# Reproducibility and Experimental Design

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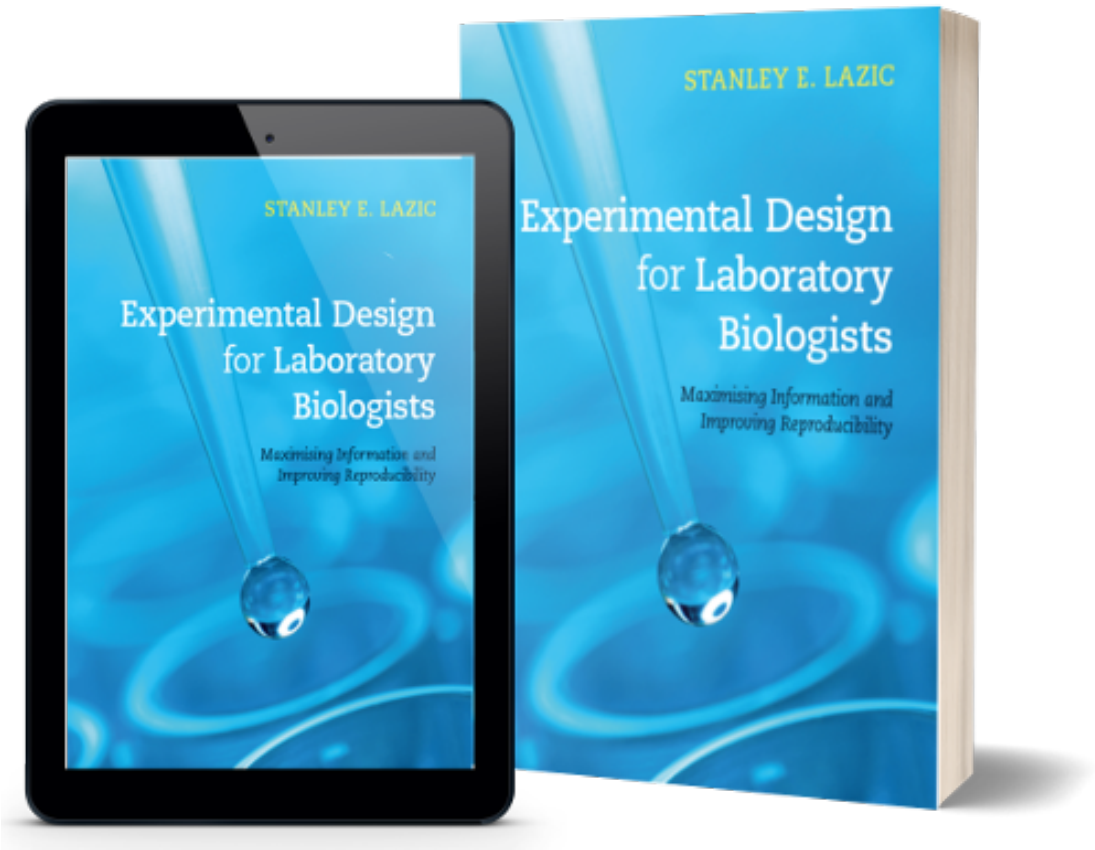
# Overview

- What is reproducibility?
- Source of bias
- Experimental Design
- Replication (N = ?)
- Recommendations

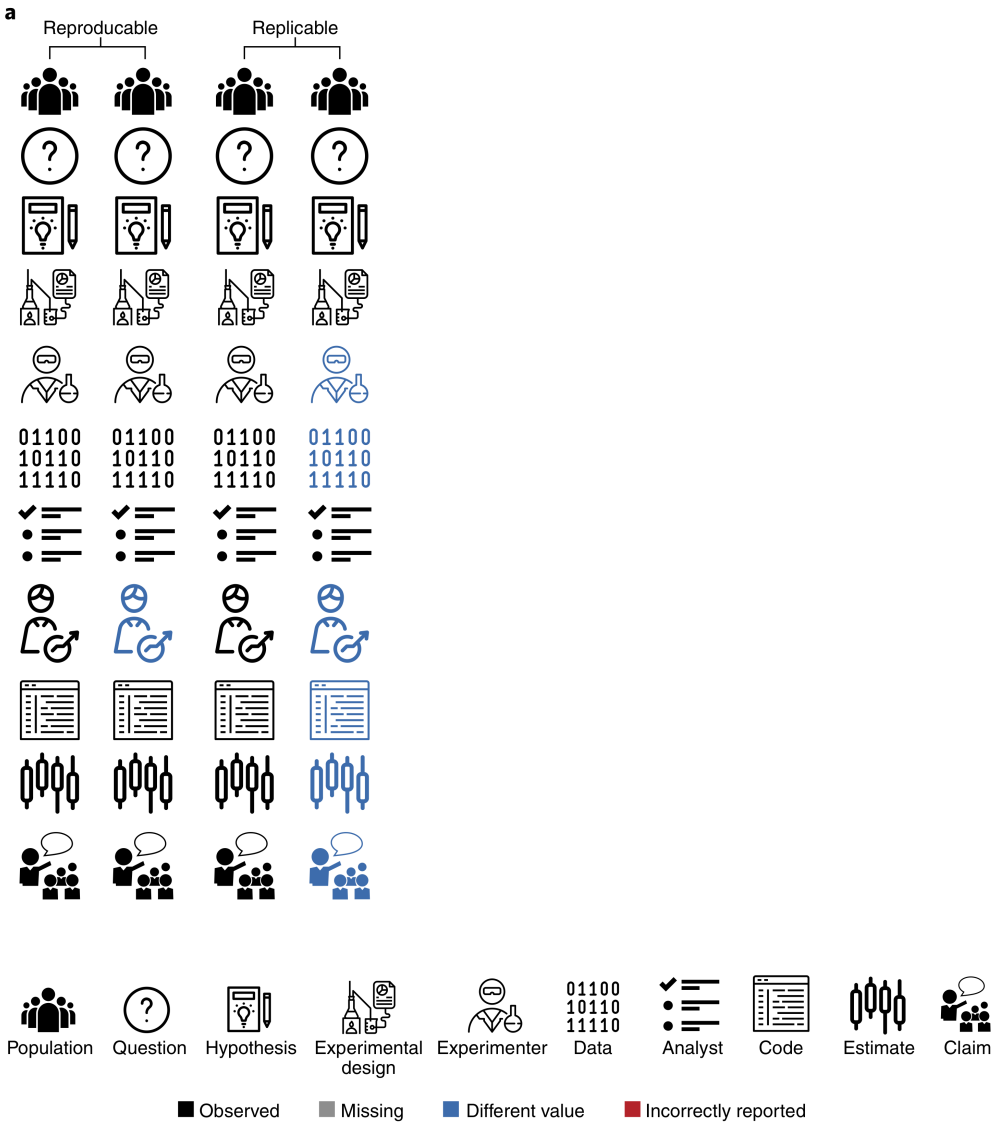
Source: https://github.com/tschauer/Reprodicibility_ExpDesign

# Reference Book



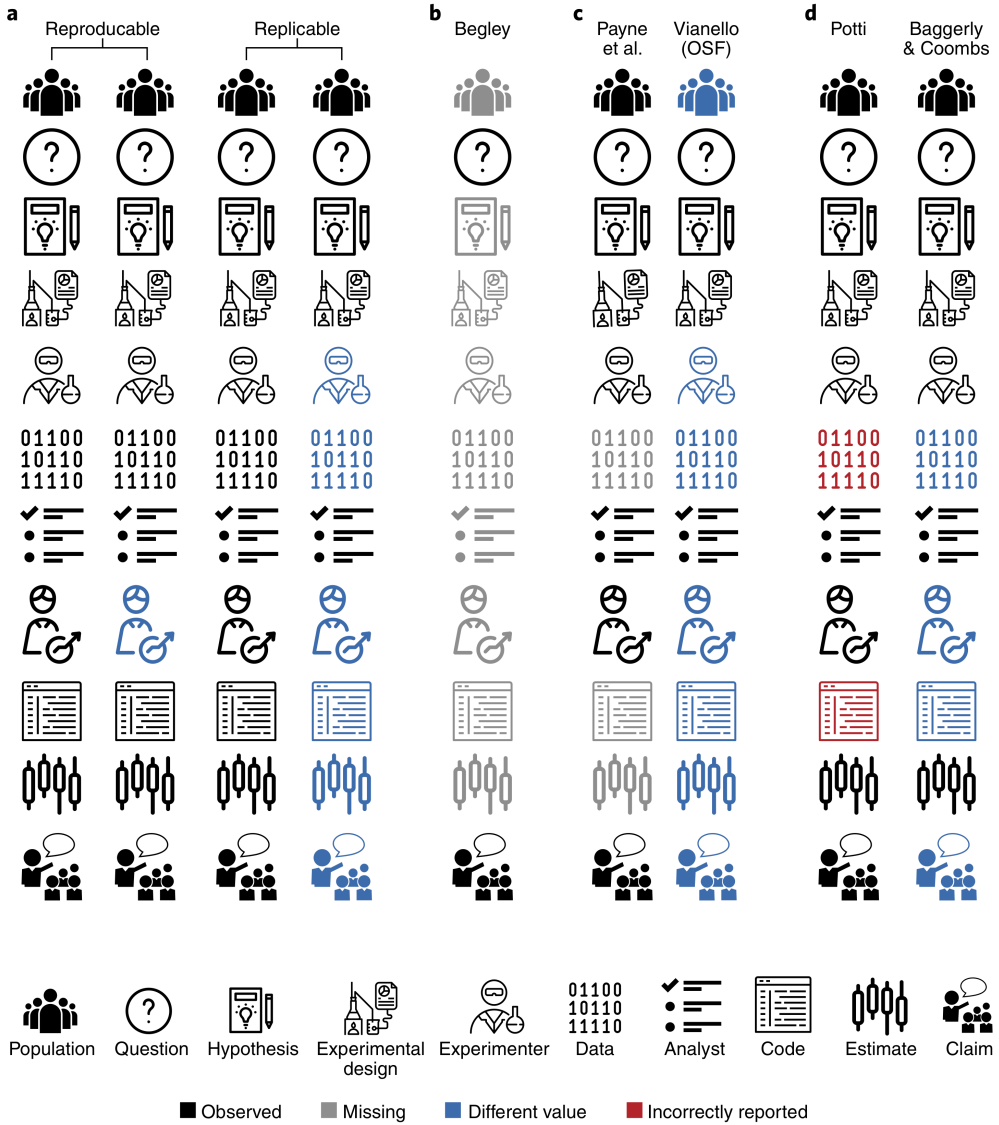

Lazic, 2016

# Reproducibility vs. replicability




Patil, Peng and Leek, 2019

# Reproducibility vs. replicability



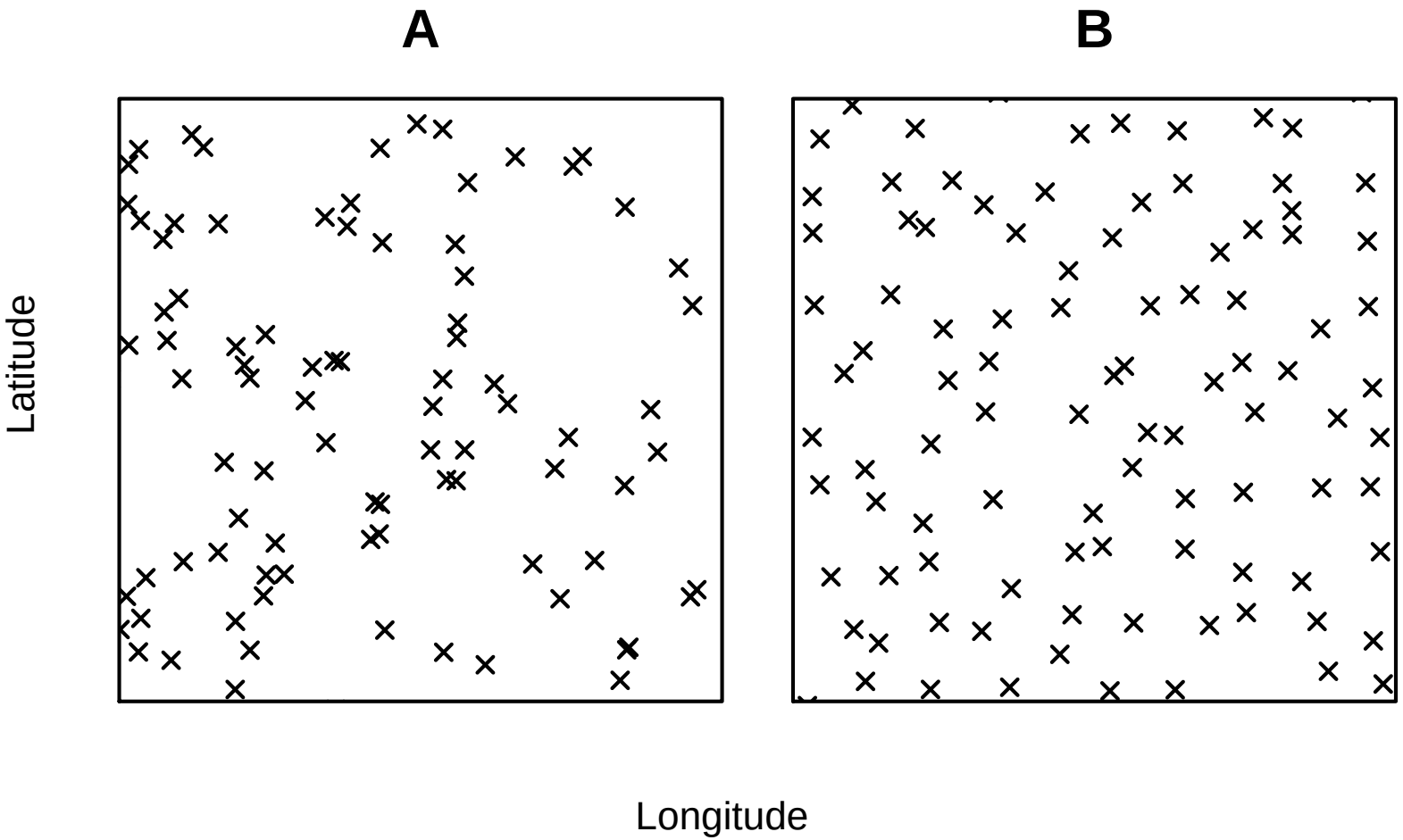

Patil, Peng and Leek, 2019

# Reproducibility by Lazic

- **analytical**: original data and analysis (code!)
- **direct**: same conditions, materials, methods
- **systematic**: different conditions (e.g. cell line, KD vs drug)
- **conceptual**: general under diverse conditions (paradigm)

# Source of bias

- Strategy: which location was bombed randomly?

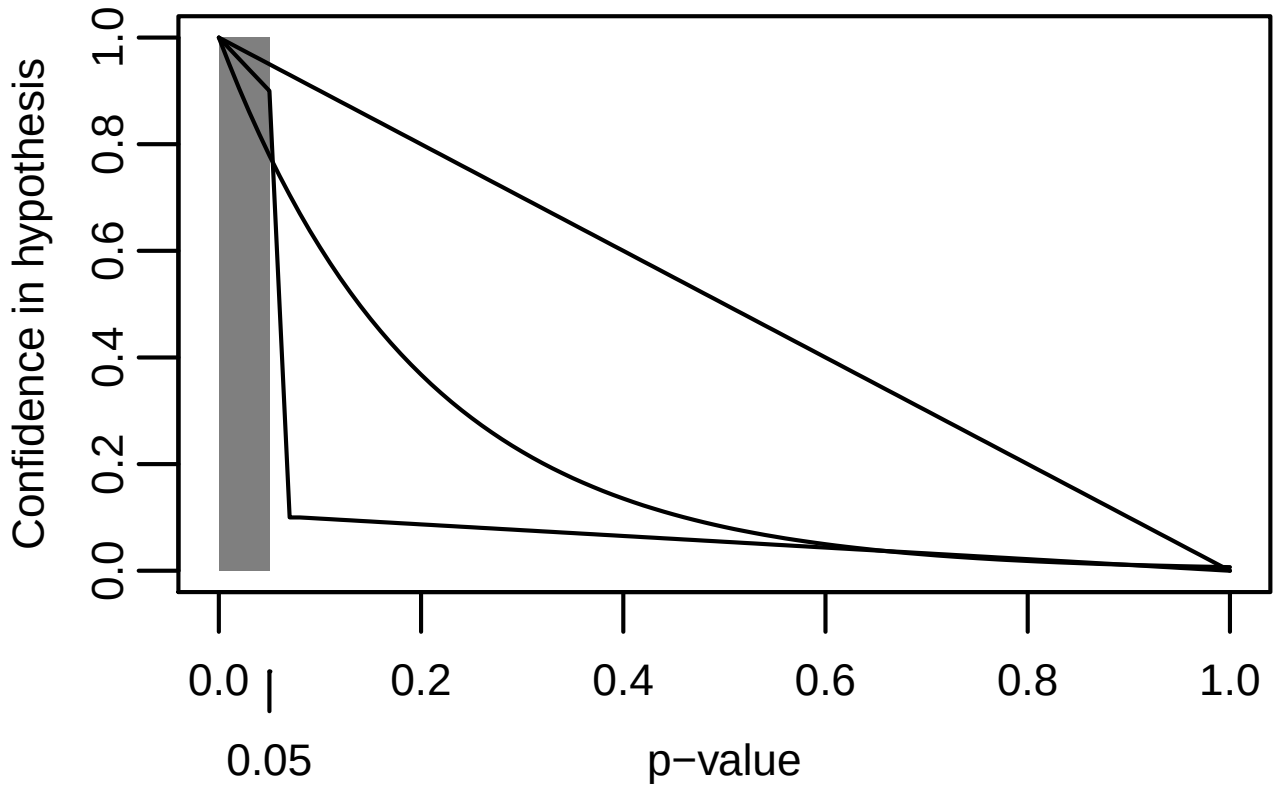


# Source of bias

- seeing pattern in randomness
- not wanting to miss anything (what else can we get out?)
- *"if a hypothesis is derived from the data, then the ability of the data to support that hypothesis is diminished"*
- exploratory vs. confirmatory research

# Source of bias

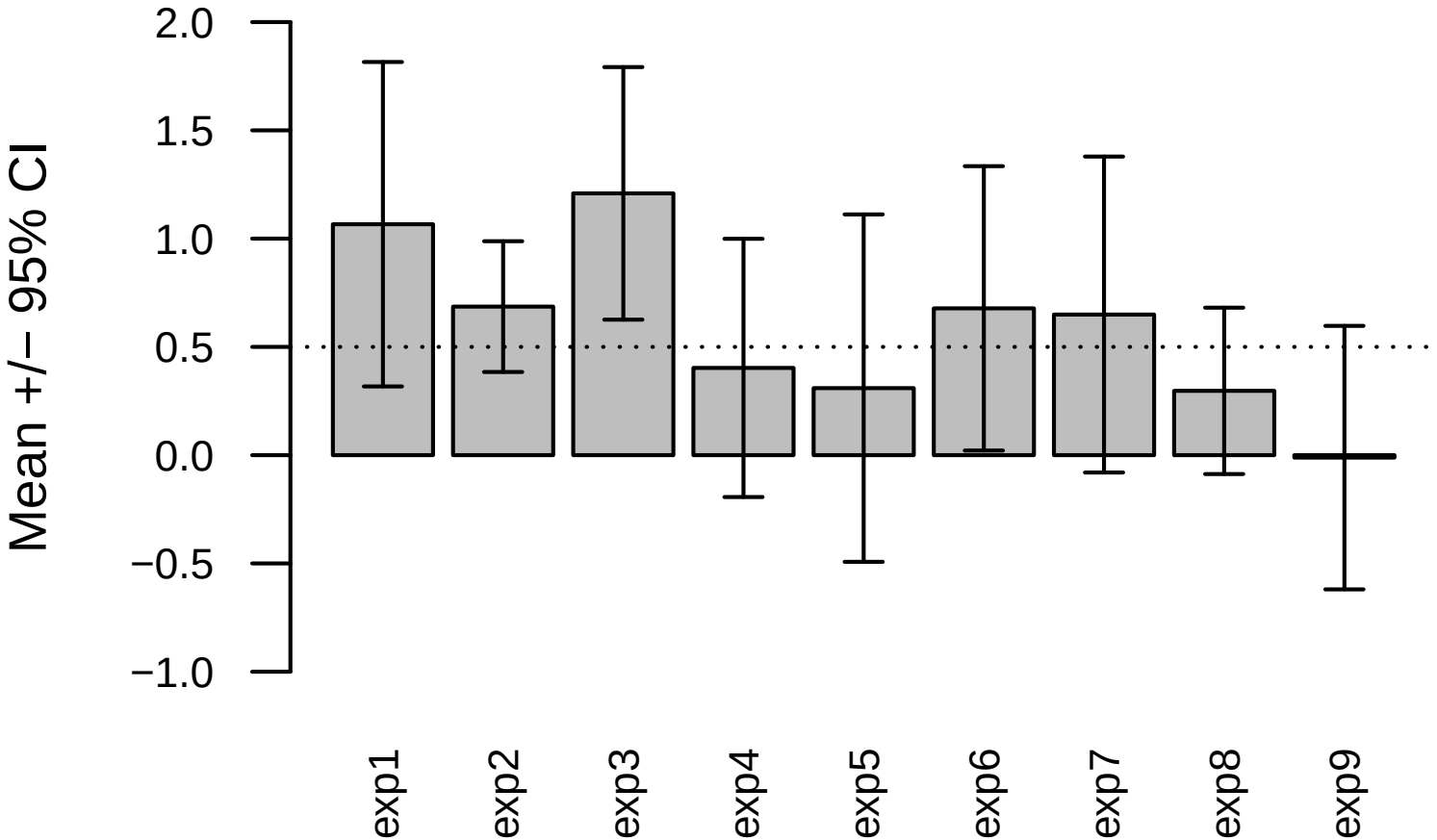
- psychological cliff at p = 0.05




original study: Poitevineau and Lecoutre, 2001

# Source of bias

- neglect of sampling variability




- mean = 0.5, standard deviation = 1, n=10 each

# Source of bias

- lack of independence
  - repeated measures
    - observations are close together in space or time
    - same animal, litter, cell culture dish, fly vial
  - correlated variables
    - different measures of a single underlying effect
    - co-regulated genes, proteins, metabolites
    - disease severity

# Source of bias

- confirmation bias
  - Pubmed search: disease + gene name
    - what about studies which do not find the association?
    - neglecting negative results
  - data transformation until it "gets" significant
  - selecting data to tell the story (data that do not fit excluded)

# Source of bias

- expectancy effects (measurements are influenced)
- hindsight bias ('I knew it all along')
- herding effect (scientific inbreeding)

# Common problems

- Experimental Design
  - confounding (conditions $\sim$ biological, technical effects)
  - experimental unit (replicates)
  - lack of randomization
  - low statistical power
- Conducting experiments
  - lack of blinding
  - lack of randomization
  - optional stopping

# Common problems

- Analysis
  - experimental unit (inflated sample size)
  - inapproriate model (normal distribution)
  - incorrect interpretation
  - selective reporting

# Experimental Goal

| | Exploratory | Confirmatory |
|---|---|---|
| Question | General | Specific |
| Hypothesis | Generating | Before |
| Order | Before | After (independent data) |
| Analysis | Data dependent | Data independent |
| Minimize | False Negatives | False Positives |
| P-value | No Diagnostic Value | Diagnostic Value |
| Power Analysis | Rarely | Yes |

# Experimental Goal

| | Exploratory | Confirmatory |
|---|---|---|
| Subjects | Heterogeneous | Homogeneous |
| Environment | Varied | Standardized |
| Treatments | Many | Few |
| Levels | Many | Few |
| Time points | Many | Few |
| Outcome | Many | Few |
| Controls | Few | Many |
| Blinding | Possibly | Yes |
| Randomization | Yes | Yes |
| Blocking | Yes | Yes |

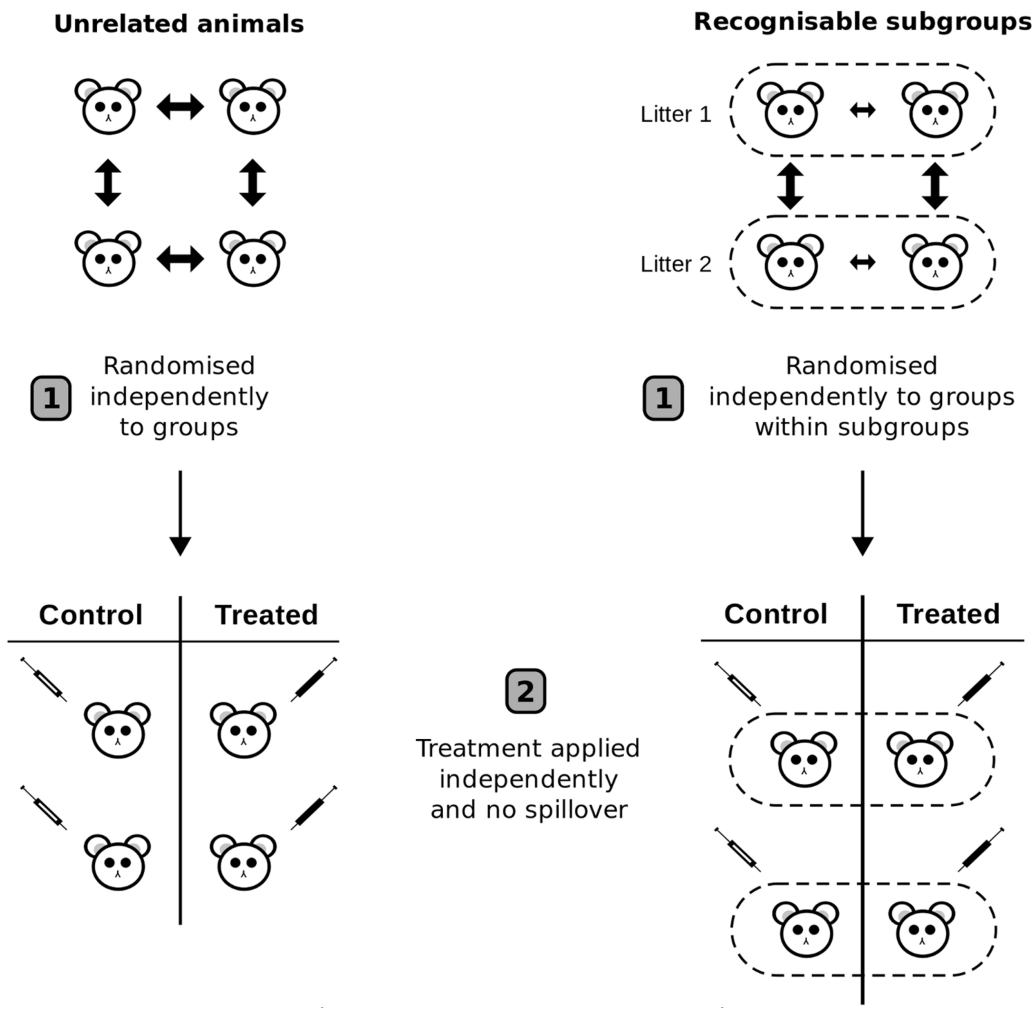
# Hypothesis testing?

- p-value?
  - A: "Given these data what is the probability that the null hypothesis is true"
  - B: "Given that the null hypothesis is true, what is the probability of these (or more extreme) data"
- "The Earth is round ($p < .05$)" Cohen, 1994
- Solution? Pre-registration: https://cos.io/prereg/

# Experimental Design Equation

| Outcome = | Treatment + | Biological + | Technical + | Error |
|---|---|---|---|---|
| Gene exp. | Environment | Sex | Person | Experimental |
| Protein | Compound | Age | Batch | Treatment |
| Cell counts | Inhibitor | Weight | Flask | Sampling |
| | siRNA | Litter | Cage | Measurement |
| | Dose | Genotype | Day | |
| | Time | Cell line | Incubator | |

# Randomization




- Completely randomized vs. randomized blocked design

Lazic, 2018

# 2-factor design

- crossed

| | Control | Treated |
|---|---|---|
| Day 1 | ***** | ***** |
| Day 2 | ***** | ***** |

- nested

| | Control | Treated |
|---|---|---|
| Cage 1 | ***** | |
| Cage 2 | ***** | |
| Cage 3 | | ***** |
| Cage 4 | | ***** |

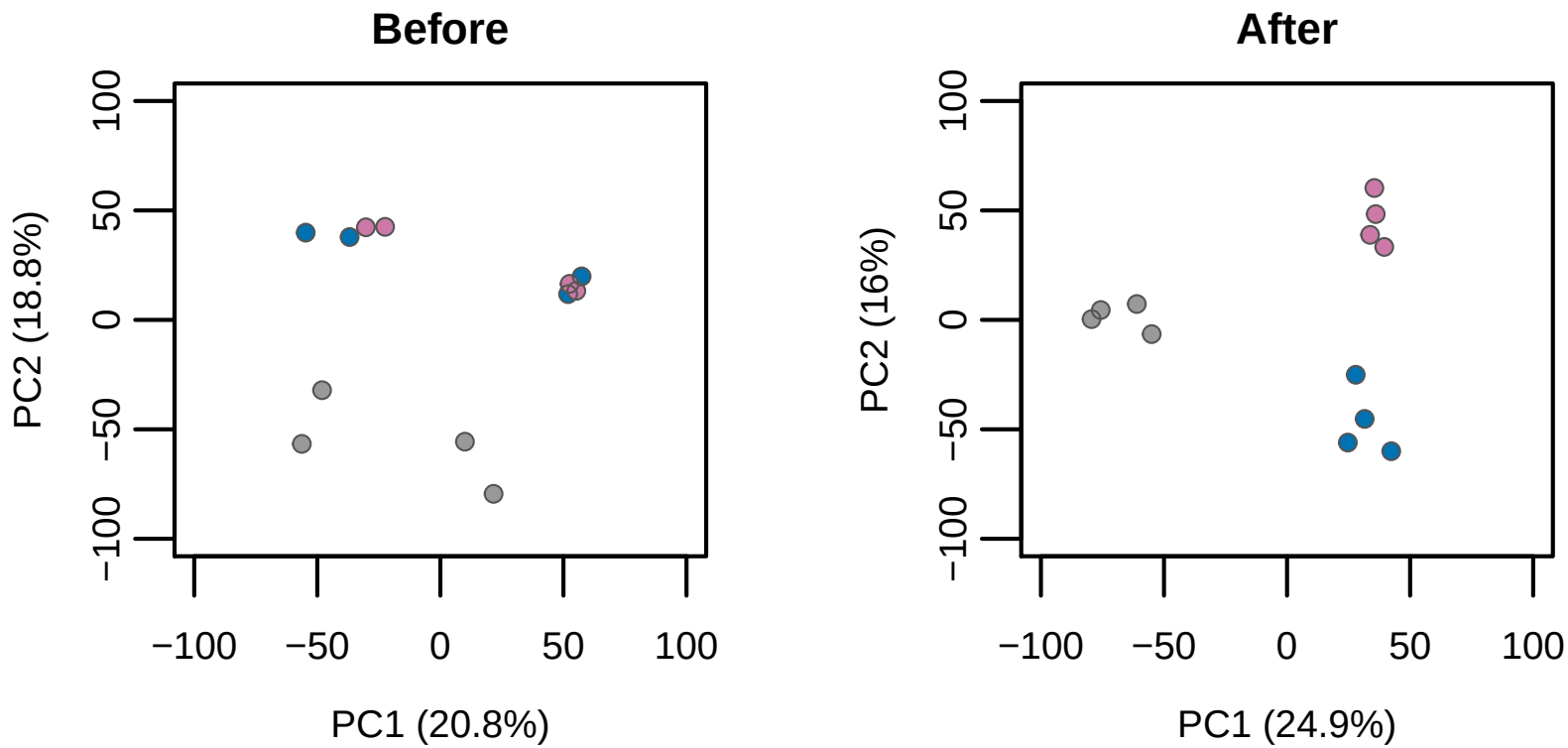
# 2-factor design

- confounded

| | Control | Treated |
|---|---|---|
| Batch 1 | ********** | |
| Batch 2 | | ********** |

# Batch effects

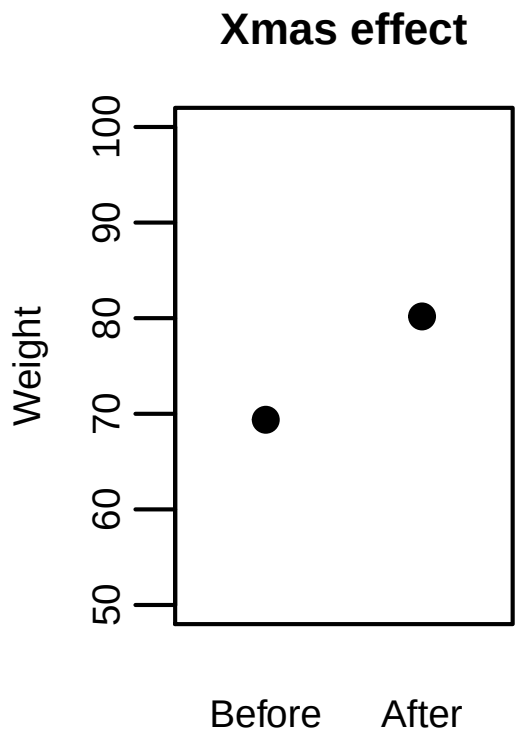
- 3 conditions, 4 reps in 2 batches




Data: Catherine Regnard

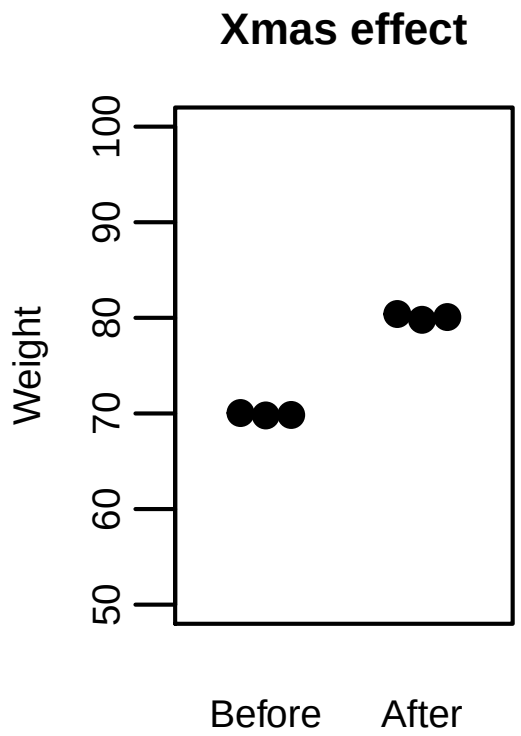
# Example

- How does Christmas affect human body weight?



# Example

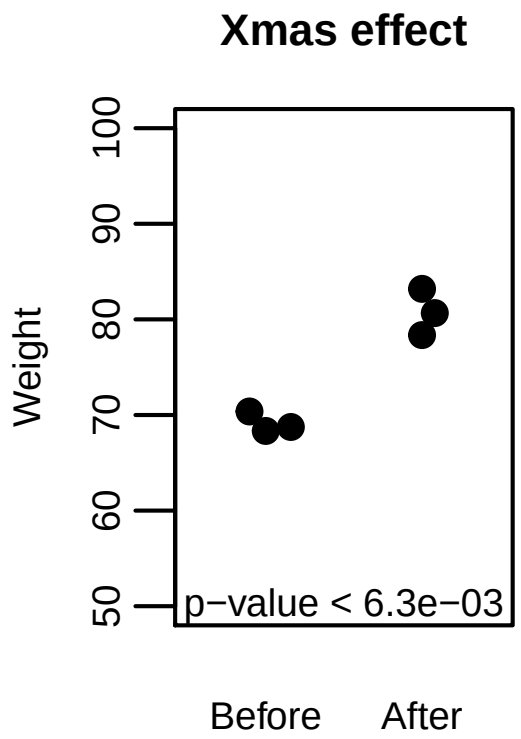
- measuerment error (3x within minutes)




- qPCR well-replicates
- sequencing the same library

# Example

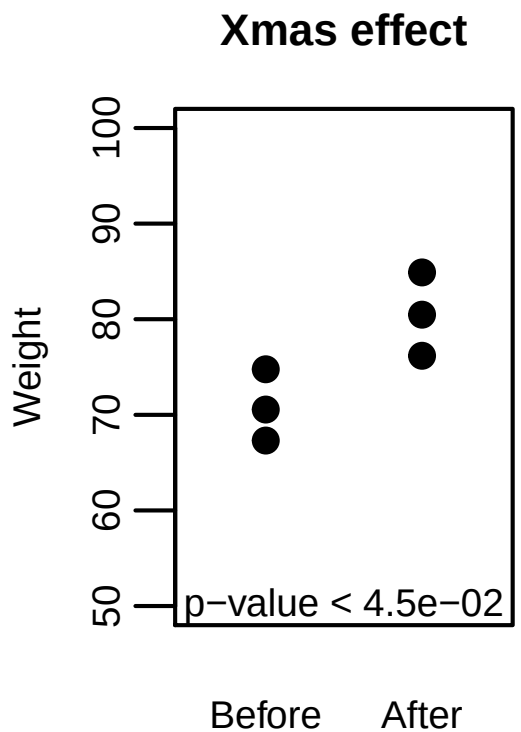
- Different days same person




- Christmas signficantly increases human body weight ???
- e.g. cell culture experiments (generalizable?)

# Example

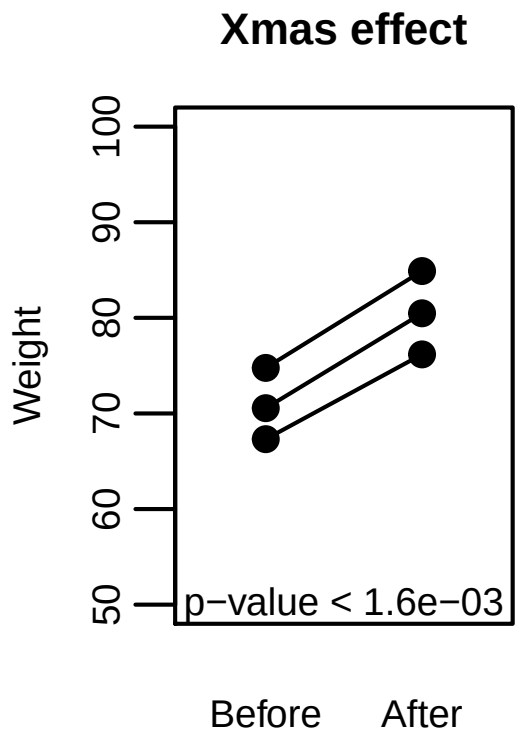
- Different years same person




- year can be used as grouping factor

# Example

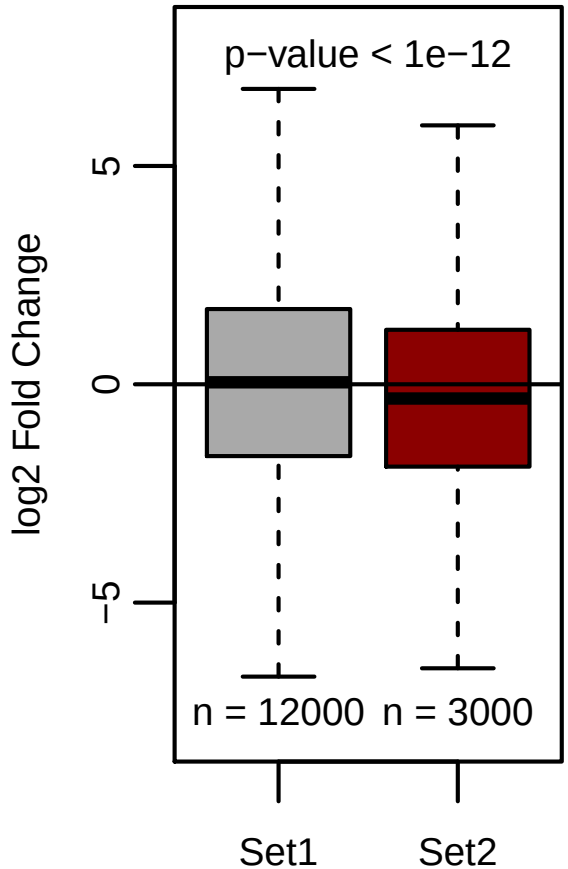
- Different years same person




- it is still just a single person
- different years not always applicable

# What is N?

- within sample testing (comparing set of genes)




- True difference: -0.25
- large number of genes inflates p-value (no diagnostic value)

# What is N?

- Biological unit (BU): the entity about which inferences are made.
- Experimental unit (EU): the entity that is randomly and independently assigned. Sample size N = EU.
  - BU of interest
  - groups of BUs
  - parts of a BU
  - sequence of observations on a BU
- Replicate EU to increase N!

definitions by Lazic, 2016

# What is N?

- Observational unit (OU): the entity on which measurements are taken.
- More OUs do not increase N
  - e.g. cells from a single aliquot, well, slide
- Multiple OUs should be averaged (or use hierarchical model)!
- Report: what is EU, OU.

definitions by Lazic, 2016

# Recommendations

- Share: lab protocol, raw data and **analysis code**
- Careful design (conditions, batches, confounding)
- Quantitative data: effect size, variance
- Analysis: what is your sampling N?

# Acknowledgements


- BMC, Bioinformatics
  - Tobias Straub
- BMC, Molecular Biology
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