Correlation
Power analysis
Multiple hypothesis testing

Practical Statistics for Experimental Biologists
Lecture 4
Thursday, 30 July 2020
10:00am - 12:00pm

Correlation

Example: lipids and insulin sensitivity

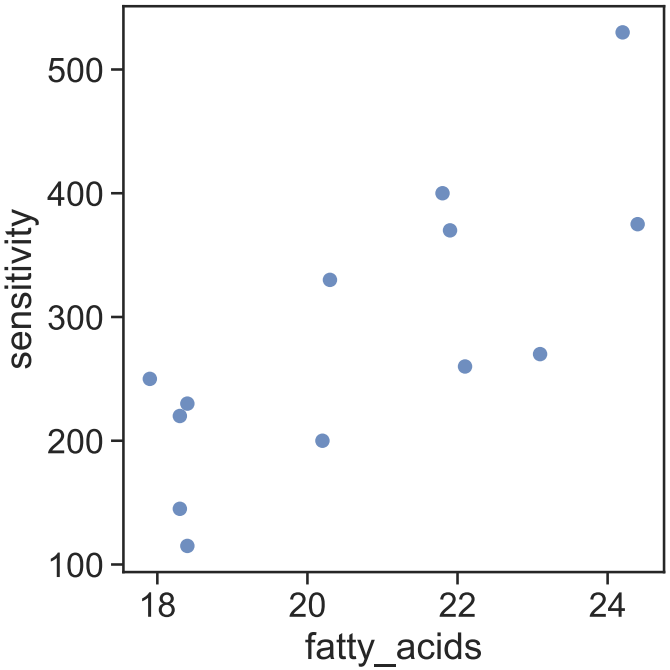
sensitivity	fatty_acid
250	17.9
220	18.3
145	18.3
115	18.4
230	18.4
200	20.2
330	20.3
400	21.8
370	21.9
260	22.1
270	23.1
530	24.2
375	24

Borkman et al. (1993) wanted to understand why insulin sensitivity varies so much among individuals. They hypothesized that the lipid composition of the cell membranes of skeletal muscle affects the sensitivity of the muscle for insulin.

They determined the insulin sensitivity of $N = 13$ healthy men by infusing insulin at a standard rate (adjusting for size differences) and quantifying how much glucose they needed to infuse to maintain a constant a blood glucose level…

They also took a small muscle biopsy from each subject and measured its fatty acid composition. We'll focus on the fraction of of polyunsaturated fatty acids that have between 20 and 22 carbon atoms ("fatty_acid").

Correlation is used to describe relationships between real-numbered variables



summary statistics

	pearson
N	13
r	0.77
95% CI	[0.38, 0.93]
r^2	0.593
P-val	0.00207701

Covariance and correlation are estimated from data in the familiar manner

The formula for variance is

$$\widehat{\mathrm{var}}(x) = \sigma_x^2 = \frac{1}{N-1}\sum_i (x_i - \hat{\mu}_x)^2$$

Covariance is estimated in a manner similar to variance

$$\widehat{\mathrm{cov}}(x, y) = \frac{1}{N-1}\sum_i (x_i - \hat{\mu}_x)(y_i - \hat{\mu}_y)$$

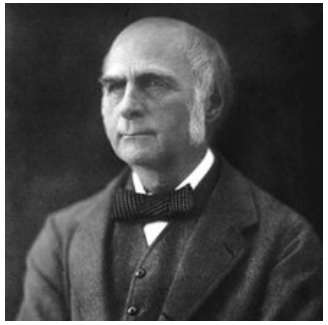
The corresponding “correlation coefficient” is

$$r = \frac{\widehat{\mathrm{cov}}(x, y)}{\hat{\sigma}_x \, \hat{\sigma}_y}$$

https://en.wikipedia.org/wiki/Pearson_correlation_coefficient

The correlation coefficient, as used today, was developed by Karl Pearson and Francis Galton in the 1880s

$$r = \frac{\widehat{\mathrm{cov}}(x, y)}{\hat{\sigma}_x \, \hat{\sigma}_y}$$ is often called "Pearson correlation"



Francis Galton
(1822-1911)

- Discovered the correlation coefficient in 1888 (independent of Auguste Bravis, in 1844) and proposed the use of "r".
- Also invented linear regression & idea of "regression toward the mean"
- Invented the term "eugenics" in 1883

Karl Pearson
(1857-1936)

- Is credited with founding the discipline of mathematical statistics.
- Further developed the correlation coefficient proposed by Galton
- Other contributions include P-values, χ^2 tests, and principal component analysis (PCA)
- Founded world's first university statistics department at University College, London
- Was a vocal and influential social Darwinist and advocate of race wars.
- Founded the "Annals of Eugenics", which is now "Annals of Human Genetics"

UCL (University College London)

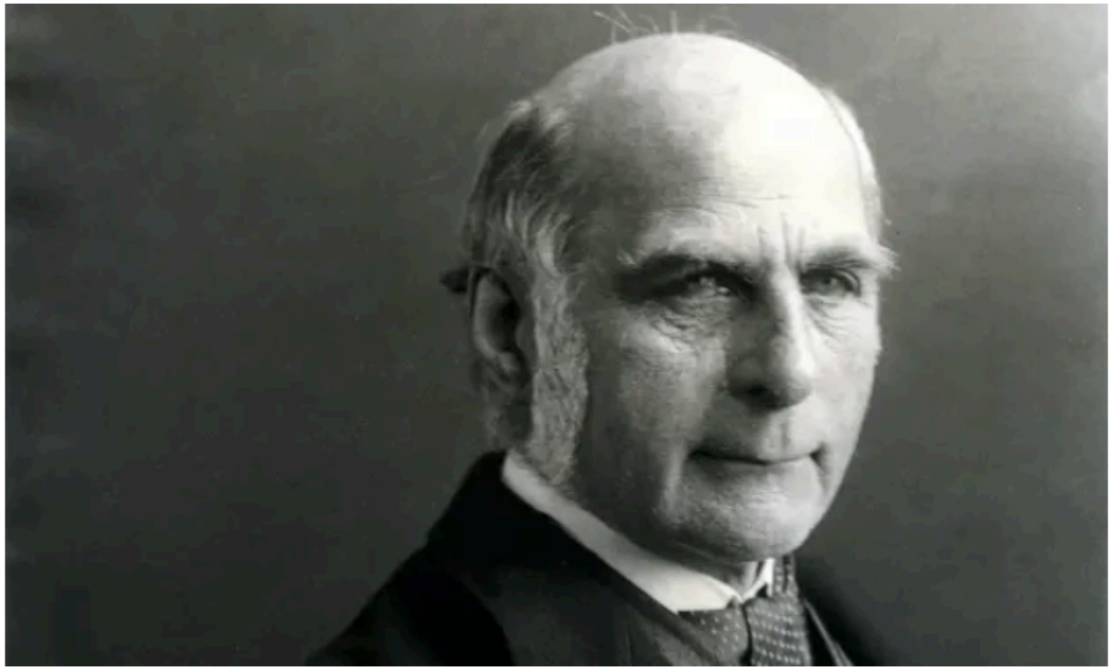
This article is more than **1 month old**

UCL renames three facilities that honoured prominent eugenicists

London university removes names of Francis Galton and Karl Pearson from two lecture theatres and a building

PA Media

Fri 19 Jun 2020 15.10 EDT



▲ Francis Galton coined the term eugenics in 1883 and endowed UCL with his personal collection and archive. Photograph: Corbis via Getty Images

UCL has renamed two lecture theatres and a building that honoured the prominent eugenicists Francis Galton and Karl Pearson.

This is what the correlation coefficient looks like

Pearson's r ranges from -1 to 1.

$r = 0$ when the two variables are independent, i.e. $p(x, y) = p(x) \cdot p(y)$.

$r = \pm 1$ when the two variables share a deterministic linear relationship.

Adding a constant to all x or all y, or a multiplicative rescaling of all x or all y, do not change r.

https://en.wikipedia.org/wiki/Pearson_correlation_coefficient

This is what the correlation coefficient looks like

In the deterministic case, r is unaffected by the magnitude of the slope relating two variables, while the sign of r is equal to the sign of the slope.

Sometimes $r = 0$ when two variables have a nonlinear relationship.

https://en.wikipedia.org/wiki/Pearson_correlation_coefficient

The coefficient of determination another name for r^2

The coefficient of determination is simply r^2, which is also often written as R^2.

r^2 is always between 0 and 1 (inclusive)

Remember that $r^2 \leq |r|$, so beware of people reporting r instead of r^2 to make a correlation seem stronger.

r^2 is commonly interpreted as the fraction of variance in y explained by x (or the other way around).

P-values correspond to the null hypothesis of no correlation in the underlying distribution

The p-value reported alongside values of r or r^2 corresponds to the null hypothesis that the underlying correlation is zero.

If the underlying correlation is zero, then the quantity

$$t = r\sqrt{\frac{N-2}{1-r^2}}$$

follows a t-distribution with $N-2$ degrees of freedom.

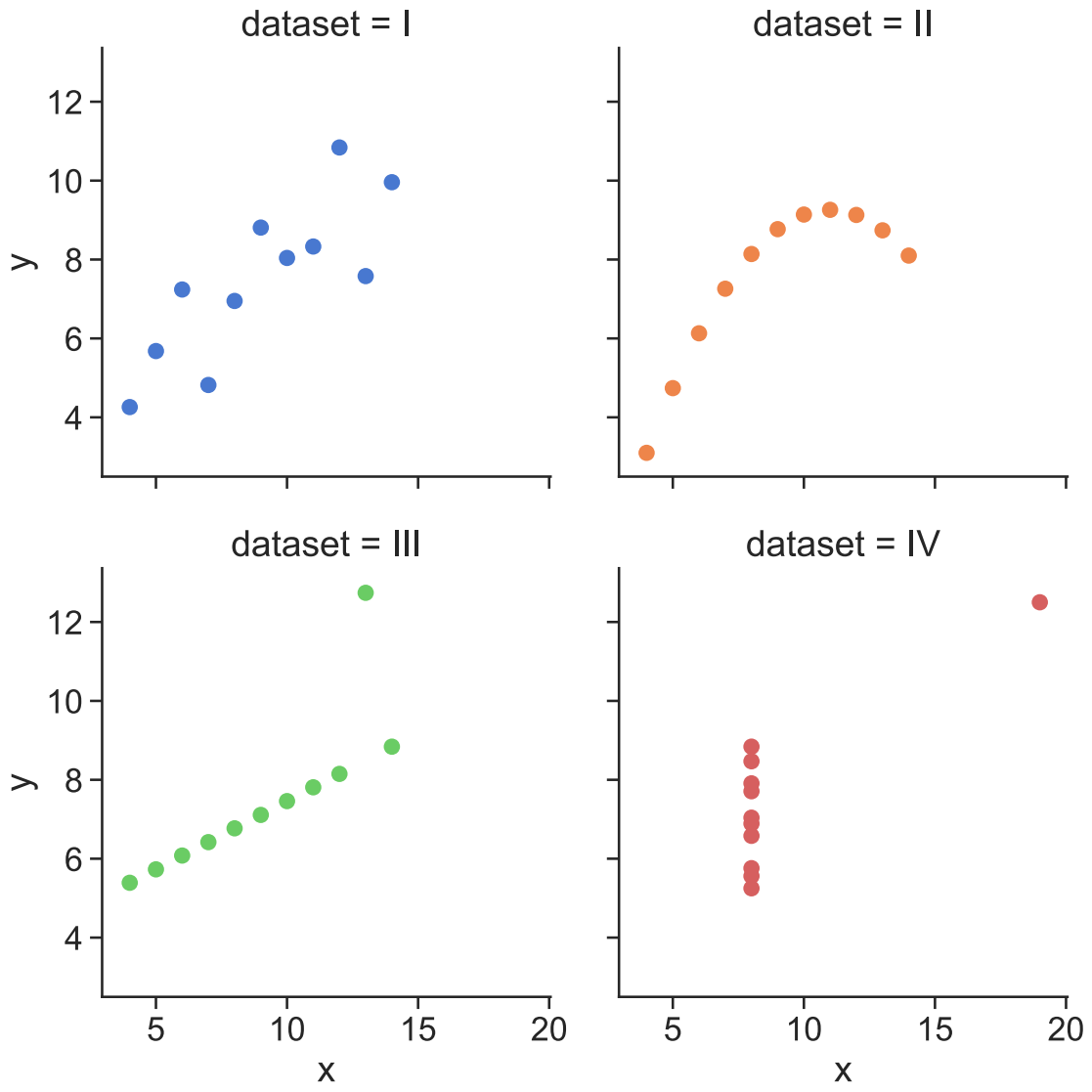
The inverse relationship

$$r = \frac{t}{\sqrt{N-2+t^2}}$$

is used to compute values for the 95% confidence interval on r.

Lots of different-looking datasets will have the same value for r.

"Anscombe's quartet": $r = 0.816$ for all 4 datasets



Anscombe, F. J. (1973). "Graphs in Statistical Analysis". American Statistician. 27 (1): 17–21.

Assumptions underlying correlation

Interpreting the correlation coefficient r, and especially the associated P-value, requires multiple assumptions:

- Each data point (x, y) is independently sampled from a 2D Gaussian distribution.
- In particular, x and y each follow a 1D Gaussian distribution
- All covariation between x and y is linear, with perfect concordance disrupted only by Gaussian noise.

There are usually many explanations for why two variables might correlate

Possible reasons for a correlation between lipid levels and insulin sensitivity:

- The lipid content of membranes affects insulin sensitivity
- The insulin sensitivity affects membrane lipid content
- Both insulin sensitivity and lipid content are under the control of some third factor, such as a hormone.
- Lipid content, insulin sensitivity, and other factors are all part of a complex molecular/biochemical/physiological network, perhaps with positive and/or negative feedback components. The correlation observed is just a peak at a much more complex set of interdependent relationships.
- Membrane lipid content and insulin sensitivity don’t actually correlate at all; the result is just a coincidence.

Welcome to GraphPad Prism

GraphPad Prism
Version 8.2.1 (279)

NEW TABLE & GRAPH

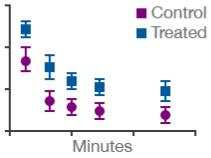
XY
Colum
Group
Contingency
Survival
Parts of Whole
Multiple variables
Nested

EXISTING FILE

Open a File
LabArchives
Clone a Graph
Graph Portfolio

XY tables: Each point is defined by an X and Y coordinate

		X	A			B		
		Minutes	Control			Treated		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title							
2	Title							
3	Title							



? Learn more

Data table:

- (•) Enter or import data into a new table
- ( ) Start with sample data to follow a tutorial

Options:

X:
- (•) Numbers
- ( ) Numbers with error values to plot horizontal error bars
- ( ) Dates
- ( ) Elapsed times

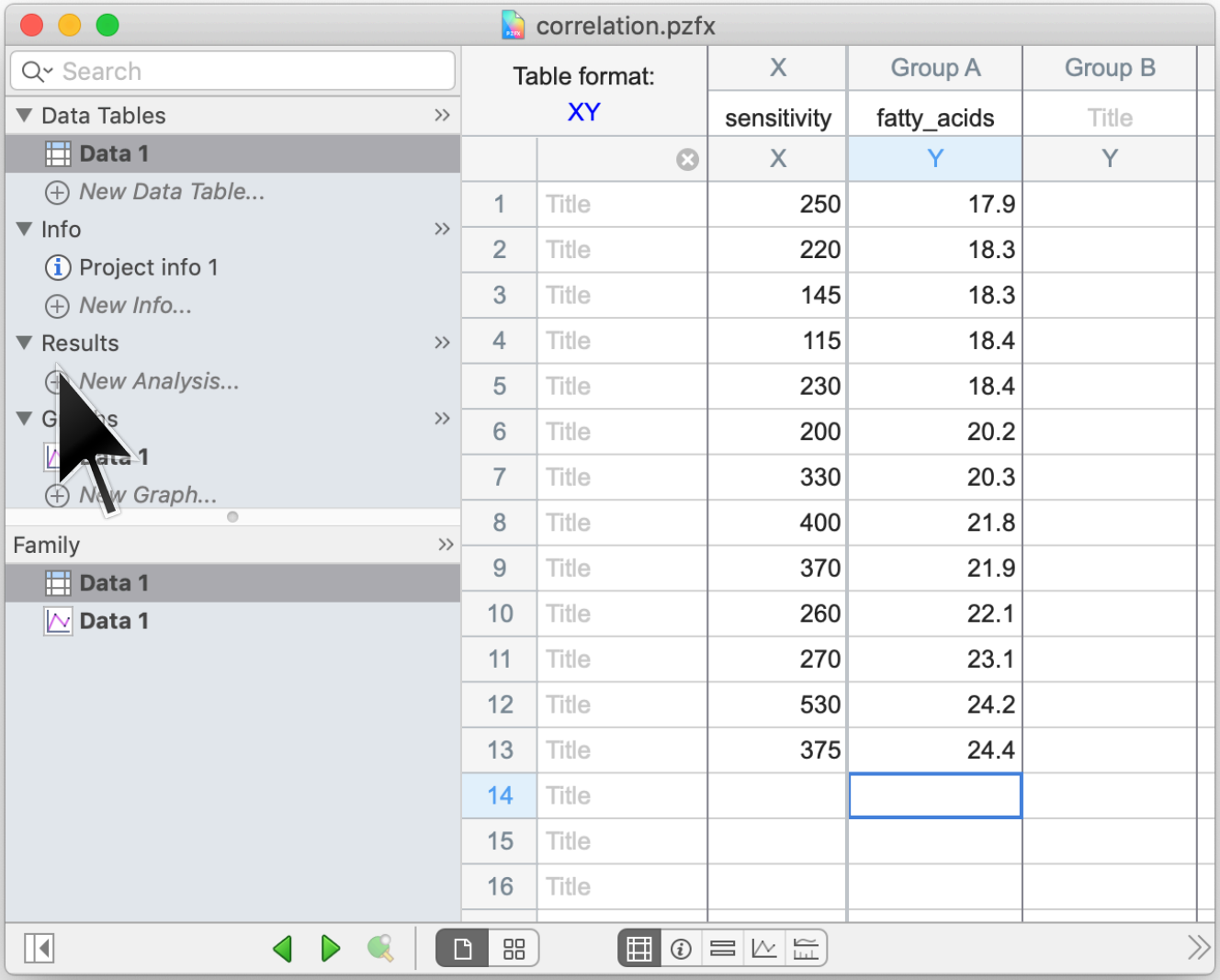
Y:
- (•) Enter and plot a single Y value for each point
- ( ) Enter 3 replicate values in side-by-side subcolumns
- ( ) Enter and plot error values already calculated elsewhere

Enter: Mean, SD, N

Prism Tips

Cancel

Create


correlation.pzfx
Search
Data Tables
Data 1
New Data Table...
Info
Project info 1
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Results
New Analysis...
New Graph...
Family
Data 1
Data 1
Table format:
XY
X
Group A
Group B
sensitivity
fatty_acids
Title
X
Y
Y
1 Title 250 17.9
2 Title 220 18.3
3 Title 145 18.3
4 Title 115 18.4
5 Title 230 18.4
6 Title 200 20.2
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12 Title 530 24.2
13 Title 375 24.4
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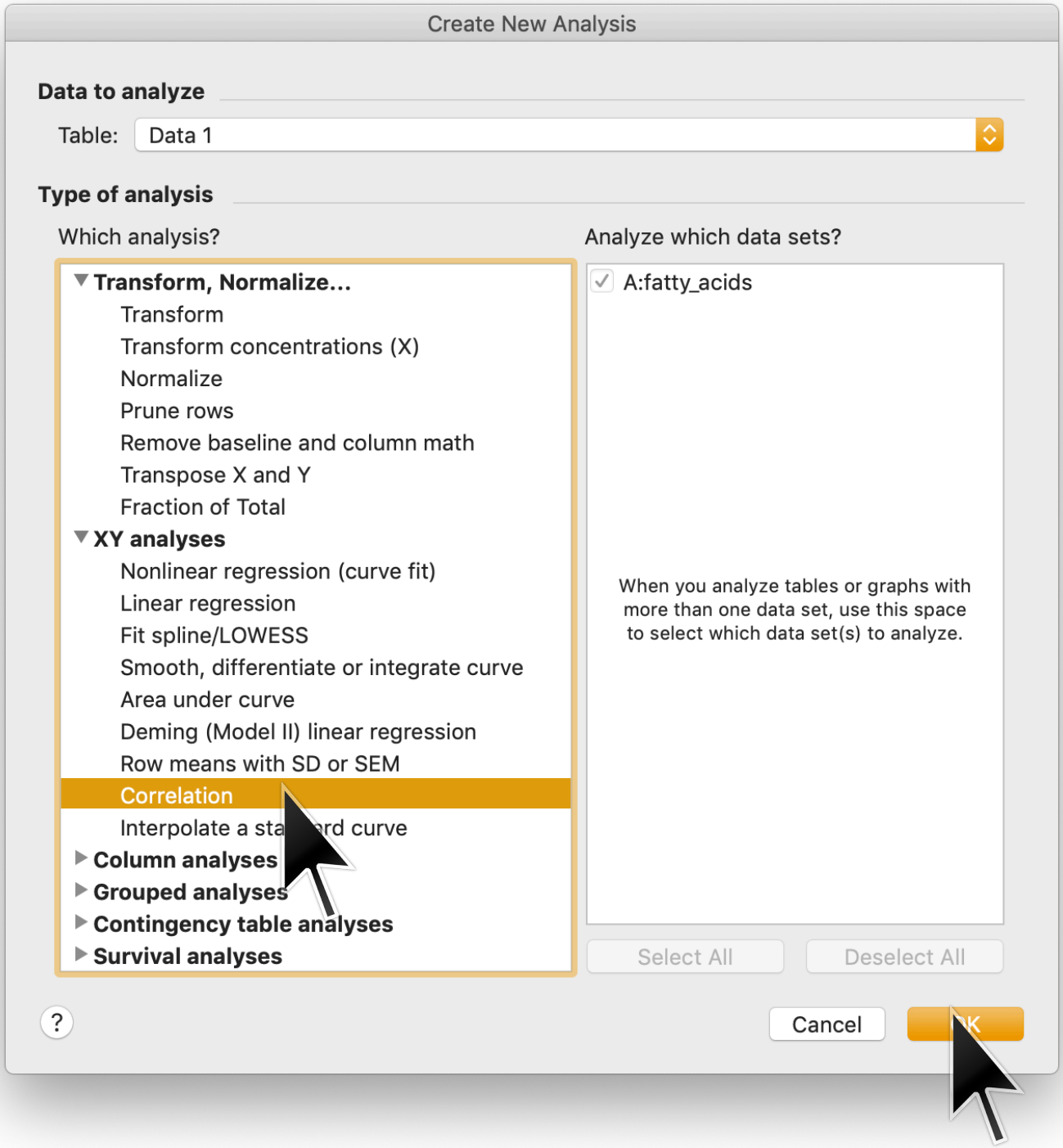
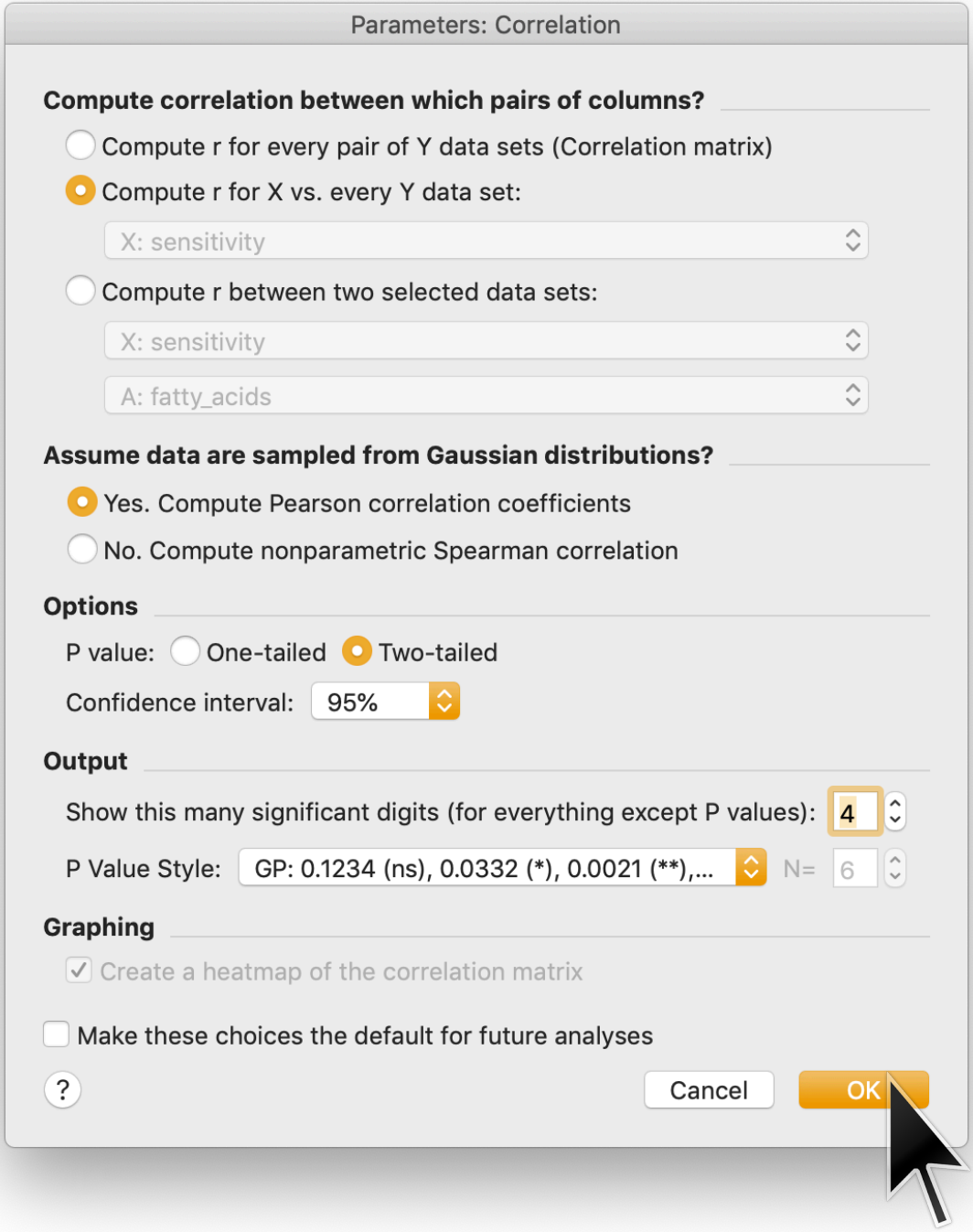
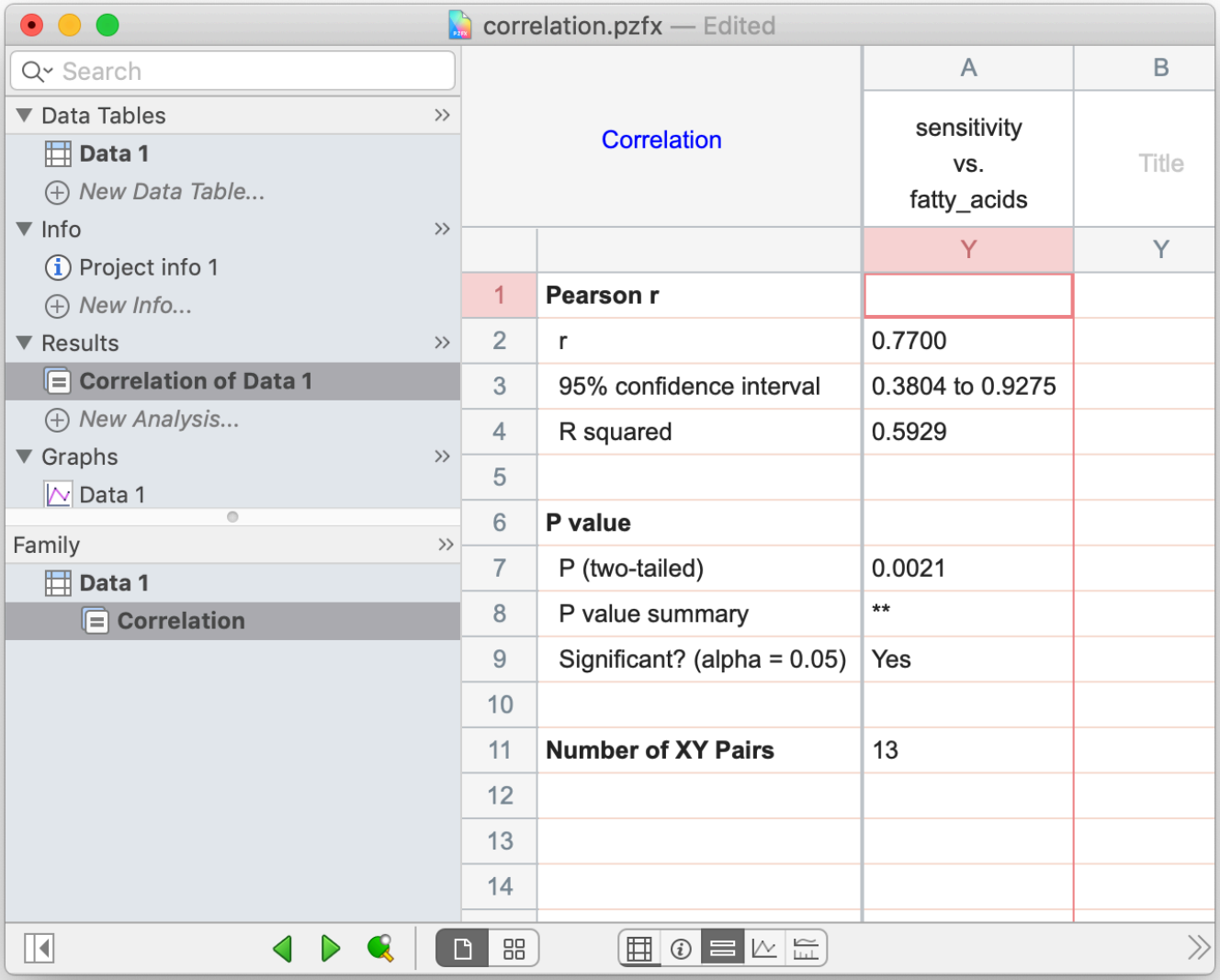

Create New Analysis
Data to analyze
Table:
Data 1
Type of analysis
Which analysis?
Analyze which data sets?
Transform, Normalize...
Transform
Transform concentrations (X)
Normalize
Prune rows
Remove baseline and column math
Transpose X and Y
Fraction of Total
XY analyses
Nonlinear regression (curve fit)
Linear regression
Fit spline/LOWESS
Smooth, differentiate or integrate curve
Area under curve
Deming (Model II) linear regression
Row means with SD or SEM
Correlation
Interpolate a sta rd curve
Column analyses
Grouped analyses
Contingency table analyses
Survival analyses
A:fatty_acids
When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.
Select All
Deselect All
?
Cancel
K

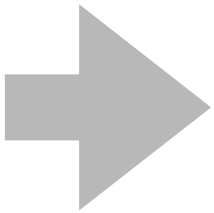

Parameters: Correlation
Compute correlation between which pairs of columns?
Compute r for every pair of Y data sets (Correlation matrix)
Compute r for X vs. every Y data set:
X: sensitivity
Compute r between two selected data sets:
X: sensitivity
A: fatty_acids
Assume data are sampled from Gaussian distributions?
Yes. Compute Pearson correlation coefficients
No. Compute nonparametric Spearman correlation
Options
P value: One-tailed Two-tailed
Confidence interval: 95%
Output
Show this many significant digits (for everything except P values): 4
P Value Style: GP: 0.1234 (ns), 0.0332 (*), 0.0021 (**),... N= 6
Graphing
Create a heatmap of the correlation matrix
Make these choices the default for future analyses
?
Cancel
OK


correlation.pzfx — Edited
Search
Data Tables
Data 1
New Data Table...
Info
Project info 1
New Info...
Results
Correlation of Data 1
New Analysis...
Graphs
Data 1
Family
Data 1
Correlation
Correlation
A
sensitivity vs. fatty_acids
B
Title
Y
Y
1 Pearson r
2 r 0.7700
3 95% confidence interval 0.3804 to 0.9275
4 R squared 0.5929
5
6 P value
7 P (two-tailed) 0.0021
8 P value summary **
9 Significant? (alpha = 0.05) Yes
10
11 Number of XY Pairs 13
12
13
14

Spearman's rank correlation is a non-parametric measure of dependence

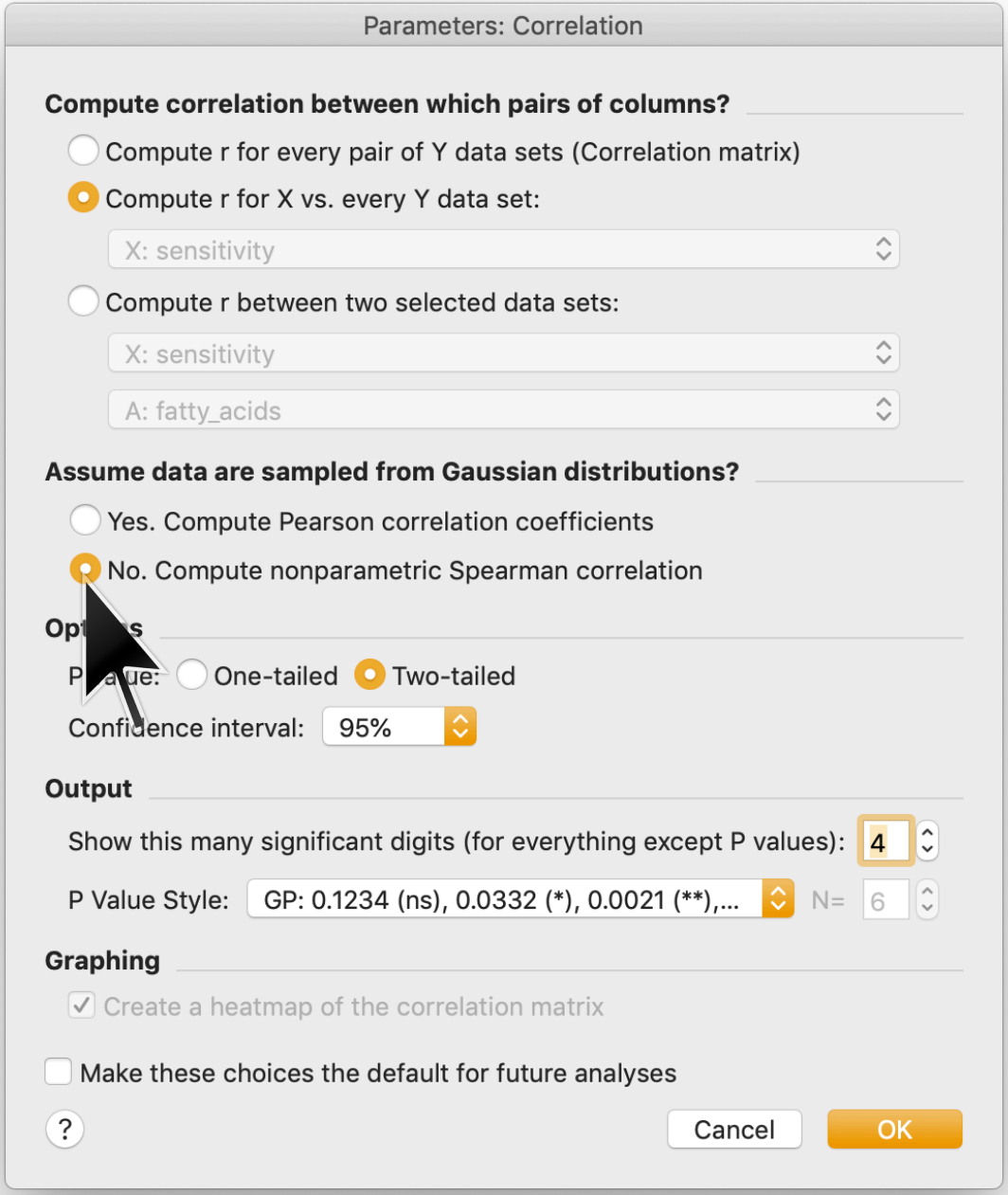
Spearman's ρ is just Pearson's r computed on the ranks of the x and y values

x	y
17.9	250
18.3	220
18.3	145
18.4	115
18.4	230
20.2	200
20.3	330
21.8	400
21.9	370
22.1	260
23.1	270
24.2	530
24.4	375



x rank	y rank
1.0	6.0
2.5	4.0
2.5	2.0
4.5	1.0
4.5	5.0
6.0	3.0
7.0	9.0
8.0	12.0
9.0	10.0
10.0	7.0
11.0	8.0
12.0	13.0
13.0	11.0

https://en.wikipedia.org/wiki/Spearman%27s_rank_correlation_coefficient


Parameters: Correlation
Compute correlation between which pairs of columns?
Compute r for every pair of Y data sets (Correlation matrix)
Compute r for X vs. every Y data set:
X: sensitivity
Compute r between two selected data sets:
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Output
Show this many significant digits (for everything except P values): 4
P Value Style: GP: 0.1234 (ns), 0.0332 (*), 0.0021 (**),... N= 6
Graphing
Create a heatmap of the correlation matrix
Make these choices the default for future analyses
?
Cancel
OK

Mutual information is a universal measure of dependence that plays a fundamental role in information theory

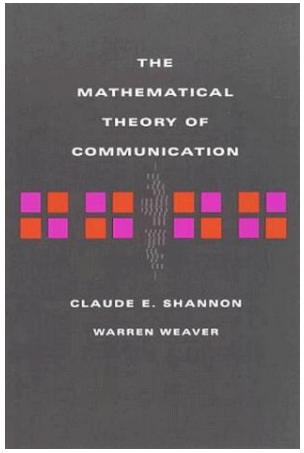
Claude Shannon

Mutual information is symmetric:

$$I[x;y] = I[y;x]$$

Mutual information can range from zero to infinity:

$$0 \leq I[x;y] \leq \infty$$

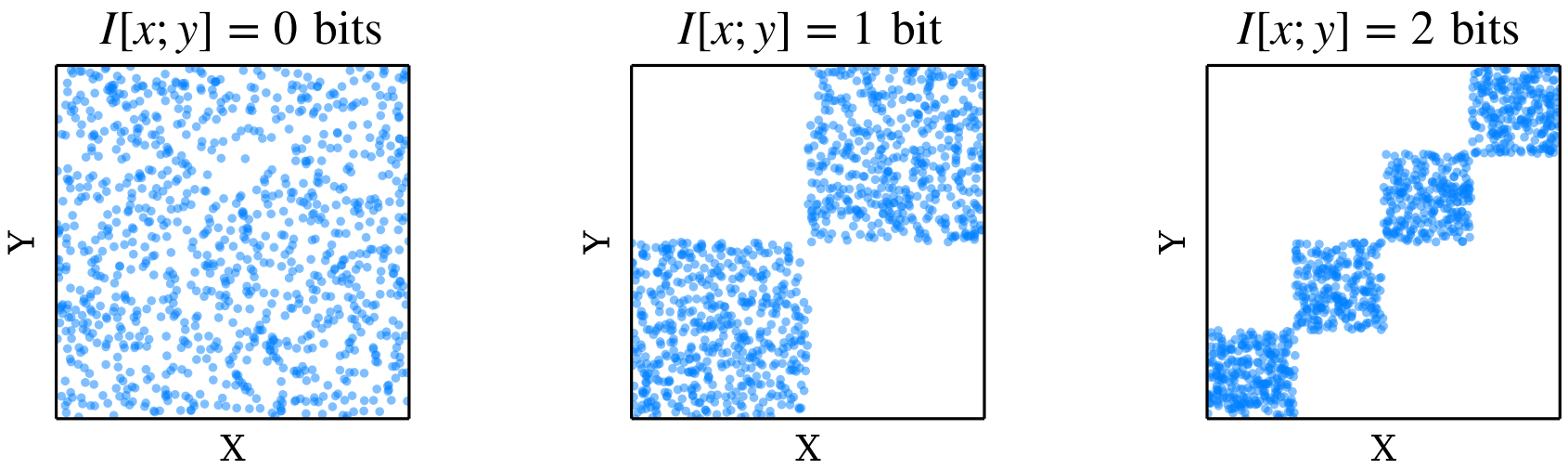


Shannon, 1948

Mutual information is zero only when the two variables are independent:

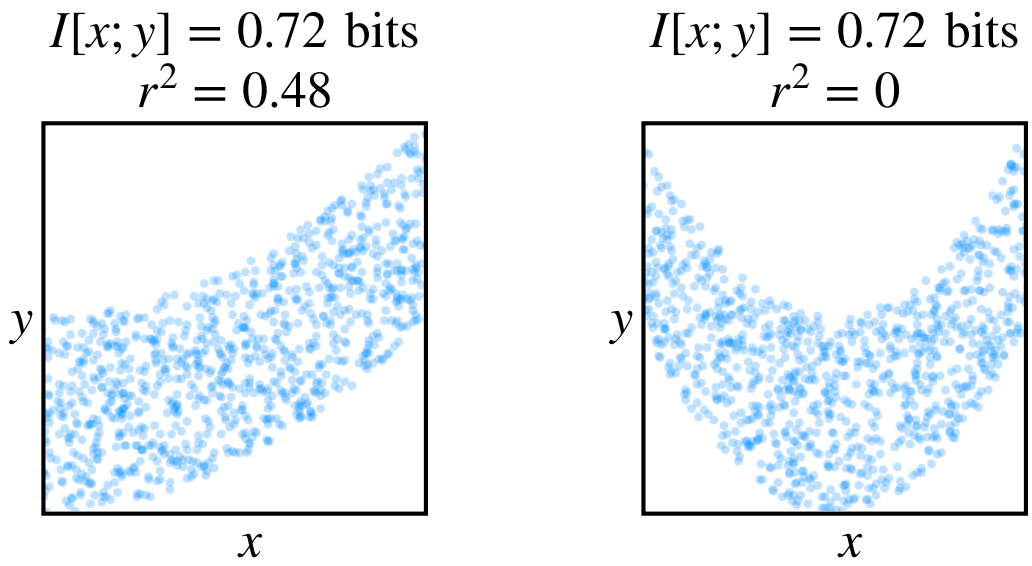
$$I[x;y] = 0 \Leftrightarrow p(x,y) = p(x)\ p(y)$$

Mutual information is the amount of information in “bits” that knowing one variable tells you about the value of another variable



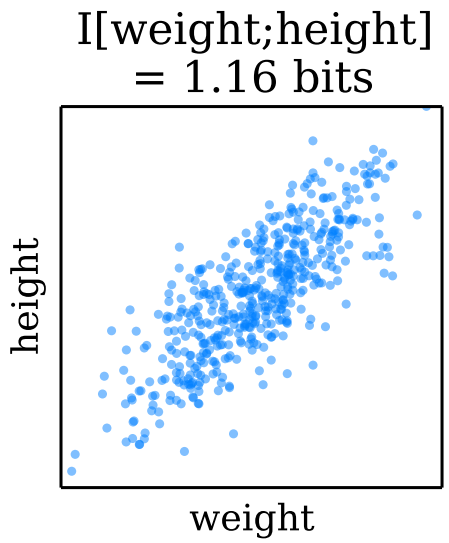
Kinney JB, Biomed Comput Rev (2014)

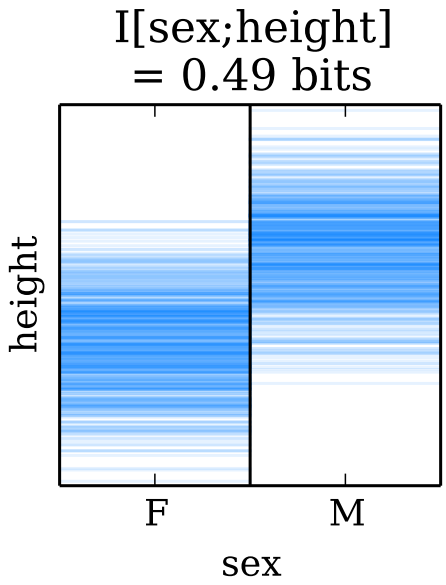
Mutual information, unlike Pearson correlation, quantifies nonlinear and non monotonic relationships in a meaningful way

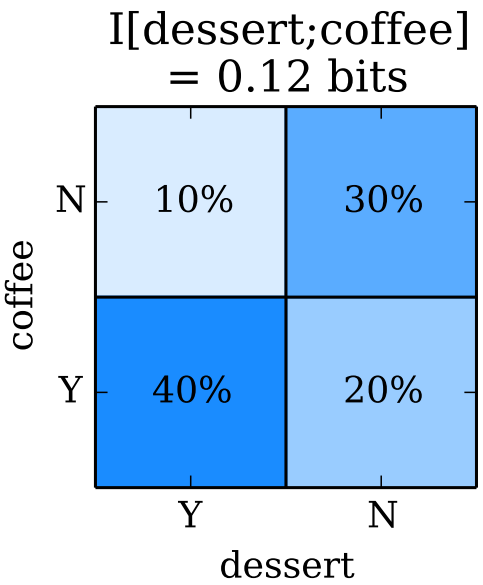


Kinney JB, Biomed Comput Rev (2014)

Mutual information information can be evaluated between any two types of variables.







Unfortunately, there is no simple plug-in formula for computing mutual information from data.

Mutual information is not commonly used in biological data analysis.

Kinney JB, Biomed Comput Rev (2014)

Power analysis

Statistical power is the probability of detecting an effect that actually does exist.

power:
The probability of getting a statistically significant result if the null hypothesis actually is actually false.

power analysis:
The process of assigning and/or computing four quantities (sometimes more) that describe one's experiment:

1. The sample size N
2. The false positive probability α (confidence = $1 - \alpha$)
3. The false negative probability β (power = $1 - \beta$)
4. The anticipated effect size

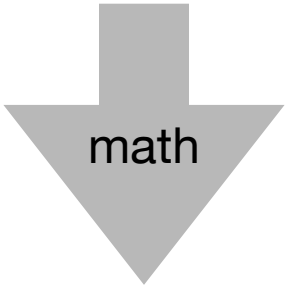
Motulsky, Ch. 20

Example: sex ratio

1. Confidence level: $1 - \alpha = 95\,\%$
2. Number of birth records: $N = 19500$
3. Hypothesized effect size: $|p(\text{boy}) - p(\text{girl})| = 2\,\%$

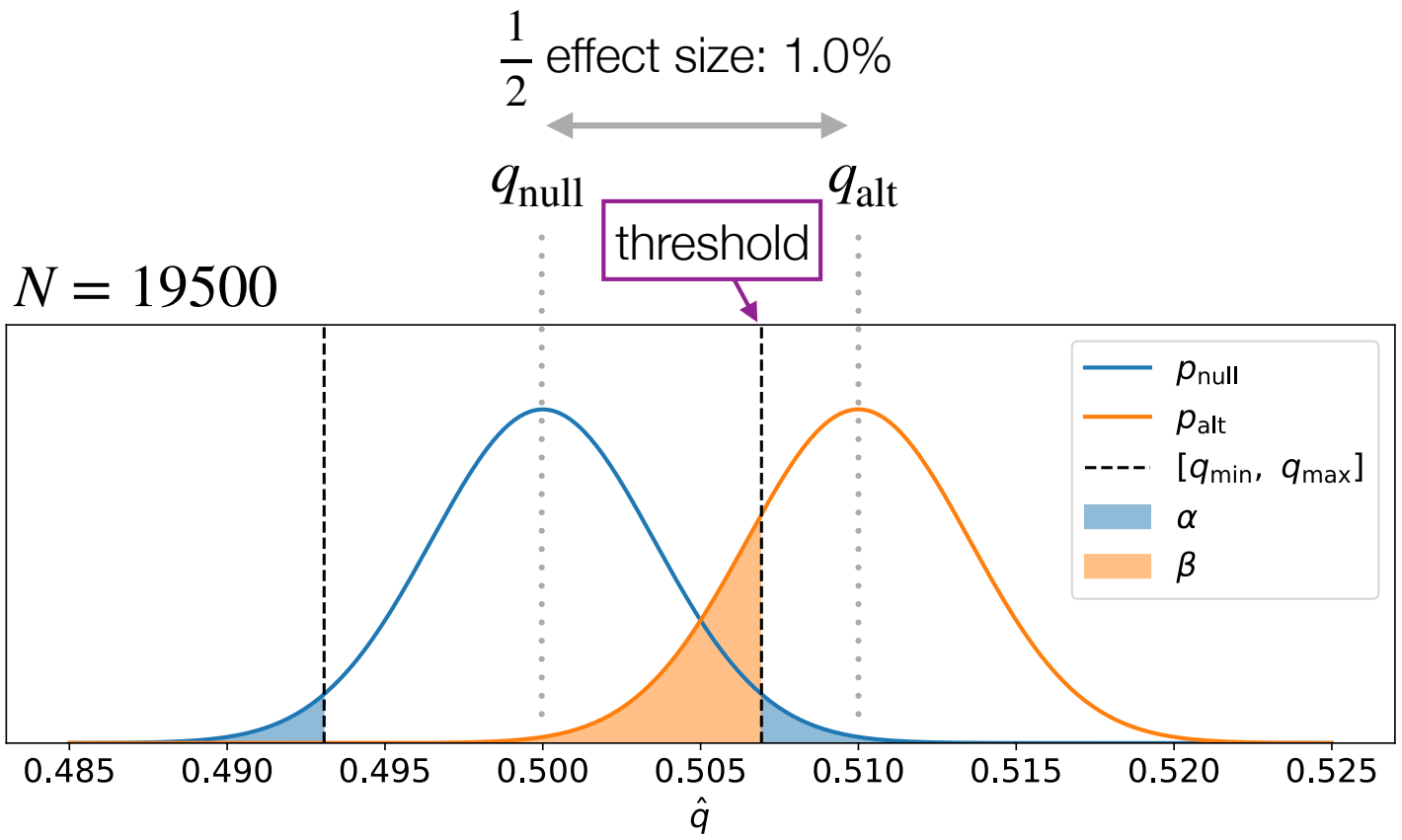
The key parameter is $q = p(\text{boy})$, so we use

$$q_{\text{null}} = 50\,\%\,, \quad q_{\text{alt}} = 51\,\%$$



4. We compute a statistical power of: $1 - \beta = 80\,\%$

Statistical power example: sex ratio data



False Positive Probability: $\alpha = 0.05$

False Negative Probability: $\beta = 0.20$
(or 80% power)

Power analysis claims come in different forms

There are four relevant parameters: N, α, β, and effect size.

Power analysis involves assuming values for any three parameters and computing the value of the forth

"Controlling the false positive rate at $\alpha = 5\,\%$, the statistical power at $1 - \beta = 80\,\%$, and assuming an effect size of $2\,\%$, our study will require using $N = 19500$ birth records."

"Using $N = 19500$ birth records, controlling the false positive rate at $\alpha = 5\,\%$, and assuming a $2\,\%$ effect size, our study will have $1 - \beta = 80\,\%$ power."

"Controlling the false positive rate at $\alpha = 5\,\%$, the statistical power at $1 - \beta = 80\,\%$, and using $N = 19500$ birth records, our study will be sensitive to an effect size of $2\,\%$."

"Using $N = 19500$ birth records, assuming an effect size of $2\,\%$, and holding the statistical power to $1 - \beta = 80\,\%$, our study will be able to hold the false positive rate to $\alpha = 5\,\%$."

You will most likely do one of these two things:

You are supposed to do this:

1. Assume a false positive rate of $\alpha = 5\,\%$ (standard)
2. Assume a power of $1 - \beta = 80\,\%$ (standard)
3. Assume what you consider to be a biologically significant effect size
4. Compute & use the required sample size N.

You'll actually probably do this:

1. Assume a false positive rate of $\alpha = 5\,\%$ (standard).
2. Assume a power of $1 - \beta = 80\,\%$ (standard)
3. Assume a reasonable / affordable sample size N
4. Compute & report the detectable effect size.

If the detectable effect size is too small

Power analysis example: body temperature

1. Assume a false positive rate of $\alpha = 5\,\%$ (standard).
2. Assume a power of $1 - \beta = 80\,\%$ (standard)
3. Assume what you consider to be a biologically significant effect size: ~~$\Delta\mu = 0.1$ F.~~ $\Delta\mu = 0.2$ F

   The key parameter is the "normalized effect size": $\dfrac{\Delta\mu}{\sigma}$

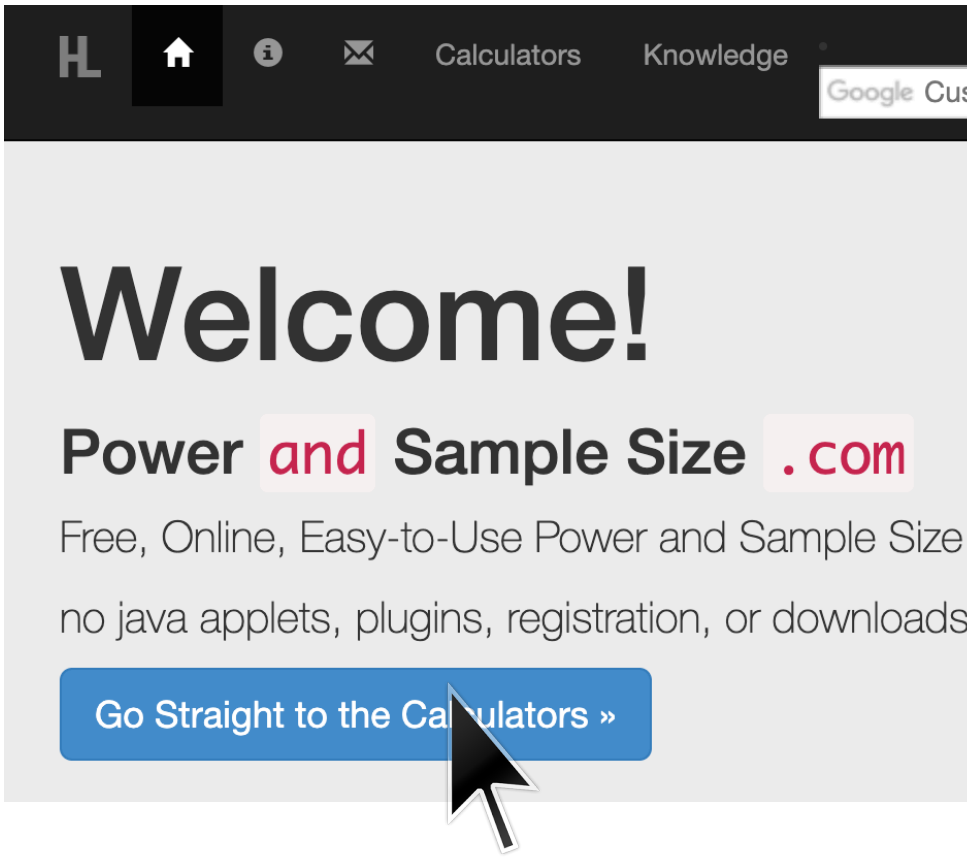
   From preliminary data, we know $\sigma \approx 0.7$ F

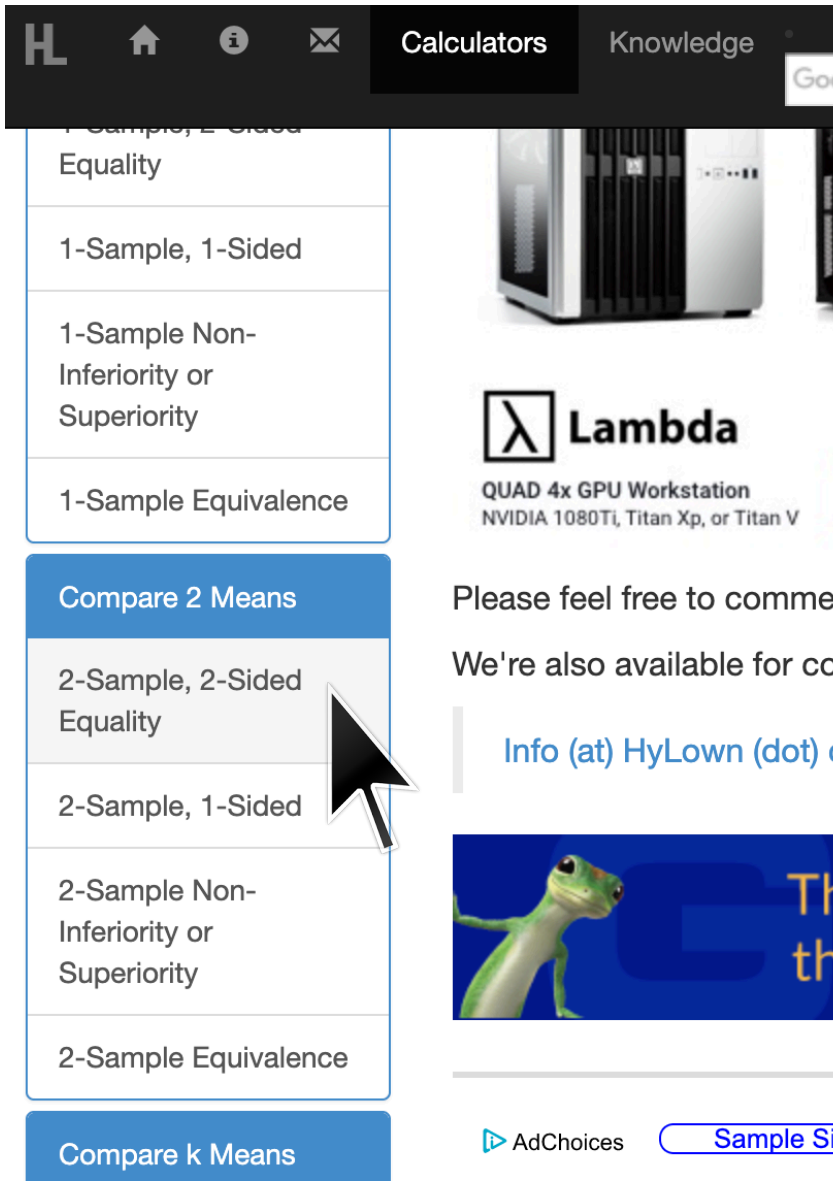
4. Compute the required sample size: ~~$N = 1540$~~ $N = 386$

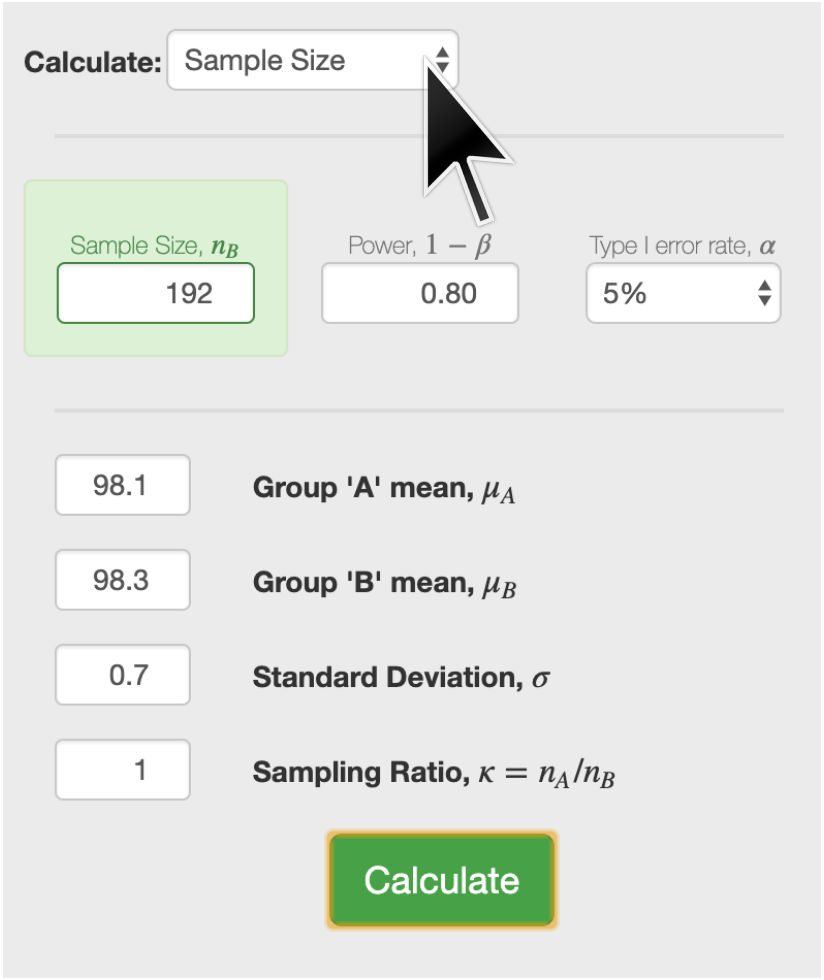
   Too big! OK.

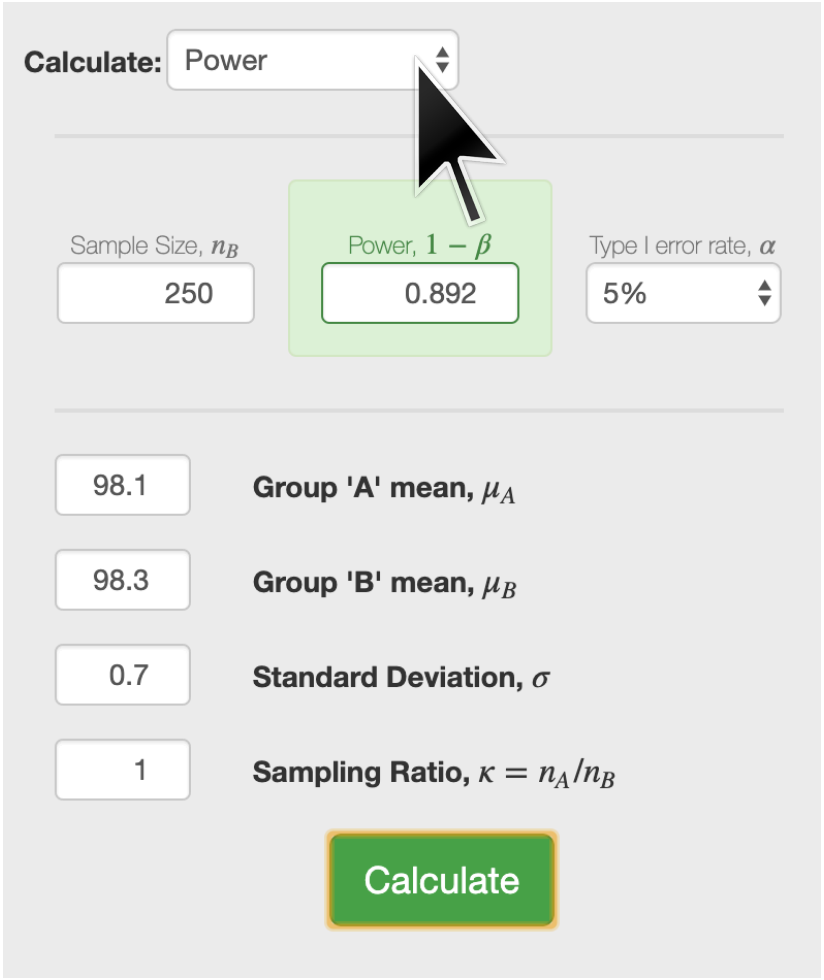
There are a number of online power analysis calculators

http://powerandsamplesize.com/



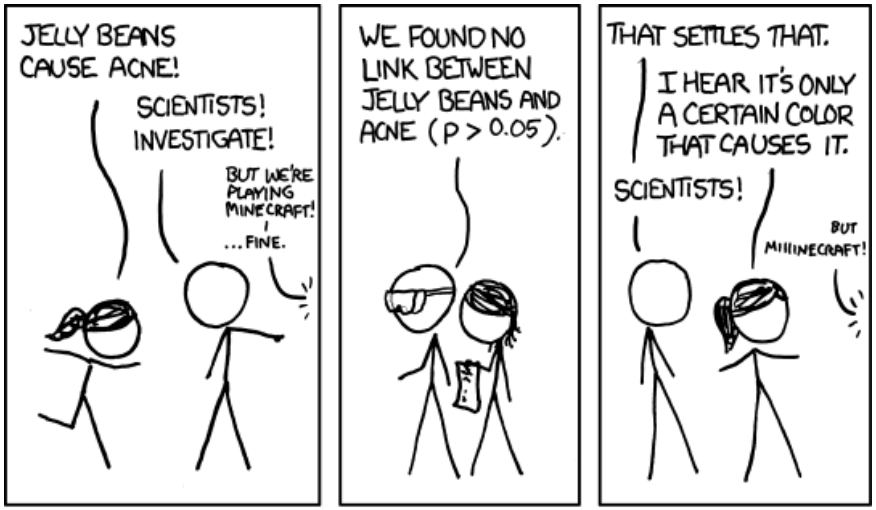


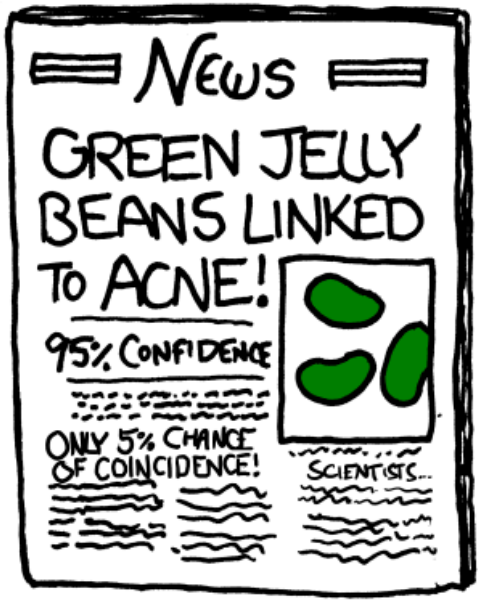

Calculate: Sample Size
Sample Size, n_B
192
Power, $1-\beta$
0.80
Type I error rate, α
5%
98.1
Group 'A' mean, μ_A
98.3
Group 'B' mean, μ_B
0.7
Standard Deviation, σ
1
Sampling Ratio, $\kappa = n_A/n_B$
Calculate

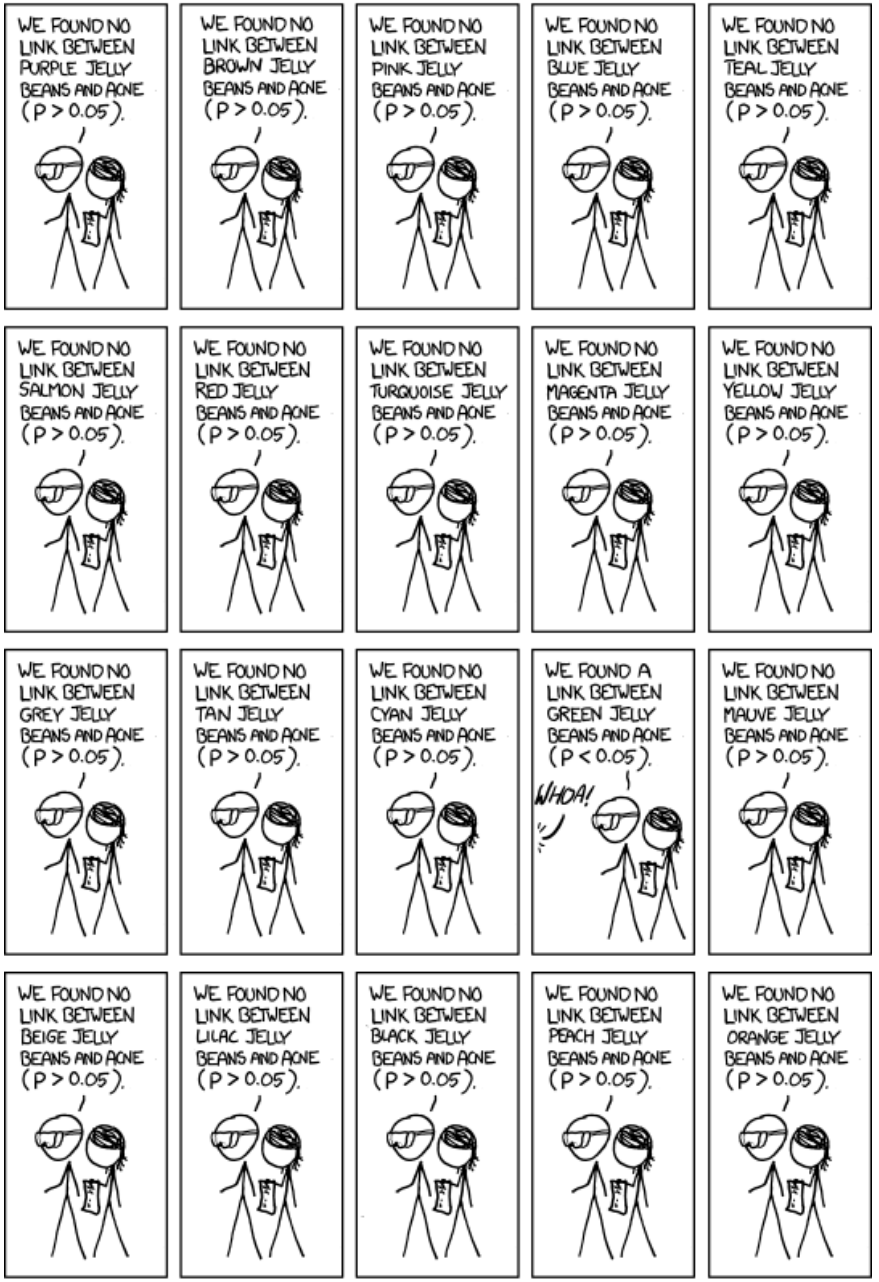

Calculate: Power
Sample Size, n_B
250
Power, $1-\beta$
0.892
Type I error rate, α
5%
98.1
Group 'A' mean, μ_A
98.3
Group 'B' mean, μ_B
0.7
Standard Deviation, σ
1
Sampling Ratio, $\kappa = n_A/n_B$
Calculate

Multiple hypothesis testing

The problem of multiple subgroups







https://xkcd.com/882/

The family-wise error rate increases rapidly with the number of tests performed

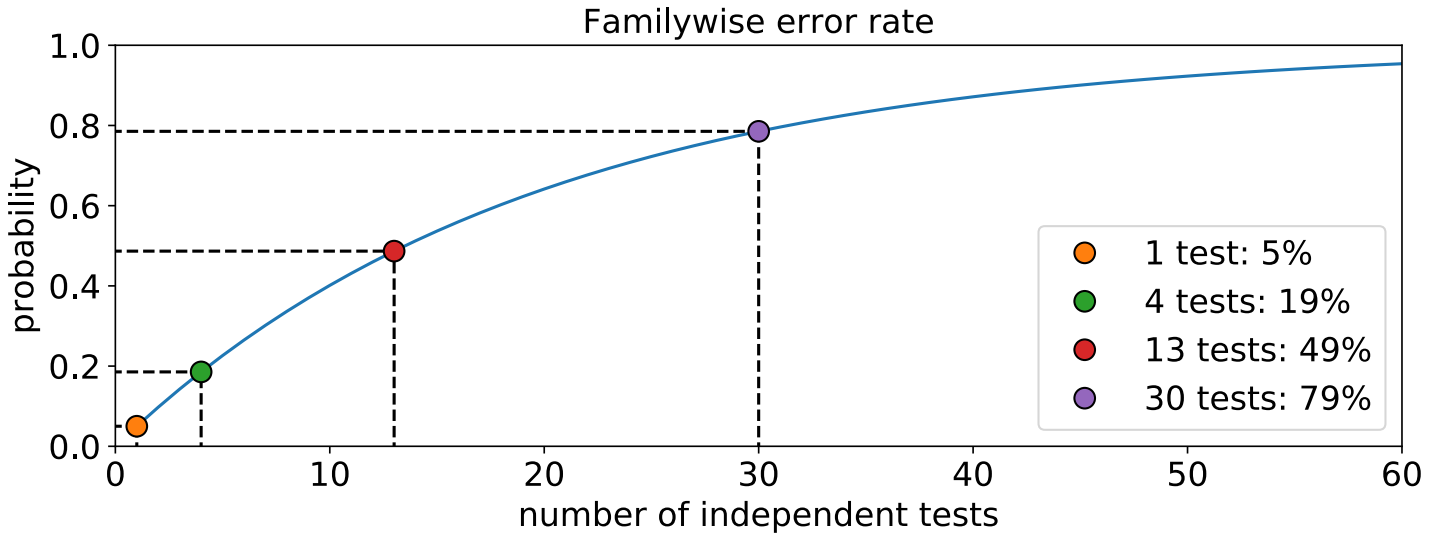
Scenario:

we perform null hypothesis tests on K independent datasets, for each of which the null hypothesis is true.

Family-wise error rate:

the probability of at least one false positive (FP)

$$p(\text{FP} \geq 1 \,|\, \text{null hypothesis}) = 1 - \text{confidence}^K$$



Summary of multiple hypothesis correction techniques

Approach	What you control	Expression
No correction	α: if all null hypotheses are true, the fraction of tests that produce a significant result	$\alpha = \frac{\mathrm{FP}}{\mathrm{FP} + \mathrm{TN}}$
Bonferroni / Dunn-Sidak	α: if all null hypotheses are true, the chance of obtaining one or more significant results	$\alpha = p(\#\mathrm{FP} > 0)$
False discovery rate (FDR)	Q: the fraction of all discoveries for which the null hypothesis is actually true	$Q = \frac{\mathrm{FP}}{\mathrm{FP} + \mathrm{TP}}$

Lucas et al. (2005)

Simple ways to counteract the multiple hypothesis problem

Bonferroni correction:

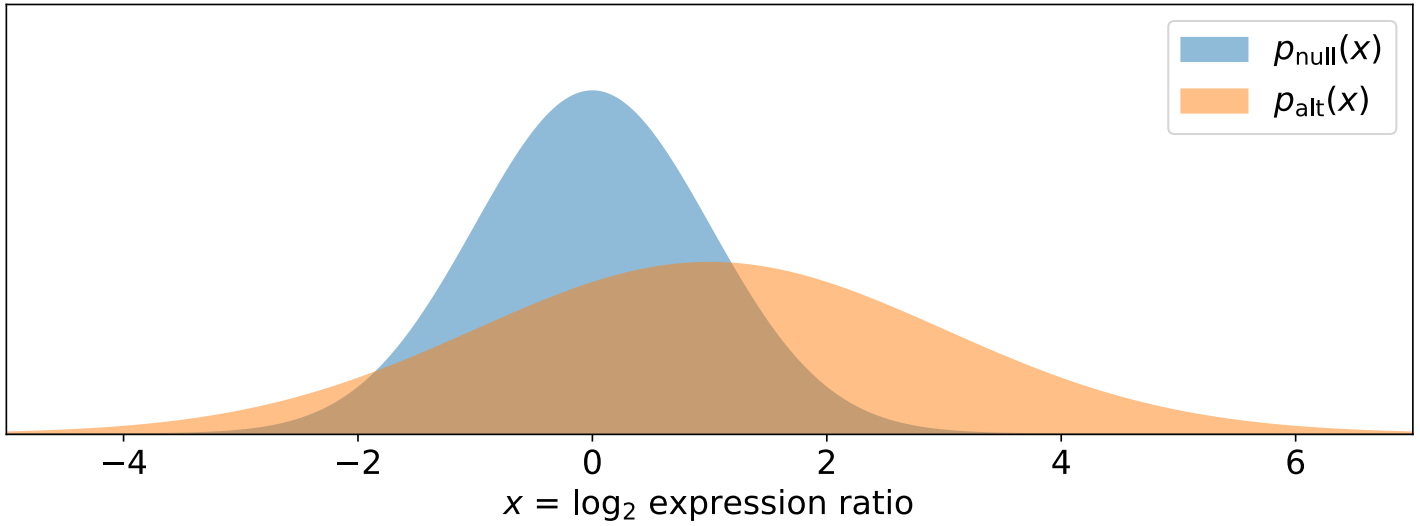
$$\alpha_{\text{Bonferroni}} = \frac{\alpha}{K}$$

Dunn-Sidak correction:

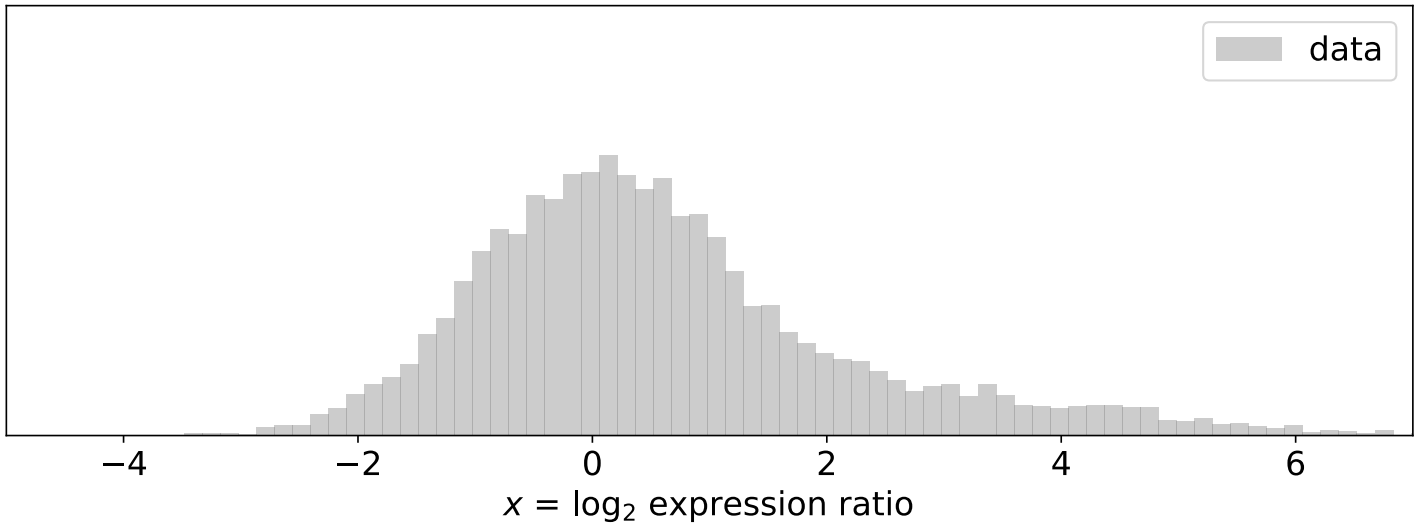
$$\alpha_{DS} = 1 - (1 - \alpha)^{1/K}$$

Dunn-Sidak is the exact solution; Bonferroni is an approximation

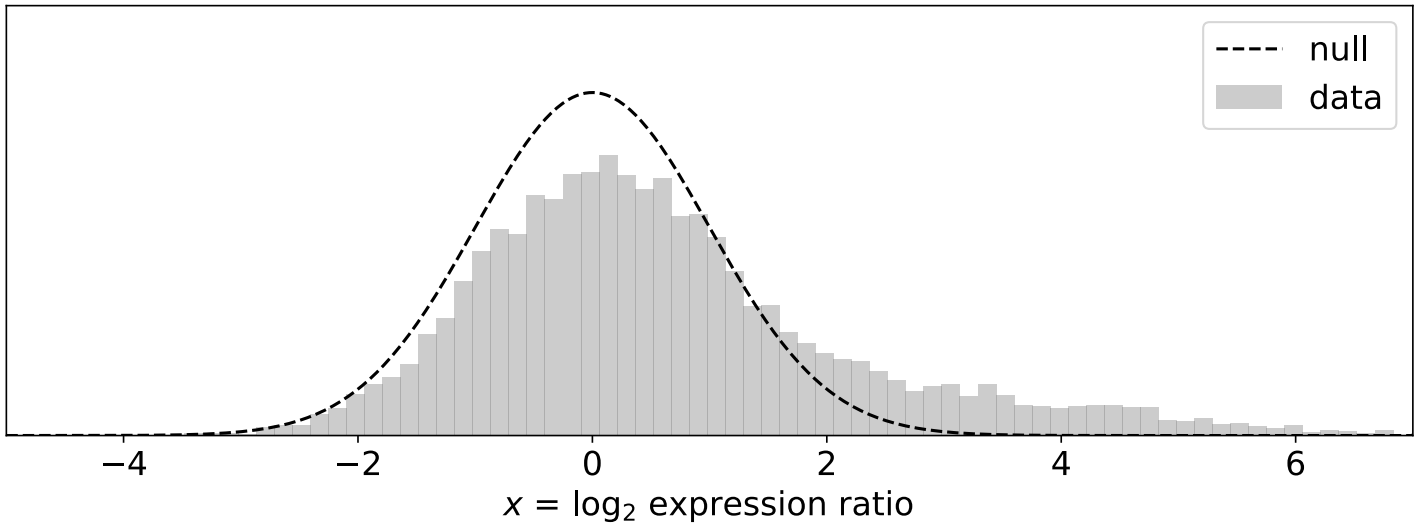
Example: differential expression (simulation)



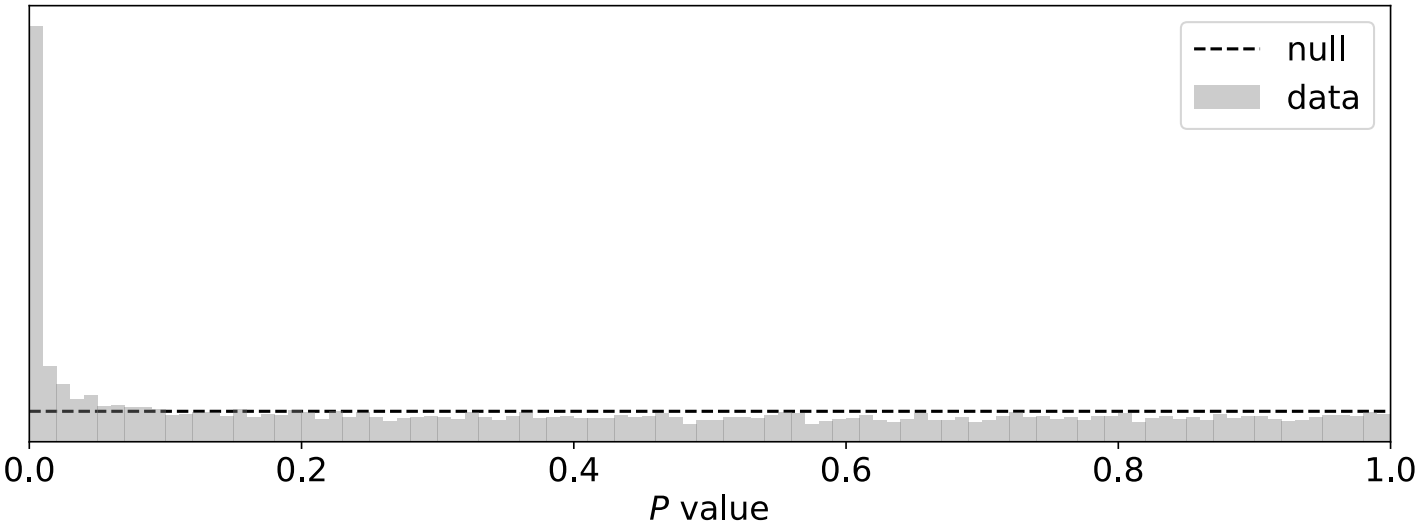
7,000 xs from $p_{\text{null}}(x)$
+ 3,000 xs from $p_{\text{alt}}(x)$



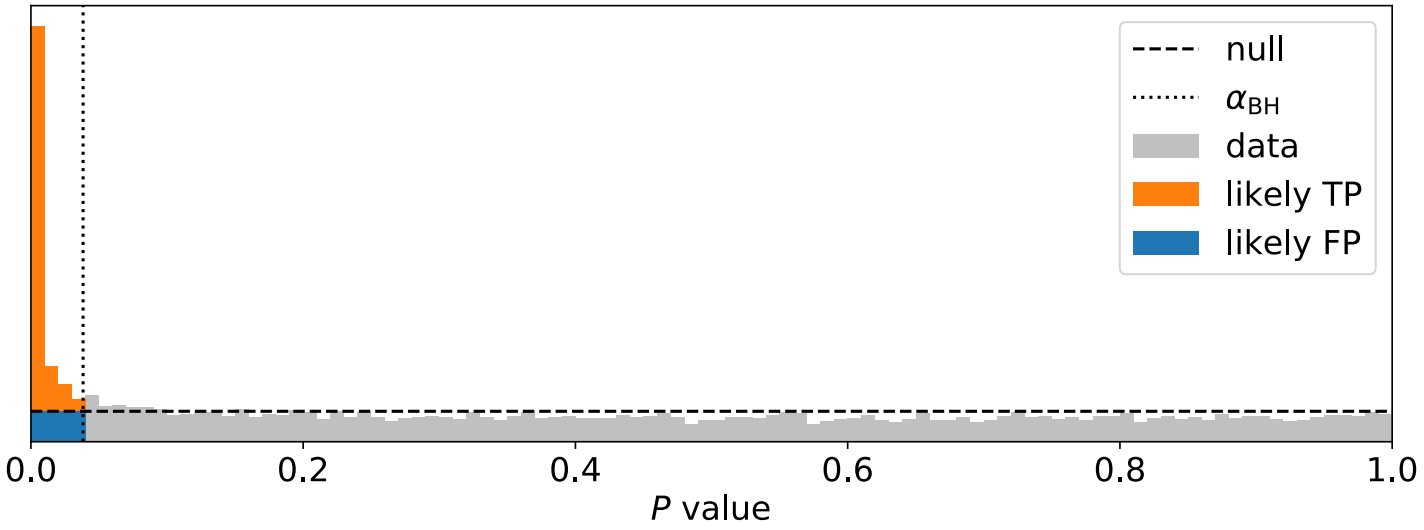
First, convert data to p-values



use knowledge of $p_{\text{null}}(x)$ to
compute a p-value for each datapoint

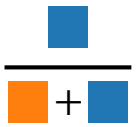


Benjamini–Hochberg procedure



Choose α_{BH} such to match the target False Discovery Rate (10% here):

$$\mathrm{FDR} = Q = \frac{\mathrm{FP}}{\mathrm{TP} + \mathrm{FP}} =$$



Declare all P-values below α_{BH} as “discoveries”.

Multiple comparisons are ubiquitous and insidious

"Most scientists are oblivious to the problems of multiplicities. Yet they are everywhere. In one or more of its forms, multiplicities are present in every statistical application. They may be out in the open or hidden. And even if they are out in the open, recognizing them is but the first step in a difficult process of inference. Problems of multiplicities are the most difficult that we statisticians face. They threaten the validity of every statistical conclusion."

from Berry (2007, p. 155), in Motulsky, Ch. 23

Multiple comparisons arise in many many contexts

multiple subgroups:
You perform tests on multiple subgroups of your data.

multiple ways to dichotomize:
You do pairwise comparisons between different combinations of subgroups.

multiple sample sizes:
You keep collecting data until you find $P < 0.05$. **DO NOT DO THIS.**

multiple ways to preprocess the data:
You analyze data preprocessed in multiple different ways.

multiple statistical tests:
You use different statistical tests on the same data before finding $P < 0.05$.

Motulsky, Ch. 23

Multiple comparisons arise in many, many contexts

multiple ways to select relevant variables:
You try to model your data using different subsets of possible variables.

multiple ways to analyze your data ("garden of forking paths"):
You try lots of qualitatively different analysis strategies.

outcome switching:
You change the quantity you care about after you've looked at the data.

multiple geographic areas:
E.g., you investigate a "cancer cluster" you hear about in the news.

Motulsky, Ch. 23

Correcting for multiple comparisons is not always needed

Scenario 1:
If readers can be reasonably expected to account for multiple comparisons on their own.

Scenario 2:
Before looking at the data, you have clearly defined one outcome as primary and others as secondary.

Scenario 3:
You make only a few planned comparisons and your P-values are not marginal.

Scenario 4:
A large fraction the tests you perform are significant.

Motulsky, Ch. 19

Practical advice of avoiding multiple hypothesis pitfalls

Raise your standards: use $\alpha = 0.01$, not $\alpha = 0.05$.

Separate exploratory data analysis from confirmatory data analysis.

Distinguish critical p-values from ancillary p-values.

Don't spend too much time analyzing a small dataset.

When generating small expensive datasets (e.g. mice), blind your experiments as best you can, and plan your analysis ahead of time

When in doubt, double-check your hypothesis with new data

Don't worry about informal multiple hypothesis testing when $P < 10^{-4}$.