# Elements of Bioinformatics Autumn 2010

VELI MÄKINEN

HTTP://WWW.CS.HELSINKI.FI/EN/COURSES/582606/2010/S/K/1

# Lecture Thu 25.11.

GENE PREDICTION CONTINUED
AND
COMPLETING THE PIPELINES

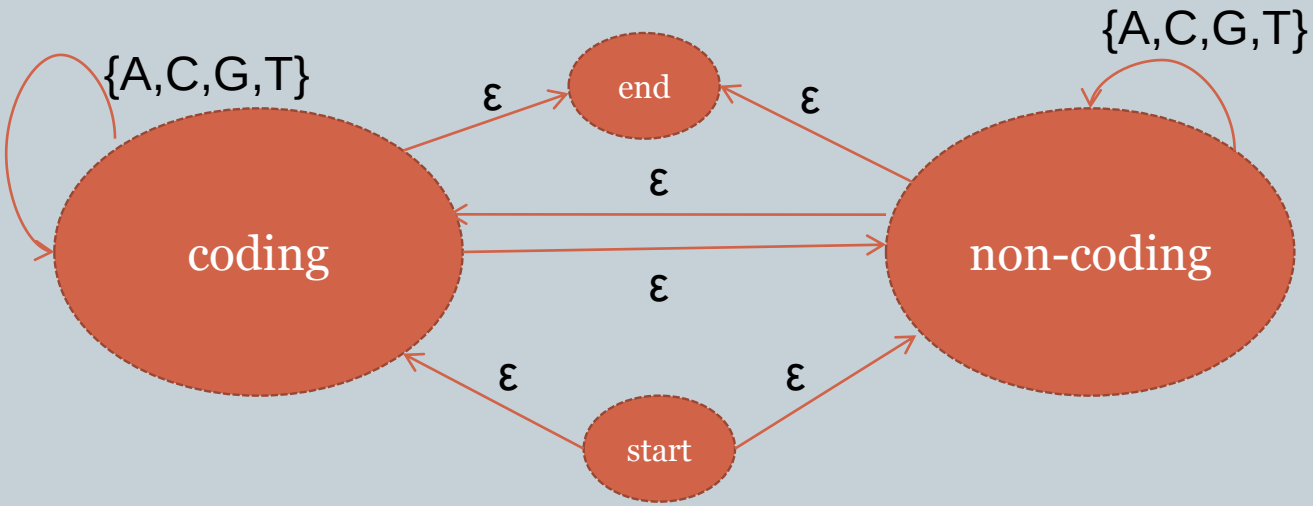
# Part I

## STATISTICAL APPROACHES TO GENE PREDICTION:

## HMMS, VITERBI, FORWARD, BACKWARD

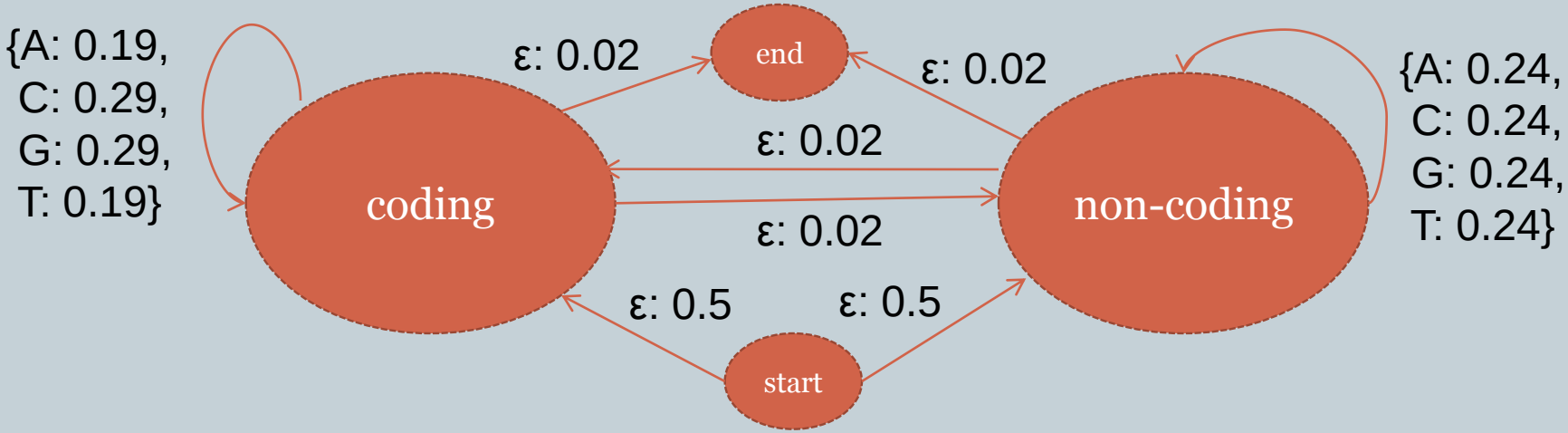
# Hidden Markov Models : Idea

- Consider using GC content to segment a DNA sequence into coding and non-coding parts.
- A natural way to model this is to study ”two”-state finite automaton reading the DNA, where one state corresponds to being in coding part and the other to being in non-coding part:



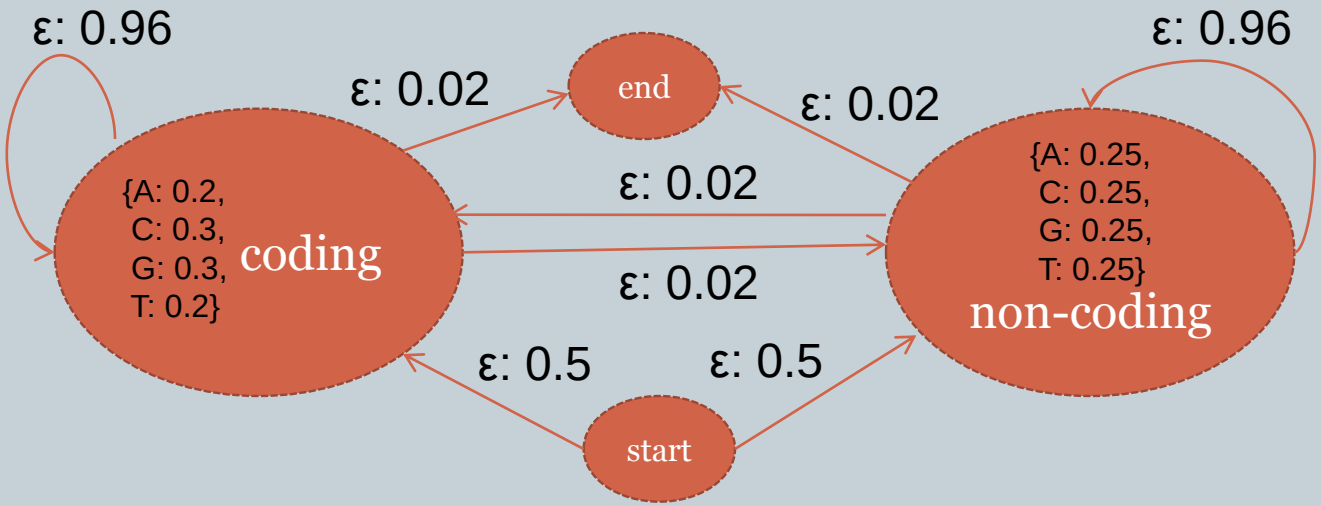
# Hidden Markov Models: Idea

- Now, the above automaton just recognizes any DNA sequence and the path taken through the automaton labels each position as coding or non-coding.
  - Plug in the state-dependent probabilities of emitting a symbol and small probabilities to change the state.
  - The most probable path through the automaton corresponds to the most probable segmentation given the parameters (transition and emission probabilities).



# Hidden Markov Models: Definition

- Hidden Markov Models could be defined as the probabilistic extension of finite automaton like the previous example suggests, but the convention is to separate emissions and transitions:
  - Nodes emit symbols and all transitions are epsilon-transitions.
  - Equivalent notion in terms of expressibility.



# Hidden Markov Models: Definition

- *Hidden Markov Model (HMM)* is a tuple (H, Σ, T, E, p), where H={1,…,|H|} is the set of states, Σ the set of symbols, T the set of transitions, E the set of emissions, and p the probability distribution for elements of T and E, with the following conditions:
  - Let p(t) and p(e) denote the probability for transition t:=(h,h') in T and emission e:=(h,c) in E, respectively, where h,h' in H and c in Σ:
    - $\sum_{h' \text{ in } H} p(h,h')=1$, $\sum_{c \text{ in } \Sigma} p(h,c)=1$ for all h in H.
  - There is a single *start state* $h_{start}$ in H such that there is no transition $(h, h_{start})$ in T.
  - There is a single *end state* $h_{end}$ in H such that there is no transition $(h_{end}, h)$ in T.

# Hidden Markov Models: Definition

- A *path through HMM* is a sequence **h** of hidden states $\mathbf{h}=h_0,h_1,h_2,\ldots,h_n,h_{n+1}$, where $h_0=h_{start}$, $h_{n+1}=h_{end}$, and $h_i$ in $H\backslash\{h_{start}, h_{end}\}$. The probability of path **h** given a sequence $\mathbf{c}=c_1,c_2,\ldots,c_n$, $c_i$ in $\Sigma$, is

$$P(h \mid c) = \prod_{i=0}^{n} p(h_i, h_{i+1}) \prod_{i=1}^{n} p(h_i, c_i).$$

- Let us denote
  - by $H(n)$ the set of all paths through HMM of length $n + 2$ including start ($h_0$) and end ($h_{n+1}$) states,
  - by $H^p(n)$ the set of all (*prefix*) paths of length $n+1$ inside HMM excluding end state ($h_{n+1}$),
  - by $H^s(n)$ the set of all (*suffix*) paths of length $n+1$ inside HMM excluding start state ($h_0$) ,
  - and by $H^*(n)$ the set of all (*local*) paths of length $n$ inside HMM excluding start ($h_0$) and end ($h_{n+1}$) states.

# Three common problems studied on HMM

- *Most probable path*:
  - Given sequence **c**=$c_1,c_2,\ldots,c_n$ , $c_i$ in $\Sigma$, find the path **h*** having the highest probability:

$$h^* = \arg\max_{h \in H(n)} P(h \mid c) = \arg\max_{h \in H(n)} \prod_{i=0}^{n} p(h_i, h_{i+1}) \prod_{i=1}^{n} p(h_i, c_i).$$

- *Probability of sequence being generated by HMM*:
  - Given sequence **c**=$c_1,c_2,\ldots,c_n$ , $c_i$ in $\Sigma$, compute the probability

$$\sum_{h \in H(n)} P(h \mid c) = \sum_{h \in H(n)} \prod_{i=0}^{n} p(h_i, h_{i+1}) \prod_{i=1}^{n} p(h_i, c_i).$$

- *Probability of* $c_j$ *matching state* k:
  - Given sequence **c**=$c_1,c_2,\ldots,c_n$ , $c_i$ in $\Sigma$, compute the probability

$$\sum_{h \in H(n)} P(h \mid c, h_j = k) = \sum_{h \in H(n), h_j = k} \prod_{i=0}^{n} p(h_i, h_{i+1}) \prod_{i=1}^{n} p(h_i, c_i)$$

# Three common problems studied on HMM

- All three problems can be solved using dynamic programming:
  - First one with an algorithm called *viterbi*.
  - Second one with an algorithm called *forward*.
  - Third one with a combination of executing forward algorithm on **c** and its reverse (called *backward* algorithm then).
  - The difference in viterbi and forward is simply to replace max with sum.

# Viterbi algorithm

- Compute a matrix V[0…n+1,1…|H|] such that V[j,k] equals

$$\max_{h \in H^p(j), h_j = k} P(h \mid c_1 \ldots c_j) = \max_{h \in H^p(j-1)} \left( P(h \mid c_1 \ldots c_{j-1}) \max_{(h_{j-1}, k) \in T} p(h_{j-1}, k)\, p(k, c_j) \right).$$

- That is, $V[j,k] = \max_{(k',k) \in T} V[j-1,k']\, p(k',k)\, p(k,c_j)$.
- Let k=1 denote the start state and k=|H| the end state.
  - Initialization: $V[0,1] = 1$.
  - Finalization: $V[n+1,|H|] = \max_{(k',|H|) \in T} V[n,k']\, p(k',|H|)$.
- Probability of the most probable path is V[n+1,|H|].
- The most probable path can be traced back checking which V[n,k']p(k',|H|) equals V[n+1,|H|], and so on.
- Running time O(n |T|).

# Forward algorithm

- Compute a matrix F[0…n+1,1…|H|] such that F[j,k] equals

$$\sum_{h \in H^p(j), h_j = k} P(h \mid c_1 \ldots c_j) = \sum_{h \in H^p(j-1)} \left( P(h \mid c_1 \ldots c_{j-1}) \sum_{(h_{j-1}, k) \in T} p(h_{j-1}, k)\, p(k, c_j) \right).$$

- That is, $F[j,k] = \sum_{(k',k) \in T} F[j-1,k']\, p(k',k)\, p(k,c_j).$

- Let k=1 denote the start state and k=|H| the end state.
  - Initialization: $F[0,1] = 1.$
  - Finalization: $F[n+1,|H|] = \sum_{(k',|H|) \in T} F[n,k']\, p(k',|H|).$

- Probability of the sequence is F[n+1,|H|].

# Backward algorithm

- Compute a matrix B[0…n+1,1…|H|] such that B[j,k] equals

$$\sum_{h \in H^s(j)} P(h \mid c_{n-j+1} \ldots c_n) = \sum_{h \in H^s(j-1)} \left( P(h \mid c_{n-j+2} \ldots c_n) \sum_{(k,h_1) \in T} p(k,h_1)\, p(k, c_{n-j+1}) \right).$$

- That is, $B[j,k] = \sum_{(k,k') \in T} B[j-1,k']\, p(k,k')\, p(k, c_{n-j+1}).$

- Let k=1 denote the start state and k=|H| the end state.
  - Initialization: $B[0, |H|] = 1.$
  - Finalization: $B[n+1,1] = \sum_{(1,k') \in T} B[n,k']\, p(1,k').$

- Probability of the sequence is B[n+1,1].

# Probability of $c_j$ matching state $k$

- Can be computed through F and B:

$$\sum_{h \in H(n)} P(h \mid c_1 \ldots c_n, h_j = k)$$

$$= \sum_{h \in H^p(j), h_j = k} \left( P(h \mid c_1 \ldots c_j) \sum_{(k,k') \in T} p(k,k') \sum_{h' \in H^s(n-j), h'_1 = k'} P(h' \mid c_{j+1} \ldots c_n) \right)$$

$$= F[j,k] \sum_{(k,k') \in T} p(k,k') B[n-j+1, k']$$

# Log transform

- Multiplication is the source of numerical problems in HMM algorithms.
- However, easy to go over by transforming series of multiplications into summation of logarithms:

$$\log \prod_i p_i = \sum_i \log p_i$$

- For example, in viterbi we can fill in table VL[] with rule

$$VL[j,k] = \max_{(k',k)\in T} VL[j-1,k'] + \log p(k',k) + \log p(k,c_j).$$

- Then

$$V[j,k] = 2^{VL[j,k]}$$

with initialization VL[0,1]=1.

# Where the probabilities?

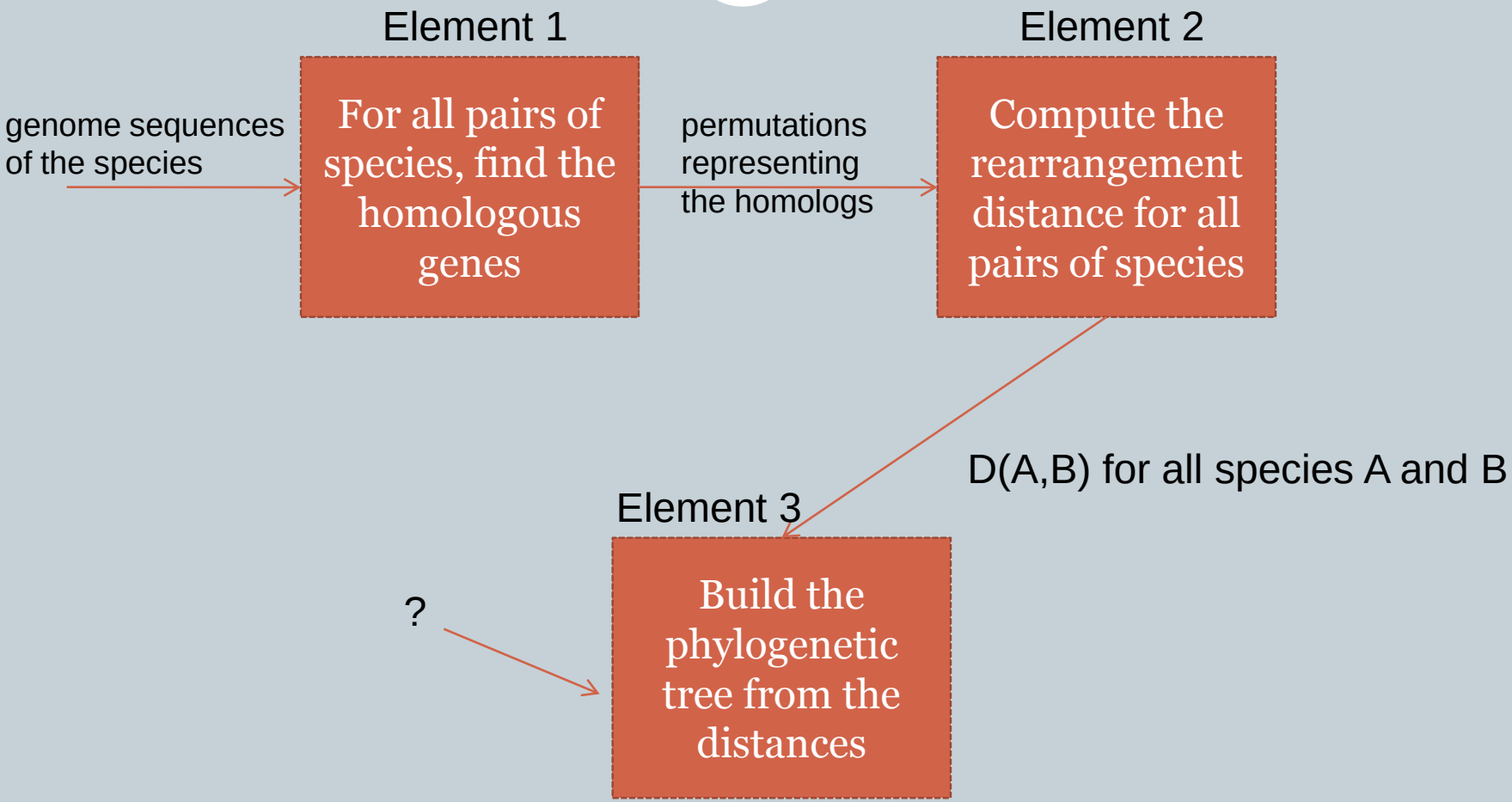
- Learn from the data (Introduction to Machine Learning course).
- Given a set of valid annotated hidden paths (like DNA sequences with exon/intron annotation), it is easy to compute the frequencies of different emissions and transitions taken.
  - Add pseudocounts to cope with too sparse training data.
- Without the annotation, the task of optimizing the parameters is hard:
  - General local optimization routines such as EM algorithm / simulated annealing are used.

→ Biological sequence analysis course

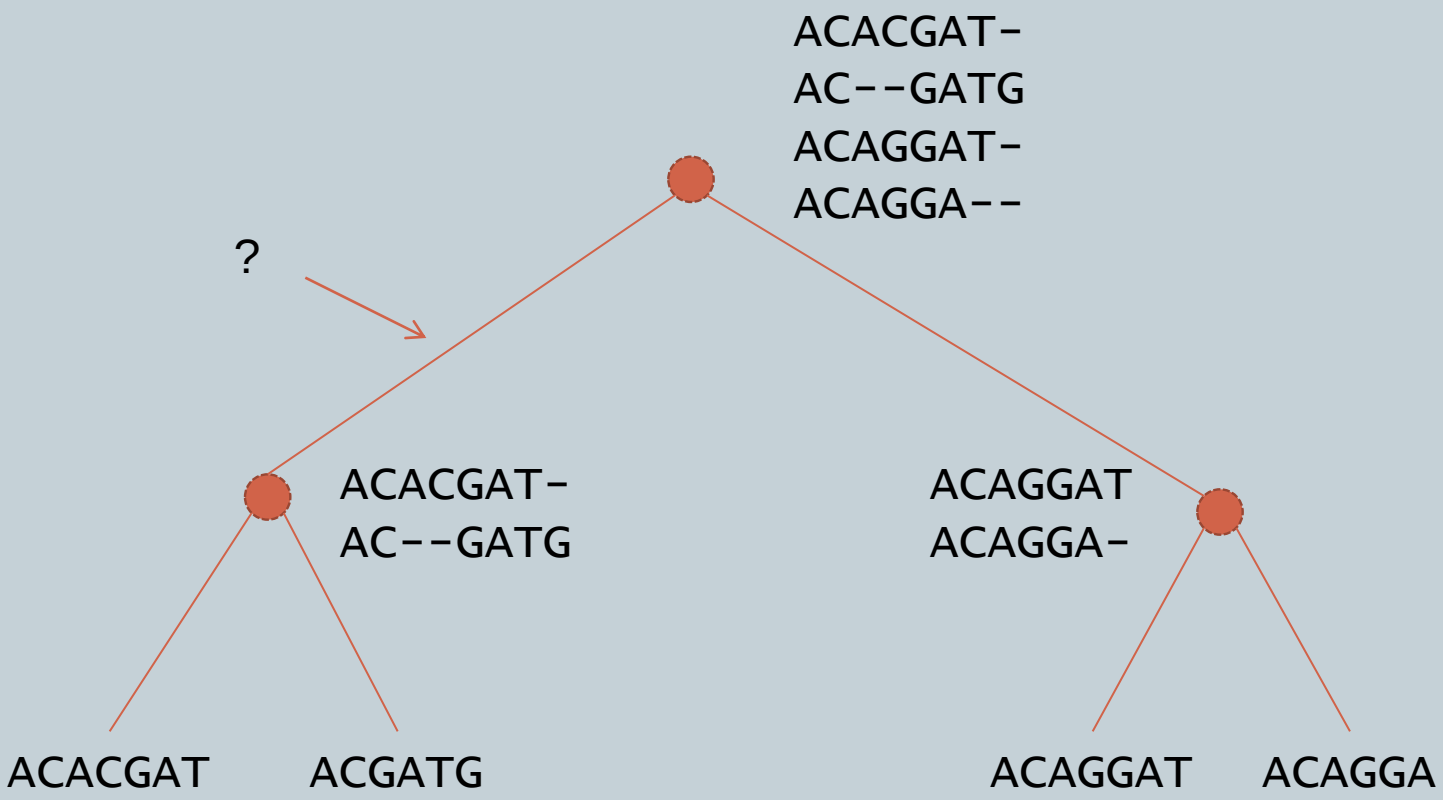
# Part II

## COMPLETING THE PIPELINES: CLUSTERING, UPGMA, NEIGHBOR JOINING

# **Recall:** Phylogeny by distance method pipeline



# **Recall:** Progressive multiple alignment example



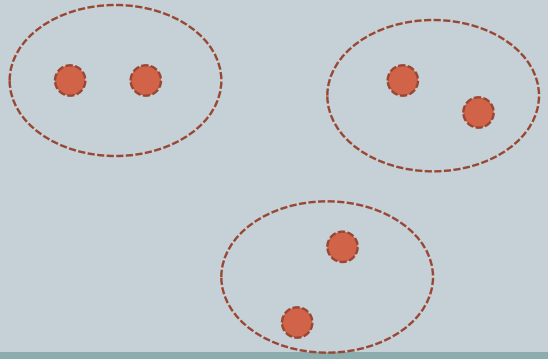
# **Recall:** Time series expression profiling

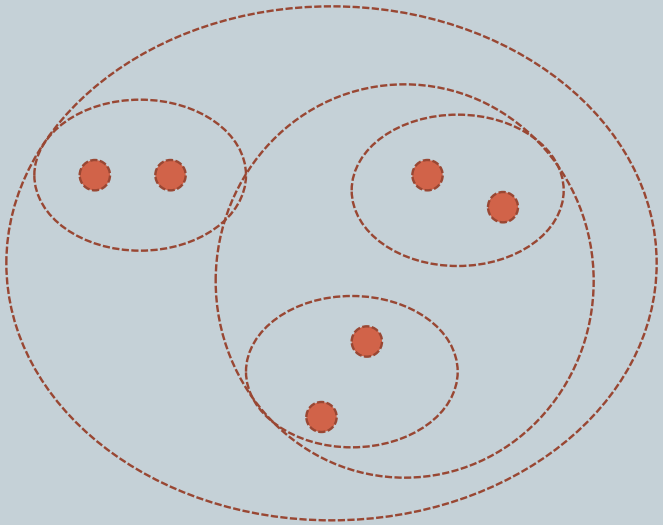
- It is possible to make a series of microarray experiments to obtain a time series expression profile for each gene.

- *Cluster* similarly behaving genes.

# Clustering

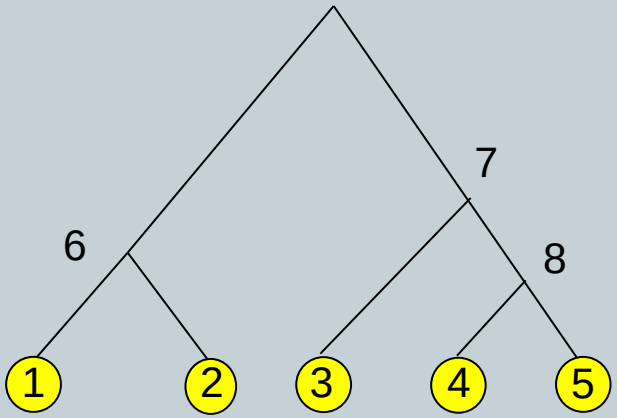
- Hierarchical clustering
  - Iteratively join two closest clusters until forming a tree hierarchy (agglomerative… also divisive version exists)
  - Distance between clusters can be e.g. max pair-wise distance (complete linkage), min (single-linkage), UPGMA (average linkage), neigbor joining
- Partitional clustering
  - k-means, etc.





# Distances in a phylogenetic tree

- Distance matrix $D = (d_{ij})$ gives pairwise distances for *leaves* of the phylogenetic tree
- In addition, the phylogenetic tree will now specify distances between leaves and internal nodes
  - Denote these with $d_{ij}$ as well




Distance $d_{ij}$ states how far apart species i and j are evolutionary

# Distances in evolutionary context

- Distances $d_{ij}$ in evolutionary context satisfy the following conditions
  - Positivity: $d_{ij} \geq 0$
  - Identity: $d_{ij} = 0$ if and only if $i = j$
  - Symmetry: $d_{ij} = d_{ji}$ for each $i, j$
  - Triangle inequality: $d_{ij} \leq d_{ik} + d_{kj}$ for each $i, j, k$
- Distances satisfying these conditions are called *metric*
- In addition, evolutionary mechanisms may impose additional constraints on the distances
  - ▷ *additive* and *ultrametric* distances

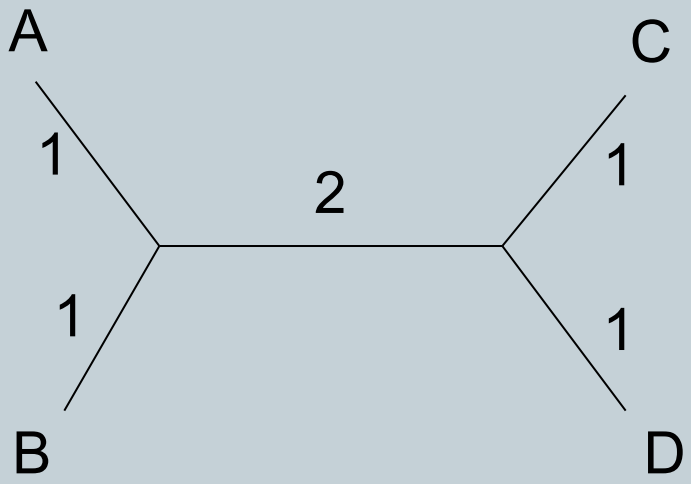
# Additive trees

- A tree is called *additive*, if the distance between any pair of leaves (i, j) is the sum of the distances between the leaves and a node k on the shortest path from i to j in the tree

$$d_{ij} = d_{ik} + d_{jk}$$

# Additive trees: example

| | A | B | C | D |
|---|---|---|---|---|
| A | 0 | 2 | 4 | 4 |
| B | 2 | 0 | 4 | 4 |
| C | 4 | 4 | 0 | 2 |
| D | 4 | 4 | 2 | 0 |



# Ultrametric trees

- A rooted additive tree is called an *ultrametric tree*, if the distances between any two leaves i and j, and their common ancestor k are equal

$$d_{ik} = d_{jk}$$

- Edge length $d_{ij}$ corresponds to the time elapsed since divergence of i and j from the common parent
- In other words, edge lengths are measured by a *molecular clock* with a constant rate

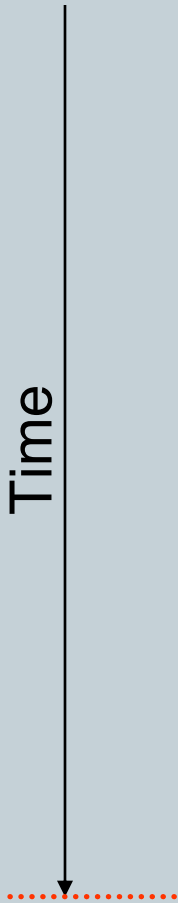
# Identifying ultrametric data

- We can identify distances to be ultrametric by the three-point condition:

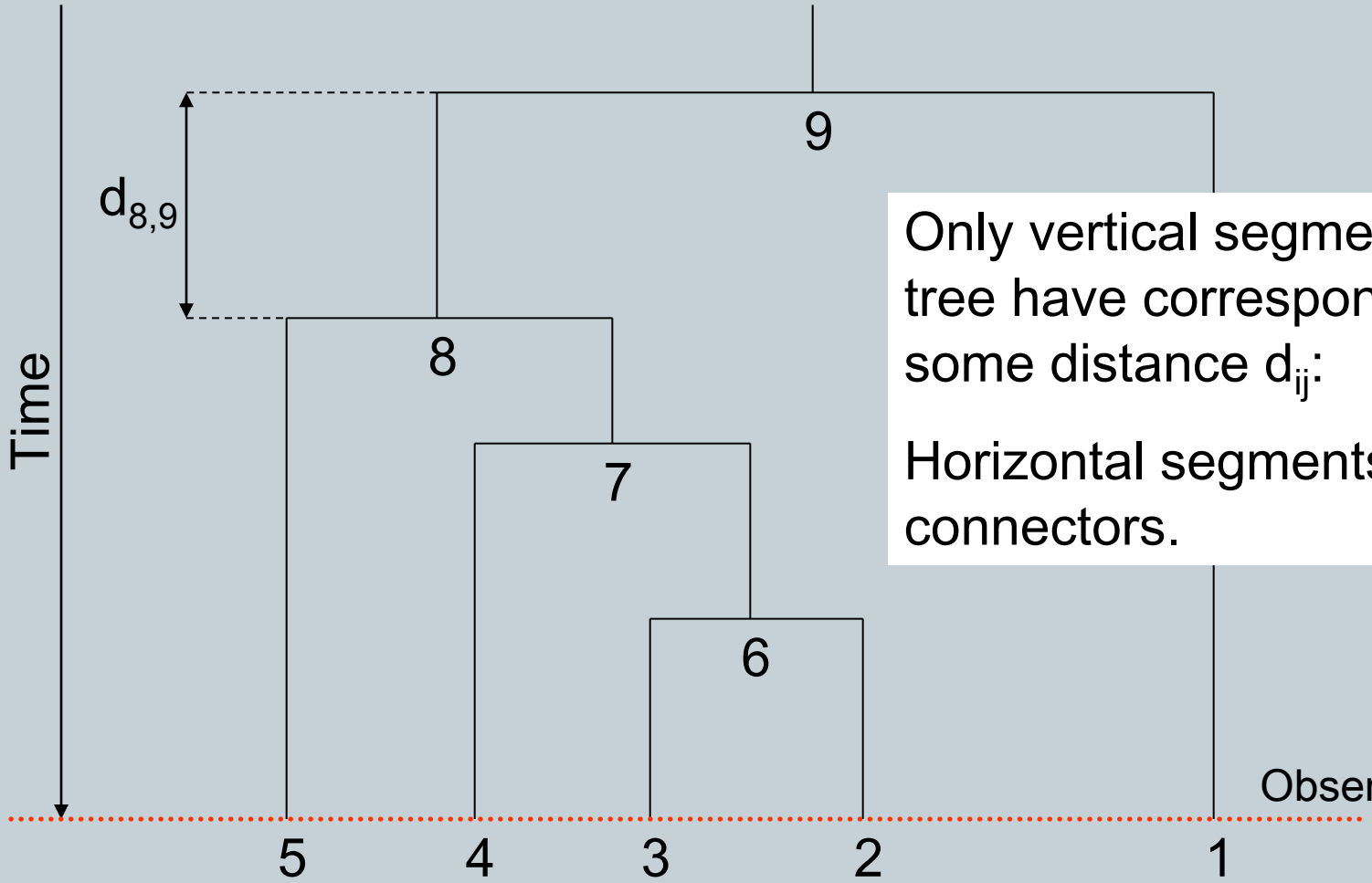
  D corresponds to an ultrametric tree if and only if for any three **species** i, j and k, the distances satisfy $d_{ij} \leq \max(d_{ik}, d_{kj})$

- If we find out that the data is ultrametric, we can utilise a simple algorithm to find the corresponding tree

# Ultrametric trees



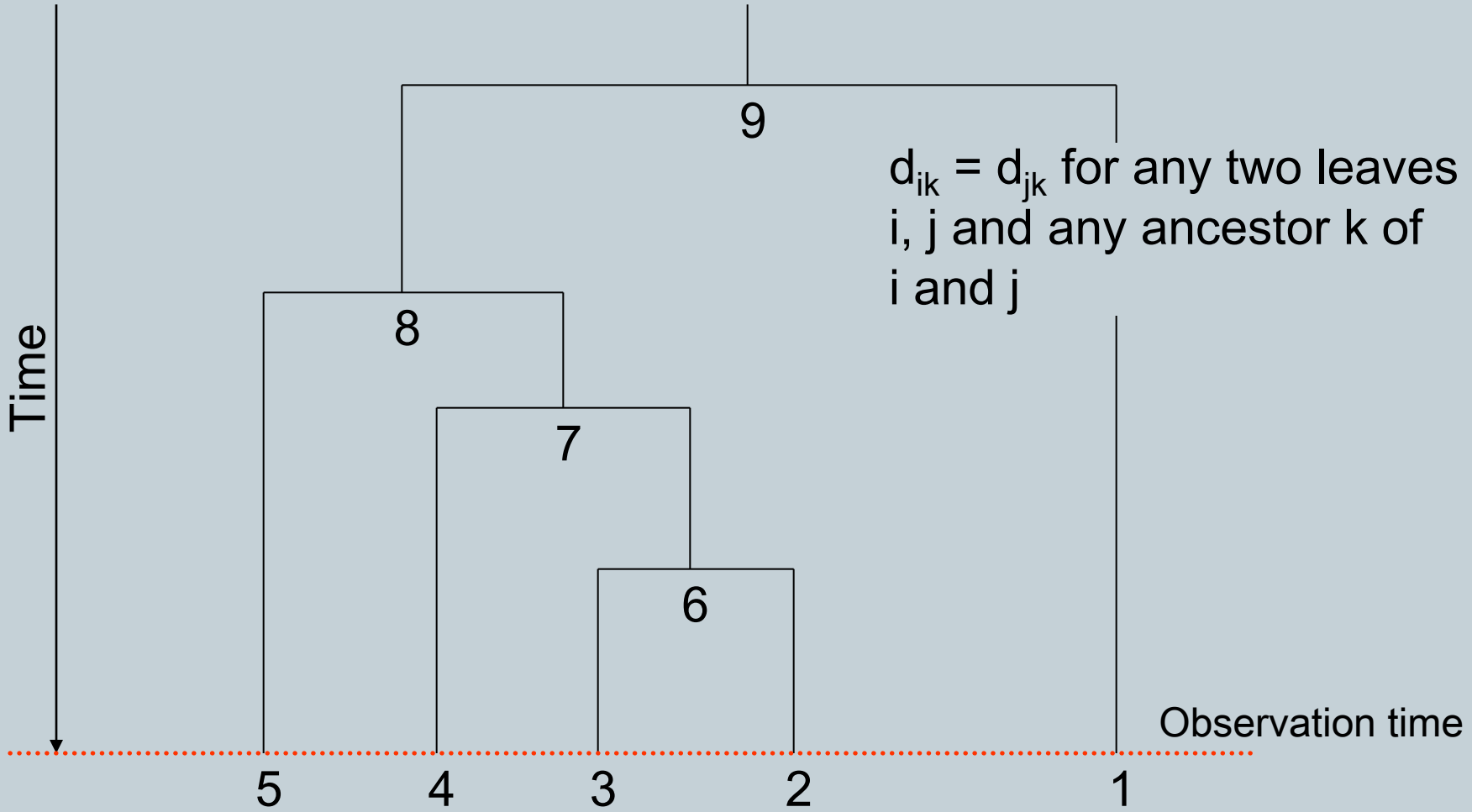
# Ultrametric trees




Only vertical segments of the tree have correspondence to some distance $d_{ij}$:

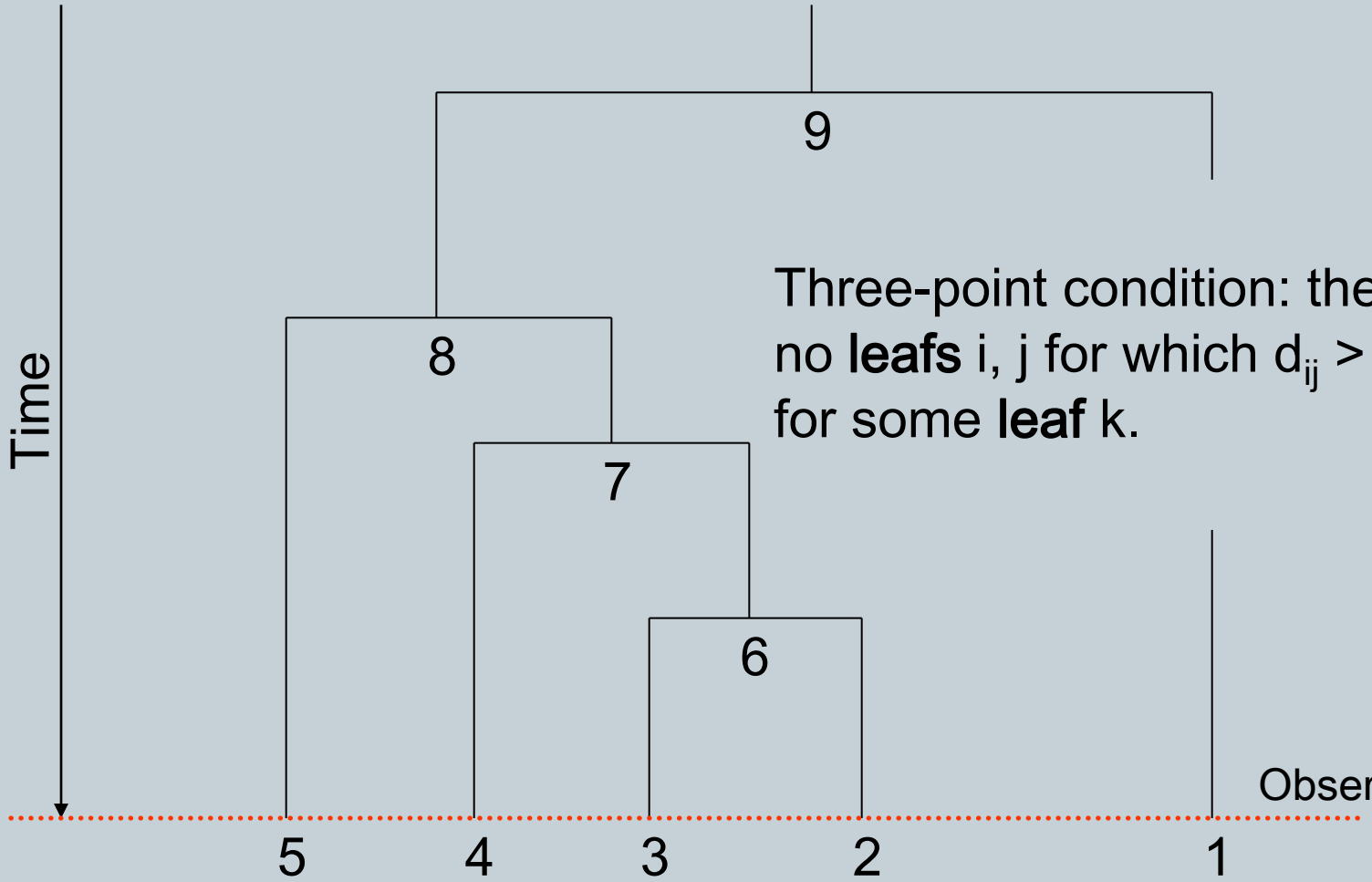
Horizontal segments act as connectors.

# Ultrametric trees




$d_{ik} = d_{jk}$ for any two leaves i, j and any ancestor k of i and j

# Ultrametric trees




Three-point condition: there are no **leafs** i, j for which $d_{ij} > \max(d_{ik}, d_{jk})$ for some **leaf** k.

# UPGMA algorithm

- UPGMA (unweighted pair group method using arithmetic averages) constructs a phylogenetic tree via clustering
- The algorithm works by at the same time
  - Merging two clusters
  - Creating a new node on the tree
- The tree is built from leaves towards the root
- UPGMA produces a ultrametric tree

# Cluster distances

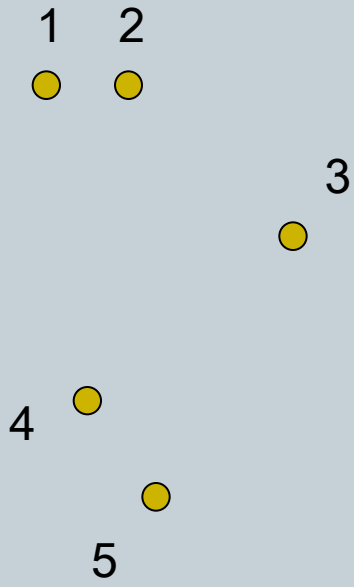
- Let distance $d_{ij}$ between clusters $C_i$ and $C_j$ be

$$d_{ij} = \frac{1}{|C_i||C_j|} \sum_{p \in C_i, q \in C_j} d_{pq},$$

that is, the average distance between points (species) in the cluster.

# UPGMA algorithm

- Initialisation
  - Assign each point $i$ to its own cluster $C_i$
  - Define one leaf for each sequence, and place it at height zero
- Iteration
  - Find clusters $i$ and $j$ for which $d_{ij}$ is minimal
  - Define new cluster $k$ by $C_k = C_i \cup C_j$, and define $d_{kl}$ for all $l$
  - Define a node $k$ with children $i$ and $j$. Place $k$ at height $d_{ij}/2$
  - Remove clusters $i$ and $j$
- Termination:
  - When only two clusters $i$ and $j$ remain, place root at height $d_{ij}/2$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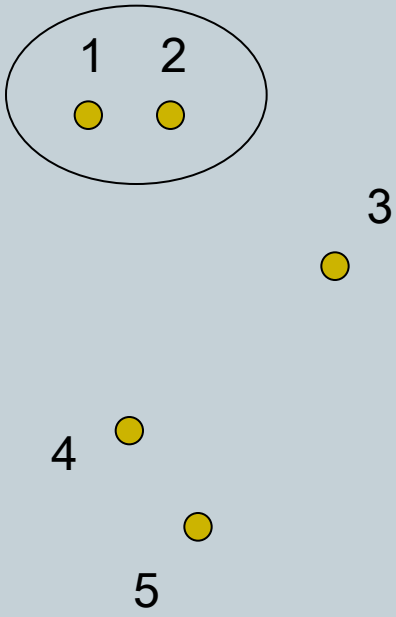
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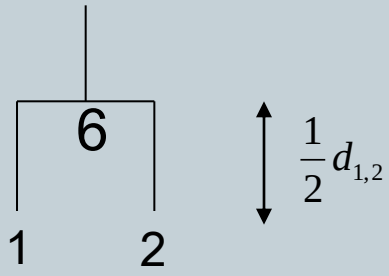
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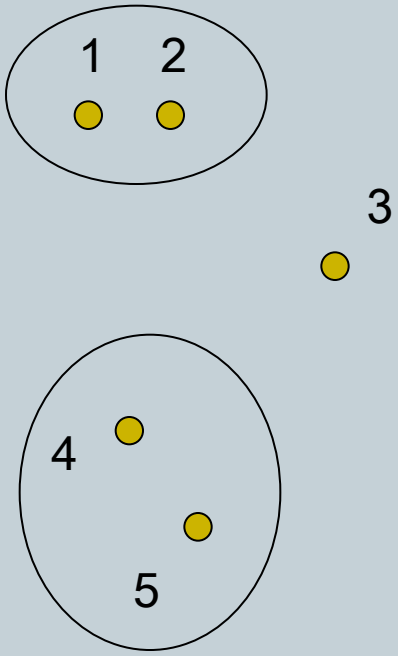
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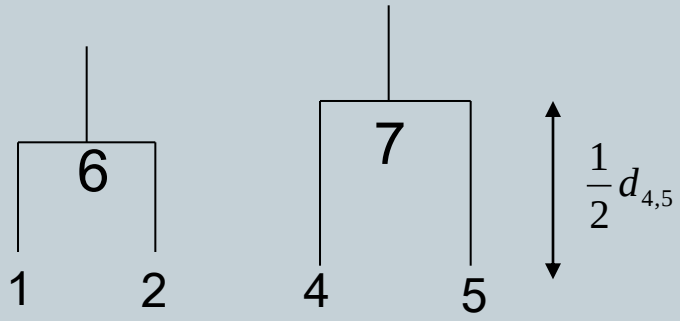
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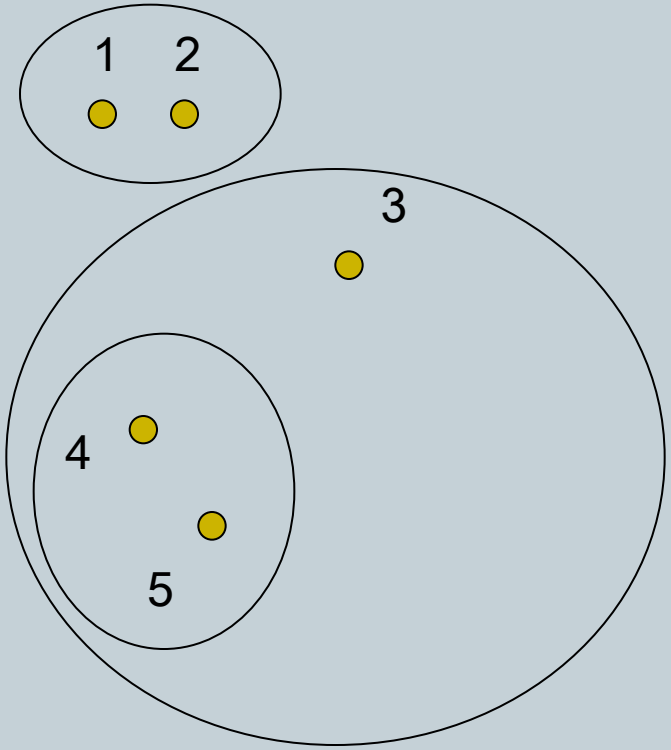
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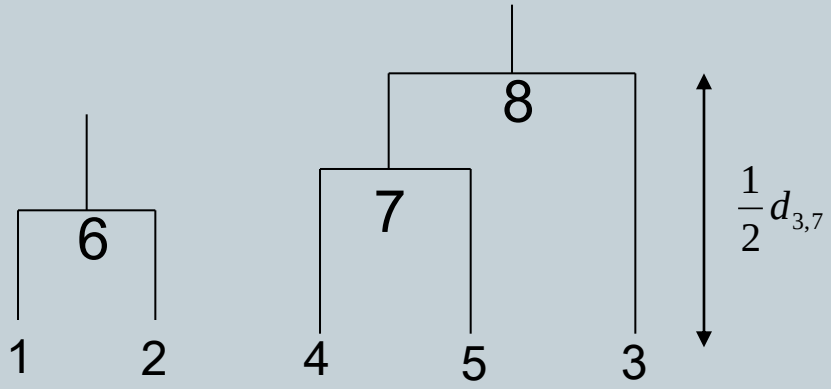
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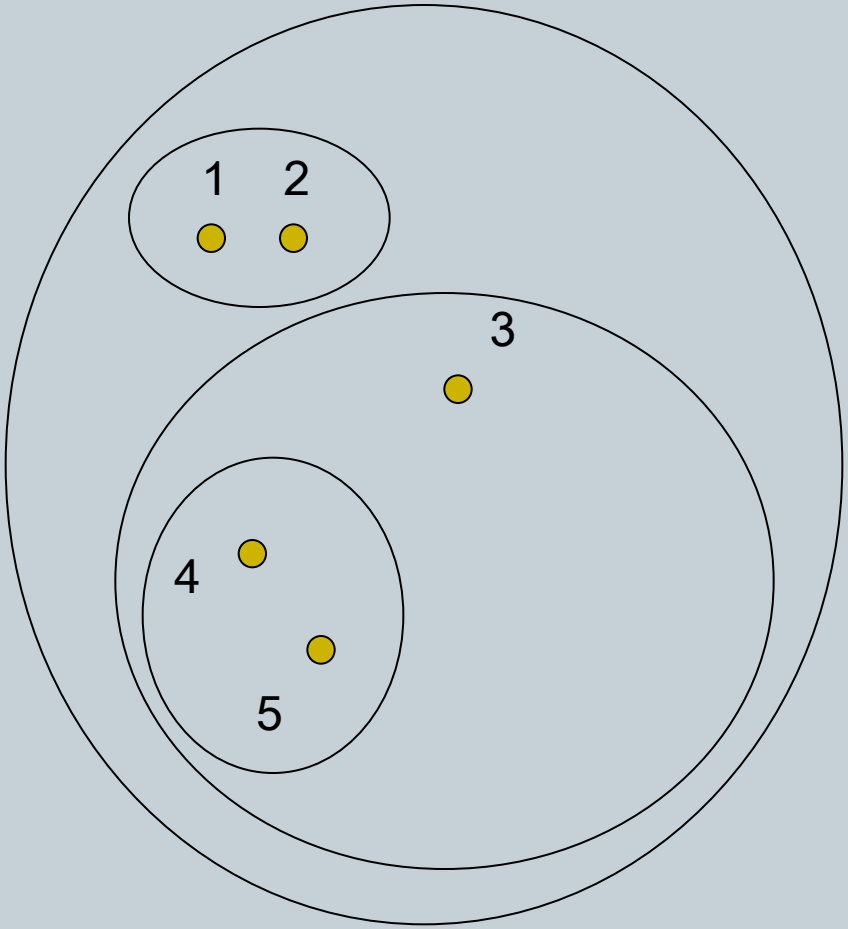
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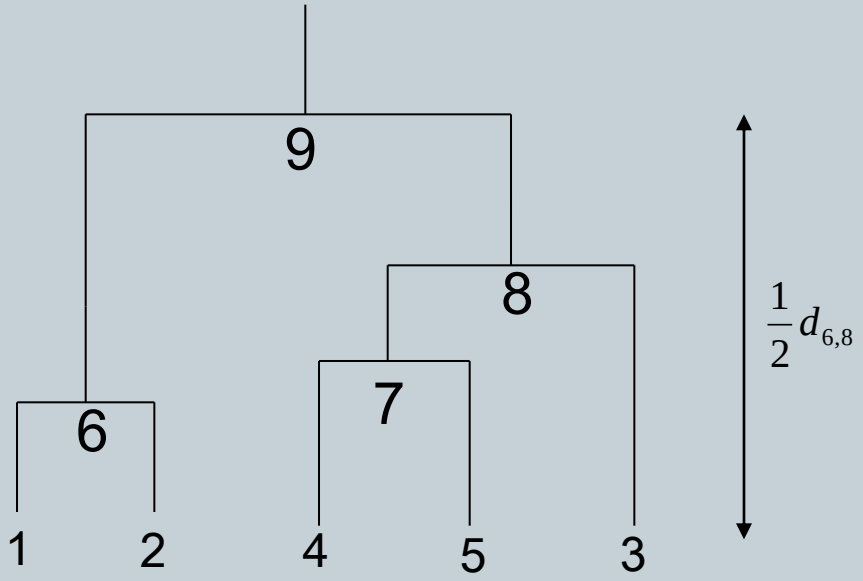
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$\frac{1}{2}d_{6,8}$

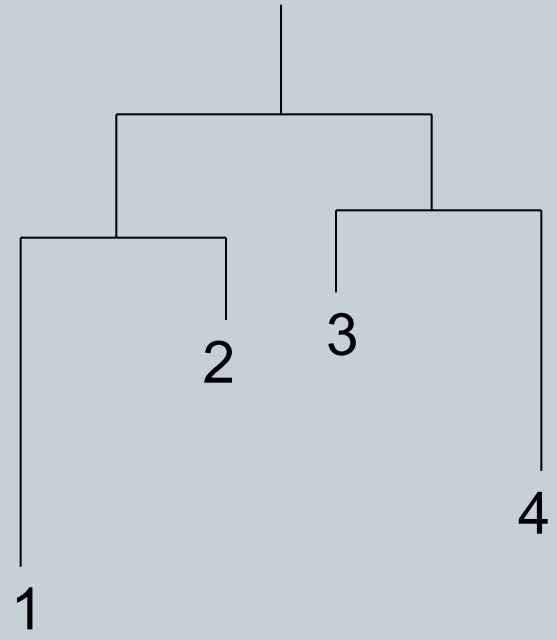
# UPGMA implementation

- In naive implementation, each iteration takes $O(n^2)$ time with $n$ sequences => algorithm takes $O(n^3)$ time
- The algorithm can be implemented to take only $O(n^2)$ time (see Gronau & Moran, 2006, for a survey)

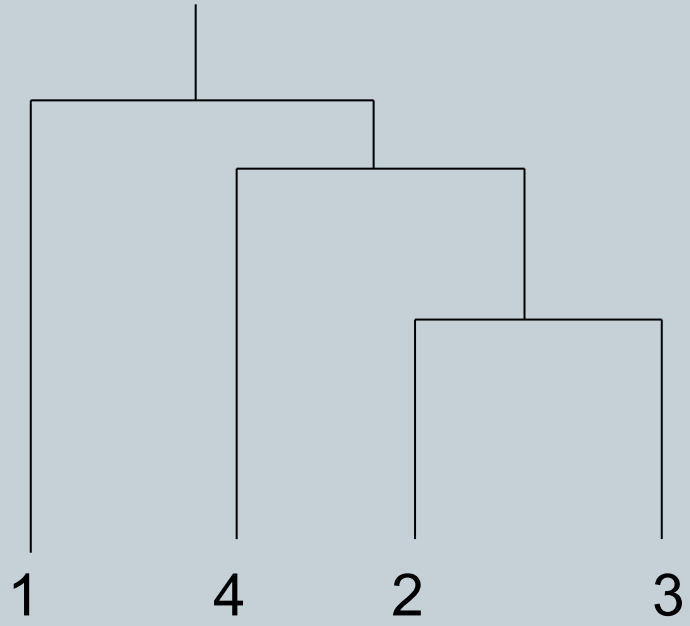
# Problem solved?

- We now have a simple algorithm which finds a ultrametric tree
  - If the data is ultrametric, then there is exactly one ultrametric tree corresponding to the data (proof left as an exercise)
  - The tree found is then the ”correct” solution to the phylogeny problem, if the assumptions hold
- Unfortunately, the data is not ultrametric in practice
  - Measurement errors distort distances
  - *Basic assumption of a molecular clock does not hold usually very well*

# Incorrect reconstruction of non-ultrametric data by UPGMA




Tree which corresponds to non-ultrametric distances




Incorrect ultrametric reconstruction by UPGMA algorithm