# Biological Data Analysis

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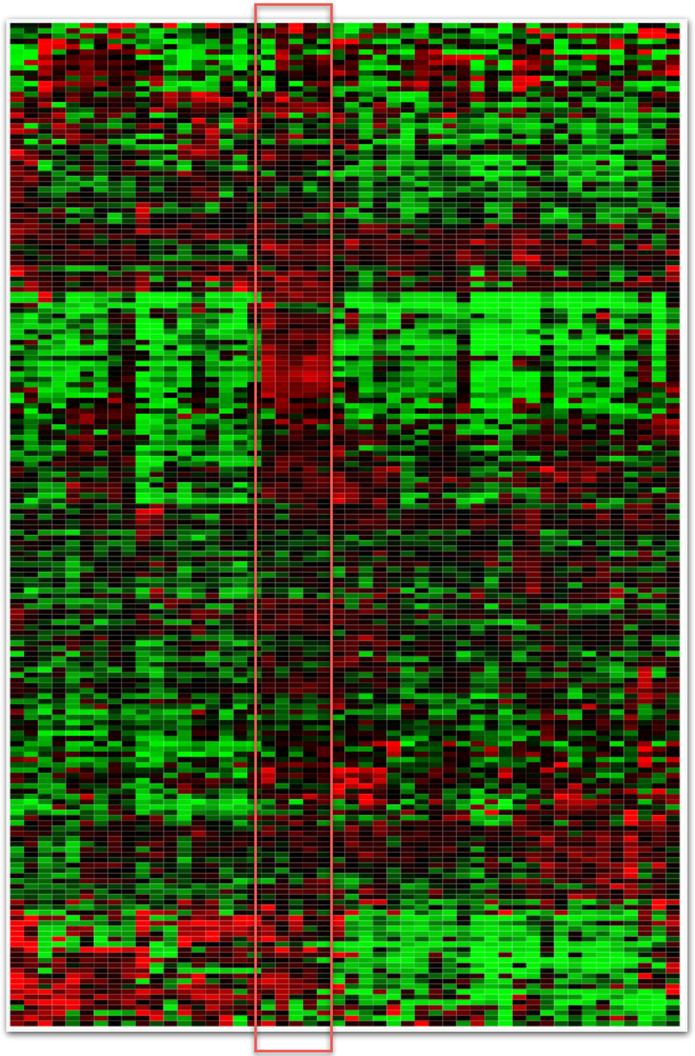
# 4. finding structure in the data
## introduction to clustering

# Finding structure in the data

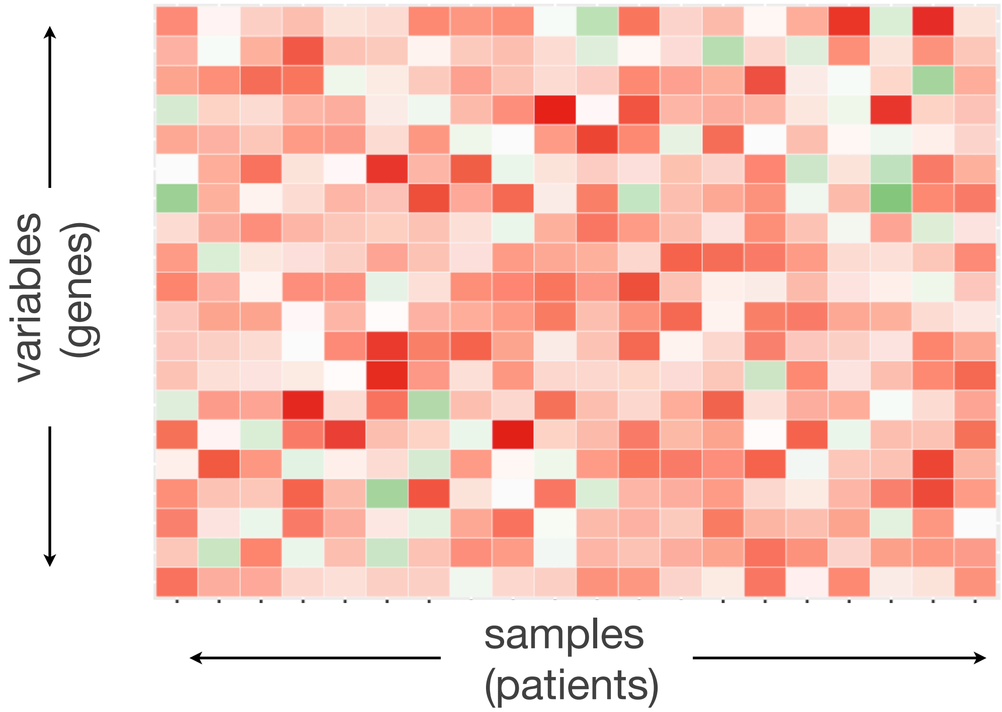
- Goal:
  use a dataset to identify groups of samples
  which seem to be similar
- Example
  - gene expression data
  - clinical data
  - ...
- Important for molecular subtyping

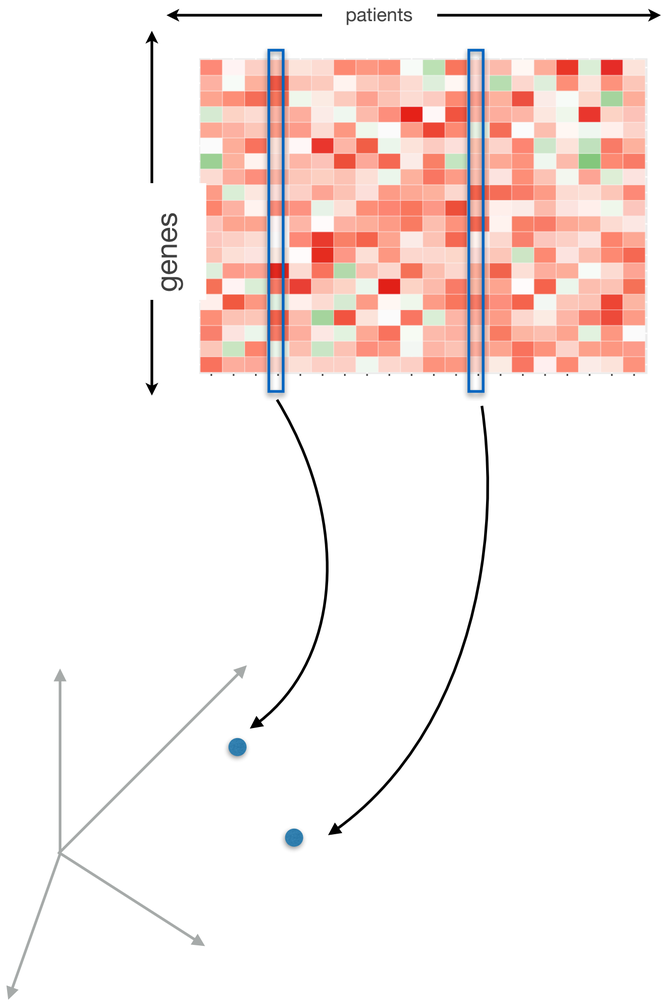


# Finding structure in the data

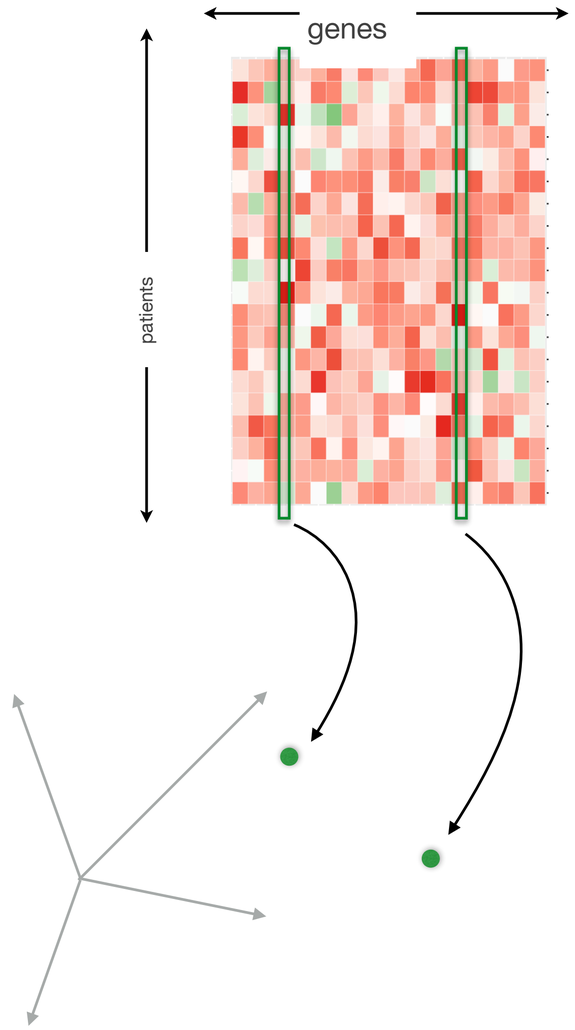
- So far, we have looked at individual variables (distribution, mean, spread,…) and the relationship between **variables**
- We now want to find a structure between **samples** (groups!)

  - group similar samples
    → **clustering**
  - show variables with high variance
    → **principal component analysis**






Points = **patients** in 20,000 dimensional **gene space**




Points = **genes** in 500 dimensional **patient space**

# Distances / dissimilarities

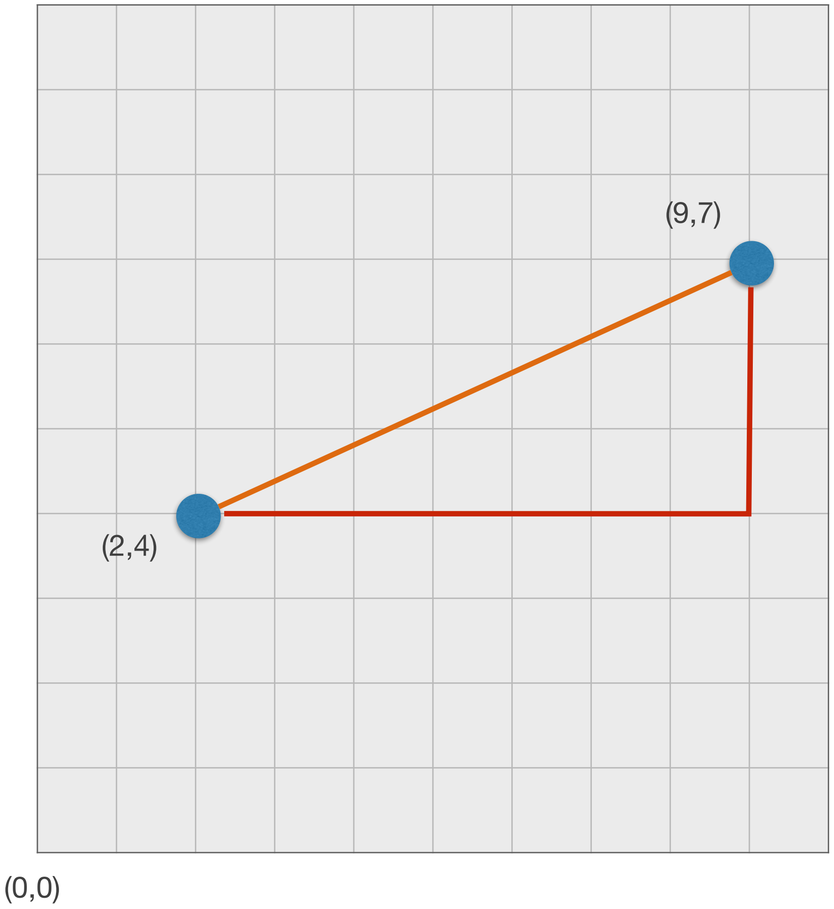
- Distances should be
  - positively defined : *d(a,b) >= 0*
  - *d(a,a) = 0*
  - d(a,c) $\leq$ d(a,b) + d(b,c)
- Several distances can be defined in n-dimensional space
  - **Euclidean distance**

    $d^2 = (9-2)^2+(7-4)^2 = 58$
  - **Manhattan distance**

    d = 7 + 3 = 10
  - **Correlation distance** (when dimension >2)

$$d = \frac{1}{2}(1 - r)$$

$$r = \sum_{i=1,2} \frac{(x_i - \bar{x})(y_i - \bar{y})}{sd(x)sd(y)}$$




***Can be generalized to any number of dimensions!***

# Distances / dissimilarities in any dimensions

- Euclidean distance

$$d_{Euclidean}(x,y) = \sqrt{\sum_{i=1}^{n}(x_i - y_i)^2}$$

- Manhattan distance

$$d_{Manhattan}(x,y) = \sum_{i=1}^{n} x_i - y_i$$

- Correlation distance

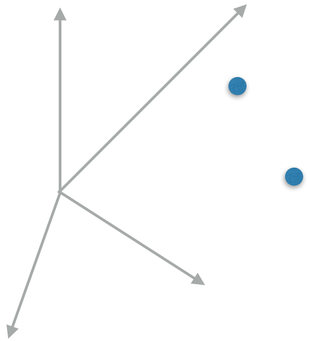
$$r = \frac{1}{n-1}\sum_{i=1}^{n}\frac{(x_i - \bar{x})(y_i - \bar{y})}{sd(x)sd(y)} \qquad d_{corr}(x,y) = \frac{1}{2}(1-r)$$

- Maximum distance

$$d_{Max}(x,y) = max\ x_i - y_i$$

# Example

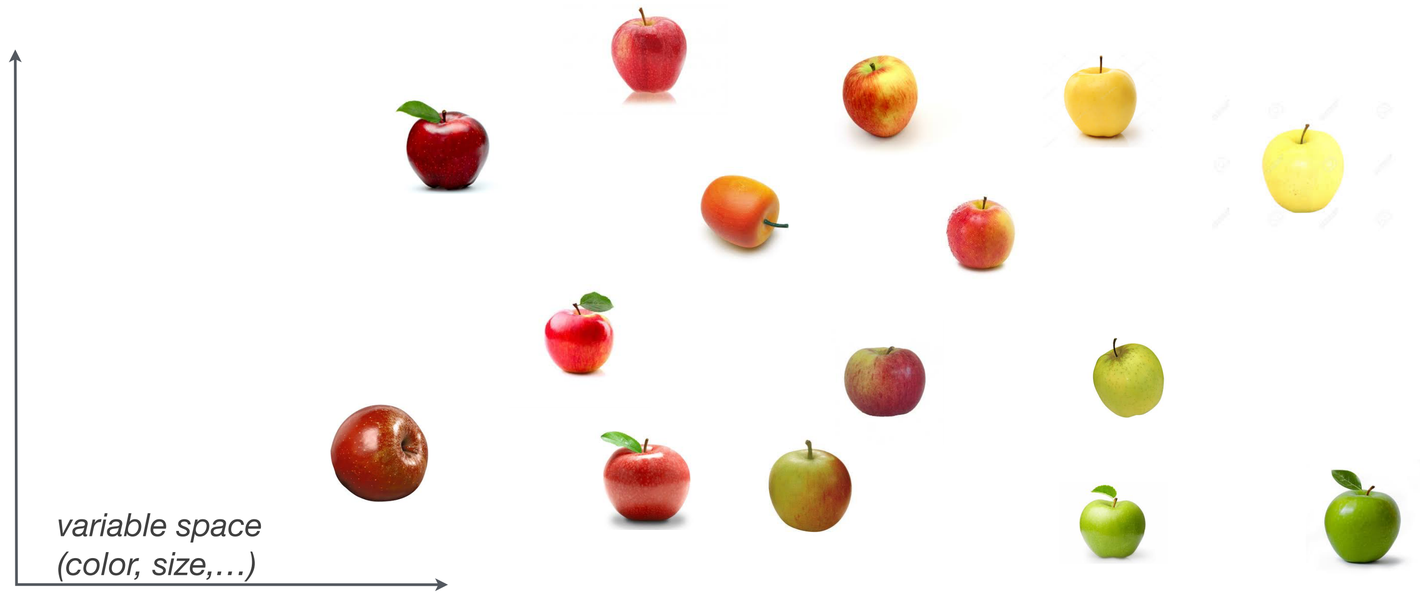
| | variable 1 | variable 2 | variable 3 | variable 4 |
|---|---|---|---|---|
| x | -0.7152422 | -0.7526890 | -0.9385387 | -1.0525133 |
| y | -0.4371595 | 0.3311792 | -2.0142105 | 0.2119804 |



| Distance (x,y) | Value |
|---|---|
| euclidean | 2.00 |
| manhattan | 3.70 |
| maximum | 1.26 |
| correlation distance | 0.5(1-0.16) = 0.42 |

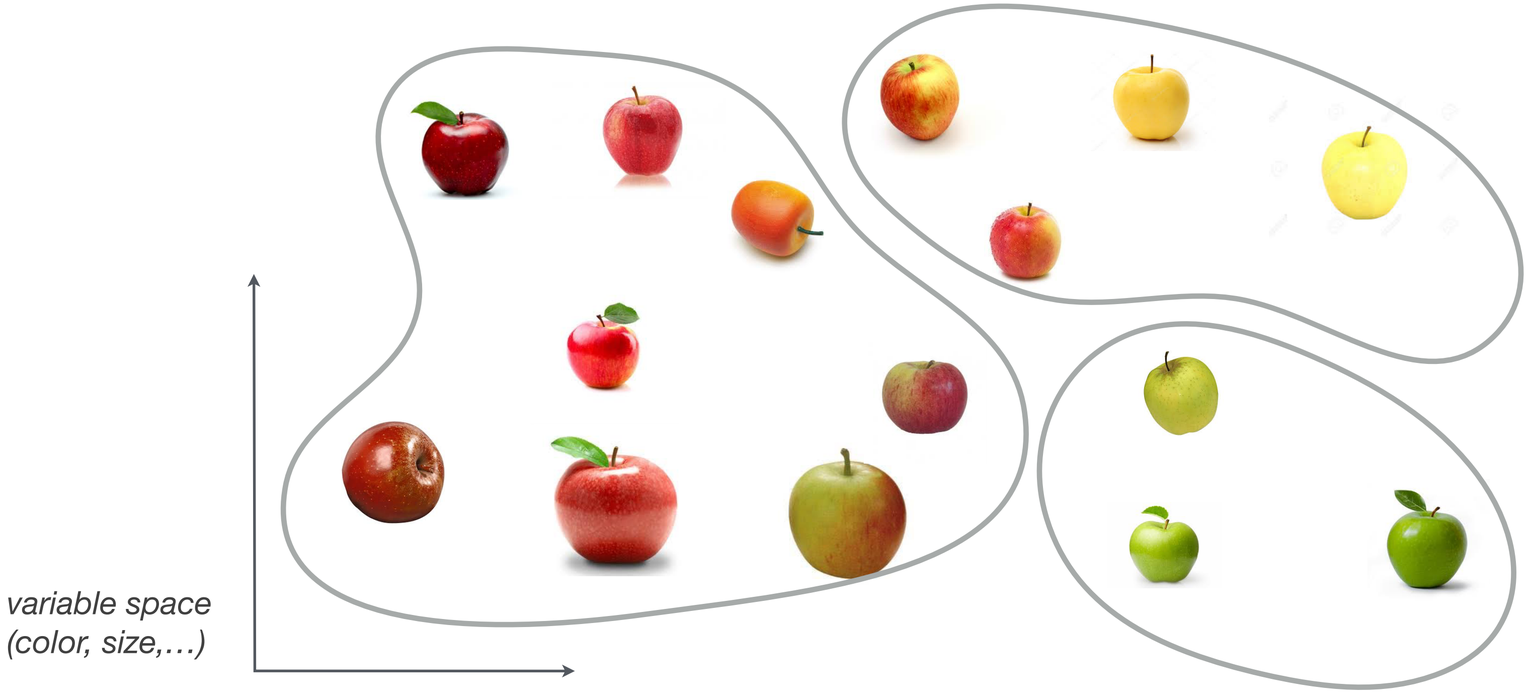
# Clustering data

- Idea: group objects ( = samples) which are **close to each other** in the variable space
- this implies the definition of a **distance between objects**
- **unsupervised approach:** we do **NOT** know to which group which sample belongs!



# Flat clustering

- **Partition samples** into k-groups
- Issues
  - how to define a **distance**?
  - optimal **number *k* of groups**?
  - how to deal with **ambiguous** samples?



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# 4. finding structure in the data
## k-means clustering

# k-means clustering

- **Objects** in a d-dimensional space
  - points in a 2d euclidean space
  - patients in a 20000d space of gene expression, …

- Distance between object is the **euclidean metric**

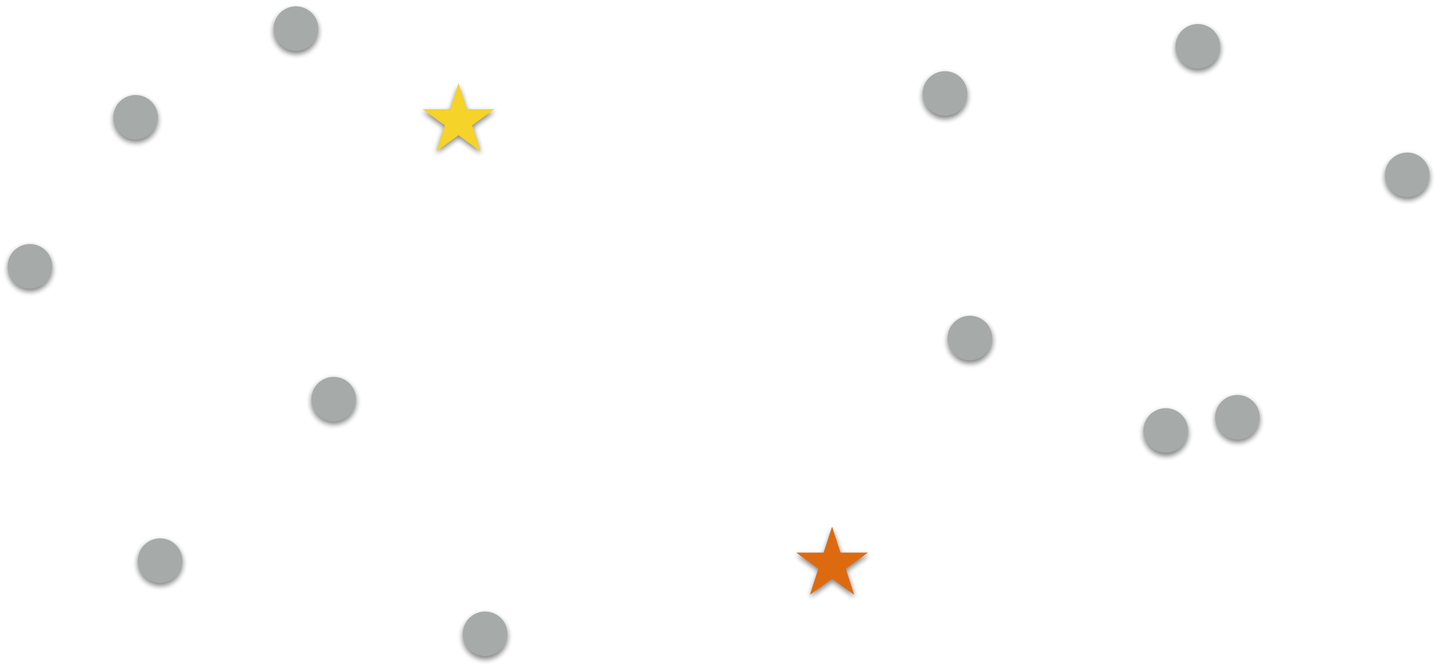
- Define an **expected number *k* of clusters**
  - try out different numbers k
  - use external knowledge

- See demo here
  http://stanford.edu/class/ee103/visualizations/kmeans/kmeans.html

# k-means

# k-means

- **Step 1:** define k random centers (here: *k*=2)
- assign each point to the closest one of these centers



# k-means

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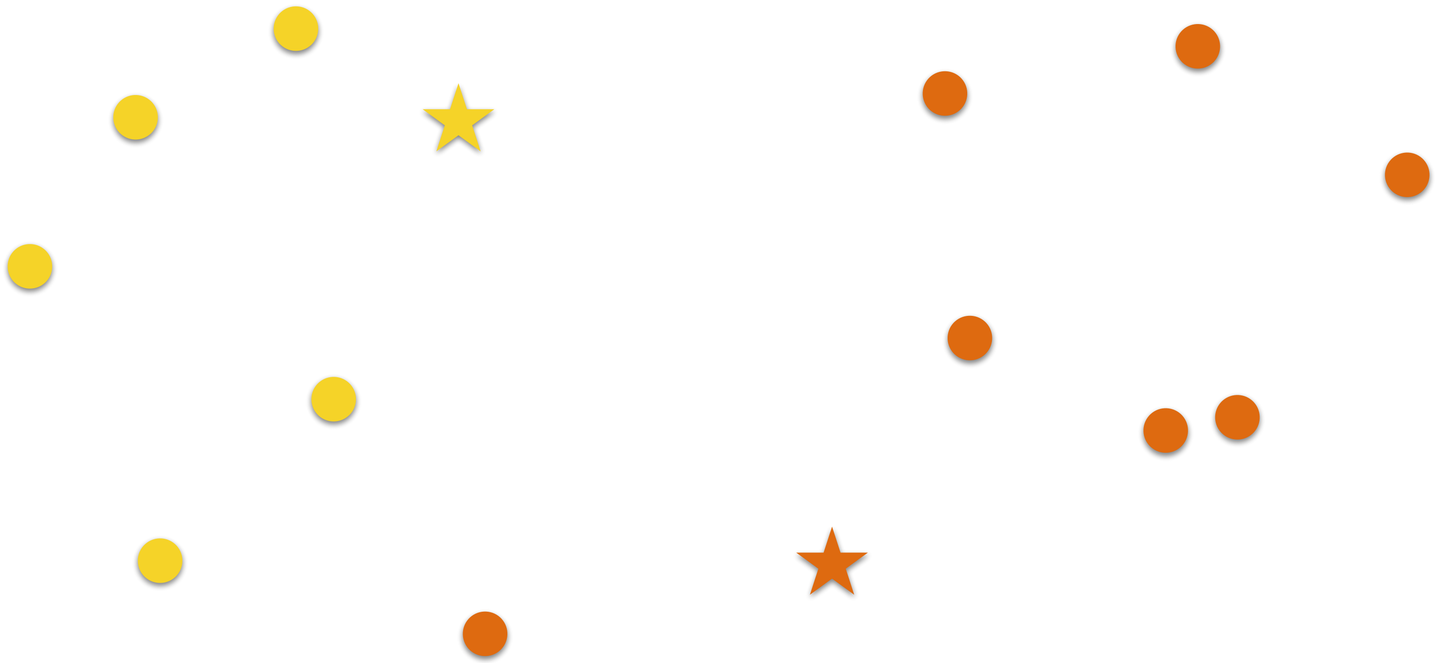
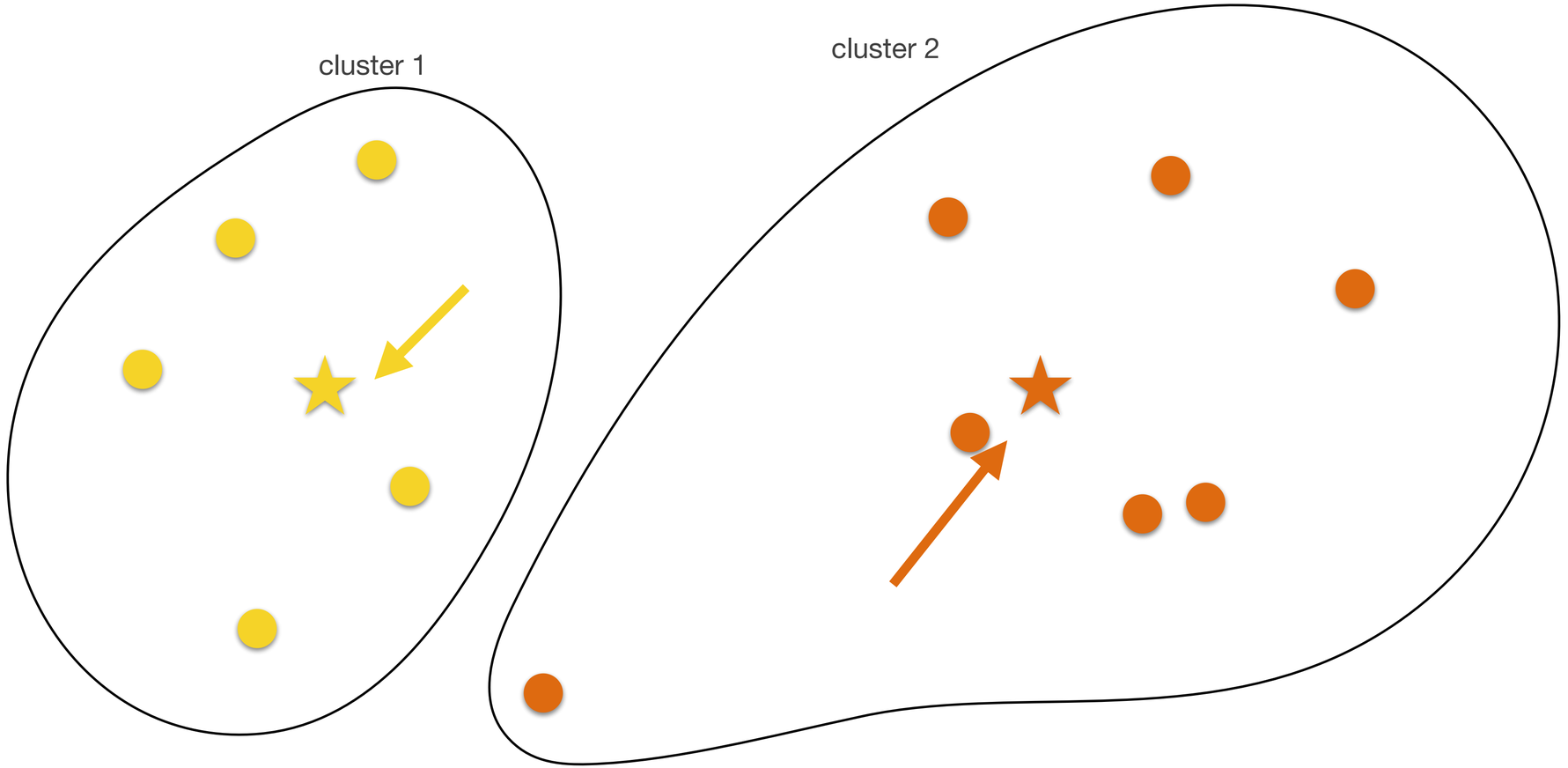


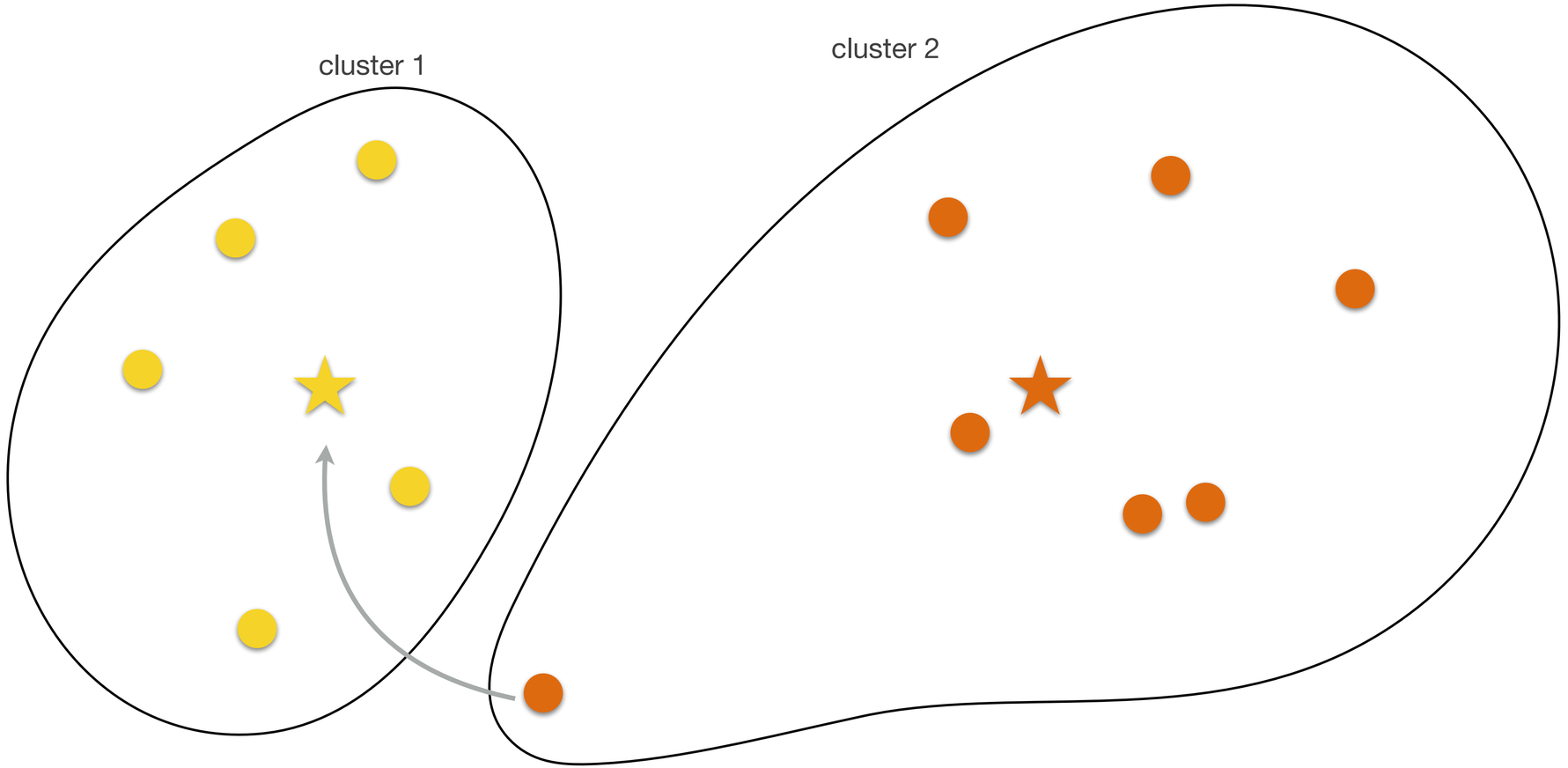
# k-means

- **Step 2:** determine the **center of gravity** of these 2 groups of objects
- assign each point to the closest one of these centers



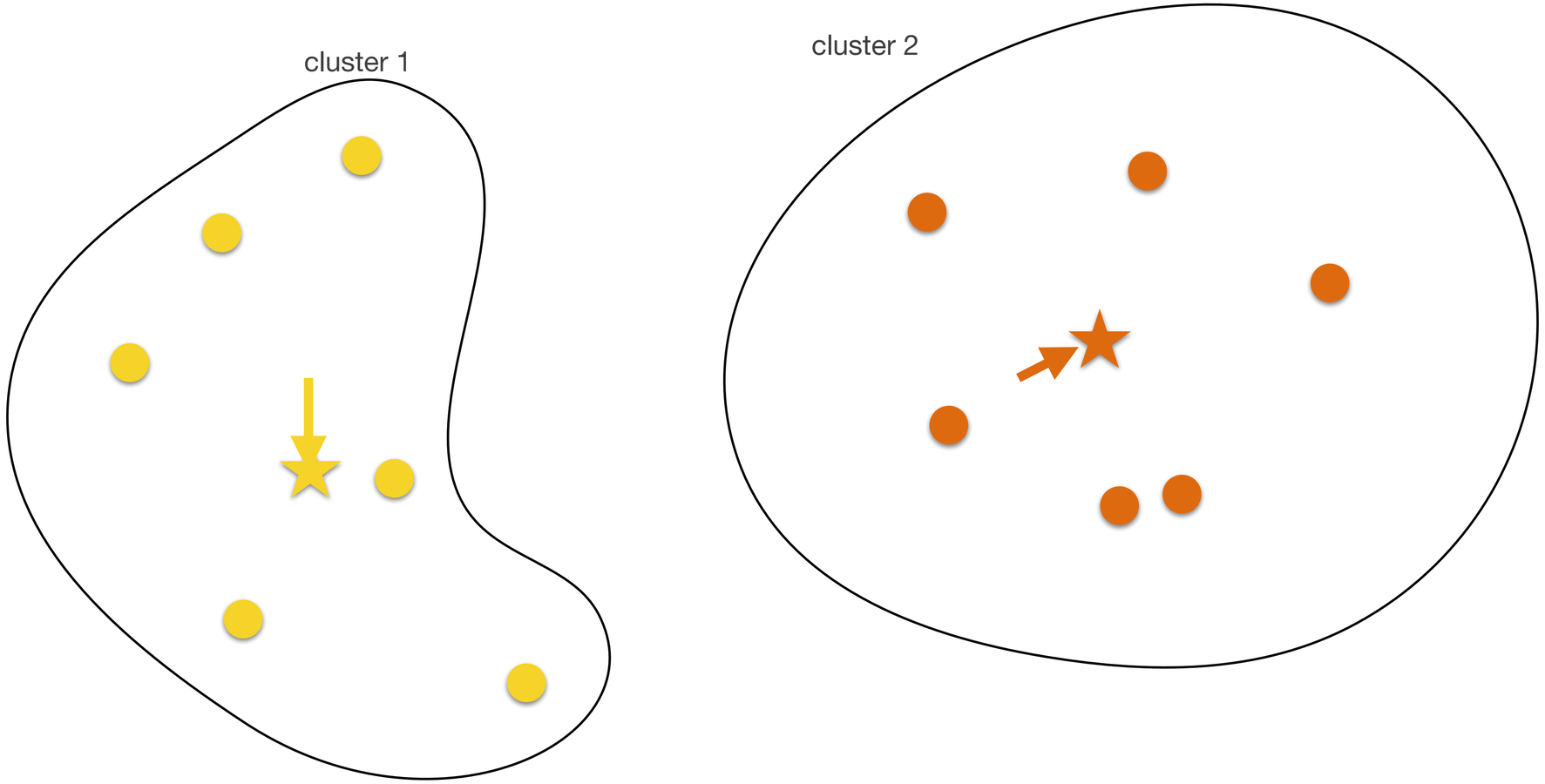
# k-means

- **Step 3:** assign again each object to the updated centers
- assign each point to the closest one of these centers



# k-means

- **Step 4:** update the centers according to the new assignment, and start over



# k-means

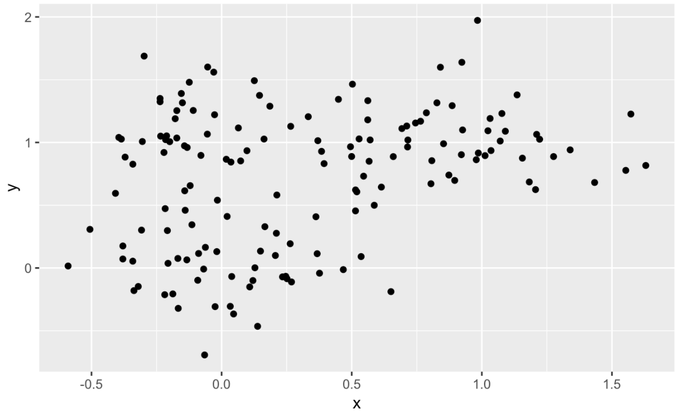
- When to stop:
  - when no object chances its cluster assignment anymore
  - or: when a certain number of max iterations are reached

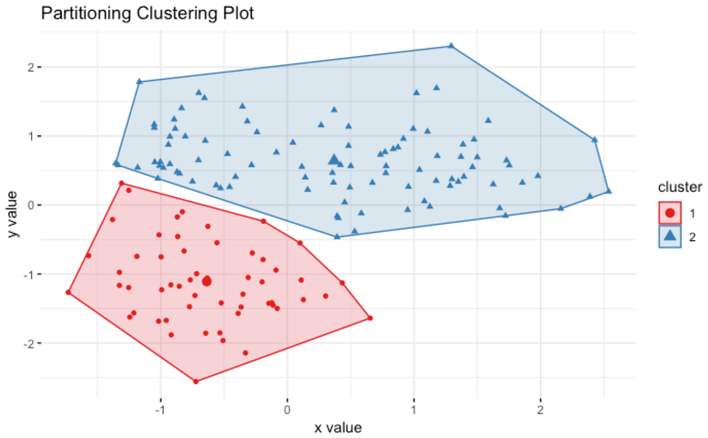
- Process should be repeated over a number of random initial conditions!
- Verify the consistency of the clustering obtained using several random conditions
  - identical = very strong cluster structure
  - fluctuating = no clear cluster structure

- **Clusters will be identified, even if there is no group structure in the data!**

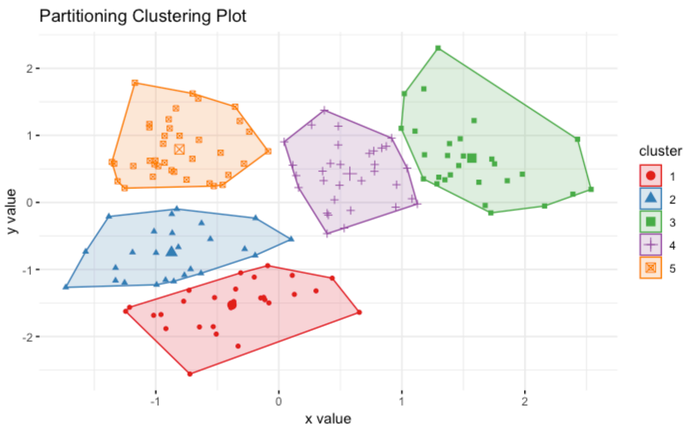
# k-means

## choosing k






Which of these clusterings is better and why?

# k-means
## choosing k

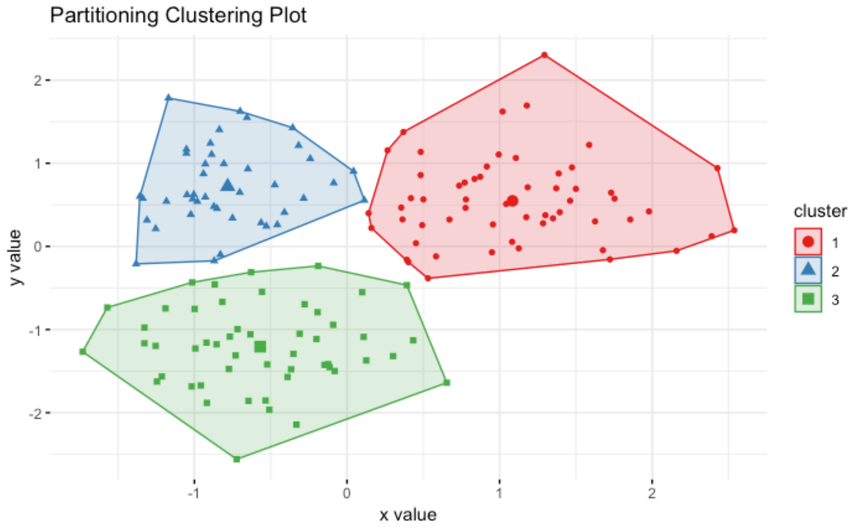
- **Fundamental idea**
  in a good clustering, the **distance whithin clusters** should be much smaller that the **distance between clusters**
- Several methods implement this principle
  - **elbow** method:
  - **silhouette** method
  - (many other methods exist to define optimal cluster number…)

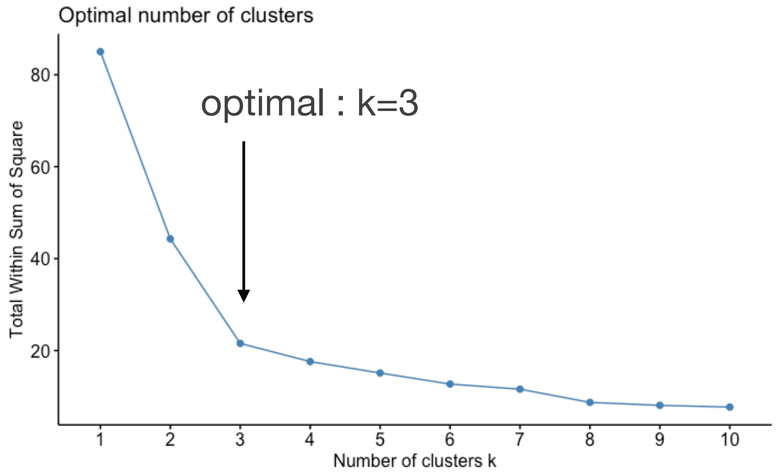
# k-means
## elbow method

- Sum all **pairwise distances squared** between members of the same cluster (within square distance = WSS)

$$WSS = \sum_{i,j \in \text{same cluster}} x_i - x_j \ ^2$$

- If each point is its own cluster, then WSS = 0
- WSS decreases with increasing k
- when optimal number of clusters is reached, adding additional clusters does not improve the WSS that much
  → kink (or “elbow”) in the curve



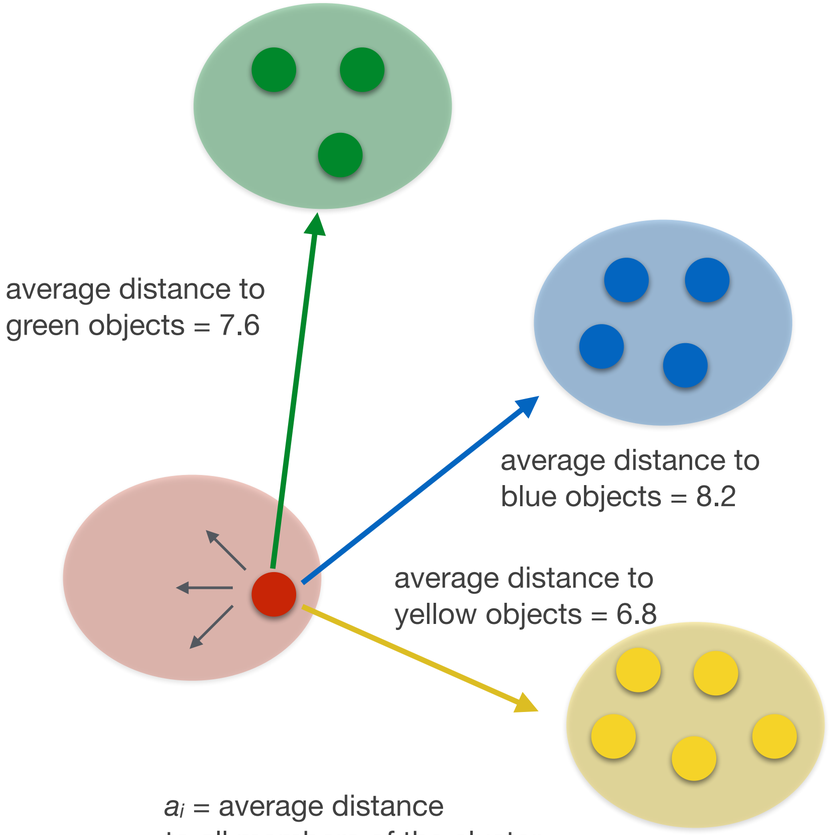
# k-means
## silhouette method

- for each element i, compute
  - mean distance $a_i$ to all members of his cluster
  - smallest average distance $b_i$ to members of all other clusters
- Define silhouette value as

$$s_i = \frac{b_i - a_i}{max(a_i, b_i)}$$

$s_i = 0$ if only one point in cluster

- Properties
  - $s_i$ ~ 1 : object very well clustered
  - $s_i$ ~ 0 : ambiguous object
  - $s_i$ <0 : wrongly assigned
  - Convention : $s_i$ = 0 if only one element in the cluster!

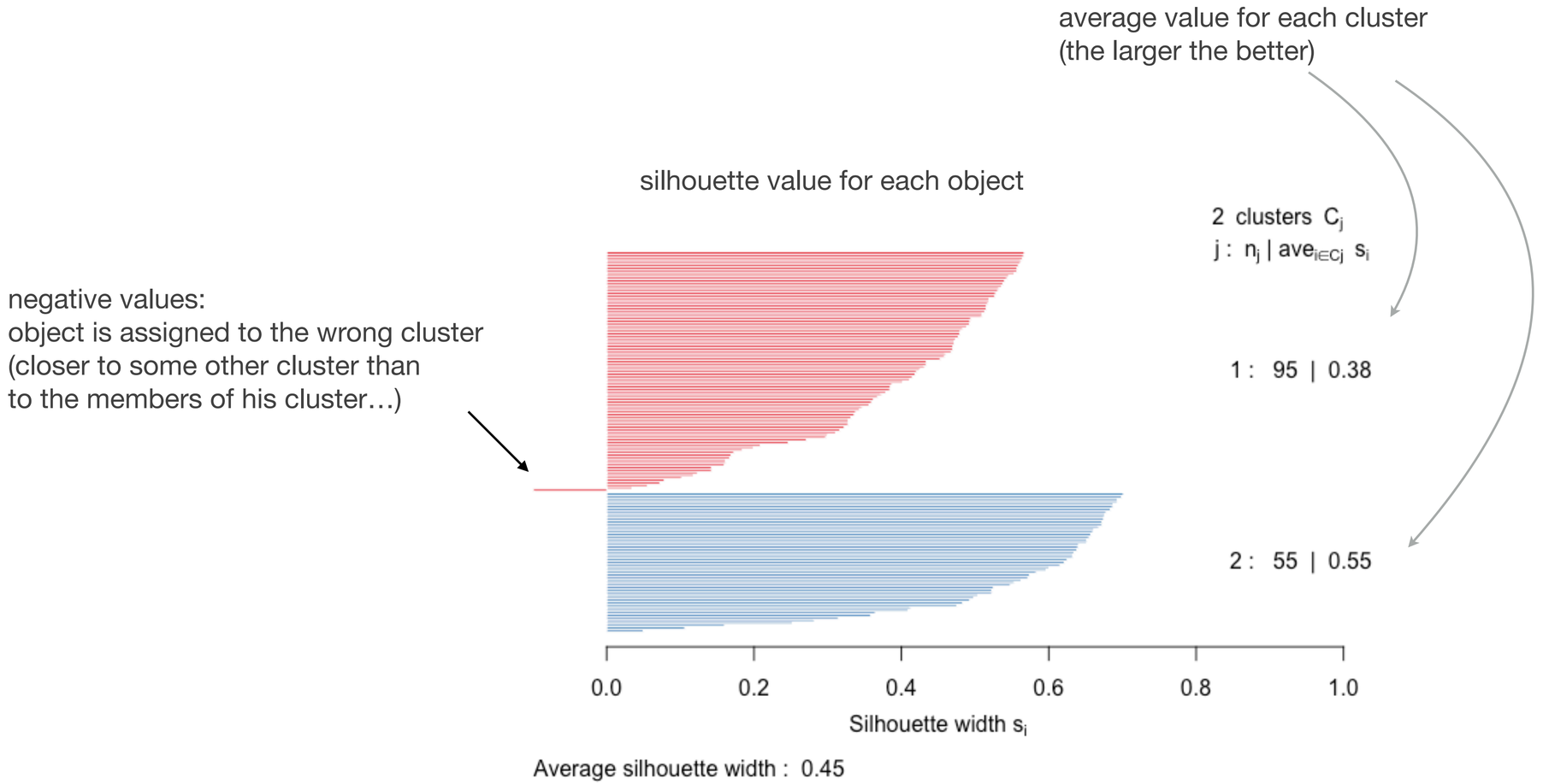



$a_i$ = average distance
to all members of the cluster
ex.: $a_i$ = 4.6

$b_i$ = min(7.6, 8.2, 6.8) = 6.8

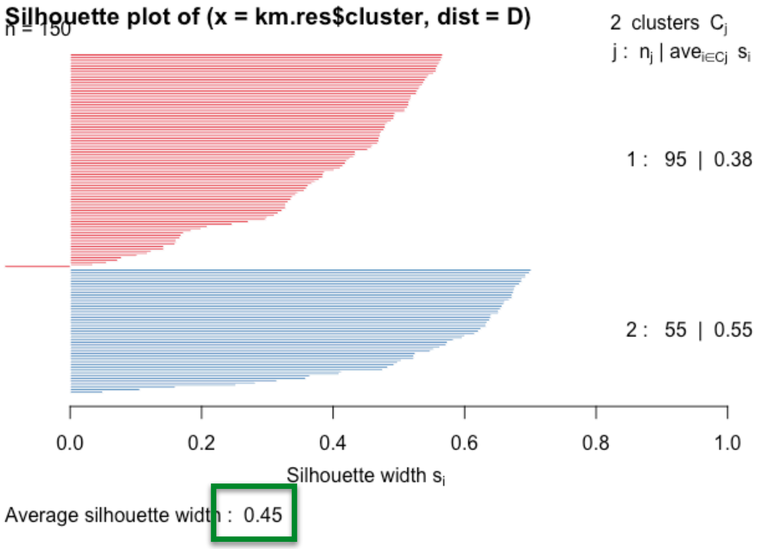
$s_i$ = (6.8 - 4.6)/6.8 = 0.32

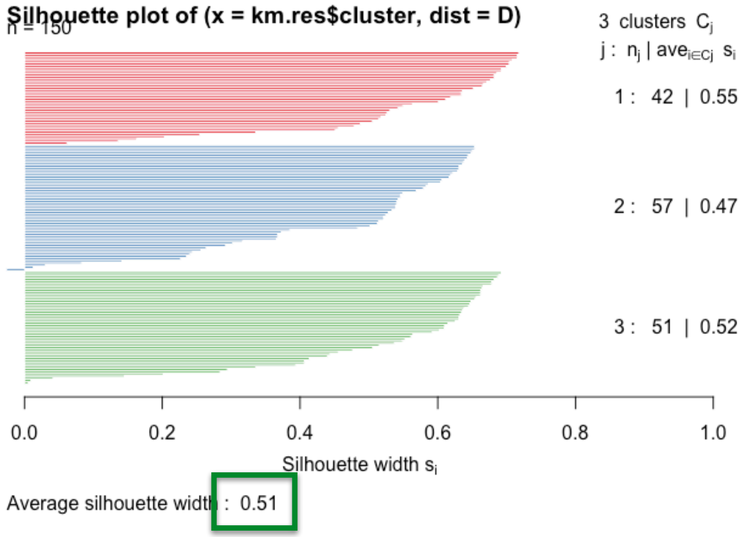
# k-means
## silhouette method

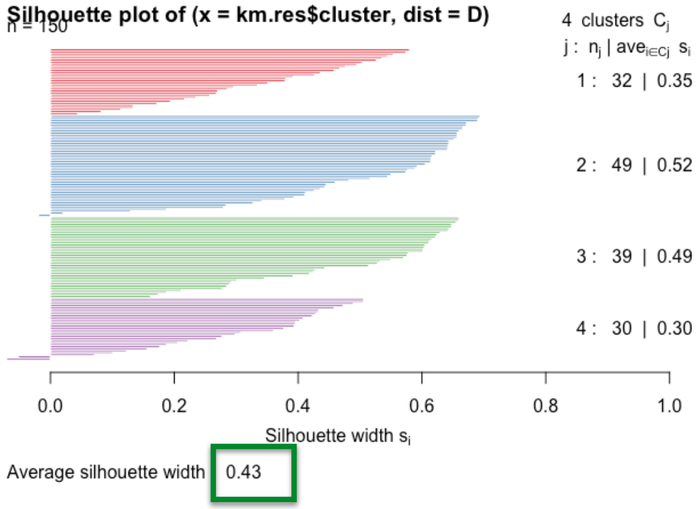


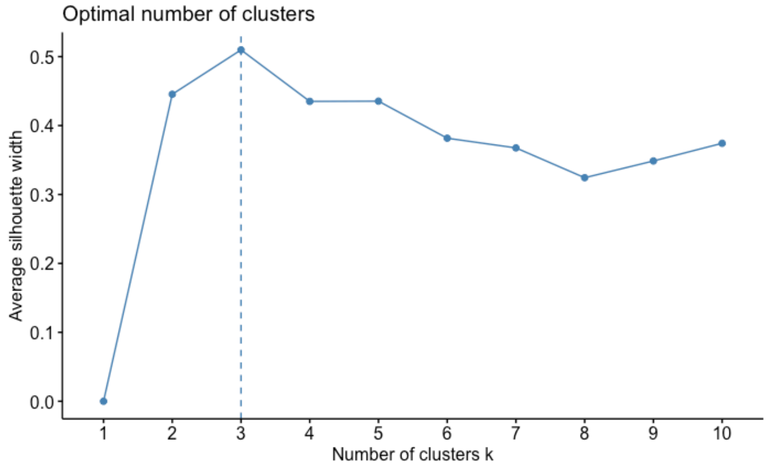
# k-means

## silhouette method



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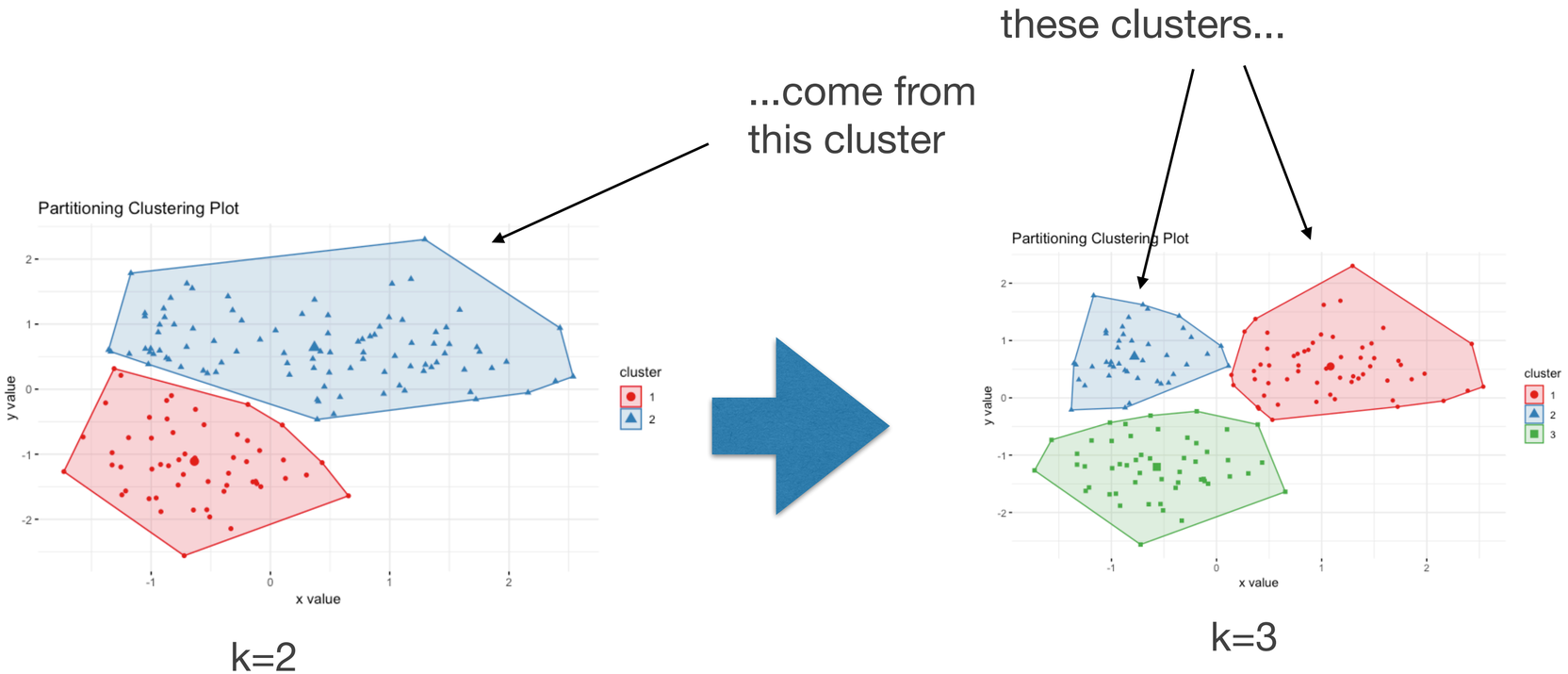
# 4. finding structure in the data
## hierarchical clustering

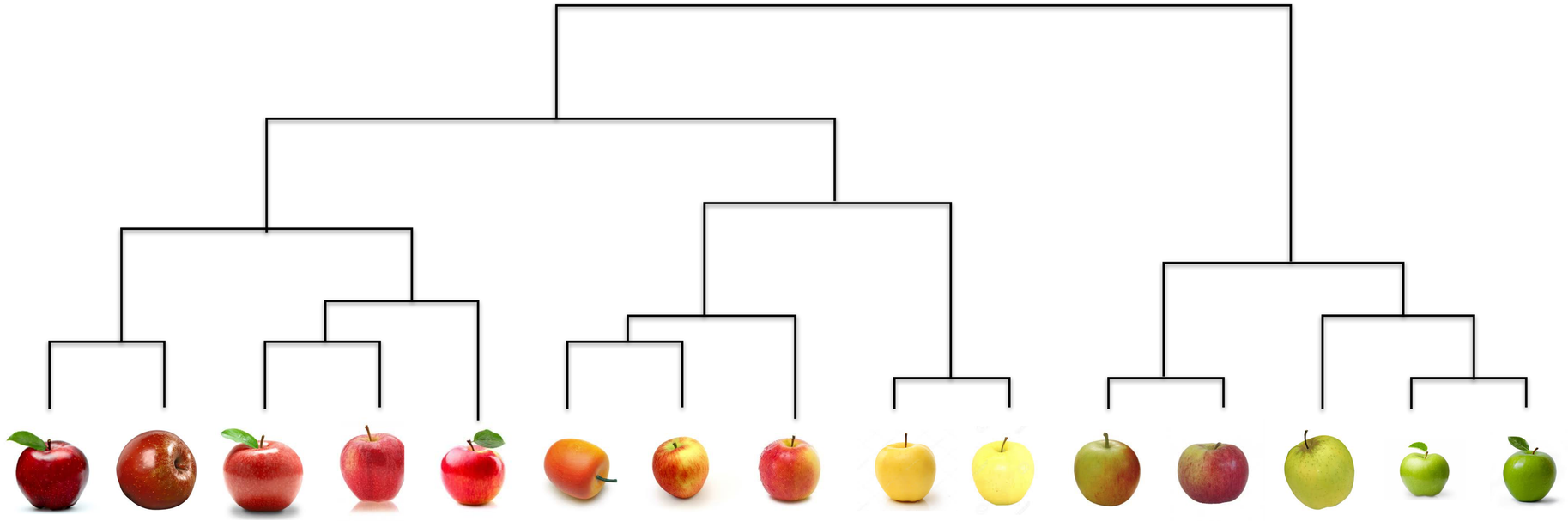
# Clustering

- There is often a hierarchical structure in the data that is not captured by the k-means clustering



# Hierarchical clustering

- **Divisive clustering** (also "top-down"): iteratevely split the full group into sub-groups
- **Agglomerative clustering** (also "Bottom-up"): group iteratively objects from most similars to less similar groups
- tree-like representation of the relationships/distances between objects



# Hierarchical clustering

- Starting point: **distance / similarity matrix** between all objects
- **Agglomerative** method (“bottom up”)
  - Step 1 : find closest pair (x,y)
  - Step 2 : join objects to form a cluster (x,y)
  - Step 3 : replace x and y in the distance matrix by cluster (x,y) and recompute all distances
  - Step 4 : Repeat from Step1
  - Stop when all objects have been merged!

- Distance between single objects: based on distance measure
- Distance between object and cluster?
  → several possible definitions
  (= “linkage methods”)

| | a | b | c | d | e |
|---|---|---|---|---|---|
| a | 0 | 7.1 | 2.7 | 5.1 | 3.8 |
| b | | 0 | 3.2 | 2 | 1.1 |
| c | | | 0 | 4.3 | 4.1 |
| d | | | | 0 | 2.5 |
| e | | | | | 0 |

smallest distance

→ **join (b,e)**

| | a | c | d | **(b,e)** |
|---|---|---|---|---|
| a | 0 | 2.7 | 5.1 | ? |
| c | | 0 | 4.3 | ? |
| d | | | 0 | ? |
| **(b,e)** | | | | 0 |

# Linkage methods

- Several possible definitions of the distance between two clusters of objects (or an object and a cluster)
- **single-linkage**
  d(A,B) = **minimal** distance between all elements of A and B

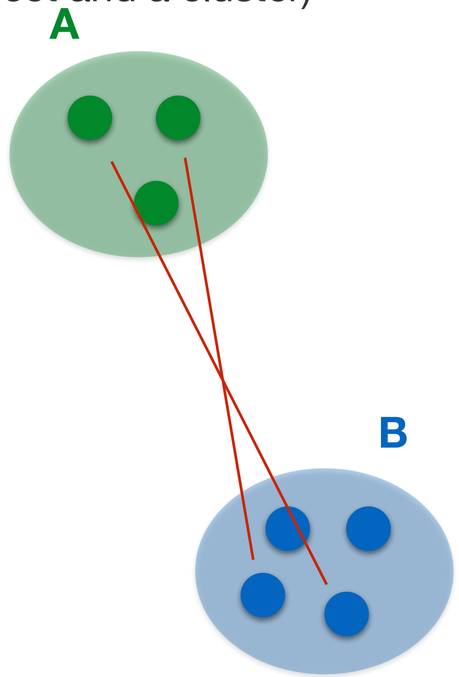
$$d_{\text{single linkage}} = \min_{i,j}(d(a_i, b_j))$$

- **complete-linkage**
  d(A,B) = **maximal** distance between all elements of A and B

$$d_{\text{complete linkage}} = \max_{i,j}(d(a_i, b_j))$$

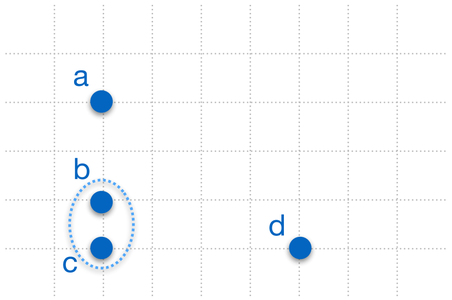
- **average linkage (UPGMA)**
  d(A,B) = **average** of all pairwise distances between elements of A and B

$$d_{\text{average linkage}} = \frac{1}{A \quad B} \sum_i \sum_j d(a_i, b_j)$$

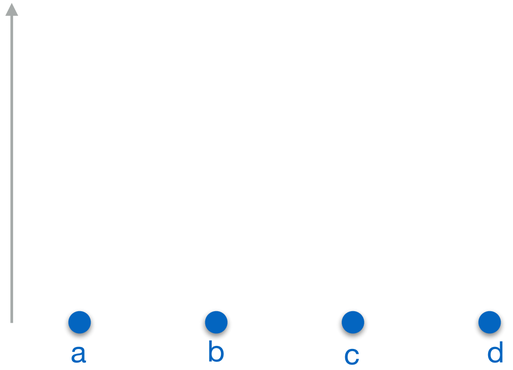


# Hierarchical clustering

(single-linkage)

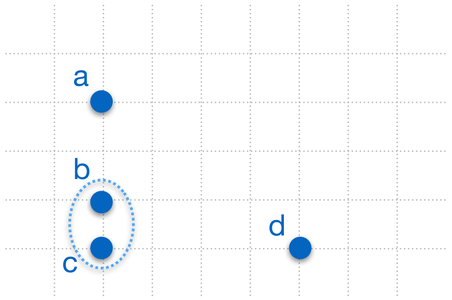



| | a | b | c | d |
|---|---|---|---|---|
| a | 0 | 2 | 3 | 5 |
| b | | 0 | **1** | 4.1 |
| c | | | 0 | 4 |
| d | | | | 0 |

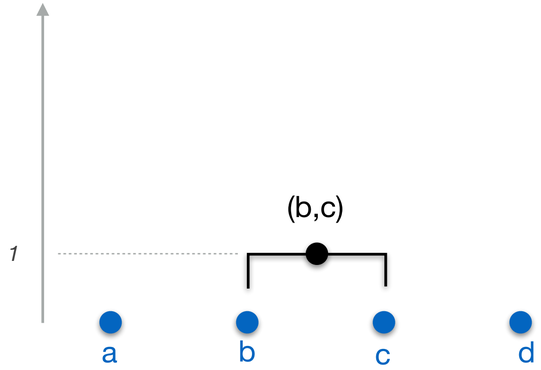


# Hierarchical clustering

(single-linkage)

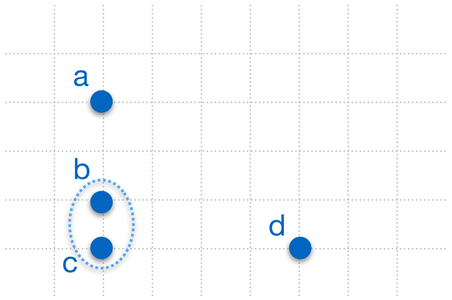



| | a | b | c | d |
|---|---|---|---|---|
| a | 0 | 2 | 3 | 5 |
| b | | 0 | **1** | 4.1 |
| c | | | 0 | 4 |
| d | | | | 0 |

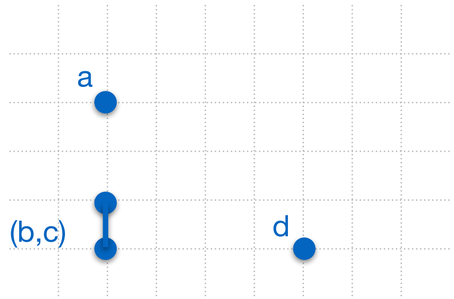


# Hierarchical clustering

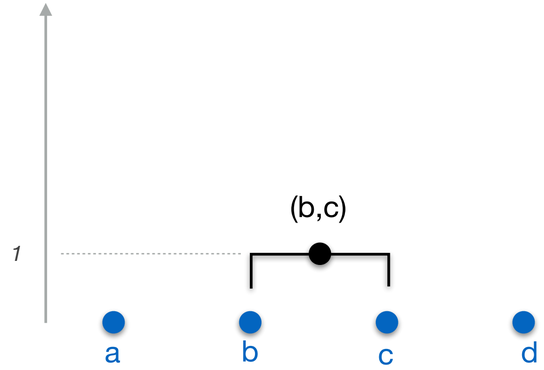
(single-linkage)




| | a | b | c | d |
|---|---|---|---|---|
| a | 0 | 2 | 3 | 5 |
| b | | 0 | **1** | 4.1 |
| c | | | 0 | 4 |
| d | | | | 0 |

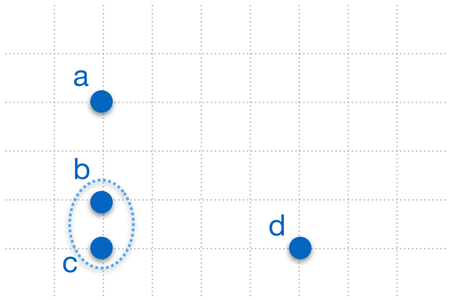



| | a | (b,c) | d |
|---|---|---|---|
| a | 0 | **2** | 5 |
| (b,c) | | 0 | **4** |
| d | | | 0 |

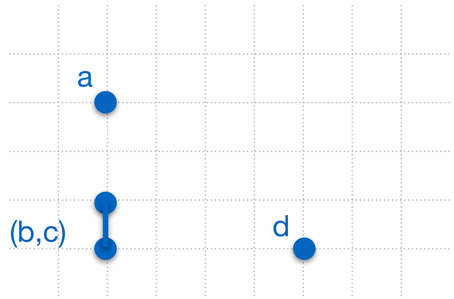


# Hierarchical clustering

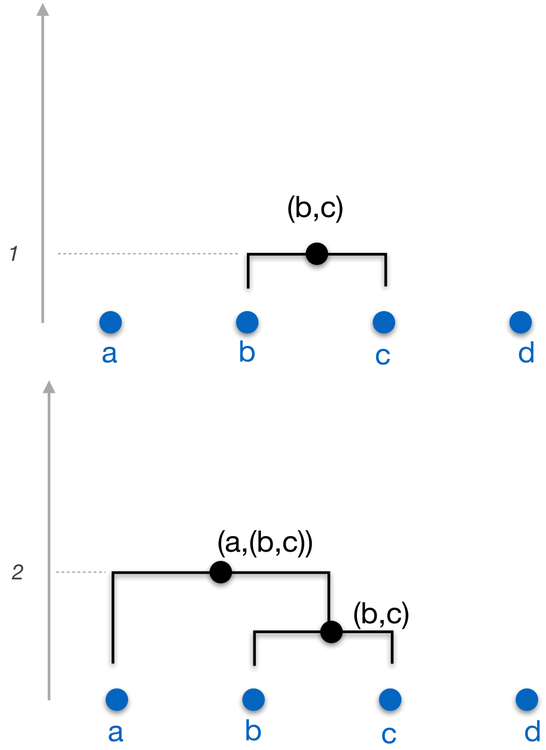
(single-linkage)




| | a | b | c | d |
|---|---|---|---|---|
| a | 0 | 2 | 3 | 5 |
| b | | 0 | **1** | 4.1 |
| c | | | 0 | 4 |
| d | | | | 0 |

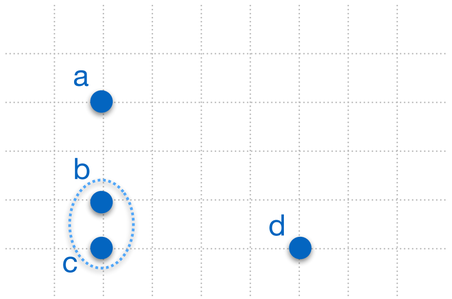



| | a | (b,c) | d |
|---|---|---|---|
| a | 0 | **2** | 5 |
| (b,c) | | 0 | **4** |
| d | | | 0 |

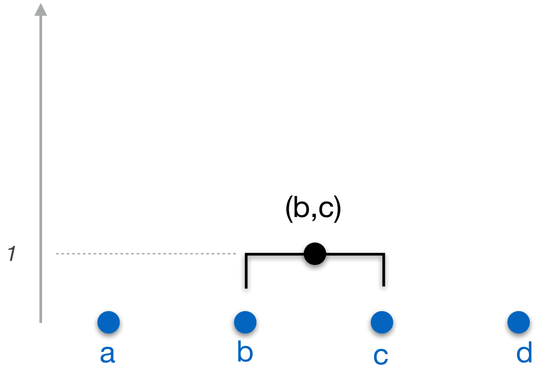


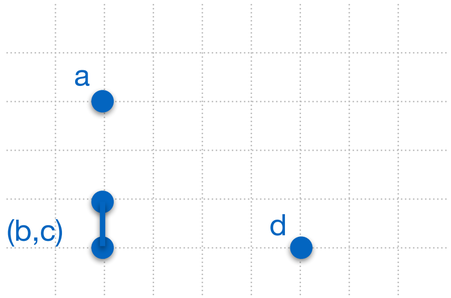
# Hierarchical clustering

(single-linkage)

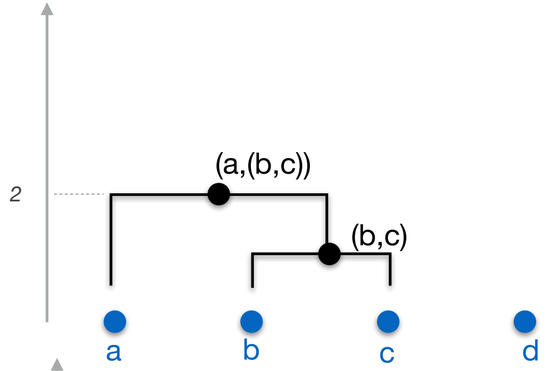


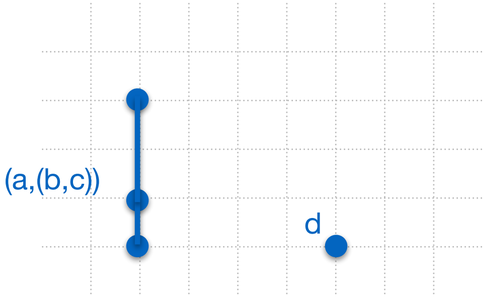

| | a | b | c | d |
|---|---|---|---|---|
| a | 0 | 2 | 3 | 5 |
| b | | 0 | **1** | 4.1 |
| c | | | 0 | 4 |
| d | | | | 0 |



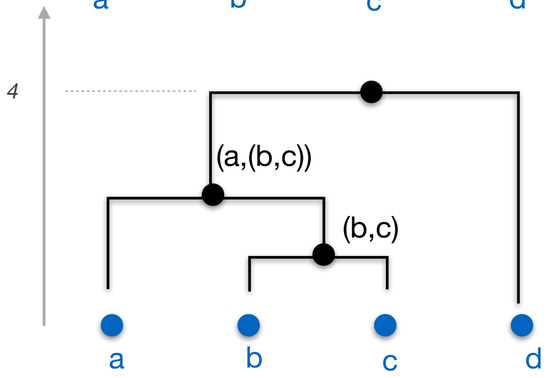




| | a | (b,c) | d |
|---|---|---|---|
| a | 0 | **2** | 5 |
| (b,c) | | 0 | **4** |
| d | | | 0 |



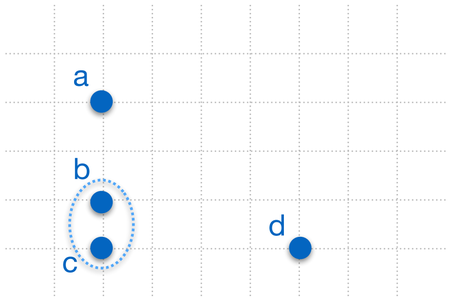




| | (a,(b,c)) | d |
|---|---|---|
| (a,(b,c)) | 0 | **4** |
| d | | 0 |

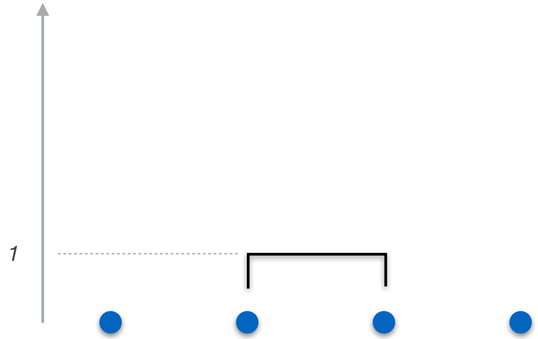


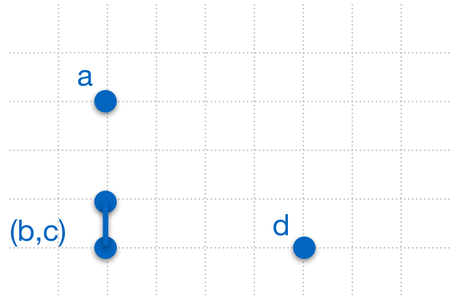
# Hierarchical clustering

(complete-linkage)

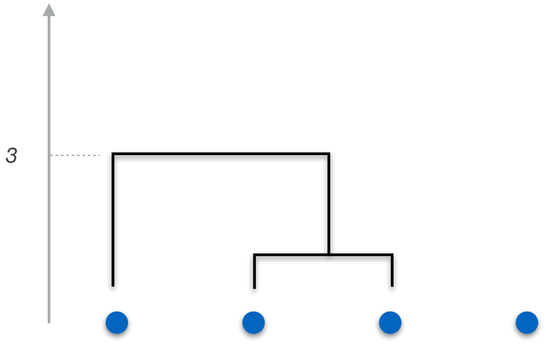


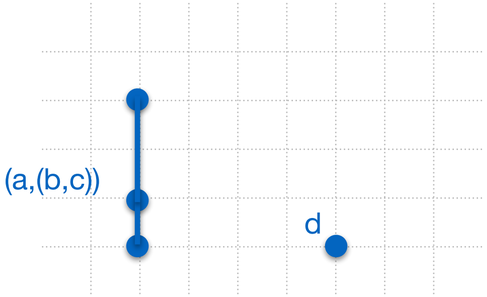

| | a | b | c | d |
|---|---|---|---|---|
| a | 0 | 2 | 3 | 5 |
| b | | 0 | **1** | 4.1 |
| c | | | 0 | 4 |
| d | | | | 0 |



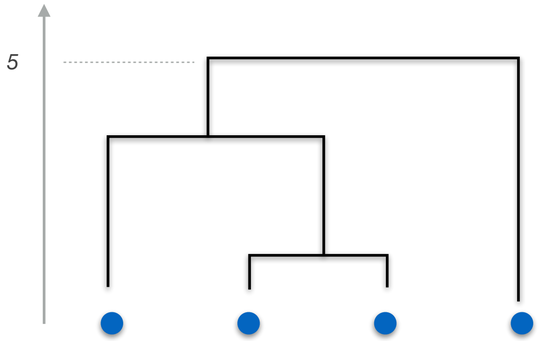




| | a | (b,c) | d |
|---|---|---|---|
| a | 0 | **3** | 5 |
| (b,c) | | 0 | **4.1** |
| d | | | 0 |



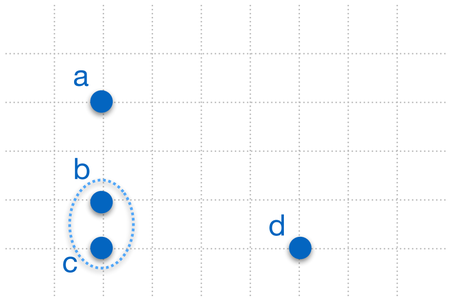




| | (a,(b,c)) | d |
|---|---|---|
| (a,(b,c)) | 0 | **5** |
| d | | 0 |

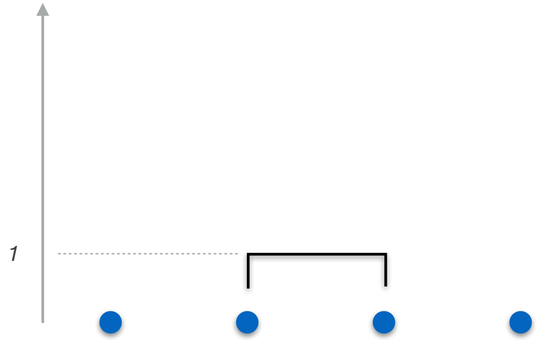


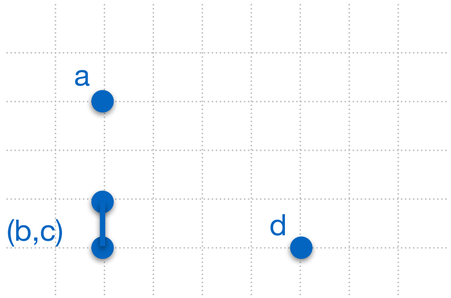
# Hierarchical clustering

(average-linkage)

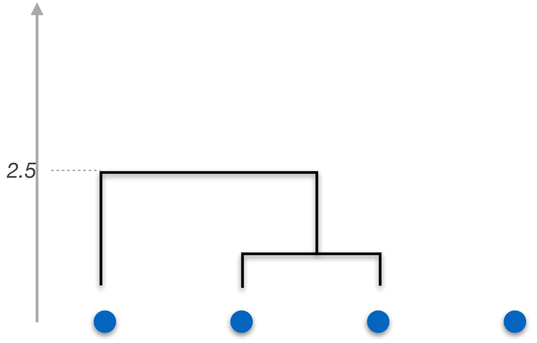


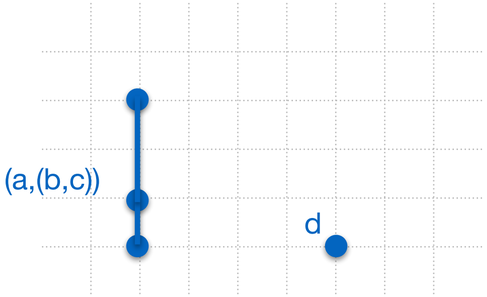

| | a | b | c | d |
|---|---|---|---|---|
| a | 0 | 2 | 3 | 5 |
| b | | 0 | **1** | 4.1 |
| c | | | 0 | 4 |
| d | | | | 0 |



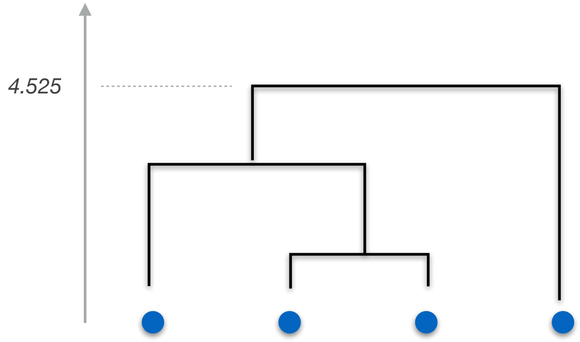




| | a | (b,c) | d |
|---|---|---|---|
| a | 0 | **2.5** | 5 |
| (b,c) | | 0 | **4.05** |
| d | | | 0 |



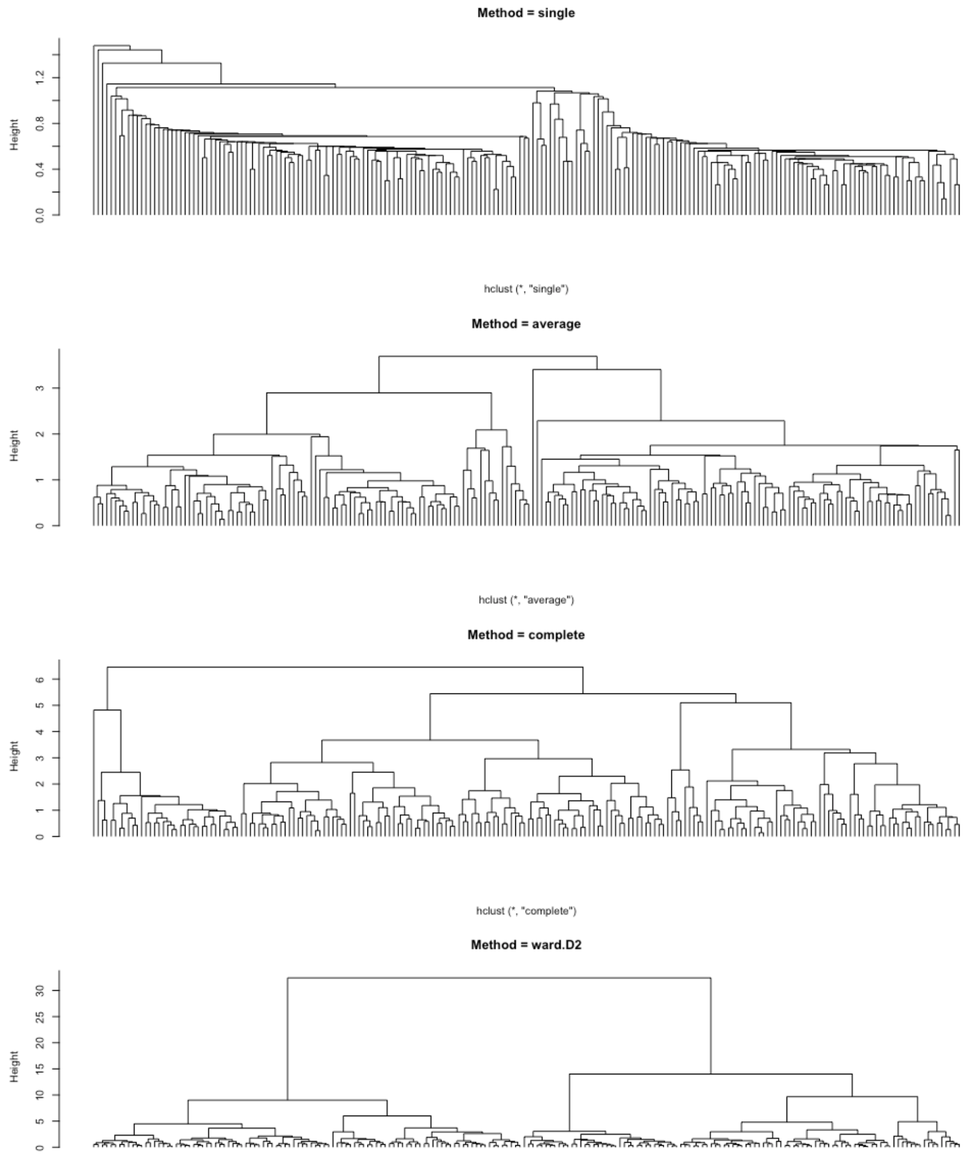




| | (a,(b,c)) | d |
|---|---|---|
| (a,(b,c)) | 0 | **4.525** |
| d | | 0 |



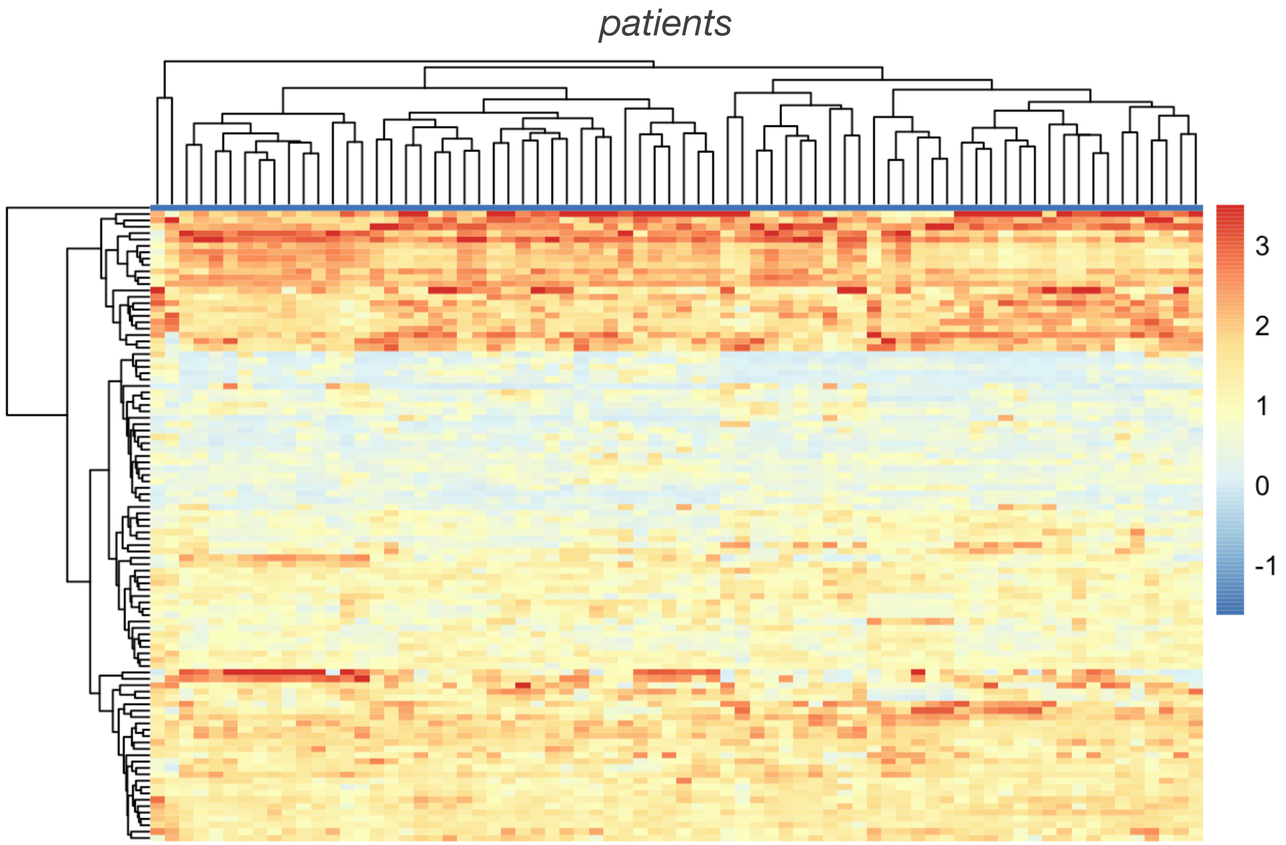
# Effect of linkage

- Further linkage methods
  - **centroid-linkage**: distance between the clusters is given by the distance to the centers of the clusters
  - **Ward linkage**: Distance between two clusters is equal to the increase of the variance of the combined cluster compared to individual clusters
  - ...

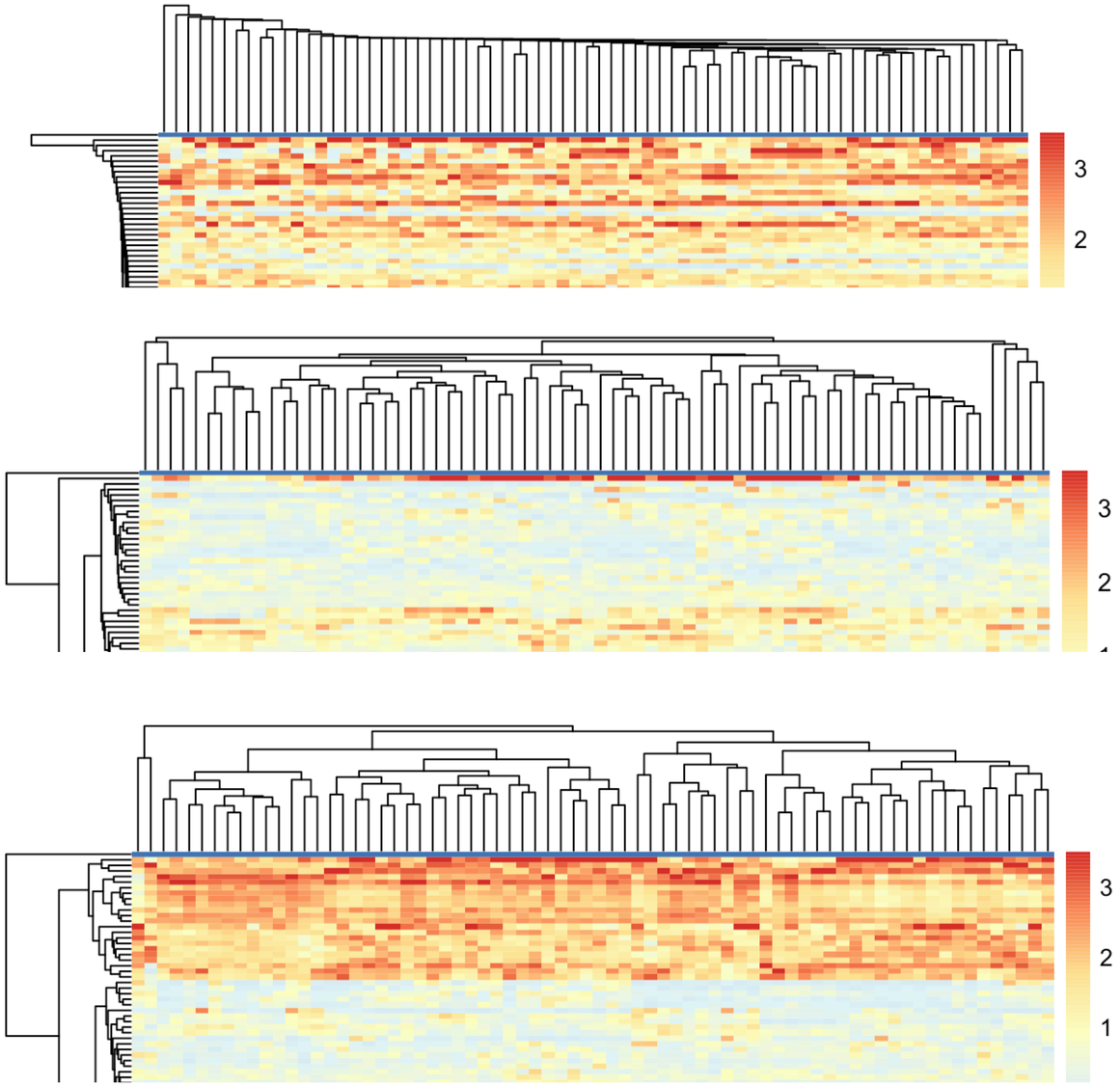


# Real world example

- expression data from 72 AML / ALL patients (Golub et al. 1999)
- clustering of rows and columns: **complete linkage**
- distance between rows / columns: pearson correlation coefficient



# Effect of linkage



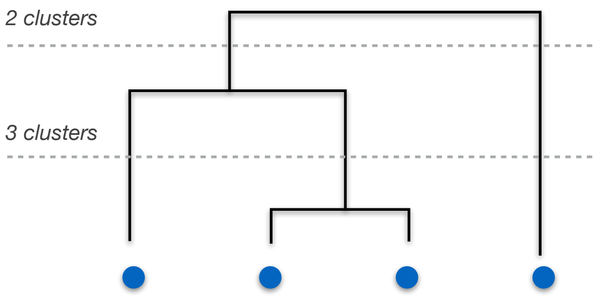

single linkage

average linkage

complete linkage

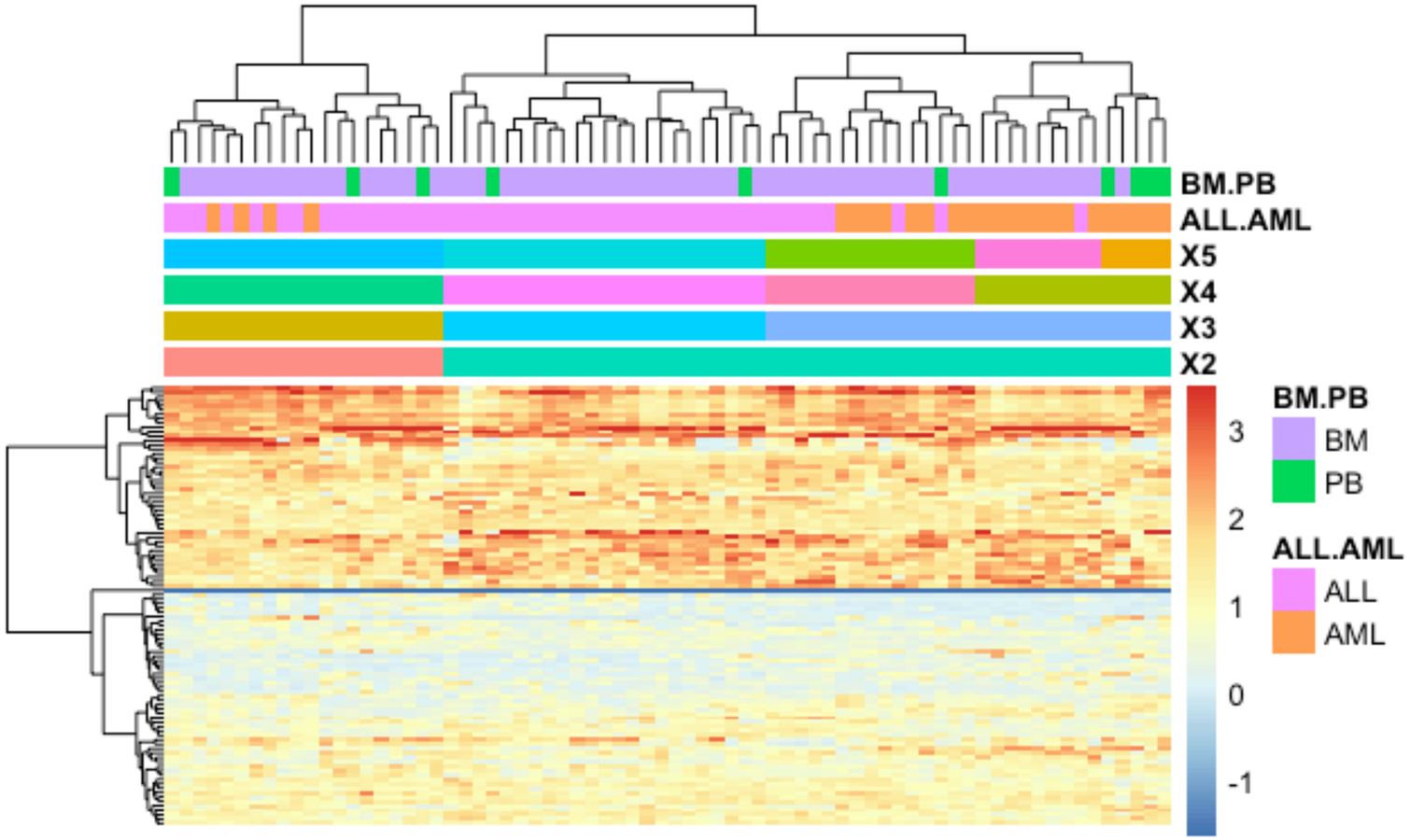
# Hierarchical clustering

- Dendrogram (= rooted tree diagram)
- height of a branch = distance between the clusters it joins
- **Different linkage methods** can lead to **different tree topologies**!
- **Different similarity measures** can lead to **different tree topologies**!
- Tree does not yet define clusters:
  tree can be cut at different branch height to define clusters
- Where to cut? How many clusters?
  → see criteria discussed previously!
  (elbowplot, silhouette,…)



# Real world example

## Clustering patients




BM.PB: bone marrow or peripheral blood
ALL.AML : acute lymphoid leukemia / acute myeloid leukemia
X2,...X5 : clusters obtained by cutting the tree

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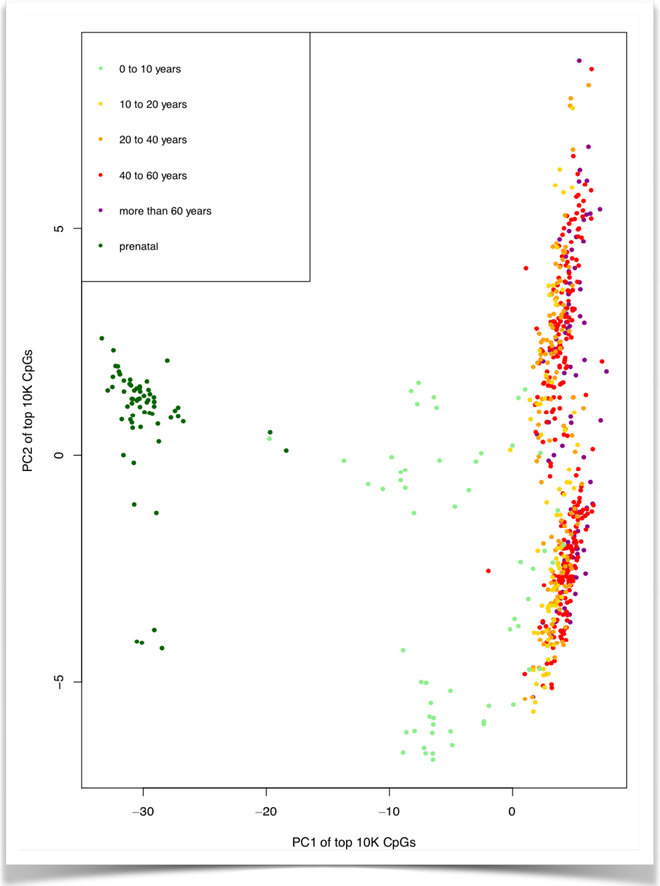
# 4. finding structure in the data
## Principal Component Analysis

# Reducing dimensions

- Dataset have a very high dimensionality (e.g. number of genes)
- Need to reduce this large number of dimensions to a smaller number of relevant variables
- Relevant variables = variables which carry most of the information ( or variance) of a dataset
- Goal:
  - identify **directions** in the data corresponding to **biological effects**

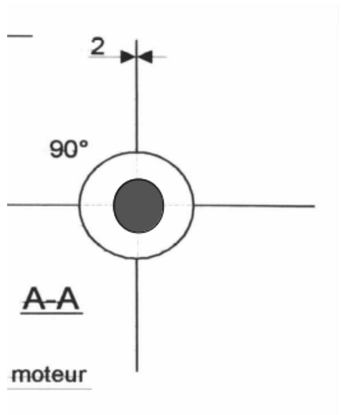



Example of DNA methylation of blood samples
in patient cohort (Jana Dalhoff)
data matrix : 400.000 CpG positions / 250 patients

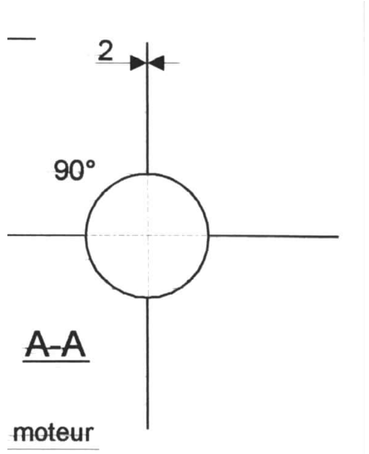
# 3d → 2d

- How to draw ( = 2d ) a rocket ( = 3d ) ?

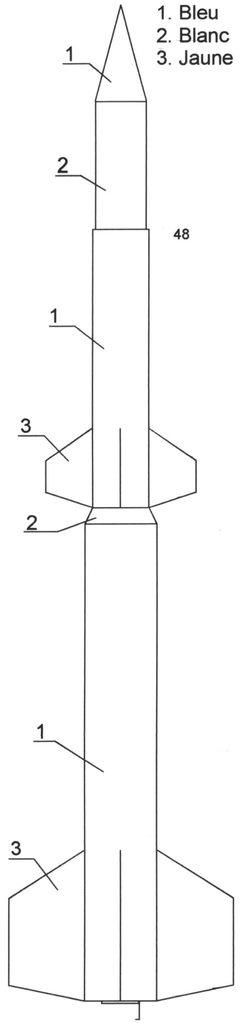
*from above*




*from below*



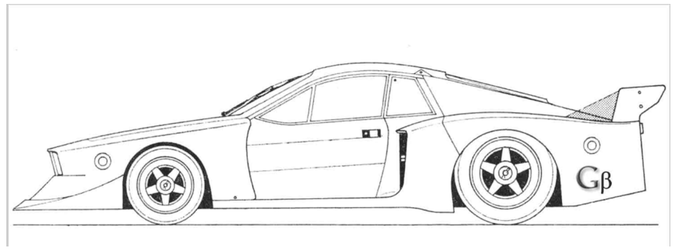

*from the side*



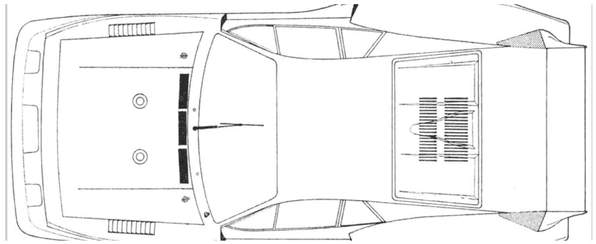
# 3d → 2d

- How to draw ( = 2d ) a car ( = 3d) ?

*from the side*



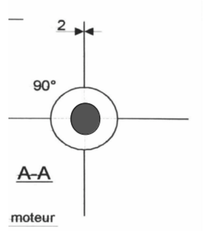
*from above*



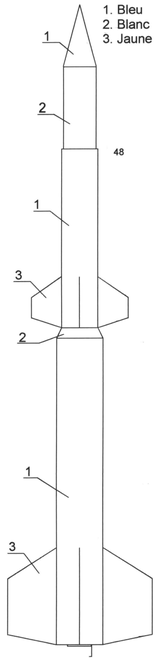
*from the front*

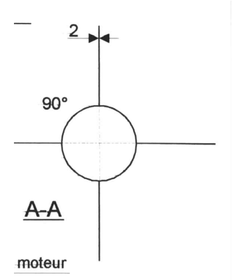
# How to choose?

*10% information*



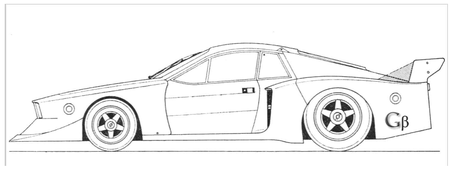

*80% information*



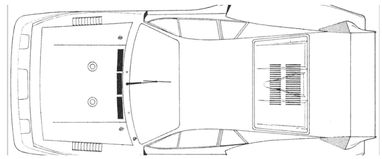




*10% information*

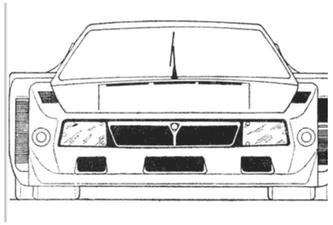
*45% information*




*30% information*



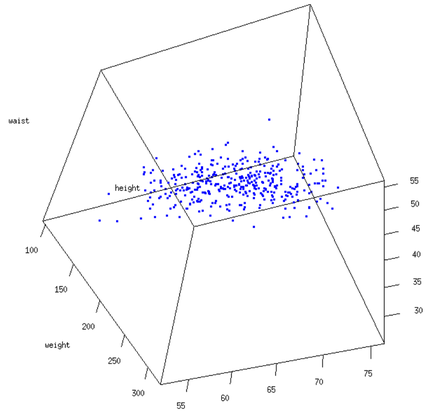
*25% information*



***This is principal component analysis!***

# Principal Component Analysis

- Idea of principle component analysis: **reduce a multi-dimensional dataset to a lower number of informative dimensions** (“Principal Components”)
- Example diabetes dataset with chol / age / height / weight / waist
- each patient is a dot in a 5d space
  → how to choose new coordinates?



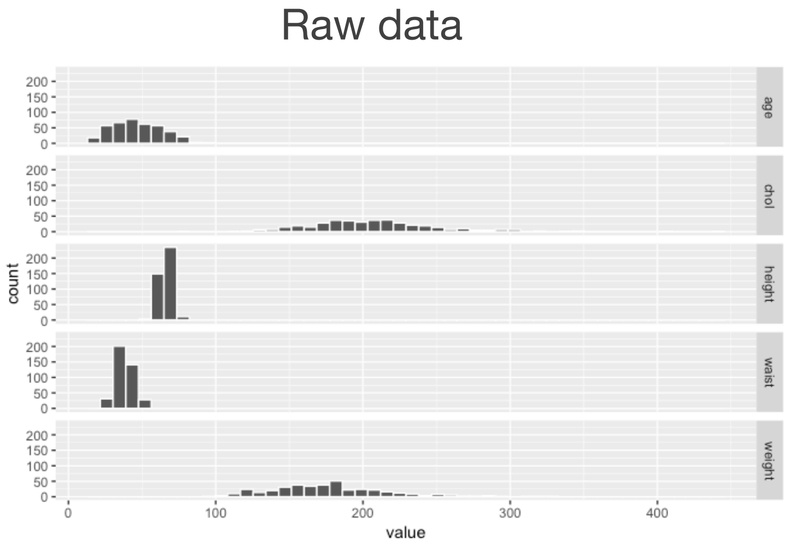

| id | chol | age | height |
|---|---|---|---|
| 1000 | 203 | 46 | |
| 1001 | 165 | 29 | |
| 1002 | 228 | 58 | |
| 1003 | 78 | 67 | |
| 1005 | 249 | 64 | |
| 1008 | 248 | 34 | |
| 1011 | 195 | 30 | |
| 1015 | 227 | 37 | |
| 1016 | 177 | 45 | |
| 1022 | 263 | 55 | |
| 1024 | 242 | 60 | |
| 1029 | 215 | 38 | |
| 1030 | 238 | 27 | |
| 1031 | 183 | 40 | |
| 1035 | 191 | 36 | |
| 1036 | 213 | 33 | |
| 1037 | 255 | 50 | |

k=17 patients, n=5 variables

# Preparing the dataset

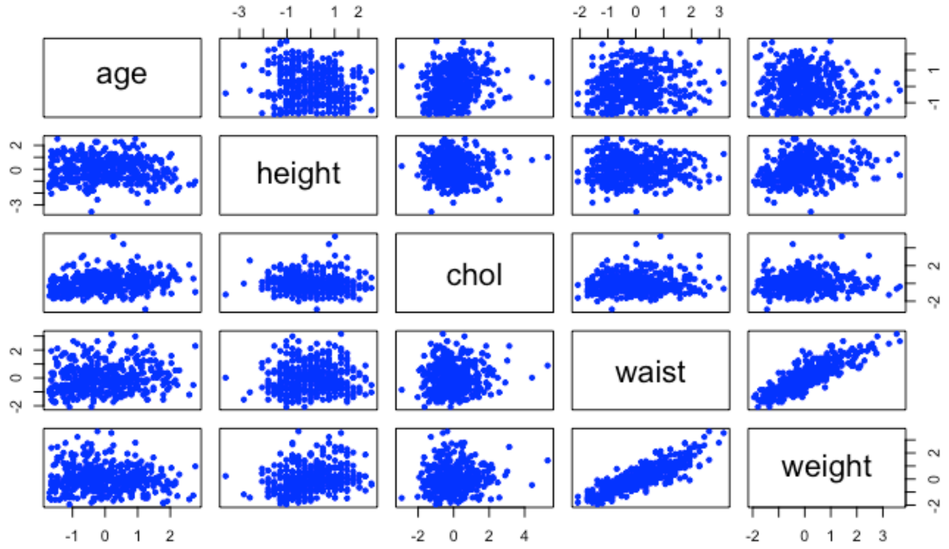
- Each variable has a totally different **range of variation**
- **Solution:** make variables dimensionless using a z-transformation
  → **centering** and **scaling**

$$Z = \frac{x - \bar{x}}{\sigma_x}$$




# Correlation structure

- Some variables are **highly correlated** : knowing one gives a lot of information about the second
- Some are **unrelated** : no information gain from one over the other




| | age | height | chol | waist | weight |
|---|---|---|---|---|---|
| **age** | 1.000 | -0.095 | 0.240 | 0.153 | -0.063 |
| **height** | -0.095 | 1.000 | -0.059 | 0.057 | 0.253 |
| **chol** | 0.240 | -0.059 | 1.000 | 0.112 | 0.059 |
| **waist** | 0.153 | 0.057 | 0.112 | 1.000 | **0.850** |
| **weight** | -0.063 | 0.253 | 0.059 | **0.850** | 1.000 |

# Correlation structure

- if two variables are **strongly correlated**, they are partly redundant: knowing the variation of one, you have information about how the second variables changes
  → having 2 variables does not add much information w.r.t. a single variable

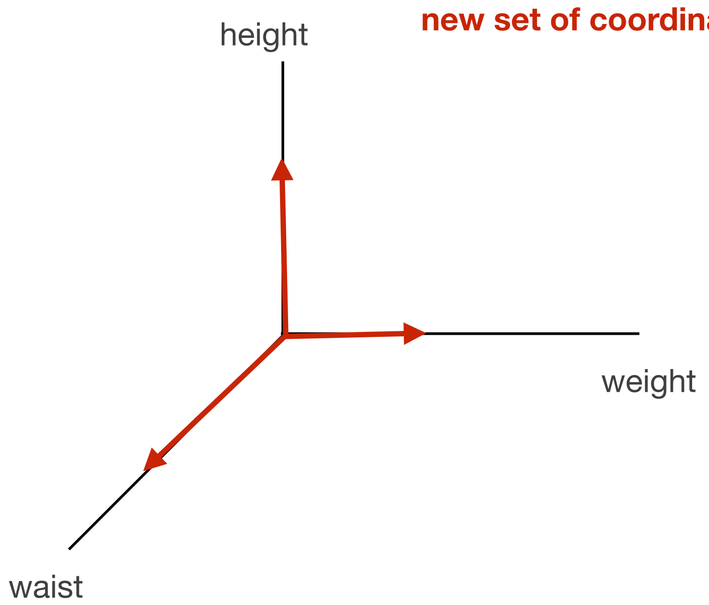
- if two variables have **little correlation**, each variable carries information not contained in the other
  → we need to keep these 2 variables to have information about the dataset

**The more diagonal a correlation matrix is, the more information is revealed by the variables**

| | age | height | chol | waist | weight |
|---|---|---|---|---|---|
| **age** | 1.000 | -0.095 | 0.240 | 0.153 | -0.063 |
| **height** | -0.095 | 1.000 | -0.059 | 0.057 | 0.253 |
| **chol** | 0.240 | -0.059 | 1.000 | 0.112 | 0.059 |
| **waist** | 0.153 | 0.057 | 0.112 | 1.000 | **0.850** |
| **weight** | -0.063 | 0.253 | 0.059 | **0.850** | 1.000 |

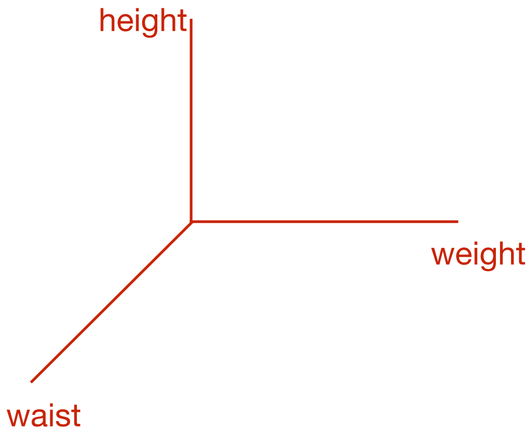
# Defining new variables

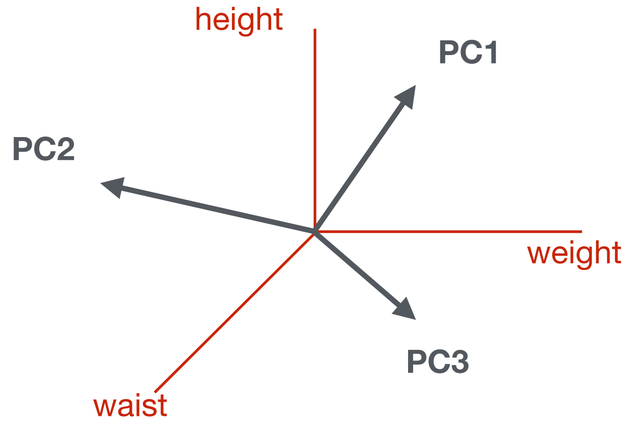
- We want to express the dataset in a new set of coordinates
- for each of these coordinate systems, the correlation matrix will change
- **Goal**: find the rotation that makes the correlation matrix diagonal

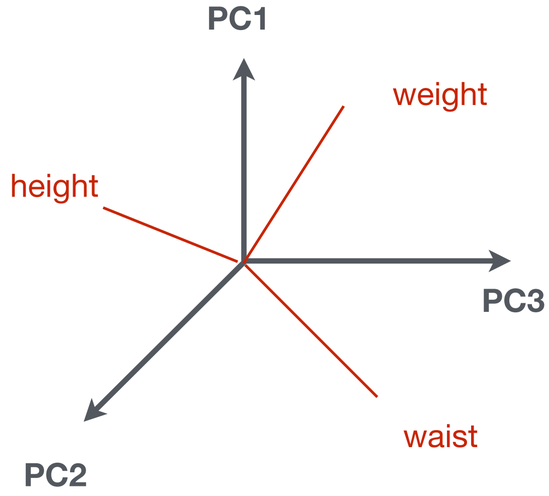



***Hello, matrix diagonalization !***

# New coordinate system



# New coordinate system

patients

| | age | height | chol | waist | weight |
|---|---|---|---|---|---|
| [1,] | -0.056039494 | -1.023316184 | -0.118183417 | -1.563795253 | -1.418763531 |
| [2,] | -1.090068507 | -0.514242224 | -0.978388626 | 1.405952579 | 0.990046092 |
| [3,] | 0.673863338 | -1.277853164 | 0.447741063 | 1.930025726 | 1.933703470 |
| [4,] | 1.221290463 | 0.249368716 | -2.947805816 | -0.865031057 | -1.468429708 |
| [5,] | 1.038814754 | 0.503905697 | 0.923117626 | 1.056570481 | 0.120887981 |
| [6,] | -0.785942327 | 1.267516637 | 0.900480647 | -0.340957910 | 0.294719603 |
| [7,] | -1.029243271 | 0.758442677 | -0.299279250 | 1.405952579 | 0.319552692 |
| [8,] | -0.603466618 | -1.786927124 | 0.425104084 | -0.690340008 | -0.201942175 |
| [9,] | -0.116864730 | 0.758442677 | -0.706744876 | -0.690340008 | -0.301274530 |
| [10,] | 0.491387630 | -0.768779204 | 1.240035335 | 1.231261530 | 0.592716670 |

patients

| | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| [1,] | -2.2724017805 | -0.394780391 | -0.37314376 | 0.141536224 | 0.2090433700 |
| [2,] | 1.3055985070 | 1.119481681 | -1.50495414 | 0.143832964 | -0.3513275630 |
| [3,] | 2.4741433771 | -1.132974842 | -1.49880577 | 0.037584884 | 0.3143758721 |
| [4,] | -1.8399173774 | 0.920620520 | -0.30773345 | -2.959165349 | -0.2523118706 |
| [5,] | 1.1108365939 | -1.098650879 | 0.71155625 | -0.241751636 | -0.5571448997 |
| [6,] | 0.3019126963 | 0.582590104 | 1.33455719 | 1.004709292 | 0.1312818611 |
| [7,] | 1.2128762055 | 1.111191009 | -0.06294882 | 0.315416477 | -0.9968437164 |
| [8,] | -0.9786990772 | -0.575259493 | -1.28325003 | 1.043051917 | 0.4815398584 |
| [9,] | -0.6156836153 | 0.831267656 | 0.56093836 | -0.500214895 | 0.1441444663 |
| [10,] | 1.2805031592 | -1.405669315 | -0.45963453 | 0.587442537 | -0.2560111460 |

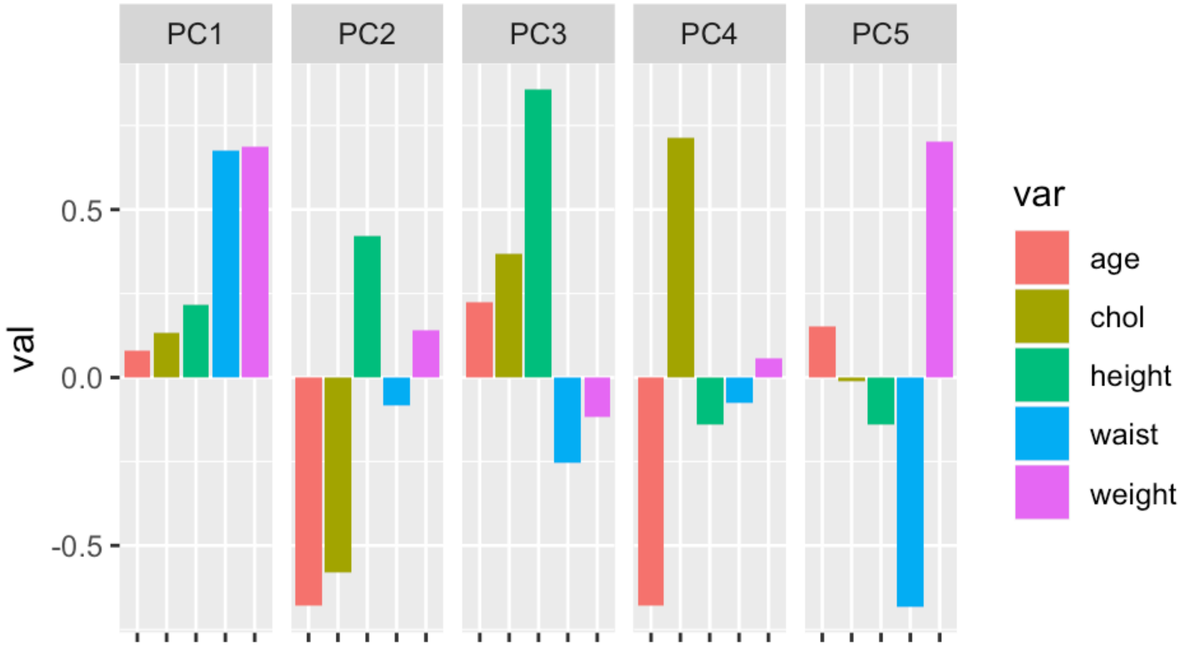
# PCA biplot

- each **dot** is a sample / patient
- new coordinate system is (PC1,PC2)
- **Red arrows** indicate the contribution of each “old" coordinate to the PCs

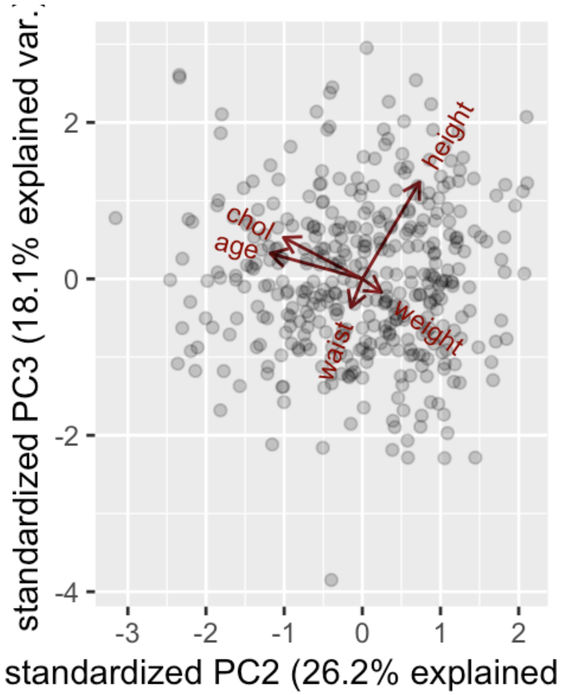
# Principal components

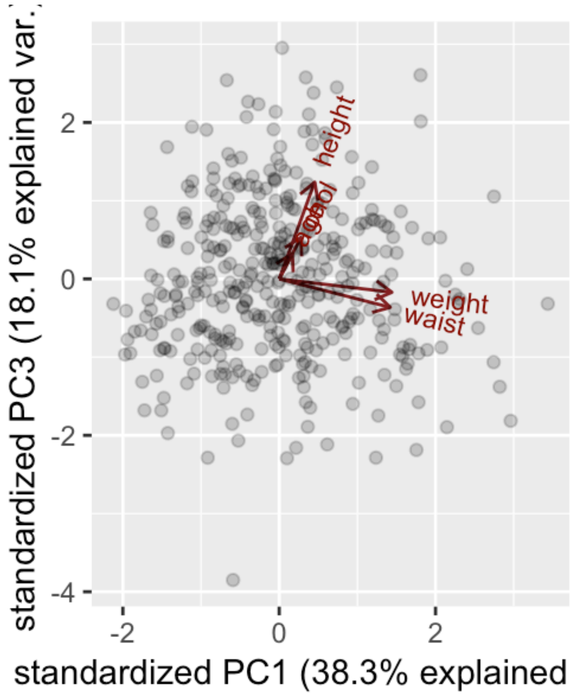
$$PC_i = \alpha_i \cdot \text{age} + \beta_i \cdot \text{chol} + \gamma_i \cdot \text{height} + \delta_i \cdot \text{waist} + \epsilon_i \cdot \text{weight}$$

- contribution of each variable to the principal components (coefficients are called "***loadings***")
- some variables contribute in the same direction to some PCs (e.g. waist and height for PC1), but opposite to others (PC5)



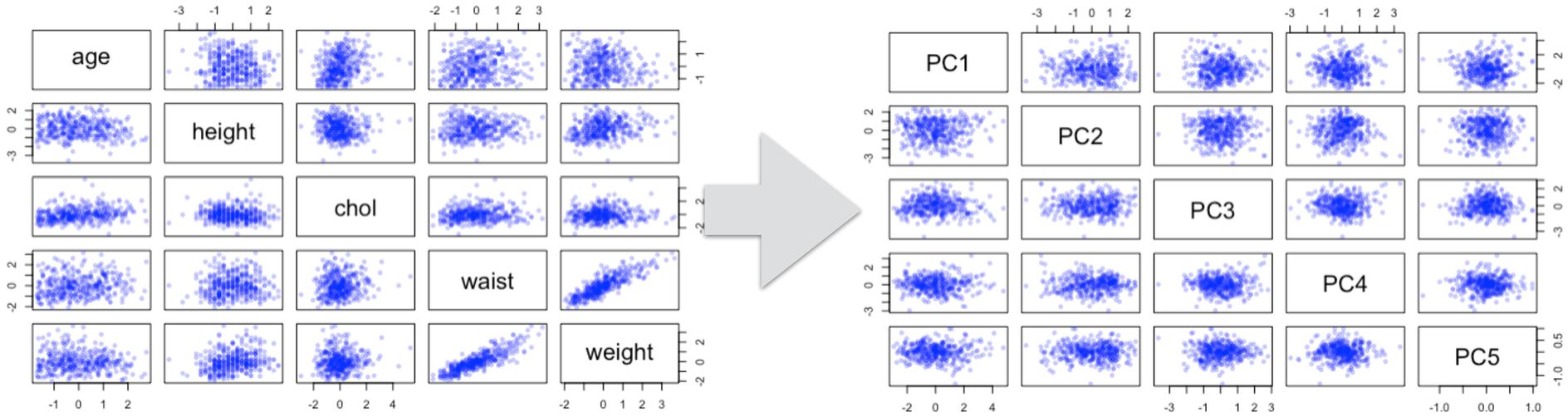
# Principal components

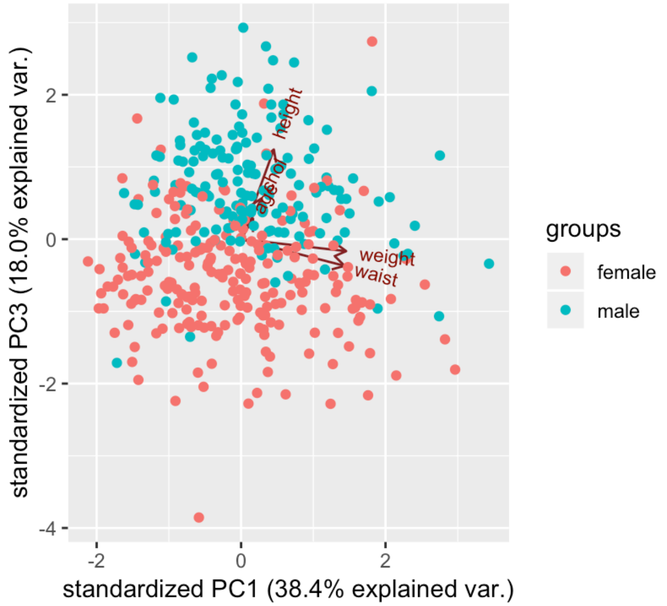
# Principal components

- by construction, **Principal Components have no correlation to each other!**



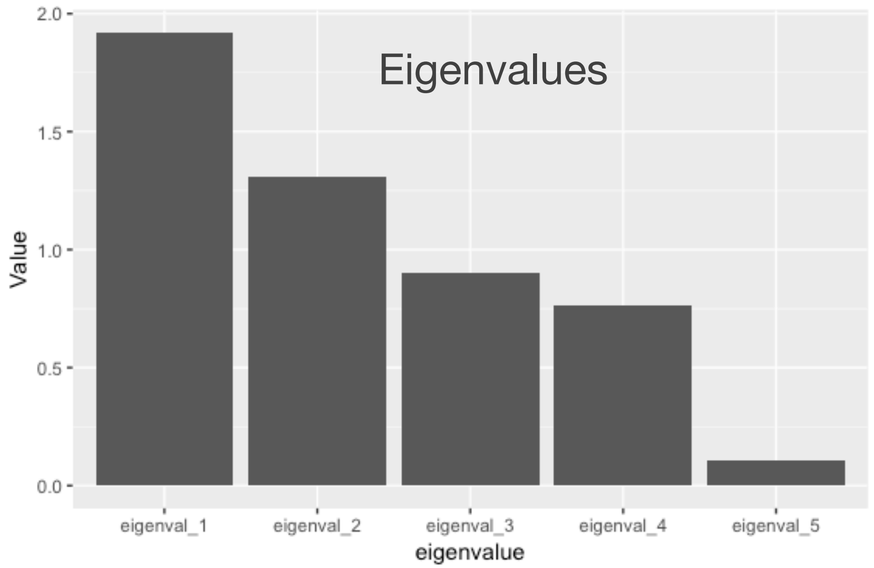
# Identifying interesting PCs

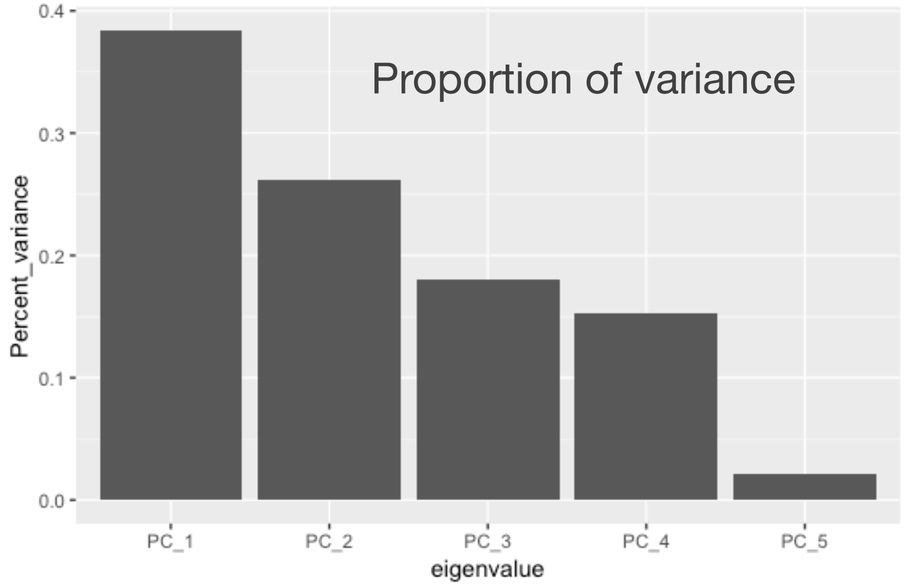
- PC plots can highlight a new group structure
- Example: **PC3** seems very associated to gender
- indicates that a combination of height and cholesterol does separate men /women




# Number of PCs?

- Each PC explains some part of the **total variance** of the dataset
- This amount is proportional to the corresponding **eigenvalue**
- PCs are ordered by **decreasing eigenvalue** (hence variance)




*Considering PC1 & PC2 explains 63% of the total vairance*

# Choosing the number of PCs

- several criteria to select the optimal subset of PCs, without loosing too much information

- **Proportion of variance:**
  keep PCs such that the cumulative variance is above threshold $\sum_{i=1}^{k} \frac{\lambda_i}{\sum \lambda_i} \geq \text{var}_{min}$

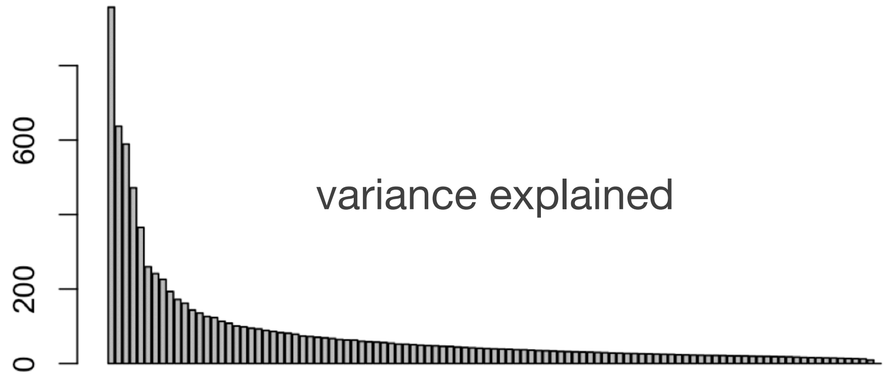
- **Average eigenvalue criteria:**
  keep PCs which have eigenvalue larger than
  - mean eigenvalue (Kaiser rule) or
  - 70% of mean eigenvalue (Jottclife rule)

  $\lambda_i \geq \bar{\lambda}$

# Application to gene expression

- Gene expression dataset of **breast cancer patients**
- 2 groups: ER+ and ER- patients
- Dimension: k = 105 patients / n = 8534 genes (here: n >> k)
- pre-processing:
  - **scale** the gene expression across patients
  - **center** the gene expression across patients
- How many principal components do we get?
  → **k-1** (this has to do with the rank of the data matrix)

[M. Ringner, Nature Biotech. (2008)]



# Application to gene expression

- PC1 separates ER+ from ER- patients