DESIGN FOUNDATIONS

DEAR · CLASS 1

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TODAY'S THREE ACTIVITIES

1. Why experimental design matters
2. Experimental and observational units
3. Replication, subsampling and pseudoreplication

> How many independent pieces of experimental information do we really have?

WHY DESIGN MATTERS

ACTIVITY 1 · 20 MINUTES

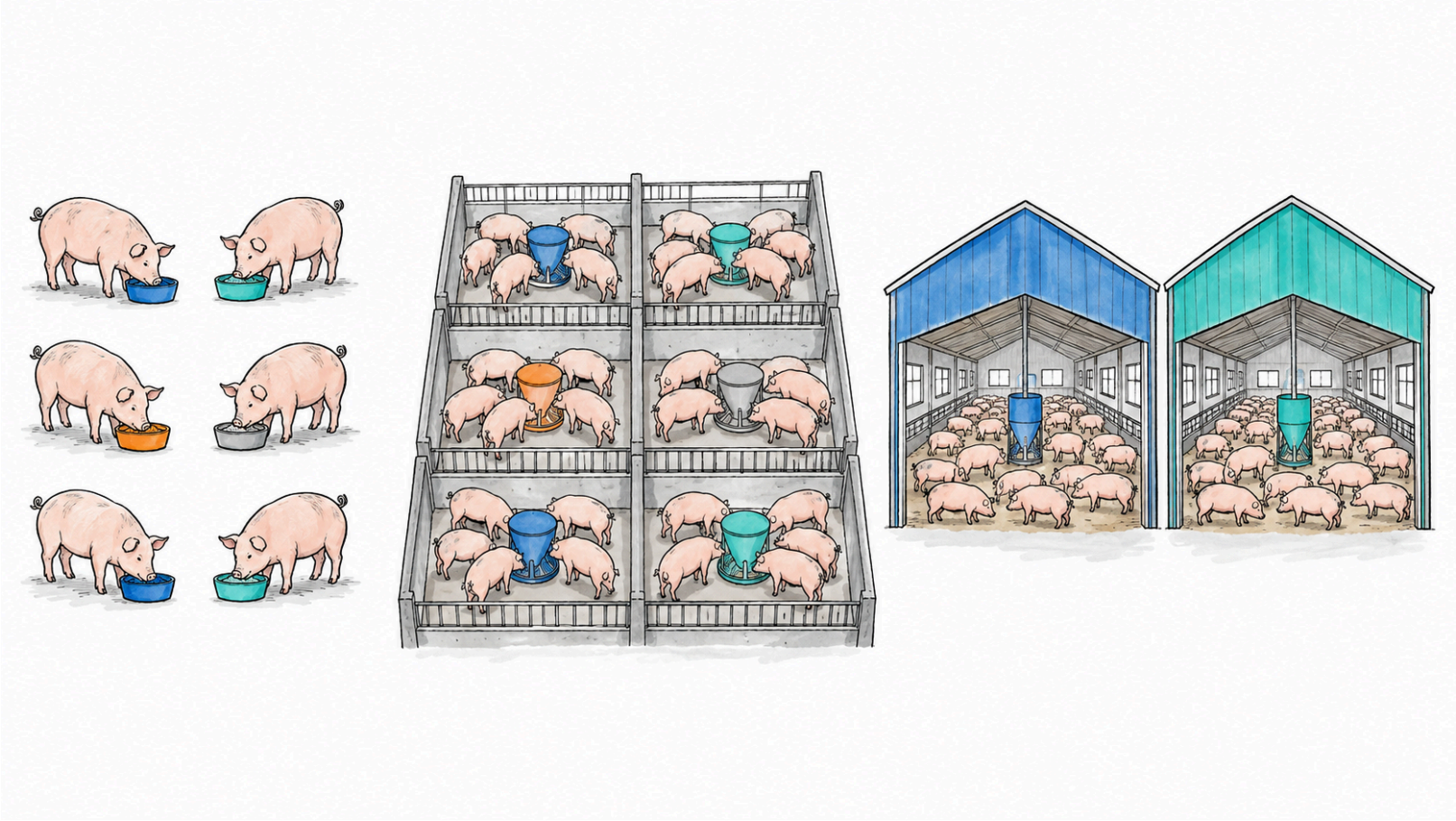
ONE BIOLOGICAL QUESTION

> Does a feed additive increase pig growth?

The question stays the same, but researchers could apply the diets to:

- individual pigs fed separately
- pens with a shared feeder
- whole barns

THREE POSSIBLE STUDIES



EVIDENCE DIFFERS

Study	Independent treatment applications	What many pig weights add
Pigs fed separately	Many pigs per diet	Information about pigs and their variation
Several pens per diet	Several pens per diet	Information about pigs within each pen
One barn per diet	One barn per diet	Detailed description of each barn

> The number of animals measured does not by itself tell us how strongly the treatment has been tested.

THE TREATMENT COMPARISON

Before collecting data, the design determines:

- which treatments can be compared
- what receives each treatment independently
- where biological variation enters the comparison
- which observations belong together
- how far the conclusion can reasonably extend

LIMITS OF ANALYSIS

An analysis can describe the data collected from two barns in great detail.

It cannot create additional barns that received the treatments independently.

> If the barns differ, which difference should we attribute to the feed additive?

COSTS OF POOR DESIGN

- The treatment effect may remain inseparable from another difference.
- A large dataset may give unwarranted confidence.
- Time, money and animals may be used without answering the intended question.
- A later statistical adjustment may require assumptions the design could have avoided.

> Good design creates a defensible treatment comparison before measurement begins.

FIRST DIAGNOSIS

For each pig study, discuss:

1. What receives the feed treatment?
2. Could another pig, pen or barn receive the other diet independently?
3. How many independent applications of each diet are present?
4. What alternative explanation remains?

UNITS

ACTIVITY 2 · 25 MINUTES

EXPERIMENTAL AND OBSERVATIONAL UNITS

EXPERIMENTAL UNIT

The **experimental unit** is the smallest unit that can be independently assigned to, or independently receive, a treatment.

The experimental unit follows the application of the treatment. It need not be an individual animal.

OBSERVATIONAL UNIT

The **observational unit** is the unit on which a response is measured or observed.

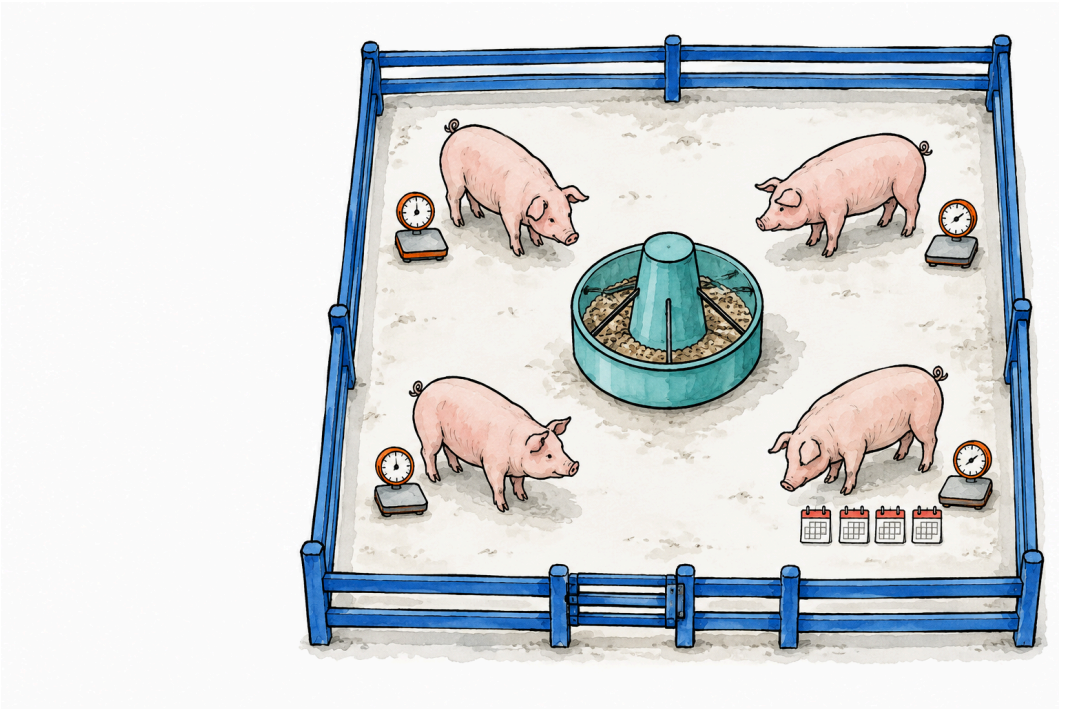
The experimental and observational units may be the same, but they do not have to be.

PEN TREATMENT

Thirty-two pigs live four per pen.

Eight pens receive one of two diets. Every pig is weighed weekly.

- Treatment: diet
- Experimental unit: **pen**
- Observational unit: **pig**



ONE DESIGN, SEVERAL UNITS

Unit	Role in the pen study	Number
Pen	Receives a diet independently	8
Pig	Provides a body-weight observation	32
Pig at a week	Provides a repeated observation	256 if measured for 8 weeks

All three counts describe the study. Only the eight pens count independent treatment assignments.

INDIVIDUAL TREATMENT

Twenty-four dogs are randomly allocated to three diets. Each dog eats separately from its own bowl. Body weight is recorded weekly.

Question	Answer
What receives diet independently?	Each dog
Experimental unit	**Dog**
Observational unit	Dog at each weighing time
Independent treatment replication	8 dogs per diet

TREATMENT WITHIN AN ANIMAL

Twelve dogs each have two similar affected skin areas. Within every dog, one area receives cream A and the other receives cream B.

- The treated skin area is the experimental unit.
- The response is the severity score for each area.
- The two areas form a paired comparison within a dog.

> The experimental unit can be part of an animal when treatment can be applied independently at that level.

MATERNAL TREATMENT

Sixteen sows receive either a prenatal supplement or the standard diet. Birth weight and survival are recorded for every piglet.

Design element	Unit
Prenatal treatment applied to	**Sow**
Experimental unit	**Sow**
Outcomes observed on	Piglets within each litter

> Could two piglets from the same sow receive different prenatal assignments?

TWO DIAGNOSTIC QUESTIONS

1. What was independently assigned to treatment?
2. What would appear as one row in the randomisation list?

Then ask separately:

- What was measured?
- How often was it measured?
- How are observations grouped within experimental units?

> Begin with treatment allocation, not with the longest column in the dataset.

UNIT CHECK

Six tanks contain ten trout each. Three tanks receive cool water and three receive warm water. Every fish is weighed after six weeks.

Name the treatment, experimental unit, observational unit and number of independent replicates per temperature.

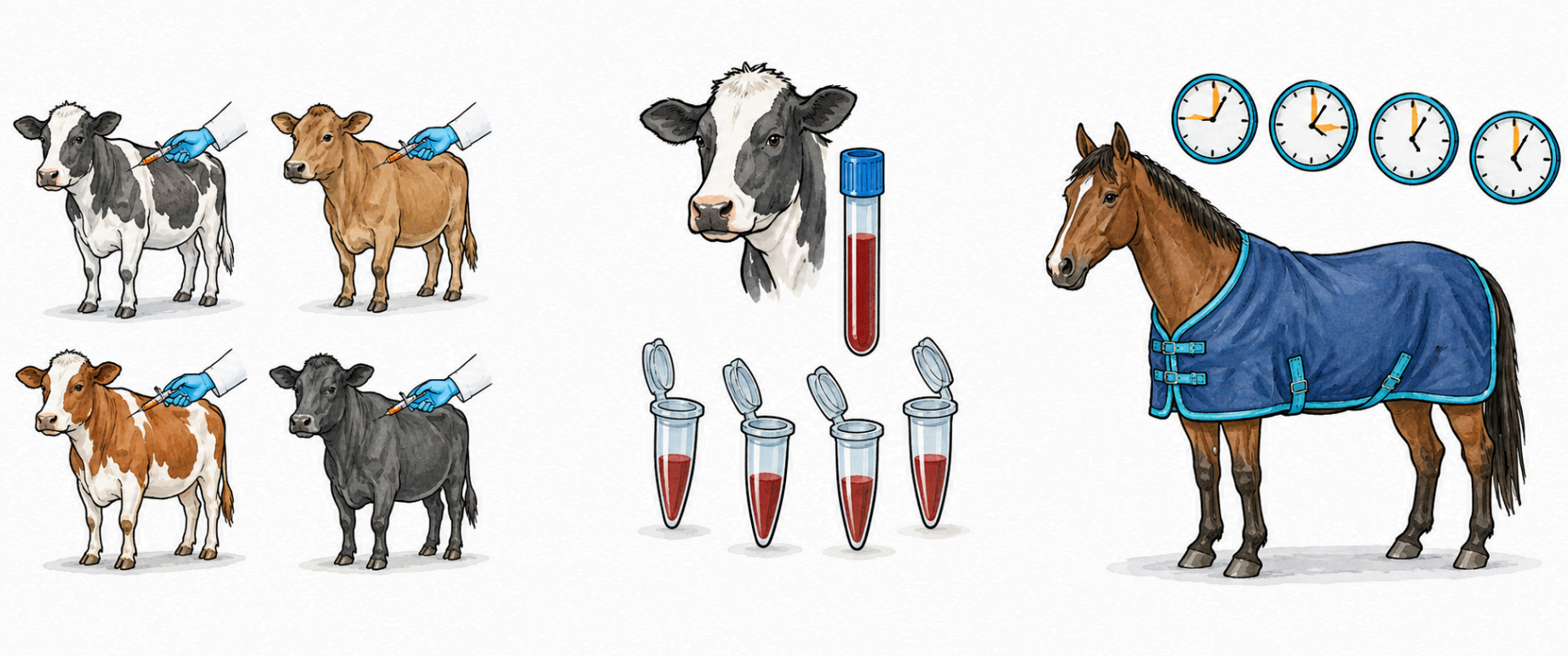
Treatment: temperature. Experimental unit: tank. Observational unit: fish. Replication: three tanks per temperature.

REPLICATION

ACTIVITY 3 · 15 MINUTES

SUBSAMPLING AND PSEUDOREPLICATION

THREE KINDS OF INFORMATION



TRUE REPLICATION

Replication means applying each treatment independently to more than one experimental unit.

Replication lets us compare the treatment pattern with variation among experimental units receiving the same treatment.

SUBSAMPLING

A **subsample** is an observation taken from within an experimental unit.

Subsampling can:

- describe variation within an experimental unit
- improve the precision of a unit-level response
- reveal measurement or sampling error

It does not add independent applications of the treatment.

REPEATED OBSERVATIONS

Measurements through time describe change within the same experimental unit.

For a horse measured at 0, 10, 20 and 30 minutes:

- the four heart-rate values describe its recovery profile
- values from the same horse are related
- the number of horses determines treatment replication

COUNT THE ASSIGNMENTS

Eight pens contain ten pigs each. Four pens receive diet A and four receive diet B. Every pig is weighed weekly for eight weeks.

8 pens, 80 pigs, 640 weight records

> Treatment sample size: eight independently treated pens, four per diet.

PSEUDOREPLICATION

Pseudoreplication occurs when observations that are not independent treatment replicates are analysed or interpreted as though they were independent replicates.

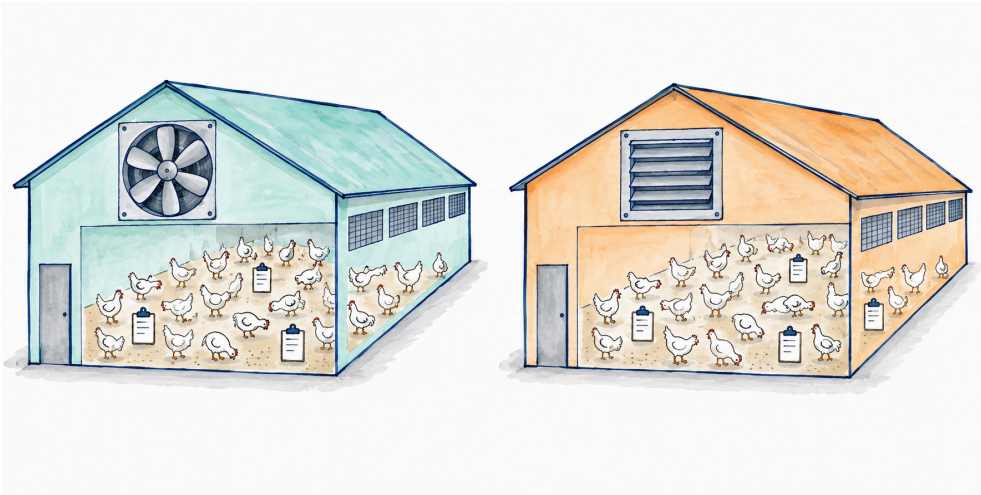
The problem is the mismatch between treatment allocation and the claimed evidence, not the collection of multiple observations itself.

ONE HOUSE PER TREATMENT

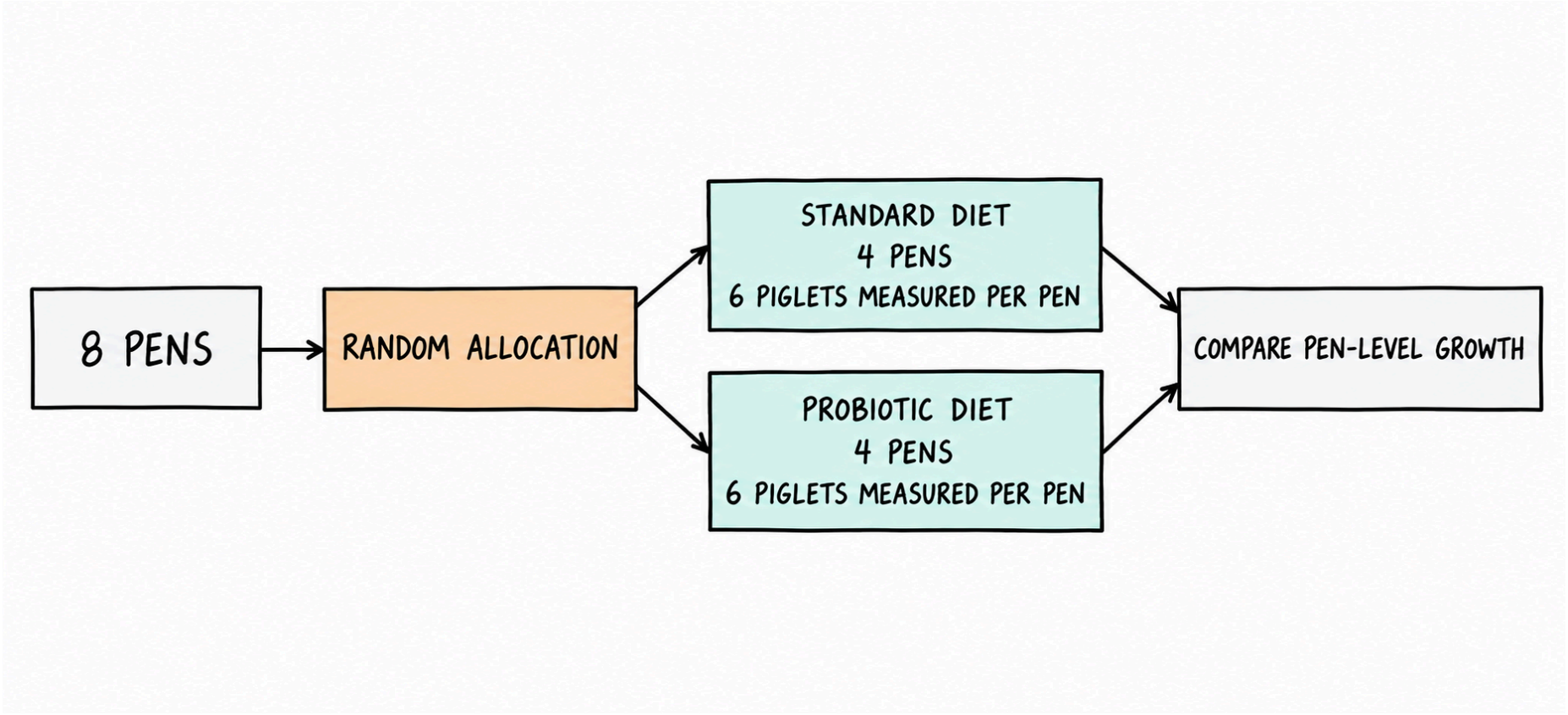
Two broiler houses each contain 1,000 birds.

- House 1 receives ventilation A.
- House 2 receives ventilation B.
- Every bird is measured.

The comparison has **one house per treatment**.



A DEFENSIBLE REPAIR



CLASSIFY THE INFORMATION

For each case, decide whether the extra observations provide treatment replication, subsampling or repeated measurement.

1. Twelve cows independently receive an injection, with one blood value per cow.
2. One blood sample from each cow is divided into three aliquots.
3. Each horse is measured four times during recovery.

> Case 1 provides replication. Case 2 provides subsampling. Case 3 provides repeated measurement.

THE DIAGNOSTIC TO KEEP

- Identify the treatment and how it is applied.
- Find the smallest unit that receives treatment independently.
- Count independent experimental units in each treatment.
- Identify observations, subsamples and repeated measurements within those units.
- Make the analysis and conclusion respect that structure.

> How many independent pieces of experimental information do we really have?

SOURCES

Ruxton, Graeme D., and Nick Colegrave. 2016. *Experimental Design for the Life Sciences*. 4th ed. Oxford: Oxford University Press.

- Chapter 1, sections 1.1–1.5, pp. 1–11
- Chapter 4, sections 4.1–4.2, pp. 46–57
- Chapter 5, sections 5.1–5.6, pp. 67–79
- Chapter 11, section 11.2.1, pp. 157–159

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