ASCA in one hour

Morten Arendt Rasmussen

Heads up!

We are recording this webinar

Kudos to

- Age Smilde

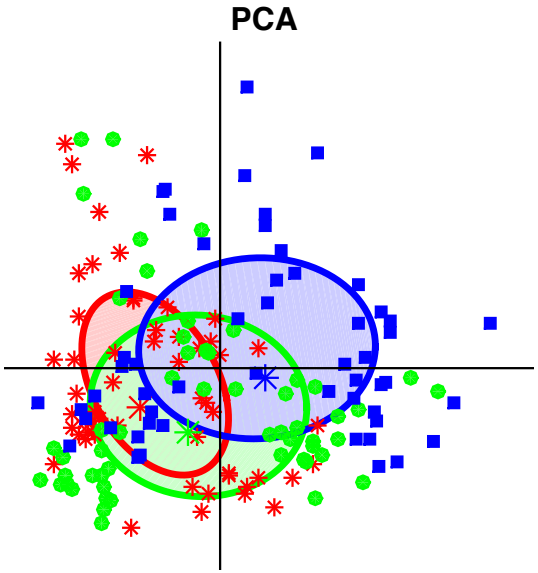
Jeroen Jansen

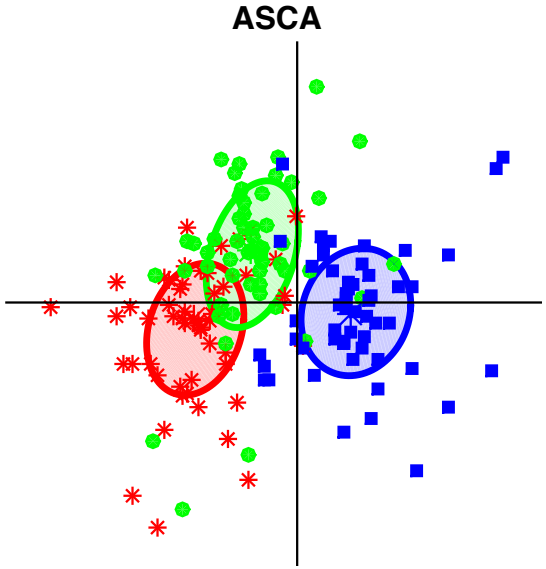
Take home

- ASCA combines the best of two worlds: **ANOVA** from statistics and **PCA** from chemometrics

- The **dominating variance** in data is often **trivial**, while our **questions of interest** are way more **subtle**.
- ASCA can *dig* such information out

A Motivating Example



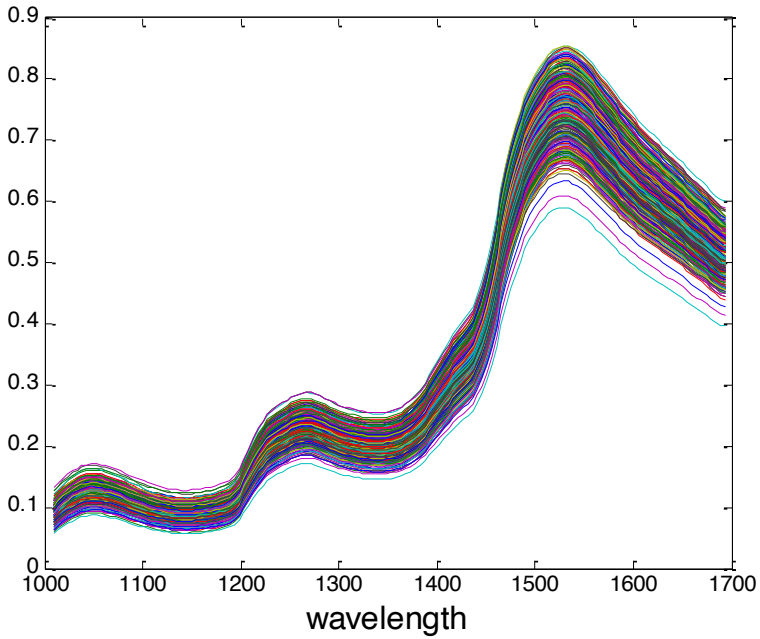


Design of Experiments

- Example – Bread

- Three factors:
- - **Type** (different enzymes): A, B, C
- - **Days** after production: 1,..,6
- - **Position**: Top, middle, bottom

- ***Full factorial with 6 replicates***
- ***N = 324***



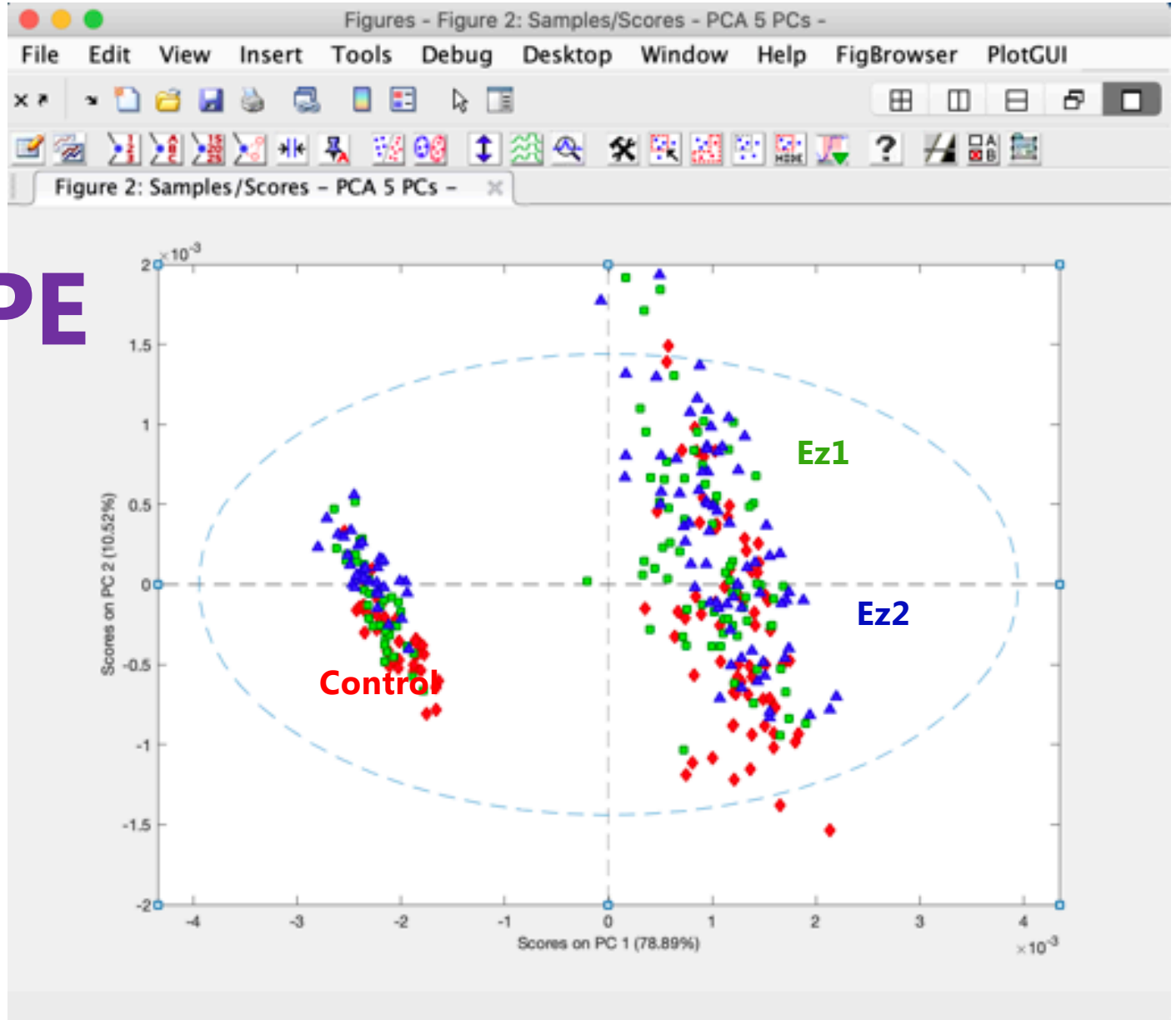
Data can be found here:
https://github.com/mortenarendt/ASCA

Staling of Bread

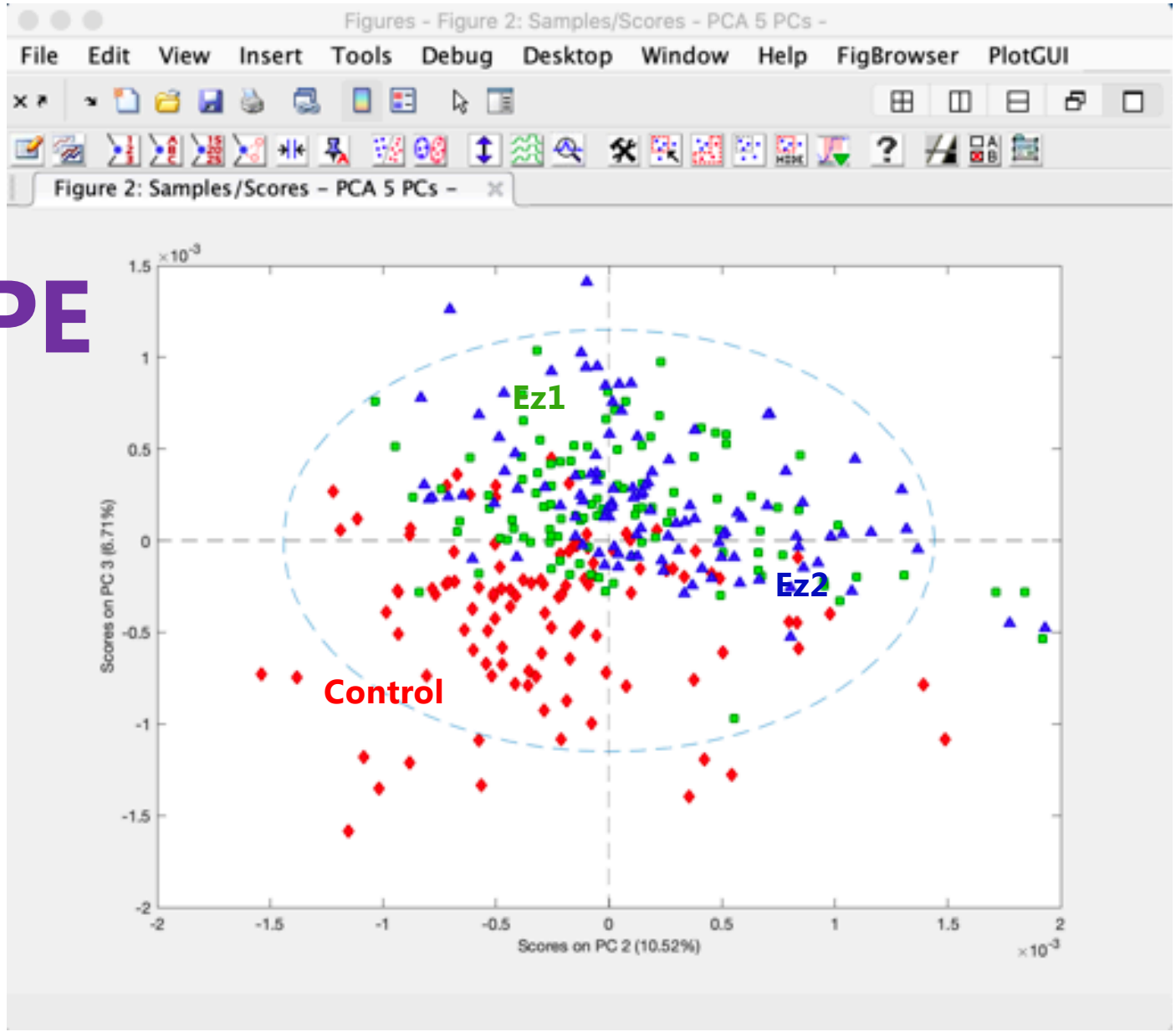
- Crumb firming
- Migration of moisture
- Crystalization of starch (amylose and amylopectine)
- …

Anti-staling enzyme:
α-amylases

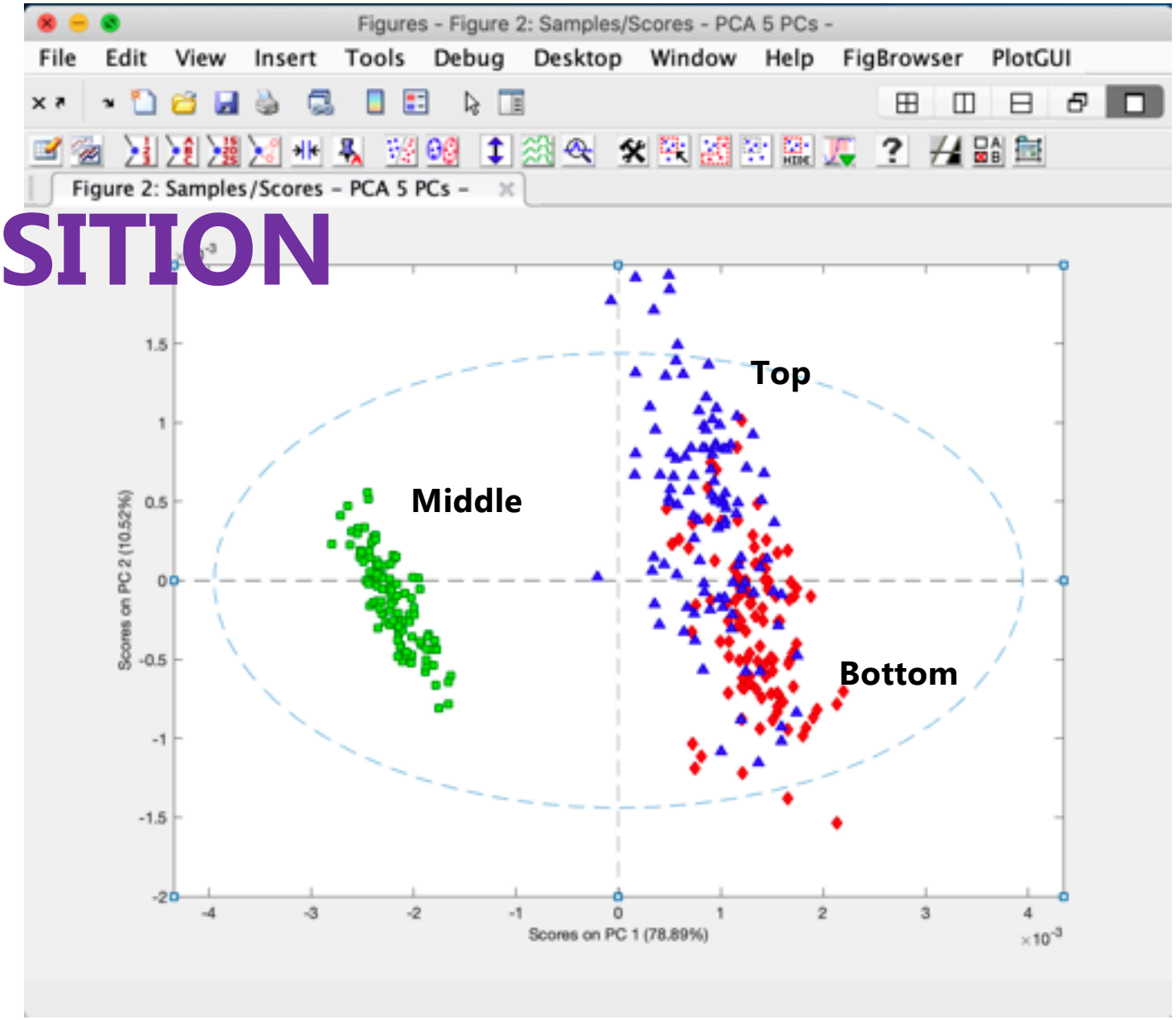
TYPE


Figures - Figure 2: Samples/Scores - PCA 5 PCs -
File Edit View Insert Tools Debug Desktop Window Help FigBrowser PlotGUI
Figure 2: Samples/Scores - PCA 5 PCs -
Ez1
Ez2
Control
Scores on PC 2 (10.52%)
Scores on PC 1 (78.89%)
×10^-3

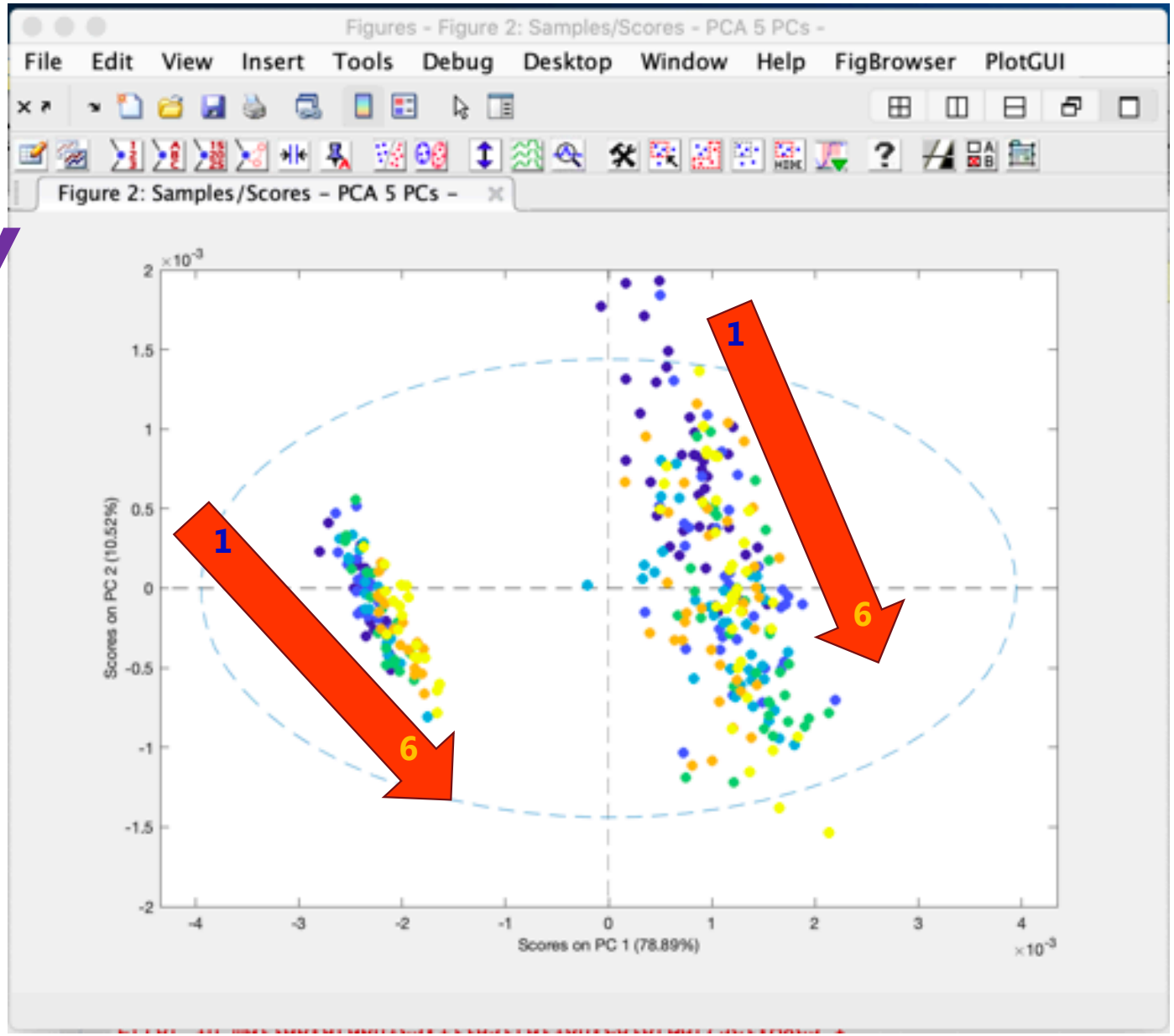
TYPE


Figures - Figure 2: Samples/Scores - PCA 5 PCs -
File Edit View Insert Tools Debug Desktop Window Help FigBrowser PlotGUI
Figure 2: Samples/Scores - PCA 5 PCs -
Ez1
Ez2
Control
Scores on PC 3 (6.71%)
Scores on PC 2 (10.52%)
×10^-3

POSITION


Figures - Figure 2: Samples/Scores - PCA 5 PCs -
File Edit View Insert Tools Debug Desktop Window Help FigBrowser PlotGUI
Figure 2: Samples/Scores - PCA 5 PCs -
Top
Middle
Bottom
Scores on PC 2 (10.52%)
Scores on PC 1 (78.89%)
×10^-3

DAY


Figures - Figure 2: Samples/Scores - PCA 5 PCs -
File Edit View Insert Tools Debug Desktop Window Help FigBrowser PlotGUI
Figure 2: Samples/Scores - PCA 5 PCs -
Scores on PC 2 (10.52%)
Scores on PC 1 (78.89%)
1
6
1
6

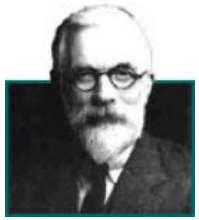
Where is the information?

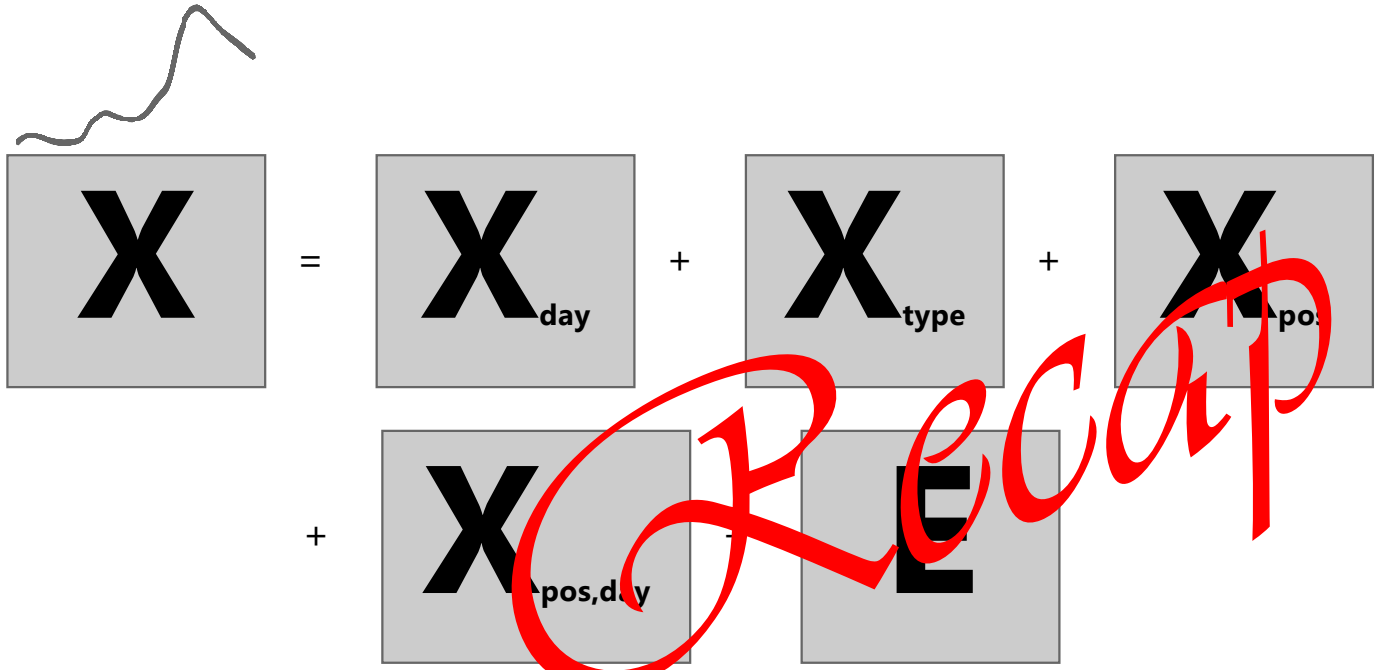
Three factors:

- **Type** PC2 & PC3
- **Days** PC1 & PC2
- **Position** PC1 & PC2

- **PCA is powerful in extracting information**
- ***But….***
- **In a normal PCA we do not *at all* utilize that there were an experimental design**

ANOVA



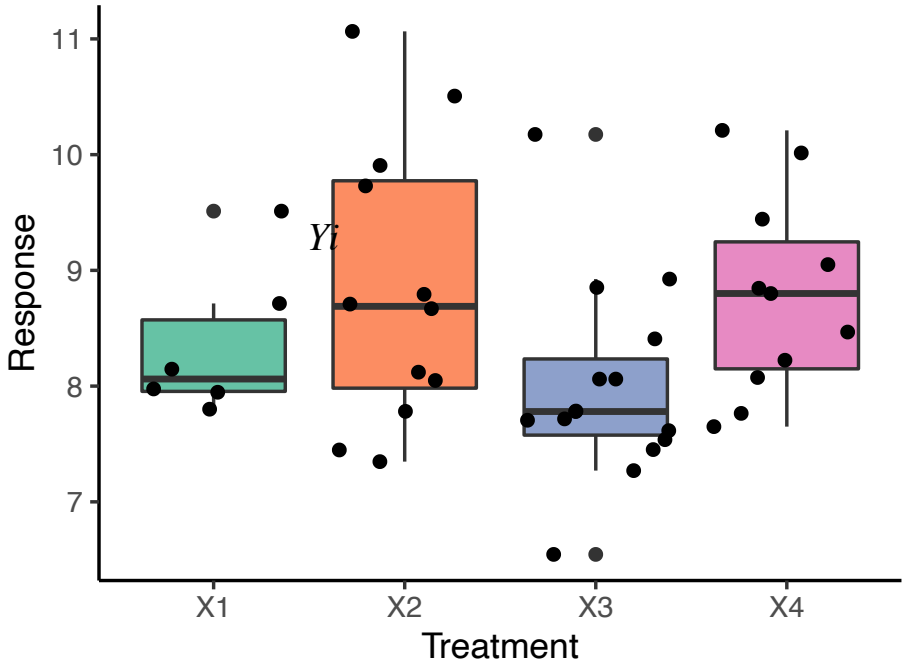


Recap

ANOVA *(oneway)*

$$Y_i = \mu + \alpha_{Treatment_i} + e_i$$

$e_1, .., e_n$
Comes from the same distribution



ANOVA

$$Y_i = \mu + \alpha_{Treatment_i} + \beta_{Time_i} + \gamma_{Treatment_i, Time_i} + e_i$$

Treatment	Time	Y
1	1	12,2
1	2	14,5
1	3	22,4
1	1	9,2
1	2	13,0
1	3	27,1
1	1	6,9
1	2	19,2
1	3	21,5
2	1	12,5
2	2	16,6
2	3	24,7
2	1	10,7
2	2	24,0
2	3	28,9
2	1	10,9
2	2	18,8
2	3	24,6

ANOVA *– some features*

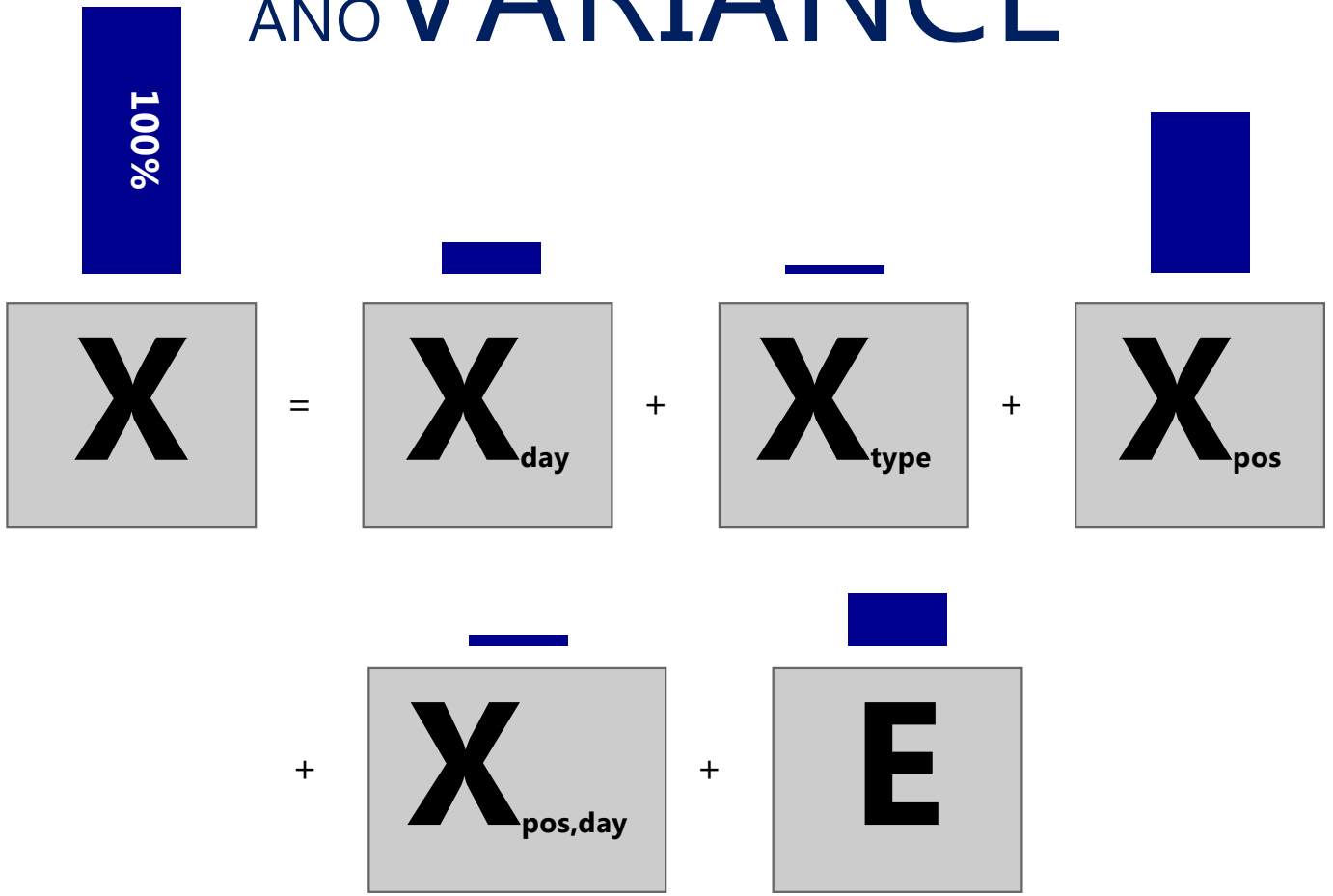
- Particular useful for analysis of **experimental data**.
- Analysis of comparative experiments—those in which only the ***difference*** in outcomes is of interest.
- The calculations of ANOVA can be characterized as computing a number of means and variances.
- ANOVA can be performed for a single factor or multiple factors.

•Assumptions

- Response variables are normally distributed (or approximately normally distributed).
- Samples are independent.
- Variances of populations (different groups in the data) are equal.
- This is in statistics known as the **iid assumption**

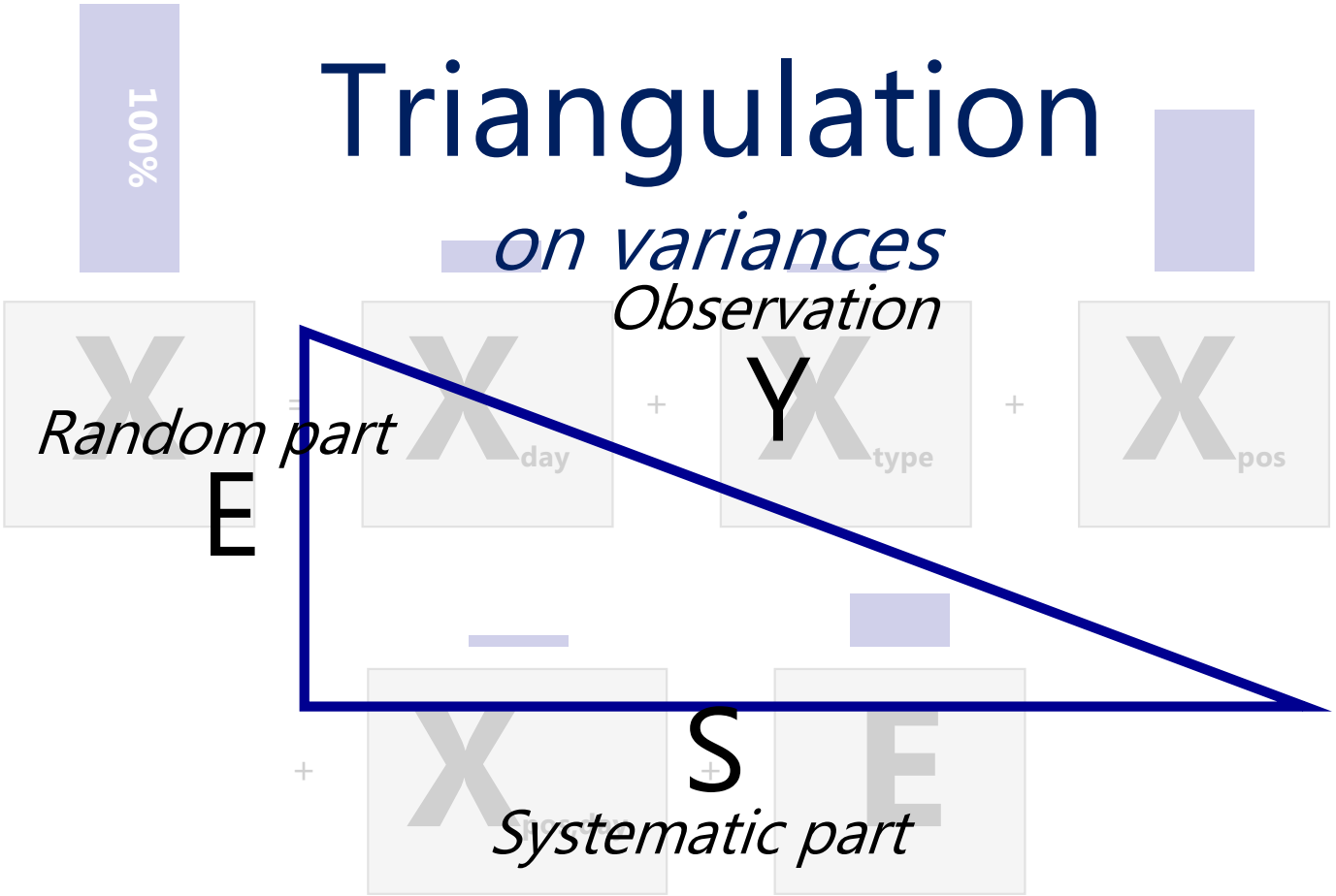
iid = **i**ndependent and **i**dentically **d**istributed

ANOVARIANCE

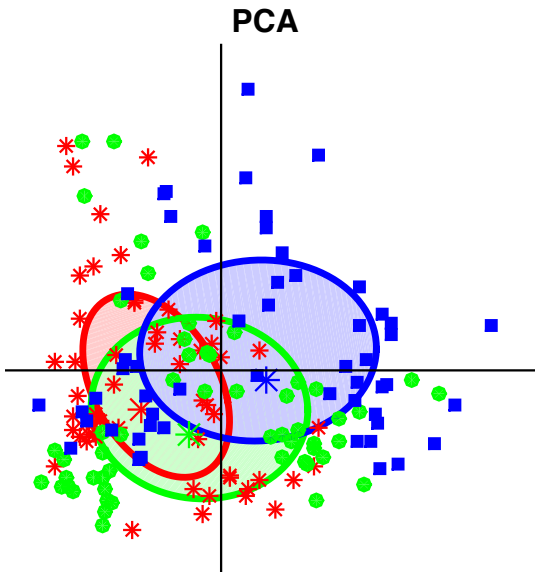


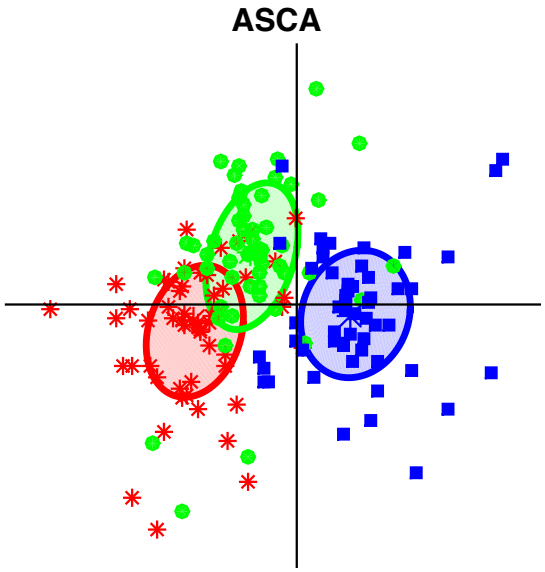
Triangulation

on variances



Example in PLStb



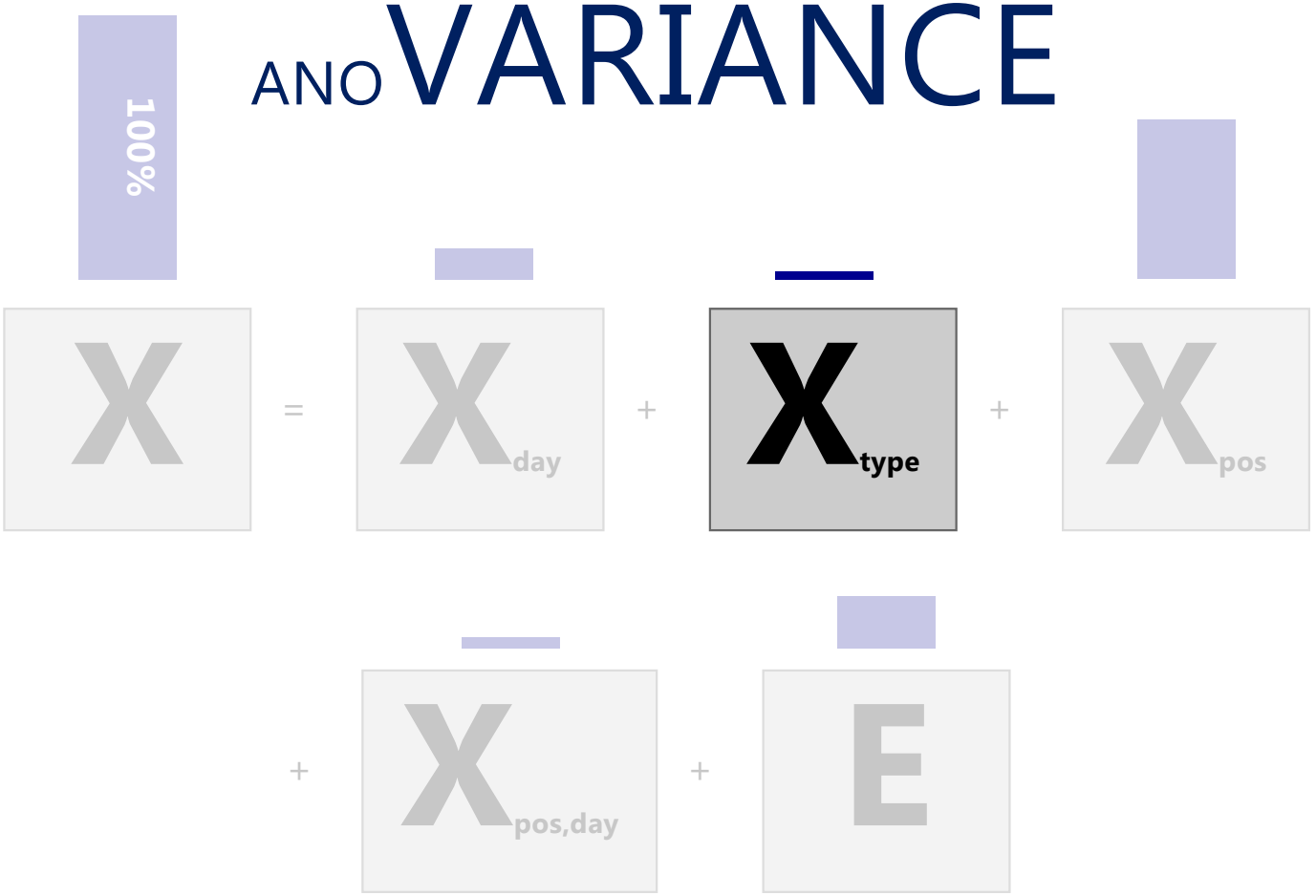


ASCA

A two step procedure

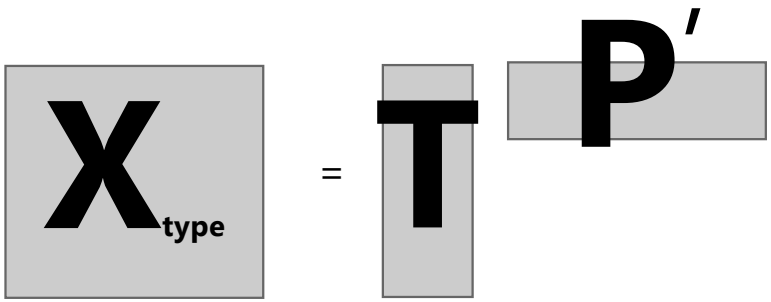
1. **Split** the data into design related matrices and a random term
   - This procedure is basically univariate ANOVA for each variable.
   - Testing of the individual design effects is a straight-forward extension of the normal ANOVA test.
2. Use **PCA** on the effect of interest.
   - A bilinear model is calculated directly on the systematic effect matrix
   - In order to get an idea about the spread (uncertainty) the random term is projected on this part.

ANOVARIANCE



Simultaneous Component Analysis

"A PCA model on the part of interest"

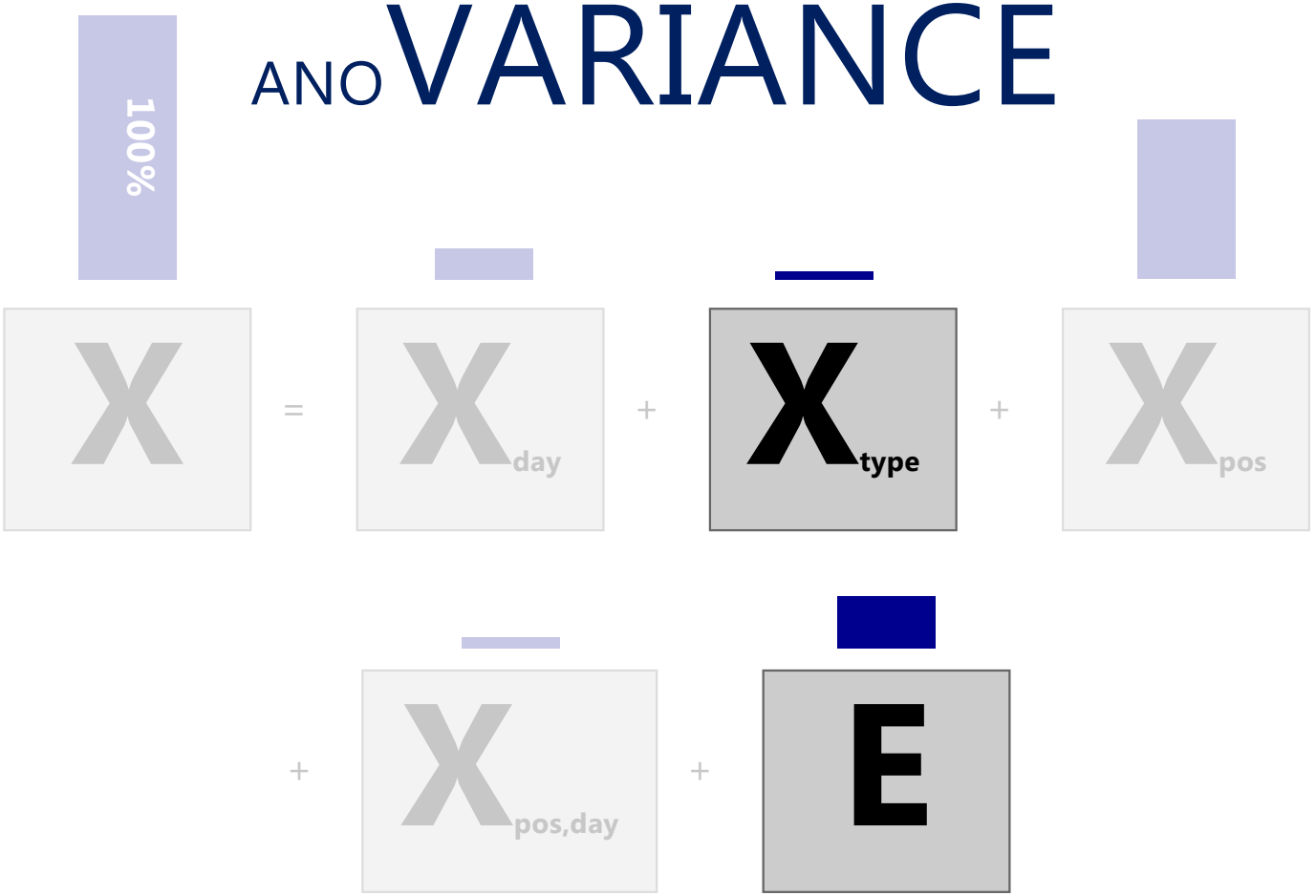


But!... What about the error?

This has rank =
number of classes -1

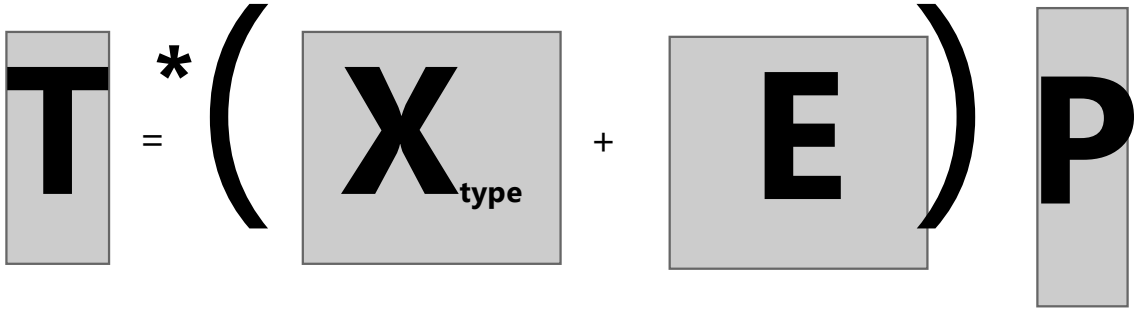
Smilde, Age K., et al. "ANOVA-simultaneous component analysis (**ASCA**): a new tool for analyzing designed metabolomics data." *Bioinformatics* 21.13 (2005): 3043-3048.

ANOVA VARIANCE



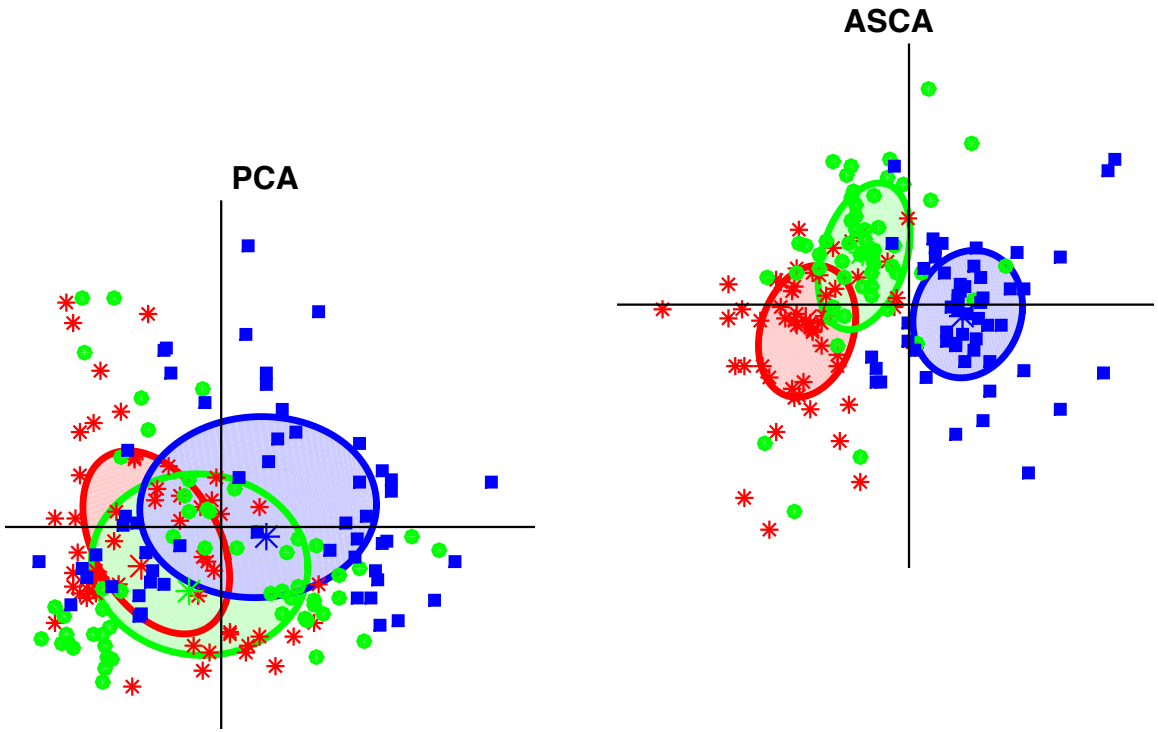
Simultaneous Component Analysis

Score plots

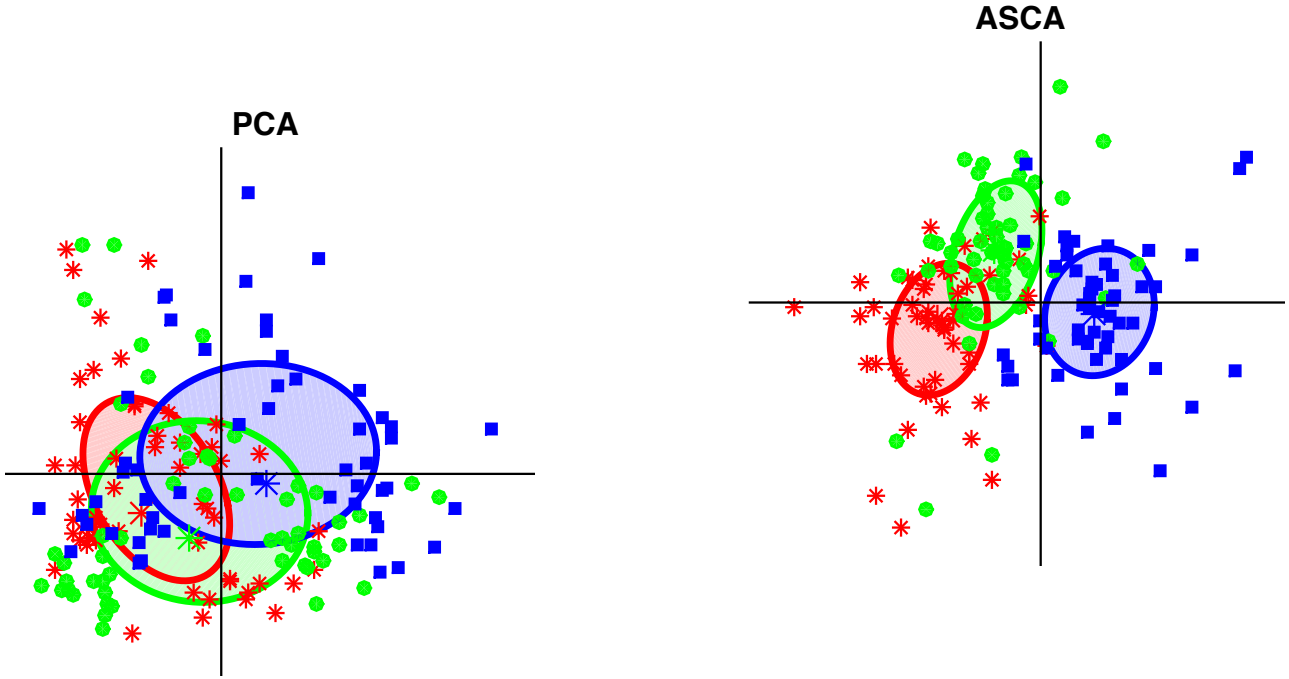


T, **T*** and **P** are used for interpretation

Example continued



Hypothesis Testing



Null Hypothesis Testing

The general idea

•State a question

•…in relation to the design.

•Central statistics

•Figure out which number that encapsulate the differences observed in the data (with respect to the design variable of interest)

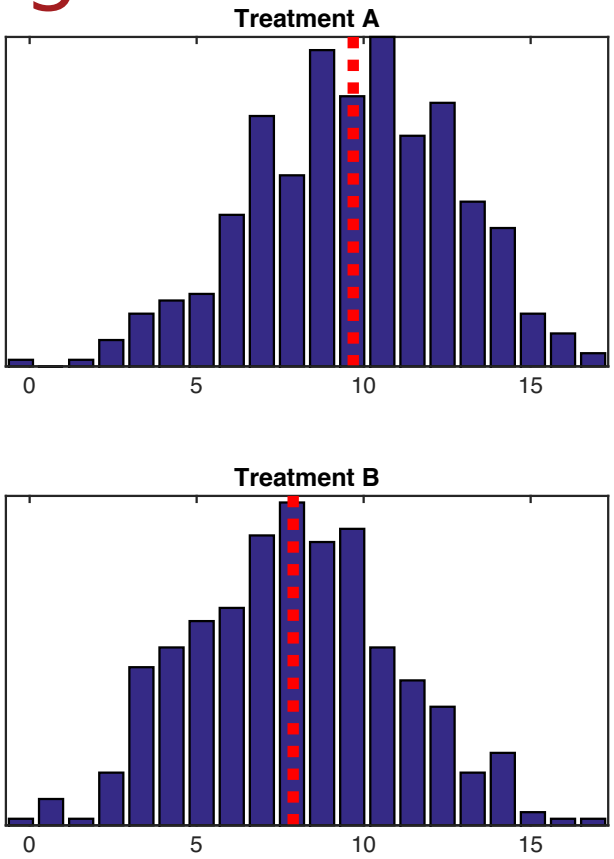
•*e.g. The difference between means*

•Test

•The central statistics measures the ***distance*** to the null hypothesis.

•Find out whether this number is a big or small.

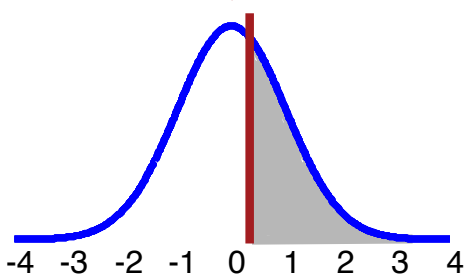
•We can use analytical *null-distributions* – if this one is known! Or we can create it from data.



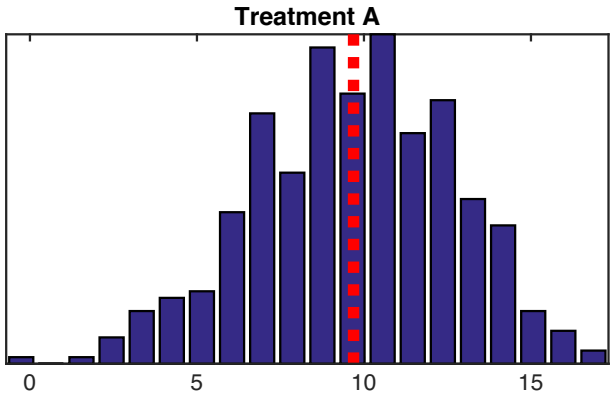
Null Hypothesis Testing

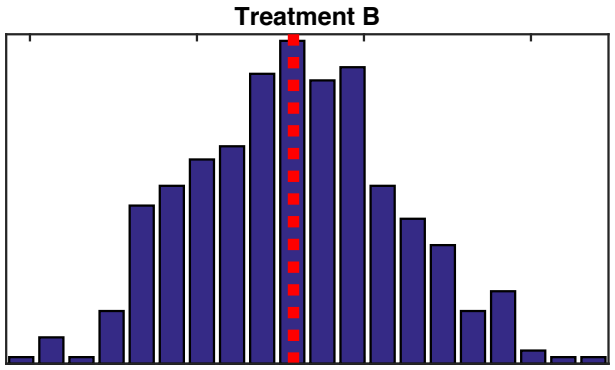
T-test

$$t = \frac{\bar{x}_A - \bar{x}_B}{\sqrt{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}}}$$



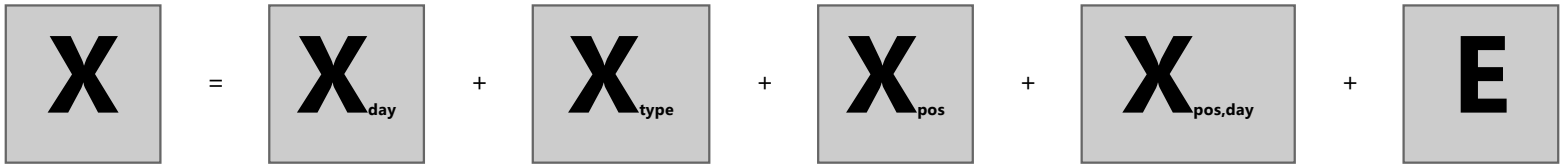
t_{df}



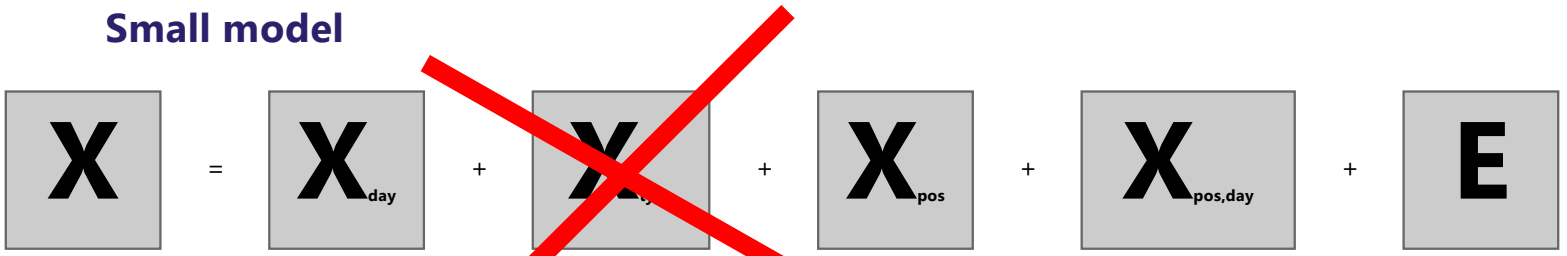


Testing individual effects

Big model



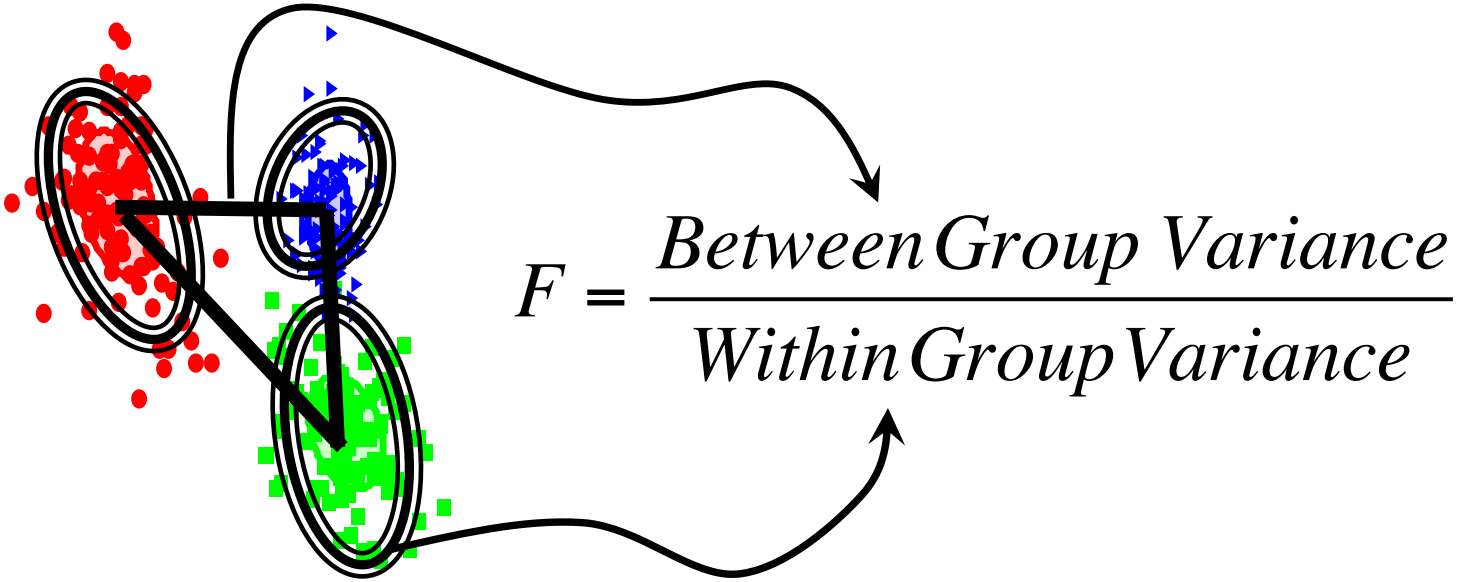
Small model



Central Statistics

$$F = \frac{(SSE_{small\,m} - SSE_{big\,m}) / (dfe_{small\,m} - dfe_{big\,m})}{SSE_{big\,m} / dfe_{big\,m}}$$

Testing individual effects

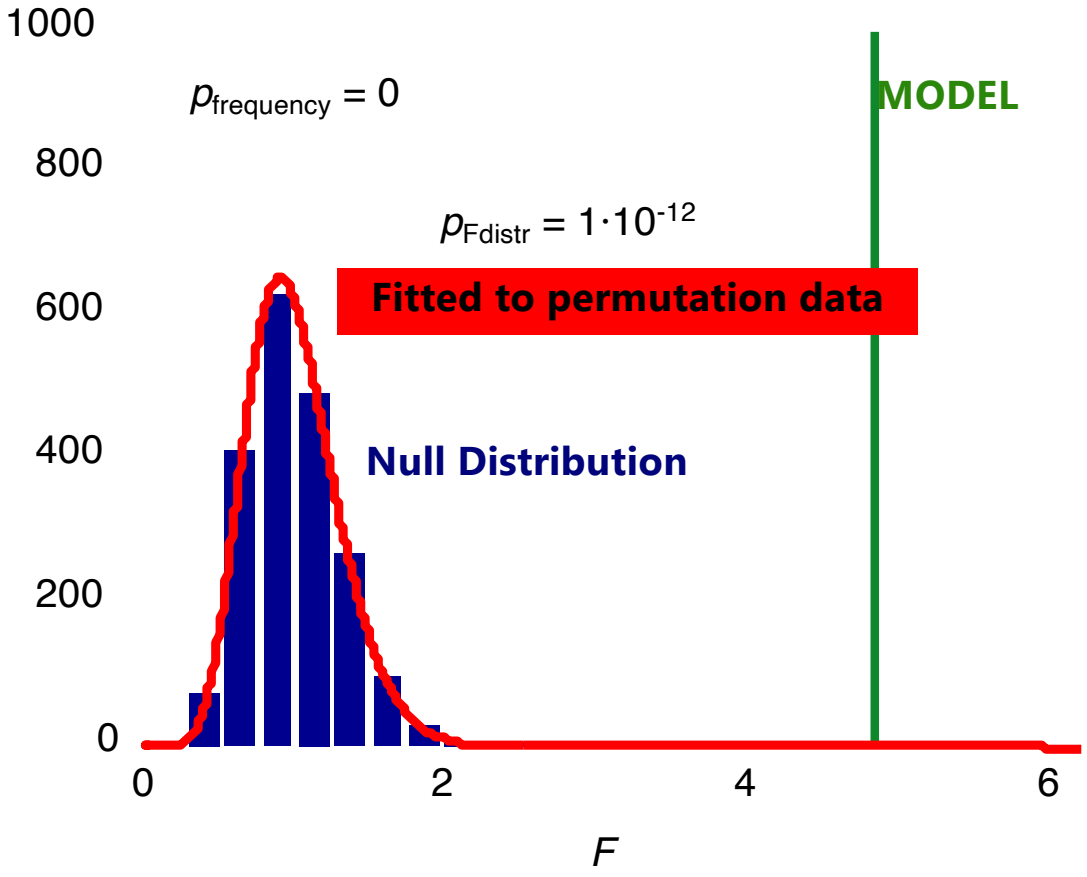


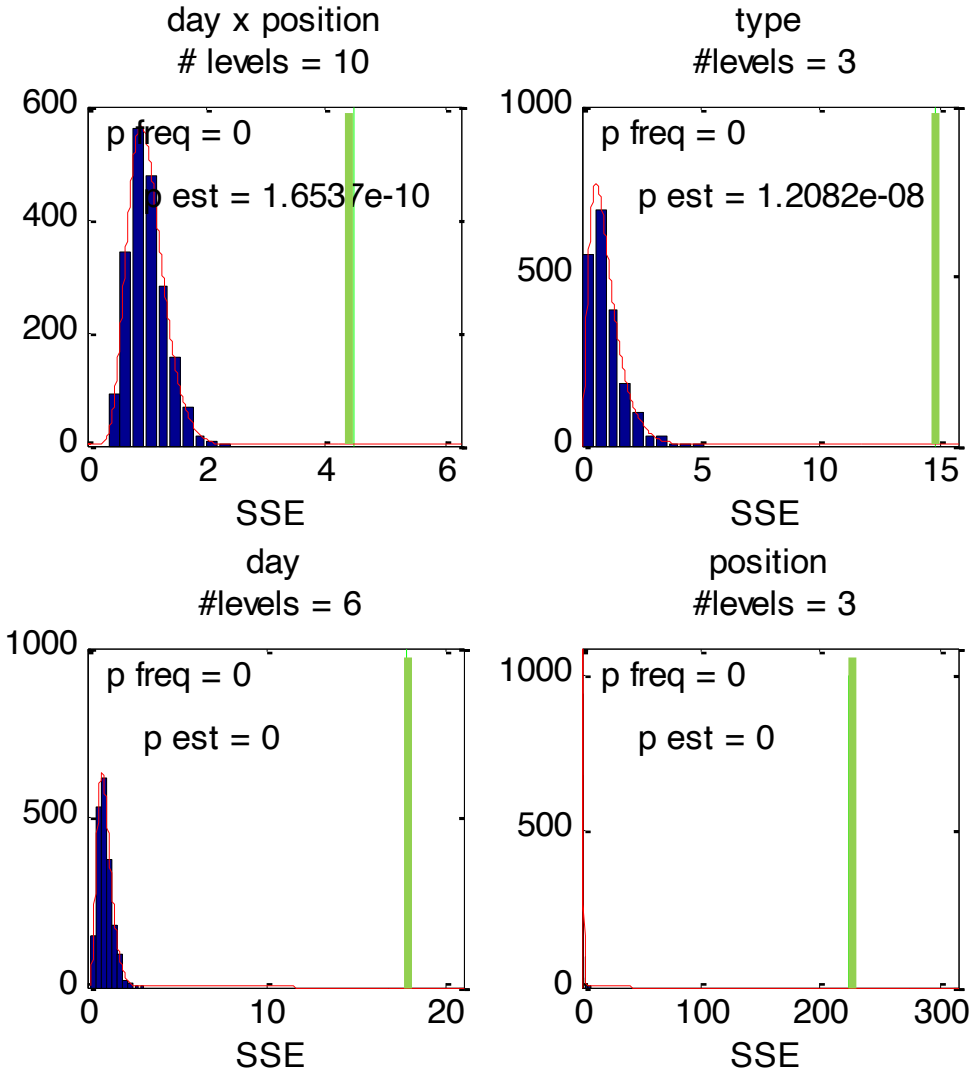
$$F = \frac{Between\,Group\ Variance}{Within\,Group\,Variance}$$

Central Statistics

$$F = \frac{(SSE_{small\,m} - SSE_{big\,m}) / (dfe_{small\,m} - dfe_{big\,m})}{SSE_{big\,m} / dfe_{big\,m}}$$

Permutation testing




day x position
levels = 10
p freq = 0
p est = 1.6537e-10
SSE
type
#levels = 3
p freq = 0
p est = 1.2082e-08
SSE
day
#levels = 6
p freq = 0
p est = 0
SSE
position
#levels = 3
p freq = 0
p est = 0
SSE

Summing up

ASCA is

- Powerful in disentangling subtle variation from multivariate data
- Basically ANOVA extended to multivariate data.
  - That is:
    - Splitting of each variable into design related variance and error
    - Effects are tested by comparing the individual design variance terms with the error variance. This is done by permutation testing in lack of an analytical distribution.

- Design related variance is interpreted by a *focused* PCA model, with uncertainty imposed by the residuals.

Do you want to know more?
Do you have projects with tricky data?

Contact us:

Frans van den Berg	fb@food.ku.dk
Rasmus Bro	rb@food.ku.dk
Åsmund Rinnan	aar@food.ku.dk
Morten Arendt Rasmussen	mortenr@food.ku.dk


Thank you
for the attention