BIOMETRY 222

Introduction to Experimental Design

Complied by H M Dicks
Former Senior Lecturer
Department of Statistics and Biometry
University of Natal, Pietermaritzburg. South Africa.
(02/2006)

Chapter 1 : Introduction to Experimental Design

1.0 INTRODUCTION.

An experiment should provide :

i. Unbiased estimates of treatment differences.
ii. Unbiased estimate of the experimental error to which the estimates of treatment differences are subject.

Two Components of Experimental Design:

i. Structure of experimental units.
ii. Structure of treatment set.

1.1 STRUCTURE OF EXPERIMENTAL UNITS.

Experimental units are those units of experimental material to which the different treatments are allocated in consequence of each single act of randomisation. The structure of experimental units is concerned with identifying the units and describing the pattern of variation between the units which would be observed if a single treatment were applied to all units.

CONSIDER:

- Representativeness
  – the extent to which the experimental material, i.e., the units to which the treatments are applied, are representative of the units or material for which inferences are to be made. This may well be the most important point in the validity of an experiment; if the material used in the experiment is not representative of the material to which the conclusions are to be applied, then there is probably little merit in the experiment.
- Replication
  – the repetition of of plots of a treatment within a single experiment. Replication in any experiment will be denoted by r (r = 1 implies an unreplicated experiment).

  Role of Replication:
  (i) Permits estimation of error variance (σ^2) where σ^2= Var (ε), for the appropriate model. The Error Mean Square, s^2, is our estimate of error variance.
  (ii) Increase in r increases precision of parameter estimation
    (a) Var(treatment mean) = $\frac{\sigma^2}{r}$ hence reduced S.E's for treatment means.
    (b) More d.f. available for estimation of σ^2
- Randomization
  – the allocation by chance so that each treatment has an exactly equal chance of being allocated to any one experimental unit. A physical process is insisted upon through, for example the use of tables of random numbers.
  Some arguments for randomisation may be found in the following references:
  * The Design of Experiments, chapter 2 – R A Fisher.
  * The American Statistician (1973) – R Hader.
  * Planning of Experiments, page 85 – D R Cox.

  It not at all unusual for a resesrcher to record several observations on each experimental unit. In such instances one must concentrate on the nature of the randomisation since eventually the randomisation used when the experiment is set up will dictate the proper analysis.
- Blocking
  – the grouping of experimental units into groups (blocks) within which units treated alike will show less variation within that grouping (block) than over the whole set of experimental units (experimental area). To a large extent, the efficiency of any experimental design will depend on the ability of the researcher identifying such grouping (blocks).

It may be further noted that the efficiency of an experiment is determined by:

- S.E (treatment mean)
- d.f availabe for the estimation of the Error M.S.
- replication.

1.2 DESIGNS.

1.2.1 Regular order designs.

A	B	C	D	E	F	G	H	Etc

H → L (?)

1.2.2 Balanced allocation.

Concept: Each treatment to have a set of plots of approximately equal average "fertility".

(a) Uniformity data method.

(b) Systematic Designs.

A	B	C	D	D	C	B	A	A	B	C	D	etc

1.2.3 Valid designs.

Valid experimental designs are ones in which the randomisation of treatments to plots is such that, for the appropriate method of analysis, on the Null Hypothesis the mean of the Treatment M.S over all randomisations is equal to the mean of the Error M.S's over all randomisations. For valid designs the Error M.S of the appropriate A.O.V. is an unbiased estimate of the Error Variance (σ^2). In addition, one must take account of the conditions and assumptions implicit in the model for any design. For example, consider the model for the analysis of a Simple Random Design, (completly randomised design CRD):

$$y_{ij} = \mu + \tau_i + \epsilon_{ij} \qquad \textbf{(Model 1)}$$

Assumptions to be fulfilled are :

i. Effect of i^{th} treatment must be constant for all plots with this treatment, irrespective of magnitude of ϵ_{ij} (- the assumption of ***Additivity***)

ii. ϵ's must be a sample from an infinite population.

iii. ϵ's must be uncorrelated.

iv. ϵ's must have the same variance, irrespective of treatment;
(- the assumption of ***Homogeneity of Variance***).

v. For F- and t-tests, population must be N.D, i.e., ϵ's must be N.I.D $(0, \sigma^2)$

1.3 TERMINOLOGY.

Field Experiments	General
experimental area	experimental material
plot	experimental unit
yield	observation, determination, reading, etc.

1.4 PLOT ARRANGEMENTS

a) General Considerations

i. Experimental area (material) should be chosen to be as UNIFORM as possible – uneven sites cause high error and do not add to generality.

ii. Plots (field experiments):
– usually square or rectangular
– equal in area.
– consistent shape
– same orientation, i.e. longer sides of rectangular plots parallel.

iii. Data from uniformity trials can be used to compare different plot sizes and shapes : i.e., build up multiples of smallest units into plots of various sizes and shapes, superimpose dummy treatments in typical design and analyse to obtain CV%.

b) Factors affecting plot size (field experiments).

i. Relationship between plot size and CV% -
(%CV decreases with increasing plot size)

Note: If doubling plot size reduces CV% from 10% to 9%, extra land would have been better invested in doubling the number of replications giving a reduction in the SE (treatment mean) in ratio $\sqrt{2} : 1$

ii. Common Plot Sizes – (Field Experiments):
$\frac{1}{50}$ to $\frac{1}{250}$ hectare — larger plots are sometimes needed, e.g., forest trials, pastures with grazing stock, irrigation methods, etc.
If a plot results in too great a bulk of harvested produce to be weighed rather sample than reduce plot size.

ii Very Small Plots:
For a given area of land, the smallest plot is usually the most efficient (i.e. lowest S.E of treatment mean). CV% is high, but so is number of replications.
However :
– very small plots are often unrealistic, especially if the site is to be used for demonstrations.
– too many separate plots to harvest.
– borders take up large proportion of gross plot.
– use of very small plots without many replications is unjustified – high CV%.

iii. Experimental area restricted.
It is better to maintain a satisfactory number of replications and reduce plot size below norm than vice versa.

iv. Large Plots (i.e., above optimal):
– pointless in ordinary crop experiments unless to facilitate use of machinery.
– too much produce to bag and weigh.
– experimental area should be compact or it may not keep desired uniformity.

Note: Change in aspect can also affect variability, and interpretation of results.

1.5 FACTORS GOVERNING NUMBER OF REPLICATION

(a) Adequate number of D.F. for error.
A minimum 12 df for "error", with 20 or more being desirable. Treatments also generate error d.f; with a large number of treatments fewer replications are needed for the same number of error d.f. (Example: for CRD (Completely random design) error df = t(r-1))

(b) Labour, costs, time, etc. – An average field experiment has between 40 and 60 plots; over 100 plots is a large experiment, which can become unwieldy to manage.

(c) Inverse Square Root Law Applies:
Increase in (r) from 3 to 12 halves S.E. of treatment mean, i.e., 9 replications extra, but to halve S.E. again would need an extra 36 replications.

1.6 FACTORS AFFECTING SHAPE OF PLOT

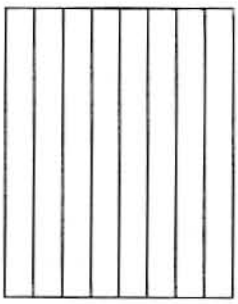
1.6.1 GRADIENTS.

General Principle:
Plots should be set out so as to be as alike in fertility as possible. Thus, for example, by using uniformity data, odd shaped plots could be constructed according to this principle. In practice, the choice is between square and oblong plots and if oblong, which orientation. The magnitude of experimental error is influenced by this choice.

Case I : No Fertility Gradient

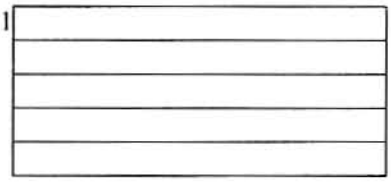
Long narrow plots alongside ;

a) Have their centres closer together and hence could vary less than square plots.
b) Tend to slice through areas more likely to be of similar fertility.



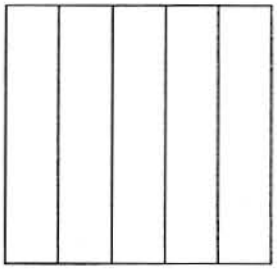
Case II: Fertility Gradient Present

a) Long narrow plots lying alongside parallel to gradient give lowest error, but if at right angles to gradient give highest error.

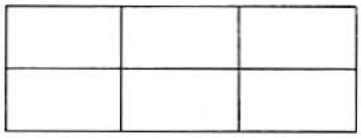


(a) Lowest Error (b) Highest Error

Gradient ⟶



b) Squarish plots on the same area would be intermediate :



Summary:

i) If fertility gradient is known, long narrow plots placed parallel to gradient will give lowest error.

ii) If fertility gradient is unknown (which is usual) squarish plots may be preferred as a middle course.

1.6.2 SHAPE OF PLOTS (Practical considerations)

a) Convenience at planting/harvesting – with row crops it is easier to have oblong plots with rows parallel to longest side.

b) Squarish plots are unsuitable for machinery (too many short runs).

c) "Border Areas" – square plots have the lowest perimeter for a given size (area) and so need the least area devoted to borders (with a nett plot consisting of a single row with single row borders less than a third of the gross plot counts for yield).

d) Sloping Ground – there is usually a fertility gradient running down the slope but to counter washaways, plots should lie across the slope.

e) Contoured Land – plot orientation may vary.

Chapter 2 : Randomised (Complete) Blocks Design (RCBD)

2.0 PRINCIPLE OF LOCAL CONTROL

- Not all variation is due to chance.
- Variation is often non-random or systematic of character and in field experiments can manifest itself in two main ways:
  a) Identically treated adjacent plots tend to yield alike as a rule than plots further apart; this is ofetn called:

     "THE PRINCIPLE OF LOCAL CONTROL"

  b) There is often a tendency for the general level of fertility to change in a particular direction, generally referred to as

     "FERTILITY" GRADIENTS.

- The possibility of high experimental error with a simple random design may be countered by dividing the experimental area into a number of equal rectangular areas called BLOCKS, within which the treatments allotted to a particular block are randomised. Plots are likely to vary less within a smaller area of a block than over the whole experimental area.
- Differences between blocks are eliminated from experimental error by means of certain D.F. in the ANOVA.

The simplest example of the use of *local control* is the Randomised Complete Blocks Design (RCBD).

2.1 DEFINITIONS

- Number of Blocks = Number of Replications
- In in simplest from, each block contains a single replication, i.e., each treatment appears once and only once in each block, i.e., number of plots per block is equal to the number of treatments
- The order of treatments within <u>each</u> block is randomised.

Example: r = 4 (number of blocks = numbre of replications), t = 6 (number of plots per block = number of treatments)

Rep 1

F	B	E
C	D	A

Rep 2

D	F	A
C	B	E

Rep 3

E	D	B
F	C	A

Rep 4

C	B	E
A	D	F

Treatments :
A = Control
B = N
C = N+P
D = N+L
E = N+P+L
F = "Special NPK" Mix

2.2 STATISTICAL MODEL FOR RANDOMIZED COMPLETE BLOCKS DESIGN

Let y_{ij} be the yield for the i^{th} treatment in the j^{th} block (replication). This may be represented as:

$$y_{ij} = \mu + \beta_j + \tau_i + \epsilon_{ij} \qquad \textbf{(MODEL 2)}$$

where β_j and τ_i the block and treatment effects are both regarded as FIXED EFFECTS. τ_i is a purely additive component, independent of the magnitude of ϵ_{ij} and β_j - more generally however the β_j (block effects) are considered to be random.

NOTE:

i. The assumption of ADDITIVITY in a Randomised Blocks Design implies that the treatment effects are constant over the different blocks. Also, that the block effects are constant for all plots within a block.

ii) Also, it is usual to assume that the ϵ_{ij} are NID $(0, \sigma^2)$

2.3 PARTITIONING OF TOTAL S.S. IN A RANDOMISED COMPLETE BLOCKS DESIGN

Consider the following rectangular array of the yields (y_{ij}) from 4 replications of 5 treatments.
y_{ij}= yield of the i^{th} treatment in the j^{th} block, $i = 1 \ldots t\ (=5)$; $j = 1 \ldots r\ (=4)$.

	Blocks				Treatment	
Treatments	1	2	3	4	Means	**Totals**
1	y_{11}	y_{12}	y_{13}	y_{14}	y_{10}	$\mathbf{T_1}$
2	y_{21}	y_{22}	y_{23}	y_{24}	y_{20}	$\mathbf{T_2}$
3	y_{31}	y_{32}	y_{33}	y_{34}	y_{30}	$\mathbf{T_3}$
4	y_{41}	y_{42}	y_{43}	y_{44}	y_{40}	$\mathbf{T_4}$
5	y_{51}	y_{52}	y_{53}	y_{54}	y_{50}	$\mathbf{T_5}$
Means	y_{01}	y_{02}	y_{03}	y_{04}	$\bar{y}$	**GT.**
Totals	$\mathbf{B_1}$	$\mathbf{B_2}$	$\mathbf{B_3}$	$\mathbf{B_4}$		

Now for Completely Random Design (CRD) our model, ignoring the distinction between random and fixed effects is:

$$y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

From which we derived the following partitioning of the sums of squares in an ANOVA table as:

Total S.S. = Treatment S.S. + Error S.S.

i.e. $\sum_i \sum_j (y_{ij} - \bar{y})^2 = r\sum_i (y_{io} - \bar{y})^2 + \sum_i \sum_j (y_{ij} - y_{io})^2$ [Note: in this example, r=4]

For the Randomised Complete Blocks Design (RCBD) our model is now extended to include a term for the Blocks component, (see also 2.3 below):

$$y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij}$$

And in the partitioning of the TOTAL SS it is necessary to separate out an S.S. for Blocks from $\sum_i \sum_j (y_{ij} - y_{io})^2$, the Error SS in the Completely Random Design.

Now $\sum x^2 = n\bar{x}^2 + \sum (x - \bar{x})^2$
if we let $x = y_{i1} - y_{io}$, so that $\bar{x} = y_{o1} - \bar{y}$
then $\sum_i (y_{i1} - y_{io})^2 = 5(y_{o1} - \bar{y})^2 + \sum_i (y_{i1} - y_{io} - y_{o1} + \bar{y})^2$
If we generalize and now sum over all j (i.e. blocks) we have : –
$\sum_i \sum_j (y_{ij} - y_{io})^2 = 5\sum_j (y_{oj} - \bar{y})^2 + \sum_i \sum_j (y_{ij} - y_{io} - y_{oj} + \bar{y})^2$
Generally we have:

$$\sum_i \sum_j (y_{ij} - \bar{y})^2 = r\sum_i (y_{io} - \bar{y})^2 + t\sum_j (y_{oj} - \bar{y})^2 + \sum_i \sum_j (y_{ij} - y_{io} - y_{oj} + \bar{y})^2$$

i.e. **Total S.S. = Treatment S.S. + Blocks S.S. + Error S.S.**
If the y_{ij} are statistical variates, the subdivision of the Total S.S. being exact, is orthogonal.
(Note: the concept of orthogonality in experimental design will be discussed later.)

The d.f. for Total S.S. $= (rt - 1)$ may be partitioned as:

$$(rt - 1) = (t - 1) + (r - 1) + (t - 1)(r - 1)$$
$$= \text{Treat (d.f.)} + \text{Block (d.f.)} + \text{Error(d.f.)}$$

SYMBOLIC ANOVA TABLE FOR RCBD:

Source	d.f.	S.S.		M.S.
Blocks	$r - 1$	$t\sum (y_{oj} - \bar{y})^2$		
Treatments	$t - 1$	$r\sum (y_{io} - \bar{y})^2$	$= A$	$\frac{A}{(t-1)}$
Error	$(r - 1)(t - 1)$	$\sum\sum (y_{ij} - y_{io} - y_{oj} - \bar{y})^2$	$= B$	$\frac{B}{(r-1)(t-1)}$
TOTAL	$rt - 1$	$\sum\sum (y_{ij} - \bar{y})^2$		

NOTE: We are usually not interested in testing the significance of differences between blocks, (i.e. block effects). In practice, S.S. Blocks and Treatments are calculated from TOTALS not means.

CALCULATIONS:

i. $CF = \frac{GT^2}{n}$ $(= rt)$

ii. S.S. Treatments $= r\sum_i (y_{io} - \bar{y})^2$

$= \frac{1}{r}\sum_i T_i^2 - CF$ where $T_i = i^{th}$ treatment total, $i = 1 \ldots t$

iii. S.S. Blocks $= t\sum_i (y_{oj} - \bar{y})^2$

$= \frac{1}{t}\sum_i B_j^2 - CF$ and $B_j = j^{th}$ Block Total : $(j = 1 \ldots r)$

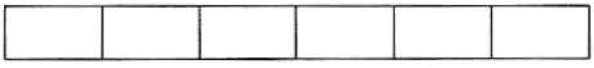
2.4 FIELD LAYOUT — RCBD

i. Experimental area should be as uniform as possible.
ii. Objective is NOT to make the BLOCKS as different from each other in average fertility as possibile.
iii. Objective should be to see that plots within a block vary in fertility as little as possible.
iv. Squarish or Compact blocks are preferred.
v. Shape of block, and plot arrangement with a block should be identical for all blocks.
vi. All blocks should be similarly orientated.
vii. Shape of block depends to some extent on:
   - shape of the plot.
   - area available (of a given shape)
   - topography
   - cultivation practices

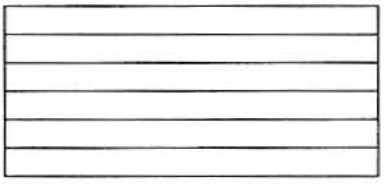
   e.g., mechanical planting/harvesting, side dressing, etc.

2.4.1 ARRANGEMENT OF 4 PLOTS IN A LONG NARROW BLOCK

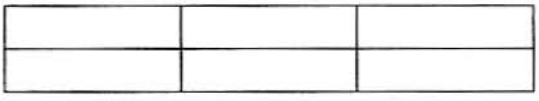
Example 1:



(a)



(b)



(c) (2)

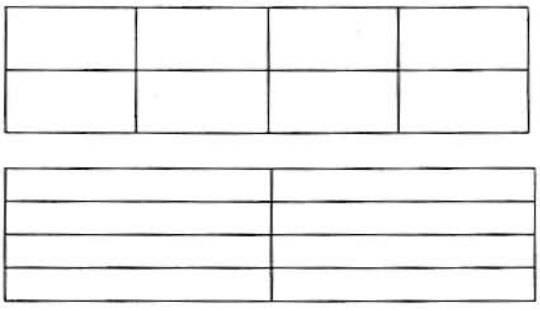
(1)

NOTE: i) If gradient (1) operates then:
Variability within layout (a) >> Variability within layout (b)

ii) If gradient (2) operates then:
Variability within (b) > Variability within layout (a)
i.e. not as great a difference as plots are narrower.

iii) Layout (c) is intermediate, but variability is high if gradient (1) operates.

Example 2:



(a)

(b)

↓ (2)

——————————→ (1)

NOTE: i) Layout (a) will be approximately equal in effect to layout (b) whatever the direction of fertility.

ii) For gradient (1), layouts (a) and (b) should have approximately the same Error M.S., although S.S. Blocks for (b) will be greater than S.S. Blocks for (a).

ii) Layout (b) for example, facilitates the use of heavy machinery in weed control, planting, harvesting, etc.

2.5 ORTHOGONALITY OF THE RANDOMIZED BLOCKS DESIGN

DEFINITION:

Orthogonality is that property of the design which ensures that the different classes of effects to which the experimental material is subject shall be capable of direct and separate estimation without any entanglement.

– F Yates –

In partitioning the Total S.S. for the RCBD we have :

Total S.S. = Block S.S. + Treatment S.S. + Error S.S.

This partitioning is orthogonal.

In the RBD model : $y_{ij} = \mu + \beta_j + \tau_i + \epsilon_{ij}$

Let the estimates of μ, β_j and τ_i be denoted by m, b_j and t_i respectively, then expected values for table are :

		Treatments: 1	2	...	4	Block Totals
Blocks:	1	$\mu + \beta_1 + \tau_1$	$\mu + \beta_1 + \tau_2$	...	$\mu + \beta_1 + \tau_4$	$4\mu + \sum\tau_i + 4\beta_1$
	2	$\mu + \beta_2 + \tau_1$	$\mu + \beta_2 + \tau_2$	...	$\mu + \beta_2 + \tau_4$	$4\mu + \sum\tau_i + 4\beta_2$
	⋮	⋮	⋮		⋮	⋮
	5	$\mu + \beta_5 + \tau_1$	$\mu + \beta_5 + \tau_2$	...	$\mu + \beta_5 + \tau_4$	$4\mu + \sum\tau_i + 4\beta_5$
Treatment totals		$5\mu + \sum\beta_j + 5\tau_1$	$5\mu + \sum\beta_j + 5\tau_2$	...	$5\mu + \sum\beta_j + 5\tau_4$	$20\mu + 5\sum\tau_i + 4\sum\beta_j$

i.e., Expected value of Total for treatment 1 is, $E(Y_{10}) = 5\mu + \sum\beta_j + 5\tau_1$

Similarly, $E(Y_{20}) = 5\mu + \sum\beta_j + 5\tau_2$ etc. To estimate the parameters, μ, β, τ etc., we equate the numerical values of these quantities to their expectations.

2.5.1 ESTIMATION OF INDIVIDUAL TREATMENT EFFECTS

Now $t_1 - t_2 = \frac{1}{5}(Y_{10} - Y_{20}) = y_{10} - y_{20}$

Similarly $t_1 - t_3 = y_{10} - y_{20}$

$t_1 - t_y = y_{10} - y_{40}$

$t_2 - t_3 = y_{20} - y_{30}$

$t_2 - t_y = y_{20} - y_{40}$

i.e., the equations are not independent, e.g.,

$(t_1 - t_3) - (t_1 - t_2) = t_2 - t_3$

number of independent equations = 3 (i.e., d.f.)

To obtain a single solution apply a linear restriction to the 3 independent equations, i.e., $\sum t_i = 0$. This is logical since earlier we imposed the restriction that $\sum\tau_i = 0$, and summing the first 3 equations, since they are independent we get,

Thus : $3t_1 - t_2 - t_3 - t_4 = 3y_{10} - y_{20} - y_{30} - y_{40}$ and $4t_1 - \sum t_i = 4y_{10} - \sum y_{i0}$

i.e., $4t_1 = 4y_{10} - \sum y_{i0}$ and $t_1 = y_{10} - \bar{y}$

similarly, $t_2 = y_{20} - \bar{y}$, $t_3 = y_{30} - \bar{y}$, and $t_4 = y_{40} - \bar{y}$ etc.

Thus for example: $5m + \sum b_j + 5t_1 = y_{10}$ and $5m + \sum b_j + 5t_2 = y_{20}$,
and $(t_1 - t_2) = \frac{1}{5}(Y_{10} - Y_{20})$
Thus, the estimates of the difference of any two treatment effects can be computed free from any entanglement with the block effects.
Similarly it can be shown that: $4m + 4b_1 + \sum t_i = Y_{01}$ and $4m + 4b_2 + \sum t_i = Y_{02}$
Thus $b_1 - b_2 = \frac{1}{4}(Y_{01} - Y_{02})$ and the estimate of the differences of any two block effects can be computed free from any entanglement with the treatment effects.

2.6 COMPARISONS BETWEEN CRD AND RCBD.

- The efficiency of a RCBD relative to a Completely Random Design on the same plots, can vary from equal efficiency to much greater efficiency according as the blocks happen to remove the effects of a "fertility gradient" from error or not.
- The RCBD is an "insurance" against unfavourable (fertility) gradients which might cause the error of a Completely Random Design to be very large.
- In a RCBD there is a loss of d.f. from Error to blocks, but this is usually trivial and almost invariable more than counter-balanced by the greatly reduced error.

To compare two experimental designs, one compares the amounts of information available for each of these designs, this gives the relative efficiency. In this case where we are comparing the CRD and RCBD designs this is equivalent to a comparison of the estimates of the error mean squares for the two designs, namely: S^2_{RCBD} and S^2_{CRD} .

Let $S^2_{RCBD} = MS.E$

Then it may be shown that a reasonable estimate of the Error mean square for the CRD is given by :

$$S^2_{CRD} = \frac{(b-1)\,MS.b + b(t-1)MS.E}{(bt-1)}$$

(Ref: Kempthorne, *Design of Experiments,* Chap 9,)

Where b and t are the number of blocks and treatment respectively, and MS.B is the Mean Square for Blocks from the ANOVA table for the RCBD.

Thus Relative Efficiencey is calculated as:

$$\textbf{RCBD vs. CRD} = \frac{\textbf{(b-1) MS.B + b(t-1)MS.E}}{\textbf{(bt-1) MS.E}}$$

Chapter 3 : Latin Square Design

3.1 INTRODUCTION

Consider a RCBD with 4 treatments and 4 replications. Let the arrow (→) denote the direction of the "fertility" gradient.

A	B	C	D

etc.

——————————→

Blocks eliminate a considerable proportion of error due to 'soil heterogeneity' as compared with a Completely Random Design. However, variability within blocks cannot be reduced with the above layout, the following layout being equally efficient.

B	C	A	D

etc.

——————————→

What happens if the "gradient" is also in the direction (↓) ?

3.2 LATIN SQUARE DESIGN – Two systems of blocks in a single design.

Consider the order of treatments within each of the 4 blocks so that each treatment occupies the four possible positions in a block once and once only:

Column Blocks :

Row blocks:

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

Now, not only may each "row" serve as a block but also each "column". We are thus in a position to eliminate from error, "fertility" difference in two directions (at right angles) simultaneously.

A design laid out in Rows and Columns in this way is called a **LATIN SQUARE**: i.e., each treatment occurs once and once only in each row block and each column block.

Thus:

Number of Replications = Number of Rows = Number of Columns = Number of Treatments.

3.3 STATISTICAL MODEL — LATIN SQUARE DESIGN

Let y_{ijk} be the yield for the k^{th} treatment in the i^{th} row-block and j^{th} column block. This may be represented as.

$$y_{ijk} = \mu + \beta_i + \gamma_{jk} + \tau_k + \epsilon_{ijk} \qquad \textbf{MODEL LATIN SQUARE}$$

where τ_k, β_i and γ_j , the row, column and treatment effects are regarded as FIXED EFFECTS. τ_k is a purely additive component, independent of the magnitude of ϵ_{ijk} , β_i and γ_j.

NOTE:

The assumption of ADDITIVITY in a Latin Square Design implies that the treatment effects are constant over the different block types (rows and columns). Also, that the row and column effects are constant for all plots within that block type. In other words, treatments and rows, treatments and columns, and rows and columns do not interact.

3.4 PARTITIONING OF TOTAL S.S. — LATIN SQUARE DESIGN

Consider a 5 × 5 square array :

						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}
Col. means	y_{01}	y_{02}	y_{03}	y_{04}	y_{05}	$\bar{y}$

Where y_{ij} represents the yield in the i^{th} row and j^{th} column.
By the partitioning of S.S. for R.B. Design we have:

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

Total S.S. = S.S. Rows + S.S. Columns + S.S. Residuals

Now the S.S(Residuals) may be further sub-divided as,
S.S. (Residuals) = S.S. (Treatments) + S.S. (Error)
Let y_{ijk} have a third suffix, k, representing the k^{th} Treatment.
Row means are now denoted by y_{i00}, column means by y_{0j0}, and treatment means by y_{00k}.
Thus :

	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}

Note: each value of k occurs once in each row and column.
Residual S.S. $= \sum_i\sum_j(y_{ijk} - y_{i00} - y_{0j0} + \bar{y})^2$
For k = 1, the residuals residuals are:

	y_{111}	−	y_{100}	−	y_{010}	+	$\bar{y}$
	y_{241}	−	y_{200}	−	y_{040}	+	$\bar{y}$
	y_{331}	−	y_{300}	−	y_{030}	+	$\bar{y}$
	y_{451}	−	y_{400}	−	y_{050}	+	$\bar{y}$
	y_{521}	−	y_{500}	−	y_{020}	+	$\bar{y}$
Mean :	y_{001}	−	$\bar{y}$	−	$\bar{y}$	+	$\bar{y}$ = $y_{001} - \bar{y}$

As for the RCBD applying, $\sum x^2 = n\bar{x}^2 + \sum(x - \bar{x})^2$,
we get $\sum_i\sum_j(y_{ij1} - y_{i00} - y_{0j0} + \bar{y})^2 = 5(y_{001} - \bar{y})^2 + \sum_i\sum_j(y_{ij1} - y_{i00} - y_{001} + 2\bar{y})^2$
and summing over all k,

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

= Treatments(S.S.) + Error (S.S.).

Generally, for a square of order (r) we have :

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

Total(S.S.) = Row(S.S.) + Column(S.S.) + Treatment(S.S.) + Error(S.S.)
If the square is of order (r), then the partitioning of **Total d.f.** is:

$$r^2 - 1 = (r-1) + (r-1) + (r-1) + (r-1)(r-2)$$

SYMBOLIC ANOVA TABLE FOR LATIN SQUARE DESIGN:

Source	d.f.	S.S.
Rows	$r-1$	$r\sum_i(y_{i\circ\circ}-\bar{y})^2$
Columns	$r-1$	$r\sum_i(y_{\circ i\circ}-\bar{y})^2$
Treatments	$r-1$	$r\sum_i(y_{\circ\circ i}-\bar{y})^2$
Error	$(r-1)(r-2)$	$\sum_i\sum_j\sum_k(y_{ijk}-y_{i\circ\circ}-y_{\circ j\circ}-y_{\circ\circ k}+2\bar{y})^2$
Total	r^2-1	$\sum_i\sum_j\sum_k(y_{ijk}-\bar{y})^2$

NOTE: i) On the usual assumption of errors N.I.D $(0, \sigma^2)$, the F-test of, $\frac{\text{Treatment(M S)}}{\text{Error(M S)}}$, may be made.

ii) S.S. Treatments, Rows, Columns are usually calculated using TOTALS.

3.5 LIMITATIONS OF THE LATIN SQUARE DESIGN

a) Since t = r always, with large t we could have too many replications, and thus possible problems with the layout of the experiment, especially with "field" experiments.

b) Squares of size 3 x 3 and 4 x 4 are usually ruled out as they have too few degrees of freedom for the estimation of error; 2 and 6 d.f. respectively. This shortcoming can often be overcome by repeating squares in order to obtain adequate degrees of freedom for the *residual*.

c) Squares of size larger than 9 x 9 usually impose limitations on space for field experiments. This is not usually the case with non-field experiments. For field experiments at least, squares for t = 5 to 9, i.e. squares (5 x 5) to (9 x 9) can be considered.

d) Cannot drop a row or column without seriously affecting the orthoganality of the design. Compare this with dropping a complete replication in a randomised complete blocks design.

3.6 RELATIVE EFFICIENCY

When it come to "relative efficiency" that is, answering the question: "Should a Latin Square Design have been used?", several possibilities arise. First there is the possibility of ignoring the column classification and comparing the Latin Square to a RCBD with rows as blocks. An alternative, is the case where row blocks are ignored and the Latin Square is compared with a RCBD with columns as blocks.
In these two cases the ***Realtive Efficiency*** is calculated as:

i) $$\textbf{Latin Square vs. RCBD}_{\textbf{row blocks}} = \frac{(t-1)\text{ x C.MS} + (t-1)^2\text{ x E.MS}}{t(t-1)\text{ x E.MS}}$$

ii) $$\textbf{Latin Square vs. RCBD}_{\textbf{column blocks}} = \frac{(t-1)\text{ x R.MS} + (t-1)^2\text{ x E.MS}}{t(t-1)\text{ x E.MS}}$$

A third possibility is to ignore both row and column groupings, and compare the Latin Square Design with the experiment as a CRD, completely randomised design.

iii) $$\textbf{Latin Square vs. CRD} = \frac{(t-1)\text{ x C.MS} + (t-1)\text{ x R.MS} + (t-1)^2\text{ x E.MS}}{(t+1)(t-1)\text{ x E.MS}}$$

Chapter 4 : Treatment Design
(Factorial Treatment Structures)

4.1 INTRODUCTION

Any Experimental Design comprises two components:

(i) Structure (nature) of experimental units.
This has been discussed in some detail in the previous three chapters.

(ii) Structure of Treatment Set.
The decision which treatments to include in an experiment is closely linked to the aims and objectives of the experiment. This chapter will look at a particular aspect of treatment design (selection), namely, the factorial treatment set.

4.2 DEFINITIONS:

FACTOR: A type of treatment, e.g., Variety, Fertiliser, Animal Feed, Drug, etc.
LEVEL: A particular treatment of a factor set, e.g., Variety `A', etc.

4.2.1 QUANTITATIVE AND QUALITATIVE FACTORS

Factors may be **QUALITATIVE** :
e.g. Variety – with levels : variety A, variety B, etc.
OR
Factors may be **QUANTITATIVE** :
e.g. Nitrogen – with levels : 0, 50, 100 kg N/ha. etc.

4.3 CASE # 1: TWO FACTOR Experiments

Consider an experiment in which one is interested in whether four different varieties (V) have different phosphate (P) requirements. It is to be noted that we have an experiment with both a quantitative and qualitative factor.
Let V = Varieties at 4 levels: v_1 , v_2 , v_3 , v_4
and P = Phosphate at 3 levels: p_0, p_1 , p_2 , corresponding to nil, 100, 200 kg superphosphate/ha.
It is to be noted that factors are usually denoted by capital letters, for example: V, P above, while factor levels and treatment combinations are represented by lower case letters, e.g. v_1, v_2; and v_1p_0, etc.

The TREATMENTS consist of all possible combinations of each level of each factor viz :

v_1p_0 , v_1p_1 , v_1p_2
v_2p_0 , v_2p_1 , v_2p_2
v_3p_0 , v_3p_1 , v_3p_2
v_4p_0 , v_4p_1 , v_4p_2 i.e. $(3 \times 4) = 12$ treatment combinations.

In this situation we speak of a (3 x 4) factorial experiment.

4.3.1 INDEPENDENCE AND INTERACTION

Two factors A and B are said to be **independent** if the effects of A, i.e., responses to the levels of A, are the same at all levels of factor B, similarly the effects of B are the same at all levels of A.
When factors A and B are not independent, i.e., the effects of A vary over the levels of B and vice versa, we say that A and B, **interact,** and we speak of the A × B (or AB) interaction.
In order to investigate the independence or interaction of two factors we draw up the appropriate **InteractionTable.**

Example : Variety x Phosphate Interaction Table

	Phosphate:			
Variety:	p_0	p_1	p_2	Means
v_1	v_1p_0	v_1p_1	v_1p_2	$\bar{v}_1$
v_2	v_2p_0	v_2p_1	v_2p_2	$\bar{v}_2$
v_3	v_3p_0	v_3p_1	v_3p_2	$\bar{v}_3$
v_4	v_4p_0	v_4p_1	v_4p_2	$\bar{v}_4$
Means	$\bar{p}_0$	$\bar{p}_1$	$\bar{p}_2$	

Consider two factors A and B each at two levels :

Treatment Combinations are :

	b_1	b_2
a_1	a_1b	a_1b_2
a_2	a_2b_1	a_2b_2

With yields represented by :

	b_1	b_2
a_1	y_{11}	y_{12}
a_2	y_{21}	y_{22}

Example : **ADDITIVE** (Independence) **Model I.**

Let $M.V\,(y_{11}) = \theta$

$M.V\,(y_{21}) = \theta + \alpha$

$M.V\,(y_{12}) = \theta + \beta$;

then the (true) response to Factor A at level b_1 of B is α, similarly, the response to B at level a_1 of A is β and we get:

Mean yield:

	b_1	b_2
a_1	θ	$\theta + \beta$
a_2	$\theta + \alpha$	$\theta + \alpha + \beta$

In this case the effects of the factors are **ADDITIVE** i.e. the combined response is the sum of their separate response, and we say the two factors are **INDEPENDENT.**

It is to be noted that the response to A at level $b_2 = \theta + \alpha + \beta - (\theta + \beta) = \alpha$, i.e., the same as at level b_1. Similarly for the response to β.

Example : Alternative Model for Independence (Additivity) – Model II.

Let $M.V\,(y_{ij}) = \mu + \alpha_i + \beta_j$, then we have:

Mean yields:

	b_1	b_2
a_1	$\mu + \alpha_1 + \beta_1$	$\mu + \alpha_1 + \beta_2$
a_2	$\mu + \alpha_2 + \beta_1$	$\mu + \alpha_2 + \beta_2$

where μ = common component

α_1 , α_2 = effects of factor A at levels 1 and 2 respectively.

β_1 , β_2 = effects of factor B at levels 1 and 2 respectively

We may express the relationship between Model I and Model II by:

$\theta = \mu + \alpha_1 + \beta_1$; $\alpha = \alpha_2 - \alpha_1$ and $\beta = \beta_2 - \beta_1$

Effects of A and B are additive :

Response to A i.e. $(\alpha_2 - \alpha_1)$ is the same for both levels of B

Response to B i.e. $(\beta_2 - \beta_1)$ is the same for both levels of A

Example:

Let $\theta = 12$, $\alpha = 3$, $\beta = 4$.

	(i) b_1	(i) b_2	(ii) b_1	(ii) b_2	(iii) b_1	(iii) b_2
a_1	12	16	12	16	12	16
a_2	15	19	15	25	15	15

These three tables represent examples of :

(i) **No Interaction,** i.e., the effects are additive/independent.

(ii) **$+^{ve}$ Interaction,** i.e., the effects are <u>not</u> additive/independent.

(iii) **$-^{ve}$ Interaction,** i.e., the effects are <u>not</u> additive/independent.

These **"types of interaction"** will be considered in detail below

4.3.2 MAIN EFFECTS AND INTERACTIONS.

It will be assumed that environmental effect. for example, blocks/rows/columns, etc., affect all treatments equally and can therefore be ignored.

Furthermore, on hypothesis of no interaction we may consider the following model :

$$y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij} \qquad \textbf{Model A}$$

where: y_{ij} = mean of the $(ij)^{th}$ treatment combination.

α_i = effect of the i^{th} level of factor A $\qquad i = 1 \ldots m$

β_j = effect of the j^{th} level of factor B $\qquad j = 1 \ldots n$

ϵ_{ij} = mean of (r) random components each with mean = 0, variance σ^2 ,

i.e., ϵ_{ij} has mean = 0, variance = $\frac{\sigma^2}{r}$

Consider the following 2-way table of means of treatment combinations for Factors A and B at m and n levels respectively :

TABLE 4.1: TREATMENT MEANS : (r) replications.

	b_1	b_2	... ...	b_n	Means
a_1	y_{11}	y_{12}	.....	y_{1n}	y_{10}
a_2	y_{21}	y_{22}	.....	y_{2n}	y_{20}
⋮	⋮	⋮		⋮	⋮
a_m	y_{m1}	y_{m2}	.....	y_{mn}	y_{m0}
Means	y_{01}	y_{02}	.....	y_{0n}	$\bar{y}$

Now for a randomised complete blocks design (i.e., a 2-way analysis) we have :

Total S.S. = Blocks S.S. + Treatment S.S. + Error S.S.

$$\sum\sum(y_{ij} - \bar{y})^2 = t\sum_j(y_{0j} - \bar{y})^2 + r\sum_i(y_{i0} - \bar{y})^2 + \sum\sum(y_{ij} - y_{i0} - y_{0j} + \bar{y})^2$$

Thus for the above Table 4.1 of (mn) treatment combinations, a similar partitioning may be considered corresponding to:

$$\text{SS (Factor A)} = n\sum_i(y_{i0} - \bar{y})^2$$

$$\text{SS (Factor B)} = m\sum_j(y_{0j} - \bar{y})^2$$

As we will see later (4.3.3), SS (A) and SS(B) are equivalent to:

SS (due to fitting $\alpha_1\alpha_2 \ldots \alpha_m$) and SS(due to fitting $\beta_1\beta_2 \ldots \beta_n$), respectively.

In addition, we have SS("residual effects"), i.e., SS (due to "lack of fit" of the additive model),

namely $= \sum_i\sum_j(y_{ij} - y_{i0} - y_{0j} + \bar{y})^2$

Now on the assumption that there are r observations (replications) for each of the observed means in Table 4.1, the "Total S.S." for the above table is equivalent to:

$r\sum_i(y_{i0} - \bar{y})^2$ the "Treatments S.S." in the partitioning of the Total S.S. for a RCBD with

t treatments and r replications; in this case, t = (mn) treatment combinations. Thus all S.S.. must be multiplied by r, and for the subdivision of the Treatment S.S. we have:

$$r\sum_i\sum_j(y_{ij} - \bar{y})^2 = rn\sum_i(y_{i0} - \bar{y})^2 + rm\sum_j(y_{0j} - \bar{y})^2 + r\sum_i\sum_j(y_{ij} - y_{i0} - y_{0j} + \bar{y})^2$$

i.e. **Treatment S.S. = S.S. (A) + S.S. (B) + S.S. ("residual effects")**

With a corresponding partitioning of d.f., namely,

$$(mn - 1) = (m - 1) + (n - 1) + (m - 1)(n - 1) \qquad \text{since } t = mn$$

Thus the Treatments S.S. has been partitioned exactly (i.e. orthogonally) into three component S.S. with a corresponding partitioning of the Treatments d.f.

The above partitioning of the Treatments S.S. can be considered in 2 parts, namely:

4.3.3 MAIN EFFECTS

S.S.(A) $= nr\sum(y_{io} - \bar{y})^2$ represents deviations of marginal means for levels of Factor A about $\bar{y}$. $(y_{io} - \bar{y})$ is called the **MAIN EFFECT** of the **i^{th} level of A** since it represents an average over all the levels of Factor B.

Hence $\mathbf{nr\sum_i (x_{io} - \bar{y})^2}$ is called the **S.S. (Main Effect A)** or S.S.(A).

Similarly $(y_{oj} - \bar{y})$ is the **MAIN EFFECT** of the **j^{th} level of B**

and $\mathbf{mr\sum_j (y_{oj} - \bar{y})^2}$ is the **S.S. (Main Effect B)** or S.S.(B)

It may be shown that with **equal** replication for each treatment combination, $(y_{io} - \bar{y}) = \hat{\alpha}_i$ and $(y_{oj} - \bar{y}) = \hat{\beta}_j$ are the "least squares estimates" of α_i and β_j in Model A above, subject to the single linear restrictions that $\sum_i \alpha_i = \sum_j \beta_j = 0$.

4.3.4 INTERACTION OF 2 FACTORS

$r\sum_i\sum_j (y_{ij} - y_{io} - y_{oj} + \bar{y})^2$ the last S.S. in the partitioning of the Treatment S.S., may be represented as $r\sum_i\sum_j \left\{(y_{ij} - y_{io}) - (y_{oj} - \bar{y})\right\}^2$, i.e., SS of $(y_{ij} - y_{io})$ about their means, $(y_{oj} - \bar{y})$. In other words the variation in the effect of of the i^{th} level of factor A over the different levels of of factor B. Summation over all i and j thus measures the variation in the "effects of A" over the levels of factor B, and vice versa, i.e. **INTERACTION**; refer definitions in 4.3.1 above. This sum of squares is called **SS(Interaction)** and from another point of view it may be seen to be a measure of deviations from the "Additive (independence) Model A", above. SS(Interaction) is written alternatively as: S.S.(A × B) or simply S.S.(AB).

Ignoring block effects, it is instructive therefore to make allowance for interaction in the additive model, i.e. Model A, above, namely,

$$\mathbf{y_{ij} = \mu + \alpha_i + \beta_i + \epsilon_{ij}} \qquad \textbf{Model A.}$$

and include an interaction term $(\alpha\beta)_{ij}$ i.e.,

$$\mathbf{y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ij}} \qquad \textbf{Model B.}$$

Where, as before, α_i and β_j represent the main effects of the i^{th} level of factor A and the j^{th} level of factor B respectively, $(\alpha\beta)_{ij}$ represents the deviations due to treatment by which the expected value of y_{ij} differs from the additive model (A).

4.3.5 ANOVA TABLE FOR FACTORIAL TREATMENT SET

[Factor A (m levels), Factor B (n levels) – Design: RCBD with r replications.]

For this analysis it is necessary to include a term for blocks in Model B above, namely,

$$\mathbf{y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \epsilon_{ijk}}$$

where in addition to the definitions for α and β above,

γ_k = effect of the k^{th} block (replicarion) $\qquad k = 1 \ldots r$

Source	d.f.	S.S.
Blocks	$(r - 1)$	$mn\sum_k (y_{ook} - \bar{y})^2$
Treatments:	$(mn - 1)$	$r\sum_i\sum_j (y_{ijo} - \bar{y})$
A	$(m - 1)$	$rn\sum_i (y_{ioo} - \bar{y})^2$
B	$(n - 1)$	$rm\sum_j (y_{ojo} - \bar{y})^2$
AB	$(m - 1)(n - 1)$	$r\sum_i\sum_j (y_{ijo} - y_{ioo} - y_{ojo} + \bar{y})^2$
Error	$(r - 1)(mn - 1)$	$\sum_i\sum_j\sum_k (y_{ijk} - y_{ijo} - y_{ook} + \bar{y})^2$
Total	$mnr - 1$	$\sum_i\sum_j\sum_k (y_{ijk} - \bar{y})^2$

4.3.5.1 NOTES ON THE ANALYSIS OF VARIANCE:

a) S.S. (Error) is usually obtained by subtraction.

b) Check: S.S. (Treatments) = S.S.(A) + S.S.(B) + S.S.(AB)

c) The test $\frac{\text{Treatment M.S.}}{\text{Error M.S.}}$ has very little relevance.

d) The important tests are:

i) $\frac{\text{M.S.(A)}}{\text{Error M.S.}}$ (ii) $\frac{\text{M.S.(B)}}{\text{Error M.S.}}$ (ii) $\frac{\text{M.S.(AB)}}{\text{Error M.S.}}$

e) Interpretation of results:

i) Significant Main Effect (e.g., A):
- marginal means differ more than expected by chance.

ii) Significant (AB) interaction:
- response to Factor A not consistent over levels of Factor B, and vice versa.

iii) In the presence of interaction the main effects are likely to be of very limited interest since:
- the effects of the factors in combination cannot be described solely in terms of marginal means of the interaction table. (i.e. the effects of neither factor are consistent over the levels of the other). In other words the additive model is inappropriate and it is necessary to examine individual combinations within the body of the interaction table.
- In the absence of interaction the main effects (i.e. marginal means) provide the whole story of treatment effects.
- In practice, the F test of the Interaction M.S. decides the critical issue of whether the **Additive model** (A) $y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$ or the **Interactive model** (B) $y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ij}$ should be adopted.

4.3.6 "STRUCTURED" AND "UNSTRUCTURED" FACTORS

a) **FACTOR A — "UNSTRUCTURED"**

Assuming the AB interaction non-significant, the significance of A means that differences between marginal means may be tested using multiple comparison procedures such as Duncan's Multiple Range, Scheffe, Dunnett, etc. If the interaction AB is significant then attention is directed to comparisons within the body of the interaction table.

b) **FACTOR A — "STRUCTURED"**

If A is structured, a further partitioning of S.S.(A), for the predetermined comparisons, using for example, t- or F-tests, can be performed. These tests **do not** depend on the significance of the "main effect" of factor A. When for example a factor (A say) is a quantitative factor at k-levels (k>2), it is usual to consider, at least as an appproximation, some polynomial relationship between the response variable, Y say, and the quantitaive factor A. For this purpose one can use "orthogonal polynomials" up to degree $(k-1)$, in which case the $(k-1)$ degrees of freedom for the testing of the "main effect" of A can now be subdivided into k single degrees of freedom for the testing of "linear", "quadratic", "cubic", etc., effects over the levels for that factor.

4.3.7 PARTITIONING OF MAIN EFFECT AND INTERACTION SS.

Corresponding to any (orthogonal) partitioning of the S.S. of the main effect of a factor, there exists a conformable partitioning of the S.S. for any interaction involving that factor.

OBJECTIVES OF THE PARTITIONING.

i) The nature of an interaction may be clarified and attention drawn to particular comparisons within the interaction table.

ii) A component of the interaction S.S. may show up significant when the interaction as a whole, is non – significant. Significance of any component of the interaction means that the simple additive hypothesis cannot be accepted.

EXAMPLE 1: One factor "structured", the other "unstructured".

Variety : 4 levels

Phosphates : 3 levels (quantitative).

Then : S.S. (P) = S.S. (P′) + S.S. (P′) with : 1 d.f. + 1 d.f. = 2 d.f.

Where P′ and P″ represent the linear and quadratic effects of Phosphate

SYMBOLIC ANOVA TABLE:

Source of Variation		d.f.	
Main effect Variety		3	i.e., (v − 1)
Main effect Phosphate		(2)	i.e., (p − 1)
phosphate, linear	(P′)	1	
phosphate, quadratic	(P″)	1	
Variety x Phosphate		(6)	i.e., (v − 1)(p − 1)
variety x P-linear	(VP′)	3	
variety x P-quadratic	(VP″)	3	
Treatments		11	i.e., (vp − 1)

4.3.7.1 NOTES ON THE ANALYSIS

a) The ratio $\frac{\text{M.S.(VP')}}{\text{Error M.S.}}$ tests whether the linear response to phosphate is consistent over the different varieties.

b) Similarly, $\frac{\text{M.S.(VP'')}}{\text{Error M.S.}}$ tests whether the quadratic response to phosphate is consistent over the different varieties.

4.3.7.2 INTERPRETATION OF SIGNIFICANCE OF PARTITIONED MEAN SQUARES.

Examples :

	P′	P″	VP′	VP″
result 1.	sig.	n.s.	n.s.	n.s.
result 2.	sig.	n.s.	Sig.	n.s.
result 3.	sig.	n.s.	n.s.	sig.
result 4.	sig.	sig.	sig.	n.s.

EXPLANATION/INTERPRETATION:

1. Consistent upward (or downward) trend; the same for all varieties, i.e., **P′**, significant.
2. For each variety there is a consistent upward (or downward) trend, but the magnitude of this varies with varieties, i.e., **VP′**, significant.
3. Averaged over all varieties, the effect of P shows a consistent upward (or downward) trend i.e., **P′** significant. But for individual varieties the trend is not always consistent, and sometimes curvature is in opposite directions, i.e., **VP″**, significant.
4. The response to P is curvilinear **P″** significant but with different average slopes for different varieties, i.e., **VP′**, significant.

4.4 CASE # 2: THREE FACTOR Experiments.

Consider the situation where there are three factors:

V = Varieties (4 levels) : v_1 v_2 v_3 v_4

P = Phosphate (3 levels) : p_0 p_1 p_2

K = Potash (2 levels) : k_0 k_1

The treatment set (t) consists of all possible combinations of these levels, i.e., (4 × 3 × 2) = 24 treatment combinations namely:

$v_1p_0k_0$	$v_1p_0k_1$	$v_1p_1k_0$	$v_1p_1k_1$	$v_1p_2k_0$	$v_1p_2k_1$
$v_2p_0k_0$	$v_2p_0k_1$	$v_2p_1k_0$	$v_2p_1k_1$	$v_2p_2k_0$	$v_1p_2k_1$
$v_3p_0k_0$	$v_3p_0k_1$	$v_3p_1k_0$	$v_3p_1k_1$	$v_3p_2k_0$	$v_3p_2k_1$
$v_4p_0k_0$	$v_4p_0k_1$	$v_4p_1k_0$	$v_4p_1k_1$	$v_4p_2k_0$	$v_4p_2k_1$

In general we will consider three factors A,B,C with m, n, p levels respectively, i.e. (m × n × p) treatment combinations in all.

The **ADDITIVE MODEL** now becomes :

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \epsilon_{ijk} \qquad \textbf{Model : } A_1$$

While the **INTERACTIVE MODEL** now becomes :

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{ik} + \epsilon_{ijk} \qquad \textbf{Model : } B_1$$

Partitioning of d.f. according to Model B_1 is as follows :

Source	d.f.
Main Effect A	$m - 1$
Main Effect B	$n - 1$
Main Effect C	$p - 1$
Interaction AB	$(m - 1)(n - 1)$
Interaction AC	$(m - 1)(p - 1)$
Interaction BC	$(n - 1)(p - 1)$
Deviations Model : B_1	$(m - 1)(n - 1)(p - 1)$
Total Treatments	$mnp - 1$

i.e. $t = mnp$ = no. of treatments.

As for the two factor case it can be shown that :

$$\begin{aligned} e_{ijk} &= y_{ijk} - \hat{y}_{ijk} \\ &= y_{ijk} - \hat{\mu} - \hat{\alpha}_i - \hat{\beta}_j - \hat{\gamma}_k - (\alpha\beta)_{ij} - (\alpha\hat{\gamma})_{ik} - (\beta\gamma)_{jk} \\ &= y_{ijk} - \bar{y} - (y_{i\circ\circ} - \bar{y}) - (y_{\circ j\circ} - \bar{y}) - (y_{\circ\circ k} - \bar{y}) - \{(y_{ij\circ} - y_{i\circ\circ}) - (y_{\circ j\circ} - \bar{y})\} \\ &\quad - \{(y_{i\circ k} - y_{i\circ\circ}) - (y_{\circ\circ k} - \bar{y})\} - \{(y_{\circ jk} - y_{\circ j\circ}) - (y_{\circ\circ k} + y)\} \end{aligned}$$

i.e., $e_{ijk} = (y_{ijk} - y_{ij\circ} - y_{i\circ k} - y_{\circ jk} + y_{i\circ\circ} + y_{\circ j\circ} + y_{\circ\circ k} - \bar{y})$

and S.S. (residuals) $= r\sum_i\sum_j\sum_k e_{ijk}^2$

Also $e_{ijk} = (y_{ijk} - y_{i\circ k} - y_{\circ jk} + y_{\circ\circ k}) - (y_{ij\circ} - y_{i\circ\circ} - y_{\circ j\circ} + \bar{y})$

$$= \left\{ (\alpha\hat{\beta})_{ij(k)} - (\alpha\hat{\beta})_{ij} \right\}$$

This is a measure of the AB interaction at the k^{th} level of C, for combination a_ib_j. Thus $\sum_k e_{ijk}^2$ represents variation of this interaction effect over the levels of C, and $\sum_i\sum_j\sum_k e_{ijk}^2$ represents variation of AB over the levels of C, i.e. the ABC interaction; tahat is deviations from Model (B_1) above. Similarly it can be shown that $\sum_i\sum_j\sum_k e_{ijk}^2$ is the variation of AC over the levels of B, and BC over the levels of A, respectively. In other words :

$$\textbf{ABC} \equiv \{\textbf{AB} \times \textbf{C}\} = \{\textbf{AC} \times \textbf{B}\} \equiv \{\textbf{BC} \times \textbf{A}\}$$

and $\textbf{d.f. (ABC)} = \textbf{d.f.(A)} \times \textbf{d.f.(B)} \times \textbf{d.f.(C)}$

TERMINOLOGY:

(i) AB, AC, BC — are called **Two Factor** or **First Order** interactions.

(ii) ABC is called a **Three Factor** or **Second Order** interaction.

Thus: $y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \epsilon_{ijk}$

is a **FIRST ORDER INTERACTION** model.

This may be modified to include the second order interaction term $(\alpha\beta\gamma)_{ijk}$, namely

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijk} \qquad \textbf{Model C}$$

i.e., a **SECOND ORDER INTERACTION** model.

4.5 CASE # 3: FOUR FACTOR Experiments (ABCD)

a) Main Effects: A. B. C. D

b) 1st Order Interactions:
AB, AC, AD, BC, BD, CD

c) 2nd Order Interactions:
ABC, ABD, ACD, BCD

d) 3rd Order Interactions : ABCD

NOTE: ABCD ≡ ABC × D ≡ ABD × C ≡ ACD × B ≡ BCD × A

GENERALLY:

	Number of Factors (k)			
Order of interaction (p)	2	3	4	5
1	2	3	4	5
2	1	3	6	10
3		1	4	10
4			1	5
5				1

i.e., any entry = $\binom{k}{p}$ tables (effects)

4.6 HIGH ORDER INTERACTIONS AS ESTIMATES OF ERROR

Interactions of order higher than the **Second**, are often assumed non-existent because a treatment effect of this type is considered unlikely. Thus, on the assumption that the S.S. for any such interaction represents error, it may be pooled with the error S.S. (obtained by subtraction). The objective of pooling is thus to gain d.f. for Error. An objection raised against pooling is that if the assumption is wrong, the estimate of error will be biased upwards.

ADDITIONAL NOTES:

a) In certain exploratory experiments even second order interactions may be pooled.

b) Also, less easily interpretable interaction components such as (Cubic × Quadratic × Linear) etc, may also be considered of the order of error and thus pooled with error.

4.7 SPECIAL CASE: SINGLE REPLICATE DESIGNS.

In this case there is no estimate of Error from the usual source, i.e. as the "Blocks × Treatments" interaction, instead an estimate is obtained from high order interactions designated for this purpose (*a-priori*). This type of design is particularly useful for large factorials for which even 2 replications would be impossible.

Using this approach, even *fractional* (eg half-replicate) designs have been developed to accommodate large factorial treatment sets.

Note: "Half replicate" design contain a selected half of the total treatment combinations, etc.

4.8 APPRAISAL OF THE FACTORIAL METHOD.

4.8.1. "HIDDEN REPLICATION"

Compare a 4 × 3 (Variety × Phosphate) experiment with 6 replications (i.e. 72 plots) with the single factor alternatives :

a) A variety trial at some guessed optimal level of P (36 plots) i.e. 9 reps.

b) A fertiliser trial, 3 levels of P, with a selected variety (36 plots) i.e. 12 reps.

At first glance the alternative experiments seem to have the advantage of more replications, however in the factorial experiment, Variety comparisons are based on means of 18 plots (compared with 9) in the case of the alternative experiment), while P comparisons are based on means of 24 plots (instead of 12) even though there are only **6** absolute replications. Of course the advantage of "hiddenreplication" disappears with the detection of interaction.

4.8.2. DETECTION OF INTERACTION.

The advantage of hidden replication vanishes in the presence of interaction (since main effects are of little importance) and it is necessary to fall back on the treatment means in the body of the table (6 reps). It is to be noted that the detection of interaction is a finding of great importance, which could not be obtained from the alternative experiments.

4.8.3. CONCLUSIONS HAVE A WIDER INDUCTIVE BASIS.

Example: If phosphate responses are similar for all varieties, a recommended optimal level for P is sounder than if based on a single variety. If interaction is present, an optimal level can be specified for each variety. With the single – factor approach, a recommended optimal based on one variety could be wrong for another. On this principle, therefore, as many factors as are thought to be important should be included. Thus, rather than have factorial with 2 replications, is may be better to ADD a factor at 2 levels and have a single replicate design with the same number of plots.

4.8.4. "TOO MANY TREATMENT COMBINATIONS"

This causes serious problems since : –

a) The Latin Square design can seldom be used.
b) Block size in randomised blocks design may be excessively large.
   Solution: Use "Incomplete Block Designs".
c) A reasonable number of replications can make the experiment too large.
   Solution: "Hidden replication".

It is to be noted that in practice the need for many factors each at several levels is not often felt, the usual situation being:

a) Exploratory Experiments :
   i.e. many factors at few levels (e.g. 2 levels: nil vs. some/plenty)
b) Few factors, many levels – for careful determination of the optimal combinations after irrelevant factors have been eliminated.

4.8.5 GENERAL OBSERVATIONS/RESTRICTIONS.

The factorial method can result in:

a) Complex field layout
b) "silly" treatment combinations
c) comparisons between individual treatment combinations being of low precision.

These objections are, however, regarded as trivial.

Chapter 5 : Covariance Analysis

5.1 INTRODUCTION.

At times a researcher will have access to some variable associated with each experimental unit, a variable that gives some indication of yield to be observed but which for some reason or other is not, or cannot be used to construct a blocking scheme. It may be that while this variable indexes variability, it may not be possible to construct suitably homogeneous blocks. Or, it may be that this variable only becomes available after the treatments have been assigned. Some examples of such concomitant variables are:

- Field experiments :
  - previous year's crop yields
  - pH of soil from each plot before start of experiment
  - degree of weed/disease, etc., infestation on each plot
  - "dummy variate" (score) of damage caused by, for example, soil wash/erosion, etc.
- Animal experiments :
  - initial body mass, or age
  - milk yield prior to application of treatments.
- Human experiments :
  - performance on a pre-test
- Laboratory experiments :
  - ambient temperature, humidity, atmospheric pressure, etc.

In all such cases the **ANALYSIS OF COVARIANCE** will be a tool that allows the researcher to incorporate exactly such information into the analysis of data of his/her experiment.
A good discussion of the Analysis of Covariance can be found in the special covariance issue of Biometrics (1957).

5.1.2 REDUCTION OF VARIANCE DUE TO REGRESSION (covariance).

Example :
An analysis of bivariate data (x,y), where x = water content, y = nitrogen content, resulted in the following:
S.S.(x) = 6.3073, S.S.(y) = 0.8944, SP(xy) = 2.2665, $S_y^2 = 0.0344$ $n = 27$
If we consider the regression of y on x, then, $S_{y.x}^2 = 0.0032$
Now $s_y^2 = 0.0344$ is an estimate of the variance of the marginal distribution of y while, $s_{y.x}^2 = 0.0032$ is an estimate of the variance of the conditional distribution of y for fixed values of x. Thus in an experiment, if variates x and y are correlated, it would be possible to reduce the variance of y by holding x constant, provided of course this was a feasible procedure.

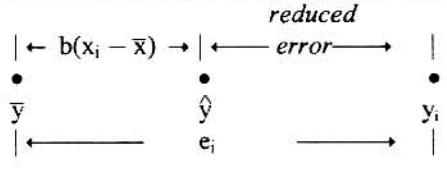
Consider the random variate y_i , i.e., $y_i = \bar{y} + e_i$, where e_i = random residual deviation. If, corresponding to each y_i there is a variate value x_i and it is suspected that part of e_i is due to the deviation of x_i from $\bar{x}$ according to the regression model :

$$\hat{y}_i = \bar{y} + b(x_i - \bar{x})$$

then, to obtain an estimate of what y_i would have been if $x_i = \bar{x}$, we consider subtracting the quantity $b(x_i - \bar{x})$ from y_i i.e.,

$$\hat{y}_{adj} = y_i - b(x_i - \bar{x})$$

This reduction in error may be illustrated as follows,



Thus without adjustment, S.S.(Error) = $\sum(y_i - \bar{y})^2$ i.e., ignoring possible relationship with covariate (x).
With adjustment, S.S.(Error) = $\sum(y_i - \hat{y}_i)^2$ i.e., adjusted for significant linear relationship between y and x.

Consider the formula for $\hat{y}$ (i.e. $y_{adjusted}$), i.e., $\mathbf{y_{adj} = y_i - b(x_i - \bar{x})}$.

(i) If x and y are uncorrelated, b will be zero (or close to zero). Thus it is necessary to have some evidence of correlation ($+^{ve}$ or $-^{ve}$) before considering the regression adjustment. In other words it will be necessary to test for $\beta = 0$ before proceeding with a detailed analysis of covariance (i.e., analysis of adjusted treatment means).

(ii) The error variance can be divided into 2 parts, namely,
Error S.S. = S.S(due to regression) + S.S(deviations from regression).

5.2 THE COVARIANCE MODEL

The usual assumption is that the concomitant variable is available prior to the experiment, but strictly speaking this is not always the case. However, one assumption of considerable importance is that **treatment does not affect the covariate.** It is further assumed that the covariate is measured without error. There is no concomitant probability structure.

The analysis of covariance can be adapted to any design and, in addition, can be modified to accommodate several covariates, "multiple covariance analysis".

Consider the following details for the CRD with one covariate. Assume t treatments with r observations on treatment i. Let the observations and covariates be y_{ij} and x_{ij} respectively for j = 1... r and i = 1... t. The model is thus :

$$\mathbf{y_{ij} = \mu + \tau_i + \beta\, x_{ij} + \epsilon_{ij}} \qquad \textbf{COVARIANCE MODEL}$$

where β denotes the regression coefficient of y on x.

It is implicit in the model that β **does not change** from treatment to treatment, or from block to block, etc.
$\epsilon_{ij} \sim$ N.I.D. $(0, \sigma^2_{y.x})$, where $\sigma^2_{y.x}$ is assumed **constant** for all values of x.

Estimates of the parameters (for example, τ_1^*), **will not be the same** as the corresponding parameters in the ordinary model (see revised model below).

We can rewrite this model as follows:

$$\begin{aligned} y_{ij} &= \mu + \tau_i + \beta\, x_{ij} + \epsilon_{ij} \\ &= \mu + \tau_i + \beta(x_{ij} - \bar{x}_{io}) + \beta(\bar{x}_{io} - \bar{x}) + \beta\bar{x} + \epsilon_{ij} \\ &= \underbrace{\mu + \beta\bar{x}}_{\mu^*} + \tau_i + \underbrace{\beta(\bar{x}_{io} - \bar{x})}_{\text{appears to contribute to treatment}} + \underbrace{\beta(x_{ij} - \bar{x}_{io})}_{\text{contributes to error}} + \epsilon_{ij} \end{aligned}$$

$$\underbrace{\qquad\qquad\qquad\qquad}_{\text{We wish to remove both of these}}$$

$$y_{ij} = \mu^* + \tau^* + \beta(x_{ij} - \bar{x}_{io}) + \epsilon_{ij}$$

5.3 ANALYSIS OF COVARIANCE (reducing experimental error)

5.3.1 PARTITIONING OF SUM OF SQUARES/PRODUCTS

For any design the total sum of squares of a variate (y) in an analysis of variance may be partitioned into the required component S.S., e.g for a randomised blocks design,
Block S.S. + Treatment S.S. + Error S.S., etc., using the basic formula :

$$\sum x^2 = n\bar{x}^2 + \sum(x - \bar{x})^2$$

For bivariate data (x, y) there corresponds an exactly homologous partitioning of the total sum of products, namely,

$$\sum yx = n\bar{x}\,\bar{y} + \sum(y - \bar{y})(x - \bar{x}).$$

The partitioning of d.f, SP's and S.S. in this manner for the two variates (x and y) is termed an **ANALYSIS OF COVARIANCE.**

It is to be noted that for each category of the analysis (e.g. RCBD) there are S.S. of the two variates and their SP. This is illustrated in the table below.

TABLE 5.1 : SYMBOLIC ANALYSIS OF COVARIANCE TABLE – (Design: RCBD)

Source	d.f.	S.S.(x)	SP(xy)	S.S.(y)
Blocks	$r-1$	Bxx	Bxy	Byy
Treatments	$t-1$	Txx	Txy	Tyy
Error	$(r-1)(t-1)$	Exx	Exy	Eyy
Total	$rt-1$	Sxx	Sxy	Syy

The only application of Analysis of Covariance to be considered will be where a variate (y) in an experiment is adjusted for variations in a variate (x) by means of **Linear Regression**.

Note:

- The variate (x) is measured for each "plot" and is considered likely to be correlated with y in such a way that some of the otherwise unaccountable variation in y may be associated with variations in x. If this hypothesis proves to be well founded, it will be possible to correct for these variations in x by reducing the error variance and by adjusting the treatment means.
- The covariate (x) **must be entirely uninfluenced by treatments**.

5.3.2. CALCULATION OF THE S.S.'s and SP's

a) Calculate the S.S. for both y and x and the SPs for each category of the appropriate analysis (e.g., RCBD, Latin Square, etc.).

Revised Covariance Model:

$$y_{ij} = \mu + \gamma_j + \tau_i + \beta\, x_{ij} + \epsilon_{ij}$$

where in addition, γ_j is the effect associated with the j^{th} block, $j = 1 \ldots r$.

b) **FORMULA for SP's**

For example consider a RCBD design with t-treatments and r-replications :

Let X_{oj} and Y_{oj} be the respective block **TOTALS** of the j^{th} block then:

$$\textbf{Block SP} = \frac{1}{t}\left\{ X_{01} \,.\, Y_{01} + X_{02} \,.\, Y_{02} \ldots + X_{or} \,.\, T_{or} \right\} - \textbf{CF}$$

where $CF = \frac{(GT_x \,.\, GT_y)}{rt}$

Similarly, if X_{io} and Y_{io} are the respective treatment TOTALS for the i^{th} treatment then,

$$\textbf{Treatment SP} = \frac{1}{r}\left\{ X_{10} \,.\, Y_{10} + \ldots + X_{to} \,.\, Y_{to} \right\} - \textbf{CF}$$

Likewise,

$$\textbf{Total SP} = \sum_i \sum_j x_{ij} \,.\, y_{ij} - \textbf{CF}$$

As usual, Error SP is obtained by subtraction.

5.3.3 CALCULATION of $\hat{\beta}$, the "Error Regression Coefficient"

Generally $\hat{\beta} = b = \frac{SP_{xy}}{SS_x}$

Thus for "Error Regression", $\mathbf{b = \frac{E_{xy}}{E_{xx}}}$ (refer, Table 5.1 above)

5.3.4 ESTIMATION OF REDUCED ERROR VARIANCE.

i) **ANALYSIS OF REGRESSION**

S.S. (due to Error Regression) $= \frac{(E_{xy})^2}{E_{xx}}$

S.S. (deviations from Regression) $= E_{yy} - \frac{(E_{xy})^2}{E_{xx}}$

ii) **TABLE 5.2: SYMBOLIC ANOVA FOR ANALYSIS OF ERROR REGRESSION**

Test : H_0: $\beta = 0$

Source	d.f.	S.S.		M.S.	
Due to Regn.	1	$\frac{(E_{xy})^2}{E_{xx}}$	$= A$	A	
Residual	$(f_e - 1)$	$\left\{ E_{yy} - \frac{(E_{xy})^2}{E_{xx}} \right\}$	$= B$	$\frac{B}{(f_e-1)}$	$(= s^2_{y.x})$
Total	f_e	E_{yy}			

NOTE:
- f_e = "Error" df form Table 5.1 above.
- The reduction of Error Variance entails the "loss" of 1 d.f.
- The ratio, $\frac{A}{s^2_{y.x}} \sim F_{1,(f_e-1)}$ is a a test of, H_0: $\beta = 0$

5.3.5 CALCULATION Of ADJUSTED TREATMENT MEANS

Status of test of significance of error regression – (refer also 5.4.2).

The test of significance of error regression may be a useful guide as to whether the regression adjustments are worthwhile. It is generally accepted that the procedure to continue with the analysis should be adopted if the test is significant at the **5% level**, otherwise to abandon it as failing to bring about a significant reduction in the Error M.S. Adjusted treatment means are obtained using the formula :

$$\hat{y}_{i\circ} = y_{i\circ} - b(x_{i\circ} - \bar{x})$$

where, $\hat{y}_{i\circ}$ is the adjusted treatment mean, **b** is the error regression coefficient, $x_{i\circ}$ is the **mean** of the r-variate values associated with $y_{i\circ}$, i.e., $(x_{i\circ}, y_{i\circ})$ is the bivariate mean for the i^{th} treatment.

5.3.6 SE (ADJUSTED TREATMENT MEAN).

$$SE(\hat{y}_{i\circ}) = \sqrt{S^2_{y.x}\left\{\frac{1}{r} + \frac{(x_{i\circ}-\bar{x})^2}{E_{xx}}\right\}}$$

which of course varies from treatment to treatment due to variation in $x_{i\circ}$.

5.3.7 TESTS OF SIGNIFICANCE BETWEEN 2 ADJUSTED TREATMENT MEANS

$$SE(y_{1\circ} - y_{2\circ}) = \sqrt{\sigma^2_{y.x}\left\{\frac{2}{r} + \frac{(x_{1\circ}-x_{2\circ})^2}{E_{xx}}\right\}}$$

where $\sigma^2_{y.x}$ is replaced by its estimate $s^2_{y.x}$, the reduced error variance , with $(f_e - 1)$ d.f.

$$t_{(f_e-1)} = \frac{y_{1\circ}-y_{1\circ}}{SE(y_{1\circ}-y_{2\circ})}$$

More generally, any comparison among the adjusted means determined by coefficients, $[\lambda_1, \lambda_2, \ldots \lambda_t]$, has variance :

$$\sigma^2_{y.x}\left\{\frac{\sum\lambda_i^2}{r} + \frac{(\sum\lambda_i x_{i\circ})^2}{E_{xx}}\right\}$$

5.3.8 APPROXIMATE STANDARD ERRORS

Exact tests of significance require a separate S.E. for each comparison under test, this can be tiresome and in most cases an approximate or EFFECTIVE Mean Square can be calculated using the following formula:

$$(S'_{y.x})^2 = S^2_{y.x}\left\{1 + \frac{\text{Treatment M.S.(x)}}{E_{xx}}\right\}$$

where $(S'_{y.x})^2$ is the "*Effective mean square*". This in turn permits the calculation of the following approximations:

- "Approximate" SE (diff 2 adj means) = $S'_{y.x}\sqrt{2/r}$
- "Approximate" SE (adj mean) = $\frac{S'_{y.x}}{\sqrt{r}}$
- Effective C.V.% = $\frac{S'_{y.x}}{\bar{y}} \times 100\%$

5.3.9 REDUCED TREATMENT S.S.

a) If an overall test for significant differences among the adjusted means is required, it will be necessary to calculate a S.S. representing differences between these means. Now it can be shown that the estimates of τ_i and γ_i in the Covariance Model (for the RCBD for example) are not orthogonal and it is necessary to compute the "Adjusted Treatment S.S." in a roundabout method, namely:

$$\textbf{Adjusted Treatment SS} = \left\{ (Tyy + Eyy) - \frac{(Txy+Exy)^2}{Txx+Exx} \right\} - \left\{ E_{yy} - \frac{(Exy)^2}{Exx} \right\}$$

with $$\left\{ (t-1) + (f_e - 1) \right\} - \left\{ (f_e - 1) \right\} = (t-1)\,df$$

The ratio : $$\frac{\text{Adjusted treatment MS}}{s^2_{y.x}} \sim F_{(t-1)df,-1)}$$

is a test of $H_0 : \tau_1 = \tau_2 = \ldots \tau_t$, eliminating all other effects (such as blocks, β, etc.).

b) When an analysis calls for a partitioning of the Treatment S.S. into component S.S. in order to make the tests indicated by a structured set of treatments, the procedure to be followed is that each component must be considered in exactly the same way as was illustrated to obtain "adjusted treatment S.S." for each component of the subdivision

.

Example: A x B factorial treatment structure (laid out as a RCBD).

In this case one would obtain: SS(A | blocks, B, and A.B)
SS(B | blocks, A, and A.B)
SS(AB | blocks, A, B), etc.

5.4 COVARIANCE vs BLOCKING AS A MEANS OF CONTROLLING ERROR.

It is well to remind ourselves of the following:

COVARIANCE – *statistical* control of error using "*regression*".

BLOCKING – applies the "*Principle of Local Control*" for the control of error.

5.4.1 NATURAL BLOCKS.

Analysis of Covariance based on uniformity data for example, may still bring about a sizable reduction of error.

5.4.2 ARTIFICIAL BLOCKS

If blocking is based on a variate x_1 say, then covariance which is also based on x_1 is unlikely to reduce error variance to any appreciable extent. However, if a second such variate x_2 were available, covariance may be used to adjust for initial variability in x_2.

The problem of deciding whether to set up blocks using the covariates or to run a RCBD and use covariance to adjust is discussed in detail by, *D R Cox, Biometrika, Vol. 44, 1957.*

Generally:

Expected correlation between x and y	Recommendation
If $0.0 < \rho < 0.3$	ignore covariate
If $0.3 < \rho < 0.6$	use covariate to construct blocks
If $0.6 < \rho$	use covariance.

5.5 MISCELLANEOUS COMMENTS

5.5.1 CORRECTION OF PLOT YIELDS FOR PLANT NUMBER.

- Varying plant numbers per plot are likely to add to experimental error.
- A proportional correction for the missing plants is equivalent to analysing mean yield per plant i.e. y/x.
- Corrections based on stand are not always satisfactory.
- If too great a proportion of plants in a aplot are missing, it will be better to reject the plot (refer also: "missing plots").
- A correction for a high yielding treatment is unlikely to be suitable for a low yielding treatment, (Note: covariance assumption – "β constant for all treatments")
- Stand may be affected by treatment, e.g., seed "burn" with too high application of fertiliser at planting, etc., (Note: covariance assumption – "covariate not affected by treatment")

5.5.2 USE OF UNIFORMITY DATA IN COVARIANCE ANALYSIS

SHORT TERM EXPERIMENTS.

- Uniformity data are not usually employed in short term experiments.
- There is usually a delay of one year in the production of experimental results.
- The effort involved may be more profitably devoted to increasing the number of replications.
- The correlation between successive years' yield is not always as high as one might expect.

LONG TERM EXPERIMENTS.

- The delay of a year in the production of experimental results is not so serious.
- With perennial crops the adjustments may be intended as a correction for inherent differences in the plants on the different plots, as well as for soil fertility.
- There will be a tendency for the correlation to diminish in successive years.

5.6 ANALYSIS OF COVARIANCE FOR SPLIT-PLOT AND SPLIT-BLOCK DESIGNS.

[Reference: Chapter 6, for details on Split-plot and Split-block designs]

An easy to read article on the application of covariance for the split-plot, split-split-plot and split-block experiments is:

- "Covariance Analysis for Split-Plot and Split-Block Designs"
  W T Federer and M P Meridith, The American Statistician, Vol 46, May 1992, (155 – 162).

Chapter 6 : The Split — Plot Design

6.1 INTRODUCTION

It is common practice to refer to a particular experimental design as a "split-plot design". Technically this is incorrect or at least incomplete, for split-plot designs are really a class or type of design since one can superimpose the "split-plot" feature across any other design. Split-plot experiments are very common, the reason being no doubt, that they lead to such sensible experimental plans or designs. However, researchers often set them up inadvertently without being aware of it and then understandably analyse them incorrectly. This can lead to all sorts of difficulties, not least of which is that the analyst grossly underestimates the experimental error since as we shall see, the experimental error at the "split-plot" level is generally very much smaller then the experimental error at the "whole-plot". In addition, split-plot designs are often misused for, as stated by A A Rayner (*Biometry for Agriculture Students*), they become the "lazy-man's design", a last resort when more careful attention to detail in the initial planning stages of an experiment could have resulted in a more efficient selection of design.

To illustrate the basic idea of the split-plot consider the following example:

A RCBD with 3 replications (r), factor A at 3 levels (m) assigned at random to mr plots. In addition, each of the mr plots is "split" to accommodate the random allocation of 5 levels of factor B (n). The schematic layout for this experiment is as follows:

Factor A at m = 3 levels (whole-plot treatments : 1, 2, 3)
Factor B at n=5 levels (sub-plot treatments: A, B, C, D, E)
Design : RCBD, r = 3, replications

1	
2	Block 1
3	
3	
1	Block 2
2	
3	
2	Block 3
1	

With a separate randomisation for the five sub-plot treatments within each whole-plot the revised layout is as follows:

B1	E1	A1	D1	C1	
D2	B2	C2	A2	E2	Block 1
A3	E3	D3	C3	B3	
A3	C3	E3	B3	D3	
E1	B1	C1	D1	A1	Block 2
D2	B2	A2	C2	E2	
E3	C3	B3	D3	A3	
B2	D2	E2	A2	C2	Block 3
C1	E1	A1	B1	D1	

With reference to the above layout the following is to be noted:

- Theoretically, any field experiment can have an additional factor super-imposed by dividing each plot (now termed the "whole plot") into a number of "sub-plots" equal to the number of levels of the added factor.

- There are two distinct randomisations, namely:
  – the randomisation of treatments to the whole-plots must be that specified for the whole-plot design, (e.g., RCBD, Latin Square, etc.)
  – the sub-plot treatments are randomised to the sub-plots within each whole-plot separately for each whole-plot.
- It is unnecessary to think of the split-plot factor as "additional", the split-plot design is simply an alternative design for an experiment with two (or more) factors.

6.2 SYMBOLIC ANALYSIS OF VARIANCE FOR SPLIT-PLOT EXPERIMENT

One can think of the example in 6.1 as two experiments, one superimposed on the other. This is reflected in the symbolic analysis below.

a) **Whole plot Analysis (mr – 1) d.f.**

Source	d.f.	
Blocks	r – 1	
A	m – 1	} whole plot analysis as for RCBD
Error	(r – 1)(m – 1)	
Between Whole Plots	mr – 1	

b) **Sub-plot Analysis mr(n – 1) d.f.**

Source	d.f.	
B	n – 1	
AB	(m – 1)(n – 1)	} within whole plot analysis
Error	m(n – 1)(r – 1)	
Within Whole Plots	mr(n – 1)	

c) TOTAL SUBPLOTS mrn – 1

6.2.1 DISCUSSION OF SUB-PLOT ANALYSIS

a) For the standard split-plot experiment the type of basic analysis for the sub-plot section of the design is invariable in form.

b) As is apparent from the randomisation procedure the whole plots act as "blocks" for the sub-plot design, the analysis of which must therefore follow that for a randomised complete blocks design. However, there is one important difference:
   For a RCBD: S.S. (Error) = S.S. (Blocks × Treatments)
   For the Sub-Plot Analysis:
   i) Whole-Plots receiving different levels of Factor A and Factor B yield a measure of the AB Interaction,
   ii) Whole-Plots receiving the same level of Factor A and Factor B give rise to a "sum of squares" pure error.

6.2.2 DISCUSSION OF WHOLE-PLOT ANALYSIS

a) In the Sub-Plot analysis the "blocks" (or Whole Plot) S.S. will have a divisor of n (levels of sub plot factor). But, the Whole-Plot S.S. = "total S.S." for the whole plot analysis. Thus, if the two sections of the analysis are to be combined into a single analysis, all the S.S. in the whole plot analysis will have to be divided by an extra n.

b) Thus the divisors are based on the number of sub-plots irrespective of which section of the analysis is being considered. The correction factor (CF) for whole-plots is the same as for sub-plots and is used throughout the analysis.

6.2.3 COMBINED ANALYSIS OF VARIANCE FOR SPLIT-PLOT EXPERIMENT

(given that the whole plot design is a RCBD).

Source	d.f.
Blocks	$r - 1$
A	$m - 1$
Error (a)	$(r - 1)(m - 1)$
Between whole plots	$mr - 1$
B	$n - 1$
AB	$(m - 1)(n - 1)$
Error (a)	$m(n - 1)(r - 1)$
Total subplots	$mrn - 1$

6.2.4 TWO (or more) EXPERIMENTAL ERRORS

From the symbolic analysis in 6.2.3, it is to be noted that a split-plot experiment has (at least) **2 Errors**, namely :

- **Error (a)** – which reflects random variability between whole plots.
- **Error (b)** – which represents random variability of sub-plots within whole plots.

6.2.5 TESTS OF SIGNIFICANCE (assuming Factors A and B are "fixed effects")

a) The F-test for the significance of "main effect (A)" is referred to Error (a)

b) The F-tests of "main effect (B)" and "interaction (AB)" are referred to Error (b).

6.3 EXAMPLES :

6.3.1.

Whole Plot Design : Latin Square (# columns = #rows = #levels, Factor A = r)

Sub-Plot Factor B at (n) levels.

SYMBOLIC ANOVA TABLE:

Source	d.f.	
Rows	$(r - 1)$	
Columns	$(r - 1)$	} i.e. whole plot analysis as for Latin Square.
A	$(r - 1)$	
Error (a)	$(r - 1)(r - 2)$	
Whole Plots	$r^2 - 1$	
B	$(n - 1)$	
AB	$(r - 1)(n - 1)$	} i.e sub – plot analysis is invariable.
Error (b)	$r(r - 1)(n - 1)$	
Total sub-plots	$nr^2 - 1$	

6.3.2.

Whole plot design: RCBD with (r) replications

Whole plot Treatments : Factors A and B at (m) and (n) levels, respectively.

Sub-plot treatments : Factor C at (p) levels

SYMBOLIC ANOVA TABLE:

Source	d.f.	
Blocks	$(r-1)$	
A	$(m-1)$	} whole plot analysis as for RCBD with two factors A and B.
B	$(n-1)$	
AB	$(m-1)(n-1)$	
Error (a)	$(mn-1)(r-1)$	
Whole Plots	$mnr-1$	
C	$(p-1)$	
AC	$(m-1)(p-1)$	
BC	$(n-1)(p-1)$	} sub-plot analysis invariable.
ABC	$(m-1)(n-1)(p-1)$	
Error (b)	$mn(r-1)(p-1)$	
Total sub-plots	$mnpr-1$	

6.4 STATISTICAL MODEL

Let the whole plots comprise (r) randomised blocks then:

$$y_{ijk} = \mu + \rho_j + \alpha_i + \beta_k + (\alpha\beta)_{ij} + \eta_{ij} + \epsilon_{ijk} \qquad \textbf{SPLIT-PLOT MODEL}$$

y_{ijk} = yield associated with the k^{th} level of the sub-plot factor within the whole-plot in the j^{th} block receiving the i^{th} level of the whole-plot factor.
ρ_j = component common to all sub-plots in the j^{th} block.
α_i = Main Effect component of i^{th} level of A.
β_k = Main Effect component of k^{th} level of B.
$(\alpha\beta)_{ik}$ = Interaction component.
η_{ij} = random component common to all sub-plots in the $(i,j)^{th}$ whole plot.
ϵ_{ijk} = random component peculiar to the sub-plot with the k^{th} level of B in the $(i,j)^{th}$ whole-plot.

6.4.1 ASSUMPTIONS:

It is assumed that :

a) i) $\eta_{ij} \sim$ N.I.D. (o, σ_w^2)
   ii) $\epsilon_{ijk} \sim$ N.I.D. (o, σ^2)
   iii) η_{ij} and ϵ_{ijk} are independent.

b) ϵ_{ijk} represents random variability of sub-plot yields within a whole plot and has variance (σ^2) estimated by Error(b)M.S., i.e s_b^2.

c) Error(a)M.S., i.e s_a^2, is an estimate of the Error variance of a whole plot total (expressed on a sub-plot basis).

Thus the random components in the <u>total</u> of the $(i,j)^{th}$ whole-plot are:

$$Y_{ijo} = n\eta_{ij} + \sum_k \epsilon_{ijk},$$

with variance, $Var(Y_{ijo}) = n^2\sigma_w^2 + n\sigma^2$.
Thus S_a^2 is an estimate of $(n\sigma_w^2 + \sigma^2)$.
Now since S_a^2 is an estimate of $(n\sigma_w^2 + \sigma^2)$ and s_b^2 is an estimate of σ^2 we have:

$$\tfrac{1}{n}(s_a^2 - s_b^2) = \sigma_w^2$$

d) Thus, in theory, $\boldsymbol{\sigma_w^2 > 0}$. If $s_a^2 < s_b^2$, then $\sigma_w^2 = 0$.

6.5 STANDARD ERRORS FOR SPLIT-PLOT DESIGN

6.5.1. MARGINAL TOTALS/MEANS:

a) **WHOLE PLOT FACTOR (A):**

SE (diff of 2 marginal whole plot **TOTALS**) $= \sqrt{2nrs_a^2}$

OR

SE (diff. of 2 marginal whole plot **MEANS**) $= \sqrt{2\,\frac{s_a^2}{nr}}$

PROOF:

In terms of the statistical model (refer 6.4 above), and ignoring design terms e.g. blocks, etc., two A – factor totals may be represented as :

$$Y_{100} = nr\mu + nr\alpha_1 + n\sum_j \eta_{1j} + \sum_j \sum_k \epsilon_{1jk}$$

$$Y_{200} = nr\mu + nr\alpha_2 + n\sum_j \eta_{2j} + \sum_j \sum_k \epsilon_{2jk}$$

$$\text{Thus, } (Y_{100} - Y_{200}) = nr(\alpha_1 - \alpha_2) + n\sum_j^r (\eta_{1j} - \eta_{2j}) + \sum_j^r \sum_k^n (\epsilon_{1jk} - \epsilon_{2jk})$$

$$\text{and Variance}(Y_{100} - Y_{200}) = 2n^2 r\sigma_w^2 + 2nr\sigma^2 = 2nr\,(n\sigma_w^2 + \sigma^2) = 2nr\sigma_a^2$$

Replacing σ_a^2 by its estimate s_a^2 we obtain

SE (diff of 2 marginal whole plot Totals) $= \sqrt{2nrs_a^2}$

and,

SE (diff. of 2 marginal whole plot Means) $= \sqrt{2\,\frac{s_a^2}{nr}}$

b) **SUB-PLOT FACTOR (B):**

S.E. (Diff. of 2 sub-plot marginal Totals) $= \sqrt{2mr\,s_b^2}$

and,

S.E. (Diff. of 2 sub-plot marginal Means) $= \sqrt{\frac{2\,s_b^2}{mr}}$

PROOF:

Two sub-plot factor totals may be represented as :

$$Y_{001} = mr\mu + mr\beta_1 + \sum_i \sum_j \eta_{ij} + \sum\sum \epsilon_{ij1}$$

$$Y_{002} = mr\mu + mr\beta_2 + \sum_i \sum_j \eta_{is} + \sum\sum \epsilon_{ij2} \quad \text{respectively.}$$

$$\text{Thus, } (Y_{001} - Y_{200}) = mr(\beta_1 - \beta_2) + \sum_i^m \sum_j^r (\epsilon_{ij1} - \epsilon_{ij2})$$

$$\text{and Variance } (Y_{001} - Y_{002}) = 2mr\sigma^2$$

Substituting s_b^2 for σ^2 we get :

S.E. (Diff. of 2 sub-plot marginal Totals) $= \sqrt{2mr\,s_b^2}$

and,

S.E. (Diff. of 2 sub-plot marginal Means) $= \sqrt{\frac{2\,s_b^2}{mr}}$

6.5.2 TWO TREATMENT TOTALS/MEANS WITH SAME LEVEL OF Factor A:

SE (diff of 2 TOTALS with SAME level of A) $= \sqrt{2r\,s_b^2}$

and,

SE (diff of 2 MEANS with SAME level of A) $= \sqrt{\frac{2\,s_b^2}{r}}$

PROOF:

$Y_{101} = r\mu + r\alpha_1 + r\beta_1 + r(\alpha\beta)_{11} + \sum_j \eta_{1j} + \sum_j \epsilon_{1j1}$

$Y_{102} = r\mu + r\alpha_1 + r\beta_2 + r(\alpha\beta)_{12} + \sum_j \eta_{1j} + \sum_j \epsilon_{1j2}$

thus $(Y_{101} - Y_{102}) = r\{\beta_1 - \beta_2 + (\alpha\beta)_{11} - (\alpha\beta)_{12}\} + \sum_j (\epsilon_{1j1} - \epsilon_{1j2})$

and Variance $(Y_{101} - Y_{102}) = 2r\sigma^2$; the result follows.

6.5.3 S.E. (Diff. Two treatment TOTALS/MEANS with SAME LEVEL of FACTOR B)

SE (diff of 2 totals with same level of B) $= \sqrt{\frac{2r}{n}\{s_a^2 + (n-1)s_b^2\}}$

and,

SE (diff of 2 Means, with same level of B) $= \sqrt{\frac{2}{nr}\{\mathbf{s_a^2 + (n-1)s_b^2}\}}$

PROOF:

$Y_{101} = r\mu + r\alpha_1 + r\beta_1 + r(\alpha\beta)_{11} + \sum\eta_{1j} + \sum\epsilon_{1j1}$

$Y_{201} = r\mu + r\alpha_2 + r\beta_1 + r(\alpha\beta)_{21} + \sum\eta_{2j} + \sum\epsilon_{2j1}$

$(Y_{101} - Y_{201}) = r\{\alpha_1 - \alpha_2 + (\alpha\beta)_{11} - (\alpha\beta)_{21}\} + \sum_j(\eta_{1j} - \eta_{2j}) + \sum(\epsilon_{1j1} - \epsilon_{2j1})$

with Variance$(Y_{101} - Y_{201}) = 2r\sigma_w^2 + 2r\sigma^2$

now $\sigma_w^2 = \frac{1}{n}(s_a^2 - s_b^2)$

Thus Variance $(Y_{101} - Y_{201}) = 2r\{\frac{1}{n}(s_a^2 - s_b^2) + s_b^2\} = \frac{2r}{n}\{s_a^2 + (n-1)s_b^2\}$

i.e., SE (diff of 2 totals with same level of B) $= \sqrt{\frac{2r}{n}\{s_a^2 + (n-1)s_b^2\}}$

and

SE (diff of 2 Means, with same level of B) $= \sqrt{\frac{2}{nr}\{s_a^2 + (n-1)s_b^2\}}$

6.5.4 SE (Diff. Two Treatment Totals/Means without a level of either Factor in common)

As for 6.5.3, it can be shown that:

SE (Diff of 2 Totals without a level of either factor in common) $= \sqrt{\frac{2r}{n}\{s_a^2 + (n-1)s_b^2\}}$

and,

SE (Diff of 2 Means without a level of either factor in common) $= \sqrt{\frac{2}{nr}\{s_a^2 + (n-1)s_b^2\}}$

6.6 TESTS OF SIGNIFICANCE OF LINEAR FUNCTIONS OF MEANS

6.6.1 Where the SE of the linear function involves only s_a^2 or s_b^2, the usual t-test is made with the d.f. of s_a^2 (= f_a) or the d.f. of s_b^2 (= f_b).

6.6.2 Where the SE involves both s_a^2 or s_b^2 an approximate least significant value of t (called t′), which lies between that of f_a d.f. (say t_a) and that for f_b d.f. (say t_b) must be calculated., (c.f comparisons of type 6 .6.3 and 6 .6.4 above).

$$t' = \frac{s_a^2 t_a + (n-1)s_b^2 t_b}{s_a^2 + (n-1)s_b^2}$$

NOTE:

Since $t_a > t' > t_b$, there is often no need to compute t′ at all, for if,

(i) $t_{calculated} > t_a$, this implies that $t_{calc.} > t'$ and the result is declared significant.

(ii) Similarly if $t_{calculated} < t_b$ the result will be declared non-significant.

6.7 SOME VALID REASONS FOR ADOPTING A SPLIT-PLOT DESIGN

- Certain types of treatments are troublesome or impossible to apply to small areas (plots) and may therefore be applied to whole-plots, e.g., methods of cultivation, irrigation treatments, frequency of grazing, veld burning, etc.
- There may be no real interest in the main effect of one of the factors, but only in its interaction with the other factors. Under such circumstances the factor concerned should be allocated to whole-plots.
- One of the factors may be such that its main effects are expected to be significantly large; such a factor can be allocated to whole-plots.
- There may be more interest in one factor than another, the less important factor under such circimstances being applied to whole-plots.

6.8 SOME VARIANTS OF THE SPLIT-PLOT DESIGN.

6.8.1 SPLIT-SPLIT-PLOT EXPERIMENTS

Any split-plot design can have a further factor added by introducing an additional stage of "splitting", i.e., the subplots are split into sub-sub-plots ant the levels of the extra factor are, randomised over the sub-sub-plots within each sub-plot. In theory there is nothing to prevent the extension of this process, so that after a third stage of splitting we would have a split-split-split-plot design with sub-sub-sub-plots as the ultimate experimental units. In practice, however, more than two stages of splitting would be very rarely appropriate. It is well to remember also that with each split there is an extra "error mean square", and a series of standard errors even more awkward then those outlined in {6.5} above.

Example:
Whole plot Factor A at m-levels, (Whole plot design RCBD with r replications)
Sub-plot Factor B at n-levels,
Sub-sub-plot Factor C at p-levels

SYMBOLIC ANOVA TABLE:

Source	d.f.
Blocks	$r - 1$
A	$m - 1$
Error (a)	$(r - 1)(m - 1)$
between whole plots	$mr - 1$
B	$n - 1$
AB	$(m - 1)(n - 1)$
Error (b)	$m(n - 1)(r - 1)$
total sub-plots	$mnr - 1$
C	$p - 1$
AC	$(m - 1)(p - 1)$
BC	$(n - 1)(p - 1)$
ABC	$(m - 1)(n - 1)(p - 1)$
Error (c)	$mn(p - 1)(r - 1)$
Total sub-sub-plots	$mnpr - 1$

6.8.2 SPLIT-PLOT EXPERIMENTS WITH SUB-PLOTS IN A LATIN SQUARE.

In this design(which is, of course, entirely different from that illustrated in {6.3.1})there must be as many replications as there are levels of the sub-plot factor.

6.8.3 SPLIT-BLOCK (STRIP-BLOCK) DESIGN.

If in a split-plot design the sub-plot treatments are **NOT** separately randomised, for each whole-plot, but are allocated to strips of sub-plots cutting across each replication, we have what is variously called a "strip-plot" or "split-block" design. This is illustrated below.

B1	E1	A1	D1	C1	
B2	E2	A2	D2	C2	Replication 1
B3	E3	A3	D3	C3	
A3	C3	E3	B3	D3	
A1	C1	E1	B1	D1	Replication 2
A2	C2	E2	B2	D2	
E3	D3	B3	A3	C3	
E2	D2	B2	A2	C2	Replication 3
E1	D1	B1	A1	C1	

Here there are two types of whole-plots corresponding to the two factors, the levels of each factor being separately randomised in each block. This is a valid design and has a use, especially in field experiments, when it is convenient to apply both factors to large areas. However, corresponding to the **three** types of unit (2 types of whole-plot, and subplots), there are three error terms, one for each type of main effect and one for the interaction. These three errors give rise to some awkward S.E's in some types of comparisons.

Revised 02/2006/hmd