Multivariate projection methodologies for the exploration of large biological data sets

Methods

Agenda

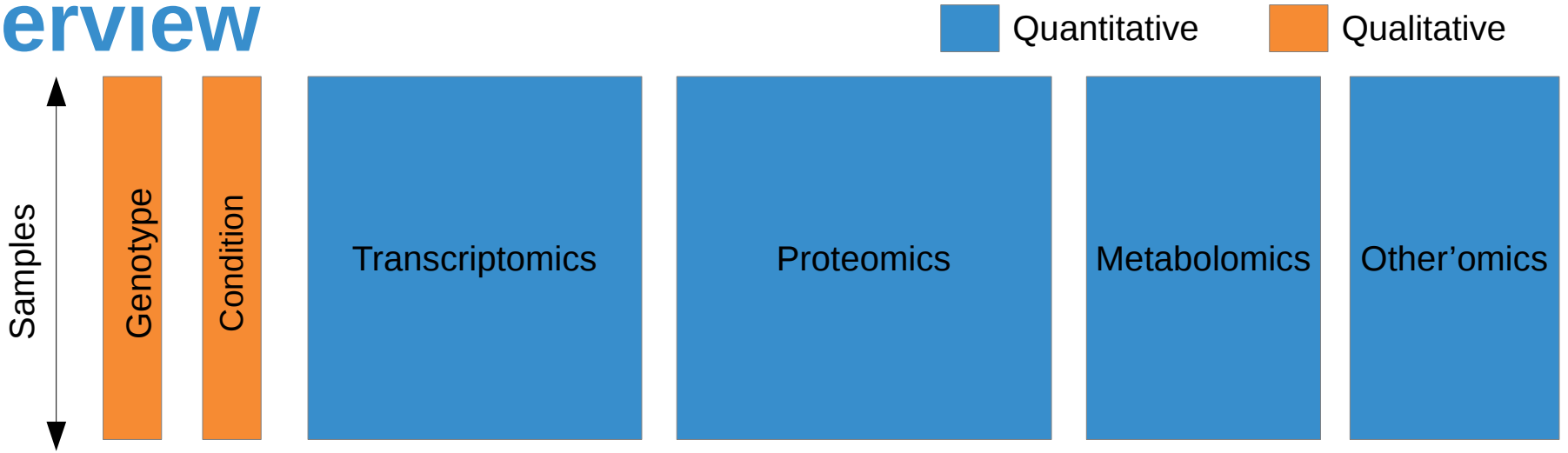
- Introduction
- Reminders (?)

- Explore one data set (PCA)
- Discriminant analysis (LDA, PLS-DA)
- Data integration (PLS, CCA, GCCA)

- Graphical outputs
- Sparsity
- Conclusion

Introduction

Overview



- Univariate
  Mean, median, standard deviation...
- Bivariate: 2 quantitatives or 1 quantitative + 1 qualitative or 2 qualitatives
  Correlation, statistical test (Student, ANOVA, Chi2)
- Multivariate unsupervised
  PCA
- Multivariate supervised
  PLS-DA
- Multi-block unsupervised
  PLS (2 blocks), GCCA
- Multi-block supervised
  GCC-DA

Guidelines

- I want to explore one single data set (e.g. microarray data):
  - I would like to identify the trends or patterns in your data, experimental bias or, identify if your samples 'naturally' cluster according to the biological conditions: Principal Component Analysis (PCA)
- I want to want to unravel the information contained in two data sets, where two types of variables are measured on the same samples (e.g. metabolomics and transcriptomics data)
  - I would like to know if I can extract common information from the two data sets (or highlight thecorrelation between the two data sets). The total number of variables is less than the number of samples: Canonical Correlation Analysis (CCA) or Projection to Latent Sructures (PLS) canonical mode. The total number of variables is greater than the number of samples: Regularized Canonical Correlation Analysis (rCCA) or Projection to Latent Sructures (PLS) canonical mode
- I have one single data set (e.g. microarray data) and I am interested in classifying my samples into known classes:
  - Here X = expression data and Y = vector indicating the classes of the samples. I would like to know how informative my data are to rightly classify my samples, as well as predicting the class of new samples: PLS-Discriminant Analysis (PLS-DA)

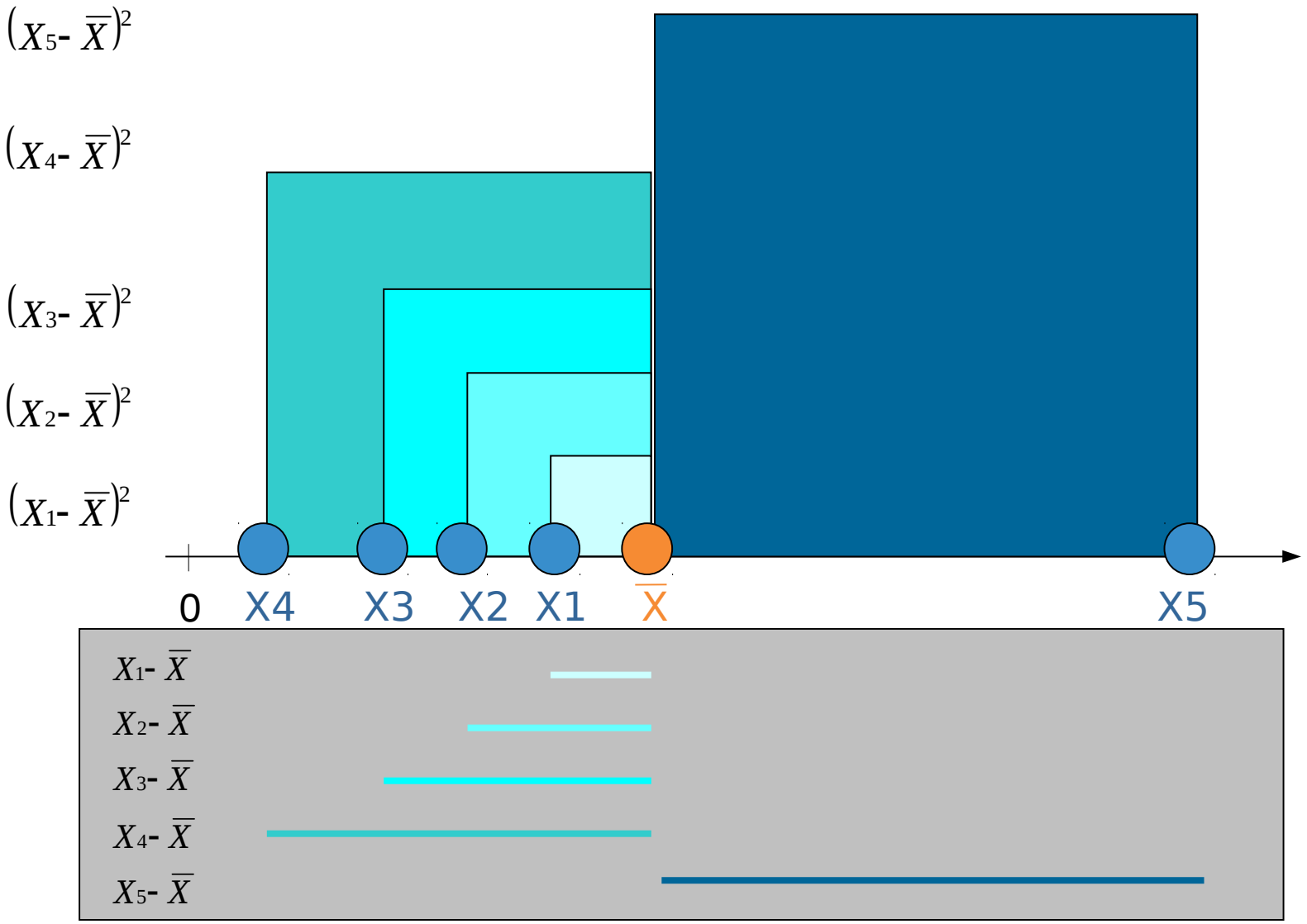
The mixOmics story

- Started with two phD projects in Université de Toulouse:
  - Ignacio González (2004-2007): rCCA
  - Kim-Anh Lê Cao (2005-2008): sPLS
- The Australian mixOmics immigration processed began in 2008 ...
  - K-A moved to UQ for a postdoc (IMB)
  - Core team established: Kim-Anh Lê Cao (FR, AUS), Ignacio González (FR), Sébastien Déjean (FR)
- First R CRAN release in May 2009
- Today
  - 21,000 downloads (unique IP adress) in 2016 (4,000 in 2014, 10,000 in 2015)
  - Website: `www.mixomics.org`
  - Two web-interfaces (shiny and PHP, also Galaxy but not advertised)
  - 19 multivariate methodologies and sparse variants (13 are our own methods)
  - Team: 3 core members, 4 key contributors, many others...

Reminders (?)

Variance and Standard Deviation

Square root of the mean of the squared deviation to the mean



$$\mathrm{var}(X)=\frac{1}{n}\sum_{i=1}^{n}(X_i- \overline{X})^2$$

Variance

Standard deviation

$$\sigma(X)=\sqrt{\mathrm{var}(X)}$$

In the same unit as the data (as the mean but unlike the variance)

Covariance

Covariance $\text{cov}(X,Y)=\frac{1}{n}\sum_{i=1}^{n}(X_i-\bar{X})(Y_i-\bar{Y})$

cov(X,X)=var(X)

Sign of the product $(Xi-\bar{X})(Yi-\bar{Y})$

Intuitively :

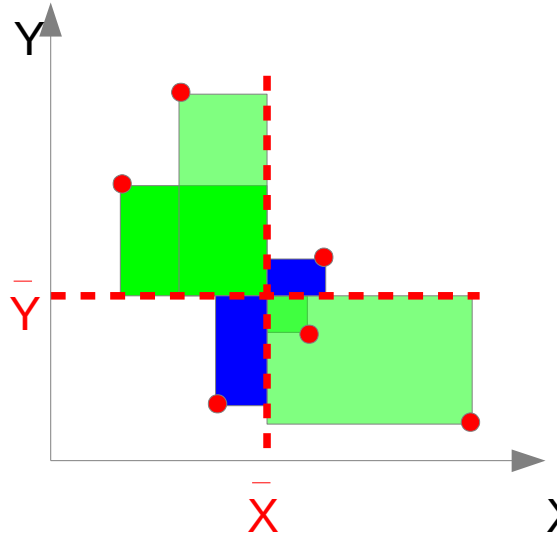
- If the **+** win

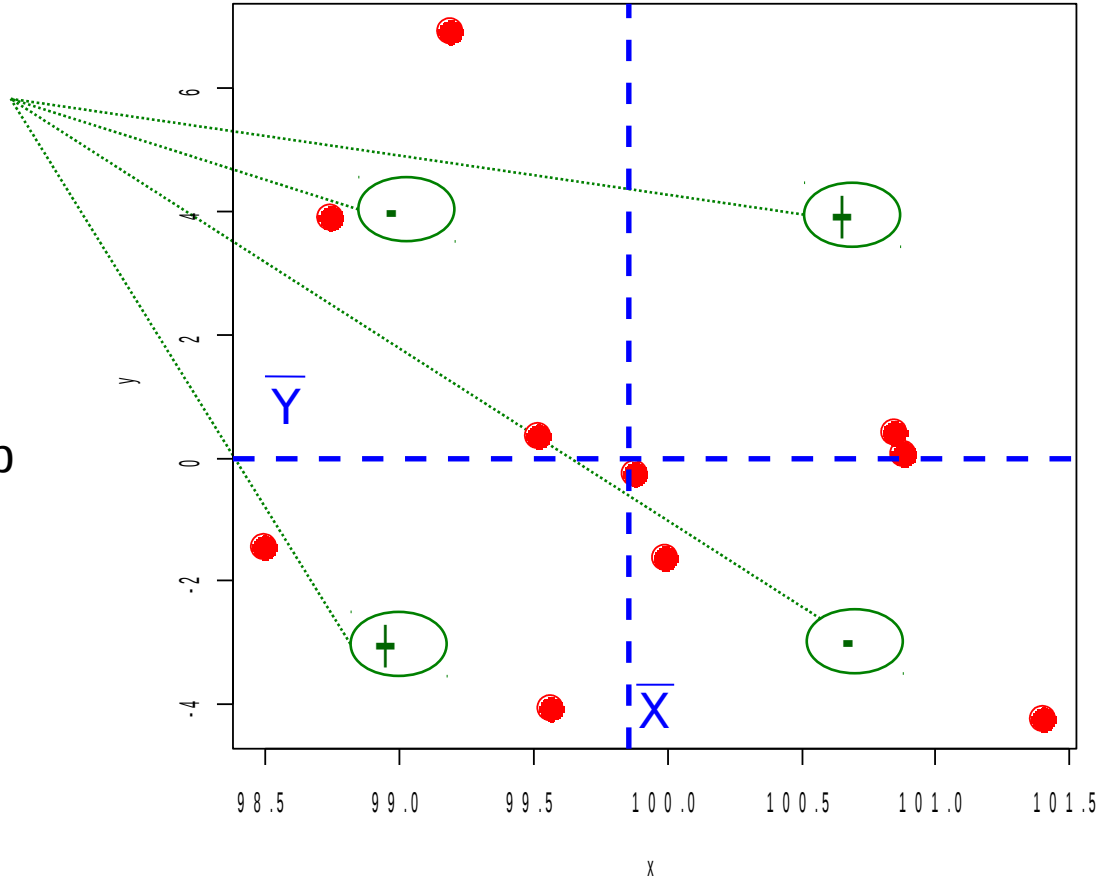
→positive linear relationship

- If the **–** win

→négative linear relationship

*On this example : cov(X,Y)=**-1.36***





The covariance depends on the physical units

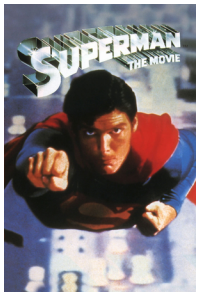
➔ correlation coefficient

Correlation

- Pearson correlation coefficient: $\rho(X,Y)=cov(X,Y)/(\sigma_X \sigma_Y)$

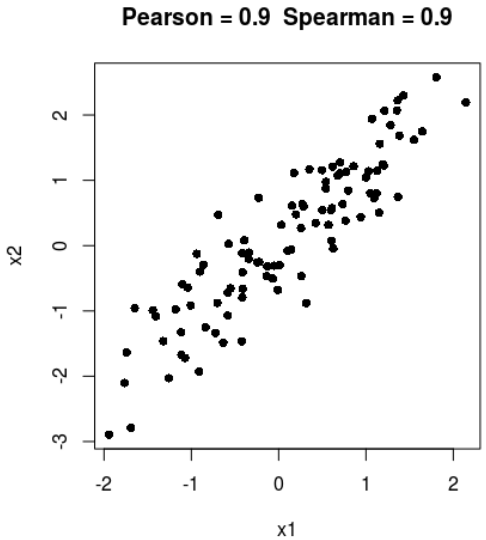
linear relationship

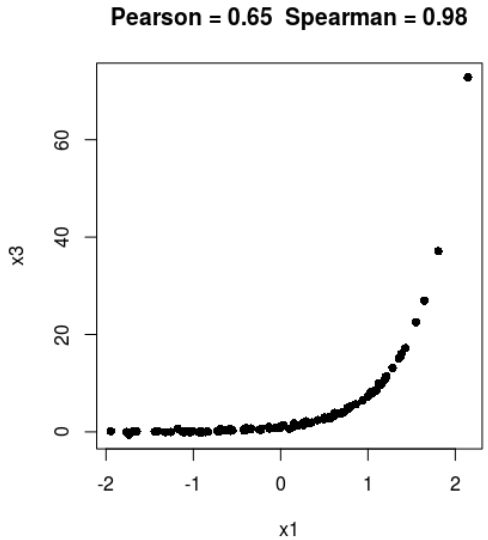
- Spearman correlation coefficient (ranks): $\rho_s(X,Y)=\rho(RX,RY)$

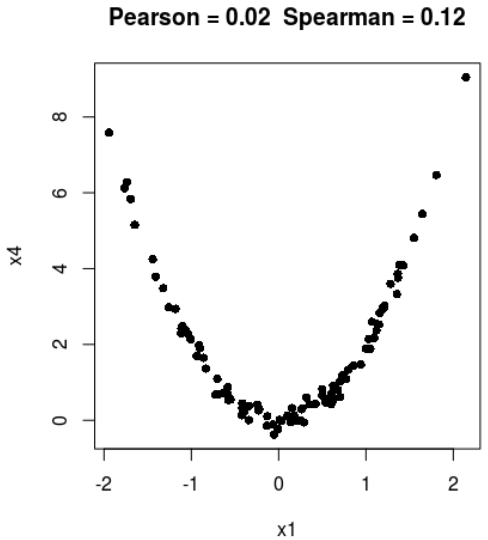


monotone relationship

- Between –1 and 1
- If the coefficient is positive : when a variable is high the other is also high. Replace high with low.
- If the coefficient is negative : when a variable is high the other is low. Replace high with low and inversely.







Linear combination

2 variables

2 coefficients : c1 = 0.5 ; c2 = 2 $\quad \mathbf{W} = \begin{pmatrix} 0.5 \\ 2 \end{pmatrix}$

Height	Weight
174.0	65.6
175.3	71.8
193.5	80.7
186.5	72.6
187.2	78.8
181.5	74.8
184.0	86.4
184.5	78.4
175.0	62.0
184.0	81.6

X

Linear combination of the 2 variables Height and Weight with coefficients c1 and c2

$$LC = 0.5 \begin{pmatrix} 174.0 & 65.6 \\ 175.3 & 71.8 \\ 193.5 & 80.7 \\ 186.5 & 72.6 \\ 187.2 & 78.8 \\ 181.5 & 74.8 \\ 184.0 & 86.4 \\ 184.5 & 78.4 \\ 175.0 & 62.0 \\ 184.0 & 81.6 \end{pmatrix} + 2 = \begin{pmatrix} 218.20 \\ 231.25 \\ 258.15 \\ 238.45 \\ 251.20 \\ 240.35 \\ 264.80 \\ 249.05 \\ 211.50 \\ 255.20 \end{pmatrix}$$

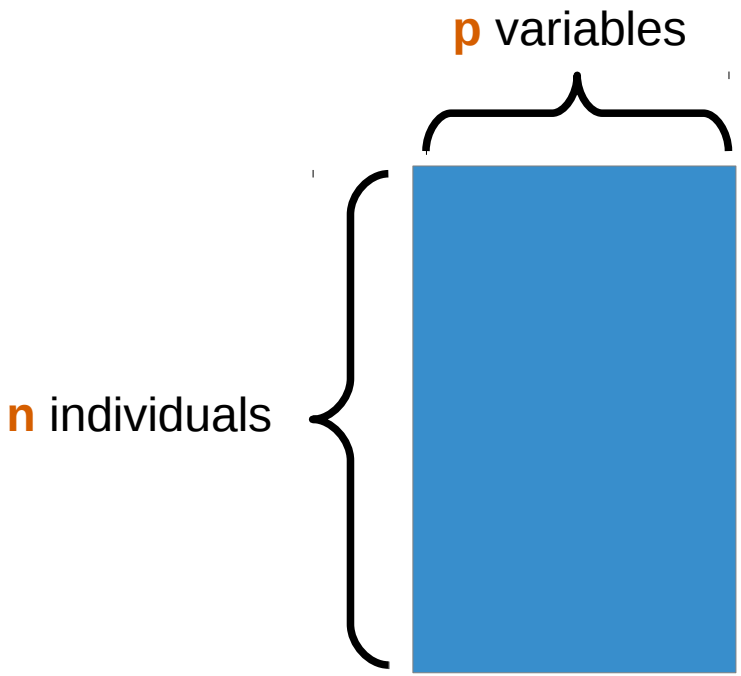
Matrix notation: **LC** = **XW**

A principal component is a linear combination of the initial variables.

Explore one data set

Principal Components Analysis

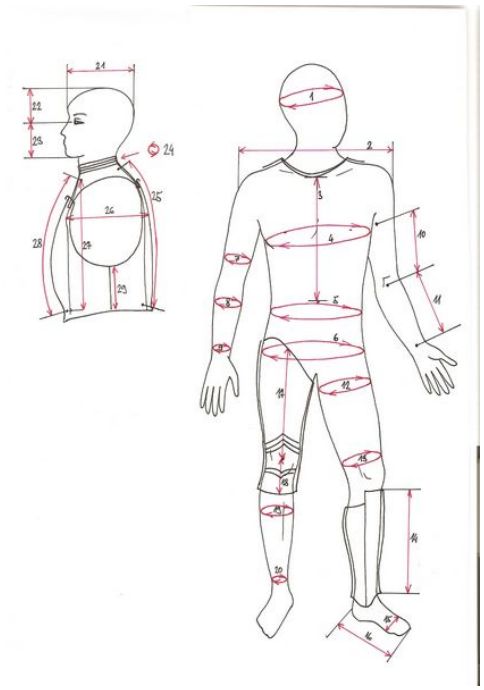
Describe with no prior a data set exclusively composed of **quantitatives** variables



Body data set

- 20 individuals
- 5 variables

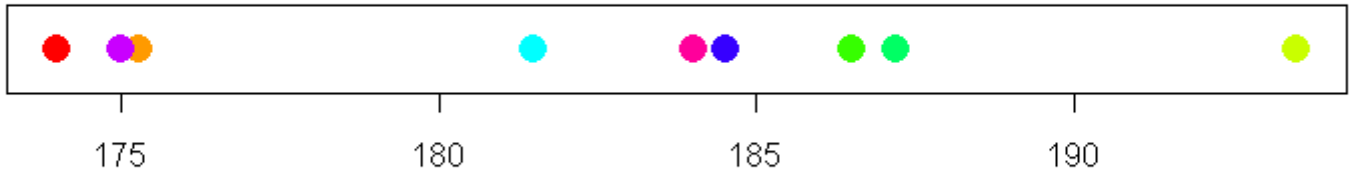
V1 : shoulder girth (cm)
V2 : chest girth (cm)
V3 : waist girth (cm)
V4 : weight (kg)
V5 : height (cm)



	V1	V2	V3	V4	V5
H 1	106.2	89.5	71.5	65.6	174.0
H 2	110.5	97.0	79.0	71.8	175.3
H 3	115.1	97.5	83.2	80.7	193.5
H 4	104.5	97.0	77.8	72.6	186.5
H 5	107.5	97.5	80.0	78.8	187.2
H 6	119.8	99.9	82.5	74.8	181.5
H 7	123.5	106.9	82.0	86.4	184.0
H 8	120.4	102.5	76.8	78.4	184.5
H 9	111.0	91.0	68.5	62.0	175.0
H 10	119.5	93.5	77.5	81.6	184.0
F 1	105.0	89.0	71.2	67.3	169.5
F 2	100.2	94.1	79.6	75.5	160.0
F 3	99.1	90.8	77.9	68.2	172.7
F 4	107.6	97.0	69.6	61.4	162.6
F 5	104.0	95.4	86.0	76.8	157.5
F 6	108.4	91.8	69.9	71.8	176.5
F 7	99.3	87.3	63.5	55.5	164.4
F 8	91.9	78.1	57.9	48.6	160.7
F 9	107.1	90.9	72.2	66.4	174.0
F 10	100.5	97.1	80.4	67.3	163.8

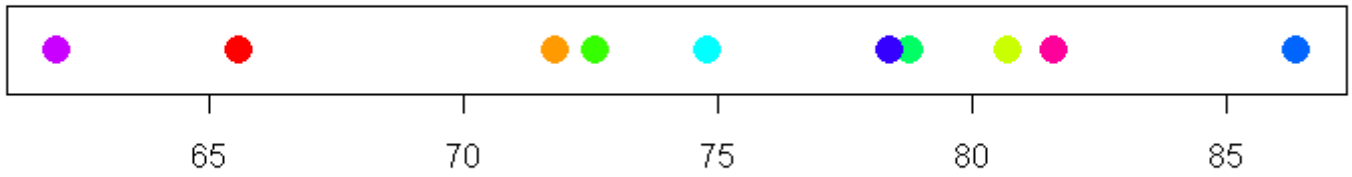
1D graphical output: stripchart

Height 174.0 175.3 193.5 186.5 187.2 181.5 184.0 184.5 175.0 184.0



Height in cm

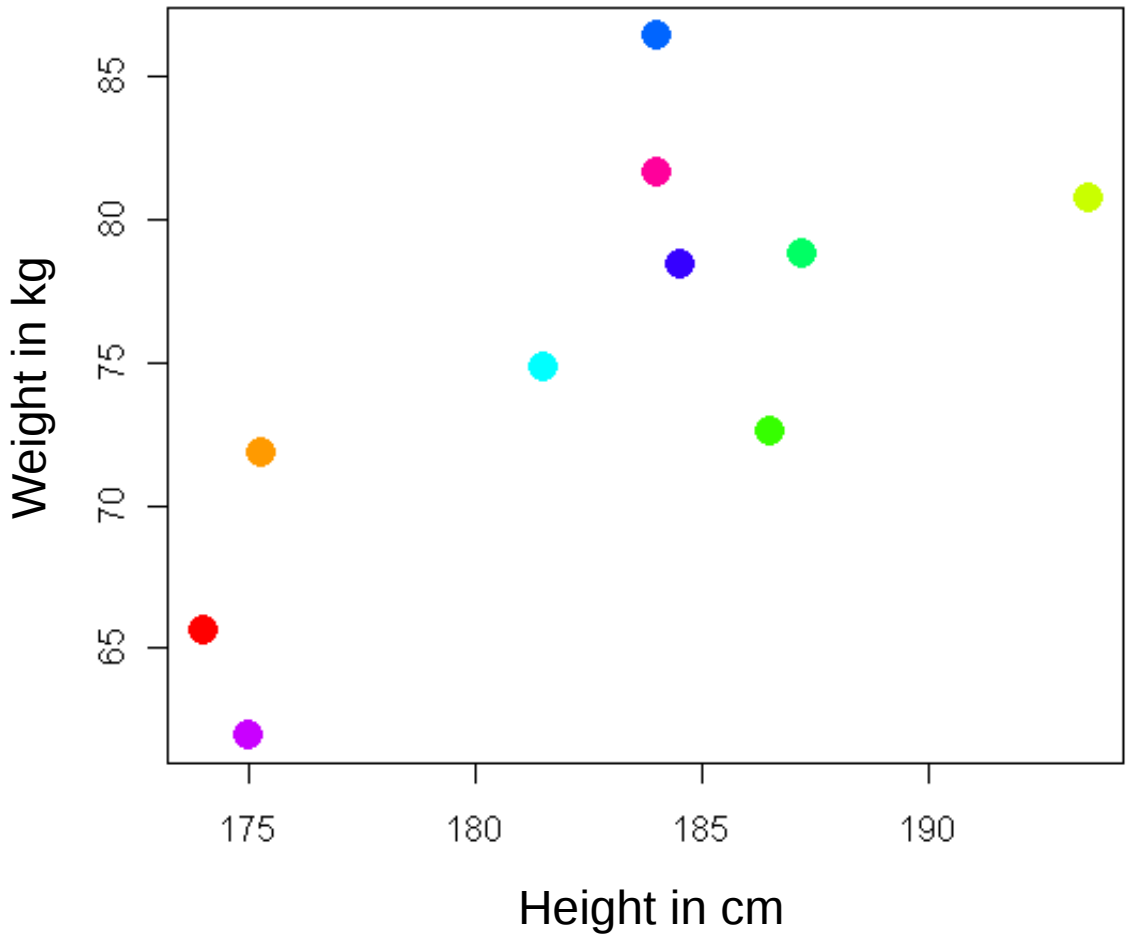
Weight 65.6 71.8 80.7 72.6 78.8 74.8 86.4 78.4 62.0 81.6



Weight in kg

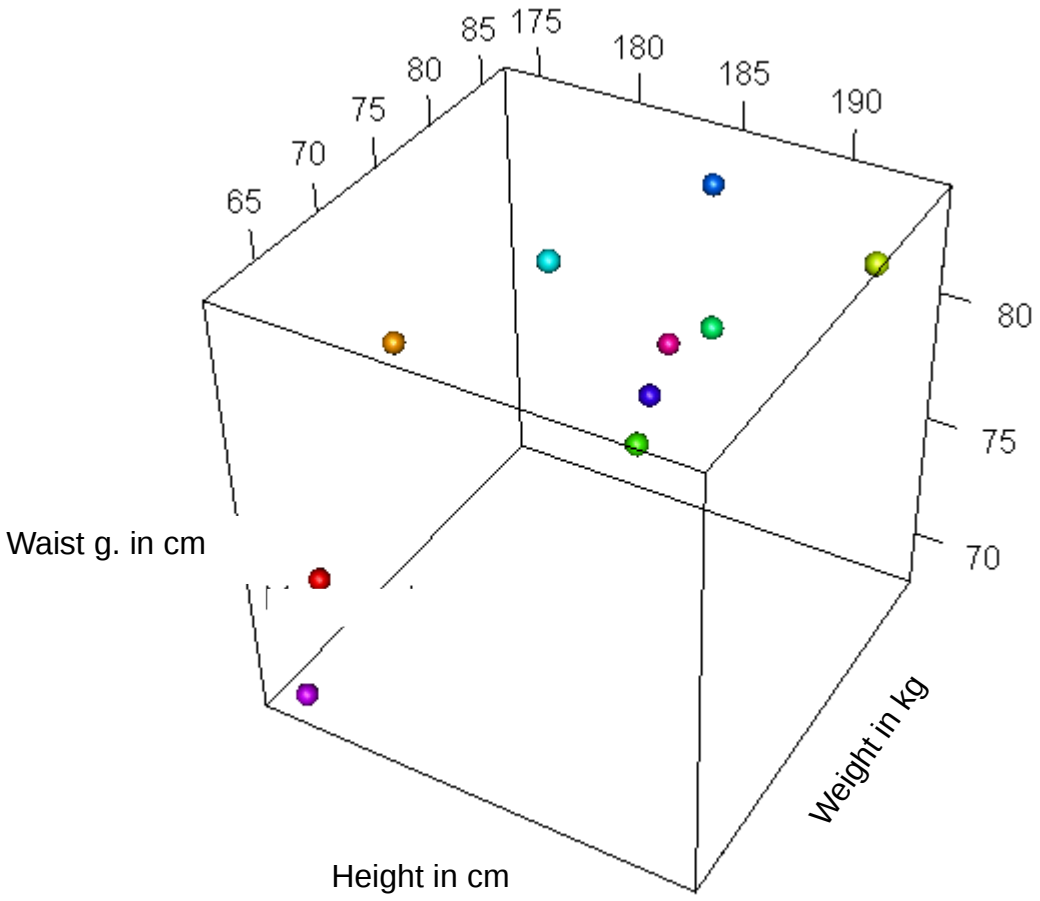
2D graphical output: scatter plot

Height	174.0	175.3	193.5	186.5	187.2	181.5	184.0	184.5	175.0	184.0
Weight	65.6	71.8	80.7	72.6	78.8	74.8	86.4	78.4	62.0	81.6

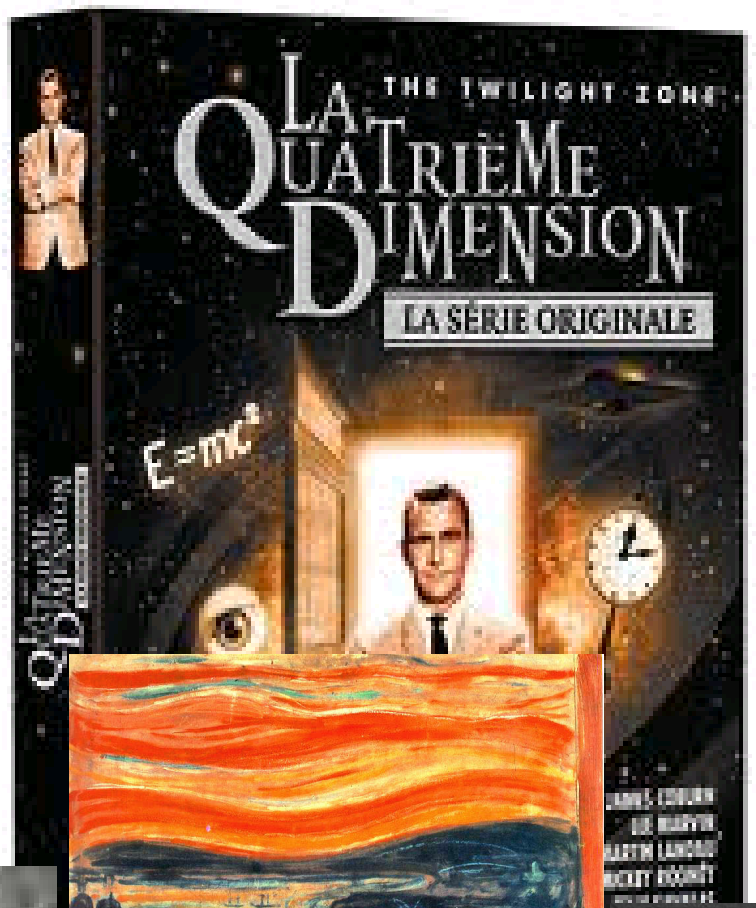


3D graphical output: scatter plot

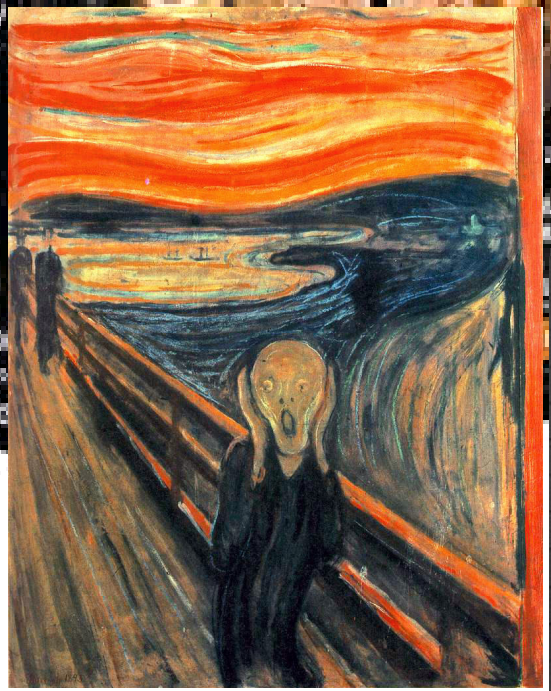
Height	174.0	175.3	193.5	186.5	187.2	181.5	184.0	184.5	175.0	184.0
Weight	65.6	71.8	80.7	72.6	78.8	74.8	86.4	78.4	62.0	81.6
Waist g.	71.5	79.0	83.2	77.8	80.0	82.5	82.0	76.8	68.5	77.5



4D ?

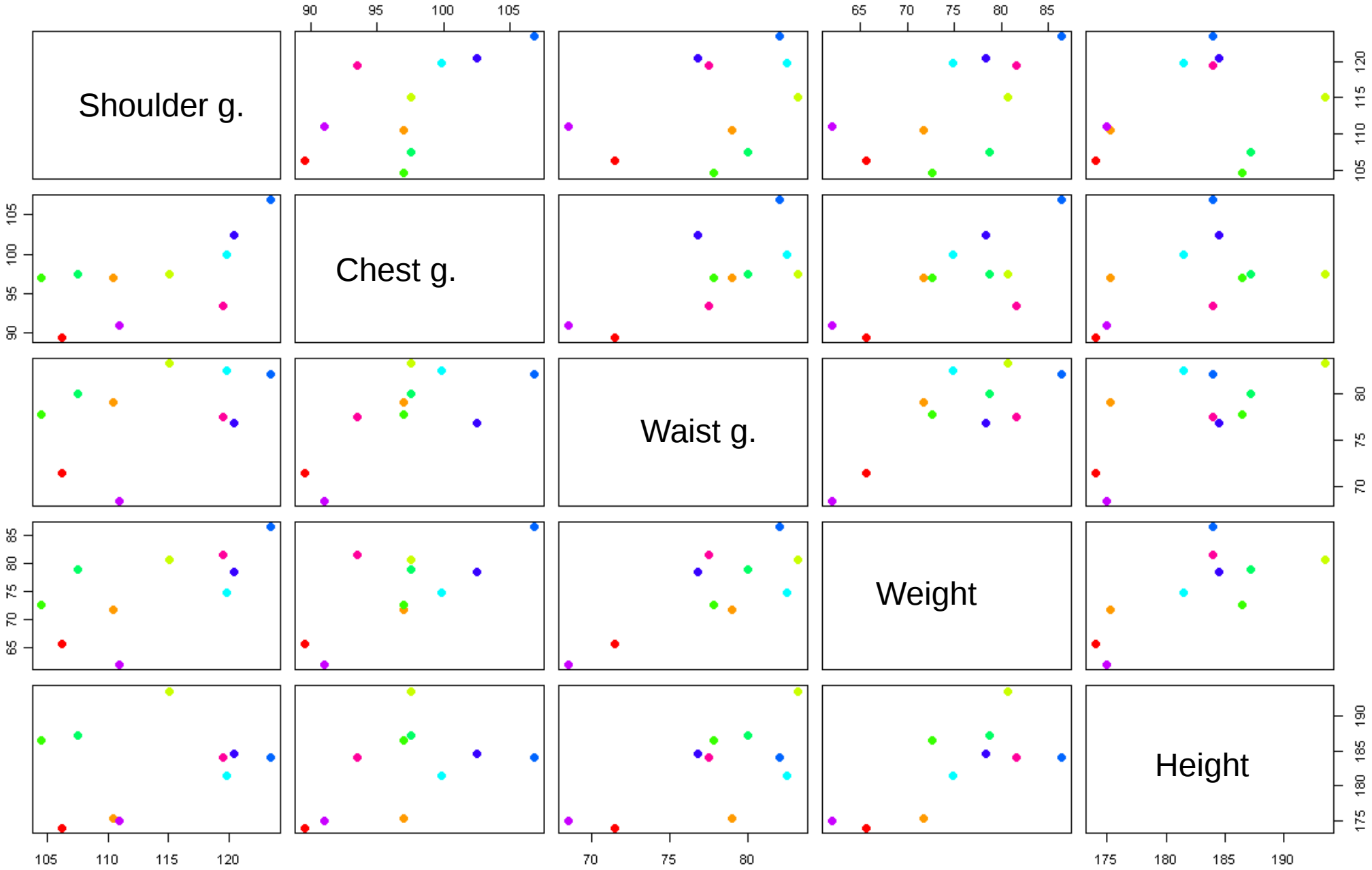

THE TWILIGHT ZONE
LA QUATRIÈME DIMENSION
LA SÉRIE ORIGINALE
E=mc²

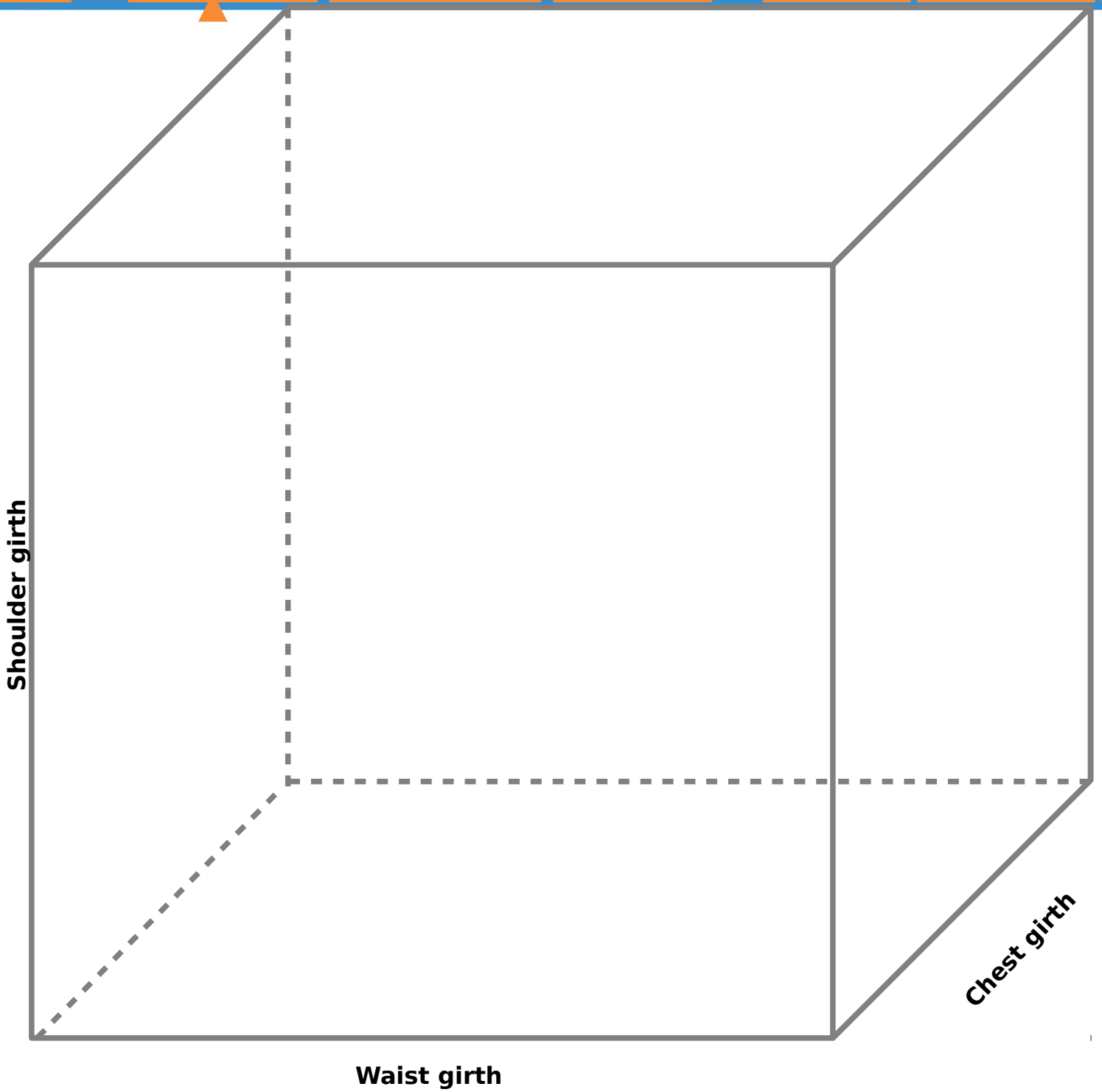

DAY!

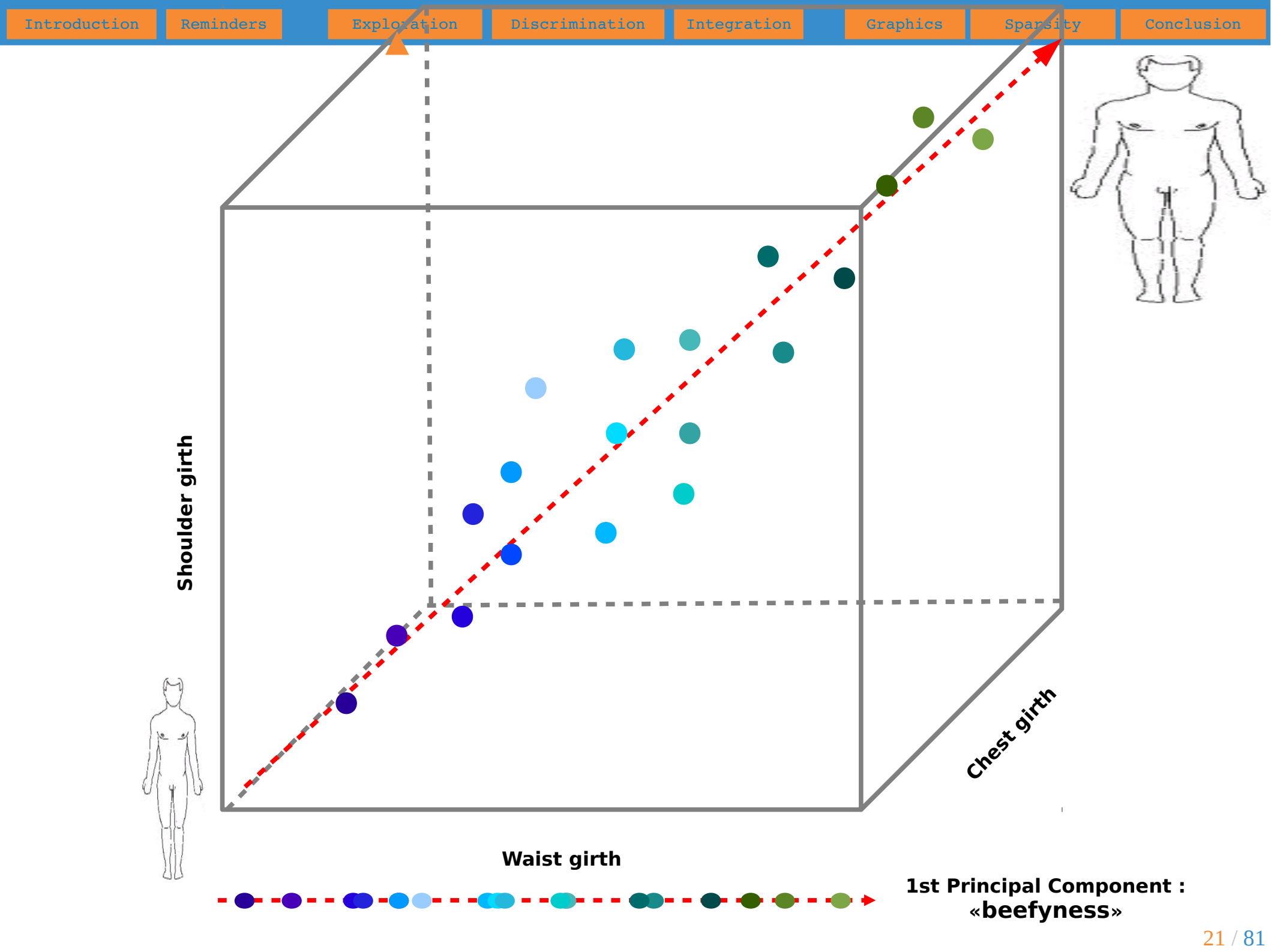



Ship Stolen
Need Money
To Get Home

Alternative to 4D (or more)

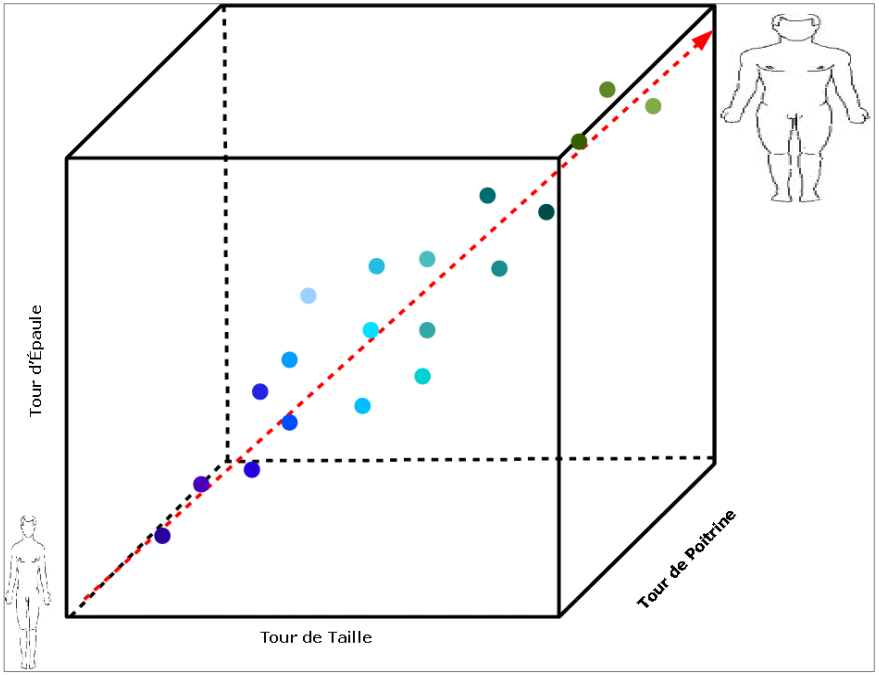



Shoulder girth
Waist girth
Chest girth


Shoulder girth
Chest girth
Waist girth
1st Principal Component :
«beefyness»

Comments

The measurements are rather **strongly correlated**. Indeed, one can assume that a person with a high shoulder girth will also have high chest girth (even if exceptions exist...). In these conditions, the information brought by the 5 variables are **redundant**. Graphically, in the cube determined by shoulder girth, chest girth and waist girth, there are nearly empty areas. One variable calculated as a **combination** of these 3 variables (represented as the dotted arrow) would be enough to represent the individuals with a **minimal loss in information** because all the points are located along these direction that is the first principal component.



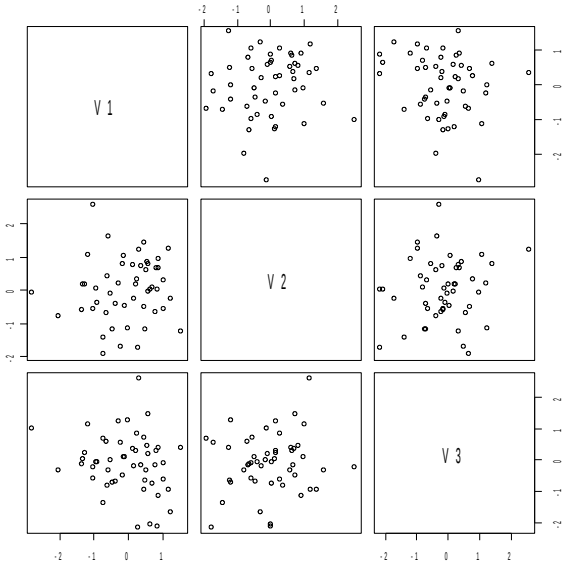
In other words

- PCA allow determine the sub-spaces of lower dimension than the initial space on which the projection of the individuals is the **least modified**, that is to say, the sub-spaces that keep the **greatest part of the information** (i.e. **variability**).
- The principle of PCA consists in finding a direction (the first PC), calculated as a **linear combination of the initial variables**, such that the **variance** of the points around this direction is **maximal**. Iterate this process in orthogonal directions to determine the following principal components. The number of PC that can be calculated is equal to the number of initial variables.
- Concerning the variables, the PCA keeps at best the **correlation structure** between the initial variables.

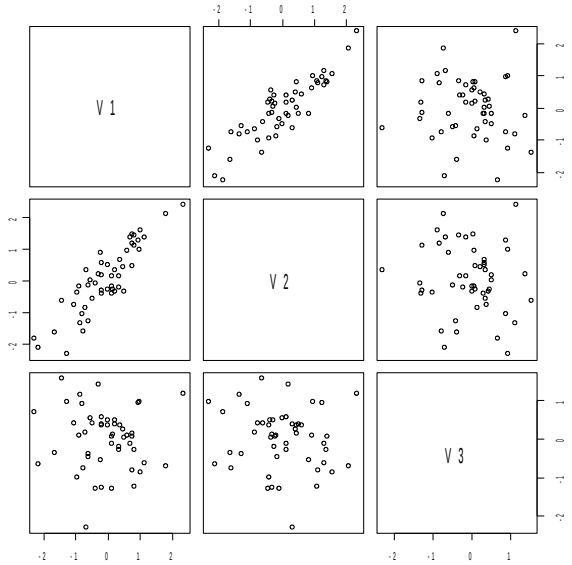
PCA: simulated examples

Data set : 50 observations, 3 variables (V1 – V2 - V3)

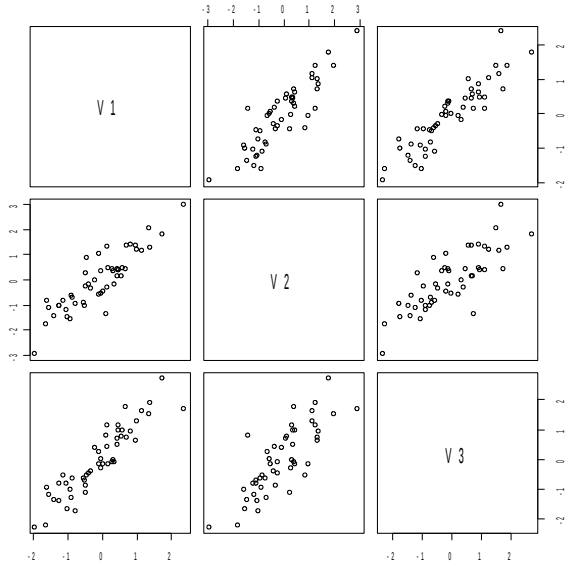
Case 1)
{V1} - {V2} - {V3}



Case 2)
{V1 - V2} - {V3}



Case 3)
{V1 - V2 - V3}



Pearson Correlation matrices

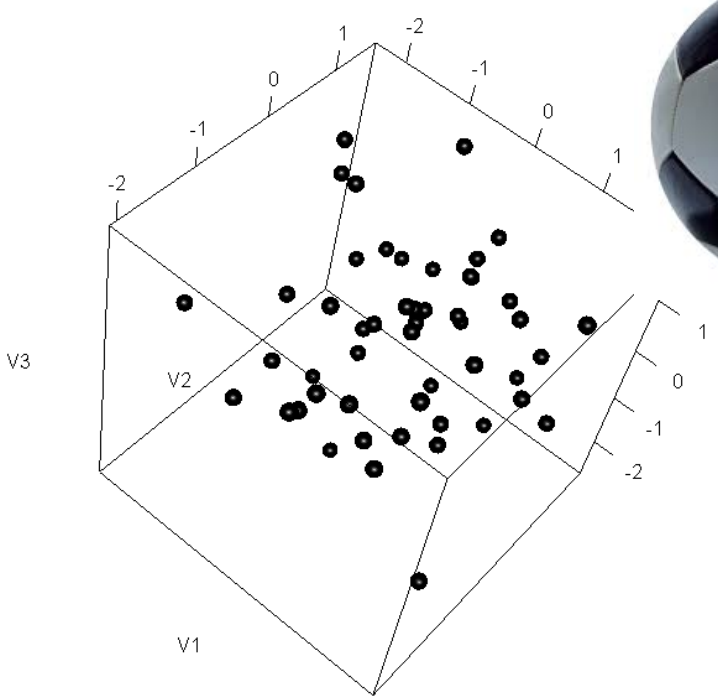
1)	V1	V2	V3
V1	1.0	-0.10	0.00
V2	-0.1	1.00	-0.12
V3	0.0	-0.12	1.00

2)	V1	V2	V3
V1	1.00	0.88	-0.05
V2	0.88	1.00	-0.11
V3	-0.05	-0.11	1.00

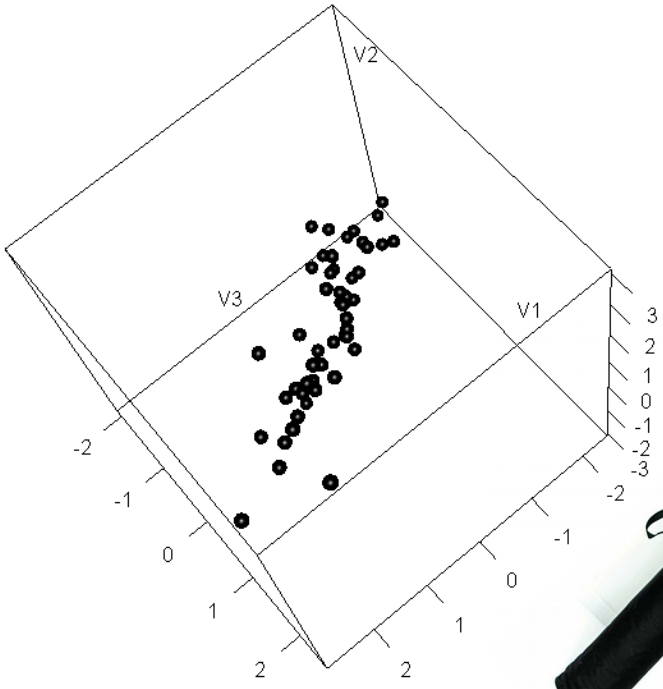
3)	V1	V2	V3
V1	1.00	0.88	0.92
V2	0.88	1.00	0.81
V3	0.92	0.81	1.00

Example: 3D scatter plots

Case 1)

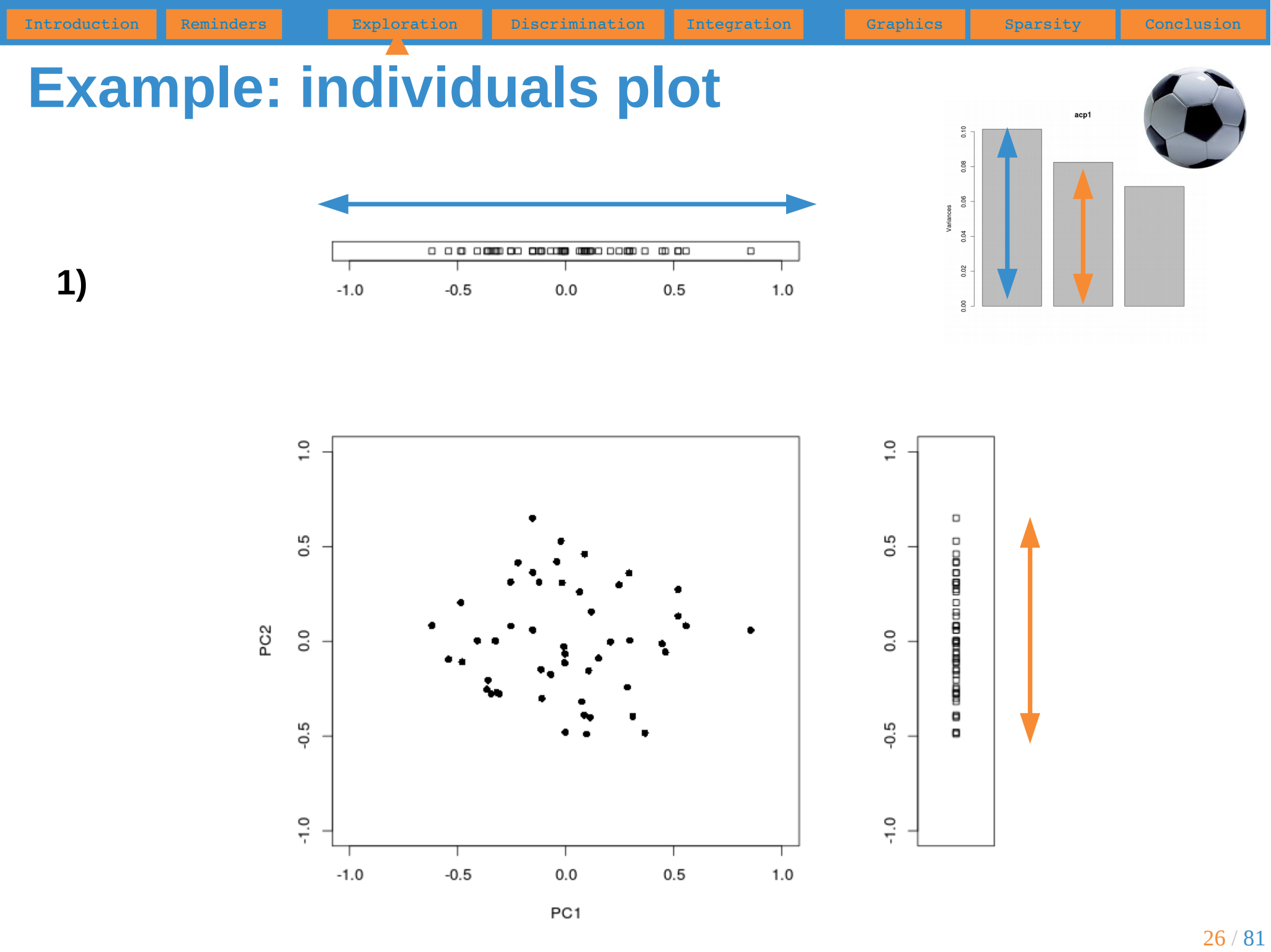


Case 2)



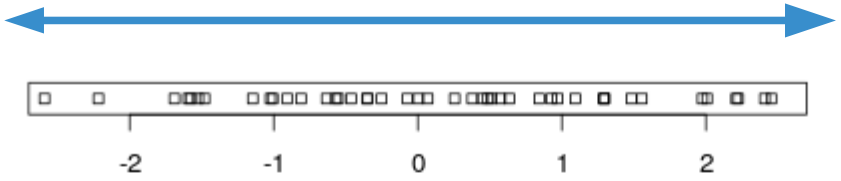
Case 3)

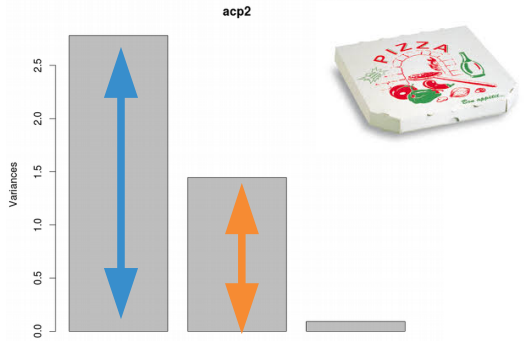
Example: individuals plot

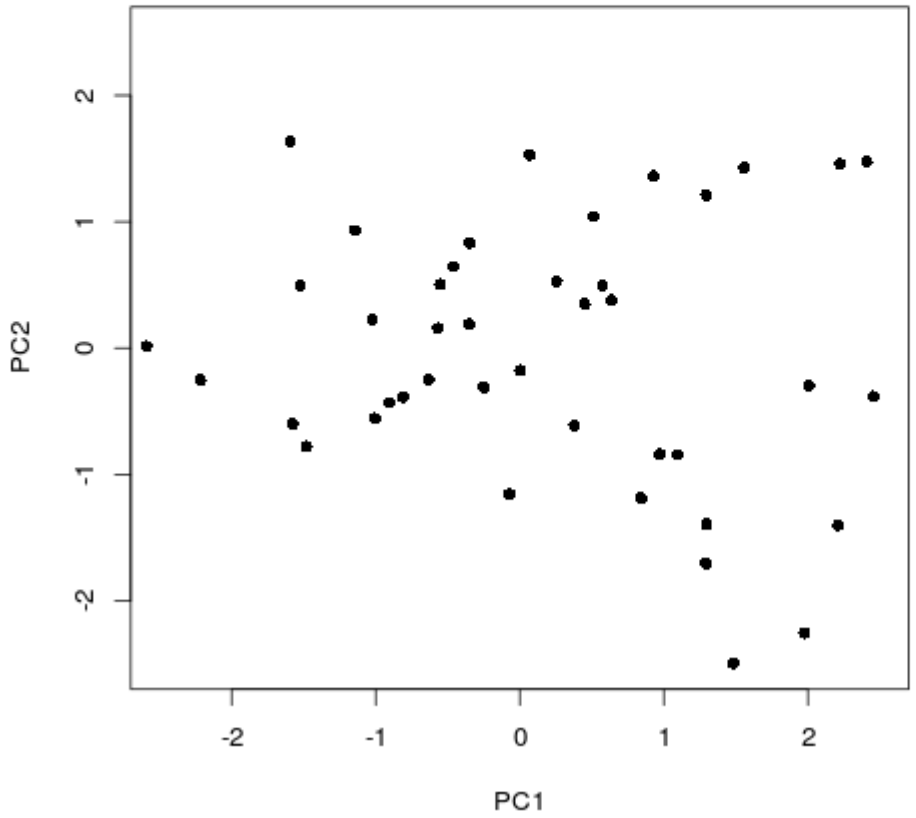


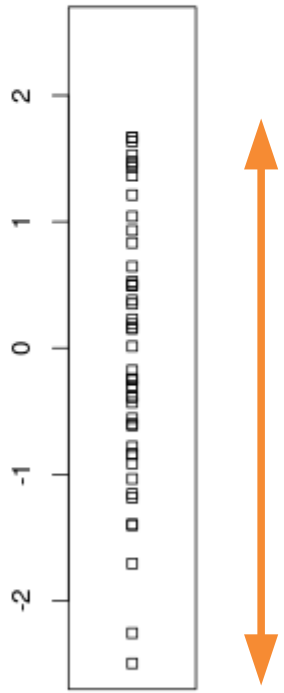
Example: individuals plot

2)

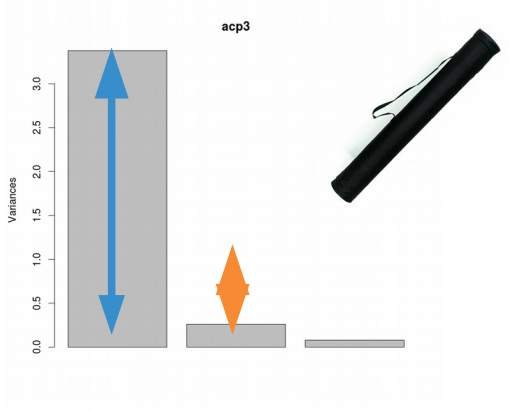




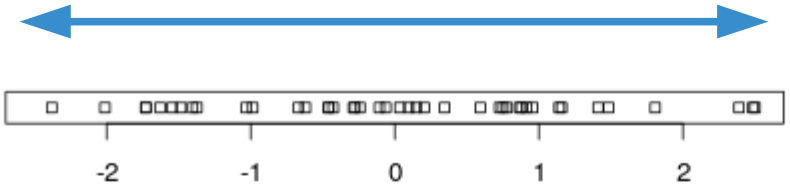


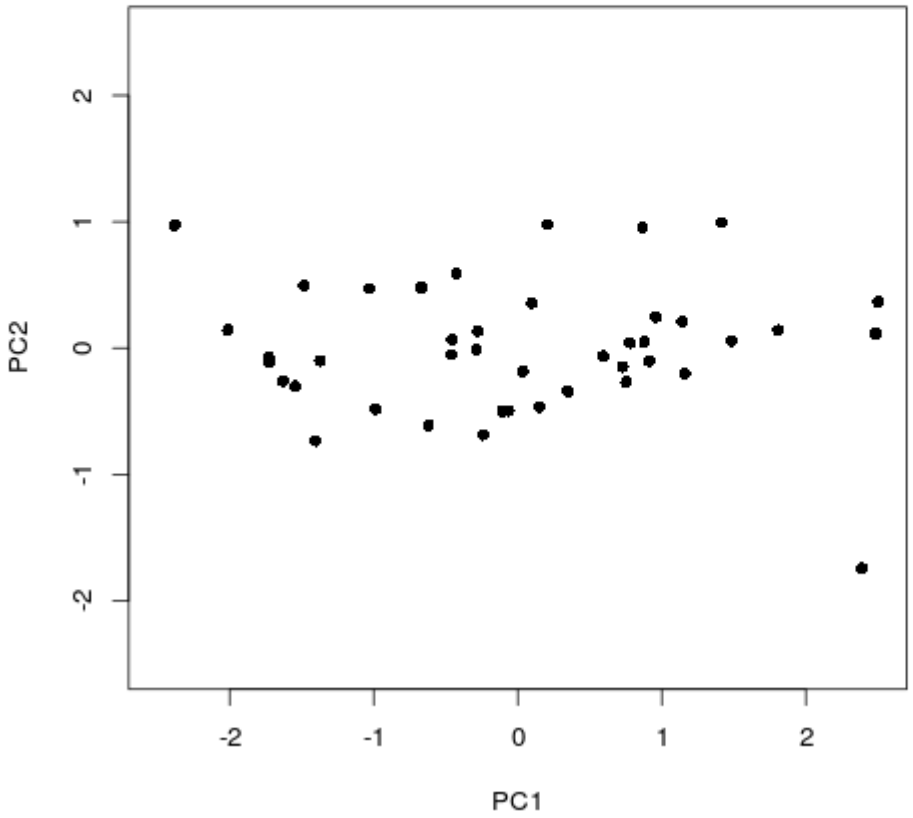


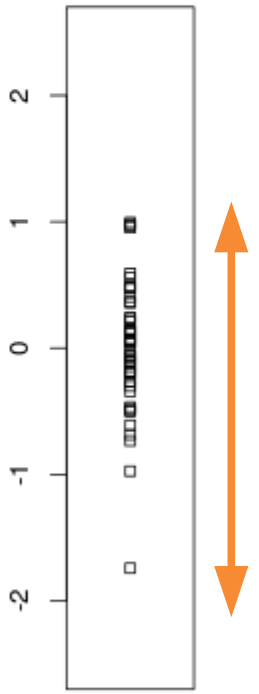
Example: individuals plot



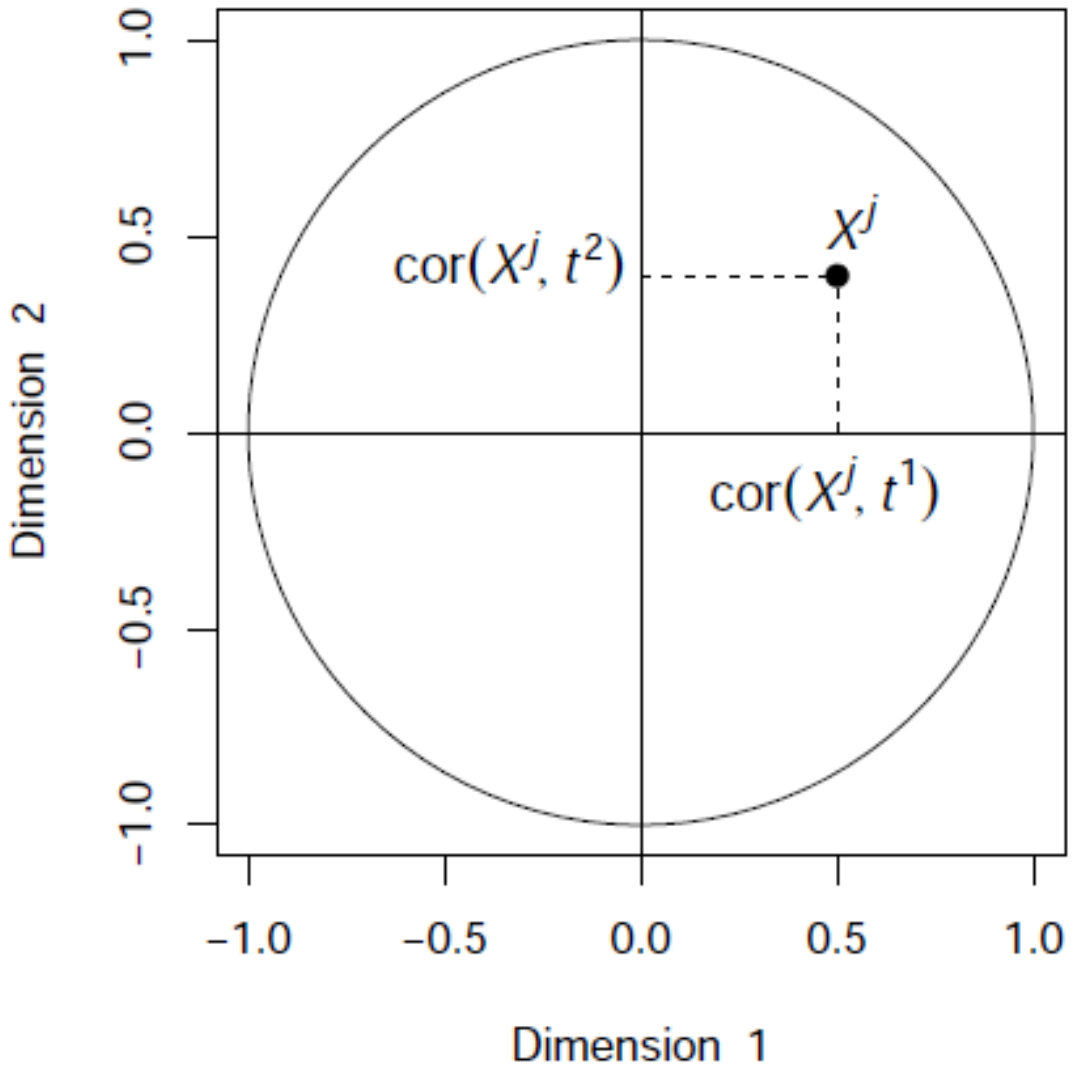
3)







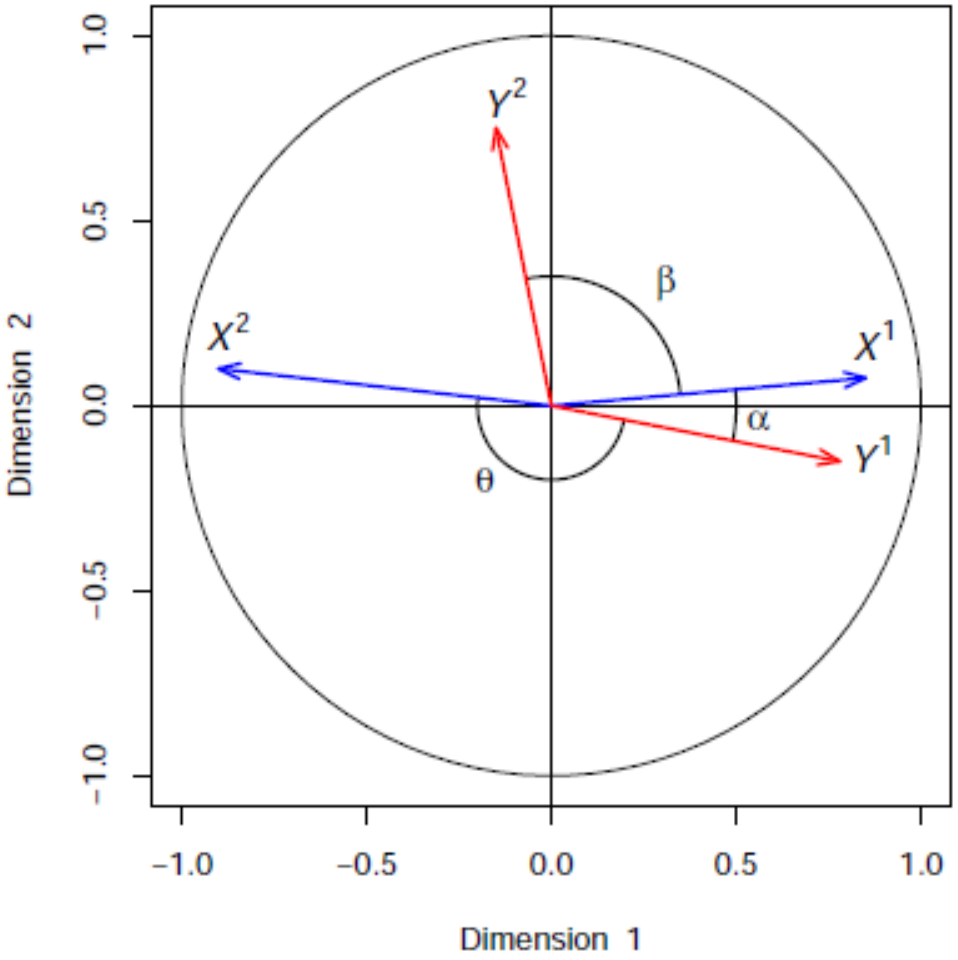
Variables plot



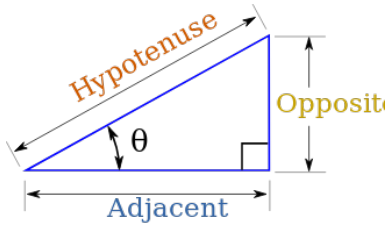
The coordinates of a variable X^j on a principal component PC^i is given by the correlation between this variable and the component PC^i.

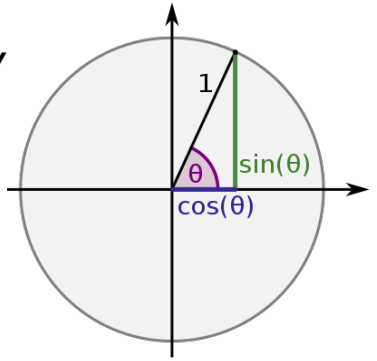
Variables plot

Correlation ≈ cosine



Remember trigonometry and right triangles:





The correlation between two variables is represented as:

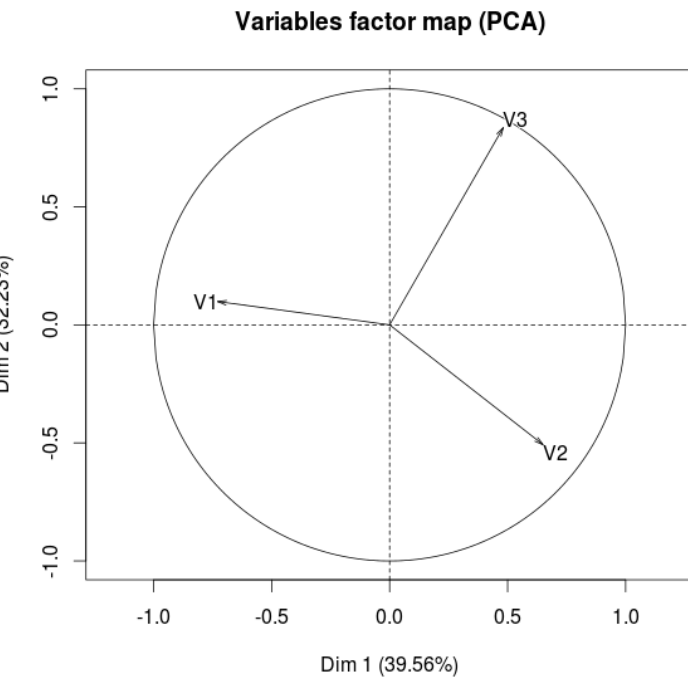
- An acute angle ($\cos(\alpha) > 0$) if it is positive
- An obtuse angle ($\cos(\theta) < 0$) if it is negative
- A right angle ($\cos(\beta) \approx 0$) if it is near zero

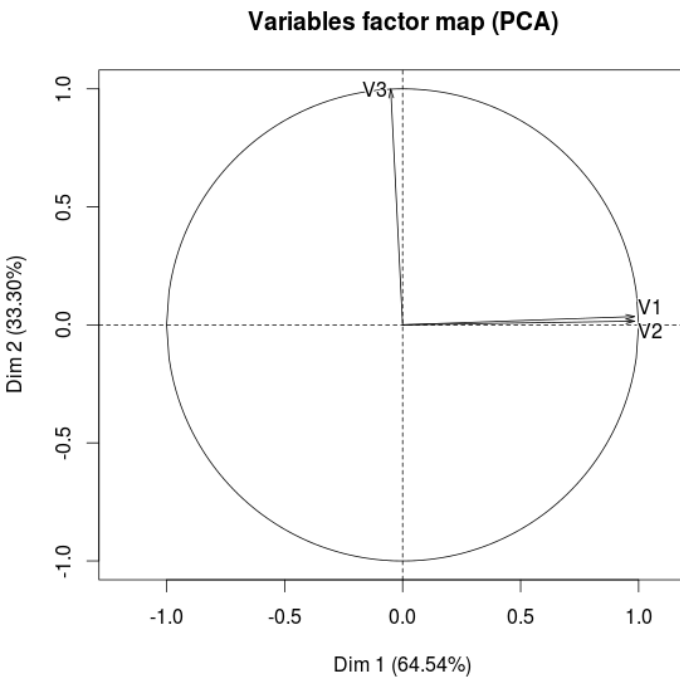
Variables plot

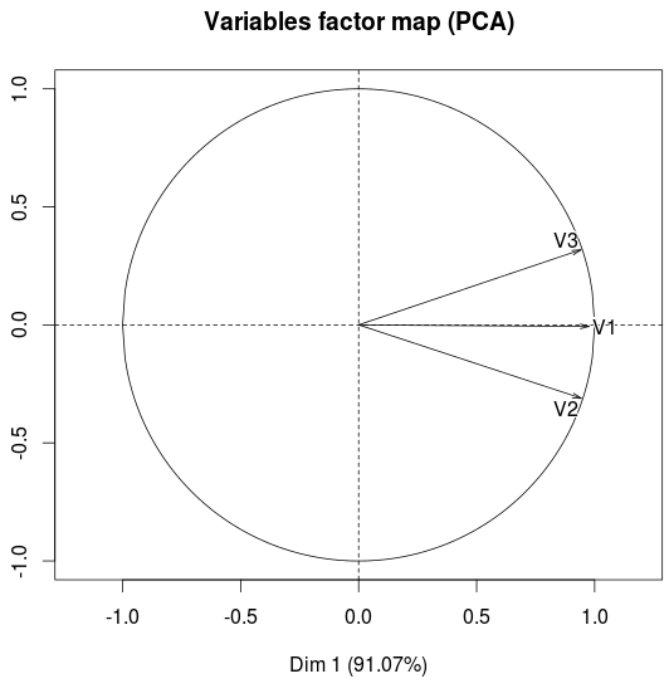
1) 

2) 

3) 







Correlation matrices

1)	V1	V2	V3
V1	1.0	-0.10	0.00
V2	-0.1	1.00	-0.12
V3	0.0	-0.12	1.00

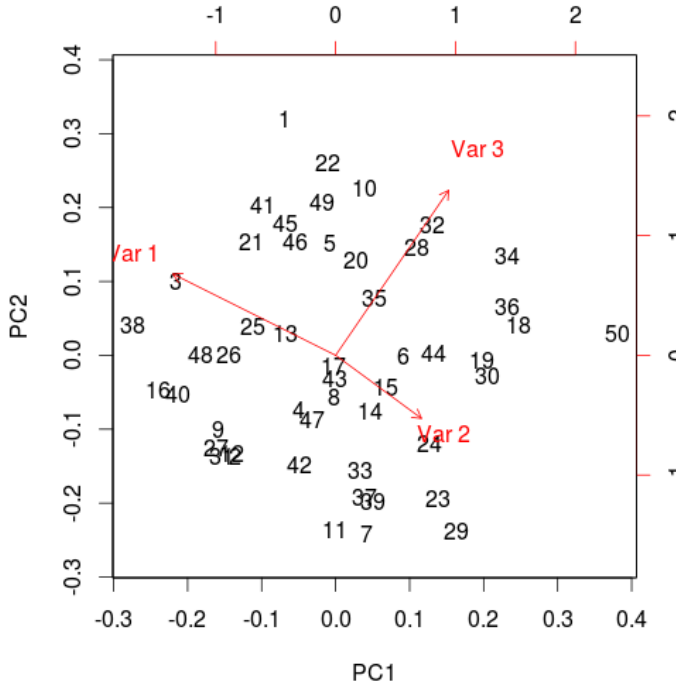
2)	V1	V2	V3
V1	1.00	0.88	-0.05
V2	0.88	1.00	-0.11
V3	-0.05	-0.11	1.00

3)	V1	V2	V3
V1	1.00	0.88	0.92
V2	0.88	1.00	0.81
V3	0.92	0.81	1.00

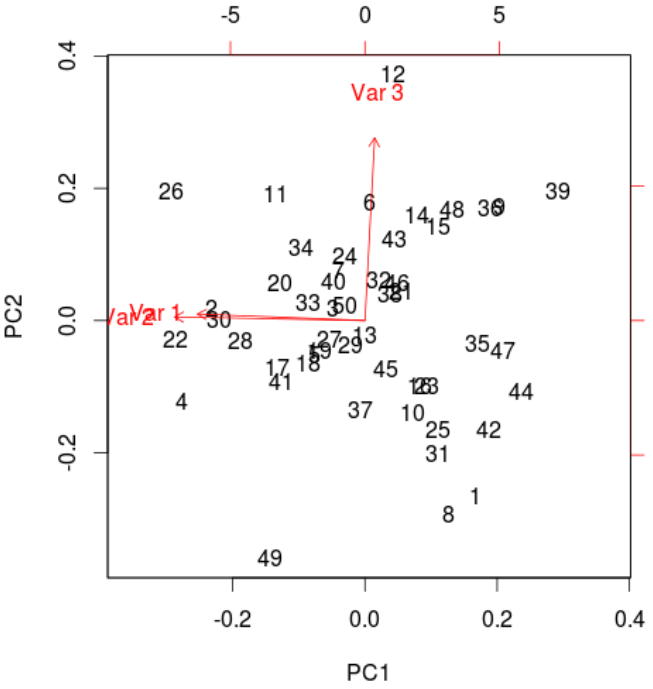
Example: biplot representation

Individuals and variables are plotted on the same graph

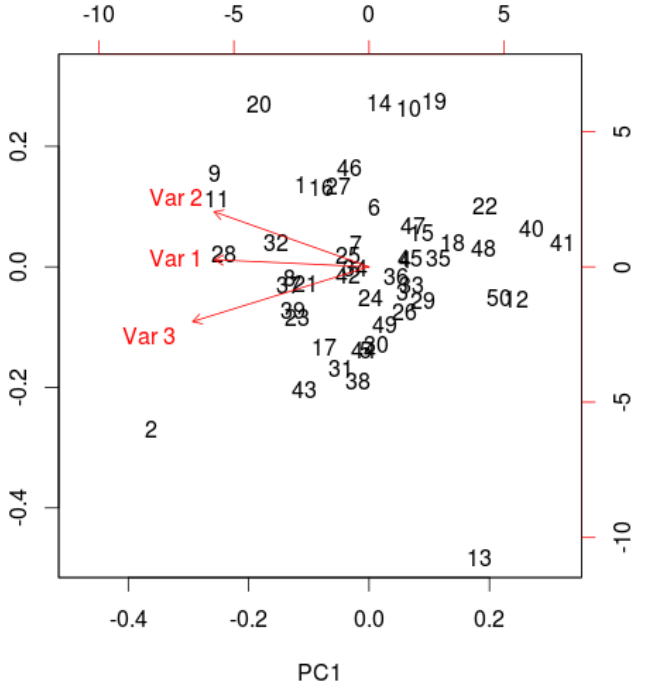
1) 



2) 



3)



And what about PCA?

- Mathematically, to perform a PCA consists in diagonalising the covariance (or the correlation for scaled PCA) matrix.
- Indeed, it can be shown that the sub-space in which the projected points have a maximal variance is given by the first eigen vectors of the covariance (or correlation) matrix ; the variance are given by the corresponding eigen values.
- The first eigen vector provides the direction (via the coefficients of the linear combination to apply to initial variables) that explains the greatest part of variability. The second explains the greatest past of the remaining variance and so on...

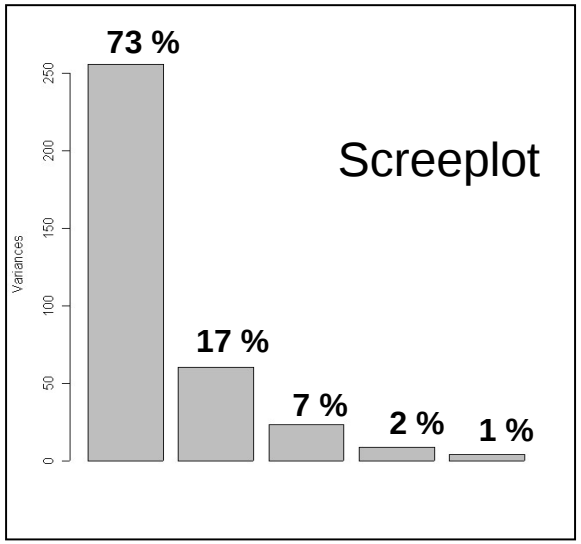
PCA: practical aspects

- Should I scale my data before performing PCA?
  - Without scaling: one variable with high variance will structure nearly alone the first principal component
  - With scaling: one noisy variable with low variability will be given the same variance as others meaningful variables
- Can I perform PCA with missing values?
  - Specific algorithms to deal with missing values exist (for instance, NIPALS - implemented in mixOmics). It can be used to impute missing values but it requires « many » components.

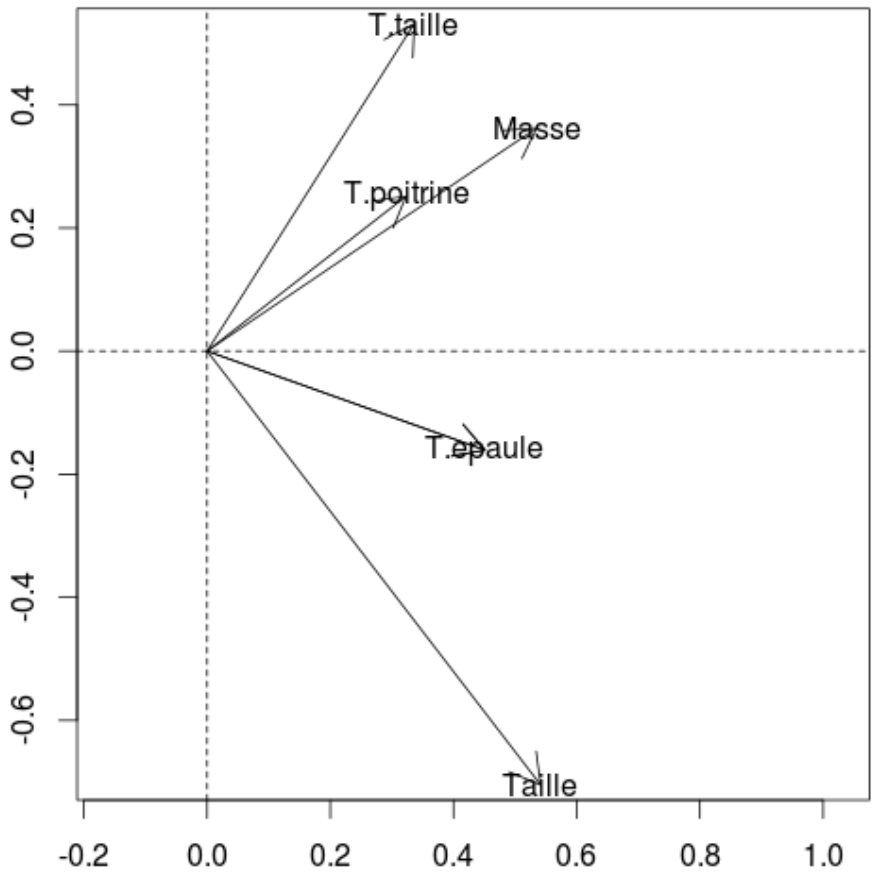
The best thing to do about missing data is not to have any.

Gertrude Cox, 1900-1978, American statistician

PCA: *body* data set



Variables plot

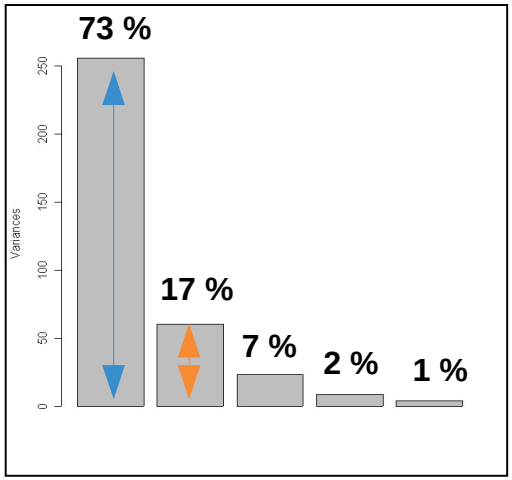


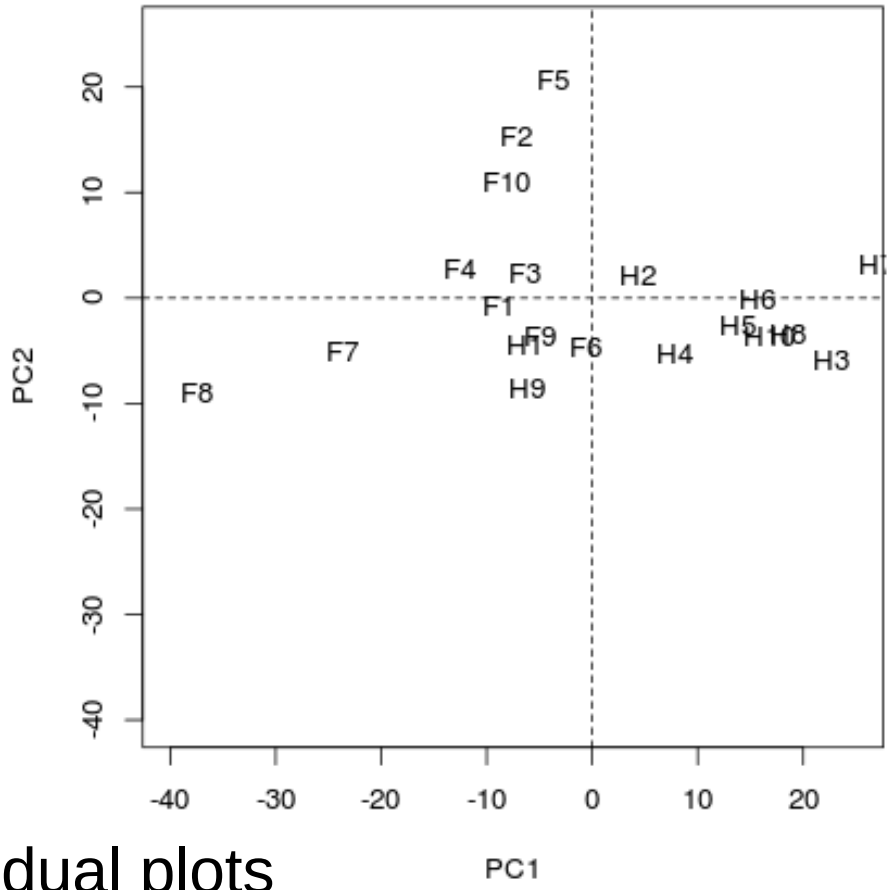
- 90% of the variability is explained by the first two PCs
- 10% of the information is lost when projecting from 5 to 2 dimensions.
- PC 1 «beefyness»: separation of beefy people on the right (high values for the 5 variables) and weakling ones on the left.
- PC 2 «fatness, rotundity»: bottom, variables linked to height and shoulders; top, weight, waist and chest girth.

Correlation matrix

	T.ep	T.p	T.t	M	T
T.ep	1.00	0.74	0.48	0.72	0.71
T.p	0.74	1.00	0.78	0.81	0.51
T.t	0.48	0.78	1.00	0.86	0.37
M	0.72	0.81	0.86	1.00	0.61
T	0.71	0.51	0.37	0.61	1.00

PCA: *body* data set

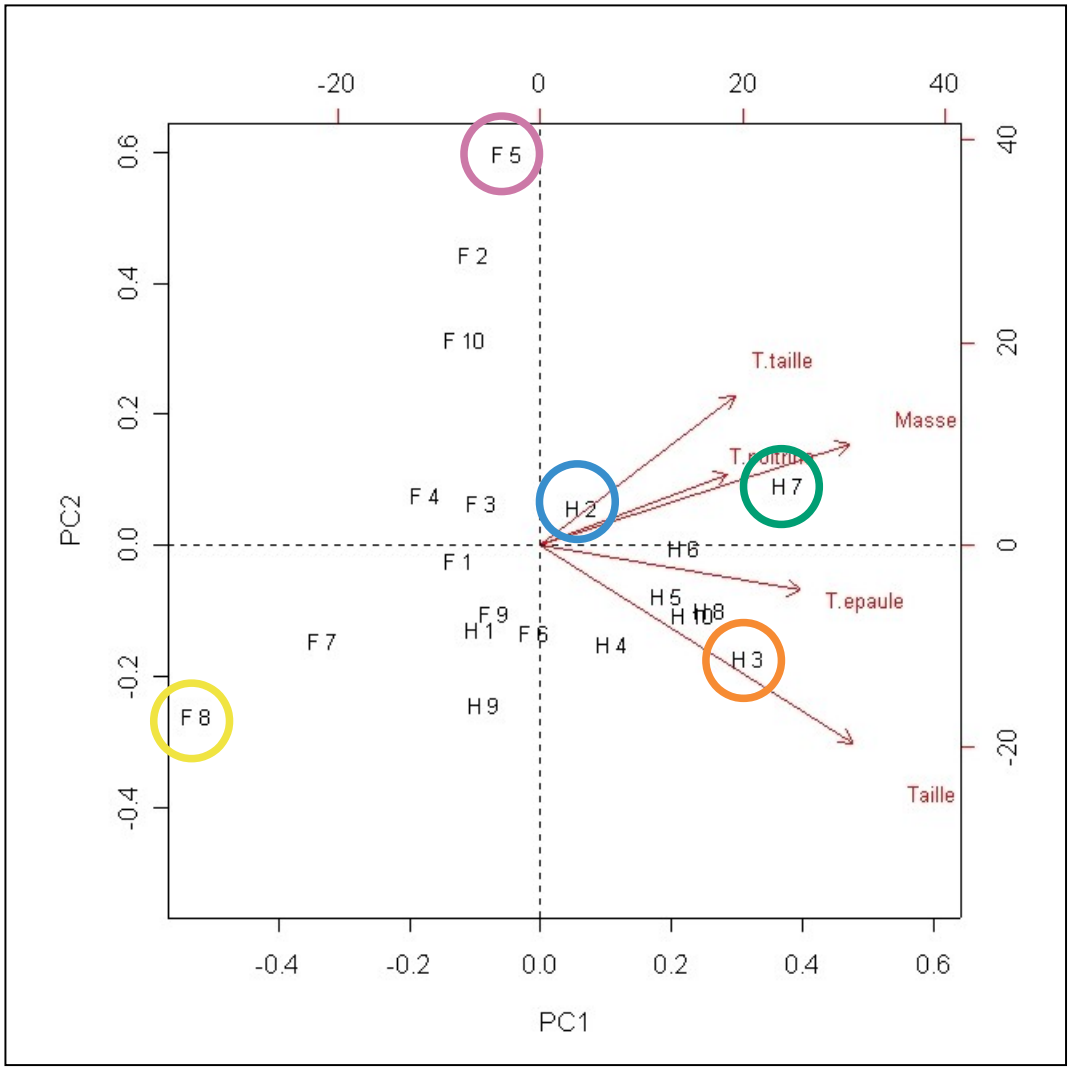




Individual plots

PCA: *body* data set

	s.g	c.g	w.g	w	h
H 1	106.2	89.5	71.5	65.6	174.0
H 2	110.5	97.0	79.0	71.8	175.3
H 3	115.1	97.5	83.2	80.7	193.5
H 4	104.5	97.0	77.8	72.6	186.5
H 5	107.5	97.5	80.0	78.8	187.2
H 6	119.8	99.9	82.5	74.8	181.5
H 7	123.5	106.9	82.0	86.4	184.0
H 8	120.4	102.5	76.8	78.4	184.5
H 9	111.0	91.0	68.5	62.0	175.0
H 10	119.5	93.5	77.5	81.6	184.0
F 1	105.0	89.0	71.2	67.3	169.5
F 2	100.2	94.1	79.6	75.5	160.0
F 3	99.1	90.8	77.9	68.2	172.7
F 4	107.6	97.0	69.6	61.4	162.6
F 5	104.0	95.4	86.0	76.8	157.5
F 6	108.4	91.8	69.9	71.8	176.5
F 7	99.3	87.3	63.5	55.5	164.4
F 8	91.9	78.1	57.9	48.6	160.7
F 9	107.1	90.9	72.2	66.4	174.0
F 10	100.5	97.1	80.4	67.3	163.8



Origin (coordinate (0,0)): average individual

s.g	c.g	w.g	w	h
108.1	94.2	75.4	70.6	174.4

PCA: *body* data set

Data

	s.g	c.g	w.g	w	h
H 1	106.2	89.5	71.5	65.6	174.0
H 2	110.5	97.0	79.0	71.8	175.3
H 3	115.1	97.5	83.2	80.7	193.5
H 4	104.5	97.0	77.8	72.6	186.5
H 5	107.5	97.5	80.0	78.8	187.2
H 6	119.8	99.9	82.5	74.8	181.5
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H 8	120.4	102.5	76.8	78.4	184.5
H 9	111.0	91.0	68.5	62.0	175.0
H 10	119.5	93.5	77.5	81.6	184.0
F 1	105.0	89.0	71.2	67.3	169.5
F 2	100.2	94.1	79.6	75.5	160.0
F 3	99.1	90.8	77.9	68.2	172.7
F 4	107.6	97.0	69.6	61.4	162.6
F 5	104.0	95.4	86.0	76.8	157.5
F 6	108.4	91.8	69.9	71.8	176.5
F 7	99.3	87.3	63.5	55.5	164.4
F 8	91.9	78.1	57.9	48.6	160.7
F 9	107.1	90.9	72.2	66.4	174.0
F 10	100.5	97.1	80.4	67.3	163.8
Mean	108.1	94.2	75.3	70.6	174.4
Var.	68.6	37.5	50.8	85.7	109.3

Covariance matrix

	s.g	c.g	w.g	w	h
Shoulder.g	**68.64**	37.74	28.08	55.32	61.19
Chest.g	37.74	**37.51**	33.90	45.70	32.40
Waist.g	28.08	33.90	**50.77**	56.58	27.70
Weight	55.32	45.70	56.58	**85.71**	59.52
Height	61.19	32.40	27.70	59.52	**109.31**

68.64 + 37.51 + 50.77 + 85.71 + 109.31 = 351.94

351.94 represents (somehow) the quantity of information contained in the data.

PCA: *body* data set

Coefficients (optimally calculated) to build principal components

	PC1	PC2	PC3	PC4	PC5
shoulder.g	0.45	-0.16	0.78	-0.18	0.36
chest.g	0.32	0.25	0.26	0.72	-0.49
waist.g	0.34	0.53	-0.33	0.24	0.66
weight	0.54	0.36	-0.17	-0.60	-0.44
height	0.54	-0.70	-0.43	0.17	0.02

$$PC1 = 0.45*shoulder.g + 0.32*chest.g + 0.34*waist.g + 0.54*weight + 0.54*height$$

$$PC2 = -0.16*shoulder.g + 0.25*chest.g + 0.53*waist.g + 0.36*weight - 0.70*height$$

$$PC3 = ...$$

255.7 is the greatest value of variance that we can obtain on the individuals with a linear combination of the initial variables.

Coordinates of the individuals on the PCs

	PC1	PC2	PC3	PC4	PC5
H1	-6.50	-4.48	-0.37	-1.03	1.27
H2	4.40	2.04	0.81	1.87	1.38
H3	22.66	-5.94	-6.18	0.11	1.97
H4	7.78	-5.24	-8.38	4.10	-1.74
H5	13.73	-2.67	-8.02	0.82	-2.15
H6	15.67	-0.15	4.49	2.33	4.40
H7	26.99	3.19	6.29	0.04	-3.08
H8	18.41	-3.43	5.63	1.09	-1.96
H9	-6.25	-8.48	4.97	0.79	1.86
H10	16.78	-3.67	1.99	-7.08	1.22
F1	-8.83	-0.78	0.28	-3.02	0.07
F2	-7.28	15.41	-2.31	-3.00	-2.35
F3	-6.45	2.25	-7.60	0.95	1.15
F4	-12.51	2.68	8.91	4.27	-1.53
F5	-3.65	20.76	-0.30	-2.45	1.99
F6	-0.63	-4.62	0.34	-3.46	-2.80
F7	-23.61	-5.07	2.20	1.19	-1.15
F8	-37.50	-9.07	-1.33	-1.89	-0.02
F9	-4.98	-3.61	0.33	-0.50	1.02
F10	-8.24	10.89	-1.74	4.86	0.44
Mean	0	0	0	0	0
Var.	255.7	60.2	23.5	8.61	4.0

Covariance matrix between PCs

	PC1	PC2	PC3	PC4	PC5
PC1	255.66	0.00	0.00	0.00	0.00
PC2	0.00	60.18	0.00	0.00	0.00
PC3	0.00	0.00	23.48	0.00	0.00
PC4	0.00	0.00	0.00	8.61	0.00
PC5	0.00	0.00	0.00	0.00	4.01

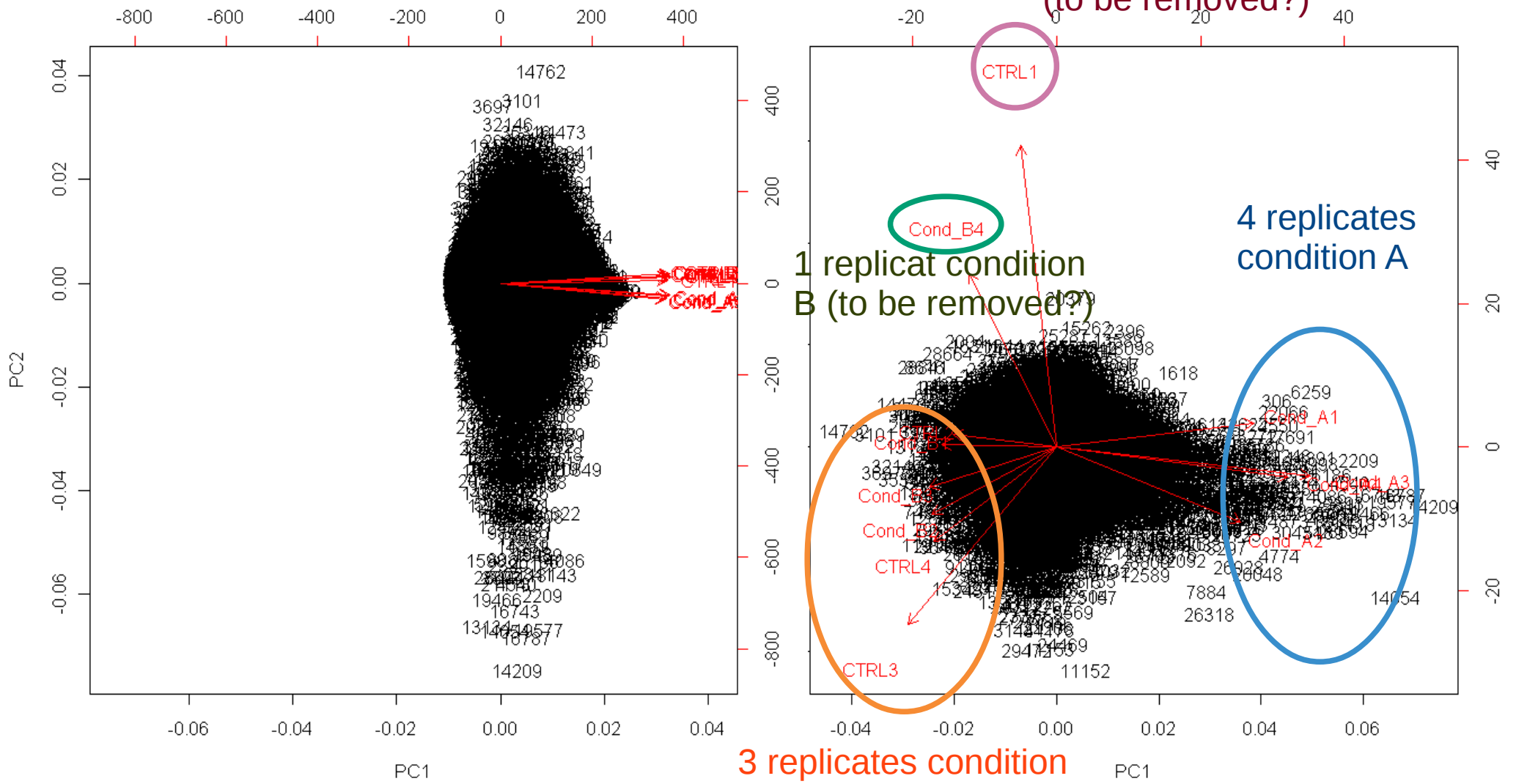
$$255.66 + 60.18 + 23.48 + 8.61 + 4.01 = 351.94$$

The same quantity of information (**351.94**) is kept but it is ``optimally'' allocated.

Biological data set

PCA for quality control!

3 conditions, 4 replicates, 38000 genes, chip Affymetrix



PCA = projection

- To interpret the graphical results of PCA must be done keeping in mind that one is looking at a projection on a plane (or in a volume for 3D representation).
- Be careful when interpreting visual proximities
- Illustration in comics with the *only true super-heros* ...

PCA = projection

★ *I'm TWO-D boy. The boy X-Y who doesn't care about the Z !*

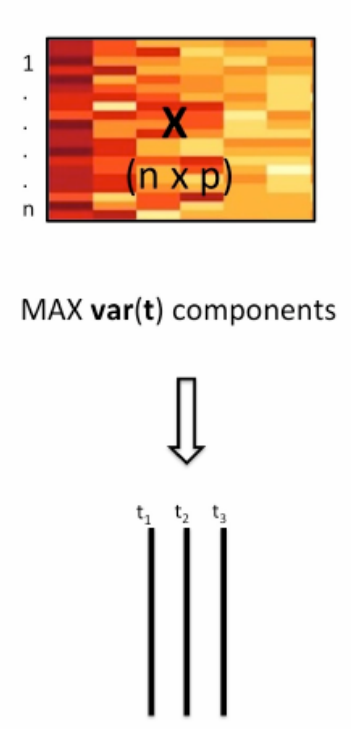
Scenario & illustration
Pascal Jousselin

Colour
Laurence Croix

Web
`pjousselin.free.fr`

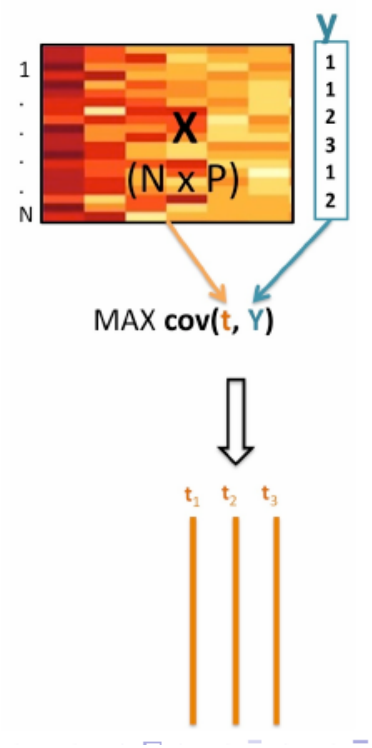
Spoiler alert

Explore one data set



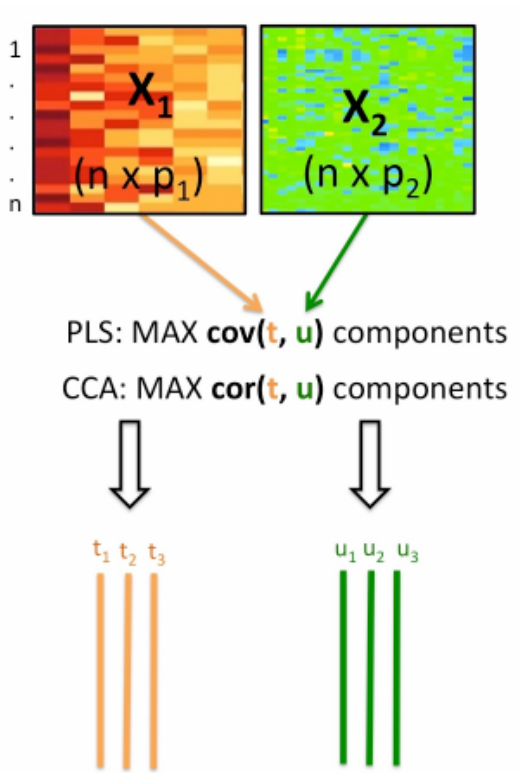
PCA

Discriminant analysis



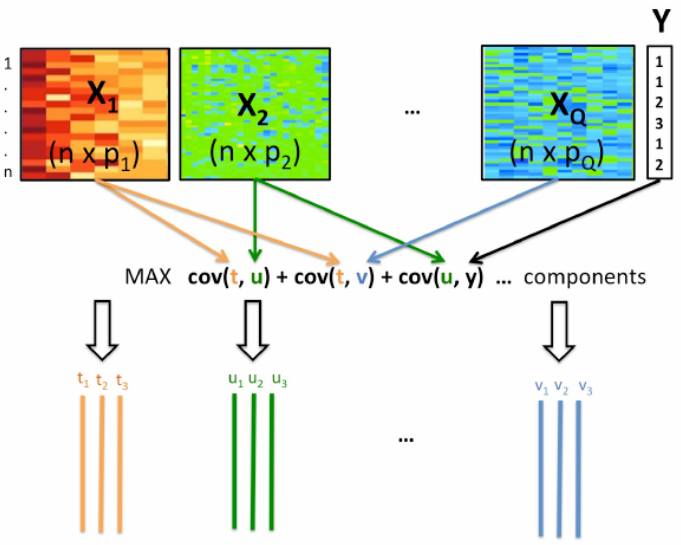
PLS-DA

Integrative 2-blocks analysis



PLS / CCA

Integrative multi-blocks analysis

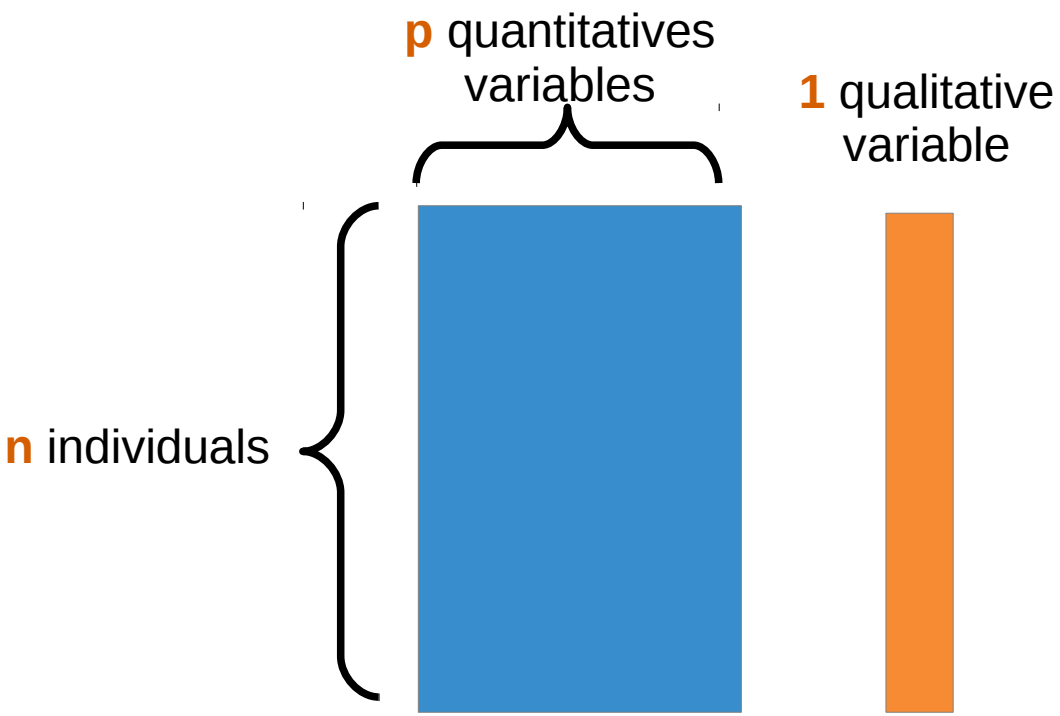


Generalized PLS / CCA

Discriminant analysis

Linear Discriminant Analysis (LDA)

Explore a data set composed of **quantitative** variables and **one qualitative** variable in order to separate the individuals based on their membership to the categories of the qualitative variable.



Body data set

Can we found a space where the projection of the individuals will separate men and women (qualitative variable S) according to the 5 body measurements (V1 to V5)?

	V1	V2	V3	V4	V5	S
I 1	106.2	89.5	71.5	65.6	174.0	M
I 2	110.5	97.0	79.0	71.8	175.3	M
I 3	115.1	97.5	83.2	80.7	193.5	M
I 4	104.5	97.0	77.8	72.6	186.5	M
I 5	107.5	97.5	80.0	78.8	187.2	M
I 6	119.8	99.9	82.5	74.8	181.5	M
I 7	123.5	106.9	82.0	86.4	184.0	M
I 8	120.4	102.5	76.8	78.4	184.5	M
I 9	111.0	91.0	68.5	62.0	175.0	M
I 10	119.5	93.5	77.5	81.6	184.0	M
I 11	105.0	89.0	71.2	67.3	169.5	W
I 12	100.2	94.1	79.6	75.5	160.0	W
I 13	99.1	90.8	77.9	68.2	172.7	W
I 14	107.6	97.0	69.6	61.4	162.6	W
I 15	104.0	95.4	86.0	76.8	157.5	W
I 16	108.4	91.8	69.9	71.8	176.5	W
I 17	99.3	87.3	63.5	55.5	164.4	W
I 18	91.9	78.1	57.9	48.6	160.7	W
I 19	107.1	90.9	72.2	66.4	174.0	W
I 20	100.5	97.1	80.4	67.3	163.8	W

LDA: simulated example

Data set

- 50 individuals, 4 variables
- 3 quantitatives V1 – V2 – V3
- 1 qualitative Group with 2 categories A and B

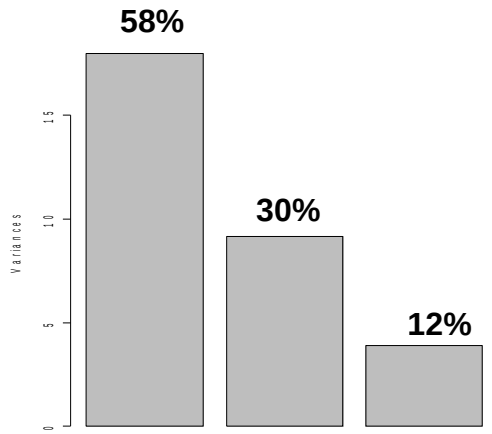
Can we find a space where the projections of the individuals from groups A and B are well separated?

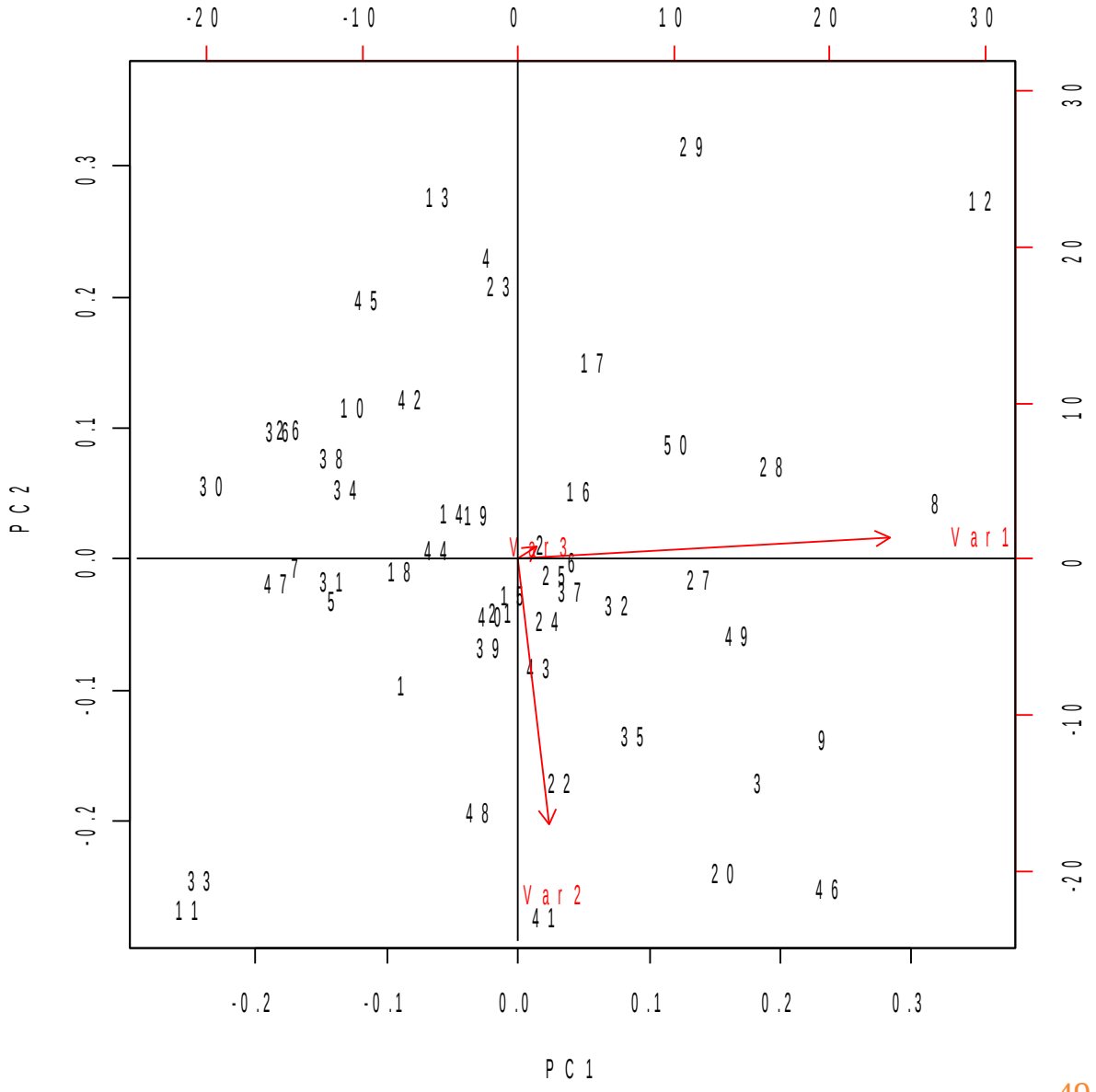
	V1	V2	V3
Mean	0	0	0
Variance	20	10	2

	V1	V2	V3	Groupe
1	-2.02	1.93	2.09	A
2	1.37	-0.12	2.01	A
3	6.02	4.15	1.77	A
4	0.50	-4.84	2.63	A
5	-3.46	0.40	2.04	A
6	2.03	0.22	2.09	A
7	-4.27	-0.19	1.84	A
8	10.44	-0.08	1.43	A
9	7.53	3.55	1.59	A
10	-2.75	-2.69	2.06	A
11	-7.16	5.18	2.00	A
12	11.82	-4.89	2.25	A
13	-0.52	-5.94	2.05	A
14	-0.62	-0.77	1.97	A
15	0.67	0.64	1.76	A
16	2.34	-0.93	1.74	A
17	2.79	-2.98	2.07	A
18	-1.87	0.05	2.02	A
19	-0.09	-0.69	2.32	A
20	5.07	5.57	2.08	A
21	0.38	0.90	1.69	A
22	1.50	3.79	1.96	A
23	0.78	-4.40	1.81	A
24	1.40	1.16	2.13	A
25	1.64	0.38	1.77	A
26	-4.00	-2.60	-1.95	B
27	5.15	0.59	-1.94	B
28	6.98	-1.14	-2.17	B
29	5.57	-6.49	-2.15	B
30	-5.84	-1.83	-1.82	B
31	-3.20	-0.07	-2.14	B
32	3.20	0.87	-1.50	B
33	-6.63	4.56	-1.92	B
34	-2.80	-1.53	-1.70	B
35	3.43	2.98	-2.14	B
36	-4.24	-2.61	-2.18	B
37	2.20	0.55	-1.89	B
38	-3.07	-2.07	-1.97	B
39	0.26	1.30	-1.85	B
40	0.32	0.79	-1.78	B
41	1.14	5.79	-1.64	B
42	-1.21	-2.88	-1.50	B
43	1.38	1.71	-2.11	B
44	-0.80	-0.38	-1.99	B
45	-2.04	-4.60	-2.00	B
46	7.67	5.84	-2.09	B
47	-4.50	-0.15	-1.85	B
48	-0.19	3.95	-1.89	B
49	5.92	1.54	-1.72	B
50	4.82	-1.70	-2.41	B

LDA: simulated example

Results of a **PCA** applied only on the quantitative variables (**without considering the qualitative variable**).

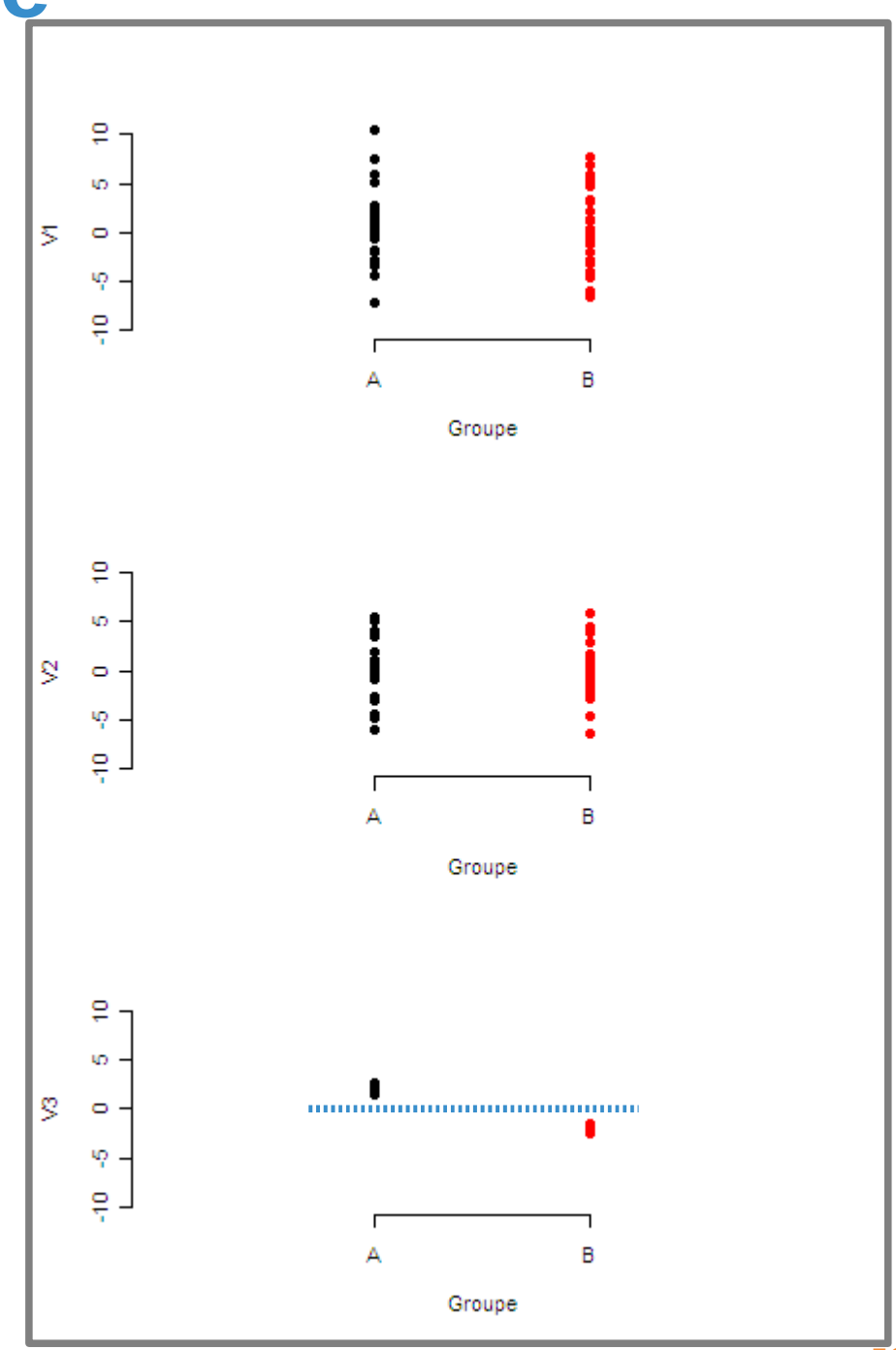




- The 3 PC are clearly identified to the 3 initial variables.
- Most part of the variability in the data is explained by V1, then by V2, then by V3.

LDA: simulated example

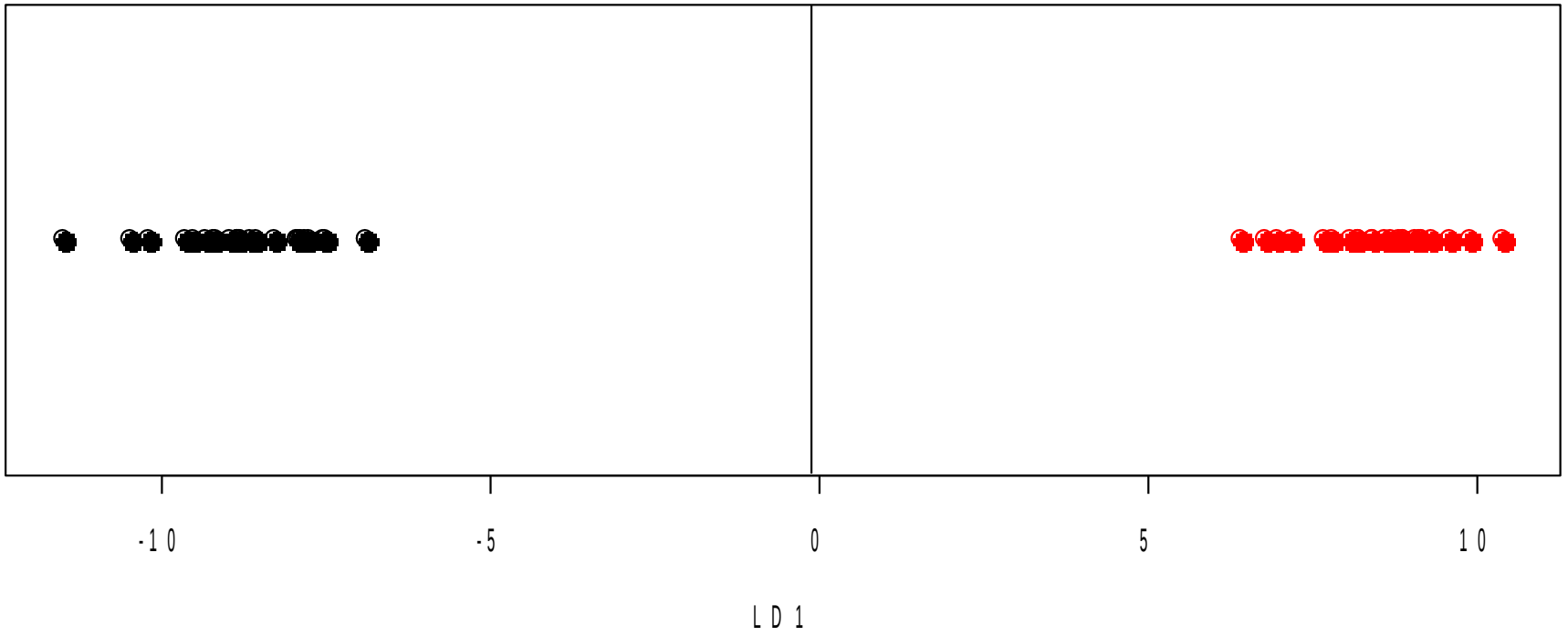
Representation of the 50 individuals according the 3 variables. Color depends on the categorie (A-black or B-red)



Although displaying the smallest variability, V3 is relevant when addressing a discrimination purpose.

LDA: simulated example

LDA result



- 2 categories → 1 discriminant variable (graphical representation in)
- Linear combination of the initial variables:

$$LD1 = -0.058 * V1 - 0.028 * V2 \mathbf{- 4.41} * V3$$

- LD1 roughly corresponds to V3 (with negative coefficient, but the sign doesn't matter).

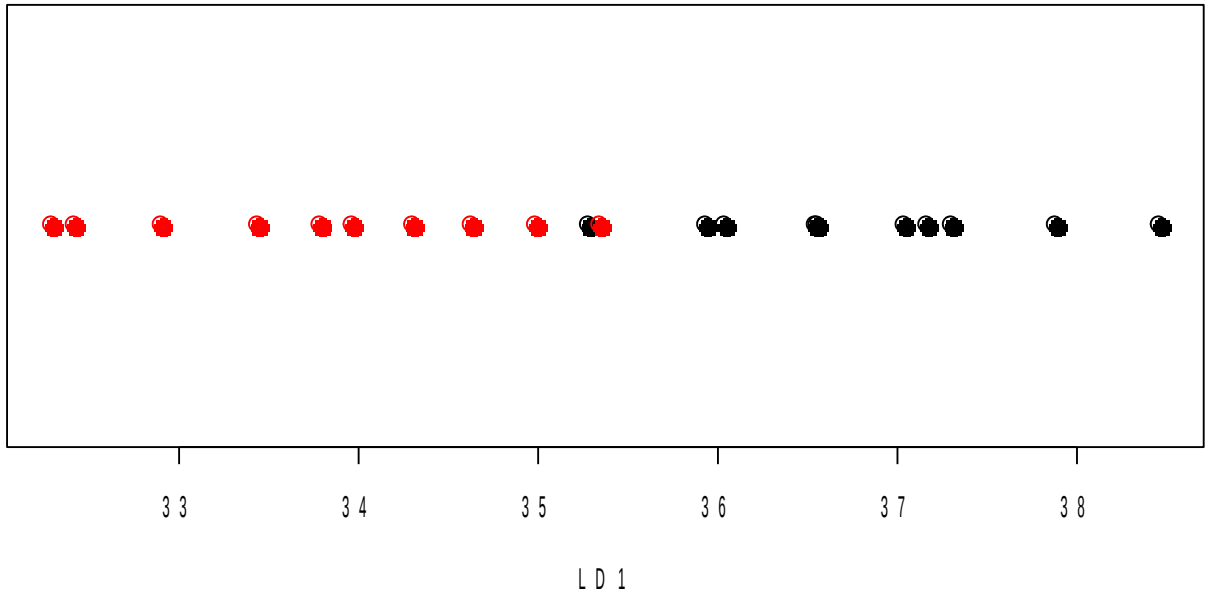
LDA: body data set

Centroïd of the 2 groups

	s.g	c.g	w.g	w	h	LD1
F	102.31	91.15	72.82	65.88	166.17	**33.81**
H	113.80	97.23	77.88	75.27	182.55	**36.82**

Coefficients of linear discriminants:

	LD1
shoulder g.	0.12
chest g.	-0.02
waist g.	0.11
weight	-0.11
height	0.14



The coefficients indicates that chest girth is the less discriminant variable (loading -0.02)... The other variables participate nearly in the same way (loadings around 0.1 in absolute value).

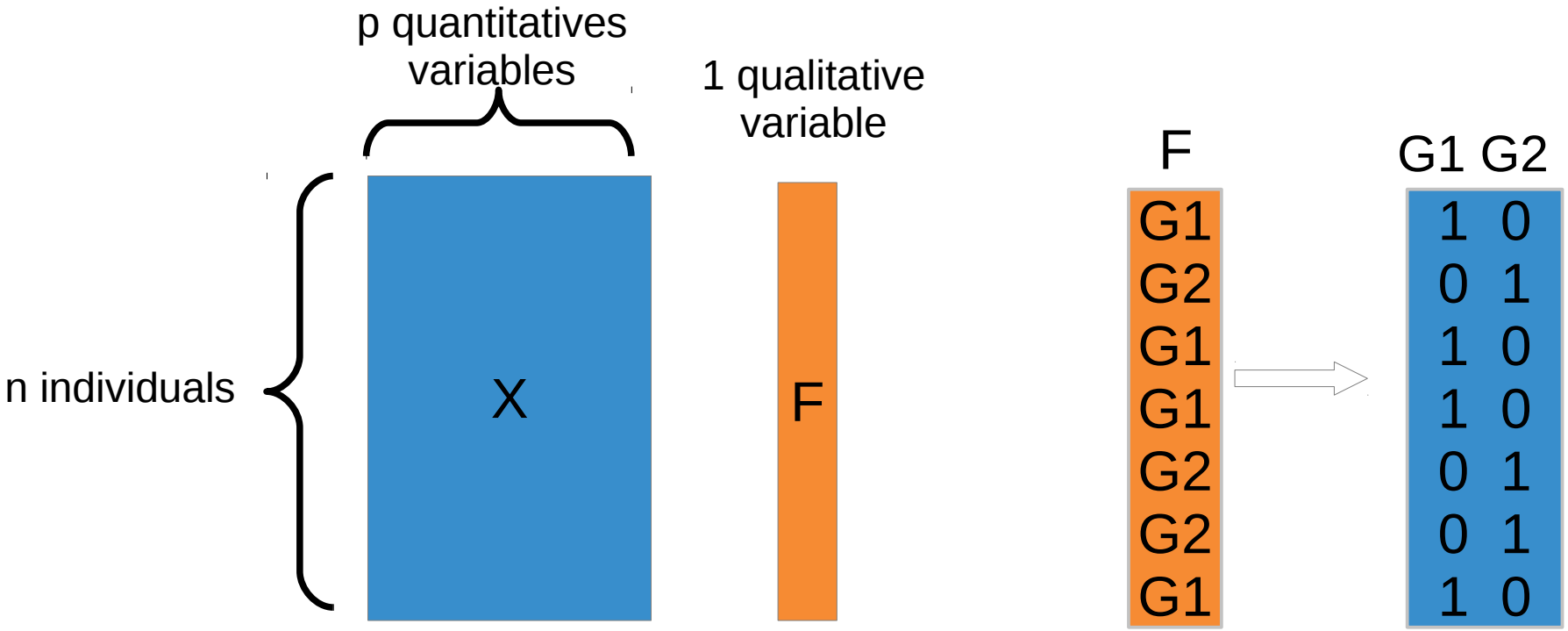
LDA: principle

- LDA is similar to a PCA performed on the centroid of the groups determined by the categories of the qualitative variable.
- Thus, we are looking for a sub-space of small dimension in which the centroids are the furthest possible (having a maximal variability)
- If the number of categories is 2, then the dimension the sub-space is 1; so LDA will provide only LD1.

Decision-making with LDA

- For a supplementary individual, when knowing the quantitative variables, the decision-making problem relies on the affectation of this individual to a categorie of the qualitative variable.
- Naive (and not so bad) rule: affect the new individual to the categorie whose centroid is the closest (many others more sophisticated rules exist).
- Application: credit scoring, quality control, diagnostic...

Projection to Latent Structure Discriminant Analysis (PLS-DA)



The PLS regression(*) has been extended to deal with discrimination issues. To do that, the qualitative variable is converted into a dummy matrix (composed of 0 and 1) with as many rows as individuals and as many columns as catagories of the qualitative variable.

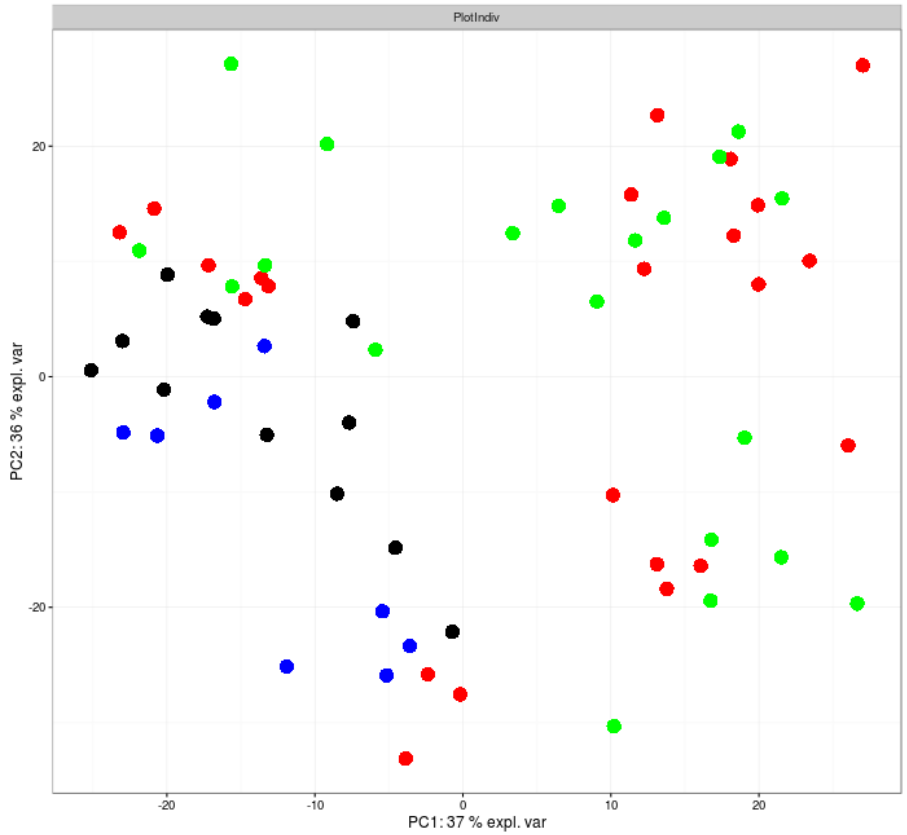
Please wait for few slides (section integration) to know more about PLS!

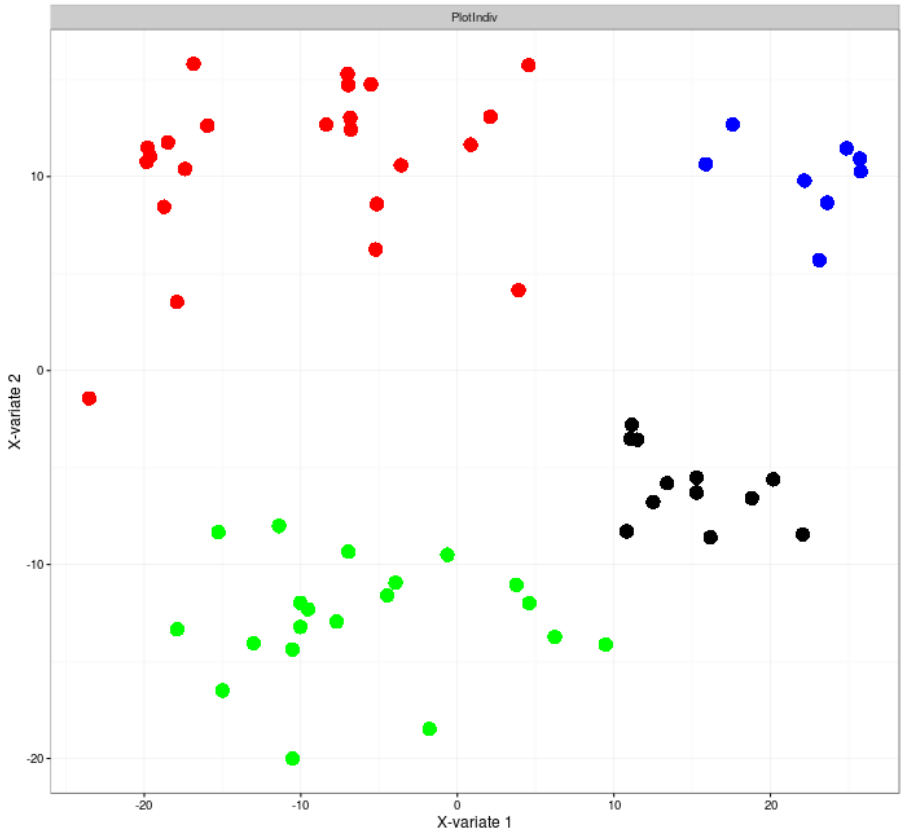
Comparison PCA-PLSDA

PCA

Small Round Blue Cell Tumors dataset from Khan et al., (2001). **63** samples, **2308** genes. **4** classes.

PLS-DA



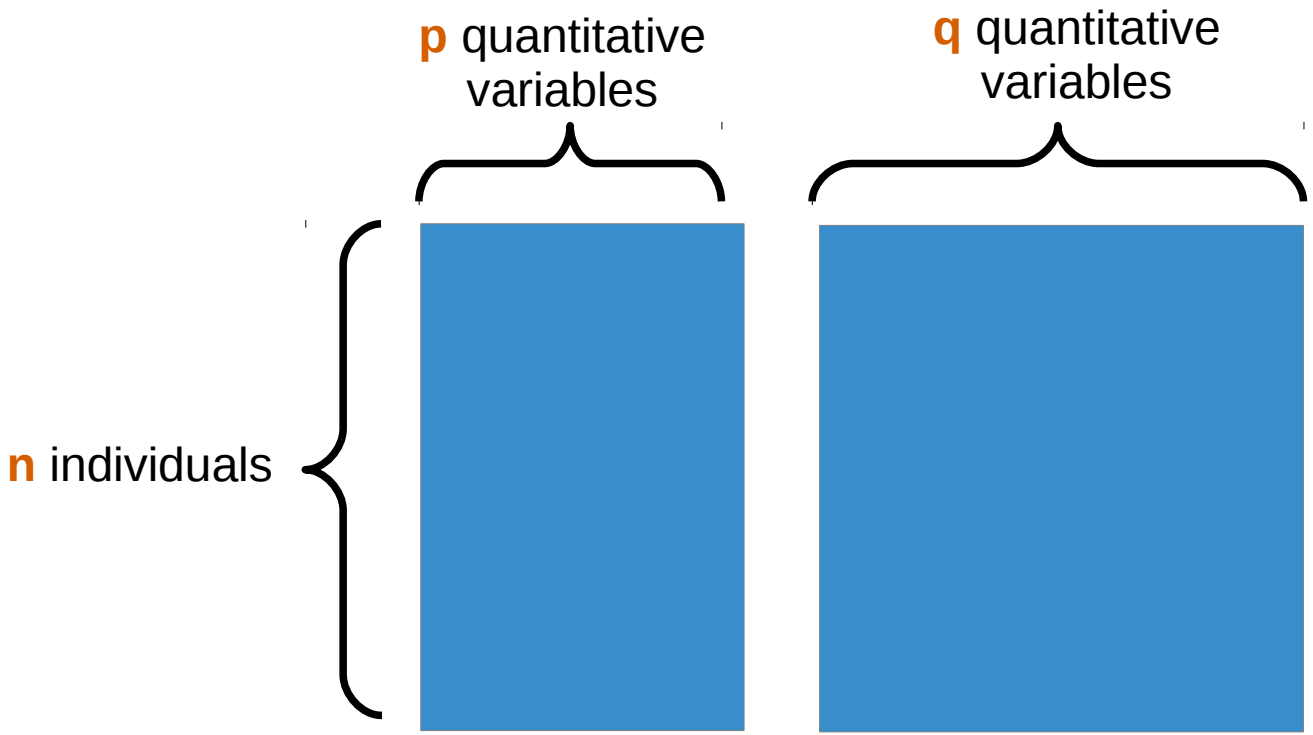


- PCA does not reveal clusters associated to classes → the main source of variability in the data is not due to the classes of the samples
- PLS-DA (supervised method) clearly highlights the 4 classes of samples → it is its job! (unlike PCA)

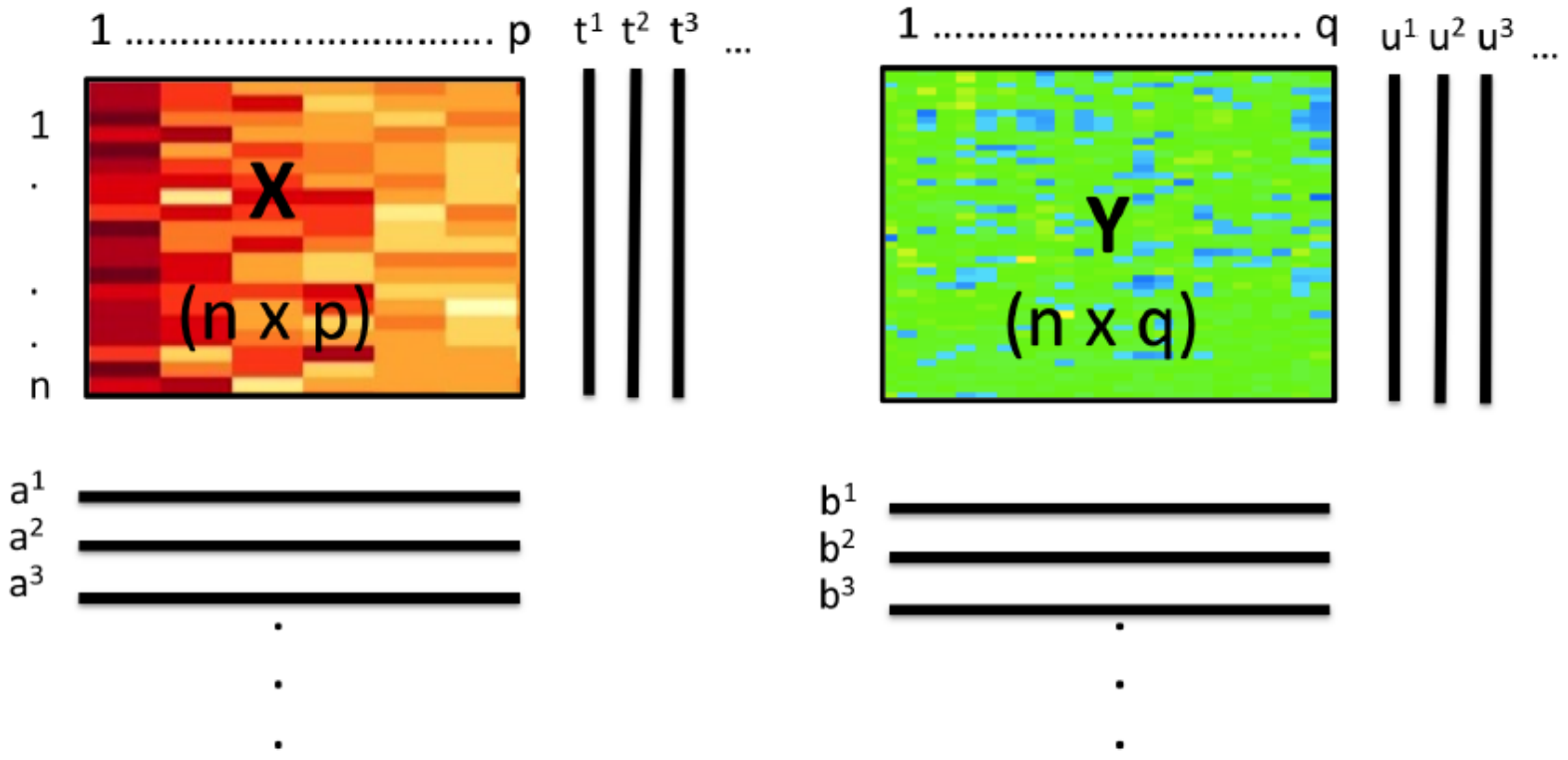
Data integration

Data integration

Understand the correlation/covariance structure between two data sets composed of **quantitative** variables



Principle



- Methods generate a set of components(*) and loading(*) vectors associated to each dataset and are unsupervised.
- Canonical Correlation Analysis: method to maximize **cor(t^1,u^1)**…
- Projection to Latent Structures: algorithm to maximize **cov(t^1,u^1)**...

(*) annoyingly they have different names for different methods

CCA: simulated example

X

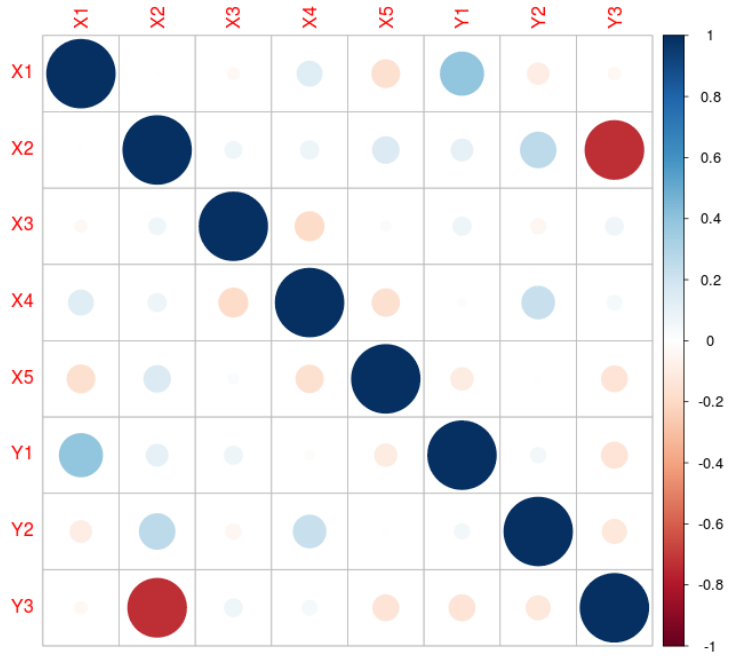
X1	X2	X3	X4	X5
0.87	0.31	0.24	0.06	0.29
0.76	0.8	0.52	0.1	0.95
0.65	0.76	0.57	0.1	0.17
0.86	0.47	0.00	0.21	0.75
0.65	0.46	0.41	0.23	0.86
0.11	0.56	0.84	0.14	0.49
0.85	0.81	0.42	0.65	0.39
0.74	0.73	0.15	0.81	0.80
0.75	0.30	0.72	0.48	0.99
0.55	0.06	0.30	0.87	0.67
0.41	0.52	0.21	0.51	0.59
0.59	0.87	0.99	0.67	0.28
0.34	0.35	0.56	0.03	0.56
0.07	0.02	0.59	0.04	0.54
0.17	0.08	0.50	0.37	0.89
0.39	0.54	0.53	0.65	0.46
0.06	0.17	0.28	0.82	0.46
0.22	0.83	0.90	0.17	0.49
0.83	0.27	0.51	0.38	0.55
0.02	0.51	0.56	0.34	0.99
0.04	0.46	0.81	0.47	0.46
0.32	0.95	0.65	0.10	0.43
0.42	0.27	0.17	0.36	0.37
0.39	0.68	0.94	0.79	0.87
0.48	0.30	0.83	0.60	0.22
0.84	0.25	0.54	0.00	0.52
0.31	0.14	0.33	0.48	0.38
0.15	0.80	0.09	0.87	0.29
0.99	0.07	0.81	0.96	0.01
0.26	0.21	0.20	0.24	0.66
0.99	0.07	0.86	0.84	0.36
0.91	0.19	0.82	0.04	0.25
0.46	0.17	0.48	0.38	0.02
0.95	0.94	0.41	0.83	0.48
0.80	0.34	0.54	0.72	0.58
0.09	0.01	0.81	0.02	0.63
0.93	0.75	0.54	0.79	0.90
0.78	0.99	0.67	0.08	0.84
0.83	0.05	0.04	0.70	0.41
0.97	0.68	0.37	0.88	0.34
0.13	0.35	0.16	0.95	0.81
0.5	0.04	0.17	0.49	0.15
0.37	0.64	0.55	0.96	0.14
0.01	0.98	0.48	0.94	0.76
0.40	0.44	0.80	0.40	0.94
0.44	0.67	0.67	0.42	0.20
0.92	0.07	0.48	0.92	0.06
0.30	0.39	0.54	0.23	0.92
0.60	0.75	0.22	0.60	0.50
0.25	0.77	0.02	0.51	0.18

Y

Y 1	Y2	Y3
0.71	0.33	-0.53
0.62	0.07	-0.78
0.77	0.10	-0.52
0.49	0.57	-1.09
0.76	0.67	-0.30
0.53	0.84	-0.55
0.71	0.57	-0.75
0.24	0.89	-0.50
1.62	0.18	-0.80
-0.51	0.16	0.25
0.29	0.72	-0.61
1.11	0.80	-0.95
0.49	0.27	-0.06
0.51	0.02	-0.46
0.20	0.48	0.36
0.27	0.88	-0.48
0.61	0.98	-0.51
0.02	0.82	-0.74
0.40	0.08	-0.39
-0.53	0.46	-0.69
0.49	0.59	-0.28
0.07	0.61	-1.19
0.06	0.51	-0.31
0.05	0.76	-0.18
-0.25	0.25	-0.13
0.96	0.11	-1.58
0.24	0.74	0.41
0.23	0.89	-1.57
0.06	0.76	-0.29
0.42	0.61	-0.22
0.64	0.09	0.12
1.44	0.08	0.12
1.12	0.70	0.18
1.29	0.58	-1.37
1.60	0.51	-0.38
-0.02	0.23	0.05
-0.01	0.65	-1.20
1.12	0.81	-1.12
1.53	0.87	0.09
1.15	0.71	-0.52
0.28	0.23	-0.07
-0.89	0.20	0.25
1.15	0.73	-0.48
0.60	0.01	-1.49
0.28	0.64	0.23
0.71	0.61	-1.18
0.98	0.24	0.71
1.01	0.83	-0.51
0.09	0.56	-1.04
0.67	0.15	-0.87

Correlation matrix (X,Y)

	X1	X2	X3	X4	X5	Y1	Y2	Y3
X1	1.00	0.00	-0.03	0.13	-0.17	**0.40**	-0.10	-0.03
X2	0.00	1.00	0.06	0.07	0.15	0.10	0.27	**-0.74**
X3	-0.03	0.06	1.00	-0.18	0.02	0.07	-0.05	0.07
X4	0.13	0.07	-0.18	1.00	-0.16	-0.02	0.23	0.05
X5	-0.17	0.15	0.02	-0.16	1.00	-0.11	0.01	-0.14
Y1	**0.40**	0.10	0.07	-0.02	-0.11	1.00	0.05	-0.15
Y2	-0.10	0.27	-0.05	0.23	0.01	0.05	1.00	-0.12
Y3	-0.03	**-0.74**	0.07	0.05	-0.14	-0.15	-0.12	1.00

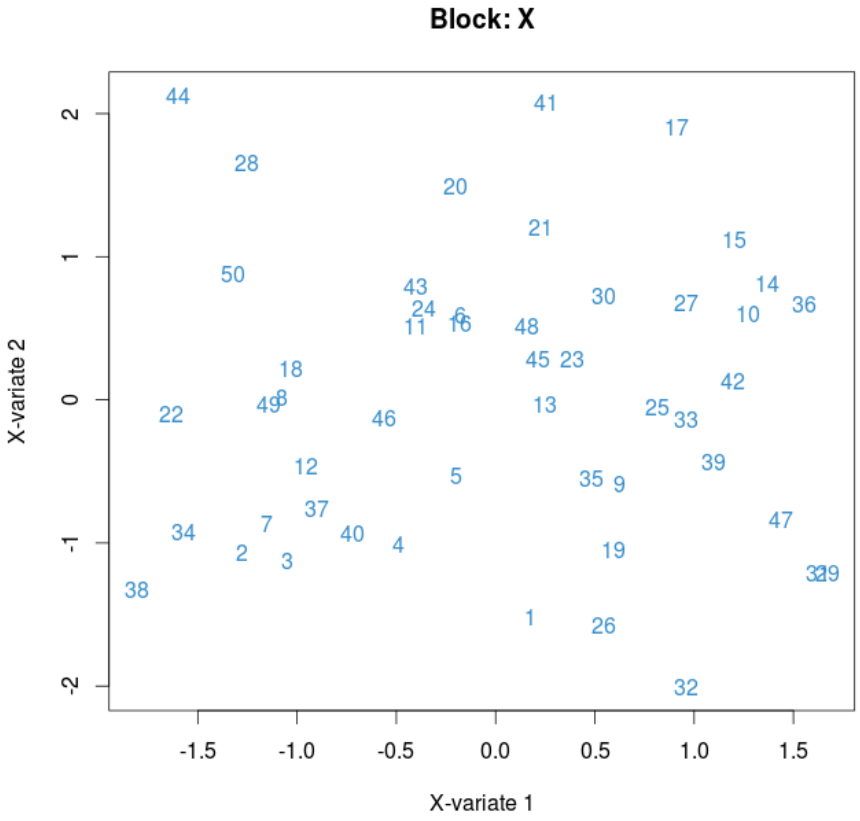


Package R
corrplot

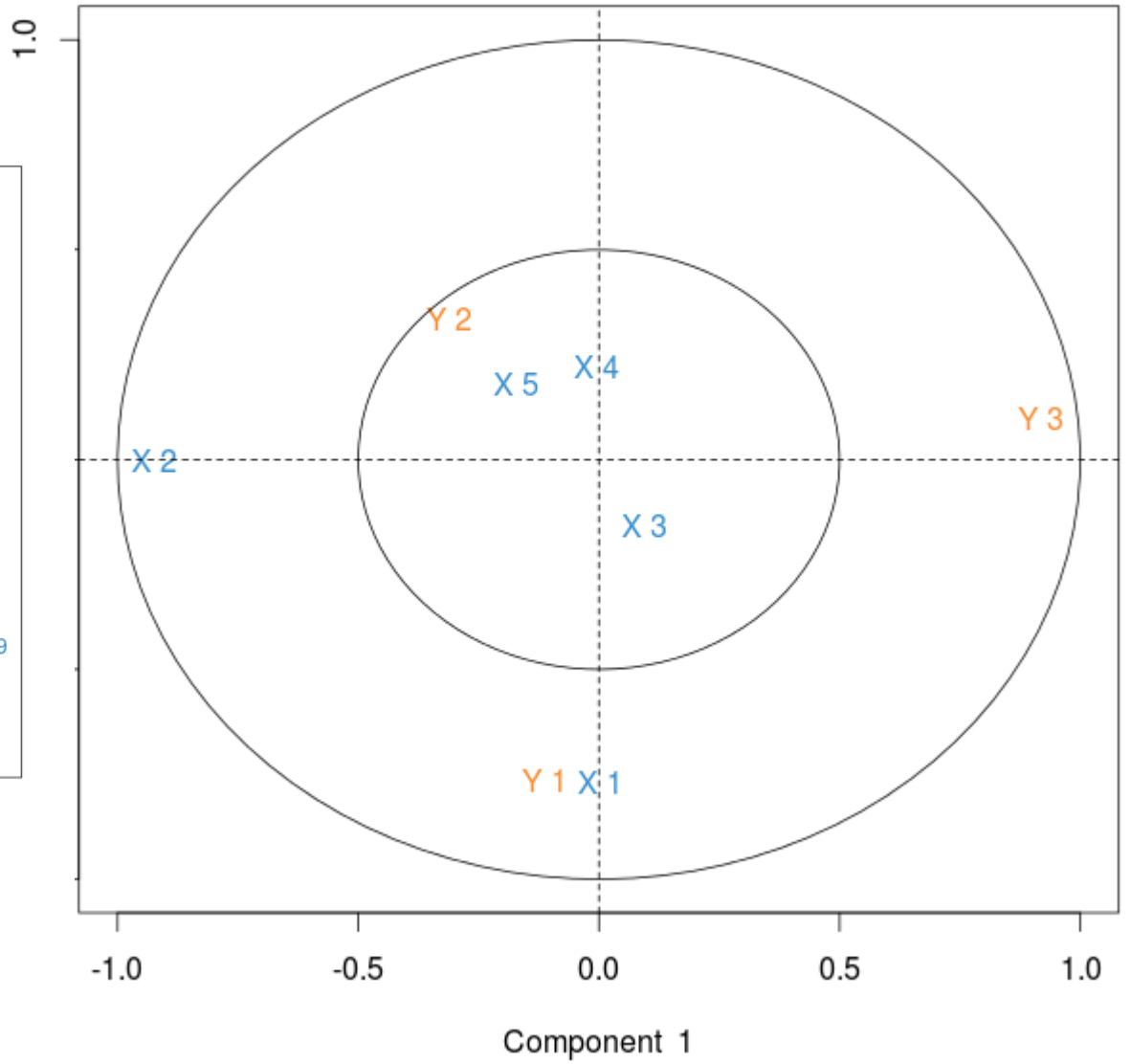
CCA: simulated example

Graphical outputs

Individuals plot



Variables plot



CCA: principle

- The CCA can be viewed as an interative algorithm (like PCA)
  - Maximize the correlation (ρ_1) between two linear combinations: one from variables X (t_1), the other from variables Y (u_1).

$$t_1 = a_{11}X_1 + a_{12}X_2 + \ldots + a_{1p}X_p$$

$$u_1 = b_{11}Y_1 + b_{12}Y_2 + \ldots + b_{1q}Y_q$$

$$\rho_1 = cor(t_1,u_1) = \max_{t,u} cor(t,u)$$

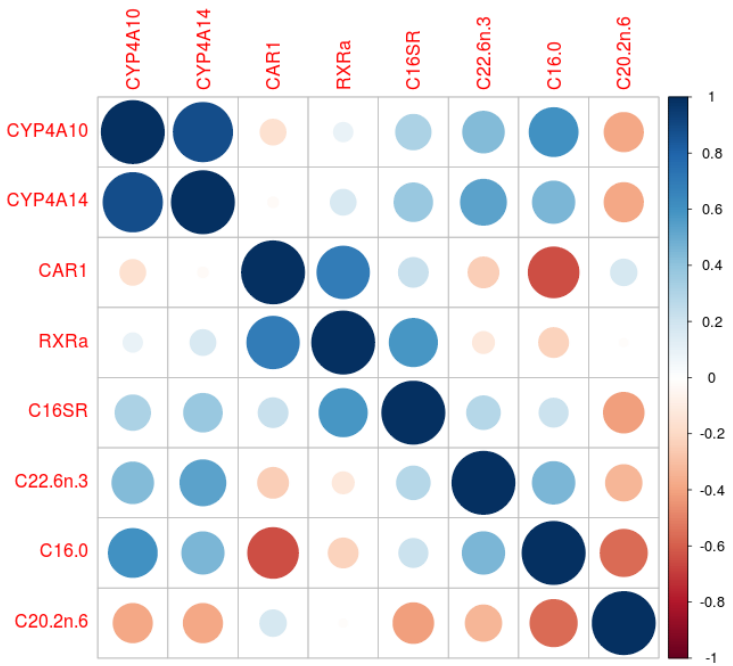
t_1 and u_1 are the first canonical variates and p_1 is the first canonical correlation.

  - For next levels, iterate the process under orthogonality constraints
- CCA is analog to PCA for the production and the interpretation of graphical outputs.
- Mathematical aspects are in the same vein as PCA (eigen decomposition of matrices)

CCA: nutrimouse data set

- 40 mice (2 genotypes)
- Expression of 5 genes
- Concentration of 3 lipids

Question: are there any genes related to lipids?



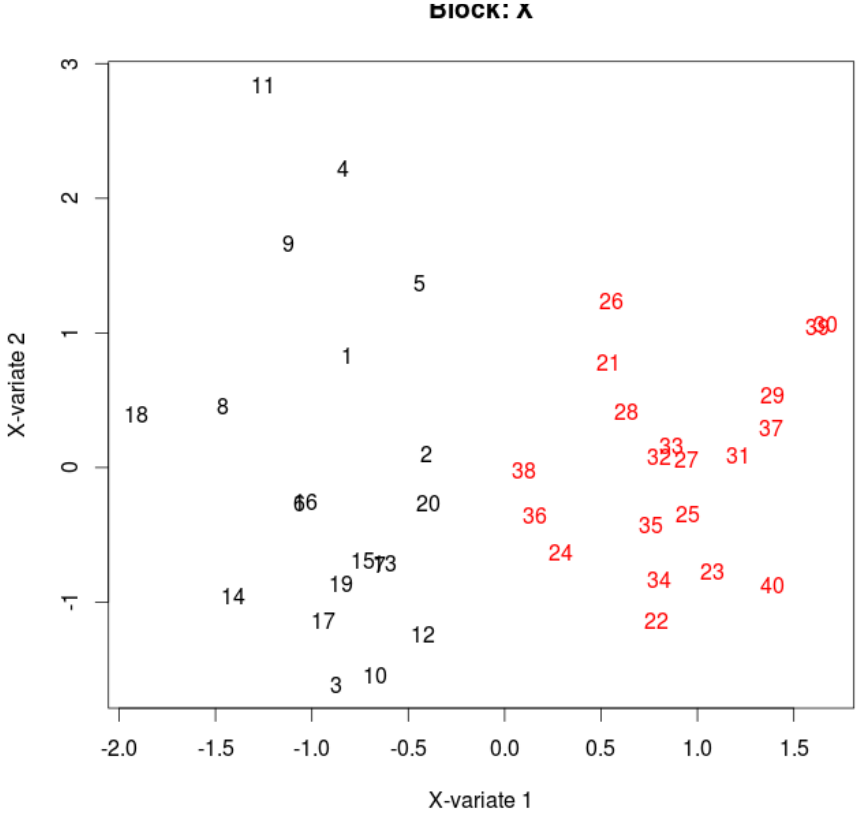
Correlation matrix

CYP4A10	CYP4A14	CAR1	RXRa	C16SR	C22.6n.3	C16.0	C20.2n.6
-0.81	-0.81	-0.97	-0.67	1.66	10.39	26.45	0.00
-0.88	-0.84	-0.92	-0.59	1.65	2.61	24.04	0.30
-0.71	-0.98	-0.98	-0.68	1.57	2.51	23.70	0.33
-0.65	-0.41	-0.97	-0.72	1.61	14.99	25.48	0.00
-1.16	-1.16	-1.06	-0.78	1.66	6.69	24.80	0.23
-0.99	-1.09	-1.03	-0.62	1.70	2.56	26.04	0.00
-0.62	-0.76	-0.91	-0.65	1.58	9.84	25.94	0.00
-0.82	-0.87	-1.11	-0.76	1.62	10.40	28.63	0.00
-0.48	-0.37	-0.85	-0.55	1.72	16.36	25.34	0.00
-0.79	-0.95	-0.99	-0.67	1.55	1.86	28.49	0.00
-0.51	-0.15	-0.92	-0.60	1.69	16.21	25.73	0.00
-1.00	-1.13	-1.02	-0.69	1.57	6.61	24.28	0.21
-0.88	-0.99	-0.99	-0.67	1.60	3.27	24.63	0.36
-1.05	-1.15	-1.19	-0.75	1.59	7.04	26.04	0.19
-0.72	-0.73	-0.93	-0.58	1.61	2.71	24.76	0.35
-0.67	-0.85	-0.99	-0.72	1.60	10.96	26.46	0.00
-1.19	-1.22	-1.15	-0.69	1.60	1.99	23.45	0.00
-0.56	-0.73	-0.95	-0.55	1.78	17.35	29.72	0.00
-1.03	-1.10	-1.02	-0.59	1.67	2.44	27.00	0.00
-1.01	-1.06	-1.01	-0.70	1.60	5.97	24.09	0.23
-1.21	-1.17	-0.91	-0.67	1.65	0.64	23.59	0.05
-1.15	-1.29	-0.90	-0.69	1.55	2.16	19.95	0.31
-1.22	-1.25	-0.88	-0.67	1.55	1.70	17.64	0.61
-1.15	-1.19	-0.90	-0.58	1.65	11.56	22.73	0.27
-1.16	-1.18	-0.87	-0.67	1.57	0.91	14.65	0.83
-0.93	-0.90	-0.73	-0.52	1.74	1.22	20.49	0.32
-1.13	-1.10	-0.83	-0.62	1.61	3.44	18.44	0.09
-1.09	-1.08	-0.85	-0.63	1.64	4.02	17.72	0.12
-1.33	-1.22	-0.85	-0.66	1.60	13.26	21.70	0.24
-1.18	-1.08	-0.74	-0.63	1.62	4.45	16.25	0.10
-1.18	-1.14	-0.84	-0.67	1.57	1.16	22.91	0.00
-0.96	-1.05	-0.70	-0.49	1.72	0.28	23.27	0.00
-1.07	-1.03	-0.83	-0.63	1.60	1.41	20.25	0.33
-1.12	-1.11	-0.84	-0.57	1.60	1.11	20.18	0.54
-1.22	-1.15	-0.90	-0.62	1.59	11.57	20.71	0.24
-1.05	-0.96	-0.88	-0.53	1.65	0.64	21.79	0.07
-1.07	-1.03	-0.73	-0.58	1.62	2.29	21.57	0.11
-1.23	-1.18	-0.98	-0.64	1.64	16.28	25.23	0.26
-1.08	-1.12	-0.63	-0.53	1.72	3.87	16.20	0.13
-1.13	-1.14	-0.79	-0.61	1.55	1.83	20.70	0.59

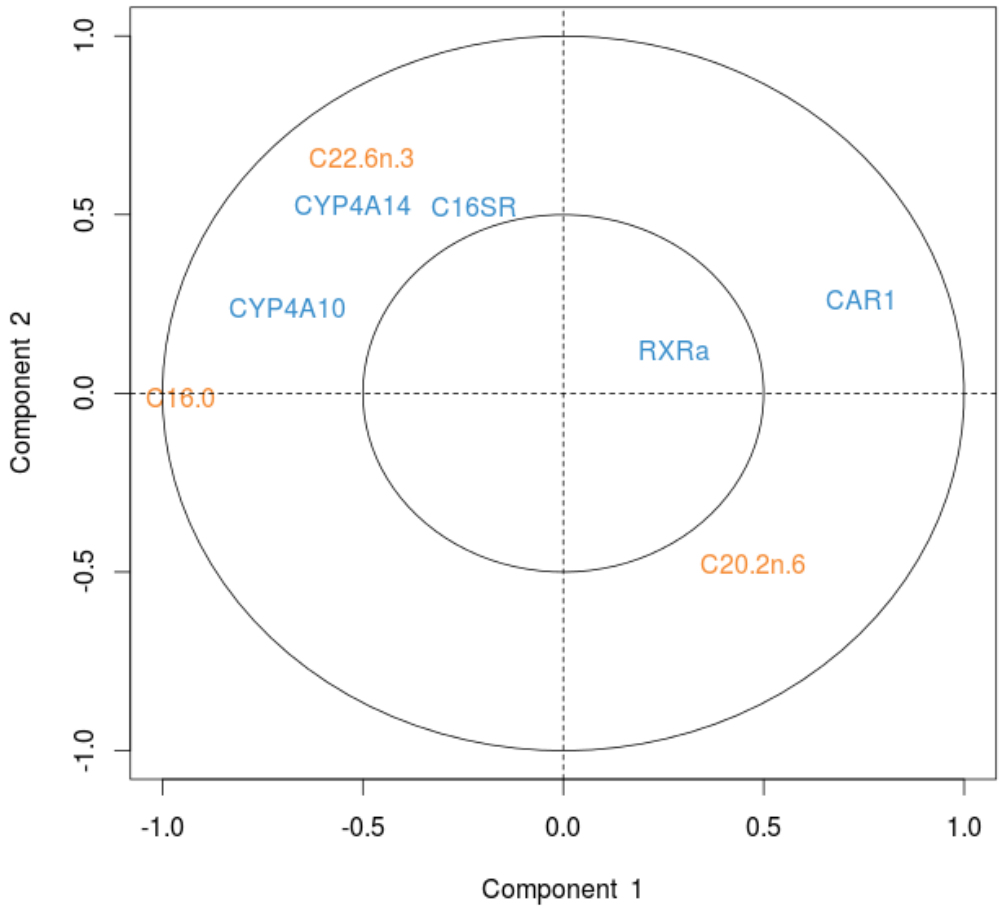
CCA: nutrimouse data set

Individual plot

color depending on the genotype added a posteriori



Variable plot



Canonical correlations : `0.853 0.627 0.253`

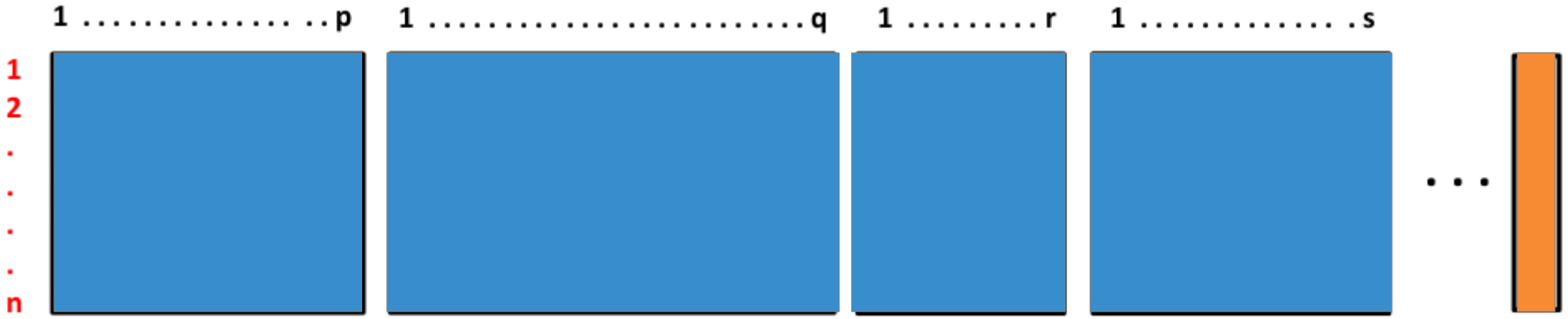
CCA: a fundamental method...

- If one data set has only one quantitative variable, CCA is equivalent to **multiple linear regression**.
- If one data set is a dummy matrix corresponding to the categories of a qualitative variable, CCA is equivalent to **Linear Discriminant Analysis**.
- If the two data sets are dummy matrices corresponding to the categories of two qualitative variables, CCA is equivalent to **Correspondance Analysis**.

… with limits

- CCA can only be perfomed with « enough » observations: n >> p+q (sounds like a joke regarding ‘omics data...)
- Variables X and Y must not be « too » correlated
- Alternative: **regularised CCA, PLS, sparse PLS**

Generalisation

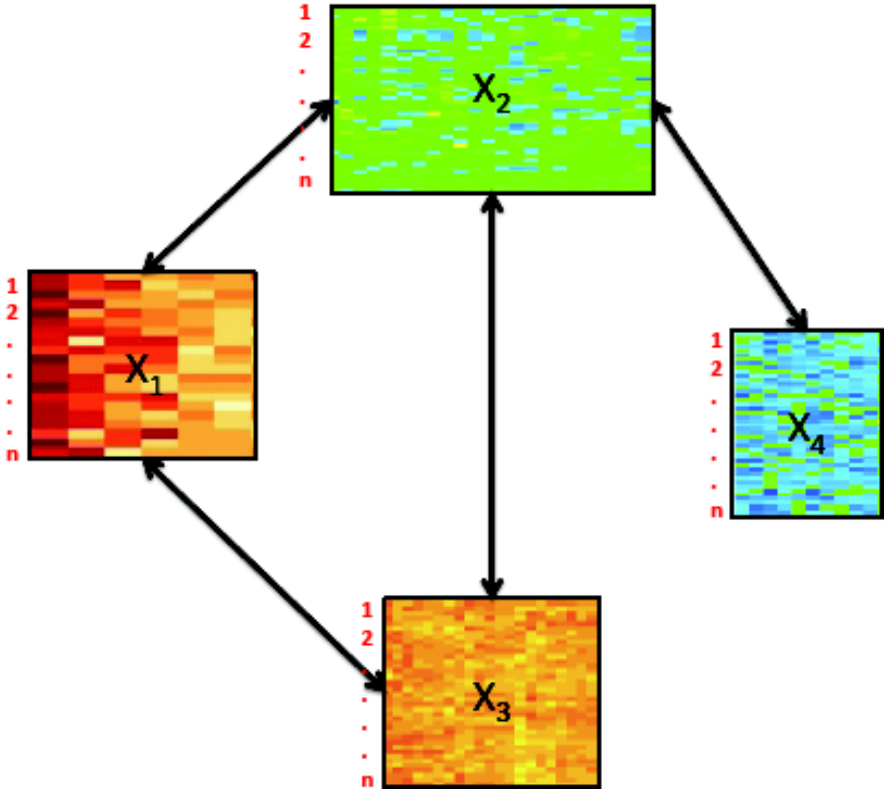


- **Generalized** CCA (GCCA): integration of more than 2 data sets ; maximizes the sum of every pairwise covariances between two components.
- Sparse (see *extensions*) GCCA (SGCCA): variable selection is performed on each data set

Tenenhaus, A., Philippe, C., Guillemot, V., Lê Cao K-A., Grill, J., Frouin, V. 2014, Variable selection for generalized canonical correlation analysis, Biostatistics

Define links between data sets

A link between 2 data sets indicates that the covariance between these two data sets has to be considered in the criterion to maximise.

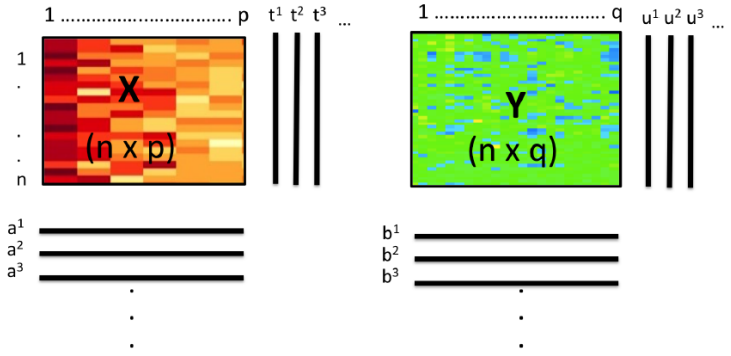


Design matrix

	X1	X2	X3	X4
X1	0	1	1	0
X2	1	0	1	1
X3	1	1	0	0
X4	0	1	0	0

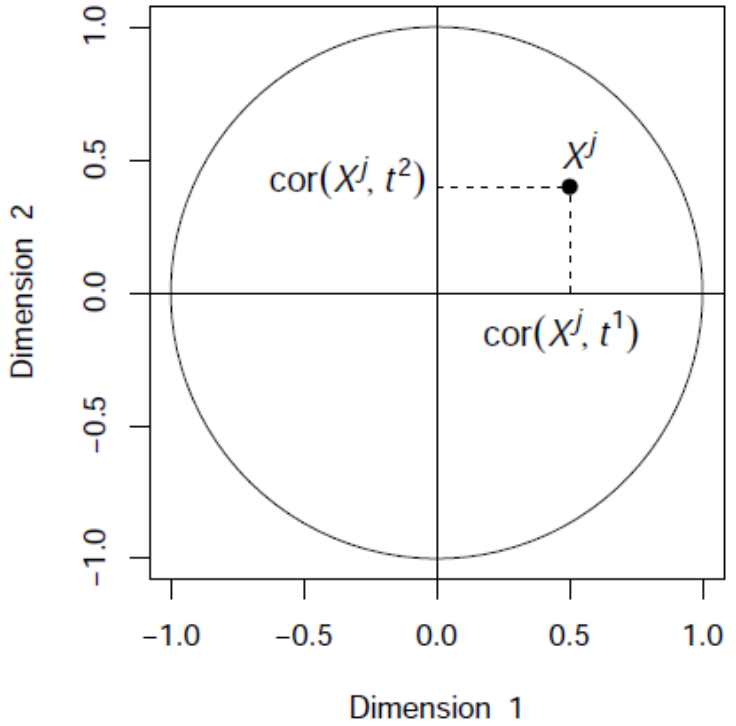
Graphical outputs

The same principles as those for PCA are still true for other multivariate methods mentioned here:



- **Individuals plots**: the coordinates of the individuals are given by the **components** calculated with the method (PCA, PLS-DA, PLS, CCA...)

- **Variables plot**: the variables are usually represented using their **correlation with the components** defining the axes. In other words, the coordinate of one variable X^j on the component t^i is given by $cor(X^j, t^i)$



Alternative graphical outputs

Motivations: usual plots are difficult to read and interpret when

- The numer of variables is too high
- The number of relevant components is greater than 2 inducing a « more than 2D » representation space.

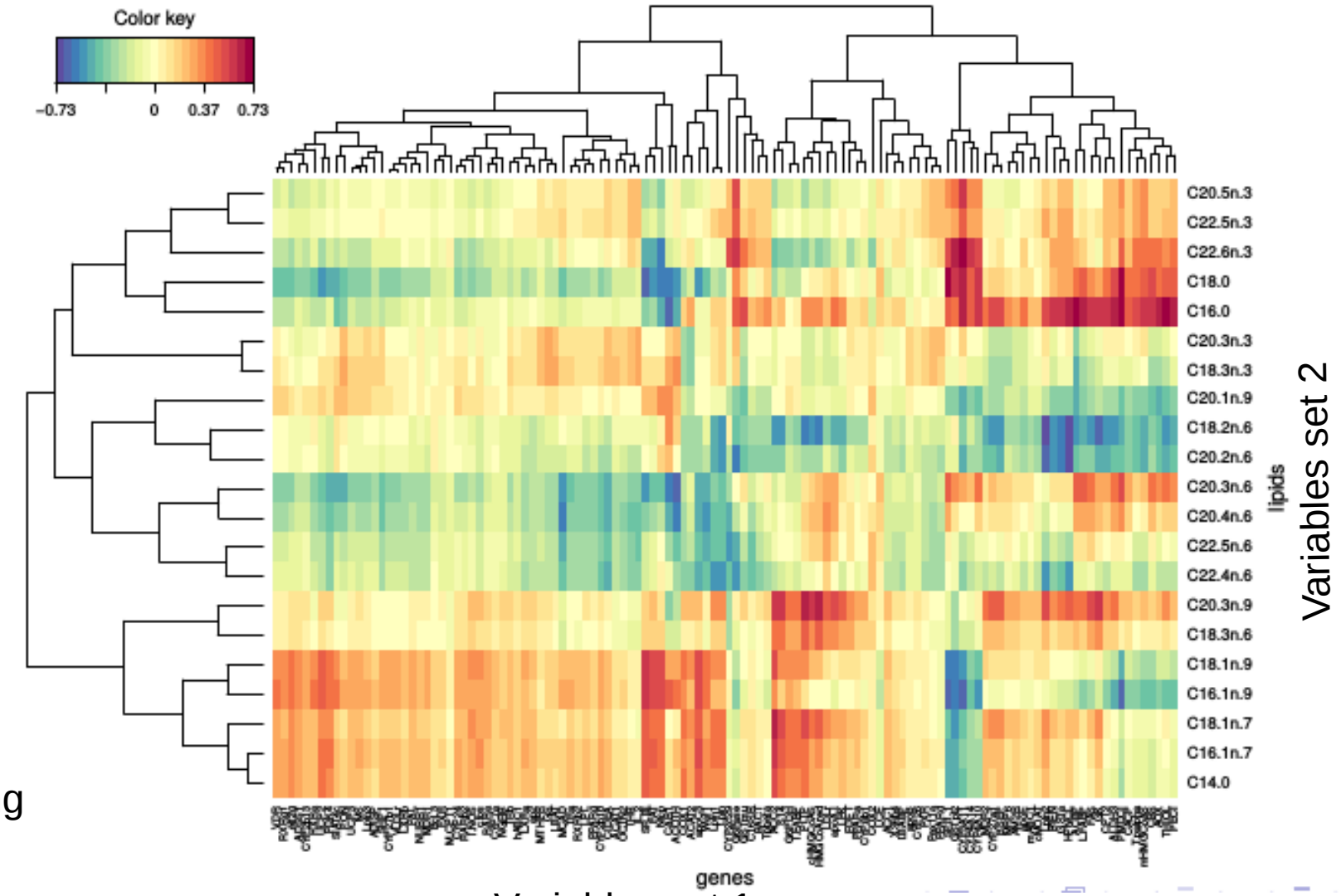
Propositions:

- Identify the pairs of highly related variables
- Produce graphical display making easy the interpretation

I. González, K-A. Lê Cao, M. Davis, S. Déjean (2012) – *Visualising associations between paired 'omics' data sets*. BioData Mining

Alternative graphical outputs

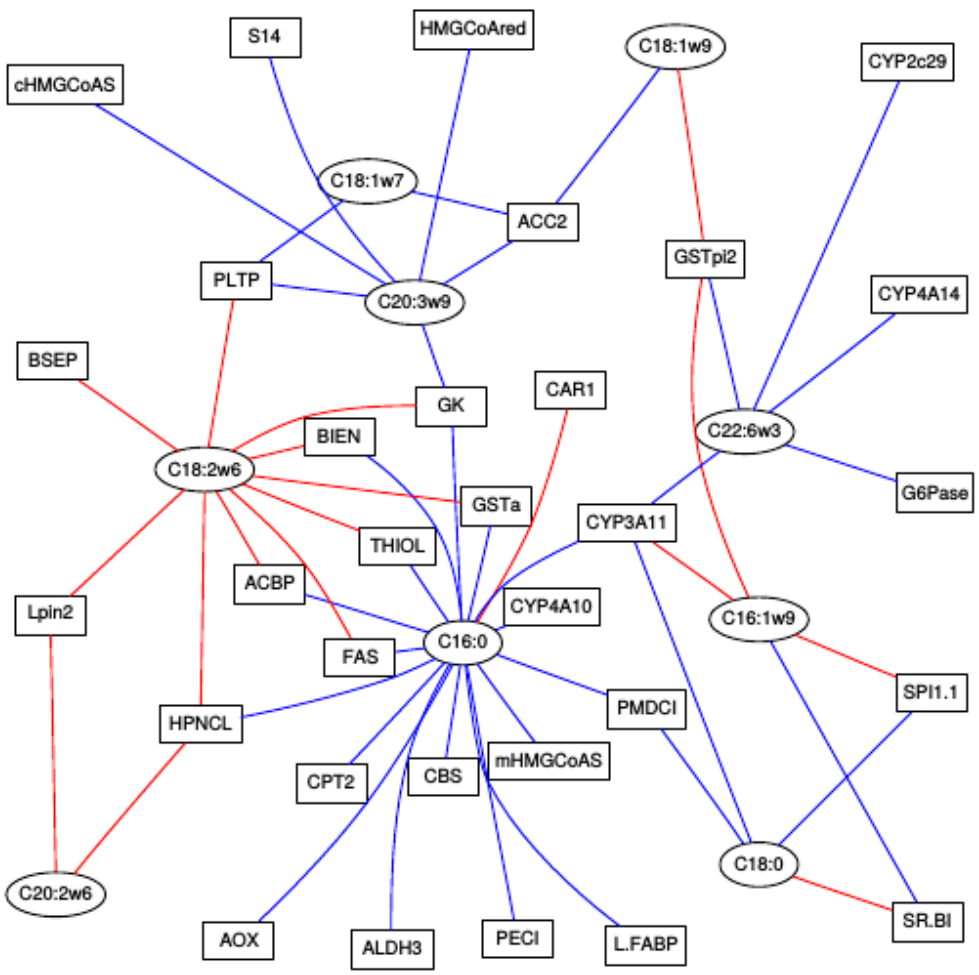
- Clustered Image Maps (CIM), Weinstein et al. (1997)
- Heatmaps, Eisent et al. (1998)

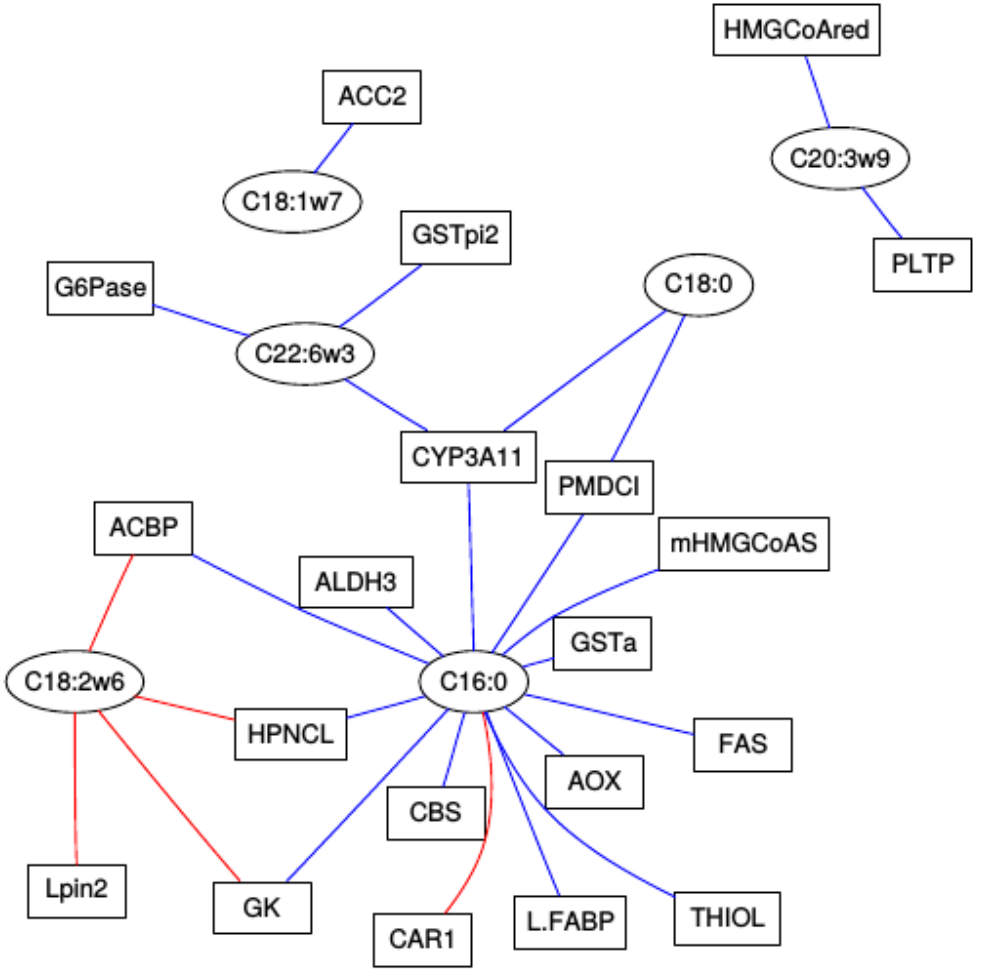


Differ from usual heatmaps crossing individuals and variables

Alternative graphical outputs

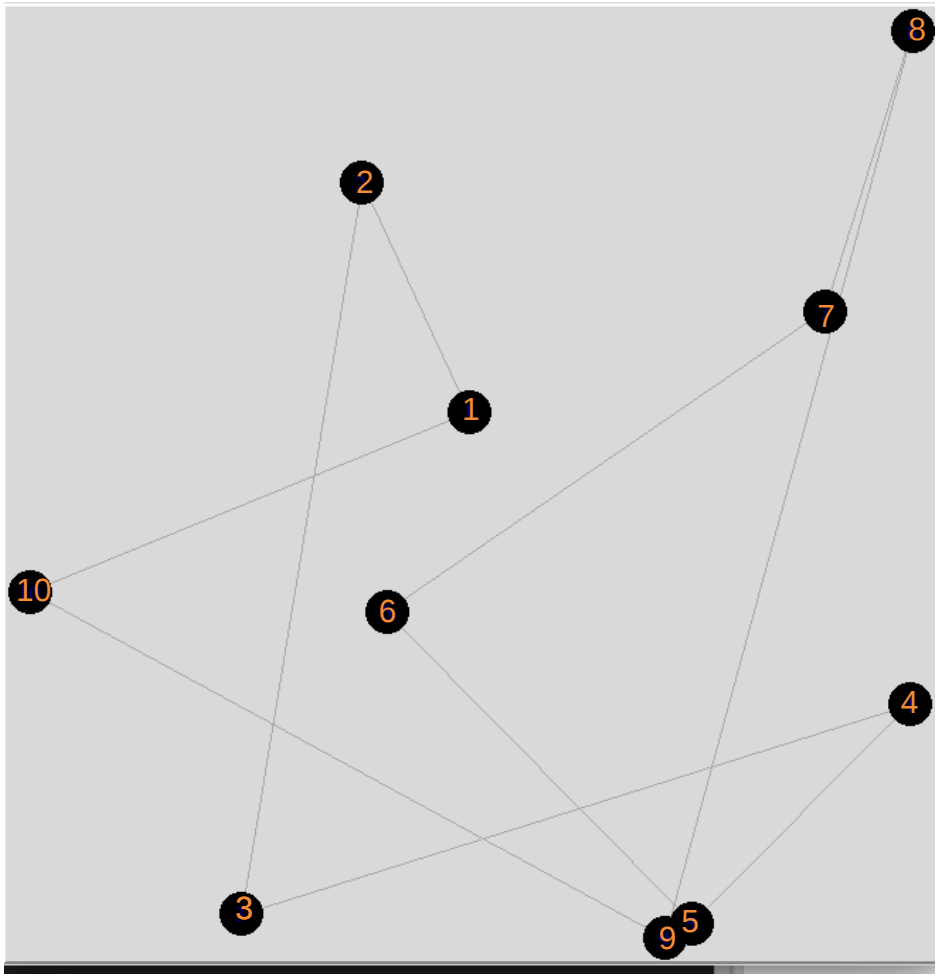
- Covariance Graph, Cox et Wermuth (1993)
- Relevance Network, Butte et al. (2000)

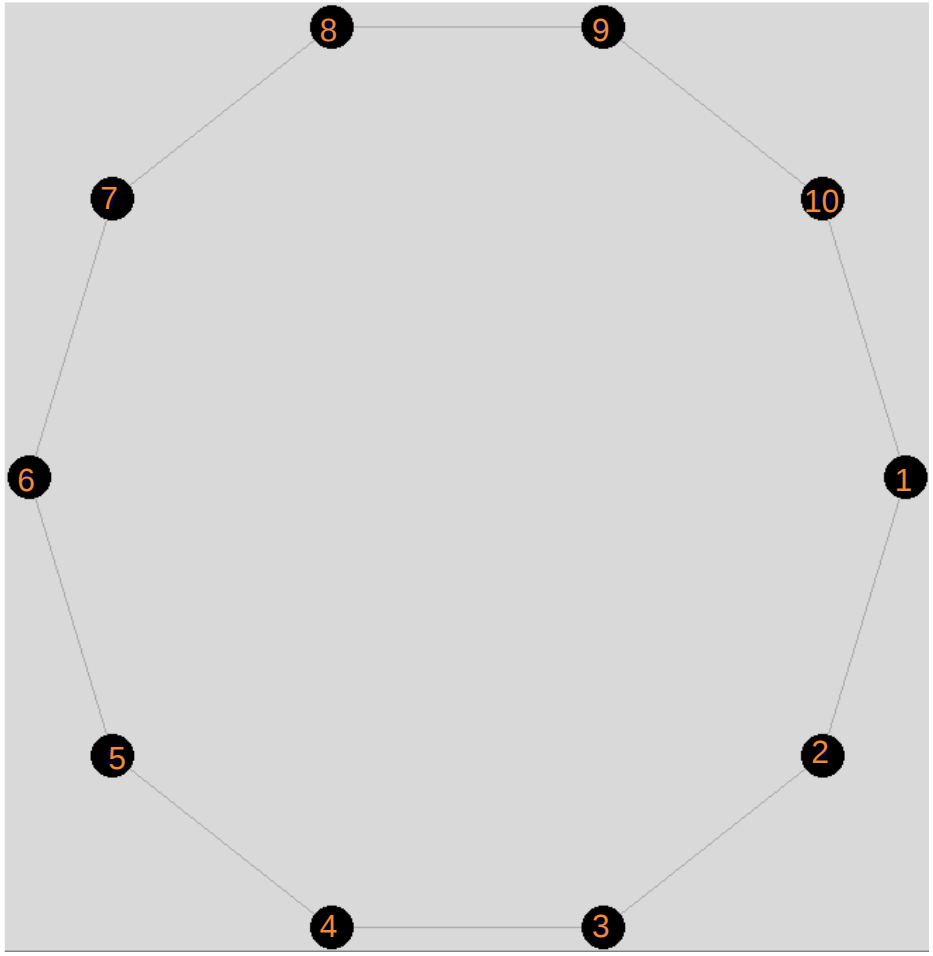




Alternative graphical outputs

- Be careful when interpreting network-based visualisation!
- The same network (same links between same edges) can be represented in very different ways.





Sparsity

Curse of dimensionality

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

The curse of dimensionality refers to various phenomena that arise when analyzing and organizing data in **high-dimensional spaces** (often with hundreds or thousands of dimensions) that do not occur in low-dimensional settings such as the three-dimensional physical space of everyday experience. The expression was coined by Richard E. Bellman when considering problems in dynamic optimization.

→ **Sparse** methods aim at dealing with problems related to the high dimension of the data.

Occam's razor (law of parsimony): this principle states that among competing hypotheses, the one with the fewest assumptions should be selected.

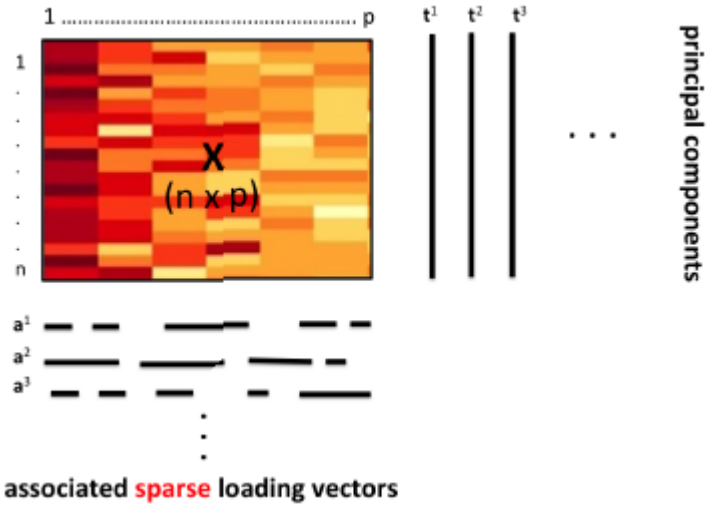
https://en.wikipedia.org/wiki/Occam's_razor

Sparse PCA

- High throughput experiments: too many variables, noisy or irrelevant. PCA is difficult to visualise and understand.

- Clearer signal if some of the variable weights were set to 0 for the 'irrelevant' variables (small weights):

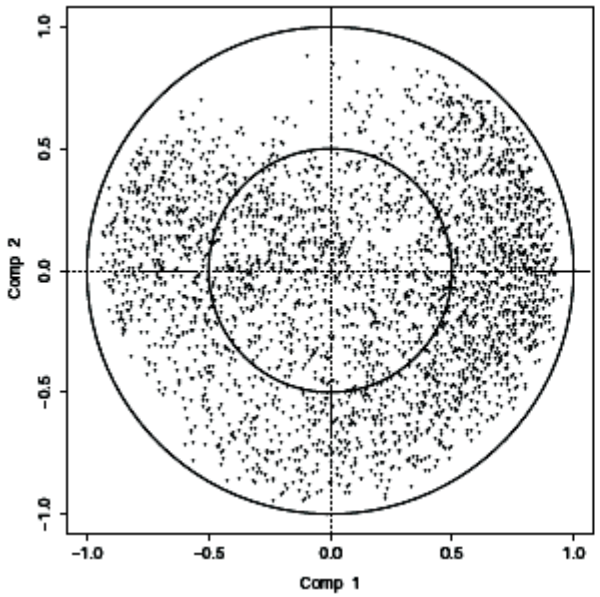
$$t = 0.x1 + a_2.x_2 + \ldots + 0.x_p$$



- **Important weights**: important contribution to define the Pcs.
- **Null weights**: those variables are not taken into account when calculating the PCs

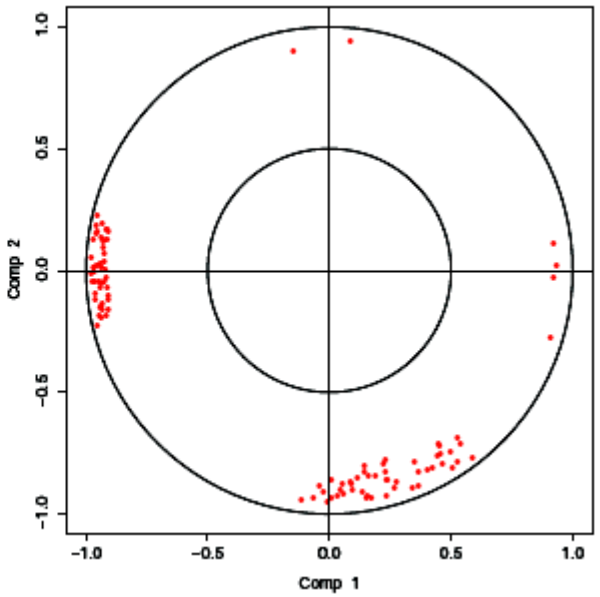
Graphical outputs

PCA

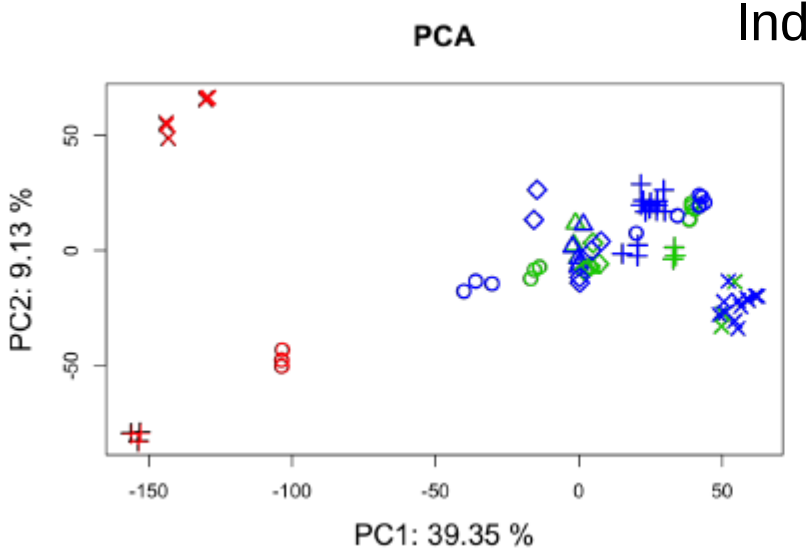


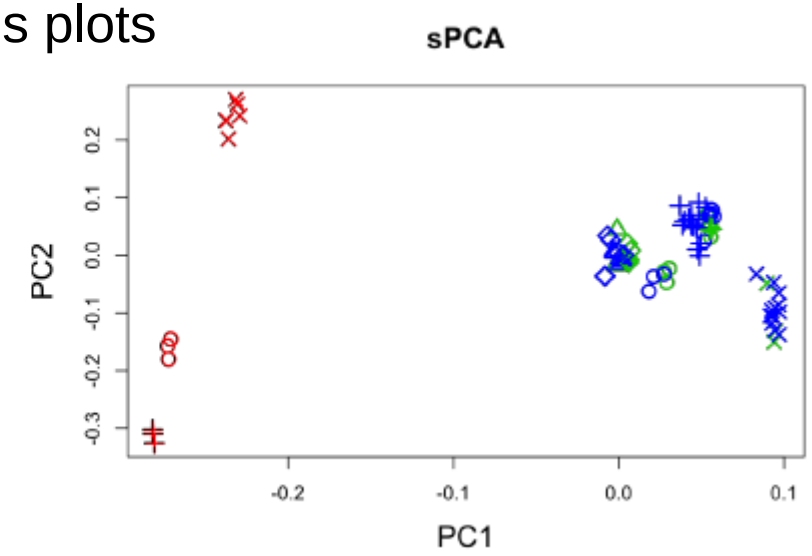
Variables plots

Sparse PCA



Individuals plots





Sparse PLS, PLS-DA...

- **Sparse PLS**: select the variables in each data set that are the most important to define the covariance structures. The selection can be performed on only one data set.

- **Sparse PLS-DA**: select the most discriminating variables in the quantitative data set.

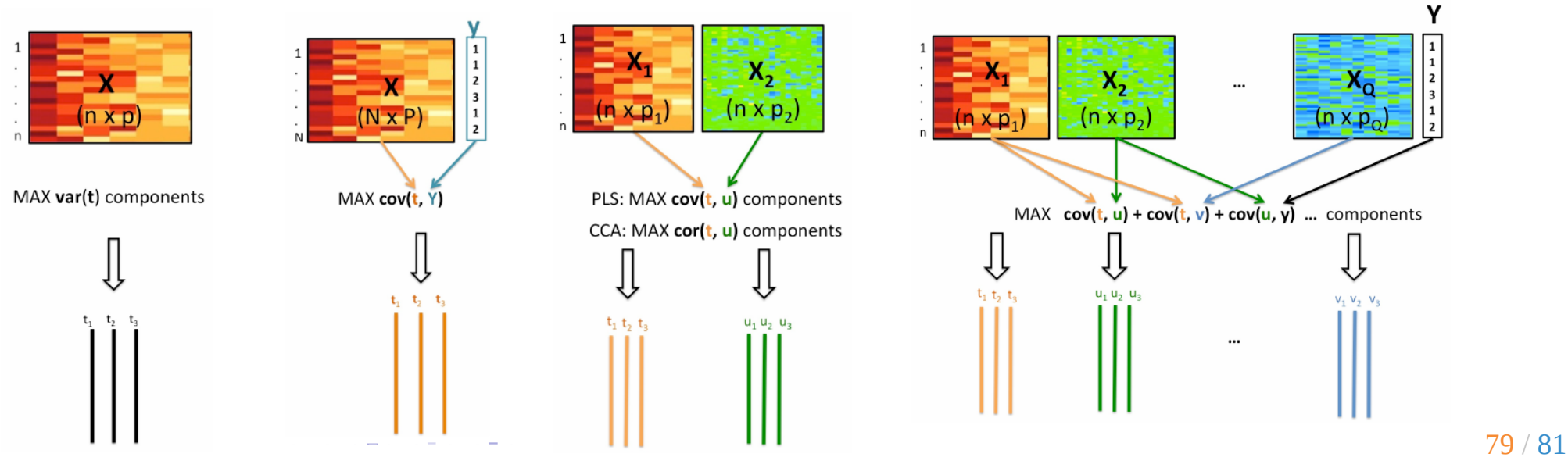
- **Sparse multi-block PLS**: same as Sparse PLS.

To put it in a nustshell

- Multivariate linear methods enables to answer a wide range of biological questions
  - data exploration
  - classification
  - integration of multiple data sets
- Variable selection (*sparse*)
- Cross-over design (*multilevel*)

- Principles

PCA : max var(aX) → a ?
PLS1 : max cov(aX, by) → a, b ?
PLS2 : max cov(aX, bY) → a, b ?
CCA : max cor(aX,bY) → a, b ?
PLSDA → PLS2
GCCA : max Σ cov($a_i X_i$,$b_j X_j$) → a_i, b_i ?



Questions, *feedback*

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And many many mixOmics users and attendees!