

# Scientific Inquiry

## Part II: Experimental Design and Data Analysis

Charlotte Soneson & Michael Stadler



**FMI**

Friedrich Miescher Institute  
for Biomedical Research



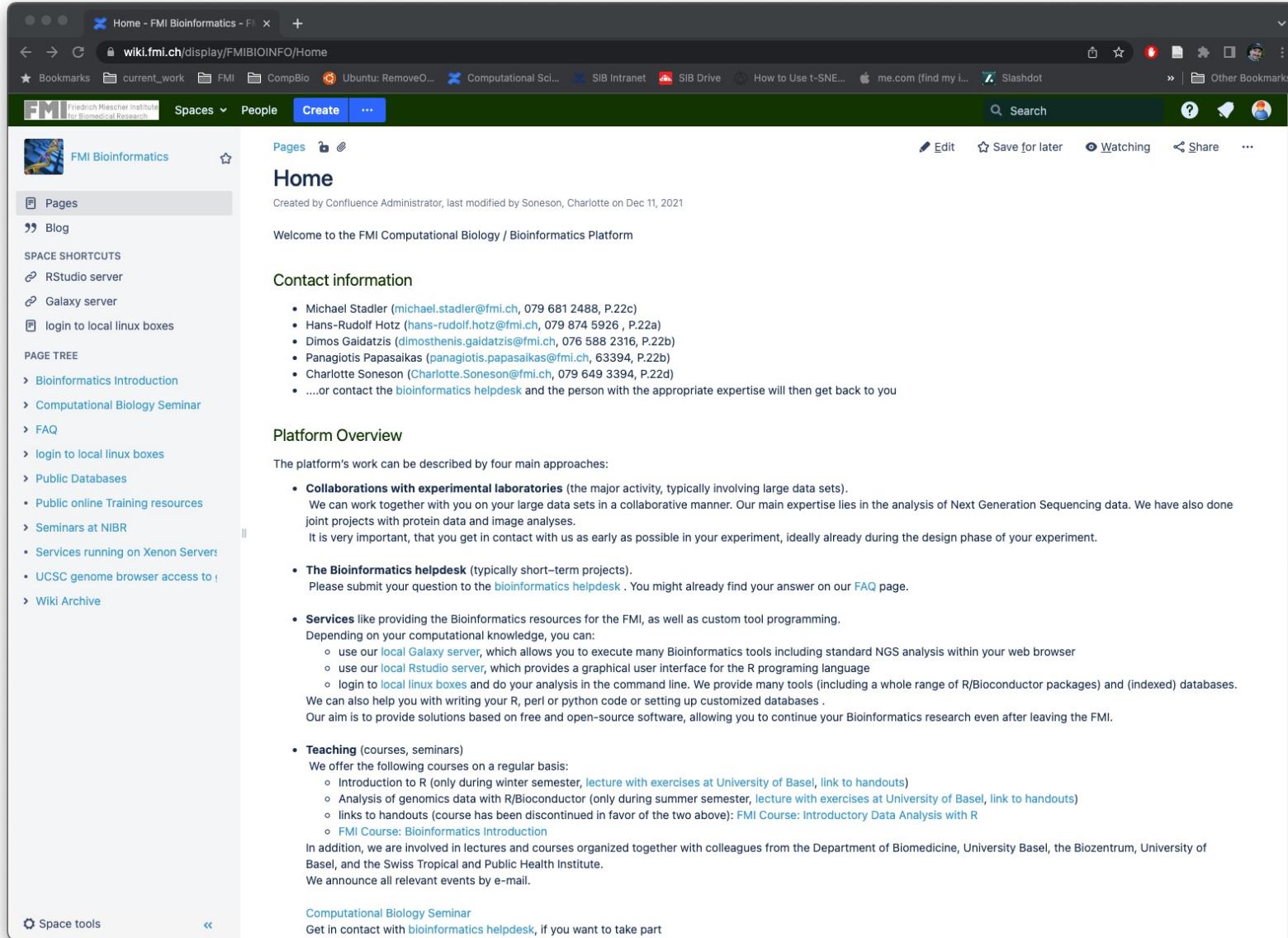
# Where I come from

- 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](http://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 Confluence Home page. The page has a dark header with the FMI logo and the text "Friedrich Miescher Institute for Biomedical Research". The main content area is titled "Home" and includes sections for "Contact information", "Platform Overview", and "Teaching (courses, seminars)". The "Contact information" section lists several staff members with their email addresses. The "Platform Overview" section describes the platform's work in four main approaches: collaborations with experimental laboratories, the bioinformatics helpdesk, services like RStudio and Galaxy servers, and teaching courses. The "Teaching (courses, seminars)" section lists courses like "Introduction to R", "Analysis of genomics data with R/Bioconductor", and "FMI Course: Bioinformatics Introduction". The footer contains links to the "Computational Biology Seminar" and the "bioinformatics helpdesk".

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 Papasaikas ([panagiotis.papasaikas@fmi.ch](mailto:panagiotis.papasaikas@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.

Space tools

Computational Biology Seminar

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

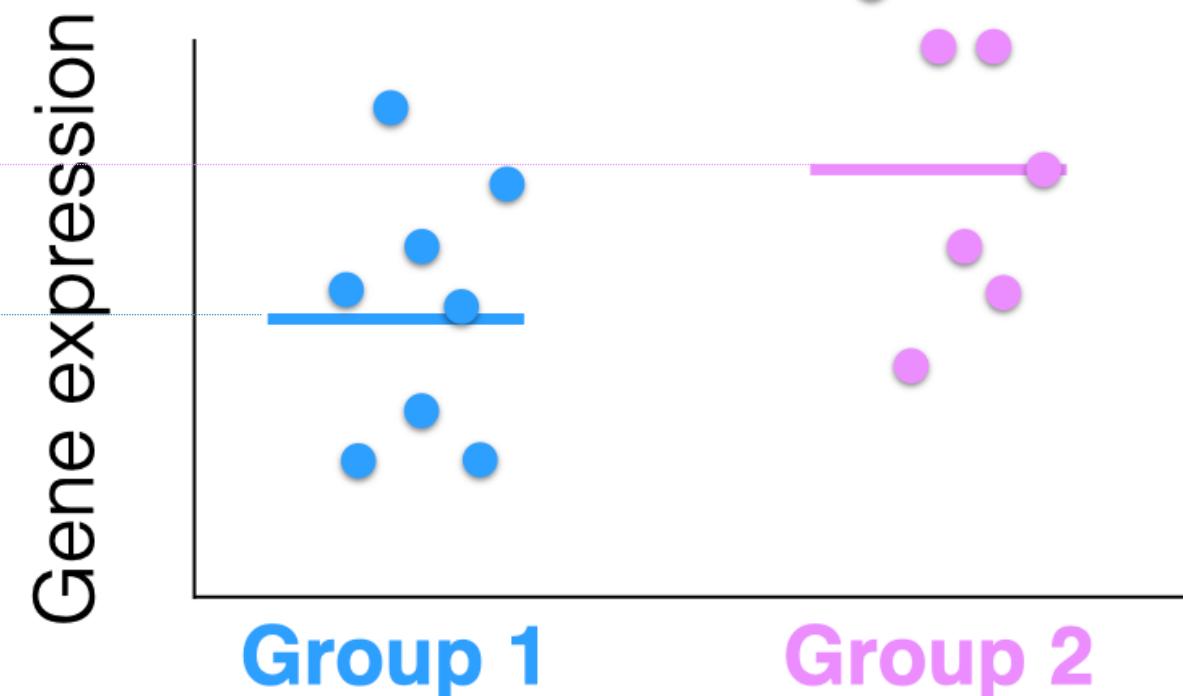
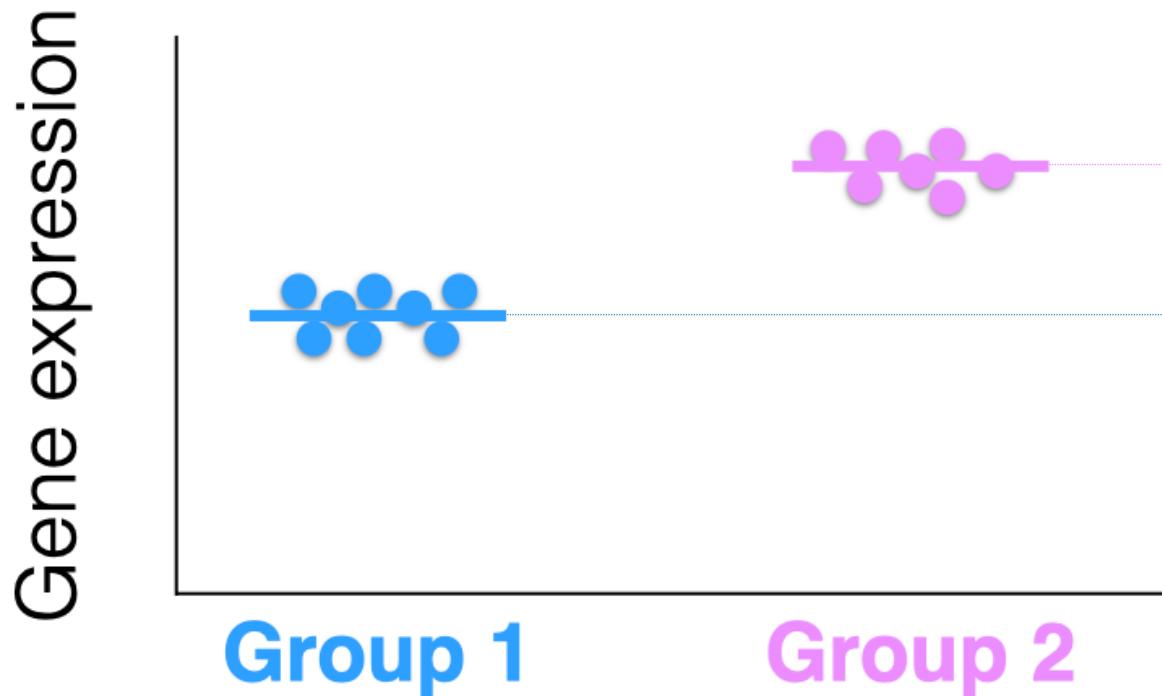
# Episodes

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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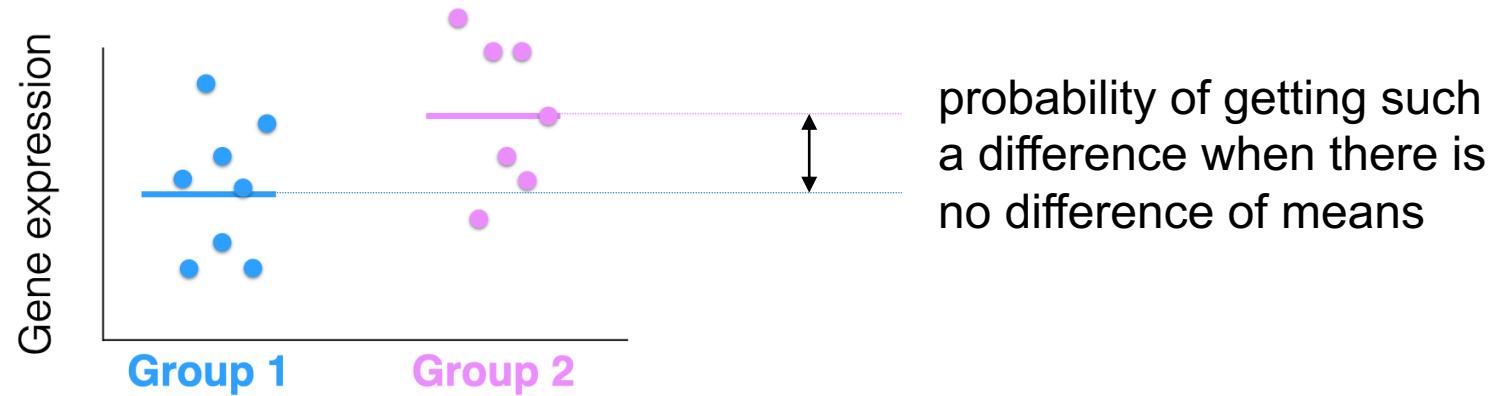
# 1. Statistical significance



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

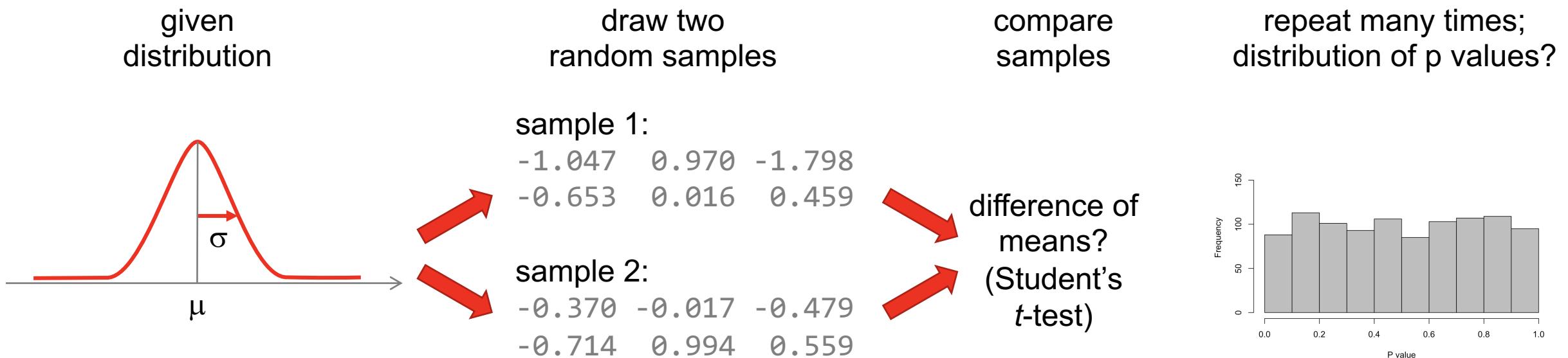
# Episodes

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:



## 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
}

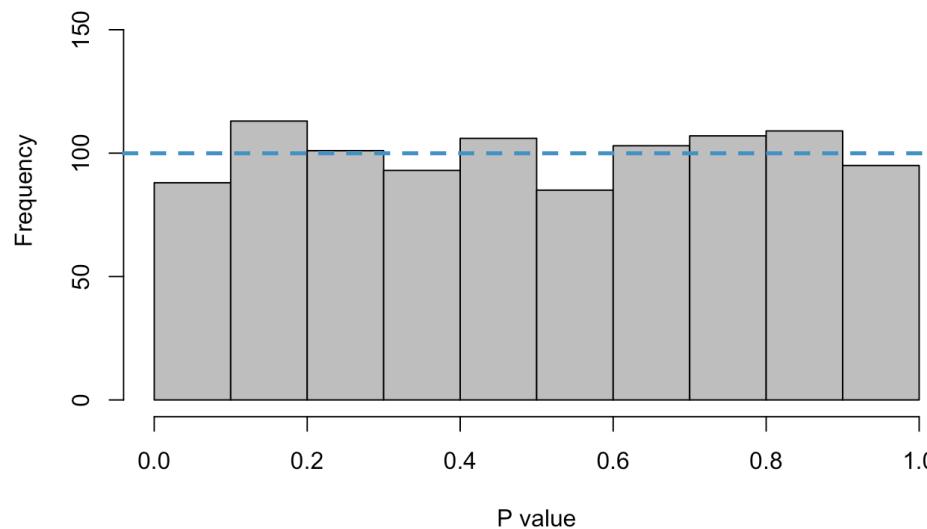
mean(pvals <= 0.1) # 0.088 (~0.1)
padj <- p.adjust(pvals, method = "fdr")
mean(padj <= 0.1) # 0
```

```
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
}

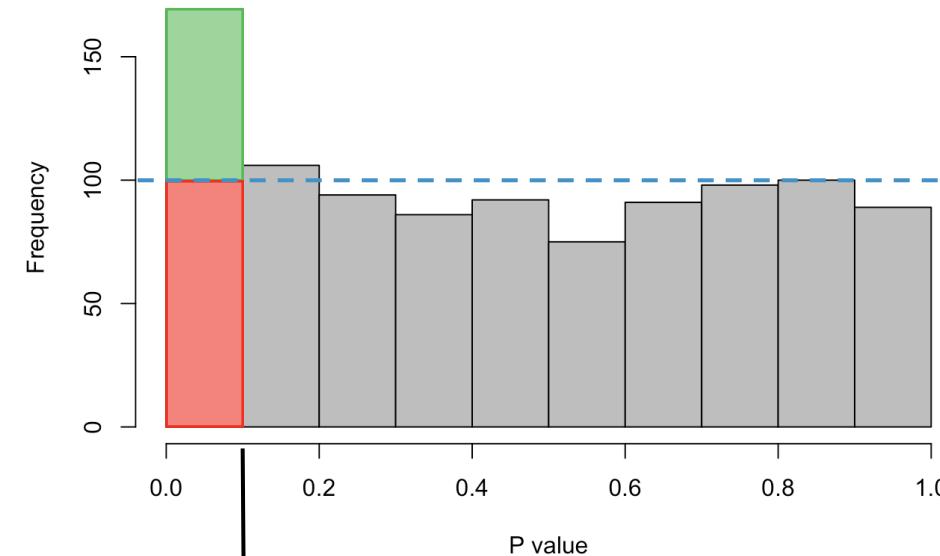
mean(pvals <= 0.1) # 0.169
padj <- p.adjust(pvals, method = "fdr")
mean(padj <= 0.1) # 0.067
```

## 2. Multiple testing correction

histogram of P values (under  $H_0$ )



histogram of P values (10% true effect)



false discovery rate (FDR) =

$$\frac{\text{red}}{\text{green} + \text{red}} = \frac{100}{100 + 69} = 0.59$$

# Episodes

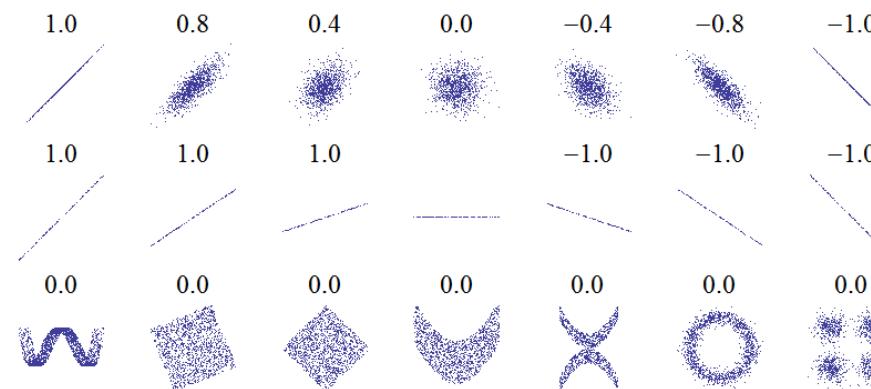
1. Statistical significance
2. Multiple testing correction
3. **Correlation and causation (or “Eat more chocolate!”)**
4. Good practices for data visualization

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

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

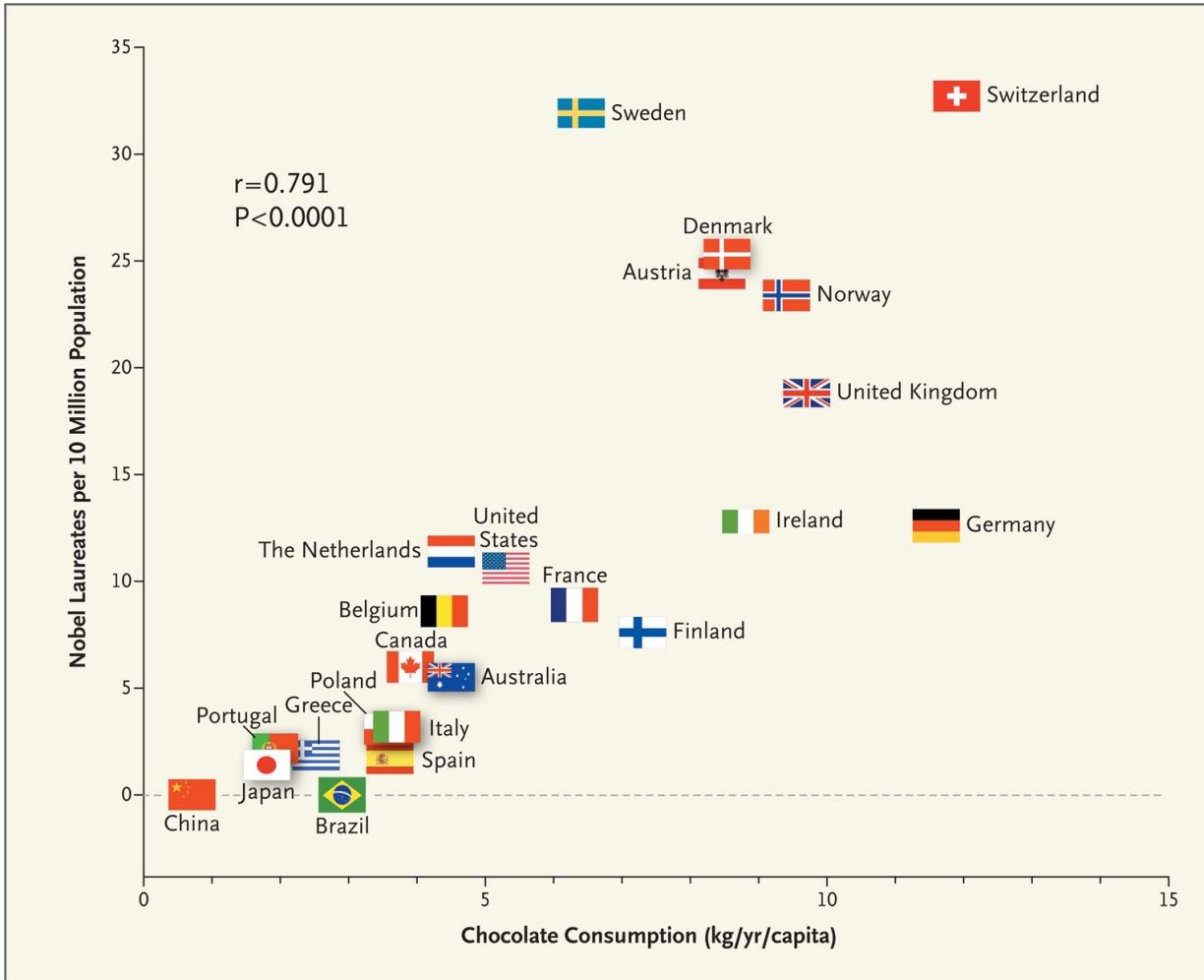
Pearson’s correlation coefficient  $r$  [-1, +1]

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



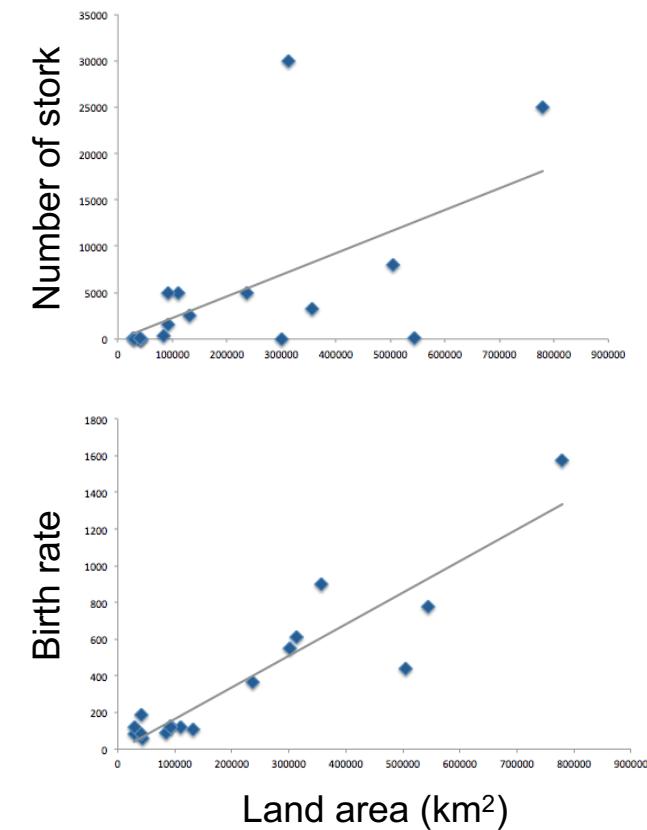
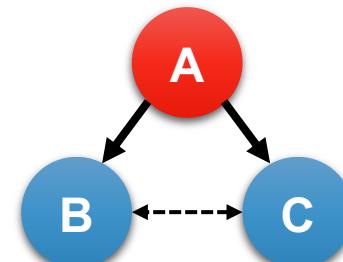
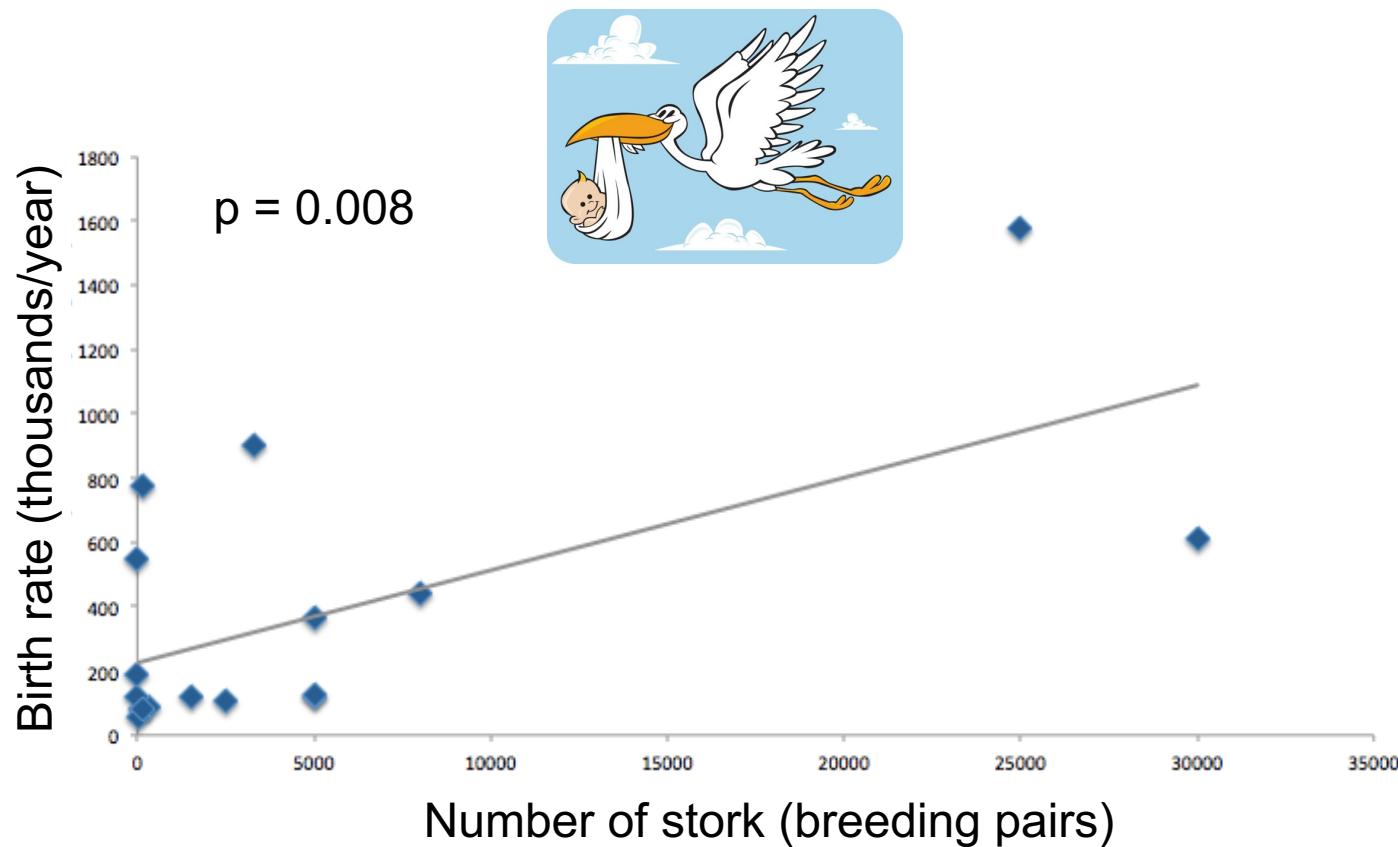
“Correlation implies association, but not causation.”

# 3. Eat more chocolate!



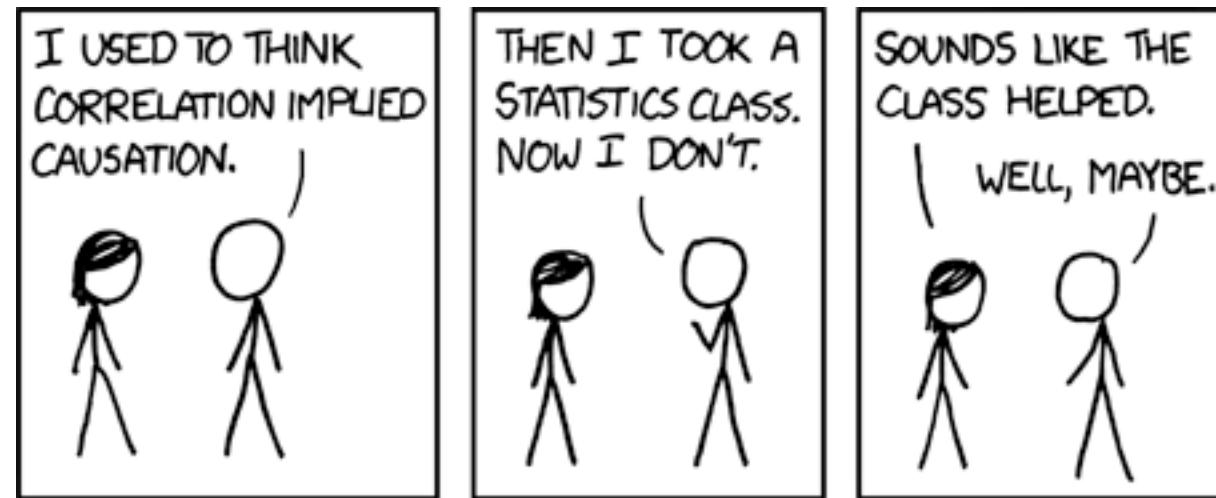
“The slope of the regression line allows us to estimate that it would take about 0.4 kg of chocolate per capita per year to increase the number of Nobel laureates in a given country by 1.”

# 3. Do Storks deliver babies?

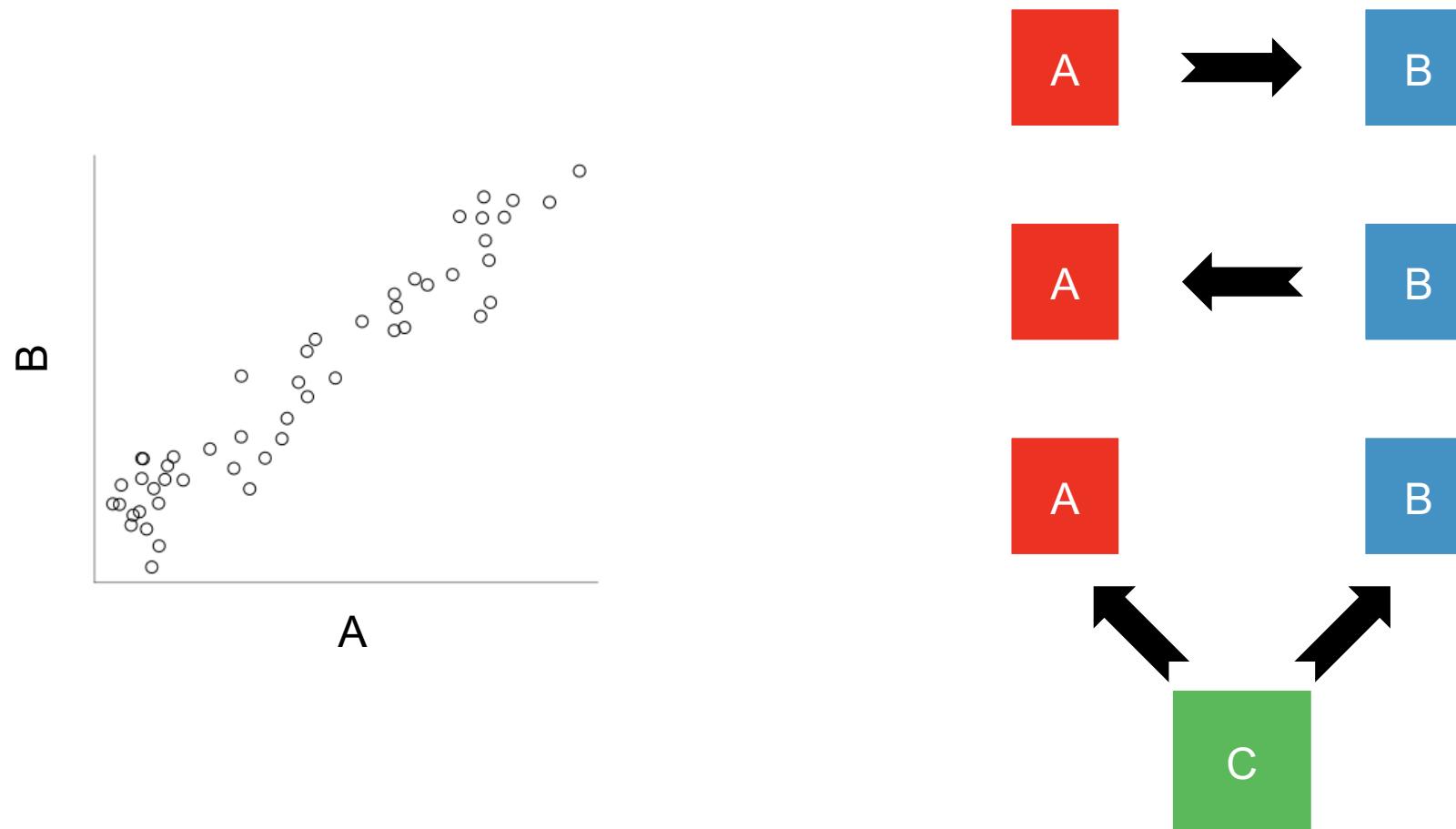


“Storks deliver babies (p=0.008)” doi: 10.1111/1467-9639.00013  
[priceconomics.com/do-storks-deliver-babies/](http://priceconomics.com/do-storks-deliver-babies/)

### 3. Interpreting correlations in general



### 3. Interpreting correlations in general



# 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...

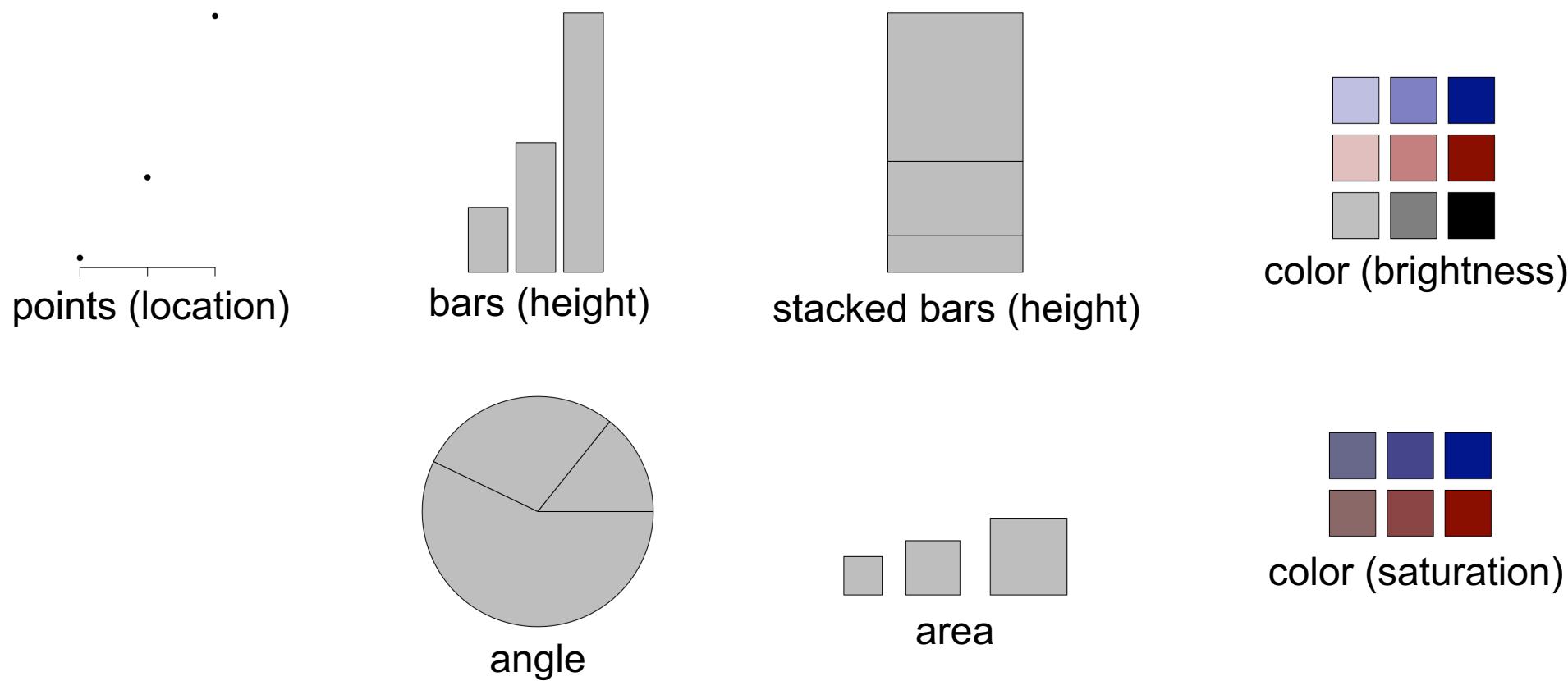
- geometrical primitives (lines, points, ...) that represent data sets
- scales (length, position, color, angle, ...) that encode the data values

(“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)

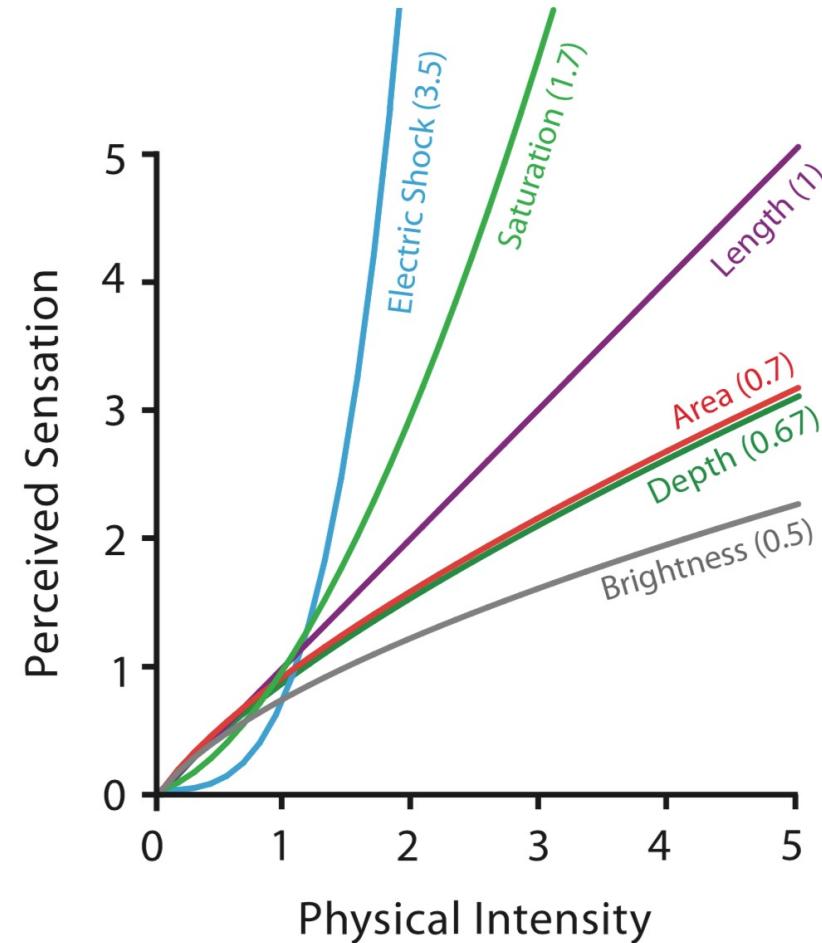


Which one best represents the 1-2-4 ratios?

# 4. Human perception is not linear

... except for length

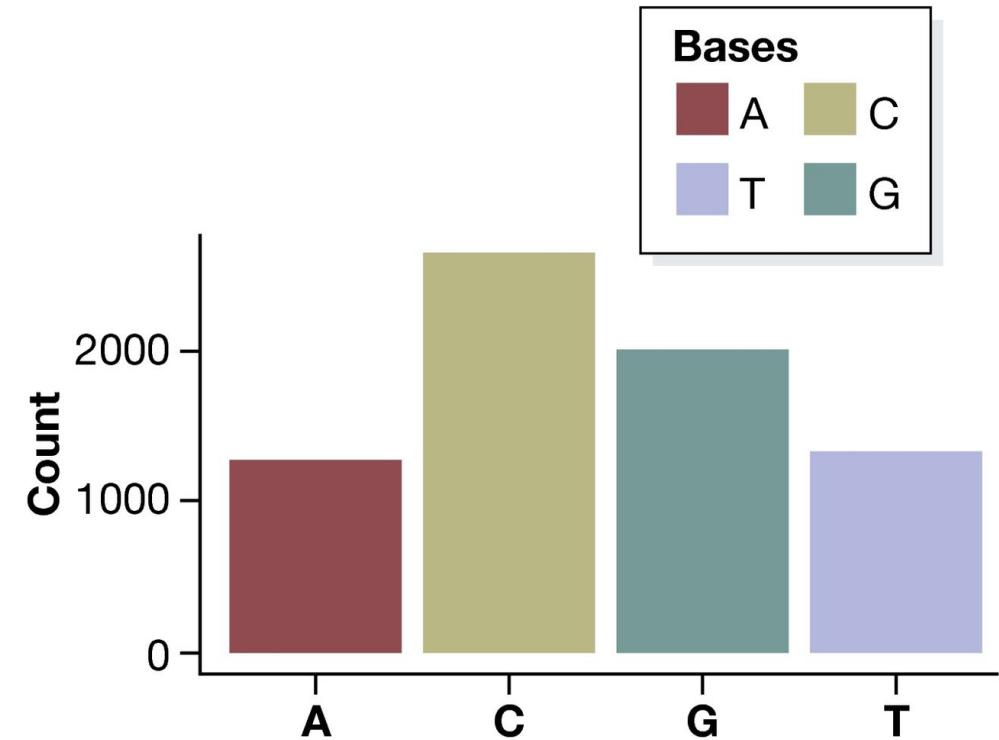
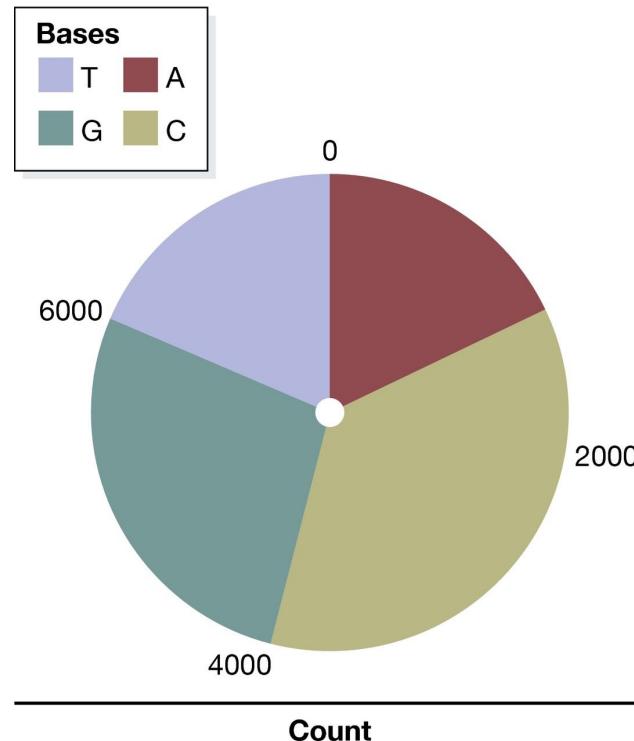
Steven's Psychophysical Power Law:  $S = I^N$



# 4. What wrong with pie charts?

base composition  
in the Zyxin gene

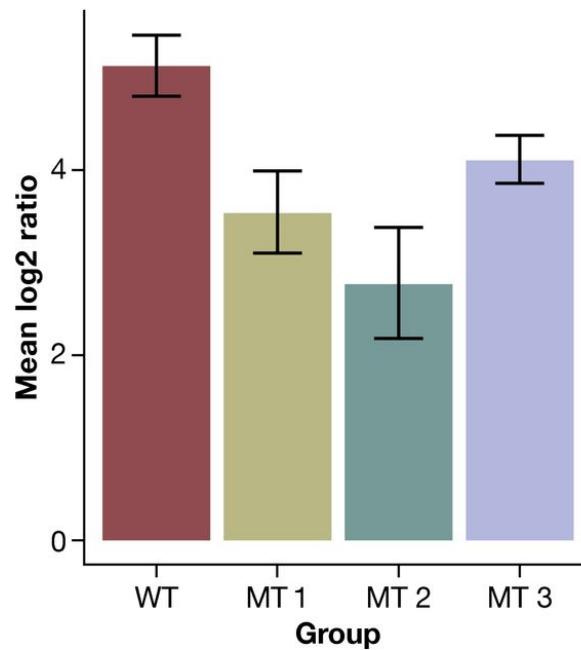
A	1,285
C	2,635
G	2,013
T	1,332



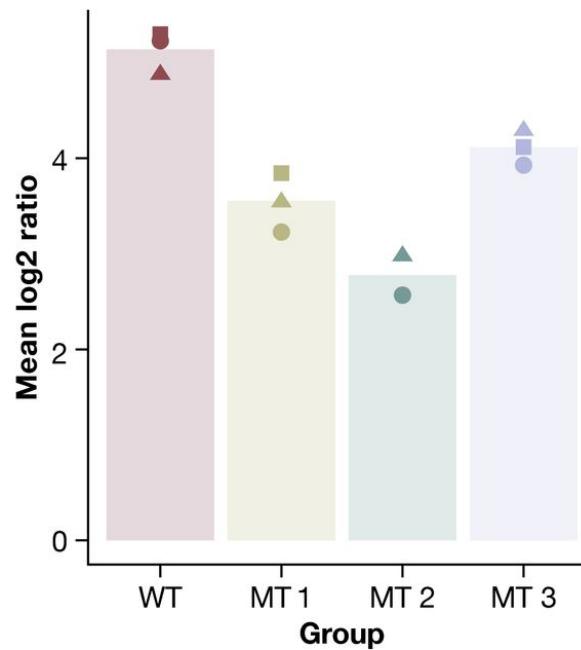
human perception: better to use length than area

# 4. What wrong with bar plots?

**A Barplot**



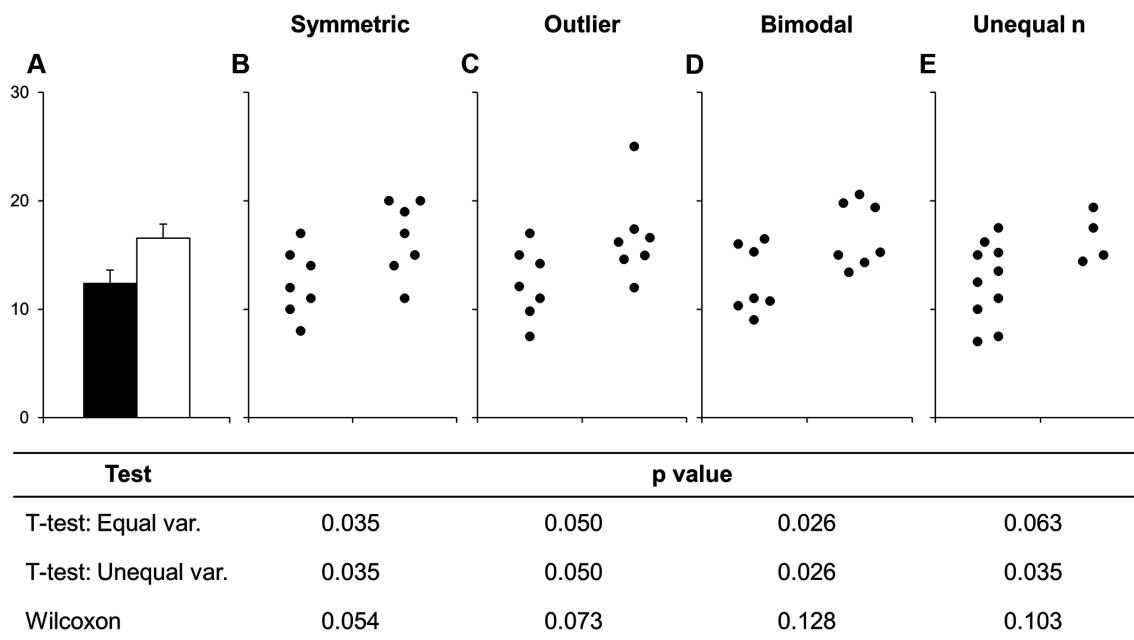
**B Scatterplot**



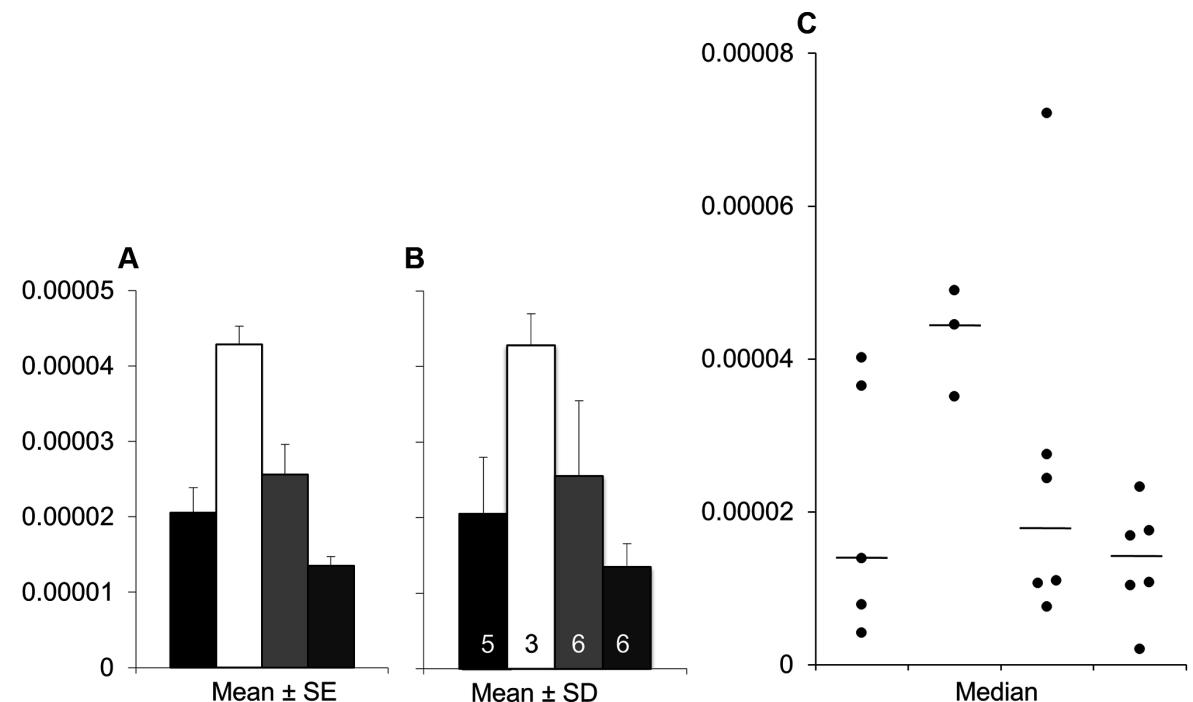
- barplot only show one number per group (e.g. mean)
- can be used to hide information (e.g. outlier, multimodal distribution)
- many types of error bars (s.d., s.e.m., 95%-confidence intervals)

# 4. Examples of misleading bar plots

Various data produce the same barplot

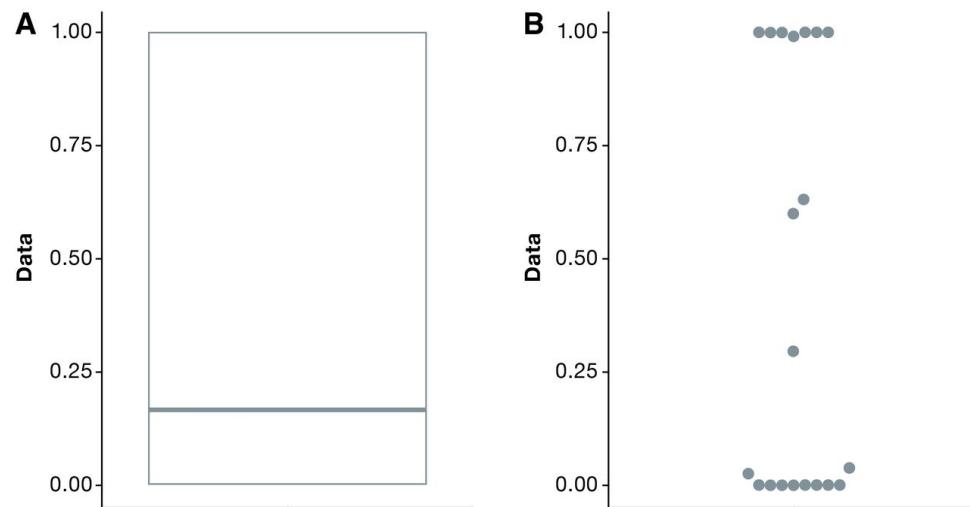


Placental endothelin 1 (EDN1) mRNA data

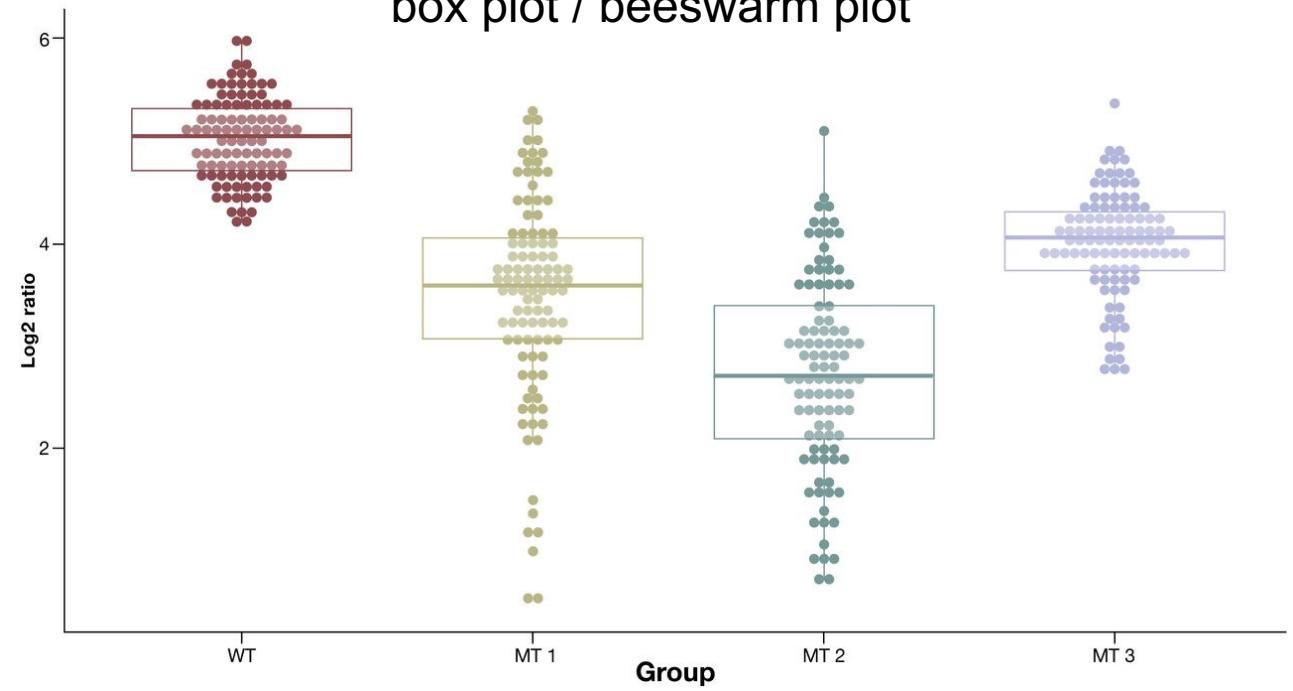


# 4. Plots for more data points

boxplot may also hide information

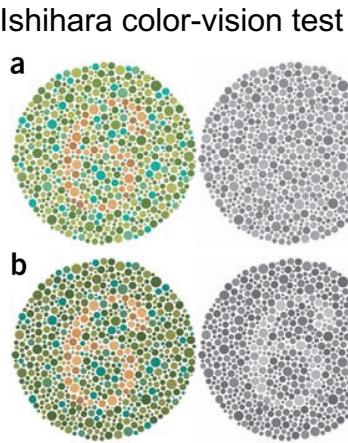


box plot / beeswarm plot



# 4. On the use of colors

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



Colors optimized for color-blind individuals

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

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

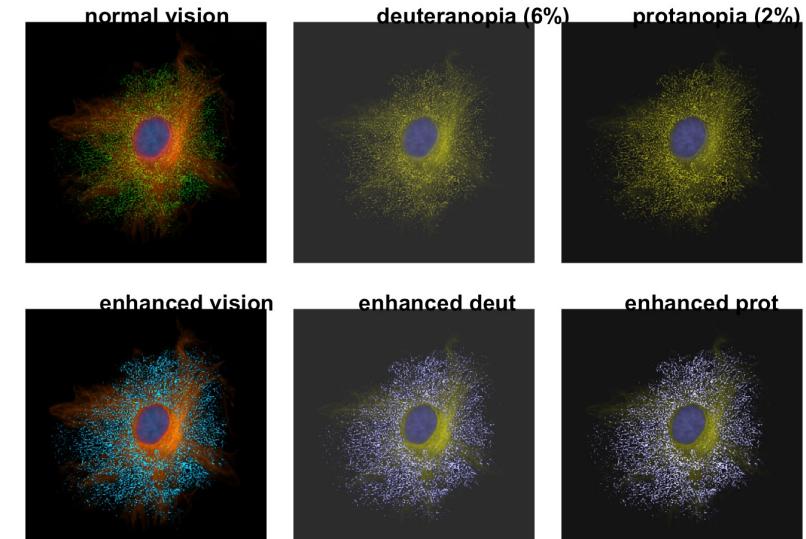
[nowosad.github.io/colorblindcheck/](http://nowosad.github.io/colorblindcheck/)

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

palette_check(rainbow_pal, plot = TRUE)

Normal
Deutanopia
Protanopia
Tritanopia
```

	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	deutanopia	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



# 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/embj.201592958.
- Klaus B. Statistical relevance – relevant statistics, part II. EMBO J. 2016;35(16):1726-9. doi: 10.15252/embj.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