

# Experimental Design

LECTURE 16

## Objectives

- ▶ Define terms.
- ▶ Explain why experimental design is necessary and why it is particularly important for researchers in the life sciences.
- ▶ Describe how the question and hypothesis influence experimental design.
- ▶ Explain the role of randomization and replication in experimental design.
- ▶ Define and identify examples of pseudoreplication.
- ▶ Discuss factors that influence sampling and describe sampling systems.

## Objectives

- ▶ Discuss the different types of control treatments and the applicability to different problems.
- ▶ Explain blocking and its uses.
- ▶ Define the different experimental designs.

## Overview

- ▶ This is a brief introduction to the possibility of more complex research and the need to more seriously consider experimental design.
  - ▶ The scientific method we discussed earlier is very basic.
  - ▶ Expand the portion on developing and executing experiments to setup more complex analyses.
  - ▶ Multivariate Statistics

## Overview

- ▶ Appropriate design of an experiment is the key to the successful answering of a research question.

## Overview

- ▶ Good experimental design is particularly important to life scientists.
  - ▶ Minimize within population variation (random variation or noise)
  - ▶ Account for confounding factors (control variables)
- ▶ Experimental design is less critical for other sciences.
  - ▶ Less random variation
  - ▶ Simpler systems – easier to identify confounding variables and control them.

## Overview

- ▶ Experimental design is about removing or controlling variation due to factors that we are not interested in (random variation or confounding variables) so that we can observe the effects of those factors that do interest us.

## Question/Hypothesis

- ▶ Good experimental design begins with a good question and a good hypothesis.
- ▶ Question
  - ▶ Definable, measurable, and controllable.
- ▶ Hypothesis
  - ▶ Falsifiable
  - ▶ Allows the gathering of data to support or refute the explanation.

## Question/Hypothesis

- ▶ Hypotheses
  - ▶ Supported by observation and understanding of how the system might work.
    - ▶ Read
  - ▶ Alternate hypothesis
  - ▶ Null hypothesis
- ▶ Hypothesis sets
  - ▶ Exhaustive
  - ▶ Exclusive

## Question/Hypothesis

- ▶ Question → Hypothesis → Prediction
- ▶ Useful exercise.
- ▶ We test the prediction. (Statistics are useful here)
  - ▶ If the prediction is correct, then the hypothesis is supported.
  - ▶ If the prediction is incorrect, then the hypothesis is refuted.
- ▶ The prediction needs to give us something we can measure.
  - ▶ Indirect measures
    - ▶ Surrogate measures
  - ▶ Direct measures

## Question/Hypothesis

- ▶ Be careful doing an experiment when only one result (prediction) is interesting.
  - ▶ Experiments that confirm our understanding of the system and do not offer new perspectives are generally considered uninteresting or unpublishable.
  - ▶ Interesting questions
    - ▶ New areas – new questions (non-trivial)
    - ▶ Overturn our understanding
    - ▶ New perspective on our current understanding.

## Experimental vs. Observational

- ▶ Different types of studies – Affects the design of the study
  - ▶ Experimental
    - ▶ Manipulative
    - ▶ Preference
  - ▶ Observational
    - ▶ Comparative
    - ▶ Correlative

## Experimental vs. Observational

- ▶ Advantages of observational studies
  - ▶ Often easier to conduct
  - ▶ Reduced possibility of unintended consequences due to the study.
  - ▶ Biologically relevant variation (level of treatment).

## Experimental vs. Observational

- ▶ Advantages of experimental studies
  - ▶ More certain identification of third variables.
  - ▶ More certain recognition of reverse causation.

## Experimental vs. Observational

- ▶ In some situations, experimental studies may be undesirable.
  - ▶ Unethical
  - ▶ Impossible
    - ▶ Resources
    - ▶ Scale

## Replication

- ▶ One way to cope with random variation is to measure a number of different experimental subjects rather than just one – replicates.
  - ▶ Pseudoreplication – Non-independent replicates
    - ▶ Common environment
    - ▶ Relatedness
    - ▶ Pseudoreplication of manipulation
    - ▶ Temporal

## Randomization

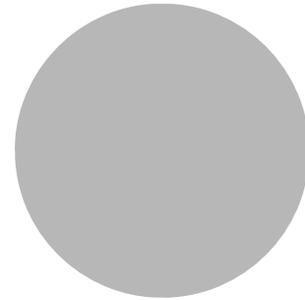
- ▶ Any individual has the same chance of finding itself in each experimental group.
- ▶ Helps control the effect of confounding variables.
- ▶ Problems
  - ▶ Haphazard/arbitrary sampling

## Sampling

- ▶ A subset of the population used to represent the population.
- ▶ Sample unit
  - ▶ What are you trying to compare?
- ▶ Sample size
  - ▶  $\alpha$
  - ▶  $\beta$
  - ▶  $\delta$
  - ▶  $\sigma^2$

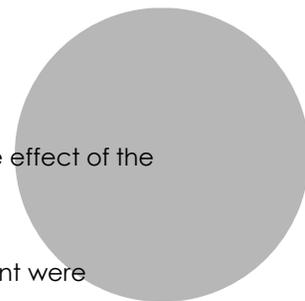
## Sampling

- ▶ Stratified Random Sample
  - ▶ Better in a heterogeneous environment.
  - ▶ Make sure that each strata is sampled adequately.
- ▶ Timing of samples
  - ▶ Introduction of temporal variation in the environment.



## Control Treatments

- ▶ Control treatment
- ▶ Procedural control
  - ▶ Distinguish between the effect of the manipulation and the effect of the procedure.
- ▶ Temporal control
  - ▶ Making sure that the control and the manipulated treatment were comparable prior to one another prior to the manipulation.



## Control Treatments

- ▶ Experimental control
  - ▶ Manipulation of all factors in the environment that might influence the dependent variable.
  - ▶ Artificiality
- ▶ Statistical control

## Terms

- ▶ Balanced – Equal numbers of observations in each treatment.
- ▶ Factors – Independent variables
- ▶ Levels – Treatments under a single factor
- ▶ Covariate – A ratio or interval factor that you would like to control or eliminate the effect.

## Blocking

- ▶ It is a way to control (eliminate) the effect of a variable that might influence the dependent variable.
- ▶ Divide the variable whose effect we want to eliminate in to ordinal groups (blocks) and then randomly assign subjects of each treatment group to the blocks.
- ▶ Somewhat like pairing the samples.
- ▶ Demonstrate

## Blocking

- ▶ Better control of the blocked variable.
- ▶ Decrease in power
- ▶ Blocking is better with larger sample sizes, but is not advised with smaller sample sizes.
- ▶ Not an actual design – an element of designs

## Paired Design

## Cross-Over Design

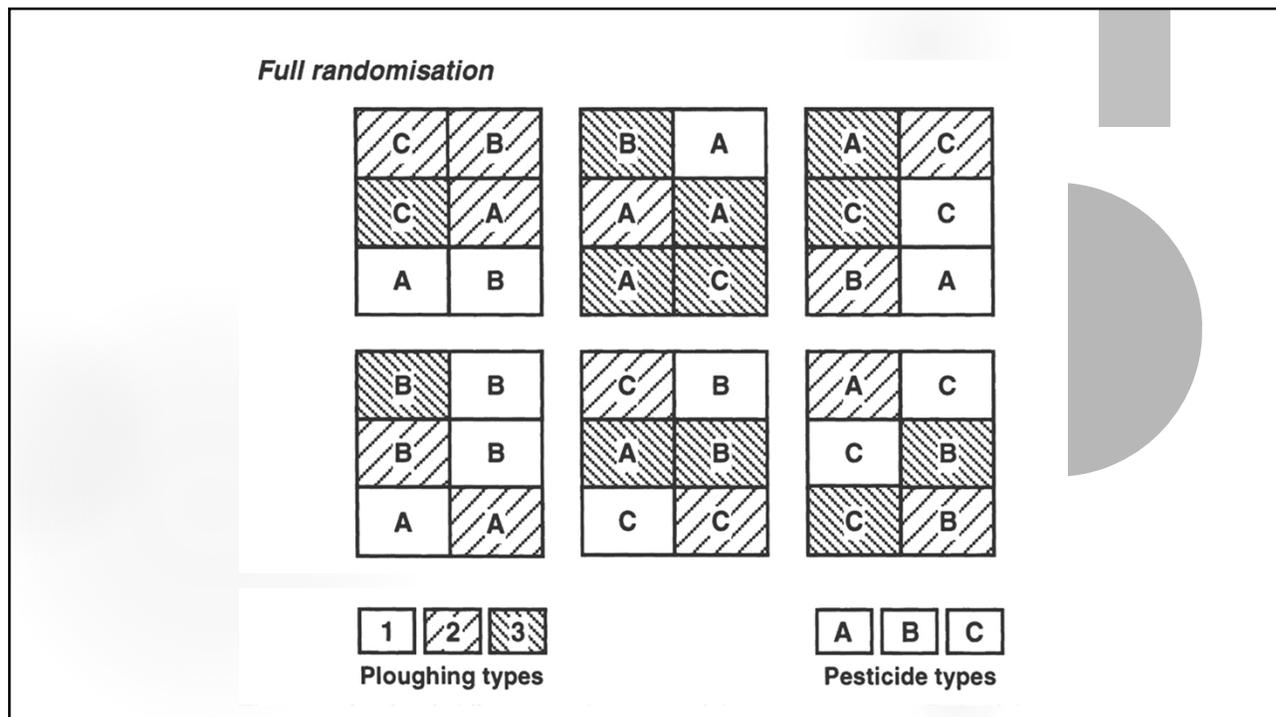
- ▶ Individual subjects are sequentially exposed to all treatments.
- ▶ Some researchers call this a repeated measures design – it is not. Repeated measures is something different (in a moment).
- ▶ Problems with independence of samples – handled with advanced statistics.
  - ▶ Similar to pairing

## Repeated Measures Design

- ▶ Individuals are assigned to one treatment, but are measured multiple times during the experiment.
- ▶ Problems with independence of samples – handled with advanced statistics.
  - ▶ Similar to pairing

## Completely Randomized Design

- ▶ Experimental subjects/units are randomly assigned to treatment groups.
- ▶ Simple to design.
- ▶ Problems with comparisons across individuals
  - ▶ Reduced if among between individual variation is low.



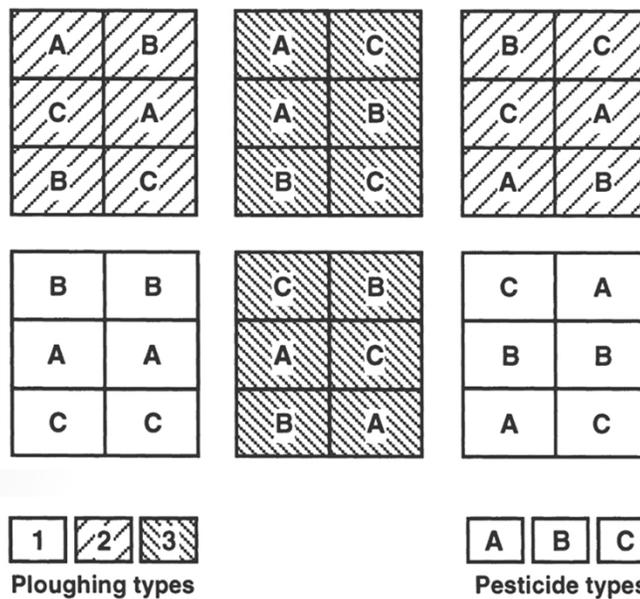
## Split Plot Designs

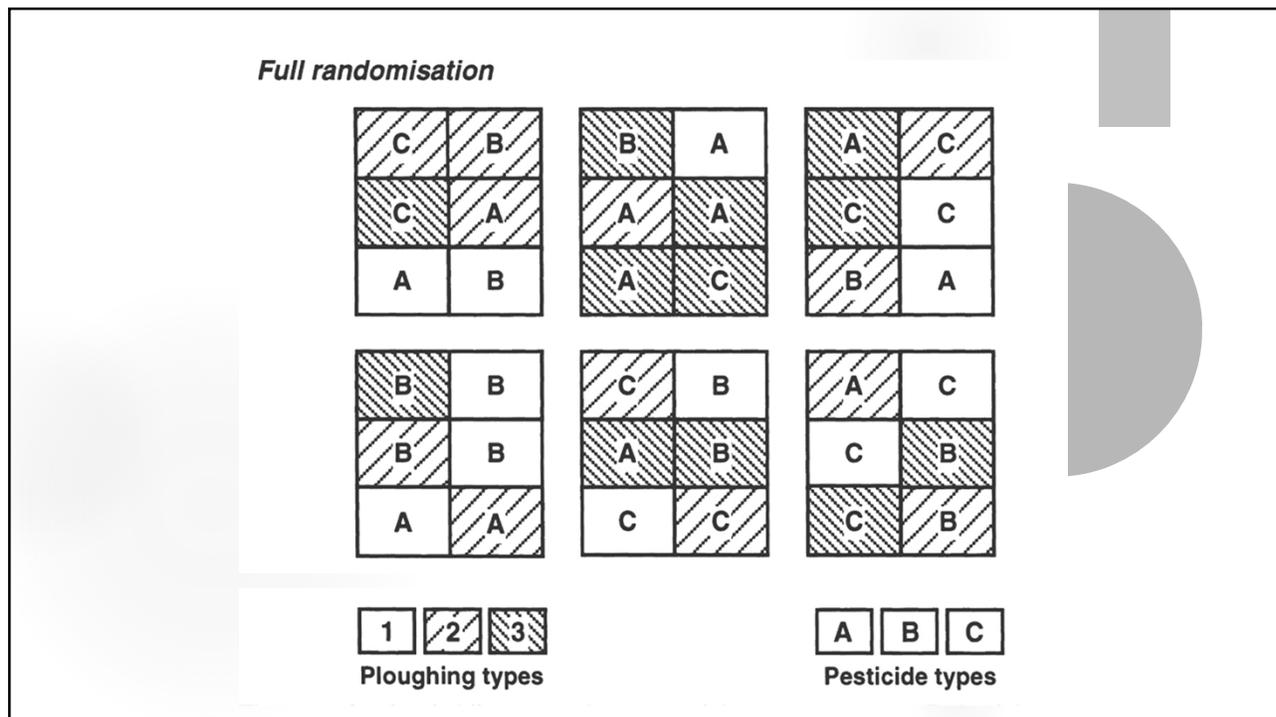
- ▶ Two factors in which we are interested.
  - ▶ Main-plot factor – Usually the factor that we expect to have the greater effect.
  - ▶ Sub-plot factor – Usually the factor that we expect to have less effect (more difficult to detect)
  - ▶ Design increases the ability to detect a sub-plot factor effect.

## Split Plot Designs

- ▶ Large groups are randomly assigned to the main-plot treatments and then within those groups randomly assigned to sub-plot treatments.
- ▶ Analyzed with a modified Two-way ANOVA
- ▶ Example
- ▶ Contrast with a complete randomized design.

*Split plot*





## Latin Square Design

- ▶ A variation on the complete randomized design.
- ▶ Blocked on two variables.
- ▶ The number of levels in the independent variable dictates the number of levels on both of the blocked variables.
- ▶ Very efficient design
- ▶ Very constraining and rarely the appropriate design because of those constraints.