Machine Learning for Bioinformatics & Systems Biology

4. Clustering & hidden Markov models

Perry Moerland *Amsterdam UMC, University of Amsterdam*
Marcel Reinders *Delft University of Technology*
Lodewyk Wessels *Netherlands Cancer Institute*

Some material courtesy of Robert Duin and David Tax

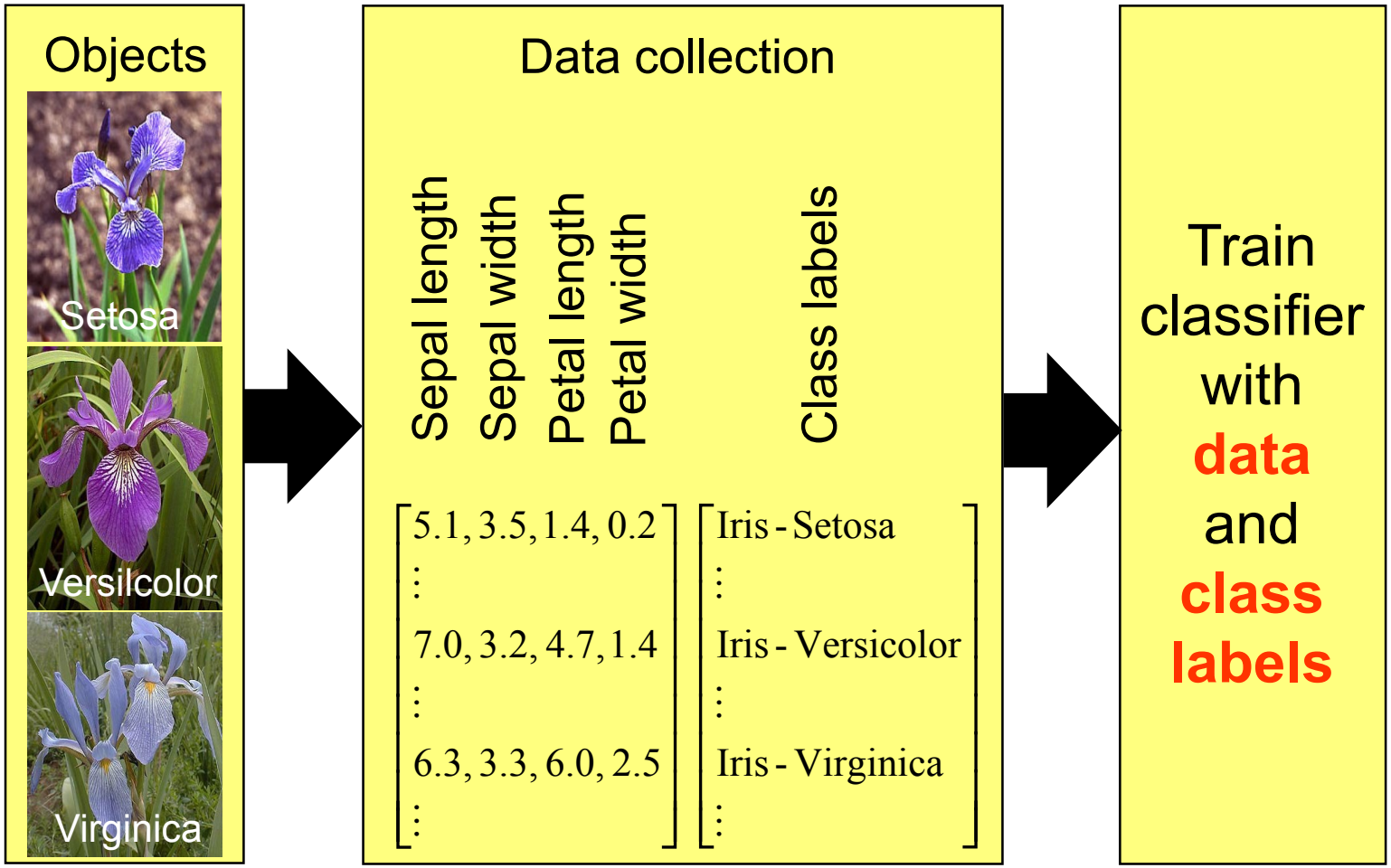
Clustering

- Supervised vs. unsupervised learning

- Hierarchical clustering
- Sum-of-squares clustering (k-means)
- Cluster validation
- Mixtures-of-Gaussians clustering (EM algorithm)

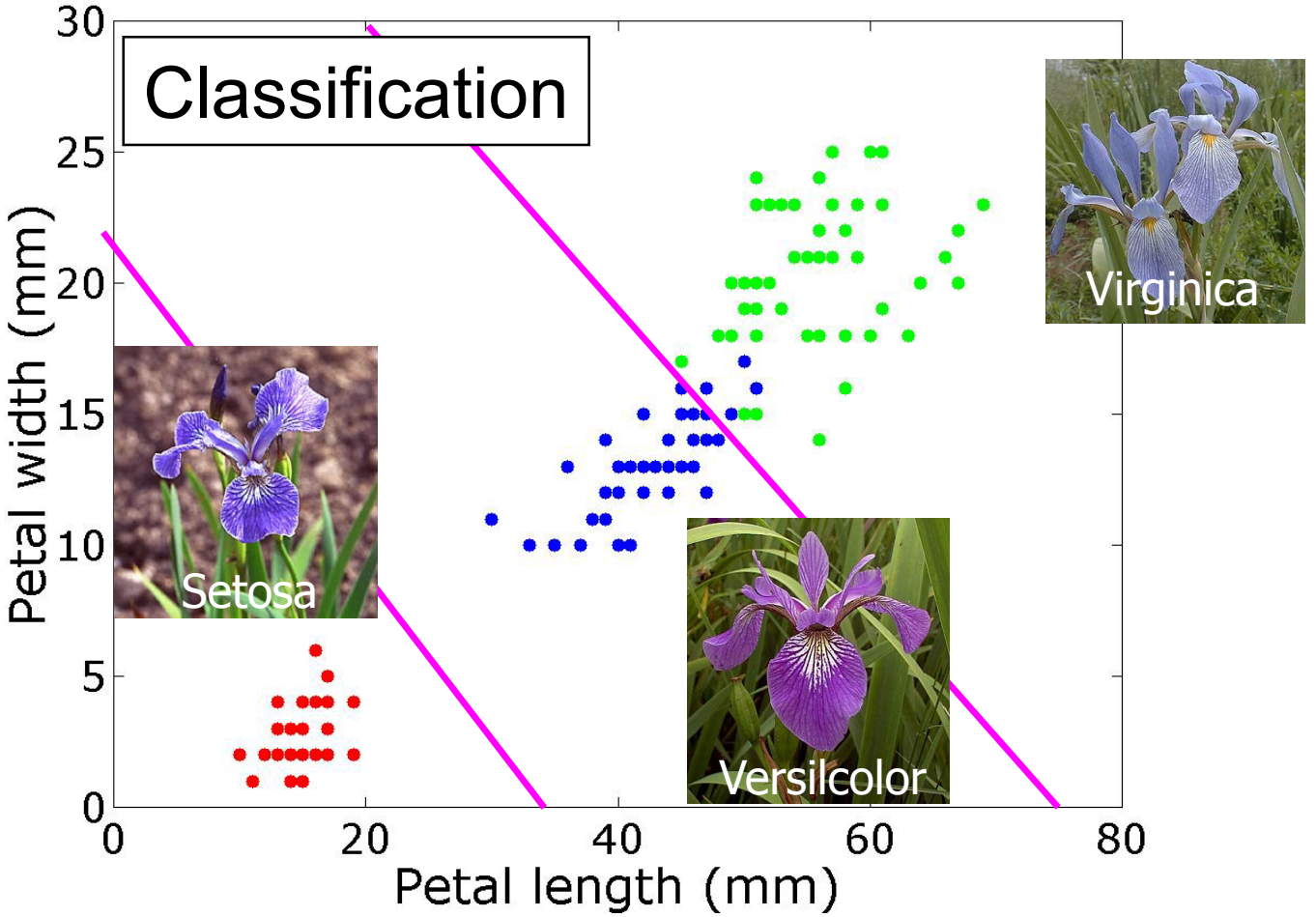
BioSB

Supervised learning

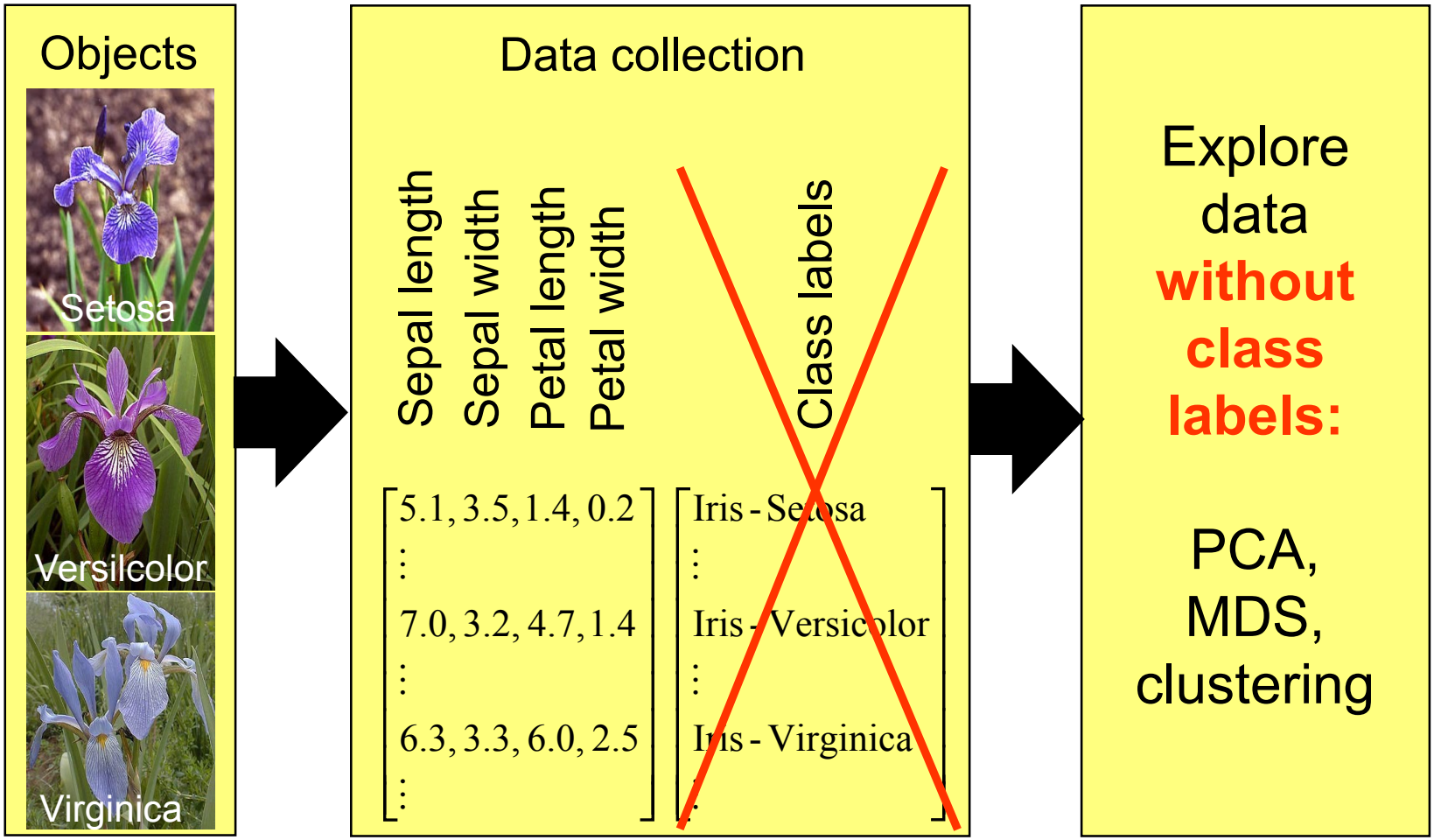


BioSB

Supervised learning (2)

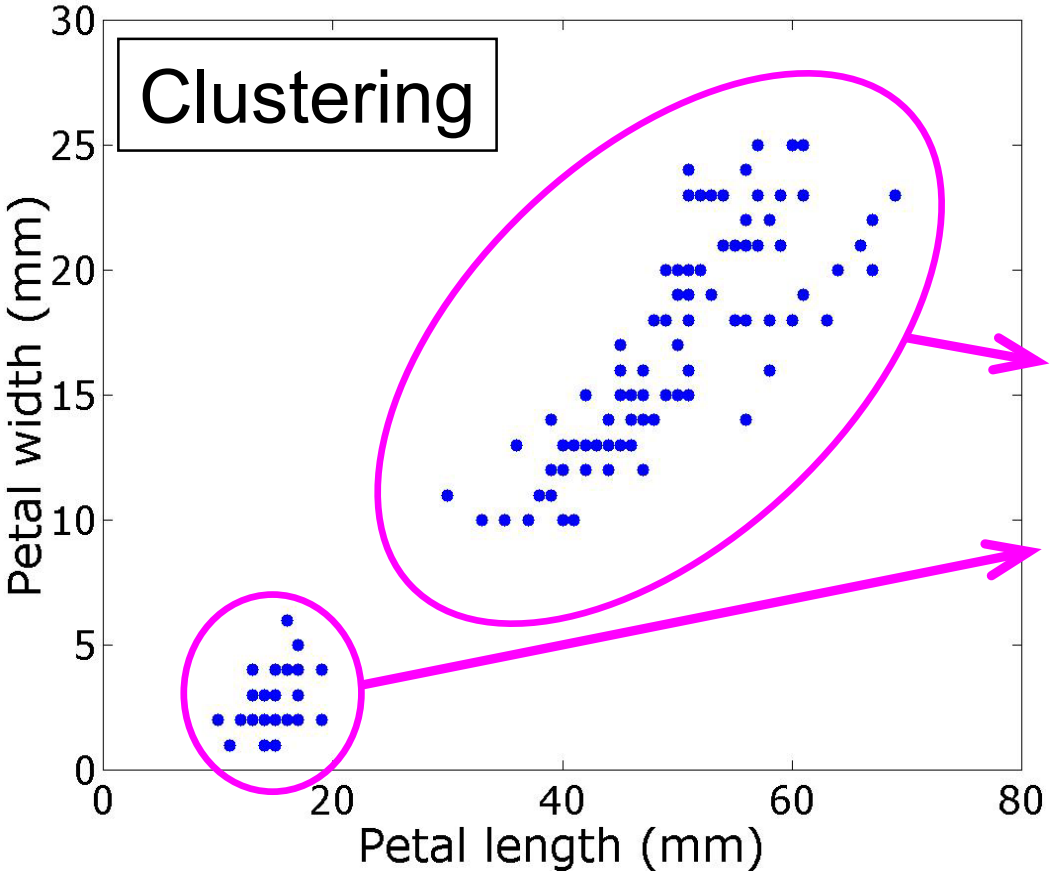


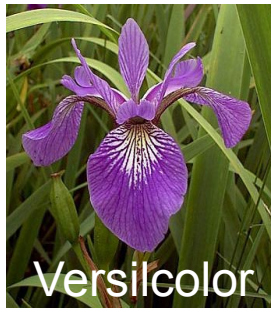
Unsupervised learning



BioSB

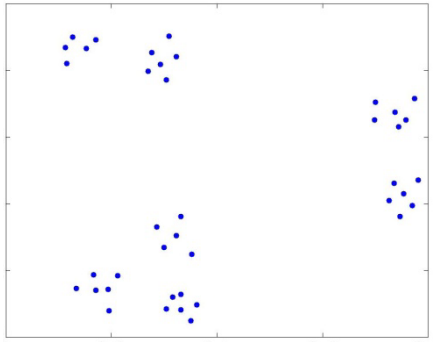
Unsupervised learning (2)



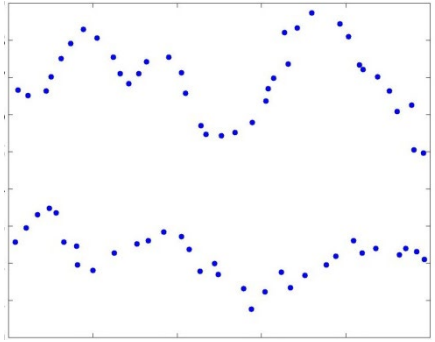


BioSB

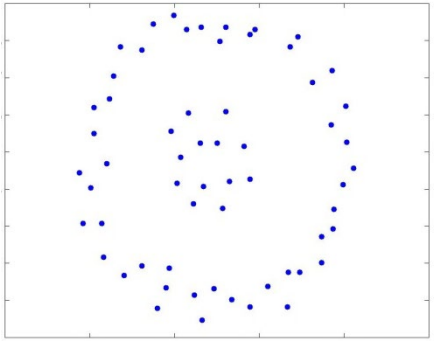
What is a cluster?



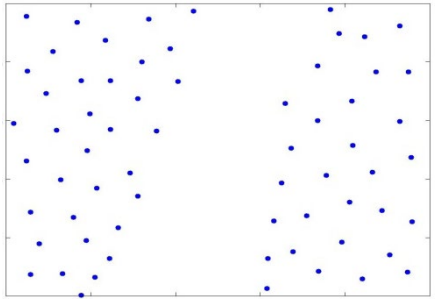
Shape: compact, convex
Separation: large



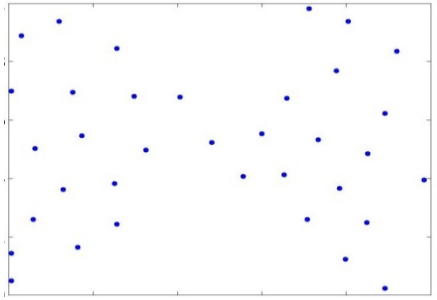
Shape: strings
Separation: large?



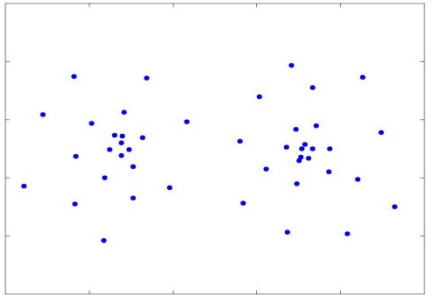
Shape: convex and circular
Separation: large?



Shape: ?
Separation: large?



Shape: loose, convex
Separation: small



Shape: loose, convex
Separation: small

BioSB

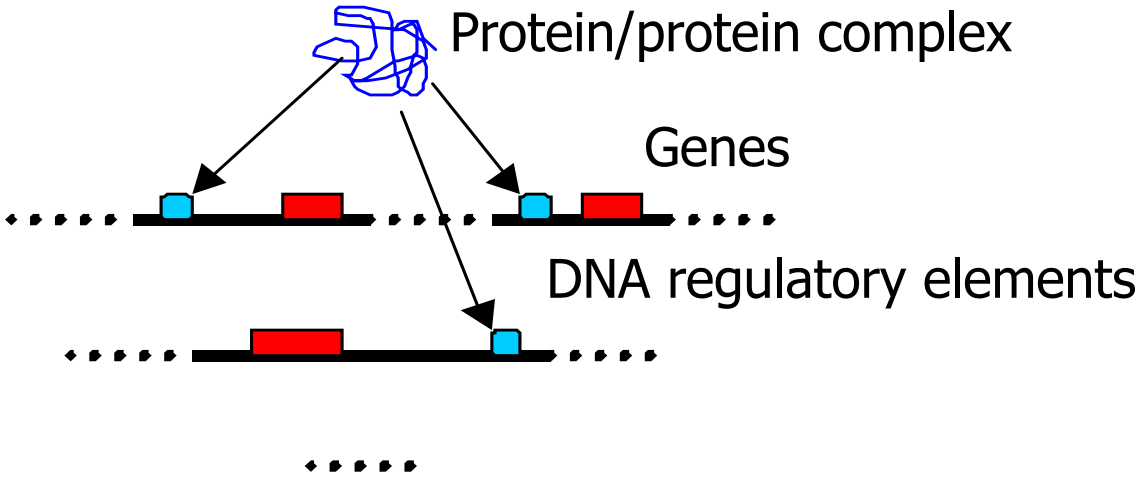
What is a cluster? (2)

- Clustering: finding natural groups in data...
  - which themselves are far apart
  - in which objects are close together
- Define what is “far apart” and “close together”:
  - Need a distance measure or dissimilarity measure
  - This measure should capture what we think is important for the grouping
  - The choice for a certain distance measure is often the most important choice in clustering!
- There is no such thing as *the objective clustering*

BioSB

What is a cluster in bioinformatics?

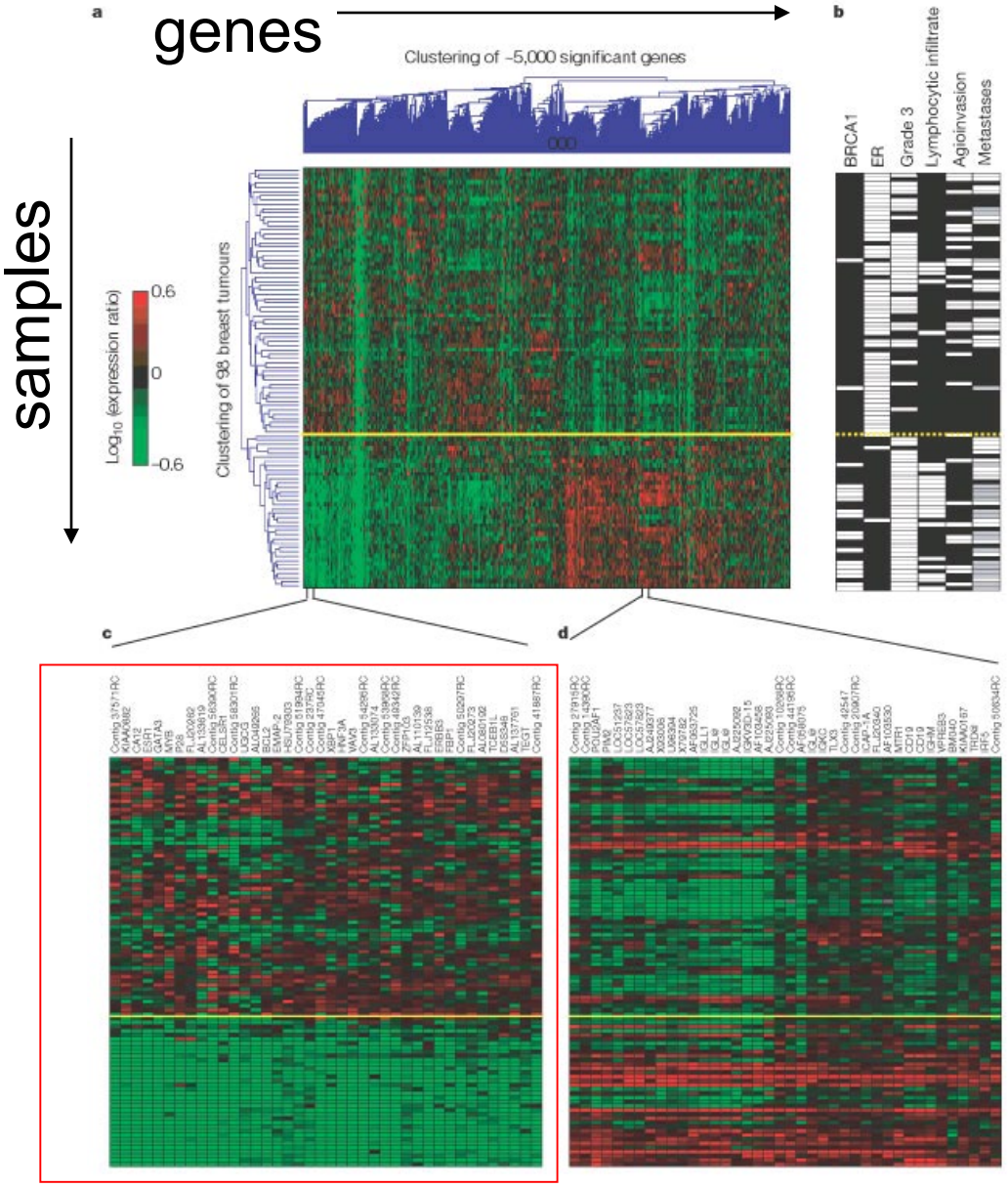
- Clustering gene expression data:
- Genes: similar ~ co-expression ~ co-regulation ~
  same pathway / same function



- Samples: similar ~ same type of tissue
- Used for discovery of new subclasses (*subtypes*) in tumors

BioSB

Example: genes (and samples)



negative

positive

histopathological data

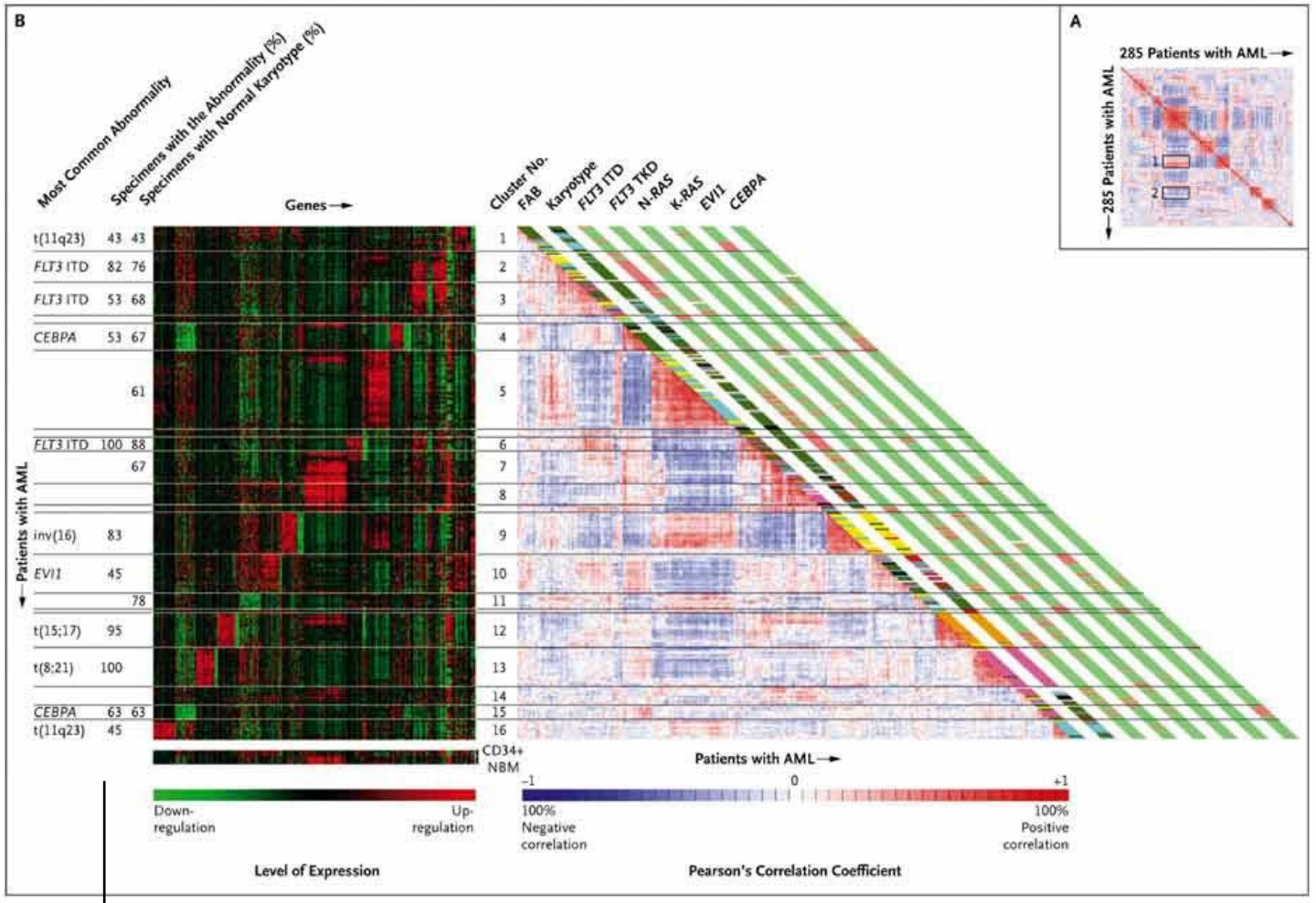
ER gene *(ESR1)* and genes co-regulated with ER, some of which are known ER target genes

Van 't Veer et al, Nature 415: 530-536 (2002)

BioSB

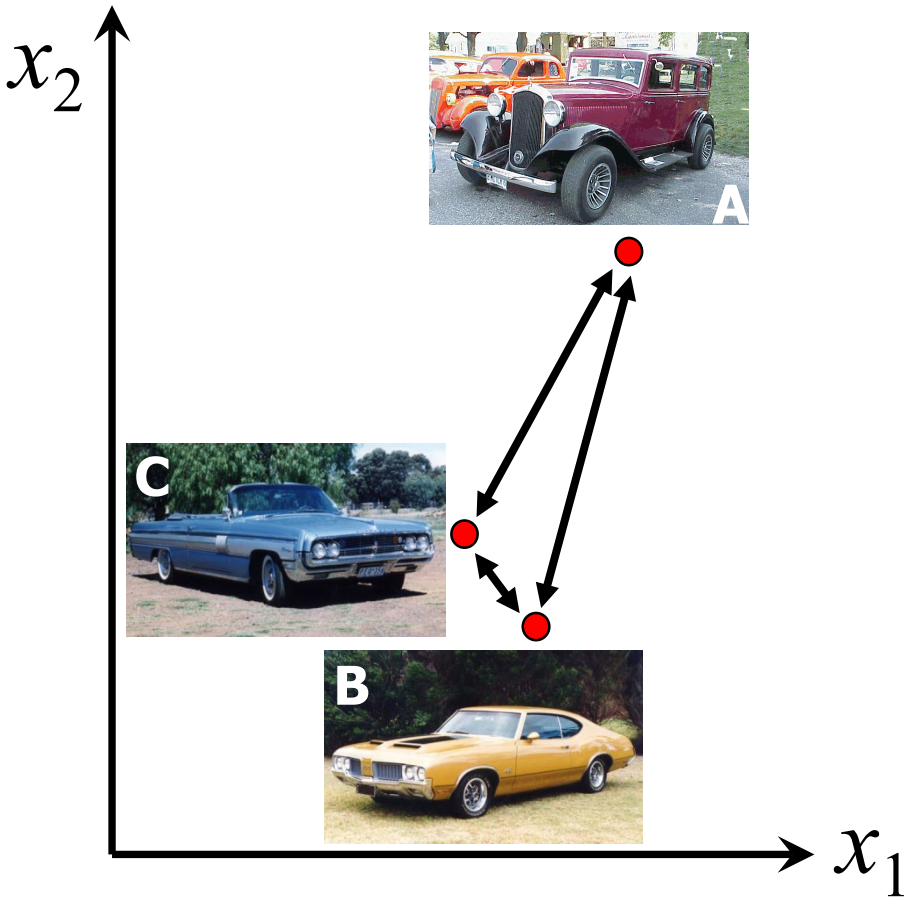
Example: samples

Valk et al, N Engl J Med. 2004 Apr 15;350(16):1617-28.



Identified 16 groups of patients with acute myeloid leukemia

Dissimilarity measures



$$\boldsymbol{D} = \begin{bmatrix} 0 & d(\mathbf{A},\mathbf{B}) & d(\mathbf{A},\mathbf{C}) \\ & 0 & d(\mathbf{B},\mathbf{C}) \\ & & 0 \end{bmatrix}$$

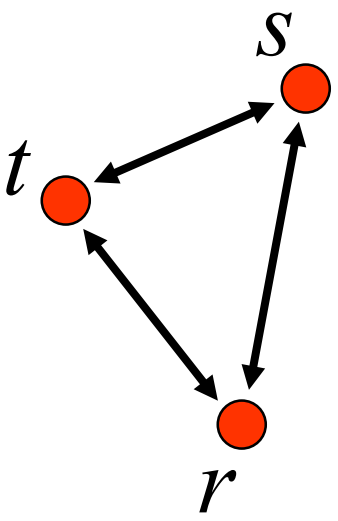
$$= \begin{bmatrix} 0 & 10 & 11 \\ & 0 & 2 \\ & & 0 \end{bmatrix}$$

BioSB

Dissimilarity measures (2)

- Let $d(r,s)$ be the dissimilarity between objects r and s
- Formally, dissimilarity measures should satisfy

$$d(r,s) \geq 0, \forall\, r,s$$
$$d(r,r) = 0, \forall\, r$$
$$d(r,s) = d(s,r), \forall\, r,s$$



- If in addition, the triangle inequality holds, the measure is a *metric*

$$d(r,t) + d(t,s) \geq d(r,s), \forall\, r,s,t$$

- Most often used: Euclidean distance (metric)

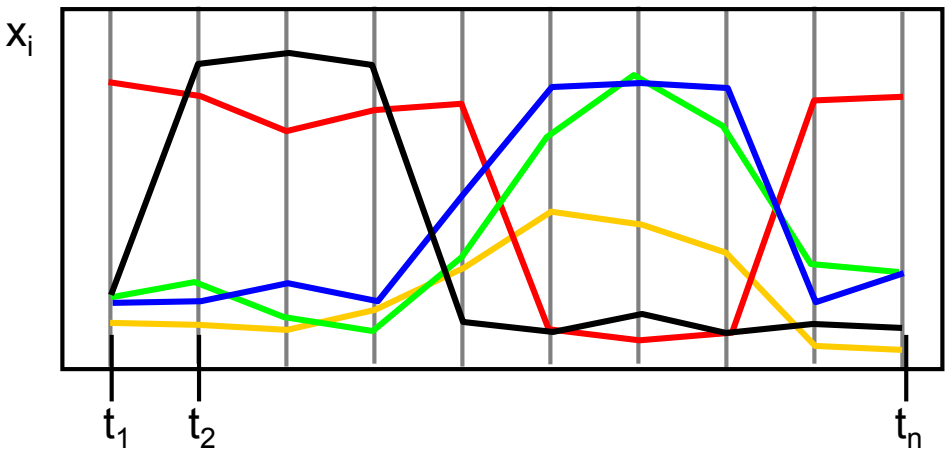
BioSB

Dissimilarity measures (3)

- Example: time series data

(squared) Euclidean distance

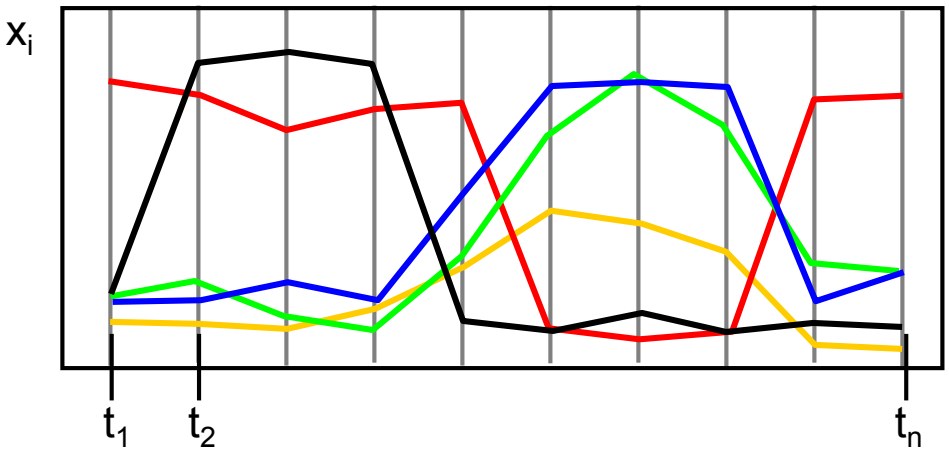
$$d(\mathbf{x}_i, \mathbf{x}_j) = \sum_{t=1}^{n} (x_{i,t} - x_{j,t})^2$$



BioSB

Dissimilarity measures (3)

- Example:
  time series data



Euclidean distance
match exact shape

$$d(\mathbf{x}_i, \mathbf{x}_j) = \sum_{t=1}^{n} (x_{i,t} - x_{j,t})^2$$

d(● , ●) < d(● , ●)

d(● , ●) << d(● , ●)

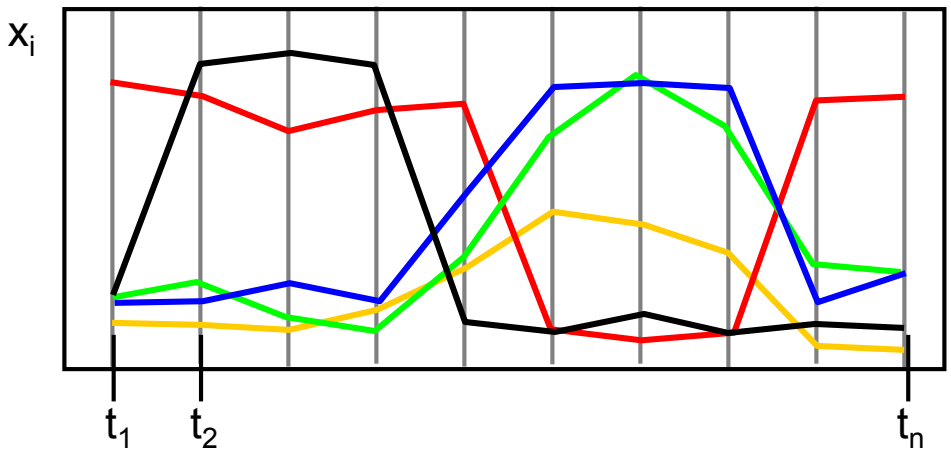
d(● , ●) << d(● , ●)

Dissimilarity measures (3)

- Example: time series data

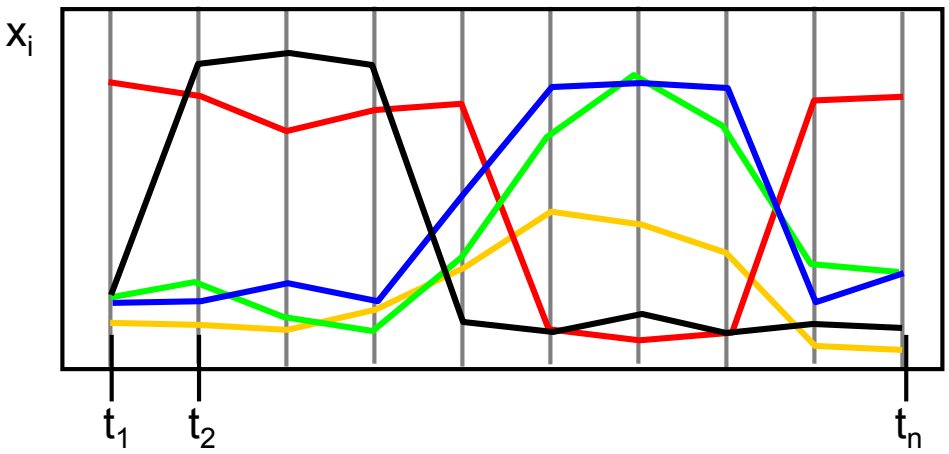
Euclidean distance
match exact shape

$$d(\mathbf{x}_i, \mathbf{x}_j) = \sum_{t=1}^{n} (x_{i,t} - x_{j,t})^2$$



Dissimilarity measures (3)

- Example: time series data



Euclidean distance
match exact shape

$$d(\mathbf{x}_i, \mathbf{x}_j) = \sum_{t=1}^{n} (x_{i,t} - x_{j,t})^2$$

d(● , ●) < d(● , ●)
d(● , ●) << d(● , ●)
d(● , ●) << d(● , ●)

Pearson correlation
ignore amplitude

$$\rho_{ij} = \sum_{t=1}^{n} (x_{i,t} - \mu_i)(x_{j,t} - \mu_j) \Big/ \sigma_i \sigma_j$$

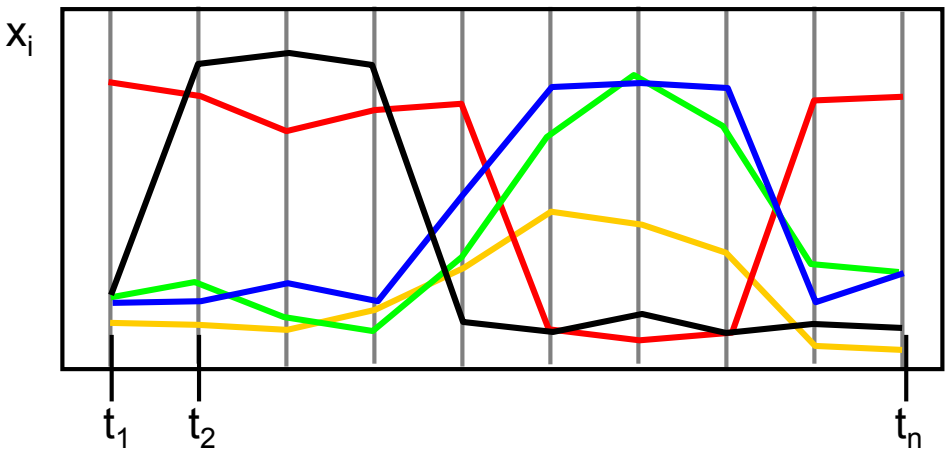
$$1 - \rho_{ij}$$

d(● , ●) ≈ d(● , ●)
d(● , ●) << d(● , ●)
d(● , ●) << d(● , ●)

BioSB

Dissimilarity measures (3)

- Example: time series data



Euclidean distance
match exact shape

$$d(\mathbf{x}_i, \mathbf{x}_j) = \sum_{t=1}^{n} (x_{i,t} - x_{j,t})^2$$

d(● , ●) < d(● , ●)
d(● , ●) << d(● , ●)
d(● , ●) << d(● , ●)

Pearson correlation
ignore amplitude

$$\rho_{ij} = \sum_{t=1}^{n} (x_{i,t} - \mu_i)(x_{j,t} - \mu_j) \Big/ \sigma_i \sigma_j$$

$$1 - \rho_{ij}$$

d(● , ●) ≈ d(● , ●)
d(● , ●) << d(● , ●)
d(● , ●) << d(● , ●)

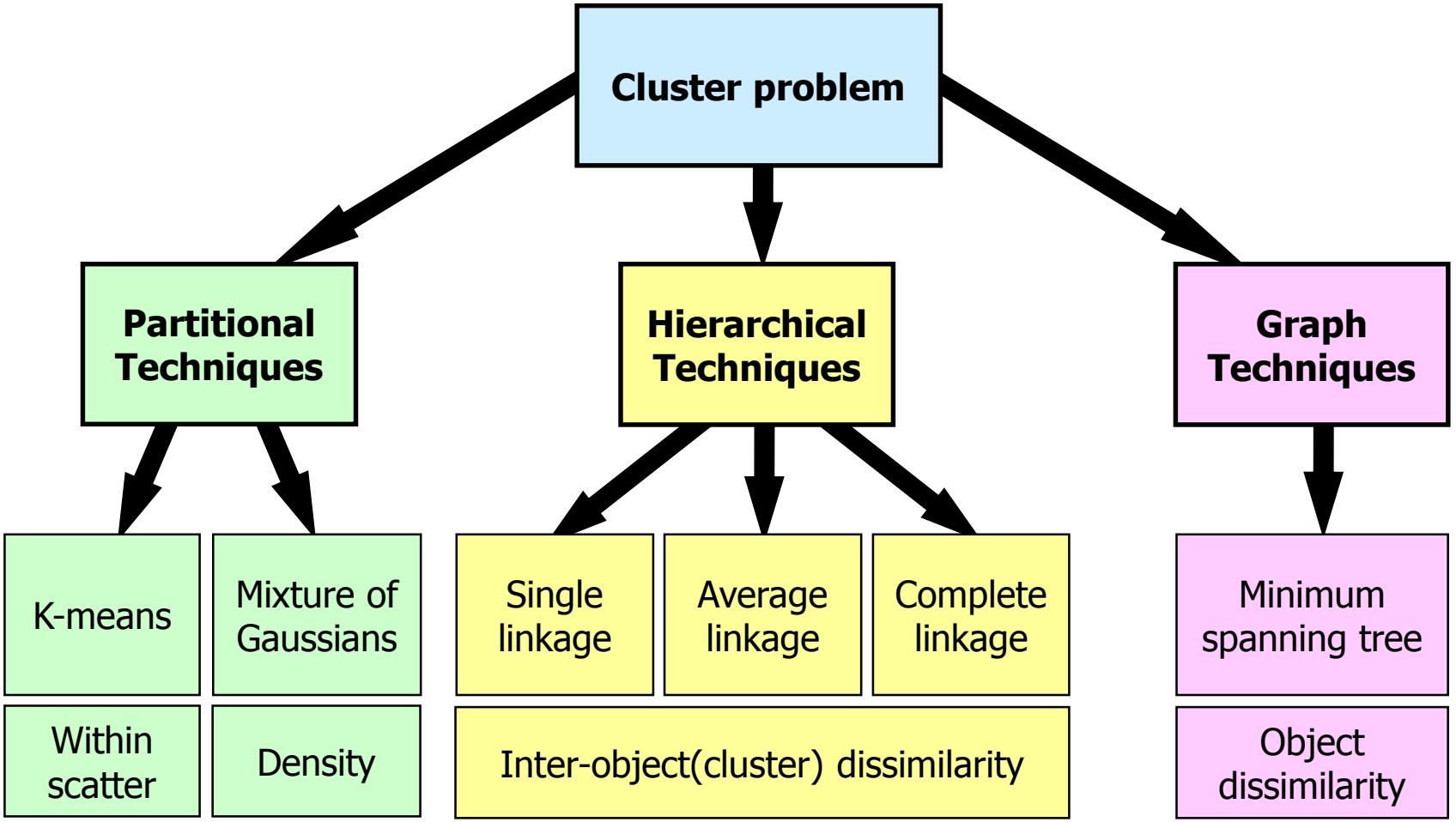
Absolute correlation
ignore amplitude & sign

$$1 - |\rho_{ij}|$$

d(● , ●) ≈ d(● , ●)
d(● , ●) ≈ d(● , ●)
d(● , ●) << d(● , ●)

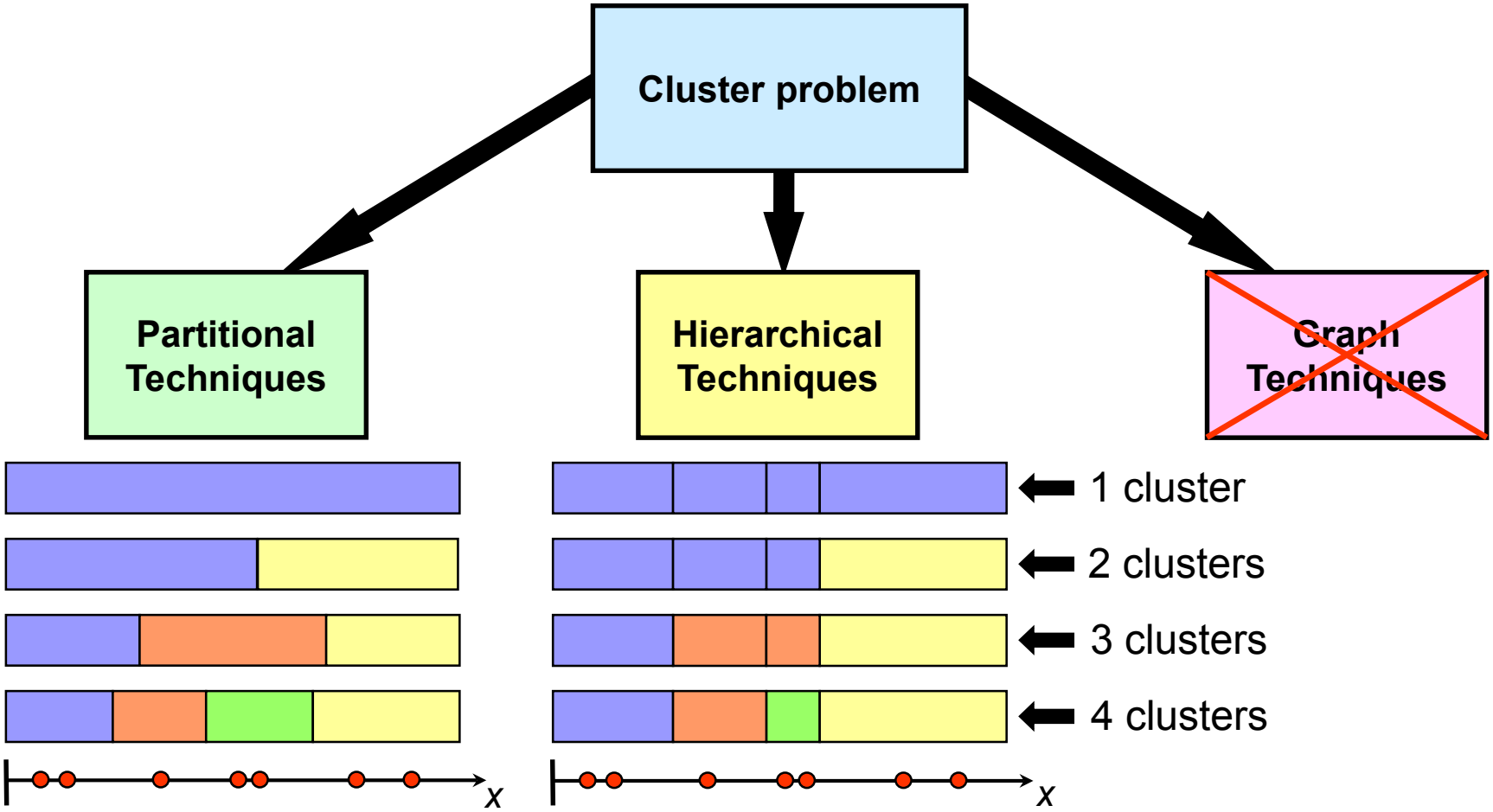
BioSB

Clustering techniques



BioSB

Clustering techniques (2)



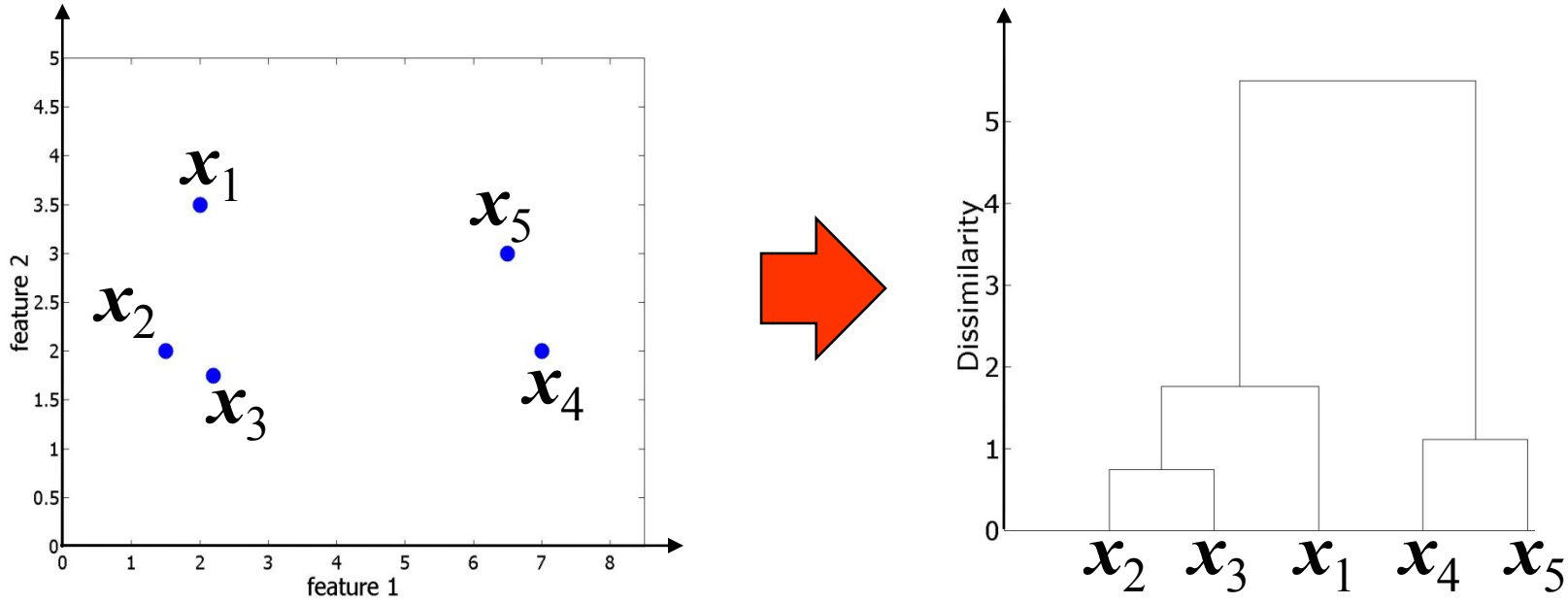
Hierarchical clustering

Input:

- dataset, ***X***: [*n* x *p*], or directly:
- dissimilarity matrix, ***D***: [*n* x *n*]
- linkage type

Output:

- dendrogram



Hierarchical clustering (2)

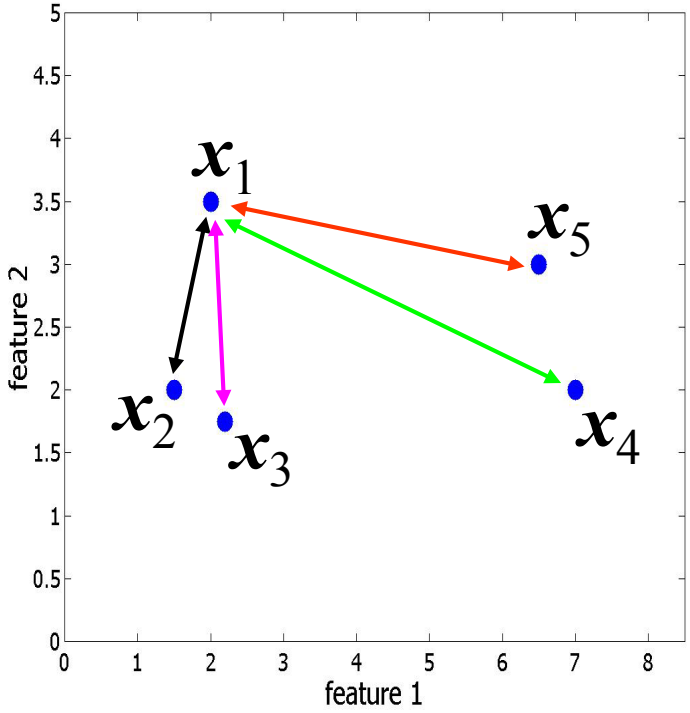
- **Algorithm** (agglomerative clustering)
  - Start: all objects of ***X*** in a separate cluster
  - Clustering: combine the 2 clusters with

    the shortest distance in dissimilarity matrix, ***D***
  - Distance between clusters is based on linkage type:
    - single, complete, average, …
  - Repeat until only 1 cluster is left

BioSB

Hierarchical clustering (3)

Dataset

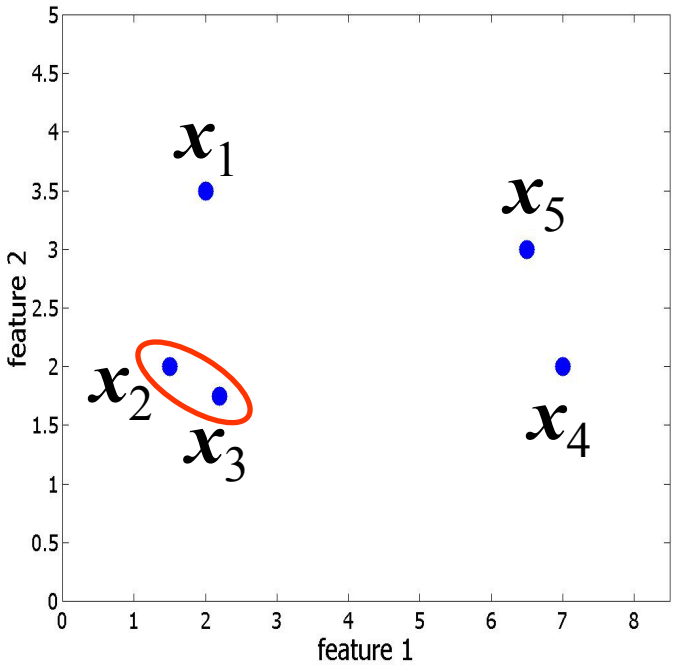


Euclidean distance matrix, ***D***

	x_1	x_2	x_3	x_4	x_5
x_1	0.00	1.58	1.76	5.22	4.53
x_2		0.00	0.74	5.50	5.10
x_3			0.00	4.81	4.48
x_4				0.00	1.12
x_5					0.00

Hierarchical clustering (4)

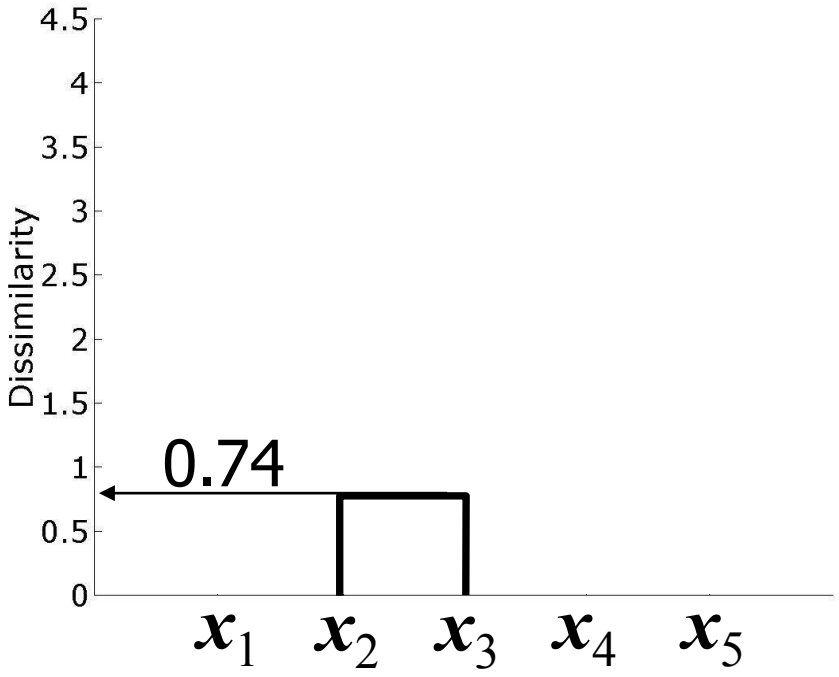
- **Step 1:**
  Find the most similar pair of objects: $\min_{(i,j)}\{d(i,j)\} = d(2,3)$



	x_1	x_2	x_3	x_4	x_5
x_1	0.00	1.58	1.76	5.22	4.53
x_2		0.00	0.74	5.50	5.10
x_3			0.00	4.81	4.48
x_4				0.00	1.12
x_5					0.00

Hierarchical clustering (5)

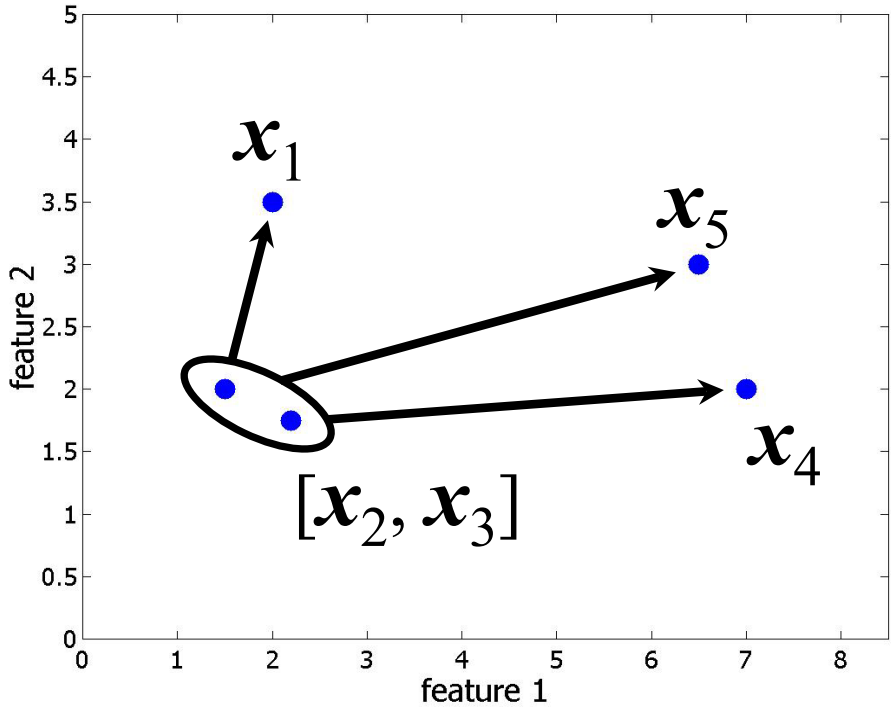
- **Step 2:**
  Merge x_2 and x_3 into a single object, $[x_2, x_3]$;



BioSB

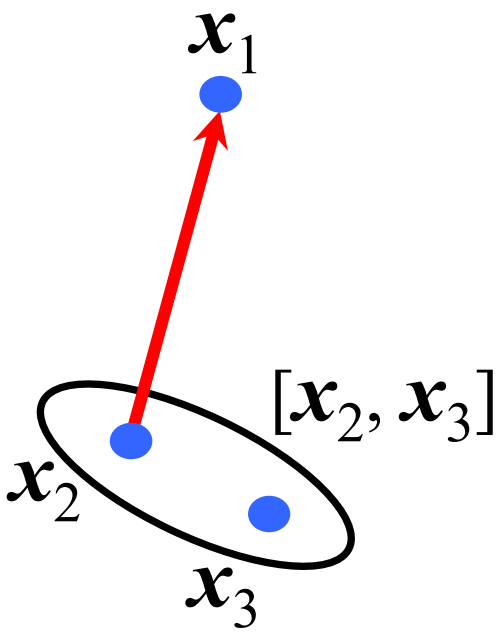
Hierarchical clustering (6)

- **Step 3:**
  Recompute $\boldsymbol{D}$ –
  what is the distance between $[x_2, x_3]$ and the rest?



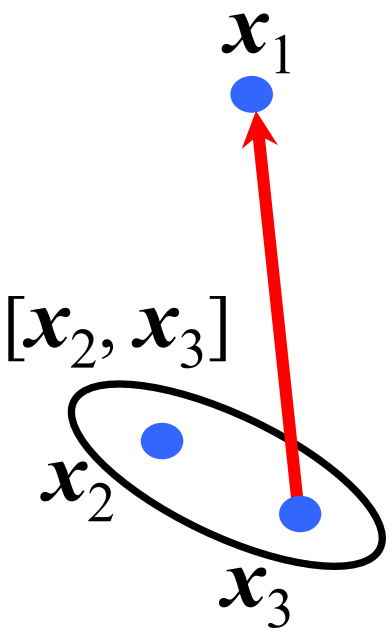
Hierarchical clustering (7)

- **Step 3:**
  Recompute $\boldsymbol{D}$ –
  single linkage: $d([\boldsymbol{x}_2,\boldsymbol{x}_3],\boldsymbol{x}_1) = \min(d(\boldsymbol{x}_1,\boldsymbol{x}_2),d(\boldsymbol{x}_1,\boldsymbol{x}_3))$



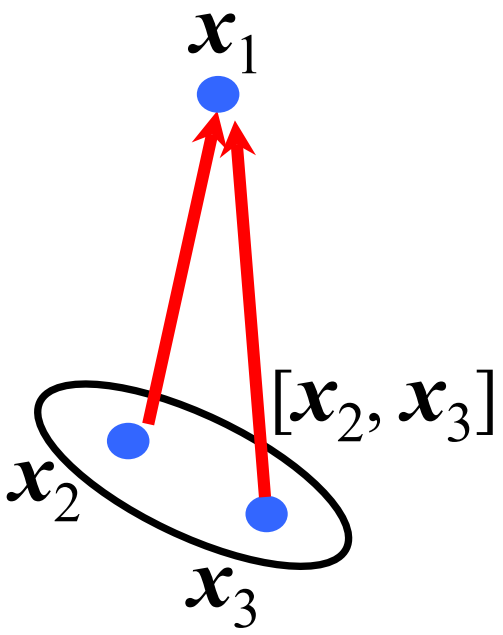
Hierarchical clustering (8)

- **Step 3:**
  Recompute $\boldsymbol{D}$ –
  complete linkage: $d([\boldsymbol{x}_2,\boldsymbol{x}_3],\boldsymbol{x}_1) = \max(d(\boldsymbol{x}_1,\boldsymbol{x}_2),d(\boldsymbol{x}_1,\boldsymbol{x}_3))$



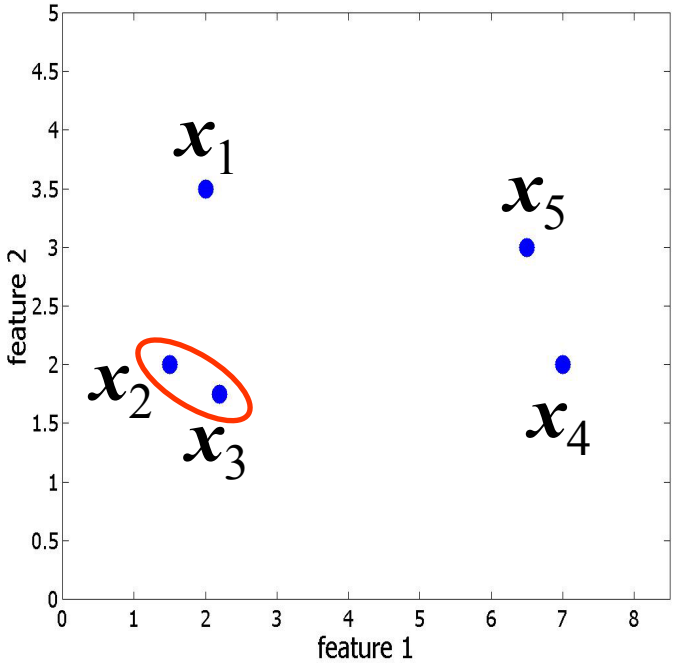
Hierarchical clustering (9)

- **Step 3:**
  Recompute $\boldsymbol{D}$ –
  average linkage: $d([\boldsymbol{x}_2,\boldsymbol{x}_3],\boldsymbol{x}_1) = \text{mean}(d(\boldsymbol{x}_1,\boldsymbol{x}_2),d(\boldsymbol{x}_1,\boldsymbol{x}_3))$



Hierarchical clustering (10a)

- **Step 3:**
  Recompute D – **single linkage:**



	x_1	x_2	x_3	x_4	x_5
x_1	0.00	1.58	1.76	5.22	4.53
x_2		0.00	0.74	5.50	5.10
x_3			0.00	4.81	4.48
x_4				0.00	1.12
x_5					0.00

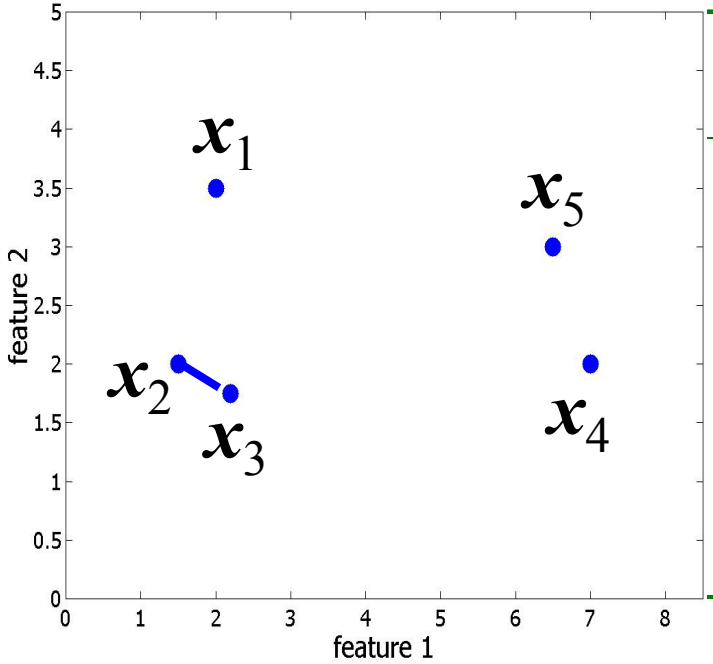
Hierarchical clustering (10b)

- **Step 3:**
  Recompute D – **single linkage:**

	x_1	$[x_2,x_3]$	x_4	x_5
x_1	0.00	1.58	5.22	4.53
$[x_2,x_3]$		0.00	4.81	4.48
x_4			0.00	1.12
x_5				0.00

Hierarchical clustering (11)

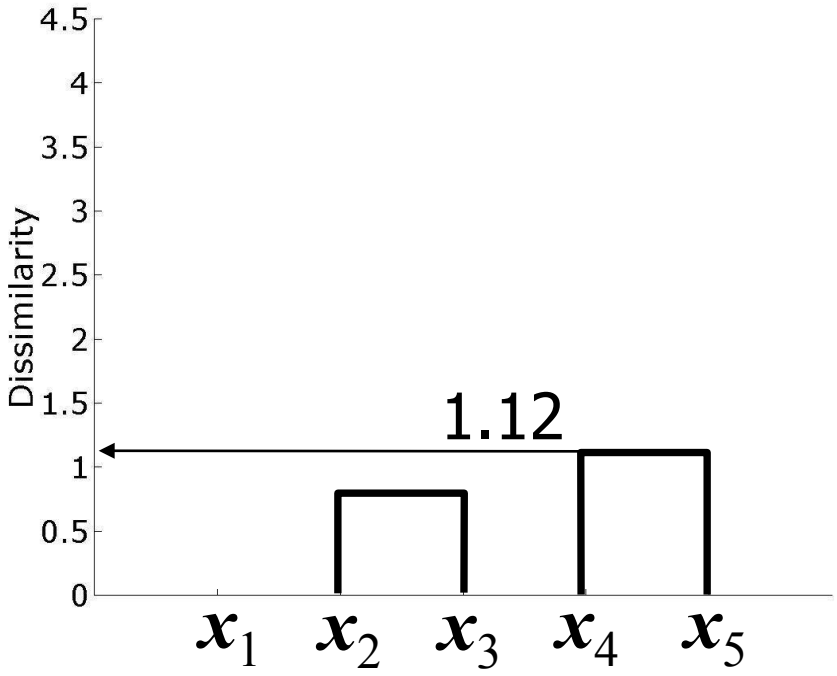
- **Repeat, step 1:**
  Find the most similar pair of objects: $\min_{(i,j)}\{d(i,j)\} = d(4,5)$



	x_1	$[x_2,x_3]$	x_4	x_5
x_1	0.00	1.58	5.22	4.53
$[x_2,x_3]$	1.58	0.00	4.81	4.48
x_4	5.22	4.81	0.00	1.12
x_5	4.53	4.48	1.12	0.00

Hierarchical clustering (12)

- **Repeat, step 2:**
  Merge x_4 and x_5 into a single object, $[x_4, x_5]$;



BioSB

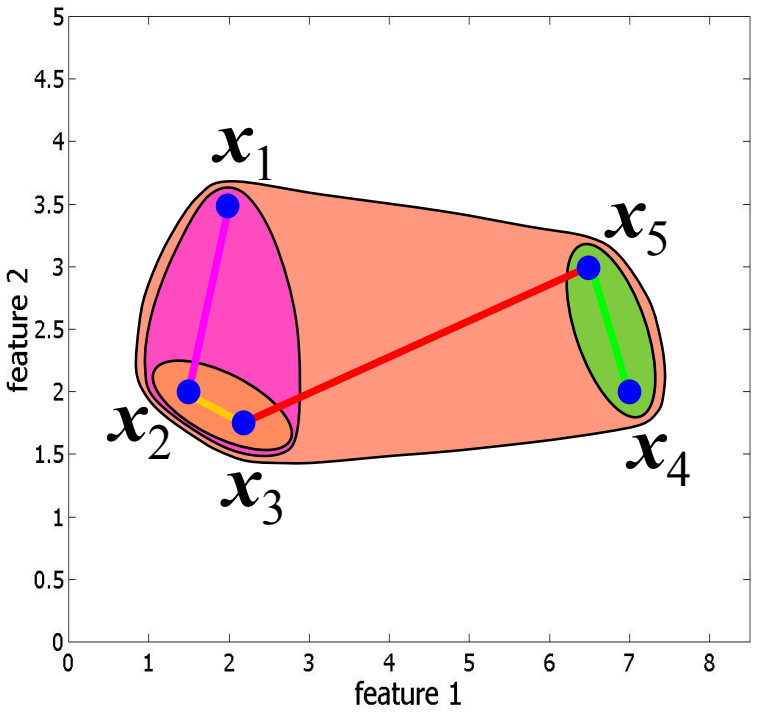
Hierarchical clustering (13)

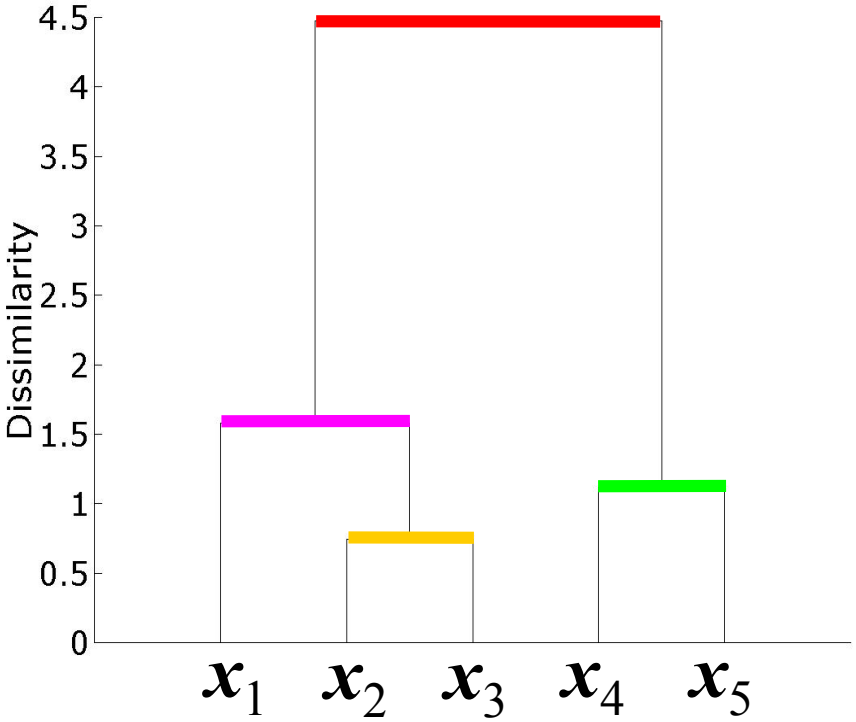
- **Repeat, step 3:**
  Recompute D (single linkage):

	x_1	$[x_2,x_3]$	$[x_4,x_5]$
x_1	0.00	1.58	4.53
$[x_2,x_3]$		0.00	4.48
$[x_4,x_5]$			0.00

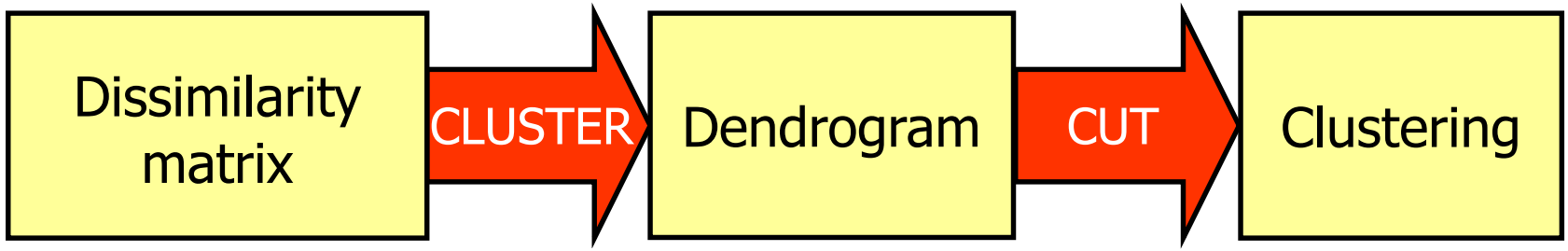
Hierarchical clustering (14)

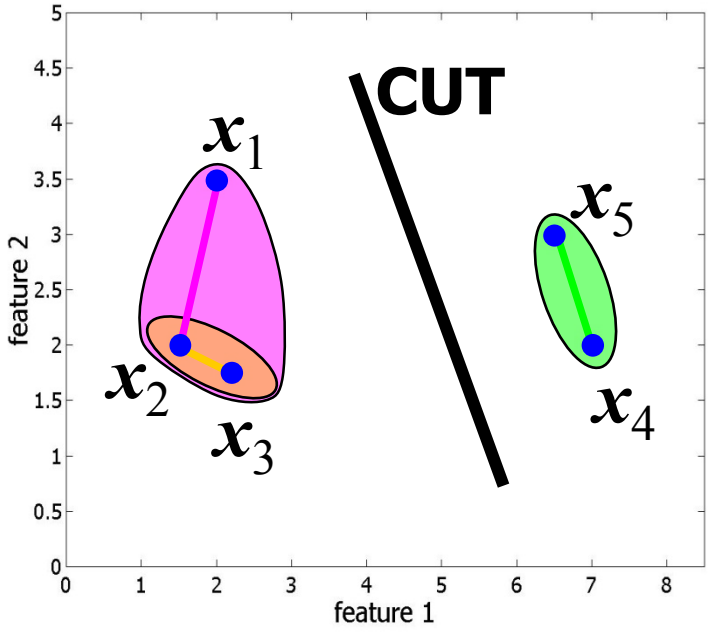
- Repeat steps 1-3 until a single cluster remains

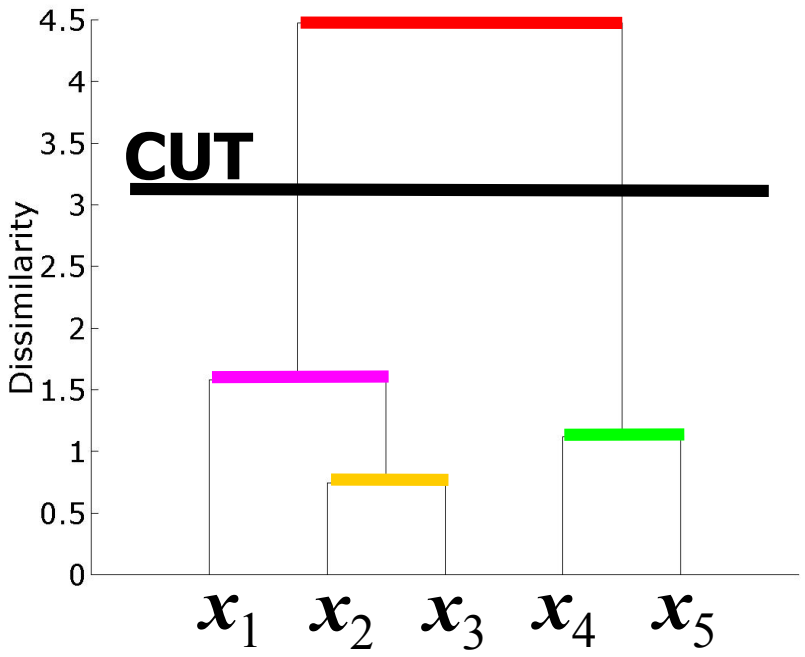




Hierarchical clustering (15)



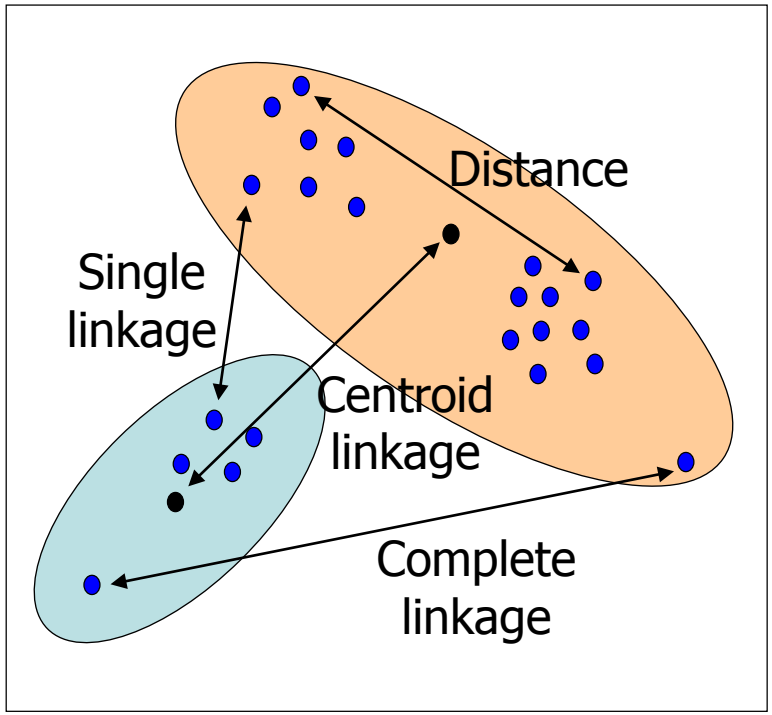




Hierarchical clustering (16)

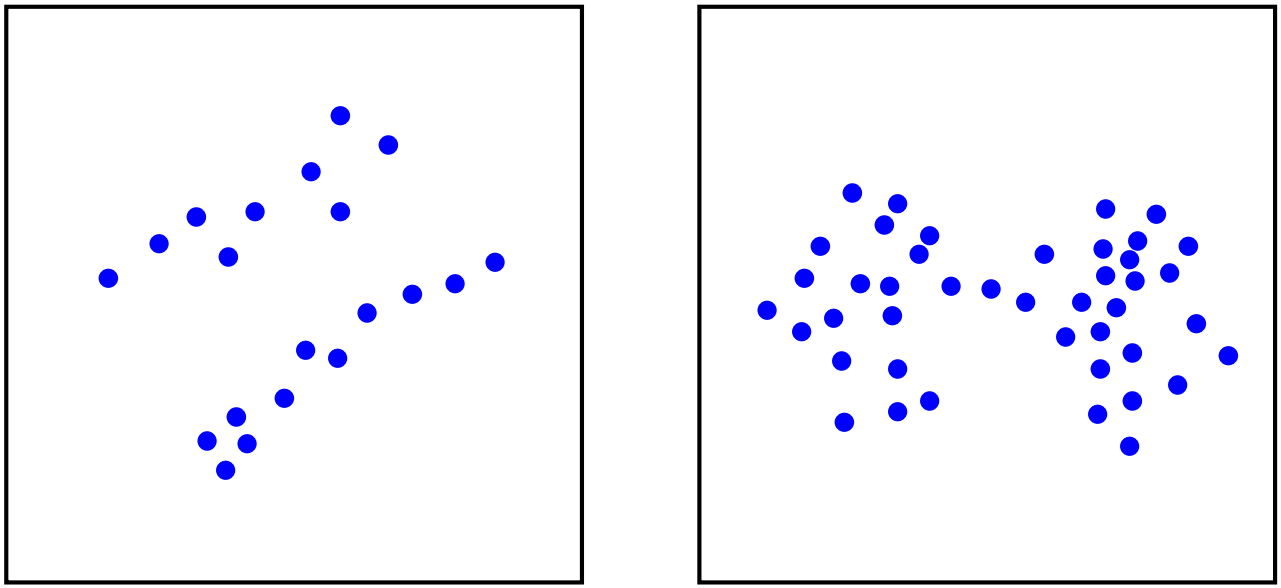
- Hierarchical clustering: repeatedly group closest clusters

- Important choices:
  - *Distance measure*
    between objects:
    Euclidean, correlation,
    Hamming, Minkowski, ...
  - *Linkage*
    between clusters:
    single, average (centroid), complete



BioSB

Linkage and cluster shape

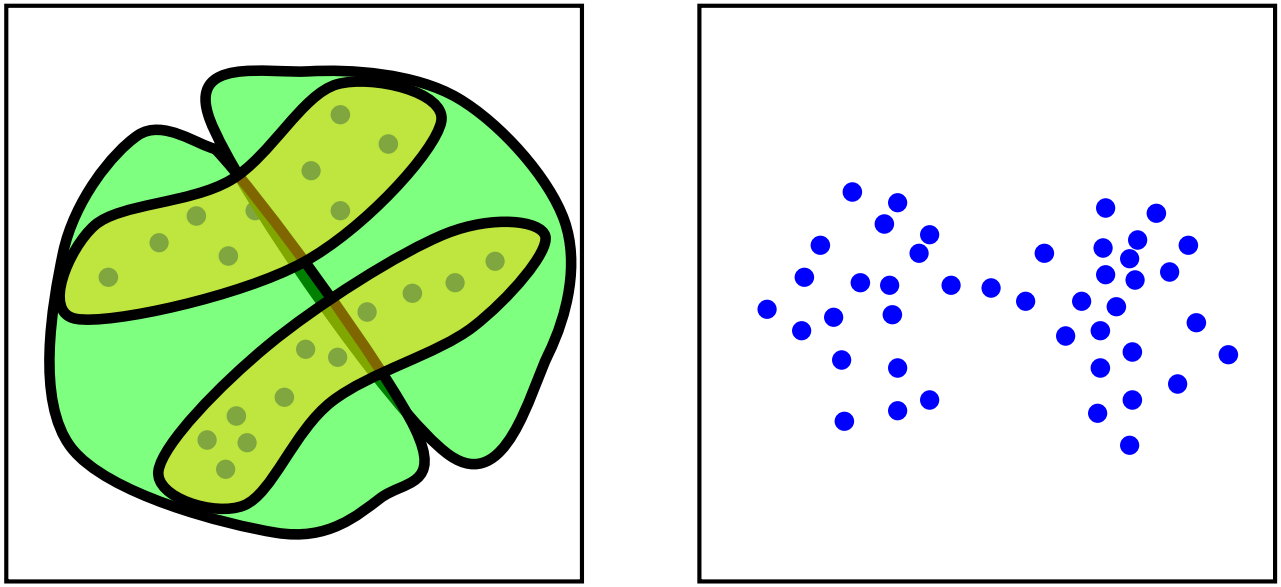


Complete linkage

Single linkage

BioSB

Linkage and cluster shape (2)

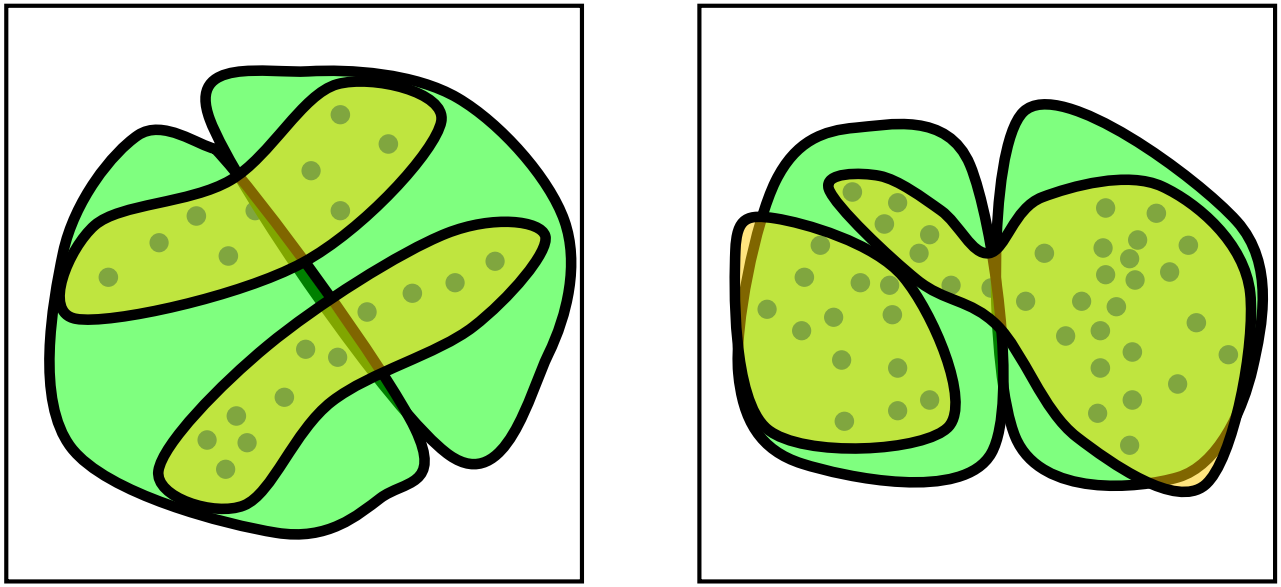


Complete linkage

Single linkage

BioSB

Linkage and cluster shape (3)



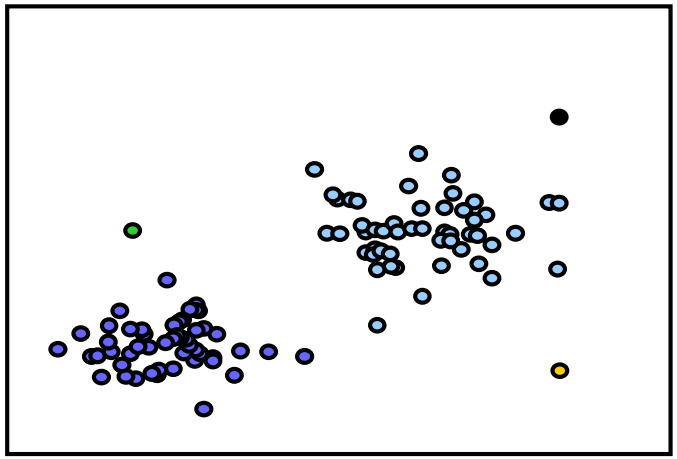
Complete linkage

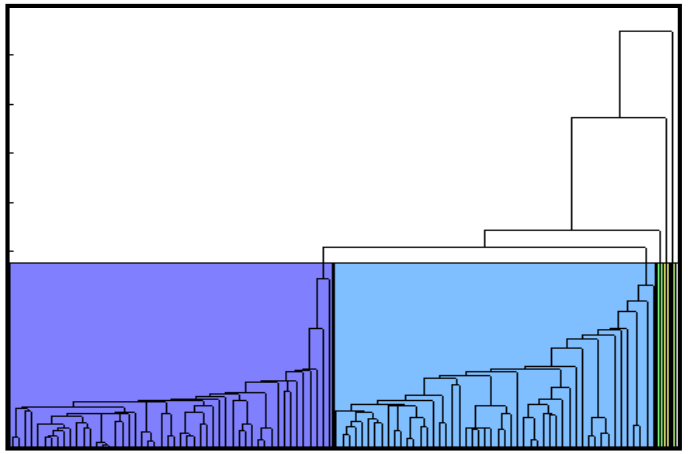
Single linkage

BioSB

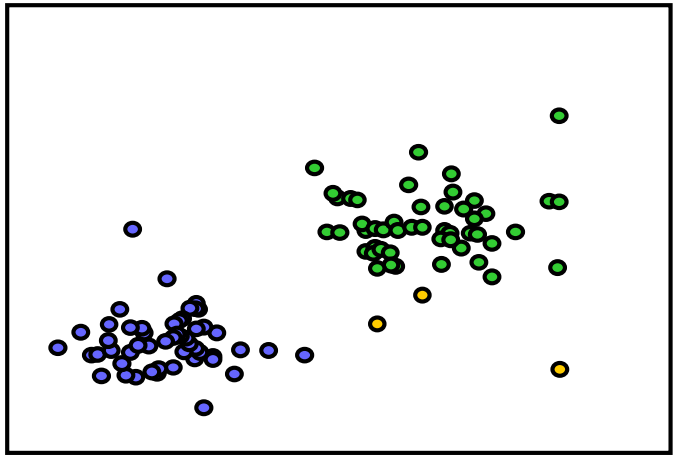
Linkage and outliers

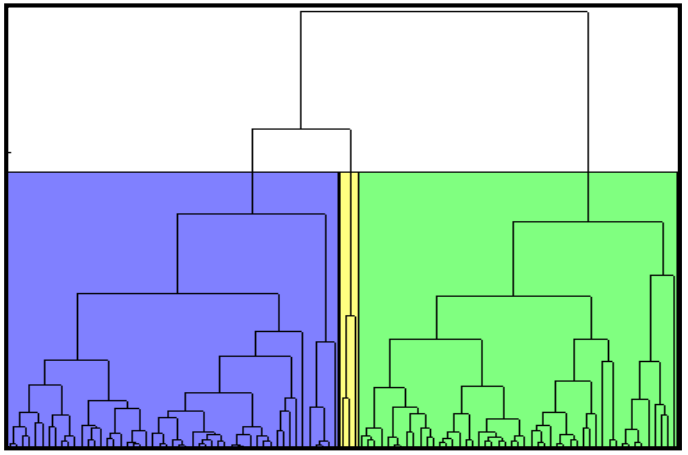
Single linkage





Complete linkage

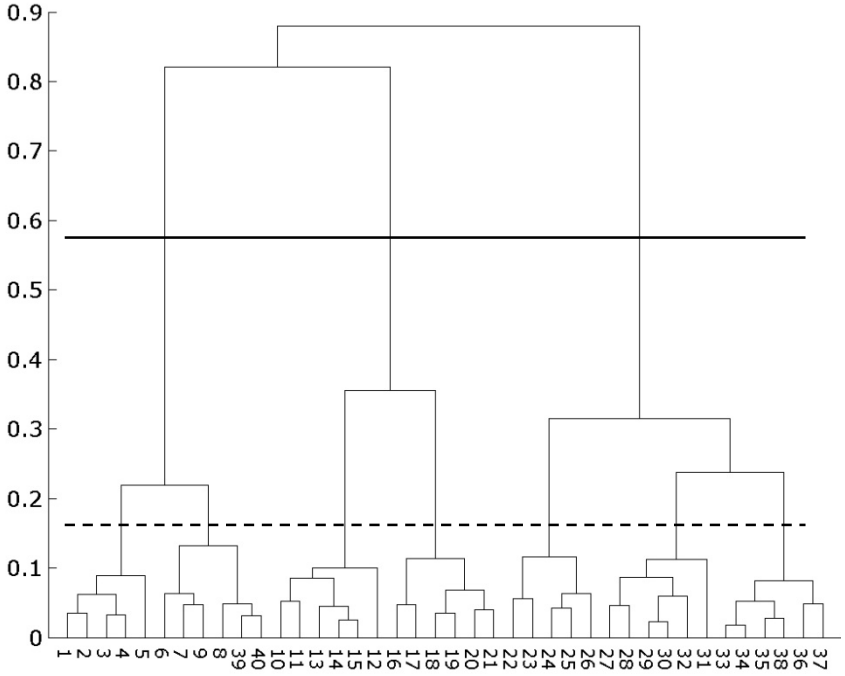


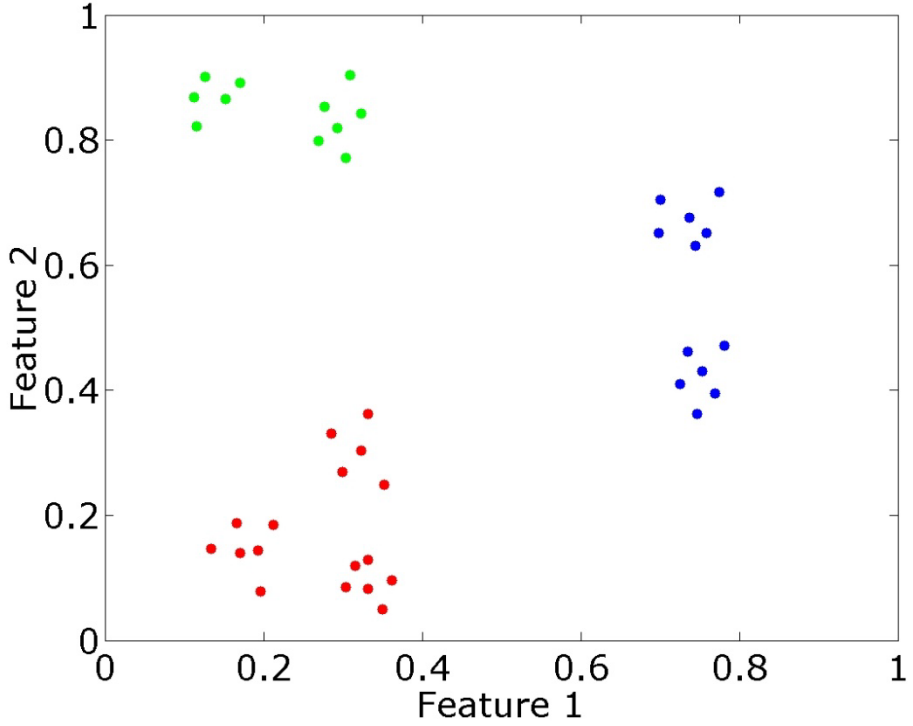


BioSB

Hierarchical clustering examples

Euclidean, complete linkage

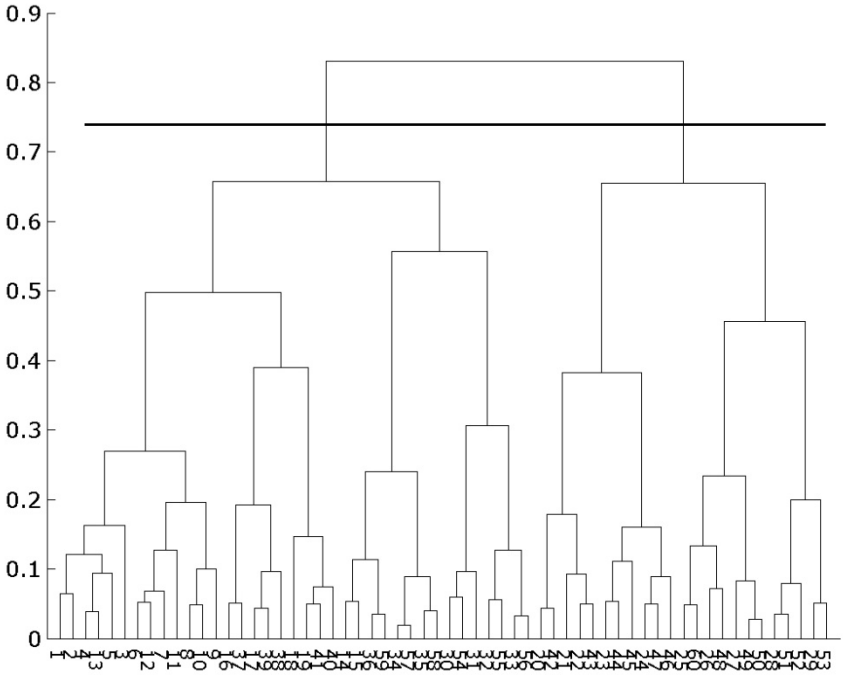


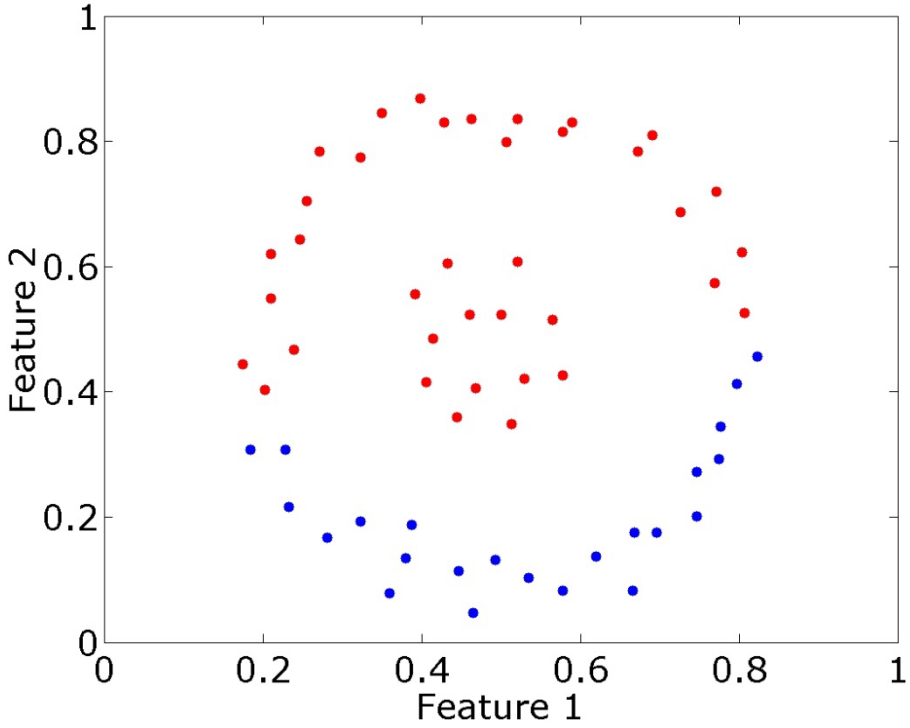


BioSB

Hierarchical clustering examples (2)

Euclidean, complete linkage

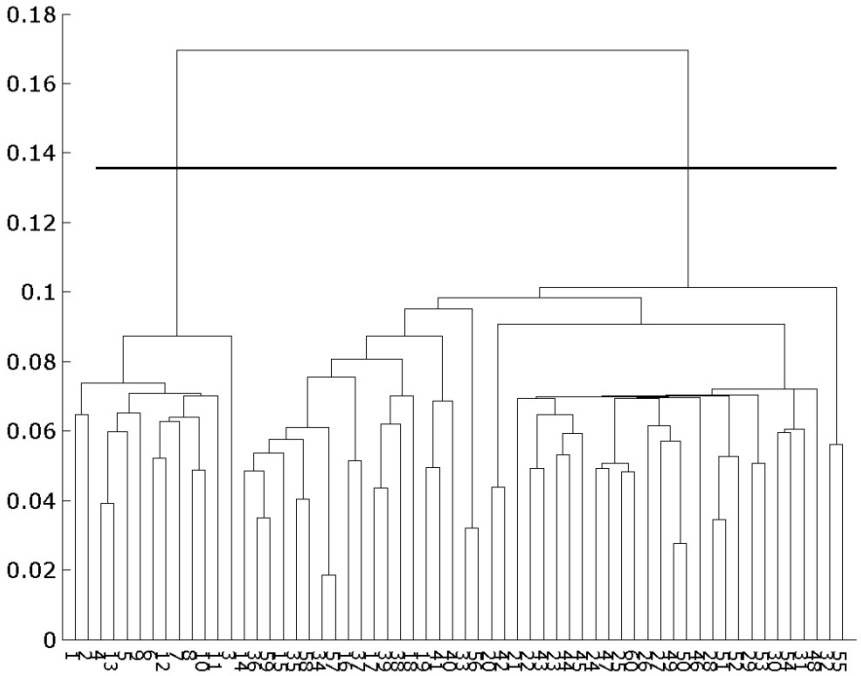


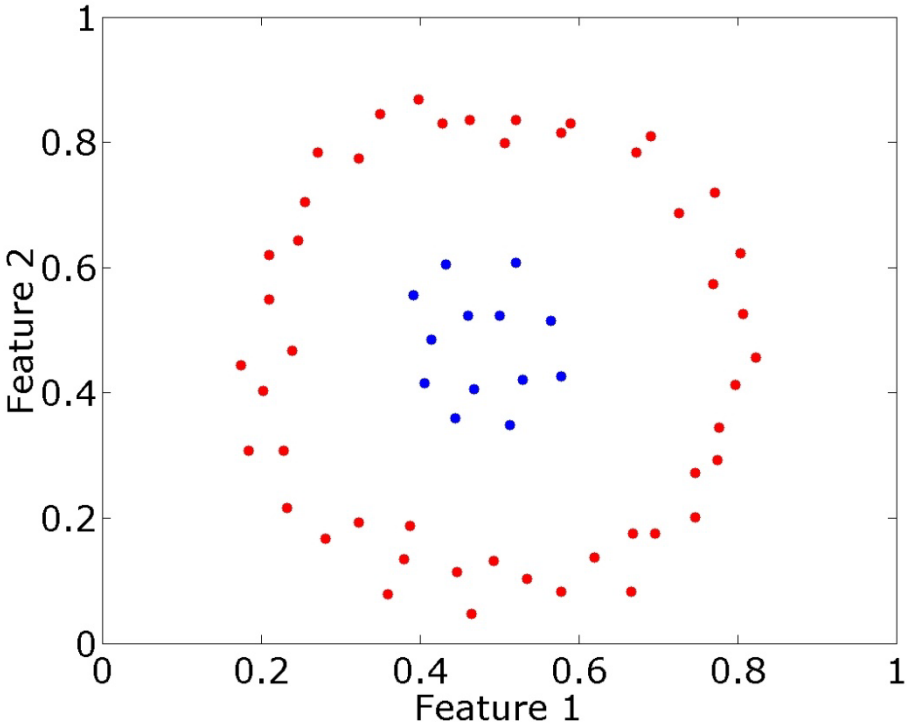


BioSB

Hierarchical clustering examples (3)

Euclidean, single linkage





BioSB

Hierarchical clustering (17)

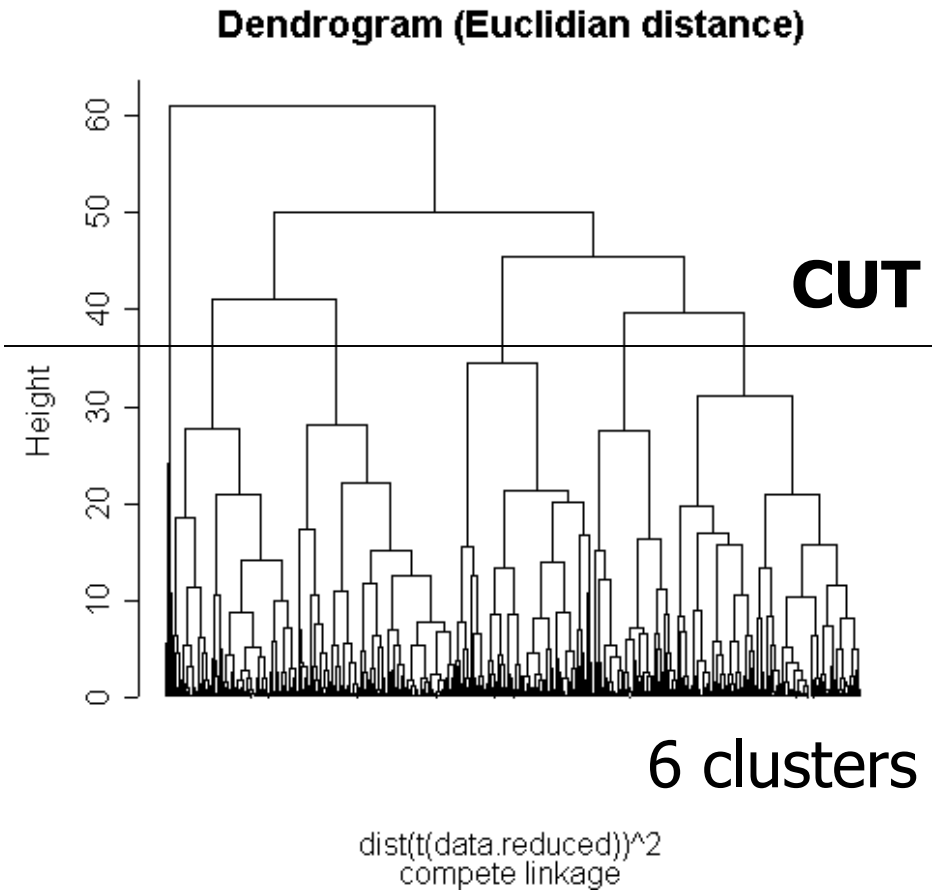
- Advantages:
  - dendrogram gives overview of all possible clusterings
  - linkage type allows to find clusters of varying shapes (convex and non-convex)
  - different dissimilarity measures can be used

- Disadvantages:
  - computationally intensive:
    $O(n^2)$ in complexity and memory
  - clusterings limited to “hierarchical nestings”

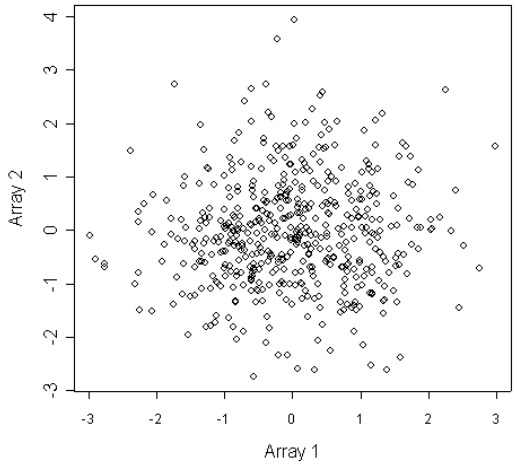
BioSB

Hierarchical clustering: warning

- Cluster 500 genes, 5 arrays:



Data were random …



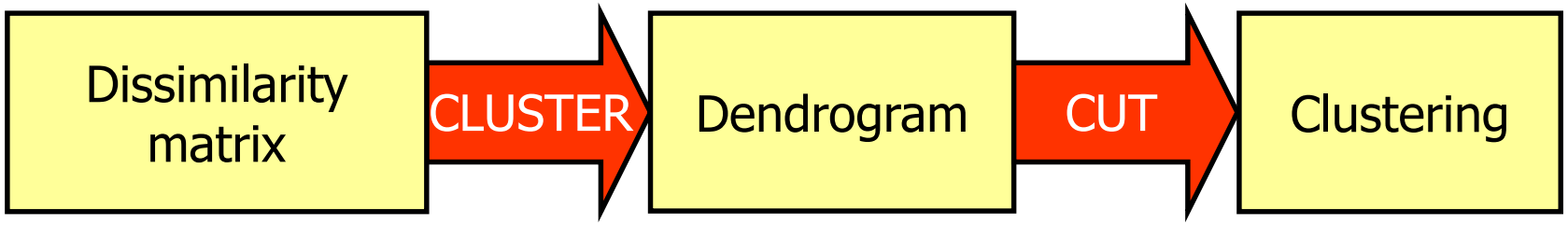
Validation is needed

BioSB

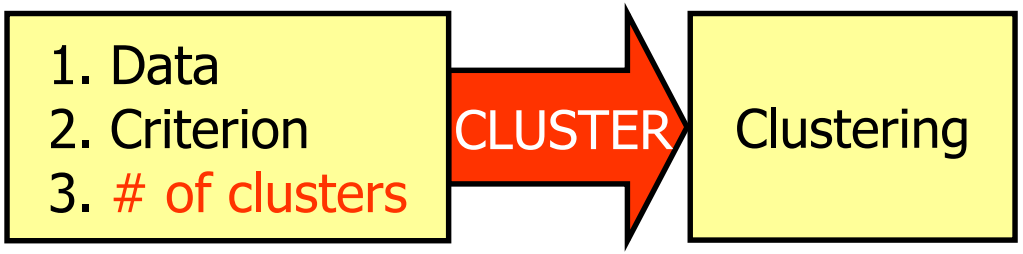
10min break
Exercise 4.1-4.7

Sum-of-squares clustering

- Hierarchical:



- Sum-of-squares:



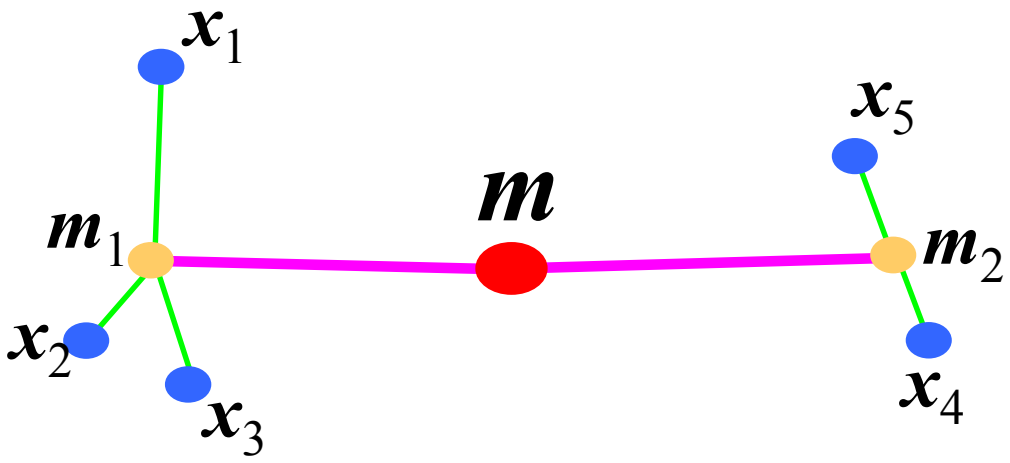
BioSB

Sum-of-squares clustering (2)

- Recall from Day 2 (& 3) (Fisher: within and between scatter):

$$\boldsymbol{S}_w = \sum_{i=1}^{C} \frac{n_i}{n} \Sigma_i \quad (n_1 = 3, n_2 = 2, n = 5, C = 2)$$

$$\boldsymbol{S}_B = \sum_{i=1}^{C} \frac{n_i}{n} (\boldsymbol{m}_i - \boldsymbol{m})(\boldsymbol{m}_i - \boldsymbol{m})^T, \; \boldsymbol{m} = \sum_{i=1}^{C} \frac{n_i}{n} \boldsymbol{m}_i$$



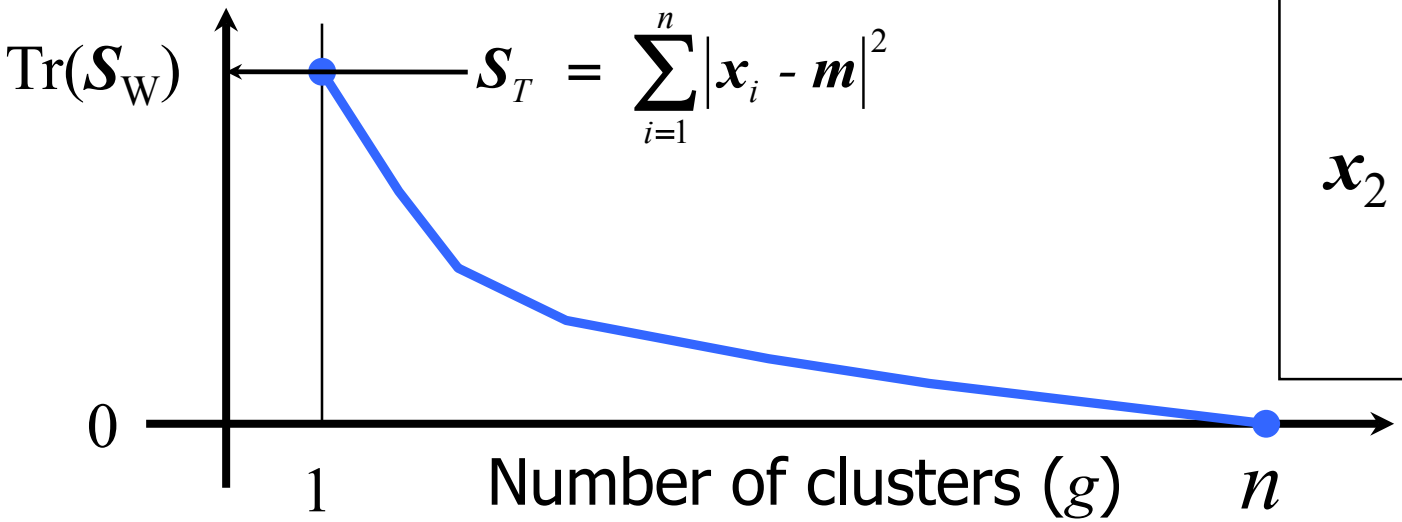
BioSB

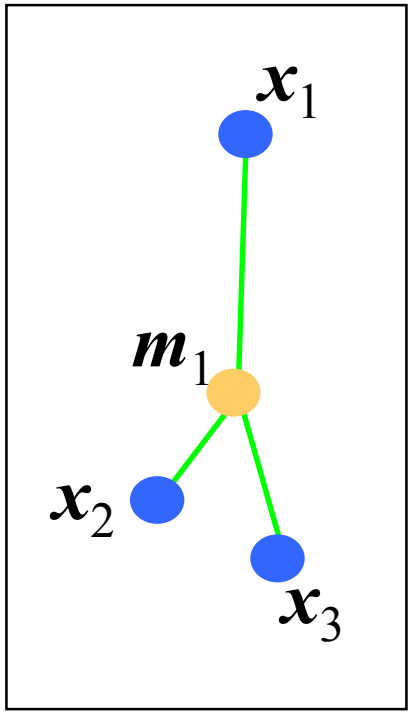
K-means

- Minimize: $\mathrm{Tr}(\mathbf{S}_\mathrm{W}) = \frac{1}{n}\sum_{j=1}^{g}\mathbf{S}_j$

$$\boldsymbol{S}_j = \sum_{i=1}^{n_j}\left|\boldsymbol{x}_i - \boldsymbol{m}_j\right|^2$$

(sum of per cluster variances)





K-means (2)

- Iterative procedure to search for $\min(\mathrm{Tr}(\boldsymbol{S}_\mathrm{W}))$:
  1. choose number of clusters (g)
  2. position prototypes $(m_j,\ j=1,\ldots,g)$ randomly
  3. assign samples to closest prototype
  4. compute mean of samples assigned to same prototype: new prototype position

  Repeat steps 3 and 4 as long as prototypes move

BioSB

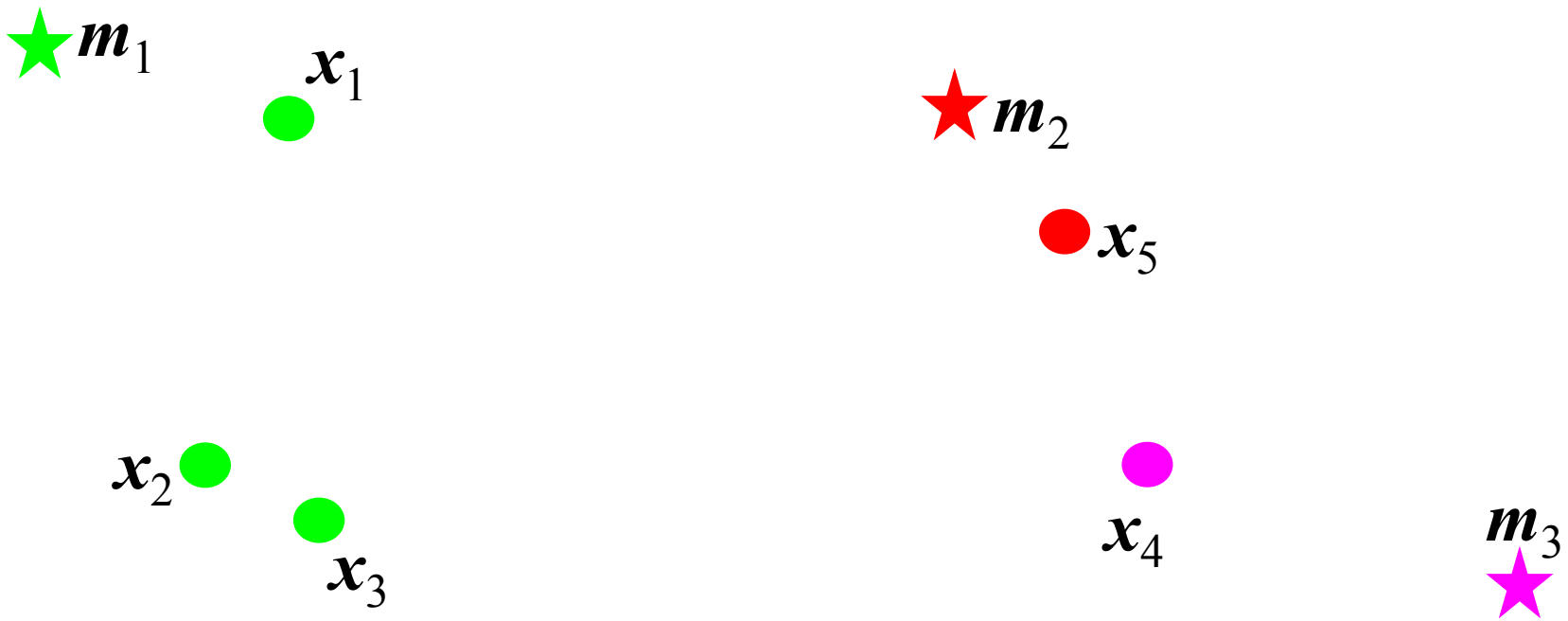
K-means (3)

- **Step 1:** Choose number of clusters/prototypes
- **Step 2:** Position prototypes randomly

BioSB

K-means (4)

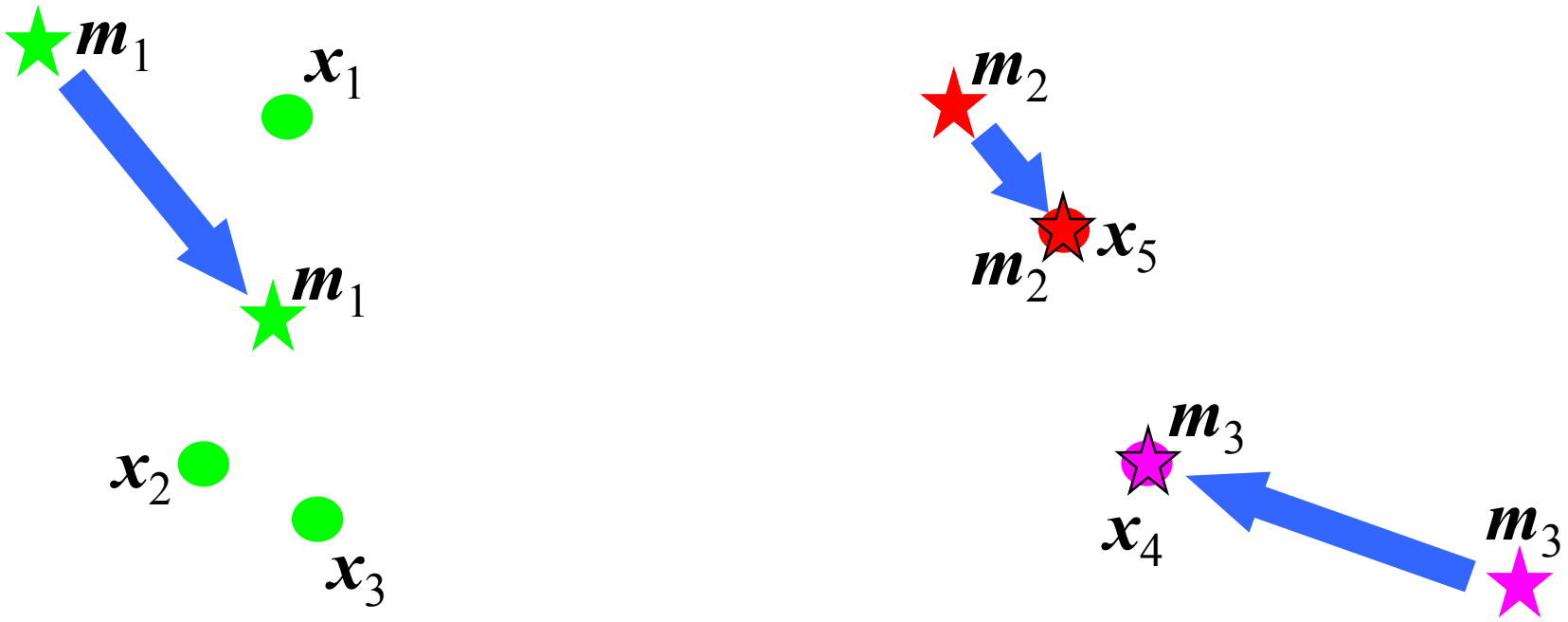
- **Step 3:** Assign samples to closest prototype



BioSB

K-means (5)

- **Step 4:** Compute mean of samples assigned to same prototype: new prototype positions



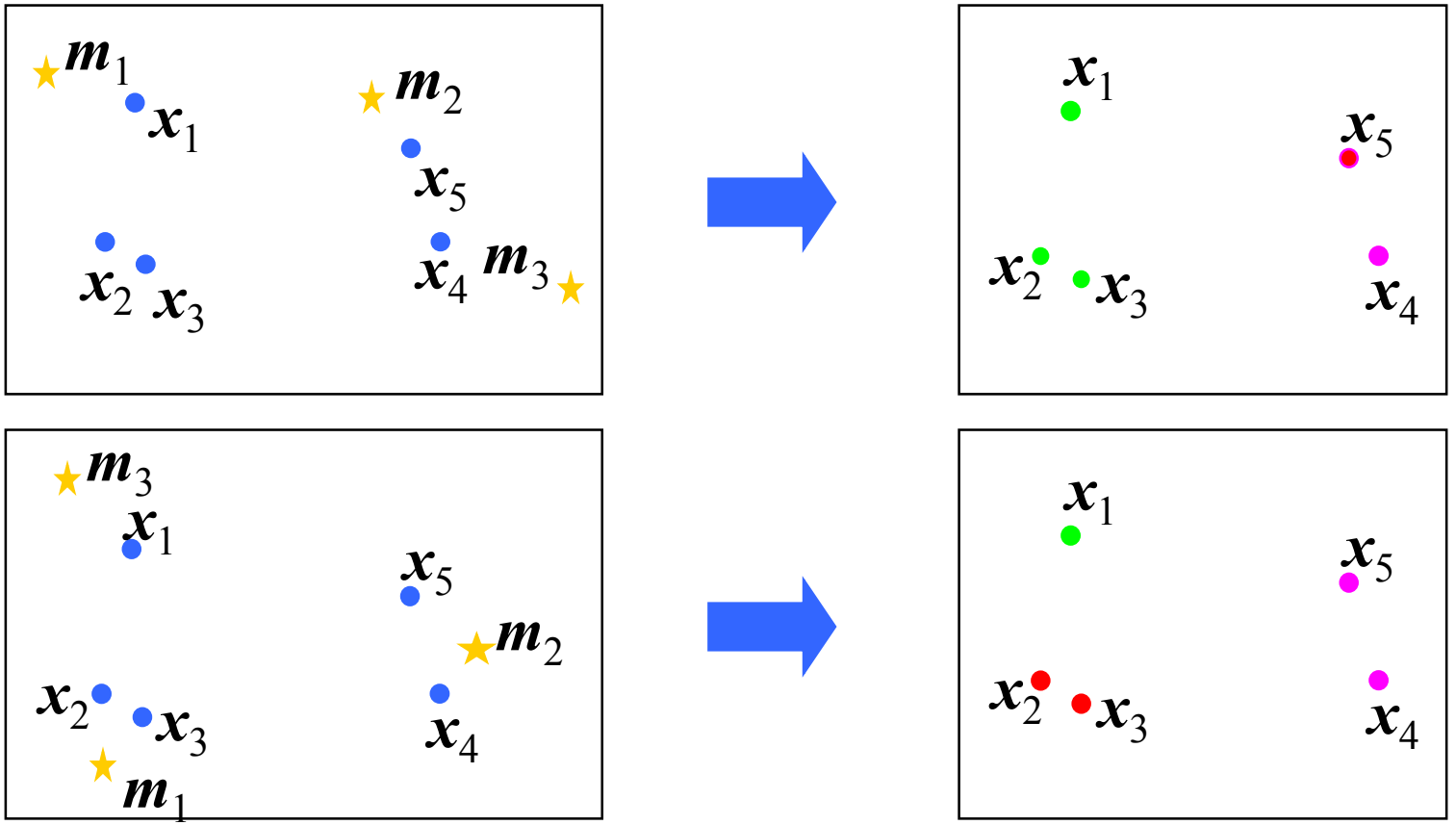
K-means (6)

- **Repeat** as long as prototype positions change**:**
  - **Step 3:** Assign samples
  - **Step 4:** Recompute prototype positions

BioSB

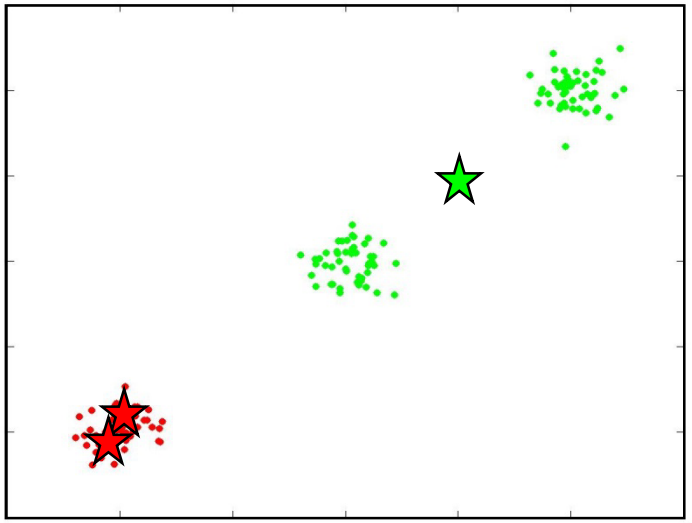
K-means problems

- Clustering depends on initialization



K-means problems (2)

- Algorithm can get stuck in local minima

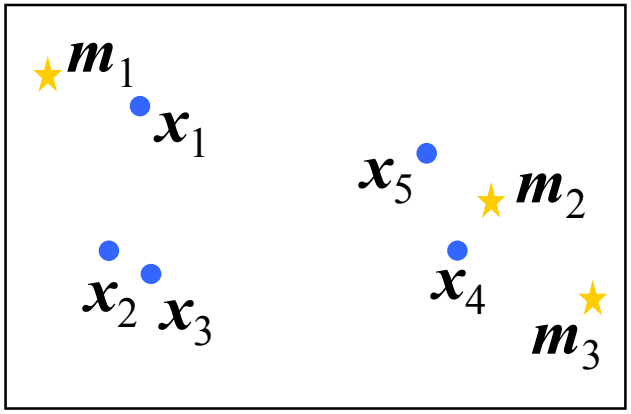


- Solution:
  - start from I different random initialisations
  - keep the best clustering (lowest $\mathrm{Tr}(\boldsymbol{S}_\mathrm{W})$)
  - For high-dimensional data, many restarts can be necessary (e.g. I = 100)

BioSB

K-means problems (3)

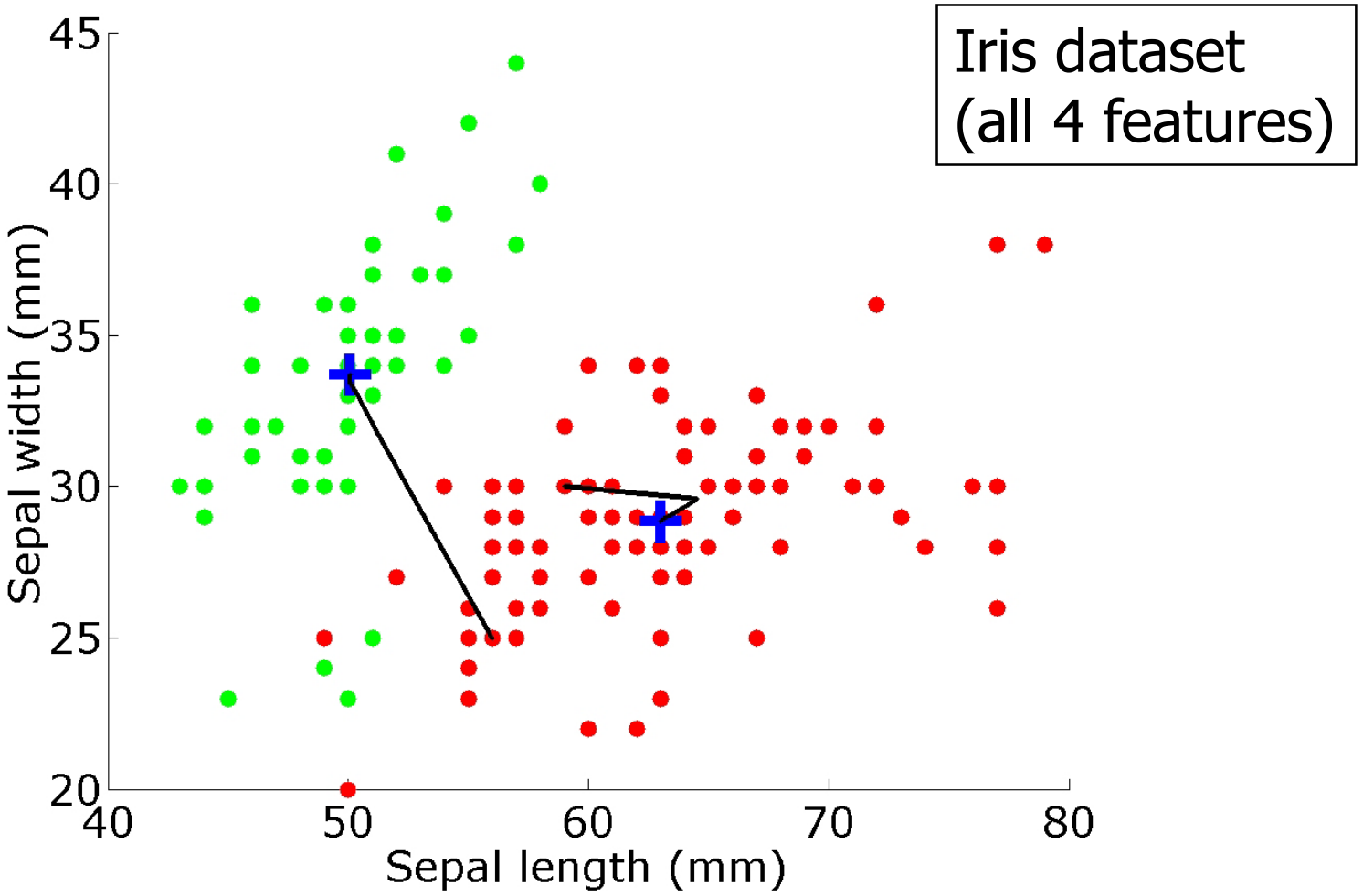
- Clusters can loose all samples



- Possible solution:
  - remove cluster and continue with $g - 1$ means
  - alternatively, split largest cluster into two
    or add a random cluster to continue with g means

BioSB

K-means example



BioSB

Advantages/disadvantages: *K*-means

- Disadvantages:
  - Finds only convex clusters (“round shapes”)
  - Sensitive to initialization
  - Can get stuck in local minima

- Advantages:
  - Very simple
  - Fast

BioSB

Recapitulation

- Clustering is way to detect *natural* groups in data
- What is natural is partly subjective
- We looked at:
  - *Hierarchical clustering*
  - *Sum of squares* (k-means) clustering
- Hierarchical clustering:
  - *dendrogram* shows a complete hierarchy of possible clusterings
  - computionally intensive
- *K*-means
  - fast
  - sensitive to *initialization* and *local minima*

BioSB

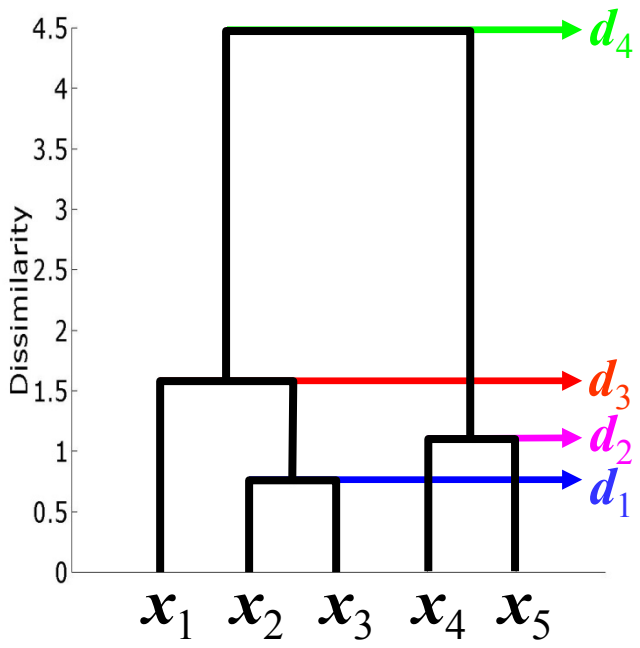
Cluster validation

- Cluster validation:
  - Checking whether grouping is really present
  - Choosing the optimal number of clusters
- A difficult problem – the ground truth is not known (since we do not know the object labels)!
- Methods:
  - Distortion measures:
    - Does clustering approximate structure in data?
  - Validity measures:
    - Davies-Bouldin index
  - Fusion graph
  - Gap statistic

BioSB

Distortion measures

- How well does a dendrogram capture structure in data?



d^*	x_1	x_2	x_3	x_4	x_5
x_1	0	d_3	d_3	d_4	d_4
x_2		0	d_1	d_4	d_4
x_3			0	d_4	d_4
x_4				0	d_2
x_5					0

Distortion measures (2)

- Measure of distortion: Pearson correlation of $\boldsymbol{d}$ and $\boldsymbol{d}^*$

$$\rho(\boldsymbol{d},\boldsymbol{d}^*) = \frac{\mathrm{cov}(\boldsymbol{d},\boldsymbol{d}^*)}{\sqrt{\mathrm{var}(\boldsymbol{d})\mathrm{var}(\boldsymbol{d}^*)}} \in [-1,1]$$

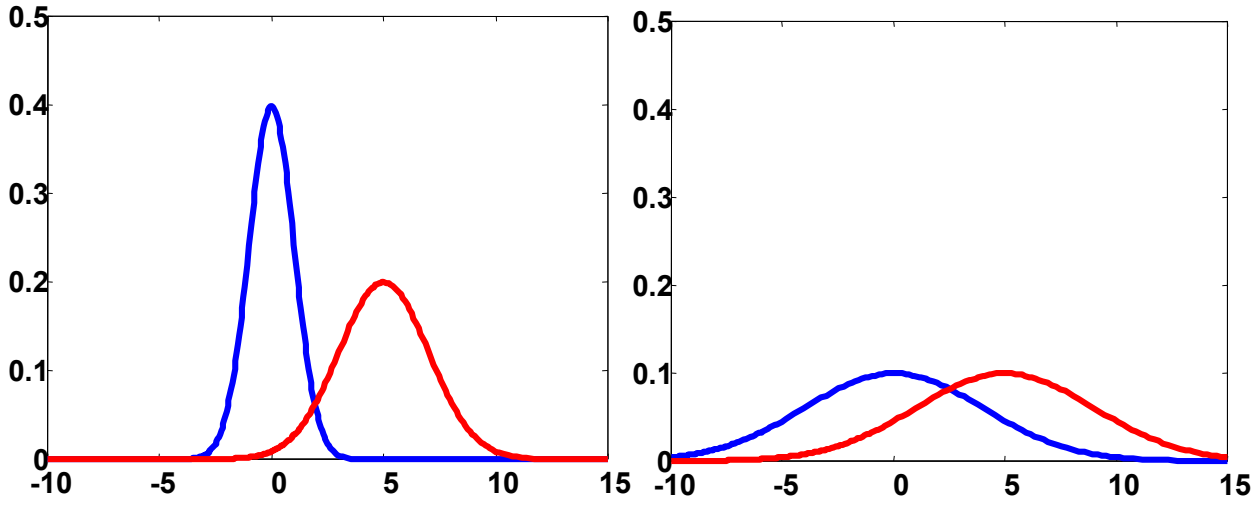
$\boldsymbol{d}$	x_1	x_2	x_3	x_4	x_5
x_1	0.00	1.58	1.76	5.22	4.53
x_2		0.00	0.74	5.50	5.10
x_3			0.00	4.81	4.48
x_4				0.00	1.12
x_5					0.00

$\boldsymbol{d}^*$	x_1	x_2	x_3	x_4	x_5
x_1	0	d_3	d_3	d_4	d_4
x_2		0	d_1	d_4	d_4
x_3			0	d_4	d_4
x_4				0	d_2
x_5					0

BioSB

Validity measures

- Many are based on within and between group scatter
- The larger the between group scatter and the smaller the within group scatter, the better
- Example: Davies-Bouldin



BioSB

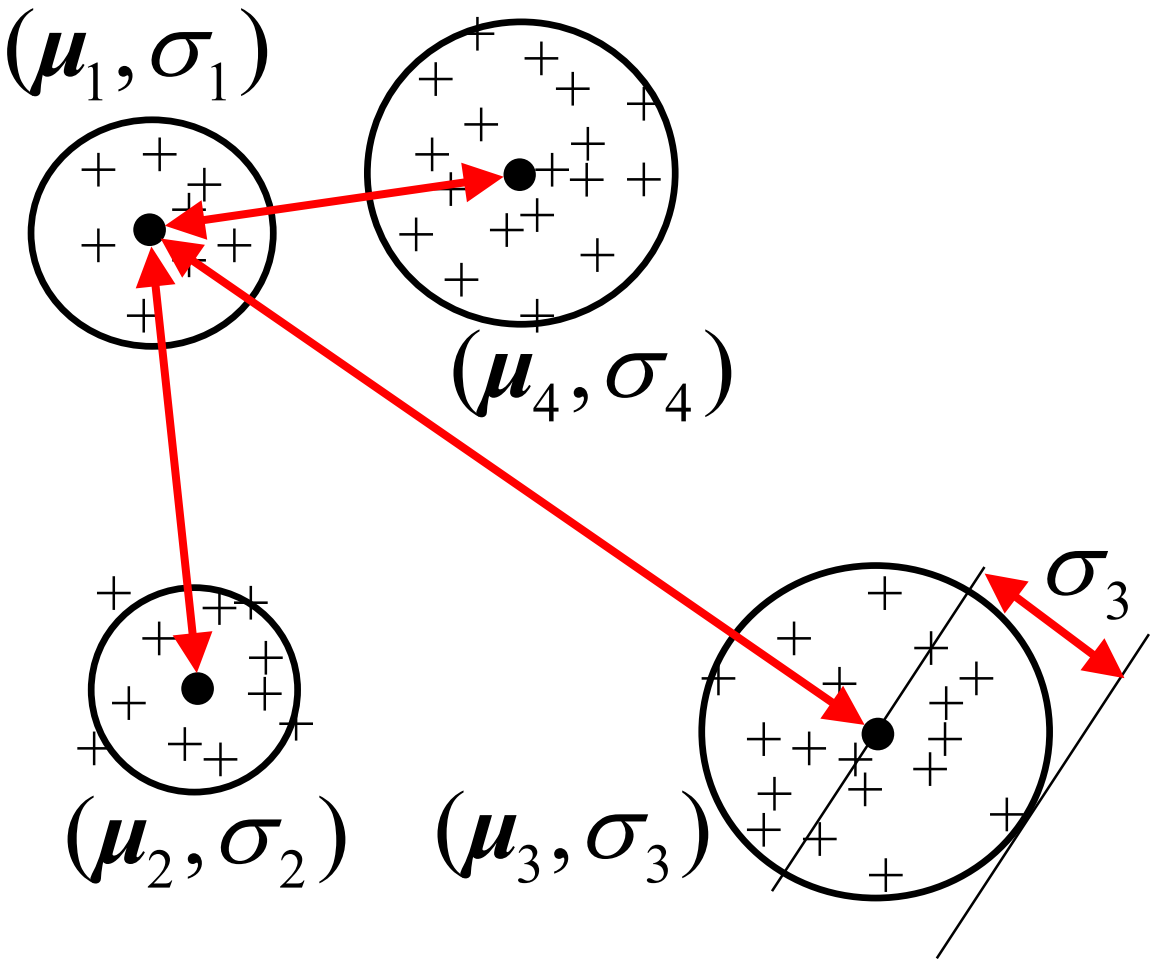
Davies-Bouldin index

- Assumption: clusters are spherical
- For a good clustering, it should hold that:
  - objects are compactly organized within a cluster
  - clusters are far apart

- D.L. Davies and D.W. Bouldin, IEEE Transactions on Pattern Analysis and Machine Intelligence 1, pp. 224-227, 1979

BioSB

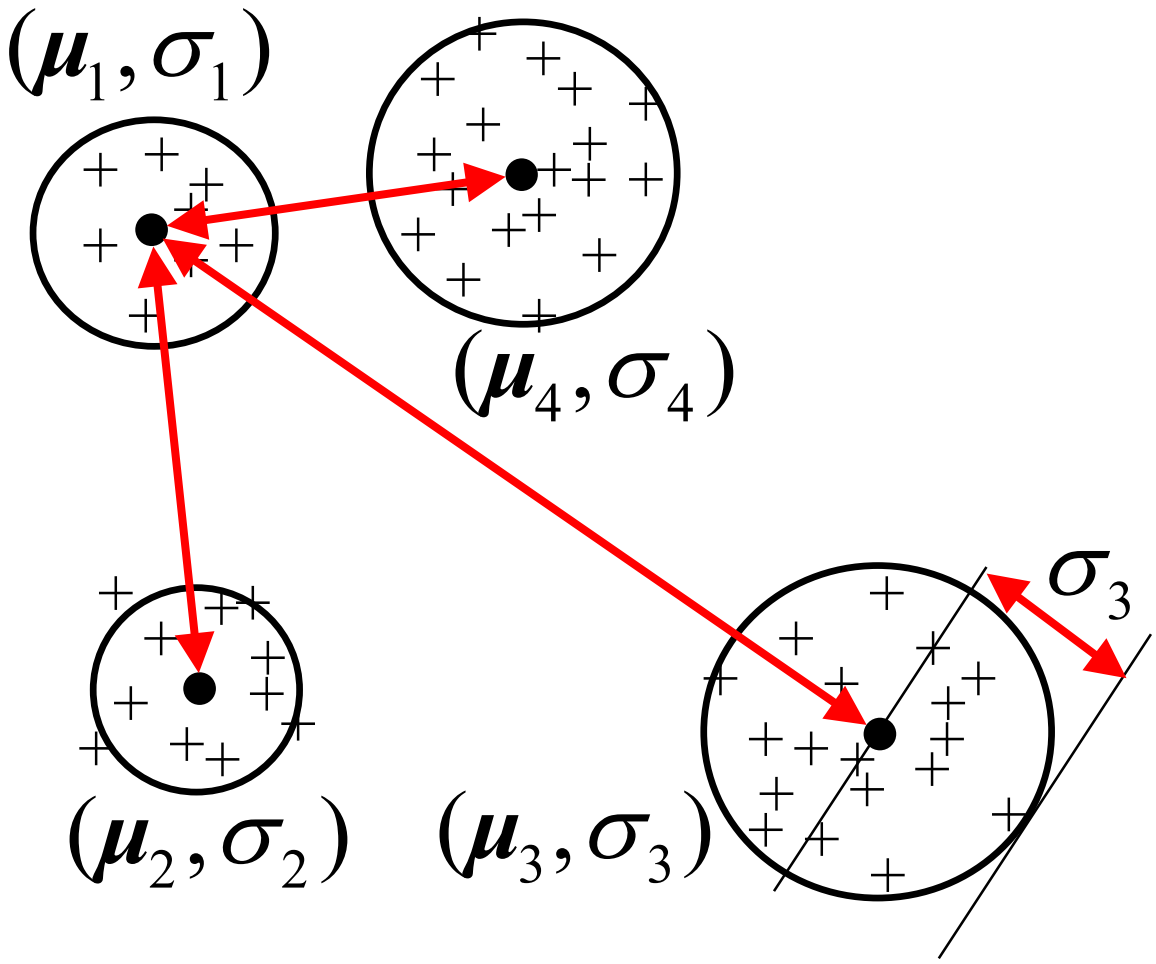
Davies-Bouldin index (2)



$$\sigma_j = \sqrt{\frac{1}{n_j} \sum_{x_i \in C_j} \left\| x_i - \mu_j \right\|^2}$$

$$\mu_j = \frac{1}{n_j} \sum_{x_i \in C_j} x_i$$

Davies-Bouldin index (3)



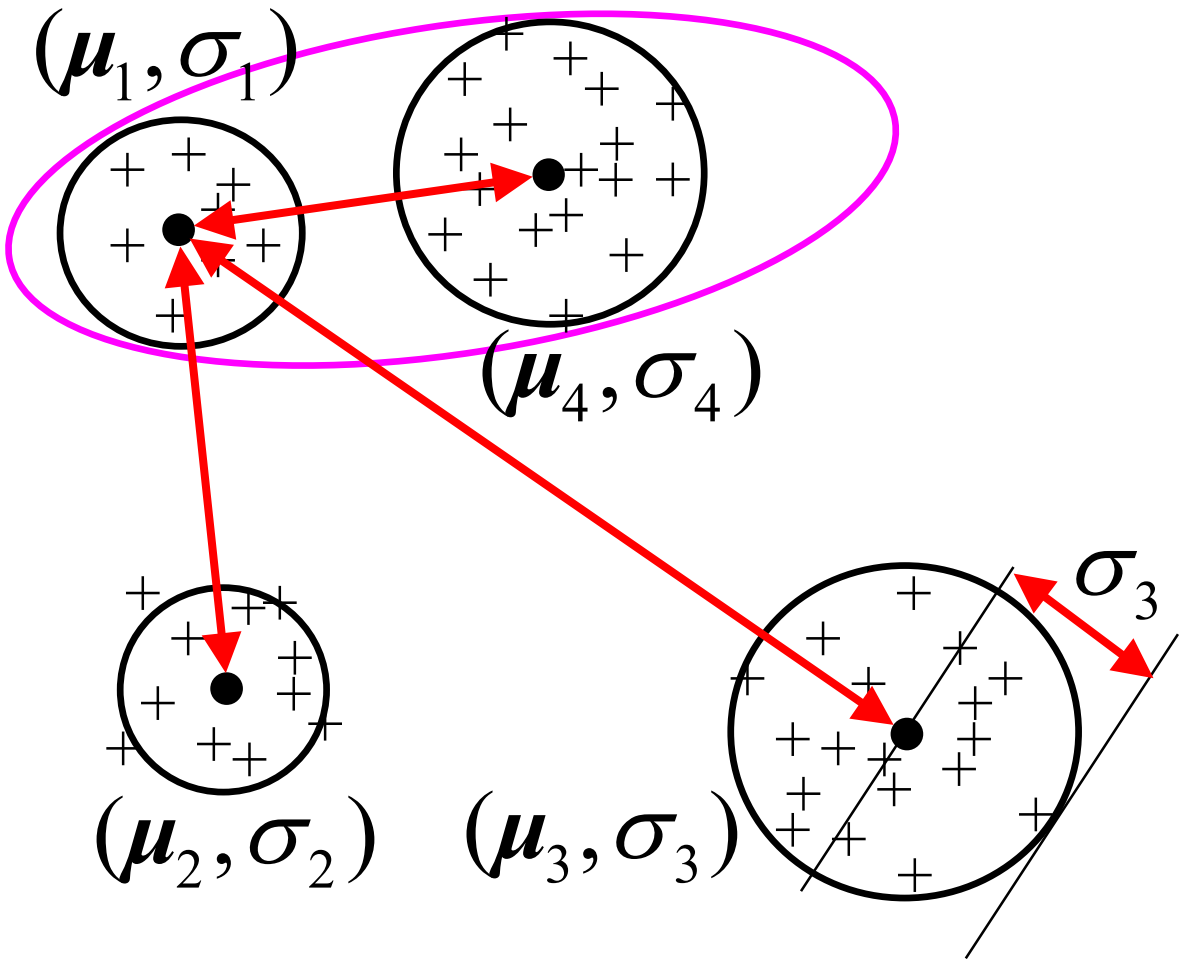
$$R_{jk} = \frac{\sigma_j + \sigma_k}{\left\| \boldsymbol{\mu}_j - \boldsymbol{\mu}_k \right\|}$$

$$\sigma_j = \sqrt{\frac{1}{n_j} \sum_{\boldsymbol{x}_i \in C_j} \left\| \boldsymbol{x}_i - \boldsymbol{\mu}_j \right\|^2}$$

$$\boldsymbol{\mu}_j = \frac{1}{n_j} \sum_{\boldsymbol{x}_i \in C_j} \boldsymbol{x}_i$$

BioSB

Davies-Bouldin index (4)



$$R_{jk} = \frac{\sigma_j + \sigma_k}{\left\| \boldsymbol{\mu}_j - \boldsymbol{\mu}_k \right\|}$$

$$R_j = \max_{k=1,..g;k\neq j} R_{jk}$$

Davies-Bouldin index (5)

$$R_{jk} = \frac{\sigma_j + \sigma_k}{\left\| \boldsymbol{\mu}_j - \boldsymbol{\mu}_k \right\|}$$

Paired cluster criterion

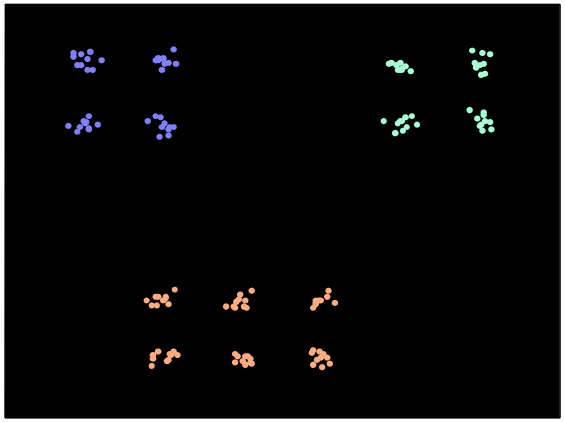
$$R_j = \max_{k=1,..g;k\neq j} R_{jk}$$

Worst-case value per cluster

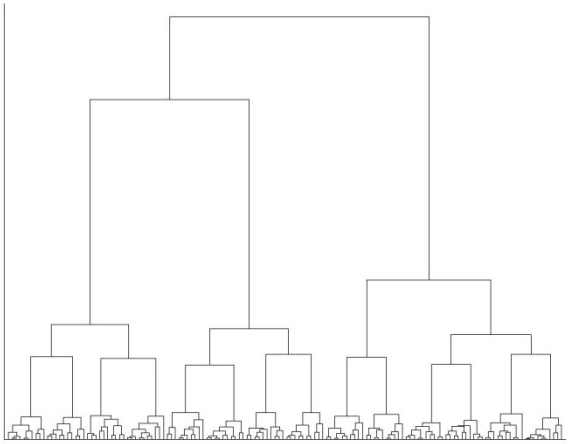
$$I_{DB} = \frac{1}{g}\sum_{j=1}^{g} R_j$$

Average worst-case

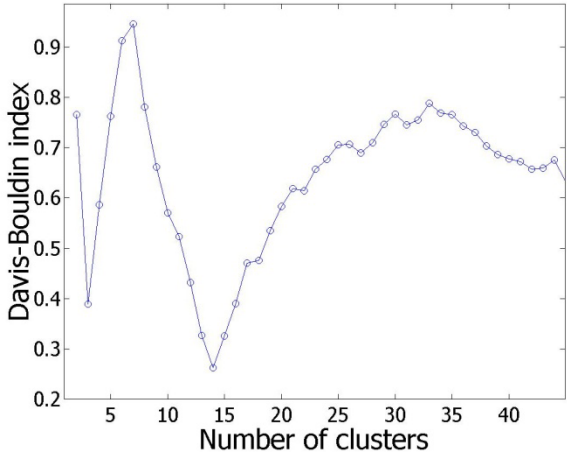
Davies-Bouldin index (5)



Dataset



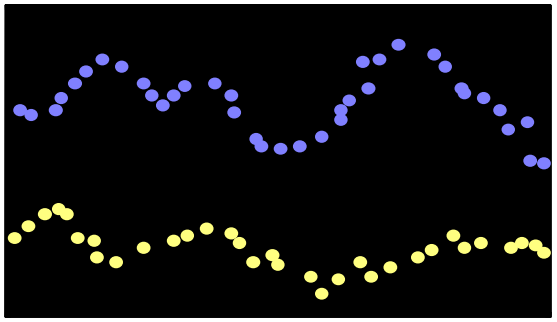
Complete link



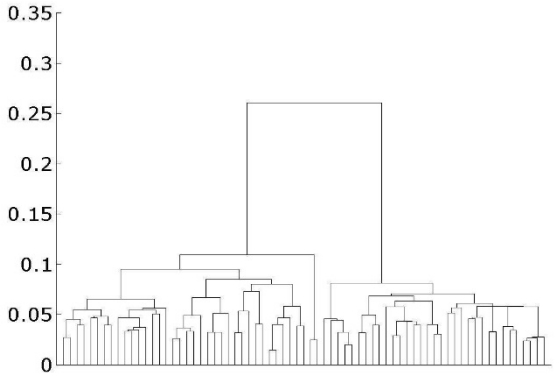
Davies-Bouldin:
3 or 14 clusters

BioSB

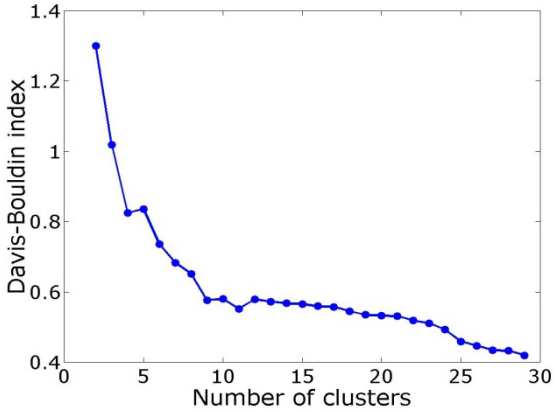
Davies-Bouldin index (7)



Single link

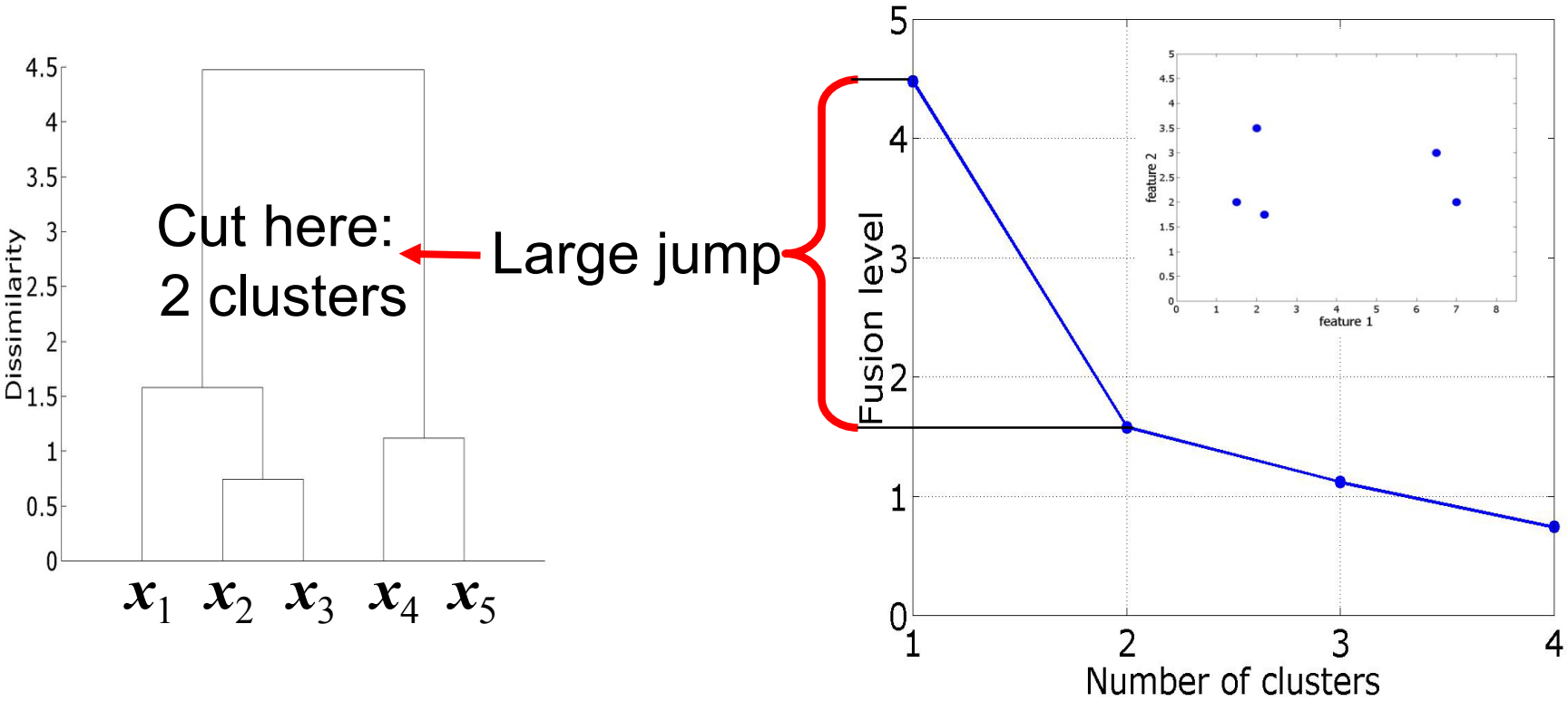


Davies-Bouldin:



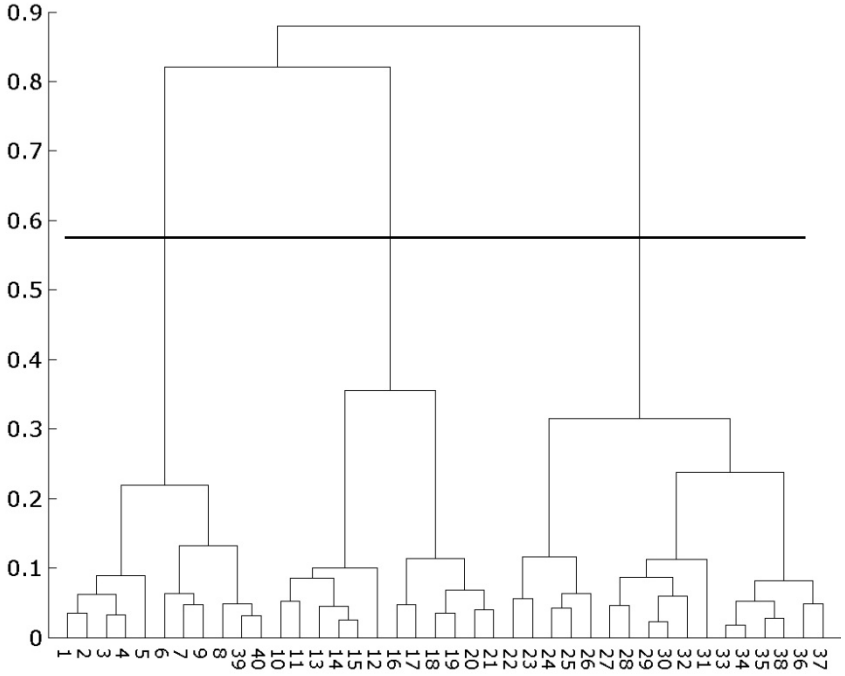
Fusion graph

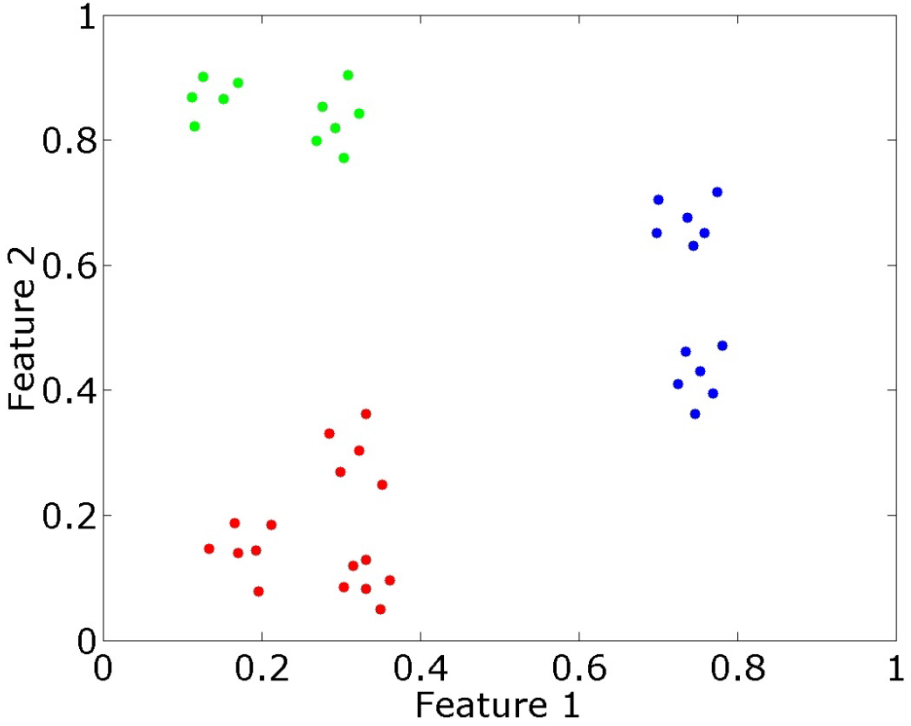
- Heuristic approach: fusion level



Fusion graph (2)

(Euclidean; complete linkage)

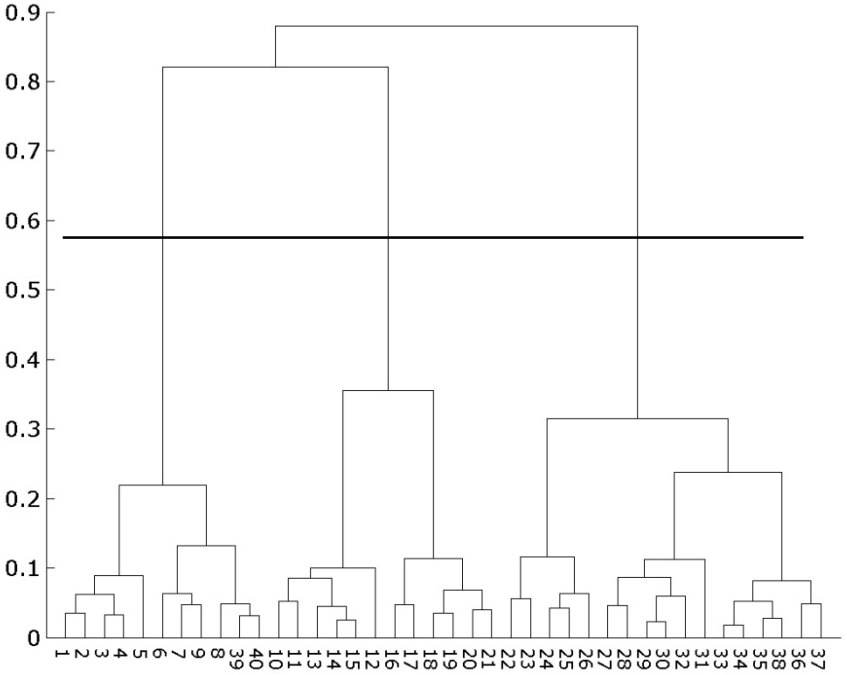


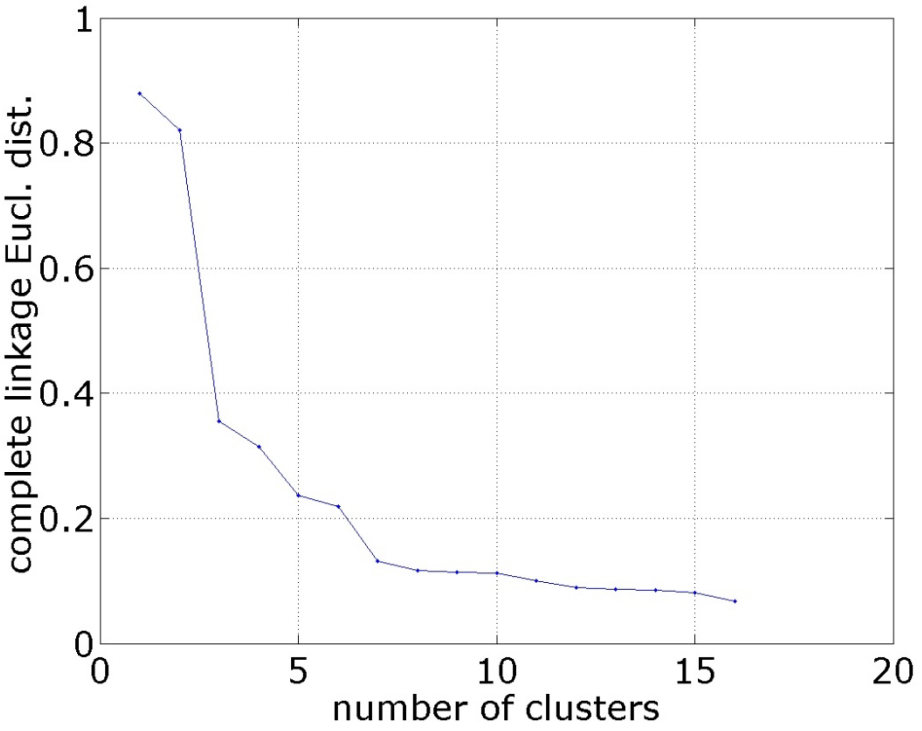


BioSB

Fusion graph (3)

(Euclidean; complete linkage)

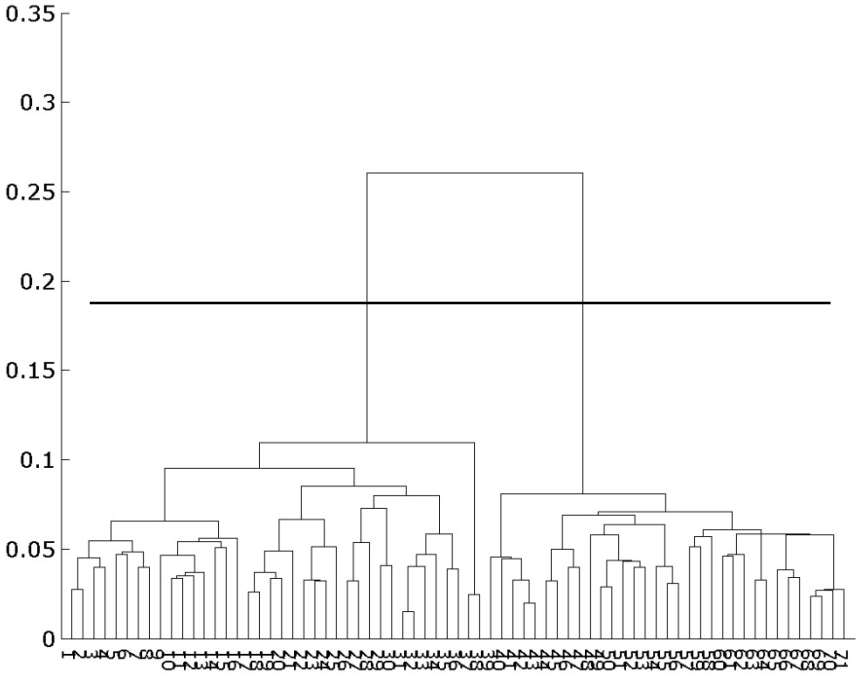


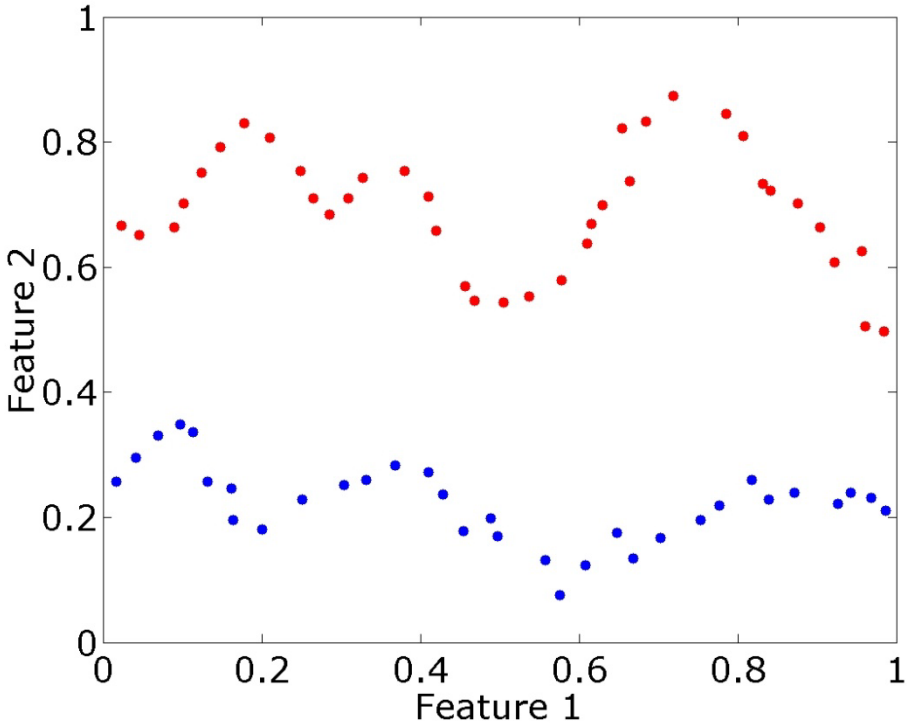


BioSB

Fusion graph (4)

(Euclidean; single linkage)

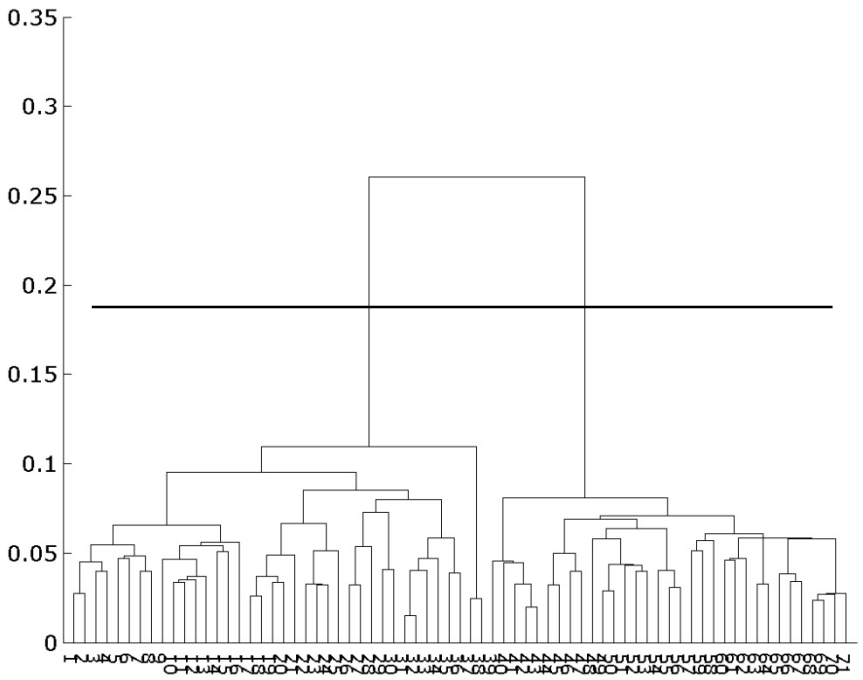


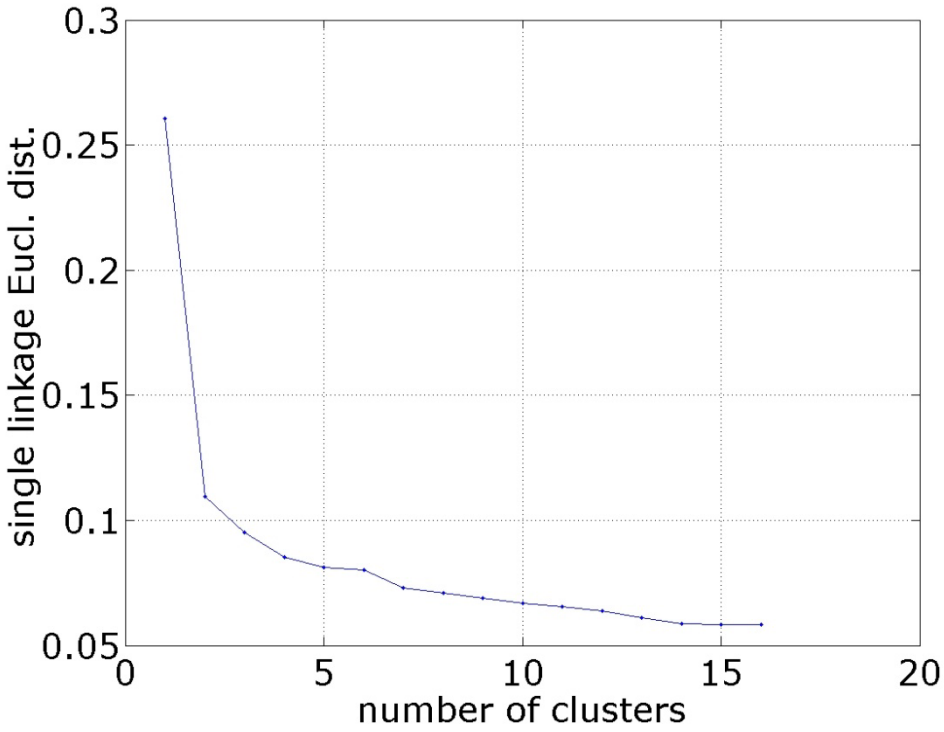


BioSB

Fusion graph (5)

(Euclidean; single linkage)

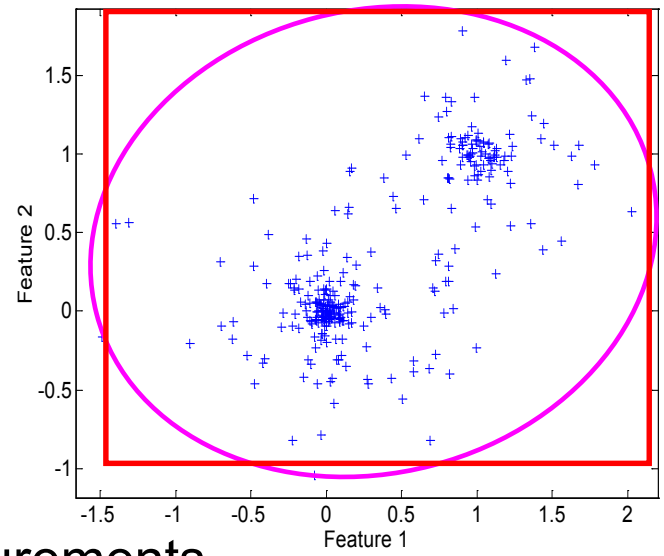




BioSB

What is a large jump?

- Compare the fusion graph of the dataset with a *null hypothesis*, i.e. a dataset where the clustering structure has been destroyed
- Different approaches:
  - Generate random data within bounding box or convex hull of data;
  - Preferable to shuffle data, i.e. not generate new data, but perturb relationships between measurements
  - For example, randomly match feature values, i.e. permute values within columns



BioSB

The gap statistic

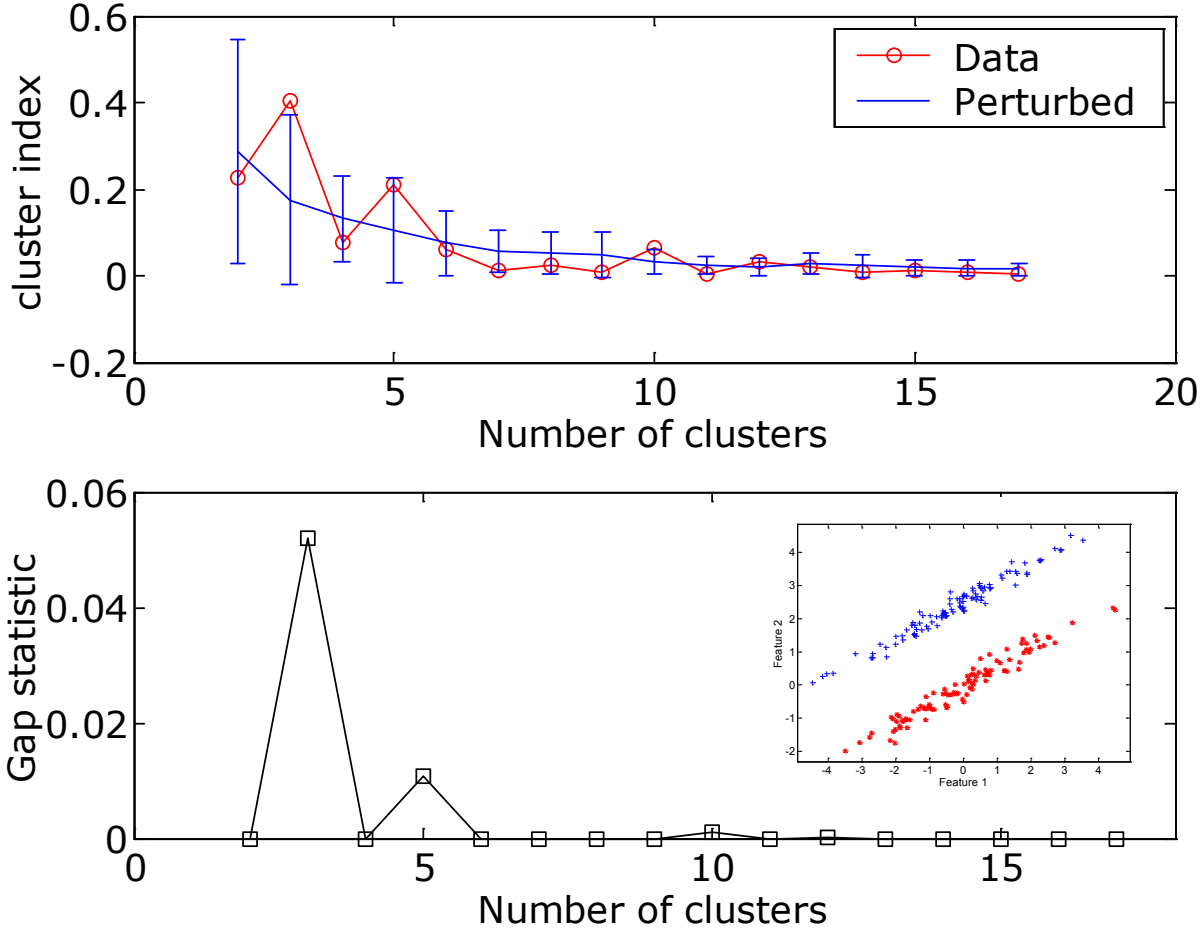
1. Generate dendrogram and extract fusion graph, f_j
2. Repeat r times
   1. Perturb columns
   2. Generate dendrogram and fusion graph, $f^*_{j,r}$
3. Compute average μ^*_j and standard deviation σ^*_j of these perturbed graphs
4. Compute the difference between the data fusion graph and the average perturbed fusion graph (*gap statistic*):

$$g_j^{gap} = \max\left\{f_j - \mu_j^*, 0\right\}, j = 1, 2, ..., g$$

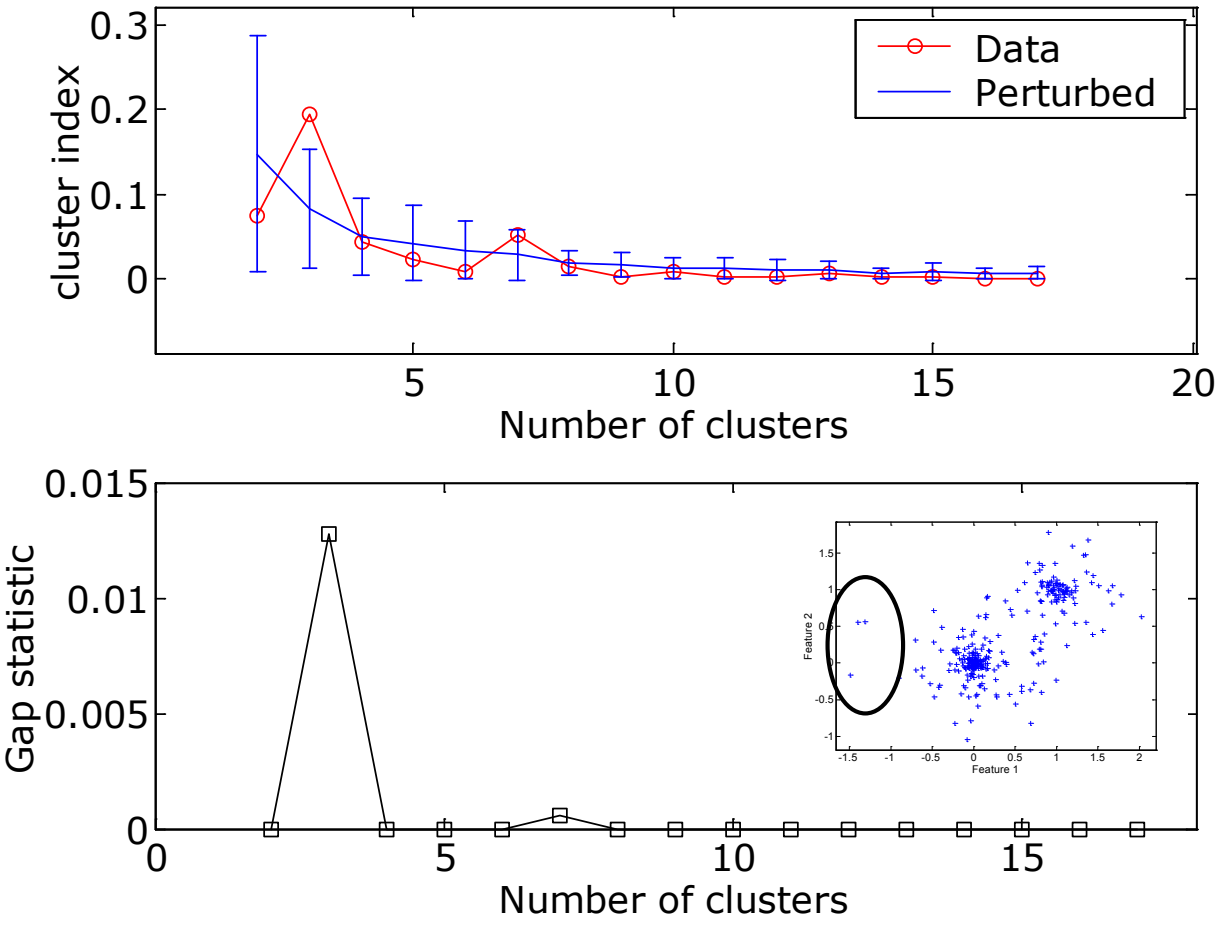
5. Look for large values of gap statistic $g_j^{gap} = f_j$

BioSB

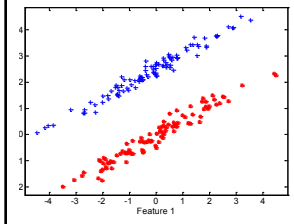
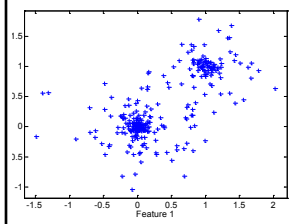
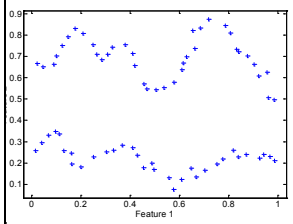
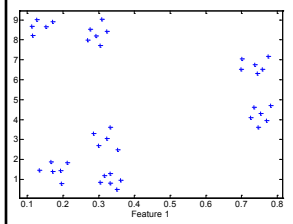
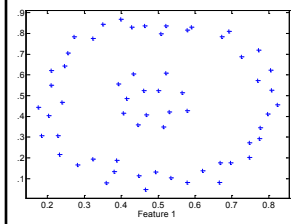
Gap fusion graph (single linkage)



Gap fusion graph (single linkage) (2)



DBI vs. fusion graphs

					
DBI (s)	?	3/4	?	4	4+
DBI (c)	8+	2	5+	4	8+
Gap fusion graph (s)	3	3	2	3	2
Gap fusion graph (c)	2 (?)	2	4	3	3

BioSB

Recapitulation

- *Cluster validation* is used for:
  - Assessing clustering
  - Deciding on the number of clusters

- Methods:
  - *Distortion* measures (dendrogram)
  - *Davies-Bouldin index*
  - *Fusion graph* and *gap statistic*

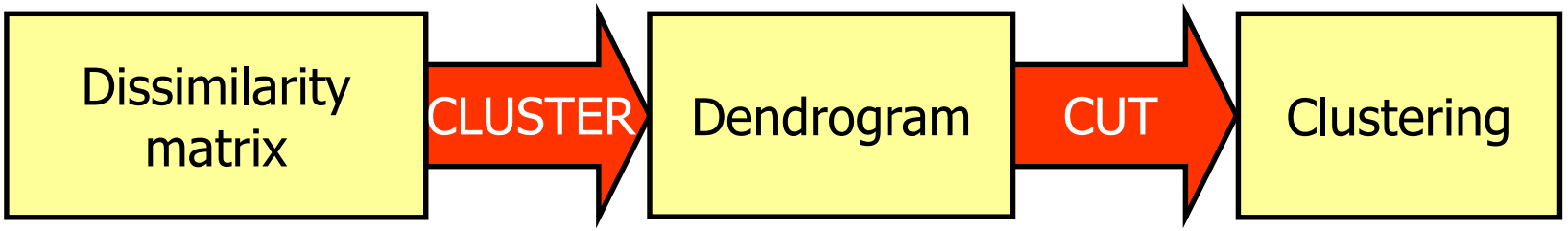
- When applying cluster validation, one also needs to define what a good cluster is – like in clustering itself.
  There's no free lunch...

BioSB

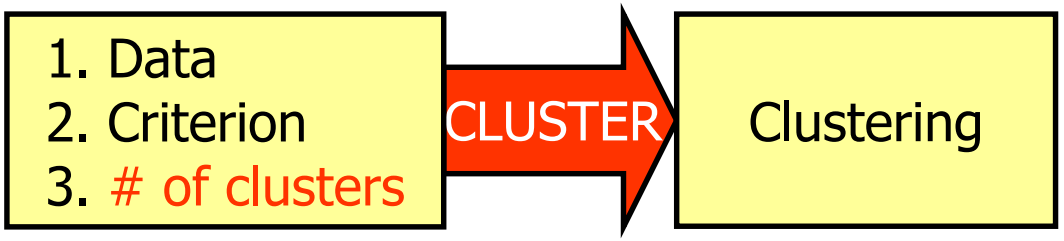
Lunch break
Exercise 4.8-4.16

Clustering overview

1. Hierarchical:



2. K-means:

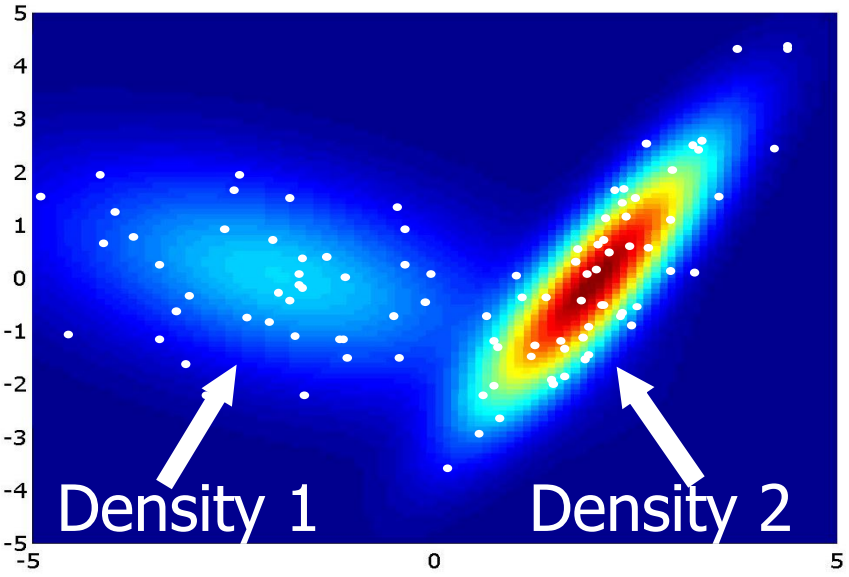


3. Density-based:

BioSB

Density-based clustering

- Each cluster is described by a probability density function
- Total dataset described by a *mixture* of density functions
- Clustering = maximizing the mixture fit
- Clusters are based on *a posteriori probabilities*

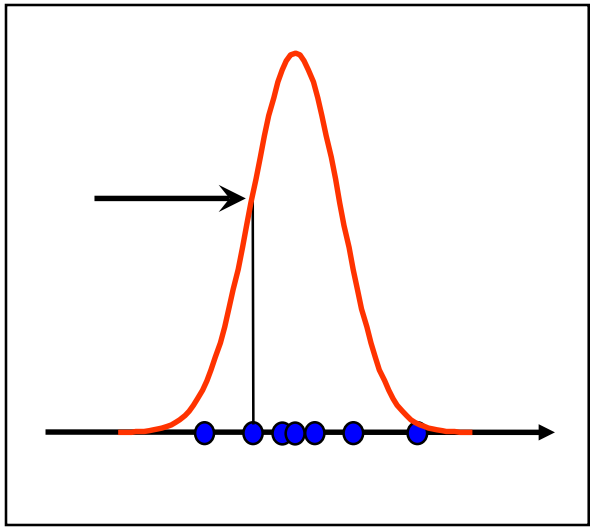


BioSB

Density-based clustering (2)

- Given:
  - n independent objects: $\{\boldsymbol{x}_1,\ldots,\boldsymbol{x}_n\}$
  - probability density function model:

$$p(\boldsymbol{x}\,|\,\theta) \sim N(\boldsymbol{\mu},\boldsymbol{\Sigma})$$



- Estimate parameters $\theta = \{\mu, \Sigma\}$ such that model *fits* data
- Use *likelihood* as criterion: probability of observing the data set, given the model (as on Day 1, for kernel width h in Parzen density estimation)

BioSB

Estimation: maximum likelihood

- General method to estimate parameters θ of probability distribution from data $D = \{x_1, ..., x_n\}$. How?
- Maximize joint probability of the data

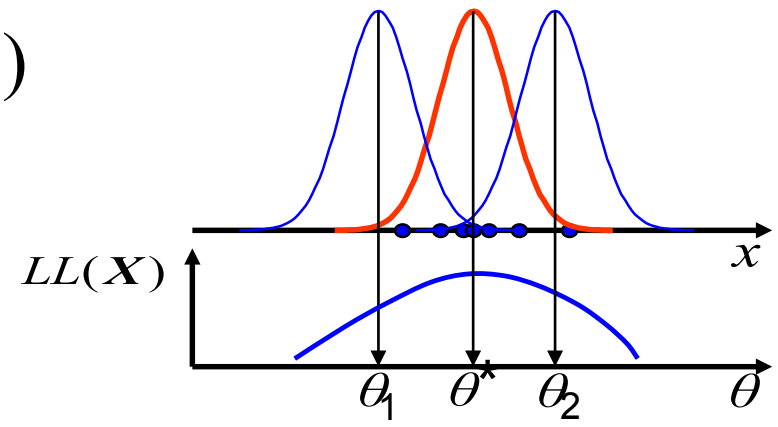
independence

likelihood:

$$L = p(x_1, ..., x_n \mid \theta) = \prod_{i=1}^{n} p(x_i \mid \theta)$$

log-likelihood:

$$LL = \sum_{i=1}^{n} \log \sum_{Q} p(x_i, Q \mid \theta)$$



same solution since log is monotonic

BioSB

Estimation: maximum likelihood (2)

Two possible outcomes: $x = 0$ or $x = 1$.
Success ($x = 1$) occurs with probability p

Bernoulli distribution: $P(x) = p^x (1-p)^{1-x}$

Likelihood: $P(X_1 = x_1, \ldots, X_n = x_n \mid p) = p^{x_1}(1-p)^{1-x_1} \ldots p^{x_n}(1-p)^{1-x_n}$

$$= p^{n_1}(1-p)^{n-n_1}$$

n_1: # of successes

$$\frac{d(p^{n_1}(1-p)^{n-n_1})}{dp} = 0$$

Maximum at $p = n_1/n$

BioSB

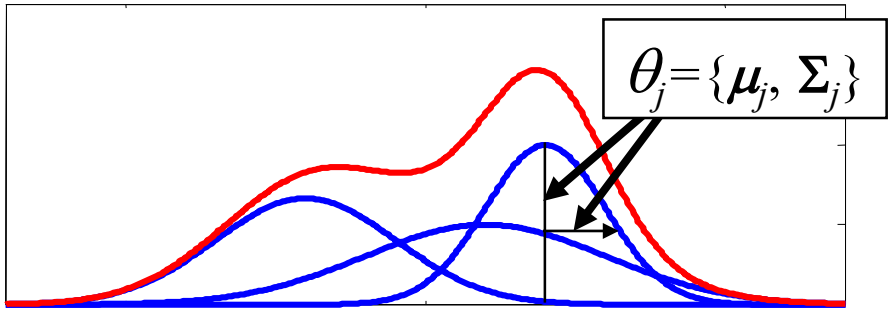
Mixture-of-Gaussians

- Choose Gaussian as component density $p(\boldsymbol{x};\theta_j)$:

$$p(\boldsymbol{x};\theta_j) = \frac{1}{\sqrt{2\pi^p \det(\boldsymbol{\Sigma}_j)}} \exp\left(-\frac{1}{2}(\boldsymbol{x}-\boldsymbol{\mu}_j)^{\mathrm{T}} \boldsymbol{\Sigma}_j^{-1} (\boldsymbol{x}-\boldsymbol{\mu}_j) \right)$$

- Describe complete data set as a mixture of $p(\boldsymbol{x};\theta)$'s:

$$p(\boldsymbol{x};\Psi) = \sum_{j=1}^{g} \pi_j p(\boldsymbol{x};\theta_j) \quad \text{with} \quad \sum_{j=1}^{g} \pi_j = 1$$



BioSB

Mixture-of-Gaussians (2)

$$p(\boldsymbol{x};\Psi) = \sum_{j=1}^{g} \pi_j \, p(\boldsymbol{x};\theta_j) \quad \text{with} \quad \sum_{j=1}^{g} \pi_j = 1$$

- Parameters:
  - Set number of clusters, g
  - Estimate other parameters by maximum-likelihood:

$$\Psi = (\boldsymbol{\pi}, \theta = \{\boldsymbol{\mu}_j, \boldsymbol{\Sigma}_j\}_{j=1\ldots g})$$

mixture coefficients

component density parameters

log-likelihood: $$LL(\boldsymbol{X};\Psi) = \sum_{i=1}^{n} \log \sum_{j=1}^{g} \pi_j \, p(\boldsymbol{x}_i;\theta_j)$$

BioSB

EM algorithm

- **Problem:** need to simultaneously estimate two interdependent things...
  - Cluster membership of each object
  - Density parameters of each cluster: $\pi_j, \boldsymbol{\mu}_j, \boldsymbol{\Sigma}_j$

- **Expectation-Maximization algorithm:**
  - General class of algorithms for this type of problem
  - Repeatedly:
    - Recalculate cluster membership of each object *(E)*
    - Recalculate density parameters of each cluster *(M)*

- Introduce a hidden variable ***z*** to explicitly indicate mixture components

$$\pi_j = p(z = j)$$

BioSB

Intermezzo: probabilities

$n = 20$

	1	2	3	4	5	6
die 1	•	• •	• • •	• • •	•	•
die 2			•	•	• • •	• • • •

sum rule: $P(x) = \sum_y P(x, y)$

$$1/5 = P(3) = P(3,\ \text{die 1}) + P(3,\ \text{die 2}) = 3/20 + 1/20$$

product rule: $P(x, y) = P(x \mid y)P(y) = P(y \mid x)P(x)$

$$3/20 = P(3,\ \text{die 1}) = P(3 \mid \text{die 1})P(\text{die 1}) = (3/11)(11/20) = 3/20$$
$$= P(\ \text{die 1} \mid 3)P(3) = (3/4)(4/20) = 3/20$$

BioSB

Intermezzo: Bayes' theorem

From product rule

$$P(x \mid y)P(y) = P(y \mid x)P(x)$$

$$\textbf{Bayes}: \; P(x \mid y) = \frac{P(y \mid x)P(x)}{P(y)} = \frac{P(y \mid x)P(x)}{\sum_x P(y \mid x)P(x)}$$

$$P(\text{die 1} \mid \; 3) = \frac{P(3 \mid \text{die 1})P(\text{die 1})}{P(3)} = \frac{(3/11)(11/20)}{4/20} = 3/4$$

BioSB

EM algorithm (2)

arbitrary distribution hidden variable

$$\log p(D) = \sum_x \log p(x) = \sum_x \sum_z q(z) \log p(x)$$

$$= \sum_{x,z} q(z) \log \frac{p(x,z)}{p(z \mid x)} = \sum_{x,z} q(z) \log \left(\frac{p(x,z)}{p(z \mid x)} \times \frac{q(z)}{q(z)} \right)$$

$$= \sum_{x,z} q(z) \log \left(\frac{p(x,z)}{q(z)} \right) + \sum_{x,z} q(z) \log \left(\frac{q(z)}{p(z \mid x)} \right)$$

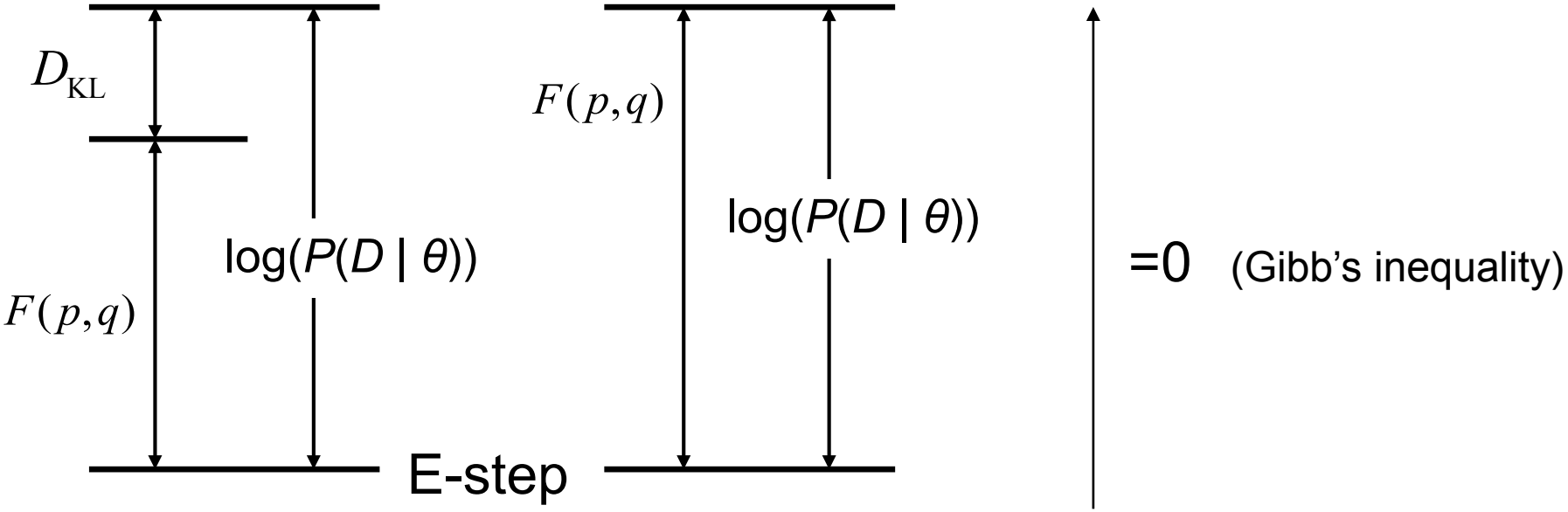
$$= F(p_{\text{joint}}, q) + D_{KL}(q \,\|\, p_{\text{post}})$$

free energy relative entropy (≥ 0)

BioSB

EM algorithm: E-step

$$\log p(D) = \sum_{x,z} q(z) \log \left(\frac{p(x,z)}{q(z)} \right) + \sum_{x,z} q(z) \log \left(\frac{q(z)}{p(z \mid x)} \right)$$



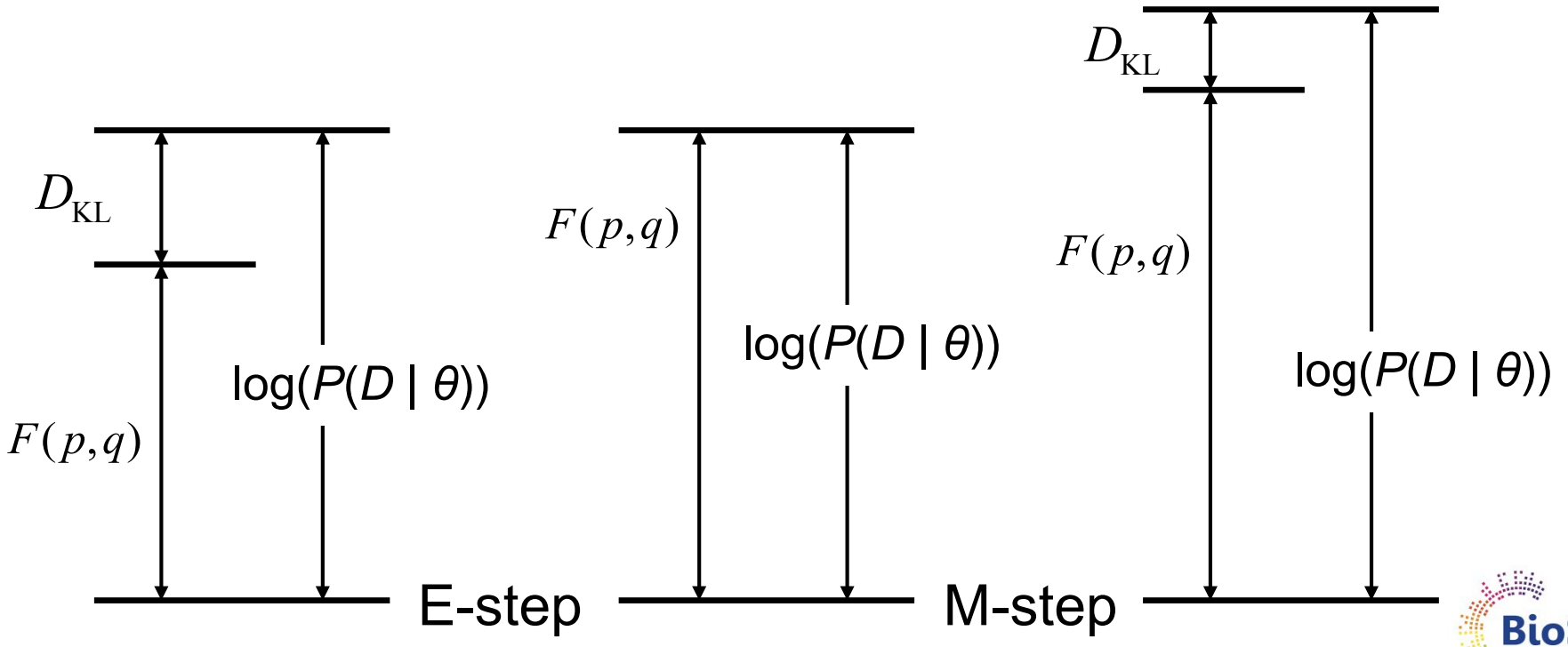
E-step: $q^{\mathrm{new}}(z \mid x) = p_{\mathrm{post}} = p(z \mid x)$

BioSB

EM algorithm: M-step

$$\log p(D) = \sum_{x,z} p(z \mid x) \log \left(\frac{p(x,z)}{p(z \mid x)} \right)$$

M-step: maximize log[$p(D)$] with respect to the parameters



EM algorithm (3)

Iterate to maximize likelihood:

E-step: $p_{\text{post}} = p(z \mid x, \theta)$

Calculate the distribution of the hidden variables given the data and the model parameters

M-step: $\theta^{new} = \arg\max_{\theta} \sum_{x,z} p(z \mid x) \log p(x, z \mid \theta)$

Maximize the expected (with respect to hidden variables) log-likelihood of the complete data.

Compare M-step with MoG log-likelihood: $\sum_{i=1}^{n} \log \sum_{j=1}^{g} \pi_j p(\mathbf{x}_i; \theta_j)$

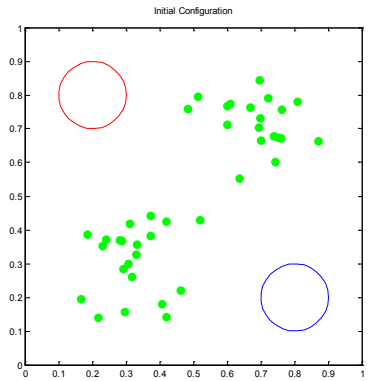
M-step is easier: log within sum

BioSB

EM: mixture model

Very simple example of a model with hidden variables:

2-component mixture model



$$p(x) = \pi_1 p_1(x \mid \theta) + \pi_2 p_2(x \mid \theta)$$

hidden variable z = 1,2 - component label

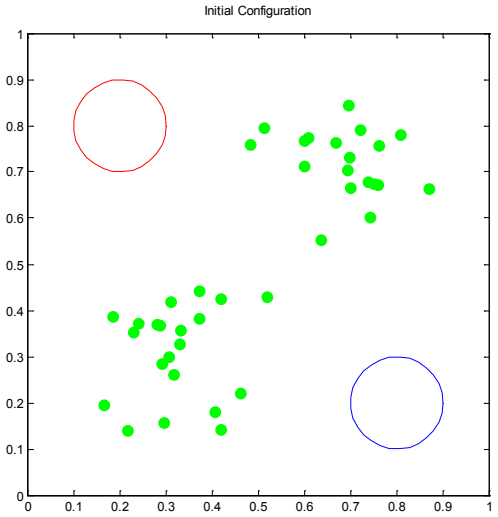
E-step: $p(z = j \mid x, \theta) = \dfrac{p(z = j \mid \theta) p(x \mid z = j, \theta)}{p(x \mid \theta)} = \dfrac{\pi_j p_j(x \mid \theta)}{p(x)}$

responsibility

M-step: maximize $\sum_{x, z \in \{1,2\}} p(z \mid x) \log p(x, z \mid \theta)$

BioSB

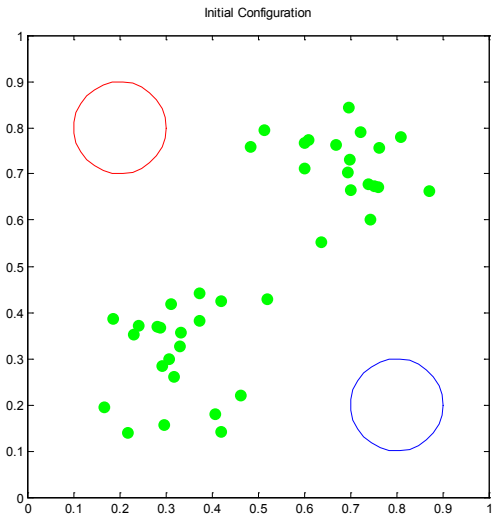
EM: mixture model (2)



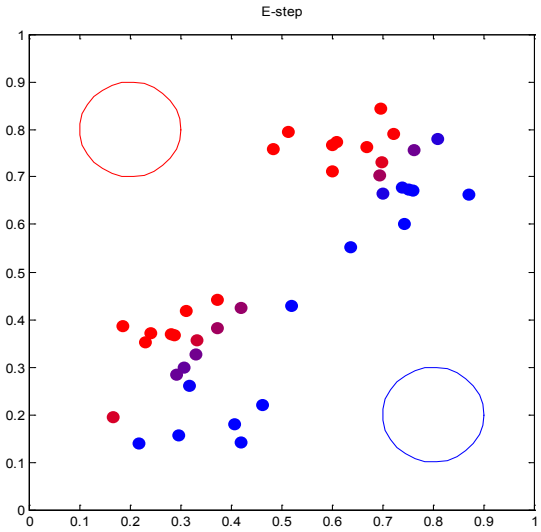
Initialization

BioSB

EM: mixture model (3)



Initialization



E-step

BioSB

EM: mixture model (4)

- **M-step**: Maximization

  Maximize the expected complete LL by updating

  - mixture coefficients π_j
  - cluster means and covariances $\theta_j=\{\mu_j, \Sigma_j\}$, $j=1,...,g$:

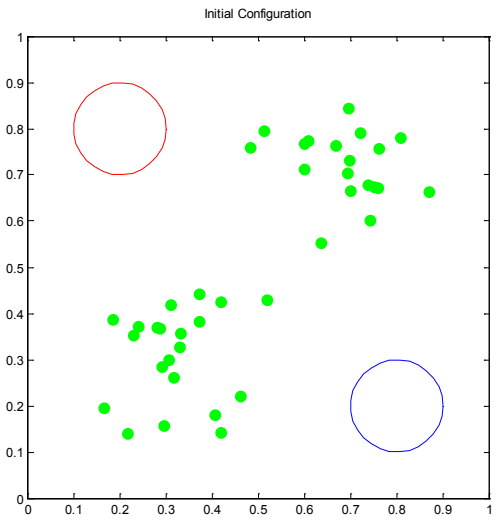
$$\hat{\pi}_j = \frac{1}{n}\sum_{i=1}^{n} p(z=j \mid x_i) = \frac{1}{n}\sum_{i=1}^{n} w_{ij}$$ } "total membership"

$$\hat{\mu}_j = \frac{\sum_{i=1}^{n} w_{ij}\boldsymbol{x}_i}{\sum_{i=1}^{n} w_{ij}}$$

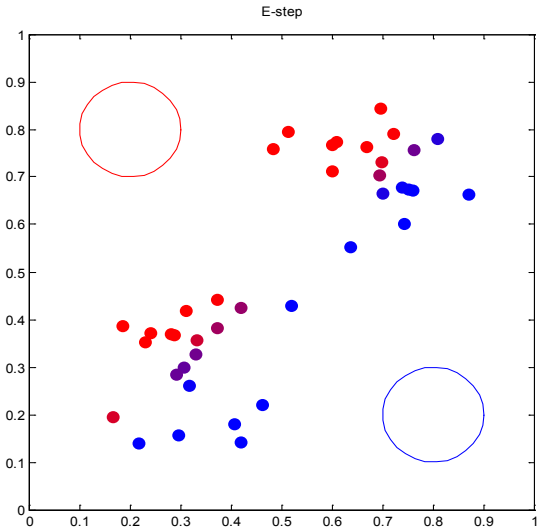
$$\hat{\Sigma}_j = \frac{\sum_{i=1}^{n} w_{ij}(\boldsymbol{x}_i - \hat{\mu}_j)(\boldsymbol{x}_i - \hat{\mu}_j)^T}{\sum_{i=1}^{n} w_{ij}}$$ } weighted sums

BioSB

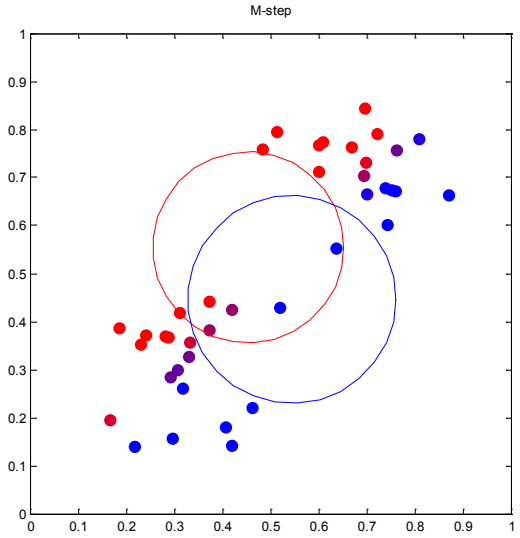
EM: mixture model (5)



Initialization

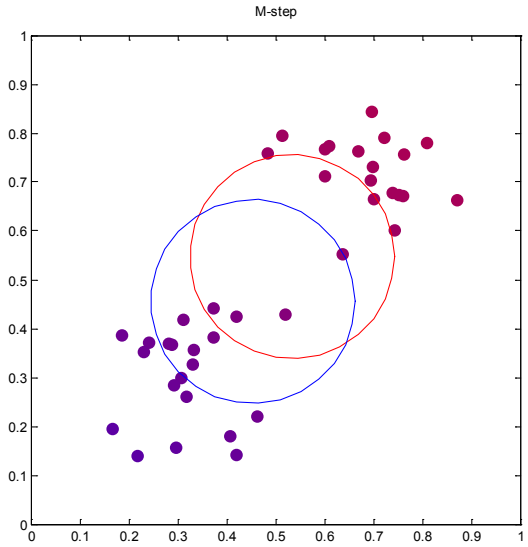


E-step



M-step

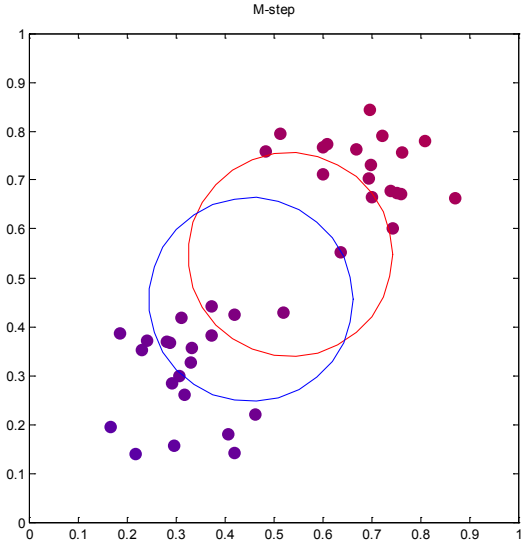
EM: mixture model (6)



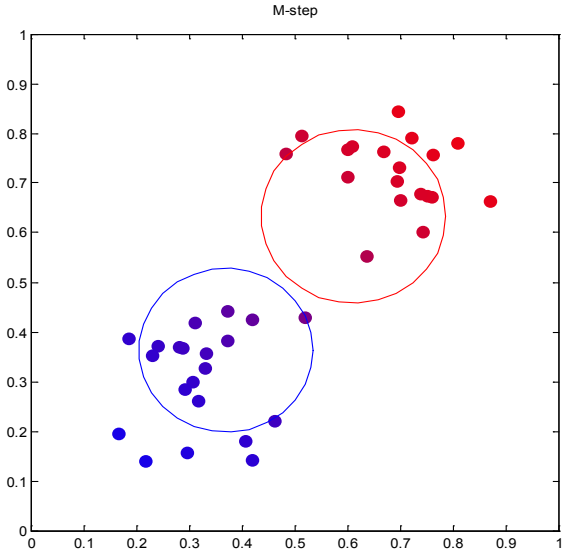
M-step: 3

BioSB

EM: mixture model (7)



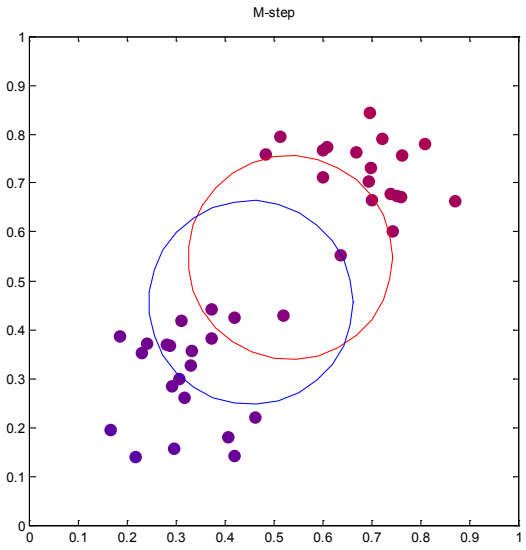
M-step: 3



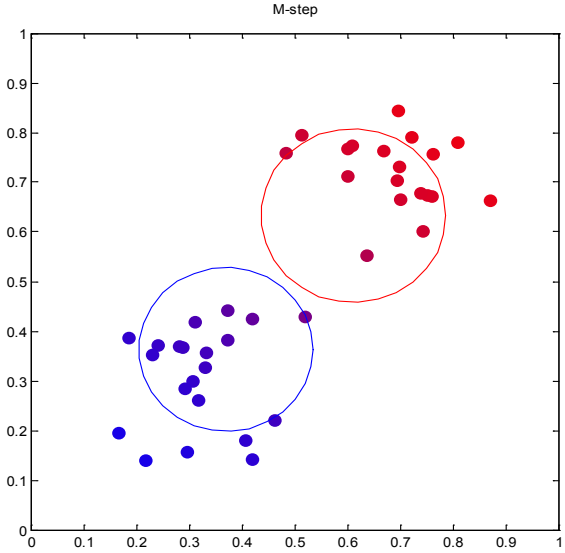
M-step: 5

BioSB

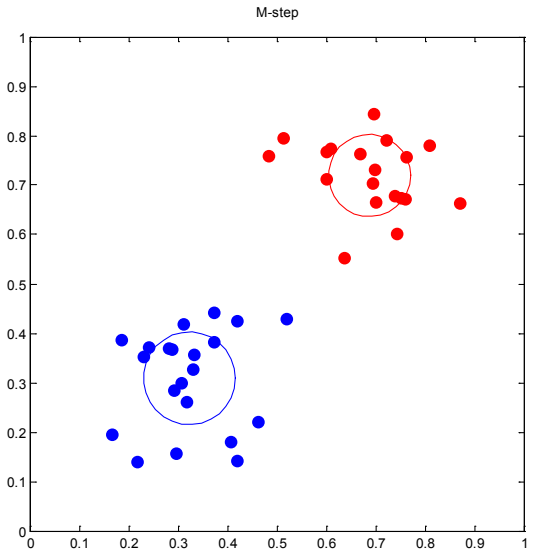
EM: mixture model (8)



M-step: 3



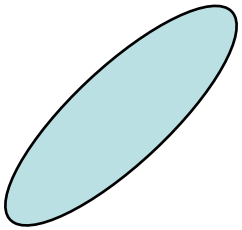
M-step: 5



M-step: 9

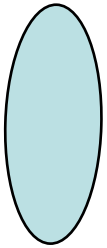
Mixture-of-Gaussians (3)

- ‘Gauss’:



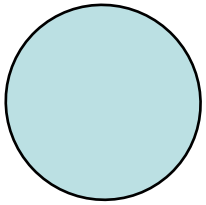
$$\Sigma = \begin{bmatrix} \sigma_{11} & \sigma_{12} \\ \sigma_{21} & \sigma_{22} \end{bmatrix}$$

- ‘Aligned’:



$$\Sigma = \begin{bmatrix} \sigma_{11} & 0 \\ 0 & \sigma_{22} \end{bmatrix}$$

- ‘Circular’:



$$\Sigma = \begin{bmatrix} \sigma & 0 \\ 0 & \sigma \end{bmatrix}$$

BioSB

EM: mixture model (9)

- If...
  - all clusters are spherical
  - the variance of each cluster is infinitely small

$$\boldsymbol{\Sigma} = \begin{bmatrix} \varepsilon^2 & 0 & 0 \\ 0 & \varepsilon^2 & 0 \\ 0 & 0 & \varepsilon^2 \end{bmatrix}, \qquad \varepsilon \to 0$$

then the EM algorithm simplifies to the *K*-means algorithm (samples are always assigned to the closest cluster!)

BioSB

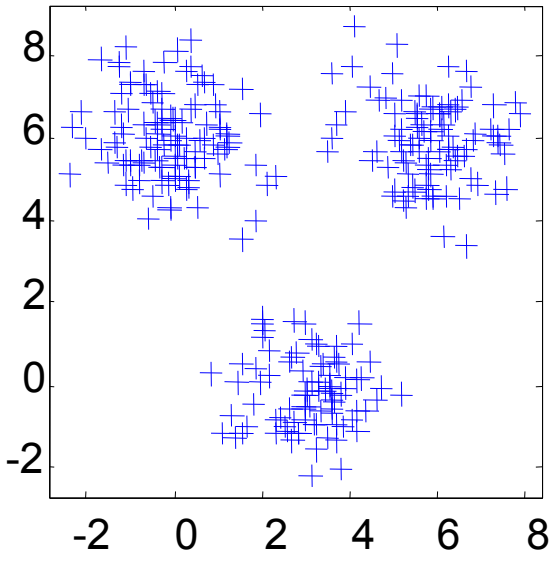
EM algorithm (4)

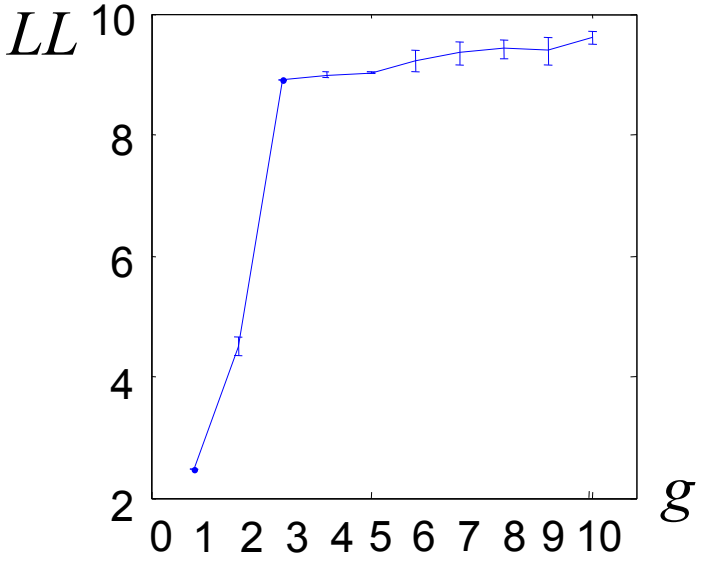
- Disadvantages:
  - can get stuck in local minima
  - depends on initial conditions
  - convergence can be slow
  - problems with covariance estimates:
    if too few samples are members of a cluster,
    there will not be enough data to base estimate on
- Advantages:
  - simple to implement

BioSB

Cluster validation: log-likelihood

- For probabilistic models (e.g. mixture-of-Gaussians):
  - Log-likelihood will probably not increase anymore when too many clusters are used
  - Look for “plateau” in log-likelihood graph





- Problem: when $g = n$, the log-likelihood is infinite;

  Solution: information criteria (Day 5)

BioSB

Recapitulation

- Density based clustering:
  - Assume a *probability density function* per cluster
  - Train using the *EM algorithm*

- Example:
  - *Mixture of Gaussians*
  - But many probability densities fit in the same framework principal component analysis, factor analysis, …

- EM algorithm:
  - problem *decomposition*: simple to implement
  - sensitive to *local minima*

BioSB

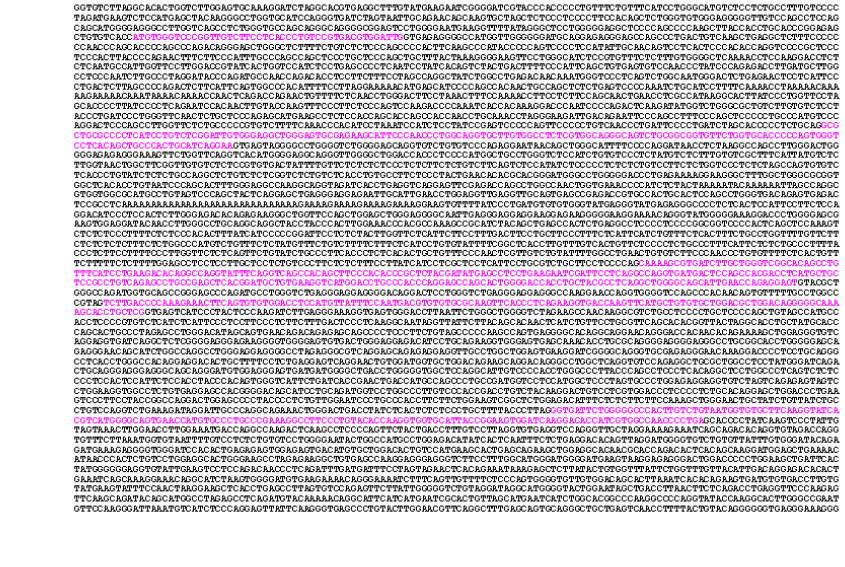
15min break
Exercise 4.17

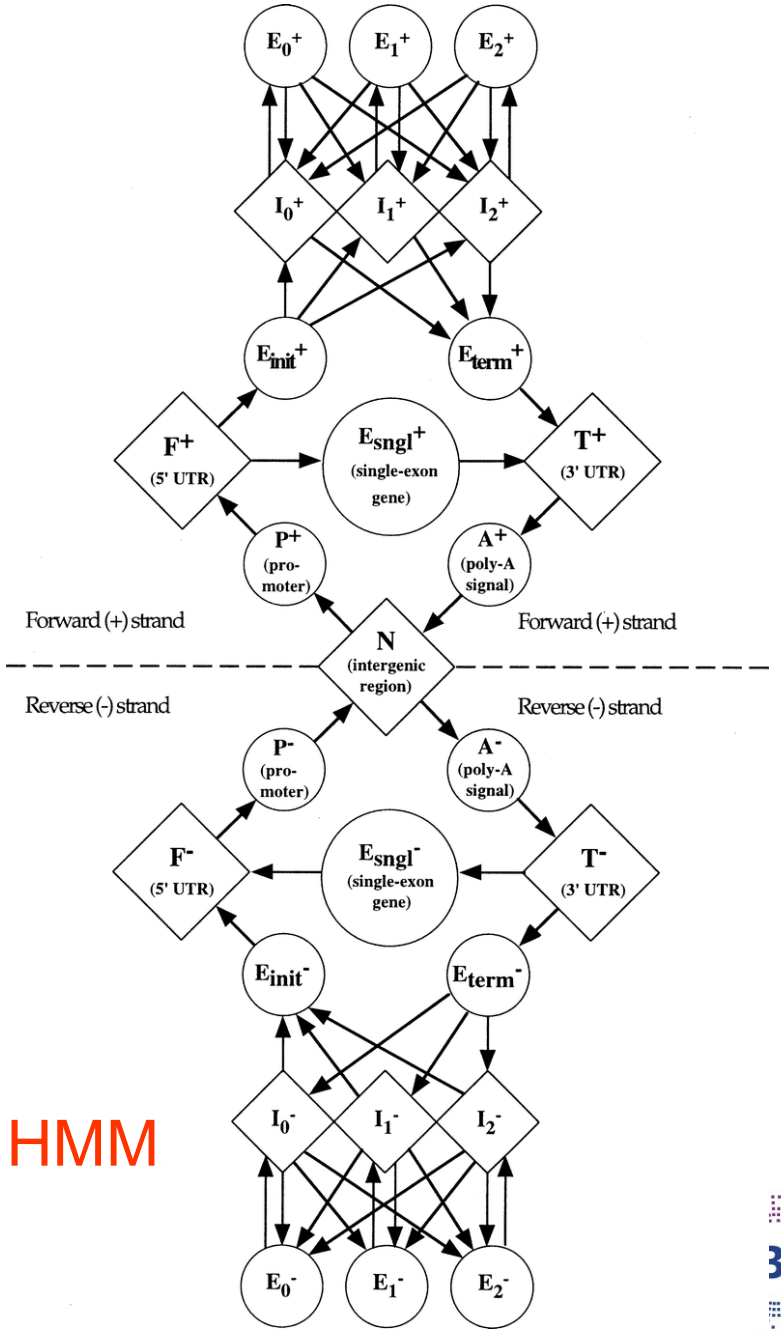
Hidden Markov models

- Regular expressions & weight matrices
- Dependencies & Markov chains
- Hidden Markov models
- HMMs & EM
- Profile HMMs
- Genefinding

BioSB

Application: genefinding

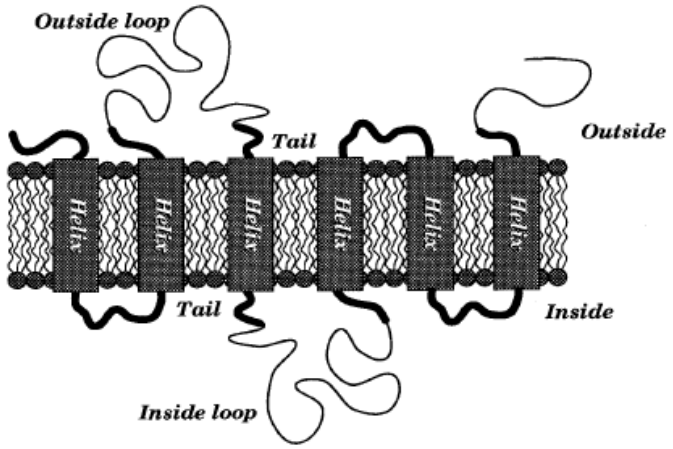


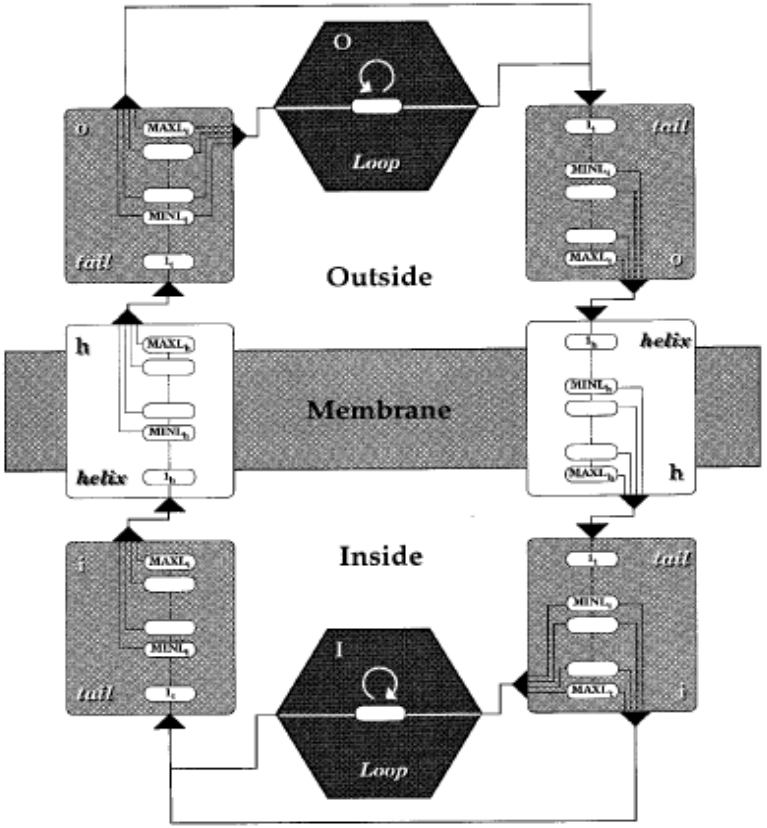


generalized HMM

BioSB

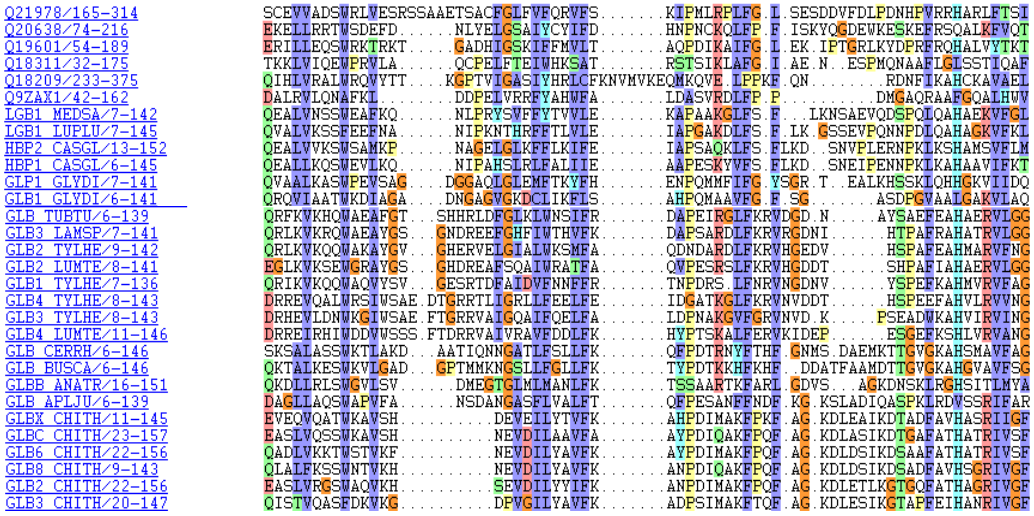
Application: transmembrane proteins



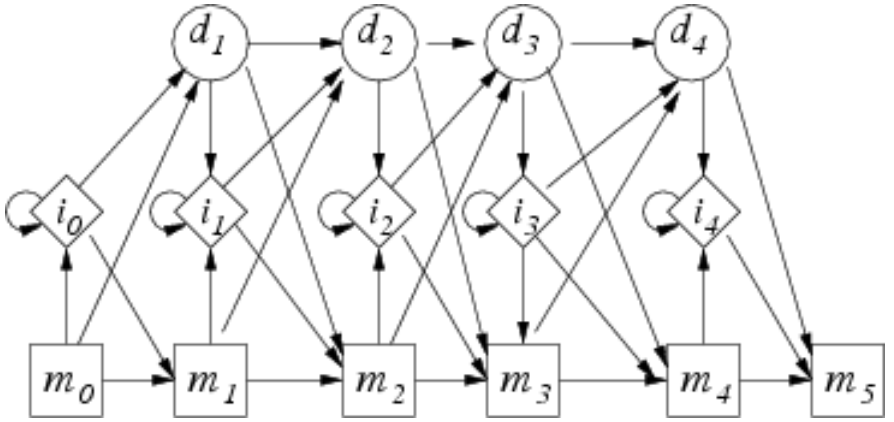


HMM

Application: protein domains



Profile HMM



BioSB

Outline

- Regular expressions & weight matrices
- Dependencies & Markov chains
- Hidden Markov models
- HMMs & EM
- Profile HMMs
- Genefinding

BioSB

Sites

Site: short sequence containing some signal

A	C	A	A	T	G
T	C	A	A	T	C
A	C	A	A	G	C
A	G	A	A	T	C
A	C	C	A	T	C

Examples: intron splice sites, transcription start site, transcription factor binding sites

Goals: - give a mathematical description (model) of a site

- find possible sites in a long sequence

BioSB

Consensus sequence

majority vote:

A C A A T C

A	C	A	A	T	G
T	C	A	A	T	C
A	C	A	A	G	C
A	G	A	A	T	C
A	C	C	A	T	C

W S M A K S from IUPAC code:

M	A/C
R	A/G
W	A/T
S	C/G
Y	C/T
K	G/T
B	C/G/T
D	A/G/T
H	A/C/T
V	A/C/G
N	A/C/G/T

BioSB

Regular expressions

[ab] : union {a,b}
ab : concatenation {ab}
ε : empty string
a* : Kleene star {ε,a,aa,aaa, …}

A	C	A	A	T	G
T	C	A	A	T	C
A	C	A	A	G	C
A	G	A	A	T	C
A	C	C	A	T	C

[AT][CG][AC]A[TG][GC]

A C A A T C , but also T G C A G G

See also http://prosite.expasy.org

BioSB

Weight matrices

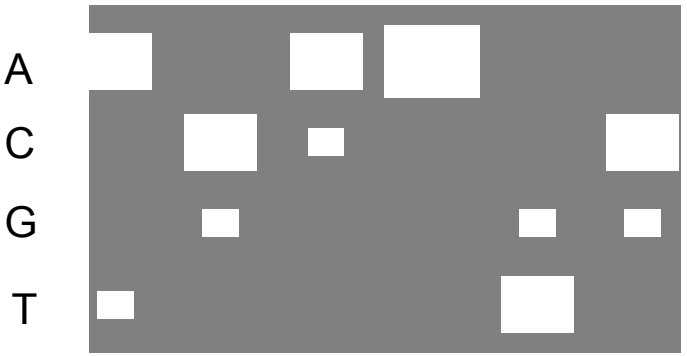
$$\begin{array}{c} \\ A \\ C \\ G \\ T \end{array}\begin{array}{c} \begin{array}{cccccc} 1 & 2 & 3 & 4 & 5 & 6 \end{array} \\ \begin{pmatrix} 4 & 0 & 4 & 5 & 0 & 0 \\ 0 & 4 & 1 & 0 & 0 & 4 \\ 0 & 1 & 0 & 0 & 1 & 1 \\ 1 & 0 & 0 & 0 & 4 & 0 \end{pmatrix} \end{array}$$

counts

A	C	A	A	T	G
T	C	A	A	T	C
A	C	A	A	G	C
A	G	A	A	T	C
A	C	C	A	T	C

probabilities

$$W = \begin{array}{c} \\ A \\ C \\ G \\ T \end{array}\begin{array}{c} \begin{array}{cccccc} 1 & 2 & 3 & 4 & 5 & 6 \end{array} \\ \begin{pmatrix} 0.8 & 0.0 & 0.8 & 1.0 & 0.0 & 0.0 \\ 0.0 & 0.8 & 0.2 & 0.0 & 0.0 & 0.8 \\ 0.0 & 0.2 & 0.0 & 0.0 & 0.2 & 0.2 \\ 0.2 & 0.0 & 0.0 & 0.0 & 0.8 & 0.0 \end{pmatrix} \end{array}$$



aka position specific score matrix

Weight matrices (2)

Sequence: $x = x_1 x_2 \ldots x_N$

independence

$$P(x_1 x_2 \ldots x_N \mid W) = \prod_{i=1}^{N} w_{x_i,i} = \prod_{i=1}^{N} P_i(x_i \mid W)$$

$$P(\mathtt{ACAATC} \mid W) = P_1(\mathtt{A}) P_2(\mathtt{C}) P_3(\mathtt{A}) P_4(\mathtt{A}) P_5(\mathtt{T}) P_6(\mathtt{C})$$

$$= 0.8 \times 0.8 \times 0.8 \times 1 \times 0.8 \times 0.8 = 0.33$$

	1	2	3	4	5	6
A	**0.8**	0.0	**0.8**	**1.0**	0.0	0.0
C	0.0	**0.8**	0.2	0.0	0.0	**0.8**
G	0.0	0.2	0.0	0.0	0.2	0.2
T	0.2	0.0	0.0	0.0	**0.8**	0.0

BioSB

Weight matrices (3)

Sequence: $x = x_1 x_2 \ldots x_N$

independence

$$P(x_1 x_2 \ldots x_N \mid W) = \prod_{i=1}^{N} w_{x_i,i} = \prod_{i=1}^{N} P_i(x_i \mid W)$$

$$P(\mathtt{CCAATC} \mid W) = P_1(\mathtt{C}) P_2(\mathtt{C}) P_3(\mathtt{A}) P_4(\mathtt{A}) P_5(\mathtt{T}) P_6(\mathtt{C})$$

$$= 0 \times 0.8 \times 0.8 \times 1 \times 0.8 \times 0.8 = 0$$

	1	2	3	4	5	6
A	0.8	0.0	**0.8**	**1.0**	0.0	0.0
C	**0.0**	**0.8**	0.2	0.0	0.0	**0.8**
G	0.0	0.2	0.0	0.0	0.2	0.2
T	0.2	0.0	0.0	0.0	**0.8**	0.0

BioSB

Weight matrices: pseudocounts

$$P(x) = \frac{\#x + \boxed{1}}{\sum_i (\#i + \boxed{1})}$$ pseudocount (Laplace)

A	C	A	A	T	G
T	C	A	A	T	C
A	C	A	A	G	C
A	G	A	A	T	C
A	C	C	A	T	C

$$\begin{array}{c} A \\ C \\ G \\ T \end{array}\begin{pmatrix} 5 & 1 & 5 & 6 & 1 & 1 \\ 1 & 5 & 2 & 1 & 1 & 5 \\ 1 & 2 & 1 & 1 & 2 & 2 \\ 2 & 1 & 1 & 1 & 5 & 1 \end{pmatrix} \qquad W' = \begin{array}{c} A \\ C \\ G \\ T \end{array}\begin{pmatrix} \boxed{0.56} & 0.11 & \boxed{0.56} & \boxed{0.67} & 0.11 & 0.11 \\ 0.11 & \boxed{0.56} & 0.22 & 0.11 & 0.11 & \boxed{0.56} \\ 0.11 & 0.22 & 0.11 & 0.11 & 0.22 & 0.22 \\ 0.22 & 0.11 & 0.11 & 0.11 & \boxed{0.56} & 0.11 \end{pmatrix}$$

$$P(\mathtt{ACAATC} \mid W') = P_1(\mathtt{A})P_2(\mathtt{C})P_3(\mathtt{A})P_4(\mathtt{A})P_5(\mathtt{T})P_6(\mathtt{C}) = 0.56^5 \times 0.67 = 0.037$$

$$P(\mathtt{CCAATC} \mid W') = P_1(\mathtt{C})P_2(\mathtt{C})P_3(\mathtt{A})P_4(\mathtt{A})P_5(\mathtt{T})P_6(\mathtt{C}) = 0.11 \times 0.56^4 \times 0.67 = 0.0072$$

BioSB

Bayes' rule: odds

class A: sites class B: non-sites

$$x \text{ is assigned to class } A \Leftrightarrow \frac{P(x \mid \text{class } A)P(A)}{P(x)} > \frac{P(x \mid \text{class } B)P(B)}{P(x)}$$

$$\Leftrightarrow \frac{P(x \mid \text{class } A)}{P(x \mid \text{class } B)} > \frac{P(B)}{P(A)}$$

priors

equal priors: $\frac{P(x \mid \text{class } A)}{P(x \mid \text{class } B)} > 1 \Leftrightarrow \log\left(\frac{P(x \mid \text{class } A)}{P(x \mid \text{class } B)}\right) > 0$

odds log-odds

unequal priors, e.g.: $\log\frac{P(B)}{P(A)} = \log\frac{0.7}{0.3} = 1.22$

BioSB

Weight matrices: odds

W: weight matrix, *R*: background model (independent of position)

$$\frac{P(x_1 x_2 \ldots x_N \mid W)}{P(x_1 x_2 \ldots x_N \mid R)} = \frac{\prod_{i=1}^{N} P_i(x_i \mid W)}{\prod_{i=1}^{N} P(x_i \mid R)}$$

$$\log_2\left(\frac{P(x_1 x_2 \ldots x_N \mid W)}{P(x_1 x_2 \ldots x_N \mid R)}\right) = \log_2\left(\frac{\prod_{i=1}^{N} P_i(x_i \mid W)}{\prod_{i=1}^{N} P(x_i \mid R)}\right) = \sum_{i=1}^{N} \log_2\left(\frac{P_i(x_i \mid W)}{P(x_i \mid R)}\right)$$

log-odds

BioSB

Weight matrices: log-odds

R uniform: $P(\mathtt{A}|R) = P(\mathtt{C}|R) = P(\mathtt{G}|R) = P(\mathtt{T}|R) = 0.25$

A	**1.16**	-1.17	**1.16**	**1.42**	-1.17	-1.17
C	-1.17	**1.16**	-0.17	-1.17	-1.17	**1.16**
G	-1.17	-0.17	-1.17	-1.17	-0.17	-0.17
T	-0.17	-1.17	-1.17	-1.17	**1.16**	-1.17

$\log(0.56/0.25)$

$$\text{log-odds}(\mathtt{ACAATC}) = 1.16+1.16+1.16+1.42+1.16+1.16 = 7.22$$

$$\text{log-odds}(\mathtt{TGCAGG}) = -0.17-0.17-0.17+1.42-0.17-0.17 = 0.57$$

$$\text{log-odds}(\mathtt{CTTGAT}) = 6\times -1.17 = -7.02$$

BioSB

Outline

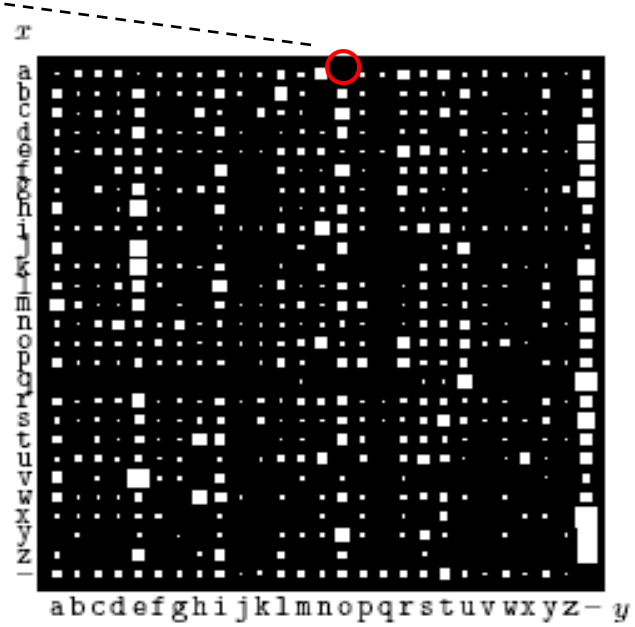
- Regular expressions & weight matrices
- Dependencies & Markov chains
- Hidden Markov models
- HMMs & EM
- Profile HMMs
- Genefinding

BioSB

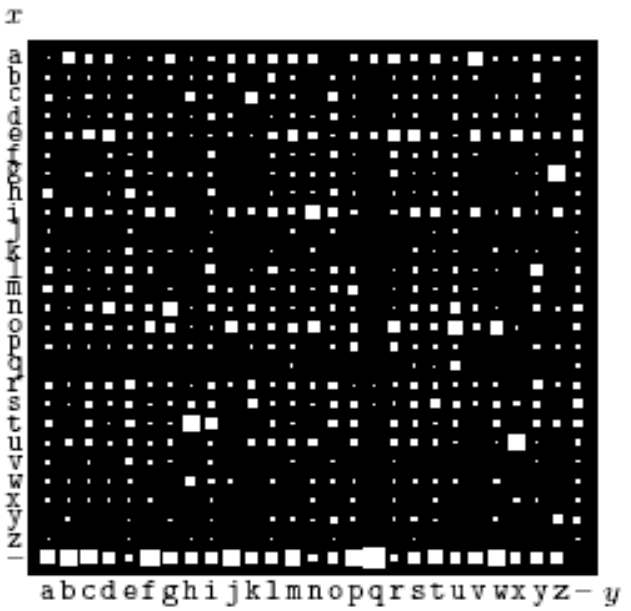
Dependencies: language

Probability (in English) of “o” given that previous letter is “a”

i	a_i	p_i
1	a	0.0575
2	b	0.0128
3	c	0.0263
4	d	0.0285
5	e	0.0913
6	f	0.0173
7	g	0.0133
8	h	0.0313
9	i	0.0599
10	j	0.0006
11	k	0.0084
12	l	0.0335
13	m	0.0235
14	n	0.0596
15	o	0.0689
16	p	0.0192
17	q	0.0008
18	r	0.0508
19	s	0.0567
20	t	0.0706
21	u	0.0334
22	v	0.0069
23	w	0.0119
24	x	0.0073
25	y	0.0164
26	z	0.0007
27	–	0.1928



(a) $P(y \mid x)$



(b) $P(x \mid y)$

BioSB

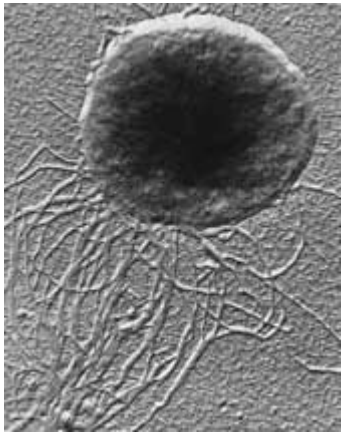
Dependencies: biology

P_i : probability of nucleotide i

P_{ij} : probability of dinucleotide ij

$$s_{ij} = \frac{P_{ij}}{P_i P_j}$$

independent $\Leftrightarrow$ $s_{ij} = 1$



M. jannaschii

$$S = \begin{array}{c} \\ A \\ C \\ G \\ T \end{array} \begin{array}{c} \begin{array}{cccc} A & C & G & T \end{array} \\ \begin{pmatrix} 1.13 & 0.73 & 1.10 & 0.94 \\ 1.03 & 1.37 & 0.32 & 1.11 \\ 1.05 & 1.12 & 1.39 & 0.71 \\ 0.83 & 1.05 & 1.13 & 1.14 \end{pmatrix} \end{array}$$

BioSB

Markov chains

Sequence: $q = q_1 q_2 \ldots q_N$

$$P(q_N, q_{N-1}, \ldots, q_1) = P(q_N \mid q_{N-1}, \ldots, q_1) P(q_{N-1} \mid q_{N-2}, \ldots, q_1) \ldots P(q_1) = \prod_{t=2}^{N} P(q_t \mid q_{t-1}, \ldots, q_1) P(q_1)$$

Only dependent on previous symbol:

$$P(q_N, q_{N-1}, \ldots, q_1) = \prod_{t=2}^{N} P(q_t \mid q_{t-1}) P(q_1)$$

First-order Markov chain

state: value of q_j

transition probability: $P(q_t = j \mid q_{t-1} = i)$

BioSB

Markov chains: language

Zero-order approximation (symbols independent but with frequencies of English text).

```
OCRO HLI RGWR NMIELWIS EU LL NBNESEBYA TH EEI
ALHENHTTPA OOBTTVA NAH BRL.
```

First-order Markov (transition probabilities as in English).

```
ON IE ANTSOUTINYS ARE T INCTORE ST BE S DEAMY
ACHIN D ILONASIVE TUCOOWE AT TEASONARE FUSO
TIZIN ANDY TOBE SEACE CTISBE.
```

Second-order Markov (transition probabilities as in English).

```
IN NO IST LAT WHEY CRATICT FROURE BIRS GROCID
PONDENOME OF DEMONSTURES OF THE REPTAGIN IS
REGOACTIONA OF CRE.
```

C.E. Shannon (1948)

BioSB

Markov chains: language

Zero-order word approximation. Words are chosen independently but with their appropriate frequencies.

REPRESENTING AND SPEEDILY IS AN GOOD APT OR COME CAN DIFFERENT NATURAL HERE HE THE A IN CAME THE TOOF TO EXPERT GRAY COME TO FURNISHES THE LINE MESSAGE HAD BE THESE.

First-order Markov (on words). Word transition probabilities are as in English.

THE HEAD AND IN FRONTAL ATTACK ON AN ENGLISH WRITER THAT THE CHARACTER OF THIS POINT IS THEREFORE ANOTHER METHOD FOR THE LETTERS THAT THE TIME OF WHO EVER TOLD THE PROBLEM FOR AN UNEXPECTED.

C.E. Shannon (1948)

BioSB

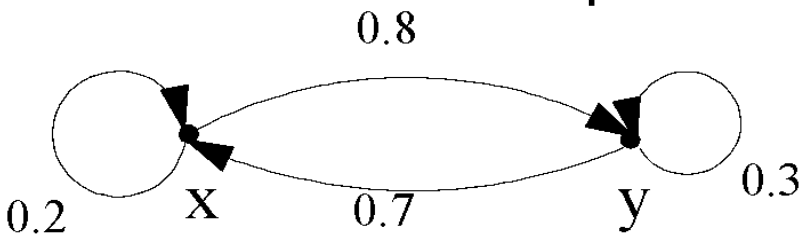
Markov chain: graphical representation

Two states: *x* and *y*

Matrix

$$A = \begin{array}{c} \\ x \\ y \end{array} \begin{array}{c} \begin{array}{cc} x & y \end{array} \\ \begin{pmatrix} 0.2 & 0.8 \\ 0.7 & 0.3 \end{pmatrix} \end{array}$$

Graph: arrows for transition probability



a_{ij} : transition probability from *i* to *j*

Generative model (example): *xyyxyxyyxxyxyxx*...

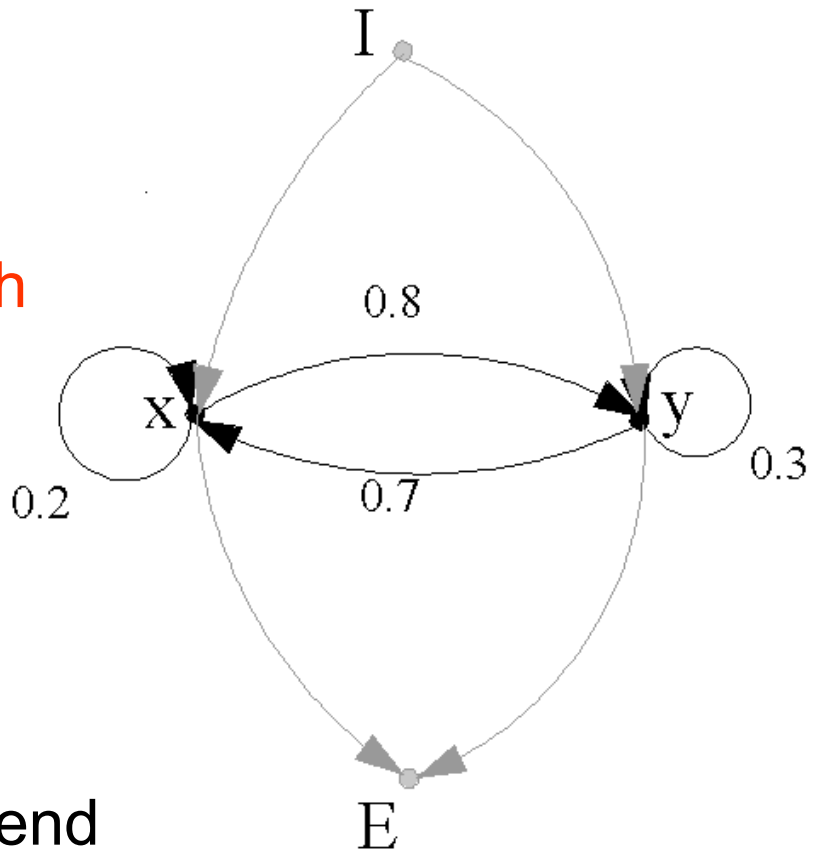
$$P(xyyxy) = P(x)P(y \mid x)P(y \mid y)P(x \mid y)P(y \mid x) = P(x) \times 0.8 \times 0.3 \times 0.7 \times 0.8$$

BioSB

Markov chain: graphical representation (2)

Two states: *x* and *y*

Graph



Add begin state q_0

$$P(q_N, q_{N-1}, \ldots, q_1) = \prod_{t=1}^{N} P(q_t \mid q_{t-1})$$

and end state q_{N+1} to model end of sequence

BioSB

Markov chain: estimation

a_{ij} : transition probability from *i* to *j*

Estimation: simply by counting

$$a_{ij} = \frac{\#\ \text{of}\ t\ \text{such that}\ q_{t-1} = i, q_t = j}{\#\ \text{of}\ t\ \text{such that}\ q_{t-1} = i}$$

Begin state:

$$a_{0i} = \frac{\#\ \text{of}\ t\ \text{such that}\ q_t = i}{N}$$

BioSB

Markov chains: log-odds

Sequence: $x = x_1 x_2 \ldots x_N$

A,*B* : Markov chains for class *A* and *B*, respectively

$$\log\left(\frac{P(x \mid \text{class } A)}{P(x \mid \text{class } B)}\right) = \log\left(\frac{\prod_{t=1}^{N} P_A(x_t \mid x_{t-1})}{\prod_{t=1}^{N} P_B(x_t \mid x_{t-1})}\right) = \sum_{t=1}^{N} \log\left(\frac{P_A(x_t \mid x_{t-1})}{P_B(x_t \mid x_{t-1})}\right)$$

$$= \sum_{t=1}^{N} \log\left(\frac{a^A_{x_{t-1},x_t}}{a^B_{x_{t-1},x_t}}\right)$$

BioSB

Markov chains: limitations

For biological sequences:

- Mononucleotide repeats (due to polymerase slippage) are more frequent than predicted by Markov chain

  Reason: probability of *d* consecutive *i* 's is $(a_{ii})^{d-1}(1-a_{ii})$ (geometric distribution)

- Codon (position) biases are not taken into account

BioSB

Outline

- Regular expressions & weight matrices
- Dependencies & Markov chains
- Hidden Markov models
- HMMs & EM
- Profile HMMs
- Genefinding

BioSB

Multiple alignment

Sequence ensemble as before but now with some insertions and gaps

```
A C A - - - A T G
T C A A C T A T C
A C A C - - A G C
A G A - - - A T C
A C C G - - A T C
```

regular expression: [AT][CG][AC][ACGT]*A[GT][CG]

insertions and gaps ←

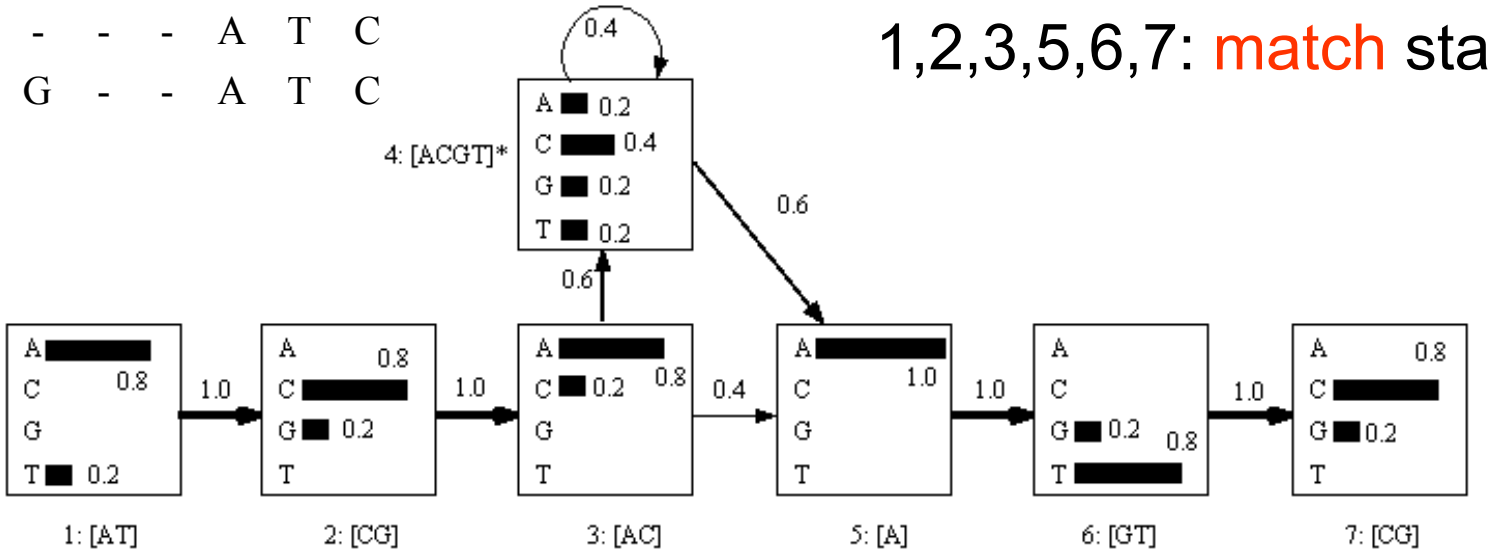
A different representation

A	C	A	-	-	-	A	T	G
T	C	A	A	C	T	A	T	C
A	C	A	C	-	-	A	G	C
A	G	A	-	-	-	A	T	C
A	C	C	G	-	-	A	T	C

boxes: states

4: insert state

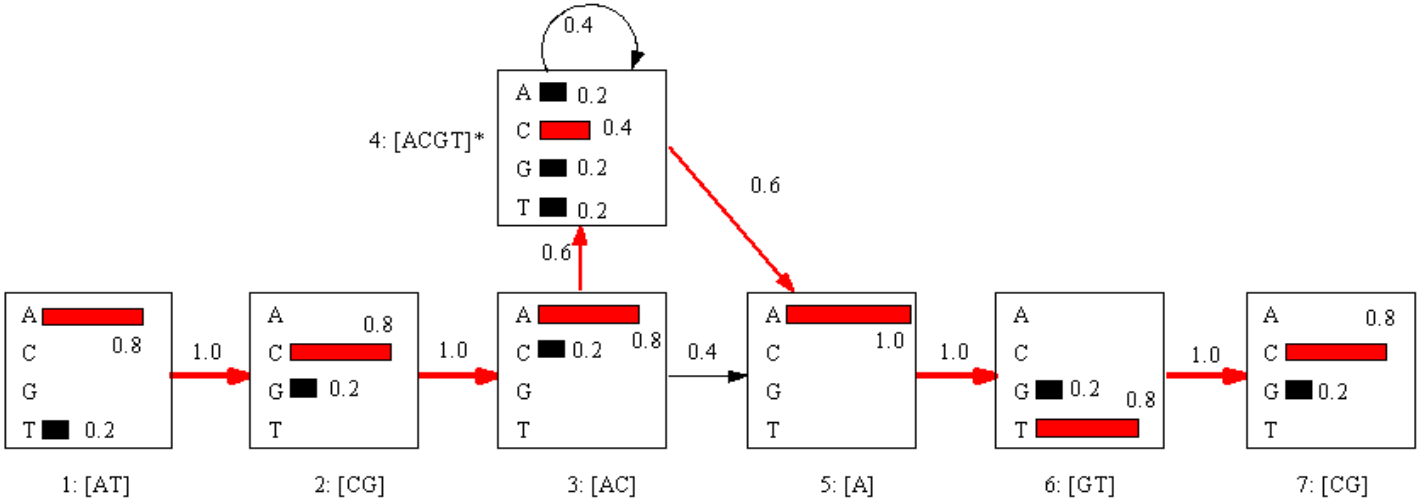
1,2,3,5,6,7: match state



mix of weight matrices and Markov chains

BioSB

Probability of consensus sequence



$$P(\mathrm{ACACATC}) = 0.8 \times 1 \times 0.8 \times 1 \times 0.8 \times 0.6 \times 0.4 \times 0.6 \times 1 \times 1 \times 0.8 \times 1 \times 0.8 = 0.047$$

Markov chain: one state = one symbol

Here: C can be generated by states 2,3,4 or 7 – states are hidden

BioSB

Hidden Markov models

Alphabet *K* of (observed) symbols

States: *Q* = {0,1,2, …, S} 0: begin state (non-emitting)

transition probability: $a_{ij} = P(q_t = j \mid q_{t-1} = i) \qquad 0 \le i, j \le S$

emission probability: $b_i(x) = P(x \mid i) \quad x \in K,\ 1 \le i \le S$

→ probability of emitting symbol *x* in state *i*

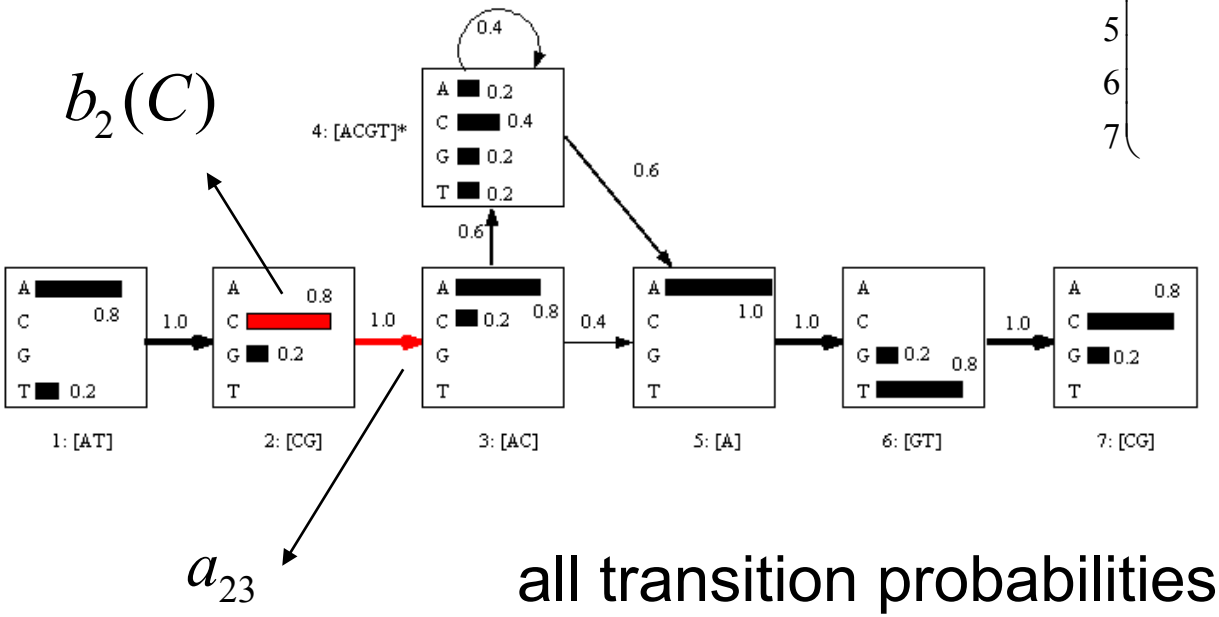
BioSB

Hidden Markov models (2)

Alphabet of 4 symbols {A,C,G,T}

$Q = \{1,2, \ldots, 7\}$

$$A = \begin{array}{c} \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \end{array} \begin{array}{c} \begin{array}{ccccccc} 1 & 2 & 3 & 4 & 5 & 6 & 7 \end{array} \\ \begin{pmatrix} 1.0 & & & & & & \\ & 1.0 & & & & & \\ & & & 0.6 & 0.4 & & \\ & & & 0.4 & 0.6 & & \\ & & & & & 1.0 & \\ & & & & & & 1.0 \\ & & & & & & \end{pmatrix} \end{array}$$



all transition probabilities = 1: weight matrix

HMM: three problems

Evaluation: probability of an observed sequence, given the model, e.g., to calculate odds.

Decoding: optimal state sequence for an observed sequence

Estimation: of transition and emission probabilities from a given set of sequences

BioSB

HMM evaluation: known state sequence

State sequence: $Q = q_0 q_1 q_2 ... q_N$

Observed sequence: $x = x_1 x_2 ... x_N$

$$P(x,Q) = P(x \mid Q)P(Q) \xrightarrow{\text{Markov}} P(Q) = \prod_{t=1}^{N} P(q_t \mid q_{t-1})$$

$$\downarrow$$

$$P(x \mid Q) = P(x_N \mid x_{N-1},...,x_1,Q)P(x_{N-1} \mid x_{N-2},...,x_1,Q)...P(x_1 \mid Q) = \prod_{t=1}^{N} P(x_t \mid q_t)$$

$$P(x,Q) = \prod_{t=1}^{N} P(q_t \mid q_{t-1}) \prod_{t=1}^{N} P(x_t \mid q_t) = \prod_{t=1}^{N} a_{q_{t-1},q_t} \prod_{t=1}^{N} b_{q_t}(x_t)$$

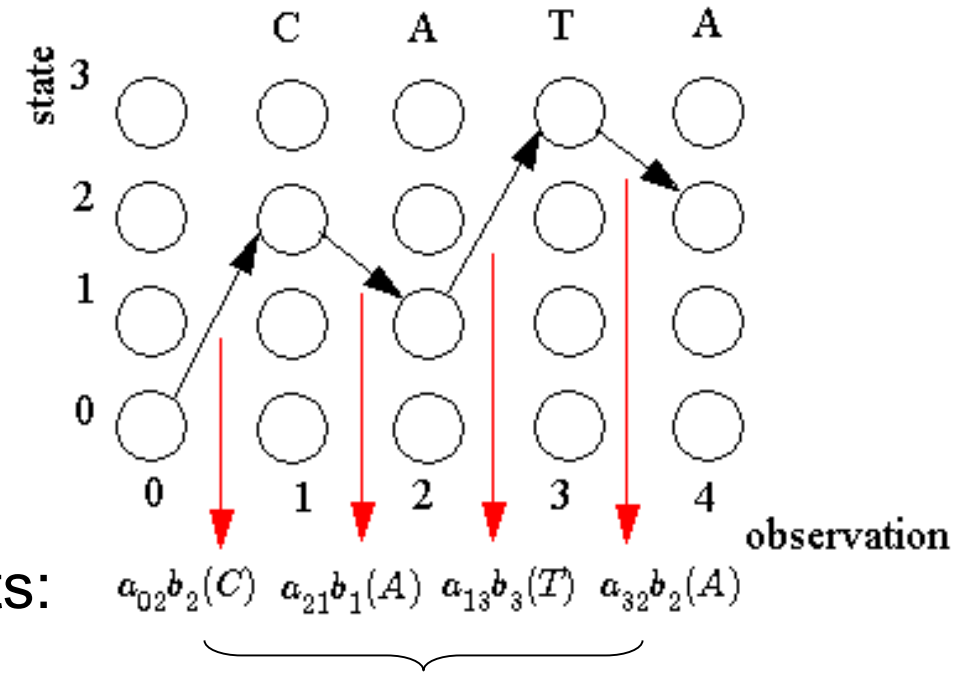
$$P(\texttt{ACACATC}) = 0.8 \times 1 \times 0.8 \times 1 \times 0.8 \times 0.6 \times 0.4 \times 0.6 \times 1 \times 1 \times 0.8 \times 1 \times 0.8 = 0.047$$

BioSB

HMM evaluation: graphical representation on a trellis

$$P(x,Q) = \prod_{t=1}^{N} P(q_t \mid q_{t-1}) \prod_{t=1}^{N} P(x_t \mid q_t) = \prod_{t=1}^{N} a_{q_{t-1},q_t} \prod_{t=1}^{N} b_{q_t}(x_t)$$

state sequence = path



$$P(\text{CATA}, 0-2-1-3-2)$$

BioSB

HMM evaluation: forward algorithm

State sequence unknown: $P(x) = \sum_{Q} P(x, Q)$

Sum over all paths through trellis: ~ S^N state sequences!

Smarter: $P(x) = \sum_{i=0}^{S} P(x, q_N = i) = \sum_{i=0}^{S} \alpha(N, i)$ forward variable

$\alpha(t, i) = P(x_1 x_2 \ldots x_t, q_t = i)$, that is, probability of having observed $x_1 x_2 \ldots x_t$ and being in state *i* at step *t*

BioSB

HMM evaluation: forward algorithm (2)

$$\alpha(t,i) = P(x_1 x_2 \ldots x_t, q_t = i)$$

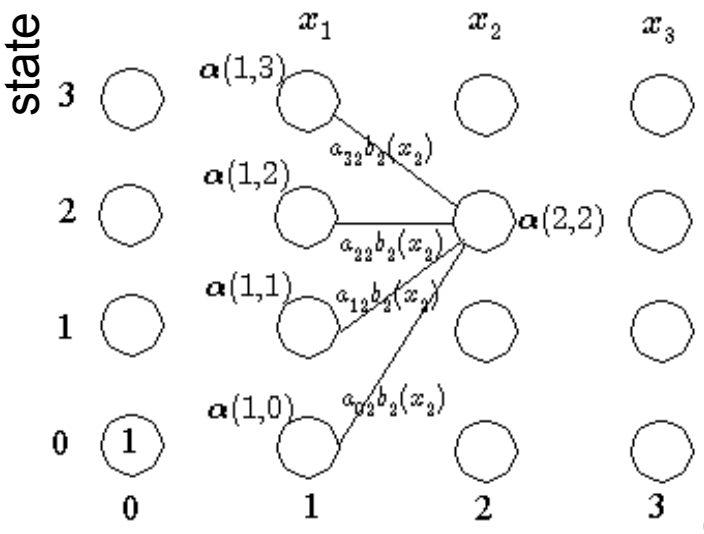
initialization: $\alpha(0,0) = 1\ ,\ \alpha(0,j) = 0 \qquad 1 \le j$

recursion : $\alpha(t,i) = \sum_j \alpha(t-1,j) a_{ji} b_i(x_t) \qquad 1 \le t \le N, 0 \le i, j \le S$

$$P(x) = \sum_{i=0}^{S} \alpha(N,i)$$

Complexity: *S*×*N*

$$\alpha(2,2) = \sum_{j=0}^{3} \alpha(1,j) a_{j2} b_2(x_2)$$



BioSB

Forward algorithm: proof

$$x_{1..t} = x_1 x_2 \ldots x_t$$

$$1 \le t \le N, 0 \le i, j \le S$$

$$\alpha(t,i) = P(x_{1..t}, q_t = i) = \sum_j P(x_{1..t}, q_{t-1} = j, q_t = i)$$

$$= \sum_j P(x_{1..t-1}, q_{t-1} = j) P(x_t, q_t = i \mid x_{1..t-1}, q_{t-1} = j)$$

.

Observed symbol and the state depend only on previous state:

$$= \sum_j P(x_{1..t-1}, q_{t-1} = j) P(x_t, q_t = i \mid q_{t-1} = j)$$

$$= \sum_j \alpha(t-1, j) P(q_t = i \mid q_{t-1} = j) P(x_t \mid q_t = i)$$

$$= \sum_j \alpha(t-1, j) a_{ji} b_i(x_t)$$

recursion

BioSB

HMM: three problems

Evaluation: probability of an observed sequence, given the model, e.g., to calculate odds.

Decoding: optimal state sequence for an observed sequence

Estimation: of transition and emission probabilities from a given set of sequences

BioSB

HMM decoding: Viterbi algorithm

Decoding: find state sequence which best explains observed sequence.

Viterbi: best = most probable

Viterbi variable

$$V(x) = \max_Q P(Q \mid x) = \max_Q \frac{P(x,Q)}{P(x)} = \max_Q P(x,Q)$$

$$V(x) = \max_Q P(x,Q) = \max_i \left[\max_{Q_{0..N-1}} P(x, Q_{0..N-1}, q_N = i) \right] = \max_i \left[v(N,i) \right]$$

$$v(t,i) = \max_{Q_{0..t-1}} \left[P(x_{1..t}, Q_{0..t-1}, q_t = i) \right]$$

probability of having observed $x_1 x_2 \ldots x_t$ along most probable path ending in state *i* at step *t*

BioSB

HMM decoding: Viterbi algorithm (2)

$$v(t,i) = \max_{Q_{0..t-1}} \left[P(x_{1..t}, Q_{0..t-1}, q_t = i) \right]$$

initialization : $v(0,0) = 1 \ , \ v(0,j) = 0$ $\quad 1 \le j$

recursion : $v(t,i) = \max_j \ [v(t-1,j)a_{ji}]b_i(x_t)$ $\quad 1 \le t \le N, 0 \le i, j \le S$

$p(t,i) = \text{argmax}_j \ [v(t-1,j)a_{ji}]$

end : $V(x) = \max_i \ [v(N,i)]$

$q_N^* = \arg\max_i [v(N,i)]$

backtracking : $q_t^* = p(t+1, q_{t+1}^*)$ $\quad 0 \le t \le N-1$

BioSB

Dishonest casino: Viterbi

Casino switches between a fair (F) die and a loaded (L) die

transition probabilities

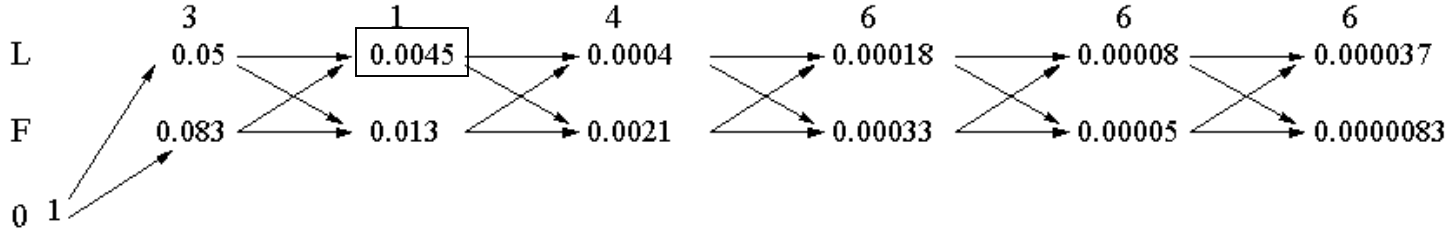
$$A = \begin{array}{c} \\ 0 \\ F \\ L \end{array} \begin{array}{c} \begin{array}{ccc} 0 & F & L \end{array} \\ \begin{pmatrix} 0 & 0.5 & 0.5 \\ 0 & 0.95 & 0.05 \\ 0 & 0.1 & 0.9 \end{pmatrix} \end{array}$$

$$\begin{array}{c} \\ F: \\ L: \end{array} \begin{array}{c} \begin{array}{cccccc} 1 & 2 & 3 & 4 & 5 & 6 \end{array} \\ \begin{array}{cccccc} \frac{1}{6} & \frac{1}{6} & \frac{1}{6} & \frac{1}{6} & \frac{1}{6} & \frac{1}{6} \\ \frac{1}{10} & \frac{1}{10} & \frac{1}{10} & \frac{1}{10} & \frac{1}{10} & \boxed{\frac{1}{2}} \end{array} \end{array}$$

emission probabilities

observations: 3-1-4-6-6-6

Viterbi



$v(2,\mathrm{L}) = \max[v(1,\mathrm{F})a_{\mathrm{FL}}b_{\mathrm{L}}(1)\ ,\ v(1,\mathrm{L})a_{\mathrm{LL}}b_{\mathrm{L}}(1)]$

$= \max[(0.083\times0.05\times0.1\ ,\ 0.05\times0.9\times0.1] = 0.0045$

BioSB

Dishonest casino: Viterbi (2)

Casino switches between a fair (F) die and a loaded (L) die

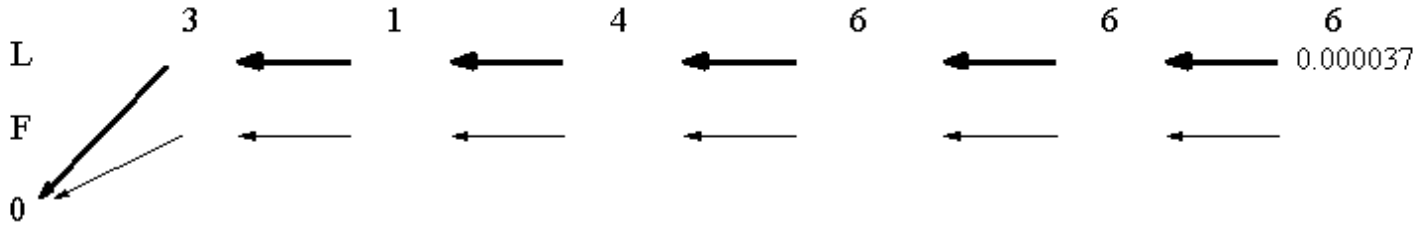
transition probabilities

$$A = \begin{array}{c} \\ 0 \\ F \\ L \end{array} \begin{array}{c} \begin{array}{ccc} 0 & F & L \end{array} \\ \begin{pmatrix} 0 & 0.5 & 0.5 \\ 0 & 0.95 & 0.05 \\ 0 & 0.1 & 0.9 \end{pmatrix} \end{array}$$

$$\begin{array}{c} \\ F: \\ L: \end{array} \begin{array}{c} \begin{array}{cccccc} 1 & 2 & 3 & 4 & 5 & 6 \end{array} \\ \begin{array}{cccccc} \frac{1}{6} & \frac{1}{6} & \frac{1}{6} & \frac{1}{6} & \frac{1}{6} & \frac{1}{6} \end{array} \\ \begin{array}{cccccc} \frac{1}{10} & \frac{1}{10} & \frac{1}{10} & \frac{1}{10} & \frac{1}{10} & \boxed{\frac{1}{2}} \end{array} \end{array}$$

emission probabilities

Backtracking



Optimal state sequence: 0-L-L-L-L-L-L

Outline

- Regular expressions & weight matrices
- Dependencies & Markov chains
- Hidden Markov models
- HMMs & EM
- Profile HMMs
- Genefinding

BioSB

HMM: three problems

Evaluation: probability of an observed sequence, given the model, e.g., to calculate odds

Decoding: optimal state sequence for an observed sequence

Estimation: of transition and emission probabilities from a given set of sequences

BioSB

HMM: estimation

Sequences: $\{x^1, \ldots, x^n\}$

Likelihood:
$$P(x^1, \ldots, x^n \mid \theta) = \prod_{i=1}^{n} P(x^i \mid \theta)$$

state sequence

$$= \prod_{i=1}^{n} \sum_{Q} P(x^i, Q \mid \theta)$$

Log-likelihood:
$$\sum_{i=1}^{n} \log \sum_{Q} P(x^i, Q \mid \theta)$$

same solution since log is monotonic

Maximization of this log-likelihood is difficult because of sum over hidden (state) variables

BioSB

HMM estimation: EM

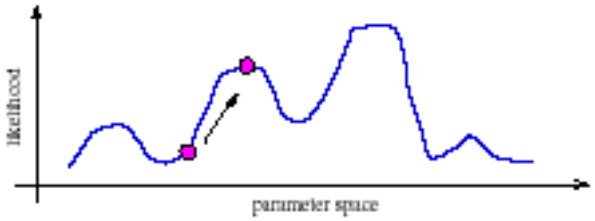
1. If we know the state sequence, parameter estimation is easy: just counting as in Markov chains
2. Can estimate state path using the forward-backward algorithm (not shown)
3. EM: estimate (probability of) states, then estimate parameters, re-estimate the states etc.

This maximizes the likelihood (see MoG)

HMM estimation: remarks

See references in lecture notes for EM for HMM (aka Baum-Welch algorithm) in full detail

EM converges only to a local maximum of the likelihood.
Good initial values are important!



How to choose the structure of an HMM? Black magic …

BioSB

Outline

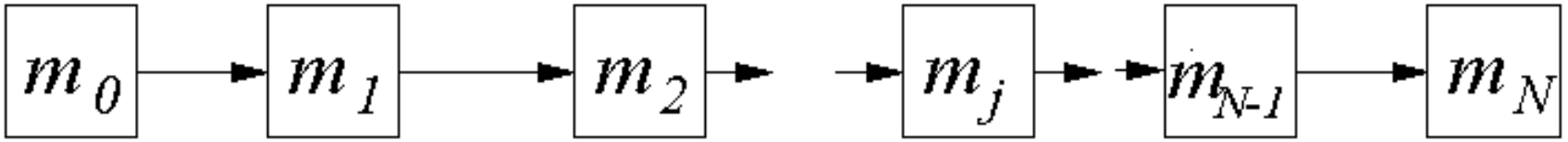
- Regular expressions & weight matrices
- Dependencies & Markov chains
- Hidden Markov models
- HMMs & EM
- Profile HMMs
- Genefinding

BioSB

Profile HMMs

A	C	A	-	-	-	A	T	G
T	C	A	A	C	T	A	T	C
A	C	A	C	-	-	A	G	C
A	G	A	-	-	-	A	T	C
A	C	C	G	-	-	A	T	C

We saw that a weight matrix can be represented as a very simple HMM

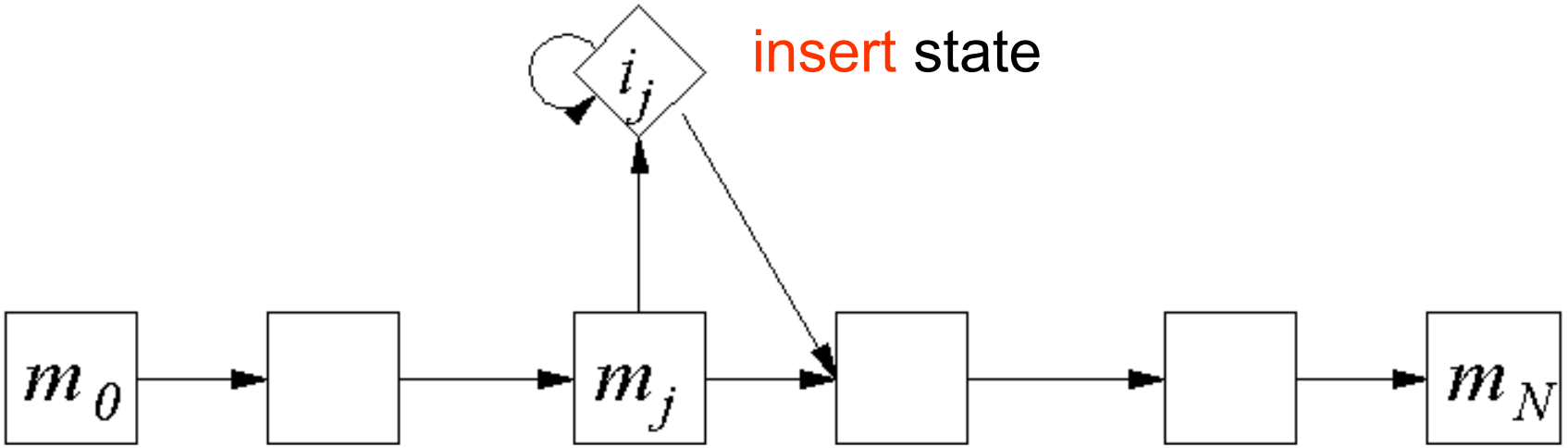


transition probabilities = 1

BioSB

Profile HMMs: insertions

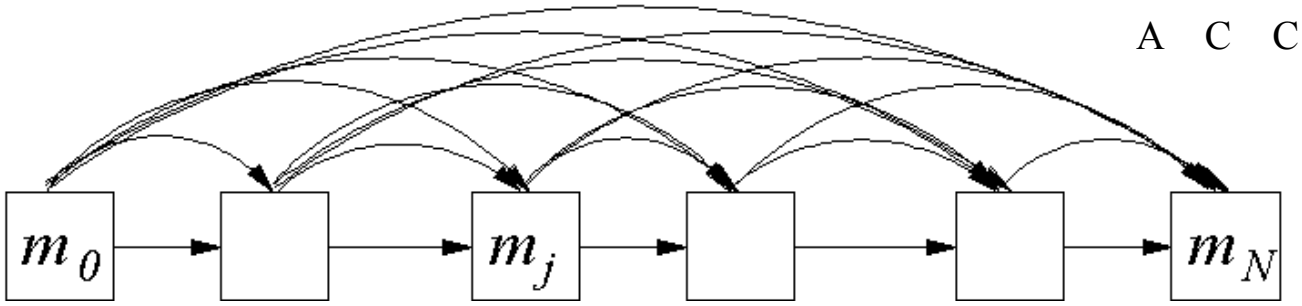
A	C	A	-	-	-	A	T	G
T	C	A	A	C	T	A	T	C
A	C	A	C	-	-	A	G	C
A	G	A	-	-	-	A	T	C
A	C	C	G	-	-	A	T	C



Model insertion(s) between position *j* and *j*+1

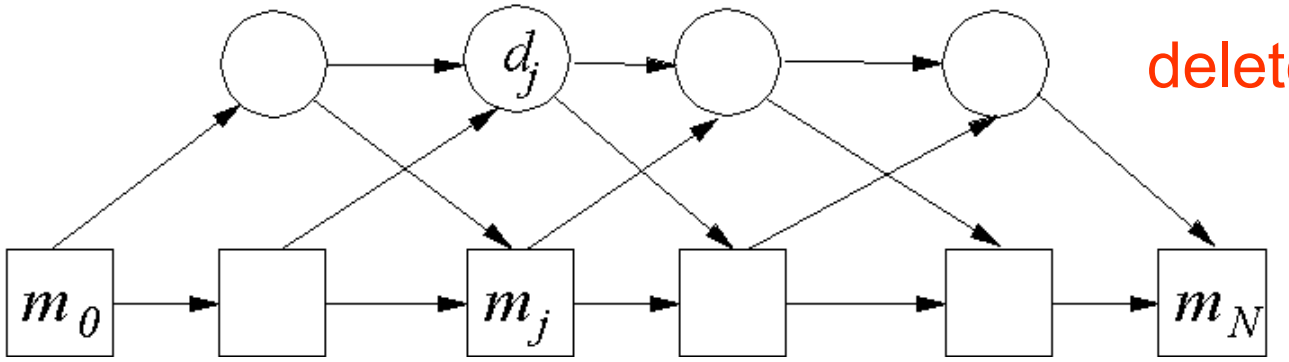
Profile HMMs: deletions

A	C	A	-	-	-	A	T	G
T	C	A	A	C	T	A	T	C
A	C	A	C	-	-	A	G	C
A	G	A	-	-	-	A	T	C
A	C	C	G	-	-	A	T	C



Many transitions = many parameters, but limited data

Solution: introduce silent (=non-emitting) delete states



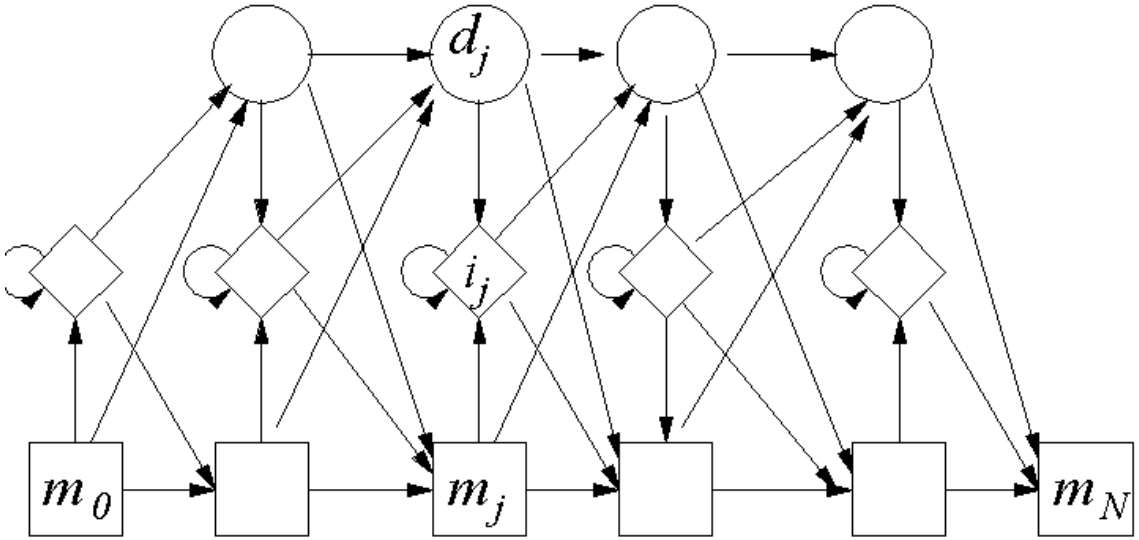
delete states

BioSB

Profile HMMs (2)

A	C	A	-	-	-	A	T	G
T	C	A	A	C	T	A	T	C
A	C	A	C	-	-	A	G	C
A	G	A	-	-	-	A	T	C
A	C	C	G	-	-	A	T	C

Put everything together:



Applications:

http://pfam.xfam.org/

- searching for remote homologs (Forward)

- align a protein to a protein family (Viterbi)

BioSB

Outline

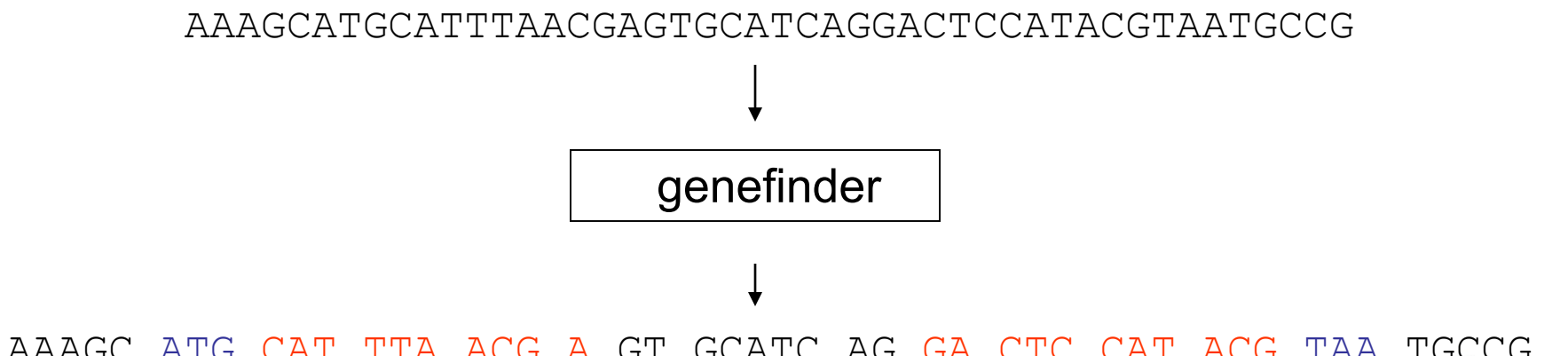
- Regular expressions & weight matrices
- Dependencies & Markov chains
- Hidden Markov models
- HMMs & EM
- Profile HMMs
- Genefinding

BioSB

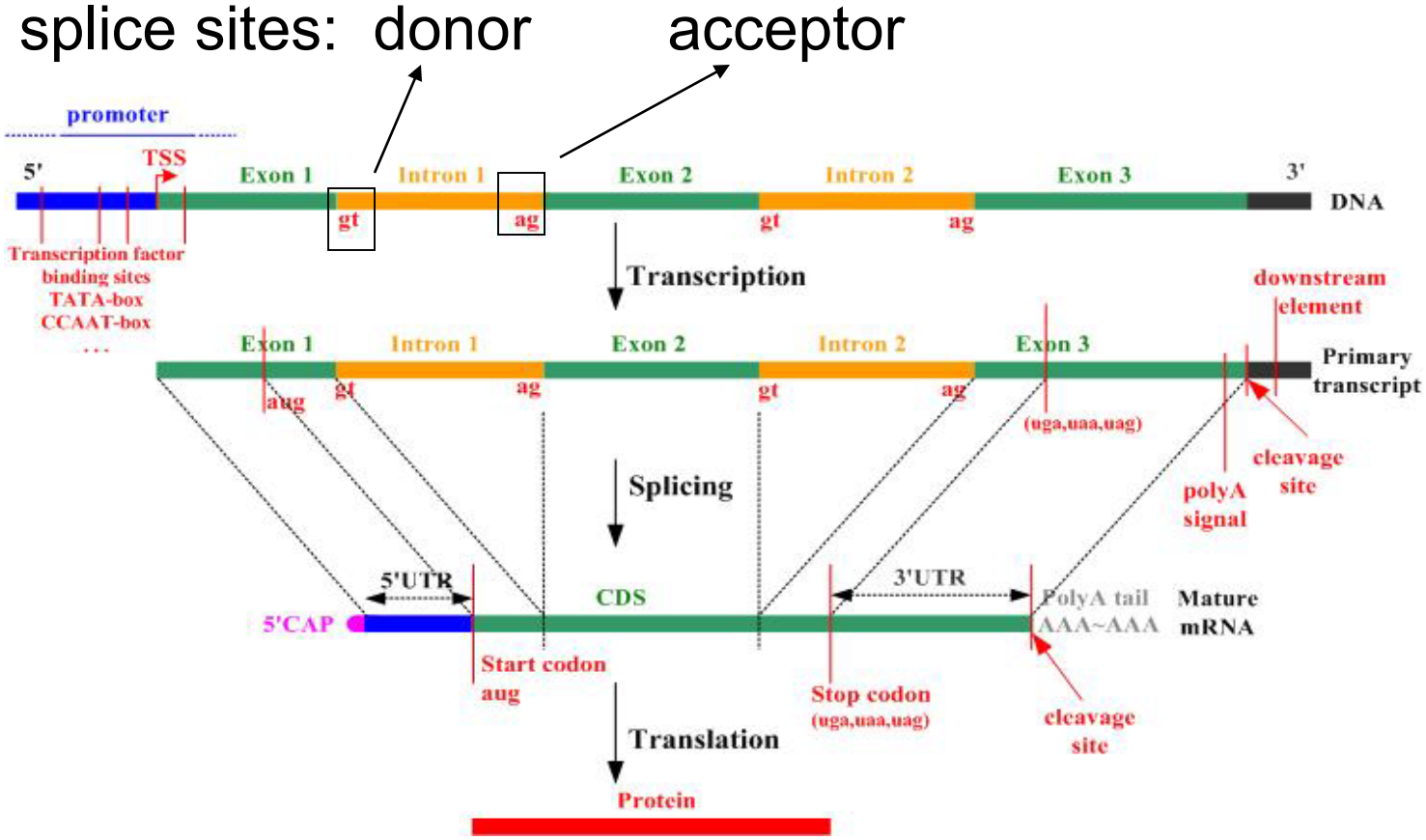
Genefinding

Input: DNA string $S \in \{A, C, G, T\}^*$

Output: annotation of string S showing for each nucleotide whether it is coding or non-coding



Genefinding: eukaryotes



More complex than for prokaryotes: lower coding density (<25% instead of >80%), splicing

BioSB

Genefinding: many signals

Possible signals: splice sites, promoter, codon bias, polyA site, dinucleotide usage ...

Possible models: everything you've seen before ...

How to integrate all these models in one consistent model that can be used for genefinding?

Solution: HMMs again!

Building blocks (=states): weight matrices, (inhomogeneous, higher-order, interpolated) Markov chains, ...

BioSB

Genefinding: HMM

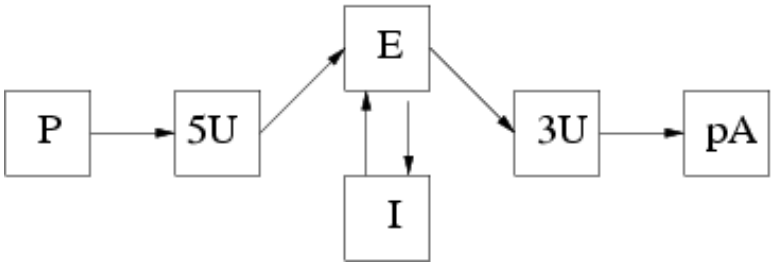
Genes have a certain structure/grammar

… exon – intron – exon – intron – exon …

Regular expression of gene structure:

promoter 5'UTR exon (intron exon)* 3'UTR polyA

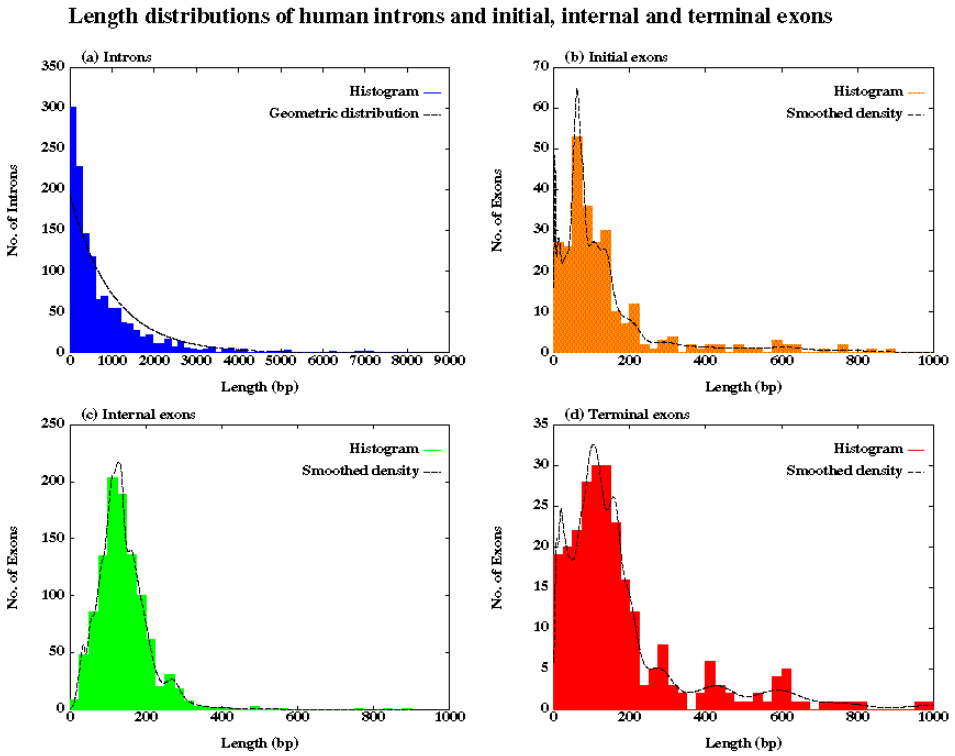
HMM →



Genefinding = annotation with states: Viterbi

BioSB

Length distributions



Standard HMM: length ~ geometric distribution

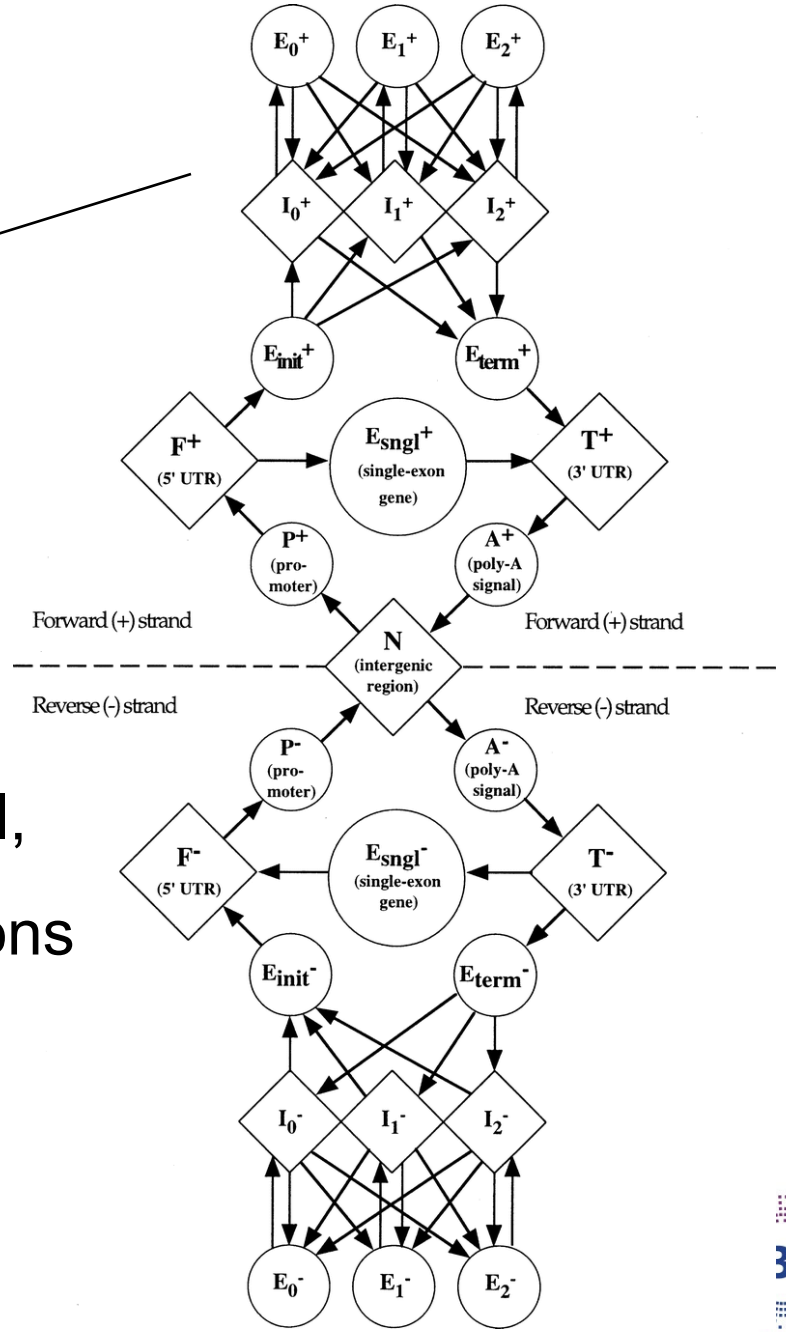
Generalized HMM: states emit sequences + length

BioSB

Genefinding: GenScan

frame-aware

both strands



Exons: separate states for initial, terminal, single and internal exons

GenScan: generalized HMM

BioSB

Recapitulation

- Hidden Markov models:
  flexible models for modeling *sequences*
  - *Evaluation*: forward algorithm
  - *Decoding*: Viterbi
  - *Estimation*: EM

- Applications:
  - Genefinding
  - Modeling protein families
  - Segmentation of array CGH data
  - SNP imputation in GWAS
  - Error correction in nanopore sequencing data

BioSB

10min break
Exercise 4.18-4.20