

# Applied Statistics Hypothesis Testing and Simple Tests

G. Bacaro

Design and Analysis of Environmental Monitoring and Experiments

Master Degree in Global Change Ecology

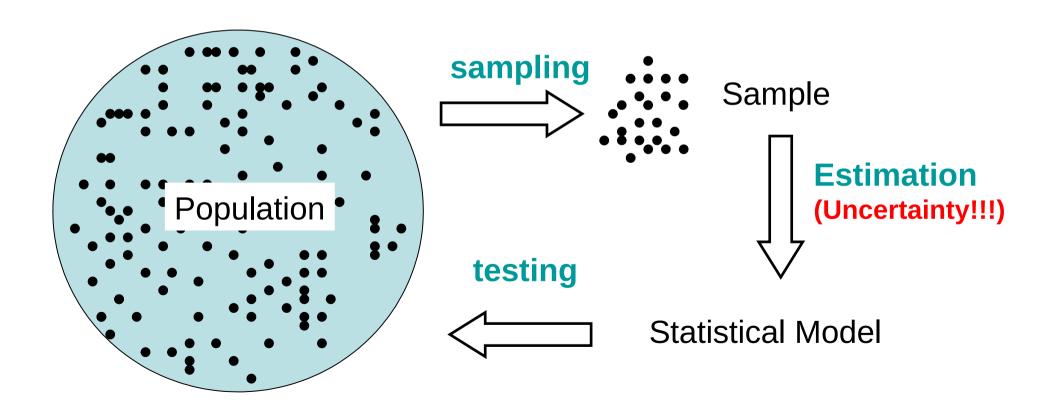
I Year, I term

#### Inference



#### Hypothesis testing

A **statistical hypothesis test** is a method of making statistical decisions from and about experimental data. Null-hypothesis testing just answers the question of "how well do the findings fit the possibility that chance factors alone might be responsible?".



# Inferential statistics: logics

#### Hypothesis testing

## Statistical testing in five steps:

- 1. Construct a null **hypothesis** (H0) (RESEARCH QUESTION)
- E.g. H0: Does spruce productivity depend on soil fertility?
- 2. Choose a statistical analysis
- E.g. Regression between N and P and biomass
- 3. Collect the data (sampling)
- E.g. Sampling of 145 sites with different level of fertility



- 4. Calculate **P-value** and test statistic Test of our regression model (F-test)
- 5. **Reject/accept (H0)** if p is small/large (ANSWER THE QUESTION)

#### **Common error**

Sampling before (1) constructing the hypothesis and (2) choosing the statistical analysis

# Key concepts

#### **Hypothesis testing**

#### **Statistical testing in five steps:**

- 1. Construct a null **hypothesis** (H0)
- 2. Choose a statistical analysis (assumptions!!!)
- 3. Collect the data (sampling)
- 4. Calculate **P-value** and test statistic
- 5. **Reject/accept (H0)** if P is small/large

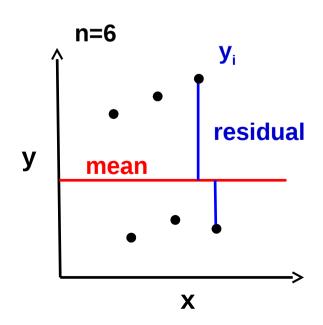


#### Concept of replication vs. pseudoreplication

- 1. Spatial dependence (e.g. spatial autocorrelation)
- 2. Temporal dependence (e.g. repeated measures)
- 3. Biological dependence (e.g. siblings)

#### **Key quantities**

mean = 
$$\frac{\sum y_i}{n}$$
 deviance =  $SS = \sum (y_i - mean)^2$   
var =  $\frac{\sum (y_i - mean)^2}{(n-1)}$ 



# Hypothesis testing

#### **Hypothesis testing**

- 1 Hypothesis formulation (Null hypothesis H0 vs. alternative hypothesis H1)
- 2 Compute the probability P that H0 is false;
- 3 If this probability is lower than a defined threshold we can reject the null hypothesis

# **Statistical Analyses**

#### **Hypothesis testing**

#### Mean comparisons for 2 populations

Test the difference between the means drawn by two samples

#### Correlation

In <u>probability theory</u> and <u>statistics</u>, correlation, (often measured as a correlation coefficient), indicates the strength and direction of a linear relationship between two <u>random variables</u>. In general statistical usage, correlation refers to the departure of two variables from independence.

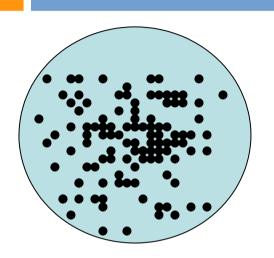
#### Introduction to Statistical Modelling

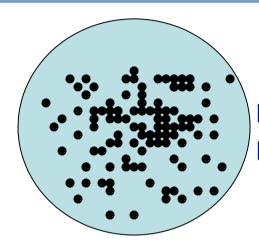
Basic concept



# **Mean Comparison for 2 Samples**

#### **Hypothesis testing**





H0: means do not differ

H1: means differ

## **Assumptions**

- <u>Independence</u> of cases this is a requirement of the design.
- Normality the distributions in each of the groups are <u>normal</u>
- <u>Homogeneity of variances</u> the variance of data in groups should be the same (use Fisher test or <u>Fligner's test</u> for homogeneity of variances).
- These together form the common assumption that the <u>errors</u> are independently, identically, and normally distributed



# **Normality**

#### **Hypothesis testing**

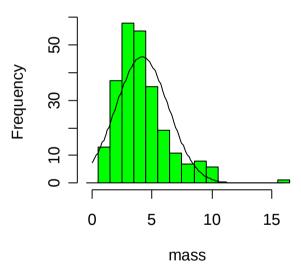
Before we can carry out a test assuming normality of the data we need to test our distribution (not always before!!!)

In many cases we must check this assumption after having fitted the model (e.g. regression or multifactorial

**RESIDUALS MUST BE NORMAL** 

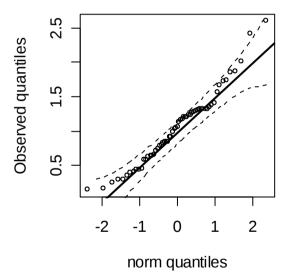
ANOVA)

## **Graphics analysis**



hist(y) lines(density(y))

#### Normal applot



library(car) qq.plot(y) or qqnorm(y)

#### **Test for normality**

Shapiro-Wilk Normality Test

shapiro.test()

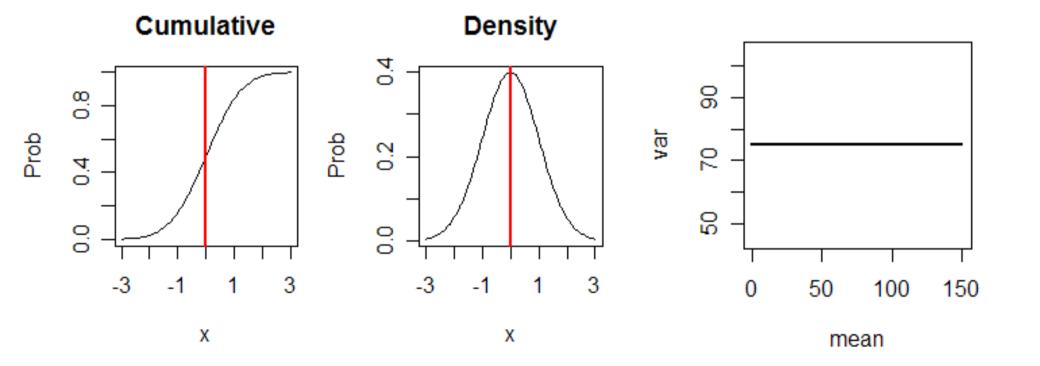




# Basic concepts: Normal distribution



The <u>normal distribution</u> is ubiquitous in nature and statistics due to the <u>central limit theorem</u>: every variable that can be modelled as a sum of many small independent variables is approximately normal.



## Basic concepts: Poisson distribution



10

The <u>Poisson distribution</u>, which describes a very large number of individually <u>unlikely</u> events that happen (count data)

Non-negative values

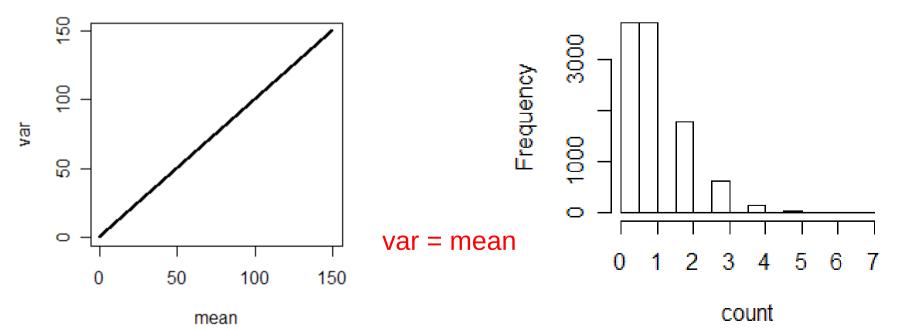
Variance=mean

Right skewed

1 Parameter: λ (mean=variance)

Use: count data

Sample from a Poisson distribution (n=1000, mean=variance=0.2)

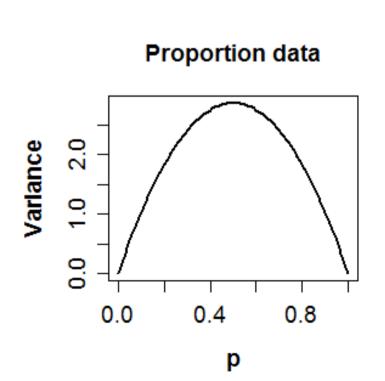


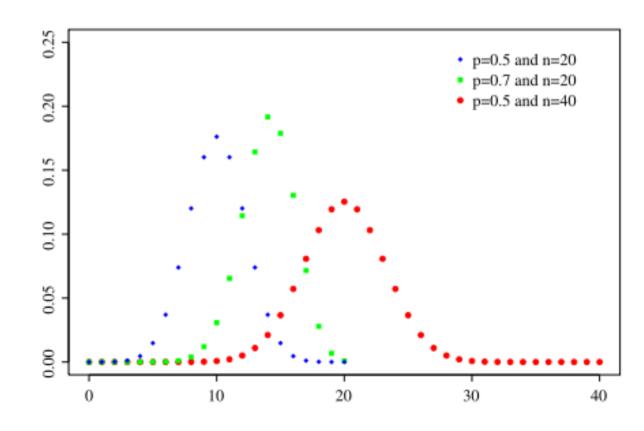
## Basic concepts: Binomial distribution



The <u>binomial distribution</u> describes the **number of** successes in a finite series of independent Yes/No experiments.

2 Parameters: sample size, probability Use: proportion data and power analysis

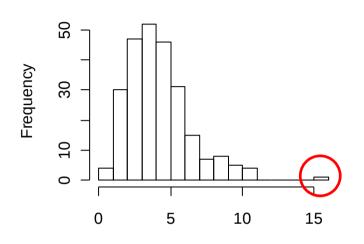


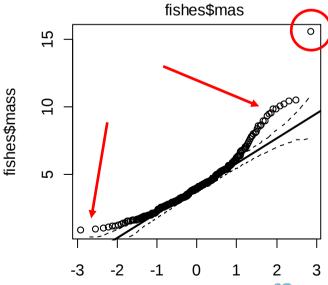


# Normality: Histogram and QQ plot

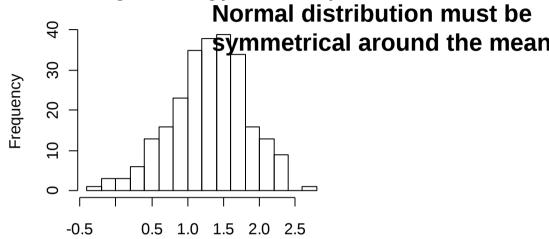
#### **Hypothesis testing**

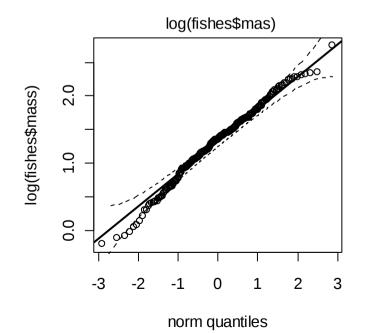
#### **Histogram of fishes\$mas**





#### **Histogram of log(fishes\$mas)**





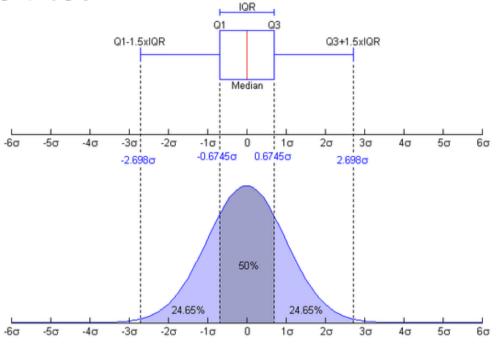


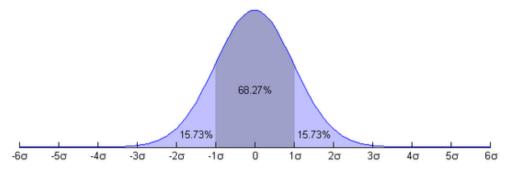
# Normaliy: Quantile-Quantile Plot

#### Hypothesis testing

Normality: Quantile-Quantile Plot

Quantiles are points taken at regular intervals from the cumulative distribution function (CDF) of a random variable. The quantiles are the data values marking the boundaries between consecutive subsets







# Not Normal...what to do?

#### **Hypothesis testing**

In case of non-normality: 2 possible approaches

#### 1. Change the distribution (use GLMs)

**Advanced statistics** 

- E.g. Poisson (count data)
- E.g. Binomial (proportion)

#### 2. Data transformation

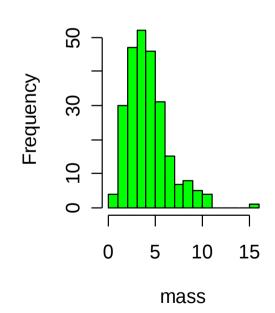
Log

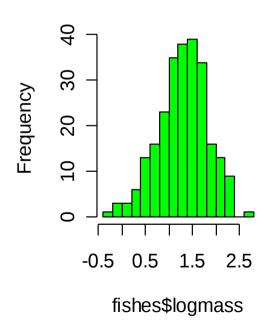
Square-root

Arcsin (percentage)

Probit (proportion)

**Box-Cox transformation** 







# **Box - Cox Normalization**

#### **Hypothesis testing**

## Lambda Transformed

$$Y_{tr} = Y^{\lambda}$$

## E.g.

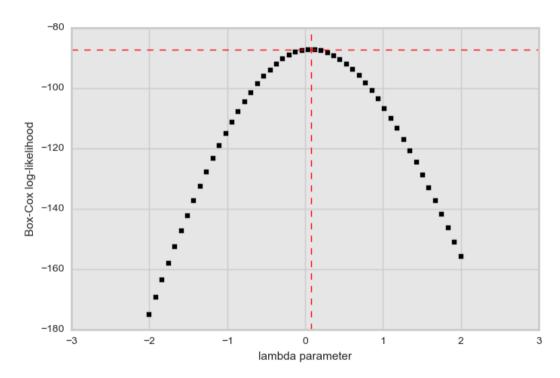
$$\lambda = 2$$
  $Y_{tr} = Y^2$ 

$$\lambda = 0.5$$
  $Y_{tr} = Y^{1/2} = Y$ 

$$\lambda = 0$$
  $Y_{tr} = log_e(Y)$ 

$$\lambda = -0.5$$
  $Y_{tr} = 1/Y^{1/2}$ 

$$\lambda = -1$$
  $Y_{tr} = 1/Y$ 



$$L = -\frac{\nu}{2} \ln s_{TRAS}^2 + (\lambda - 1) \frac{\nu}{n} \sum \ln X$$

$$X_{TRAS} = \frac{X^{\lambda} - 1}{\lambda}$$

$$\lambda \neq 0$$

$$X_{TRAS} = \log(X)$$

$$\lambda = 0$$

# Homogeneity of Variance for two samples

#### **Hypothesis testing**

Before we can carry out a test to compare two sample means, we need to test whether the sample variances are significantly different. The test could not be simpler. It is called Fisher's *F* 

To compare two variances, all you do is divide the larger variance by the smaller variance.

E.g. Students from A group vs. Students from B group

F calculated

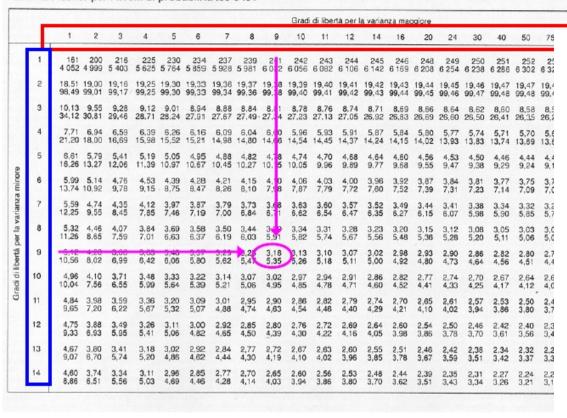
$$qf(0.975, n_A-1, n_B-1)$$

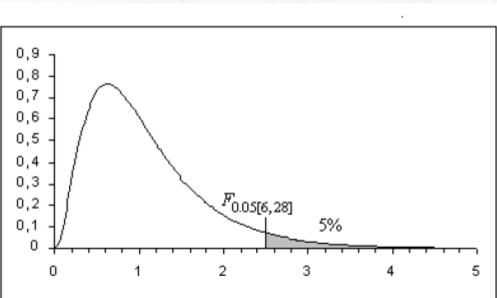
F critical

if the calculated F is larger than the critical value, we reject the null hypothesis Test can be carried out with the var.test()



TAVOLA A Valori di Fischer per i livelli di probabilità .05 e .01





0,08

0.07

0.06

0.05

0,04 0,03 0,02

0,01

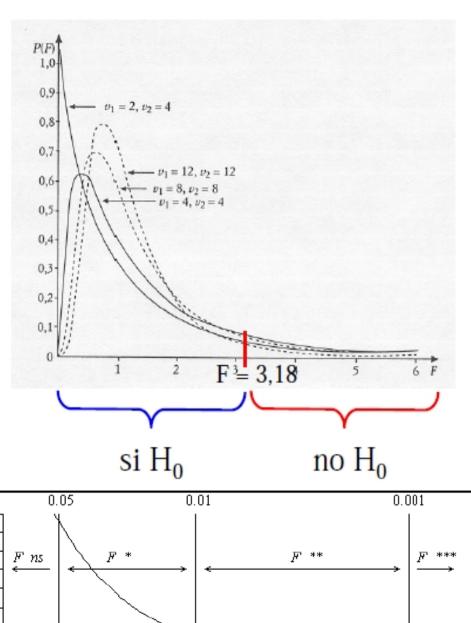
2.5

3

3.5

4.5

5.5



# Homogeneity of Variance > two samples

#### **Hypothesis testing**

It is important to know whether variance differs significantly from sample to sample. Constancy of variance (homoscedasticity) is the most important assumption underlying regression and analysis of variance. For multiple samples you can choose between the

Bartlett test and the Fligner-Killeen test.

Bartlett.test(response, factor)

Fligner.test(response, factor)

There are differences between the tests: Fisher and Bartlett are very sensitive to outliers, whereas Fligner–Killeen is not



# **Mean Comparisons**

#### **Hypothesis testing**

In many cases, a researcher is interesting in gathering information about two populations in order to compare them. As in statistical inference for one population parameter, <u>confidence intervals</u> and <u>tests of significance</u> are useful statistical tools for the difference between two population parameters.

Ho: the two means are the same

H1: the two means differ

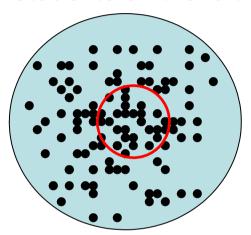
- All Assumptions met? Parametric t.test()
- t test with independent or paired sample
- -Some assumptions not met? Non-parametric Wilcox.test()
- The **Wilcoxon signed-rank test** is a <u>non-parametric</u> alternative to the <u>Student's t-test</u> for the case of two samples.



# Mean Comparison: Two independent sample

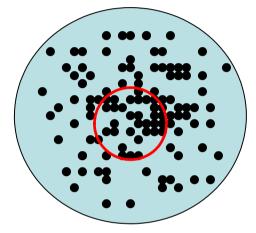
#### **Hypothesis testing**

#### Students on the left



$$mean_a = \frac{\sum y_i}{n}$$

Students on the right



$$mean_b = \frac{\sum y_i}{n}$$

$$SE_{diff} = \sqrt{\frac{\text{var}_a}{n_a} + \frac{\text{var}_b}{n_b}}$$

$$t = \frac{mean_a - mean_b}{SE_{diff}}$$

The two samples are statistically independent

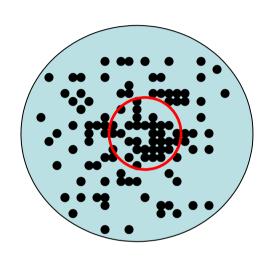
Test can be carried out with the t.test() function





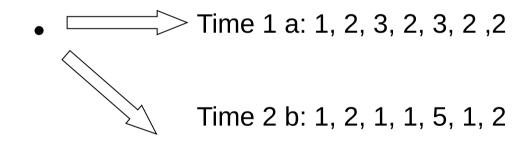
# T-Test for paired samples

#### **Hypothesis testing**



$$t = \frac{(\sum a_i - b_i)/n}{SD_{diff}/\sqrt{n}}$$

E.g. Test your performance before or after the course. I measure twice on the same student



If we have information about dependence, we have to use this!!!

We can deal with dependence



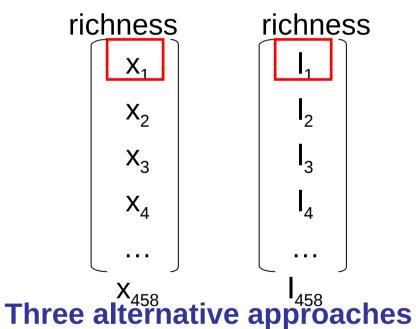
Test can be carried out with the t.test() function

# Correlation

#### **Hypothesis testing**

Correlation, (often measured as a correlation coefficient), indicates the strength and direction of a linear relationship between two random variables

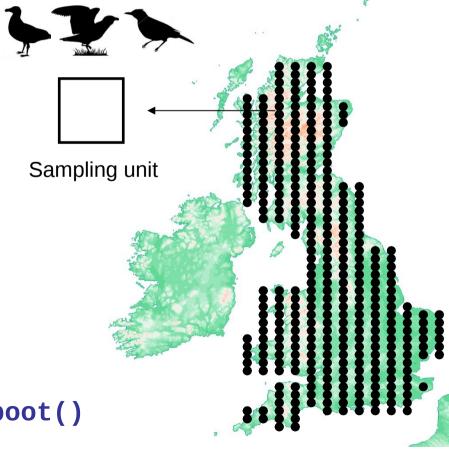
Bird species Plant species



- 1. Parametric cor()
- 2. Nonparametric cor()

STUDI DI TRIESTE

Bootstrapping - replicate(), boot()

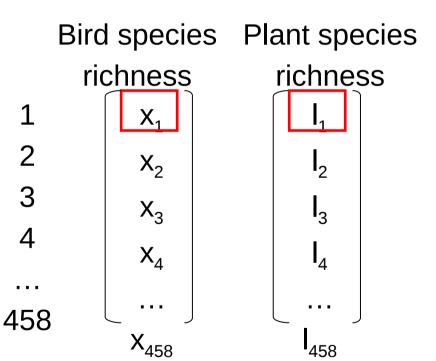


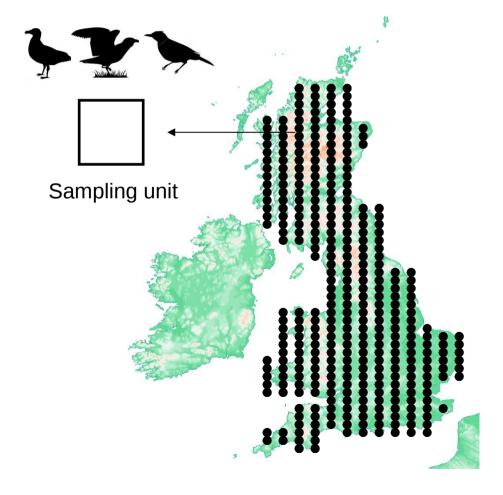
# Correlation: causal relationship?



#### **Hypothesis testing**

Which is the response variable in a correlation analysis?

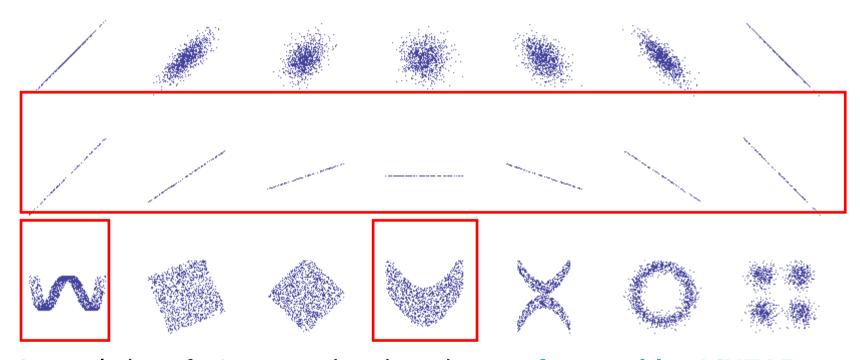




## Correlation

#### **Hypothesis testing**

#### Plot the two variables in a Cartesian space



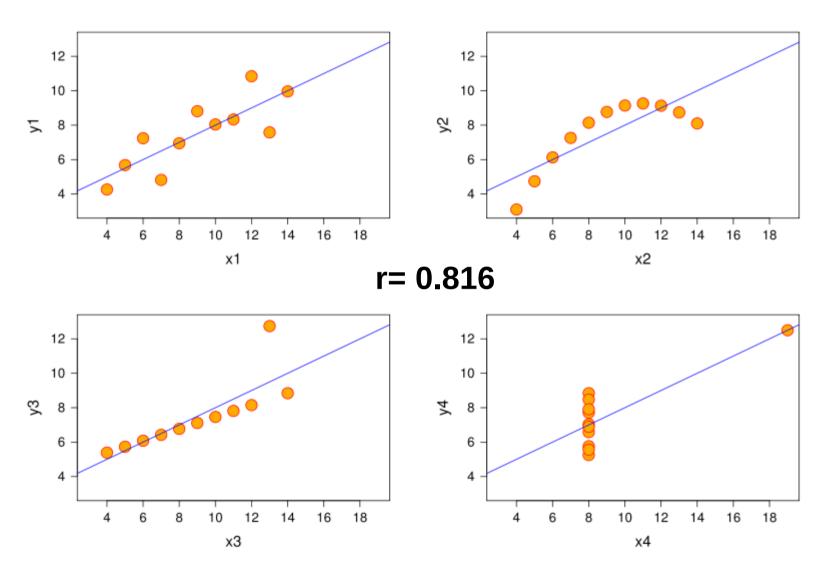
A correlation of +1 means that there is a **perfect positive LINEAR relationship** between variables.

A correlation of -1 means that there is a **perfect negative LINEAR relationship** between variables.

A correlation of 0 means there is **no LINEAR relationship** between the two variables.

#### **Hypothesis testing**

#### Same correlation coefficient!



# Parametric correlation: when is significant?

#### Hypothesis testing

#### Pearson product-moment correlation coefficient

Correlation coefficient:

$$cor = \frac{\sum (xy)}{\sqrt{\sum x^2 \sum y^2}} \qquad SE_{cor} = \sqrt{\frac{(1 - cor^2)}{n - 2}}$$

Hypothesis testing using the t distribution:

Ho: Is cor = 0

H1: Is  $cor \neq 0$ 

$$t = \frac{cor}{SE_{cor}}$$
 t critic value for d.f. = n-2

#### **Assumptions**

- Two random variables from a random populations
- cor() detects ONLY linear relationships

# Nonparametric correlation

#### Hypothesis testing

#### Rank procedures

# Distribution-free but less power

#### **Spearman correlation index**

$$cor.spearman = \frac{\sum (rank_x rank_y)}{\sqrt{\sum rank_x^2 \sum rank_y^2}}$$

#### The Kendall tau rank correlation coefficient

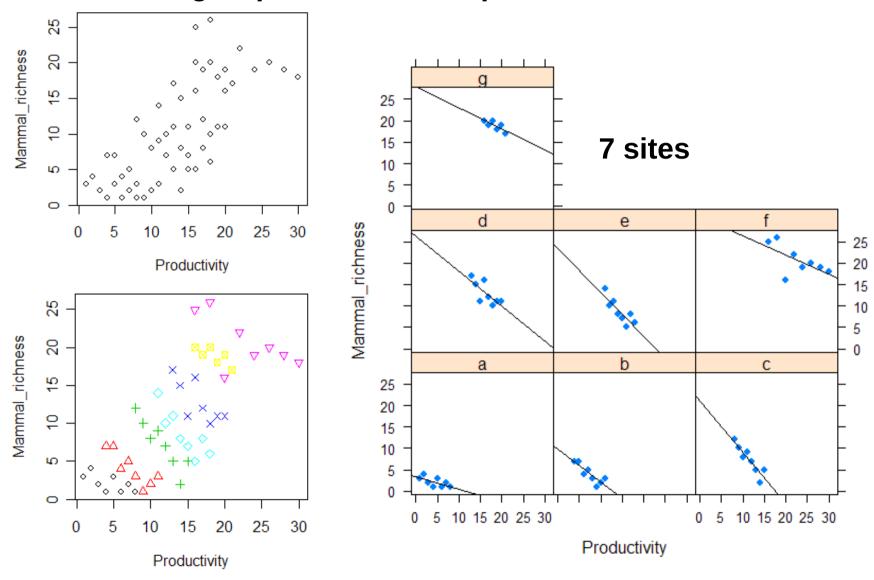
$$cor.kendall = \frac{4P}{n(n-1)} - 1$$

*P* is the number of concordant pairs *n* is the total number of pairs

# Scale-dependent correlation

#### **Hypothesis testing**

#### NB Don't use grouped data to compute overall correlation!!!



#### **Hypothesis testing**

#### **MODEL**

Generally speaking, a statistical model is a function of your explanatory variables to explain the variation in your response variable (y)

E.g. 
$$Y=a+bx_1+cx_2+dx_3$$

Y= response variable (performance of the students)

 $x_i$ = explanatory variables (ability of the teacher, background, age)

The object is to determine the values of the parameters (a, b, c and d) in a specific model that lead to the best fit of the model to the data

The best model is the model that produces the least unexplained variation (the *minimal residual deviance*), subject to the constraint that all the parameters in the model should be statistically significant (many ways to reach this!)

deviance = 
$$SS = \sum (y_i - mean)^2$$

#### **Hypothesis testing**

## Getting started with complex statistical modeling

It is essential, that you can answer the following questions:

- Which of your variables is the response variable?
- Which are the explanatory variables?
- Are the explanatory variables continuous or categorical, or a mixture of both?
- What kind of response variable do you have: is it a continuous measurement, a count, a proportion, a time at death, or a category?

## Statistical modelling: multicollinearity

#### **Hypothesis testing**

## 1. Multicollinearity

Correlation between predictors in a non-orthogonal multiple linear models

**Confounding effects difficult to separate** 

Variables are not independent

This makes an important difference to our statistical modelling because, in orthogonal designs, the variation that is attributed to a given factor is constant, and does not depend upon the order in which factors are removed from the model.

In contrast, with non-orthogonal data, we find that the variation attributable to a given factor *does* depend upon the order in which factors are removed from the model

The order of variable selection makes a huge difference

#### **Hypothesis testing**

## Getting started with complex statistical modeling

It is essential, that you can answer the following questions:

- Which of your variables is the response variable?
- Which are the explanatory variables?
- Are the explanatory variables continuous or categorical, or a mixture of both?
- What kind of response variable do you have: is it a continuous measurement, a count, a proportion, a time at death, or a category?



#### **Each analysis estimate a MODEL**

You want the model to be *minimal* (parsimony), and *adequate* (must describe a significant fraction of the variation in the data)

It is very important to understand that *there is not just one model*.

- given the data,
- and given our choice of model,
- what values of the parameters of that model make the observed data most likely?

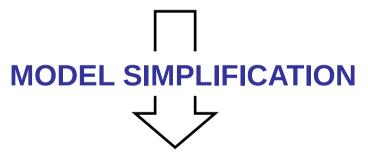
Model building: estimate of parameters (slopes and level of factors)

Occam's Razor



#### Occam's Razor

- Models should have as few parameters as possible;
- linear models should be preferred to non-linear models;
- experiments relying on few assumptions should be preferred to those relying on many;
- models should be pared down until they are minimal adequate;
- simple explanations should be preferred to complex explanations.



The process of model simplification is an integral part of hypothesis testing in R. In general, a variable is retained in the model only if it causes a significant increase in deviance when it is removed from the current model.

# Statistical modelling: model simplification



Parsimony requires that the model should be as simple as possible. This means that the model should not contain any redundant parameters or factor levels.

#### **Model simplification**

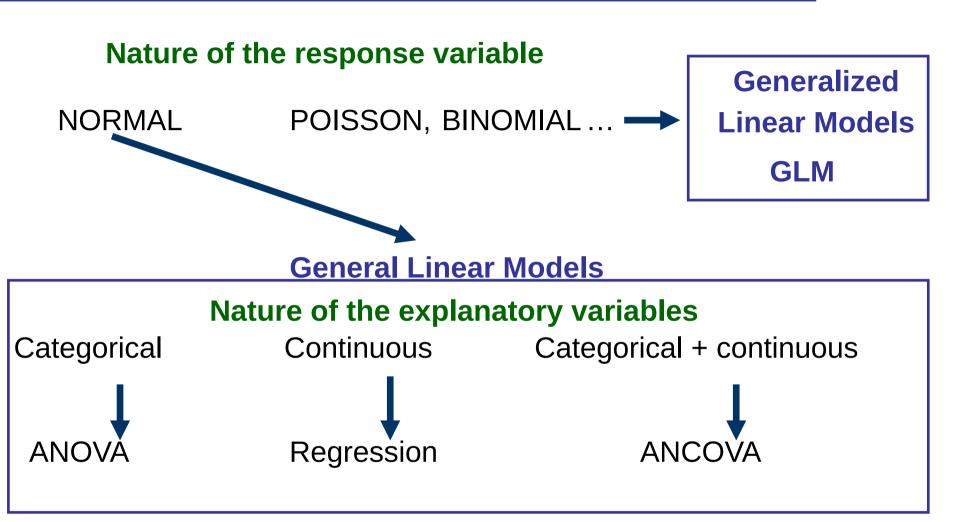
- remove non-significant interaction terms;
- remove non-significant quadratic or other non-linear terms;
- remove non-significant explanatory variables;
- group together factor levels that do not differ from one another;
- in ANCOVA, set non-significant slopes of continuous explanatory variables to zero.

# Statistical modelling: model simplification



Step	Procedure	Interpretation
1	Fit the maximal model	Fit all the factors, interactions and covariates of interest. Note the residual deviance. If you are using Poisson or binomial errors, check for overdispersion and rescale if necessary.
2	Begin model simplification	Inspect the parameter estimates (e.g. using the R function summary(). Remove the least significant terms first (using update -,) starting with the highest-order interactions.
3	If the deletion causes an insignificant increase in deviance	Leave that term out of the model. Inspect the parameter values again. Remove the least significant term remaining.
4	If the deletion causes a significant increase in deviance	Put the term back in the model (using <b>update</b> +). These are the statistically significant terms as assessed by deletion from the maximal model.
5	Keep removing terms from the model	Repeat steps 3 or 4 until the model contains nothing but significant terms.  This is the minimal adequate model (MAM).  If none of the parameters is significant, then the minimal adequate model is the null model.

## Statistical modelling: more than one parameter



### LINEAR REGRESSION 1m()

Regression analysis is a technique used for the modeling and analysis of numerical data consisting of values of a <a href="mailto:dependent variable">dependent variable</a> (response variable) and of one or more <a href="mailto:independent continuous variables">independent continuous variables</a> (explanatory variables)

#### **Assumptions**

**Independence**: The Y-values and the error terms must be independent of each other.

**Linearity** between Y and X.

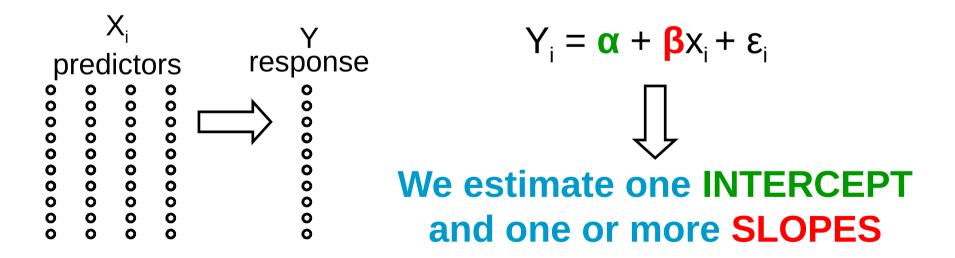
**Normality**: The populations of Y-values and the error terms are normally distributed for each level of the predictor variable x

Homogeneity of variance: The populations of Y-values and the error terms have the same variance at each level of the predictor variable x. (don't test for normality or heteroscedasticity, check the residuals instead!)

### LINEAR REGRESSION 1m()

#### **AIMS**

- 1.To describe the linear relationships between Y and  $X_i$  (EXPLANATORY APPROACH) and to quantify how much of the total variation in Y can be explained by the linear relationship with  $X_i$ .
- 2. To predict new values of Y from new values of  $X_i$  (PREDICTIVE APPROACH)



#### SIMPLE LINEAR REGRESSION

#### **Estimating parameters**

$$y_i = \alpha + \beta x_i + \epsilon_i$$

$$\beta = \frac{\sum [(x_i - x_{mean})(y_i - y_{mean})]}{\sum (x_i - x_{mean})^2}$$

$$\alpha = y_{\text{mean}} - \beta * x_{\text{mean}}$$

#### **Measure of goodness-of-fit**

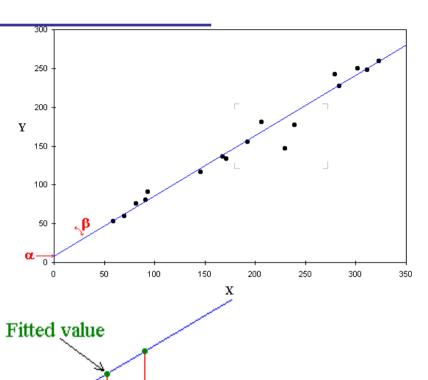
Total SS = 
$$\Sigma (y_{\text{fitted i}} - y_{\text{mean}})^2$$

Model SS = 
$$\Sigma(y_{\text{predicted i}} - y_{\text{mean}})^2$$

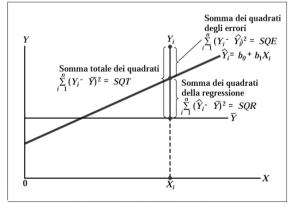
Residual SS = Total SS - Model SS

R<sup>2</sup> = Model SS /Total SS





Residual



Le misure di variabilità nel modello di regressione

#### SIMPLE LINEAR REGRESSION

### **Hypothesis testing**

Analysis of variance
Parameter t testing

Ho:  $\beta = 0$  (There is no relation between X and Y)

H1:  $\beta \neq 0$ 

**Analysis of variance** (test the model!)

 $F_{1,n-2} = (Model SS/1) / (Residual SS/n-2)$ 

Parameter t testing (test the single parameter!)

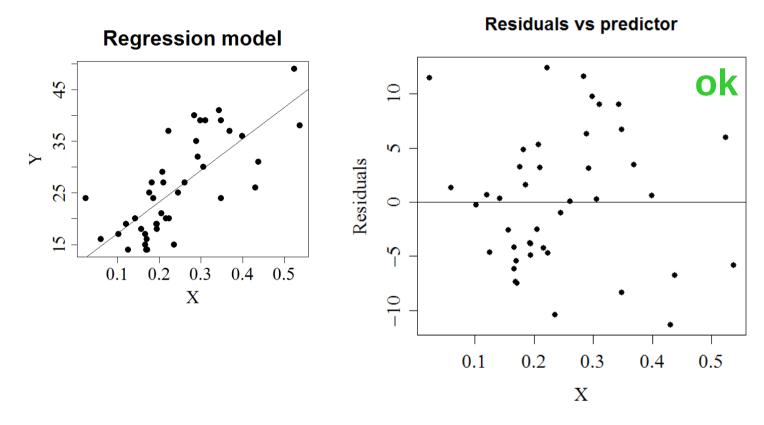
We must measure the unreliability associated with each of the estimated parameters (i.e. we need the standard errors)

$$SE(\beta) = [(residual SS/(n-2))/\Sigma(x_i - x_{mean})]^2$$
  

$$t = (\beta - 0) / SE(\beta)$$

### If the model is significant, then model checking

#### 1. Linearity between X and Y?

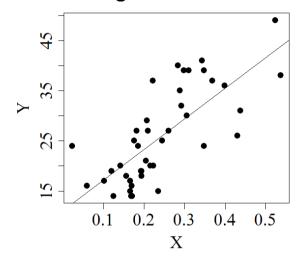


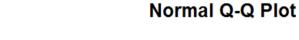
No patterns in the residuals vs. predictor plot

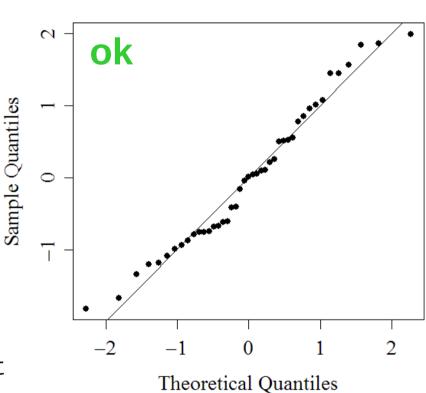
#### 2. Normality of the residuals

Q-Q plot + Shapiro-Wilk test on the residuals

#### **Regression model**







#### > shapiro.test(residuals)

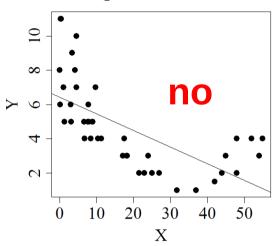
Shapiro-Wilk normality test data: residuals

W = 0.9669, p-value = 0.2461

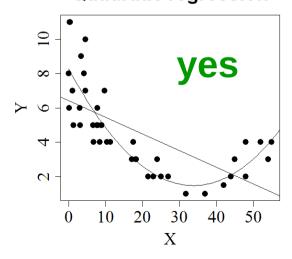
ok

#### 1. Linearity between X and Y?

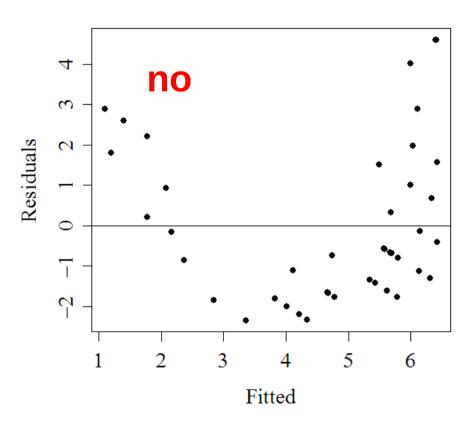
#### Regression model



#### **Quadratic regression**



#### Residuals vs fitted

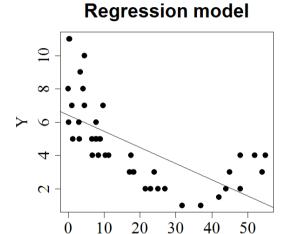


**NO LINEARITY between X and Y** 

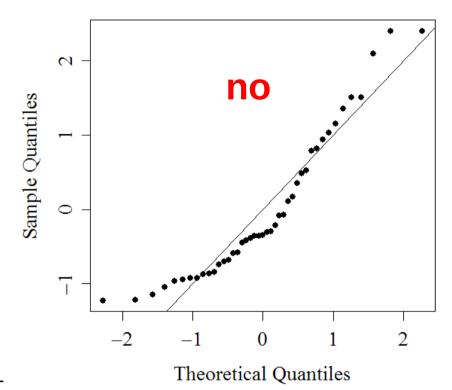
#### 2. Normality of the residuals

Q-Q plot + Shapiro-Wilk test on the residuals

#### **Normal Q-Q Plot**



0



#### > shapiro.test(residuals)

X

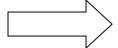
Shapiro-Wilk normality test

residuals data:

W = 0.8994, p-value = 0.001199

no

How to deal with non-linearity and non-normality situations?



### **Transformation of the data**

- -Box-cox transformation (power transformation of the response)
- -Square-root transformation
- -Log transformation
- -Arcsin transformation

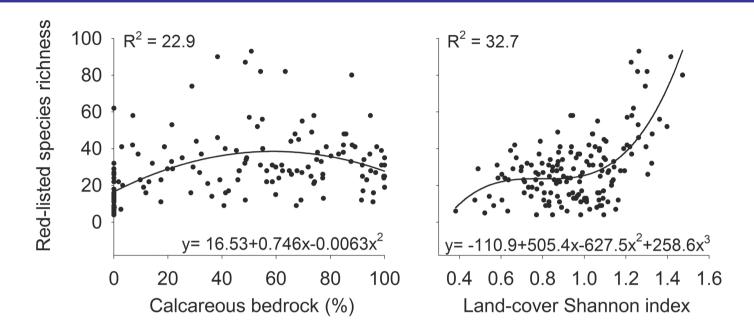
#### **Polynomial regression**

Regréssion with multiple terms (linear, quadratic, and cubic)

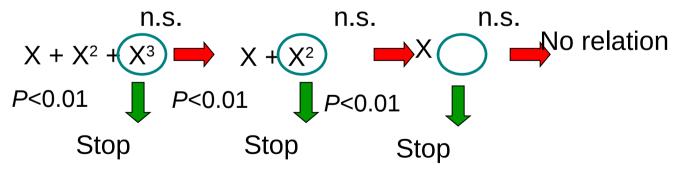
$$Y = a + b_1X + b_2X^2 + b_3X^3 + error$$

X is one variable!!!

### POLYNOMIAL REGRESSION: one x, n parameters



Hierarchy in the testing (always test the highest)!!!!



NB Do not delete lower terms even if non-significant

#### **Multiple regression**

Regression with two or more variables

$$Y = a + b_1X_1 + b_2X_2 + ... + b_iX_i + quadratic and cubic terms + interactions+ error$$

#### **Assumptions**

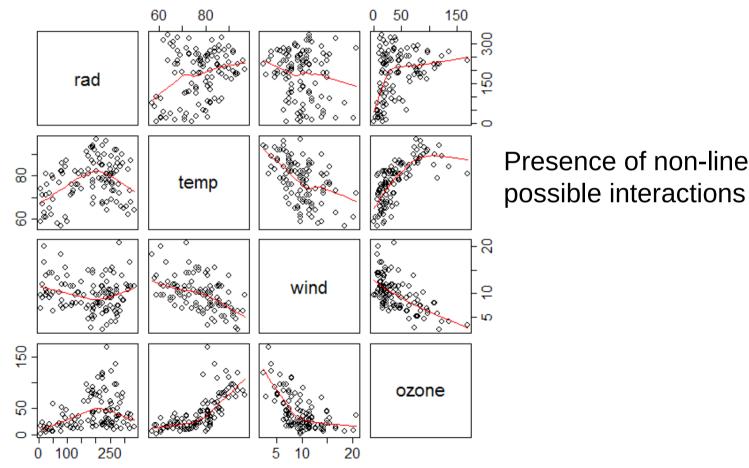
Same assumptions as in the simple linear regression!!!

#### **The Multiple Regression Model**

There are important issues involved in carrying out a multiple regression:

- which explanatory variables to include (VARIABLE SELECTION);
- NON-LINEARITY in the response to the explanatory variables;
- **INTERACTIONS** between explanatory variables;
- correlation between explanatory variables (COLLINEARITY);
- RELATIVE IMPORTANCE of variables

Let's begin with an example from air pollution studies. How is ozone concentration related to wind speed, air temperature and the intensity of solar radiation?



Presence of non-linearity and

# Start with a complex model with interactions and quadratic and cubic terms

Model simplification (Occam's razor)

Minimum Adequate Model

How to carry out a model simplification in multiple regression

- 1. Remove non-significant interaction terms.
- 2. Remove non-significant quadratic or other non-linear terms.
- 3. Remove non-significant explanatory variables.
- 4. Amalgamate explanatory variables that have similar parameter values.

### Start with the most complicate model (it is one approach)

model1<lm( ozone ~ temp\*wind\*rad+I(rad²)+I(temp²+I(wind²))</pre>

	Estimat e	Std.Erro r	t	Pr(>t)	
(Intercept)	5.7E+02	2.1E+02	2.74	0.01 **	
temp	-1.1E+01	4.3E+00	-2.50	0.01 *	
wind	-3.2E+01	1.2E+01	-2.76	0.01	• • !!!!!!
rad	-3.1E-01	5.6E-01	-0.56	0.58	We cannodelete the
I(rad^2)	-3.6E-04	2.6E-04	-1.41	0.16	terms
I(temp^2)	5.8E-02	2.4E-02	2.44	0.02 *	1111111
I(wind^2)	6.1E-01	1.5E-01	4.16	0.00 **	*
temp:wind	2.4E-01	1.4E-01	1.74	0.09	
tompired	0 45 00	7 55 00	1 10	0 27	

Delete only the highest interaction temp: wind:rad

wind:rad 2.1E-02 4.9E-02 0.42 0.68 temp:wind:ra

-4.3E-04 6.6E-04 -0.66 0.51

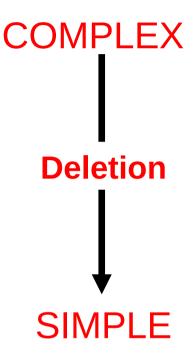
### **Manual model simplification**

(It is one of the many philosophies)
Deletion the non-significant terms one by one:

Hierarchy in the deletion:

- 1. Highest interactions
- 2. Cubic terms
- 3. Quadratic terms
- 4. Linear terms

At each deletion test: Is the fit of a simpler model worse?

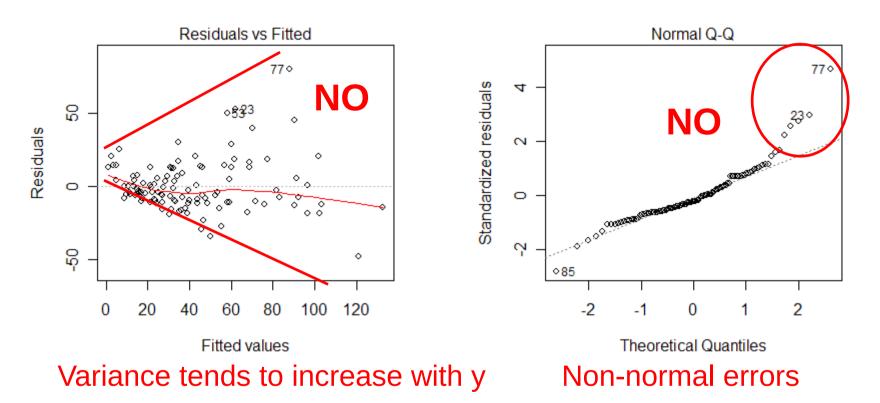


#### **IMPORTANT!!!**

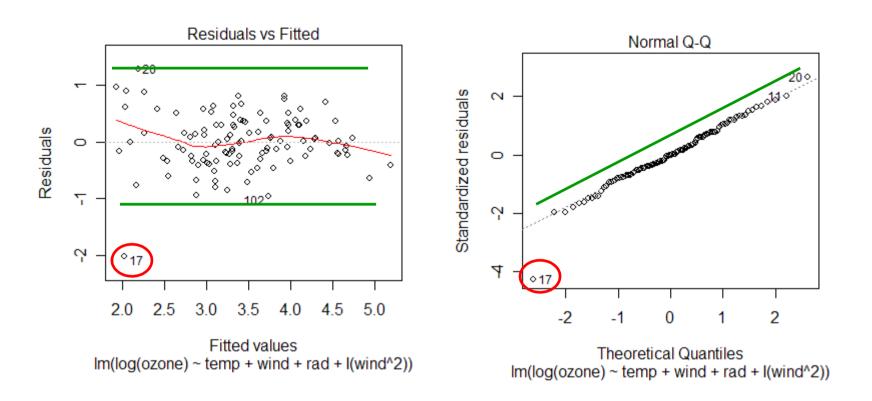
If you have quadratic and cubic terms significant you cannot delete the linear or the quadratic term even if they are not significant

If you have an interaction significant you cannot delete the main terms even if they are not significant

Once we reached the MAM we must check the assumptions



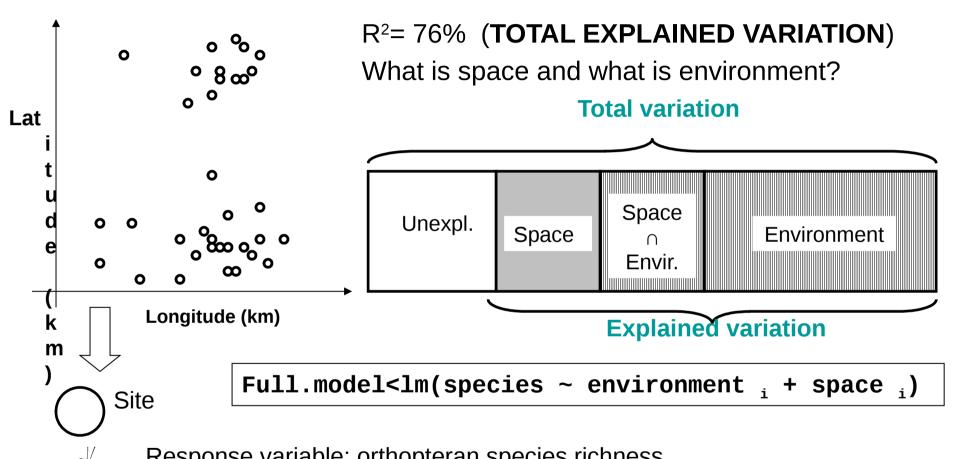
We can transform the data (e.g. Log-transformation of y)  $model < lm(log(ozone) ~ temp + wind + rad + l(wind^2))$ 



The log-transformation has improved our model but maybe there is an outlier

#### VARIATION PARTITIONING

Relative importance of groups of explanatory variables



Response variable: orthopteran species richness

Explanatory variable: SPACE (latitude + longitude) +

**ENVIRONMENT** (temperature + land-cover heterogeneity)

### VARIATION PARTITIONING: varpart (vegan)

