

Topic 7. Double grouping. Latin Squares [ST&D 9.10-9.15]

7. 1. Introduction

Latin squares are experimental designs used to remove two known sources of variations by **blocking in two ways**.

Randomization of treatments by columns and by rows.

Each row and each column receives **each treatment once**.

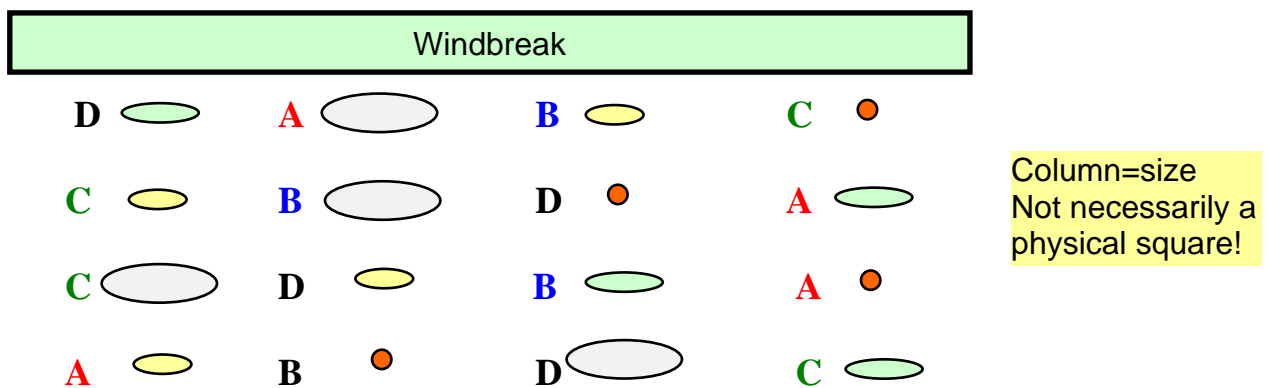
Standard square: treatments assigned to the first row and the first column in an alphabetical or numerical sequence is called a.

Fig. 1 shows the **standard squares** for 2 x 2, 3 x 3 and 4 x 4 designs.

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

7. 2. Examples:

If trees in an orchard can be classified as to size and distance from a windbreak, a Latin square may be used in treatment assignment.



16 orchard trees classified by **size** and **distance** from a windbreak to which 4 treatments (A, B, C and D) are assigned.

Other *examples*:

- Different pieces of equipment and different technicians
- In sensory testing programs, the materials or treatment to be tested can be blocked by evaluators (judges) and time periods
- Marketing studies sometimes use Latin Squares with days being rows and stores being columns.

7. 3. Randomization: crucial for the validity of any conclusions.

Number of possible different Latin squares= (# of standard LS) (K!) (K - 1)! where k is the number of treatments. k=4, N= (4) (4!) (3!) = 576. **Large!**

7. 3. 1. Manual method

The only restriction on the Latin square arrangement is that each treatment must appear in every row and every column of the table.

1.- Select any standard square for the number of treatments involved.

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

2.- From a table of random numbers or by some other procedure, select **two sets of random numbers** with size equal to the treatments involved, e.g.

	Set 1 (for columns)	Set 2 (for rows)
Random number	9 1 6 4	8 5 3 7
Rank	4 1 3 2	4 2 1 3

3.- Assign the **rank**s to the rows and columns of the standard square (Step 1). Generate a new square according to the rankings of the rows (Step 2). Generate a new square according to the rankings of the columns (Step 3).

Step 1: assign ranks

<u>4</u>	<u>1</u>	<u>3</u>	<u>2</u>
4	A	B	C
2	B	C	D
1	C	D	A
3	D	A	B

Step 2: order rows

<u>4</u>	<u>1</u>	<u>3</u>	<u>2</u>
1	C	D	A
2	B	C	D
3	D	A	B
4	A	B	C

Step 3: order columns.

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
1	D	B	A
2	C	A	D
3	A	C	B
4	B	D	C

4.- **Codes** A, B, C, and D are then **randomly assigned to the 4 treatments.**

7.4. The linear model for Latin Squares

The linear model for the Latin Square is,

$$Y_{ij(t)} = \mu + \beta_i + \gamma_j + \tau_{(t)} + \varepsilon_{ij}$$

$\beta_i = i^{\text{th}}$ **row effect**, and $\gamma_j = j^{\text{th}}$ **column effect**, and $\tau_{(t)} = t^{\text{th}}$ **treatment effect**.
The parenthesis around the t indicates that each t appears only once in each row-column combination \Rightarrow incomplete designs

The Sum of Squares equation becomes

$$\sum_{i,j} (\bar{Y}_{ij} - \bar{Y}_{..})^2 = r \sum_{i=1}^r (\bar{Y}_{i.} - \bar{Y}_{..})^2 + r \sum_{j=1}^r (\bar{Y}_{.j} - \bar{Y}_{..})^2 + r \sum_{t=1}^r (\bar{Y}_{t.} - \bar{Y}_{..})^2 + \sum_{i,j} (Y_{ij} - \bar{Y}_{i.} - \bar{Y}_{.j} - \bar{Y}_{t.} + 2\bar{Y}_{..})^2$$

$$SS_{\text{total}} = SS_{\text{rows}} + SS_{\text{col.}} + SS_{\text{trtmnt.}} + SS_{\text{error}}$$

7.5. ANOVA: The ANOVA table looks like this:

Source	df	SS	MS	F
Rows	r - 1	SSR	SSR/(r-1)	
Columns	r - 1	SSC	SSC/(r-1)	
Treatments	r - 1	SST	SST/(r-1)	MST/MSE
Error	(r-1)(r-2)	SSE	SSE/(r-1)(r-2)	
Total	r ² -1	SS		

Example (ST&D p230). 4 x 4
LS for yield of 4 wheat varieties
(A, B, C, D).

4 sources of variation: columns,
rows, varieties, & error

	Column			
Row	1	2	3	4
1	C:10.5	D: 7.7	B:12.0	A:13.2
2	B:11.1	A:12.0	C:10.3	D: 7.5
3	D: 5.8	C:12.2	A:11.2	B:13.7
4	A:11.6	B:12.3	D: 5.9	C:10.2

Source	DF	SS	MS	F Value	Pr > F
ROW	3	1.96	0.65	1.44	0.322
COL	3	6.80	2.27	5.00	0.045
TRTMT	3	78.92	26.32	58.03	<0.0001
Error	6	2.72	0.45		

- **Highly significant differences for varieties**
- Marginal differences for the columns.
- No differences for rows.

SAS program used to produce a Latin Square ANOVA.

Note that the only change to the statements in PROC ANOVA compared to a RCBD is the inclusion of one extra classification variable

```
data STp2301s;
input row col trtmt $ yield @@;
cards;
1 1 C 10.5    1 2 D  7.7    1 3 B 12.0    1 4 A 13.2
2 1 B 11.1    2 2 A 12.0    2 3 C 10.3    2 4 D  7.5
3 1 D  5.8    3 2 C 12.2    3 3 A 11.2    3 4 B 13.7
4 1 A 11.6    4 2 B 12.3    4 3 D  5.9    4 4 C 10.2
;
proc GLM;
  class row col trtmt;
  model yield = row col trtmt;
  means trtmt / lsd;
  output out=resplot p= predyiel r= resiyiel;


---


*Residual plot;
proc plot data=resplot;
  plot resiyiel*predyiel= trtmt;


---


*Normality of residuals;
proc univariate data=resplot normal;
  var resiyiel;


---


*Tukey's test for non-aditivity: rows*trt
(other tests for col* trt, & col* row also required);
proc GLM data= STp2301s;
  class row trtmt;
  model yield = row trtmt;
  output out=tukrt p= prt    r= rrt;
proc glm data=tukrt;
  class row trtmt;
  model yield = row trtmt prt*prt;


---


*Levene's test for homogeneity of var. (1 way ANOVAS);
proc GLM data= STp2301s;
  class trtmt;
  model yield = trtmt;
  means trtmt / hovtest = levене;

run; quit;
```

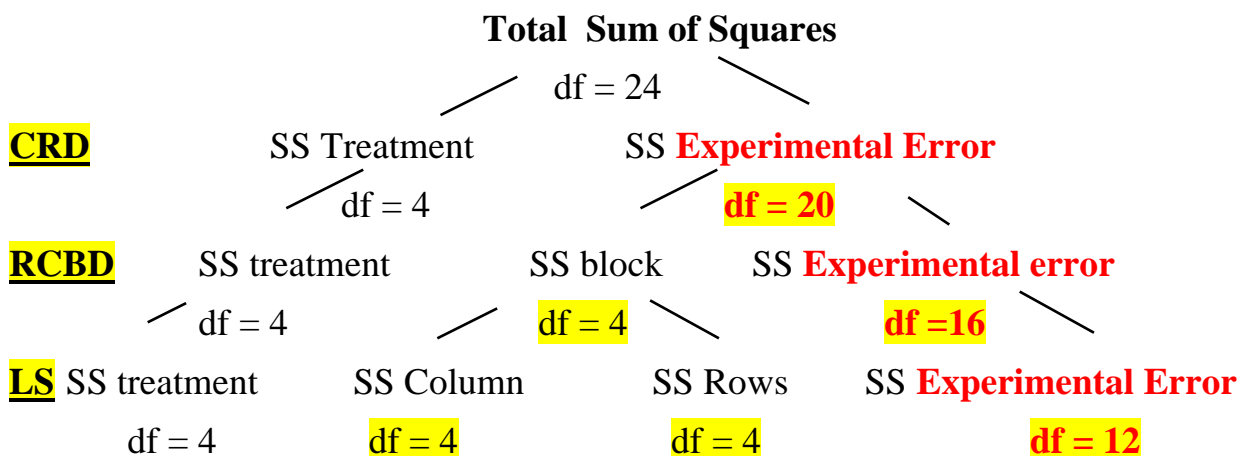
7.6 Advantages and disadvantages of the Latin square

Advantages: removes two kinds of heterogeneity from the MS error making the test more sensitive.

Disadvantages:

1. The number of treatments must be equal to the number of rows and columns. Inconvenient for large number of treatments.
2. The design is valid if there are no interactions among rows, columns, and treatments. **When there is interaction between any two among all of the criteria the F test is not sensitive.** To check this assumption: Tukey's test for non-additivity.
3. $F = \text{MST}/\text{MSE}$ but expected $\text{MSE} = \sigma^2_\epsilon + r\sigma^2_{\text{TC}} + r\sigma^2_{\text{TR}} + r\sigma^2_{\text{RC}}$
4. Squares smaller than 4 x 4 generally have too few replications and **few degrees of freedom for experimental error.**

Assume 5 treatments to be assigned to 25 experimental units with equal replication per treatment. CRD, RCBD, and Latin squares df and SS are:



This diagram illustrates the loss of degrees of freedom from the experimental error as the design becomes more complex.

Unless there are appreciable amounts of variation in the experimental error that can be removed by row and column blocking to compensate for the loss of the degrees of freedom in the experimental error, the LS design will be less efficient.

7.7. Relative Efficiency: Section 9.3 of ST&D (p237) provides a good discussion for the relative efficiencies of Latin Squares compared to RCBD.

If one of the blocking variables is not included, the MSE needs to be recalculated. In the previous example ($MSE_{LS} = 0.45$, $MS_{RLS} = 0.65$, and $MS_{CLS} = 2.27$) if **columns are not included**, MS_{col} is included in MS_{error}

$$MSE_{RCBD} \cong \frac{fcMSC + (ft + fe)MSE}{fc + ft + fe} = \frac{3 * 2.27 + (3 + 6) 0.45}{3 + 3 + 6} = 0.91$$

and the relative efficiency is

$$RE_{LS \text{ to } RCBD} = \frac{(f_{LS} + 1)(f_{RCBD} + 3)MSE_{RCBD}}{(f_{RCBD} + 1)(f_{LS} + 3)MSE_{LS}} = \frac{(6 + 1)(9 + 3)0.91}{(9 + 1)(6 + 3)0.45} = 1.89$$

The **column** blocking **increased the precision** of the model by 89%. This parallels the **significant F for columns**

If **rows are not included** in the model, $MS_{rows} (=0.65)$ is included in MS_e :

$$MSE_{RCBD} \cong \frac{3 * 0.65 + (3 + 6) 0.45}{3 + 3 + .6} = 0.50$$

and the relative efficiency is

$$RE_{LS \text{ to } RCBD} = \frac{(6 + 1)(9 + 3)0.50}{(9 + 1)(6 + 3)0.45} = 1.04$$

This indicates that the **row** grouping **did not increase much the precision** of the model (only 4%). This parallels the **NS F for rows**.

Numerical example of the effect of the missing df in the critical F :

$$LS_{3 \times 3} = df_{LS_e} = 2 \quad df_{RCBD_e} = 4 \qquad LS_{5 \times 5} = df_{LS_e} = 12 \quad df_{RCBD_e} = 16$$

$$LS \ F \ df \ 2, \ 2, \ 0.05 = \quad \mathbf{19.0} \qquad LS \ F \ df \ 2, \ 12, \ 0.05 = \quad \mathbf{3.89}$$

$$RCBD \ F \ df \ 2, \ 4, \ 0.05 = \quad \mathbf{6.94} \qquad RCBD \ F \ df \ 2, \ 16, \ 0.05 = \quad \mathbf{4.49}$$

The loss of df has a huge effect on the sensitivity of small LS!

7.7. Repeated Latin squares

Repeated LS may be used to increase the degrees of freedom for **exp. error**. Latin squares may be replicated over time or space.

There are several variations of Latin squares replications:

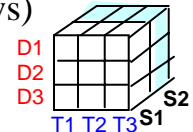
- Case-1: Latin squares that share common **rows** and **columns**
- Case-2: Latin squares that share common **columns** but **not rows**
- Case-3: Latin squares that share **rows** but **not columns**
- Case-4: Latin squares that **do not share rows** or **columns**

Example: Three gasoline additives (TREATMENTS, A B & C) were tested for gas efficiency by three **drivers (rows)** using three different **tractors (columns)**. The variable measured was the yield of carbon monoxide in a trap. The experiment was **repeated twice**. These replications can be:

Case 1: **same tractors (col)** and **drivers (col)** (3 columns and 3 rows)

PROC GLM;

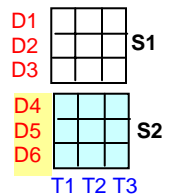
```
class square col row treat;
model yield= square col row treat;
```



Case 2: Using **different drivers (row)** but the **same tractors (col)**.

PROC GLM;

```
class square col row treat;
model yield= square col row(square) treat;
```

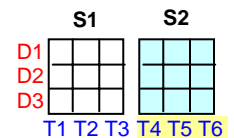


ROW(SQUARE) : row has meaning **ONLY** within square

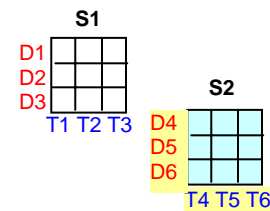
Case 3: Using **different tractors (col)** but the **same drivers (row)**

PROC GLM;

```
class square col row treat;
model yield= square col(square) row treat;
```



Case 4: Using **different drivers (row)** and **tractors (col)**



PROC GLM;

```
class square col row treat;
model yield= square col(square) row(square) treat;
```

Square 1		
B 26.0	C 25.0	A 21.3
C 28.7	A 23.6	B 28.5
A 25.3	B 28.4	C 30.1

Square 2		
C 32.4	B 28.7	A 25.8
B 31.7	A 24.3	C 30.5
A 24.9	C 29.3	B 29.2

The SAS programs will be discussed at the Lab class.

7. 8. Other complicated Latin squares

(See Chapter 8 of Box, Hunter & Hunter, Appendix 8 included)

To eliminate more than 2 sources of variability a **Greco-Latin square** (GLS) or a hyper- Greco-Latin square (HGLS) is sometimes useful.

A GLS is an $r \times r$ pattern that permits the study of r treatments simultaneously with three different blocking variables.

Example of a 4×4 Greco-Latin square could be the test of 4 different oil additives (treatments, A, B, C, and D) grouped by 4 different drivers (I, II, III, and IV), 4 different cars (1, 2, 3, and 4) and 4 different days ($\alpha, \beta, \gamma, \delta$). It is constructed from the first two 4×4 Latin Squares in the Appendix.

		Car			
		1	2	3	4
Driver	I	A α	B β	C γ	D δ
	II	B δ	A γ	D β	C α
	III	C β	D α	A δ	B γ
	IV	D γ	C δ	B α	A β

Appendix:

3×3

A B C	A B C
B C A	C A B
C A B	B C A

To form the 3×3 Graeco-Latin square superimpose the two designs. Thus, using Greek letter equivalents for the second 3×3 Latin square, we have

A α	B β	C γ
B γ	C α	A β
C β	A γ	B α

4×4

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

These three 4×4 Latin squares may be superimposed to form a hyper-Graeco-Latin square. Superimposing any pair gives a Graeco-Latin square design.

5×5

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

These four 5×5 Latin squares may be superimposed to form a hyper-Graeco-Latin square. Also, superimposing any three gives a hyper-Graeco-Latin square design. Similarly, superimposing any pair gives a Graeco-Latin square design.