

## Topic 5: Experimental Design

ENVX2001 Applied Statistical Methods

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# Introduction

## Learning Outcomes

- **Demonstrate proficiency in designing sample schemes and analysing data from them using using R;**
- **Describe and identify the basic features of an experimental design; replicate, treatment structure and blocking structure;**
- Demonstrate proficiency in the use or the statistical programming language R to apply an ANOVA and fit regression models to experimental data;
- Demonstrate proficiency in the use or the statistical programming language R to use multivariate methods to find patterns in data
- Interpret the output and understand conceptually how its derived of a regression, ANOVA and multivariate analysis that have been calculated by R;
- Write statistical and modelling results as part of a scientific report;
- Appraise the validity of statistical analyses used in publications.

# Introduction

## Outline

- Experimental unit vs sampling unit
- Replication
- Randomisation
- Control of variation
- Completely randomised design (CRD)
- Blocking – randomised complete block design (RCBD)

# Introduction

## Topic 4 Learning Outcomes

- At the end of this topic students should be able to:
  - Assess the validity of an experiment in terms of the appropriateness of replication;
  - Explain the concept of blocking;
  - Demonstrate proficiency in the use of R (and interpretation of the output) for generating experimental designs (CRD, RCBD), analysing them and interpreting the results

# Experimental and statistical modelling design process

- From:
- Fox, G. A., S. Negrete-Yankelevich, and V. J. Sosa. (2015). *Ecological statistics: contemporary theory and application*. Oxford University Press, USA.

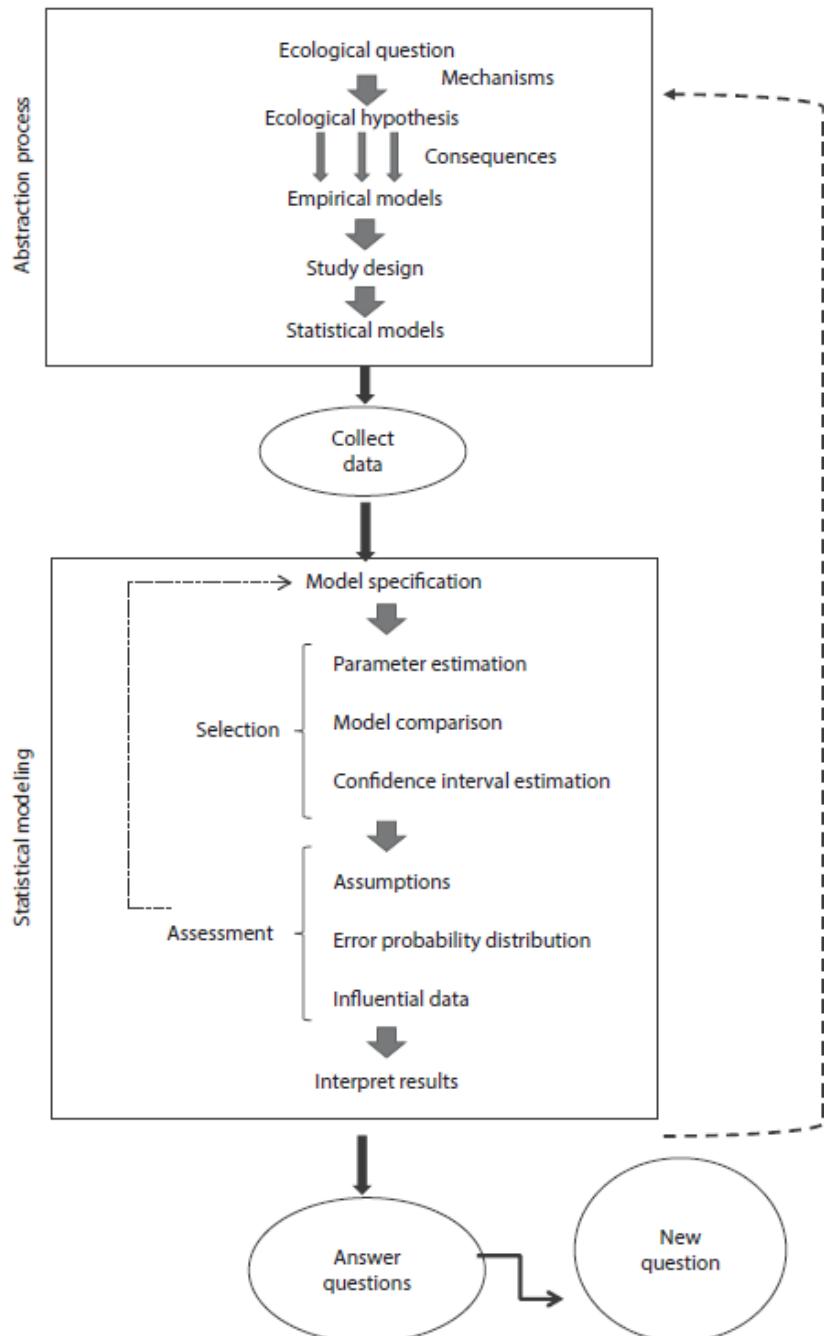


Fig. I.1 The cycle of ecological research and the role of statistical modeling.

# Introduction

*To call in a statistician after the experiment has been done may be no more than asking him to perform a post-mortem examination: he may be able to say what the experiment died of” R. Fisher*

## Experimental design

- Previously: considered statistical analysis only, not how experiment was set out
- Experimental design equally / more important than statistical analysis
- If design OK  $\Rightarrow$  analysis is (relatively) straight forward
- If design not OK  $\Rightarrow$  analysis is quite complex – or not analysable!  
 $\Rightarrow$  interpretation of results not clear
- Several principles
  - experimental units versus sampling units
  - replication
  - randomisation
  - control of variation

# Experimental vs Sampling Units

## Definitions

- Experimental unit
  - An experimental unit (e.u.) is the unit of study in our experiment, or more particularly, **the smallest unit at which a treatment is applied**
    - single animal, or herd
    - 1 m<sup>2</sup> plot of turf, or whole field
    - farm, or Rural Lands Protection Board district
- Sampling unit
  - A sampling unit is the unit at which the observations are made
  - Often, this will be the same as the experimental unit, but not always

# Replication

## Importance

- A treatment should be replicated to more than one experimental unit
- This allows treatment effects to be compared with level of biological variability of e.u.s
- Increased number of replicates:  $\Rightarrow$  smaller standard errors  $\Rightarrow$  more accuracy
- Need to balance cost with accuracy
- **There MUST be replication for everything that you have hypotheses about**
  - **replicate = experimental unit**

$$s^2 = \frac{\sum_{i=1}^n (y_i - \bar{y})^2}{n-1}$$

$$se(\bar{y}) = \sqrt{\frac{s^2}{n}} = \frac{s}{\sqrt{n}}$$

# How many replicates?

- Higher  $s$ , lower precision
- Increasing  $n$  improves precision
- However, increasing  $n$  will eventually have diminishing returns.

Scenario	Replication ( $n$ )	Standard deviation ( $s$ )	se
1	3	2	1.15
2	10	2	0.63
3	100	2	0.2
4	100	1	0.1
5	100	2	0.2
6	100	3	0.3

$$se(\bar{y}) = \frac{s}{\sqrt{n}}$$

[https://gallery.shinyapps.io/sampling\\_and\\_stderr/](https://gallery.shinyapps.io/sampling_and_stderr/)

# Replication

## Key Concepts

- Pseudo-replication:
  - artificial inflation of replicates based on sub-sampling (sampling units = experimental units)
  - lack of randomisation
- Confounding:
  - inability to determine if the difference in response is due to the treatment or other factors
  - adequate randomisation
- Hurlbert, S.H. (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* 54, 187-211

# Replication

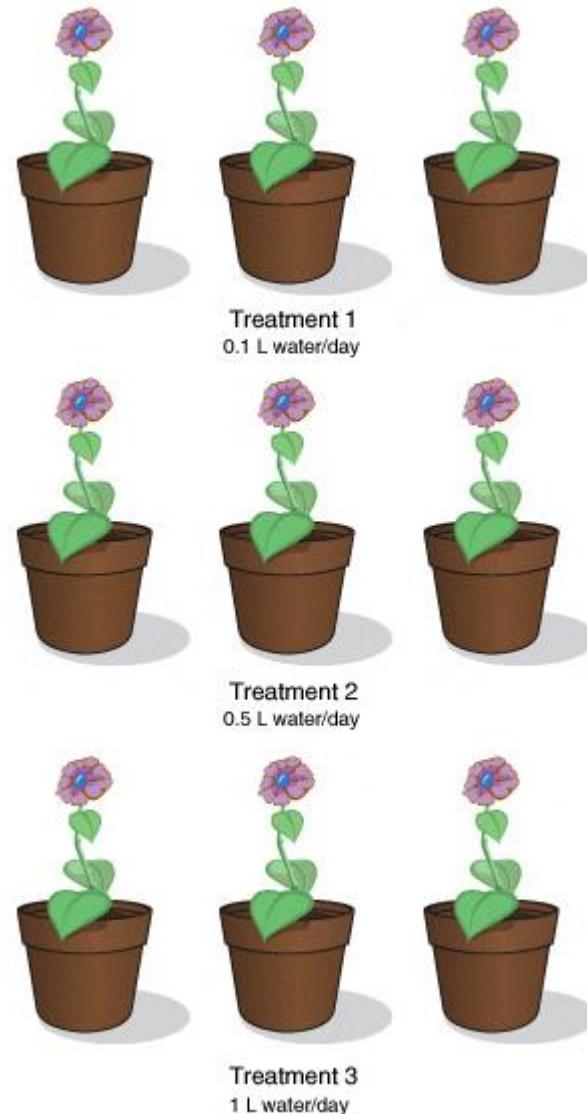
## Example

- Hypothesis: organic vs conventional agriculture leads to different weight gain in sheep
- Experiment has 40 animals
- 20 animals (randomly selected) and placed in one farm to measure weight gain; 20 animals (randomly selected) in the other farm to measure weight gain
- Experimental unit?
- Sampling unit?
- Confounding or Pseudo-replication?

# Replication

## Example

- Hypothesis: different irrigation rates lead to different plant N content
- Measure 5 leaves per plant for N content
- Experimental unit?
- Sampling unit?
- Confounding or Pseudo-replication?



# Replication

## Example

- Hypothesis: Fish fed Superfin fish food grow faster than those fed Phatbstard
- 100 fish in experiment
- Prediction & Design... 2 tanks (50 fish randomly placed in each tank), tank A apply food type S and tank B apply type P. After a period of time measure growth rate of all fish and test using t-test
- Experimental unit?
- Sampling unit?
- Confounding or Pseudo-replication?

# Randomisation

## Importance

- Random allocation of treatments amongst experimental units (e.u.s)
- Reason:
  - **Allows us to assume errors (samples) are independent which allows for hypothesis testing in normal manner**
    - i, i, d errors (independent, identically distributed errors)
  - Due to environmental & biological variability, some e.u.s perform better than others (higher response, y)
  - Need to ensure that best response to a particular treatment was obtained not just because the best e.u.s were selected
  - Otherwise, we would not know what is the effect of the treatment and what is the effect of the e.u.

# Randomisation

## Example

- A drug company needs to evaluate a treatment for nematode control. They have a list of farms that will participate in the study.
- Half of the farms will be allocated the new treatment (A), and half will be allocated the conventional treatment (B)
- Suppose that the list of farms has accumulated over time, so that the well established farms are at the top of the list, and more recent farms at the bottom of the list



Image source: <https://www.studyblue.com/notes/>



Image source: <http://nematode.unl.edu/>

# Randomisation

## Example

- Consider the two sampling methods:
  - **Systematic:**    A A A A A A . . . B B B B B  
                        Top of List      Bottom of List
  - **Random:**      A B B A B A . . . B A B B A  
                        Top of List      Bottom of List
- In the **systematic** sample, we would not know if an improvement in nematode control was due to the new treatment, or how long the farm was established
- In the long run, randomisation will remove this doubt however, in any one experiment, we can never be sure

# Randomisation

## Techniques of randomisation

- In general, we should not use our own judgement for randomisation: humans are not good at this!
- Instead we should use:
  - coin tossing (head, tail)
  - random number tables
  - computer generated numbers
  - computer generated designs

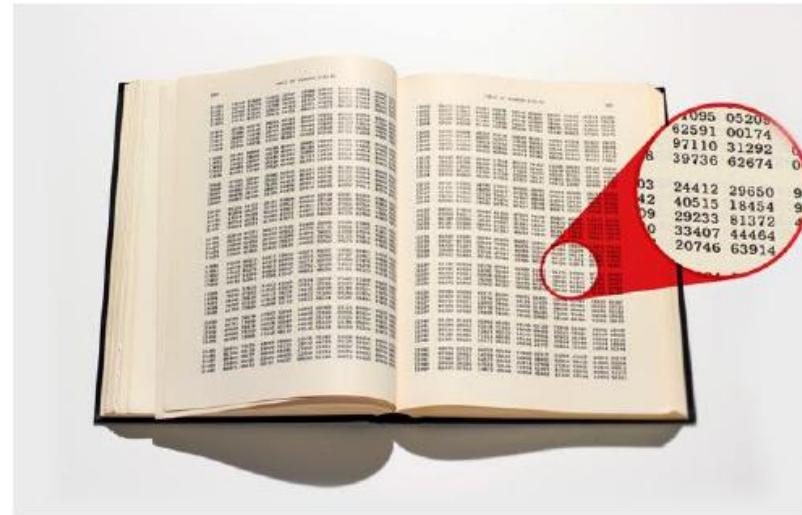


Image source: [http://www.wired.com/wiredenterprise/2012/08/st\\_randomnumbergenerators/](http://www.wired.com/wiredenterprise/2012/08/st_randomnumbergenerators/)



Image source: <http://forum.kimbucktwo.com/index.php?topic=10334.0>

# Control of variation

## Variation

- Results of experiment: analysis of a response (outcome) variable that shows variation
- Variation due to:
  - experimental treatment
  - other extraneous sources of variation
- Choosing an appropriate type of experimental design:
  - helps to isolate these extraneous sources of variation
  - variation due to the treatments can be more reliably assessed
- Some specific types of experimental designs will now be considered
  - circumstances where they are used
  - how data generated from such a design is analysed

# Completely Randomised Design (CRD)

## Features

- Simplest form of experimental design
- Randomly allocate treatments amongst all e.u.s
- This is the randomisation procedure considered until now
- Can be used when there is no reason to believe that the e.u.s differ in any systematic way (design assumption)
- Examples:
  - All animals used in a diet study are similar in terms of ages, genetics, and husbandry issues
  - All vets using a procedure have similar level of experience

# Completely Randomised Design (CRD)

## Limitations

- CRD requires that all e.u.s are similar before the treatment is applied
- Often, there are not sufficient e.u.s to guarantee this
- If we broaden the study to include more e.u.s and if there is systematic variation amongst e.u.s and we ignore this, there are two effects:
  - (1) Loss of Precision
  - (2) Incorrect Conclusions

# Completely Randomised Design (CRD)

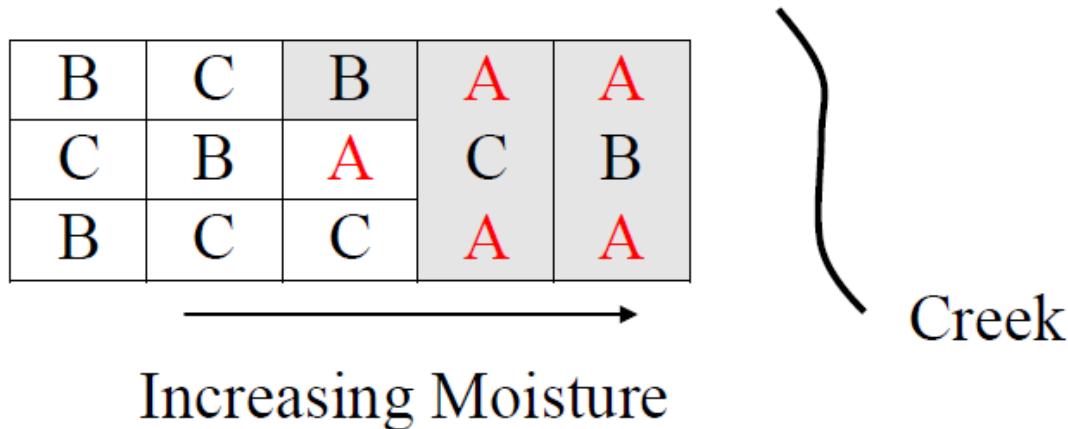
## Limitations – Loss of Precision

- The Residual SS will contain additional variability that could have been ‘explained’
  - Residual MS is larger
  - *F*-ratio is smaller
  - *P*-value is larger
  - less likely to detect significant differences

# Completely Randomised Design (CRD)

## Limitations – Incorrect Conclusions

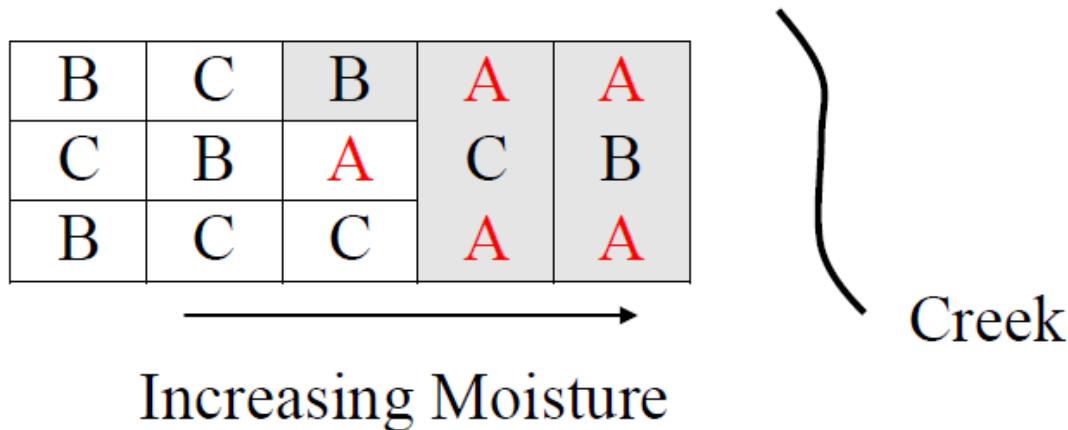
- Suppose a CRD was used to assess a crop yield experiment with three treatments, A, B, and C
- Suppose that a portion of the plots were more moist due to their proximity to a creek
- One possible randomisation is as follows:



# Completely Randomised Design (CRD)

## Limitations – Incorrect Conclusions

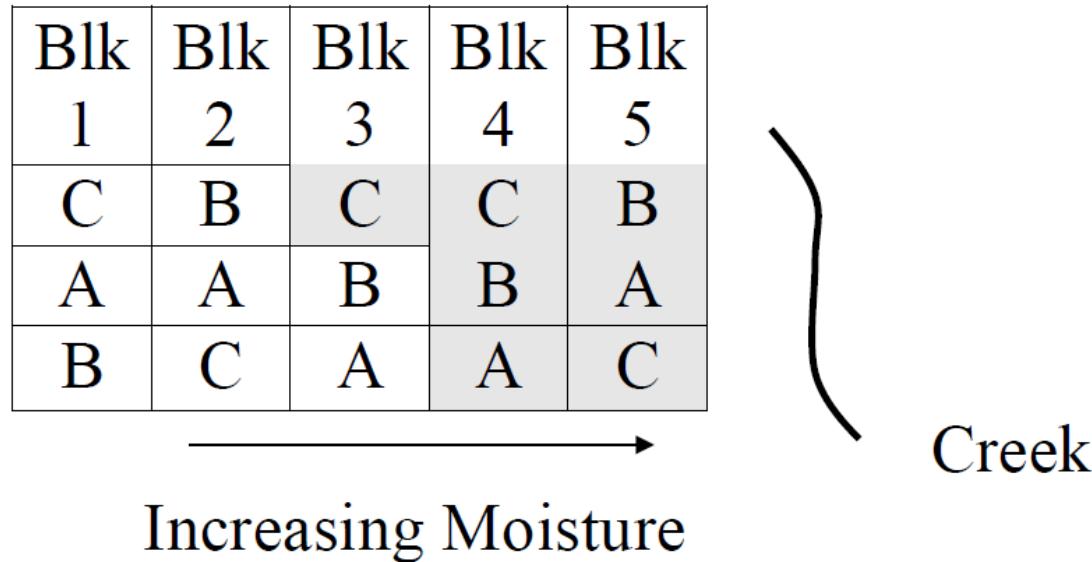
- Most of the plots receiving Treatment A are in the moist (shaded) area
- It would be difficult to separate the effect of Treatment A from the moisture effect, i.e. low growth may be due to Treatment A, or the increased moisture
- So while the treatment allocation was random, this may not be appropriate for this experiment



# Blocking

## Concept

- We need to group together e.u.s that are similar - these groups are termed **blocks**



- So each block should comprise plots of similar moisture

# Blocking

## Concept

- Within each block, a separate randomisation of treatments to plots should be made (as shown in Figure)
- Such a design is termed a Randomised Complete Block Design (RCBD)
- Complete because each block contains all the possible treatments

Blk	Blk	Blk	Blk	Blk
1	2	3	4	5
C	B	C	C	B
A	A	B	B	A
B	C	A	A	C

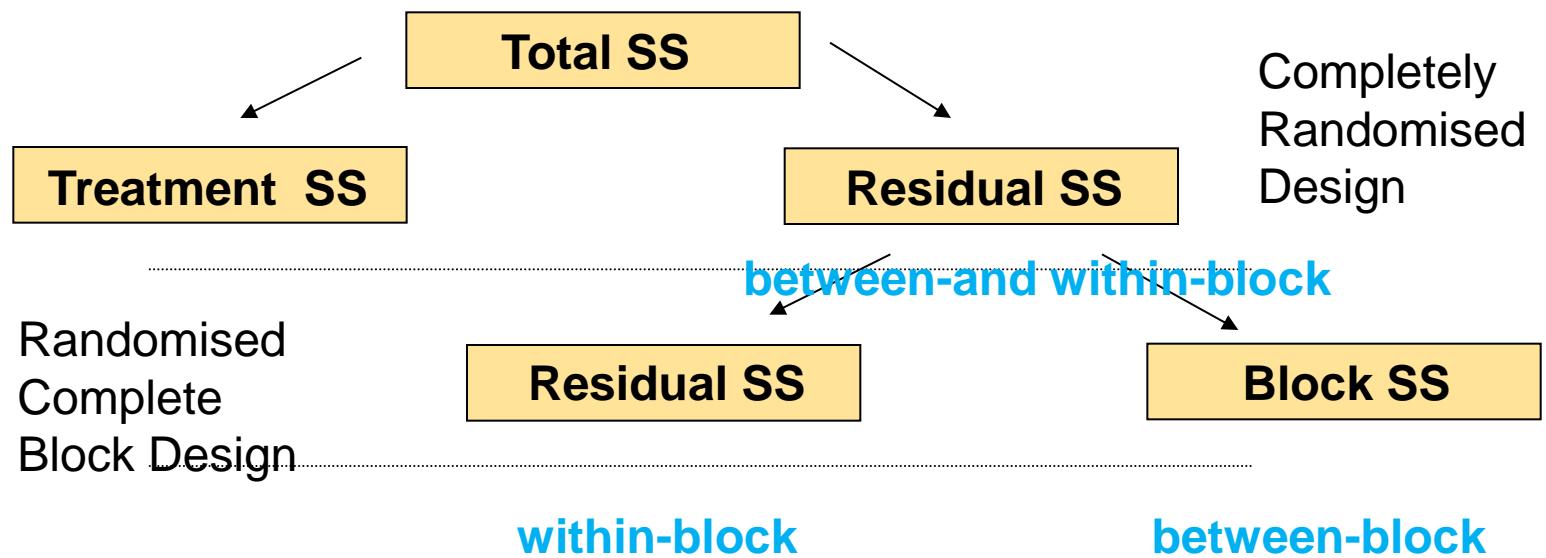
→

Increasing Moisture

Creek

# Blocking

## Concept



# Completely Randomised Design (CRD)

## Example

- A study was undertaken to compare the percentage moisture content (%) in soil subjected to four different irrigation methods (W, X, Y, Z).
- The field was divided into 100 plots in a  $10 \times 10$  array
- A CRD was used: 25 plots per irrigation treatment (random allocation)
- Summary statistics

Method	Mean	SD
W	10.60	2.23
X	11.49	1.94
Y	8.18	2.35
Z	5.96	2.35

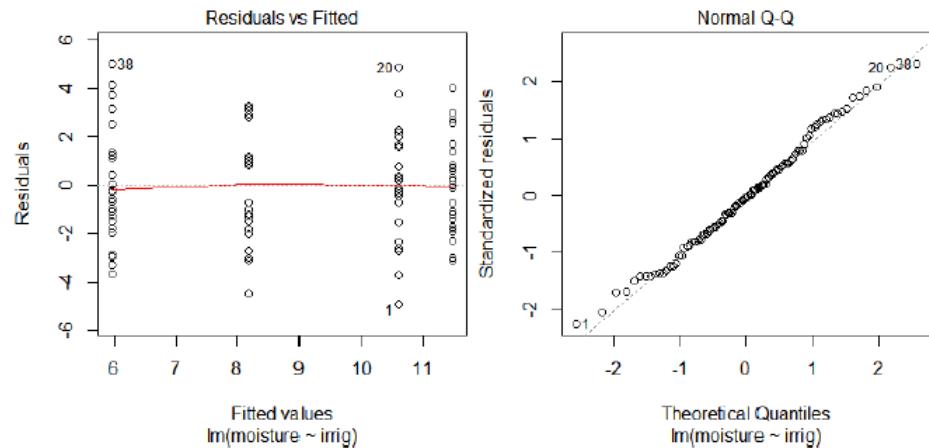
# Completely Randomised Design (CRD)

## Example

- ANOVA

Source	df	SS	MS	F	P
Irrigation	3	466.59	155.53	31.4	<0.001
Residual	96	474.88	4.95		
Total	99	941.46			

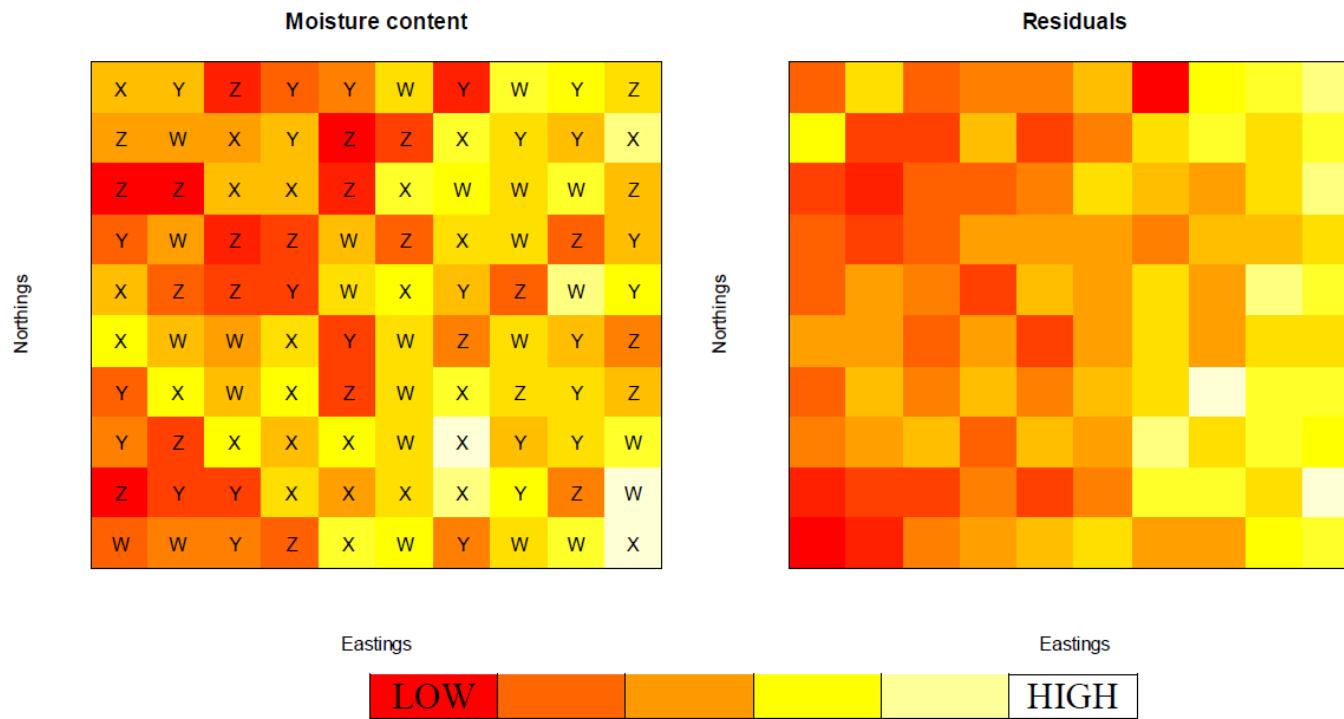
- Residual diagnostics
- What about residuals in field order?



# Completely Randomised Design (CRD)

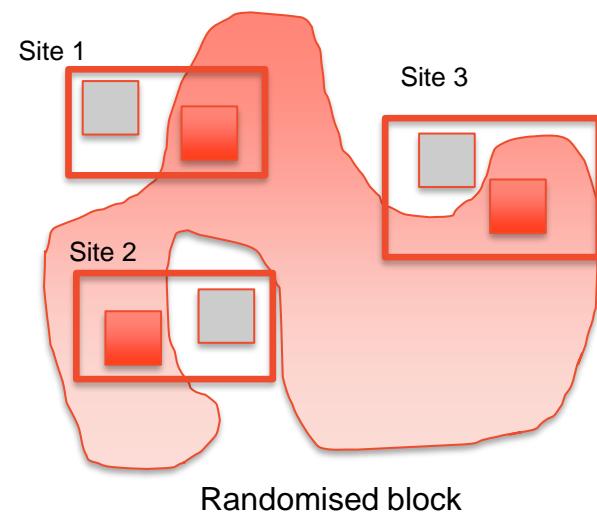
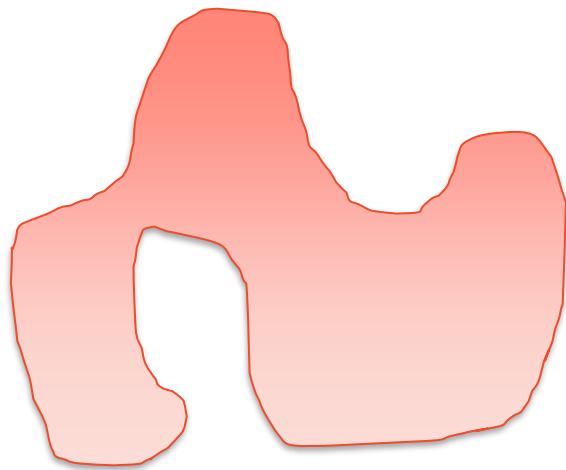
## Example

- Field layout: moisture content and residuals

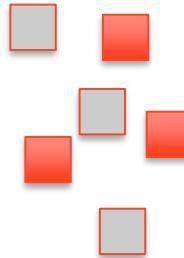


- There is a trend in residuals from West (low) to East (high)

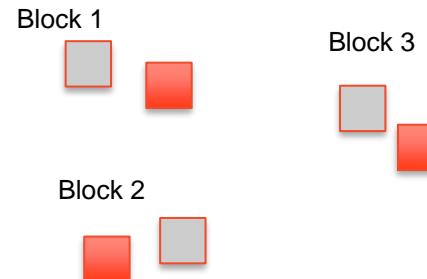
# Blocking



# Experimental Designs



Completely randomised



Randomised block

	Completely randomised	Randomised block
Source	$df$	$df$
Treatment	$t-1$	$t-1$
Block		$n-1$
Residual	$t(n-1)$	$(t-1)(n-1)$
Total	$tn-1$	$tn-1$

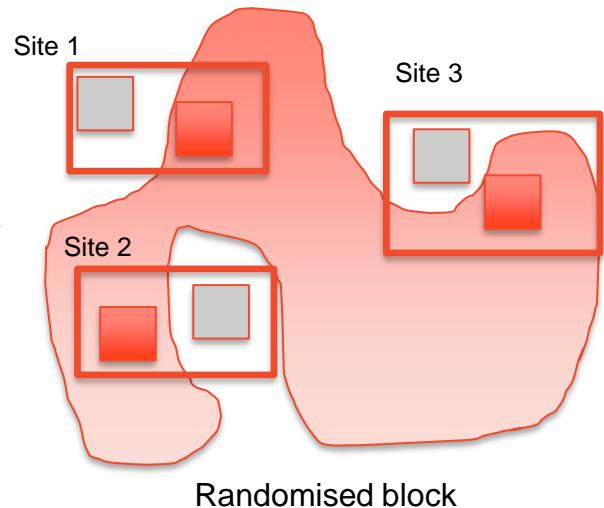
$n$  = experimental units or replicates

$t$  = number of treatments

## RANDOMISED COMPLETE BLOCK DESIGNS

### Example:

- A study was undertaken to compare the species richness between burnt and unburnt habitat.
- In this study, ten different sites were selected, each site contained a burnt and unburnt treatment close to each other to minimise spatial confounding.
- The site can be regarded as a block as we need to adjust for variability between sites, before we assess fire treatment differences.



**The following data show the species richness for each site.**

Site	Burnt	Unburnt
1	1.03	0.26
2	1.17	0.21
3	0.72	0.66
4	0.46	0.86
5	1.41	0.55
6	1.77	0.45
7	0.18	0.57
8	0.53	0.36
9	1.17	0.66
10	0.15	0.17
Mean	0.86	0.48

- Model:

$$\text{Observed data}_{ij} = \text{overall mean} + \text{block effect}_i + \text{treatment effect}_j + \text{error}_{ij}$$

*OR*

$$SpRich_{ij} = \text{overall mean} + Site_i + fire\_treatment_j + error_{ij}$$

- Need to acknowledge that “Site Effect” and “Error” capture random variation (between sites, within sites), associated with Blocking structure.
- In contrast “Overall Mean” and “Fire\_treatment” quantify things about the treatments selected, i.e. the Treatment Structure.



- Performing the ANOVA using R:

```
fire.aov <- aov(SpRich ~ Site + Fire_treatment, data = fire)
```



$$SpRich_{ij} = \text{overall mean} + Site_i + fire\_treatment_j + error_{ij}$$

# Blocking

## Creating blocks

- All the experimental units within the block should be as homogeneous (similar) as possible
- The way that e.u.s are grouped into blocks requires knowledge of the subject material (not a statistical issue) – domain knowledge
- Often a pilot study can be used to identify likely blocks

# Paired Designs

## Designs with 2 Treatments

- If each block has only two e.u.s, this is a ***paired design***
- The two treatments are randomly allocated within each pair (e.g. by coin tossing)
- The data are then analysed by using a ***paired t-test*** (one sample *t*-test on the differences)

# Paired Designs

## Example

- Two wheat varieties (A and B) were trialled on each of 8 farms (Clewer and Scarisbrick 2001, p.47-48)
- Removed farm effects: take differences between two treatments on each farm.
- Proceed with a 2-sample paired t-test (= 1-sample t-test on the differences)

Farm	Yield (kg)		Difference $A - B$
	Variety A	Variety B	
1	17.8	14.7	3.1
2	18.5	15.2	3.3
3	12.2	12.9	-0.7
4	19.7	18.3	1.4
5	10.8	10.1	0.7
6	11.9	12.2	-0.3
7	15.6	13.5	2.1
8	12.5	9.9	2.6

# Paired Designs

## Example

- Summary statistics on differences
  - Sample mean:  $\bar{y}_d = 1.525 \text{ kg}$
  - Sample SD:  $s_d = 1.516 \text{ kg}$
  - Sample size:  $n = 8$
- Therefore:
  - $se(\bar{y}_d) = \frac{1.516}{\sqrt{8}} = 0.5361 \text{ kg}$
  - $t = \frac{\bar{y}_d}{se(\bar{y}_d)} = \frac{1.525}{0.5361} = 2.84$
  - $df = n - 1 = 8 - 1 = 7, P = 0.025$
  - Variety A has a significantly higher mean yield than Variety B

# Paired Designs

```
> VarA<-c(17.8,18.5,12.2,19.7,10.8,11.9,15.6,12.5)  
> VarB<-c(14.7,15.2,12.9,18.3,10.1,12.2,13.5,9.9)  
> t.test(VarA, VarB, paired=T)
```

## Paired t-test

```
data: VarA and VarB  
t = 2.8446, df = 7, p-value = 0.02488  
alternative hypothesis: true difference in means is not equal to 0  
95 percent confidence interval:  
 0.2573084 2.7926916  
sample estimates:  
mean of the differences  
 1.525
```

# Paired Designs

## Example Conclusion

- There was a significant difference between varieties ( $t = 2.84$ ,  $P = 0.025$  , df =7)
- Variety A had a higher yield than variety B with a mean difference of 1.53 kg (SE:  $\pm 0.54$  kg) or report confidence interval (95% CI: 0.26, 2.79 kg)

# ANOVA & Blocking

## Designs with several treatments

- When there are  $\geq 3$  treatments, we use an ANOVA approach
- Each block should contain as many e.u.s as treatments (or a multiple). i.e., if 4 treatments, then 4 (or 8, 12, ...) e.u.s
- In the ANOVA procedure, we partition the total variability about the grand mean as  $\text{Total SS} = \text{Block SS} + \text{Treatment SS} + \text{Residual SS}$
- The ANOVA has one treatment factor and one blocking factor

# ANOVA & Blocking

b = number of blocks;  
t = number of treatments = number of e.u.s per block;  
N = b × t = total number of observations

## ANOVA Table

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)
Block	Blk SS	$b - 1$	Blk SS / $(b - 1)$
Treatment	Trt SS	$t - 1$	Trt SS / $(t - 1)$
Residual	Res SS	$(b-1)(t-1)$	Res SS / $(b-1)(t-1)$
Total	Tot SS	$bt - 1$	

- Test Statistic:  $F = \frac{\text{Treatment MS}}{\text{Residual MS}}$ 
  - $df = t - 1, (b - 1)(t - 1)$  or  $df = \text{treatment df, residual df}$
- We do not (usually) perform an F-test for block differences: blocks were only included to take account of known variability

# ANOVA & Blocking

## Example – Nitrogen Fertiliser on Rice

- Pearce et al. (1988) outlines an experiment where 5 levels of nitrogen are applied to 3 varieties of rice (resulting in  $5 \times 3 = 15$  treatment combinations)
- In this study, 4 blocks of land were used with 15 plots per block
- A separate randomisation is performed within each block:
  - one of the 15 treatments is randomly allocated to one of the 15 plots
- A possible randomisation for this design is (Treatments T01, T02, ..., T15):

Blk	Plot No														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	T05	T02	T11	T14	T15	T10	T09	T04	T07	T01	T06	T03	T08	T12	T13
2	T02	T07	T14	T13	T12	T04	T01	T03	T10	T05	T15	T11	T06	T08	T09
3	T08	T12	T11	T09	T14	T01	T02	T06	T03	T10	T07	T04	T15	T05	T13
4	T07	T03	T11	T10	T02	T05	T01	T08	T04	T14	T15	T09	T12	T13	T06

# ANOVA & Blocking

## Example – Nitrogen Fertiliser on Rice

- Data are rice yield ( $t \text{ ha}^{-1}$ )

Treatment	Block 1	Block 2	Block 3	Block 4	Treatment mean
1	3.852	2.606	3.144	2.894	3.124
2	4.788	4.936	4.562	4.608	4.724
3	4.576	4.454	4.884	3.924	4.460
4	6.034	5.276	5.906	5.652	5.717
5	5.874	5.916	5.984	5.518	5.823
6	2.846	3.794	4.108	3.444	3.548
7	4.956	5.128	4.150	4.990	4.806
8	5.928	5.698	5.810	4.308	5.436
9	5.664	5.362	6.458	5.474	5.740
10	5.458	5.546	5.786	5.932	5.681
11	4.192	3.754	3.738	3.428	3.778
12	5.250	4.582	4.896	4.286	4.754
13	5.822	4.848	5.678	4.932	5.320
14	5.888	5.524	6.042	4.756	5.553
15	5.864	5.264	6.056	5.362	5.637
Block mean	5.133	4.846	5.147	4.634	4.940

# ANOVA & Blocking

## Model Equation

- Note that the one-way (no blocking) model could have been written with effects rather than means since,

$$\text{Yield} = \text{Treatment mean} + \text{Error}$$

is equivalent to

$$\text{Yield} = \text{Overall mean} + \text{Treatment effect} + \text{Error}$$

## Blocking:

- $y_{i,j} = \mu + \beta_i + \tau_j + \epsilon_{i,j}$
- Observed data = Overall Mean + Block Effect + Treatment Effect + Random Error
  - $i = 1, 2, \dots, b(\text{blocks}), j = 1, 2, \dots, t(\text{treatments})$

# ANOVA & Blocking

## Interpretation of model effects

- $i^{\text{th}}$  block effect =  $i^{\text{th}}$  block mean – overall mean
- $i^{\text{th}}$  treatment effect =  $i^{\text{th}}$  treatment mean – overall mean =  $\mu_i - \mu_{\bullet}$   
where  $\mu_i = i^{\text{th}}$  treatment mean,       $\mu_{\bullet} = \text{overall mean}$

## Example: 5 varieties of wheat

- Suppose overall (population) mean yield =  $3.0 \text{ t ha}^{-1}$ .
- Suppose Varietal means are  $2.2, 2.6, 3.1, 3.5$  and  $3.6 \text{ t ha}^{-1}$
- $\therefore$  Varietal effects are

$$\text{Variety 1: } 2.2 - 3.0 = -0.8 \text{ t ha}^{-1}$$

$$\text{Variety 2: } 2.6 - 3.0 = -0.4 \text{ t ha}^{-1}$$

$$\text{Variety 3: } 3.1 - 3.0 = +0.1 \text{ t ha}^{-1} \quad \text{Note these effects sum to zero}$$

$$\text{Variety 4: } 3.5 - 3.0 = +0.5 \text{ t ha}^{-1}$$

$$\text{Variety 5: } 3.6 - 3.0 = +0.6 \text{ t ha}^{-1}$$

# ANOVA & Blocking

## Hypotheses

- Just as there are two equivalent models, so the null and alternative hypotheses have two equivalent expressions:
  - Null hypothesis:  $H_0: \mu_1 = \mu_2 = \dots = \mu_t$
  - Alternate hypothesis:  $H_1: \text{not all treatment means are equal}$
- 
- Equivalent hypotheses:
  - Null hypothesis:  $H_0: \tau_i = 0, \quad i = 1, \dots, t$
  - Alternate hypothesis:  $H_1: \text{Not all } \tau_i = 0 \text{ (not all treatment effects are equal)}$

# ANOVA & Blocking

## Example – Nitrogen Fertiliser on Rice

- ANOVA

Source of Variation	Degrees of Freedom (df)	Sum of Squares (SS)	Mean Square (MS)
Blocks	3	2.738	0.913
Treatment	14	42.952	3.068
Residual	42	6.210	0.148
Total	59	51.899	

- Blocks  $df = b - 1 = 4 - 1 = 3 \quad \checkmark$
- Treatment  $df = t - 1 = 15 - 1 = 14 \quad \checkmark$
- Residual  $df = (b - 1)(t - 1) = 3 \times 14 = 42 \quad \checkmark$
- Test statistic:  $F = 3.068 / 0.148 = 20.75$  with  $df = 14, 42$

Blocking reduced Residual SS by 1/3!

Note 2 numbers for F-test df.

# ANOVA & Blocking

## Example – Nitrogen Fertiliser on Rice

- Calculate the probability of obtaining the observed test statistic, or something larger.  $P = P(F_{14, 42} > 20.75) < 0.001$
- So there are significant differences between the mean rice yields across the 15 treatments (3 variety  $\times$  5 nitrogen)
- **If Block had been ignored**, then
  - Residual SS =  $6.210 + 2.738 = 8.948$ , with
  - Residual df =  $42 + 3 = 45$ , and hence
  - Residual MS =  $8.948 / 45 = 0.199$  (larger)
- Consequently, the test statistic would have been smaller,  
 $F = 3.068 / 0.199 = 15.43$  with  
df = 14, 45, giving  $P < 0.001$
- Still highly significant – but not as much as with blocking

# ANOVA & Blocking

## Example – Nitrogen Fertiliser on Rice

- Blocking was successful, as it captured some of the unexplained variance.
- If blocking is effective, then usually:
  - Residual MS of blocked design is smaller
  - $F$ -ratio is large
  - $P$ -value is smaller  $\Rightarrow$  easier to detect a single from the noise
- Proportion of variability accounted for by:
  - Blocks: 
$$\frac{\text{Block SS}}{\text{Total SS}} = \frac{2.738}{51.899} = 0.053 \text{ (5.3\% variation)}$$
  - Treatments: 
$$\frac{\text{Treatment SS}}{\text{Total SS}} = \frac{42.952}{51.899} = 0.828 \text{ (82.8\% variation)}$$

# ANOVA & Blocking

## Evaluating and Comparing Treatment Means

- The best estimate of  $\sigma^2$  is (as always) the Residual MS = 0.148.
- Each of the 15 treatment means is a mean of  **$b = 4$  observations (replicates)**
- SE of a mean =  $se(\bar{y}) = \sqrt{\frac{\text{Residual MS}}{\text{replicates}}} = \sqrt{\frac{0.148}{4}} = 0.192 \text{ t ha}^{-1}$
- 95% CI =  $\bar{y} \pm t_{\text{residual df}}^{0.025} \times se(\bar{y})$
- Residual df = 42 so  $t_{\text{residual df}}^{0.025} \times se(\bar{y}) = 2.018 \times 0.192 = 0.388 \text{ t ha}^{-1}$
- e.g. 95% CI for Treatment 1 mean =  $3.124 \pm 0.388 = 2.74$  to  $3.51 \text{ t ha}^{-1}$

# ANOVA & Blocking

## R Code & Output

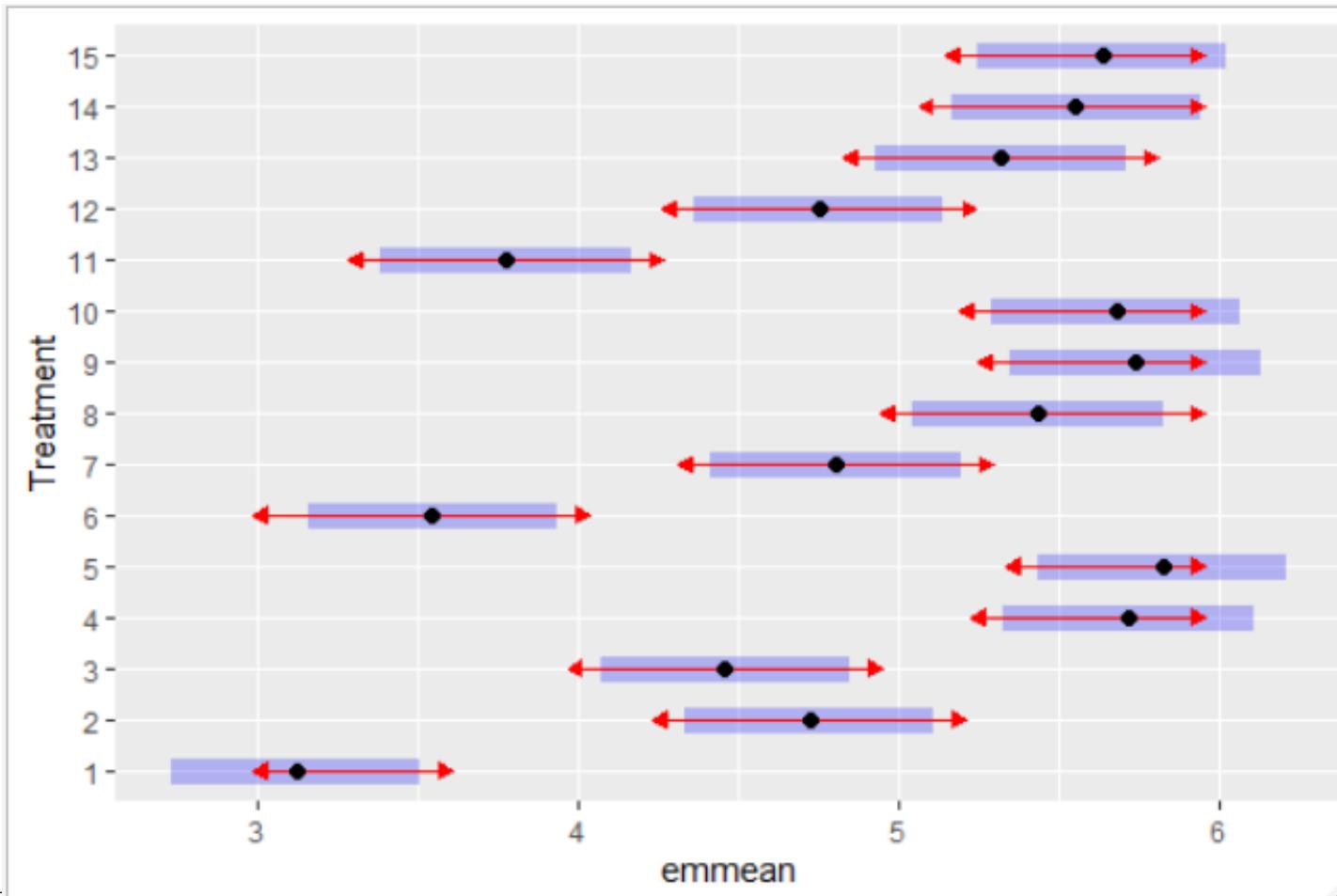
```
- rice.aov <- aov(Yield ~ Block + Treatment, data = rice)
```

```
> summary(rice.aov)
   Df Sum Sq Mean Sq F value    Pr(>F)
Block      3   2.74  0.9126   6.172  0.00143 ***
Treatment  14  42.95  3.0680  20.750 1.85e-14 ***
Residuals 42   6.21  0.1479
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

# ANOVA & Blocking

## Evaluating and Comparing Treatment Means

```
> plot(emmeans(rice.aov, ~ Treatment), comparisons = TRUE)
```

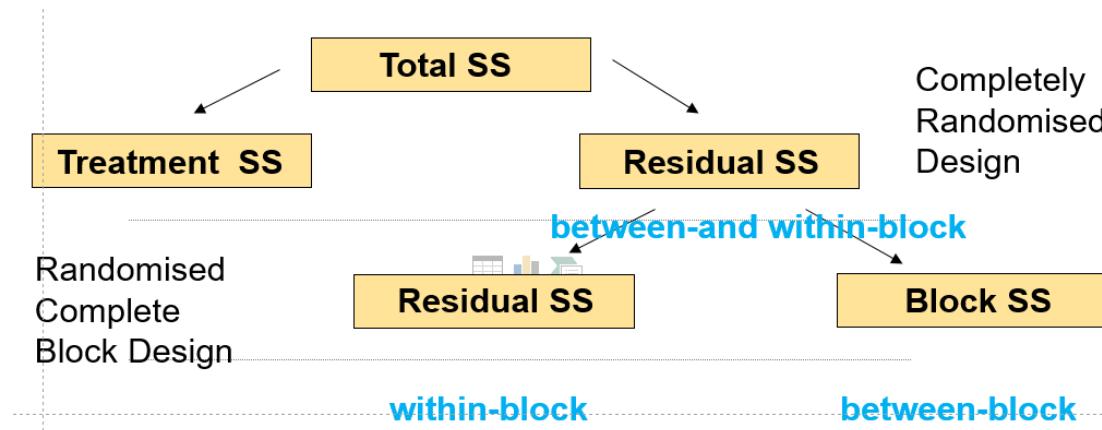


# Summary

```
> summary(rice.aov)
      Df Sum Sq Mean Sq F value    Pr(>F)
Block       3   2.74  0.9126   6.172  0.00143 ***
Treatment  14  42.95  3.0680  20.750 1.85e-14 ***
Residuals  42   6.21  0.1479
---
signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## Strata of Random Variation

- The **Blocking Structure** in the **physical layout** of the experimental design is specified.
- For each experimental unit (e.u.) or grouping of e.u.s. (e.g. Block for a RCBD) a term is required to be in the model: This takes account of random variation due to extraneous features not due to the treatment.
- There will be a separate **stratum** for each source of random variation shown in the ANOVA table



# Summary

## Definitions

- **Treatment Design** is the selection of treatments for an experiment, both the factors, as well as the levels of each factor.
- **Experimental Design** refers to the way we allocate treatments to the experimental units (e.g. plot of land, animals).
- When using analysis of variance procedures, these can also be termed the **Treatment Structure** and **Blocking Structure**.

## Resources

- Quinn & Keough (2002) or (2024). Experimental Design and Data Analysis for Biologists
- Fox, G. A., S. Negrete-Yankelevich, and V. J. Sosa. (2015). Ecological statistics: contemporary theory and application. Oxford University Press, USA.
- Logan (2010) Biostatistical design and analysis using R a practical guide
- Ebooks in library

– Questions/Feedback?



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