Distance-based methods for the construction of phylogenetic trees

Lecture 16

Problem 1

- How to measure distance between 2 DNA molecules so it reflects the time since they have separated from a common ancestor?

The relative distance between genomes can be based on the number of mutational events

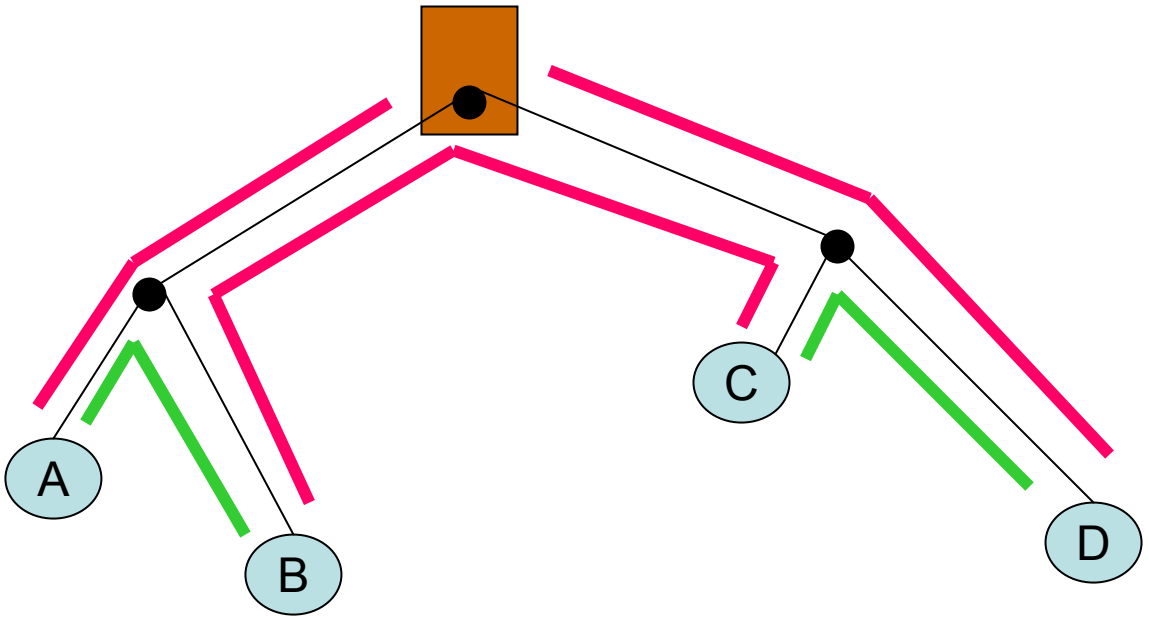
- Non computational: the melting temperature of DNA hybrids
- Computational
  - Based on DNA or protein sequences
    - Edit distance – based on point mutations
  - Gene-sequence based
    - Alignment traces – align chromosomes from the different species, connect homologous genes by an edge. The number of crosses can be used as an evolutionary distance
    - Number of breakpoints
    - Reversals distance
    - Transpositions distance
- Better to combine different events

Problem 2

- Given a set of pairwise distances, find the best tree for a given data

Additive distances

- Distances that fit onto some tree are called *additive*. To determine if the distances are additive use *the four point criterion:*

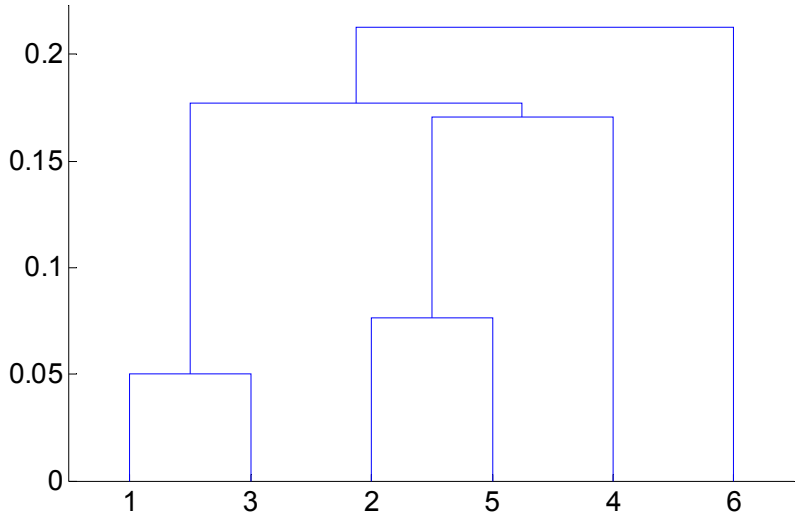


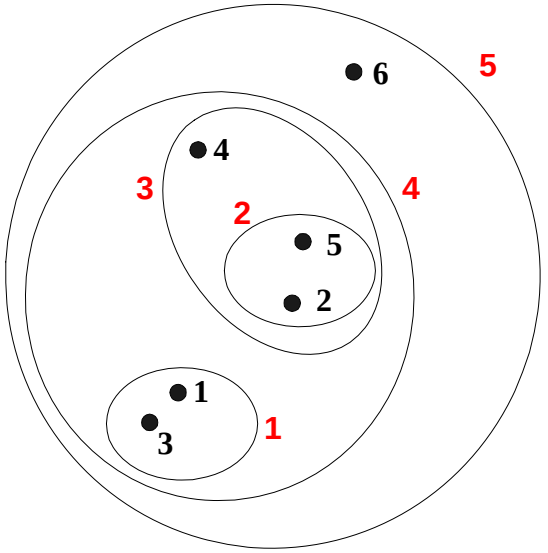
1. The sums of pairwise distances that traverse the trunk are equal
2. The sum of distances that traverse the trunk is >= the sum of remaining distances

$$d_{AD}+d_{BC}=d_{BD}+d_{AC}>=d_{AB}+d_{CD}$$

Hierarchical Clustering

- Produces a set of nested clusters organized as a hierarchical tree
- Can be visualized as a dendrogram
  - A tree like diagram that records the sequences of merges or splits





Hierarchical Clustering

- Start with the points as individual clusters
- At each step, merge the closest pair of clusters until only one cluster left.

Algorithm

Let each data point be a cluster

Compute the distance matrix

Repeat

Merge the two closest clusters

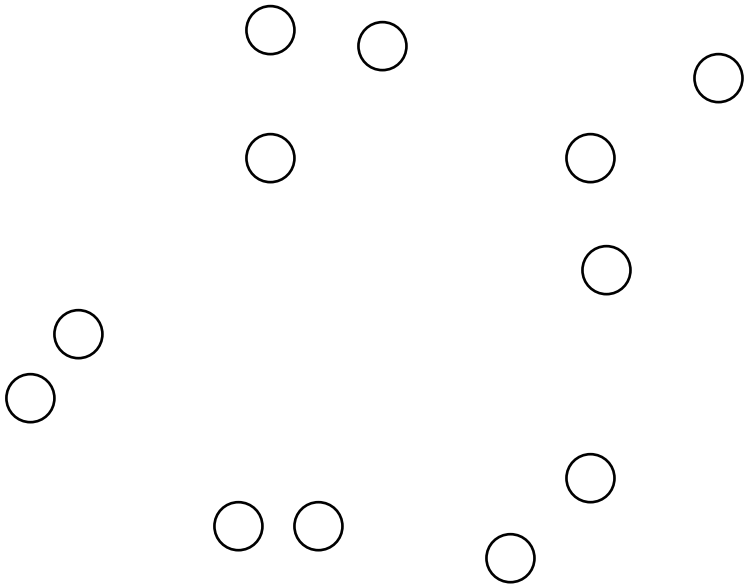
Update the distance matrix

Until only a single cluster remains

- Key operation is *the computation of the distance of two clusters.*

Starting Situation

- Start with clusters of individual points and a distance matrix

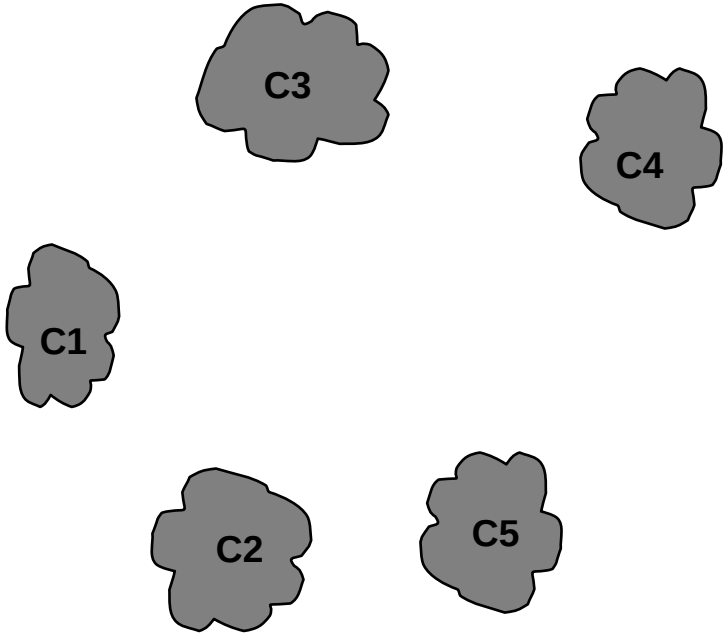


	p1	p2	p3	p4	p5	. . .
p1						
p2						
p3						
p4						
p5						
.						
.						
.						

Distance Matrix

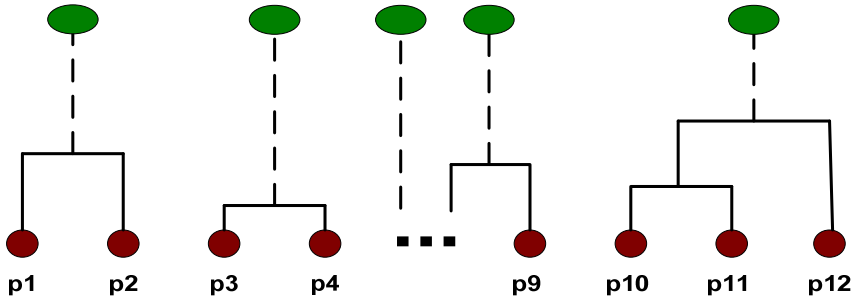
Intermediate Situation

- After some merging steps, we have some clusters



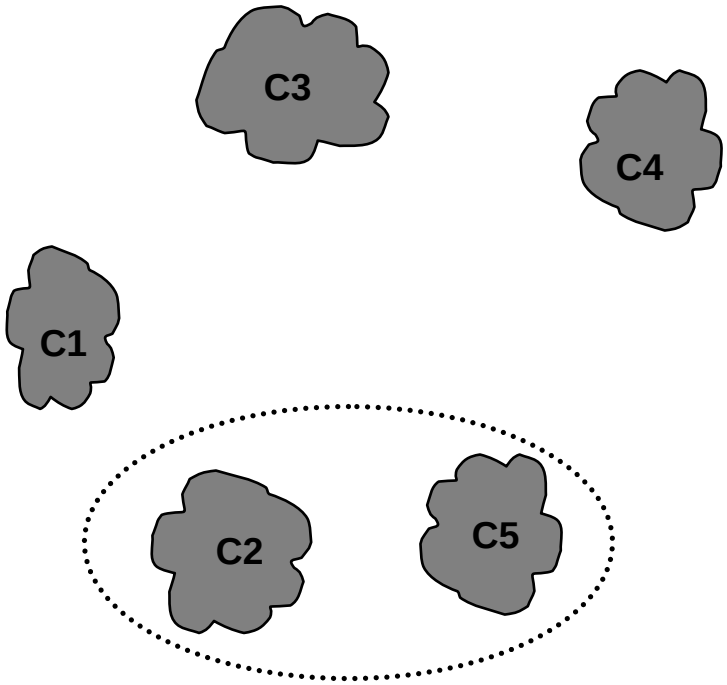
	C1	C2	C3	C4	C5
C1					
C2					
C3					
C4					
C5					

Distance Matrix



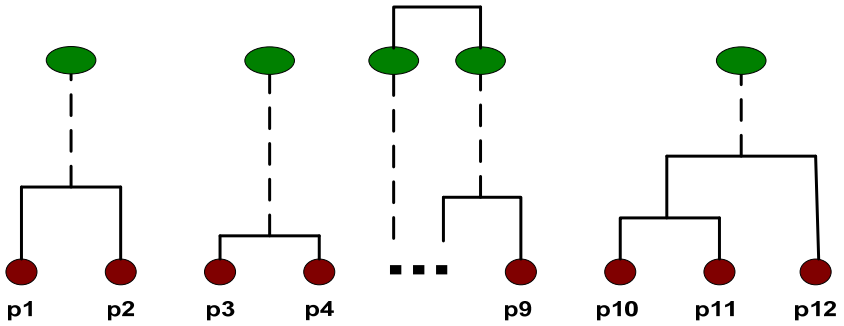
Intermediate Situation

- We want to merge the two closest clusters (C2 and C5) and update the distance matrix.



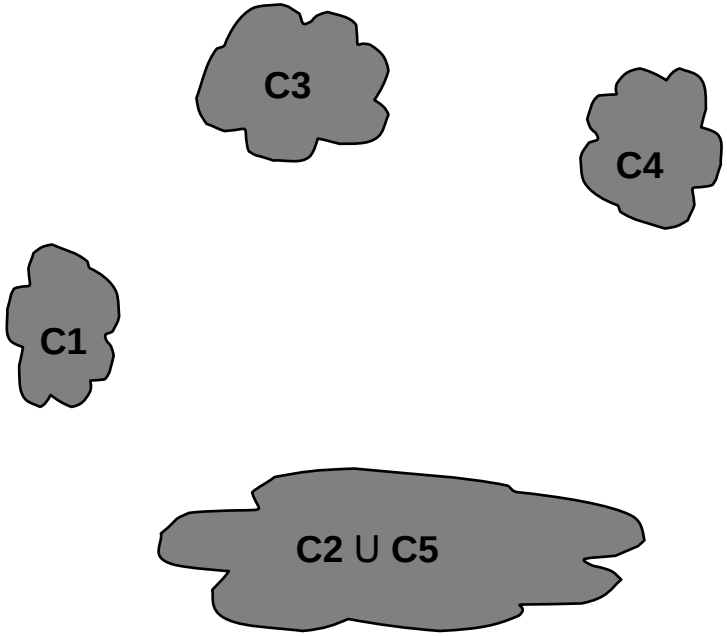
	C1	C2	C3	C4	C5
C1					
C2					
C3					
C4					
C5					

Distance Matrix



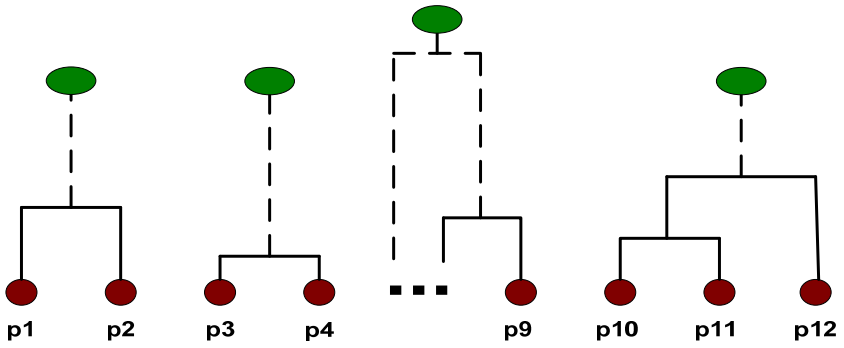
After Merging

- The question is "How do we update the distance matrix for clusters?"

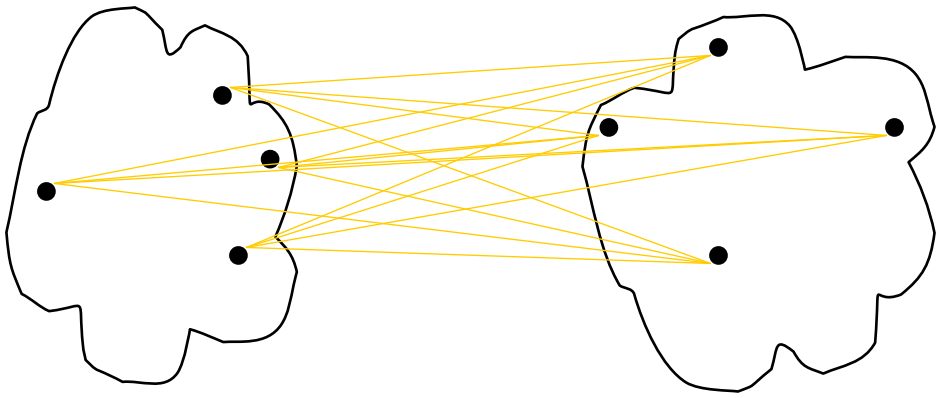


	C1	C2 U C5	C3	C4
C1		?		
C2 U C5	?	?	?	?
C3		?		
C4		?		

Distance Matrix



How to Define Inter-Cluster Distance



- MIN
- MAX
- Group Average –UPGMA:
  Unweighted **P**air-**G**roup **M**ethod using an arithmetic **A**verage

	p1	p2	p3	p4	p5	. . .
p1						
p2						
p3						
p4						
p5						
.						

.

.

Distance Matrix

Cluster Distance: Group Average

- Distance between two clusters is the average of all pairwise distances between points in the two clusters.

$$\mathbf{distance}(\mathbf{Cluster}_i, \mathbf{Cluster}_j) = \frac{\sum_{\substack{\mathbf{p}_i \in \mathbf{Cluster}_i \\ \mathbf{p}_j \in \mathbf{Cluster}_j}} \mathbf{distance}(\mathbf{p}_i, \mathbf{p}_j)}{|\mathbf{Cluster}_i| * |\mathbf{Cluster}_j|}$$

Example

	Human	Chimpanzee	Gorilla	Orangutan	Gibbon
Human					
Chimpanzee	1				
Gorilla	4	2			
Orangutan	8	7	5		
Gibbon	10	9	2	9	

The distances are determined based on the melting temperature of the DNA hybrids (mitochondrial DNA)

Distance matrix

Distance matrix

	A	B	C	D	E
A					
B	1				
C	4	3			
D	8	7	2		
E	10	9	4	6	

Are the distances additive?

ABCD

AC+BD=BC+AD=11>AB+CD=3

ABCE

AC+BE=AE+BC=13>AB+CE=5

ACDE

AD+CE=AE+CD=12>AC+DE=10

BCDE

BD+CE=CD+BE=11>BC+DE=9

	A	B	C	D	E
A					
B	1				
C	4	3			
D	8	7	2		
E	10	9	4	6	

Yes, the distances are additive, we can build the tree

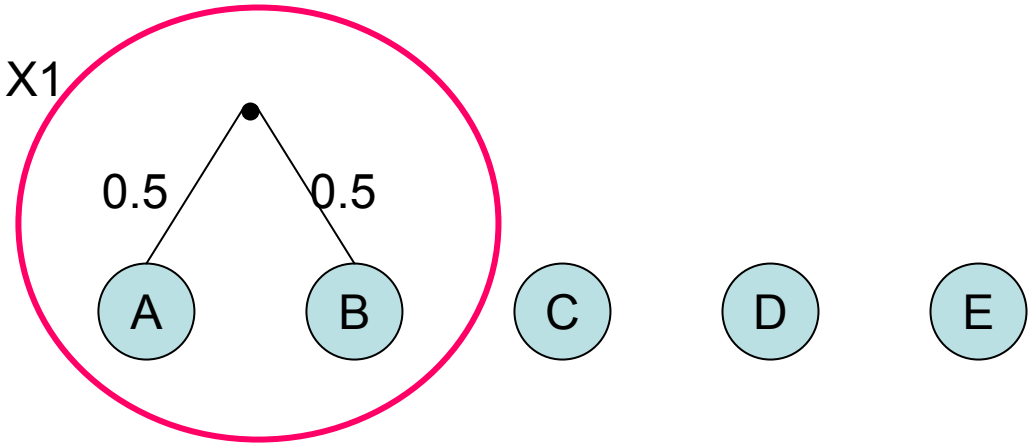
UPGMA – demo 1

	A	B	C	D	E
A					
B	1				
C	4	3			
D	8	7	2		
E	10	9	4	6	

Basic clusters – distance 0 between the elements of each cluster

UPGMA – demo 2

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0

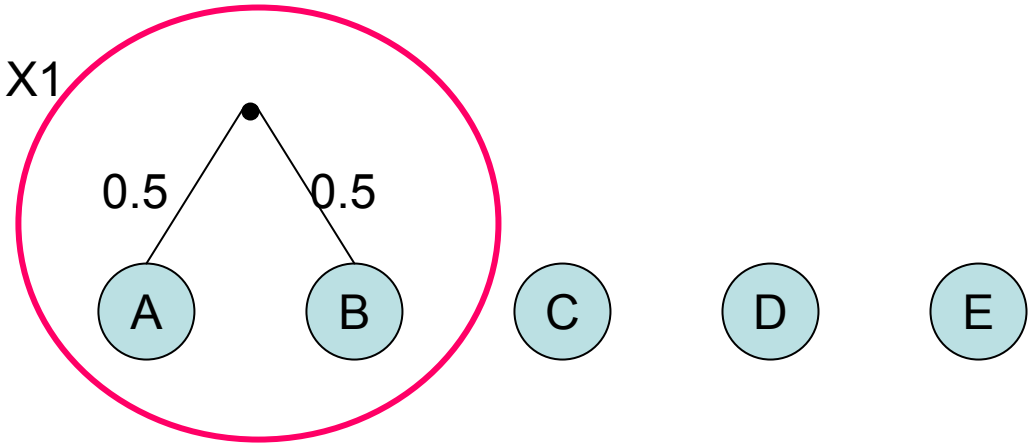


Form cluster X1 with min distance between two points

UPGMA – demo 2

	X1	C	D	E
X1	0			
C	3.5	0		
D	7.5	2	0	
E	9.5	4	6	0

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0



$dX_1C=(dAC+dBC)/(2)*1=3.5$

$dX_1D=(dAD+dBD)/(2)*1=7.5$

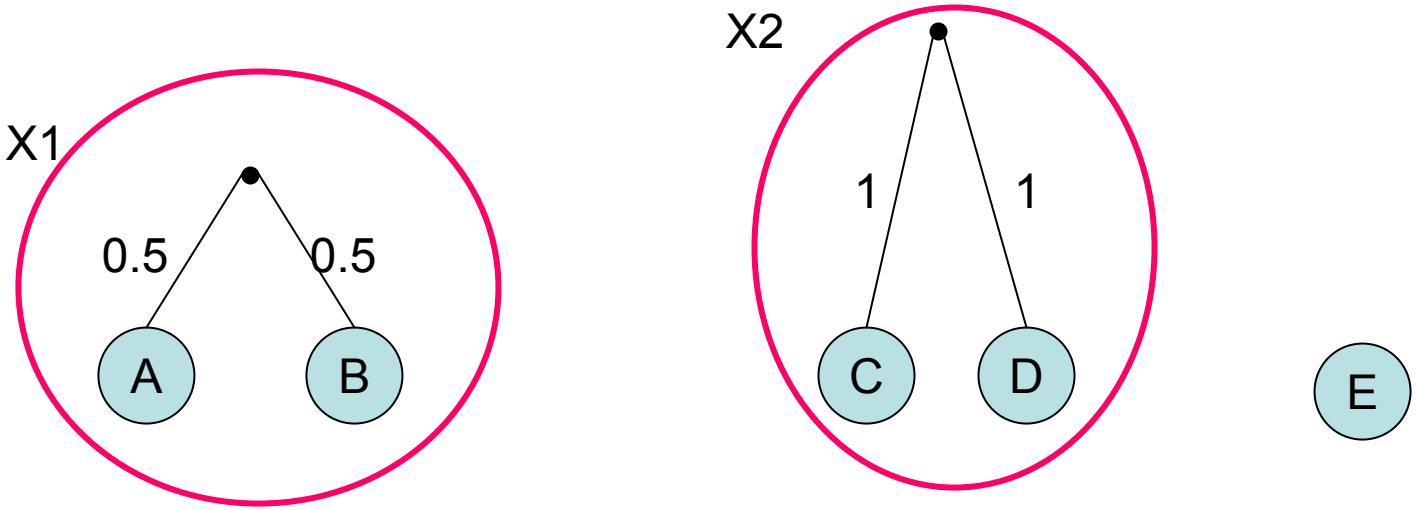
$dX_1E=(dAE+dBE)/(2)*1=9.5$

Update distance matrix

UPGMA – demo 3

	X1	C	D	E
X1	0			
C	3.5	0		
D	7.5	2	0	
E	9.5	4	6	0

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0



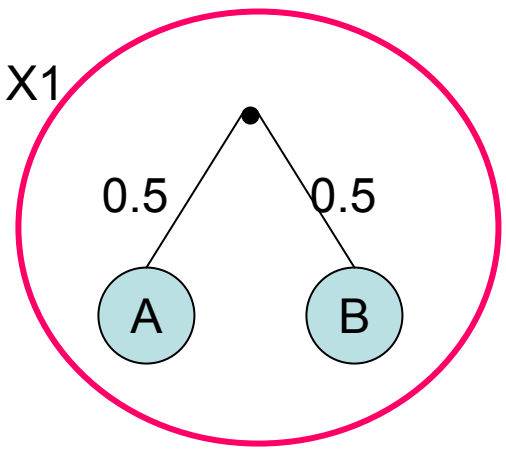
Form cluster X2 with min distance between two points

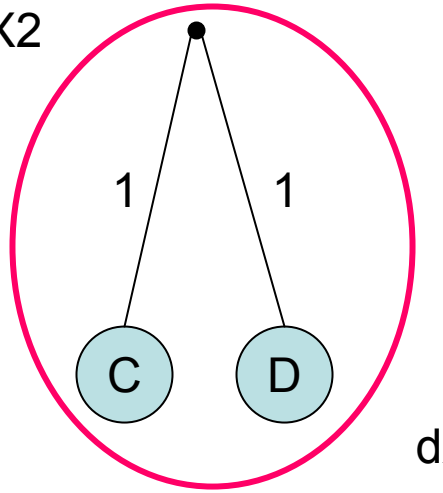
UPGMA – demo 3

	X1	X2	D
X1	0		
X2	5.5	0	
E	9.5	5	0

	X1	C	D	E
X1	0			
C	3.5	0		
D	7.5	2	0	
E	9.5	4	6	0

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0





$dX_2E=(dCE+dDE)/(2)*1=5$

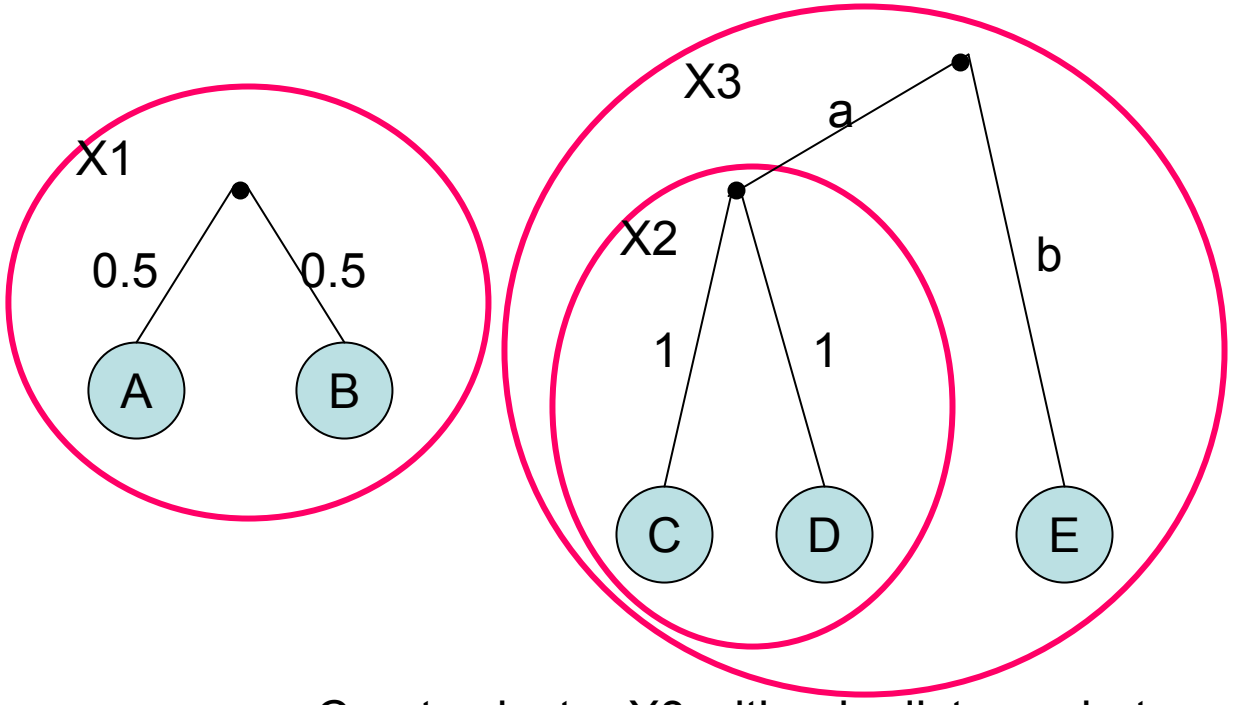
$dX_1X_2=(dAC+dBC+dAD+dBD)/(2*2)=5.5$

Update distance matrix

UPGMA – demo 4

	X1	X2	D
X1	0		
X2	5.5	0	
E	9.5	5	0

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0



a=1/2*5-1=1.5

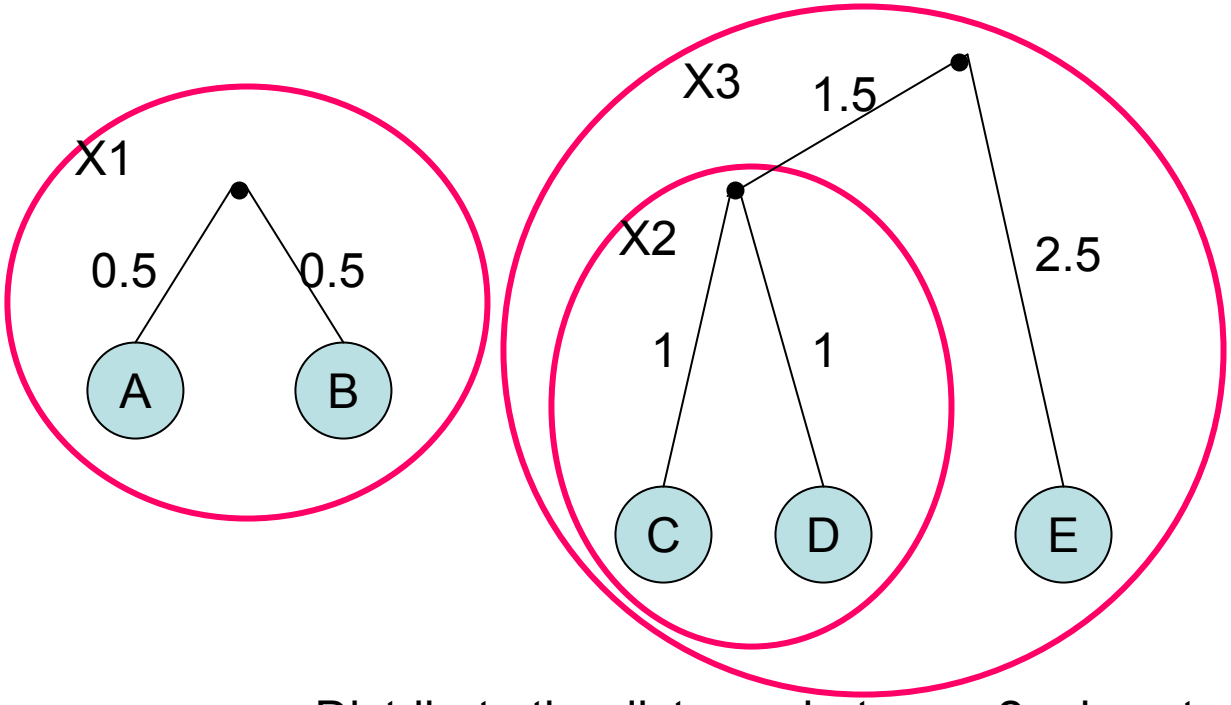
b=1/2*5=2.5

Create cluster X3 with min distance between 2 clusters

UPGMA – demo 4

	X1	X2	D
X1	0		
X2	5.5	0	
E	9.5	5	0

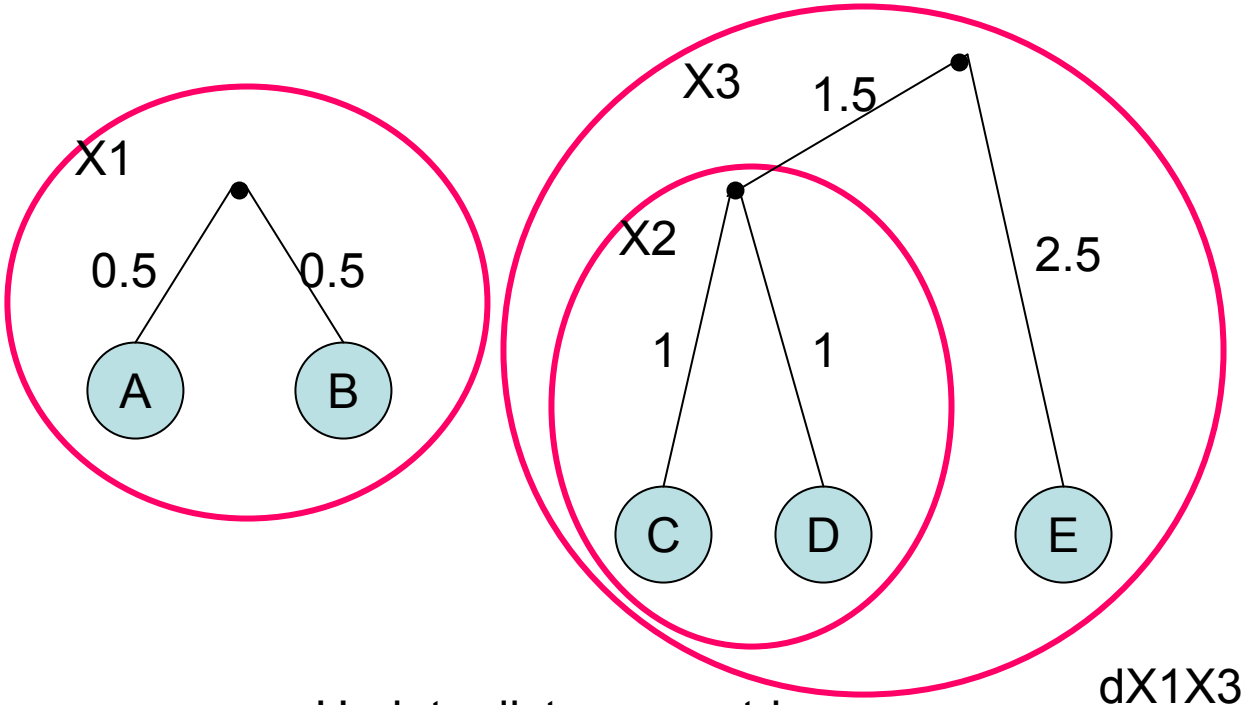
	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0



Distribute the distance between 2 edges to have half of this distance from the root to leaves in both branches

UPGMA – demo 4

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0



	X1
X1	0
X3	6.8

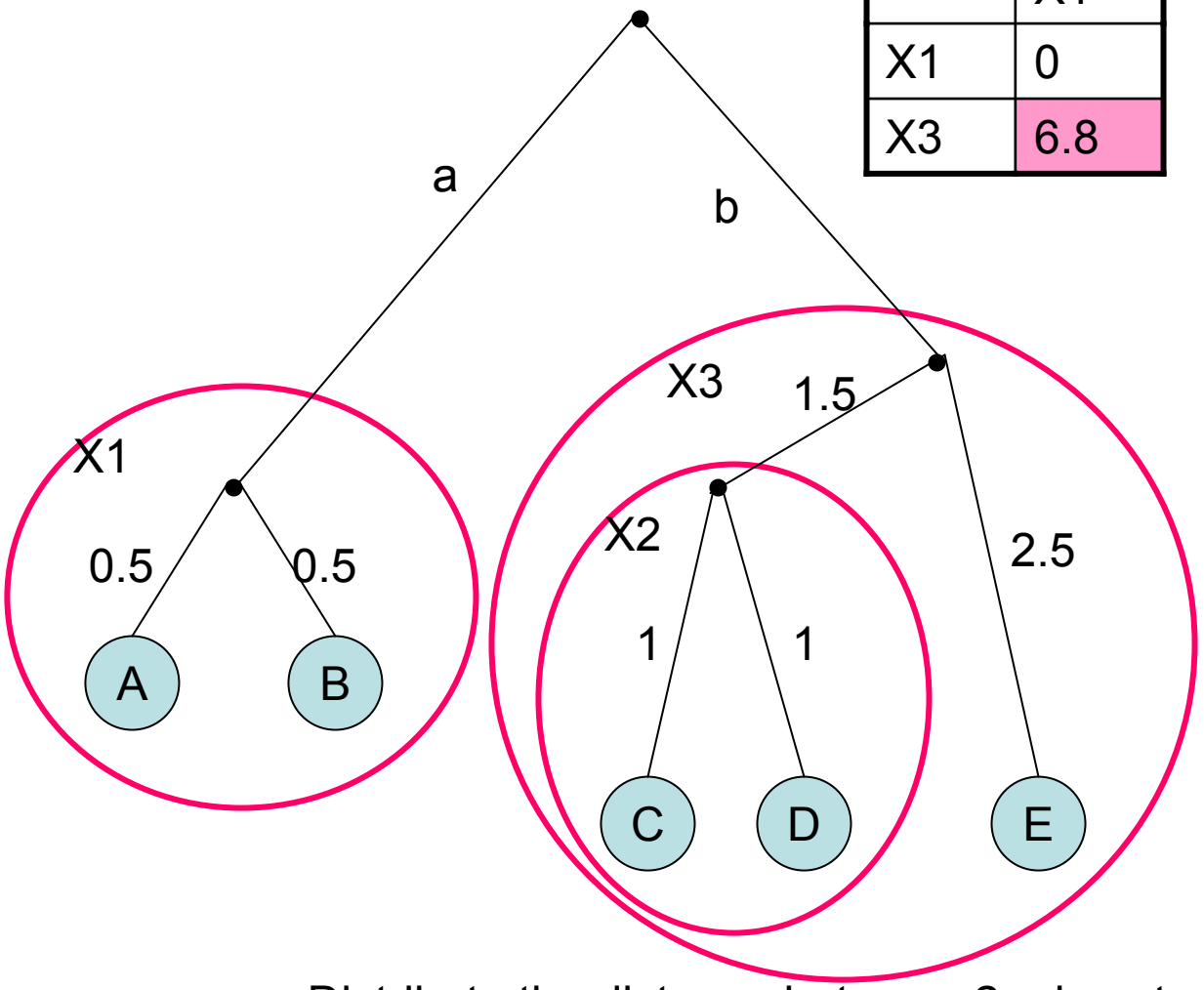
Update distance matrix

dX1X3=(dAC+dAD+dAE+dBC +dBD+dBE)/2*3=(4+8+10+3+7 +9)/6=6.8

UPGMA – demo 4

	X1
X1	0
X3	6.8

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0

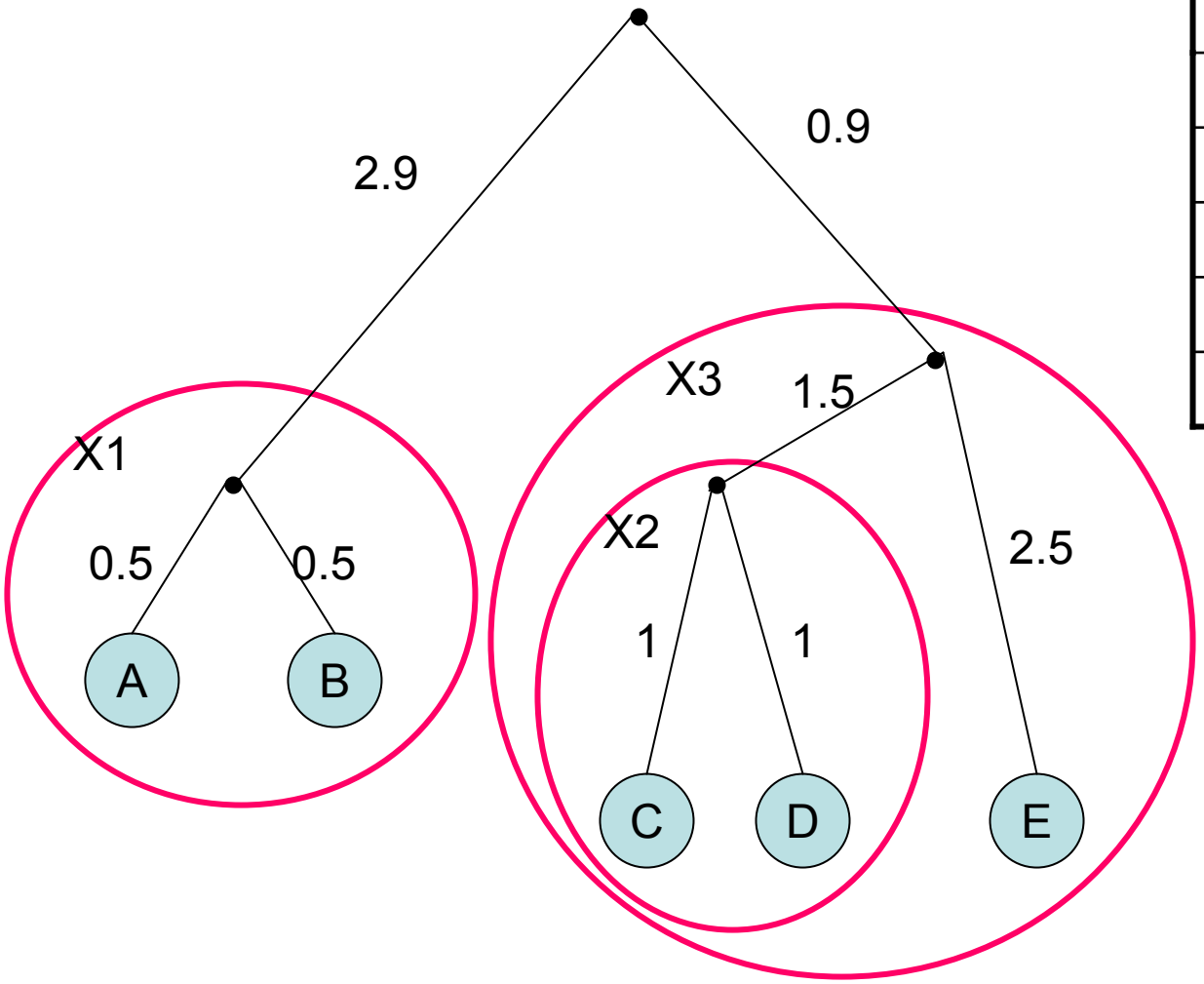


a=6.8/2-0.5=2.9

b=6.8/2-2.5=0.9

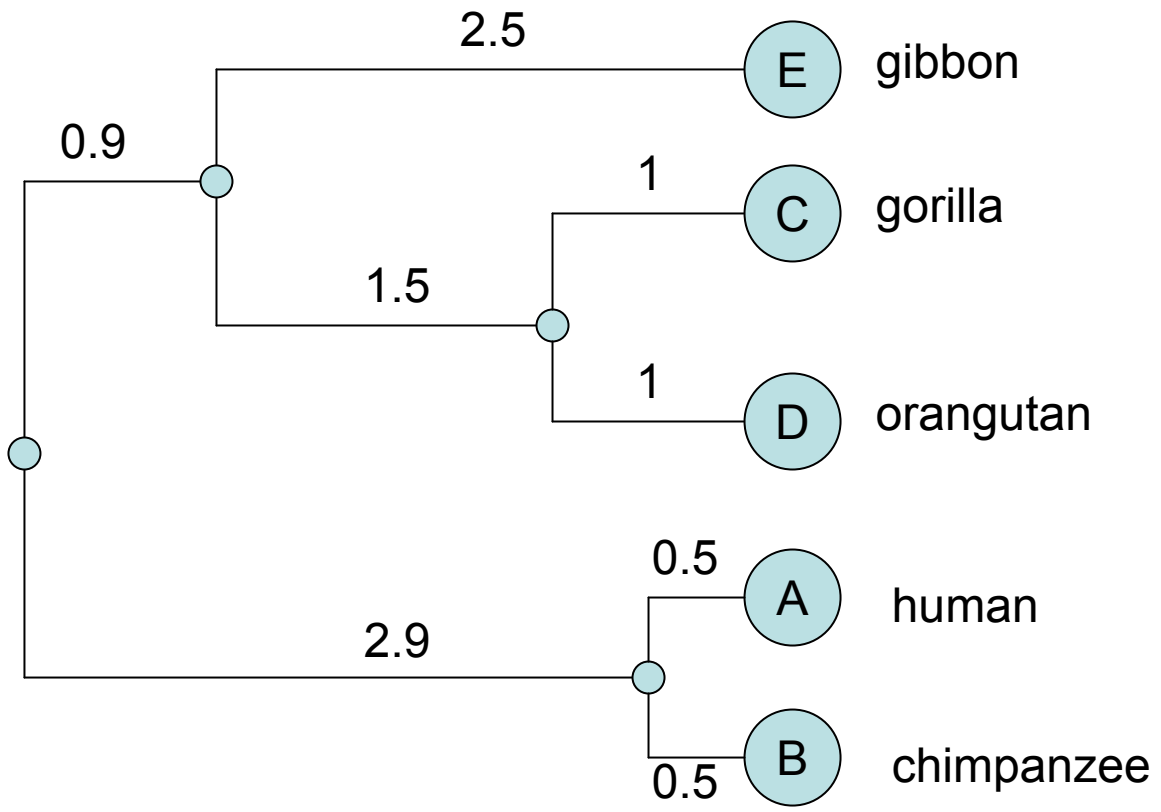
Distribute the distance between 2 edges to have half of this distance from the root to leaves in both branches

UPGMA – the resulting tree

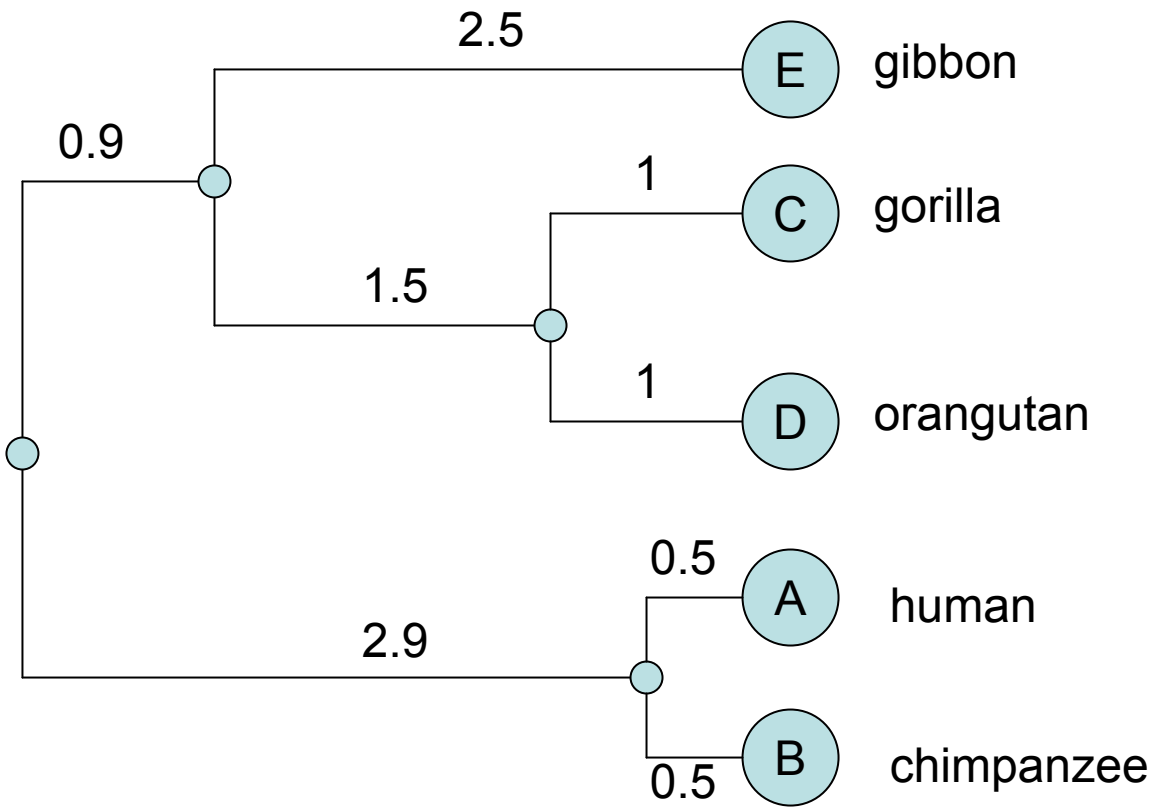


	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0

The resulting tree with distances



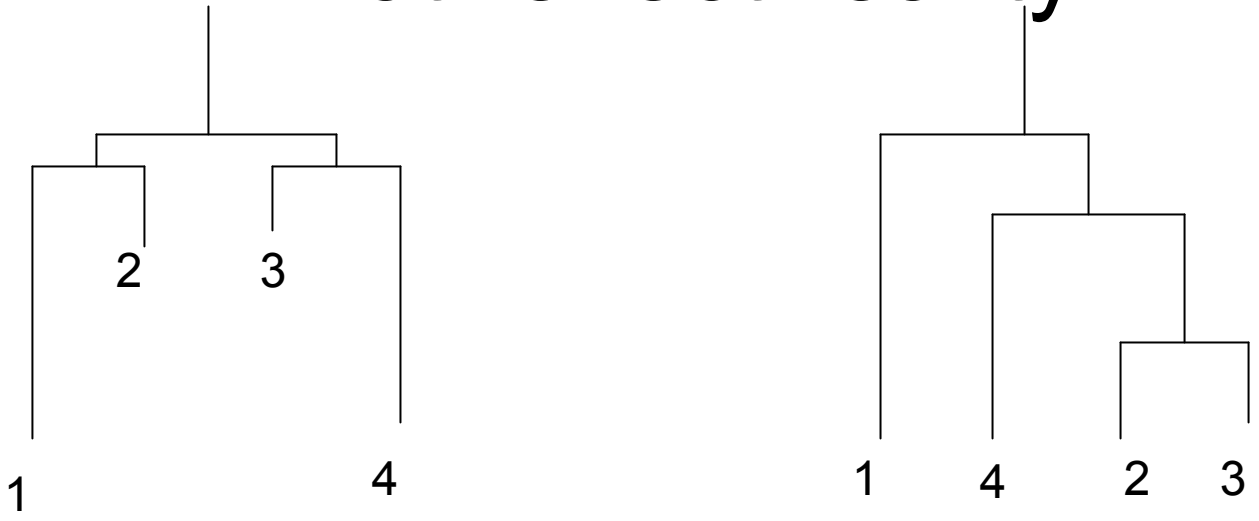
Molecular clock



The edge lengths can be viewed as times measured by *molecular clock* with a constant rate of mutational events.

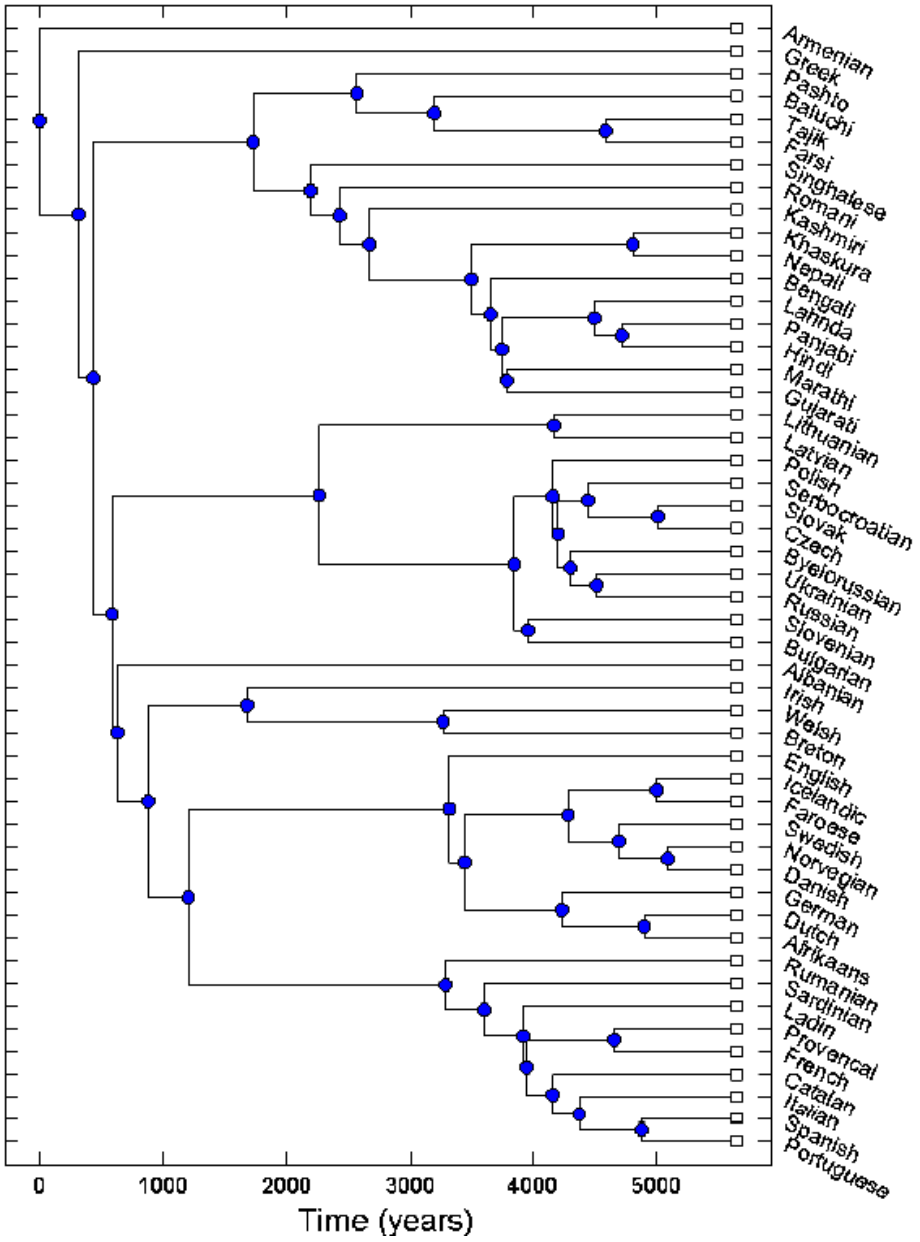
We assume that divergence occurred at the same time at all branching points, the sum of edge lengths from any node to the leaf is the same for any possible path

When the constructed tree does not reflect reality



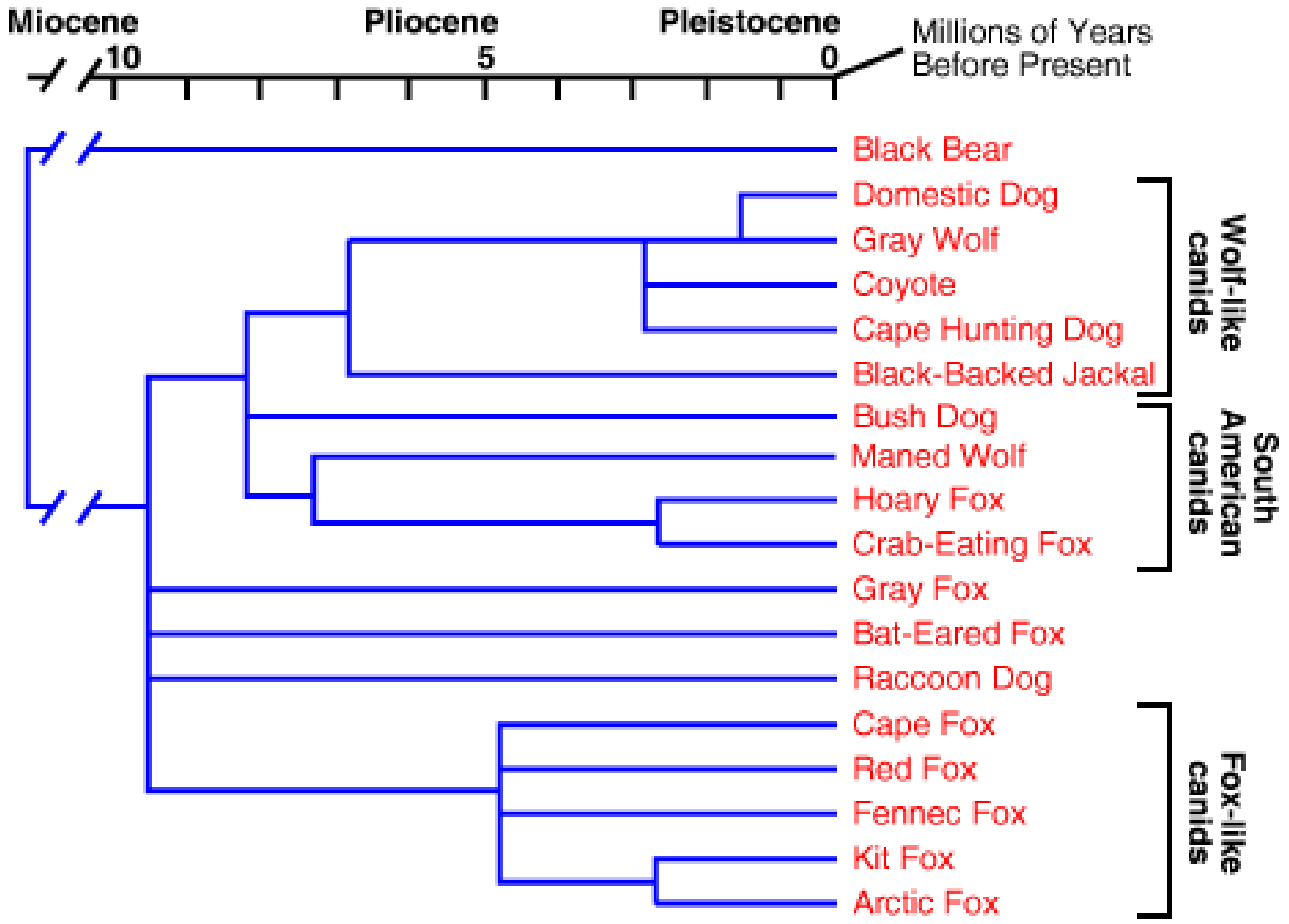
- If the original tree, which we try to reconstruct, had different path lengths to its leaves, it may be reconstructed incorrectly by UPGMA. In this case, the closest leaves (2,3) are not siblings and they do not have a common parent, which will be assigned to them by UPGMA

Tree Example 1

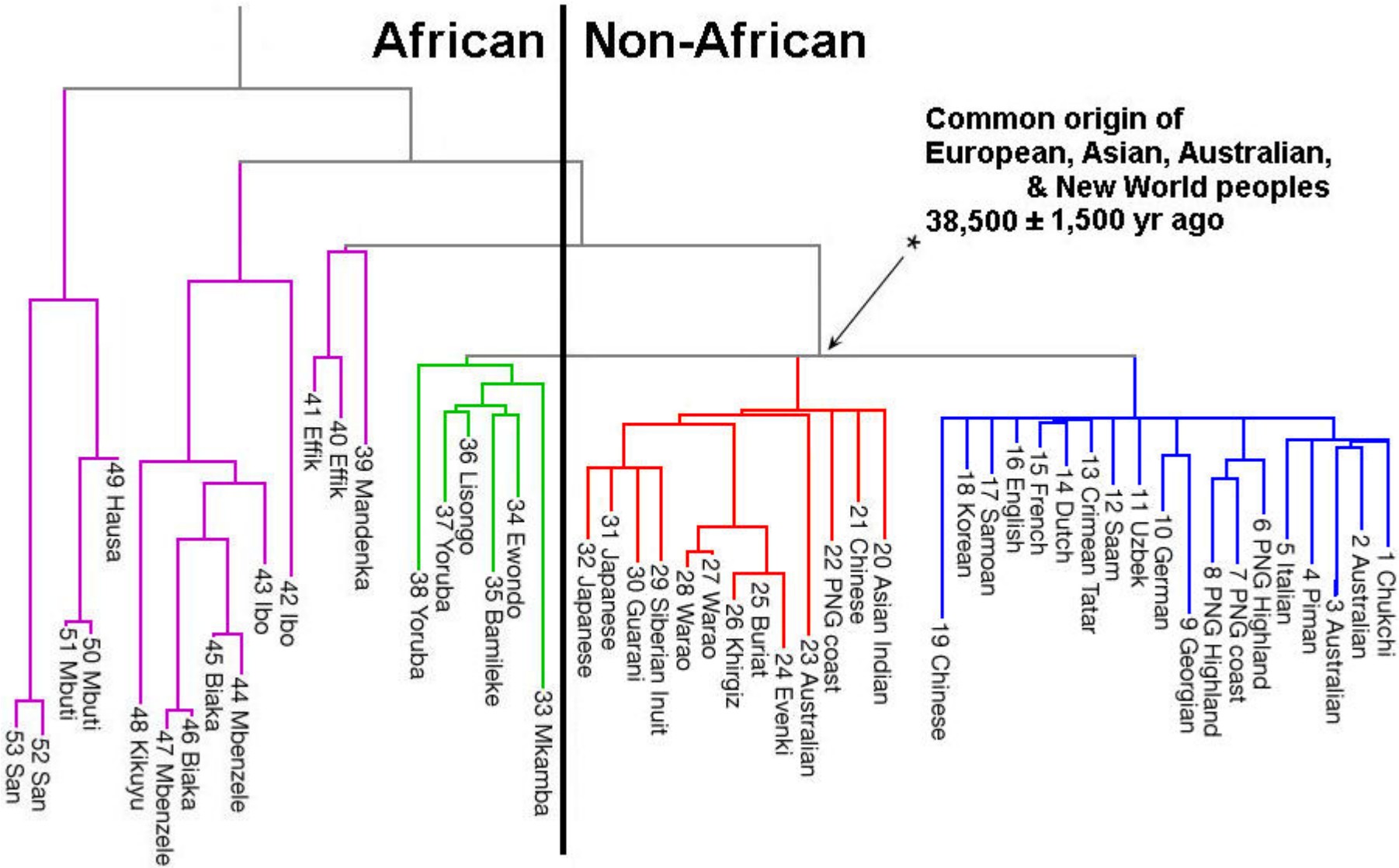


From
"Indo-European languages tree by Levenshtein distance"
by M. Serva1 and F. Petroni

Tree Example 2

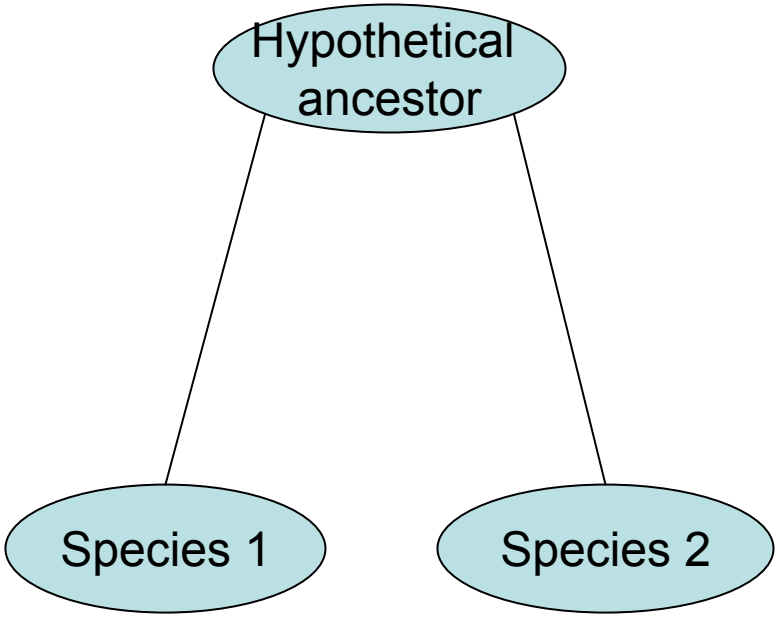


Tree Example 3



Phylogenetics - reminder

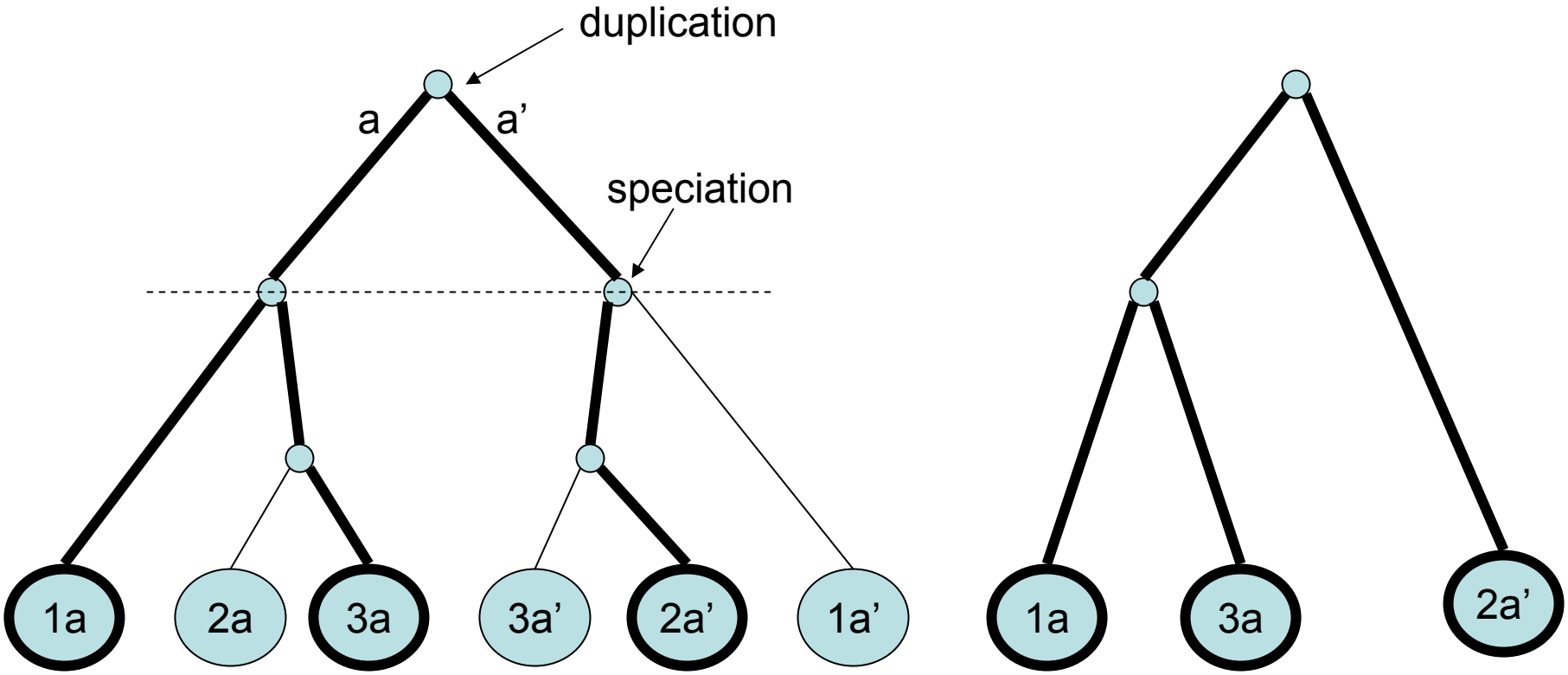
- Inferring phylogenies from a given data set



Homologous sequences (genes)

- Similar genes (encoding for some similar proteins) are called homologs
  - Orthologs – a single gene which is similar in 2 different species; implies the common ancestor
  - Paralogs – a pair of similar genes in the same organism, the result of gene duplication
  - Xenologs – a pair of similar genes imported by transposon (horizontal transfer)

Identifying orthologs can be hard



By picking for comparison genes 1a, 3a and 2a’, we construct an incorrect phylogenetic tree, where species 1 and 3 are closer to each other than to species 2, when this is not really the case

Two approaches to inferring phylogenies

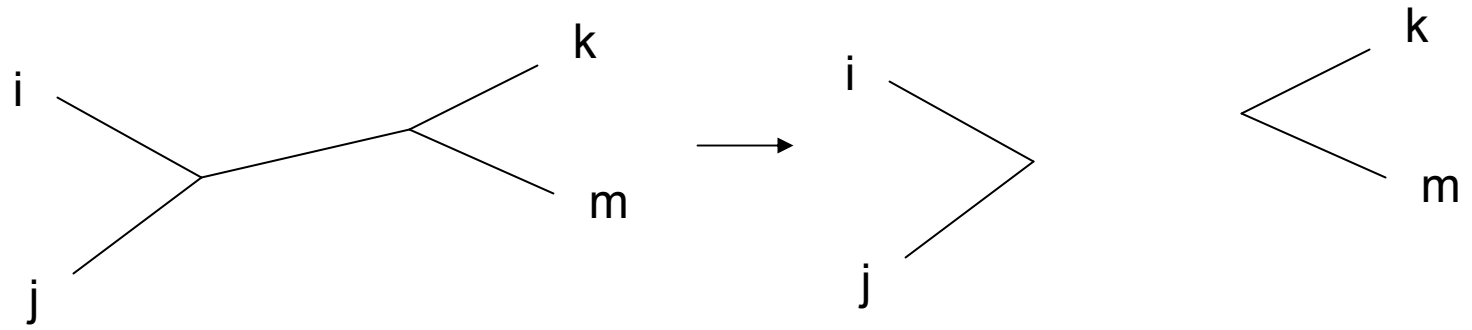
- Distance-based: for example paiwise edit distance, score of pairwise alignment etc.
- Character-based: examine each character separately for any given site in biological sequences

Distance-based - reminder

- Input: distance matrix of pairwise distances for N species
- Goal: find a tree *consistent* with the distance matrix. This means that the sum of edge lengths connecting each pair of leaves *ij* corresponds to a distance M*ij*

Additivity of distances

- For any 4 objects, i,j,k,m, there are 3 different sums of 2 distances each:
- Dij+Dkm, Dik+Djm, Dim+Djk
- From these 3 sums, 2 should be equal and greater than the third – this will allow to group the smallest pair into separate subtrees

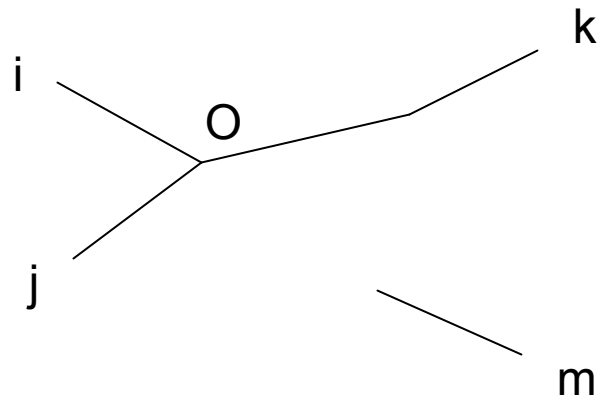


The distances are additive iff for any 4 objects there exits the following combination: Dij+Dkm<(Dim+Djk=Dik+Djm)

Why the distances have to be additive

For 3 objects, any set of distances is OK:

Let iO=a, jO=b, kO=c, then Dij=a+b, Dik=a+c, Djk=b+c

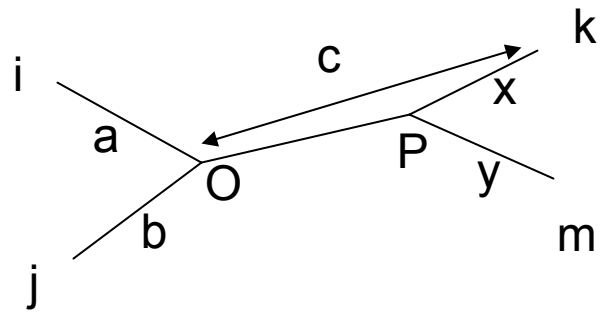


Why the distances have to be additive

Let iO=a, jO=b, kO=c

Dij=a+b, Dik=a+c, Djk=b+c

We are adding the fourth object, m, to an arbitrary position P in the tree.
Let mP=y, and kP=x, then OP=c-x



Then:

Dim+Djk=a+(c-x)+y + b+c=a+b+y+(2c-x)

Dik+Djm=a+c + b+(c-x)+y=a+b+y+(2c-x)

and

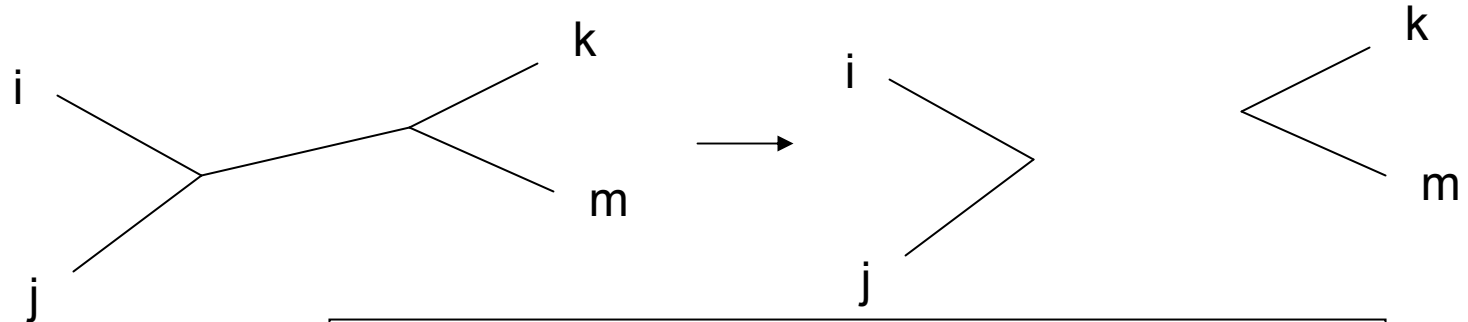
Dij+Dkm=a+b + x+y=a+b+y +x

2c-x>=x, since 2c>=2x, therefore

Dij+Dkm<=Dim+Djk=Dik+Djm

The rule of additivity

- The distances are additive if, for any 4 objects, i,j,k,m,



Dij+Dkm<(Dim+Djk=Dik+Djm)

If the distances are not additive, we CANNOT construct a phylogenetic tree

Distance-based phylogeny problem

- Input: distance matrix of pairwise distances for N species
- Goal: find a tree consistent with the distance matrix. This means that the sum of edge lengths connecting each pair of leaves ij corresponds to a distance Mij

UPGMA algorithm - summary

- Initialization:
  - Create N clusters, 1 species per cluster
  - Set the size of each cluster to 1
  - Create leaf for each cluster
- Iteration (until only 1 cluster left)
  - Find Ci and Cj with min dCiCj
  - Create a new cluster C(ij) which has $n_{(ij)}=n_i+n_j$ members
  - Connect Ci and Cj through a new parent node and set the distance from this new parent node to the leaf node of each cluster to ½ d_{CiCj}
  - Delete columns and rows that correspond to amalgamated clusters i and j
  - Add a column and a row for a new cluster
  - Compute distances from a new cluster C(ij) to all remaining clusters:

$$dC_{(ij)}C_k=(\Sigma_{\text{all } x\in C(ij),\ \text{all } y\in Ck}\ d_{xy})/(n_{(ij)}*n_k)$$

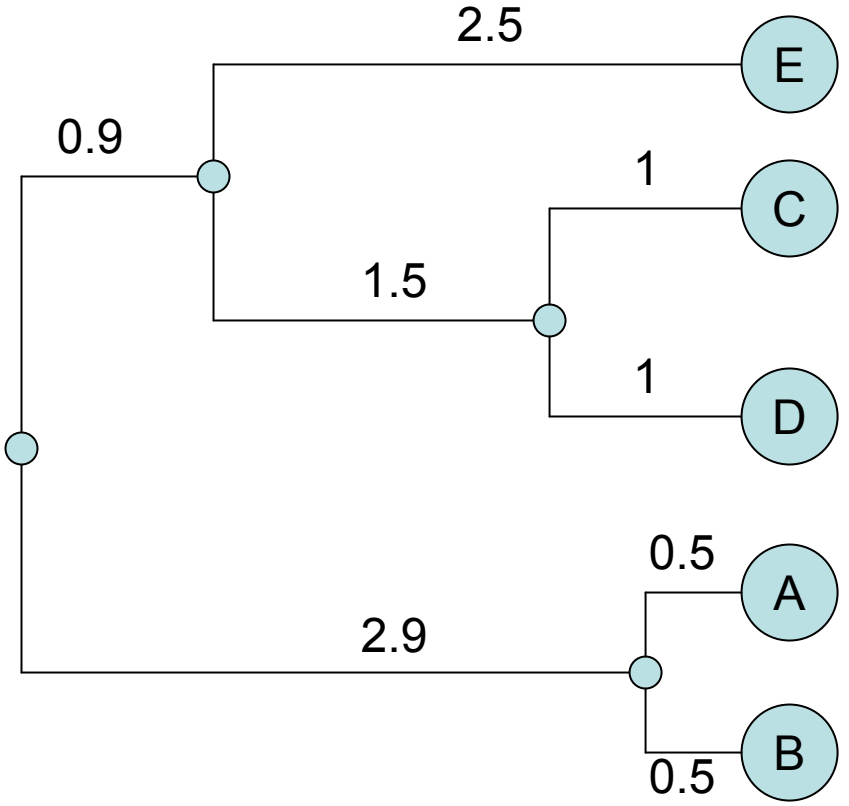
What was the goal?

- Input: distance matrix of pairwise distances for N species
- Goal: find a tree consistent with the distance matrix. This means that **the sum of edge lengths connecting each pair of leaves ij corresponds to a distance Mij**

UPGMA tree – is it consistent with M?

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0

	A	B	C	D	E
A	0				
B	1	0			
C	6.8	6.8	0		
D	6.8	6.8	2	0	
E	6.8	6.8	5	5	0



This was caused because of averaging distances between elements of the clusters

This would not happen if the molecular clock had constant speed over all branches of the tree

A less ambitious goal

- Find the tree which predicts the set of distances as closely as possible

$$SSQ(T)=\Sigma_{i \text{ from } 1 \text{ to } N}\Sigma_{j\neq i}w_{ij}(d_{ij}-TreeD_{ij})$$

dij – input distance (value Mij in the distance matrix)

wij – weight which intuitively quantifies the accuracy of distances

TreeDij – distance between leaf I and leaf j in the tree (sum of edge lengths)

The least squares method for fitting the function to the experimental curve

Distance-based phylogeny

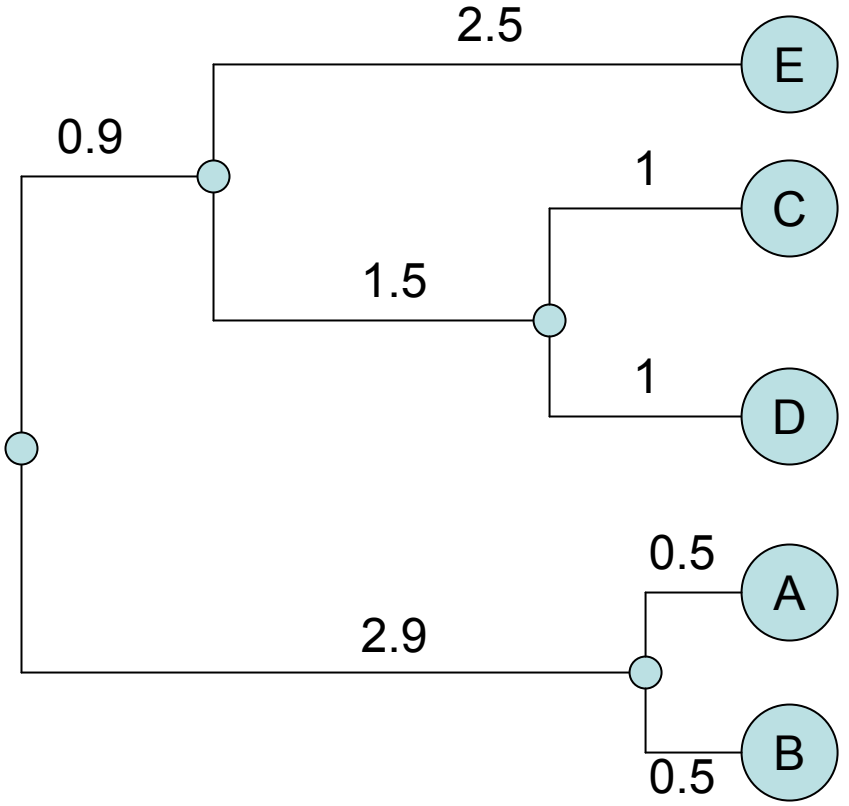
$$SSQ(T)=\Sigma_{i \text{ from } 1 \text{ to } N}\Sigma_{j\neq i} w_{ij}(d_{ij}-TreeD_{ij})$$

- Small problem: the tree is given, minimize the above expression
- Large problem: build a tree - which minimizes SSQ - from scratch (NP-complete)

Can we improve this tree?

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0

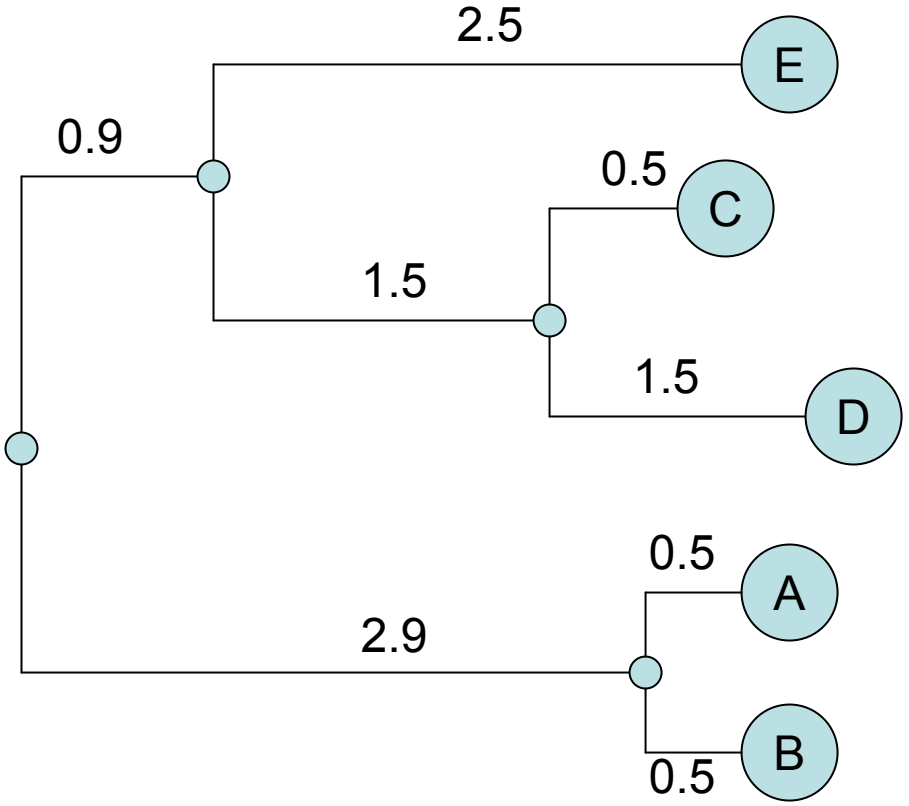
	A	B	C	D	E
A	0				
B	1	0			
C	6.8	6.8	0		
D	6.8	6.8	2	0	
E	6.8	6.8	5	5	0



Small distance-based phylogeny problem has a solution

	A	B	C	D	E
A	0				
B	1	0			
C	4	3	0		
D	8	7	2	0	
E	10	9	4	6	0

	A	B	C	D	E
A	0				
B	1	0			
C	6.8	6.8	0		
D	6.8	6.8	2	0	
E	6.8	6.8	4.5	5.5	0



We can optimize the distances in the tree as much as possible, by redistributing the length between sibling leaves

Ultrametric trees and UPGMA

- The tree of slide 48 is clocklike, ultrametric: the total length of the path from a given internal node to each leaf is the same.
- The assumption: the molecular clock of mutations ticks with a constant pace
- UPGMA reconstructs the tree based on this molecular clock assumption, that is why a new node is always created at the same distance from all the leaves

When the tree reflects reality

$$SSQ(T)=\Sigma_{i \text{ from } 1 \text{ to } N}\Sigma_{j\neq i} w_{ij}(d_{ij}-TreeD_{ij})$$

- If the solution to SSQ(T)=0, and there was a molecular clock with constant pace, then UPGMA guarantees to find an optimal solution.
- If not:
  - It can find a good enough solution, but the correctness of the tree topology is not guaranteed
  - Use *the neighbor-joining* algorithm to check the correctness of the tree topology. This algorithm relies on the additivity of distances, but does not require the distances to be ultrametric

Test for ultrametric condition

- We can predict whether the reconstruction of the real tree is likely to be correct by testing our distances for *ultrametric condition*:

The distance matrix is ultrametric if for any triplet of sequences, Xi, Xj, Xk, the distances dij, dik, djk are either all equal or two are equal and the remaining one is smaller

Thus, if distances were derived from a real tree with a molecular clock, the distance matrix has to be ultrametric

Ultrametric and non-ultrametric distance matrices

	A	B	C	D
A	0			
B	1	0		
C	4	2	0	
D	8	7	5	0

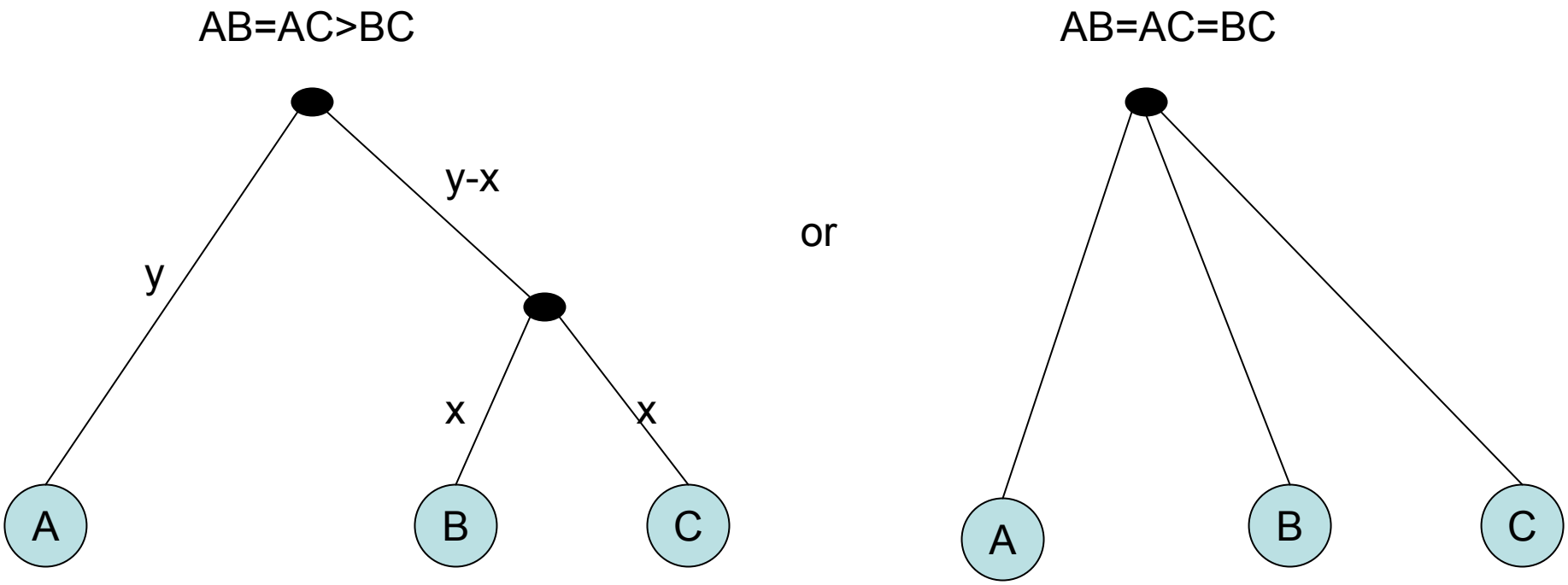
dAB=1, dAC=4, dBC=2

Non-ultrametric matrix

	A	B	C	D
A	0			
B	4	0		
C	2	4	0	
D	8	8	8	0

Ultrametric matrix

Ultrametric trees



AB=y+y-x+x=2y

AC=2y

BC=2x, x<=y, since y-x.=0 (no negative edge lengths)

The rule for ultrametric trees:

2 out of 3 distances have a tie, and are >= than the third distance