Experimental design - an introduction

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Experimental Design

What is Experimental Design?

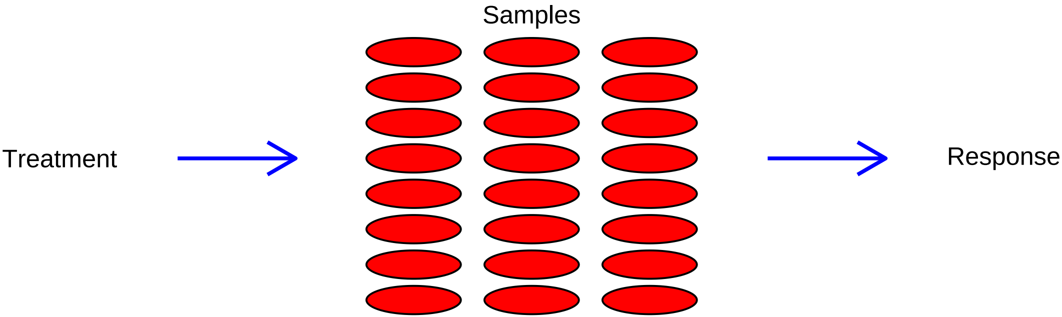
Why is Experimental Design Important?

What is Experimental Design?

- local controls
- how to assign treatments to samples? (blocking/randomisation)
- how many samples? (replication)

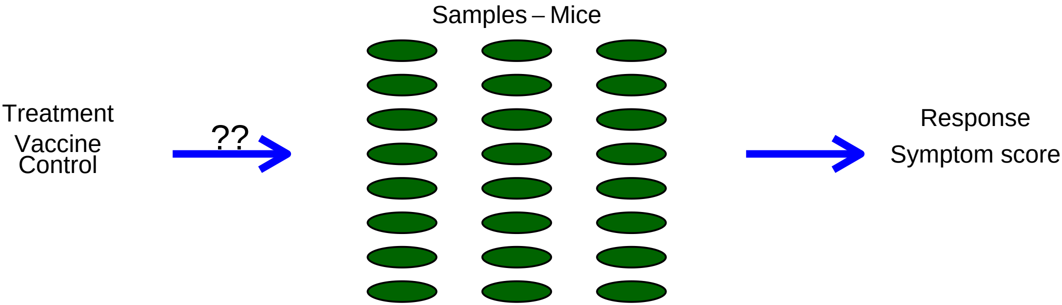
What is Experimental Design?

Assigning treatments to samples



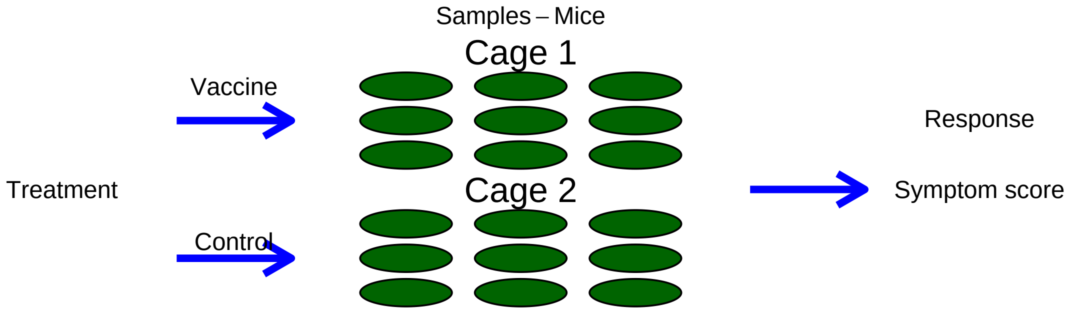
What is Experimental Design?

Assigning treatments to samples: Vaccine Challenge Study



What is Experimental Design?

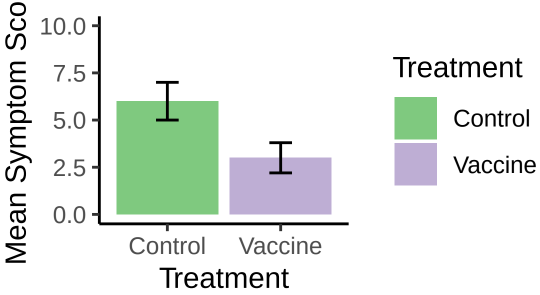
Assigning treatments to samples: Vaccine Challenge Study - by Cage?



What is Experimental Design?

Results of Vaccine Challenge Study: lm(score~Treatment)

Question: Evidence of a Vaccine effect, or a Cage effect?



What have we learned about experimental design so far?

- Cage is confounded with treatment.
- Cannot separate out the Cage effect from the Treatment effect.

Conclusion: We need to think harder about how to assign treatments to samples.

Goal: separate out Treatment Effects from effects from other factors

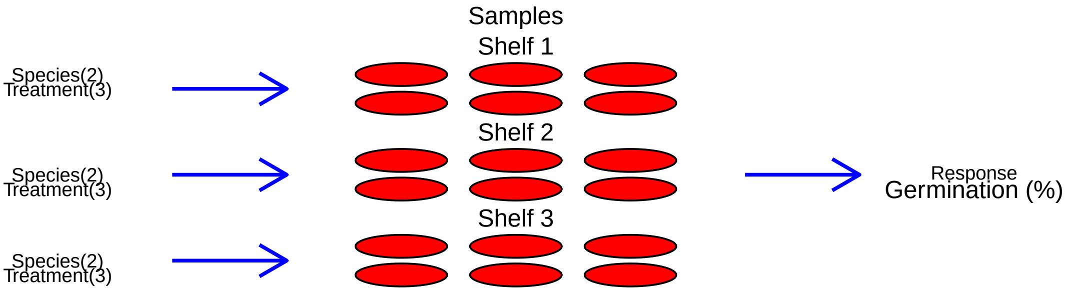
Why is Experimental Design Important?

- Designing experiment in advance saves time and energy in analysis

- Good experimental design makes analysis more robust and convincing

What is Experimental Design?

Assigning treatments to samples: Seed Germination Study



Good ideas in Experimental Design

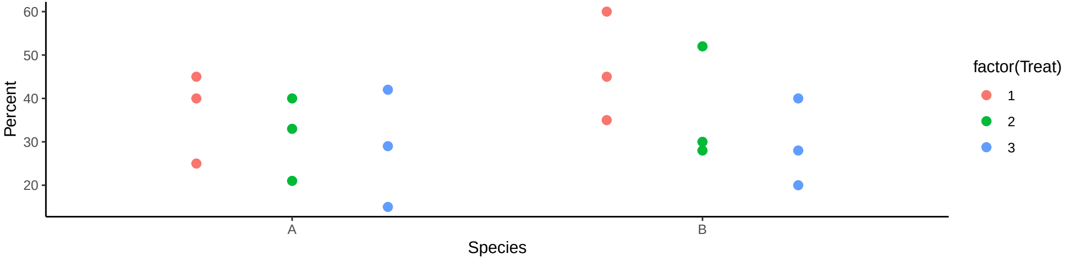
- Samples are BLOCKED into homogeneous groups
- Treatment RANDOMISED within each block
- One sample per treatment within block

Treatments are compared within blocks.

Treatment effects are averaged across blocks.

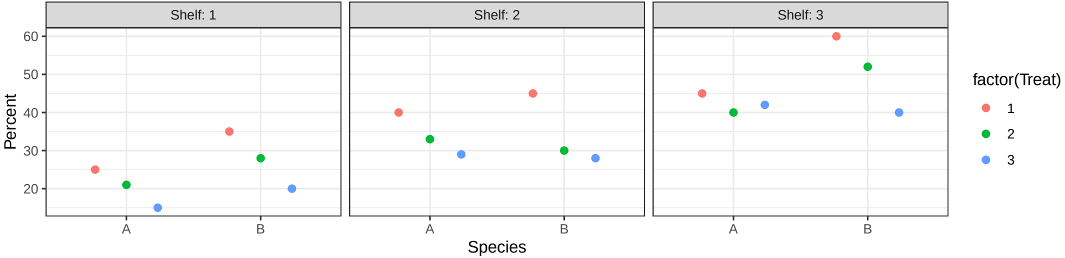
Analysing Complete Randomised Block Experiment

Exploratory Analysis



Analysing Complete Randomised Block Experiment

Exploratory Analysis



Include Experimental Design in analysis: Shelf absent

```
library(car)
Anova(lm(Percent~Species*Treat, data = dat4), type = 2)
```

```
## Anova Table (Type II tests)
##
## Response: Percent
##                Sum Sq Df F value Pr(>F)
## Species        128.00  1  0.9366 0.3523
## Treat          488.44  2  1.7870 0.2093
## Species:Treat   65.33  2  0.2390 0.7911
## Residuals     1640.00 12
```

Include Experimental Design in analysis: Shelf as Fixed Effect

```
Anova(lm(Percent~Species*Treat + Shelf, data = dat4), type = 2)
```

```
## Anova Table (Type II tests)
##
## Response: Percent
##                Sum Sq Df F value    Pr(>F)
## Species        128.00  1 10.9819 0.0078277 **
## Treat          488.44  2 20.9533 0.0002654 ***
## Shelf         1523.44  2 65.3527 1.813e-06 ***
## Species:Treat   65.33  2  2.8027 0.1080523
## Residuals      116.56 10
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Include Experimental Design in analysis: Shelf as Random Effect

```r
library(lmerTest)
anova(lmer(Percent~Species*Treat + (1|Shelf), data = dat4))
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Species        128.00 128.000     1    10 10.9819 0.0078277 **
## Treat          488.44 244.222     2    10 20.9533 0.0002654 ***
## Species:Treat   65.33  32.667     2    10  2.8027 0.1080523
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Summary so far

- In this example, Block = Shelf
- Treatments are compared within blocks
- Blocking: More power to see treatment effects
- Good experimental design: maximize information about treatment effects

Key ideas in Experimental design

<u>Block</u>: group of samples that are similar biologically and exposed to similar environmental conditions.

<u>Randomisation</u>: Samples within a block are <u>randomised</u> to different treatments.

Block effects can be separated from Treatment effects

Example: Greenhouse experiment

Response:

- Plant dry mass (g)

Experimental factors

- Nutrients (4 levels)
- Species (3 levels)

Potential Blocks

- table within greenhouse
- position on table
- tray
- position on tray

Example: Animal experiment (mice)

Response:

- Tumour size (mm^2)

Experimental factors

- Drug A (Yes/No)
- Diet (2 levels)

Potential Blocks

- litter
- cage

Example: Multi-centre clinical trial (Chronic Fatigue)

Response:

- Chronic Fatigue Score (0-100) at 6 months

Experimental factors

- Drug X (vs placebo)

Potential Blocks

- centre
- risk factors
- patient

Example: lab experiment (96 well plate): Testing chemotherapies on cancer cell line

Response:

- Cell density (OD)

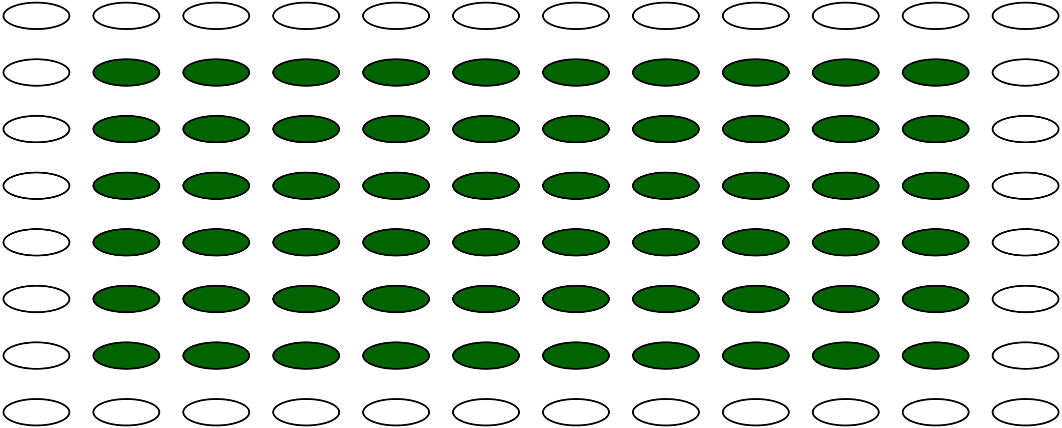
Experimental factors

- Drug A (Yes/No)
- Drug B (High/low/none)

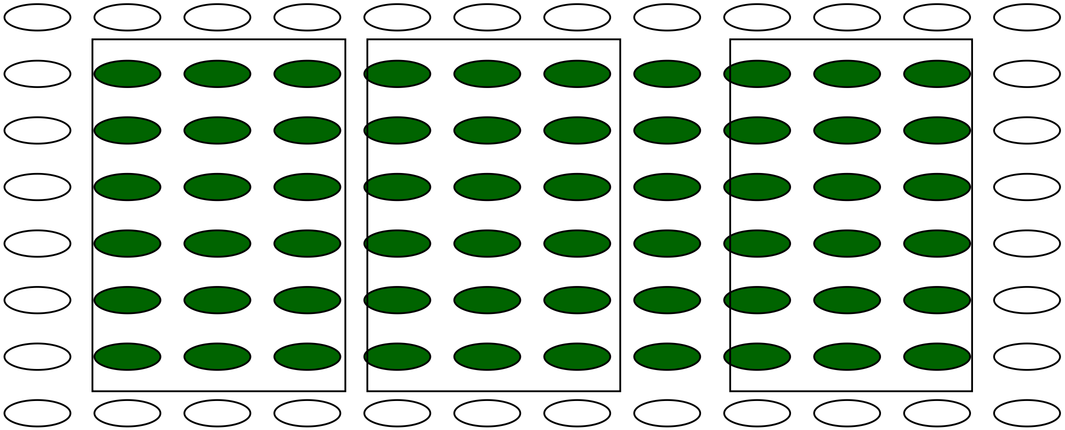
Potential Blocks

- plate
- position on plate

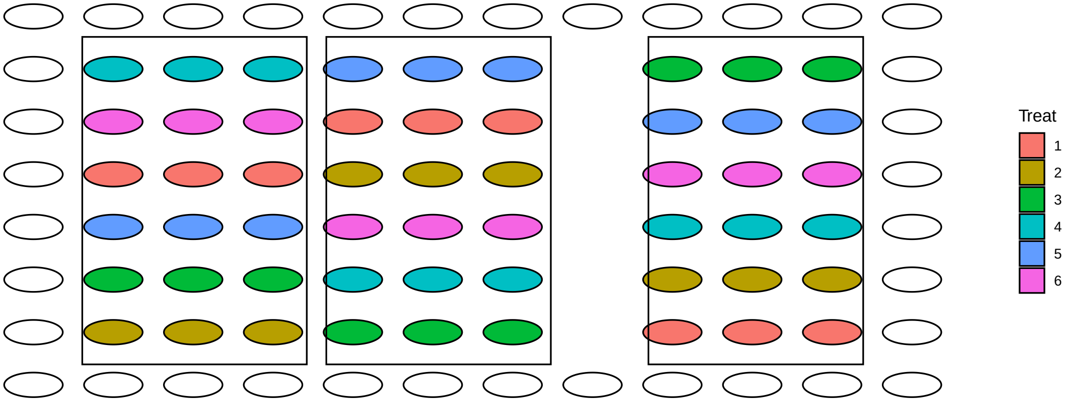
Example: lab experiment (96 well plate)



Example: lab experiment (96 well plate)



Example: lab experiment (96 well plate)



Blocks in Time and Space- Playback experiments

Birds communicating danger: Alarm calls / fleeing behaviour

Response

- Bird flees (Yes/No)

Experimental factor

- Alarm call (5 levels)

Potential blocking factors

- bird
- order of alarm call

Blocks in Time and Space - Playback experiments

Each bird (1-5) receives playback calls (A - E) in different order:

		BIRD				
		1	2	3	4	5
	1	B	D	E	C	A
	2	A	C	D	B	E
ORDER	3	C	E	A	D	B
	4	E	B	C	A	D
	5	D	A	B	E	C

Summary for today's lecture

- Experimental design matters for all biological experiments
- BLOCKING and RANDOMISATION cornerstones of good experimental design
- BLOCK first; RANDOMISE treatments within blocks
- Before you start collecting data, discuss your experimental design with colleagues.

Friday activity

APPLYING PRINCIPLES OF EXPERIMENTAL DESIGN