Lecture 8
Latin Square Design and Incomplete Block Design

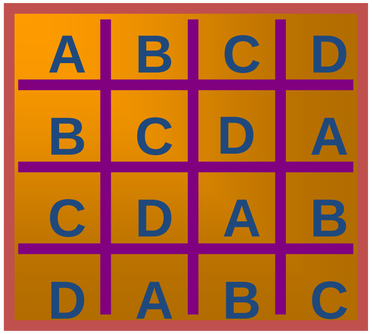
Latin Square (LS) design

Latin square (LS) design

- It is a kind of complete block designs.
- A class of experimental designs that allow for two sources of blocking.
- Can be constructed for any number of treatments, but there is a cost. If there are t treatments, then t^2 experimental units will be required.

Latin square design

- If you can block on two (perpendicular) sources of variation (rows x columns) you can reduce experimental error when compared to the RBD
- More restrictive than the RBD
- The total number of plots is the square of the number of treatments
- Each treatment appears once and only once in each row and column



Facts about the LS Design

- With the Latin Square design you are able to control variation in two directions.
- Treatments are arranged in rows and columns
- Each row contains every treatment.
- Each column contains every treatment.
- The most common sizes of LS are 5x5 to 8x8

Advantages

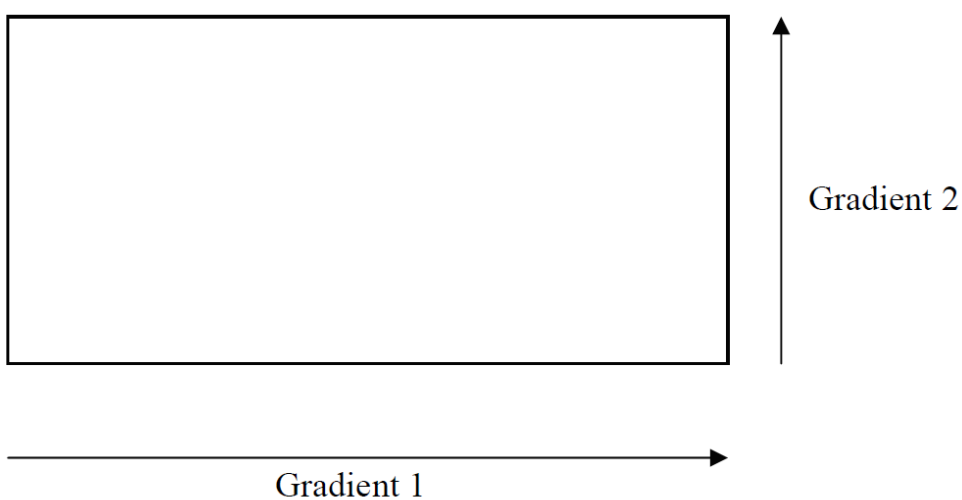
- You can control variation in two directions.
- Hopefully you increase efficiency as compared to the RBD.

Disadvantages

- The number of treatments must equal the number of replicates.
- The experimental error is likely to increase with the size of the square.
- Small squares have very few degrees of freedom for experimental error.
- You can’t evaluate interactions between:
  - Rows and columns
  - Rows and treatments
  - Columns and treatments.

Examples of Uses of the Latin Square Design

- 1. Field trials in which the experimental error has two fertility gradients running perpendicular each other or has a unidirectional fertility gradient but also has residual effects from previous trials.

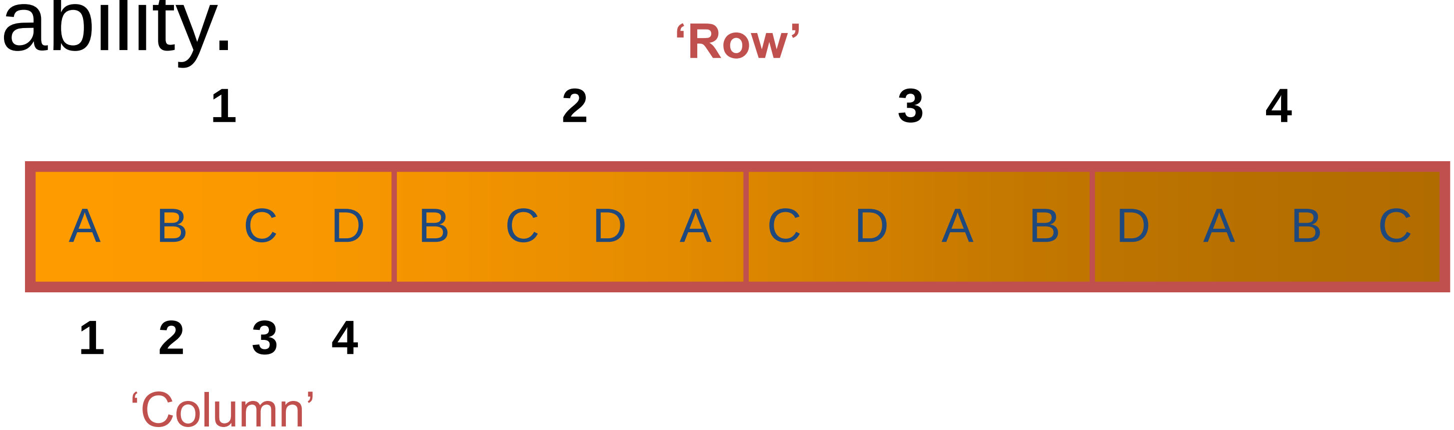


Examples of Uses of the Latin Square Design

- 2. Animal science feed trials.
- 3. Insecticide field trial where the insect migration has a predictable direction that is perpendicular to the dominant fertility gradient of the experimental field.

Examples of Uses of the Latin Square Design

- 4. Greenhouse trials in which the experimental pots are arranged in a straight line perpendicular to the glass walls, such that the difference among rows of pots and distance from the glass wall are expected to be the major sources of variability.



How to randomize the design

- If all standard Latin squares of size *t* x *t* are available, randomization is accomplished with the following steps:
  - Step 1. Randomly select one of the standard squares
  - Step 2. Randomly order all but the first row
  - Step 3. Randomly order all columns
  - Step 4. Randomly assign treatments to the letters

Example

- All possible randomizations can be generated without including the first row in Step 2 if a standard square is randomly selected.
- If all standard squares are not available for selection, then it is recommended in Step 2 that all rows be included in the randomization.
- Not all possible Latin squares are generated in this way but a number of possibilities is increased considerably. Suppose the standard square selected at Step 1 for the 4 x 4 Latin square experiment with automobile tires is

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

Step 2

- Obtain a random permutation of numbers to order the last three rows:

Permutation	Original row
3	2
1	3
2	4

- The placement of the rows for the standard square with row 1 in its original position is

Original row				
1	A	B	C	D
3	C	D	A	B
4	D	A	B	C
2	B	C	D	A

Step 3

- Obtain a random permutation of number to order the four columns from Step 2.

Permutation	Original row
1	1
4	2
3	3
2	4

- The placement of the columns for the standard square is

Original column			
1	4	3	2
A	D	C	B
C	B	A	D
D	C	B	A
B	A	D	C

Step 4

- Obtain a random permutation to assign treatments to the letters. This assignment is not necessary if the standard square has been selected at random from all possible standard squares. The method of assignment is shown here for illustration. Suppose the treatment as W, X, Y and Z. Permutation 4 (D), 2 (B), 3 (C), 1 (A).
- The treatment labels W, X, Y, Z replace the Latin square letters in the order D, B, C and A in the randomized arrangement.

Step 4

	Tire position			
Auto	1	2	3	4
1	Z	W	Y	X
2	Y	X	Z	W
3	W	Y	X	Z
4	X	Z	W	Y

Analysis for LS

Linear Model

- Linear Model: $y_{ij} = \mu + \rho_i + \gamma_j + \tau_k + \varepsilon_{ij}$
  - μ = mean effect
  - ρ_i = i^{th} row effect (i=1,…, r)
  - γ_j = j^{th} column effect (j=1, …, c)
  - τ_k = k^{th} treatment effect (k=1,…, t)
  - ε_{ij} = random error
- Each treatment occurs once in each row and once in each column
  - $r = c = t$
  - $N = t^2$

Analysis

- Set up a two-way table and compute the row and column means and deviations
- Compute a table of treatment means and deviations
- Set up an ANOVA table divided into sources of variation
  - Rows
  - Columns
  - Treatments
  - Error
- Significance tests
  - F_T tests difference among treatment means
  - F_R and F_C test if row and column groupings are effective

Size of the Square on Error Degrees of Freedom

Source	D.F.	2x2	3x3	4x4	5x5	8x8
Total	t^2-1	3	8	15	24	63
Rows	t-1	1	2	3	4	7
Columns	t-1	1	2	3	4	7
Treatments	t-1	1	2	3	4	7
Error	$(t$-1)(t-2)	0	2	6	12	42

One way to increase the Error df for small squares is to use more than one square in the experiment (i.e. repeated squares).

ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean square	Expected MS (EMS)	F
Total	t^2-1	$\sum_i \sum_j (y_{ij} - \bar{y})^2$			
Rows	t-1	$t\sum_i (\bar{y}_{i.} - \bar{y})^2$	MS_R		MS_R/MS_ε
Columns	t-1	$t\sum_j (\bar{y}_{.j} - \bar{y})^2$	MS_C		MS_C/MS_ε
Treatments	t-1	$t\sum_i (\bar{y}_k - \bar{y})^2$	MS_T	$\sigma_\varepsilon^2 + t\sigma_T^2$	MS_T/MS_ε
Error	$(t-1)(t-2)$	SS_ε	MS_ε		

ANOVA

- Where

$$SS_{\text{Total}} = \sum_i \sum_j \left(y_{ij} - \bar{y}\right)^2 = \sum_i \sum_j y_{ij}{}^2 - t^2 \bar{y}^2$$

$$SS_{\text{R}} = t \sum_i \left(\bar{y}_{i.} - \bar{y}\right)^2 = t \sum_i \bar{y}_{i.}^2 - t^2 \bar{y}^2$$

$$SS_{\text{C}} = t \sum_j \left(\bar{y}_{.j} - \bar{y}\right)^2 = t \sum_j \bar{y}_{.j}^2 - t^2 \bar{y}^2$$

$$SS_{\text{Treatment}} = t \sum_i \bar{y}_k^2 - t^2 \bar{y}^2$$

$$SS_{\varepsilon} = SS_{Total} - SS_R - SS_C - SS_{Treatment}$$

Standard errors for treatment means

- Standard Error estimate for a treatment mean $$s_{\bar{y}_k} = \sqrt{MS_\varepsilon / t}$$

- Standard Error estimate for a distance between two treatment means $$s_{\bar{y}_k - \bar{y}_m} = \sqrt{2MS_\varepsilon / t}$$

Did both blocking factors increase precision?

- The efficiency of the Latin Square design with two blocking criteria is determined relative to the randomized complete block design with only one blocking criterion.
- Relative efficiency measures can be computed separately for the row and column blocking criteria of the Latin square.

Relative efficiency of experiment designs

- Relative efficiency measures the effectiveness of blocking in experiment designs to reduce experimental error variance
- The variance of a treatment mean is a measure of the precision of the estimated treatment mean in an experiment, i.e., $\sigma_{\bar{y}}^2 = \frac{1}{r}\sigma_{\varepsilon}^2$
- Say, error variances are 1 and 2 in two designs, and replications are r_1 and r_2 $\sigma_{\bar{y}_1}^2 = \frac{1}{r_1}$ $\sigma_{\bar{y}_2}^2 = \frac{2}{r_2}$
- The two variances will be same only if $r_2 = 2\, r_1$. We say Design 1 is more efficient than Design 2 with respect to the number of replications required to have the same precision for an estimate of the treatment mean

When error variance has to be estimated from the data

- Fisher (1960) proposed the concept of Information (I), i.e.,

$$I = \frac{(f+1)}{(f+3)}\frac{1}{s^2}$$

- where s^2 is the estimated experimental error variance with f degrees of freedom

The relative efficiency

- The relative efficiency of two experiment designs is defined as the ratio of information in the two designs, i.e., $I_1 = \frac{(f_1+1)}{(f_1+3)}\frac{1}{s_1^2}$ $\quad I_2 = \frac{(f_2+1)}{(f_2+3)}\frac{1}{s_2^2}$

$$RE = \frac{I_1}{I_2} = \frac{(f_1+1)(f_2+3)}{(f_1+3)(f_2+1)}\frac{s_2^2}{s_1^2}$$

- When RE=1, the designs require the same number of replications to have the same variance of treatment mean, i.e. $\sigma_{\bar{y}}^2$
- Say RE=1.5, Design 2 requires 1.5 times as many replications as Design 1 to have the same variance of a treatment mean

Relative Efficiency of LS design

- Relative Efficiency of column blocking

$$s_{rcb}^2 = \frac{MS_{\mathrm{C}} + (t-1)MS_\varepsilon}{t} \text{ (The estimated mean square for error in RCB)}$$

$$RE_{\mathrm{C}} = \frac{MS_{\mathrm{C}} + (t-1)MS_\varepsilon}{tMS_\varepsilon}$$

- Relative Efficiency of row blocking

$$s_{rcb}^2 = \frac{MS_{\mathrm{R}} + (t-1)MS_\varepsilon}{t} \text{ (The estimated mean square for error in RCB)}$$

$$RE_{\mathrm{R}} = \frac{MS_{\mathrm{R}} + (t-1)MS_\varepsilon}{tMS_\varepsilon}$$

Relative Efficiency

- To compare with a completely randomized design

$$RE = \frac{MS_{\mathrm{R}} + MS_{\mathrm{C}} + (t - 1)MS_{\varepsilon}}{(t + 1)MS_{\varepsilon}}$$

An example

- Grain yield of a wheat variety for five different seeding rates in a Latin square design [Treatment label (A, B, C, D, or E) in parentheses following yield value]

Row	Column				
	1	2	3	4	5
1	59.45 (E)	47.28 (A)	54.44 (C)	50.14 (B)	59.45 (D)
2	55.16 (C)	60.89 (D)	56.59 (B)	60.17 (E)	48.71 (A)
3	44.41 (B)	53.72 (C)	55.87 (D)	47.99 (A)	59.45 (E)
4	42.26 (A)	50.14 (B)	55.87 (E)	58.74 (D)	55.87 (C)
5	60.89 (D)	59.45 (E)	49.43 (A)	59.45 (C)	57.31 (B)
Treatment	A	B	C	D	E
Seed rate	30	80	130	180	230

An example

Row / Column	1	2	3	4	5	Row means $\bar{y}_{i.}$
1	59.45 (E)	47.28 (A)	54.44 (C)	50.14 (B)	59.45 (D)	54.15
2	55.16 (C)	60.89 (D)	56.59 (B)	60.17 (E)	48.71 (A)	56.30
3	44.41 (B)	53.72 (C)	55.87 (D)	47.99 (A)	59.45 (E)	52.29
4	42.26 (A)	50.14 (B)	55.87 (E)	58.74 (D)	55.87 (C)	52.58
5	60.89 (D)	59.45 (E)	49.43 (A)	59.45 (C)	57.31 (B)	57.31
Column means ($\bar{y}_{.j}$)	52.43	54.30	54.44	55.30	56.16	$\bar{y}_{..} =$ 54.53
Treatment	A	B	C	D	E	
Seed rate	30	80	130	180	230	
Mean ($\bar{y}_k$)	47.13	51.72	55.73	59.17	58.88	

ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean square	F	Pr>F
Total	24	716.61			
Rows	4	99.20	24.80	5.26	0.011*
Columns	4	38.48	9.62	2.04	0.153
Seed rate	4	522.30	130.57	27.67	0.000**
Error	12	56.63	4.72		

Standard errors for treatment means

- The standard errors for a treatment mean is

$$s_{\bar{y}_k} = \sqrt{MS_\varepsilon / t} = \sqrt{4.72/5} = 0.97$$

- The standard error estimate for a difference between two treatment means is

$$s_{\bar{y}_k - \bar{y}_m} = \sqrt{2MS_\varepsilon / t} = \sqrt{2 \times 4.72/5} = 1.37$$

Relative Efficiency

- Estimated values

$$RE_{\mathrm{C}} = \frac{9.62 + 4 \times 4.72}{5 \times 4.72} = 1.21$$

$$RE_{\mathrm{R}} = \frac{24.80 + 4 \times 4.72}{5 \times 4.72} = 1.85$$

Explanation for RE_R

- There is a 85% gain in efficiency over the randomized complete block design in which only the column criterion of the Latin square design is used for blocking.
- Thus, the row blocks for soil gradients across the field effectively reduced the variance by 85%. The randomized block design without the row blocks for soil gradients would required 1.85*5=9.25 for 10 replications to have an estimated variance of the treatment mean equal to that from the Latin square design.

Explanation for RE_c

- There is a 21% gain in efficiency over the randomized complete block design in which only the row criterion of the Latin square design is used for blocking.
- Thus, the column blocks for soil gradients across the field effectively reduced the variance by 21%. The randomized block design without the column blocks for soil gradients would required 1.21*5=6 replications to have an estimated variance of the treatment mean equal to that from the Latin square design.

Correction for estimating σ^2

- The correction for estimation σ^2 by s^2 is

$$\frac{(f_{ls}+1)(f_{rcb}+3)}{(f_{ls}+3)(f_{rcb}+1)} = \frac{13\times 19}{15\times 17} = 0.97$$

- Where f_{ls} =12 and f_{rcb} =16 are the error degrees of freedom for the Latin square and randomized complete block design. The correction reduces the RE from 1.85 to 0.97*1.85=1.79 for row blocking and from 1.21 to 0.97*1.21=1.17 for column blocking. The correction has a small effect on the efficiency estimates.

Incomplete Block Design (IBD)

An introduction

- It is sometimes necessary to block experimental units into groups smaller than a complete replication of all treatments with a randomized complete block or Latin square design.
- The incomplete block design is utilized to decrease experimental error variance and provide more precise comparisons among treatments than is possible with a complete block design.

Incomplete block design

- If the block size, *k*, is less than the number of treatments *v* ($k < v$) then all treatments can not appear in each block. The design is called an Incomplete Block Design.
- For example, consider the block design with 6 treatments and 6 blocks of size two.

1	2	1	4	5	4
2	3	3	5	6	6

Balanced Incomplete Block Design (BIBD)

- The BIBD is arranged such that all treatments are equally replicated and each treatment pair occurs in the same block an equal number of times somewhere in the design.
- The balance obtained from equal occurrence of all treatment pairs in the same block results in equal precision for all comparisons between pairs of treatment means.

Balanced Incomplete Block Design (BIBD)

- There are t distinct treatments
- There are b blocks
- Each block contains exactly k distinct treatments
- Each treatments occurs in exactly r different blocks
- Every pair of distinct treatments occurs together in exactly λ blocks
- Can be expressed as (t, k, λ) or (t, b, r, k, λ)
- For last example, t= 6, b=6, r=2, k=2, λ =1

Properties

- 1. $tr=bk$ (=total number of trials = N)
- 2. $r(k\text{-}1)= \lambda(t\text{-}1)$
- Proof: For each treatment, for example A_1, it will appear in r blocks. Each of these *r* blocks contains *k*-1 non-A_1 treatments. So the total number of non-A_1 treatments in these *r* blocks is $r(k\text{-}1)$.
- In another way, the numbers that A1 with other treatments (*t*-1 treatments) should be the same. So $\lambda=r(k\text{-}1)/(t\text{-}1)$

How to randomize IBD

- After the basic design has been constructed with the treatment code numbers, the steps in randomization follow:
- Step 1. Randomize the arrangement of the blocks of treatment code number groups
- Step 2. Randomize the arrangement of the treatment code numbers within each block
- Step 3. Randomize the assignment of treatments to the treatment code numbers in the plan

An example

- t=4 treatment, b=4 blocks, k=3 experimental units each.
- Prior to randomization the plan is

Block I	1	2	3
Block II	1	2	4
Block III	1	3	4
Block IV	2	3	4

Step 1

- The treatment groups (1,2,3), (1,2,4), (1,3,4) and (2,3,4) must be randomly assigned to the runs.
- Random permutation 2, 4, 1, 3

Run				Original block
1	1	2	4	2
2	2	3	4	4
3	1	2	3	1
4	1	3	4	3

Step 2

- Assign random treatment code numbers to the three growth chambers in each run. Choose a random permutation of the numbers 1 to 4 for each chamber and omit the treatment number absent in the run.

	Chamber			
Run	A	B	C	Permutation
1	2	4	1	2 4 ~~3~~ 1
2	3	4	2	3 4 ~~1~~ 2
3	1	2	3	~~4~~ 1 2 3
4	1	4	3	1 4 ~~3~~ 2

Step 3

- Suppose treatments T1 to T4. A random permutation 2, 4, 3, 1 gives a random assign to the treatment code number with replacements 2 to T1, 4 to T2, 3 to T3, and 1 to T4.

Run	A	B	C
1	T1	T2	T4
2	T3	T2	T1
3	T4	T1	T3
4	T4	T2	T3

The model

- An appropriate linear model for observations from an incomplete block design is
- $y_{ij} = \mu + \tau_i + \rho_j + \varepsilon_{ij}$ ($i = 1, 2, \ldots, t$; $j = 1, 2, \ldots, b$),
- Where τ_i is the fixed effect of the i^{th} treatment, ρ_j the effect of the j^{th} block, and e_{ij} the error associated with the observation y_{ij}.

$$\sum_{i=1}^{t} \tau_i = \sum_{j=1}^{b} \rho_j = 0$$

Sum of squares partitions for BIBD

- The sum of squares partitions can be derived by considering alternative full and reduced models for the design. Solutions to the normal equations are obtained for the full model, $y_{ij} = \mu + \tau_i + \rho_j + \varepsilon_{ij}$, with estimates $\hat{y}_{ij} = \hat{\mu} + \hat{\tau}_i + \hat{\rho}_j$, to compute the experimental error sum of squares for the full model,

$$SS_{\varepsilon_f} = \sum_i \sum_j \left(y_{ij} - \hat{y}_{ij}\right)^2 = \sum_i \sum_j \left(y_{ij} - \hat{\mu} - \hat{\tau}_i - \hat{\rho}_j\right)^2$$

Sum of squares partitions for BIBD

- Solutions to the normal equations are obtained for the reduced model, $y_{ij} = \mu + \rho_j + \varepsilon_{ij}$, with estimates $\hat{y}_{ij} = \hat{\mu} + \hat{\rho}_j$, to compute the experimental error sum of squares for the reduced model,

$$SS_{\varepsilon_r} = \sum_i \sum_j \left(y_{ij} - \hat{y}_{ij}\right)^2 = \sum_i \sum_j \left(y_{ij} - \hat{\mu} - \hat{\rho}_j\right)^2$$

Sum of squares partitions for BIBD

- The difference SS_{ε_r}-SS_{ε_f}, is the reduction in sum of squares as a result of including τ_i in the full model. It is the sum of squares due to treatments after block effects have been considered in the model. It is referred to as SS Treatment (adjusted), implying that block effects are also considered when estimating the treatment effects in the full model.

Sum of squares partitions for BIBD

- For BIBD, the SS for treatments adjusted, SS_{ε_r}-SS_{ε_f}, can be computed directly as

$$SS_{\mathrm{T}}(\text{adjusted}) = \frac{k\sum_{i=1}^{t} Q_i^2}{\lambda t}$$

- With t-1 degrees of freedom. The quantity Q_i is an adjusted treatment total computed as

$$Q_i = y_{i.} - \frac{1}{k} B_i, B_i = \sum_{j=1}^{b} n_{ij} y_{.j}$$

- Where B_i is the sum of all block totals that include the i^{th} treatment and n_{ij}=1 if treatment i appears in block j and n_{ij}=0 otherwise.

Sum of squares partitions for BIBD

- This correction to the treatment total has the net effect of removing the block effects from the treatment total.
- The SS for blocks is derived from the reduced model with treatments ignored in the model as SS_{B}(unadjusted)=SS_{T} -SS_{ε_r}. Treatment effects are not considered when estimating the block effects, and SS is called an unadjusted SS.
- SS_{T}=SS_{B}(unadjusted)+SS_{T}(adjusted)+SS_{ε_f}

ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean square	F
Total	N-1	$\sum_i \sum_j \left(y_{ij} - \bar{y}_{..}\right)^2$		
Blocks	*b*-1	$k \sum_j \left(\bar{y}_{.j} - \bar{y}_{..}\right)^2$	MS_B(unadj.)	MS_B(unadj.) $/MS_\varepsilon$
Treatments	*t*-1	$\frac{k \sum_{i=1}^{t} Q_i^2}{\lambda t}$	MS_T(adj.)	MS_T(adj.) $/MS_\varepsilon$
Error	N-*t*-*b*-1	By subtraction	MS_ε	

Example

- Percent conversion of methyl glucoside by acetylene under high pressure in a BIBD

	Pressure (psi)					
Run	250	325	400	475	550	$y_{.j}$
1	16	18	-	32	-	66
2	19	-	-	46	45	110
3	-	26	39	-	61	126
4	-	-	21	35	55	111
5	-	19	-	47	48	114
6	20	-	33	31	-	84
7	13	13	34	-	-	60
8	21	-	30	-	52	103
9	24	10	-	-	50	84
10	-	24	31	37	-	92
$y_{i.}$	113	110	188	228	311	950
B_i	507	542	576	577	648	
Q_i	-56.0	-70.7	-4.0	35.7	95.0	
Example: $B_1 = y_{.1}+y_{.2}+y_{.6}+y_{.7}+y_{.8}+y_{.9}$						
$Q_1 = y_{1.} - B_1/3 = 113 - 507/3 = -56.0$						

ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean square	F	Pr>F
Total	29	5576.67			
Blocks	9	1394.67	154.96	5.02	0.0025**
Treatments	4	3688.58	*922.14*	29.90	0.000**
Error	16	493.42	*30.84*		

Treatment means

- The least squares estimate for a treatment mean μ_i is $\hat{\mu}_i = \hat{\mu} + \hat{\tau}_i$
- Where $$\hat{\mu} = \bar{y}_{..}, \hat{\tau}_i = \frac{kQ_i}{\lambda t}$$
- For example, Q1=-56.00,

$$\hat{\mu} = \bar{y}_{..} = 950/30 = 31.67$$

- So that

$$\hat{\tau}_1 = \frac{kQ_1}{\lambda t} = \frac{3 \times (-56.00)}{3 \times 5} = -11.20, \hat{\mu}_1 = 31.67 - 11.20 = 20.47$$

Least square estimates of treatment means

Pressure (psi)	Mean $\hat{\mu}_i$
250	20.47
325	17.53
400	30.87
475	38.80
550	50.67

Standard errors of treatment means

- The standard error for a treatment mean estimate is

$$s_{\hat{\mu}_i} = \sqrt{\frac{MS_\varepsilon}{rt}\left(1+\frac{kr(t-1)}{\lambda t}\right)} = \sqrt{\frac{30.84}{6\times 5}\left(1+\frac{3\times 6\times 4}{3\times 5}\right)} = 2.44$$

- A 95% confidence interval estimate of a treatment mean is

$$2.44\hat{\mu}_i \pm t_{0.025,16}(s_{\hat{\mu}_i}), where t_{0.025,16} = 2.120$$

- The standard error of the estimated difference between two treatment means is

$$s_{\hat{\mu}_i-\hat{\mu}_j} = \sqrt{\frac{2kMS_\varepsilon}{\lambda t}} = \sqrt{\frac{2\times 3\times 30.84}{3\times 5}} = 3.51$$