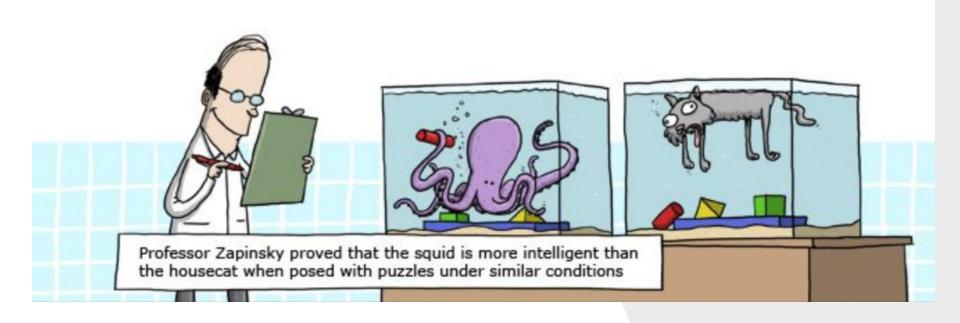


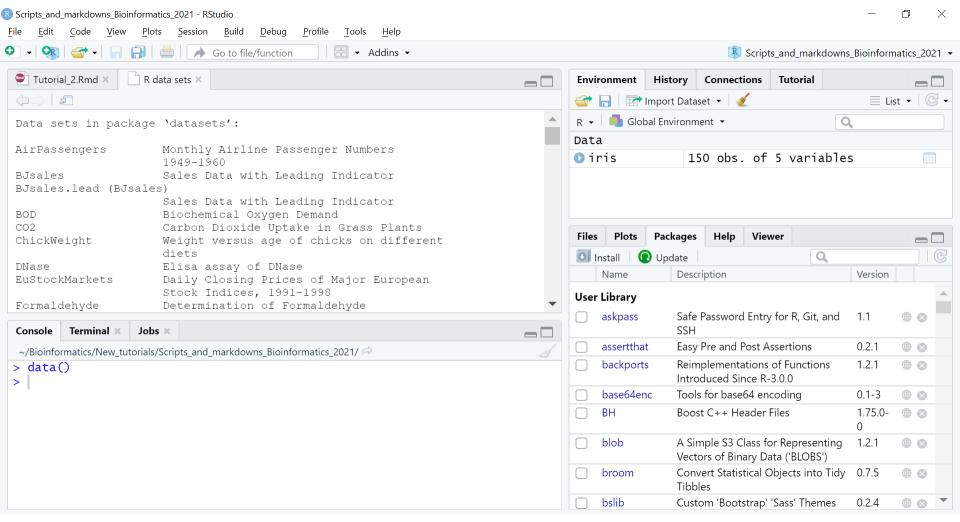
Tutorial 2

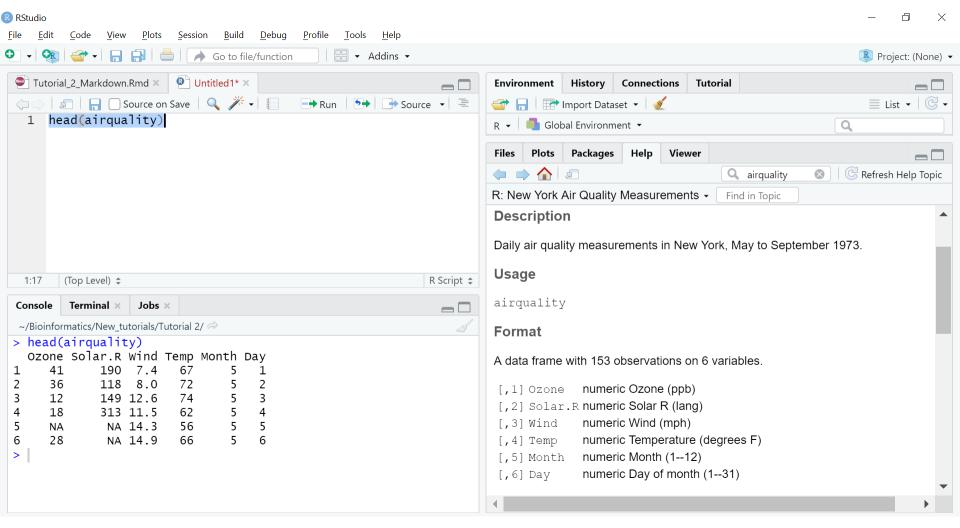
Statistics and its implementation in R



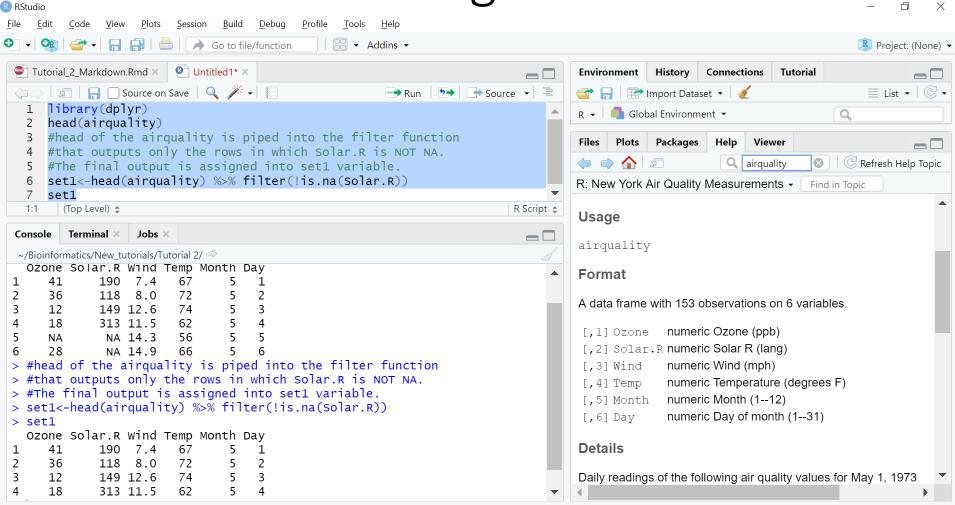
Exploratory data analysis (EDA)

- We would like to start out data analysis from exploratory analysis. This technique allows us to get the impression how does our data looks like and to act towards tiding the data and monitoring the accuracy of the analysis.
- The possible data visualization tools that allows us to perform EDA all through the analysis process include histogram, boxplots, log transforms etc.





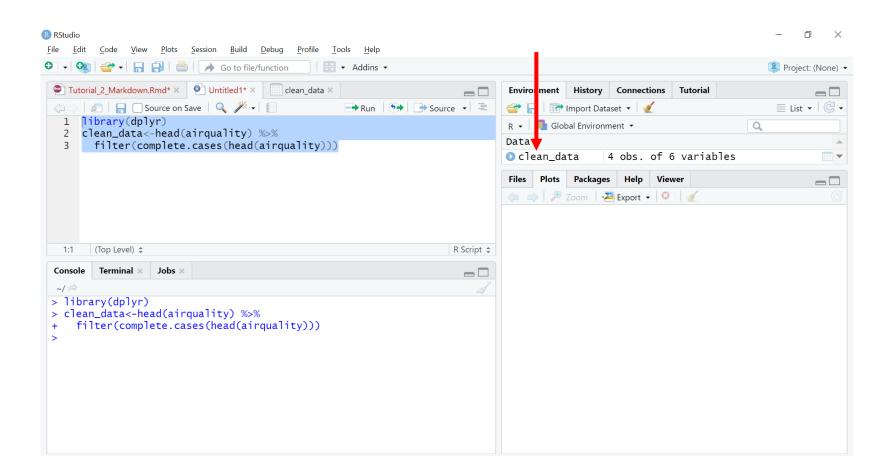
- Missing values in a vector are denoted by the letters NA. The way different functions handle missing values varies from function to function.
- Its better to pre-process your data and select the rows you want to analyze before you proceed with the data analysis.

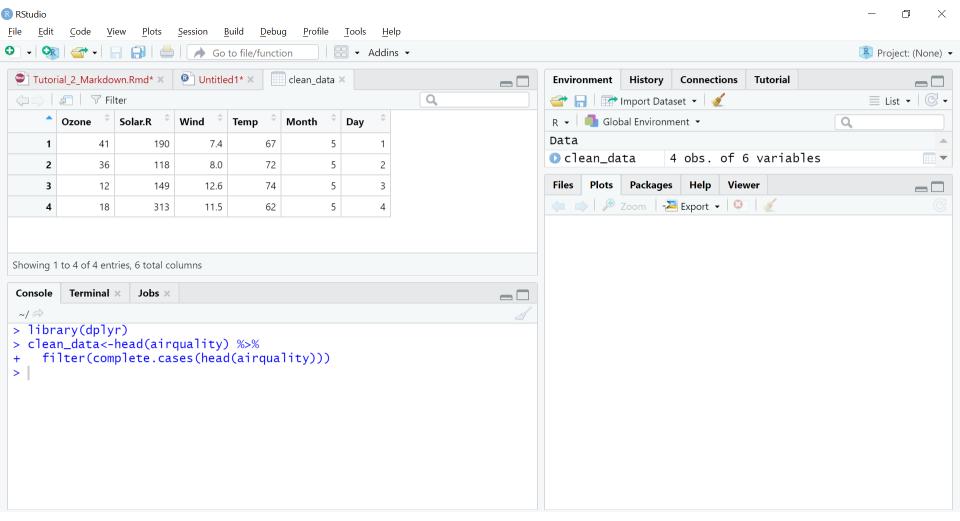




%>% operator is defined at the dplyr package. The use of this operator is similar to the pipe use in Unix.

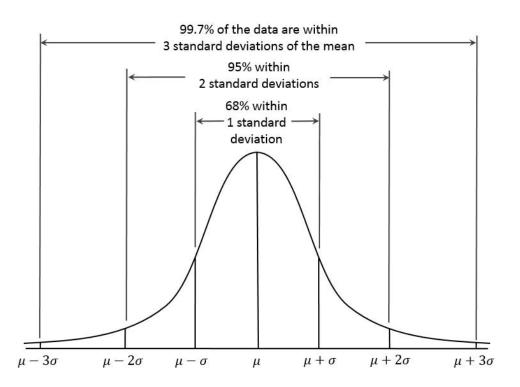
- Sometimes we want to select only rows which have no missing values, so called complete cases.
- The complete.cases() function accepts a dataframe (or matrix) and tests whether each row is complete.
 It returns a vector with a TRUE/FALSE result for each row.



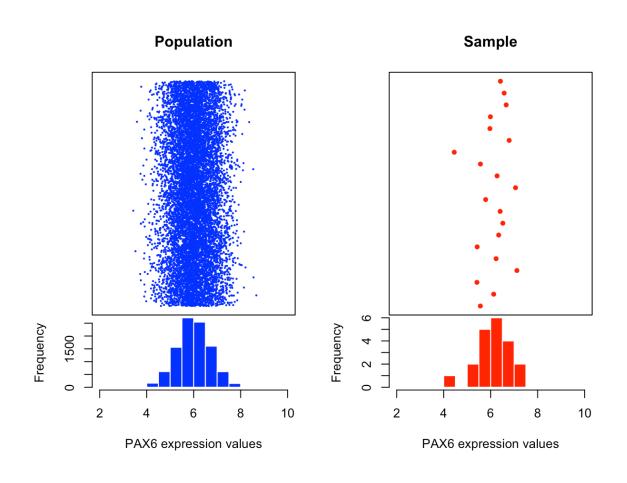


Normal (Gaussian) distribution

 Normal distribution of data can be observed in situations where data is randomly collected from independent sources.

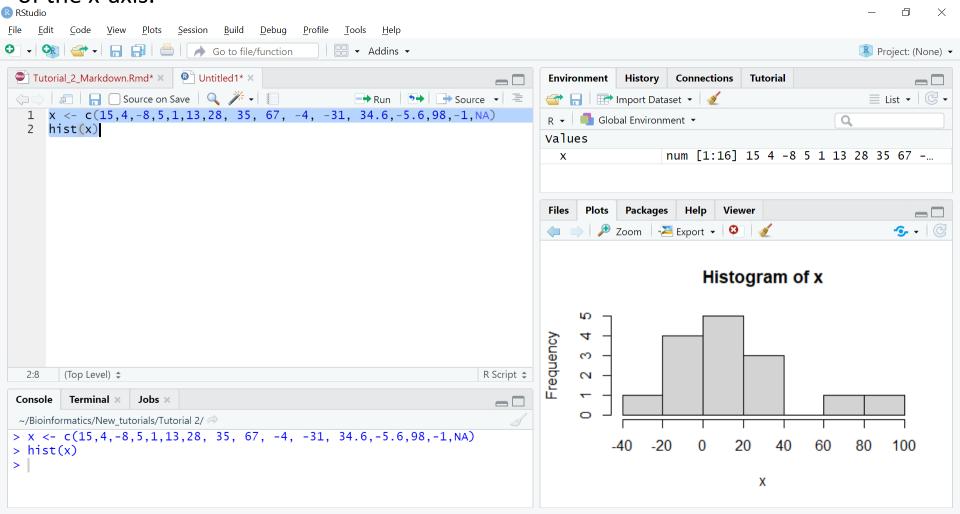


Distribution of values

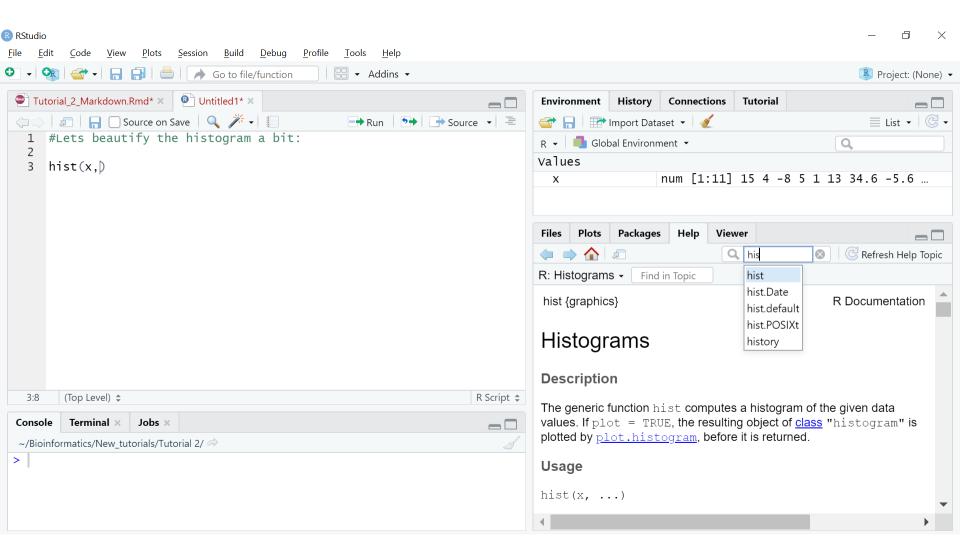


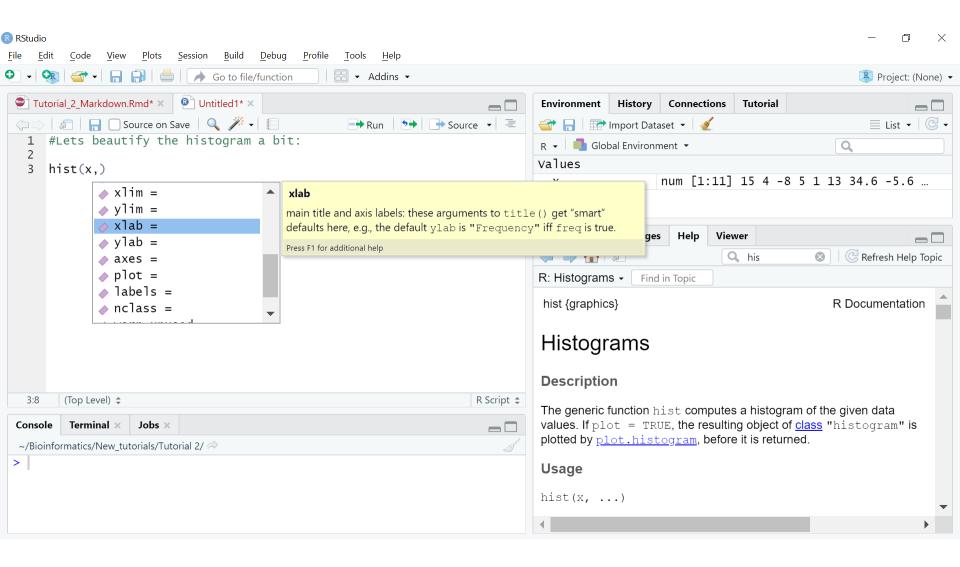


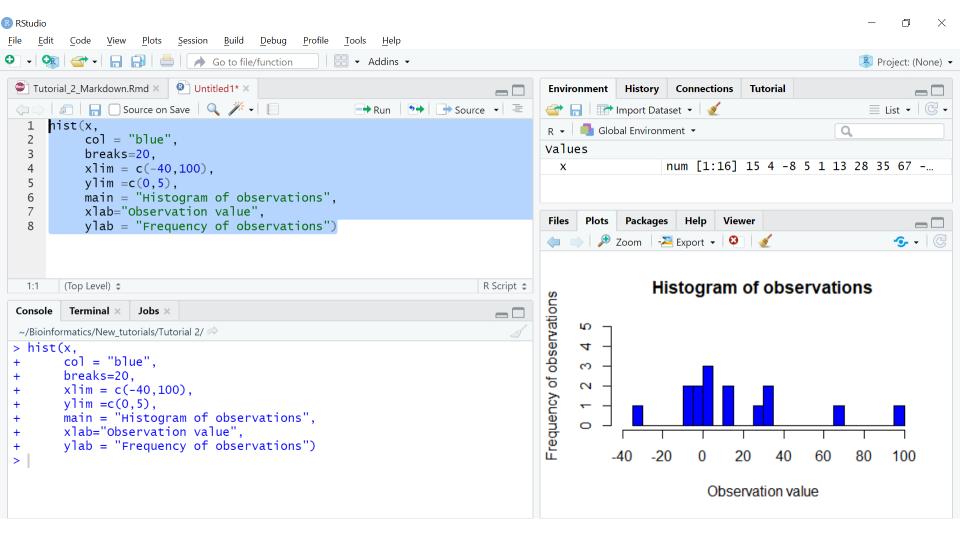
The simplest display for the shape of a distribution of data can be done using a histogram- a count of how many observations fall within specified divisions ("bins") of the x-axis.



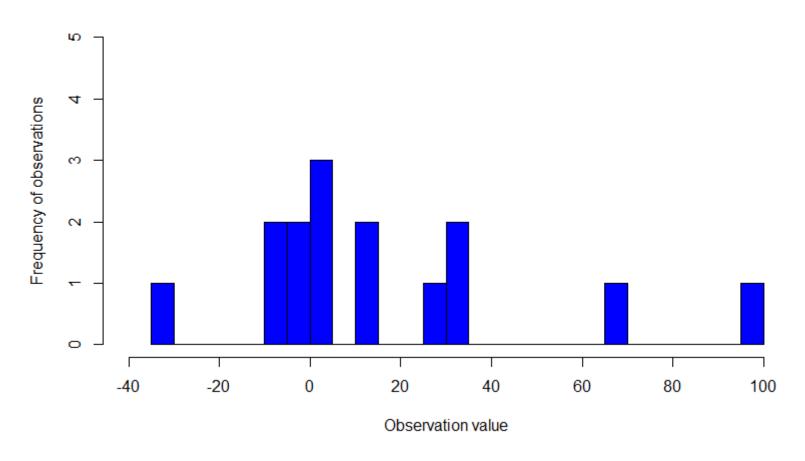
Let's beautify the histogram a bit ...







Histogram of observations



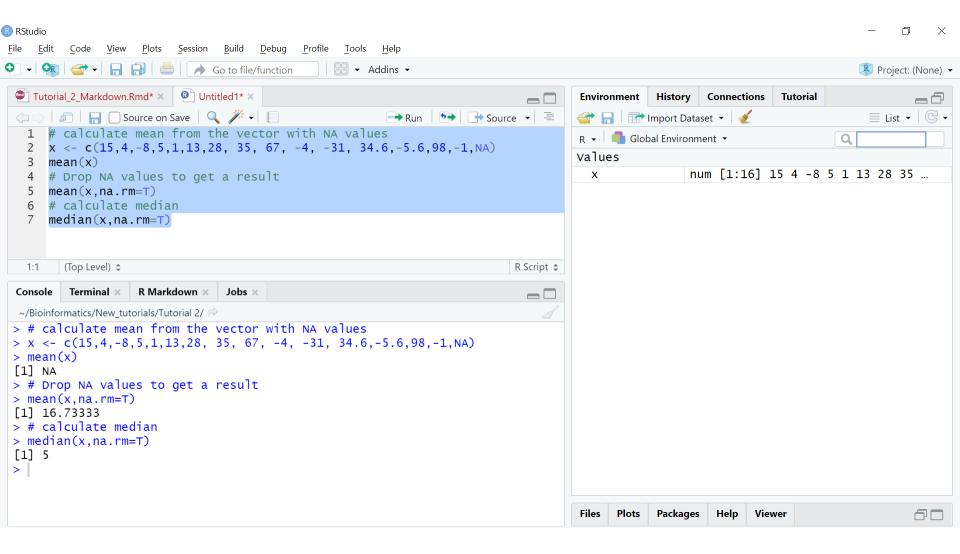
The distributions have parameters (such as mean and variance) that summarize them.

Mean and median are used to describe the central tendency of measurements.

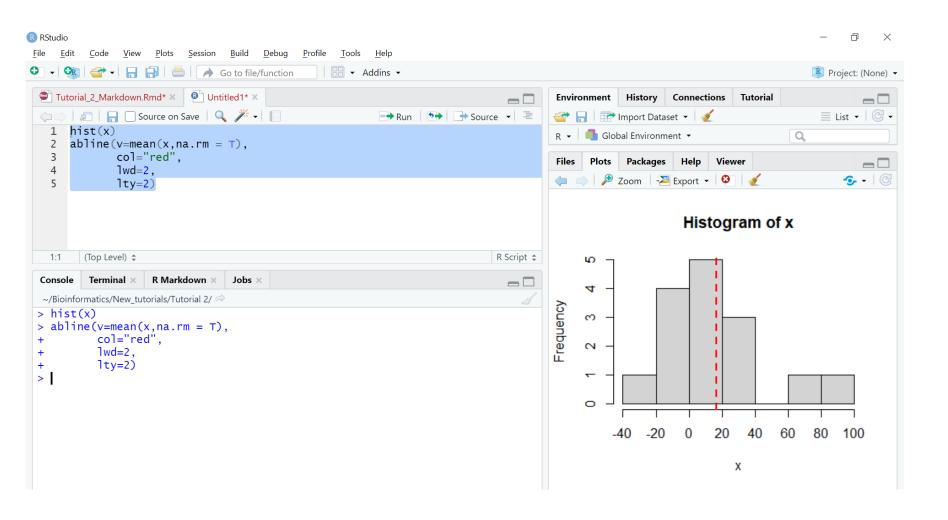
- The mean() function is used to calculate the mean of a provided vector of numbers.
- The mean is easily affected by outliers. If certain values are very high or low compared to the bulk of the sample, this will shift mean toward those outliers.
- The median() function will calculate the median.
- The median is much less affected by outliers then mean as it is the value in a distribution where half of the values are above it and the other half are below.

OUTLIER

Mean and median are used to describe the central tendency of measurements.

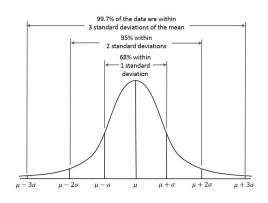


Mean and median are used to describe the central tendency of measurements.

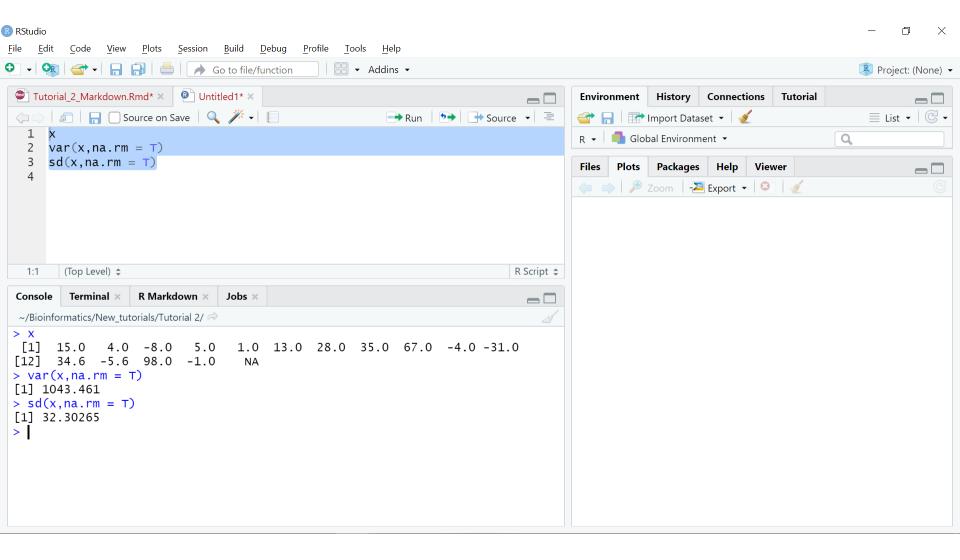


Measures that reflect variability in a distribution.

- Variance squared distance of data points from the mean.
- **Standard deviation** square root of the variance. It is measured in the same units as mean.
- A value around zero indicates there is not much variation in the values of the data points, and a high value indicates high variation in the values.



Measures that reflect variability in a distribution.



How can we test for difference between groups?

Hypothesis testing

 Hypothesis testing is used to draw inferences about the overall data behavior performing statistical tests on a sample from the population.

How can we test for difference between groups?

- Decide on a hypothesis to test, often called the "null hypothesis" (H₀). The null hypothesis assume that there is no anomaly in the tested population (ex. there is no difference between sets of samples).
- Decide on the "alternative hypothesis" (H₁). The alternative hypothesis that observation is due to real phenomena.
- Decide on a statistic test that will test the truth of the null hypothesis.
- Compare the result of the statistic test it to the observed value to establish significance, **the P-value**. Based on that, either reject or not reject the null hypothesis, H₀.

P-value

- To evaluate the significance of the difference between measurements in two groups we calculate the p-value.
- P value can be described as level of significance for the null hypothesis. By default, we will choose the level of significance as 5%.
- P-value is probability of the observed results assuming that there <u>is no difference</u> between the observations between the two samples (Ho).

Significant p-value < 0.05**

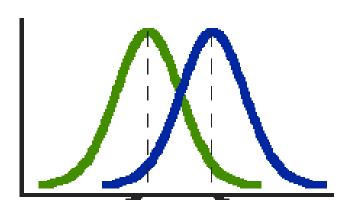
If p-value < 0.05,

there is a statistically significant difference between the groups.

How can we decide whether the difference is significant?

P-value is probability of the observed results assuming that there <u>is no difference</u> between the observations between the two samples (Ho).

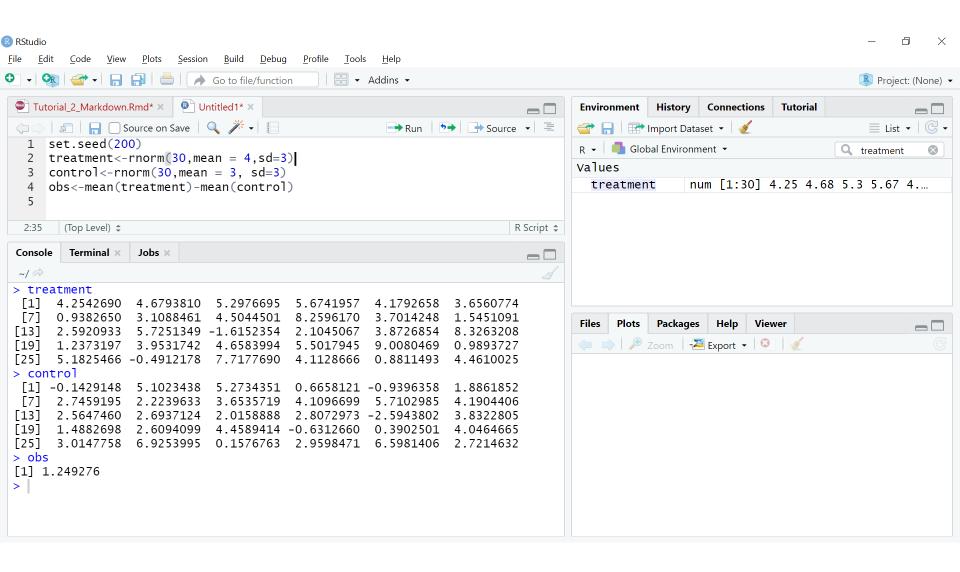
If p-value < 0.05, there is a statistically significant difference between the groups.

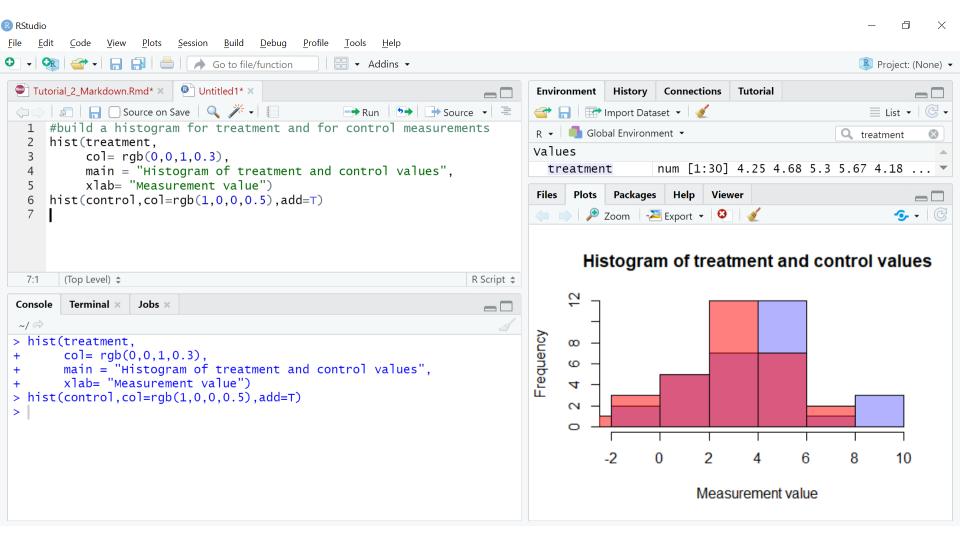


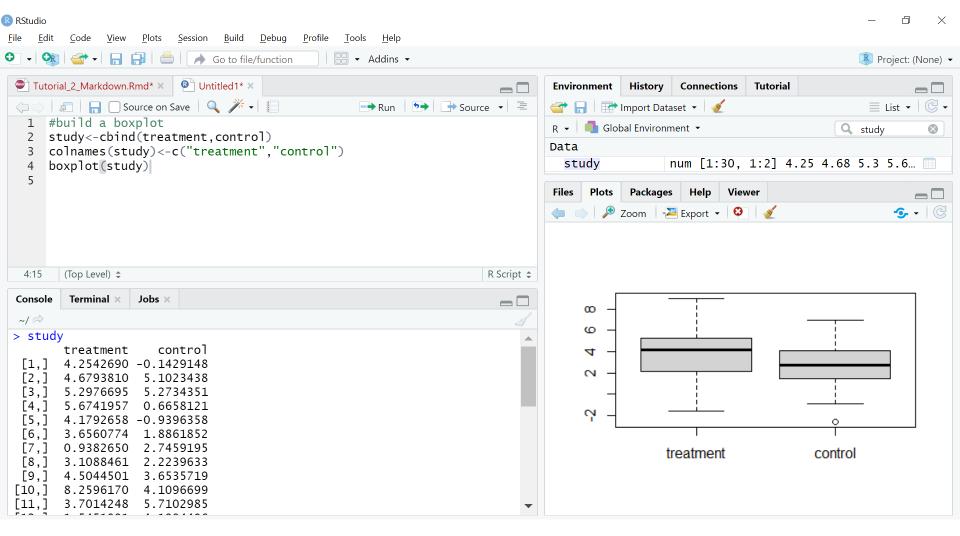
The null hypothesis is that the means (X1, X2) of two populations are equal

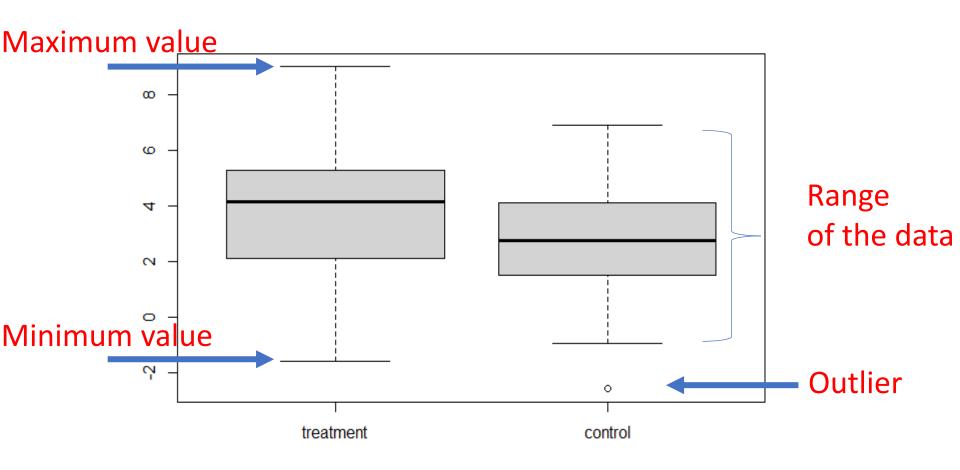
Which statistic test can we apply to find a p-value?

- Multiple tests can be applied according to the type of question you are asking.
- T-tests (t.test() function) assume that a sampled population has a normal distribution and t-test generally can tolerate deviations from normality.
- Wilcoxon signed-rank test (wilcox.text() function) is used to compare paired data and as an alternative to paired t-test when the data is not normally distributed.

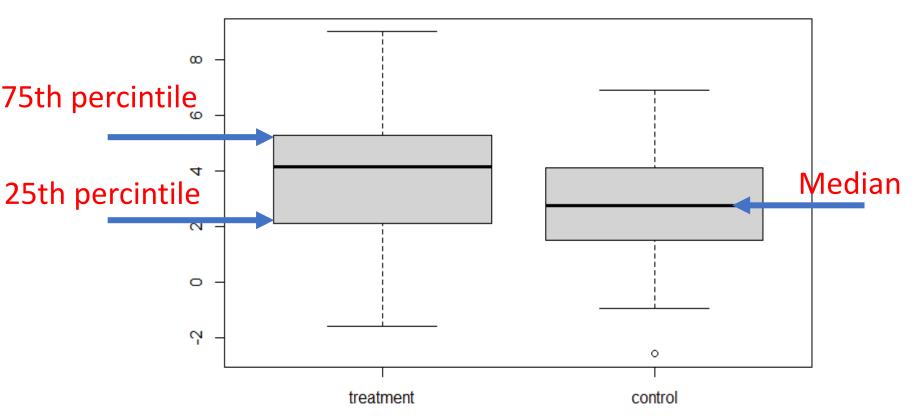






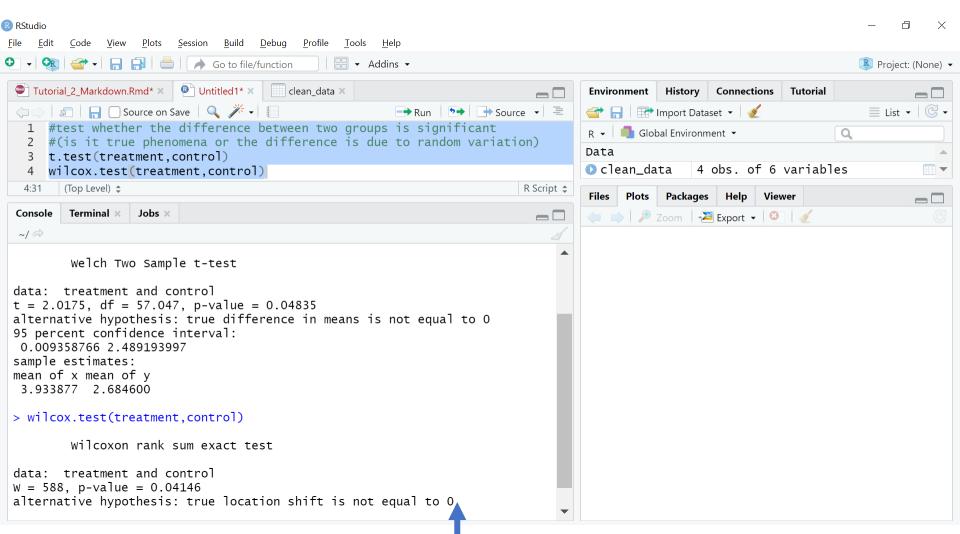


The box represents 50% of the data

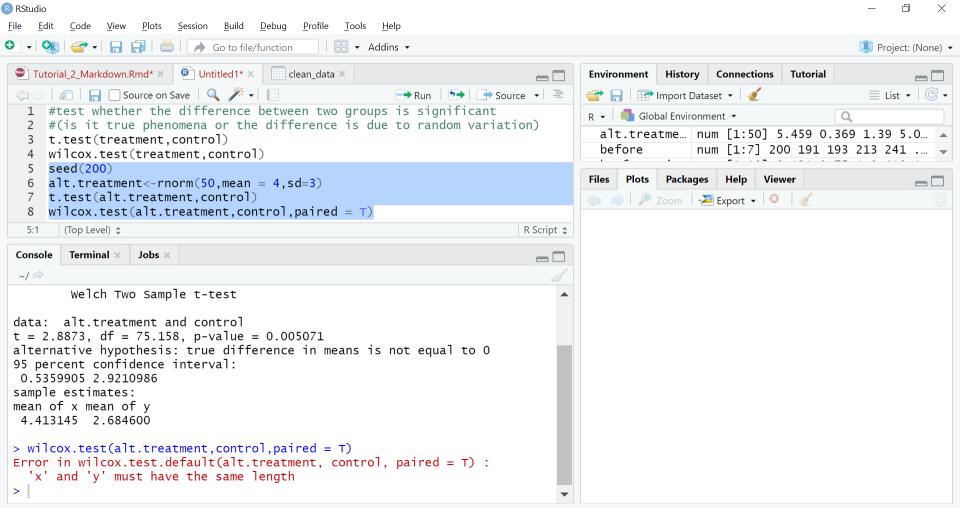


The box represents 50% of the data.

x% of data will be less than x^{th} percentile and (100% – x%) of data will be more than x^{th} percentile.



The distribution of one population is shifted to the left or right of the other, there is a difference in medians



Multiple testing correction

Significant p-value < 0.05 ***

*** For real data we must correct the p-value for multiple testing.

Why?

Because the hypothesis testing is not error-free method of making decision. As more testing you are doing the probability to get a significant p-value by chance grows.

Multiple testing correction

- False positives (Type I error) are "false discoveries" due to fact that we accept H₁ although we shouldn't.
- False negatives (Type II error) are cases in which can fail to accept the H₁ hypothesis when we should, meaning that we miss "true discoveries" by accepting Ho.

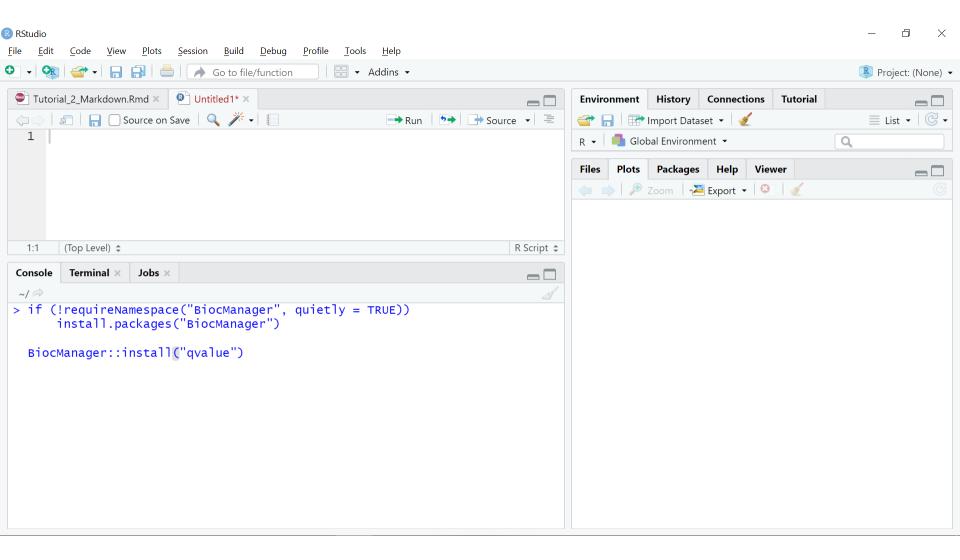
We expect to make more type I errors as the number of tests increase, which means we will reject the null hypothesis and accept H₁ by mistake.

- We perform the p-value adjustment to multiple testing by p.adjust() function. Given a set of pvalues, returns p-values adjusted using one of several methods.
- Which method to choose ?

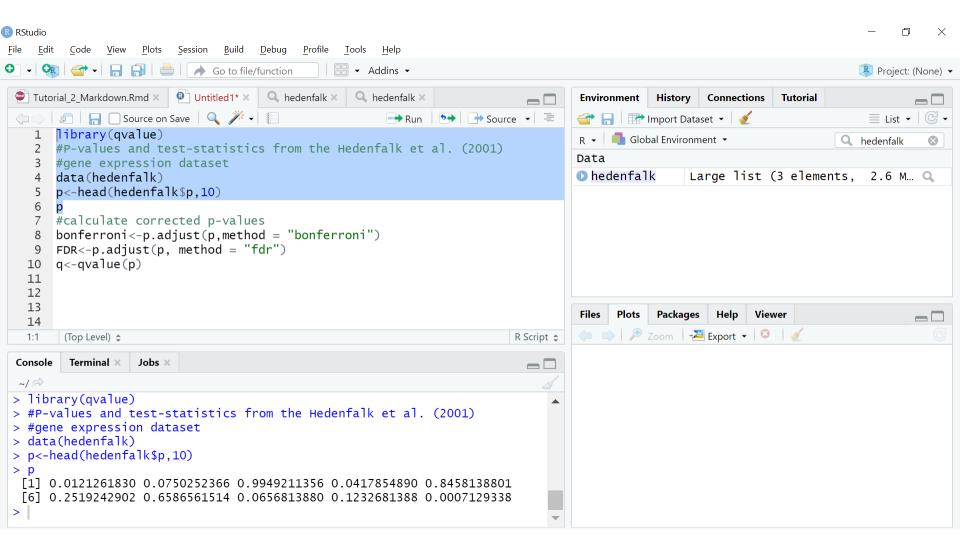
- If you concern about making even a single type 1 error, you can use **Bonferroni correction**. Using this correction you increase the type 2 error rate: you will falsely accept the null hypothesis more frequently, and so neglect findings that might be relevant.
- If your main concern is about the false positives you make, and you think it's important to you to avoid rejecting true H₁ hypothesis, you should apply Benjamini and Hochberg (BH) method which is also called FDR correction. This method allows you to control the proportion of the false discoveries.

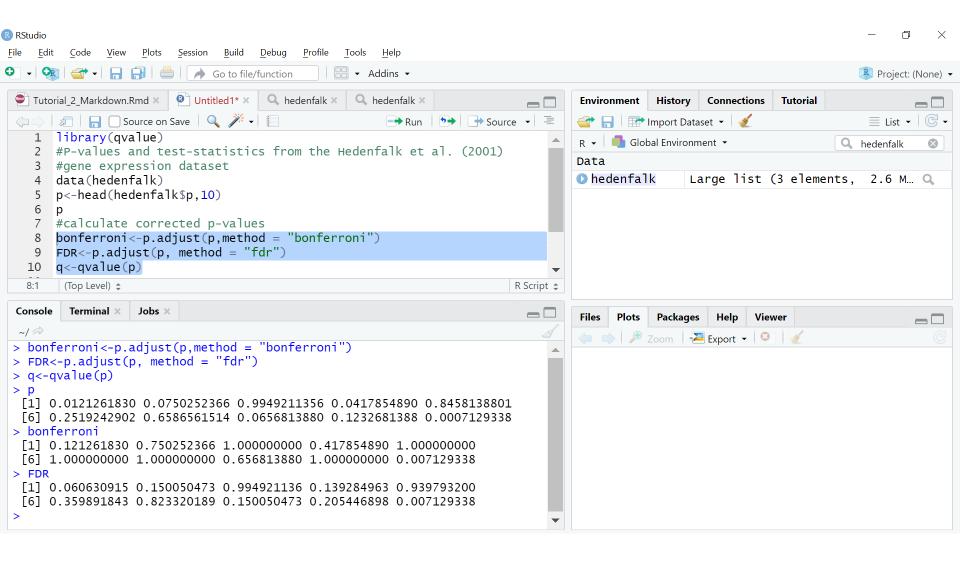
- Another method of multiple testing correction is calculation of q-value. Q-value is the proportion of significant results that turn out to be false. A q-value 0.01 would mean 1% of the tests called significant at this level will be truly null. Although they can be calculated differently the q-value and FDR adjusted Pvalue are synonymous within the genomics community.
- q-value is calculated using the qvalue package from Bioconductor.

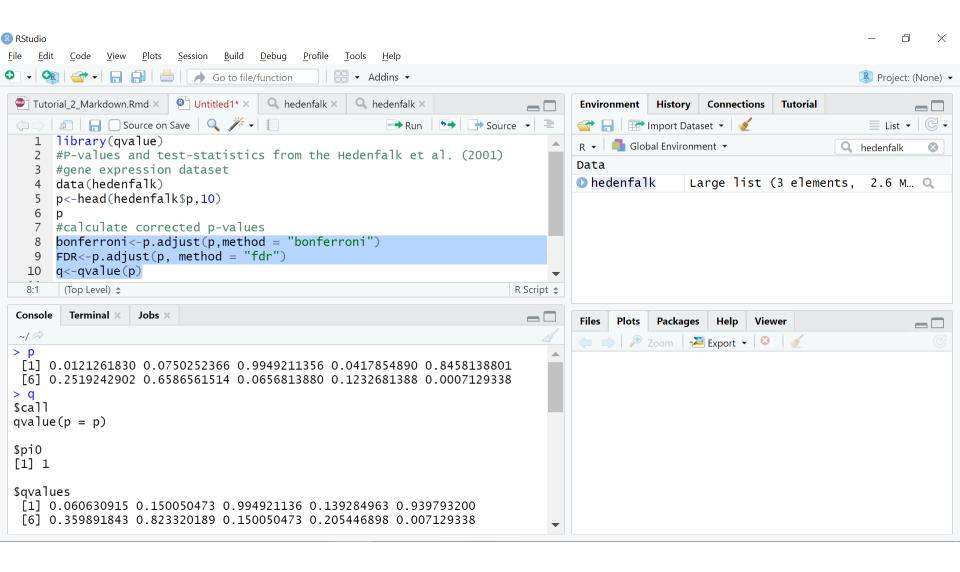
qvalue package installation



https://bioconductor.org/packages/release/bioc/html/qvalue.html



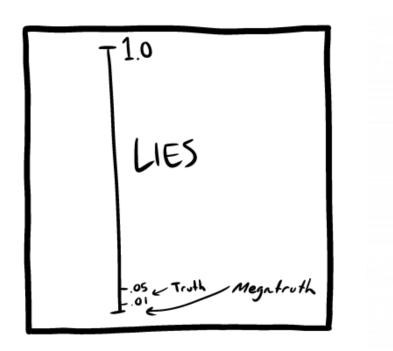




- You should choose a method familiar in your field of study. You should apply common sense and choose how you balance the probability of making a type I error relative to a type II error.
- In a preliminary study, you'll probably want to keep as many significant values as possible to not exclude potentially significant factors from future studies.
- In a clinical study that can decide the human lives fate you'd want to have a very high level of certainty before concluding that one treatment is better than another and you will use more stringent multiple testing correction, even in the price of false negatives.

Beware of p-haking!

 "P-haking is the misuse of data analysis to find patterns in data that can be presented as statistically significant when in fact there is no real underlying effect" — Wikipedia



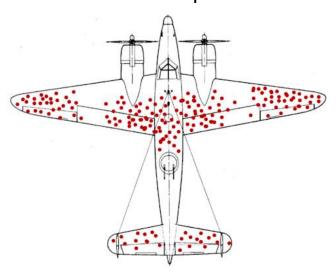


"If you'll torture the data long enough, it will confess to anything" (C. Ronald H. Coase)

Cherry picking



Survivorship bias



You are cordially invited to read more about statistical fallacies you must avoid at: https://www.geckoboard.com/best-practice/statistical-fallacies/

https://www.pinterest.com/pin/240238961352821718/

A kind reminder: Use your resources wisely.

- https://cran.r-project.org/doc/contrib/Shortrefcard.pdf
- https://stackoverflow.com/
- https://stats.stackexchange.com/
- https://community.rstudio.com/
- What statistical analysis should I use?
- https://stats.idre.ucla.edu/r/whatstat/whatstatistical-analysis-should-i-usestatistical-analysesusing-r/

Whats next?

 Next lesson we will dive into the DNA sequence analysis.