Hypothesis Testing
Correlation & Regression
as Bivariate Analyses

j3

– 14.03.24 –

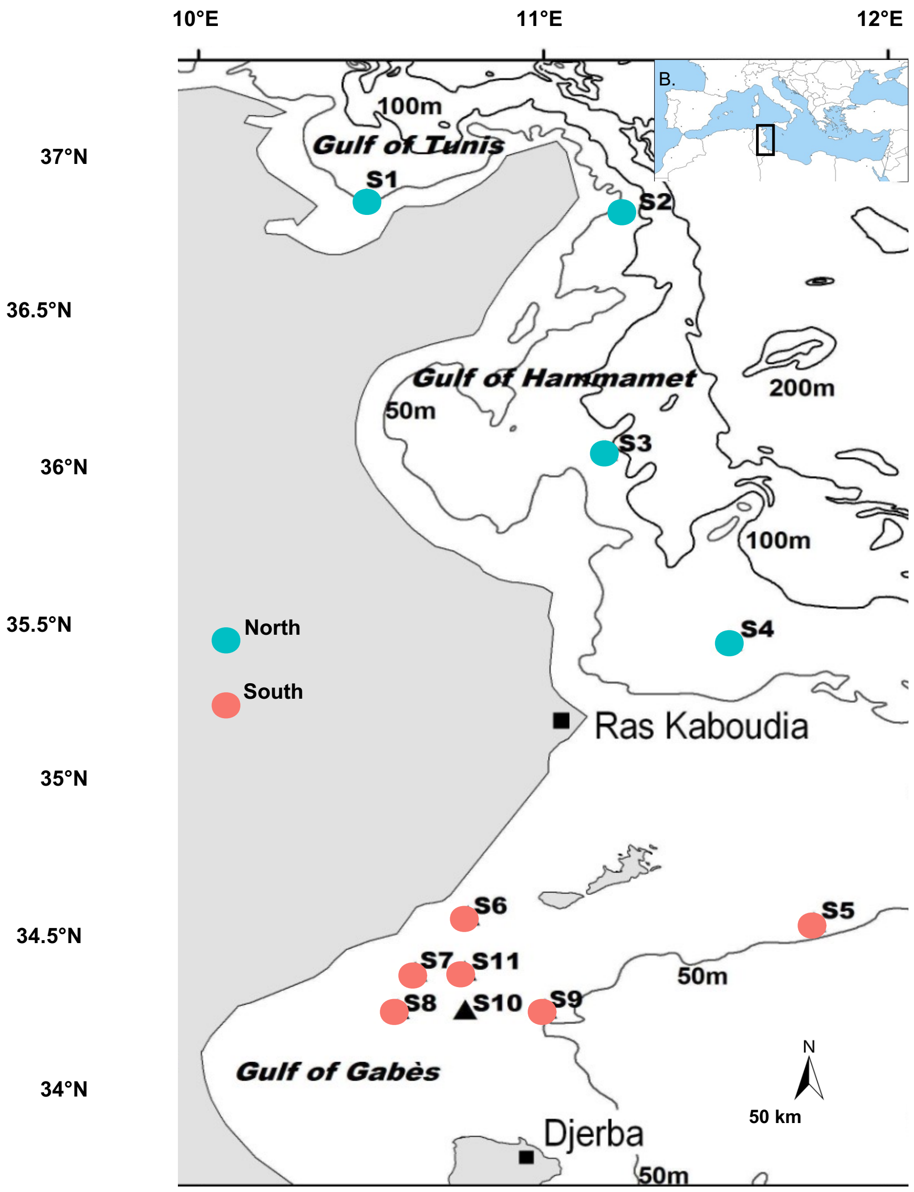
Institut Méditerranéen d'Océanologie

Institut de Recherche pour le Développement FRANCE

Plateforme OMICS - MIO

Bio-informatique & Sciences de l'Environnement : Exploration de la Diversité Taxonomique des Ecosystèmes par Metabarcoding

cnrs



Variability in species richness between North & South

Is there a real significative difference or just a coincidence ?

Using **statistics** to answer your question !!

Population VS samples

Population: set of individuals or objects of the same kind (very large or infinite)

→ We can't study an entire population: in statistics, we study a limited number of individuals, a part of the population: **a sample**

→ We try to **deduce properties** of the population from the sample

→ If we want to **study the variability** of a variable of interest in the population, we need a **representative sample** (drawn at random)

In a population, we can measure a characteristic: **a variable** that is the result of a random phenomenon.

- Qualitative
- Quantitative (continuous)

A **probability law** describes the random behavior of a phenomenon that depends on chance.

In a population, we can measure a characteristic: **a variable** that is the result of a random phenomenon.

- Qualitative
- Quantitative (continuous)

A **probability law** describes the random behavior of a phenomenon that depends on chance.

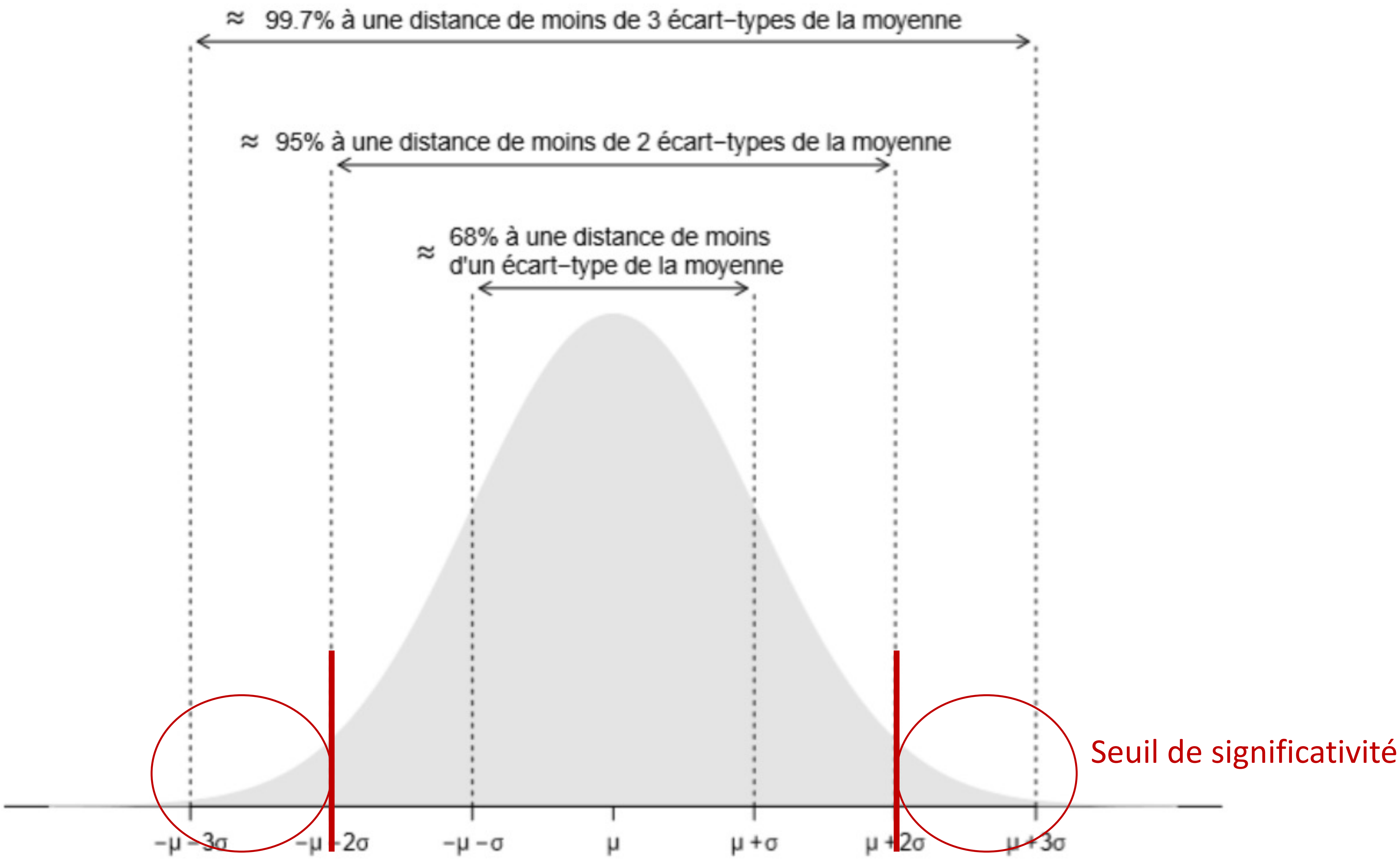
THE NORMAL LAW

If we have 1000 samples of a variable following a normal distribution, and plot the number of samples equal to each value, we obtain a "bell" curve / gaussian distribution

$X \sim N(\mu,\sigma 2)$ with μ and $\sigma 2$ the parameters of the distribution:

- μ: expectation of X
- σ: standard deviation of X = dispersion around the mean

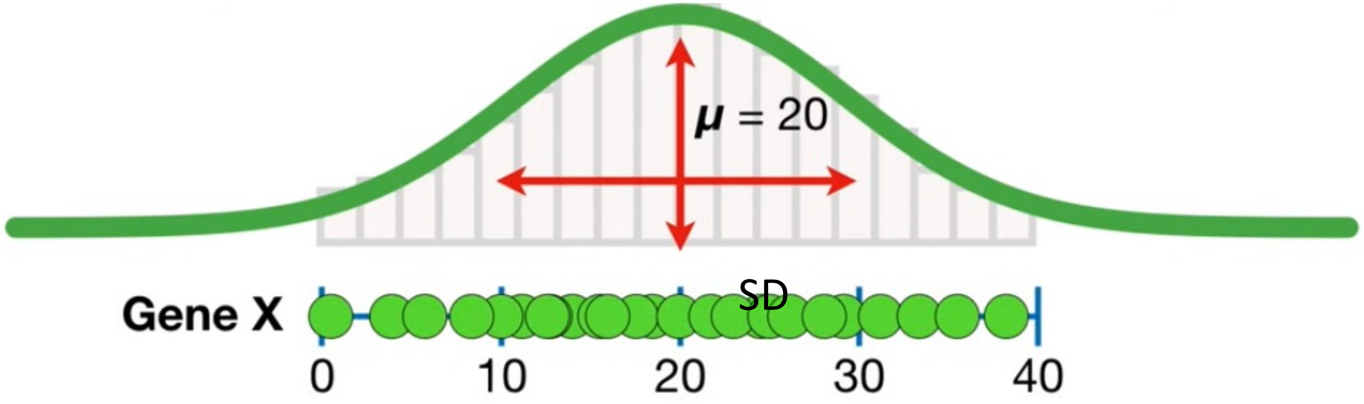
Répartition des valeurs autour de la moyenne



Remember : Descriptive statistics (Univariate analysis)

Merely describe, show and summarize collected data

- **Central tendency** (mean, mediane…)
- **Dispersion** (variance, standard deviation)
- **Frequency distribution** (count, relative, cumulative)

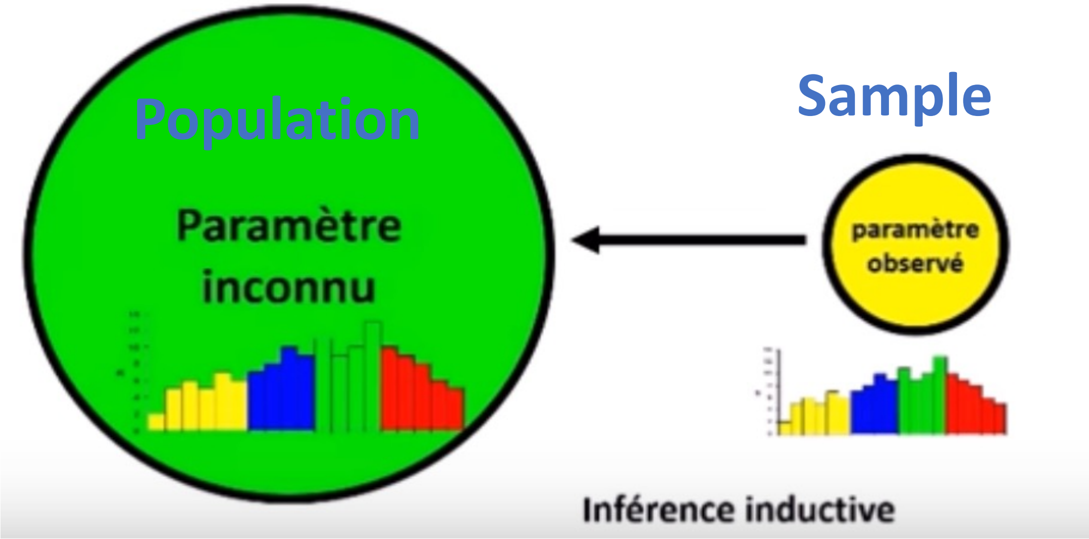


Identify the characteritics of data for each variable(s)

→ Allows you to formulate hypotheses and guide statistical analyzes

Inferential Statistics

Predictions - Generalizations



Make inferences about the population

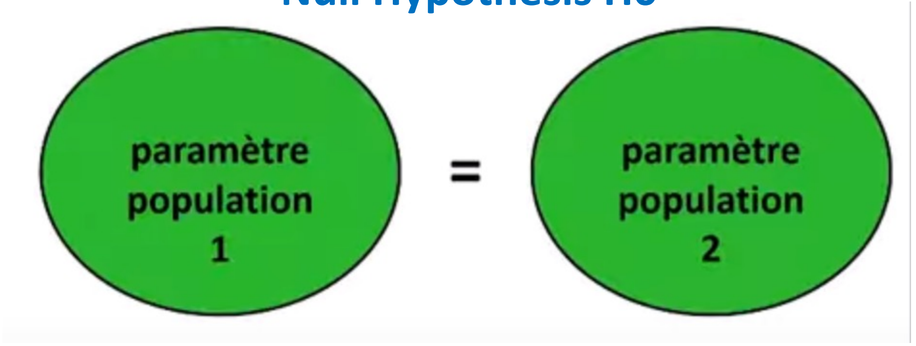
- How can I use my sample to make predictions about the population = **Estimation**
- How do I prove a theory about my data's behaviour (comparison) = **Hypothesis Testing**

Hypothesis testing approach

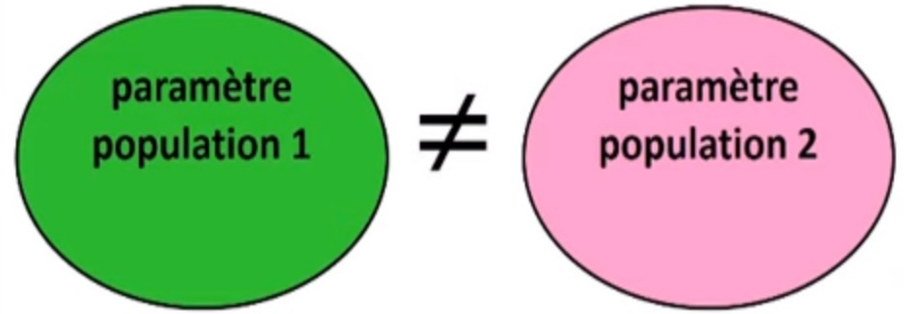
Trying to validate a hypothesis relating to a population parameter from a sample comparisons

Is there a real difference or just a coincidence (chance)

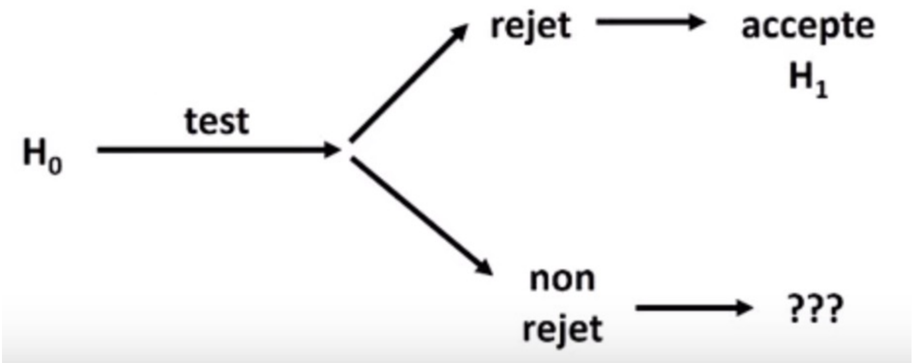
Null Hypothesis H0



Alternative hypothesis H1



We are testing the null hypothesis!



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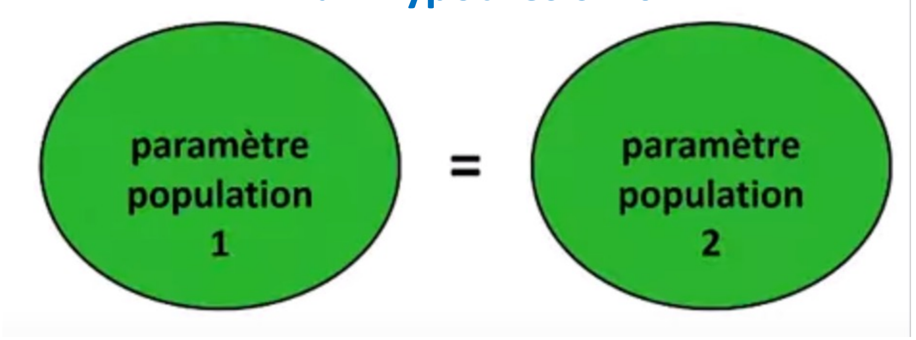

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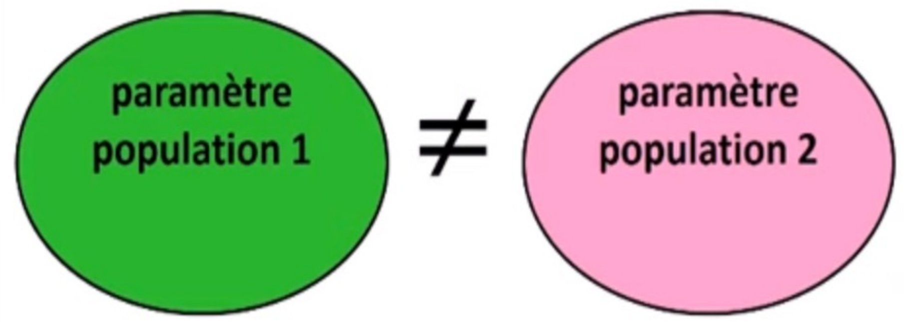
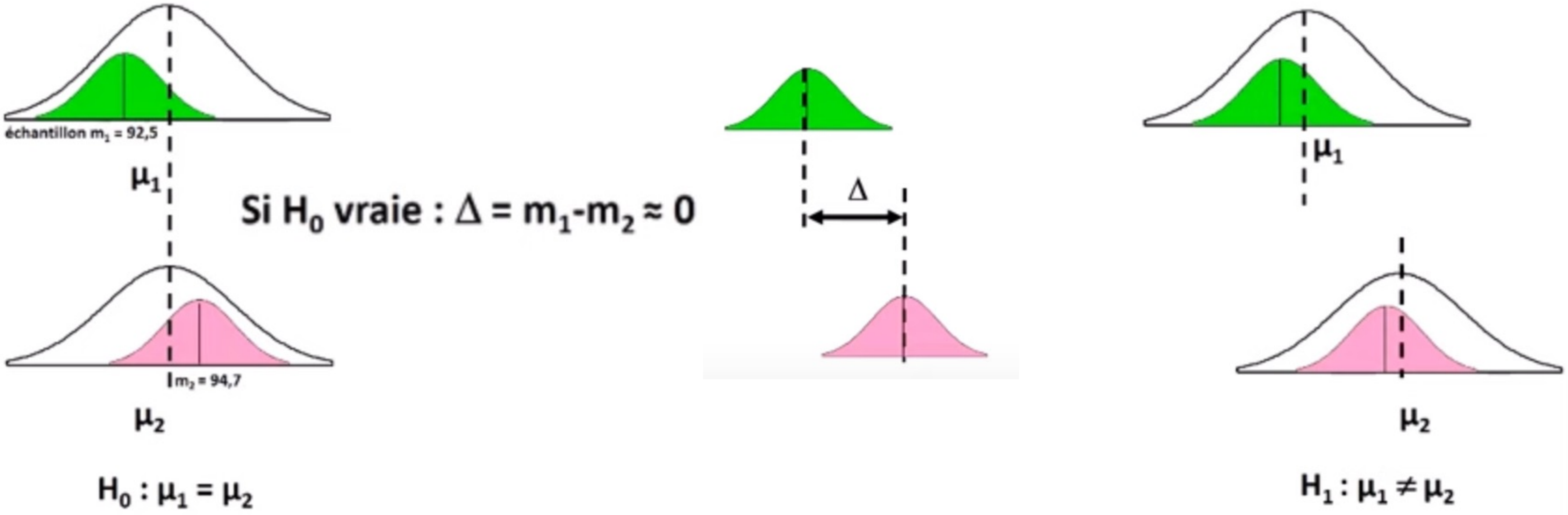


“Absence of Evidence is not Evidence of Absence”

Hypothesis testing & mean comparison

If H0 true… no difference

If H0 rejected, H1 accepted



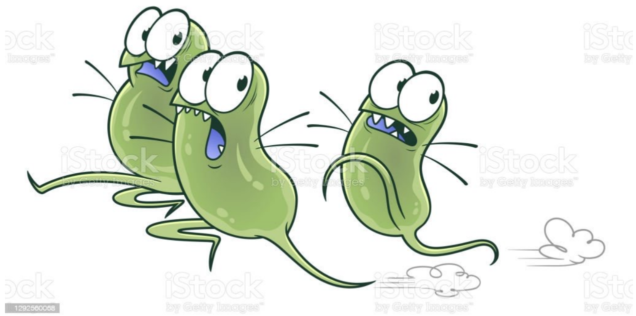
SAME distribution
→ Sampling fluctuation

Two different distributions

Inference Issue : Subjected to errors!!

The risk is linked to the result of hypothesis testing

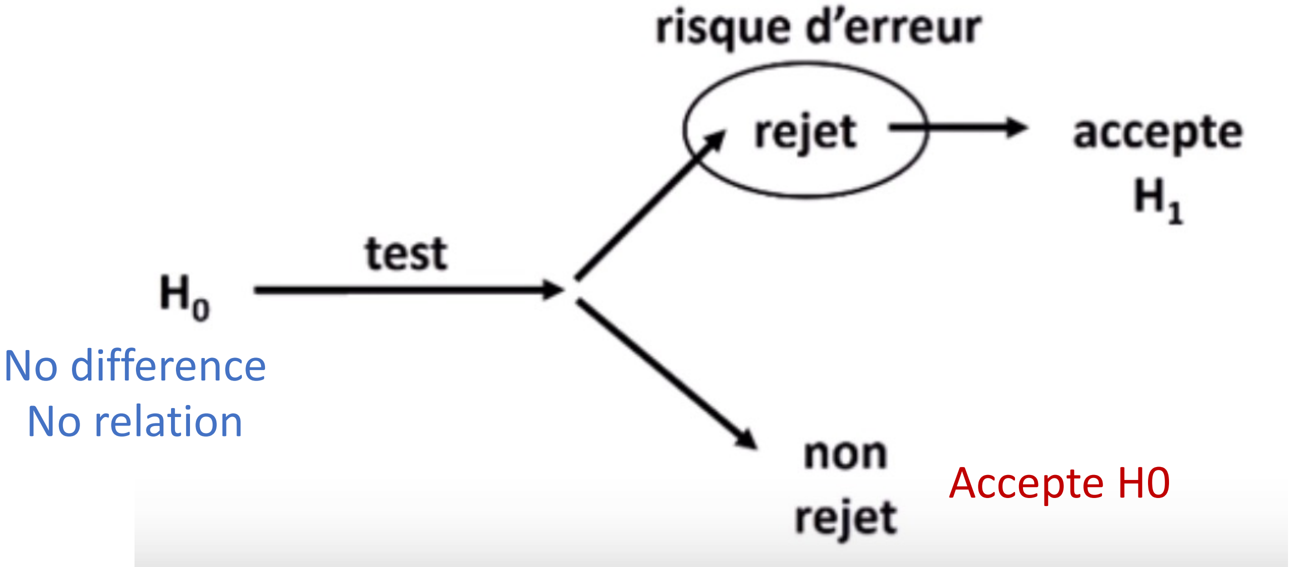
Because of your sampling!



The risk of Type I error α

- A probability between 0 and 1, or 0 and 100%

- Is when a difference is affirmed but there is none (=False positive)!!

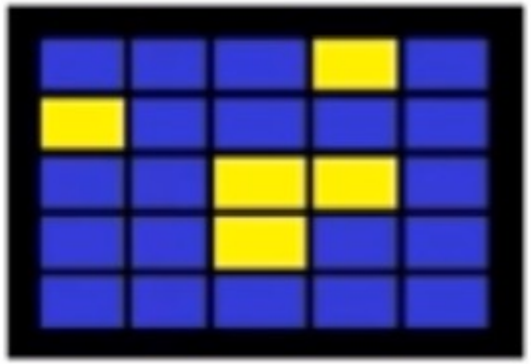


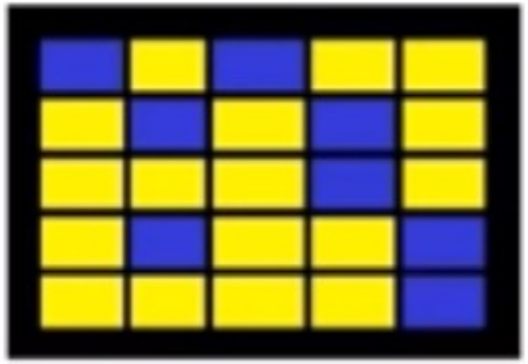
α= Risk to reject H0 if H0 is true

Sampling

25 tiles
→ 80% blue

25 tiles
→ 32% blue

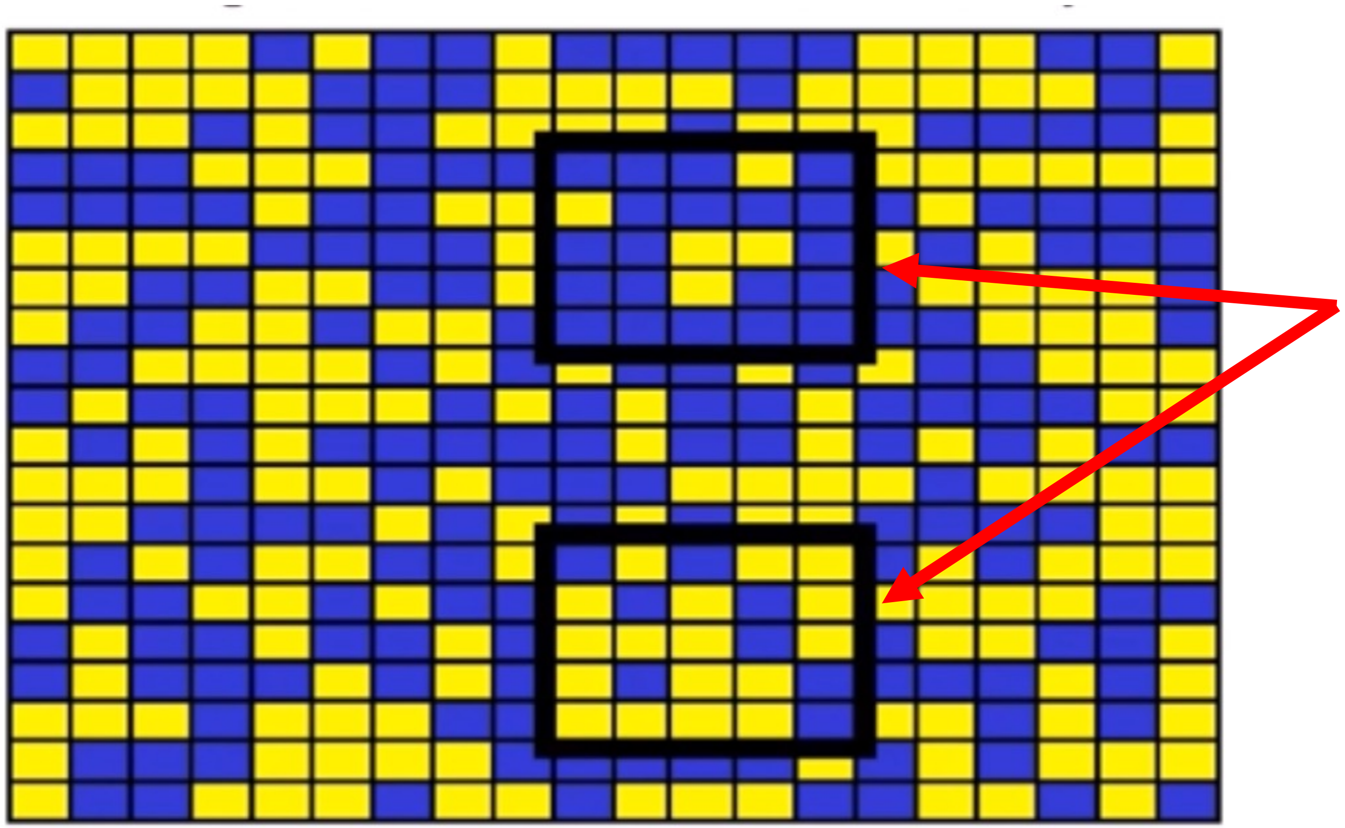




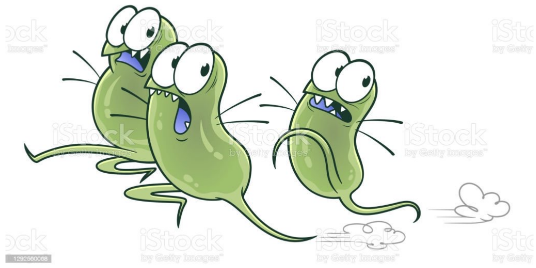
Do the two samples come from the same population? (same distribution)?

- **H0 is rejected**
- **but let's go to the store...see the population**

Come from the same population (50% blue, 50 % yellow)!!

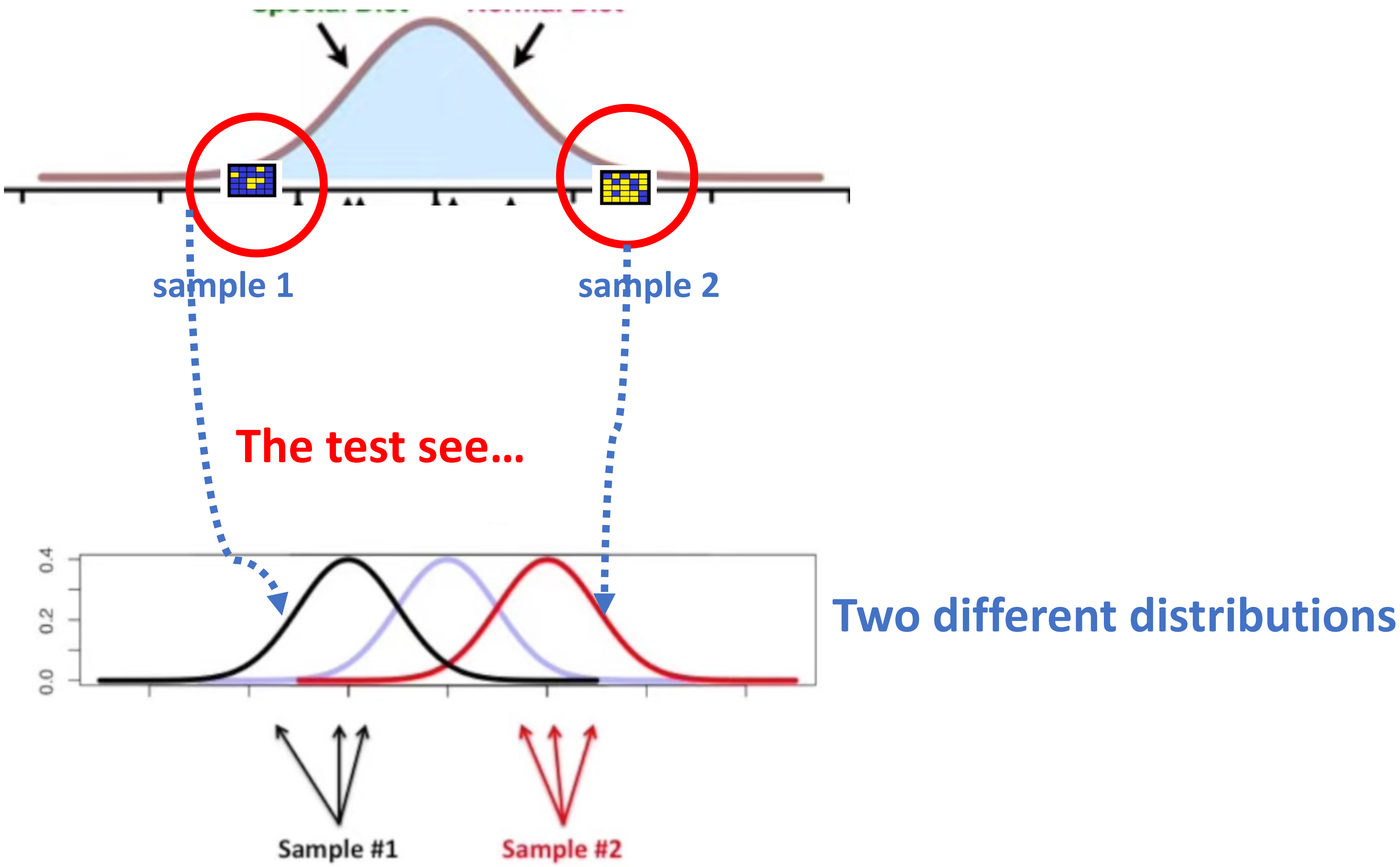


Rare sample type



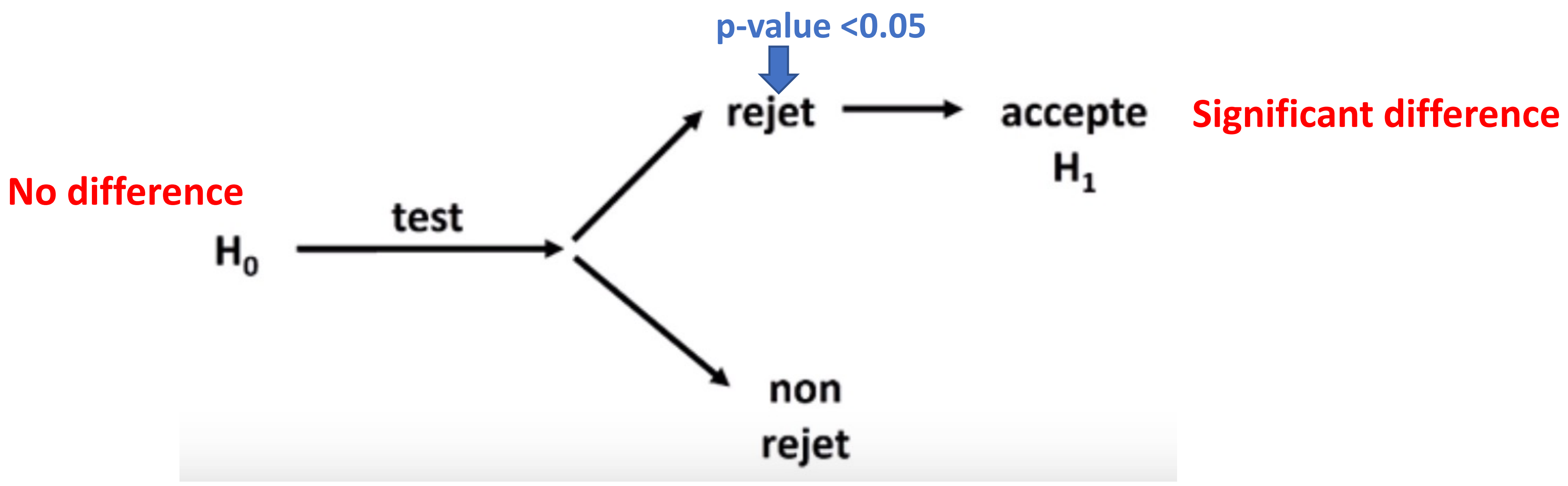
Conclude on the basis of our samples that they came from two different distributions = Type I error

Data come from the same distribution but …



- α is choosen before the test : **Significance threshold**

- α often set 5% (H0 wrongly rejected)
- In science the "almost no chance" translates to in less than 5% of cases where H0 is true = **p-value < 0.05**



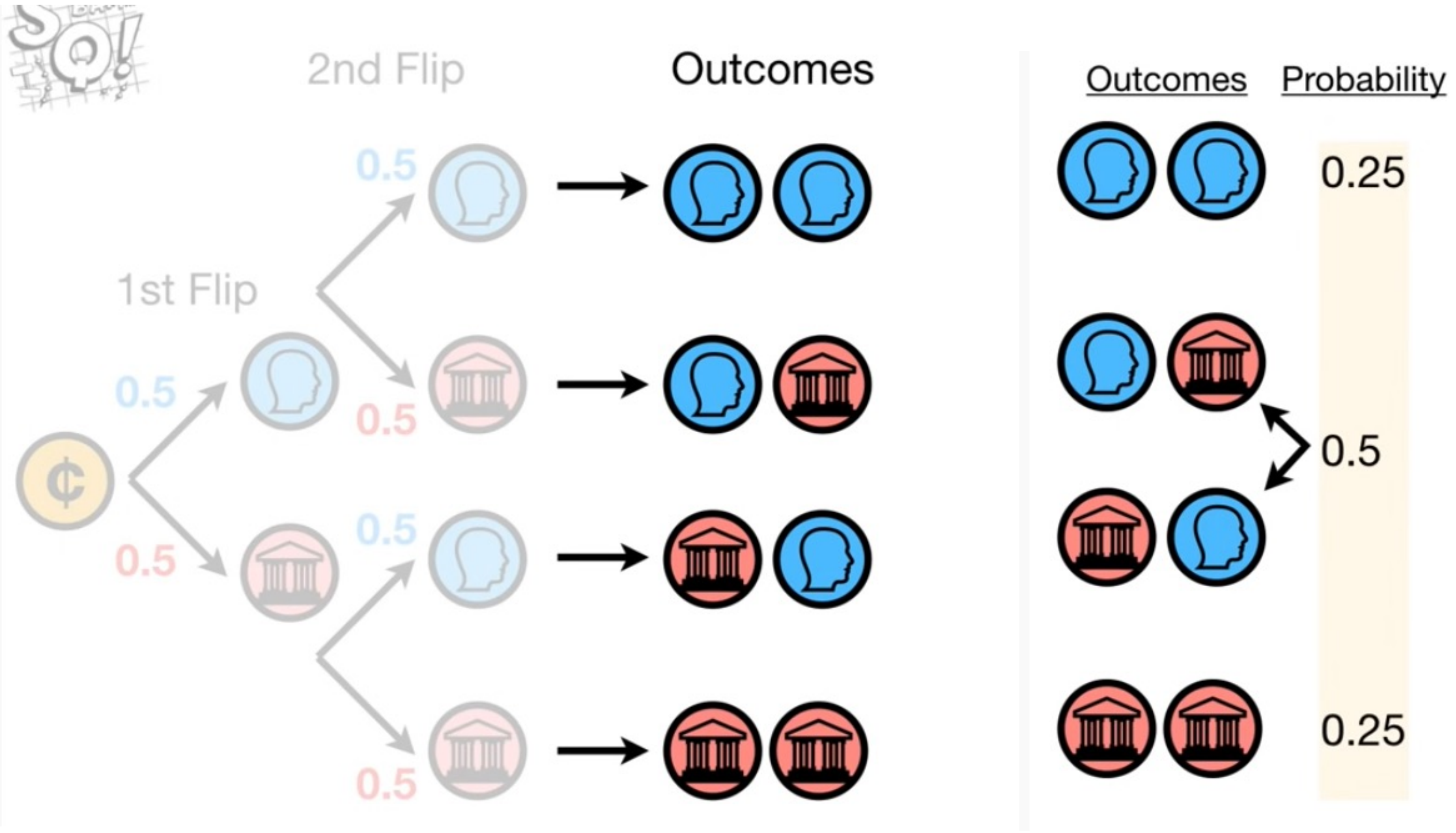
Concept of p-value…

My Coin is special: Heads twice in a row!

The Null hypothesis H0: even though I got 2 Heads in a row my coin is not different from a normal coin!

A small p-value will tell us to reject H0 (p-value <0.05)!

So let’s test the hypothesis by calculating the p-value!



$$\frac{\text{The number of times we got } \textbf{2 Heads}.}{\text{The total number of outcomes.}}$$

1st Flip 2nd Flip

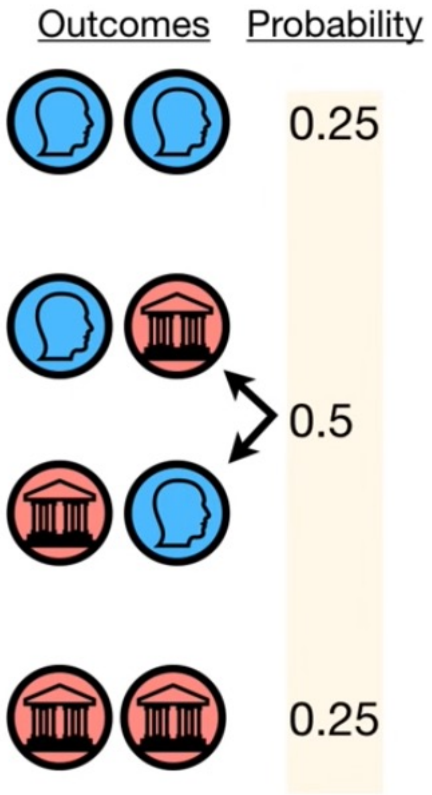
A ***p-value*** is composed of three parts:

1) The probability random chance would result in the observation.

2) The probability of observing something else that is equally rare.

3) The probability of observing something rarer or more extreme

Nothing

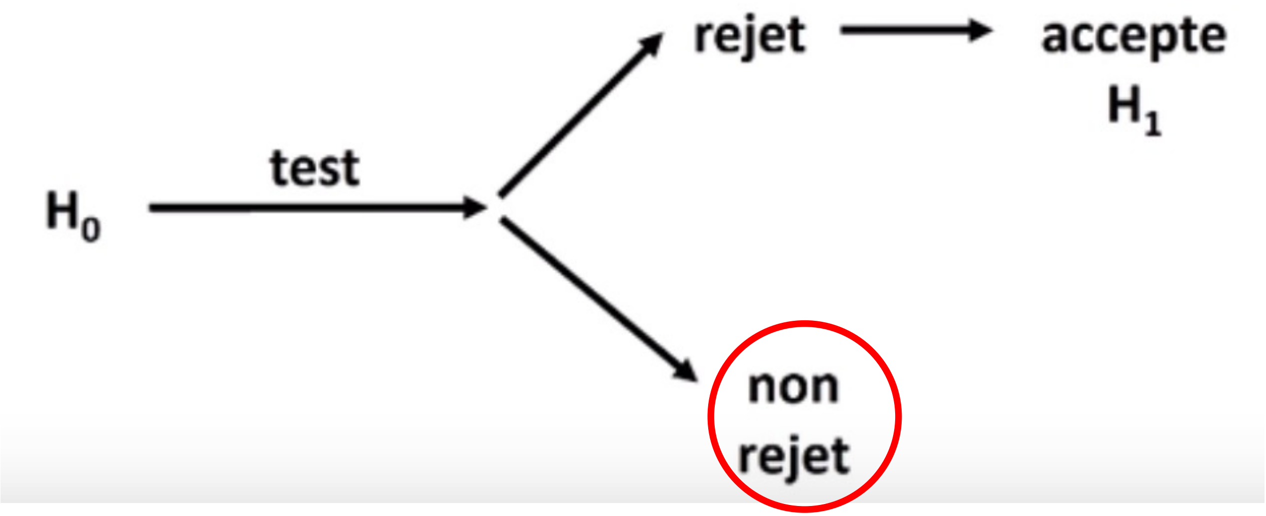


P- value for 2 Heads (Sum of three parts)= 0.25+ 0.25 + 0 = 0.50!

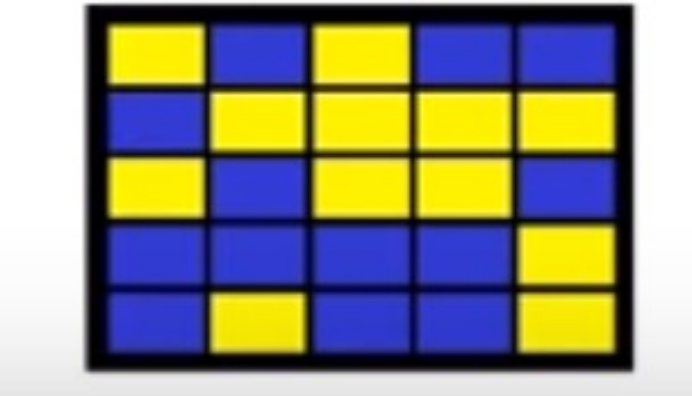
My coin is not special! p-value >>> 0.05!!!

Risk of Type II Error : β

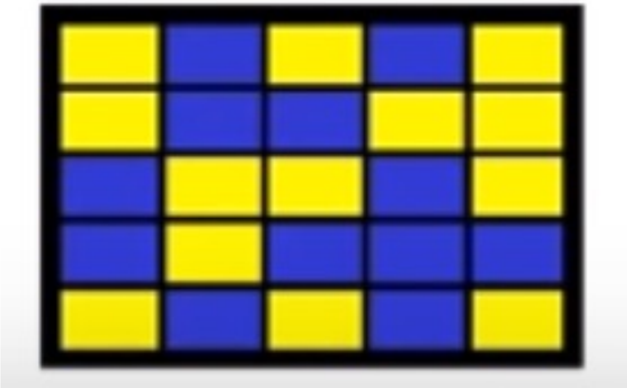
Failing to conclude **a difference when there is a true one** ("False Negative")
Probability of not rejecting H0, if H1 is true



β is not calculable

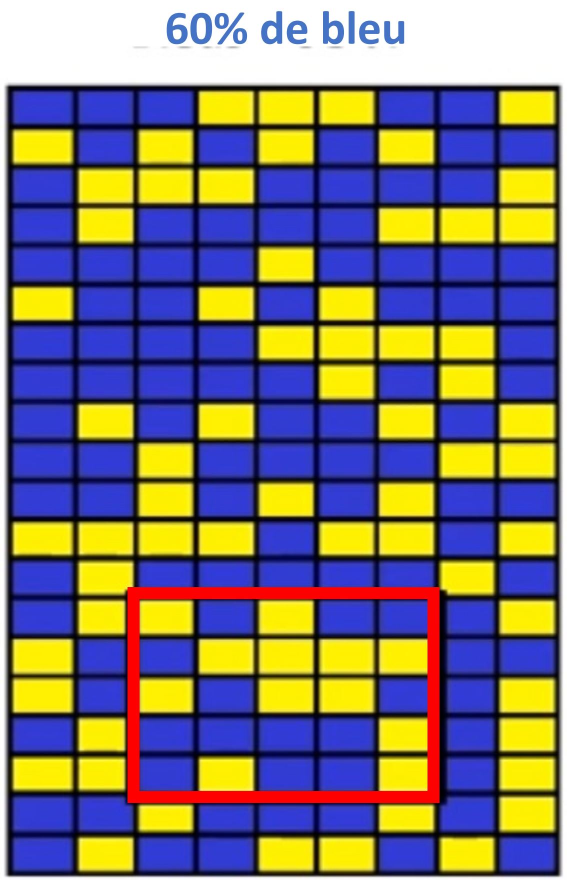


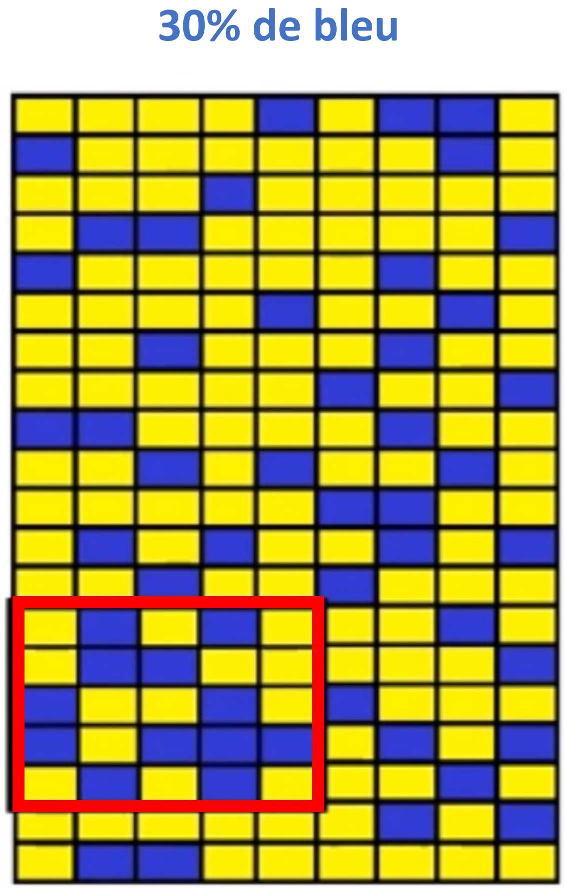
48% blue



52% blue

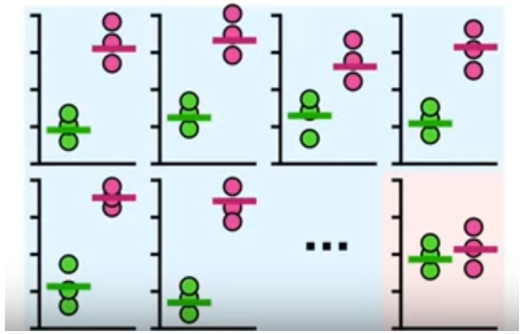
Do these two samples come from two different distributions or not?

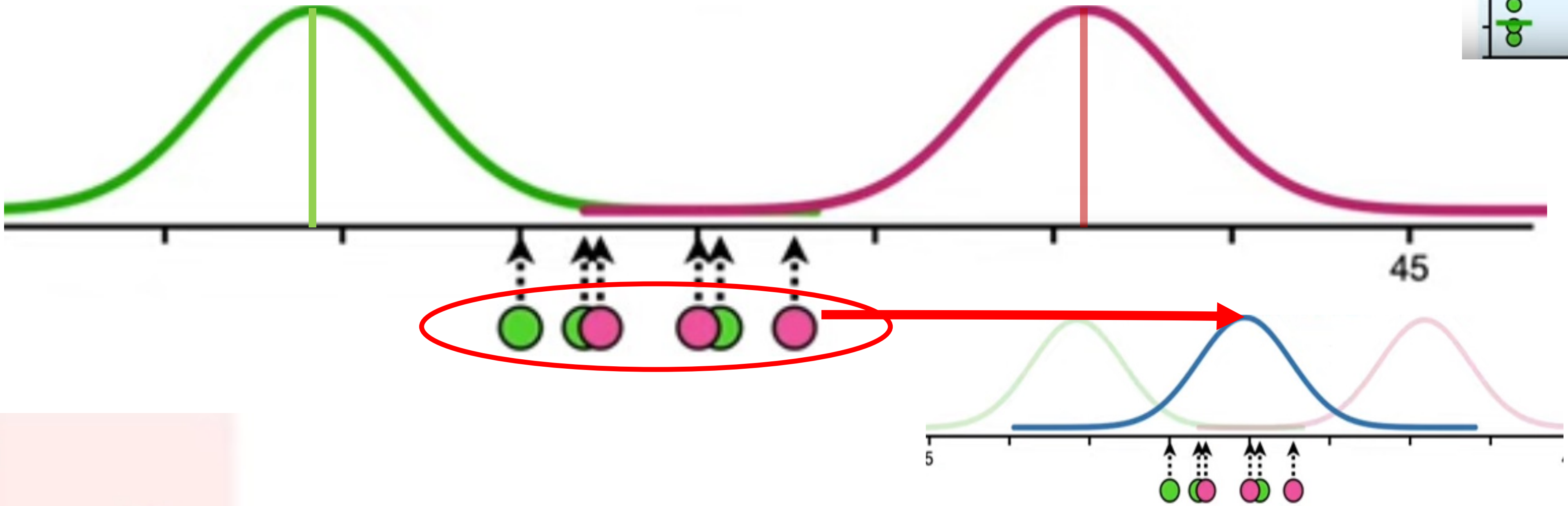


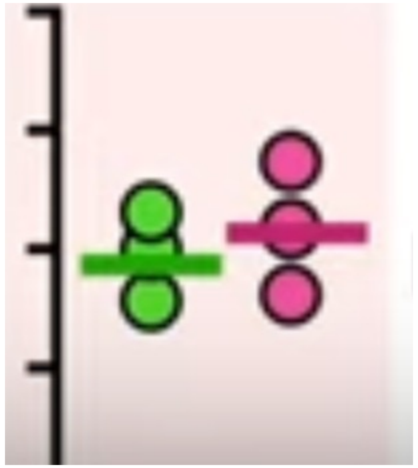


- 2 different tiles = 2 different populations, H0 should be rejected But that would not have been the case during the test with our sampling…

But sometimes…





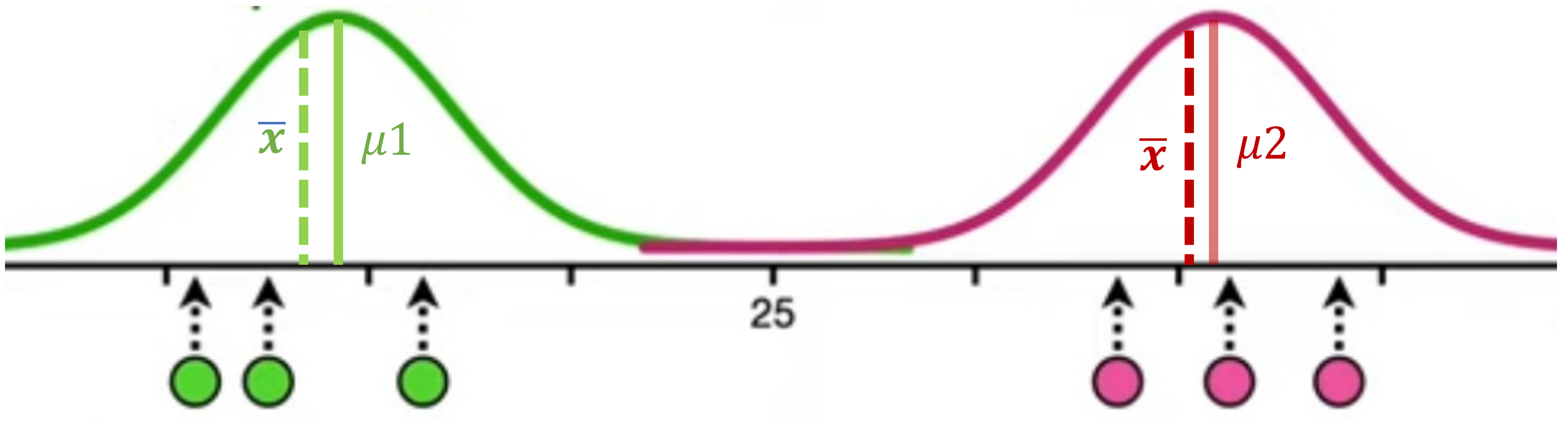


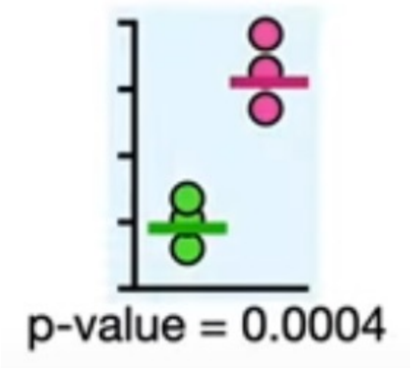
p=0.23!!!

Even if two different distributions (pop)…the test (your data) thinks they come from the SAME distribution!

Unable to correctly reject H0…

Scientifically … representative sampling of population





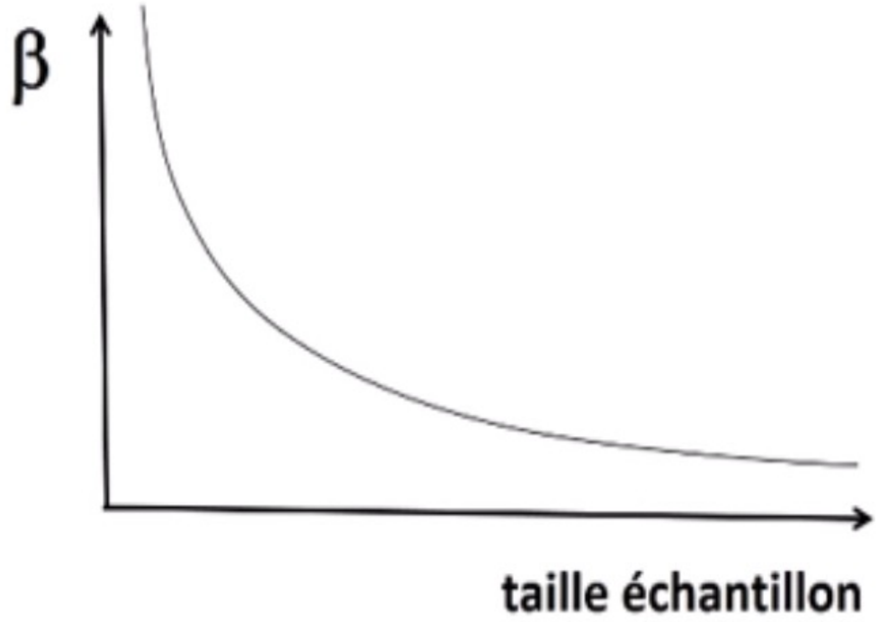
→ H0 correctly rejected

→ = Data do not belong to same distribution

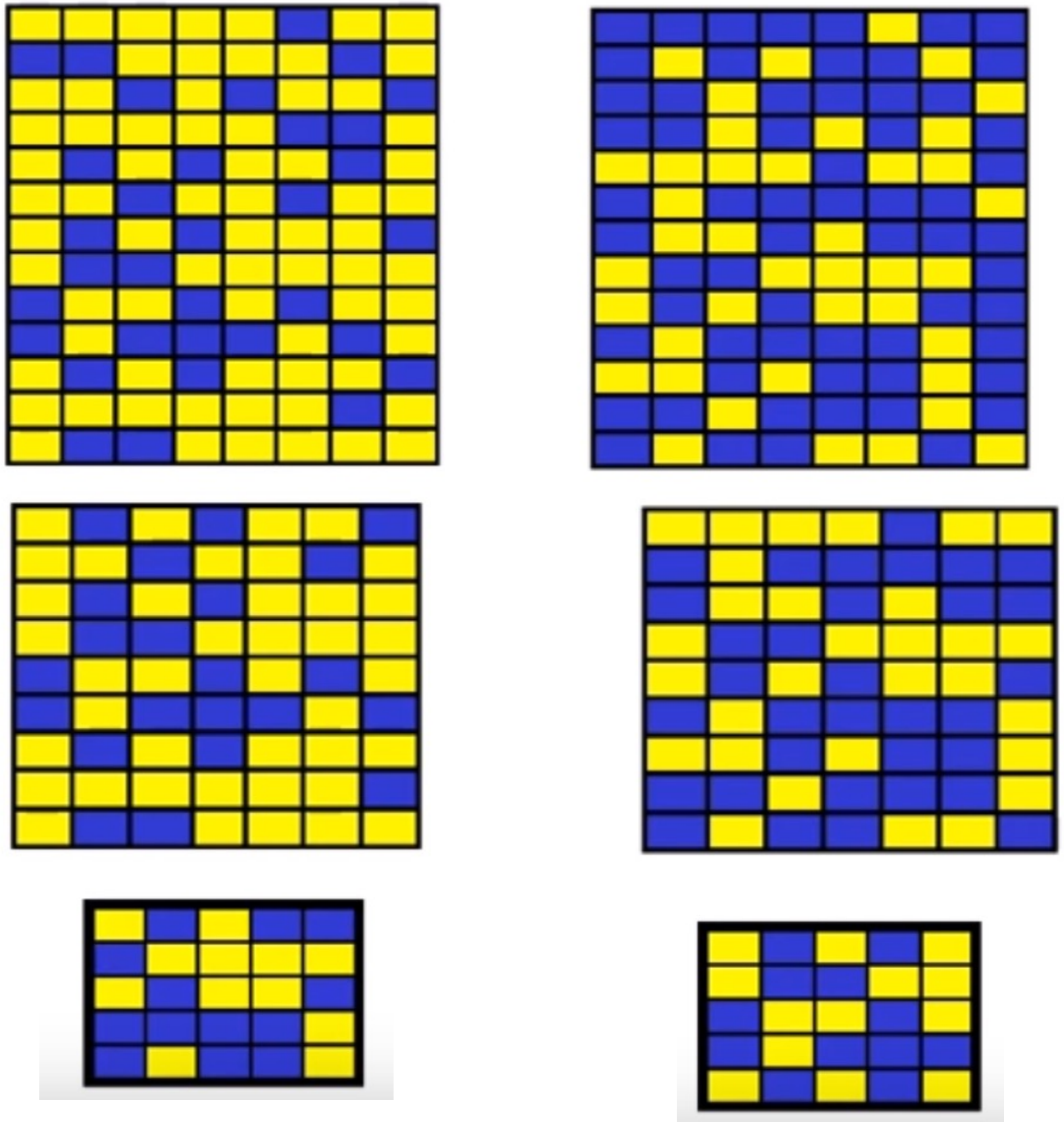
→ Two different populations

Fundamental relationship

Power = $1-\beta$



The more the size increases, the more the differences appear! The power of the test increases!



Power: Probability of correctly reject the H0 hypothesis

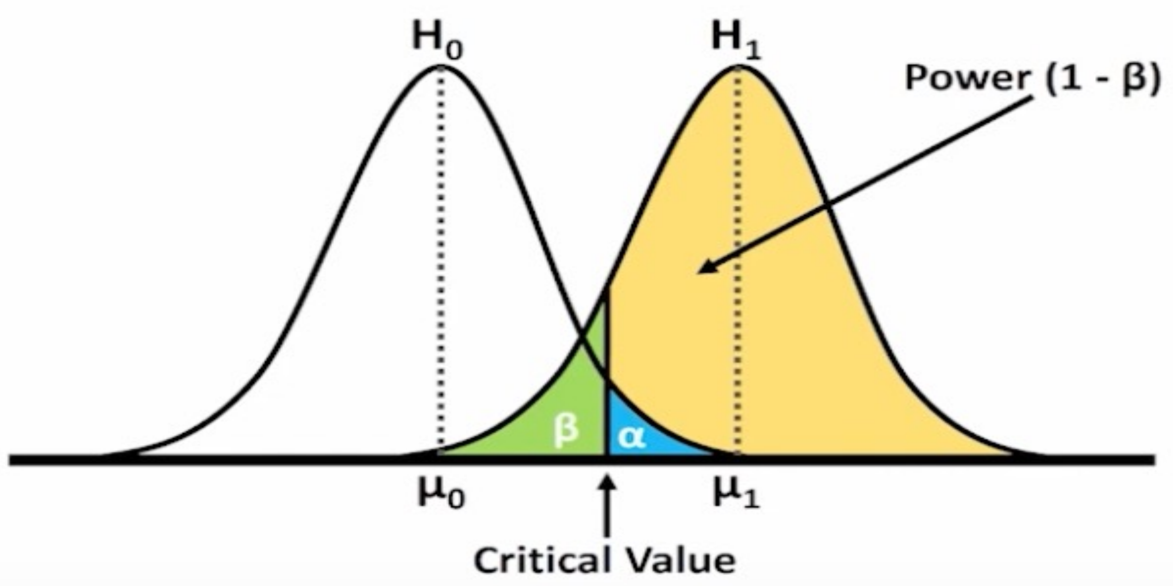
Ability of a test to detect differences

Summary

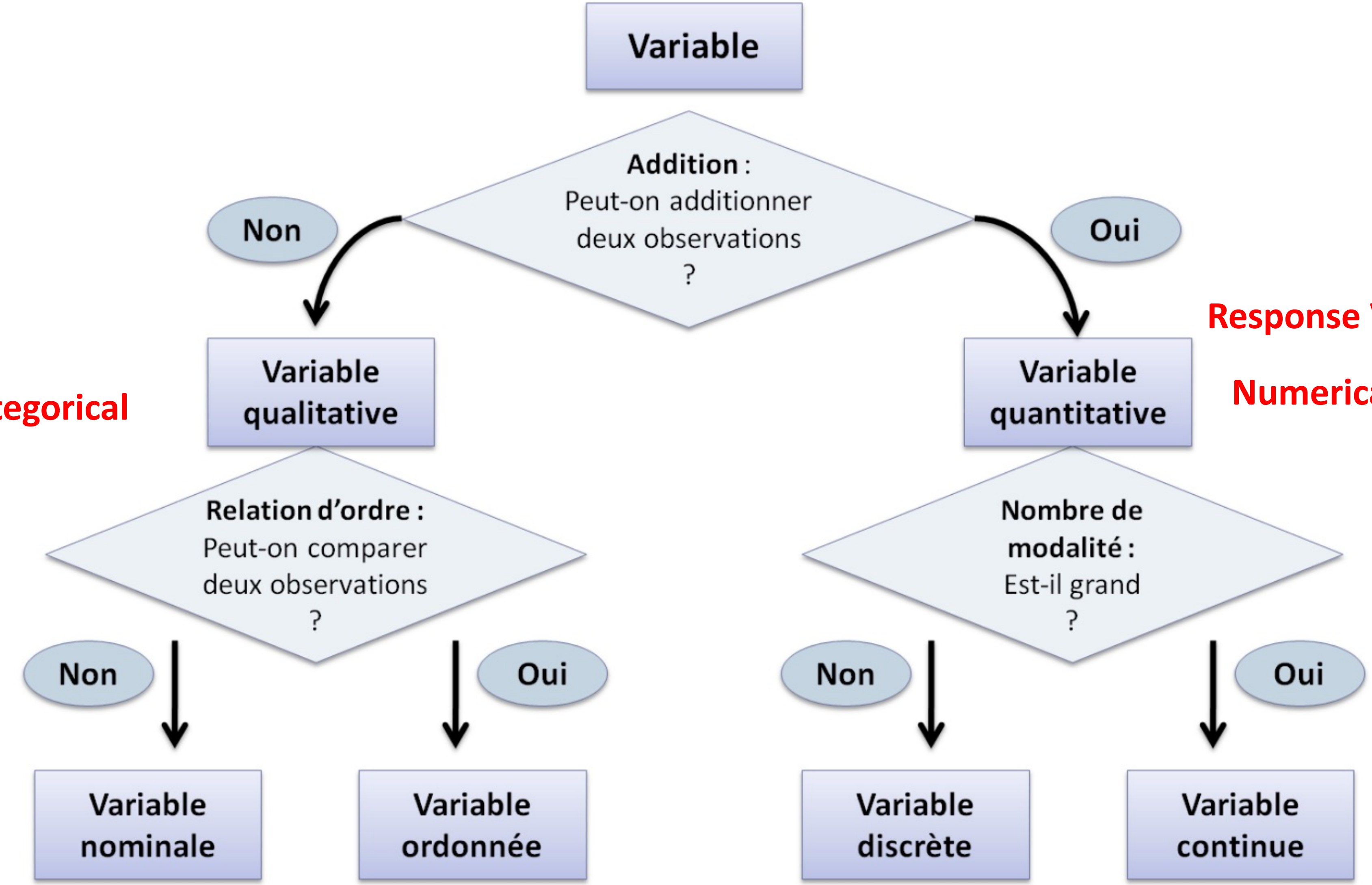
Population

TEST échantillons

	H_0 vraie	H_1 vraie
accepter H_0	OK	*erreur type 2* β Faux Négatif
rejeter H_0	*erreur type 1* α Faux positif	OK



Reminder on variables… important for statistical tests



Categorical

Response Variable

Numerical

- Maried, single…

→ No relation order

- Behaviour
- good, excellent…

Child in family (1,2,3..)
finite number of real values

Size, weight : infinite

Bivariate Hypothesis Testing

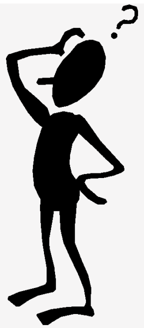
- Seek to **quantify the association** between a **variable to be explained** (response/Quantitative) and an **explanatory variable** (factor/categorical)

- **Make statistical inferences about the relationship between two variables,**
  One quantitative variable (response) & one qualitative (explicative)!

→ Can variations in species richness (response variable) be explained by the explanatory variable (factor) Treatment

→ **Comparison of mean between groups**

- **Parametric or non parametric test??**
- **which test?? significance ? (p-value)**
- **How many groups??**
- **Post hoc test required ??**



Which test for independent samples?
ONE categorical variable (H/F) & ONE continuous variable (numerical)

Normalité des données?
Shapiro, Q-Q plots

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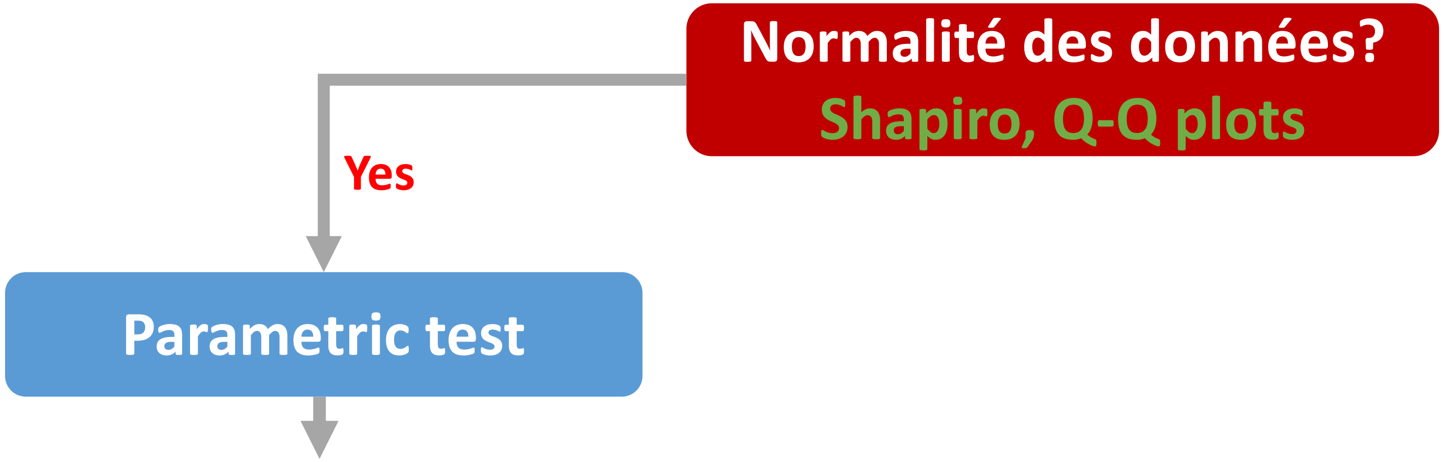
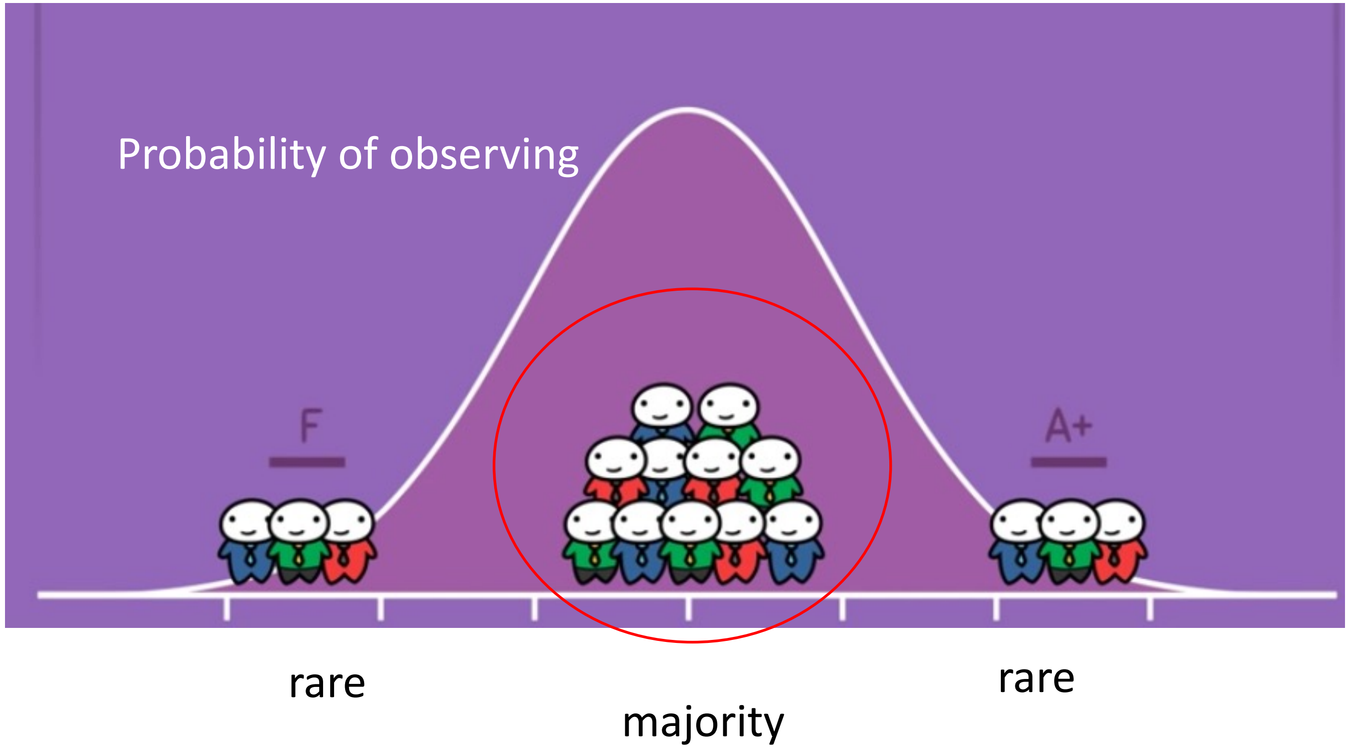


Features of Normal distribution

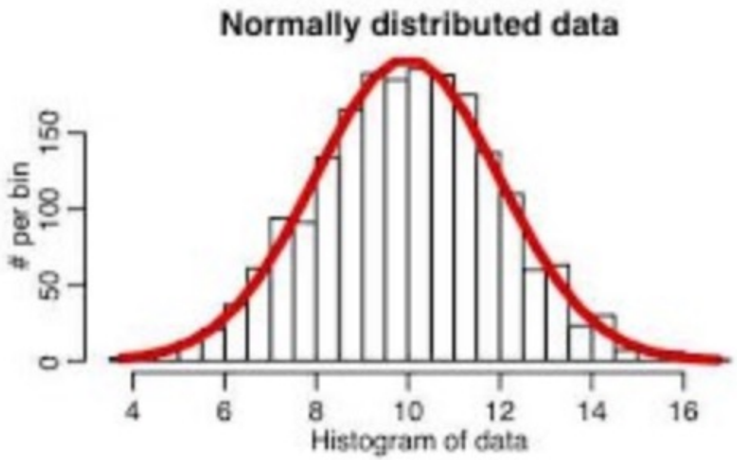
Symmetric, unimodal
- Center around the mean
- Dispertion around the mean: Standard deviation (SD)
- 95% data -/+ 2 SD

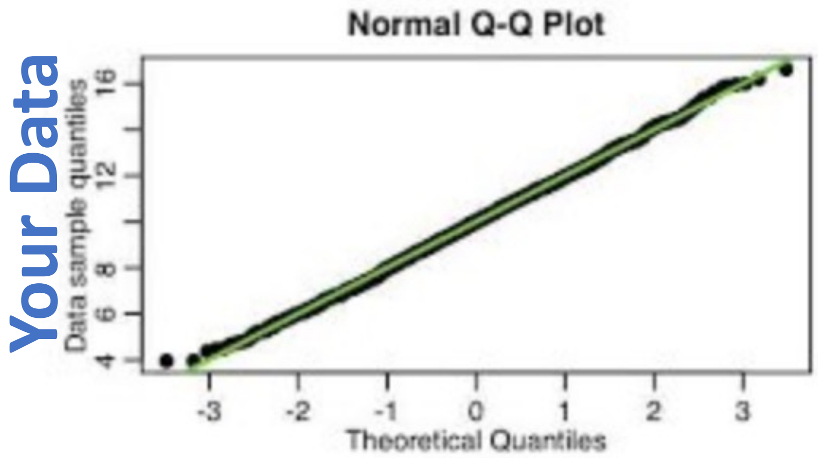


Check normality of data: Shapiro Test & QQ-plots!!

Q-Q plot normale: Compare your distribution with a normal distribution

Do my data follow a normal distribution ?



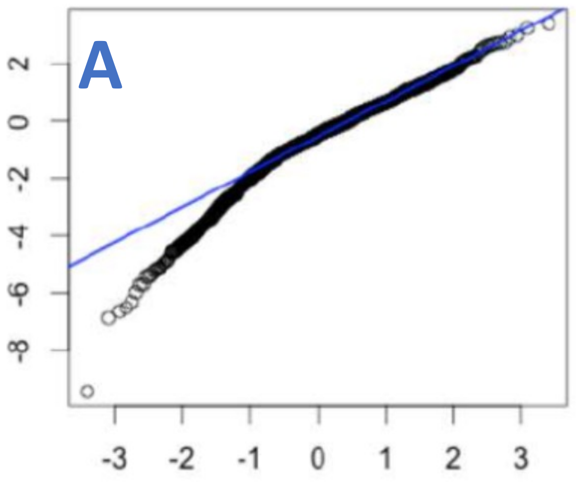


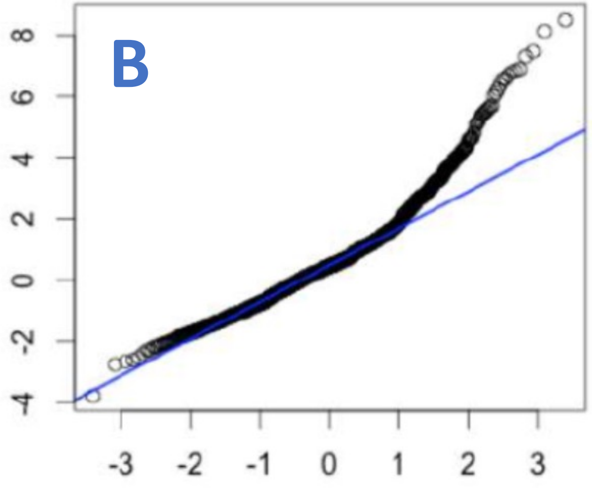
Normal Data (u= 0, SD=1)

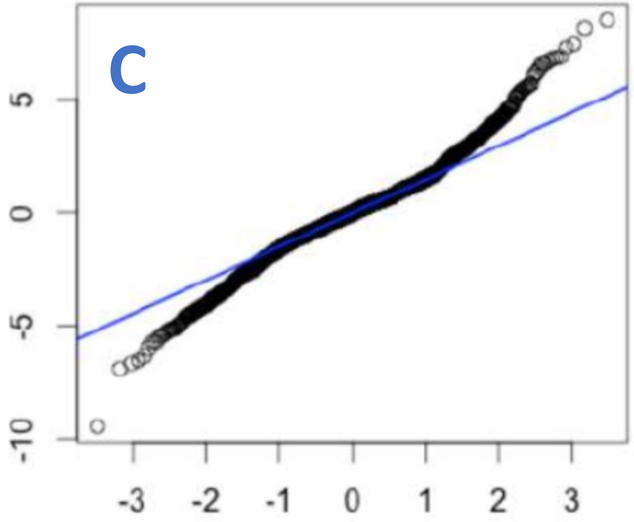
Conclusion?

The line draws by QQ-Plot indicates the position that the points must have to follow a normal distribution

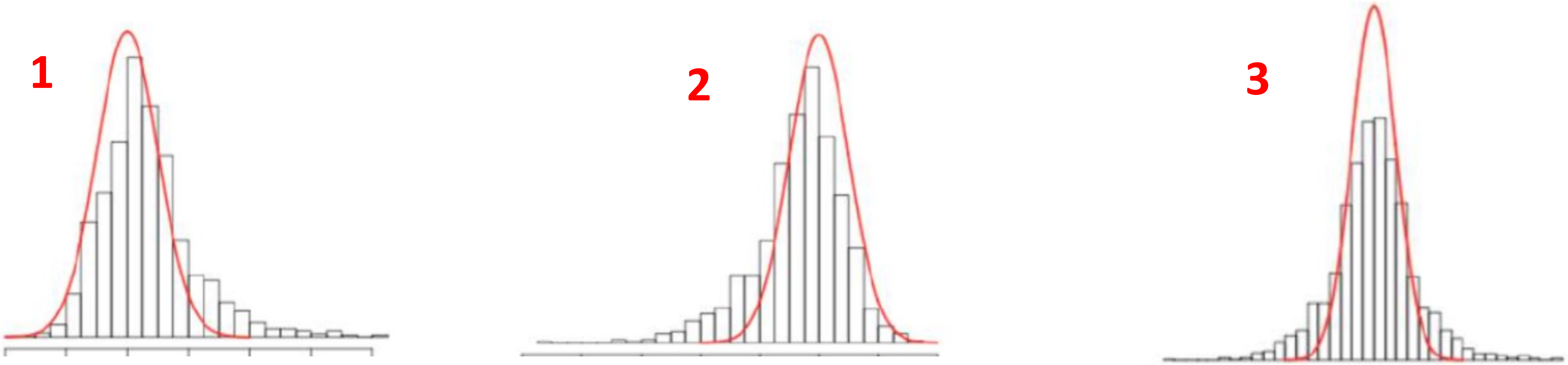
What are the distributions (bottom) corresponding to these QQ-plots?

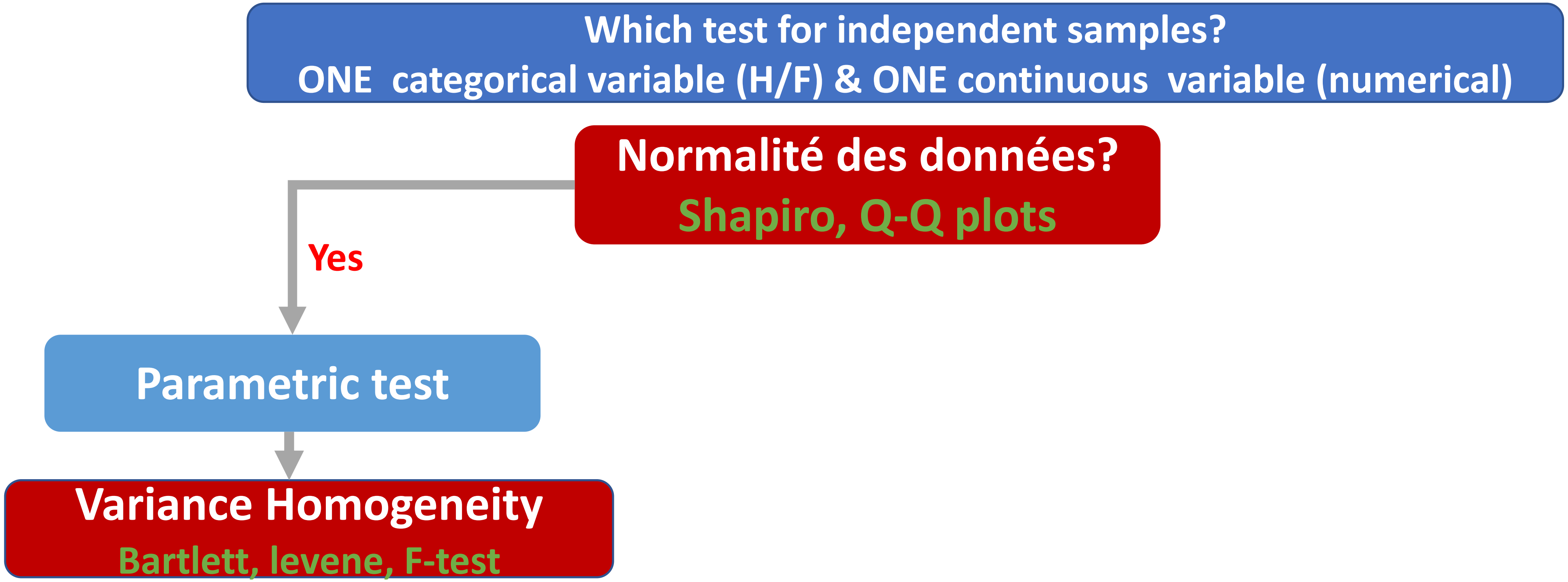






???????????

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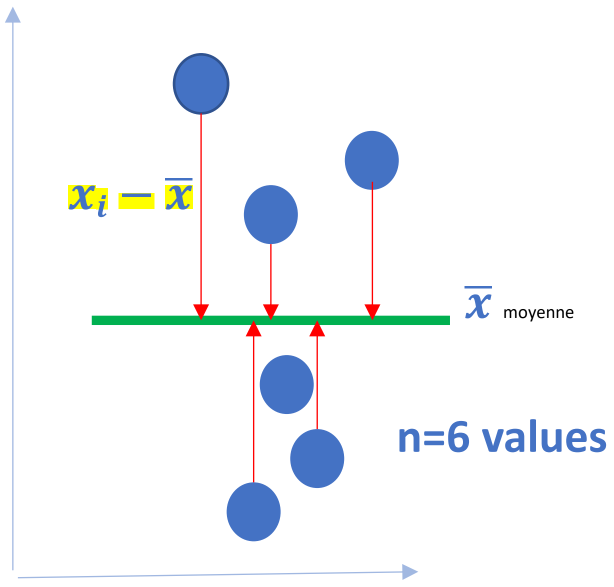
Normalité des données?
Shapiro, Q-Q plots
Yes
Parametric test
Variance Homogeneity
Bartlett, levene, F-test

Variance= S²/σ^2

- Variance measures the degree of dispersion of a data set around the mean
- Arithmetic mean of squared deviations from the mean! ☹

→ square unit

$$S^2 = \sum_{i=1}^{n} \frac{(x_i - \overline{x})^2}{n-1}$$



Standard Deviation=S/ σ

$$S = \sqrt{S^2}$$

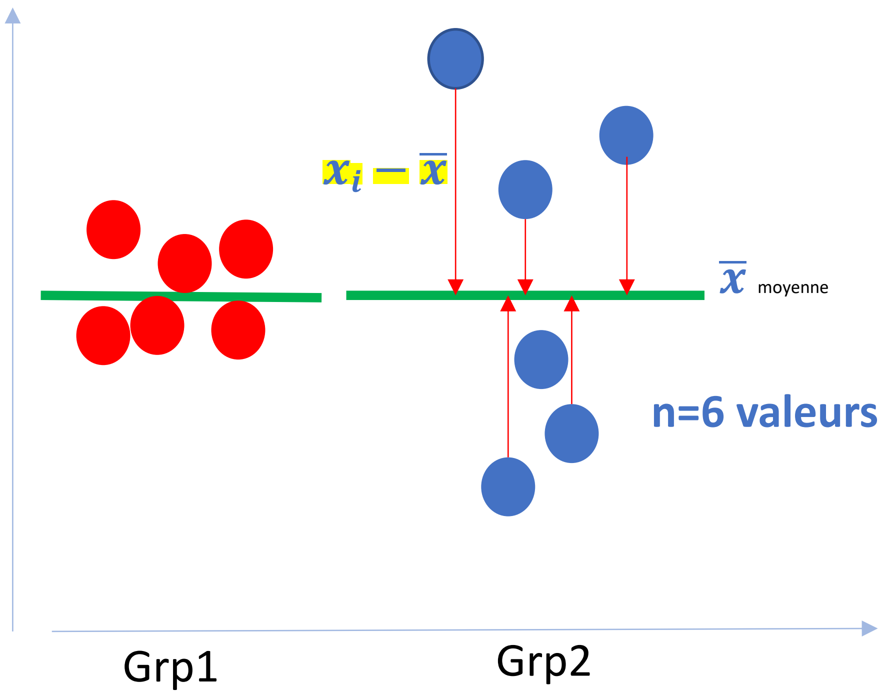
The advantage of the standard deviation : expressed in the same unit as the data series

$$S^2 = \sum_{i=1}^{n} \frac{(x_i - \overline{x})^2}{n-1} = \frac{Sum\ of\ Squares\ (SS)}{n-1}$$

SS will be greater in the sample….??

Results of test using variance :

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
groupe	3	13.03	4.343	0.211	0.887
Residuals	14	288.75	20.625		



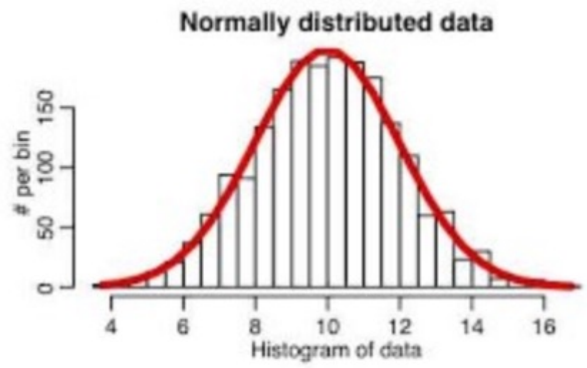
- *Sum of Squares* (= SS, Sum Sq) in your results!

→ Numerator of variance!!

- *Mean Square (= Mean Sq= VARIANCE formula!!!)*

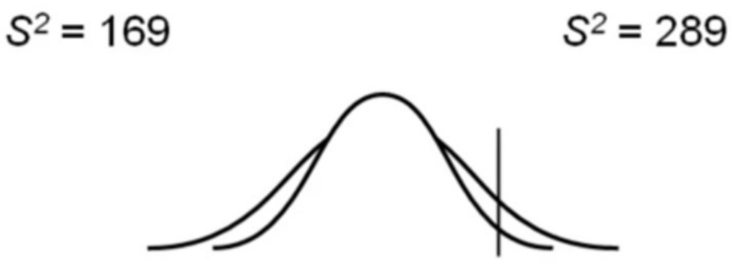
Requirement for parametric test… check-list!

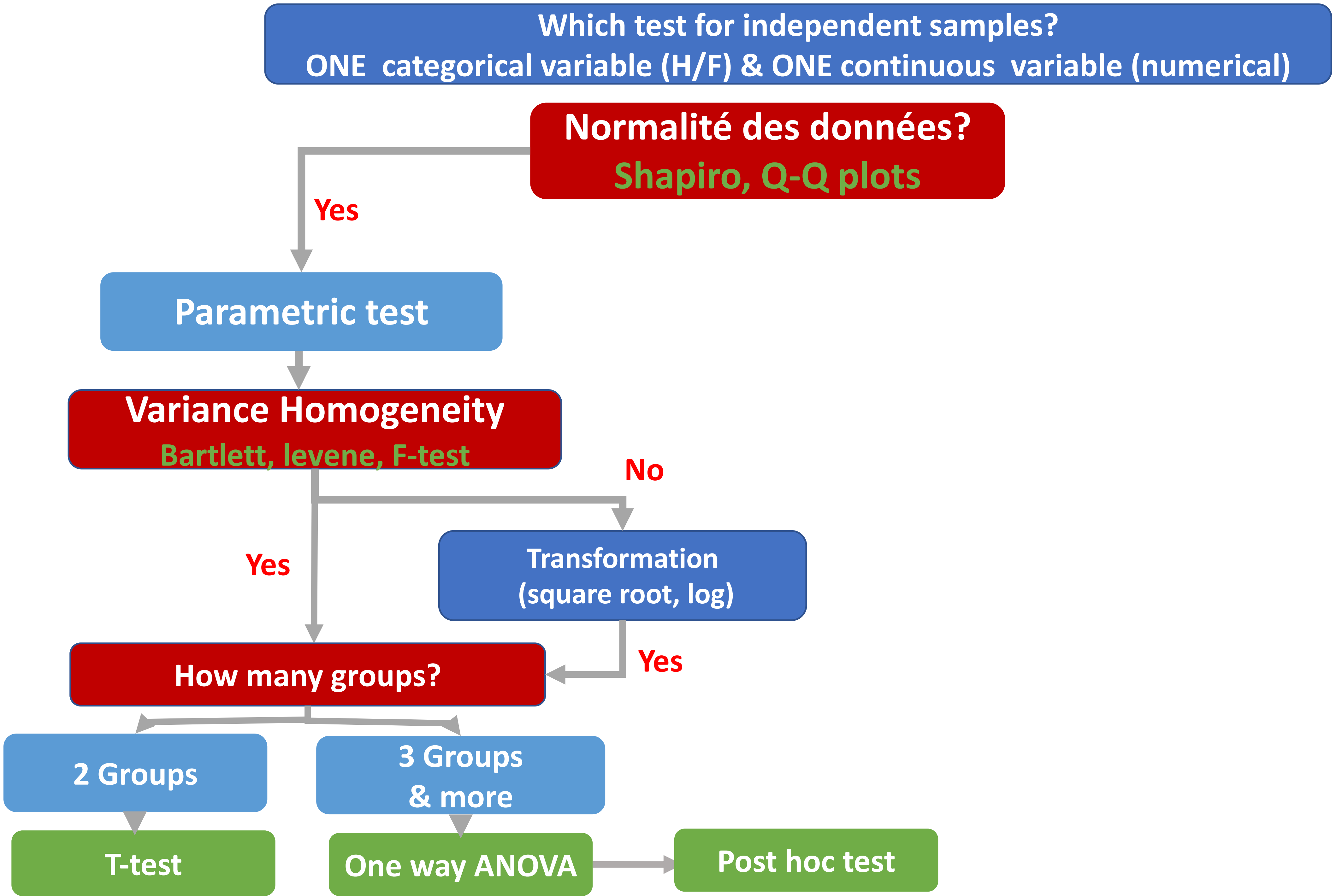
- Check normality of data: Shapiro Test & QQ-plots!!
- Shapiro: H0 is «data follow normal distribution»



- Check variance Homogeneity : F-test (2 groups), Bartlett’s & Levene’s tests

H0: « No difference »





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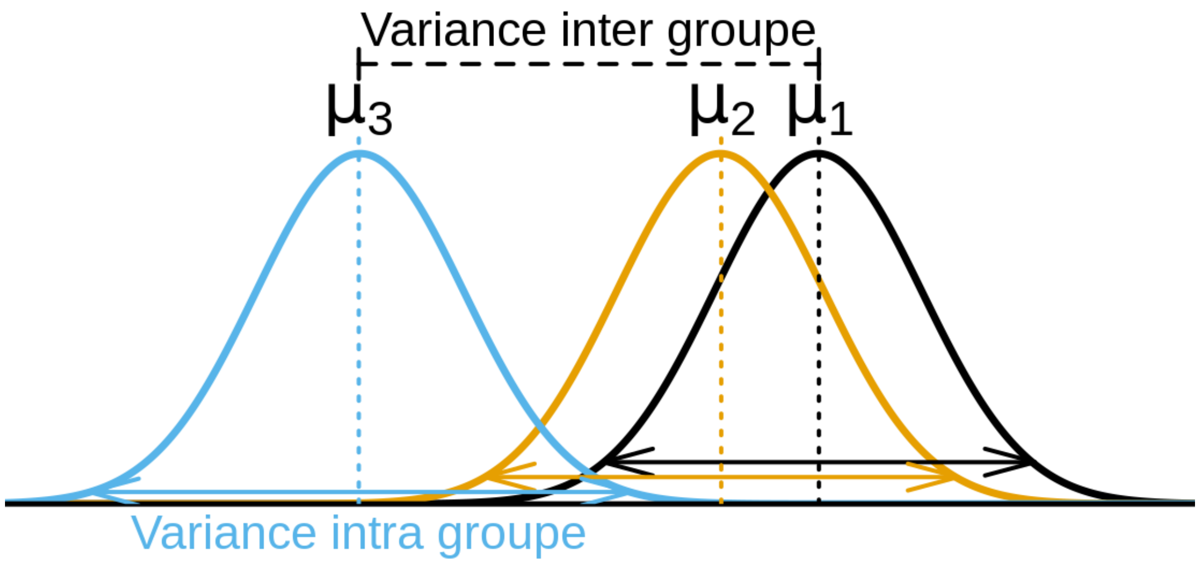
Parametric test
Variance Homogeneity
Bartlett, levene, F-test
No
Yes
Transformation
(square root, log)
Yes
How many groups?
2 Groups
3 Groups
& more
T-test
One way ANOVA
Post hoc test

ANOVA: ANalysis Of VAriance (One way Anova= Univariate) (3 groups at least)

- Compare the variance of the group means to that within groups (i.e. intra-group variance) for a single explanatory variable (qualitative)



ANOVA: ANalysis Of VAriance (One way Anova= Univariate)

- ***Postulate*** = The **VARIATIONS** observed between the **MEANS** of the different groups (AT LEAST 3) are so small that they are easily explained by chance!!!

- **Evaluation : Compare the variance of the group means to that within groups (i.e. intra-group variance)**

- **ANOVA → variations through the Variance quantity**

Variance inter-groupes + Variance intra-groupes

attribuable au facteur **attribuable à l'expérimentale (fluctuation de l'échantillonnage, hasard)**

Factor effect!

- **Statistic F** = $\dfrac{Inter-group\ Variance}{Intra-group\ Variance}$

Chance /fluctuation

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groupe	3	13.03	4.343	0.211	0.887
Residuals	14	288.75	20.625		

Idea :

if the factor really has an effect, the part of the variations that can be attributed to it = Inter-group variance will be significantly higher than the part of the variations that cannot be attributed to it = Intra-group variance!

Statistic F Follows a so-called **Fisher-Snedecor** law:

= **Distribution F used for test of variances**, distribution of variances not being normal

- Relation of an observed value of F with the a priori probability of encountering such a value (> or =) by chance!
- → probability given by the law = p-value!
- !

Denominator S^2 Numerator S^2 S^2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
groupe	3	13.03	4.343	0.211	0.887
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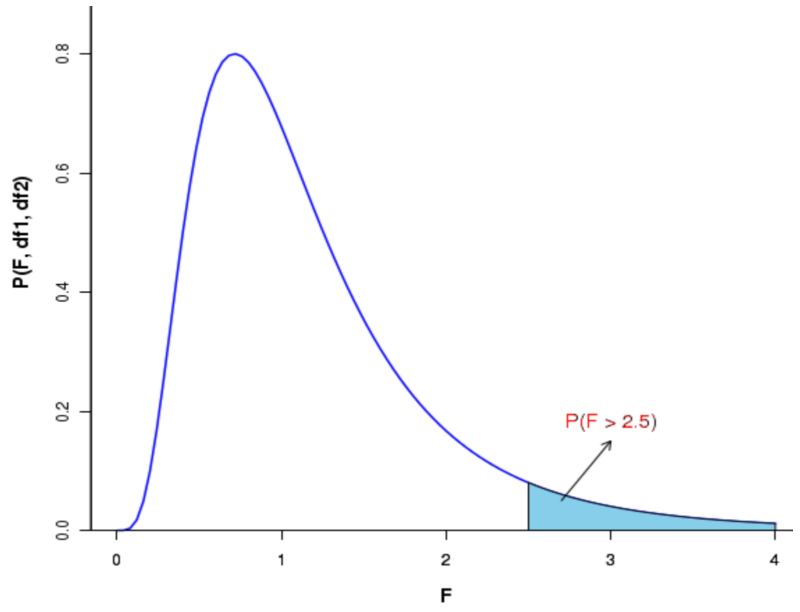

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Denominator S^2 — Numerator S^2 — S^2

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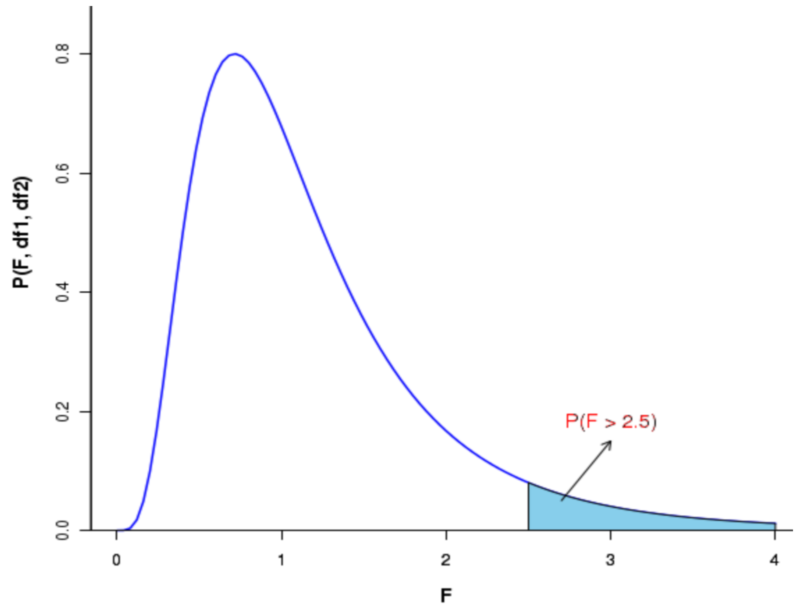


variances		ddl	F
entre k groupes	v_k	k-1	v_k / v_r
résiduelle	v_r	N - k	

Degré de liberté

- Two-ways ANOVA : Influences of TWO qualitative variables on ONE quantitative variable

Exple: Influence of soil type and degree of humidity (ordinal variable) on plant yield

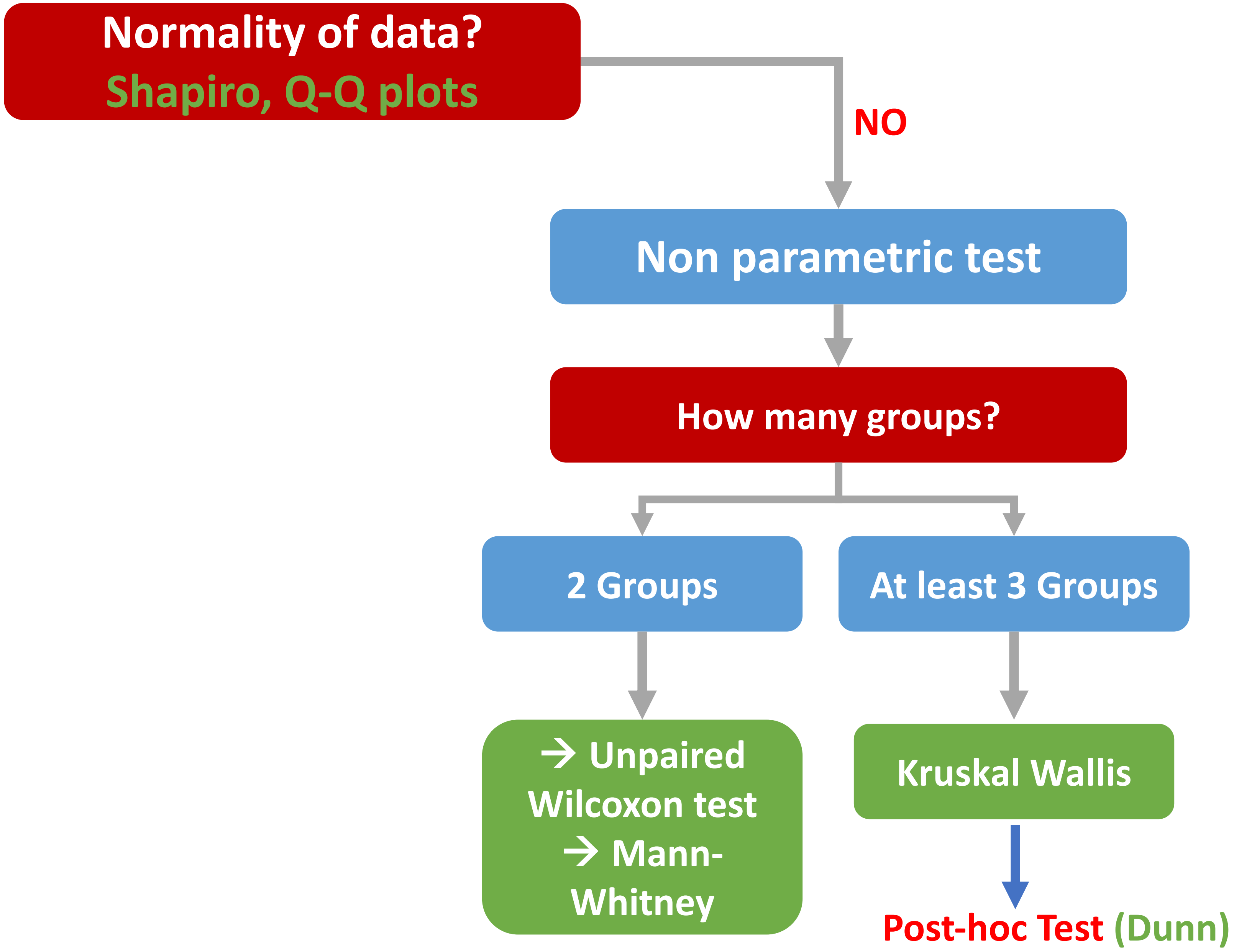
Non-parametric tests

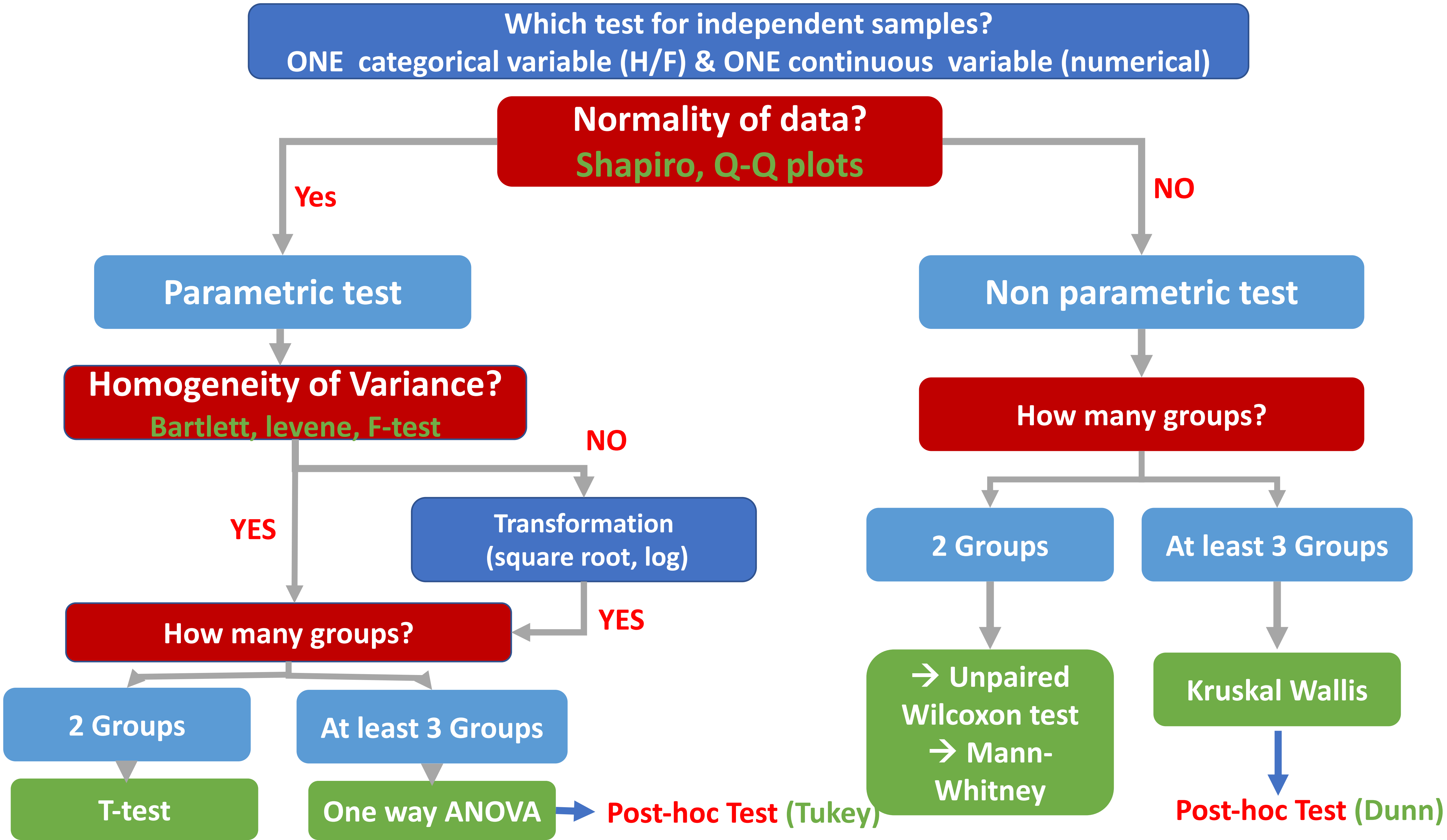
No assumptions are made for the distribution of data:
Distribution-free tests, they are alternative to parametric tests

- **Wilcoxon Rank test :** **samples are paired/unpaired, 2 sample groups**
- **Mann-Withney test: Independent samples, 2 sample groups**
- **Kruskal wallis test : Independant samples, Three or more groups**

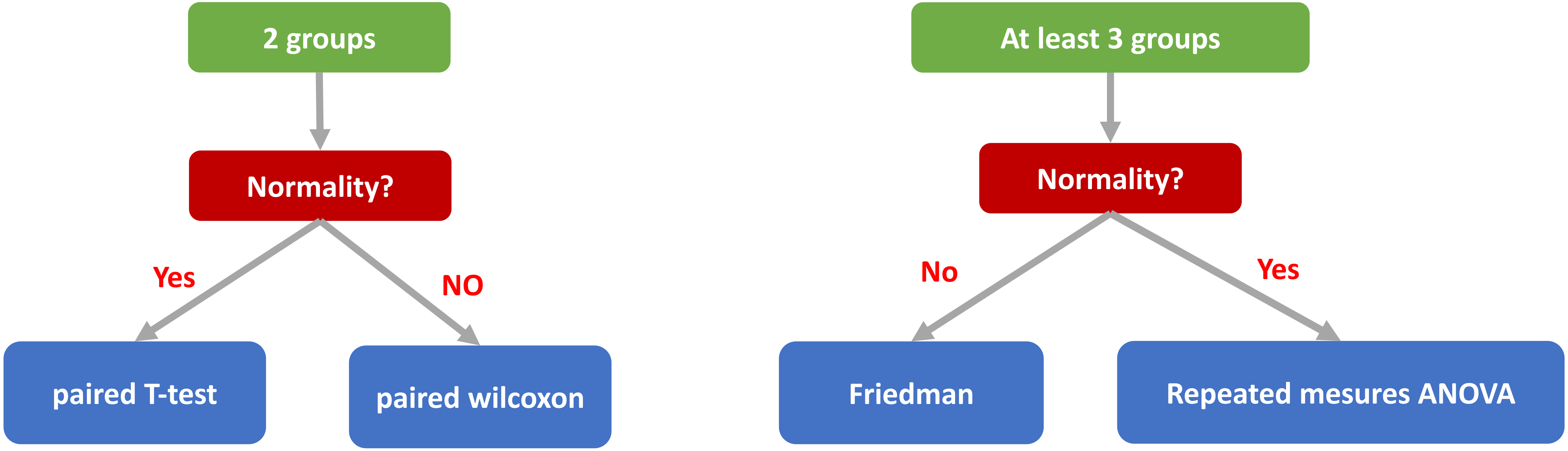
→ Based on the average ranks: we classify the values, we replace by a position (1,2 etc),
Compares the average of the ranks between the groups

Which test for independent samples?
ONE categorical variable (H/F) & ONE continuous variable (numerical)




Which test for independent samples?
ONE categorical variable (H/F) & ONE continuous variable (numerical)
Normality of data?
Shapiro, Q-Q plots
Yes
NO
Parametric test
Non parametric test
Homogeneity of Variance?
Bartlett, levene, F-test
NO
YES
Transformation
(square root, log)
YES
How many groups?
2 Groups
At least 3 Groups
T-test
One way ANOVA
Post-hoc Test (Tukey)
How many groups?
2 Groups
At least 3 Groups
→ Unpaired Wilcoxon test
→ Mann-Whitney
Kruskal Wallis
Post-hoc Test (Dunn)

Repeated measurements – paired samples Exple= time series, Before-After Treatment…



Post-hoc Test

Statistical tests with at least 3 groups!
After ANOVA, Kruskal-wallis

→ The result of an ANOVA test is an Overall p-value

Exple: You are comparing the effect of 3 soil types (A,B,C) on plant growth
ANOVA returns a p-value of 0.03
It does not tell you which pair of groups are significantly differents!!!!

→ Post-hoc Test! Multiple comparisons (eg: Gp A vs. Grp. B; GrpB vs. Grp C; Grp C vs. Grp A!)

- Parametric Post-hoc test (ANOVA) → **Tukey Test**
- Non-parametric Post-hoc test (Krukal wallis) → **Dunn Test**

Connexion à l’évènement wooclap : XSUAMN

1 Allez sur wooclap.com

2 Entrez le code d'événement dans le bandeau supérieur

Code d'événement

XSUAMN

1 Envoyez @XSUAMN au 06 44 60 96 62

2 Vous pouvez participer

Désactiver les réponses par SMS

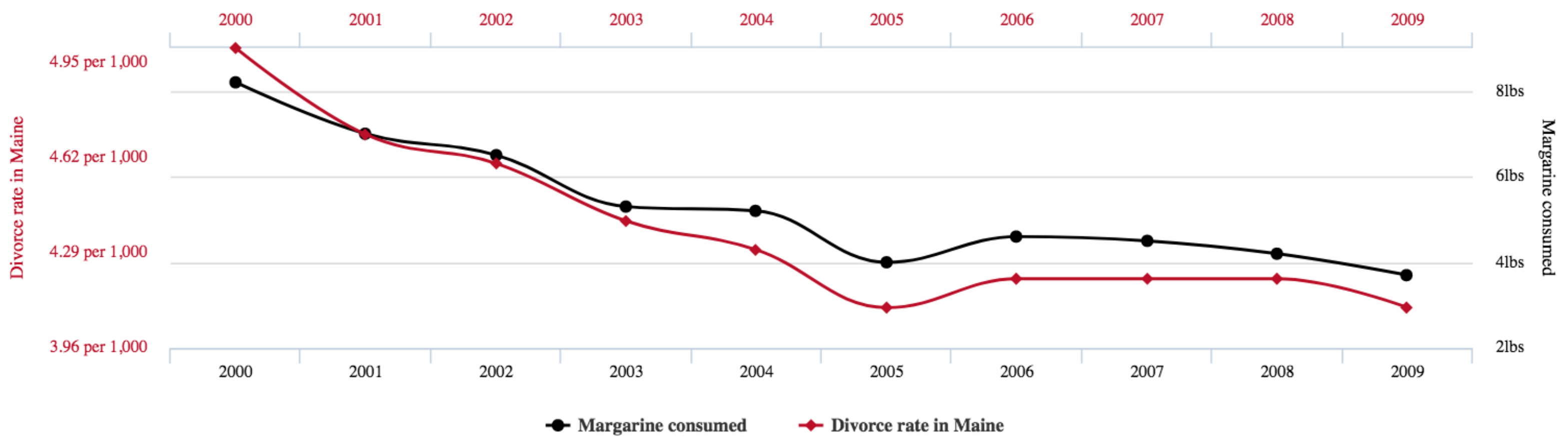
Linear Regression & Correlation (Bivariate analysis)

Objective : Analyze the **link** that may exist between **two variables** (here: quantitatives)
(Two qualitative variables -> Khi2 test)

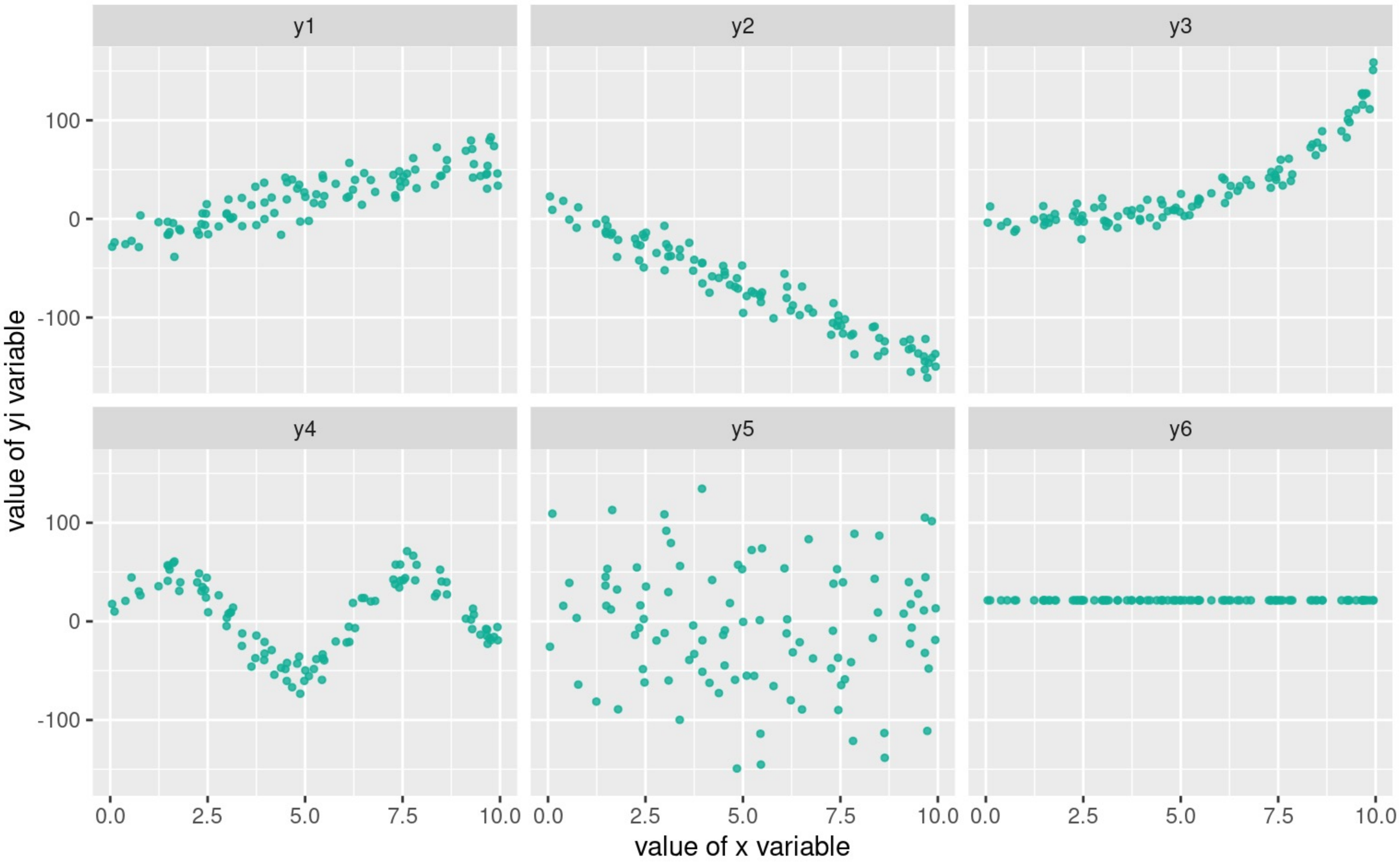
Link/relationship/dependence between the variables

→ The values of two variables **do not evolve independently** but on the contrary, present a certain form, a certain regularity

→ Intensity of the association does not indicate causality …



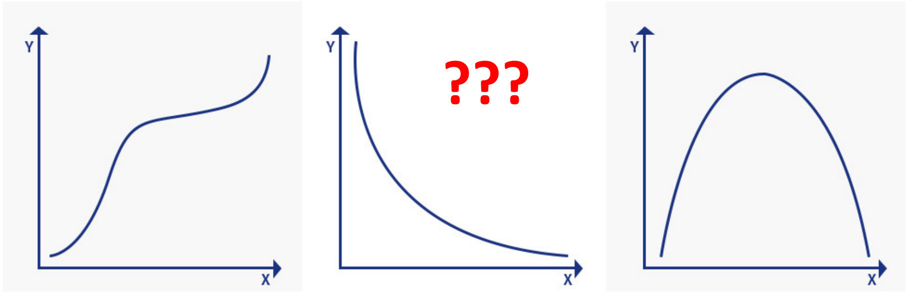
What are the relationship between the variables in each graph?

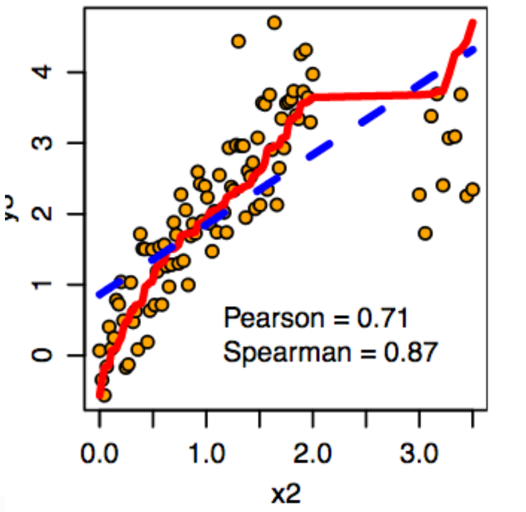


Association: Correlation Coefficient r
Intensity & Direction of the association between two variables

- **Strict Linear Relationship : Pearson** (**r**, parametric)
- **Monotonous relationship : Spearman** (**Rho**, non-parametric, rank-based)

Kendall (**Tau**, non-parametric), Alternative to Spearman (small sampling)

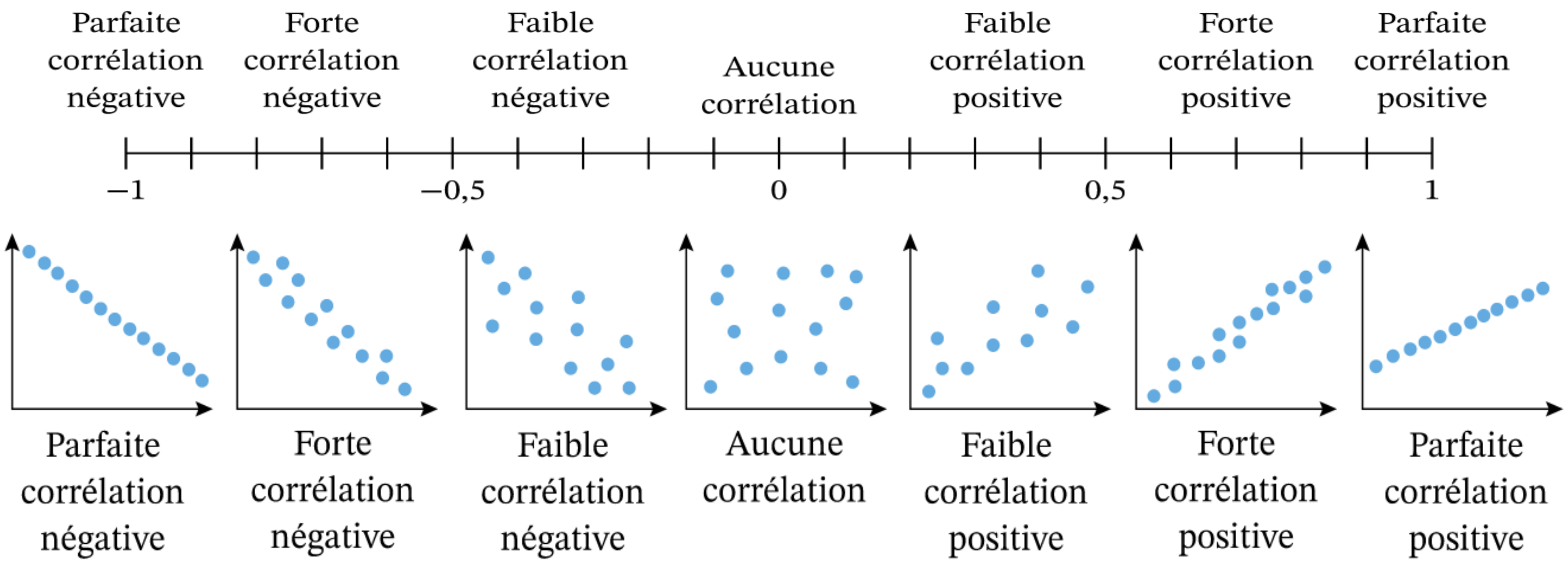




Coefficient r range between -1 et 1

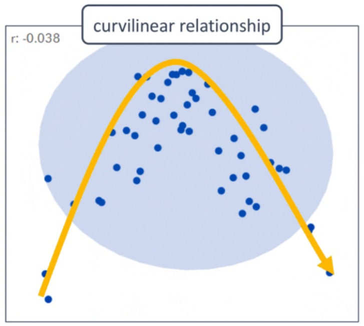
- **Positive correlation** : The values of both variables tend to increase together
- **Negative correlation** : The values of one variable tend to increase and the values of the other variable decrease
- **Zero** : no **LINEAR** association **(Pearson)**

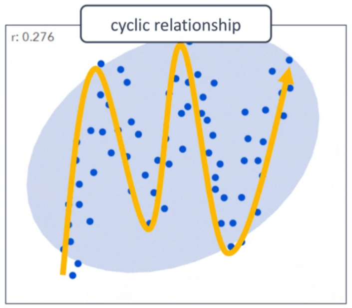
For information!!!



Because inspecting your results is never useless...

- r close to Zero: no association??





Simple Linear Regression

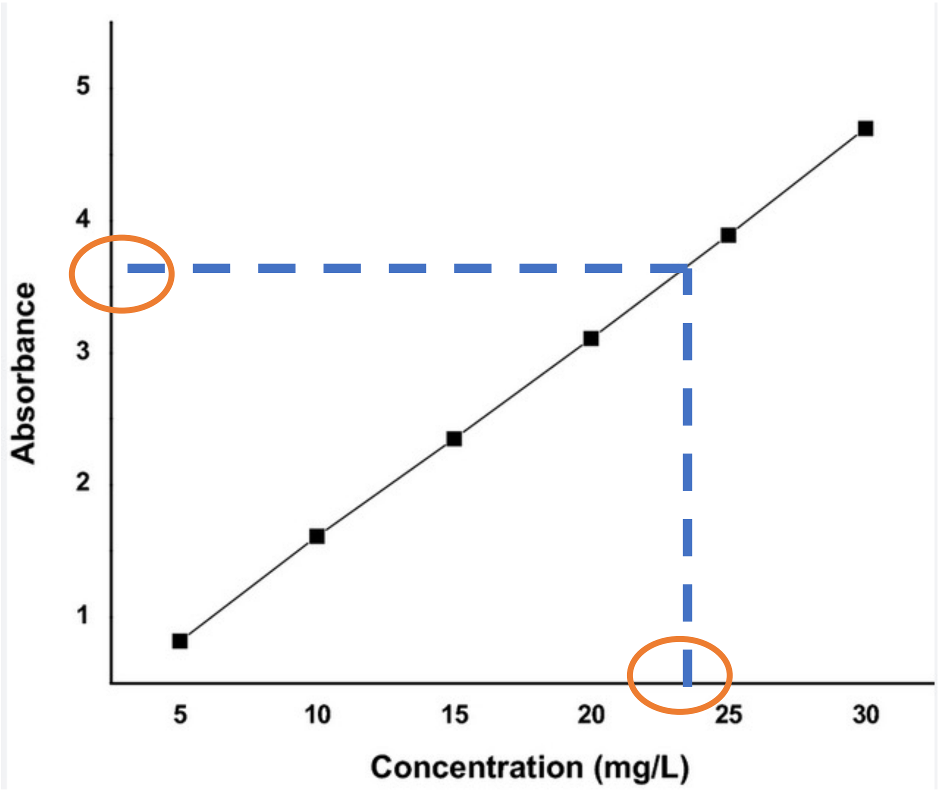
- Only for quantitative variables

- Plot the scatter plot Is there a relationship?
- Is it linear?
- What orientation (positive, negative)?
- If the association is **linear** → Make a **regression**

Requierement

- Normal distribution
- Variance homogeneity

Your favorite linear regression… calibration curve!!!

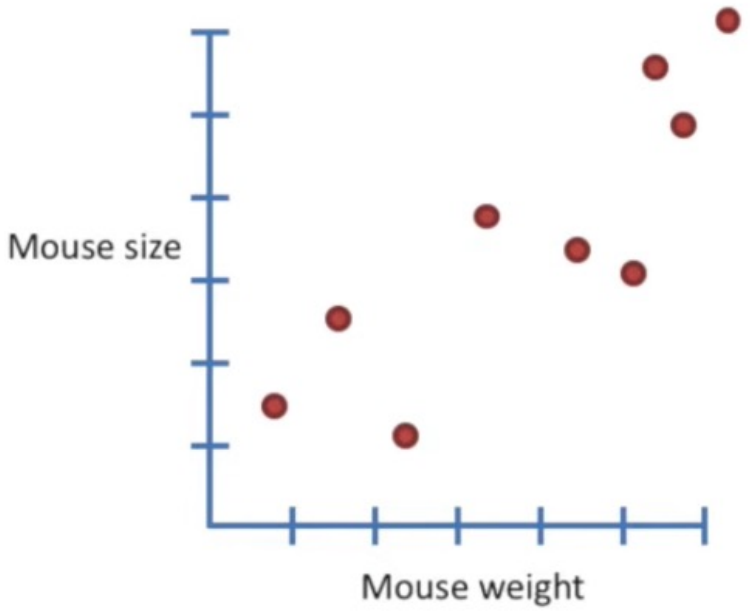


Explain and predict!

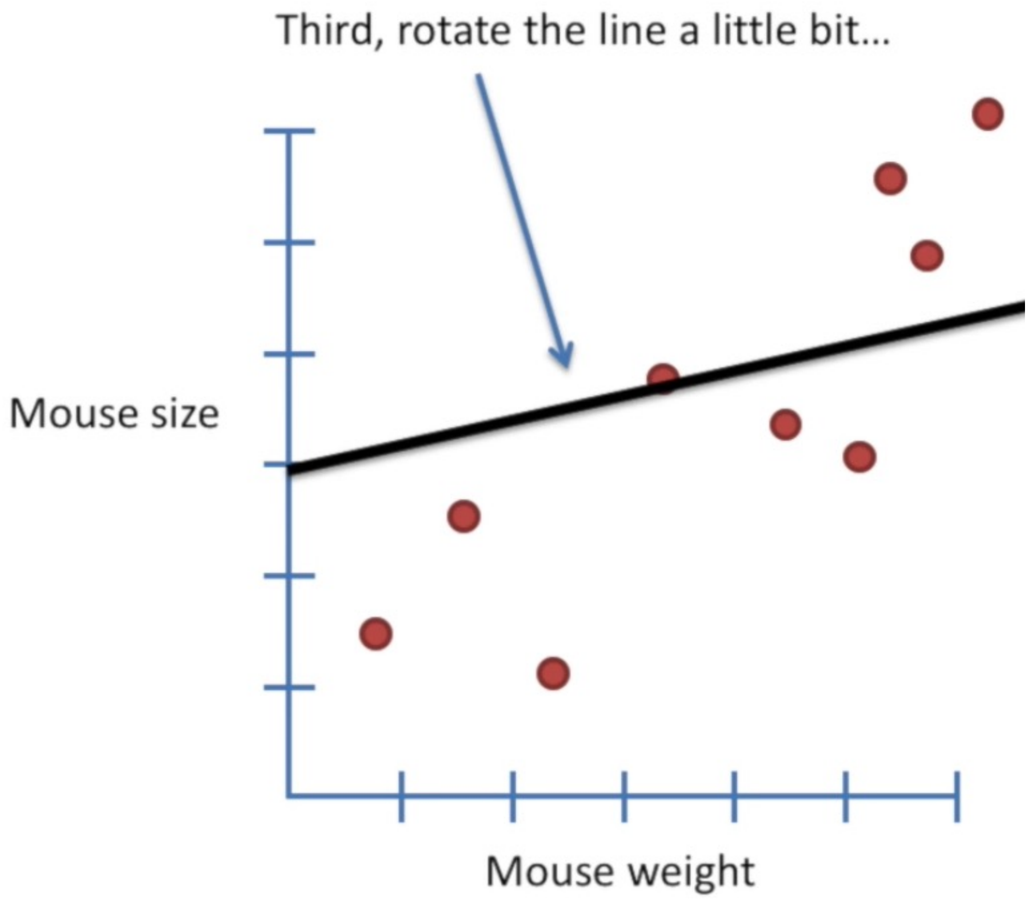
Models a linear type relationship (Y=aX+b)

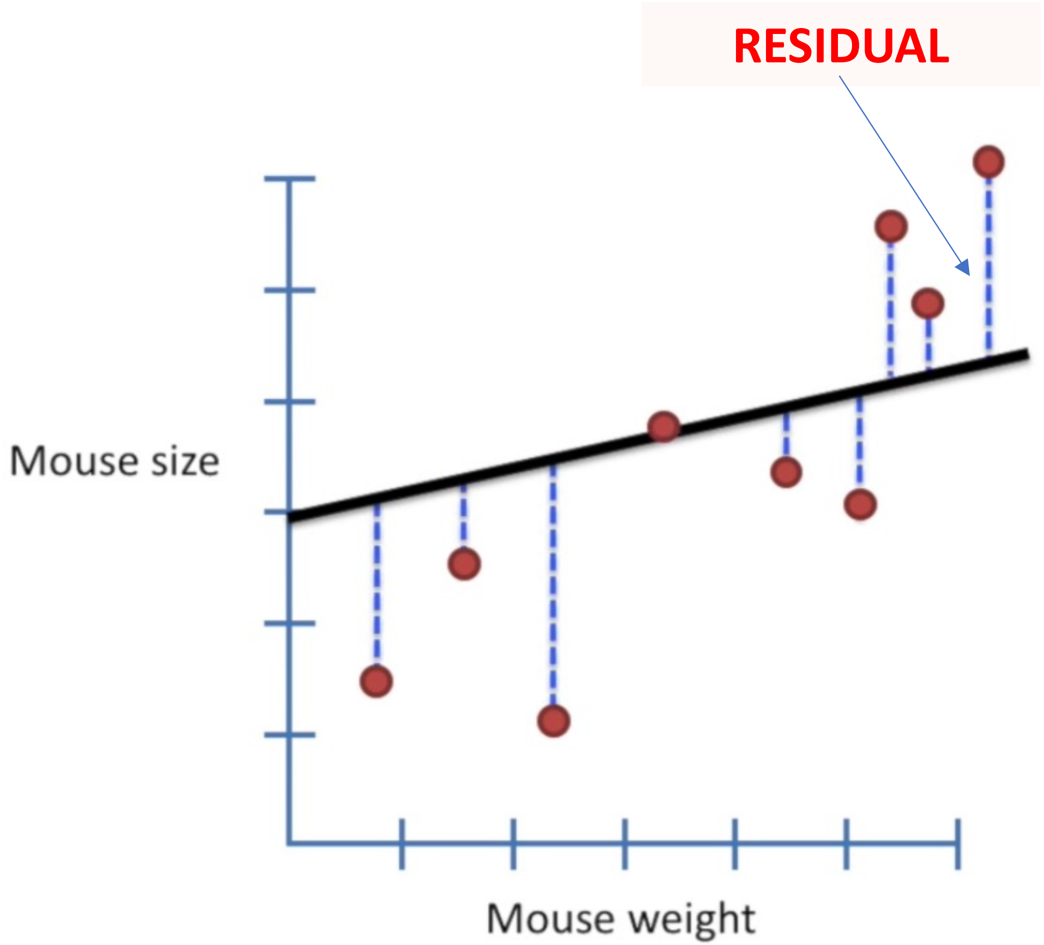
Model seeking to establish a linear relationship between a variable, called explained/dependent (Y), and another called explanatory/independent (X)

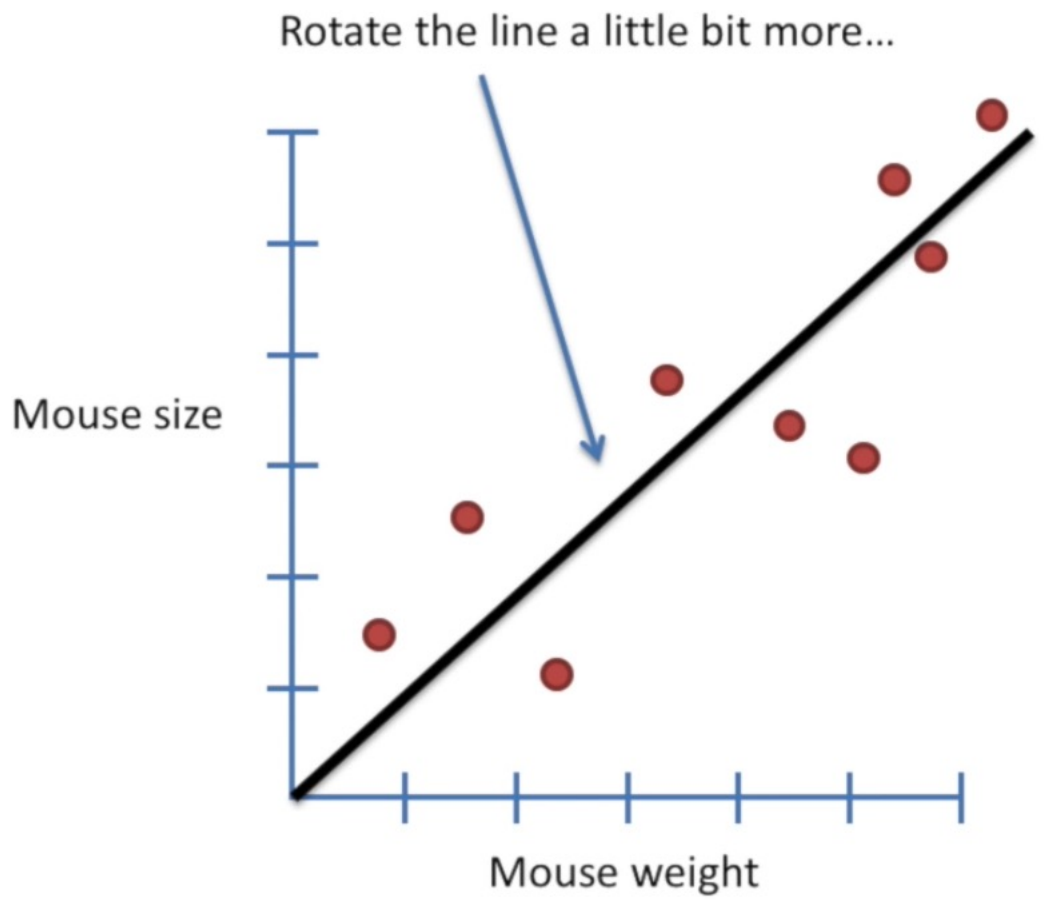
Can mouse Weight predict Size correctly? (R^2)
Relationship is due to chance? (p-value)

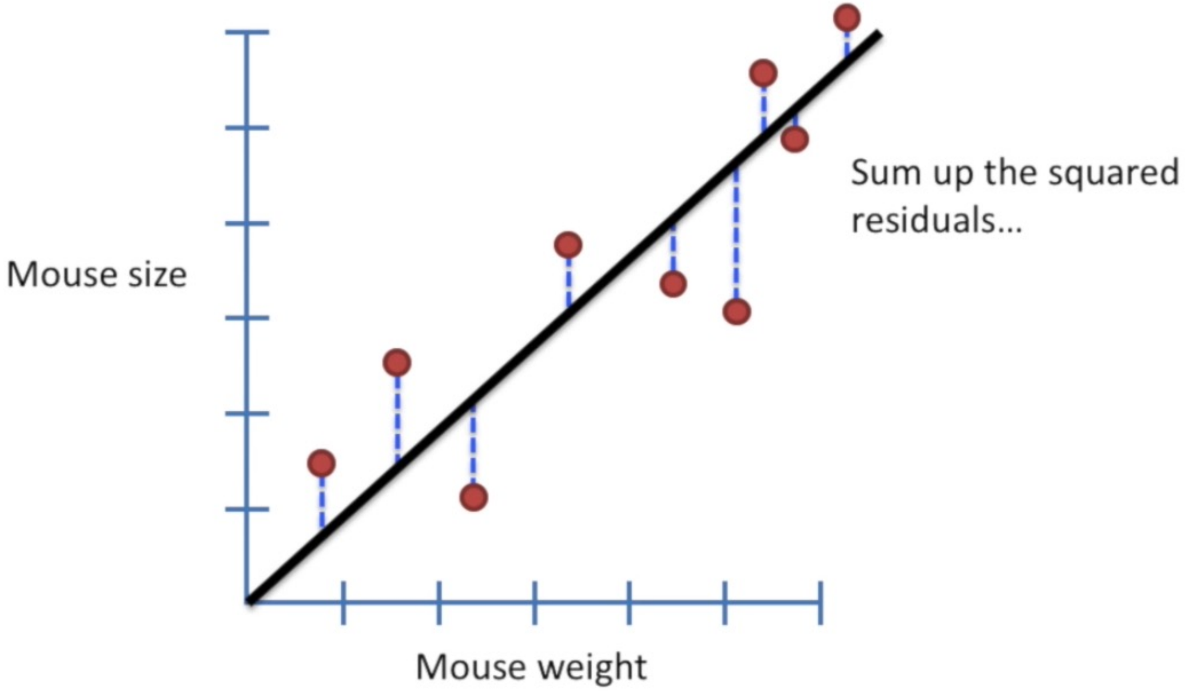


Least square method



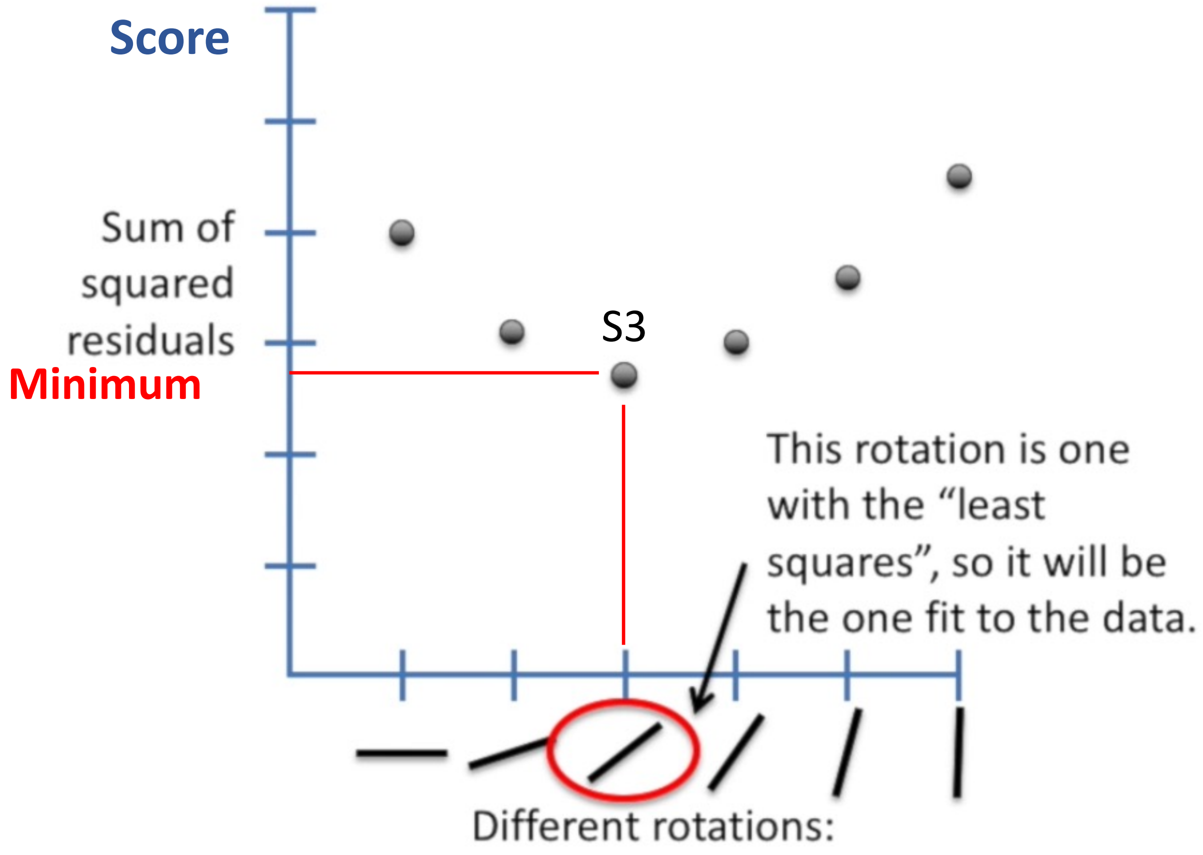




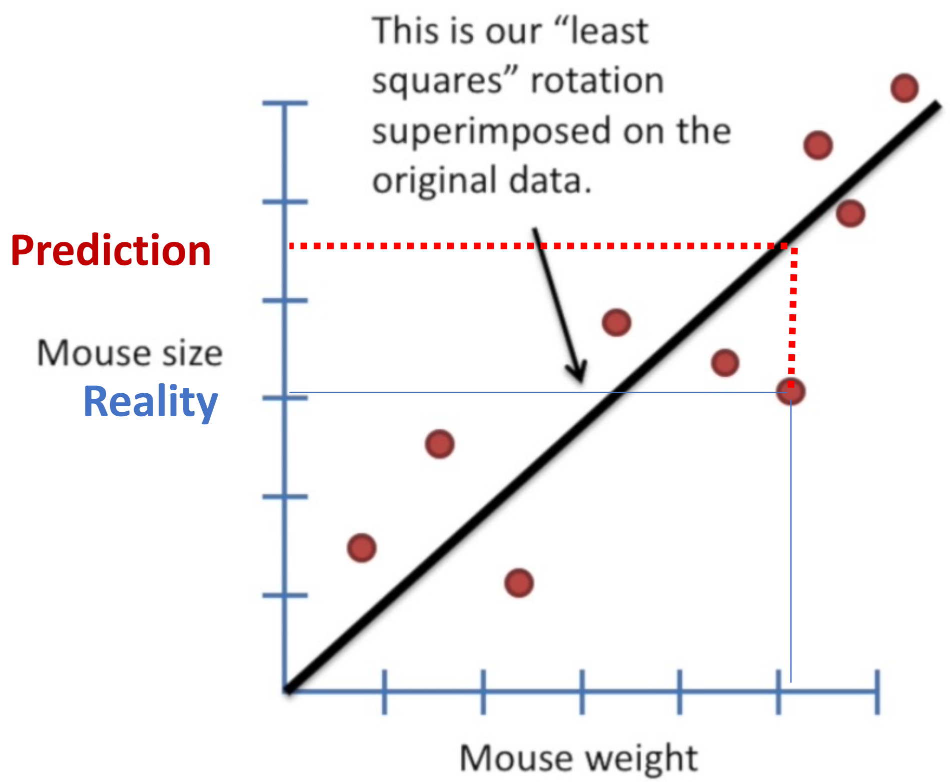


Again & again, recalculate

Resume : Sums of squared residuals for each rotation



Best rotation (=line position), the one which minimize the score of Sums of squared residuals !!!!



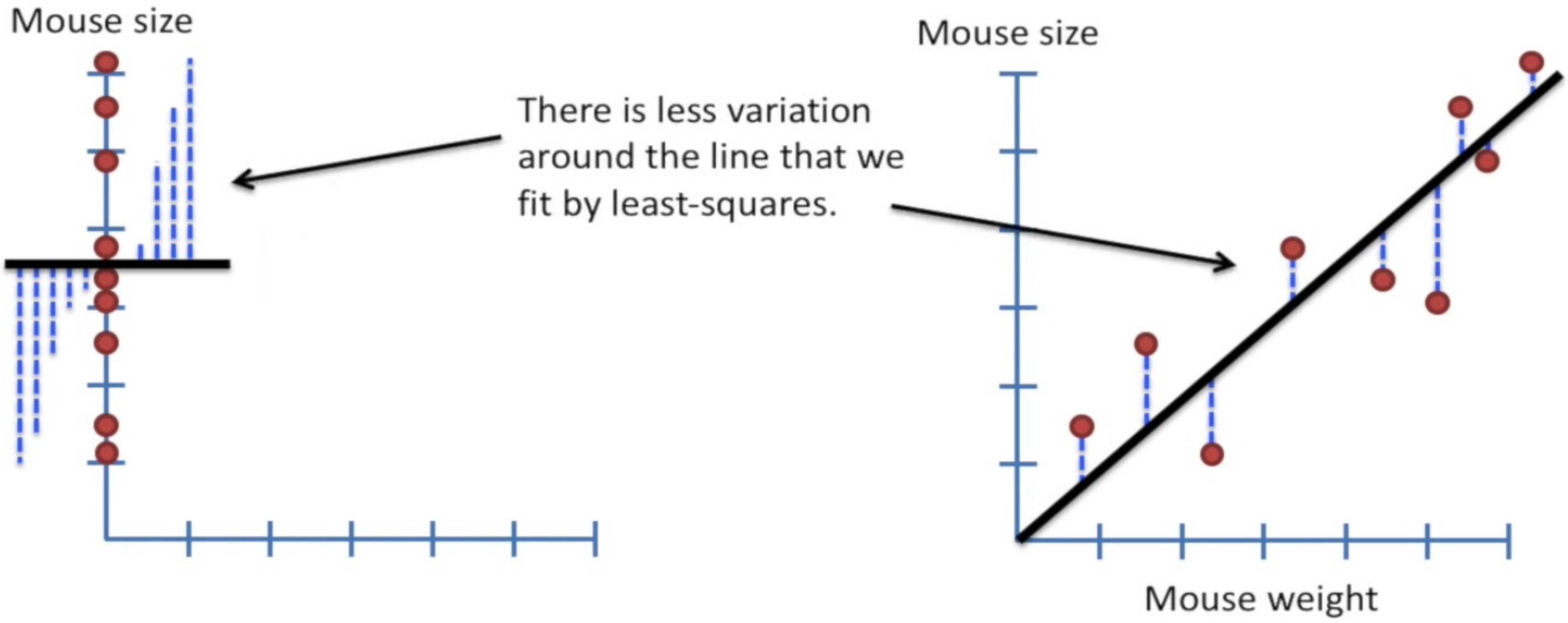
$$y = 0.1 + 0.78x$$

Dependence to « Mouse weight »

Coefficient R^2 = prediction quality

how good is the model to predict Mouse size taking into account Mouse weight!!

R^2 : Determination Coefficient



$$\text{Var(mean)} = \frac{\text{SS(mean)}}{n}$$

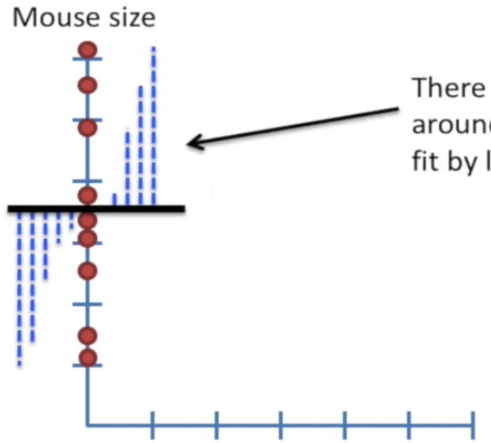
$$\text{Var(fit)} = \frac{(\text{data - line})^2}{n}$$

- **Taking into account « weight », less variations?? (SSfit < SSMean)!**

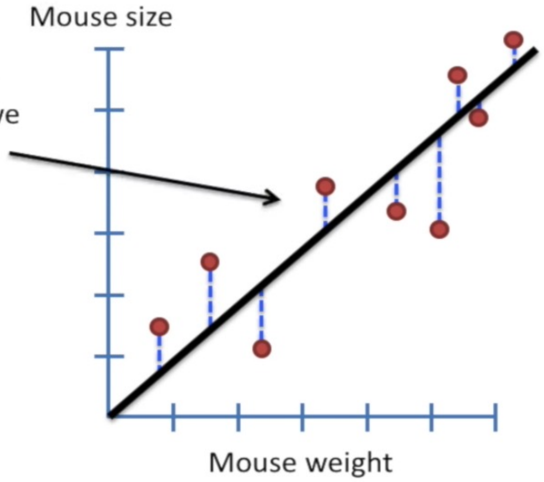
R^2 = % variation of the response variable explained by a linear model (weight variable)

$$R^2 = \frac{Var(mean) - Var(fit)}{Var(mean)}$$

Var(mean) = 11.1



There is less variation around the line that we fit by least-squares.



Var(fit) = 4.4

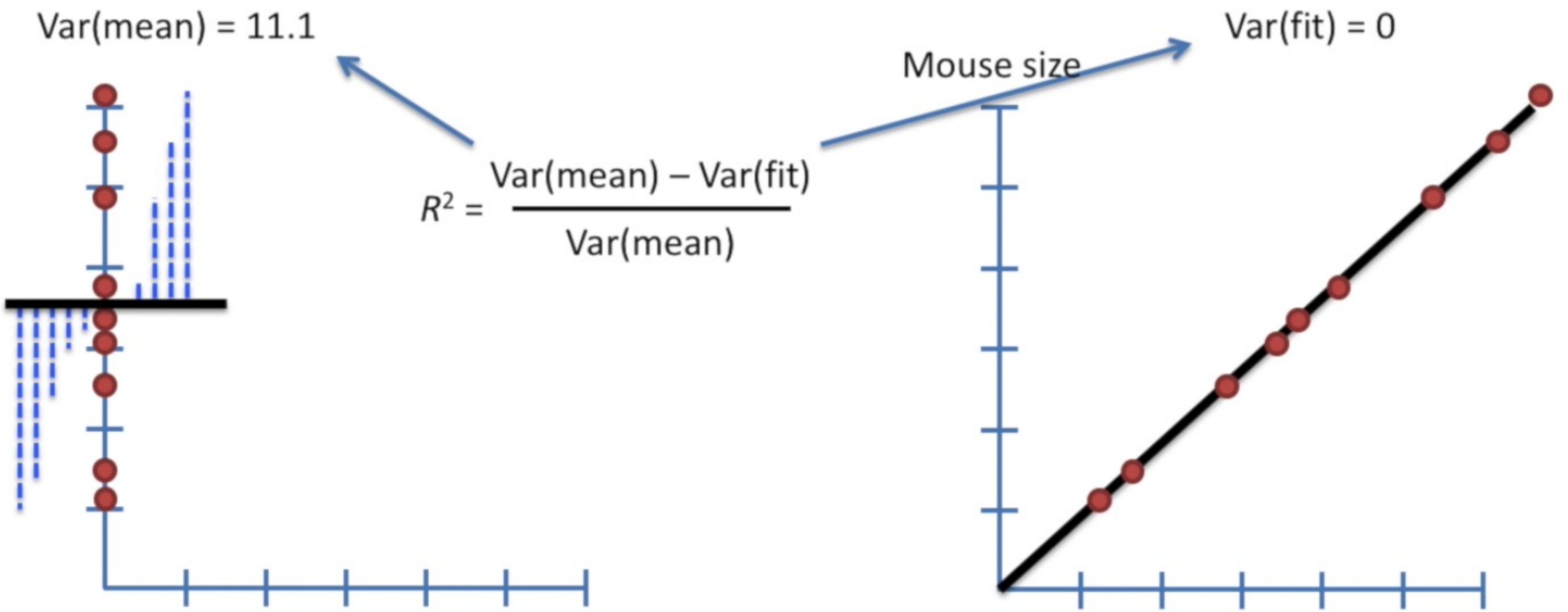
$$R^2 = \frac{11.1 - 4.4}{11.1} = 0.6 = 60\%$$

$$R^2 = \frac{Variation\ expliquée}{Variation\ totale}$$

→ The established model explains 60% of the variability/variance of the "Mouse size"

→ R^2 between 0 and 1

TO be sure …



$$R^2 = \frac{11.1 - 0}{11.1}$$

$R^2 = 1 = 100\%$

R^2 & significance?

- **Need a p-value…**
- **Variance … so p-value is given by the ratio F & distribution F**

$$F = \frac{\text{The variation in mouse size explained by weight}}{\text{The variation in mouse size not explained by weight}}$$

Relation between r & R^2

Correlation coefficent of Pearson r can be linked to linear regression R^2
Its square is the explained variance by the regression (R²)

r =0.5 -> R^2 = 0.25 -> 25% of the Y variance explained by X variable… ☹