7. Latin and Greco-Latin square design for particular prognostic variable control

§7.1. Latin square design and its special nature

- **Formation of a $g \times g$ Latin square**

  A $g \times g$ Latin square consists of three factors: two blocking factors and one treatment factor, each factor having g levels.

  The levels of the factors are arranged in the formation of a $g \times g$ square.

  The levels of the two blocking factors are arranged as rows and columns of the square respectively.

  The treatment levels are arranged such that for any level it appears once and only once in each row and in each column.

The following is the formation of a 5×5 Latin square:

	Block F2				
Block F1	1	2	3	4	5
1	4	2	5	3	1
2	2	5	1	4	3
3	1	3	2	5	4
4	3	1	4	2	5
5	5	4	3	1	2

where the numerals in the square represent the levels of the treatment.

- **Natures of Latin square design**

  Comparison between a Latin square design and a complete randomized block design

  – Complete randomized block design: All g levels of treatment appear in each block;

treatment levels are balanced within each block.

– Latin square design: Only one treatment level appears in each block; treatment levels are balanced at each level of the blocking factor.

– Latin square design is particularly suitable in situations where different treatments cannot be administered simultaneously within each block due to practical constraints.

- **Examples**

  – In agricultural experiments to compare fertilizers, to eliminating the effect of systematic gradients in fertility in the comparison, a field is divided into plots of rows and columns, fertilizers are applied to the plots according to the Latin square design.

– In animal experiments to compare diets, litters and birth order are used as blocking factors, diets as treatments.

– In clinical trials, individual patients and time are used as blocking factors, example of treatments could be dentures to be worn by the patients.

– A common nature of the above examples is that none of any treatments can be applied together with another one to a single block.

- **Randomization for Latin square design**

  A random Latin square can be obtained from a standard square by permuting its rows and columns.

  A standard square is one in which the numerals $1, 2, \ldots, g$ are in numerical order in both the first row and the first column. For example:

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

A particular standard square is the cyclic square:

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

Steps of randomization:

1. Choose at random a standard square, e.g., the first square above.
2. Permute the rows, e.g., arrange the rows by

the permuted numerals $(2, 4, 1, 3)$ to yield

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

3. Permute the columns, e.g., arrange the columns by the permuted numerals $(4, 1, 3, 2)$ to yield

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

The randomization can be easily done by the following R-code:

```
A = matrix(c(1,2,3,4, 2,1,4,3, 3,4,1,2,
       4,3,2,1), byrow=T, ncol=4)
row = sample(4)
column = sample(4)
A[row, column]
```

§7.2. Analysis of a single Latin square

- **ANOVA approach**

By using the ANOVA approach, the effects which can be analyzed must be identified and the corresponding sum of squares must be formed.

In general, in any design, all the main effects of the factors involved can be analyzed.

But the analysis of interaction between two factors is possible only when (a) all the levels of the two factors are crossed and (b) at each crossed combination of the levels more than one responses are available.

In a single Latin square, three effects can be analyzed; that is, the main effects of the rows, columns and treatments. No interaction effects can be analyzed.

The ANOVA table for a single $g \times g$ Latin square is as follows.

Source	df	SS	MS	F ratio
Row	$g-1$	$g\sum(\bar{X}_{i..} - \bar{X}_{...})^2$	RowMS	
Column	$g-1$	$g\sum(\bar{X}_{.j.} - \bar{X}_{...})^2$	CMS	
Treatment	$g-1$	$g\sum(\bar{X}_{..k} - \bar{X}_{...})^2$	TMS	TMS/RMS
Residual	$(g-1)(g-2)$	by subtraction	RMS	
Total	g^2-1	$\sum\sum(\bar{X}_{ijk} - \bar{X}_{...})^2$		

Test of treatment effect

The significance of treatment effect is tested by the F-ratio:

$$F = \frac{\text{TMS}}{\text{RMS}}.$$

Under normality assumption, F follows a F-distribution with df $g-1$ and $(g-1)(g-2)$.

Multiple comparison

When the treatment effect is significant, multiple comparison should be made. For a typical contrast $C = \sum c_k \mu_{..k}$, the test statistic is

$$L = \frac{g \sum c_k \bar{X}_{..k}}{\sqrt{\text{RMS} \sum c_k^2}}$$

which should be referred to the appropriate one of the multiple comparison criteria.

Example 1. The following table provides the data from a study comparing four formulas that were fed to newborn infants, each for one week. The response is the mean increase in weight recorded as ounces per day.

	Week			
Infant	1	2	3	4
1	0.40(2)	1.11(3)	1.16(4)	0.88(1)
2	0.20(3)	1.04(4)	0.57(1)	0.80(2)
3	1.14(1)	1.11(2)	1.32(3)	1.38(4)
4	1.08(4)	1.34(1)	1.73(2)	1.55(3)

The ANOVA table for the above data is as follows:

Source	df	SS	MS	F ratio
Infants	3	1.4407	0.4803	
Weeks	3	0.64222	0.2141	
Formulas	3	0.07762	0.0259	0.50
Residual	6	0.31288	0.0521	
Total	15	2.47349		

The F-ratio for the Formulas is not significant. No effect difference among the formulas is detected.

- **Linear model approach**

The linear model approach fit the data to the model

$$X = \mu_0 + \sum_{i=2}^{g} \alpha_i r_i + \sum_{j=2}^{g} \beta_j c_j + \sum_{k=2}^{g} \gamma_k t_k + \epsilon,$$

where r_i, c_j and t_j are dummy variables defined in the usual way as

$$r_i = \begin{cases} 1, & \text{if row } i, \\ 0, & \text{otherwise, } i = 2, \ldots, g; \end{cases}$$
$$c_j = \begin{cases} 1, & \text{if column } j, \\ 0, & \text{otherwise, } j = 2, \ldots, g; \end{cases}$$
$$t_k = \begin{cases} 1, & \text{if treatment } k, \\ 0, & \text{otherwise, } k = 2, \ldots, g. \end{cases}$$

The significance of treatment effect is tested by testing

$$H_0 : \gamma_2 = \cdots = \gamma_g = 0.$$

Multiple comparison are based on the linear combinations of the γ_k's.

For the example above, the linear model can be fitted by the following R-code:

```
x=c(0.4,0.2,1.14,1.08,
    1.11,1.04,1.11,1.34,
    1.16,0.57,1.32,1.73,
    0.88,0.8,1.38,1.55)
infant = factor(rep(c(1:4),4))
week = factor(c(rep(1,4),rep(2,4),rep(3,4),rep(4,4)))
tmt = factor(c(2,3,1,4,3,4,2,1,4,1,3,2,1,2,4,3))
options(contrasts=c("contr.treatment","contr.poly"))
lm.fit=lm(x~infant+week+tmt)
anova(lm.fit)
```

It produces the following ANOVA table:

```
          Df  Sum Sq Mean Sq F value  Pr(>F)
infant     3 1.44077 0.48026  9.2095 0.01156 *
week       3 0.64222 0.21407  4.1051 0.06674 .
tmt        3 0.07762 0.02587  0.4961 0.69822
Residuals  6 0.31289 0.05215
```

- **A Remark**

  The ANOVA approach is not easy to use when there is missing values.

  The linear model approach treat the cases of missing and non-missing values in exactly the same way.

§7.3. Replicated Latin squares

The drawback of a single Latin square is that the residual degrees of freedom is too small if g is not large, which renders the power of the significance test low.

Replicated Latin squares provide a remedy.

- **Formation of replicated Latin squares**

  In replicated Latin squares, the column levels and treatment levels are the same in all squares.

  But row levels are different from different squares.

  For instance, in the experiment of Example 1, in replicated squares, the rows in different squares will represent different infants.

Example 1(cont.) The study was augmented with three more Latin squares:

Square 1

	Week			
Infant	1	2	3	4
1	0.40(2)	1.11(3)	1.16(4)	0.88(1)
2	0.20(3)	1.04(4)	0.57(1)	0.80(2)
3	1.14(1)	1.11(2)	1.32(3)	1.38(4)
4	1.08(4)	1.34(1)	1.73(2)	1.55(3)

Square 2

	Week			
Infant	1	2	3	4
5	1.55(2)	0.89(3)	0.16(4)	0.55(1)
6	0.11(3)	1.05(4)	0.68(1)	0.98(2)
7	0.22(1)	0.96(2)	1.45(3)	0.82(4)
8	0.53(4)	1.25(1)	0.61(2)	1.91(3)

Square 3

	Week			
Infant	1	2	3	4
9	0.27(2)	1.16(3)	0.59(4)	0.45(1)
10	0.50(3)	0.70(4)	0.93(1)	0.96(2)
11	0.32(1)	1.63(2)	0.55(3)	0.79(4)
12	0.09(4)	0.30(1)	1.34(2)	1.09(3)

Square 4

	Week			
Infant	1	2	3	4
13	0.73(2)	1.21(3)	1.21(4)	0.77(1)
14	0.64(3)	1.38(4)	0.82(1)	0.79(2)
15	-0.03(1)	1.04(2)	0.57(3)	0.55(4)
16	1.05(4)	1.11(1)	1.00(2)	0.50(3)

- **Two scenarios of replicated squares**

  Scenario 1: q squares are formed at random from a total qg blocks (according to blocking factor 1, i.e., row factor) which are available for study at the same time. g levels of the second blocking factors are identified, and treatment are applied in accordance with q independently and randomly determined $g \times g$ Latin squares.

  Scenario 2: q squares represent the levels of another blocking factor. The other aspects are the same as scenario 1.

- **Analysis of scenario 1**

  ANOVA approach

  The anova approach is similar to the single Latin square case. Sums of squares due to different sources are computed and F ratios are formed for the testing of the significance of the treatment effect. The ANOVA table is as follows.

Source	df	SS	MS	F ratio
Row	$qg-1$	$g\sum\sum(\bar{X}_{i\cdot\cdot}^{(s)}-\bar{X}_{\cdots}^{(\cdot)})^2$	RowMS	
Column	$g-1$	$qg\sum(\bar{X}_{\cdot j\cdot}^{(\cdot)}-\bar{X}_{\cdots}^{(\cdot)})^2$	CMS	
Treatment	$g-1$	$qg\sum(\bar{X}_{\cdot\cdot k}^{(\cdot)}-\bar{X}_{\cdots}^{(\cdot)})^2$	TMS	TMS/RMS
Residual	$(g-1)(qg-2)$	by subtraction	RMS	
Total	qg^2-1	$\sum\sum\sum(\bar{X}_{ijk}^{(s)}-\bar{X}_{\cdots}^{(\cdot)})^2$		

The above ANOVA table is obtained by replacing $\bar{X}_{\ldots}$ with $\bar{X}_{\ldots}^{(\cdot)}$ and then add up the sums of squares of the q squares.

The F ratio has the same first df $g-1$ but a larger second df $(g-1)(qg-1)$ compared with the F ratio in a single square.

For the 4 squares in Example 1, the ANOVA table computed using the formulas of SS is given below:

Source	df	SS	MS	F ratio
Row	15	3.195644	0.2130	
Column	3	2.425906	0.8086	
Treatment	3	0.725069	0.2417	1.85
Residual	42	5.480475	0.1305	
Total	63	11.827094		

- **Linear model approach**

The linear model for q squares is the same as that for a single square, except that the row factor has qg levels. The model is as follows:

$$X = \mu_0 + \sum_{i=2}^{qg} \alpha_i r_i + \sum_{j=2}^{g} \beta_j c_j + \sum_{k=2}^{g} \gamma_k t_k + \epsilon,$$

where the dummy variables r_i, c_j and t_j are defined the same way as in the case of a single square.

The following R-code is used to fit the linear model to the data in the four squares of Example 1.

```
x1=c(0.4,0.2,1.14,1.08,
    1.11,1.04,1.11,1.34,
    1.16,0.57,1.32,1.73,
    0.88,0.8,1.38,1.55)
x2 =c(1.55, 0.11, 0.22, 0.53,
      0.89, 1.05, 0.96, 1.25,
      0.16, 0.68, 1.45, 0.61,
      0.55, 0.98, 0.82, 1.91)
x3 =c(0.27, 0.50, 0.32, 0.09,
      1.16, 0.70, 1.63, 0.30,
      0.59, 0.93, 0.55, 1.34,
      0.45, 0.96, 0.79, 1.09)
x4 =c(0.73, 0.64, -0.03, 1.05,
      1.21, 1.38, 1.04, 1.11,
      1.21, 0.82, 0.57, 1.00,
      0.77, 0.79, 0.55, 0.50)
infant1 = rep(c(1:4),4)
infant2  = rep(c(5:8),4)
infant3  = rep(c(9:12),4)
infant4  = rep(c(13:16),4)
week = factor(c(rep(1,4),rep(2,4),rep(3,4),rep(4,4)))
tmt = factor(c(2,3,1,4,3,4,2,1,4,1,3,2,1,2,4,3))
x = c(x1, x2, x3, x4)
infant = factor(c(infant1, infant2, infant3,infant4))
week = factor(rep(week,4))
tmt = factor(rep(tmt,4))
square = factor(c(rep(1,16), rep(2,16), rep(3,16),rep(4,16)))
options(contrasts=c("contr.treatment","contr.poly"))
lm.fit=lm(x~infant+week+tmt)
anova(lm.fit)
```

The computation yields the following the following ANOVA table:

```
          Df Sum Sq Mean Sq F value   Pr(>F)
infant    15 3.1956  0.2130  1.6327 0.105766
week       3 2.4259  0.8086  6.1970 0.001390 **
tmt        3 0.7251  0.2417  1.8522 0.152443
Residuals 42 5.4805  0.1305
```

Note: The linear model approach yields the same result as the ANOVA approach.

- **Analysis of scenario 2**

ANOVA approach

When squares are taken as the levels of another vector, there is an additional SS for the main effect of this factor. In addition, since this factor is crossed with both the column factor and the treatment factor with multiple response measurements at each crossed level, there are two SS's for two interactions.

Note: the row factor and square factor are not completely crossed, and the levels of row factor are nested within the levels of the square factor. Consequently, the SS for the row factor must be computed within each level of the square factor.

The ANOVA table is given below:

Source	df	SS	MS	F ratio
Square	$q-1$	$g^2\sum(\bar{X}_{\cdots}^{(s)}-\bar{X}_{\cdots}^{(\cdot)})^2$	SMS	$\frac{SMS}{R(S)MS}$
Row(S)	$q(g-1)$	$g\sum\sum(\bar{X}_{i\cdot\cdot}^{(s)}-\bar{X}_{\cdots}^{(s)})^2$	R(S)MS	
Column	$g-1$	$qg\sum\sum(\bar{X}_{\cdot j\cdot}^{(\cdot)}-\bar{X}_{\cdots}^{(\cdot)})^2$	CMS	
S by C	$(q-1)(g-1)$	$g\sum(\bar{X}_{\cdot j\cdot}^{(s)}-\bar{X}_{\cdots}^{(s)}-\bar{X}_{\cdot j\cdot}^{(\cdot)}+\bar{X}_{\cdots}^{(\cdot)})^2$	SCMS	
Treatment	$g-1$	$qg\sum(\bar{X}_{\cdot\cdot k}^{(\cdot)}-\bar{X}_{\cdots}^{(\cdot)})^2$	TMS	$\frac{TMS}{RMS}$
S by T	$(q-1)(g-1)$	$g\sum(\bar{X}_{\cdot\cdot k}^{(s)}-\bar{X}_{\cdots}^{(s)}-\bar{X}_{\cdot\cdot k}^{(\cdot)}+\bar{X}_{\cdots}^{(\cdot)})^2$	STMS	$\frac{STMS}{RMS}$
Residual	$q(g-1)(g-2)$	by subtraction	RMS	
Total	qg^2-1	$\sum\sum\sum(\bar{X}_{ijk}^{(s)}-\bar{X}_{\cdots}^{(\cdot)})^2$		

Remark:

1. In the F ratio for the square effect, the denominator is R(S)MS instead of RMS. This is because that the effect of squares and

the effect of rows are partially confounded. The SS for square also partially accounts for the variation caused by rows. The expectation of SMS is the sum of two terms, the first term measures the square effect and the second term measures the same quantity as that measured by the expectation of R(S)MS.

2. In general, to form the F ratios in an ANOVA table in complicated designs, the expectations of the MS's are derived, and the F ratios are formed such that the expected numerator differ from the expected denominator by an additional term which is the effect to be tested. For rules to determine the expected MS's, see

   Montgomery (1991). *Design and analysis of experiments*, Chapter 8. John Wiley & Sons.

3. The rows can be either fixed levels of a fac-

tor or a random sample of that factor. If the rows are random levels, the F ratio for treatment effects should be

$$F = \frac{TMS}{STMS}.$$

In Example 1, the different squares can represent different wards. Considering ward as another factor, the ANOVA table for the example is given as follows:

Source	df	SS	MS	F ratio
Square	3	0.861631	0.2872	1.48
Row(S)	12	2.334013	0.1945	
Column	3	2.425906	0.8086	
S by C	9	0.754557	0.0838	
Treatment	3	0.725069	0.2417	1.62
S by T	9	1.150394	0.1278	0.86
Residual	24	3.575524	0.1490	
Total	63	11.827094		

Computation by using `lm`

Linear model approach cannot be used for the analysis of Scenario 2. But the R function `lm` can be used to compute the anova table as follows:

- **Step 1**: Fit the linear model including only the main effects of column, treatment and squares as well as interactions between square and column and between square and treatment. The anova table of the model will provide correct CSS, TSS, SSS, SCSS and STSS. The total sum of square can be computed from the table as well.
- **Step 2**: For each square, fit a separate linear model as in the single Latin square case. Each table will provide a SS for the rows of that square.
- **Step 3**: The RowSS within squares is obtained b adding up the row SS of the individual squares.
- **Step 4**: Obtain RSS by subtraction.

Example 1 (cont.) The following code accomplishes Step 1.

```
lm.fit=lm(x~square+week+tmt+square*tmt + square*week)
anova(lm.fit)
```

Note: the other variables are generated the same as in Scenario 1.

This step produces the following ANOVA table:

```
              Df Sum Sq Mean Sq F value   Pr(>F)
square         3 0.8616  0.2872  1.7496 0.174280
week           3 2.4259  0.8086  4.9261 0.005725 **
tmt            3 0.7251  0.2417  1.4723 0.238370
square:tmt     9 1.1504  0.1278  0.7787 0.636904
square:week    9 0.7546  0.0838  0.5107 0.856969
Residuals     36 5.9095  0.1642
```

From the table, it is identified that

```
SSS = 0.8616
CSS = 2.4259
TSS = 0.7251
STSS = 1.1504
SCSS = 0.7546
```

The total sum of square is computed as

```
TTSS = 0.8616+2.4259+0.7251+1.1504+0.7546+5.9095
     = 11.8272
```

The following R code fits four separate Latin squares:

```
week = factor(c(rep(1,4),rep(2,4),rep(3,4),rep(4,4)))
tmt = factor(c(2,3,1,4,3,4,2,1,4,1,3,2,1,2,4,3))
infant1 = rep(c(1:4),4)
infant2  = rep(c(5:8),4)
infant3  = rep(c(9:12),4)
infant4  = rep(c(13:16),4)
infant = factor(infant1)
fit =lm(x1~infant+week+tmt)
anova(fit)
infant = factor(infant2)
fit =lm(x2~infant+week+tmt)
anova(fit)
infant = factor(infant3)
fit =lm(x3~infant+week+tmt)
anova(fit)
infant = factor(infant4)
fit =lm(x4~infant+week+tmt)
anova(fit)
```

Adding up the SS for infants in the four anova tables, we obtain

```
RowSS = 1.44077+0.30195+0.09457+0.49673=2.3304
```

Eventually, RSS is obtained as

```
RSS = TTSS -(RowSS+CSS+TSS+SSS+STSS+SCSS)
    = 3.57558.
```

§7.4. Greco-Latin squares

If, in addition to the three factors (row, column and treatment), there is another factor to be controlled.

A Greco-Latin square can be used.

A Greco-Latin square is obtained by imposing the levels of the additional factor on a Latin square in a way such that any level of a factor appears once and only once at each level of the other factors.

The following gives standard $g \times g$ Greco-Latin squares for $g = 3, 4$:

$g = 3$:

$$\begin{array}{ccc} A\alpha & B\beta & C\gamma \\ B\gamma & C\alpha & A\beta \\ C\beta & A\gamma & B\alpha \end{array}$$

$g = 4$:

$$\begin{array}{cccc} A\alpha & B\beta & C\gamma & D\delta \\ B\delta & A\gamma & D\beta & C\alpha \\ C\beta & D\alpha & A\delta & B\gamma \\ D\gamma & C\delta & B\alpha & A\beta \end{array} \qquad \begin{array}{cccc} A\alpha & B\beta & C\gamma & D\delta \\ B\gamma & A\delta & D\alpha & C\beta \\ C\delta & D\gamma & A\beta & B\alpha \\ D\beta & C\alpha & B\delta & A\gamma \end{array}$$

For standard Greco-Latin squares for $g = 5$,7,8,9, 11,12, see

Cochran and Cox (1957). *Experimental Designs*. 2nd ed. New York, Wiley.

Randomization of Greco-Latin squares

A random Greco-Latin squares can be obtained from a standard square in three steps: 1) permute the rows of the standard square; 2) permute the columns of the standard square; 3) permute the Greco letters.

Analysis

- The analysis of a single Greco-Latin square is similar to that of a single Latin square. The only difference is that in the ANOVA table, there is an additional SS (i.e., the Greco SS).
- Like in Latin square case, the df of the residuals of a single Greco-Latin square is too small. Replicated squares are usually needed.
- Parallel to the situation in replicated Latin squares, there are corresponding two scenar-

ios for replicated Greco-Latin squares.

- The analysis of replicated Greco-Latin squares are similar to that of Latin squares in both scenarios. The only difference is that additional main effect and interactions related to Greco factor are included in the analysis.