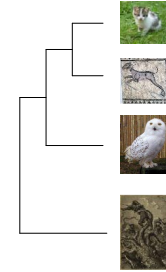


Inferring the Past: Phylogenetic Trees (chapter 12)

- *The biological problem*
- Parsimony and distance methods
- Models for mutations and estimation of distances
- Maximum likelihood methods

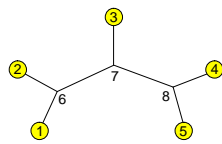
Phylogeny

- We want to study ancestor-descendant relationships, or *phylogeny*, among groups of organisms
- Groups are called *taxa* (singular: *taxon*)
- Organisms are usually called *operational taxonomic units* or *OTUs* in the context of phylogeny



Phylogenetic trees

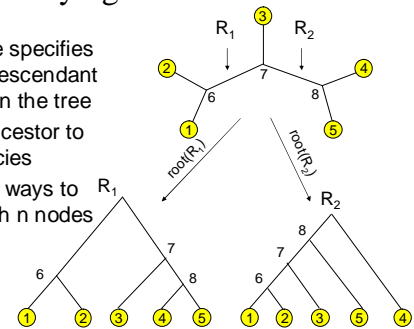
- Leaves (external nodes) ~ species, observed (OTUs)
- Internal nodes ~ ancestral species/divergence events, not observed
- Unrooted tree does not specify ancestor-descendant relationships beyond the observation "leaves are not ancestors"



Unrooted tree with 5 leaves and 3 internal nodes.
Is node 7 ancestor of node 6?

Phylogenetic trees

- Rooting a tree specifies all ancestor-descendant relationships in the tree
- Root is the ancestor to the other species
- There are $n-1$ ways to root a tree with n nodes



Questions

- Can we enumerate all possible phylogenetic trees for n species (or sequences?)
- How to score a phylogenetic tree with respect to data?
- How to find the best phylogenetic tree given data?

Finding the best phylogenetic tree: naive method

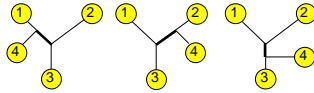
- How can we find the phylogenetic tree that best represents the data?
- Naive method: enumerate all possible trees
- How many different trees are there of n species?
- Denote this number by b_n

Enumerating unordered trees

- Start with the only unordered tree with 3 leaves ($b_3 = 1$)



- Consider all ways to add a leaf node to this tree



- Fourth node can be added to 3 different branches (edges), creating 1 new internal branch
- Total number of branches is n external and $n - 3$ internal branches
- Unrooted tree with n leaves has $2n - 3$ branches

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Enumerating unordered trees

- Thus, we get the number of unrooted trees

$$b_n = (2(n-1) - 3)b_{n-1} = (2n-5)b_{n-1}$$

$$= (2n-5) * (2n-7) * \dots * 3 * 1$$

$$= (2n-5)! / ((n-3)!2^{n-3}), n > 2$$

- Number of rooted trees b'_n is

$$b'_n = (2n-3)b_n = (2n-3)! / ((n-2)!2^{n-2}), n > 2$$

that is, the number of unrooted trees times the number of branches in the trees

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Number of possible rooted and unrooted trees

n	B_n	b'_n
3	1	3
4	3	15
5	15	105
6	105	945
7	954	10395
8	10395	135135
9	135135	2027025
10	2027025	34459425
20	2.22E+020	8.20E+021
30	8.69E+036	4.95E+038

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Too many trees?

- We can't construct and evaluate every phylogenetic tree even for a smallish number of species
- Better alternative is to
 - Devise a way to evaluate an individual tree against the data
 - Guide the search using the evaluation criteria to reduce the search space

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Inferring the Past: Phylogenetic Trees (chapter 12)

- The biological problem
- Parsimony and distance methods*
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- Maximum likelihood methods

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Parsimony method

- The parsimony method finds the tree that explains the observed *sequences* with a minimal number of substitutions
- Method has two steps
 - Compute smallest number of substitutions for a given tree with a *parsimony algorithm*
 - Search for the tree with the minimal number of substitutions

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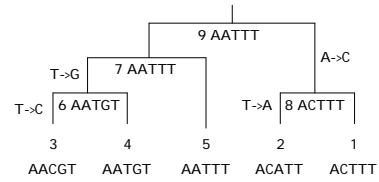
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Parsimony: an example

- Consider the following short sequences
- 1 ACTTT
- 2 ACATT
- 3 AACGT
- 4 AATGT
- 5 AATTT
- There are 105 possible rooted trees for 5 sequences
- Example: which of the following trees explains the sequences with least number of substitutions?

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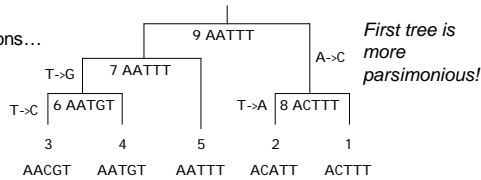


This tree explains the sequences with 4 substitutions

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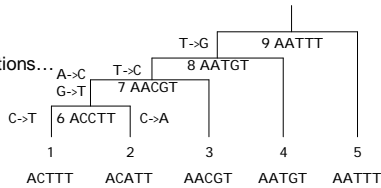
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4 substitutions...



First tree is more parsimonious!

6 substitutions...



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Computing parsimony

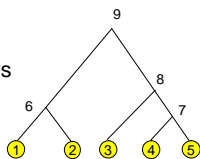
- Parsimony treats each site (position in a sequence) independently
- Total parsimony cost is the sum of parsimony costs of each site
- We can compute the minimal parsimony cost for a given tree by
 - First finding out possible assignments at each node, starting from leaves and proceeding towards the root
 - Then, starting from the root, assign a letter at each node, proceeding towards leaves

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Labelling tree nodes

- An unrooted tree with n leaves contains $2n-2$ nodes altogether
- Assign the following labels to nodes in a rooted tree
 - leaf nodes: 1, 2, ..., n
 - internal nodes: $n+1, n+2, \dots, 2n-1$
 - root node: $2n$
- The label of a child node is always smaller than the label of the parent node



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Parsimony algorithm: first phase

- Find out possible assignments at every node for each site independently. Denote site u in sequence i by $s_{i,u}$
- For $i := 1, \dots, n$ do
 - $F_i := \{s_{i,u}\}$ % possible assignments at node i
 - $L_i := 0$ % number of substitutions up to node i
- For $i := n+1, \dots, 2n-1$ do
 - Let j and k be the children of node i
 - If $F_j \cap F_k = \emptyset$ then $L_i := L_j + L_k + 1, F_i := F_j \cup F_k$
 - else $L_i := L_j + L_k, F_i := F_j \cap F_k$

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Parsimony algorithm: first phase

Choose $u = 3$ (for example)

$F_1 := \{T\}$

$L_1 := 0$

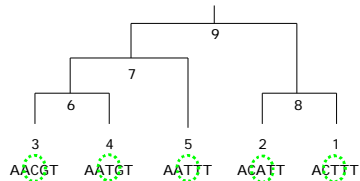
$F_2 := \{A\}$

$L_2 := 0$

$F_3 := \{C\}, L_3 := 0$

$F_4 := \{T\}, L_4 := 0$

$F_5 := \{T\}, L_5 := 0$



$F_8 := F_1 \cup F_2 = \{A, T\}$

$L_8 := L_1 + L_2 + 1 = 1$

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Parsimony algorithm: first phase

$F_6 := F_3 \cup F_4 = \{C, T\}$

$L_6 := L_3 + L_4 + 1 = 1$

$F_7 := F_5 \cap F_6 = \{T\}$

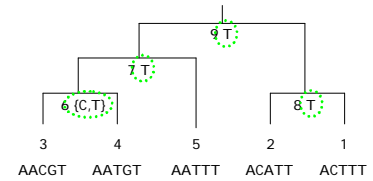
$L_7 := L_5 + L_6 = 1$

$F_8 := F_1 \cup F_2 = \{A, T\}$

$L_8 := L_1 + L_2 + 1 = 1$

$F_9 := F_7 \cap F_8 = \{T\}$

$L_9 := L_7 + L_8 = 2$



\Rightarrow Parsimony cost for site 3 is 2

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Parsimony algorithm: second phase

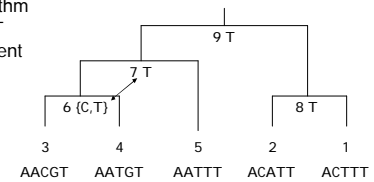
- 1 Backtrack from the root and assign $x \in F_i$ at each node
- 1 If we assigned y at parent of node i and $y \in F_i$, then assign y
- 1 Else assign $x \in F_i$ by random

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Parsimony algorithm: second phase

At node 6, the algorithm assigns T because T was assigned to parent node 7 and $T \in F_6$



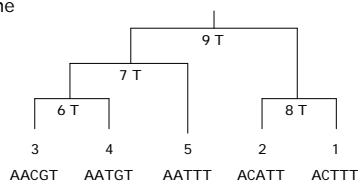
The other nodes have only one possible letter to assign

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Parsimony algorithm

First and second phase are repeated for each site in the sequences, summing the parsimony costs at each site



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Properties of parsimony algorithm

- 1 Parsimony algorithm requires that the sequences are of same length
 - First align the sequences against each other and remove indels
 - Then compute parsimony for the resulting sequences
- 1 Is the most parsimonious tree the correct tree?
 - Not necessarily but it explains the sequences with least number of substitutions
 - We can assume that the probability of having fewer mutations is higher than having many mutations

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Finding the most parsimonious tree

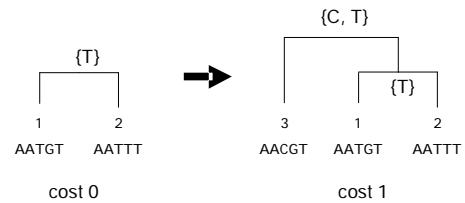
- Parsimony algorithm calculates the parsimony cost for a given tree...
- ...but we still have the problem of finding the tree with the lowest cost
- Exhaustive search (enumerating all trees) is in general impossible
- More efficient methods exist, for example
 - Probabilistic search
 - Branch and bound

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Branch and bound in parsimony

- We can exploit the fact that adding edges to a tree can only increase the parsimony cost



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Branch and bound in parsimony

Branch and bound is a general search strategy where

- Each solution is potentially generated
- Track is kept of the best solution found
- If a partial solution cannot achieve better score, we abandon the current search path

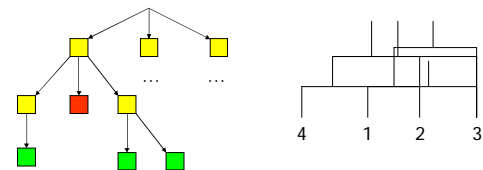
In parsimony...

- Start from a tree with 1 sequence
- Add a sequence to the tree and calculate parsimony cost
- If the tree is complete, check if found the best tree so far
- If tree is not complete and cost exceeds best tree cost, do not continue adding edges to this tree

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Branch and bound graphically



Partial tree, no best complete tree constructed yet

Complete tree: calculate parsimony cost and store

Partial tree, cost exceeds the cost of the best tree this far

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Distance methods

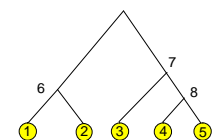
- The parsimony method works on sequence (character string) data
- We can also build phylogenetic trees in a more general setting
- Distance methods* work on a set of pairwise distances d_{ij} for the data
- Distances can be obtained from phenotypes as well as from genotypes (sequences)

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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 (e.g., number of mismatches in aligned sequences)

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Distances in evolutionary context

- | Distances d_{ij} in evolutionary context satisfy the following conditions
 - Symmetry: $d_{ij} = d_{ji}$ for each i, j
 - Distinguishability: $d_{ij} \neq 0$ if and only if $i \neq j$
 - 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

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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 the first node k that they share in the tree

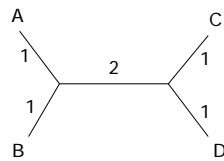
$$d_{ij} = d_{ik} + d_{jk}$$
- | "Follow the path from the leaf i to the leaf j to find the exact distance d_{ij} between the leaves."

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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



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Ultrametric trees

- | A rooted additive tree is called a *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

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Identifying ultrametric data

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

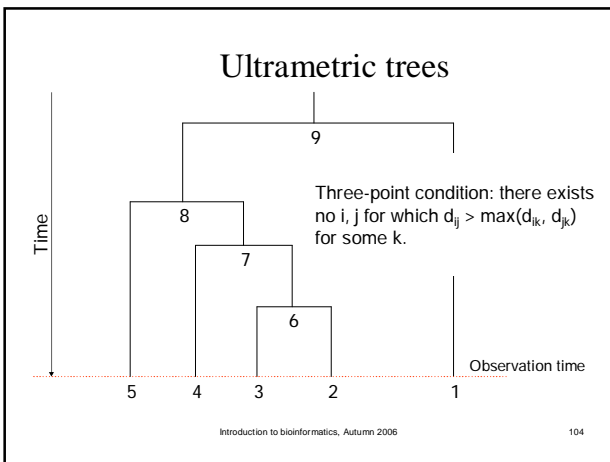
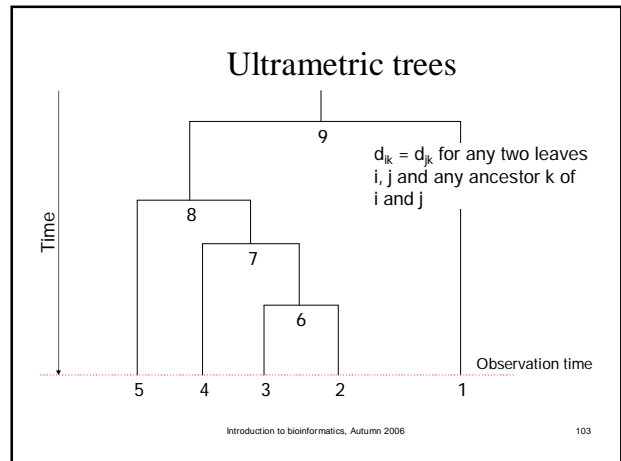
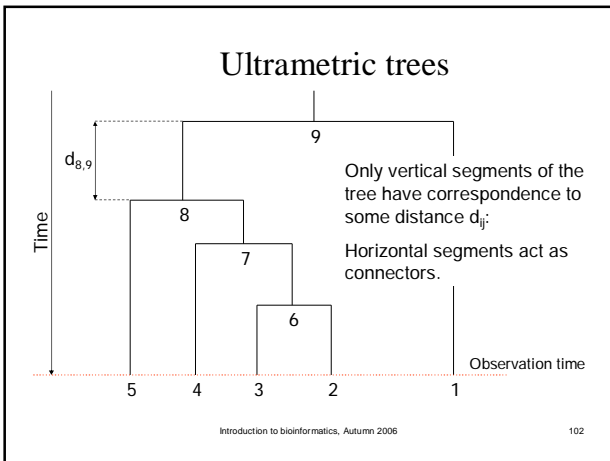
D corresponds to an ultrametric tree if and only if for any three sequences i, j and k , the distances satisfy $d_{ij} \leq \max(d_{ik}, d_{kj})$
- | If we find out that the data is ultrametric, we can utilise a simple algorithm to find the corresponding tree

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Ultrametric trees

Time



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

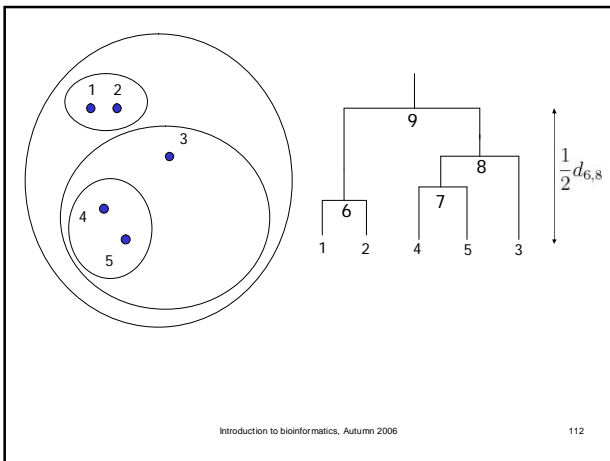
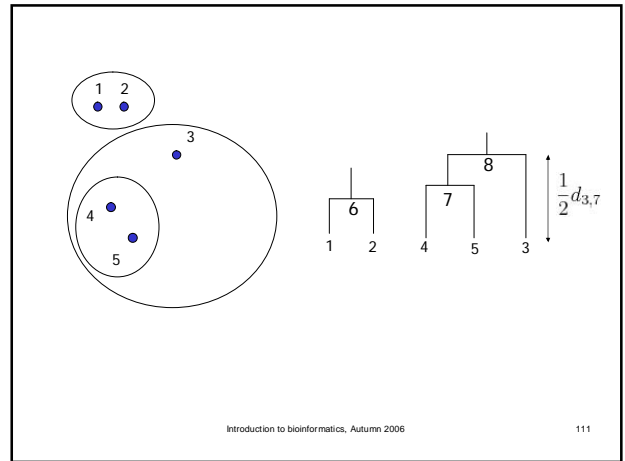
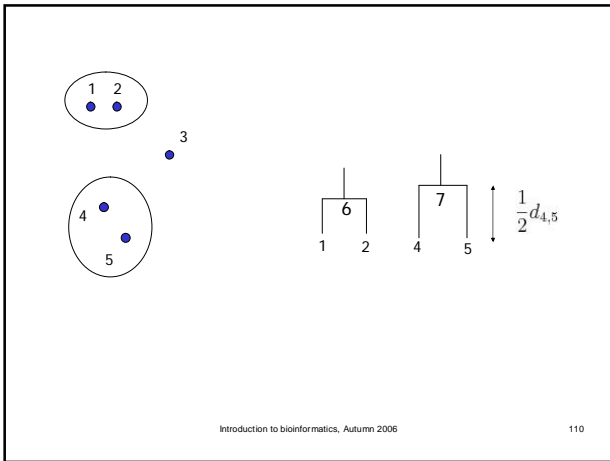
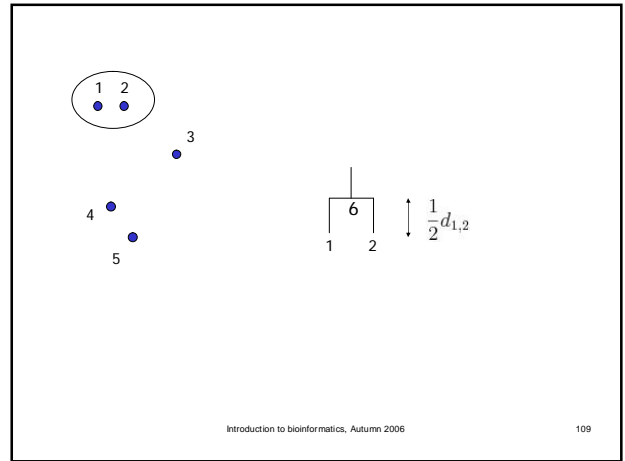
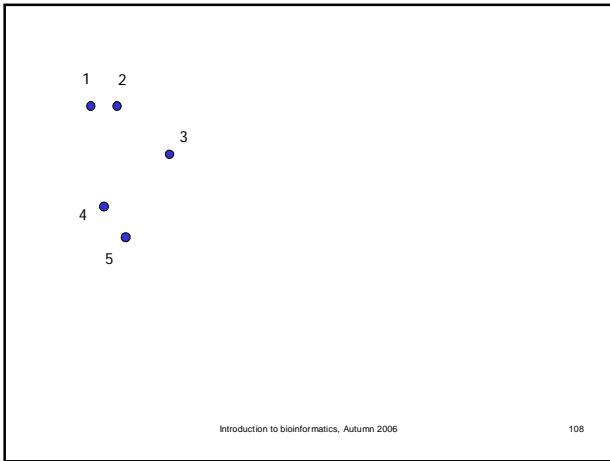
1 Let distance d_{ij} between clusters C_i and C_j be

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

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

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- ### UPGMA algorithm
- 1 Initialisation
 - Assign each point i to its own cluster C_i
 - Define one leaf for each sequence, and place it at height zero
 - 1 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
 - 1 Termination:
 - When only two clusters i and j remain, place root at height $d_{ij}/2$
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UPGMA implementation

- ▮ In naive implementation, each iteration takes $O(n^2)$ time with n sequences \Rightarrow algorithm takes $O(n^3)$ time
- ▮ The algorithm can be implemented to take only $O(n^2)$ time (Gronau & Moran, 2006)

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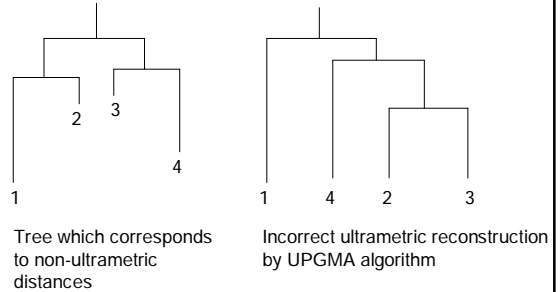
Problem solved?

- | 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 (we skip the proof)
 - 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*

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Incorrect reconstruction of non-ultrametric data by UPGMA



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Finding an additive phylogenetic tree

- | Additive trees can be found with, for example, the neighbor joining method (Saitou & Nei, 1987)
- | The neighbor joining method produces unrooted trees, which have to be rooted by other means
 - A common way to root the tree is to use an outgroup
 - Outgroup is a species that is known to be more distantly related to every other species than they are to each other
 - Root node candidate: position where the outgroup would join the phylogenetic tree
- | However, in real-world data, even additivity usually does not hold very well

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Inferring the Past: Phylogenetic Trees (chapter 12)

- | The biological problem
- | Parsimony and distance methods
- | *Models for mutations and estimation of distances*
- | Maximum likelihood methods

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