**1.1 Experimental Design: Units & Replication**

**✳️ Design Overview:**

* **50 plants**, randomly assigned to **2 treatments**:
  + **Self-pollinated (selfed)**
  + **Cross-pollinated (crossed)**
* On **each plant**, **5 flowers** are pollinated and seed count measured.

**🔍 Definitions:**

* **Experimental unit** = the smallest unit that receives a unique treatment **independently**.
  + ✅ **The plant** is the experimental unit here, because the treatment (selfed vs crossed) is applied at the plant level.
* **Measurement unit** = individual observations taken.
  + ✅ **Each flower** is a measurement unit.

**📊 Total units:**

* **Experimental units (true replicates):** 50 plants
* **Measurement units:** 50 plants × 5 flowers = **250 measurements**

**1.2 Independent Replicates per Treatment**

Assuming 25 plants per treatment:

* **25 independent replicates** in each treatment group (selfed & crossed).
* Each replicate (plant) has 5 flower-level measurements.

**1.3 Pseudoreplication: The Danger**

If you treat each **flower (n = 250)** as an **independent replicate** in your ANCOVA, you are **violating independence**—this is pseudoreplication.

**❌ Consequences:**

* **Underestimate variance** (you’re inflating sample size).
* **False positives**: higher Type I error (finding significant effects that aren't real).
* **Invalid inference** about treatment effects.

**1.4 How to Avoid Pseudoreplication**

✅ **Aggregate flower-level data at the plant level**, e.g., by using:

* **Mean seed number per plant** across 5 flowers.

Then fit ANCOVA using:

* **Treatment (selfed/crossed)**: categorical predictor
* **Plant size**: continuous covariate
* **Mean seeds per plant**: response

# Suppose you summarized the data to one row per plant

model <- lm(mean\_seed\_count ~ treatment \* plant\_size, data = plant\_summary)

summary(model)

This keeps **plant** as the true replicate, avoiding pseudoreplication.

**1.5 Word Equations for the Model**

**a) Including interaction:**

Seed production = effect of **pollination treatment** + effect of **plant size** + interaction of **treatment × size**

**b) Excluding interaction:**

Seed production = effect of **pollination treatment** + effect of **plant size**

**Definitions:**

* **Pollination treatment**: categorical predictor (selfed vs crossed)
* **Plant size**: continuous covariate
* **Interaction**: tests whether the slope of size on seed production differs between treatments

**1.6 Assumption Without Interaction**

If you **exclude the interaction**, you assume:

🔹 The **effect of plant size on seed production is the same** for both pollination types.  
In other words, both selfed and crossed plants have **parallel regression lines**.

**1.7 Graph: With Interaction**

A plot showing **different slopes** for each treatment group:

Y-axis: Seed Production

X-axis: Plant Size

Two lines:

- Selfed: positive slope

- Crossed: steeper positive slope (or maybe negative)

↳ The gap between lines changes with plant size.

**1.8 Graph: Without Interaction**

Two **parallel lines**, indicating:

* **Same slope** for both groups
* **Different intercepts** (i.e., treatment has an effect, but size influences both equally)

Y-axis: Seed Production

X-axis: Plant Size

Selfed: slope = 0.5

Crossed: slope = 0.5

→ Lines don't cross; vertical separation is constant

**.9 Graphs for Special Scenarios**

**a) No categorical effect, but continuous effect**

* **One regression line** for all data.
* Plant size influences seed production.
* Treatment has **no effect**.

Y-axis: Seed Production

X-axis: Plant Size

Single line with positive slope for both treatments; points from both overlap.

**b) No continuous effect, but categorical effect**

* **Two horizontal lines** (slopes = 0)
* Different intercepts for treatments.

Y-axis: Seed Production

X-axis: Plant Size

Selfed: flat line at 50

Crossed: flat line at 70

↳ Seed production differs by treatment, but not size.

Problem 2:

**2.1 Which model tests for equal covariate effects across groups?**

The model that **includes the interaction** between the categorical and continuous predictors does this:

Seed production = Pollination treatment + Plant size + Pollination treatment × Plant size

* ✅ This model tests if the **slope of the continuous predictor (plant size)** is **different for each treatment group** (i.e., checks for interaction).
* This allows you to **formally test** whether the covariate effect is **consistent across levels of the categorical predictor**.

➡️ So, **the model *with* the interaction term** from Problem 1.5a is the one that allows you to test this assumption.

**✅ 2.2 Which graph shows a violation of this assumption?**

The graph you drew in **Problem 1.7**—where **the slopes for selfed and crossed are not the same**—represents a **violation** of the assumption.

* In ANCOVA, we assume **parallel slopes** (i.e., same effect of plant size regardless of pollination treatment).
* **Different slopes = interaction = violation** of this core ANCOVA assumption.

**✅ 2.3a) What’s the problem if covariate ranges don’t overlap between groups?**

If the **covariate (e.g., plant size)** does **not have similar ranges** across levels of the categorical predictor (e.g., pollination type), then:

🔻 **ANCOVA cannot reliably separate the effects** of the covariate from the treatment.

**Here's why:**

* The model can’t estimate the covariate effect independently of treatment.
* The **comparison between treatments becomes confounded** by the difference in covariate values.
* **Extrapolation** occurs → the model has to guess how one group would behave in an unmeasured region.

📉 **Example problem:**  
If all large plants are cross-pollinated, and all small plants are selfed, you can't tell whether seed output is due to **size** or **treatment**.

**2.3b) Does the design from Problem 1 likely meet this assumption?**

Yes, probably ✅.

Here’s why:

* The student **measured all 50 plant sizes first**, then **randomly assigned** them to treatment.
* This randomization means the range of plant sizes is likely **evenly distributed** across both selfed and crossed groups.
* So the design **should** meet the assumption of **overlapping covariate ranges** between treatments.

**✅ 2.4 Grass Growth: Overlapping Covariate Ranges?**

*A student measured growth, soil nutrients (covariate), and grazing intensity (high/low treatment) in 20 plots.*

Not necessarily. ❌

Why?

* **This is observational**, not randomized.
* Grazing intensity and soil nutrients might be **correlated**:
  + Plots with **high grazing** could also have **low soil nutrients** (or vice versa).
* So the covariate **might not be evenly distributed** across grazing levels.

🔍 This risks **non-overlapping ranges**, which compromises the validity of ANCOVA.

**✅ 2.5 How would you check this assumption?**

Use **visual and numerical methods**:

**🔎 1. Plot the covariate by treatment:**

boxplot(plant\_size ~ pollination\_treatment, data = df)

Or for continuous data:

ggplot(df, aes(x = plant\_size, fill = pollination\_treatment)) +

geom\_density(alpha = 0.5)

This helps you **see if the covariate ranges overlap**.

2. **Check numerical overlap:**

tapply(df$plant\_size, df$pollination\_treatment, range)

 Compare min–max values per group.

 Overlap is good ✅. No overlap = problem ❌.

**3.1 Do the data meet the assumption that all treatment levels have similar covariate ranges?**

Yes, likely ✅.

Here’s why:

* The design is experimental and **balanced**, with **25 male flies per treatment**.
* The researchers **measured thorax length for all flies** and randomly assigned them to “mating present” and “mating absent” treatments.
* Assuming thorax length was measured **before** treatment assignment (as expected in a proper design), then both groups should have **comparable thorax length ranges**.

📌 So yes — the **range of thorax lengths** (covariate) is likely similar across both treatment levels.

**✅ 3.2 Can we directly interpret treatment effects in the ANCOVA?**

Yes, ✅ we **can** interpret the treatment effects.

Let’s justify that from the ANCOVA tables:

**👉 From Table 2:**

* The **interaction term** (thorax × treatment) is **not significant**:
  + F = 0.781, p = 0.3814 ❌
* This means there is **no evidence** that the effect of thorax length on longevity **differs by treatment**.

➡️ Therefore, we **do not violate the parallel slopes assumption**, which is **crucial** for interpreting the treatment effect directly.

➡️ So, you can confidently use **Table 3** (model without interaction) to interpret main effects.

**✅ 3.3 Biological interpretation of results**

Here’s a concise interpretation:

Male fruit fly longevity increases with thorax length (i.e., larger males live longer), regardless of treatment. Additionally, males allowed to mate with receptive females daily have significantly shorter lifespans than those paired with unreceptive females, even after accounting for body size. This suggests that **reproductive activity reduces male lifespan**, supporting the idea of a trade-off between reproductive effort and longevity.