares. For example, with two 4 × 4 squares the skeleton analysis of variance is:

	D.F.	
Squares	1	
Rows	6	(3 from each square)
Columns	6	(3 from each square)
Treatments	3	
Error	15	
Total	31	

14.8.6 Designs do exist which allow a two-way elimination of heterogeneity, and yet do not require the full number of replications. These designs do not, however, possess the orthogonal properties of the Latin square and are not in fact, Latin squares.

Designs based on the Latin square principle of two-way elimination of heterogeneity are not as common as designs based on the simple blocks principle.

14.9 The Latin square design in other than field experiments

14.9.1 Like the randomized blocks design, the Latin square is not restricted to use in field experiments. We might, for example, in industrial experimentation, apply different treatments to different batches of material by means of different operators, so that differences due to the different batches and operators may be eliminated. So long as the row and column groupings correspond to sources of variation over and above the random variation, the experimental error will be reduced.

14.9.2 Certain limitations of the design previously discussed in relation to field experiments may be no longer valid in other types of experiment. For example, it might be no problem to conduct a large number of replications and the question of unwieldiness need not arise. The inflexible number of replications may not always be convenient, however. Naturally there is no problem in respect of difficulty of access, for rows and columns no longer correspond to positions on the ground, and may have a logical rather than a physical basis. The infinite model [14.5] may be able to be considered as an exact possibility instead of merely a model which leads to a good approximation to the exact results derivable under randomization theory. In that case the restriction on the use of small squares would depend solely on the precision required and not on considerations of relationship to randomization theory. A 4×4 square might then be regarded as a feasible design. It is also probable that it will be desired to regard some classes of effects, e.g. operators, as random effects.

14.9.3 There is one important precaution to remember, which is hardly ever likely to warrant attention in the ordinary Latin square design in field experiments provided the variate is approximately N.D., and that is the possibility of non-additivity or interaction of row, column, and treatment effects. The presence of non-additivity would cause the error to be overestimated.

EXERCISES

(*see next page*)

EXERCISES

14.1 (Data from C. H. Goulden, *Methods of statistical analysis.*) The following are the field lay-out and yields in bushels per acre of an experiment on dusting wheat with sulphur to control stem-rust. The treatments are:

A = dusted before rains
B = dusted after rains
C = dusted once each week
D = drifting once each week
E = control or check

B 4·9	*D* 6·4	*E* 3·3	*A* 9·5	*C* 11·8
C 9·3	*A* 4·0	*B* 6·2	*E* 5·1	*D* 5·4
D 7·6	*C* 15·4	*A* 6·5	*B* 6·0	*E* 4·6
E 6·3	*B* 7·6	*C* 13·2	*D* 8·6	*A* 4·9
A 9·3	*E* 6·3	*D* 11·8	*C* 15·9	*B* 7·6

Analyse the data. (Hint: It is probable that Treatment C is a standard but difficult and expensive method, and that Treatments A, B, and D are being tested as alternatives.)

14.2 In a digestion trial carried out with 6 Shorthorn steers, each animal received each of 6 rations in 6 successive periods, the experimental design being a Latin square. Coefficients of digestibility of nitrogen were calculated as follows:

Steer	Period					
	1	2	3	4	5	6
1	61·1 (*B*)	69·3 (*D*)	67·6 (*C*)	61·9 (*F*)	58·8 (*A*)	65·2 (*E*)
2	56·9 (*A*)	59·1 (*F*)	64·0 (*D*)	61·0 (*C*)	65·7 (*E*)	56·6 (*B*)
3	66·5 (*C*)	62·2 (*A*)	61·1 (*B*)	66·2 (*E*)	62·0 (*F*)	62·2 (*D*)
4	66·7 (*E*)	67·4 (*B*)	65·1 (*F*)	65·1 (*D*)	69·6 (*C*)	52·7 (*A*)
5	67·8 (*D*)	64·7 (*C*)	63·6 (*E*)	53·2 (*A*)	61·7 (*B*)	62·0 (*F*)
6	71·4 (*F*)	67·5 (*E*)	55·8 (*A*)	63·2 (*B*)	68·0 (*D*)	62·9 (*C*)

In each case the ration concerned is given in brackets. Ration A consisted of hay alone, the other rations consisting of various mixtures of hay and barley. Analyse the results of the trial.
(N.B.: This type of design, in which treatments are given to the same unit in succession (cf. § 12.10.3), but in which provision is made for removing the effect of periods is called a **change-over design**.)