

# Scientific Inquiry

## Part II: Experimental Design and Data Analysis

Charlotte Soneson & Michael Stadler



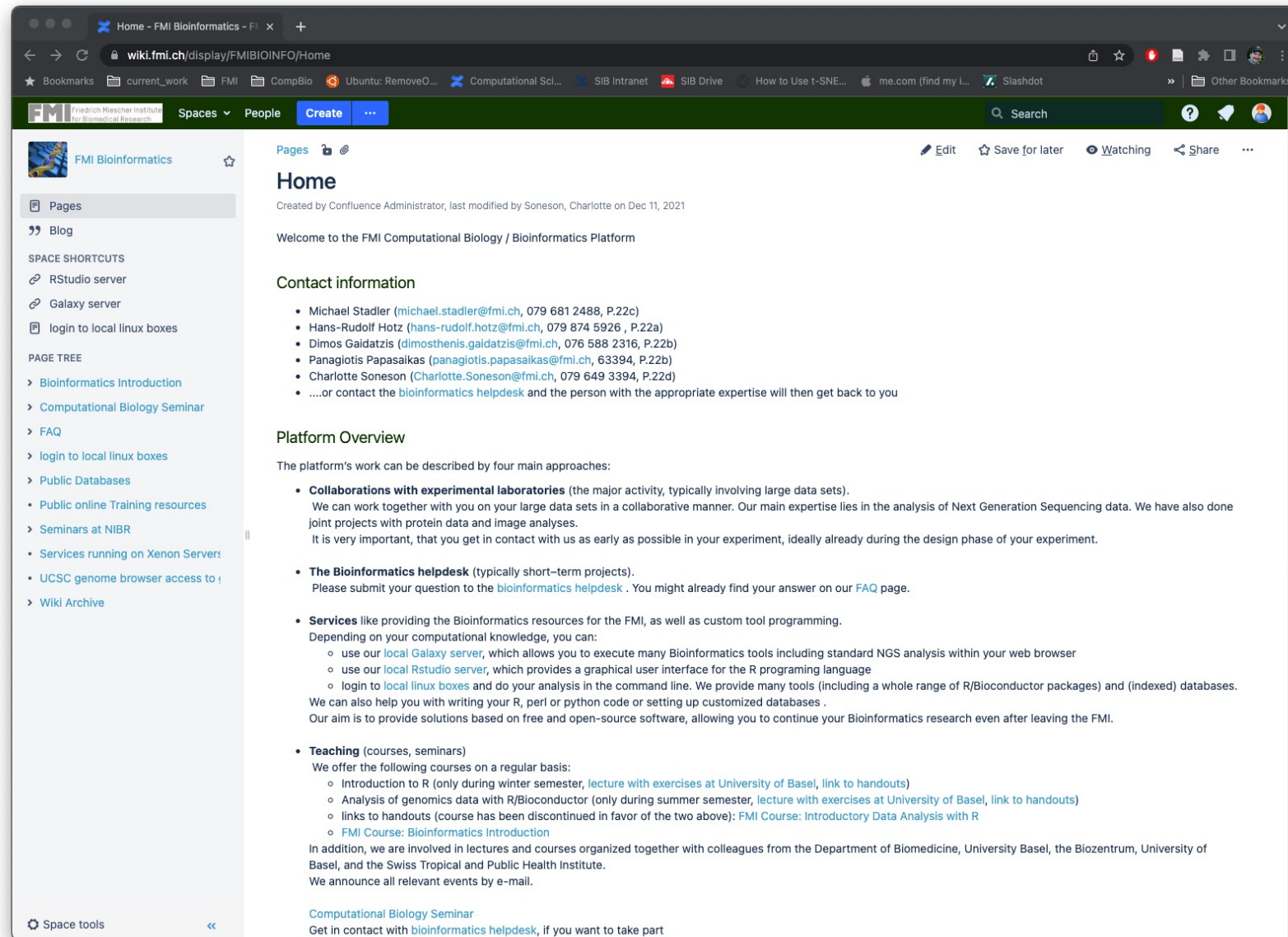
# Where I come from

- Studied “wet” biology (University of Bern) and “dry” biology (University of Geneva, EPFL, MIT)
- FMI Computational Biology platform
  - Bioinformatics infrastructure (e.g. [galaxy.fmi.ch](http://galaxy.fmi.ch), [rstudio.fmi.ch](http://rstudio.fmi.ch), xenon7/8, software)
  - Teaching (R/Bioconductor, visual data exploration, experiment design)
  - Project collaborations (often NGS)

# What we will not do today

- Learn in general how to design your experiments.  
→ specific (FMI) courses, or come talk to us
- Learn how to analyze your data,  
discover patterns and test hypotheses.  
→ specific UniBas lecture (spring/fall semester, 2 hours/week)
- Rather, we will:
  - Introduce relevant aspects of experimental design and data analysis
  - Aim: Raise your awareness, enable you to decide when to reach out

# https://wiki.fmi.ch/display/FMIBIOINFO



The screenshot shows a web browser displaying the FMI Bioinformatics Wiki Home page. The browser's address bar shows the URL `wiki.fmi.ch/display/FMIBIOINFO/Home`. The page has a dark green header with the FMI logo and navigation links like 'Spaces', 'People', 'Create', and 'Search'. A left sidebar contains a 'Pages' section with links to 'Bioinformatics Introduction', 'Computational Biology Seminar', 'FAQ', 'login to local linux boxes', 'Public Databases', 'Public online Training resources', 'Seminars at NIBR', 'Services running on Xenon Servers', 'UCSC genome browser access to', and 'Wiki Archive'. The main content area is titled 'Home' and includes a welcome message, contact information for Michael Stadler, Hans-Rudolf Hotz, Dimos Gaidatzis, Panagiotis Papasailkas, and Charlotte Soneson, and a 'Platform Overview' section. The 'Platform Overview' section describes the platform's work in four main approaches: Collaborations with experimental laboratories, The Bioinformatics helpdesk, Services, and Teaching. The 'Teaching' section lists courses on a regular basis, including 'Introduction to R', 'Analysis of genomics data with R/Bioconductor', and 'FMI Course: Bioinformatics Introduction'. The page also mentions involvement in lectures and courses organized with colleagues from the Department of Biomedicine, University Basel, the Biozentrum, University of Basel, and the Swiss Tropical and Public Health Institute.

Home - FMI Bioinformatics - FMI

wiki.fmi.ch/display/FMIBIOINFO/Home

Bookmarks current\_work FMI CompBio Ubuntu: RemoveO... Computational Sci... SIB Intranet SIB Drive How to Use t-SNE... me.com (find my l... Slashdot Other Bookmarks

FMI Friedrich Miescher Institute for Biomedical Research Spaces People Create ... Search ? ? ? ? ?

FMI Bioinformatics

Pages

Blog

SPACE SHORTCUTS

RStudio server

Galaxy server

login to local linux boxes

PAGE TREE

Bioinformatics Introduction

Computational Biology Seminar

FAQ

login to local linux boxes

Public Databases

Public online Training resources

Seminars at NIBR

Services running on Xenon Servers

UCSC genome browser access to

Wiki Archive

Space tools

Pages

Home

Created by Confluence Administrator, last modified by Soneson, Charlotte on Dec 11, 2021

Welcome to the FMI Computational Biology / Bioinformatics Platform

Contact information

- Michael Stadler ([michael.stadler@fmi.ch](mailto:michael.stadler@fmi.ch), 079 681 2488, P.22c)
- Hans-Rudolf Hotz ([hans-rudolf.hotz@fmi.ch](mailto:hans-rudolf.hotz@fmi.ch), 079 874 5926, P.22a)
- Dimos Gaidatzis ([dimosthenis.gaidatzis@fmi.ch](mailto:dimosthenis.gaidatzis@fmi.ch), 076 588 2316, P.22b)
- Panagiotis Papasailkas ([panagiotis.papasailkas@fmi.ch](mailto:panagiotis.papasailkas@fmi.ch), 63394, P.22b)
- Charlotte Soneson ([Charlotte.Soneson@fmi.ch](mailto:Charlotte.Soneson@fmi.ch), 079 649 3394, P.22d)
- ....or contact the [bioinformatics helpdesk](#) and the person with the appropriate expertise will then get back to you

Platform Overview

The platform's work can be described by four main approaches:

- Collaborations with experimental laboratories** (the major activity, typically involving large data sets).  
We can work together with you on your large data sets in a collaborative manner. Our main expertise lies in the analysis of Next Generation Sequencing data. We have also done joint projects with protein data and image analyses.  
It is very important, that you get in contact with us as early as possible in your experiment, ideally already during the design phase of your experiment.
- The Bioinformatics helpdesk** (typically short-term projects).  
Please submit your question to the [bioinformatics helpdesk](#). You might already find your answer on our [FAQ](#) page.
- Services** like providing the Bioinformatics resources for the FMI, as well as custom tool programming.  
Depending on your computational knowledge, you can:
  - use our [local Galaxy server](#), which allows you to execute many Bioinformatics tools including standard NGS analysis within your web browser
  - use our [local Rstudio server](#), which provides a graphical user interface for the R programming language
  - login to [local linux boxes](#) and do your analysis in the command line. We provide many tools (including a whole range of R/Bioconductor packages) and (indexed) databases.We can also help you with writing your R, perl or python code or setting up customized databases.  
Our aim is to provide solutions based on free and open-source software, allowing you to continue your Bioinformatics research even after leaving the FMI.
- Teaching** (courses, seminars)  
We offer the following courses on a regular basis:
  - Introduction to R (only during winter semester, [lecture with exercises at University of Basel](#), [link to handouts](#))
  - Analysis of genomics data with R/Bioconductor (only during summer semester, [lecture with exercises at University of Basel](#), [link to handouts](#))
  - links to handouts (course has been discontinued in favor of the two above): [FMI Course: Introductory Data Analysis with R](#)
  - [FMI Course: Bioinformatics Introduction](#)In addition, we are involved in lectures and courses organized together with colleagues from the Department of Biomedicine, University Basel, the Biozentrum, University of Basel, and the Swiss Tropical and Public Health Institute.  
We announce all relevant events by e-mail.

Computational Biology Seminar

Get in contact with [bioinformatics helpdesk](#), if you want to take part

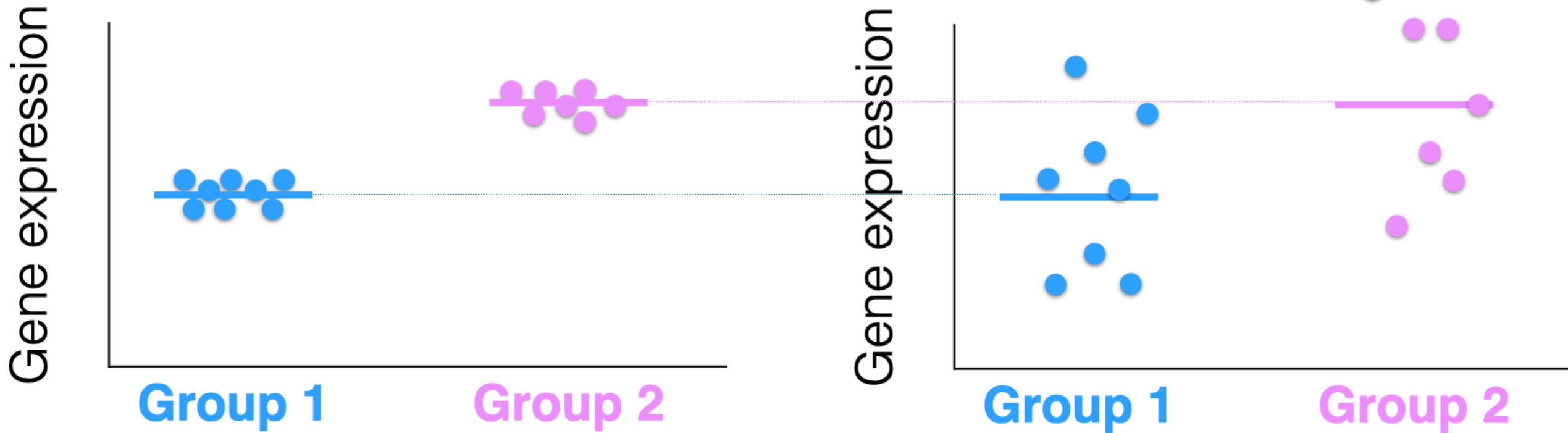
# Episodes

1. Statistical significance
2. Multiple testing correction
3. Correlation and causation (or “Eat more chocolate!”)
4. Good practices for data visualization

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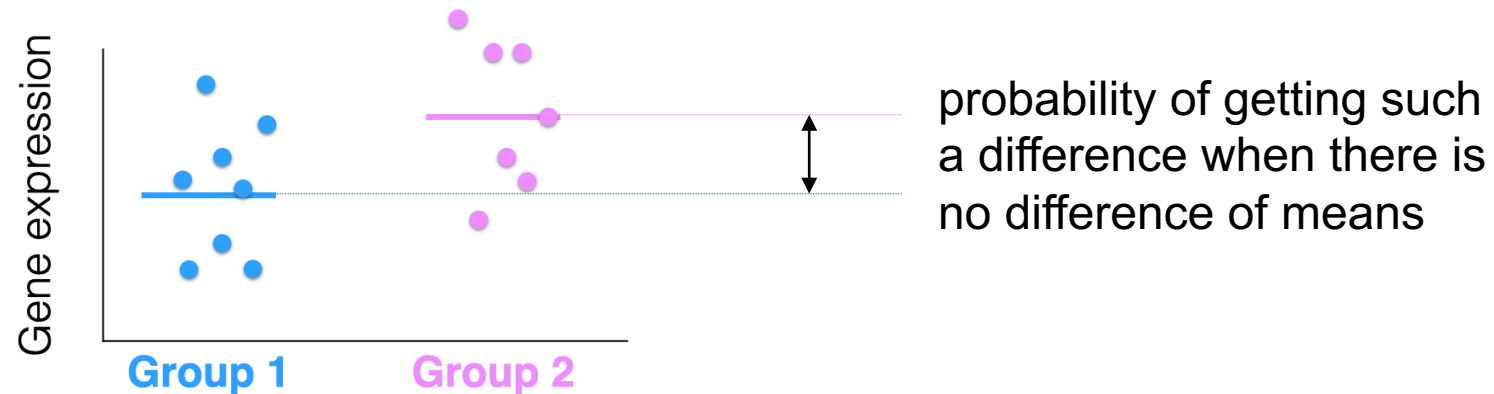
# 1. Statistical significance



Which difference of means between **Group 1** and **Group 2** do you trust more (left or right)?

# 1. What is a P value?

- A P value is the **probability** of observing **by chance** a measure **as extreme as the observed one**.



- The P value reported by tests is a probabilistic significance, not a biological one.
- With enough data, arbitrarily small effects become significant



# Episodes

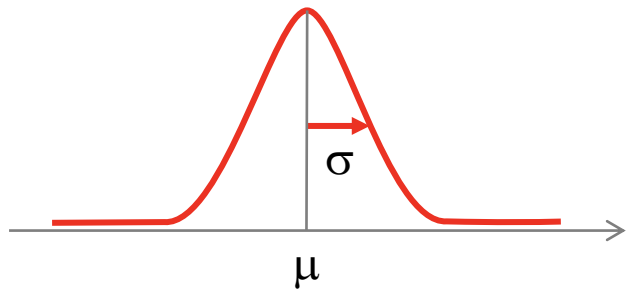
1. Statistical significance
- 2. Multiple testing correction**
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## 2. Multiple testing correction

When we perform many tests, the number of p values that are significant **by chance** increases.

Thought experiment:

given  
distribution



draw two  
random samples

sample 1:

-1.047 0.970 -1.798  
-0.653 0.016 0.459

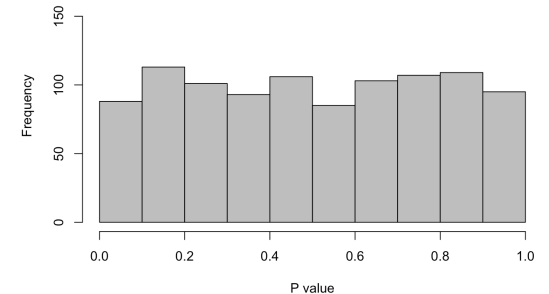
sample 2:

-0.370 -0.017 -0.479  
-0.714 0.994 0.559

compare  
samples

difference of  
means?  
(Student's  
*t*-test)

repeat many times;  
distribution of p values?



## 2. Multiple testing correction simulation in R

```
x <- rnorm(100, mean = 0, sd = 1)
y <- rnorm(100, mean = 0, sd = 1)
t.test(x, y)$p.value # 0.2653
```

```
for (i in 1:1000) {
  x <- rnorm(100, mean = 0)
  y <- rnorm(100, mean = 0)
  pvals[i] <- t.test(x, y)$p.value
}

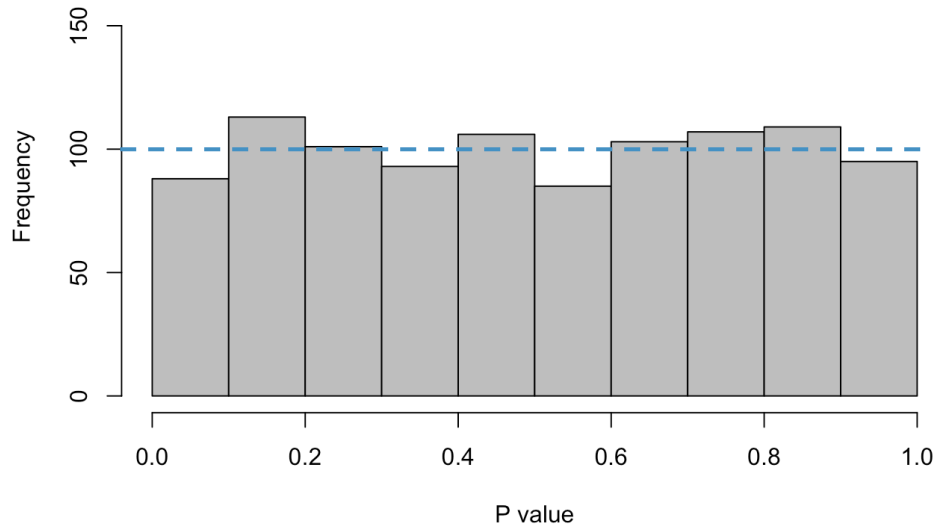
mean(pvals <= 0.1) # 0.088 (~0.1)
padj <- p.adjust(pvals, method = "fdr")
mean(padj <= 0.1) # 0
```

```
y.mu <- rep(c(0, 0.4), c(900, 100))
for (i in 1:1000) {
  x <- rnorm(100, mean = 0)
  y <- rnorm(100, mean = y.mu[i])
  pvals[i] <- t.test(x, y)$p.value
}

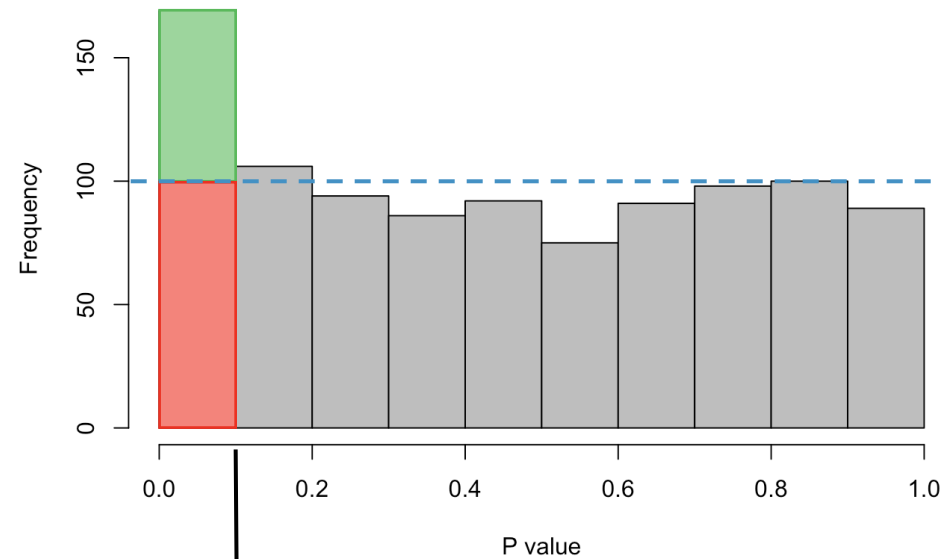
mean(pvals <= 0.1) # 0.169
padj <- p.adjust(pvals, method = "fdr")
mean(padj <= 0.1) # 0.067
```

## 2. Multiple testing correction

histogram of P values (under  $H_0$ )



histogram of P values (10% true effect)



0.1



$$\text{false discovery rate (FDR)} = \frac{\text{red box}}{\text{green box} + \text{red box}} = \frac{100}{100 + 69} = 0.59$$

# Episodes

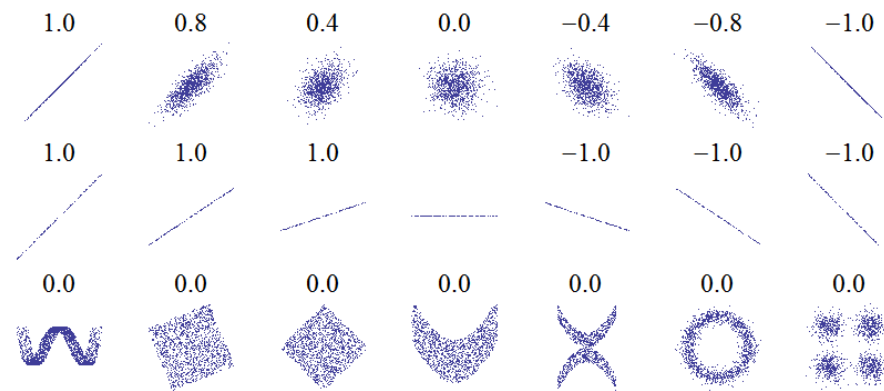
1. Statistical significance
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### 3. Correlation and causation or “Eat more chocolate!”

“Correlation measures increasing/decreasing trends [...].”

Pearson’s correlation coefficient  $r$   $[-1, +1]$

$x, y$ : independent variables  $\Rightarrow r_{xy} = 0$  (but not the inverse)

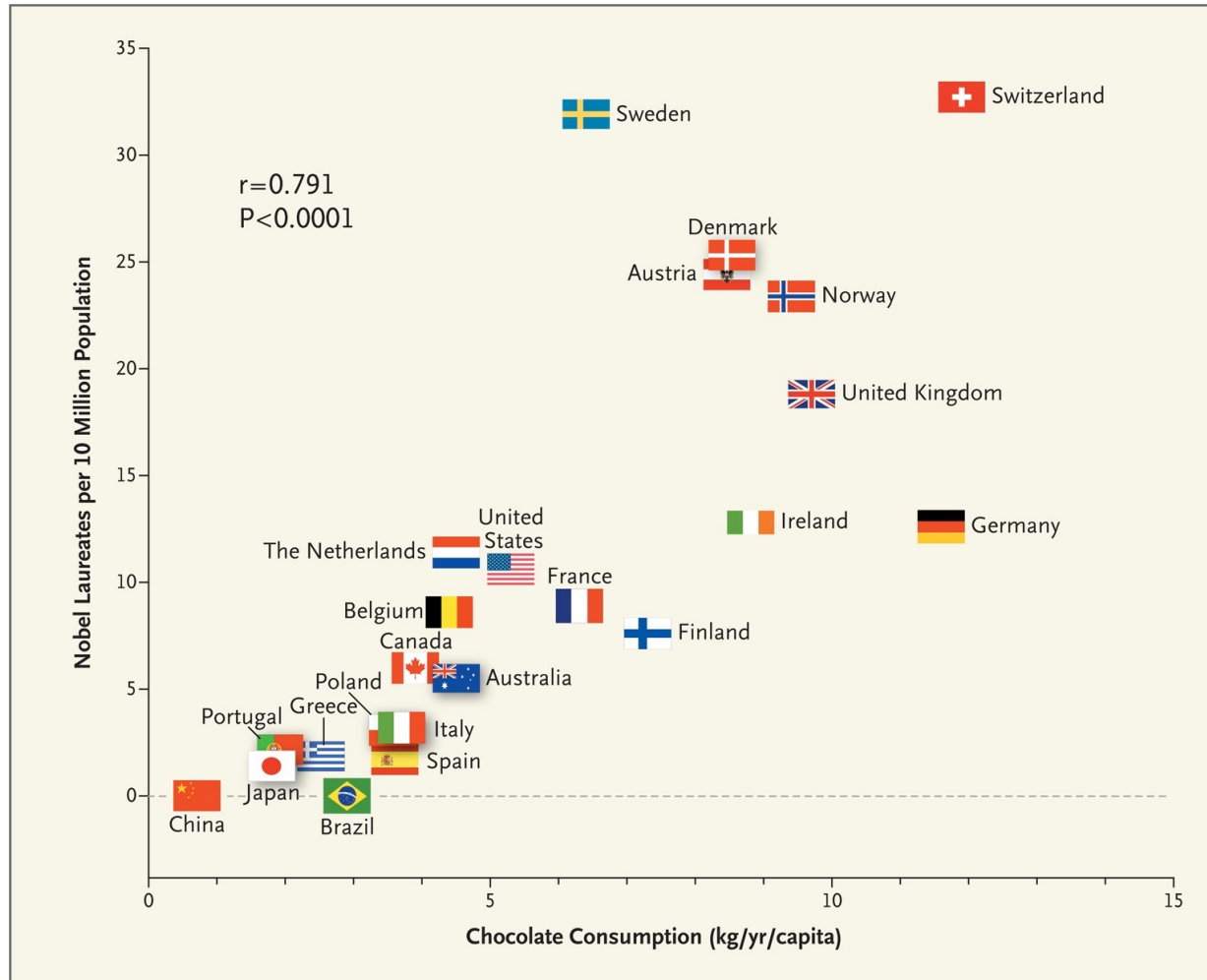


“Correlation implies association, but not causation.”

“Association, correlation and causation” doi: 10.1038/nmeth.3587

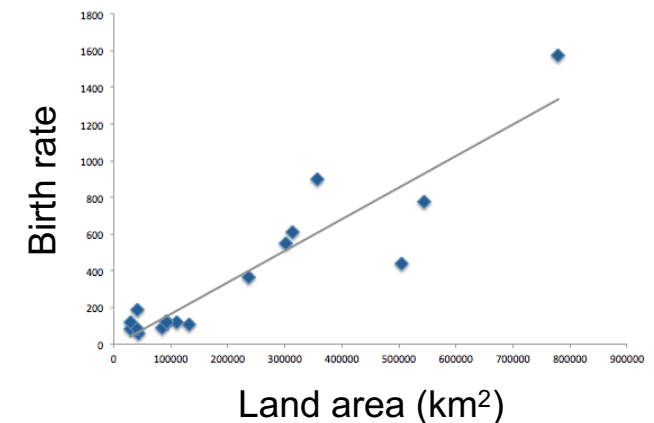
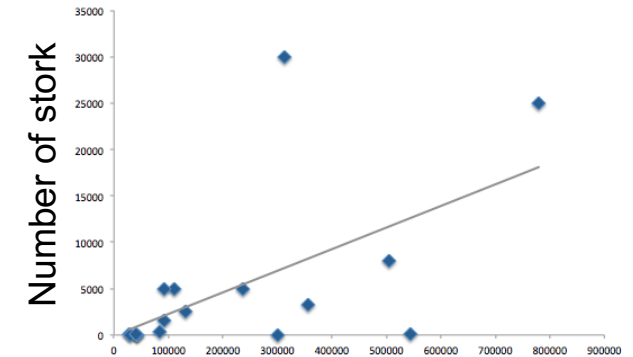
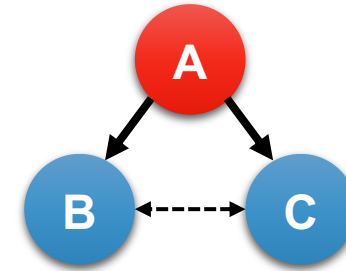
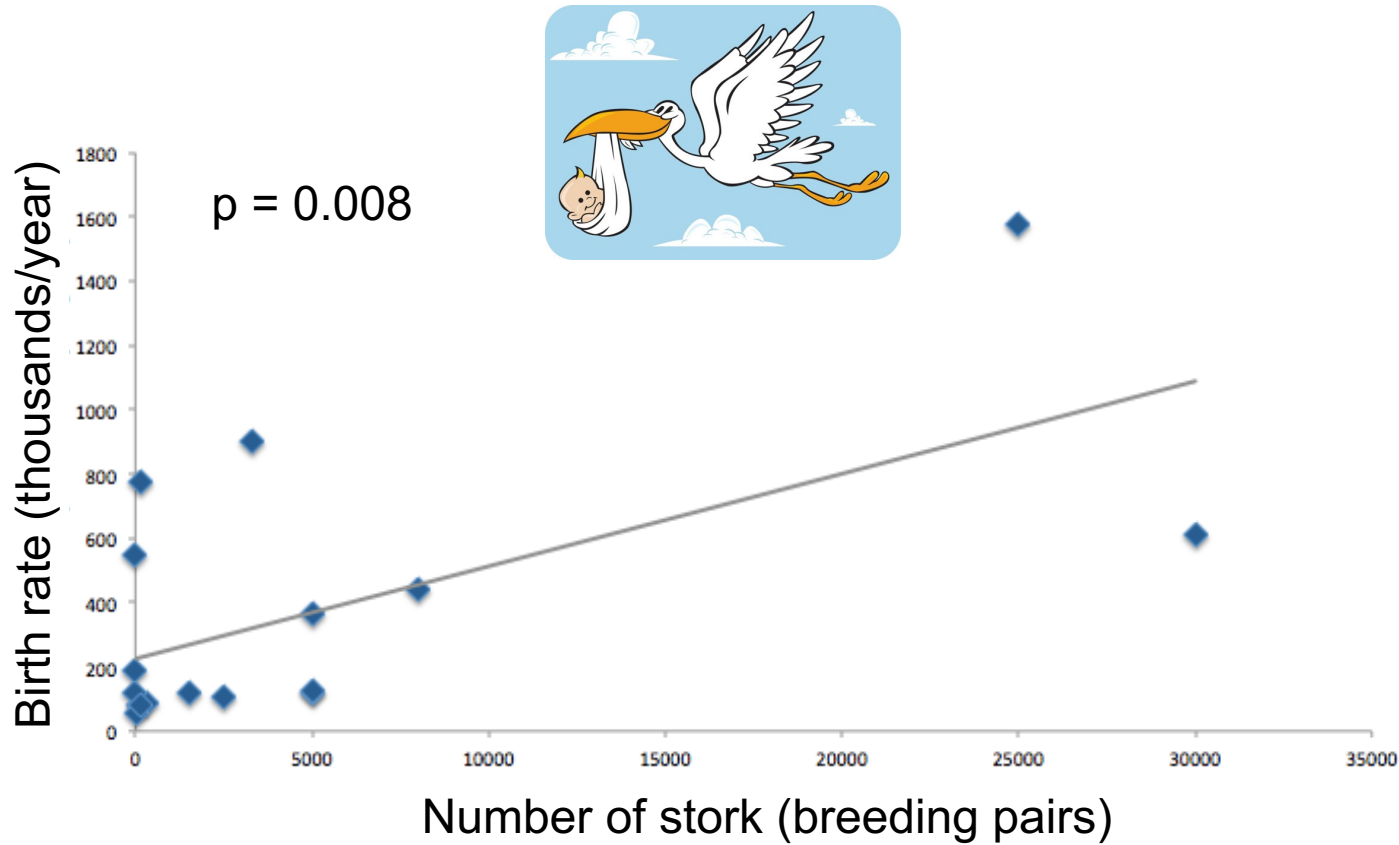
[en.wikipedia.org/wiki/Correlation\\_and\\_dependence](https://en.wikipedia.org/wiki/Correlation_and_dependence)

### 3. Eat more chocolate!



“The slope of the regression line allows us to estimate that it would take about 0.4 kg of chocolate per capita per year to increase the number of Nobel laureates in a given country by 1.”

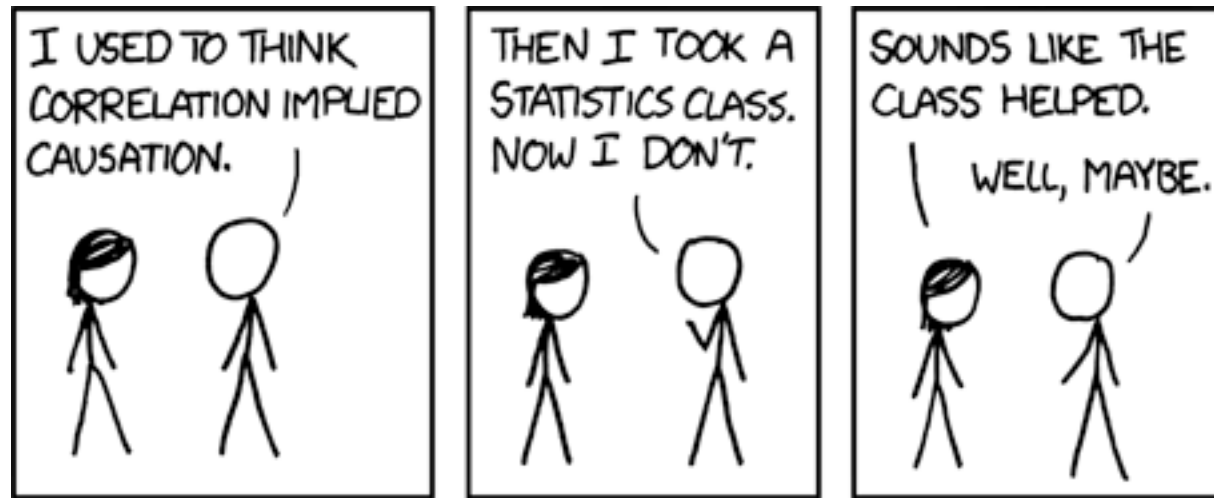
### 3. Do Storks deliver babies?



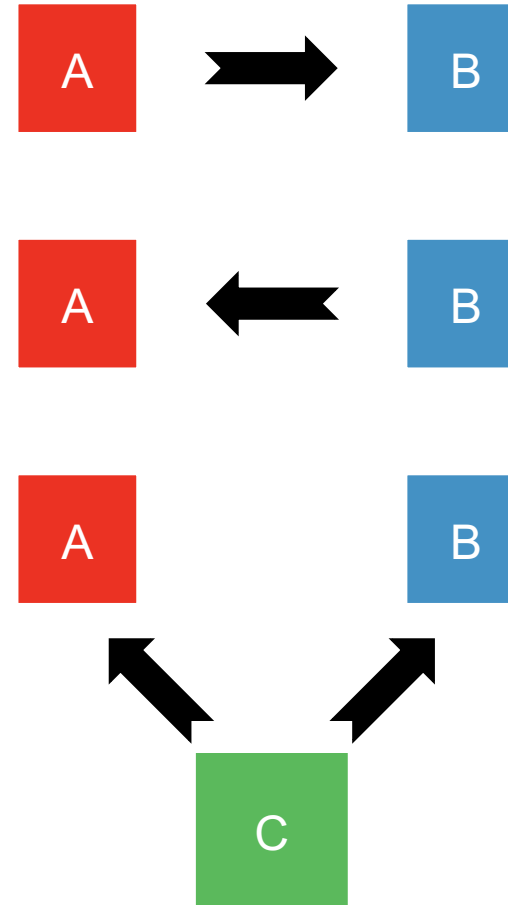
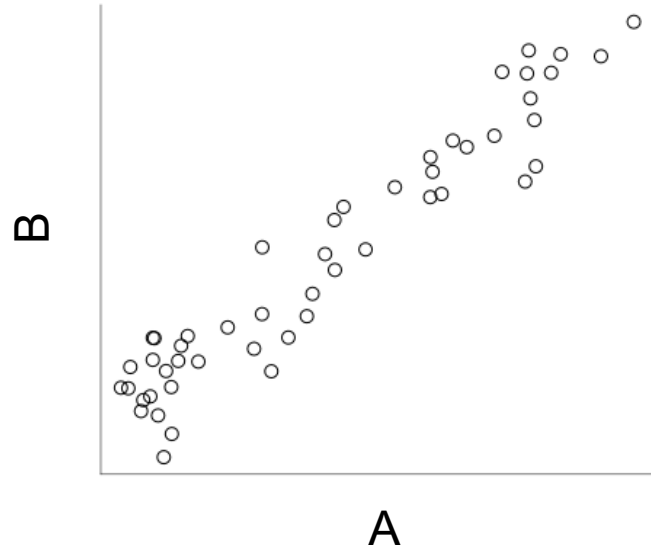
“Storks deliver babies ( $p=0.008$ )” doi: 10.1111/1467-9639.00013  
[priceconomics.com/do-storks-deliver-babies/](http://priceconomics.com/do-storks-deliver-babies/)



### 3. Interpreting correlations in general



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# Episodes

1. Statistical significance
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4. Good practices for data visualization

# 4. Good practices for data visualization

A good visualization...

- has a clear message and is focused
- is easy to interpret (summarizes the data)
- is an honest and true reflection of the data

Visualizations are made up of...

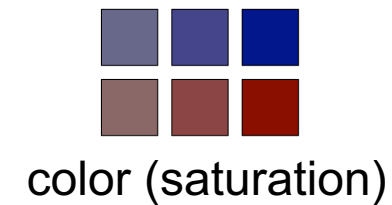
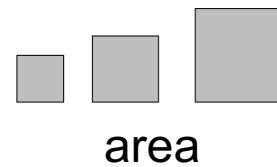
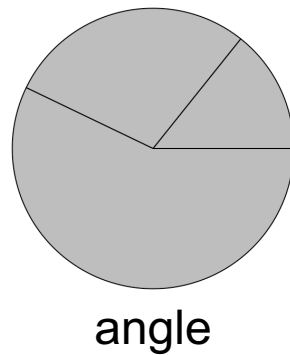
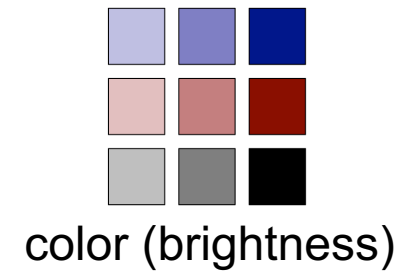
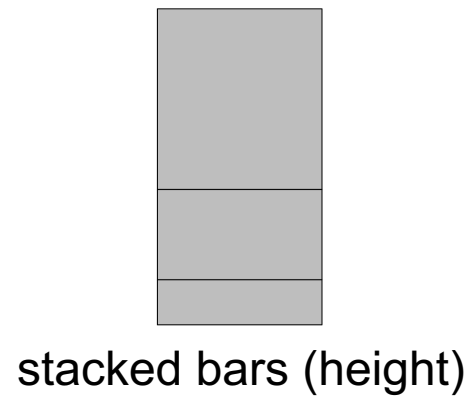
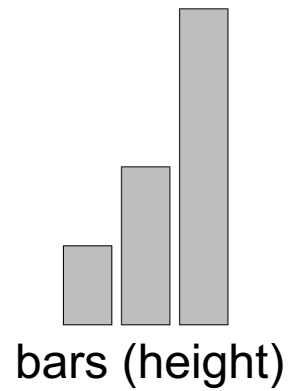
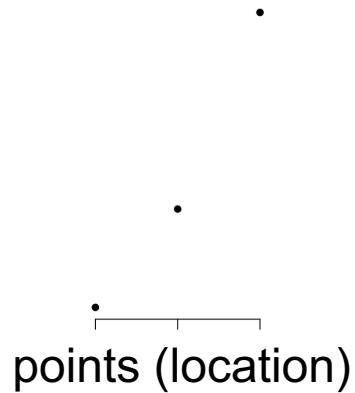
- geometrical primitives (lines, points, ...) that represent data sets
- scales (length, position, color, angle, ...) that encode the data values

(“The grammar of graphics”, ggplot2)

Encode the information with the most effective channel

# 4. Scales encode data values

All the scales below encode the same data: (1, 2, 4)

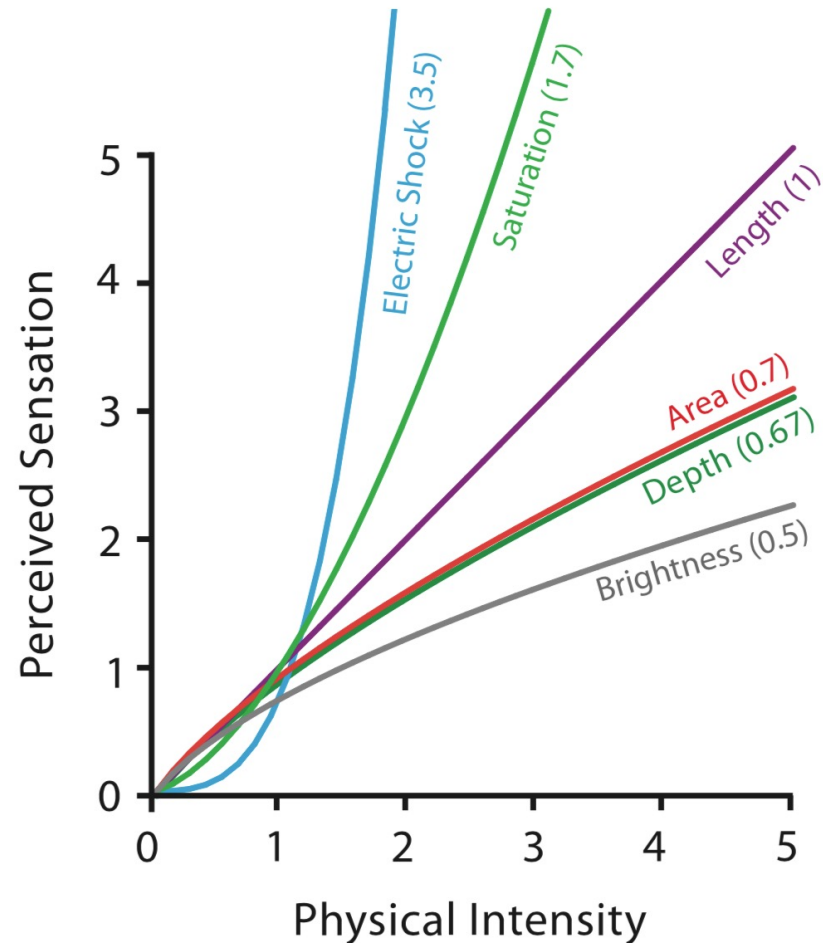


Which one best represents the 1-2-4 ratios?

# 4. Human perception is not linear

... except for length

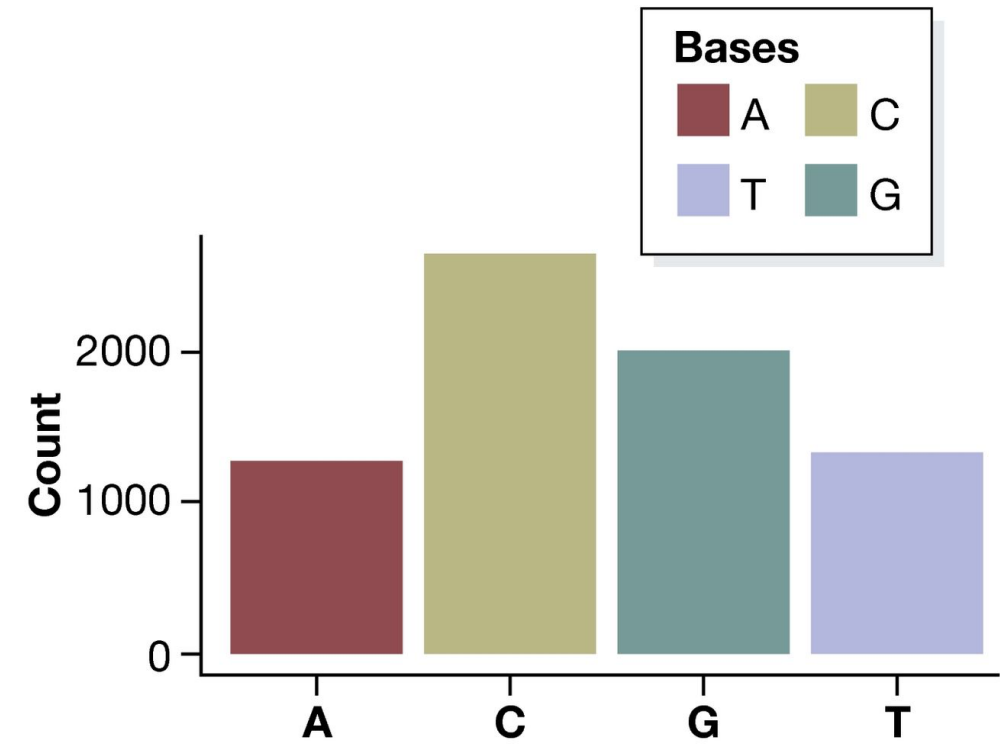
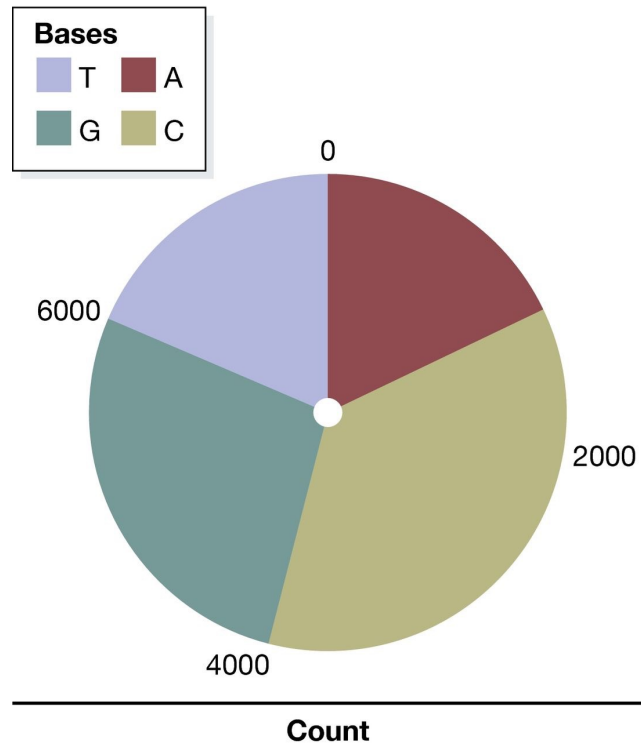
Steven's Psychophysical Power Law:  $S = I^N$



# 4. What wrong with pie charts?

base composition  
in the Zyxin gene

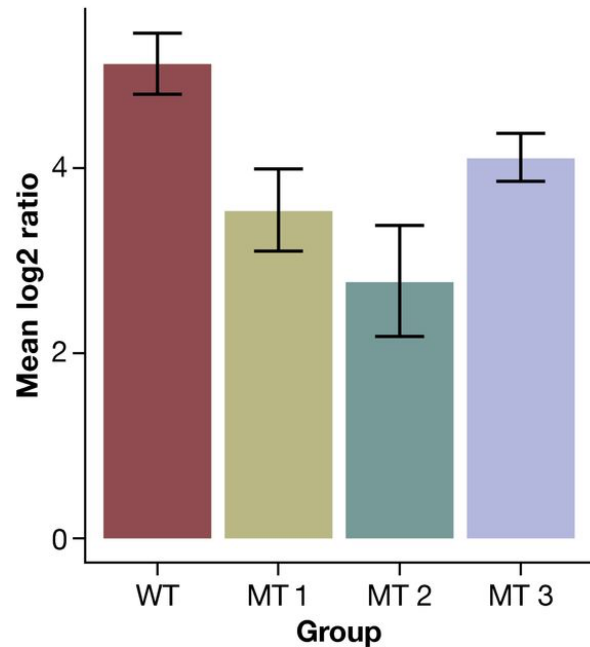
A	1,285
C	2,635
G	2,013
T	1,332



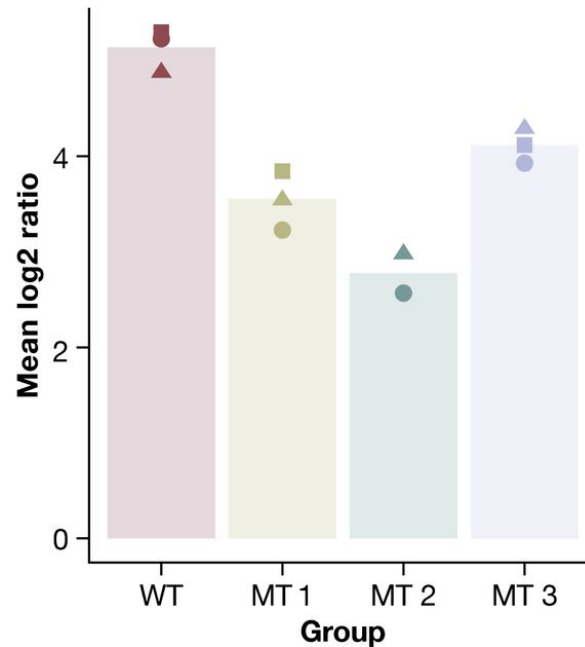
human perception: better to use length than area

# 4. What wrong with bar plots?

**A** Barplot



**B** Scatterplot

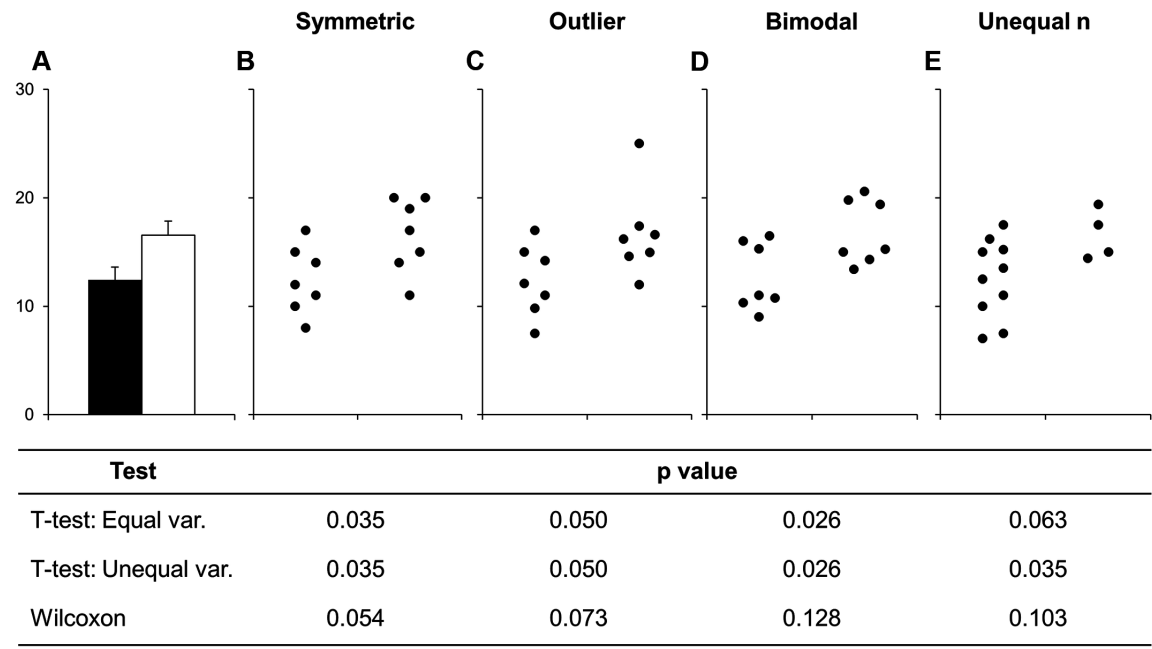


- barplot only show one number per group (e.g. mean)
- can be used to hide information (e.g. outlier, multimodal distribution)
- many types of error bars (s.d., s.e.m., 95%-confidence intervals)

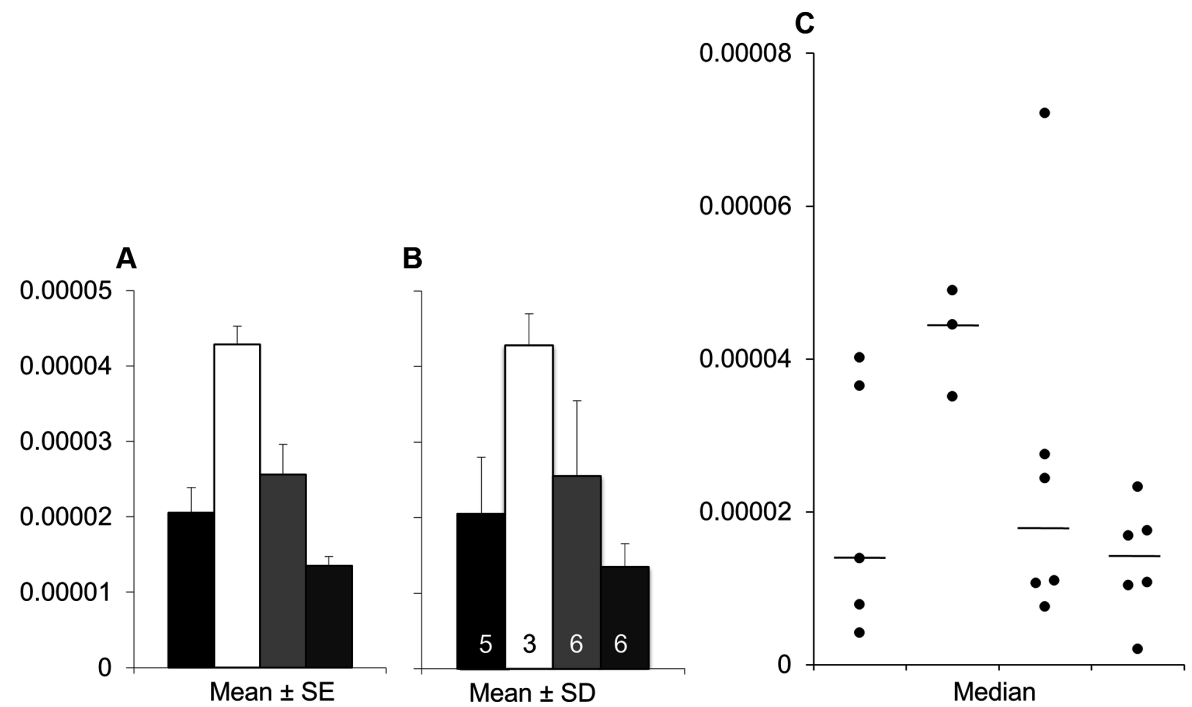


# 4. Examples of misleading bar plots

Various data produce the same barplot

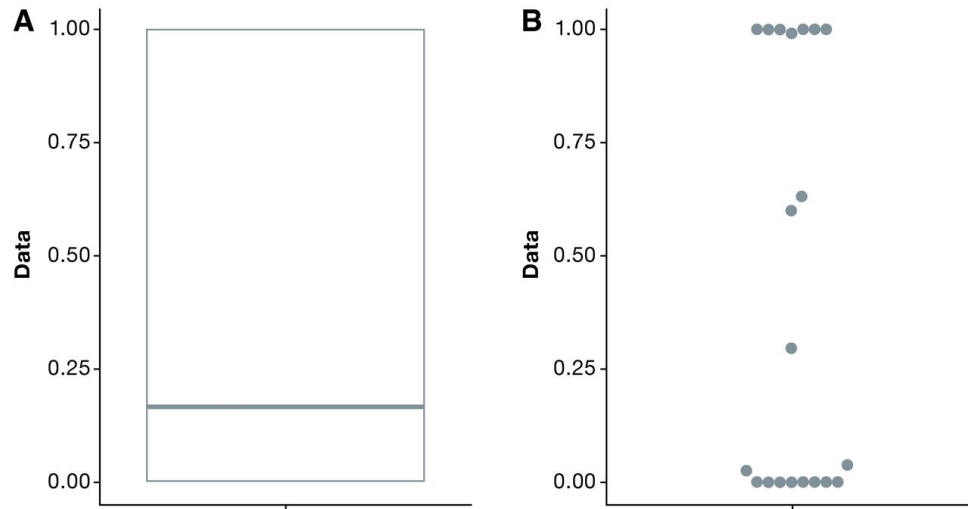


Placental endothelin 1 (EDN1) mRNA data

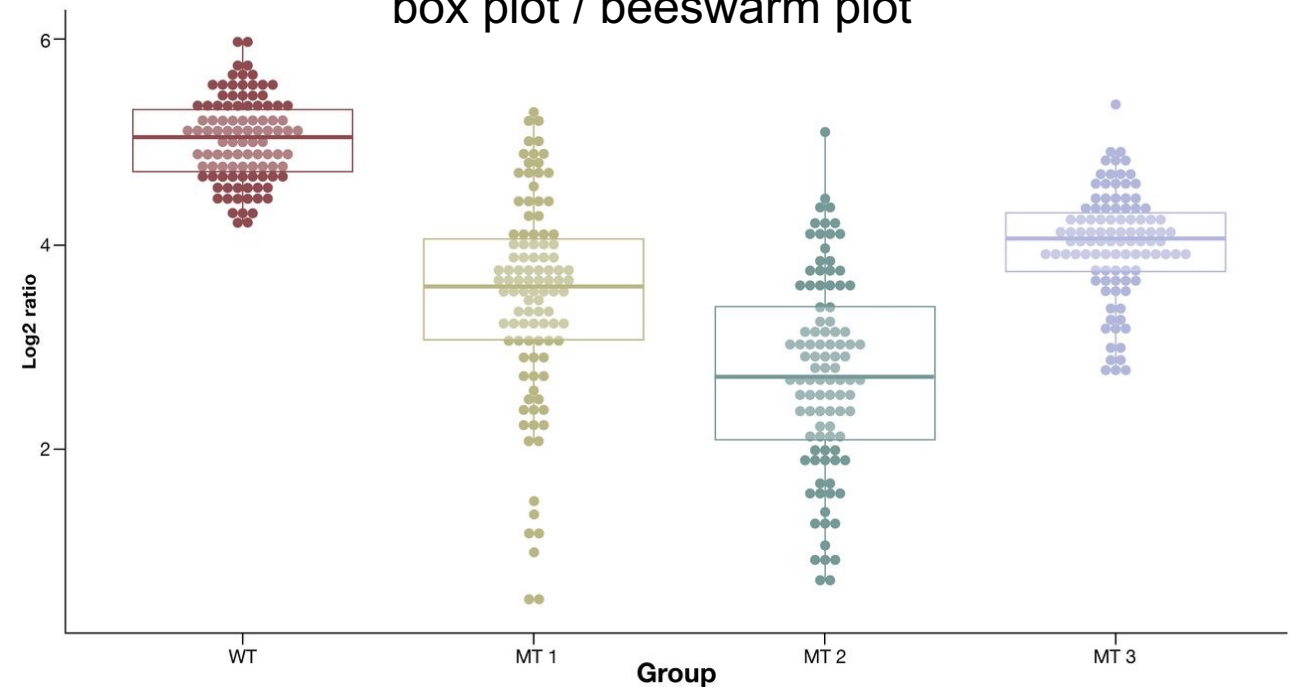


# 4. Plots for more data points

boxplot may also hide information

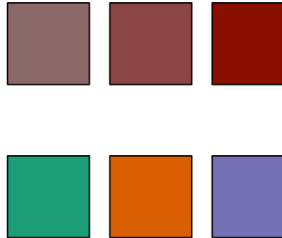


box plot / beeswarm plot



# 4. On the use of colors

- poor in encoding quantitative data
- great to represent categorical data
- don't forget the colorblind



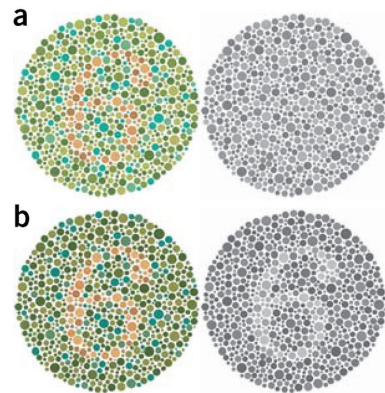
[nowosad.github.io/colorblindcheck/](https://nowosad.github.io/colorblindcheck/)

```
library(colorblindcheck)
rainbow_pal = rainbow(n = 7)
rainbow_pal
#> [1] "#FF0000" "#FFDB00" "#49FF00" "#00FF92" "#0092FF" "#4900FF" "#FF00DB"

palette_check(rainbow_pal, plot = TRUE)
```

```
#>      name n tolerance ncp ndcp min_dist mean_dist max_dist
#> 1  normal 7  12.13226  21  21 12.132257  61.06471 107.63470
#> 2 deuteranopia 7  12.13226  21  18  7.725825  50.11732  91.56339
#> 3  protanopia 7  12.13226  21  19  2.355309  55.41310  88.34820
#> 4  tritanopia 7  12.13226  21  19  8.216194  51.53678  83.10000
```

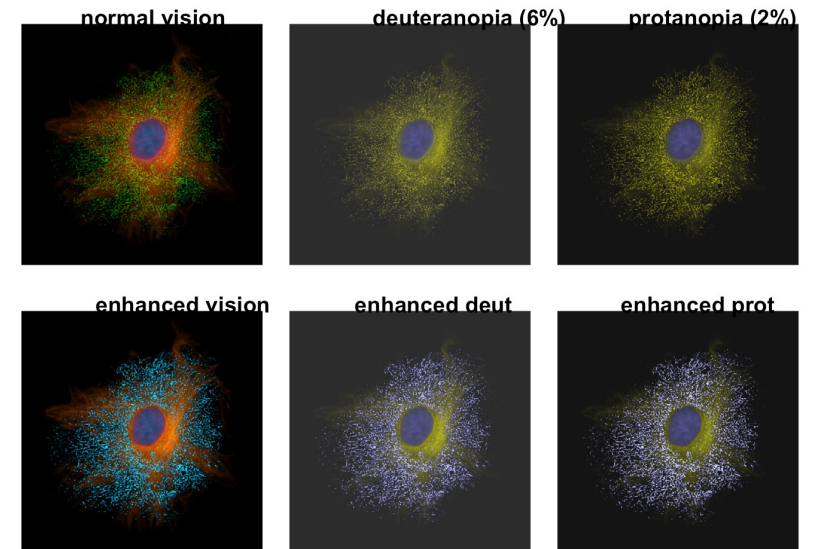
Ishihara color-vision test



Colors optimized for color-blind individuals

Color	Color name	RGB (1–255)	CMYK (%)	P	D
	Black	0, 0, 0	0, 0, 0, 100		
	Orange	230, 159, 0	0, 50, 100, 0		
	Sky blue	86, 180, 233	80, 0, 0, 0		
	Bluish green	0, 158, 115	97, 0, 75, 0		
	Yellow	240, 228, 66	10, 5, 90, 0		
	Blue	0, 114, 178	100, 50, 0, 0		
	Vermillion	213, 94, 0	0, 80, 100, 0		
	Reddish purple	204, 121, 167	10, 70, 0, 0		

P and D indicate simulated colors as seen by individuals with protanopia (red-) and deuteranopia (green-).



“Points of view: Color blindness” doi: 10.1038/nmeth.1618  
[colorbrewer2.org](https://colorbrewer2.org) [colororacle.org](https://colororacle.org) (app for macOS/Windows/Linux)

[cran.r-project.org/package=colorBlindness](https://cran.r-project.org/package=colorBlindness)

# Further information

- Nature Methods “Points of Significance”, web collection:  
<https://www.nature.com/collections/qghhqm/pointsofsignificance>
- Klaus B. Statistical relevance – relevant statistics, part I. EMBO J. 2015;34(22):2727-30. doi: 10.15252/emboj.201592958.
- Klaus B. Statistical relevance – relevant statistics, part II. EMBO J. 2016;35(16):1726-9. doi: 10.15252/emboj.201694659.
- Scientific Figure Design (1-day-course, Babraham).  
<http://www.bioinformatics.babraham.ac.uk/training.html#figuredesign>
- Munzner T. Visualization Analysis and Design. 2014 by A. K. Peters/CRC Press. ISBN 9781466508910