DESIGN AND MIXED-MODEL ANALYSIS OF EXPERIMENTS

VI. Latin squares designs

(Mead sec.8.1)

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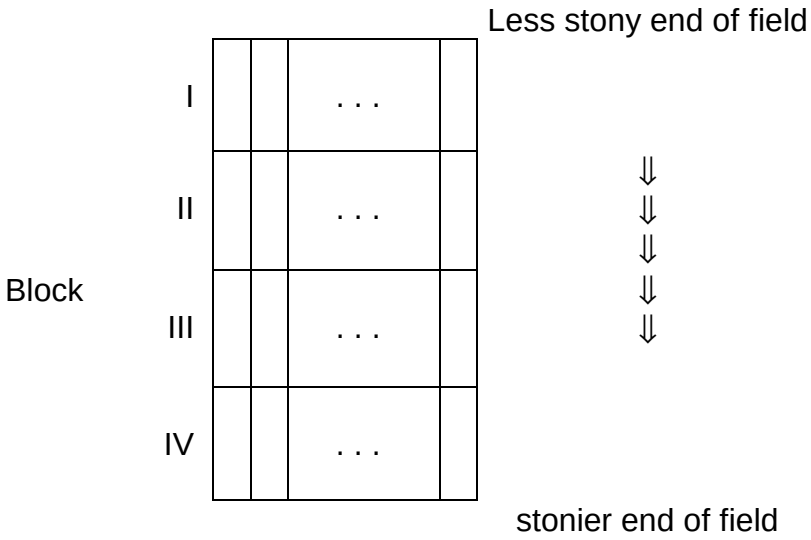
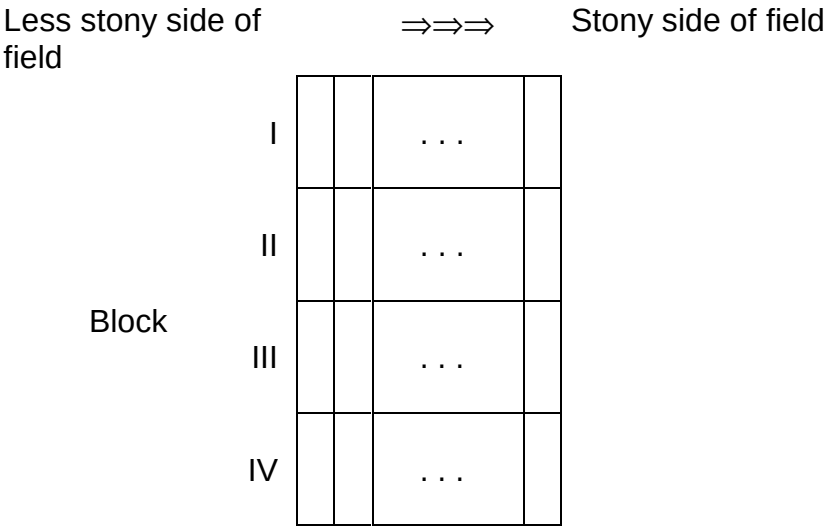
In the RCBD the plots are grouped into blocks such that the plots in a block are as similar as possible. As previously discussed, this would lead to different blocks. In the context of field experiments this means placing plots parallel to the trend and blocks perpendicular to it as illustrated in the following diagram:



It is clear that the blocks will be quite different and the plots similar. However, suppose that I had thought there was a fertility trend in a particular direction, say down the field as before, and so had laid out the trial in an RCBD to take this into account. In the event it turns out that I had got it wrong and the trend was across the field. The situation would be as follows:



Clearly, Blocks would be similar and plots different. In fact this experiment can be less sensitive than a CRD. So getting it wrong can be costly.

VI.A Design of Latin squares

In the RCBD we arranged for the isolation of one set of differences, thereby eliminating their effect on treatment differences. Thus, in the penicillin example, we arranged for the elimination of blend differences. In a field trial, if there was a trend in a particular direction, this trend could be isolated by having blocks run perpendicular to the trend.

However, suppose moisture is varying across the field and the stoniness down the field. The RCBD cannot be used to eliminate both sources of variability — a Latin square design is required.

Definition VI.1: A **Latin square design** is one in which each treatment occurs once and only once in each row and each column so that the numbers of rows, columns and treatments are all equal. ■

Clearly, the total number of observations is $n = t^2$.

Example VI.1 Fertilizer experiment

Suppose there are five different fertilizers to be compared; a Latin square design for this would be as follows:

5 x 5 Latin square

		Column					
		1	2	3	4	5	Less stony of field
	I	A	B	C	D	E	
	II	C	D	E	A	B	⇓
Row	III	E	A	B	C	D	⇓
	IV	B	C	D	E	A	⇓
	V	D	E	A	B	C	Stonier end of field

Less moisture ⇒ ⇒ ⇒ More moisture

(Fertilizers A, B, C, D, E)

■

Even if one has not identified trends in two directions, a Latin square may be employed to guard against the problem of putting the blocks in the wrong direction. Latin squares may also be used when there are two different kinds of blocking variables — for example animals and times. The major disadvantage with the Latin square is that you are restricted to having the number of replicates equal to the number of treatments.

Example VI.2 Wheat samplers

An experimenter has decided to investigate the ability of samplers to unbiasedly select a sample of wheat shoots to measure their height. Four experienced samplers are to be tested within the one day on 4 areas of wheat each containing about 80 shoots that were about 2-feet high.

The period over which the experiment was to be conducted was divided into 4 time intervals. During a particular interval, each sampler inspected a different area, chose 8 shoots they believed to be representative of their area and measured them. A Latin square was used to assign the samplers to the area they would sample during that interval. This was done by designating the rows of the design to be the areas, the columns to be the intervals and the samplers to be the treatments. Using a Latin square allows differences between intervals and differences between the areas to be eliminated so that they did not effect differences between the samplers. There is also the practical advantage that no two samplers sampled the same area at the same time. The layout was as given in the table below.

Layout for the 4 x 4 Latin square experiment

		Interval			
		1	2	3	4
	I	A	B	D	C
	II	D	C	A	B
Area	III	B	D	C	A
	IV	C	A	B	D

Sampler (A–D)

Suppose a randomized complete block design had been employed to assign the samplers. It would make sense to have Areas forming the blocks as one would want all samplers to do each area. The layout might have been as given in the table below.

A layout utilizing a randomized complete block design

		Interval			
		1	2	3	4
	I	B	A	D	C
	II	A	D	C	B
Area	III	A	C	D	B
	IV	B	A	D	C

Sampler (A–D)

Notice that with this design, all samplers do each area; however, not all samplers evaluate in a particular interval and some have to evaluate more than once.

■

The general principle is that one is interested in maximizing row and column differences so as to minimize the amount of uncontrolled variation affecting treatment comparisons. Several fundamentally different Latin squares exist for a particular t — for $t = 4$ there are three different squares. To randomize these designs appropriately involves the following:

1. randomly select one of the designs for a value of t;
2. randomly permute the rows and then the columns;
3. randomly assign letters to treatments.

The *Stat > Design > Select Design* menu command in Genstat can be used to do the first two of these steps — it can provide designs for $t = 3,4, ..., 14$. Having selected the menu command, you need to choose *Latin squares (also Graeco-Latin squares etc as feasible)* in answer to the question *Which type of design would you like to generate?* You will then be asked a series of questions to which you should respond as follows:

How many rows and columns are there in the Latin square?	number of treatments
How many treatment factors (or mutually orthogonal Latin squares) do you want to generate? (up to 3 available)	1
What would you like to call the treatment factor?	name for treats
Give the identifier to be used for the row factor?	name for rows
Give the identifier to be used for the column factor?	name for columns
Seed for randomization (0 for none)?	6-digit number
What would you like to print?	design
Do you want to check the design by ANOVA	yes

Example VI.2 Wheat samplers (continued)

The names to be used for the treats, rows and columns for this example are Samplers, Area and Interval, respectively. Also, $t = 4$. Using these values in making the responses suggested above and providing a seed for the random number generator results in the following Genstat output.

```
Genstat 5  Release 4.1  (PC/Windows NT)               28 March 2000 15:19:39
Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

                ____________________________________________________

                Genstat 5 Fourth Edition - (for Windows)
                Genstat 5 Procedure Library Release PL11
                ____________________________________________________

   4  DESIGN

 *** Treatment combinations on each unit of the design ***


 Interval   1   2   3   4
     Area
        1   4   2   1   3
        2   3   1   2   4
        3   2   4   3   1
        4   1   3   4   2

 Treatment factors are listed in the order: Samplers


4.....................................................................................

***** Analysis of variance *****
Source of variation     d.f.

Area stratum               3

Interval stratum           3

Area.Interval stratum
Samplers                   3
Residual                   6

Total                     15
```

VI.B Maximal model for a Latin square

There are a number of different maximal models for this design depending on whether each of the factors Rows, Columns and Treatments are to be regarded as fixed or random. As for the randomized complete block design, it happens that the analysis of the Latin square is essentially unaffected by which model is used.

The maximal model for the observations from a Latin square, when Rows, Columns and Treatments are fixed, is

$$\psi = E[\mathbf{Y}] = \mathbf{X}_B\beta + \mathbf{X}_C\gamma + \mathbf{X}_T\tau \text{ and } \mathbf{V} = \sigma^2\mathbf{I}_{t^2},$$

where $\mathbf{Y}$ is the *n*-vector of random variables for the observations,
β is the *t*-vector of row parameters,
γ is the *t*-vector of column parameters,
τ is the *t*-vector of treatment parameters, and
σ^2 is the variability of values from a row-column combination.

Our model also involves assuming $\mathbf{Y} \sim N(\psi, \mathbf{V})$.

This model is not of full rank in that the sum of the columns of all three **X** matrices is equal to $\mathbf{1}_n$.

VI.C Parameter estimation under the maximal model for a Latin square

As for the randomized complete block design, each of the parameters in $\theta' = [\beta' \quad \gamma' \quad \tau']$ is not estimable. So, in the next theorem, we consider the estimators for ψ — theorem V.5 tells us that these are estimable.

Theorem VI.1: Let **Y** be a *n*-vector of jointly-distributed random variables ordered so that all the variables for the same row occur consecutively in column order. Also, let

$$\psi = E[\mathbf{Y}] = \mathbf{X}_B\beta + \mathbf{X}_C\gamma + \mathbf{X}_T\tau \text{ and } \text{var}[\mathbf{y}] = \sigma^2\mathbf{I}$$

where β is the *t*-vector of parameters specifying a different mean response for each row,
$\mathbf{X}_B = \mathbf{I}_t \otimes \mathbf{1}_t$ is the $t^2 \times t$ matrix indicating the row from which an observation came,
γ is the *t*-vector of parameters specifying a different mean response for each column,
$\mathbf{X}_C = \mathbf{1}_t \otimes \mathbf{I}_t$ is the $t^2 \times t$ matrix indicating the column from which an observation came,

τ is the t-vector of parameters specifying a different mean response for each treatment,
$\mathbf{X}_T$ is the $t^2 \times t$ matrix indicating the observations that received each of the treatments.

Then $\hat{\psi} = \mathbf{B} + \mathbf{C} + \mathbf{T} - 2\mathbf{G}$ where **B**, **C**, **T** and **G** are the t^2-vectors of row, column, treatment and grand means, respectively.

Proof: According to theorem V.2,

$$\hat{\theta} = (\mathbf{X}'\mathbf{X})^{-}\mathbf{X}'\mathbf{Y}$$

Now for $\mathbf{X} = [\mathbf{X}_B \quad \mathbf{X}_C \quad \mathbf{X}_T]$,

$$\mathbf{X}'\mathbf{X} = \begin{bmatrix} \mathbf{X}_B' \\ \mathbf{X}_C' \\ \mathbf{X}_T' \end{bmatrix} [\mathbf{X}_B \quad \mathbf{X}_C \quad \mathbf{X}_T] = \begin{bmatrix} \mathbf{X}_B'\mathbf{X}_B & \mathbf{X}_B'\mathbf{X}_C & \mathbf{X}_B'\mathbf{X}_T \\ \mathbf{X}_C'\mathbf{X}_B & \mathbf{X}_C'\mathbf{X}_C & \mathbf{X}_C'\mathbf{X}_T \\ \mathbf{X}_T'\mathbf{X}_B & \mathbf{X}_T'\mathbf{X}_C & \mathbf{X}_T'\mathbf{X}_T \end{bmatrix} = \begin{bmatrix} t\mathbf{I}_t & \mathbf{J}_t & \mathbf{J}_t \\ \mathbf{J}_t & t\mathbf{I}_t & \mathbf{J}_t \\ \mathbf{J}_t & \mathbf{J}_t & t\mathbf{I}_t \end{bmatrix}$$

In particular, note that $\mathbf{X}_B'\mathbf{X}_T = \mathbf{X}_C'\mathbf{X}_T = \mathbf{J}_t$ since each treatment must occur once and only once in each row and column.

To obtain the generalized inverse of $\mathbf{X}'\mathbf{X}$ our algorithm tells us that we must take any minor of order $3t-2$ and obtain its inverse. Then the generalized inverse will be formed by replacing the minor with the transpose of its inverse and the other rows and columns with zeros. So we delete any two rows and two columns and find its inverse. Suppose we take the minor obtained by omitting the last row of $\mathbf{X}_C$ and of $\mathbf{X}_T$ in **X**. Then we require the inverse of

$$\mathbf{Z}'\mathbf{Z} = \begin{bmatrix} \mathbf{X}_B'\mathbf{X}_B & \mathbf{X}_B'\mathbf{X}_{C-1} & \mathbf{X}_B'\mathbf{X}_{T-1} \\ \mathbf{X}_{C-1}'\mathbf{X}_B & \mathbf{X}_{C-1}'\mathbf{X}_{C-1} & \mathbf{X}_{C-1}'\mathbf{X}_{T-1} \\ \mathbf{X}_{T-1}'\mathbf{X}_B & \mathbf{X}_{T-1}'\mathbf{X}_{C-1} & \mathbf{X}_{T-1}'\mathbf{X}_{T-1} \end{bmatrix} = \begin{bmatrix} t\mathbf{I}_t & \mathbf{J}_{t\times(t-1)} & \mathbf{J}_{t\times(t-1)} \\ \mathbf{J}_{(t-1)\times t} & t\mathbf{I}_{(t-1)} & \mathbf{J}_{(t-1)} \\ \mathbf{J}_{(t-1)\times t} & \mathbf{J}_{(t-1)} & t\mathbf{I}_{(t-1)} \end{bmatrix}$$

where $\mathbf{X}_{C-1}$ and $\mathbf{X}_{T-1}$ are the $t^2 \times (t-1)$ matrices formed by taking the first $t-1$ columns of $\mathbf{X}_C$ and $\mathbf{X}_T$, respectively.

To obtain this inverse, reduce **Z'Z** to a 2×2 partitioned matrix by combining the upper left 2×2 elements of **X'X** into a single matrix, say **A**. Then the upper 2 elements in column 3 of **Z'Z** form **B** and the lower right element is designated **D**. Now, as in proof of theorem V.7, we use the following expression for the inverse of a partitioned symmetric matrix **M** where

$$\mathbf{M} = \begin{bmatrix} \mathbf{A} & \mathbf{B} \\ \mathbf{B}' & \mathbf{D} \end{bmatrix} \text{ then } \mathbf{M}^{-1} = \left(\mathbf{M}^{-1}\right)' = \begin{bmatrix} \mathbf{U} & \mathbf{V} \\ \mathbf{V}' & \mathbf{W} \end{bmatrix} = \begin{bmatrix} \mathbf{A}^{-1} - \mathbf{V}\mathbf{B}'\mathbf{A}^{-1} & -\mathbf{A}^{-1}\mathbf{B}\mathbf{W} \\ -\mathbf{W}\mathbf{B}'\mathbf{A}^{-1} & \left(\mathbf{D} - \mathbf{B}'\mathbf{A}^{-1}\mathbf{B}\right)^{-1} \end{bmatrix}$$

The use of this expression requires us to find $\mathbf{A}^{-1}$. However, it is itself a partitioned matrix whose inverse can also be found using the above expression. From the proof of theorem V.7, the $\mathbf{A}^{-1}$ is obtained by substituting t for b in the expression for $\mathbf{Z}'\mathbf{Z}^{-1}$ in that theorem. That is,

$$\mathbf{A}^{-1} = \begin{bmatrix} t\mathbf{I}_t & \mathbf{J}_{t\times(t-1)} \\ \mathbf{J}_{(t-1)\times t} & t\mathbf{I}_{(t-1)} \end{bmatrix}^{-1} = \begin{bmatrix} \frac{1}{t}\mathbf{I}_t + \frac{t-1}{t^2}\mathbf{J}_t & -\frac{1}{t}\mathbf{J}_{t\times(t-1)} \\ -\frac{1}{t}\mathbf{J}_{(t-1)\times t} & \frac{1}{t}\left(\mathbf{I}_{(t-1)} + \mathbf{J}_{(t-1)}\right) \end{bmatrix}$$

The remainder of the proof follows similar lines to theorem V.7 ■

Also, note that $\mathbf{B} = \mathbf{P}_B\mathbf{Y}$, $\mathbf{C} = \mathbf{P}_C\mathbf{Y}$, $\mathbf{T} = \mathbf{P}_T\mathbf{Y}$, $\mathbf{G} = \mathbf{P}_G\mathbf{Y}$ and $\mathbf{P}_i = \mathbf{X}_i\left(\mathbf{X}_i'\mathbf{X}_i\right)^{-1}\mathbf{X}_i'$ where $\mathbf{X}_i = \mathbf{X}_B, \mathbf{X}_C, \mathbf{X}_T, \mathbf{X}_G$. That is, $\mathbf{P}_B$, $\mathbf{P}_C$, $\mathbf{P}_T$ and $\mathbf{P}_G$ are the row, column, treatment and grand mean operators, respectively. So once again the fitted values are functions of means.

Further, if the data in the vector $\mathbf{Y}$ has been arranged in the order described in the statement of theorem VI.1, the operators are:

$$\mathbf{P}_G = t^{-2}\mathbf{J}_t \otimes \mathbf{J}_t$$
$$\mathbf{P}_B = t^{-1}\mathbf{I}_t \otimes \mathbf{J}_t$$
$$\mathbf{P}_C = t^{-1}\mathbf{J}_t \otimes \mathbf{I}_t$$

In this case it is not possible to write $\mathbf{P}_T$ as a direct product of $\mathbf{I}$ and $\mathbf{J}$ matrices as the treatments will not be in a systematic order expressible in this form.

Example VI.3 A 3×3 Latin square

For example, suppose that a 3×3 Latin square with the following arrangement of treatments was being considered:

3 x 3 Latin square

		Column		
		1	2	3
Row	I	A	B	C
	II	C	A	B
	III	B	C	A

■

Then, for this example,

$$\mathbf{P}_G = \tfrac{1}{9}\mathbf{J}_3 \otimes \mathbf{J}_3$$
$$\mathbf{P}_B = \tfrac{1}{3}\mathbf{I}_3 \otimes \mathbf{J}_3$$
$$\mathbf{P}_C = \tfrac{1}{3}\mathbf{J}_3 \otimes \mathbf{I}_3$$

$$\mathbf{X}_T = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \end{bmatrix}$$

and

$$\begin{aligned} \mathbf{P}_T &= \mathbf{X}_T \left(\mathbf{X}_T' \mathbf{X}_T \right)^{-1} \mathbf{X}_T' \\ &= \frac{1}{3} \mathbf{X}_T \mathbf{I}_3 \mathbf{X}_T' \\ &= \frac{1}{3} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 \end{bmatrix} \\ &= \frac{1}{3} \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \end{bmatrix} \end{aligned}$$

VI.D Hypothesis testing using the ANOVA method for a Latin square

There are 8 possible different models for the expectation when Rows, Columns and Treatments are considered fixed:

$$\begin{aligned} \psi &= \mathbf{X}_G \mu \\ \psi &= \mathbf{X}_B \beta \\ \psi &= \mathbf{X}_C \gamma \\ \psi &= \mathbf{X}_B \beta + \mathbf{X}_C \gamma \\ \psi &= \mathbf{X}_T \tau \\ \psi &= \mathbf{X}_B \beta + \mathbf{X}_T \tau \\ \psi &= \mathbf{X}_C \gamma + \mathbf{X}_T \tau \\ \psi &= \mathbf{X}_B \beta + \mathbf{X}_C \gamma + \mathbf{X}_T \tau \end{aligned}$$

An analysis of variance will be used to choose between these models. However, to get the sums of squares in the analysis of variance, not all these models need to be fitted. A similar fitting procedure to that described for the analysis of the RCBD can be performed with the order of fitting β, γ and τ being immaterial. However, we follow the convention of fitting the terms associated with the physical structure first and so fit the following sequence of models:

$$E[\mathbf{Y}] = \mathbf{X}_G\mu$$
$$E[\mathbf{Y}] = \mathbf{X}_B\beta$$
$$E[\mathbf{Y}] = \mathbf{X}_B\beta + \mathbf{X}_C\gamma$$
$$E[\mathbf{Y}] = \mathbf{X}_B\beta + \mathbf{X}_C\gamma + \mathbf{X}_T\tau$$

The residual sums of squares (or unscaled deviance) after fitting each of these models is obtained. Let's call these D(μ), D(β), D(β,γ), and D(β,γ,τ). The test statistics on which the hypothesis testing is based can then be conveniently computed in an analysis of variance table. The hypothesis test is a follows:

Step 1: Set up hypotheses

a) H_0: $\tau_1 = \tau_2 = ... = \tau_t$
H_1: at least one pair of population treatment means is different

b) H_0: $\beta_1 = \beta_2 = ... = \beta_t$
H_1: at least one pair of population row means is different

c) H_0: $\gamma_1 = \gamma_2 = ... = \gamma_t$
H_1: at least one pair of population columns means is different

Step 2: Calculate test statistics

The analysis of variance table for a Latin square is:

Source	df	MSq		E[MSq]	F
Rows	t–1	R(β|μ)†/(t–1)	$\left(=s_B^2\right)$	$\sigma^2+f_B\left(\psi\right)^{\ddagger}$	s_B^2/s_R^2
Columns	t–1	R(γ|μ)†/(t–1)	$\left(=s_C^2\right)$	$\sigma^2+f_C\left(\psi\right)^{\ddagger}$	s_C^2/s_R^2
Rows.Columns	$(t-1)^2$	D(β,γ)			
Treatments	t–1	R(τ|β,γ)†/(t–1)	$\left(=s_T^2\right)$	$\sigma^2+f_T\left(\psi\right)^{\ddagger}$	s_T^2/s_R^2
Residual	(t–1)(t–2)	D(β,γ,τ)/(t–1)(t–2)	$\left(=s_R^2\right)$	σ^2	
Total	t^2–1				

† R(β|μ) = D(μ) – D(β) = R(β|γ) = R(β|τ) = R(β|γ,τ)
R(γ|μ) = D(μ) – D(γ) = R(γ|β) = R(γ|μ,τ) = R(γ|β,τ)
R(τ|μ) = D(μ) – D(τ) = R(τ|β) = R(τ|μ,γ) = R(τ|β,γ)

‡ $f_B\left(\psi\right)=t\sum\left(\beta_j-\bar{\beta}_.\right)^2\Big/\left(t-1\right)$,

$f_C\left(\psi\right)=t\sum\left(\gamma_j-\bar{\gamma}_.\right)^2\Big/\left(t-1\right)$,

$f_T\left(\psi\right)=t\sum\left(\tau_k-\bar{\tau}_.\right)^2\Big/\left(t-1\right)$

where ψ is the observation length vector of parameters for the expectation i.e. $\psi=\mathbf{X}\theta$

Step 3: Decide between hypotheses

If $P(F \geq F_{calc}) \leq 0.05$ then the evidence suggests that the null hypothesis be rejected.

We now derive expressions for and properties of the quantities in this analysis of variance table.

a) Expressions for the sums of squares

Computation by mean operators

We first apply the general recursive procedure based on mean operators that was described in section III.C to the Latin square.

Let $\mathbf{R}_G$, $\mathbf{R}_B$, $\mathbf{R}_C$ and $\mathbf{R}_T$ be the residual operators for the grand mean, rows, columns and treatments, respectively. Let $\mathbf{P}_G$, $\mathbf{P}_B$, $\mathbf{P}_C$ and $\mathbf{P}_T$ be the corresponding projection operators. That is, $\mathbf{P}_T\mathbf{x}$, say, is the observation length vector of treatment means calculated from the elements of $\mathbf{x}$. Hence, for each operator $\mathbf{R} = \mathbf{I} - \mathbf{P}$ and, in

particular, $\mathbf{R}_T\mathbf{x}$, $= (\mathbf{I} - \mathbf{P}_T)\mathbf{x} = \mathbf{x} - \mathbf{P}_T\mathbf{x}$; that is, $\mathbf{R}_T\mathbf{x}$ is the vector obtained by subtracting $\mathbf{P}_T\mathbf{x}$ from $\mathbf{x}$.

We surmise that the analysis can be accomplished by applying each of the four **P** mean operators in turn; that is by using the following recursive sequence of steps:

1. $\mathbf{P}_G$ is applied to $\mathbf{y}$ to form $\mathbf{P}_G\mathbf{y} = \mathbf{g}$;
   $\mathbf{g}$ subtracted from $\mathbf{y}$ to form $\mathbf{R}_G\mathbf{y} = \mathbf{y} - \mathbf{g} = \mathbf{e}_G$.
2. $\mathbf{P}_B$ is applied to $\mathbf{R}_G\mathbf{y}$ to form $\mathbf{P}_B\mathbf{R}_G\mathbf{y} = \mathbf{r}_e$;
   $\mathbf{r}_e$ is subtracted from $\mathbf{e}_G$ to form $\mathbf{R}_R\mathbf{R}_G\mathbf{y} = \mathbf{e}_G - \mathbf{r}_e = \mathbf{e}_B$.
3. $\mathbf{P}_C$ is applied to $\mathbf{R}_B\mathbf{R}_G\mathbf{y}$ to form $\mathbf{P}_C\mathbf{R}_R\mathbf{R}_G\mathbf{y} = \mathbf{c}_e$;
   $\mathbf{c}_e$ is subtracted from $\mathbf{e}_B$ to form $\mathbf{R}_C\mathbf{R}_R\mathbf{R}_G\mathbf{y} = \mathbf{e}_B - \mathbf{c}_e = \mathbf{e}_{B+C}$.
4. $\mathbf{P}_T$ is applied to $\mathbf{R}_C\mathbf{R}_R\mathbf{R}_G\mathbf{y}$ to form $\mathbf{P}_T\mathbf{R}_C\mathbf{R}_R\mathbf{R}_G\mathbf{y} = \mathbf{t}_e$;
   $\mathbf{t}_e$ is subtracted from $\mathbf{e}_{B+C}$ to form $\mathbf{R}_T\mathbf{R}_C\mathbf{R}_R\mathbf{R}_G\mathbf{y} = \mathbf{e}_{B+C+T}$.

That is, the vectors involved in this analysis are:

$$\begin{aligned} \mathbf{g} &= \mathbf{P}_G\mathbf{y} & \mathbf{e}_G &= \mathbf{R}_G\mathbf{y} \\ \mathbf{r}_e &= \mathbf{P}_B\mathbf{R}_G\mathbf{y} & \mathbf{e}_B &= \mathbf{R}_B\mathbf{R}_G\mathbf{y} \\ \mathbf{c}_e &= \mathbf{P}_C\mathbf{R}_B\mathbf{R}_G\mathbf{y} & \mathbf{e}_{B+C} &= \mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{y} \\ \mathbf{t}_e &= \mathbf{P}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{y} & \mathbf{e}_{B+C+T} &= \mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{y} \end{aligned} .$$

with $\mathbf{e}_G = \mathbf{y} - \mathbf{g}$, $\mathbf{e}_B = \mathbf{y} - \mathbf{g} - \mathbf{r}_e$, $\mathbf{e}_{B+C} = \mathbf{y} - \mathbf{g} - \mathbf{r}_e - \mathbf{c}_e$ and $\mathbf{e}_{B+C+T} = \mathbf{y} - \mathbf{g} - \mathbf{r}_e - \mathbf{c}_e - \mathbf{t}_e$.

Note that the order of the **R**s in $\mathbf{e}_{B+C+T}$ gives the order of fitting terms, although as we will show in lemma VI.1, the corresponding **P** operators commute and so the fitting can be done in any order. The first three steps of the recursive procedure are precisely the same as for the RCBD except that the Columns term is being fitted instead of the Treatments term — the $\mathbf{X}_B$ and $\mathbf{X}_C$ matrices for the Latin square are exactly the same as the $\mathbf{X}_B$ and $\mathbf{X}_T$ matrices for the RCBD, except that $b = t$. Hence, theorems III.2–4 and V.8–9 can be used to establish the following expressions for sums of squares from the analysis for the Latin square:

$$\begin{aligned} D(\mu) &= (\mathbf{Y} - \mathbf{G})'(\mathbf{Y} - \mathbf{G}) = \mathbf{Y}'\mathbf{R}_G\mathbf{Y} \\ R(\beta \mid \mu) &= R(\beta \mid \gamma) = \mathbf{B}_e'\mathbf{B}_e = \mathbf{Y}'\mathbf{P}_B\mathbf{R}_G\mathbf{Y} = \mathbf{Y}'\mathbf{P}_B\mathbf{R}_C\mathbf{R}_G\mathbf{Y} \\ R(\gamma \mid \mu) &= R(\gamma \mid \beta) = \mathbf{C}_e'\mathbf{C}_e = \mathbf{Y}'\mathbf{P}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y} = \mathbf{Y}'\mathbf{P}_C\mathbf{R}_G\mathbf{Y} \\ D(\beta, \gamma) &= \mathbf{E}_{B+C}'\mathbf{E}_{B+C} = \mathbf{Y}'\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y} \end{aligned}$$

where $\mathbf{B}_e$ and $\mathbf{C}_e$ are the n-vectors of row and column effects, respectively.

However, we still require to establish the following expressions which we suppose from the above recursive procedure to be correct: $R(\tau \mid \beta, \gamma) = R(\tau \mid \mu) = \mathbf{T}_e'\mathbf{T}_e$ and

$D(\beta,\gamma,\tau) = \mathbf{E}'_{B+C+T}\mathbf{E}_{B+C+T}$. A crucial relationship for deriving these results is that $\mathbf{P}_B\mathbf{P}_T = \mathbf{P}_C\mathbf{P}_T = \mathbf{P}_T\mathbf{P}_B = \mathbf{P}_T\mathbf{P}_C = \mathbf{P}_G$ — this relationship is proved in the following lemma. Note we already know from lemma V.1 that $\mathbf{P}_C\mathbf{P}_B = \mathbf{P}_B\mathbf{P}_C = \mathbf{P}_G$.

Lemma VI.1: Let $\mathbf{P}_G = t^{-2}\mathbf{J}_t \otimes \mathbf{J}_t$, $\mathbf{P}_B = t^{-1}\mathbf{I}_t \otimes \mathbf{J}_t$, $\mathbf{P}_C = t^{-1}\mathbf{J}_t \otimes \mathbf{I}_t$ and $\mathbf{P}_T = \mathbf{X}_T\left(\mathbf{X}'_T\mathbf{X}_T\right)^{-1}\mathbf{X}'_T$. Then

$$\mathbf{P}_B\mathbf{P}_T = \mathbf{P}_C\mathbf{P}_T = \mathbf{P}_T\mathbf{P}_B = \mathbf{P}_T\mathbf{P}_C = \mathbf{P}_G.$$

and since $\mathbf{P}_B\mathbf{P}_T = \mathbf{P}_T\mathbf{P}_B = \mathbf{P}_G$, $\mathbf{P}_T\mathbf{P}_G = \mathbf{P}_G\mathbf{P}_T = \mathbf{P}_G$

Proof: First note that since $\mathbf{X}'_T\mathbf{X}_T = t\mathbf{I}_t$, $\mathbf{P}_T = \mathbf{X}_T\left(\mathbf{X}'_T\mathbf{X}_T\right)^{-1}\mathbf{X}'_T = t^{-1}\mathbf{X}_T\mathbf{X}'_T$ and each row of $\mathbf{X}_T\mathbf{X}'_T$ is the column of $\mathbf{X}_T$ that corresponds to the treatment the observation in that row received. Now

$$\mathbf{P}_B\mathbf{P}_T = \left(t^{-1}\mathbf{I}_t \otimes \mathbf{J}_t\right)t^{-1}\mathbf{X}_T\mathbf{X}'_T,$$

But the first t rows of $\mathbf{I}_t \otimes \mathbf{J}_t$ have ones in the first t columns and zeros in the other columns. So product of $\mathbf{I}_t \otimes \mathbf{J}_t$ and $\mathbf{X}_T\mathbf{X}'_T$ will only be nonzero if there are nonzero elements in the first t rows of $\mathbf{X}_T\mathbf{X}'_T$. But every column of the first t rows of $\mathbf{X}_T\mathbf{X}'_T$ will have exactly one 1 and $(t-1)$ 0s. All elements in the first t rows of the product will be 1. Similar argument leads us to conclude that all elements of the product will be 1 so that

$$\mathbf{P}_B\mathbf{P}_T = \left(t^{-1}\mathbf{I}_t \otimes \mathbf{J}_t\right)t^{-1}\mathbf{X}_T\mathbf{X}'_T = t^{-2}\mathbf{J}_t \otimes \mathbf{J}_t = \mathbf{P}_G$$

Next,

$$\mathbf{P}_T\mathbf{P}_B = t^{-1}\mathbf{X}_T\mathbf{X}'_T\left(t^{-1}\mathbf{I}_t \otimes \mathbf{J}_t\right)$$

and a similar argument to that for $\mathbf{P}_B\mathbf{P}_T$ leads us to conclude that

$$\mathbf{P}_T\mathbf{P}_B = t^{-1}\mathbf{X}_T\mathbf{X}'_T\left(t^{-1}\mathbf{I}_t \otimes \mathbf{J}_t\right) = t^{-2}\mathbf{J}_t \otimes \mathbf{J}_t = \mathbf{P}_G$$

Similarly, $\mathbf{P}_C\mathbf{P}_T = \mathbf{P}_T\mathbf{P}_C = \mathbf{P}_G$.

Finally, multiplying $\mathbf{P}_T\mathbf{P}_B = \mathbf{P}_G$ on the left by $\mathbf{P}_T$ we have that $\mathbf{P}_T\mathbf{P}_T\mathbf{P}_B = \mathbf{P}_T\mathbf{P}_G$. But $\mathbf{P}_T\mathbf{P}_T = \mathbf{P}_T$ so that $\mathbf{P}_T\mathbf{P}_T\mathbf{P}_B = \mathbf{P}_T\mathbf{P}_B = \mathbf{P}_G = \mathbf{P}_T\mathbf{P}_G$.

Similarly by multiplying $\mathbf{P}_B\mathbf{P}_T = \mathbf{P}_G$ on the right by $\mathbf{P}_T$ we obtain $\mathbf{P}_G\mathbf{P}_T = \mathbf{P}_G$. ■

Expression for D(β,γ,τ)

Theorem VI.2: Let **Y** be a *n*-vector of jointly-distributed random variables with $\psi = E[\mathbf{Y}] = \mathbf{X}_B\beta + \mathbf{X}_C\gamma + \mathbf{X}_T\tau$ and $\text{var}[\mathbf{y}] = \sigma^2\mathbf{I}$. Also let $\mathbf{R}_T = \mathbf{I} - \mathbf{P}_T$, $\mathbf{R}_C = \mathbf{I} - \mathbf{P}_C$, $\mathbf{R}_B = \mathbf{I} - \mathbf{P}_B$ and $\mathbf{R}_G = \mathbf{I} - \mathbf{P}_G$ where $\mathbf{P}_T$, $\mathbf{P}_C$, $\mathbf{P}_B$ and $\mathbf{P}_G$ are as defined in lemma VI.1. Then the estimator for the residual sum of squares is given by

$$D(\beta,\gamma,\tau) = (\mathbf{Y} - \hat{\psi})'(\mathbf{Y} - \hat{\psi}) = \mathbf{Y}'\mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y} = \mathbf{Y}'\mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{Y} = \mathbf{E}'_{B+C+T}\mathbf{E}_{B+C+T}$$

where $\mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G$ is symmetric idempotent and $\mathbf{E}_{B+C+T} = \mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y}$.

Proof: left as an exercise ■

Expression for R($\tau|\beta,\gamma$)

Theorem VI.3: Let $D(\beta,\gamma) = \mathbf{Y}'\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y}$ and $D(\beta,\gamma,\tau) = \mathbf{Y}'\mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y}$ be the estimators for the residual sums of squares for the models $\psi = E[\mathbf{Y}] = \mathbf{X}_B\beta + \mathbf{X}_C\gamma$ and $\psi = E[\mathbf{Y}] = \mathbf{X}_B\beta + \mathbf{X}_C\gamma + \mathbf{X}_T\tau$, respectively. Then, for $R(\tau|\beta,\gamma) = D(\beta,\gamma) - D(\beta,\gamma,\tau)$,

$$R(\tau|\beta,\gamma) = \mathbf{Y}'\mathbf{P}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y} = \mathbf{Y}'\mathbf{P}_T\mathbf{R}_G\mathbf{Y} = \mathbf{T}'_e\mathbf{T}_e$$

where $\mathbf{P}_T\mathbf{R}_G$ is symmetric and idempotent and $\mathbf{T}_e = \mathbf{P}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y} = \mathbf{P}_T\mathbf{R}_G\mathbf{Y}$.

Proof: left as an exercise ■

Summary of sums of squares

The estimators for the sums of squares in the analysis of the Latin square are:

$$\begin{aligned}
D(\mu) &= \mathbf{E}'_G\mathbf{E}_G &&= \mathbf{Y}'\mathbf{R}_G\mathbf{Y} \\
R(\beta|\mu) = R(\beta|\gamma) &&&= \mathbf{B}'_e\mathbf{B}_e = \mathbf{Y}'\mathbf{P}_B\mathbf{R}_G\mathbf{Y} = \mathbf{Y}'\mathbf{P}_B\mathbf{R}_C\mathbf{R}_G\mathbf{Y} \\
R(\gamma|\mu) = R(\gamma|\beta) &= \mathbf{C}'_e\mathbf{C}_e &&= \mathbf{Y}'\mathbf{P}_C\mathbf{R}_G\mathbf{Y} = \mathbf{Y}'\mathbf{P}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y} \\
D(\beta,\gamma) &= \mathbf{E}'_{B+C}\mathbf{E}_{B+C} &&= \mathbf{Y}'\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y} \\
R(\tau|\beta,\gamma) = R(\tau|\mu) &&&= \mathbf{T}'_e\mathbf{T}_e = \mathbf{Y}'\mathbf{P}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y} = \mathbf{Y}'\mathbf{P}_T\mathbf{R}_G\mathbf{Y} \\
D(\beta,\gamma,\tau) &= \mathbf{E}'_{B+C+T}\mathbf{E}_{B+C+T} &&= \mathbf{Y}'\mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y} = \mathbf{Y}'\mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{Y}
\end{aligned}$$

b) Degrees of freedom

The following theorem establishes that the degrees of freedom of the sums of squares are as given in the analysis of variance table.

Theorem VI.4: Let $D(\mu) = \mathbf{Y}'\mathbf{R}_G\mathbf{Y}$, $R(\beta|\mu) = \mathbf{Y}'\mathbf{P}_B\mathbf{R}_G\mathbf{Y}$, $R(\gamma|\mu) = \mathbf{Y}'\mathbf{P}_C\mathbf{R}_G\mathbf{Y}$, $D(\beta,\gamma) = \mathbf{Y}'\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y}$, $R(\tau|\mu) = \mathbf{Y}'\mathbf{P}_T\mathbf{R}_G\mathbf{Y}$ and $D(\beta,\gamma,\tau) = \mathbf{Y}'\mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y}$. The degrees of freedom of $D(\mu)$, $R(\beta|\mu)$, $R(\gamma|\mu)$, $D(\beta,\gamma)$, $R(\tau|\mu)$ and $D(\beta,\gamma,\tau)$ are t^2-1, $t-1$, $t-1$, $(t-1)^2$, $t-1$ and $(t-1)(t-2)$, respectively, where t^2 is the number of observations and t is the number of rows, columns and treatments.

Proof: The main result needed for the proof is $trace(\mathbf{P}_T)$:

$$trace(\mathbf{P}_T) = t^{-1}trace(\mathbf{X}_T\mathbf{X}_T')$$

Now the ijth element of $\mathbf{X}_T\mathbf{X}_T'$ is obtained by taking the sum of products of the ith and jth rows of $\mathbf{X}_T$. For a diagonal element i = j and, since each row of $\mathbf{X}_T$ contains just a single 1, all diagonal elements are 1. Hence,

$$trace(\mathbf{P}_T) = t^{-1}trace(\mathbf{X}_T\mathbf{X}_T') = t^{-1}t^2 = t$$

The rest of the proof is left as an exercise ■

c) Expected mean squares

We here present the results for the expected mean squares under the maximal model with all factors fixed. As far as expected mean squares under reduced models is concerned, it is noted that these can be obtained by deleting the $f(\psi)$ function from the E[MSq] for any terms that are removed from the expectation model. Consequently under the minimal model $E[\mathbf{Y}] = \mathbf{X}_G\mu$ all expected mean squares are σ^2.

Also, any of the three factors can be designated as random. If a factor, say F, is designated as random, then the $f_F(\psi)$ for that factor in an expected mean square is replaced by $t\sigma_F^2$

Expected mean squares under the maximal model

Theorem VI.5: Let $\psi = E[\mathbf{Y}] = \mathbf{X}_B\beta + \mathbf{X}_C\gamma + \mathbf{X}_T\tau$, $\mathbf{V}_Y = \sigma^2\mathbf{I}_n$, $R(\beta|\mu) = \mathbf{Y}'\mathbf{P}_B\mathbf{R}_G\mathbf{Y}$, $R(\gamma|\mu) = \mathbf{Y}'\mathbf{P}_C\mathbf{R}_G\mathbf{Y}$, $R(\tau|\mu) = \mathbf{Y}'\mathbf{P}_T\mathbf{R}_G\mathbf{Y}$ and $D(\beta,\gamma,\tau) = \mathbf{Y}'\mathbf{R}_T\mathbf{R}_C\mathbf{R}_B\mathbf{R}_G\mathbf{Y}$ where $\mathbf{R}_T$, $\mathbf{R}_C$, $\mathbf{R}_B$ and $\mathbf{R}_G$ are as defined in theorem VI.2. Then,

$$
\begin{aligned}
E\left[R(\beta \mid \mu)/(t-1)\right] &= \sigma^2 + f_{\mathrm{B}}(\psi) \\
E\left[R(\gamma \mid \mu)/(t-1)\right] &= \sigma^2 + f_{\mathrm{C}}(\psi) \\
E\left[R(\tau \mid \mu)/(t-1)\right] &= \sigma^2 + f_{\mathrm{T}}(\psi) \\
E\left[\mathrm{D}(\beta,\gamma,\tau)/\{(t-1)(t-2)\}\right] &= \sigma^2
\end{aligned}
$$

where $f_{\mathrm{B}}(\psi) = \sum_{i=1}^{b} t(\beta_i - \bar{\beta}_\cdot)^2 / (t-1)$, $\bar{\beta}_\cdot = \sum_{i=1}^{b} \beta_i / t$, β_i is the ith element of the t-vector β, $f_{\mathrm{C}}(\psi) = \sum_{i=1}^{t} t(\gamma_i - \bar{\gamma}_\cdot)^2 / (t-1)$, $\bar{\gamma}_\cdot = \sum_{i=1}^{t} \gamma_i / t$, γ_i is the ith element of the t-vector γ, $f_{\mathrm{T}}(\psi) = \sum_{j=1}^{t} t(\tau_j - \bar{\tau}_\cdot)^2 / (t-1)$, $\bar{\tau}_\cdot = \sum_{j=1}^{t} \tau_j / t$, τ_j is the jth element of the t-vector τ and t is the number of treatments.

Proof: left as an exercise ■

d) Distribution of the F statistics

The sampling distribution of the F-statistics for testing row, column and treatment differences follow Snedecor's F distribution. The theorems and their proofs are similar to those for the RCBD.

e) Analysis of variance table

Gathering together the results from the previous sections, the analysis of variance table for a Latin square design is:

Source	df	SSq	MSq	E[MSq]	F
Rows	t–1	$R(\beta\|\mu) = \mathbf{r}_e'\mathbf{r}_e$	$R(\beta\|\mu)/(t-1)$	$\sigma^2 + f_{\mathrm{B}}(\psi)$	$s_{\mathrm{B}}^2/s_{\mathrm{R}}^2$
Columns	t–1	$R(\gamma\|\mu) = \mathbf{c}_e'\mathbf{c}_e$	$R(\gamma\|\mu)/(t-1)$	$\sigma^2 + f_{\mathrm{C}}(\psi)$	$s_{\mathrm{C}}^2/s_{\mathrm{R}}^2$
Rows.Columns	$(t-1)^2$	$\mathrm{D}(\beta,\gamma) = \mathbf{e}_{\mathrm{B+C}}'\mathbf{e}_{\mathrm{B+C}}$	$\mathrm{D}(\beta,\gamma)$		
Treatments	t–1	$R(\tau\|\mu) = \mathbf{t}_e'\mathbf{t}_e$	$R(\tau\|\mu)/(t-1)$	$\sigma^2 + f_{\mathrm{T}}(\psi)$	$s_{\mathrm{T}}^2/s_{\mathrm{R}}^2$
Residual	$(t-1)(t-2)$	$\mathrm{D}(\beta,\gamma,\tau) = \mathbf{e}_{\mathrm{B+C+T}}'\mathbf{e}_{\mathrm{B+C+T}}$	$\mathrm{D}(\beta,\gamma,\tau)/(t-1)(t-2)$	σ^2	
Total	t^2–1				

Comparison with traditional Latin-square ANOVA table

Again, the above analysis of variance table and the traditional Latin-square ANOVA table are essentially the same — at any rate the values of the F-statistics are exactly

the same. As illustrated in the table below, the two tables have in common four sources that are labelled differently but the tables differ in that our table includes the line Rows.Columns. Rows.Columns reflects the variation of column differences from row to row or the differences in row-column combinations after overall row and overall column differences have been removed — it is uncontrolled variation in the units. The Rows.Columns sum of squares is partitioned into Treatments and Residual sums of squares.

Source	df	Source in two-way ANOVA
Rows	t–1	Between Rows
Columns	t–1	Between Columns
Rows.Columns	$(t-1)^2$	
Treatments	t–1	Between Treatments
Residual	$(t-1)(t-2)$	Error
Total	t^2-1	Total

Again, the advantage of the table we have presented is that it exhibits the confounding in the experiment. The indenting of Treatments under Rows.Columns signifies that treatment differences are confounded or “mixed-up” with row-column differences, adjusted for overall row and overall column differences, as a result of the randomization of treatments to row-column combinations. This is not obvious from the traditional table.

f) Analysis of an example

Example VI.2 Wheat samplers (continued)

After the experiment was finished all shoots from an area were measured and the true mean height of shoots in the area determined. The response variable is then the difference (cm) between the mean height of the 8 sampled shoots and the true mean height. The results are given in the table below.

Results for the Latin square experiment

		Area			
		1	2	3	4
	I	A	B	D	C
		6	11	5	10
	II	D	C	A	B
		8	11	5	12
Interval	III	B	D	C	A
		0	–2	1	1
	IV	C	A	B	D
		2	0	5	5

Sampler (A–D)

That all values in this table, except one, are positive indicates that the samplers tend to select larger shoots and so overestimate the height.

The data decomposition can again be obtained by successive application of mean operators as described above. The decomposition for the example is

$$\mathbf{y} = \mathbf{g} + \mathbf{r}_e + \mathbf{c}_e + \mathbf{t}_e + \mathbf{e}_{B+C+T}$$

with the elements of these vectors being

Observ – ations $\mathbf{y}$		Grand Mean $\mathbf{g}$		Interval Effects $\mathbf{r}_e$		Area Effects $\mathbf{c}_e$		Samplers Effects $\mathbf{t}_e$		Interval .Area Deviations $\mathbf{e}_{B+C+T}$
6	=	5	+	3	+	–1	+	–2	+	1
11		5		3		0		2		1
5		5		3		–1		–1		–1
10		5		3		2		1		–1
8		5		4		–1		–1		1
11		5		4		0		1		1
5		5		4		–1		–2		–1
12		5		4		2		2		–1
0		5		–5		–1		2		–1
–2		5		–5		0		–1		–1
1		5		–5		–1		1		1
1		5		–5		2		–2		1
2		5		–2		–1		1		–1
0		5		–2		0		–2		–1
5		5		–2		–1		2		1
5		5		–2		2		–1		1

The sums of squares for the analysis of variance are obtained by summing the squares of the vectors $\mathbf{r}_e$, $\mathbf{c}_e$, $\mathbf{t}_e$ and $\mathbf{e}_{B+C+T}$. The sums of squares are 216, 24, 40, and 16, respectively.

VI.E Diagnostic checking and computation in Genstat

Diagnostic checking is the same as for the RCBD.

Example VI.2 Wheat samplers (continued)

The Genstat instructions for obtaining this analysis for the example are as follows:

```
"Load data from LSSample.gsh"
PRINT Area,Interval,Samplers,Error
BLOCK Area*Interval
TREAT Samplers
ANOVA [FPROB=Y] Error
"Calculate probabilities of Area and Interval F values"
CALC pA=1-FPROB(8/2.6667; 3; 6)
&    pI=1-FPROB(72/2.6667; 3; 6)
PRINT pA,pI
```

```
"Diagnostic checking"
APLOT METHOD=fit,normal
"
**** Tukey''s one-degree-of-freedom-for-non-additivity.
**** It is the term designated covariate in the following analysis
"
AKEEP [FIT=Fit]
CALC ResSq=Fit*Fit
ANOVA [PRINT=*]  ResSq; RES=ResSq
COVAR ResSq                              "A computational trick"
ANOVA [PRINT=A; FPROB=Y] Error
```

The output produced by these instructions is as follows:

```
Genstat 5  Release 4.1  (PC/Windows NT)              28 March 2000 11:38:35
Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

                ____________________________________________

                Genstat 5 Fourth Edition - (for Windows)
                Genstat 5 Procedure Library Release PL11
                ____________________________________________

   3  "Data taken from File: D:/ANALYSES/LM/ONEFAC/LSSAMPLE.GSH"
   4  DELETE [redefine=yes] Area,Interval,Samplers,Error
   5  FACTOR [modify=yes;nvalues=16;levels=4] Area
   6  READ Area; frepresentation=ordinal

    Identifier    Values   Missing    Levels
          Area        16         0         4

   8  FACTOR [modify=yes;nvalues=16;levels=4] Interval
   9  READ Interval; frepresentation=ordinal

    Identifier    Values   Missing    Levels
      Interval        16         0         4

  11  FACTOR [modify=yes;nvalues=16;levels=4] Samplers
  12  READ Samplers; frepresentation=ordinal

    Identifier    Values   Missing    Levels
      Samplers        16         0         4

  14  VARIATE [nvalues=16] Error
  15  READ Error

    Identifier   Minimum      Mean   Maximum    Values   Missing
         Error    -2.000     5.000    12.000        16         0

  17
  18  "Load data from LSSample.gsh"
  19  PRINT Area,Interval,Samplers,Error

       Area    Interval    Samplers       Error
          1           1           1       6.000
          1           2           2      11.000
          1           3           4       5.000
          1           4           3      10.000
          2           1           4       8.000
          2           2           3      11.000
          2           3           1       5.000
          2           4           2      12.000
          3           1           2       0.000
          3           2           4      -2.000
          3           3           3       1.000
          3           4           1       1.000
          4           1           3       2.000
          4           2           1       0.000
          4           3           2       5.000
          4           4           4       5.000
```

```
  20  BLOCK Area*Interval
  21  TREAT Samplers
  22  ANOVA [FPROB=Y] Error

22.....................................................................

***** Analysis of variance *****

Variate: Error

Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.

Area stratum               3    216.000     72.000   27.00

Interval stratum           3     24.000      8.000    3.00

Area.Interval stratum
Samplers                   3     40.000     13.333    5.00  0.045
Residual                   6     16.000      2.667

Total                     15    296.000


***** Tables of means *****

Variate: Error

Grand mean  5.00

 Samplers        1        2        3        4
              3.00     7.00     6.00     4.00


*** Standard errors of differences of means ***

Table             Samplers
rep.                     4
d.f.                     6
s.e.d.               1.155

  23  "Calculate probabilities of Area and Interval F values"
  24  CALC pA=1-FPROB(8/2.6667; 3; 6)
  25  &    pI=1-FPROB(72/2.6667; 3; 6)
  26  PRINT pA,pI

          pA          pI
      0.1170   0.0006987

  27  "Diagnostic checking"
  28  APLOT METHOD=fit,normal

              -
              I
              I
              I
              I
         1.0 I          2              2   *       *          2
r             I
e             I
s             I
i             I
d             I
u        0.0 I
a             I
l             I
s             I
              I
              I
              I
        -1.0 I      *       2       *           2                   *       *
             -+---------+---------+---------+---------+---------+---------+---
           -2.5       0.0       2.5       5.0       7.5      10.0      12.5
```

```
                                        fitted values

                                         Normal plot

             -
             I
             I
         1.0 I                                 *  *  * *   *  *    *       *
r            I
e            I
s            I
i            I
d            I
u        0.0 I
a            I
l            I
s            I
             I
             I
        -1.0 I *       *    *  *   * *  *  *
             -+---------+---------+---------+---------+---------+---------+---
           -1.8      -1.2      -0.6       0.0       0.6       1.2       1.8

                                    expected Normal quantiles
29  "
 -30  **** Tukey''s one-degree-of-freedom-for-non-additivity.
 -31  **** It is the term designated covariate in the following analysis
 -32  "
  33  AKEEP [FIT=Fit]
  34  CALC ResSq=Fit*Fit
  35  ANOVA [PRINT=*]  ResSq; RES=ResSq
  36  COVAR ResSq                          "A computational trick"
  37  ANOVA [PRINT=A; FPROB=Y] Error

37.....................................................................

***** Analysis of variance (adjusted for covariate) *****

Variate: Error
Covariate: ResSq

Source of variation     d.f.       s.s.       m.s.    v.r. cov.ef.  F pr.

Area stratum               3    216.000     72.000   31.42

Interval stratum           3     24.000      8.000    3.49

Area.Interval stratum
Samplers                   3     40.000     13.333    5.82    1.00  0.044
Covariate                  1      4.542      4.542    1.98          0.218
Residual                   5     11.458      2.292            1.16

Total                     15    296.000
```

Before proceeding with the hypothesis test we need to decide which factors are fixed and which are random. Presumably the Areas are intended to be representative of a large number of areas and the distribution of effects for the population of areas can conceivably be modelled using a probability distribution function. Consequently, Areas would be a random factor. Similarly, we will assume Samplers to be a random factor. Intervals on the other hand may well show a systematic pattern for reasons such as practice or fatigue and so should be designated a fixed factor.

The hypothesis test for the example is:

Step 1: Set up hypotheses

a) H_0: $\sigma_A^2 = 0$
H_1: $\sigma_A^2 \neq 0$

b) H_0: $\beta_I = \beta_{II} = \beta_{III} = \beta_{IV}$
H_1: at least one pair of population Interval means is different

c) H_0: $\sigma_S^2 = 0$
H_1: $\sigma_S^2 \neq 0$

Step 2: Calculate test statistics

Source	df	MSq	E[MSq]	F	Prob
Area	3	72.00	$\sigma^2 + 4\sigma_A^2$	27.0	<0.001
Interval	3	8.00	$\sigma^2 + f_I(\psi)$	3.0	0.117
Area.Interval	9				
Samplers	3	13.33	$\sigma^2 + 4\sigma_S^2$	5.0	0.045
Residual	6	2.67	σ^2		
Nonadditivity	1	4.54		2.0	0.228
Deviation	5	2.29			
Total	15				

Step 3: Decide between hypotheses

Areas represents a significant source of variability in this experiment, but intervals does not. There are differences between the samplers. The test for transformable nonadditivity is not significant; the residuals-versus-fitted-values plot indicates that the residuals are either –1 or 1 which is a reflection of the artificial nature of the data; further, Normal Probability Plot indicates that the data are not normal.

VI.F Treatment differences

For the purposes of the scientist the effects of rows and columns are not of primary interest Rather, attention is likely to be focused on treatment differences which can be investigated using the treatment means. The discussion of multiple comparisons and submodels for the analysis of a CRD and RCBD applies here also.

Example VI.2 Wheat samplers (continued)

In this example the treatments are the samplers and we have modelled Samplers as a variance component. We can get an estimate of its size by equating expected mean squares to the actual values of the mean squares.

$$\sigma^2 + 4\sigma_S^2 = 13.3333$$
$$\sigma^2 = 2.6667$$

and so

$$\hat{\sigma} = \sqrt{2.6667} = 1.633$$
$$\hat{\sigma}_S = \sqrt{\frac{13.3333 - 2.6667}{4}} = 1.6330$$

That is the uncontrolled variation from interval to interval by one sampler and variability between samplers are equally important as the magnitude of their contribution to the variability in the experiment is the same.

We will also examine the pattern in the Samplers means, not because we want to know exactly how the samplers differed, but to see whether there is a pattern that would invalidate modelling of Sampler effects as random effects. Note because we are looking at the means informally, we do not use multiple comparison procedures.

	Samplers			
	A	B	C	D
Means	3	7	6	4

There is not much information available with just four means, but there seems nothing unusual about them.

VI.G Sample size

The formulae for computing the power and sample size for an RCBD also apply to the Latin square except that the number of replicates is *t*, rather than *b* as in the case of the RCBD. However, there is the restriction that the number of rows, columns and treatments must be equal. So you cannot increase the treatment replication without changing the number of treatments. Consequently, the main interest will be determine if the proposed design will have the desired power, rather than determining how many replicates must be taken to achieve the desired power.