Bioinformatics

Phylogenetic trees

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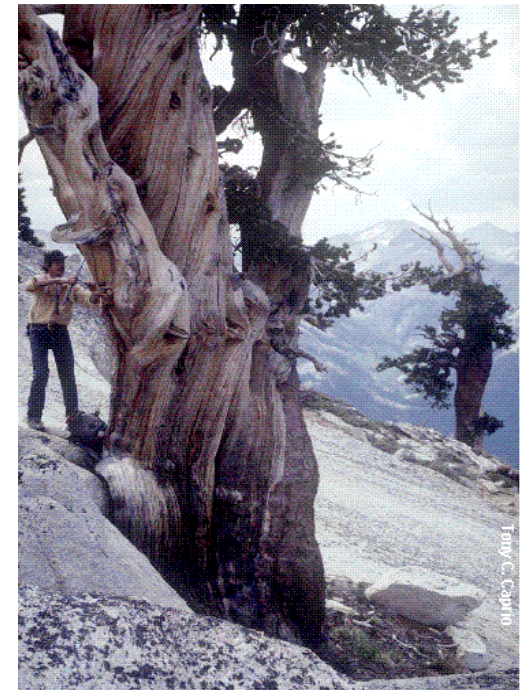Department of Computing Science, University of Glasgow

Overview

- Phylogentics
- Trees
  - Definitions
  - Properties
- Molecular clock[s]
- Tree [re]construction
- Distance methods
  - UPGMA
  - Neighbour-joining
- Character methods
  - Maximum parsimony
  - Maximum likelihood
- Assessing trees
  - Bootstrapping trees

- Mostly based on Chapter 7 'Building Phylogenetic Trees' from R. Durbin et al 'Biological Sequence Analysis' CUP 1988
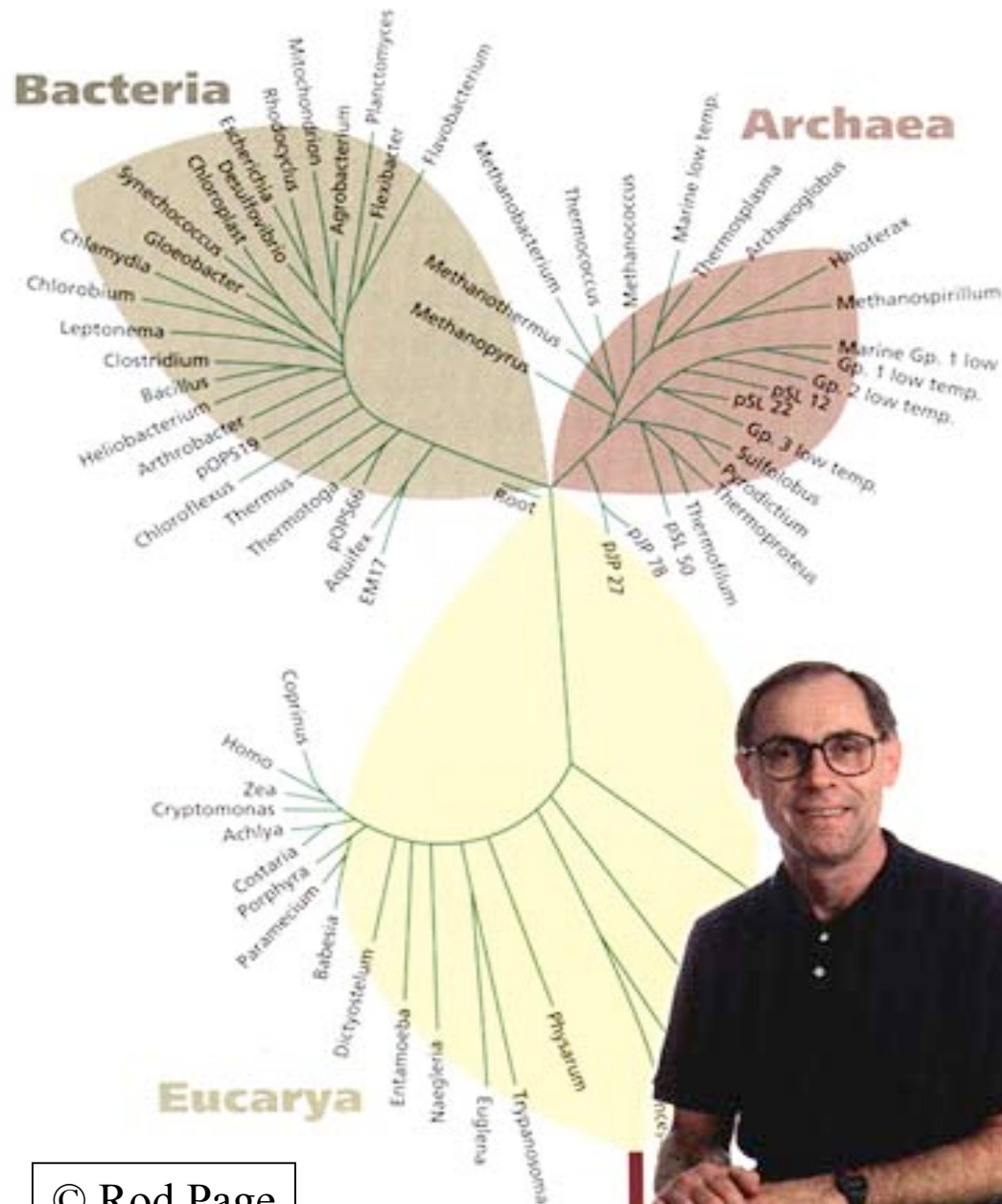
Phylogenetic Trees

- Phylogeny : *The evolutionary history and line of descent of a species*
- Phylogenetic Tree : A diagram setting out the genealogy of a species
- Purpose
  - to reconstruct the correct genealogical ties between related objects
  - To estimate the time of divergence between them since they last shared a common ancestor
- Objects typically are protein or nucleic acid sequences
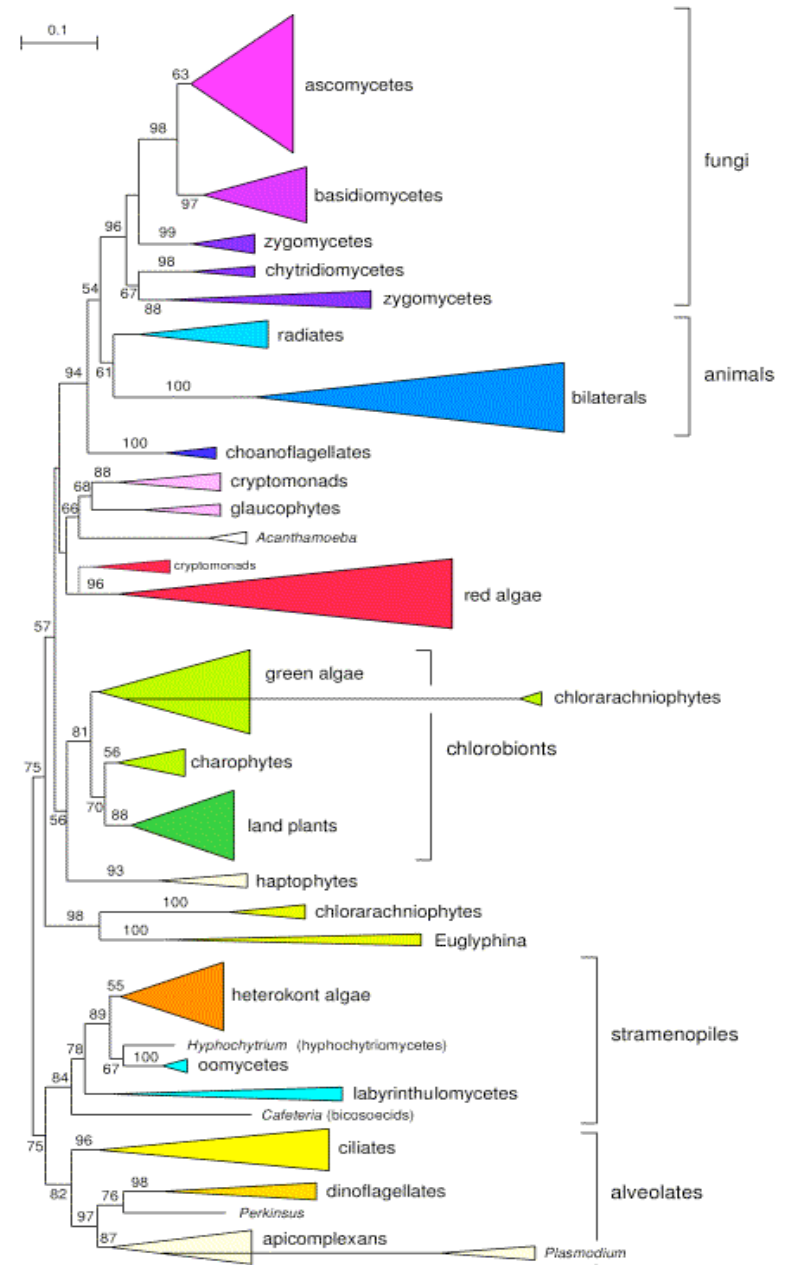
What is phylogeny good for?

- Evolutionary history ("tree of life")
- Population history
- Rates of evolutionary change
- Origins of diseases
- Prediction of sequence function
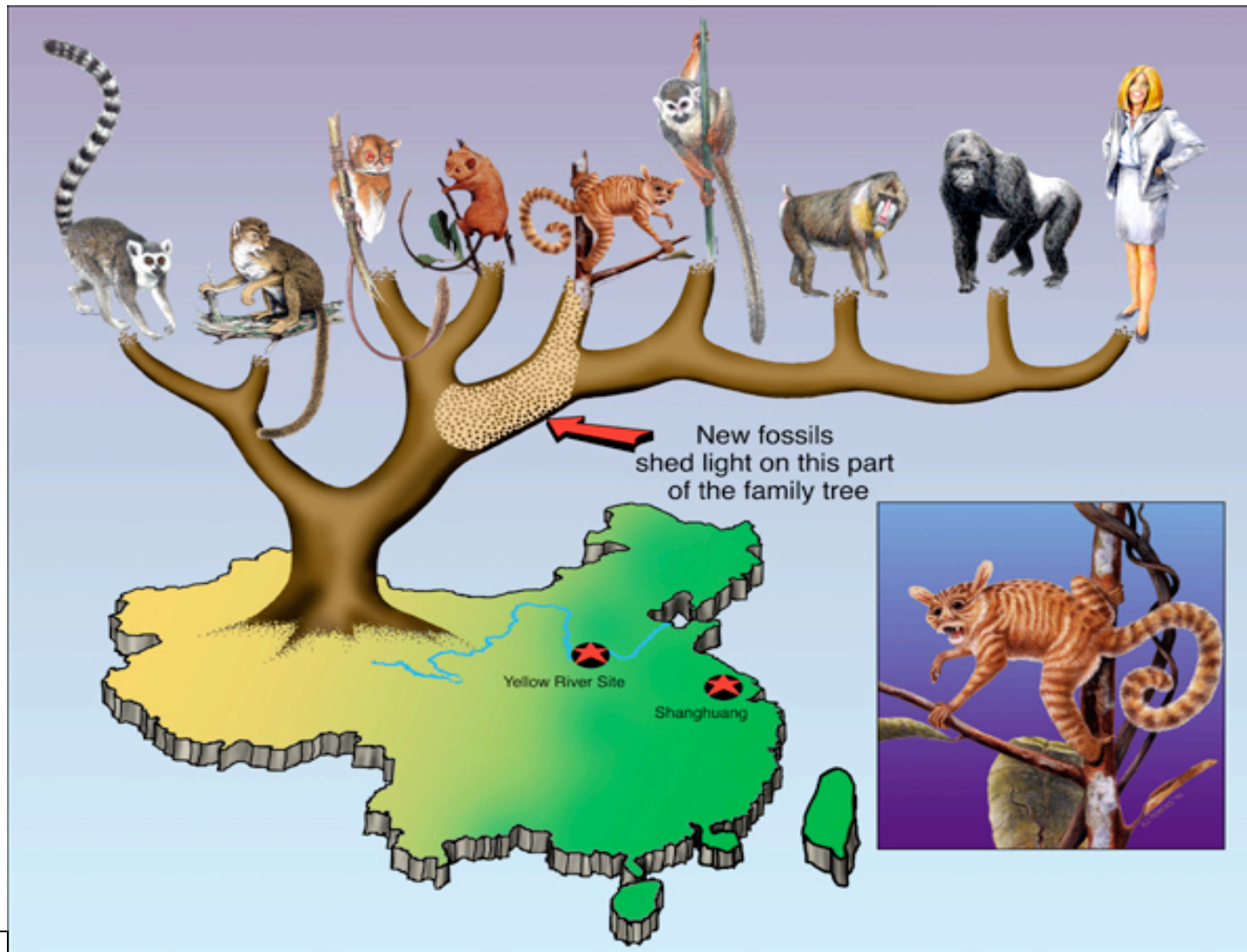- Can be applied to organisms, sequences, viruses, languages, etc.

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Phylogenetic Trees

Bacteria

Archaea

Eucarya

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Our place in Nature

New fossils shed light on this part of the family tree

Yellow River Site

Shanghuang

Phylogenetic Trees

HIV: where did it come from and how is it transmitted?

Immunodeficieny virus (Weighted tree)

Possible HIV type 1 origin
HIV type 2 origin

SIVmon Cercopithecus
SIVcpzTAN1 Chimpanze
HIV1-NDK (Zaire)
HIV-1 (Zaire)
CIVcpzUS Chimpanzee
SIVcpz Chimpanzees Ca
SIVsmSL92b Sooty Man
SIVMM239 Simian maca
SIVMM251 Macaque
HIV-2UC1 (IvoryCoast)
HIV2-MCN13
HIV-2 (Senegal)
SIVAGM3 Green monkey
SIVAGM677A Green mor
SIVmnd5440 Mandrillus
SIVlhoest L'Hoest monk

Dental clade

Branch length:
number of substitutions

0 5 10 15

Dentist-x
Patient B-x
Patient B-y
Patient A-x
Patient E-x
Patient E-y
Dentist-y
Patient C-x
Patient C-y
Patient A-y
Patient G-x
Patient G-y
LC02-x
LC03-x
LC02-y
LC09
LC35
Patient D-x
Patient D-y
LC03-y
Patient F-x
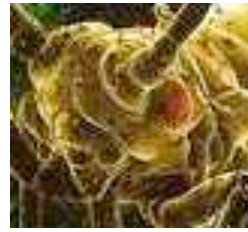Patient F-y
HIVELI

Phylogenetic Trees

Yersinia pestis

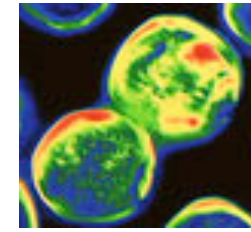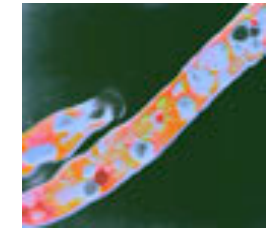**Arabidopsis thaliana**

Buchnera sp. APS

Aquifex aeolicus

Archaeoglobus fulgidus
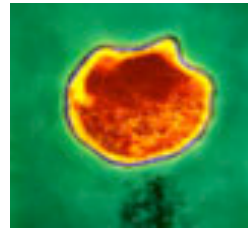
Borrelia burgorferi

Mycobacterium tuberculosis

Caenorhabitis elegans
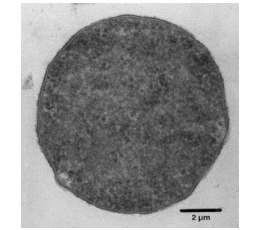
Campylobacter jejuni

Chlamydia pneumoniae

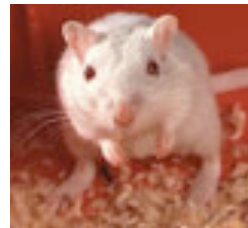**Vibrio cholerae**

Drosophila melanogaster

Escherichia coli

Thermoplasma acidophilum

Helicobacter pylori

Mycobacterium leprae

mouse

Neisseria meningitidis Z2491

Plasmodium falciparum

Pseudomonas aeruginosa
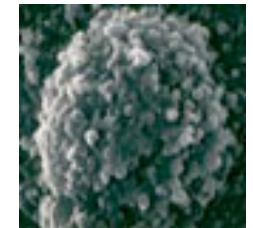
Ureaplasma urealyticum

rat
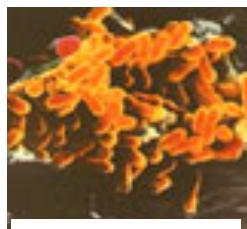
Rickettsia prowazekii
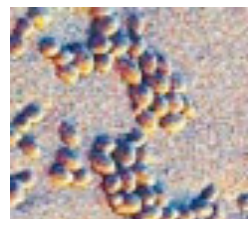
Saccharomyces cerevisiae

Salmonella enterica

Bacillus subtilis
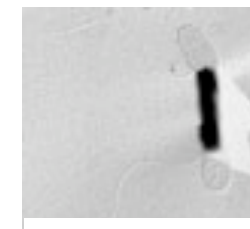
Thermotoga maritima

Xylella fastidiosa

(c) David Gilbe

8

Tree terminology

root

Internal node

leaf

Tree as text

((A , (B , C)) , D)

subtree
(A , (B , C))

Leaf label
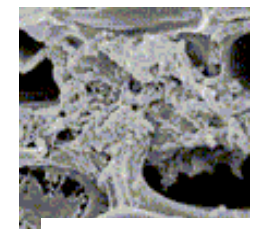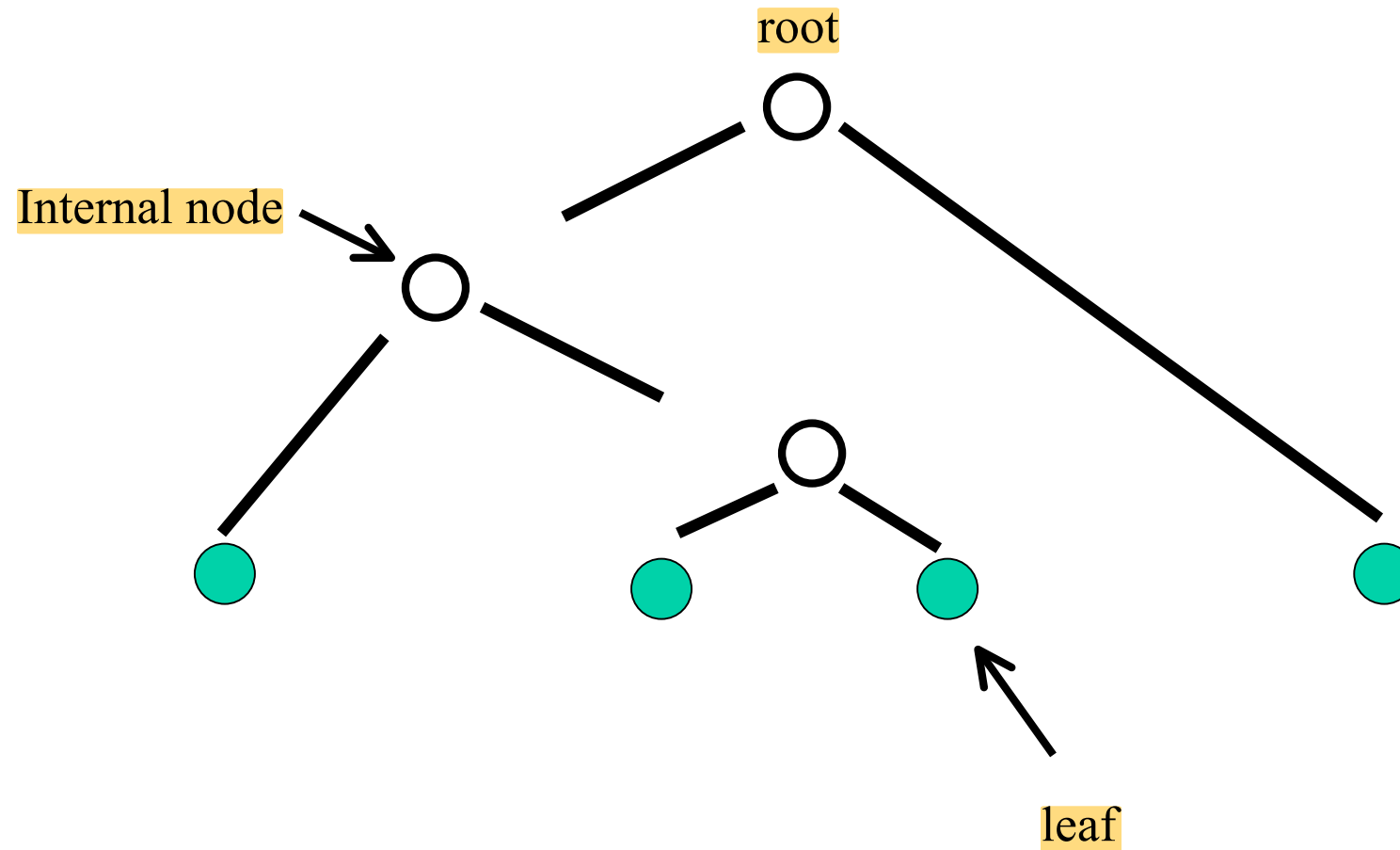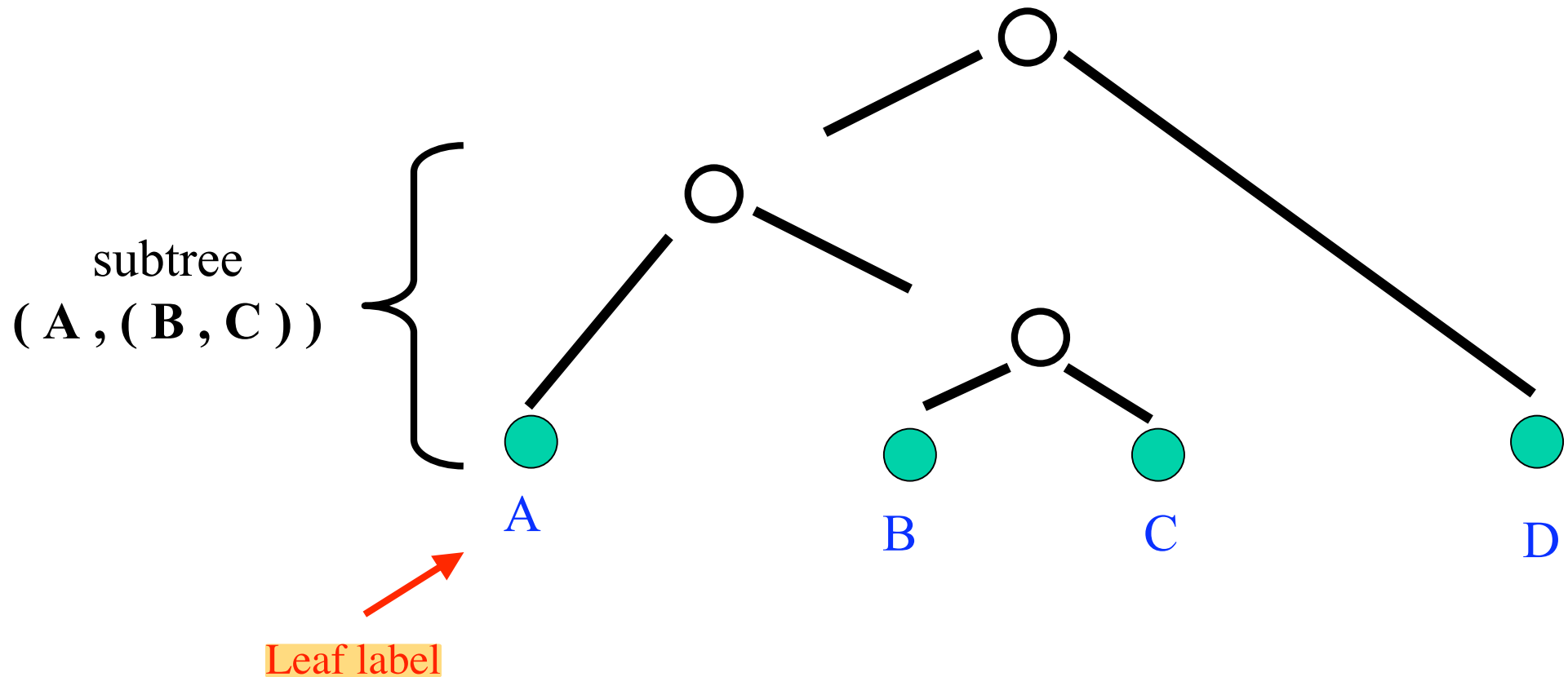
Definition of a tree

- A tree is either

    A **leaf**

    or

    (LeftTree , RightTree)

    where both LeftTree and RightTree are trees

Trees with labels

- We can add data to
  - Leaves
  - Branches
  - Nodes

(A:6 , (B : 2 , C : 2):4)

Branch level

1 Bndi?
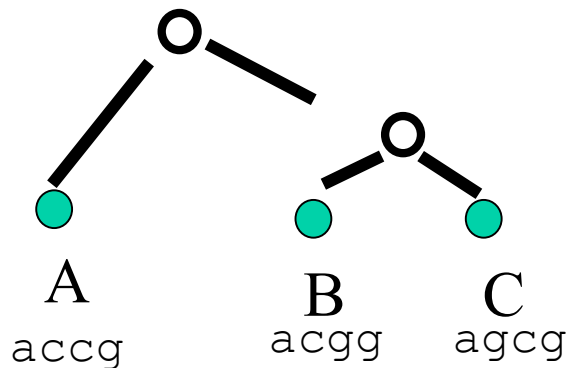
(A[accg] , (B[acgg] , C[agcg]))

Leaves   *Level 1/Binary*

6:(A , 4:(B, C))

Node level

Phylogenetic Trees
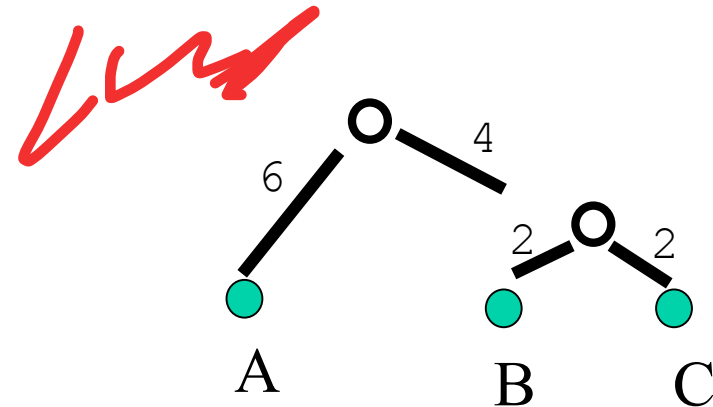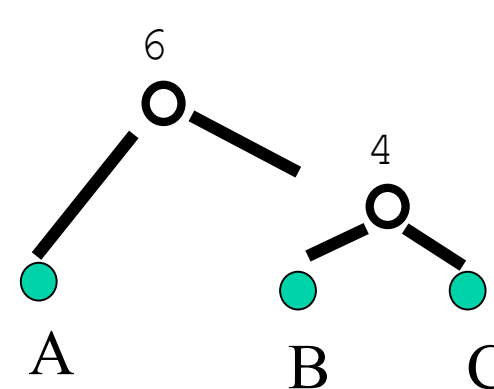
Isomorphic trees

((A , (B , C)) , D)

((A , (C , B)) , D)

Etc…
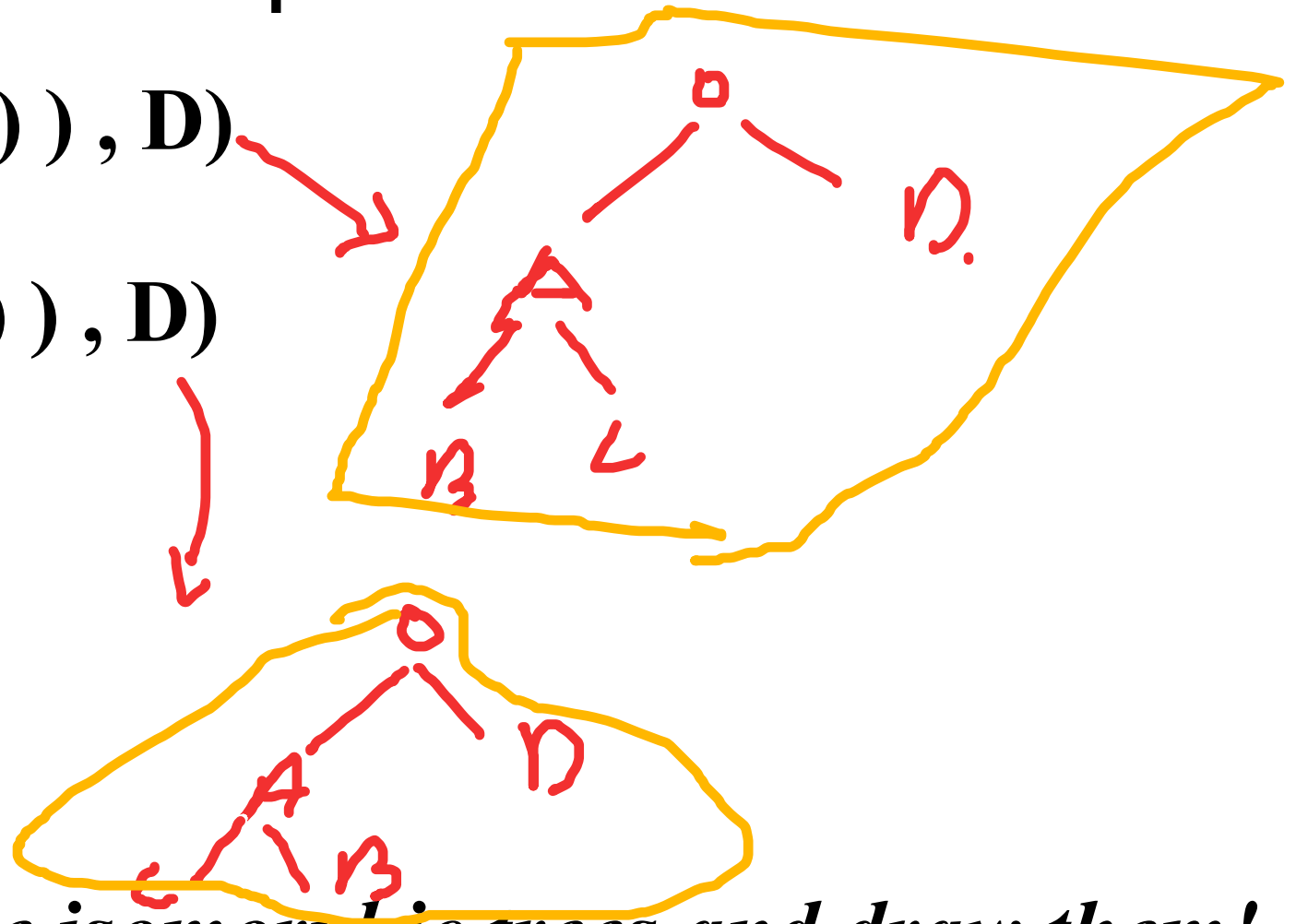
Derive [all] the isomorphic trees and draw them!

Isomorphic trees

- Isotrees(T1,T2) /Two trees T1 and T2 are *isomorphic/*
  if

  T1 is a leaf and T2 is a leaf and T1= =T2

  OR

  T1 = (L1,R1) , T2 = (L2,R2) AND

  Isotrees(L1, L2) AND Isotrees(R1, R2)

  OR

  Isotrees(L1, R2) AND Isotrees(R1, L2)

Trees can be unrooted

A

C

e   f

B

D

A   B   C   D
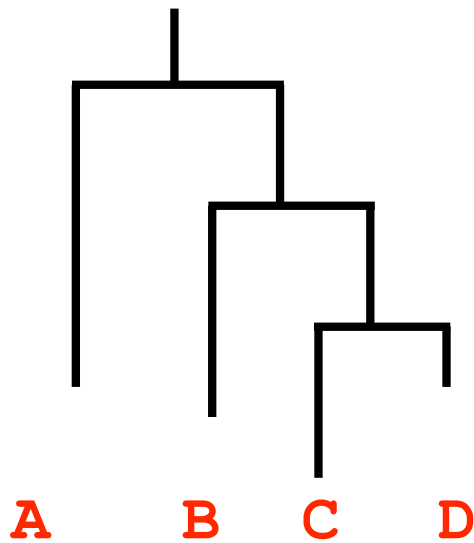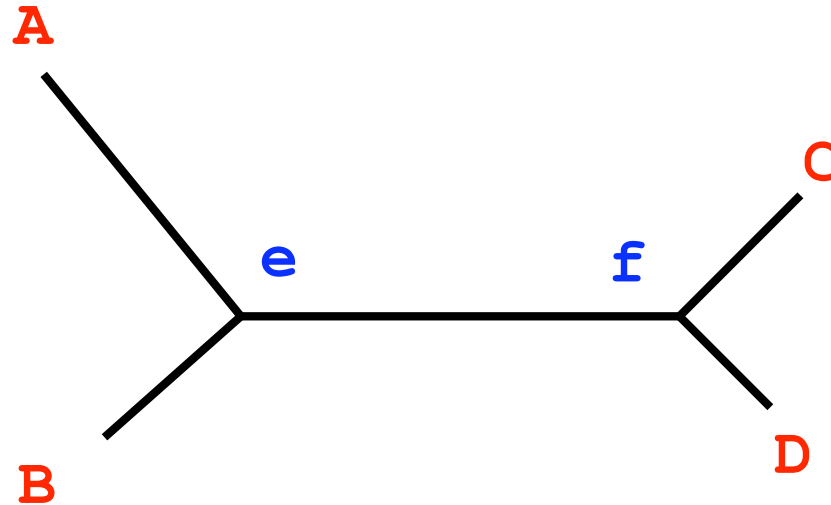
Are there alternative rootings?
Draw them…

Evolution - basic concepts

- Mutation in DNA a natural evolutionary process

- DNA *replication* errors: (nucleotide)
  - substitutions
  - insertions
  - deletions } **indels**

- Similarity between sequences
  - clue to common evolutionary origin, or
  - clue to common function

- This is a simplistic story: in fact the altered *function* of the expressed protein will determine if the organism will survive to reproduce, and hence pass on [transmit] the altered gene

Evolution - related sequences

```
                                    ggcatt
                    g→a            /      \
                                  /        \
        agcatt                                 \
       /      \                                  \
  c→g /        \                                  \
     /          \       agcata                     \
    /            \     /      \                      \
┌──────────────────────────────────────────────────────────┐
│  aggatt      agccta      agcatg          gacatt           │
└──────────────────────────────────────────────────────────┘
```
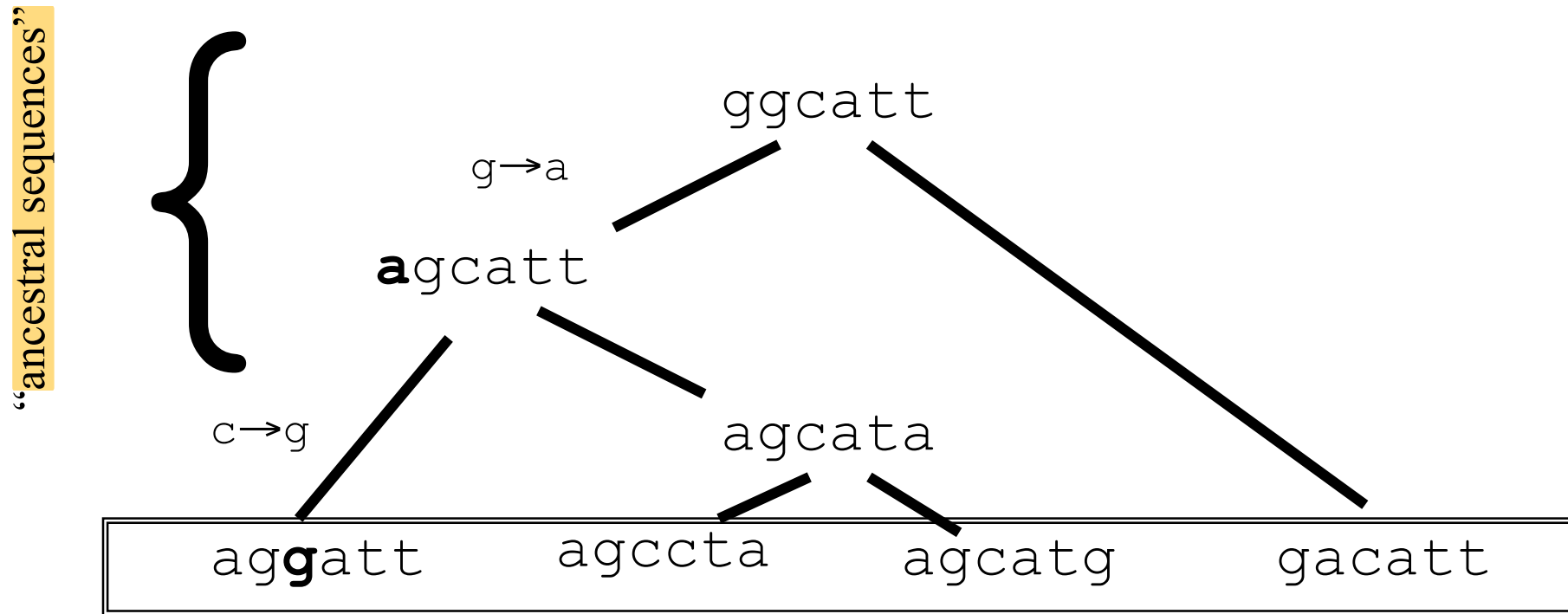
"living examples"

Convergent evolution: same sequence evolved from different ancestors

Back mutations - mutate to a previous sequence

Definitions

- **Phylum** (phyla pl): A primary division of a kingdom, as of the animal kingdom, ranking next above a class in size.

- **Phylogeny**:
  - the sequence of events involved in the evolutionary development of a species or taxonomic group of organisms
  - Evolutionary relationships within and between taxonomic levels, particularly the patterns of lines of descent. Phylogenetics -The taxonomical classification of organisms based on their degree of evolutionary relatedness. Phylogenetic tree - A variety of dendrogram (diagram) in which organisms are shown arranged on branches that link them according to their relatedness and evolutionary descent.

- **Phylogentics**: study of evolutionary relationships

- **Phylogenetic analysis**: the means of *inferring* or estimating these relationships

- **Taxonomy**: The science of naming and classifying organisms 1. [n] practice of classifying plants and animals according to their presumed natural relationships

  - 2. [n] (biology) study of the general principles of scientific classification

  - 3. [n] a classification of organisms into groups based on similarities of structure or origin etc

- **Taxon**: any named group of organisms

- **Species**: taxonomic group whose members can interbreed (but more or less able to…)

Definitions (cont)

- **Clade**: a group of biological taxa or species that share features inherited from a common ancestor; A monophyletic taxon; a group of organisms which includes the most recent common ancestor of all of its members and all of the descendants of that most recent common ancestor. From the Greek word "klados", meaning branch or twig.

- **Tree** : A data structure consisting of nodes which may contain other nodes via its branches. Unlike a tree in nature, the root node is usually represented at the top of the structure and does not have a parent node. All other nodes have a single parent. Nodes having no child nodes are called leaf nodes

- **Dendrogram**: Any branching diagram (or tree) (cf. cladogram, phylogram, phenogram); A dendrogram is a 'tree-like' diagram that summaries the process of clustering. Similar cases are joined by links whose position in the diagram is determined by the level of similarity between the cases. A treelike figure used to represent graphically a hierarchy. (dendron - greek 'tree')
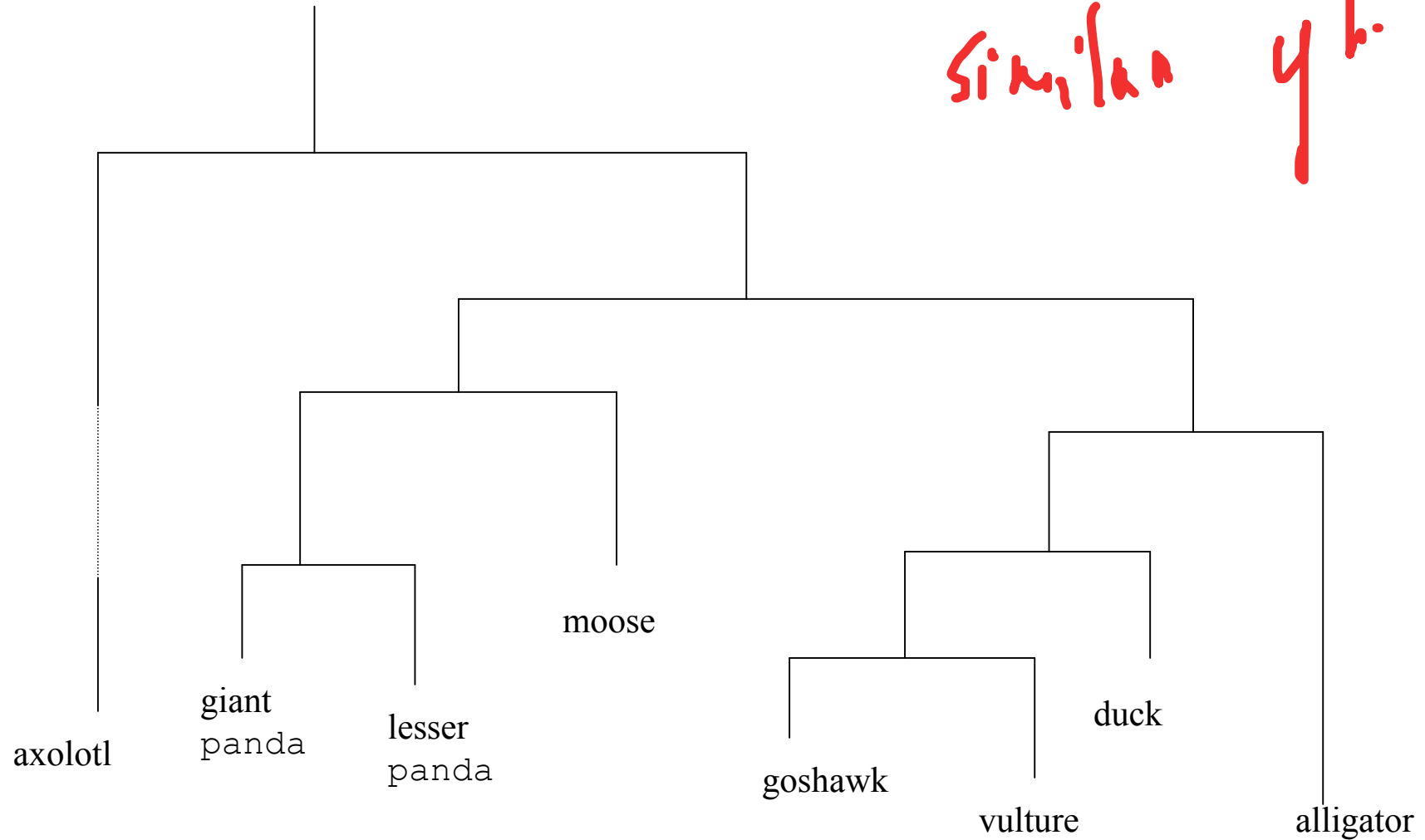
Homologues

- **Homologues**: sequences that have common origins but may or may not share common activity
- **Orthologues**: homologues produced by speciation. Tend to have similar function
- **Paralogues**: homologues produced by gene duplication. Tend to have different functions
- **Xenologues**: homologues resulting from horizontal gene transfer between 2 organisms.

Tree of orthologues based on a set of α-haemoglobins

axolotl

giant panda

lesser panda

moose

goshawk

vulture

duck

alligator

Tree of paralogues:
human haemoglobins and myoglobin

Myoglobin
(in muscles)

theta

alpha

zeta

epsilon

gamma

beta

delta
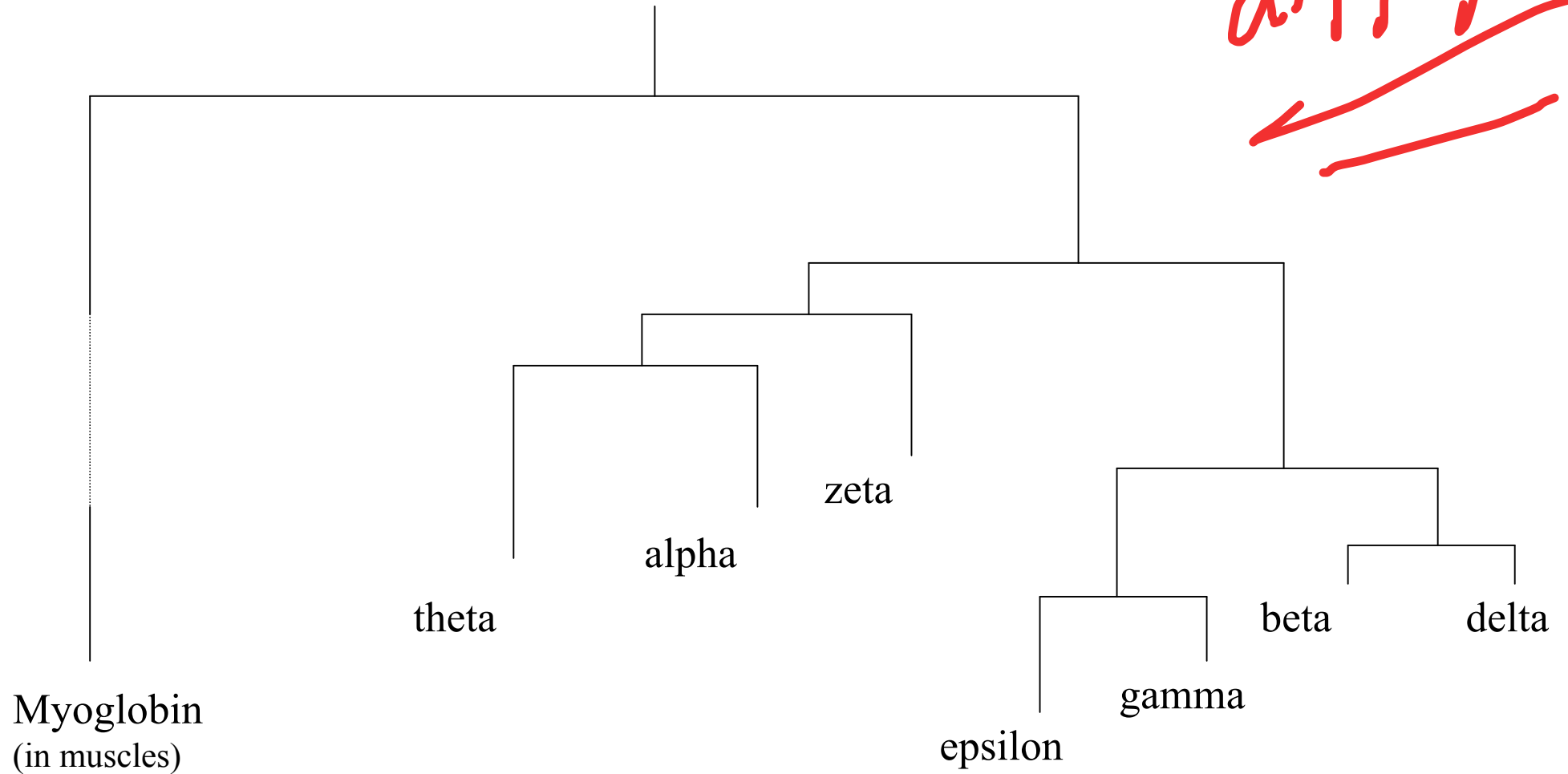
Orthologues & paralogues

G1:F1

Ancestral
organism
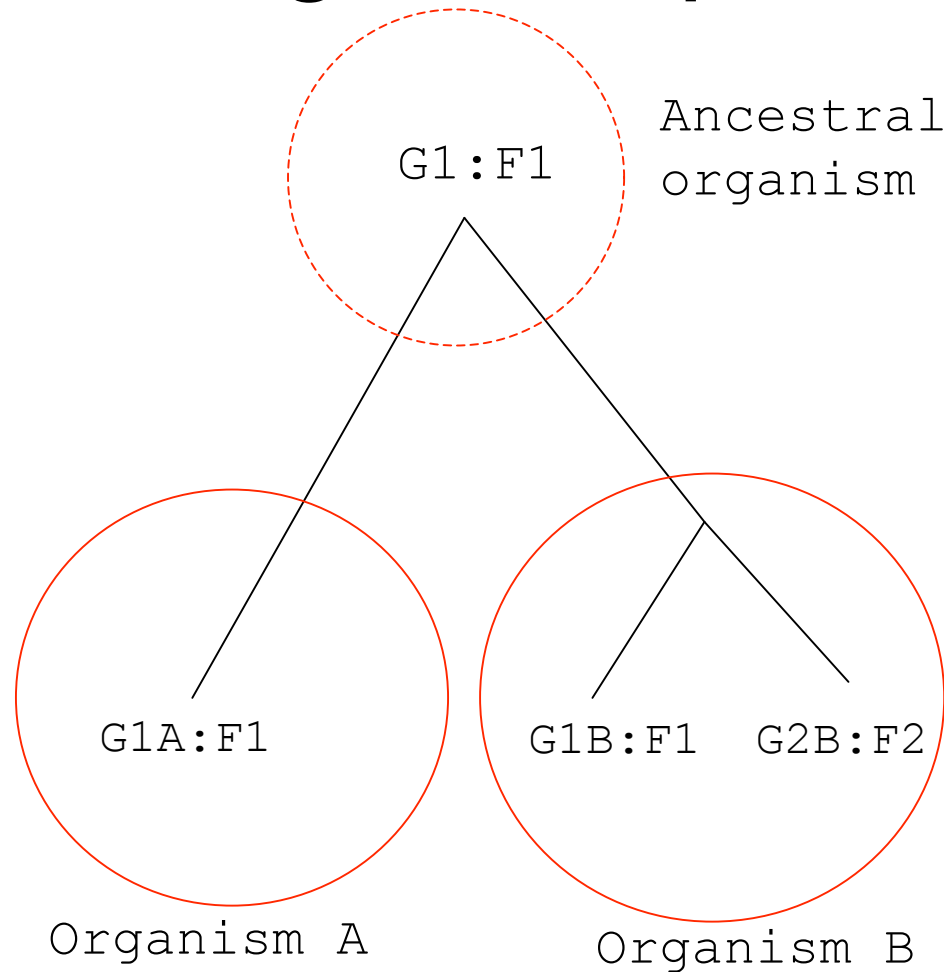
Orthologues
G1A:F1 & G1B:F1

Paralogues
G1B:F1 & G2B:F2

G1A:F1

G1B:F1   G2B:F2

Organism A

Organism B

Horizontal (lateral) gene transfer!

Bacteria can take up and spread genes in different ways

- Uptake of naked DNA directly from surroundings

- Obtain genes from infecting viruses

- Take up genes through cross-species mating

Horizontal gene transfer

from Doolittle, 1999
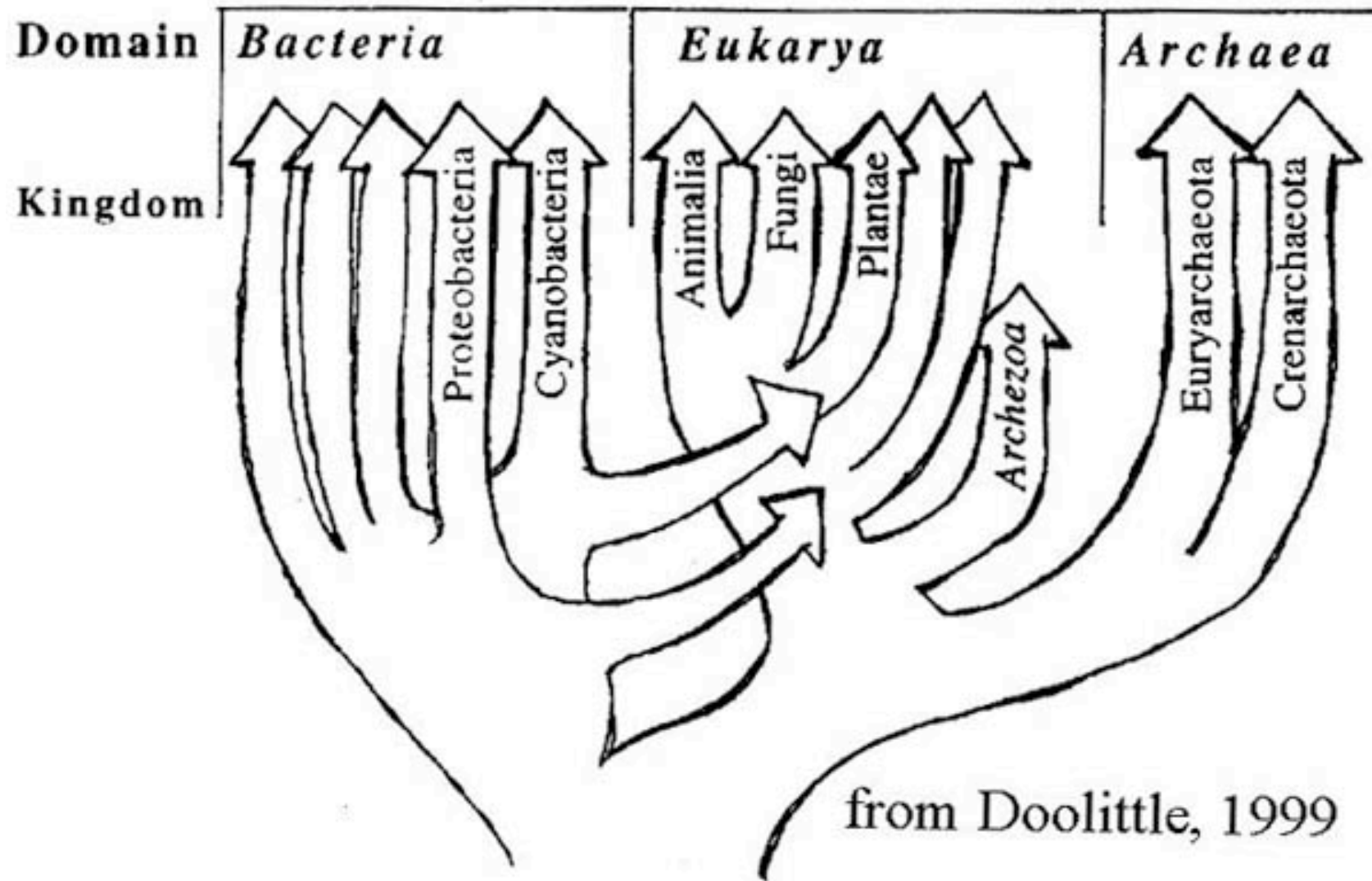
Phylogenetic Trees

Approaches to reconstructing phylogenetic trees

- Distance based methods

- Maximum parsimony methods

- Maximum likelihood methods

Phylogenetic analysis - 4 steps

1. Alignment / distance computation

2. Determine the data model

3. Tree building

4. Tree evaluation

Tree building methods

1. **Distance-based**: Transform the data into pairwise distances (dissimilarities), and then use a matrix during tree building. E.g. data from from immunology, nucleic acid hybridization, and breeding experiments, are automatically expressed as pair-wise distances but most character data need to be mathematically transformed into distances.

2. **Character-based**: Use the aligned characters, such as DNA or protein sequences, directly during tree inference – based on substitutions.

Tree building algorithms

- Distance-based:
  - input = evolutionary distance data (sequence edit dist; melting temp DNA hybridisations, strength of antibody cross reactions..)
  - Goal: construct weighted tree whose pairwise distances agree with evolutionary distances
  - Ultrametric distance data - elegant solution: UPGMA
  - Additive data - efficient solution: NJ
  - FM - Fitch-Margoliash
  - ME - minimum evolution
  - Not additive - reconstruction not guaranteed; find trees whose distances "best approximate" given data
- Rooting trees

Metric, ultrametric

- A *metric* on a set of objects O is given by the assignment of a real number $d(x,y)$ for every pair of objects x,y in O such that

  $d(x,y) > 0$ for $x \neq y$

  $d(x,y) = 0$ for $x = y$

  $d(x,y) = d(y,x)$ for all x,y

  $d(x,y) \leq d(x,z) + d(y,z)$ for all x,y,z (triangle inequality)

- In addition, for an *ultrametric*

  $d(x,y) \leq max(d(x,z) , d(y,z))$

- An *ultrametric tree* is characterised by the condition

  $d(x,y) \leq d(x,z) = d(y,z)$ (for any 3 points their pairwise distances are all equal, or 2 are equal and 1 is smaller)

Ages {Fred:20, Mary:22, Jane:24} e.g. add, subtract – are these metric / ultrametric / …?

How many pairwise comparisons?

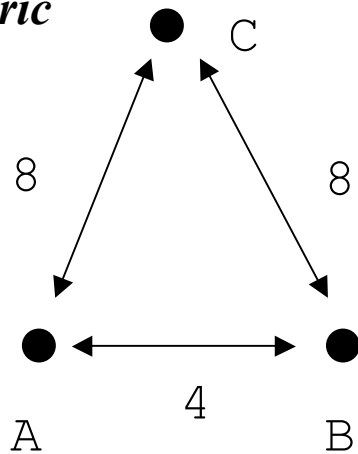|	A	B	C	D	E
A					
B					
C					
D					
E					

- How many for whole table?
- *Identity:* $(x,y) = 0$ for $x=y$
- *Symmetry:* $d(x,y) = d(y,x)$ for all x,y

Metric, Ultrametric trees
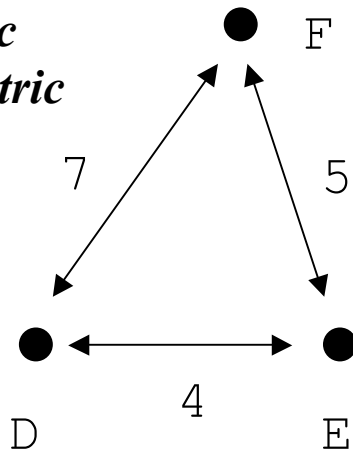
?
metric
ultrametric

Distances

Distance matrix

	A	B	C
A			
B			
C			

Tree

?
metric
ultrametric

	D	E	F
D			
E			
F			

Phylogenetic Trees

Ultrametric trees

Def: Given D a symmetric matrix n by n of real numbers; an *ultrametric tree* for D is a rooted tree T with the following properties:

1. T contains n leaves, each labelled by a unique row D

2. Each internal node of T is labelled by one entry from D and has at least 2 children

3. Along any path from the root to the leaf, the numbers labelling the internal nodes *strictly decrease*

4. For any two leaves i,j of T, D(i,j) is the label of the least common ancestor of i and j in T

Def: A *min-ultrametric tree* for D is a rooted tree T with all the properties of an ultrametric tree except that (3) is changed to:

3. Along any path from the root to the leaf, the numbers labelling the internal nodes *strictly increase*

Symmetric matrix & Ultrametric tree

	A	B	C	D	E
A	0	10	10	6	4
B		0	4	10	10
C			0	10	10
D				0	6
E					0

10 > 6 > 4

10 > 4

10

6

4

4

A   E   D   B   C
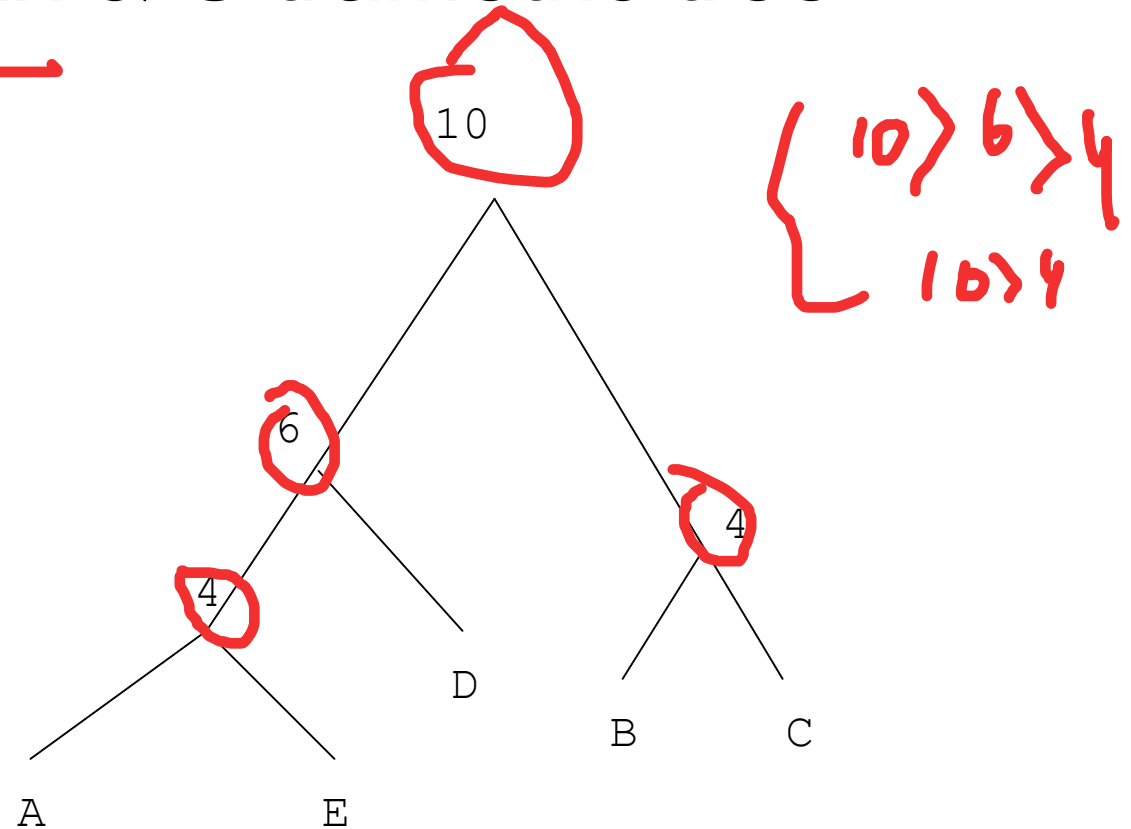
Obtaining pairwise distances

nucleic acid hybridization

- Melting temp DNA hybridisations,
- Strength of antibody cross reactions
- Protein structure comparison
- Sequences: edit distance and variations
  - $d(i,j)$: fraction f of sites u where residues x_{iu} x_{ju} differ (assume an alignment)
  - For 2 unrelated sequences : random substitutions cause f to approach fraction of differences expected by chance, hence:
  - Jukes-Cantor distance $d(i,j) = -3/4 * \log(1-4f/3)$
    - Tends to ∞ as equilibrium value of f (75% residues differ) reached

Gene sequence distance programs

- Blast (!) [but 'score' *increases* with similarity…]

- Clustal[w] – fasta format, but outputs a tree

- Phylip – its own format – sequences must be of the same length…hence use clustalw to align the sequences first [output in Phylip format, no tree]

Clustering methods: UPGMA

Unweighted Pair Group Method using Arithmetic Averages

Define the distance d(i,j) between two clusters Ci and Cj to be the average distance between pairs of sequences from each cluster:

$$d(i,j) = \frac{1}{|C_i| * |C_j|} \sum_{p \in C_i, q \in C_j} d(p,q)$$

where |Ci| and |Cj| denote the number of sequences in clusters i and j respectively

Note that if Ck = Ci ∪ Cj and Cl is any other cluster then

$$d(k,l) = \frac{d(i,l) * |C_i| + d(j,l) * |C_j|}{|C_i| + |C_j|}$$

UPGMA algorithm

Initialisation:

- Assign each sequence i to its own cluster Ci

- Define one leaf T for each sequence, and place it height zero

Iterate

- Determine the two clusters i,j for which d(i,j) [distance i-j] is minimal.
  (If there are more than two such pairs, pick one at random)

- Define a new cluster k by Ck = Ci ∪ Cj, and define d(k,l) for all l by

$$d(k,l) = \frac{d(i,l) * |C_i| + d(j,l) * |C_j|}{|C_i| + |C_j|}$$

- Define a node k with daughter nodes i and j, and place it at height d(i,j)/2

- Add k to the current clusters and remove i and j

Termination
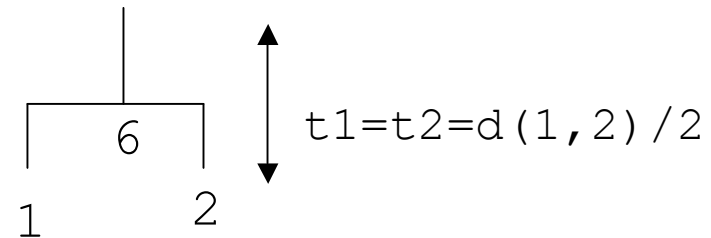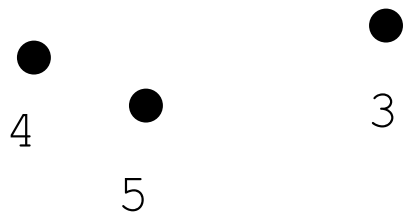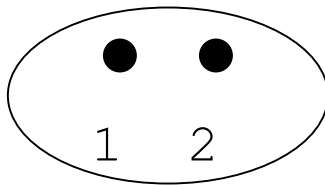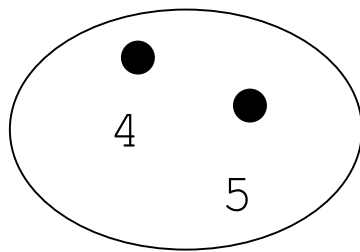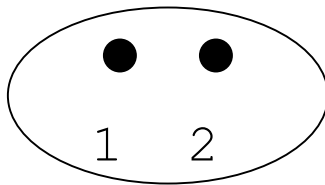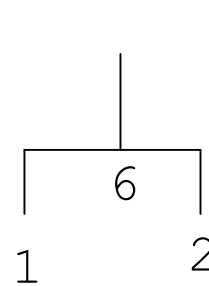- When only two clusters i,j remain, place the root at height d(i,j)/2

UPGMA example

1 2

4

5

3

UPGMA example

$t1 = t2 = d(1,2)/2$
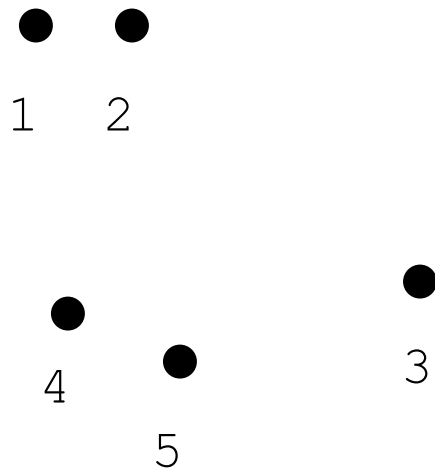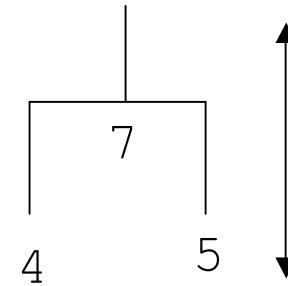
UPGMA example

$$t4=t5=d(4,5)/2$$

UPGMA example
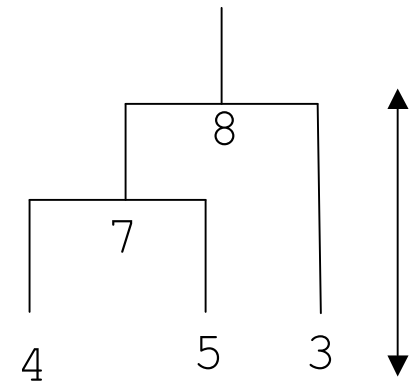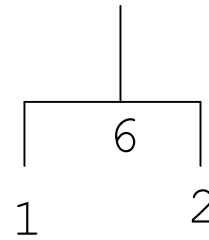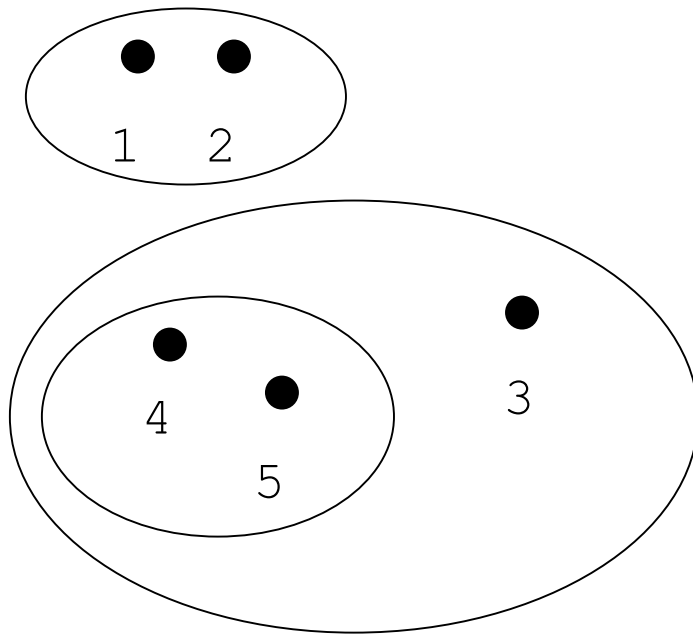
t3=d(3,7)/2

UPGMA example

d(6,8)/2
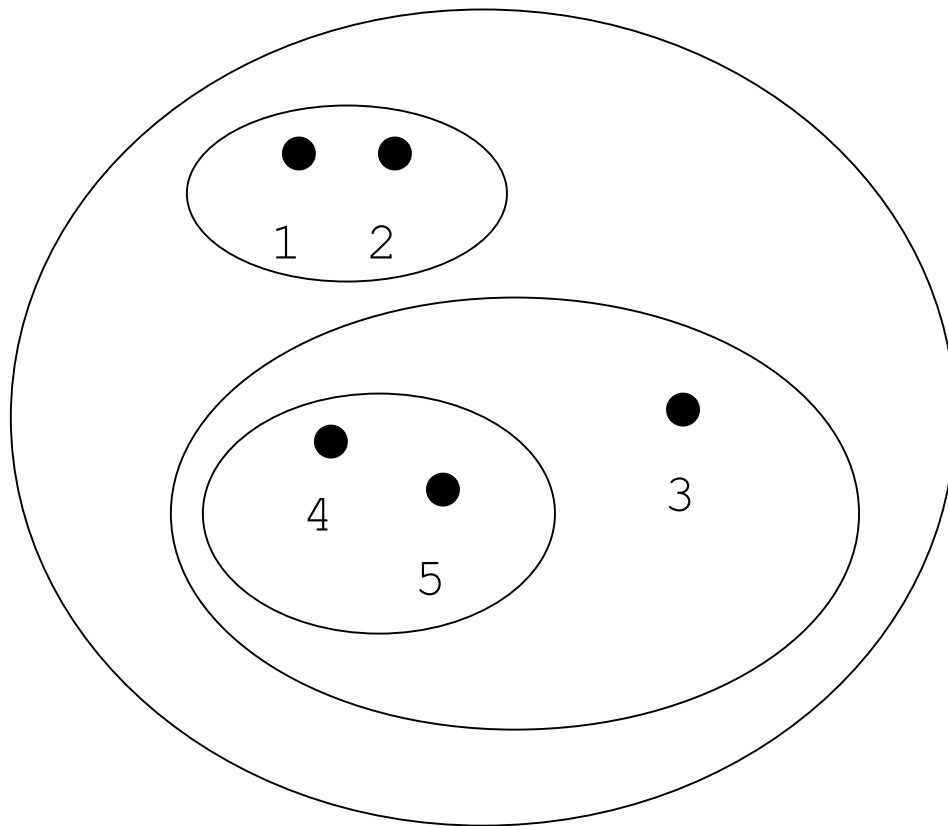
UPGMA tree construction example

	A	B	C	D	E
A	0	8	4	6	8
B		0	8	8	4
C			0	6	8
D				0	8
E					0

Like this?

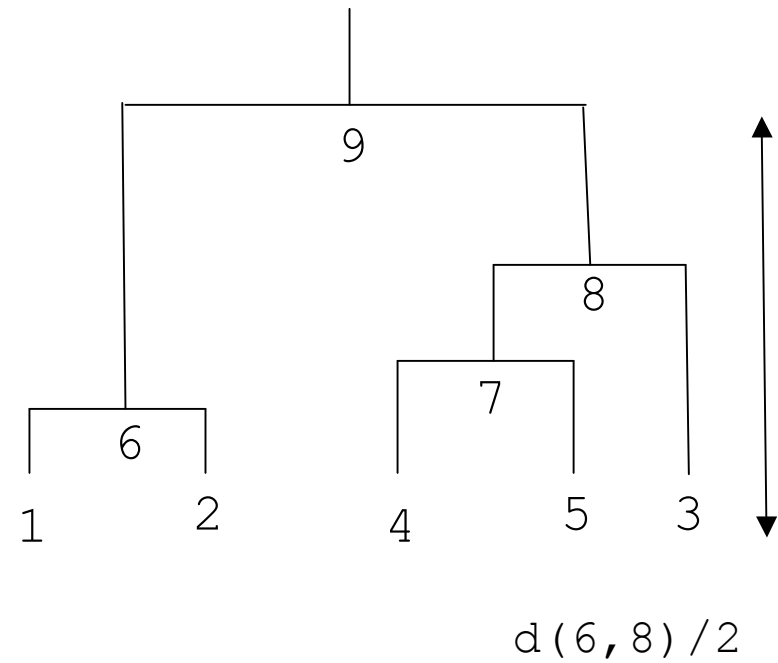|	A	B	C	D	E
A	0	8	4	6	8
B		0	8	8	4
C			0	6	8
D				0	8
E					0

	A	$\binom{B}{E}$	C	D
A	0	8	4	6
(B E)		0	8	8
C			0	6
D				0

	$\binom{A}{C}$	$\binom{B}{E}$	D
(A C)	0	8	6
(B E)		0	8
C			0

Tree (re)construction

Given: set of sequences

Assume that

    1 the pairwise sequence alignments provide a measure for the evolutionary distance between the sequences

    2. the the resulting distance matrix constitutes an ultrameric (the ideal case),

Reconstruct the phylogenetic, ultrametric tree by the general clustering procedure:

Always pick the closest pair from the distance matrix and merge these two objects into one.

Schemes differ in how the distance between a newly formed object and the other objects is defined.

If object x has been formed by merging y and z, and u is another object:

    Single linkage clustering: $d(x,u) = min(d(y,u), d(z,u))$

    maximal linkage clustering: $d(x,u) = max(d(y,u), d(z,u))$

    average linkage clustering: $d(x,u) = (d(y,u) + d(z,u)) / 2$

Assumptions underlying UPGMA

- ***Molecular clock with constant rate***
  - Edge lengths correspond to times measured by clock
  - Divergences of (sequences) assumed to occur at constant rate at all points in the tree, I.e.
  - Sum of times down a path to the leaves from any node is the same, whatever choice of path
- ***Additivity of edge lengths***
  - Distance between any pair of leaves = sum of the lengths of the edges on the paths connecting them
  - (automatic when UPGMA tree *constructed*)

Incorrect tree construction by UPGMA???

	A	B	C
A	0	3	6
B		0	5
C			0

Incorrect tree construction by UPGMA

2   3

4

1
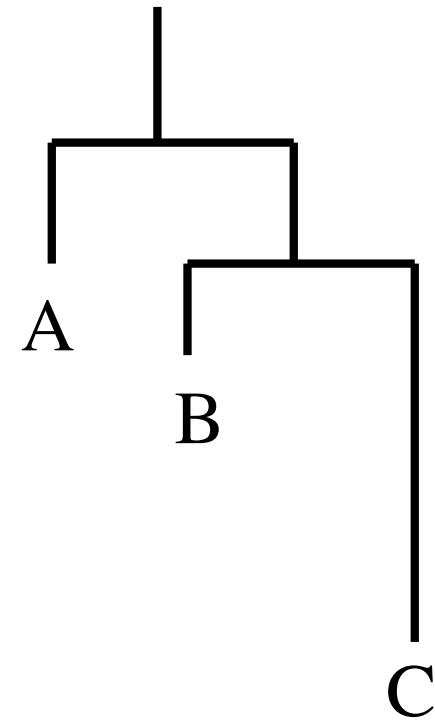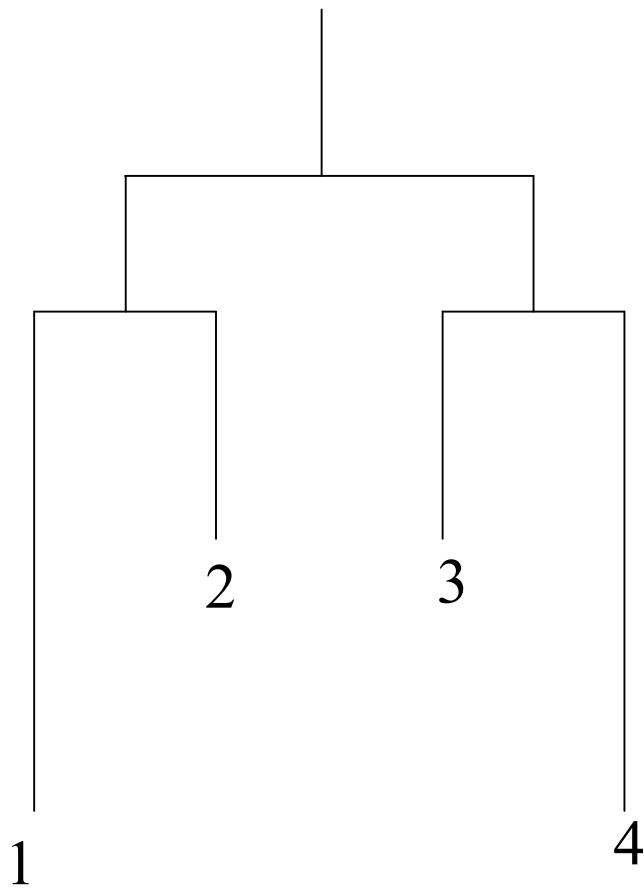
What will UPGMA give?

Incorrect tree reconstruction by UPGMA

	A	B	C	D	E
A	0	16	16	10	6
B		0	6	16	16
C			0	16	16
D				0	10
E					0

Construct the tree by UPGMA

Addivity & neighbour-joining

- Possible to have *molecular clock property fail* but *addivity hold*

- Use e.g. **neighbour-joining** algorithm

- Does *not* produce rooted trees…

Tree whose closest leaves are not neighbours.

1

2

0.1   0.1

0.1

0.4   0.4

3

4
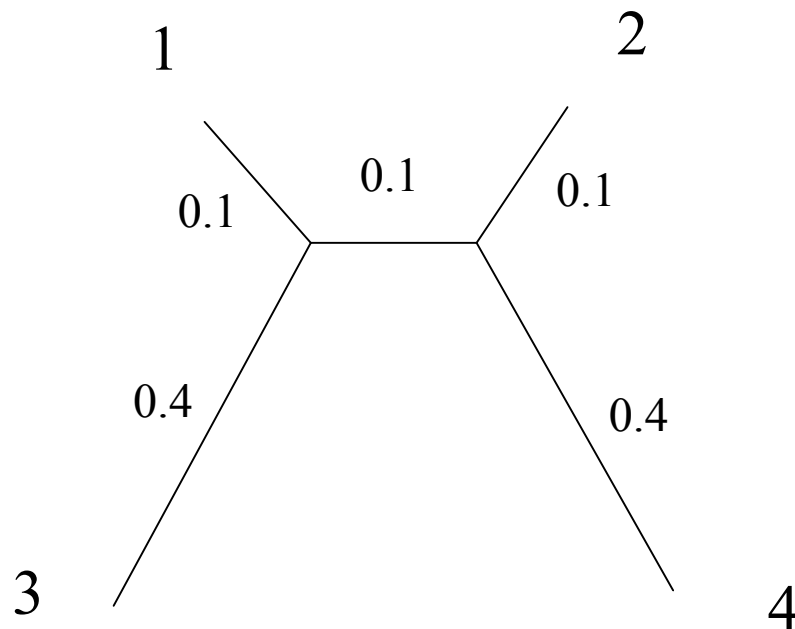
$d(1,2) =?, d(1,3)=?$

Which are
neighbouring
and which are
closest?

Distances

Hence do not pick closest leaves, but subtract the averaged distances to all other leaves to compensate for long edges

$$D(i,j) = d(i,j) - \left(r'_i + r'_j \right)$$

where

$$r'_i = \frac{1}{|L| - 2} \sum_{k \in L} d(i,k)$$

Phylogenetic Trees

Test for additivity

Four point condition:

**For every set of 4 leaves i,j,k,l
two of the distances
d(I,j)+d(k,l) d(I,k)+d(j,l), d(I,l)+d(j,k)
must be equal, and also larger to the third**

Can use NJ even if lengths are not additive, but correct reconstruction not guaranteed.

Rooting trees

- Add an ***outgroup***
- E.g. add axolotl
- If no outgroup, then ad-hoc strategies
- See also ***TreeView*** by Rod Page (Glasgow)

axolotl

giant panda

lesser panda

moose

goshawk

vulture

duck

alligator

Quick demo

http://www.dina.dk/~sestoft/bsa/Match7Applet.html
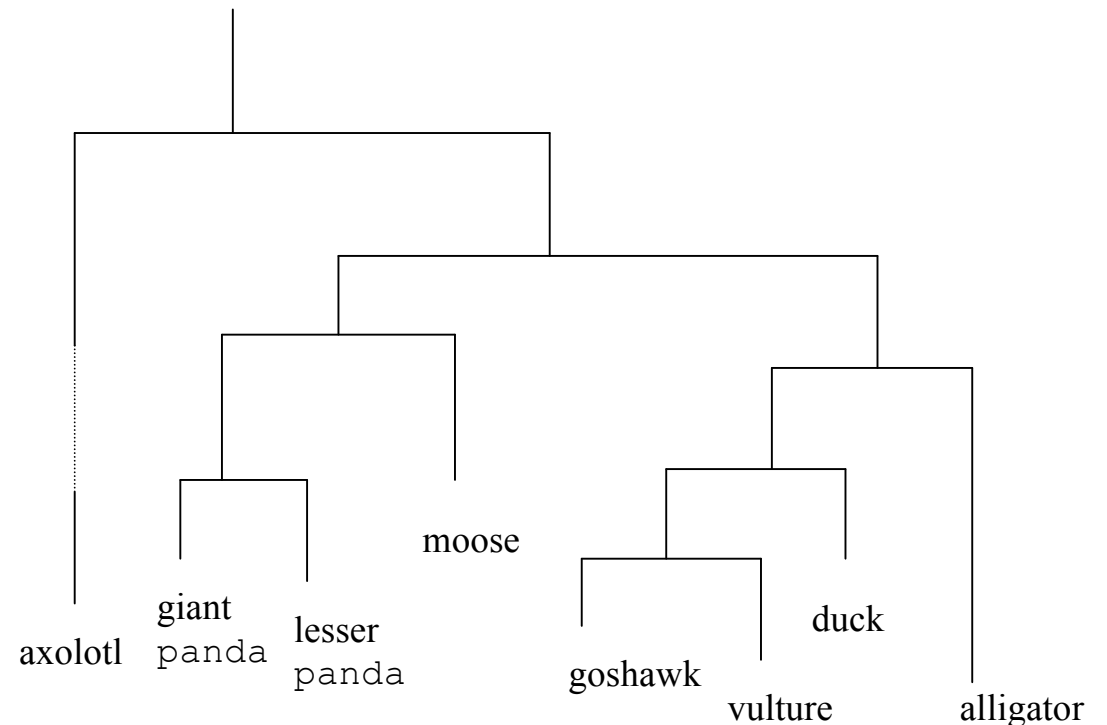
Parsimony

- Character-based method
- Finds the tree which can explain the observed sequences with a minimal number of substitutions
1. Assigns a *cost* to a given tree
2. Searches through all trees to find overall minimum of this cost

Parsimony example

AAG
AAA
GGA
AGA

Total number of changes?

AAA

| AAA | 1 | AGA |

1   AAA   1   AGA

AAG   GGA

AAA

AAA   AAA   2

1   1   GGA

AGA

AAG   AAA

AAA

AAA   AAA   1

1   2   AGA

GGA

AAG   AAA
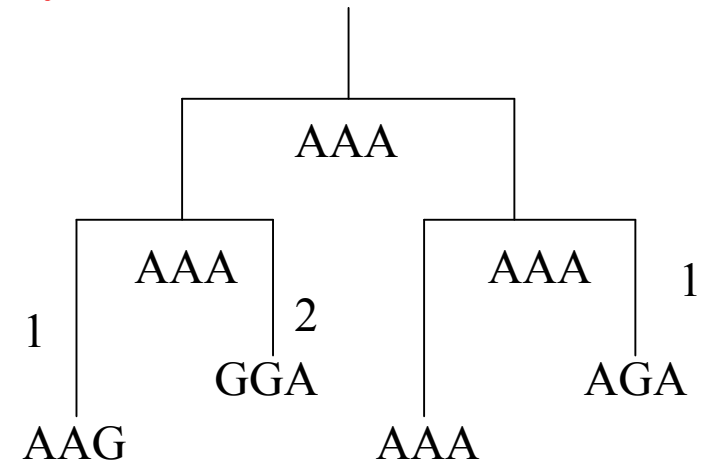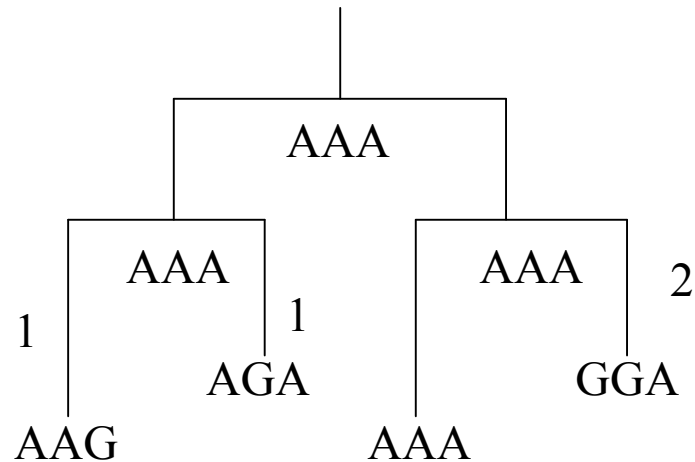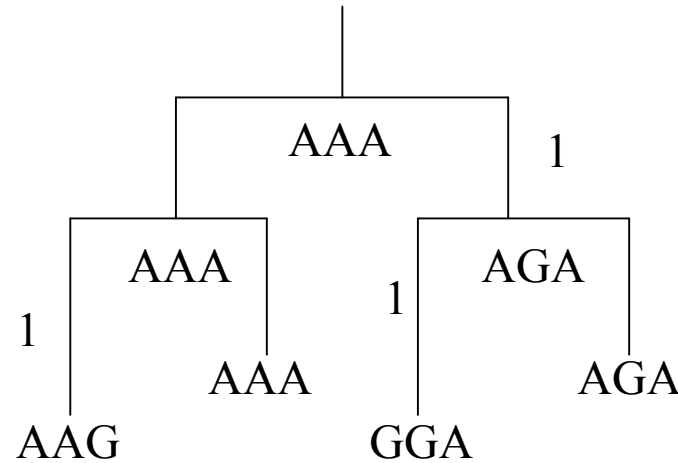
Parsimony method

- Treats each site independently

- Given a topology & assignment of residues →leaves:

- Basic step = count minimal number of changes needed at 1 site

Weighted parsimony

- Count number of subsitutions *and*

- Add costs S(a,b) for each substitution of a by b

- Aim : minimise this cost

- Weighted parsimony = traditional parsimony when S(a,b) = 1 for all a≠b

- Algorithm starts at the leaves and works up to the root : *post-order traversal*

Traditional parsimony

- Just count the number of substitutions :

- Keep a list of minimal cost residues at each node, plus current cost

Parsimony : rooted / unrooted

- Parsimony forumlated for rooted trees
- Minimal cost in trad.parsimony independent of location of root
- Root can be removed - number of trees to be searched over is reduced
- But easier to count costs in rooted tree

Search stratgeies

- Improve over simple enumeration
- Stochastic methods: randomly swap branches on tree and chose altered tree if better than current
  - Not guaranteed to find overall best tree; adding sequences in different orders can give different trees
- Build tree by adding edges 1 at time [ditto]
- Branch and bound:
  - begin systematicall building trees with increasing number of leaves
  - Abandon avenue whenever current incomplete tree has cost > smallest cost obtained so far for complete tree

Assessing trees - bootstrap

- How much trees should be trusted
- Given dataset of alignment of sequences
  - Generate artificial dataset (same size) by picking columns from alignment at random with replacement (given column in original can appear several times)
  - Apply treebuilding to new dataset
- Repeat many (1000) times
- Frequency with which phylogenetic feature appears ⇒ measure of confidence in feature

Maximum likelihood

- Rank trees according to their
  - likelihood P(data|tree)
  - Posterior probability (Bayesian view) P(tree|data)
- Define & compute the probability of a set of data given a tree
- Need a model of evolution - mutation and selection events that change sequences along the edges of a tree

Maximum likelihood

- Maximum Likelihood
- Given a probabilistic model for nucleotide or amino-acid substitution, select the tree that has the highest probability of generating the observed data – sequences
  - Give data D and model M, find tree T such that $P(D|T, M)$ maximised
- Actually what we really want is $P(T|D, M)$
  - Not so easy to obtain as summation over all possible tree topologies required to obtain posterior

$$P(T \mid D, M) = \frac{P(D \mid T, M)P(T, M)}{\sum_T P(D \mid T, M)P(T, M)}$$
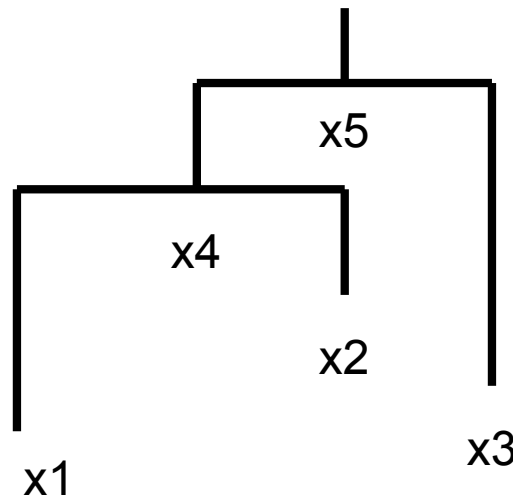
Phylogenetic Trees

- Assumptions
  - Different sites evolve independently
  - Diverged sequences evolve independently after diverging
- Consider following tree
  - *Likelihood corresponds to $P(D|T, M) = P(X1,X2,X3|T,M)$*

Phylogenetic Trees

- Note that
  - $P(D|T, M)$
    - $= P(X1,X2,X3|T,M)$
    - $= \sum_{X4, X5} P(X1,X2,X3,X4,X5|T,M)$
    - $= \sum_{X4, X5} P(X1|X4)P(X2|X4)P(X3|X5)P(X4|X5)P(X5) |T,M$
- Efficient methods to obtain required summation for likelihood (Felsenstein Algorithm)

- Have now only obtained likelihood we require maximum of likelihood over all possible topologies and models (branch lengths)

- Approximate (stochastic)search methods required

Maximum likelihood tree

- Search over tree topolgies
- For each topology: search over all possible lengths of edges
- (2n-3)!! rooted binary trees with n leaves
- N=10 :: 2 million, N=20 :: 2.2×10^{20}
- Thus need optimisation techniques

Probabilistic models of evolution

- Residue subsitution

- Deletion & insertion of groups of residues

- Simple model:
  - every site independent
  - only substitutions

Maximising likelihood

- Small number of sequences (2..5) : can enumerate all trees
  - Write down likelihood as function of edge lengths
  - Maximise likelihood by numerical technique (Kishano et al 1990, protein sequences - use PAM matrices)

- Larger number of sequences: Felsenstein's algorithm

- For protein sequences computationally demanding 20x20 substitution matrix

- Can use sampling methods

More realistic evolutionary models

- Different rates at different sites

- Models with gaps

Internet resources and programs

- Compilation of available programs:
  http://evolution.genetics.washington.edu/phylip/software.html

- http://bioweb.pasteur.fr/seqanal/phylogeny/phylip-uk.html -- phylip , e.g. for nj

- http://evolution.genetics.washington.edu/phylip.html Phylip home page [widely used, poor interface…]

- http://www.hiv.lanl.gov/content/hiv-db/TREE_TUTORIAL/Tree-tutorial.html

Phylogenetic Trees

Differences between methods

- UPGMA does not employ models of evolution

- Maximum Likelihood & Maximum Parsimony employ models of evolution

- Maximum Likelihood claimed to be best for large evolutionary distances
  - Very time consuming to build trees

Summary

- Phylogenies
- Trees, representations, rooted, unrooted
- Metrics, ultrametrics, additivity, molecular clock
- Distance methods: UPGMA (ultrametric), NJ (additive; no root)…
- Tree rooting
- Some Programs