

# Reproducibility and Experimental Design

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# Overview

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

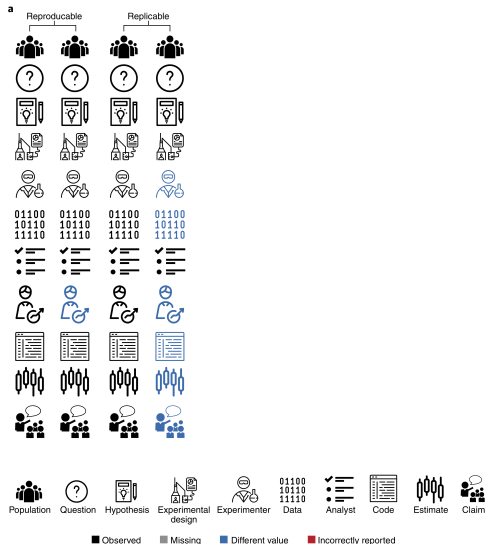
Source: [https://github.com/tschauer/Reproducibility\\_ExpDesign](https://github.com/tschauer/Reproducibility_ExpDesign)

# Reference Book

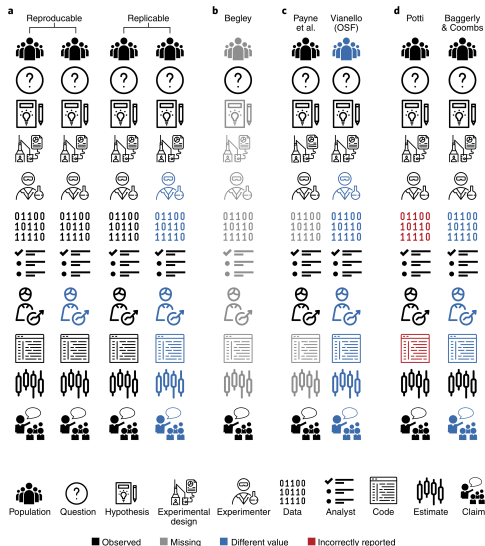


Lazic, 2016

# Reproducibility vs. replicability



# Reproducibility vs. replicability

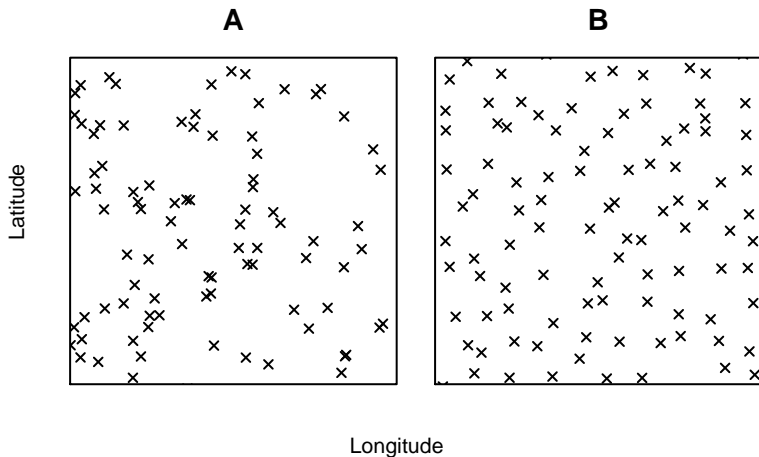


# Reproducibility by Lazic

- ▶ **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?



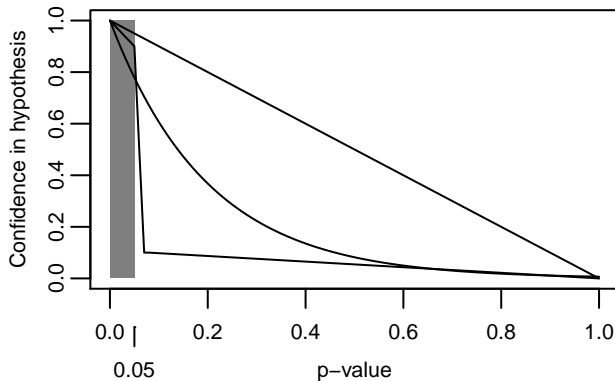
# Source of bias

- ▶ 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

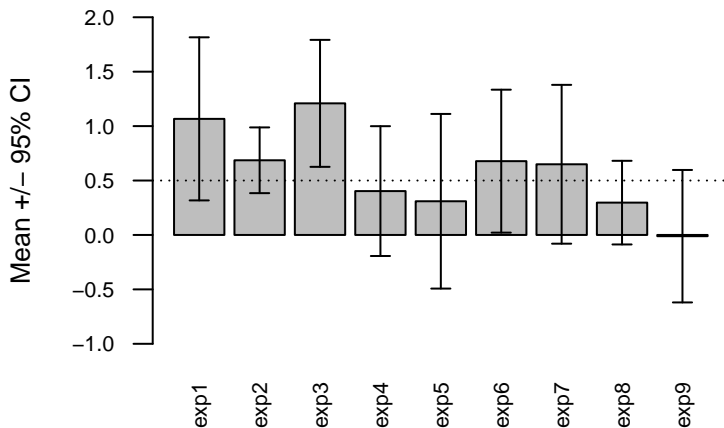
- ▶ psychological cliff at  $p = 0.05$



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)
  - ▶ inappropriate 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

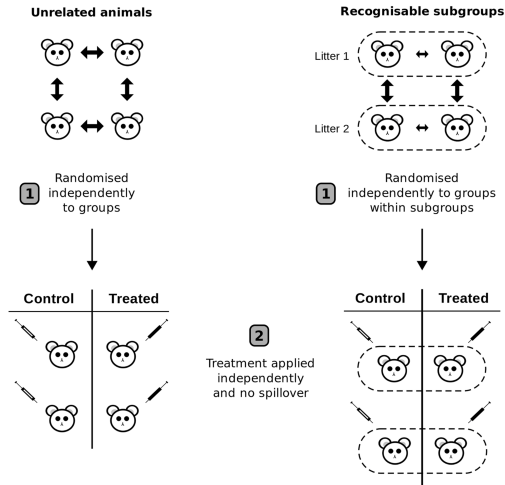
# Hypothesis testing?

- ▶ 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

## 2-factor design

► crossed

	Control	Treated
Day 1	*****	*****
Day 2	*****	*****

► nested

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

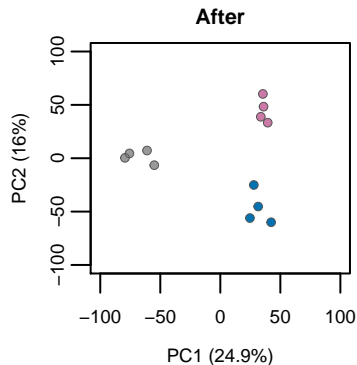
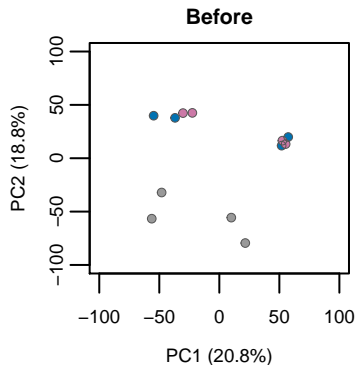
## 2-factor design

► confounded

	Control	Treated
Batch 1	*****	
Batch 2		*****

# Batch effects

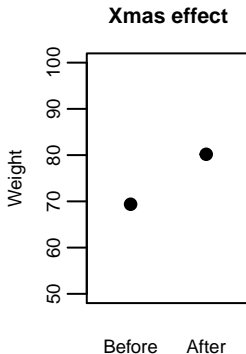
- ▶ 3 conditions, 4 reps in 2 batches



Data: Catherine Regnard

# Example

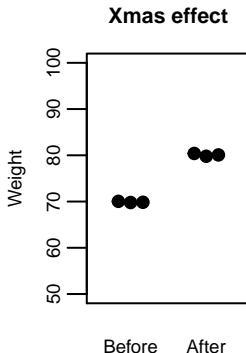
- ▶ How does Christmas affect human body weight?





# Example

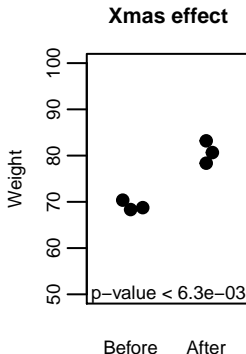
- ▶ measuerment error (3x within minutes)



- ▶ qPCR well-replicates
- ▶ sequencing the same library

# Example

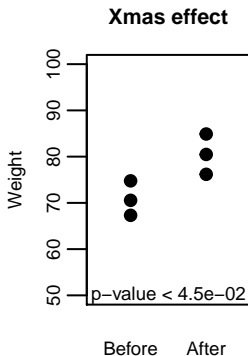
- ▶ Different days same person



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

## Example

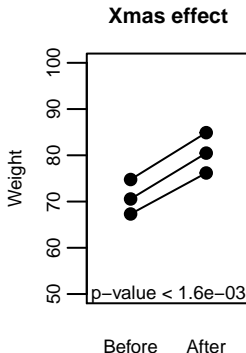
- ▶ Different years same person



- ▶ year can be used as grouping factor

# Example

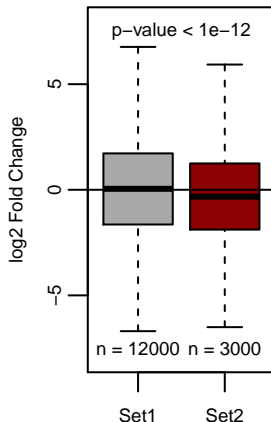
- ▶ Different years same person



- ▶ 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

- ▶ **Ask:** exploratory or confirmatory?
- ▶ **Design:** conditions, batches, confounding
- ▶ **Estimate:** effect size, variance, power
- ▶ **Report:** what is your sampling N?
- ▶ **Share:** lab protocol, raw data and analysis code



# Final Thought

WHEN YOU SEE A CLAIM THAT A  
COMMON DRUG OR VITAMIN "KILLS  
CANCER CELLS IN A PETRI DISH,"

KEEP IN MIND:



SO DOES A HANDGUN.

# Acknowledgements

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- ▶ BMC, Molecular Biology
  - ▶ collaborators
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