

# Common problems in the design of preclinical animal experiments

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REPLICATING RESULTS

# Why Biomedical Research Has A Reproducibility Problem

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SIDENOTES

One of the fundamental principles of science is *reproducibility* – the idea that a discovery is valid only if any scientist in any lab can conduct the same experiment under the same conditions and obtain the same results. Without reproducibility, we could not distinguish

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Unreliable research

# Trouble at the lab

Scientists like to think of science as self-correcting. To an alarming degree, it is not

Oct 19th 2013 | From the print edition



## Studies show only 10% of published science articles are reproducible. What is happening?

Posted on May 3, 2012 by Moshe Pritsker

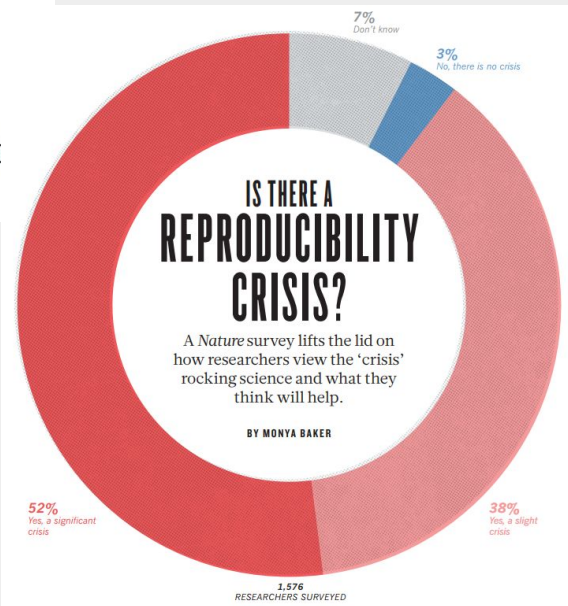
Studies show a very low reproducibility for articles published in scientific journals, often as low as 10-30%. Here is a partial list:

## In cancer science, many "discoveries" don't hold up

BY SHARON BEGLEY

NEW YORK | Wed Mar 28, 2012 2:09pm EDT

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## Biomedical research: increasing value, reducing waste

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Published: January 08, 2014 • DOI: [https://doi.org/10.1016/S0140-6736\(13\)62329-6](https://doi.org/10.1016/S0140-6736(13)62329-6)

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Of 1575 reports about cancer prognostic markers published in 2005, 1509 (96%) detailed at least one significant prognostic variable.<sup>1</sup>

However, few identified biomarkers have been confirmed by subsequent research and few have entered routine clinical practice.

<sup>2</sup> This pattern—initially promising findings not leading to improvements in health care—has been recorded across biomedical research. So why is research that might transform health care and reduce health problems not being successfully produced?

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# Main problems (selected list)

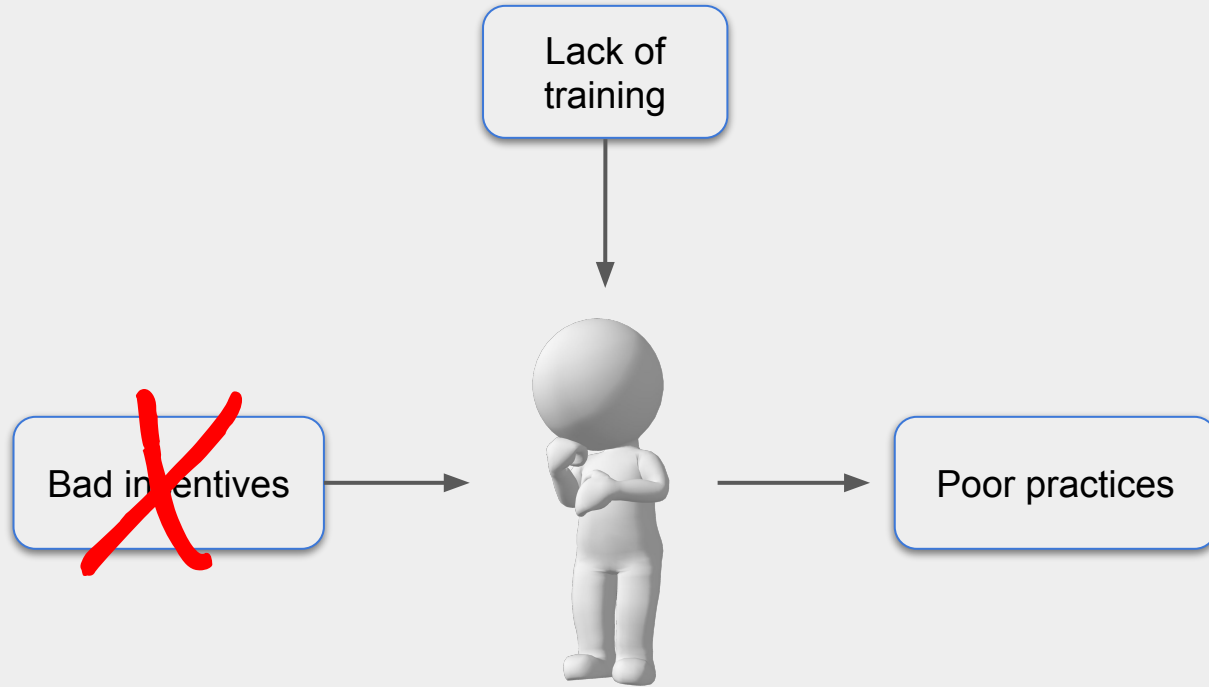
## Design

- Treatment effect confounded with biological and technical effects
- Incorrect experimental unit defined → replicating the wrong entity

## Execution

- Lack of blinding
- Lack of randomisation and/or blocking
- Optional stopping

# What's causing these problems?



# The fundamental experimental design equation

$$\text{Outcome} = \underbrace{\text{Treatment effects}} + \underbrace{\text{Biological effects}} + \underbrace{\text{Technical effects}} + \underbrace{\text{Error}}$$

Environment	Sex	Technician	Experimental
Compound	Age	Batch	Treatment
Inhibitor	Weight	Plate	Sampling
siRNA	Litter	Cage	Measurement
Dose	Genotype	Array	
Time	Species	Day	
	Cell line	Order	
		Source	

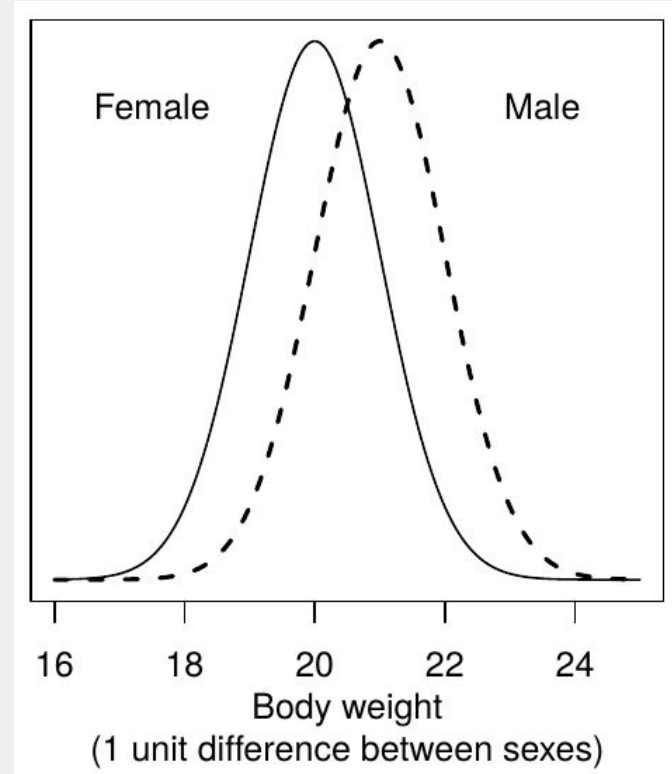
**The aim is to estimate treatment effects precisely and unambiguously.**

# Ignoring known effects in the design and analysis

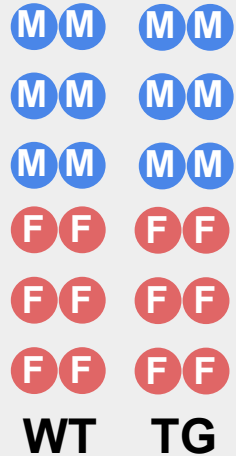
Testing the effect of genotype on body weight in males and females.

N = 24 (12 WT, 12 TG)

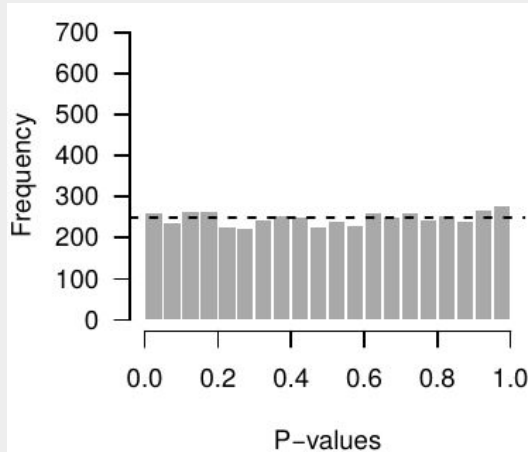
Genotype effect = 0



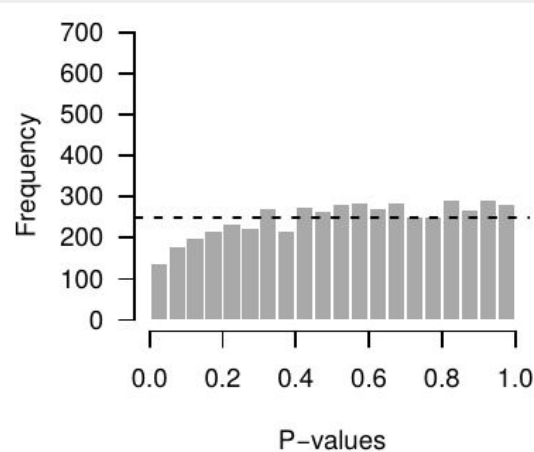
# Balanced data, ignoring sex, no genotype effect



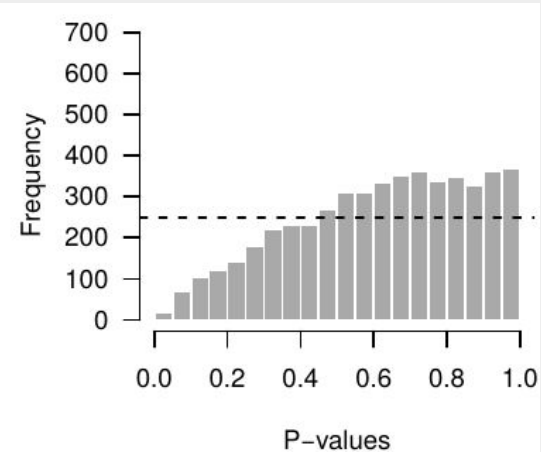
Sex effect = 0



Sex effect = 1

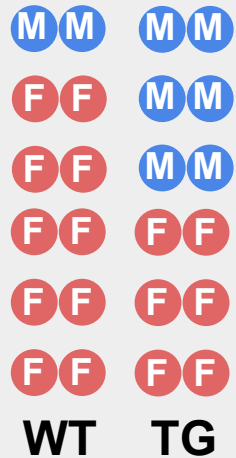


Sex effect = 2

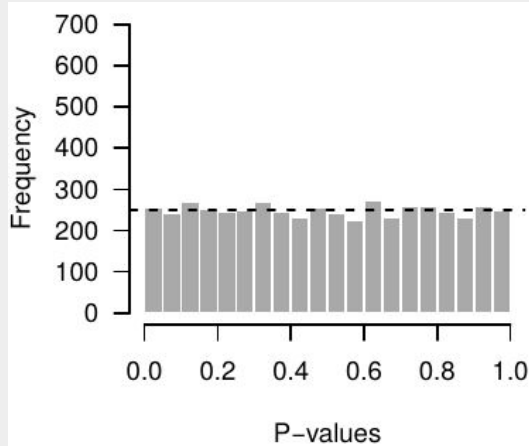




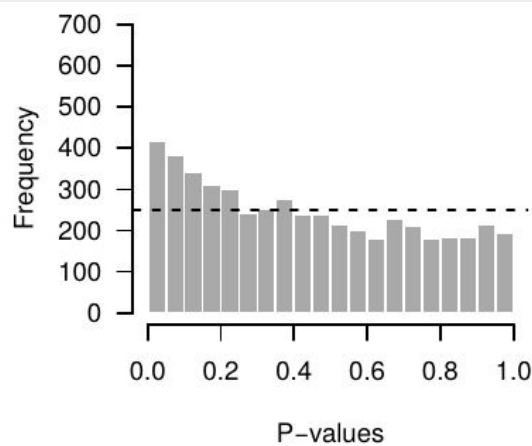
# Unbalanced data, ignoring sex, no genotype effect



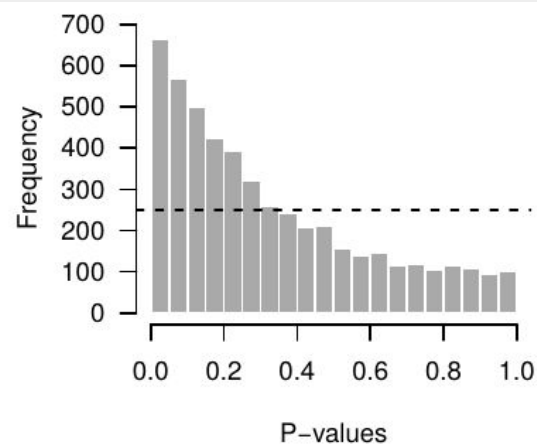
Sex effect = 0



Sex effect = 1

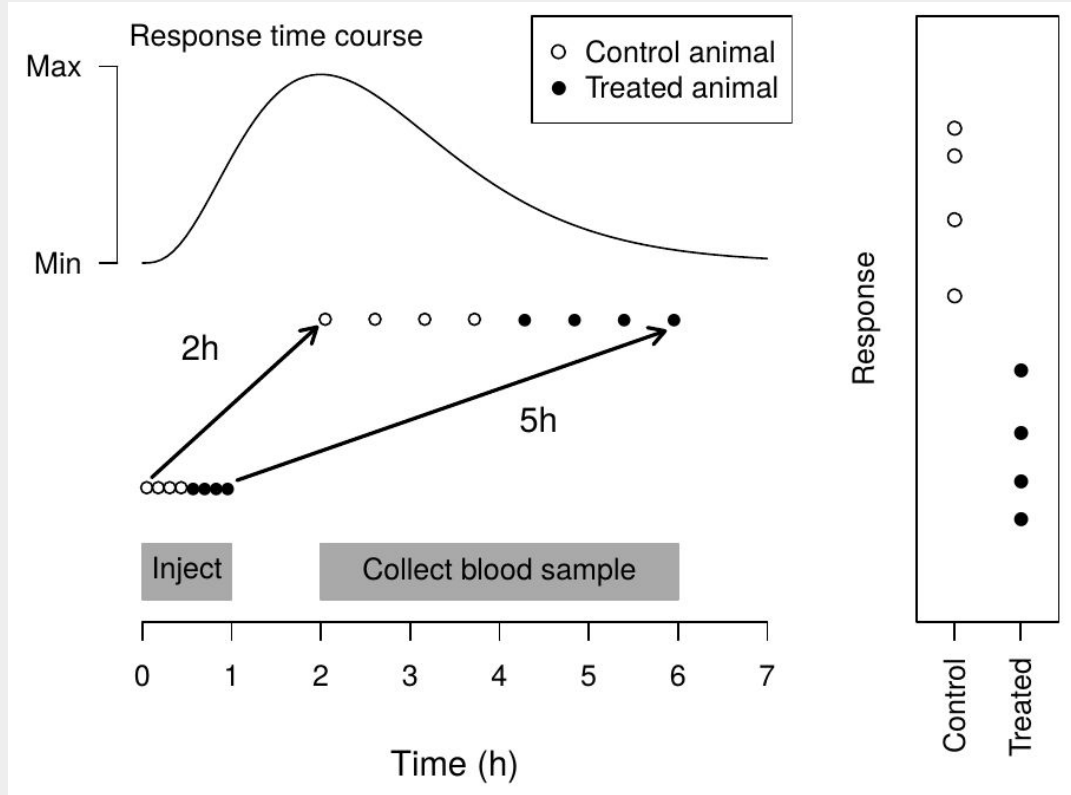


Sex effect = 2



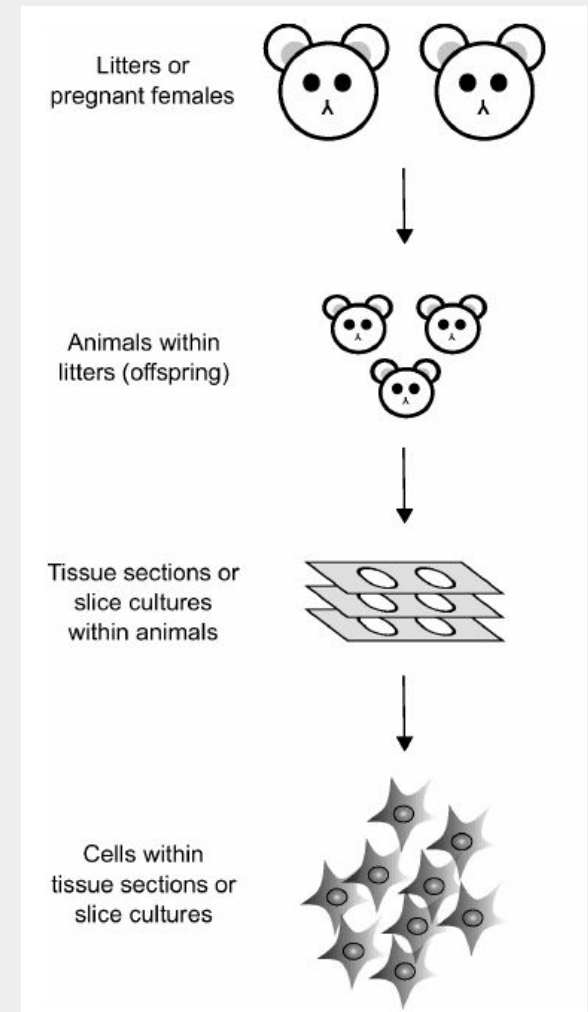
# Order effects (time-varying response)

- Inject animal with inflammatory substance
- Collect blood 2 hours after injection
- N = 4 Controls, 4 Treated
- Injecting is quick, collecting takes longer
- Treatment has no effect



# What is 'N' ?

- **Experimental Units** - Smallest biological entity that can be randomly and independently assigned to a condition .
- **Observational Units** - Biological entity on which measurements are made.
- **Biological Units** - Biological entity that a scientist wants to make an inference about.





Control



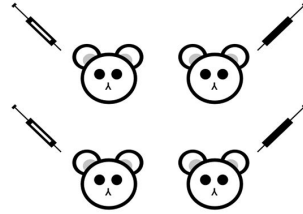
Treated

# Biological, experimental, and observational units may differ → often leads to pseudoreplication

Lazic SE, et al. (2018). What exactly is 'N' in cell culture and animal experiments? *PLoS Biol* 6(4):e2005282.

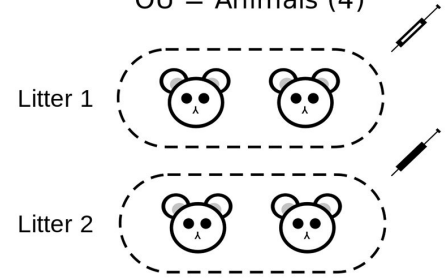
A

BU = EU = OU = Animals  
(N = 4)



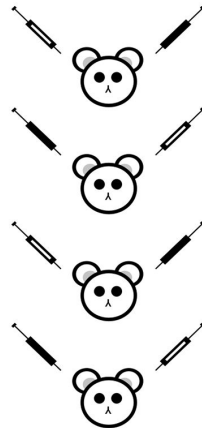
B

BU = Animals (4)  
EU = Groups of animals (N = 2)  
OU = Animals (4)



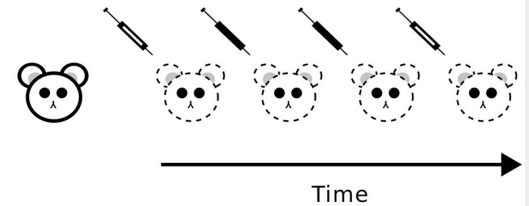
C

BU = Eyes (8)  
EU = Part of an animal (eyes,  
N = 8 with recognisable subgroups)  
OU = Eyes (8)

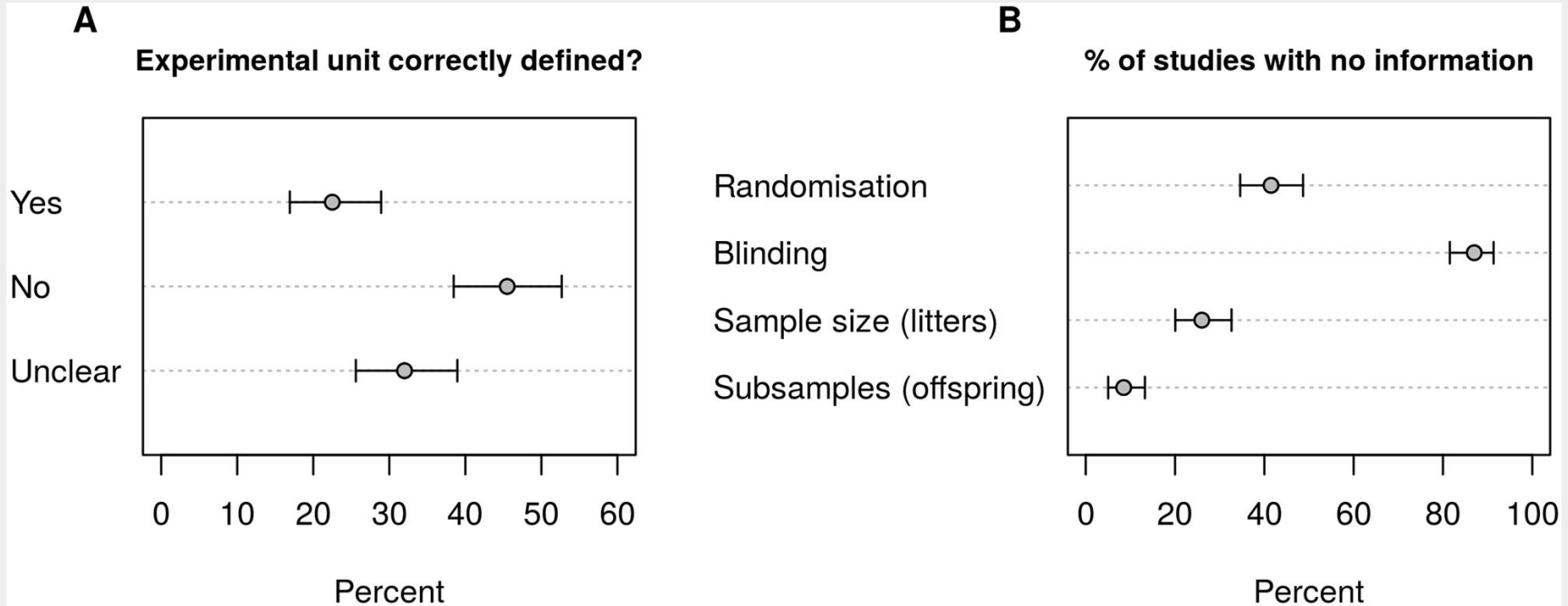


D

BU = Animal (1)  
EU = Time period (N = 4)  
OU = Animal (1)



# How common is pseudoreplication?





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