Outline

1. Randomized Complete Block Design (RCBD)
   - RCBD: examples and model
   - Estimates, ANOVA table and f-tests
   - Checking assumptions
   - RCBD with subsampling: Model
2. Latin square design
   - Design and model
   - ANOVA table
   - Multiple Latin squares

Randomized Complete Block Design (RCBD)

Suppose a slope difference in the field is anticipated. We block the field by elevation into 4 rows and assign irrigation treatment randomly within each block (row). Ex:

```
> sample(c("A","B","C","D"))
 [1] "D" "A" "B" "C"
```

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

RCBD model

response $\sim$ treatment + block + error

- Here block= , and error=variation at the level.
- no `treatment:block` interaction.
- Treatments and blocks are crossed factors.

RCBD model

Model: response $\sim$ treatment + block + error

$$Y_i = \mu + \alpha_{j[i]} + \beta_{k[i]} + e_i \quad \text{with } e_i \sim \text{ iid } \mathcal{N}(0, \sigma_e^2)$$

- $\mu =$ population mean across treatments,
- $\alpha_j =$ deviation of irrigation method j from the mean, constrained to $\sum_{j=1}^{a} \alpha_j = 0$. Fixed treatment effects.
- $\beta_k =$ fixed block effect (categorical), $k = 1, \ldots, b$ constrained to $\sum_{k=1}^{b} \beta_k = 0$. or random effect with $\beta_k \sim$ iid $\mathcal{N}(0, \sigma_\beta^2)$.

Soil moisture: $a = 4$, $b = 4$. Total of $ab = 16$ observations.

Seedling emergence example

Compare 5 seed disinfectant treatments using RCBD with 4 blocks. In each plot, 100 seeds were planted.
Response: # plants that emerged in each plot.

	Block				
Treatment	1	2	3	4	Mean ($\bar{y}_{j.}$)
Control	86	90	88	87	87.75
Arasan	98	94	93	89	93.50
Spergon	96	90	91	92	92.25
Semesan	97	95	91	92	93.75
Fermate	91	93	95	95	93.50
Mean ($\bar{y}_{.k}$)	93.6	92.4	91.6	91.0	$\bar{y}_{..} = 92.15$

Model:

$$Y_i = \mu + \alpha_{j[i]} + \beta_{k[i]} + e_i \quad \text{with } e_i \sim \text{ iid } \mathcal{N}(0, \sigma_e^2)$$

α_j: seed treatment effect, β_k: block effect.

Seedling emergence example

Population mean for trt j and block k: $\mu_{jk} = \mu + \alpha_j + \beta_k$

Predicted means, or fitted values: $\hat{\mu}_{jk} = \hat{\mu} + \hat{\alpha}_j + \hat{\beta}_k$. How?

	Block				
Trt	1	2	$\cdots$	b	$\bar{\mu}_{j\cdot}$
1	$\mu + \alpha_1 + \beta_1$	$\mu + \alpha_1 + \beta_2$		$\mu + \alpha_1 + \beta_b$	$\mu + \alpha_1$
2	$\mu + \alpha_2 + \beta_1$	$\mu + \alpha_2 + \beta_2$		$\mu + \alpha_2 + \beta_b$	$\mu + \alpha_2$
$\cdots$		$\cdots$			$\cdots$
a	$\mu + \alpha_a + \beta_1$	$\mu + \alpha_a + \beta_2$		$\mu + \alpha_a + \beta_b$	$\mu + \alpha_a$
$\bar{\mu}_{\cdot k}$	$\mu + \beta_1$	$\mu + \beta_2$		$\mu + \beta_b$	μ

Estimated coefficients (balance: 1 obs/trt/block):

- $\hat{\mu} = \bar{y}_{\cdot\cdot}$
- $\hat{\alpha}_j = \bar{y}_{j\cdot} - \bar{y}_{\cdot\cdot}$
- $\hat{\beta}_k = \bar{y}_{\cdot k} - \bar{y}_{\cdot\cdot}$ if fixed block effects

ANOVA table with RCBD

Source	df	SS	MS	$\mathbb{E}$(MS)
Block	$b-1$	SSBlk	MSBlk	$\sigma_e^2 + a\frac{\sum_{k=1}^{b}\beta_k^2}{b-1}$ (fixed) $\sigma_e^2 + a\sigma_\beta^2$ (random) f test
Trt	$a-1$	SSTrt	MSTrt	$\sigma_e^2 + b\frac{\sum_{j=1}^{a}\alpha_j^2}{a-1}$ f test
Error	$(b-1)(a-1)$	SSErr	MSErr	σ_e^2
Total	$ab-1$	SSTot		

SSBlk: involves $(\bar{y}_{.k} - y_{..})^2$ over all blocks k
SSTrt: involves $(\bar{y}_{j.} - y_{..})^2$ over all treatments j
SSErr: involves $(y_{ij} - \hat{\mu}_{ij})^2$ from all residuals
SSTot: involves $(y_{ij} - \bar{y}_{..})^2$

Why not include an interaction Block:Treatment in the model?

It would take df and there would remain df for MSErr.

Debate: fixed vs. random block effects

- Ex: does it make sense to view the 4 specific rows blocked by elevation as randomly selected from a larger population?
- Ex: 4 dosages of a new drug are randomly assigned to 4 mice in each of the 20 litters: RCBD with $a = 4$ dosage treatments and $b = 20$ litters, for a total of $ab = 80$ observations. Here, blocks (litters) can be considered as random samples from the population of all litters that could be used for the study.

- In RCBD, the choice fixed vs. random blocks does not affect the testing of the trt effect. In more complicated designs, it could.

- If we can use the simpler analysis with fixed effects, it is okay to use it!

F test for block variability

Estimation, if random block effects: $\hat{\sigma}^2_\beta = \dfrac{\text{MSBlk} - \text{MSErr}}{a}$

▸ ANOVA table

Test for the block effects (uncommon):

$$F = \frac{\text{MSBlk}}{\text{MSErr}} \text{ on df} = b - 1,\ (b-1)(a-1)$$

but even if there appears to be non-significant differences between blocks, we would keep blocks into the model, to reflect the randomization procedure.

Other commonly used blocking factors: observers, time, farm, stall arrangement etc. The general guideline to choose blocks is scientific knowledge.

F-tests for treatment effects

To test H_0: $\alpha_j = 0$ for all j (i.e., no treatment effect), use the fact that under H_0,

$$F = \frac{\text{MSTrt}}{\text{MSErr}} \sim F_{a-1,\,(b-1)(a-1)}$$

▸ ANOVA table

Source	df	SS	MS	F	p-value
Treatments	4	102.30	25.58	3.598	0.038
Blocks	3	18.95	6.32	0.889	0.47
Error	12	85.30	7.11		
Total	19	206.55			

ANOVA in R with RCBD

```
> emerge = read.table("seedEmergence.txt", header=T)
> str(emerge)
'data.frame':   20 obs. of  3 variables:
 $ treatment: Factor w/ 5 levels "Arasan","Control",..: 2 1 5 4
 $ block    : int  1 1 1 1 1 2 2 2 2 2 ...
 $ emergence: int  86 98 96 97 91 90 94 90 95 93 ...
> emerge$block = factor(emerge$block)
```

Make sure `blocks` are treated as categorical! They should be associated with $b - 1 = 3$ df in the ANOVA table or LRT.

ANOVA in R with RCBD

```
> fit.lm  = lm(  emergence ~ treatment + block, data=emerge)
> anova(fit.lm)
          Df  Sum Sq Mean Sq F value  Pr(>F)
treatment  4 102.300  25.575  3.5979 0.03775 *
block      3  18.950   6.317  0.8886 0.47480
Residuals 12  85.300   7.108

> fit.lm  = lm(  emergence ~ block + treatment, data=emerge)
> anova(fit.lm)
          Df Sum Sq Mean Sq F value  Pr(>F)
block      3  18.95  6.3167  0.8886 0.47480
treatment  4 102.30 25.5750  3.5979 0.03775 *
Residuals 12  85.30  7.1083

> drop1(fit.lm)
Single term deletions
          Df Sum of Sq    RSS    AIC F value   Pr(F)
<none>                  85.30 45.009
block      3     18.95 104.25 43.021  0.8886 0.47480
treatment  4    102.30 187.60 52.772  3.5979 0.03775 *
```

ANOVA in R with RCBD

- Here, the output of `anova()` does not depend on the order in which `treatment` and `block` are given.
- Here, type I sums of squares (sequential, `anova`) and type III sums of squares (`drop1`) are equal.
- Because the design is balanced.

- Significant effect of treatments
- Non-significant differences between blocks, but still keep blocks in the model.

- Note: aov() could have been used in place of lm().

Model assumptions

The model assumes:

1. Errors e_i are independent, have homogeneous variance, and a normal distribution.
2. Additivity: means are $\mu + \alpha_j + \beta_k$, i.e. the trt differences are the same for every block and the block differences are the same for every trt. No interaction.

Extra assumption for the ANOVA table and f-test: balance.

In particular, they assume completeness: each trt appears at least once in each block. That is $n \geq 1$ per trt and block.
Example of an incomplete block design for $b = 4$, $a = 4$:

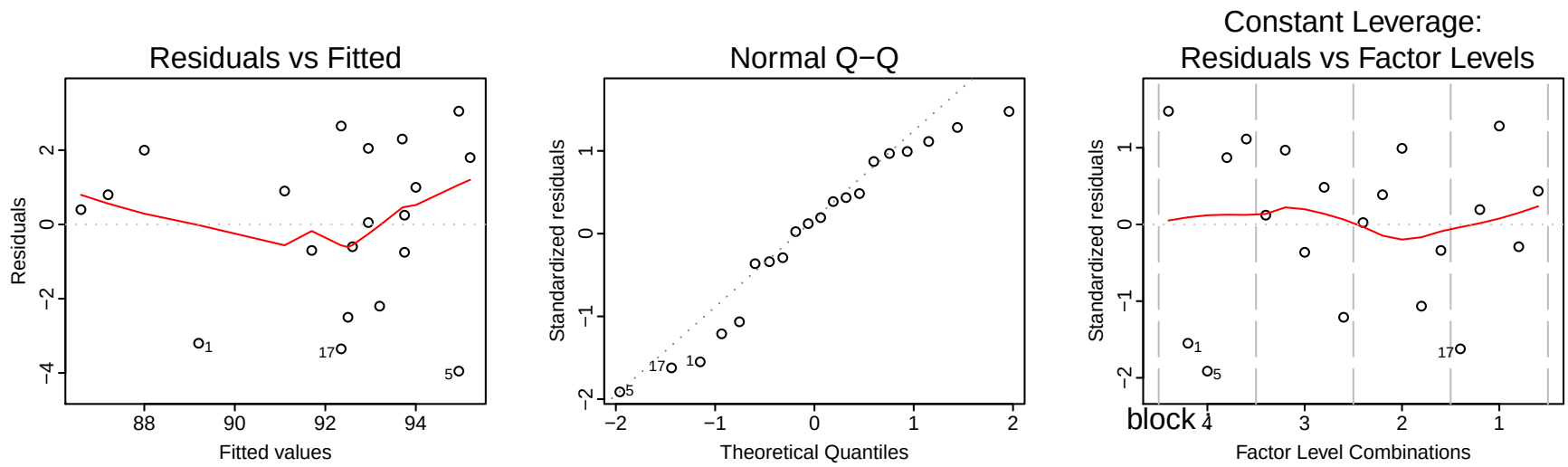
B	A	C
D	A	B
C	B	D
A	C	D

Model diagnostics

Check that residuals ($r_i = y_i - \hat{y}_i$):

- approximately have a normal distribution,
- no pattern (trend, unequal variance) across blocks.
- no pattern (trend, unequal variance) across treatments.

```
plot(fit.lm)
```

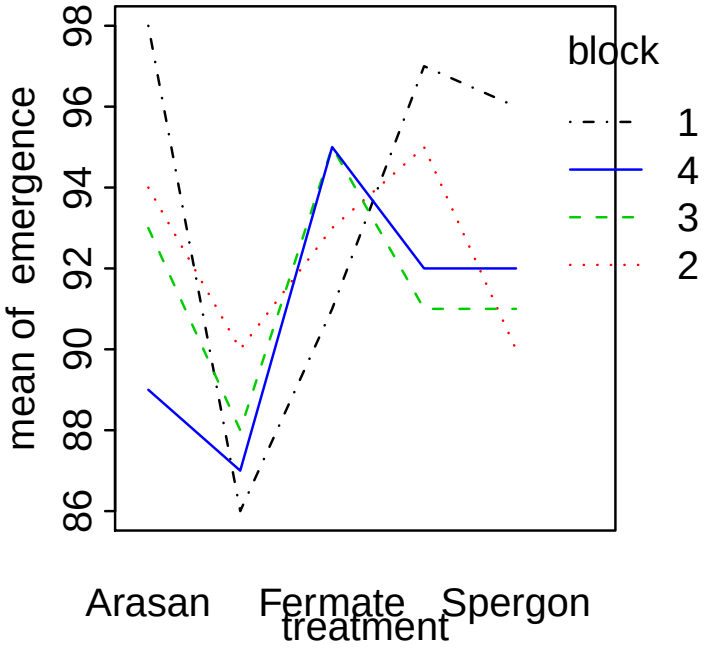


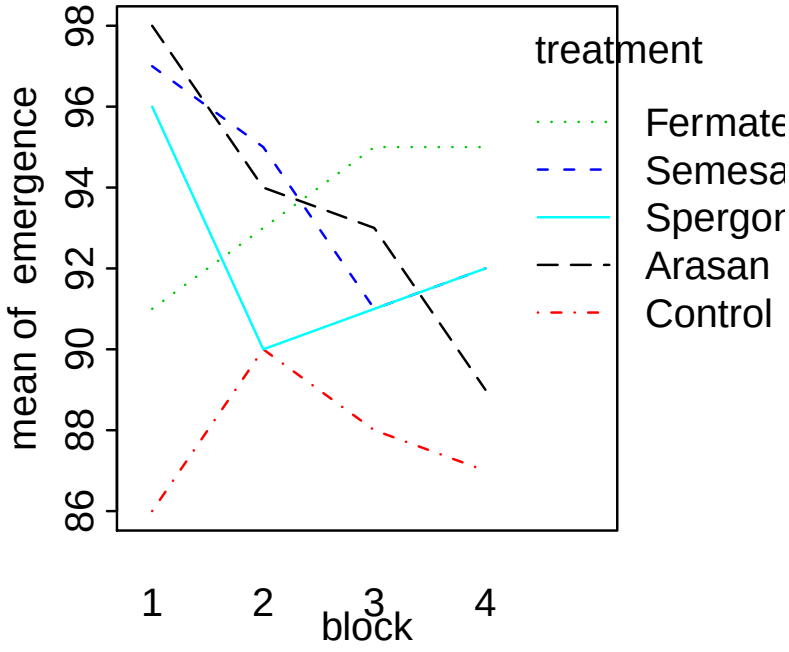
Because balanced design with factors, all observations have the same leverage. R replaces the 'residuals vs. leverage' plot by a plot of residuals vs. factor level combinations

Additivity assumption

Additivity: when each block affects all the trts uniformly.
To assess the absence of interactions visually, use a mean profile plot. Additivity should show up as parallelism.

```
with(emerge,
 interaction.plot(treatment,block,emergence, col=1:4)  )
```



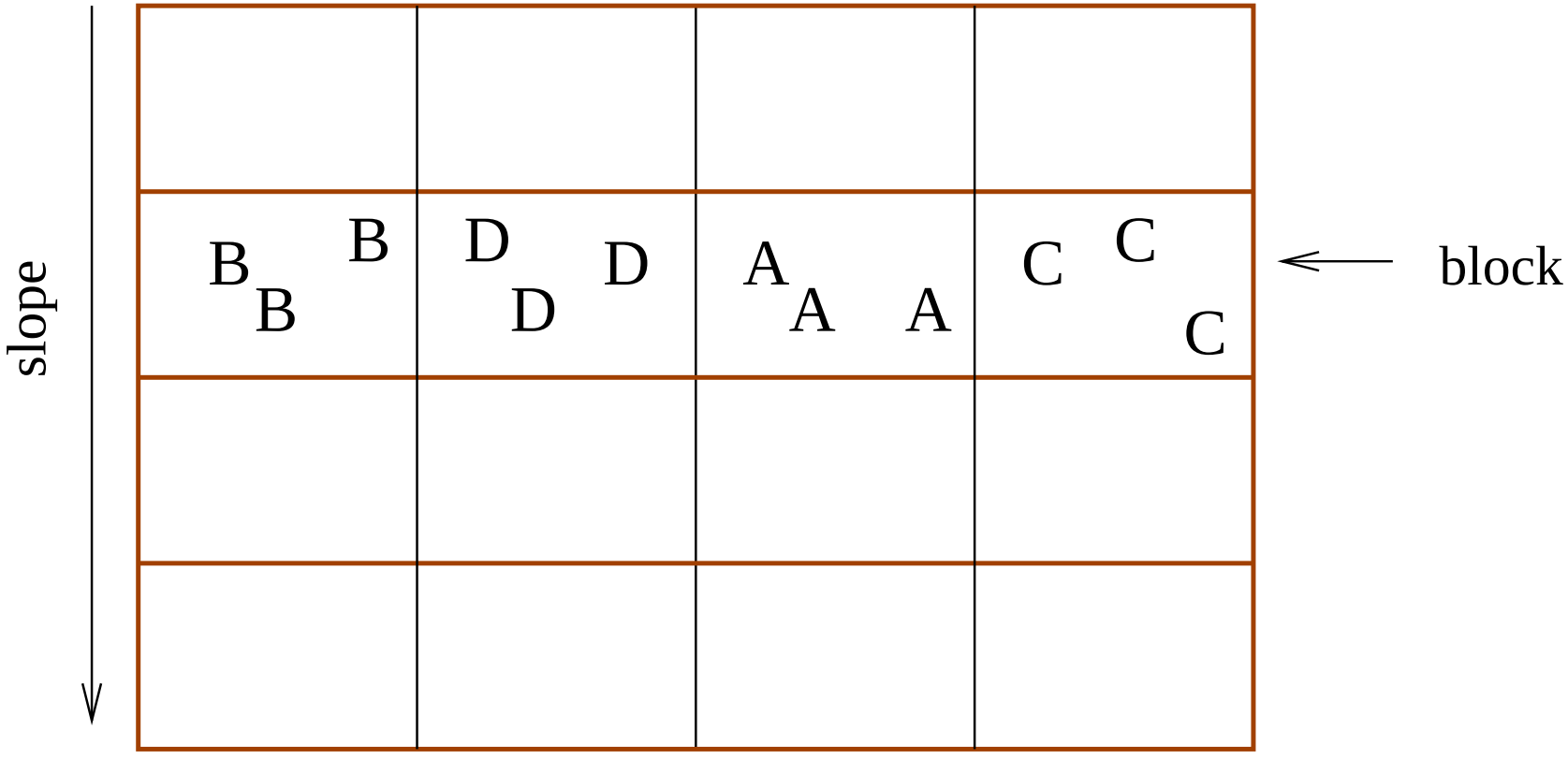


Note: each point represents only 1 measurement here.

Additivity assumption

- Tukey's additivity test can be used, but it still makes an assumption about the interaction coefficients, if they are not all 0.
- If the additivity assumption is violated, how to design an experiment differently to account for non-additivity of trt and block effects?

RCBD with subsampling



s subsamples = repeated measures in each plot

response $\sim$ treatment + block + plot + error

Here: error = variation at the level.

Subsamples nested in plots, so plot effects must be random.

RCBD with subsampling

response $\sim$ treatment + block + plot + error

$$Y_i = \mu + \alpha_{j[i]} + \beta_{k[i]} + \delta_{j[i],k[i]} + e_i$$

- μ is a population mean, averaged over all treatments,
- α_j is a fixed trt effect, constrained to $\sum_{j=1}^{a} \alpha_j = 0$
- β_k is a fixed block effect, $k = 1, \ldots, b$, $\sum_{j=1}^{b} \beta_j = 0$
- $\delta_{jk} \sim$ iid $\mathcal{N}(0, \sigma_\delta^2)$ is for variation among samples (plots) within blocks.
- $e_i \sim$ iid $\mathcal{N}(0, \sigma_e^2)$ is for variation among subsamples.

Total of *abs* observations.

ANOVA table and f-test, RCBD with subsampling

Source	df	SS	MS	$\mathbb{E}$(MS)
Blocks	$b-1$	SSBlk	MSBlk	$\sigma_e^2 + s\sigma_\delta^2 + as\frac{\sum_{j=1}^{b}\beta_k^2}{b-1}$
Treatment	$a-1$	SSTrt	MSTrt	$\sigma_e^2 + s\sigma_\delta^2 + bs\frac{\sum_{j=1}^{a}\alpha_j^2}{a-1}$
Plot Error	$(a-1)(b-1)$	SSPE	MSPE	$\sigma_e^2 + s\sigma_\delta^2$
Subsamp.	$ab(s-1)$	SSSSE	MSSSE	σ_e^2
Total	$abs-1$	SSTot		

- Plot effects take same # of df as an interaction block:treatment would.
- To test H_0: $\alpha_j = 0$ for all j (i.e., no treatment effect), use the fact that under H_0,

$$F = \frac{\text{MSTrt}}{\text{MSPE}} \sim F_{a-1,\,(b-1)(a-1)}.$$

ANOVA table and f-test, RCBD with subsampling

- Similarly to CRD with subsampling: we do not use MSSSE at the denominator.
- Same danger: do not use fixed effects for plots, do not use a fixed interactive effect block:trt instead of the random plot effect.
- We can estimate the overall magnitude of plot effects: $\hat{\sigma}^2_\delta = (\text{ MSPE} - \text{MSSSE }) / s$.
- example for this design in homework.

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   - RCBD with subsampling: Model
2. Latin square design
   - Design and model
   - ANOVA table
   - Multiple Latin squares

Latin square design

- Blocking provides a way to control known sources of variability and reduce error within blocks. We might need double-blocking.
- Ex: $a = 4$ irrigation methods and $n = 4$ plots/method. Response: soil moisture. For CRD, a possible irrigation assignment looks like:

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

- Suppose there is a North-South slope *and* a soil type difference in East-West direction.

Latin square design

- This is *a* Latin square design:
  It blocks the plots in 2 directions at the same time.

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

- Another example?

- R tools to pick one latin square at random: function `williams` in package `crossdes`, or function `design.lsd` in package `agricolae`, and probably more.

Randomization

Example: 3×3 Latin square design.

1. Start with the default design:

A	B	C
B	C	A
C	A	B

2. Randomly arrange the columns. For example, in R,

```
> sample(1:3);
[1] 3 1 2
```

3. Randomly arrange the rows, except for the first one. For example, in R,

```
> sample(2:3);
[1] 3 2
```

Model for the Latin square design

$$\text{response} \sim \text{treatment + row + column + error}$$

$$Y_i = \mu + \alpha_{j[i]} + r_{k[i]} + c_{l[i]} + e_i, \qquad \text{with } e_i \sim \text{ iid } \mathcal{N}(0, \sigma_e^2)$$

where

- μ is a population mean, averaged over treatments
- α_j is a fixed trt effect (irrigation) constrained to $\sum_{j=1}^{a} \alpha_j = 0$
- r_k is a fixed row effect (slope) constrained to $\sum_{k=1}^{a} r_k = 0$
- c_l is a fixed column effect (soil) constrained to $\sum_{l=1}^{a} c_l = 0$

Soil moisture: $a = 4$. There are a total of $a^2 = 16$ observations.

All 3 factors are crossed. No interaction.

ANOVA table for Latin square design

Source	df	SS	MS
Row	$a-1$	SSRow	MSRow
Column	$a-1$	SSCol	MSCol
Treatment	$a-1$	SSTrt	MSTrt
Error	$(a-1)(a-2)$	SSErr	MSErr
Total	a^2-1	SSTot	

To test H_0: $\alpha_j = 0$ for all j (i.e., no trt effect) use the fact that under H_0,

$$F = \frac{\text{MSTrt}}{\text{MSErr}} \sim F_{a-1,(a-1)(a-2)}$$

Why could we not include interactions?

Millet example

Yields of plots of millet, from 5 treatments (A, B, C, D, and E) arranged in a 5 by 5 Latin square.

	Column					
Row	1	2	3	4	5	Mean
1	B: 253	E: 226	A: 285	C: 283	D: 188	247.0
2	D: 255	A: 293	E: 265	B: 290	C: 260	272.6
3	E: 190	B: 260	C: 298	D: 254	A: 248	250.0
4	A: 203	C: 204	D: 237	E: 193	B: 249	217.2
5	C: 230	D: 270	B: 275	A: 333	E: 327	287.0
Mean	226.2	250.6	272.0	270.6	254.4	254.76

Treatment:	A	B	C	D	E
Mean ($\bar{Y}_{i..}$):	272.4	265.4	255.0	240.8	240.2

Millet example with R

```
> millet = read.table("millet.txt", header=T)
> str(millet)
'data.frame':   25 obs. of  4 variables:
 $ row      : int  1 2 3 4 5 1 2 3 4 5 ...
 $ column   : int  1 1 1 1 1 2 2 2 2 2 ...
 $ treatment: Factor w/ 5 levels "A","B","C","D",..: 2 4 5 1 3
 $ yield    : int  253 255 190 203 230 226 293 260 204 270 ...

> millet$row    = factor(millet$row)
> millet$column = factor(millet$column)
```

Make sure treatments, rows and columns are treated as categorical.

Millet example with R

```
> fit.lm = lm(yield ~ row + column + treatment, data=millet)
> anova(fit.lm)
          Df  Sum Sq Mean Sq F value  Pr(>F)
row        4 14256.6  3564.1  3.3764 0.04531 *
column     4  6906.2  1726.5  1.6356 0.22900
treatment  4  4156.6  1039.1  0.9844 0.45229
Residuals 12 12667.3  1055.6

> anova(  lm(yield ~ treatment + column + row, data=millet))
          Df  Sum Sq Mean Sq F value  Pr(>F)
treatment  4  4156.6  1039.1  0.9844 0.45229
column     4  6906.2  1726.5  1.6356 0.22900
row        4 14256.6  3564.1  3.3764 0.04531 *
Residuals 12 12667.3  1055.6

> drop1( fit.lm, test="F")
Single term deletions
          Df Sum of Sq   RSS    AIC F value   Pr(F)
<none>                 12667 181.70
row        4   14256.6 26924 192.55  3.3764 0.04531 *
column     4    6906.2 19573 184.58  1.6356 0.22900
treatment  4    4156.6 16824 180.79  0.9844 0.45229
```

Because of balance: the type I and type III SS are equal: the results (F and p-values) do not depend on the order.

Latin square design: notes

- It is an incomplete block design: there are not observations for each combination of row, column, and trt.

  Still, balance when we look at pairs: trt & row, trt & column, row & column.

- Main advantage: reduce variability.
- Main disadvantages:
  - lose more dfError than 1 blocking factor.
  - randomization even more restricted than RCBD with # trts = # rows = # columns.
  - Randomization procedure is more complex than CRD or RCBD.

Multiple Latin square design

An experiment is performed over 4 weeks. Each week, 3 operators evaluate one of the 3 trts on each day (MTW).
$m =$ Latin squares.

Week 1:

Operator	Mon	Tues	Wed
George	C	A	B
John	B	C	A
Ralph	A	B	C

Model:

Y = treatment + square + square:row + square:column + error

$$Y_i = \mu + \alpha_j + s_h + r_{hk} + c_{hl} + e_i \quad \text{with } e_i \sim \text{ iid } \mathcal{N}(0, \sigma_e^2)$$

where

$j = 1, \ldots, a$ indexes treatment
$h = 1, \ldots, m$ indexes square (here:)
$k = 1, \ldots, a$ indexes row within square ( )
$l = 1, \ldots, a$ indexes column within square ( )

ANOVA table for multiple Latin square design

Source	df	SS
Square	$m-1$	SSSq
Row	$m(a-1)$	SSRow
Column	$m(a-1)$	SSCol
Treatment	$a-1$	SSTrt
Error	$m(a-1)(a-2)+(m-1)(a-1)$	SSErr
Total	ma^2-1	SSTot

To test H_0: $\alpha_j = 0$ for all j (i.e., no trt effect) use the fact that under H_0,

$$F = \frac{\text{MSTrt}}{\text{MSErr}} \sim F_{a-1,\, m(a-1)(a-2)+(m-1)(a-1)}.$$