Phylogenetic Trees

Eight

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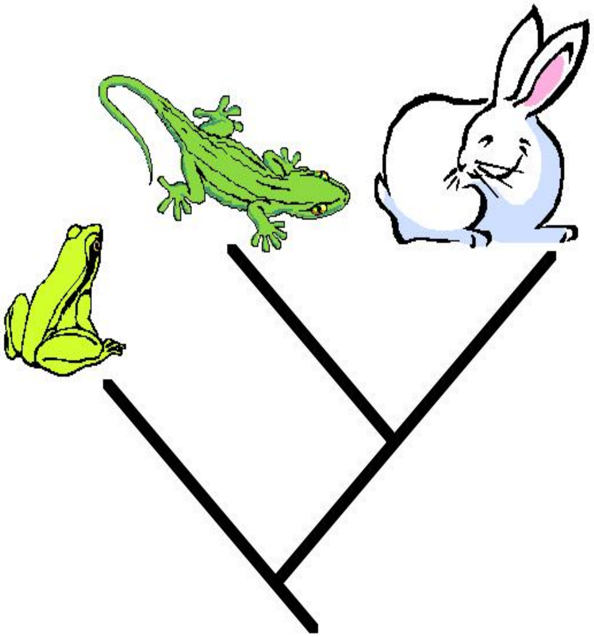
Phylogenetic Trees



- Distance Methods
- Character Methods
- Molecular Clock
- UPGMA
- Maximum Parsimony
- Maximum Likelihood
- Fitch and Margoliash

Phylogeny Terminology

- Phylogeny- the history of descent of a group of organisms from a common ancestor

  From Greek:

  – phylon = tribe, race

  – genesis = source

- Taxonomy- the science of classification of organisms

  From Greek:

  – taxis = to arrange, classify

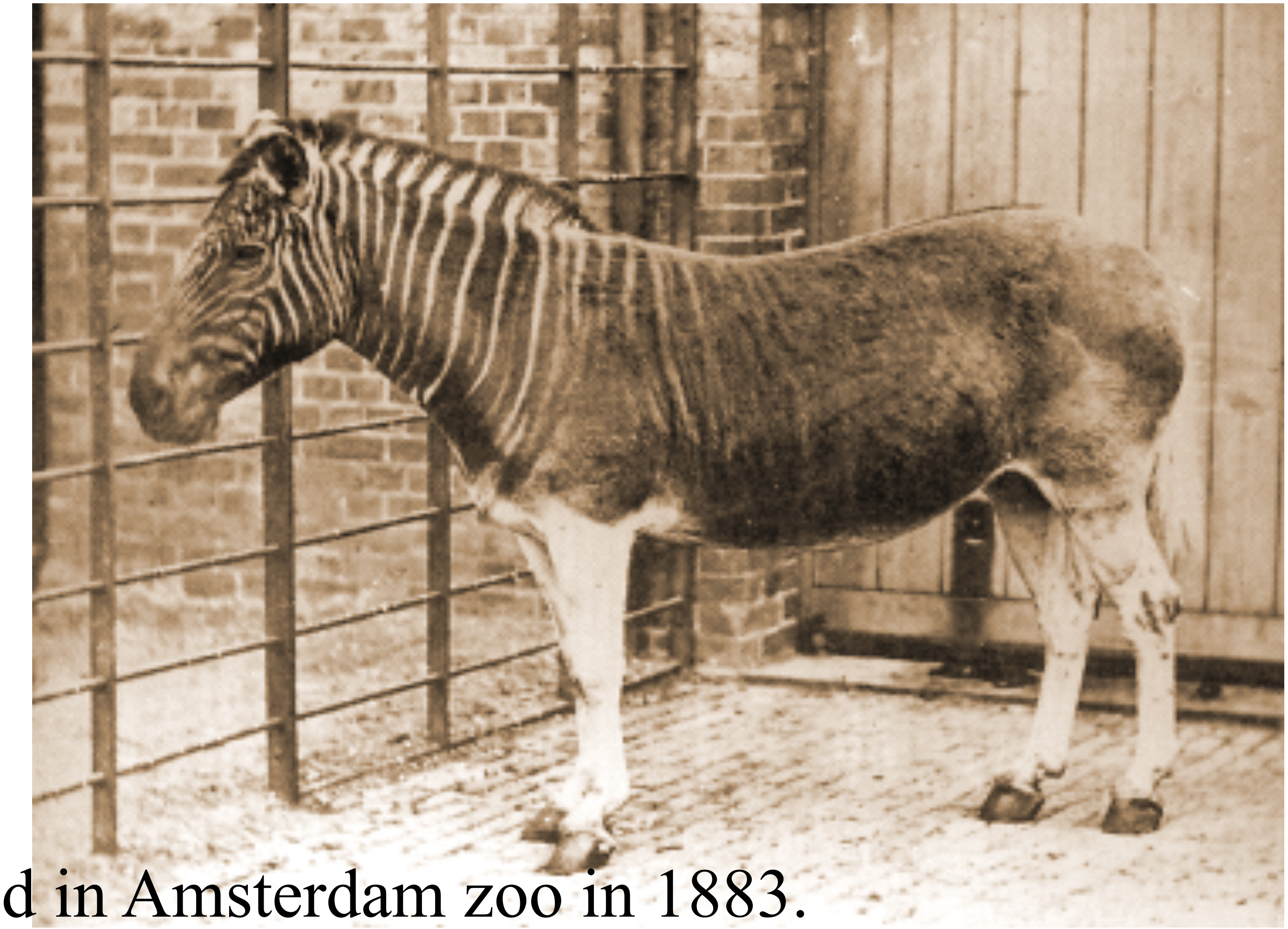
Phylogeny: Inference Tool

- Phylogeny is the inference of evolutionary relationships.
- Traditionally, phylogeny relied on the comparison of morphological features between organisms.
- Today, molecular sequence data are also used for phylogenetic analyses.

Importance of Phylogeny

- How many genes are related to my favorite gene?
- Was the extinct quagga more like a zebra or a horse?
- Was Darwin correct when he stated that humans are the closest to chimps and gorillas?
- How related are whales and dolphins to cows?
- Where and when did HIV originate?
- What is the history of life on earth?

Picture of Last Quagga



Died in Amsterdam zoo in 1883.

Phylogenetic Analysis

- A phylogenetic analysis of a family of related nucleic acid or protein sequences is a determination of how the family might have been derived during evolution.
- Two sequences that are very much alike will be located as neighboring outside branches (leaves) and will be joined by a common branch beneath them.

Aim of Phylogenetic Analysis

- The evolutionary relationships among the sequences are depicted by placing the sequences as outer branches on a tree.
- The branching relationships on the inner part of the tree then reflect the degree to which different sequences are related.
- The aim of phylogenetic analysis is to discover all of the branching relationships in the tree and the branch lengths.

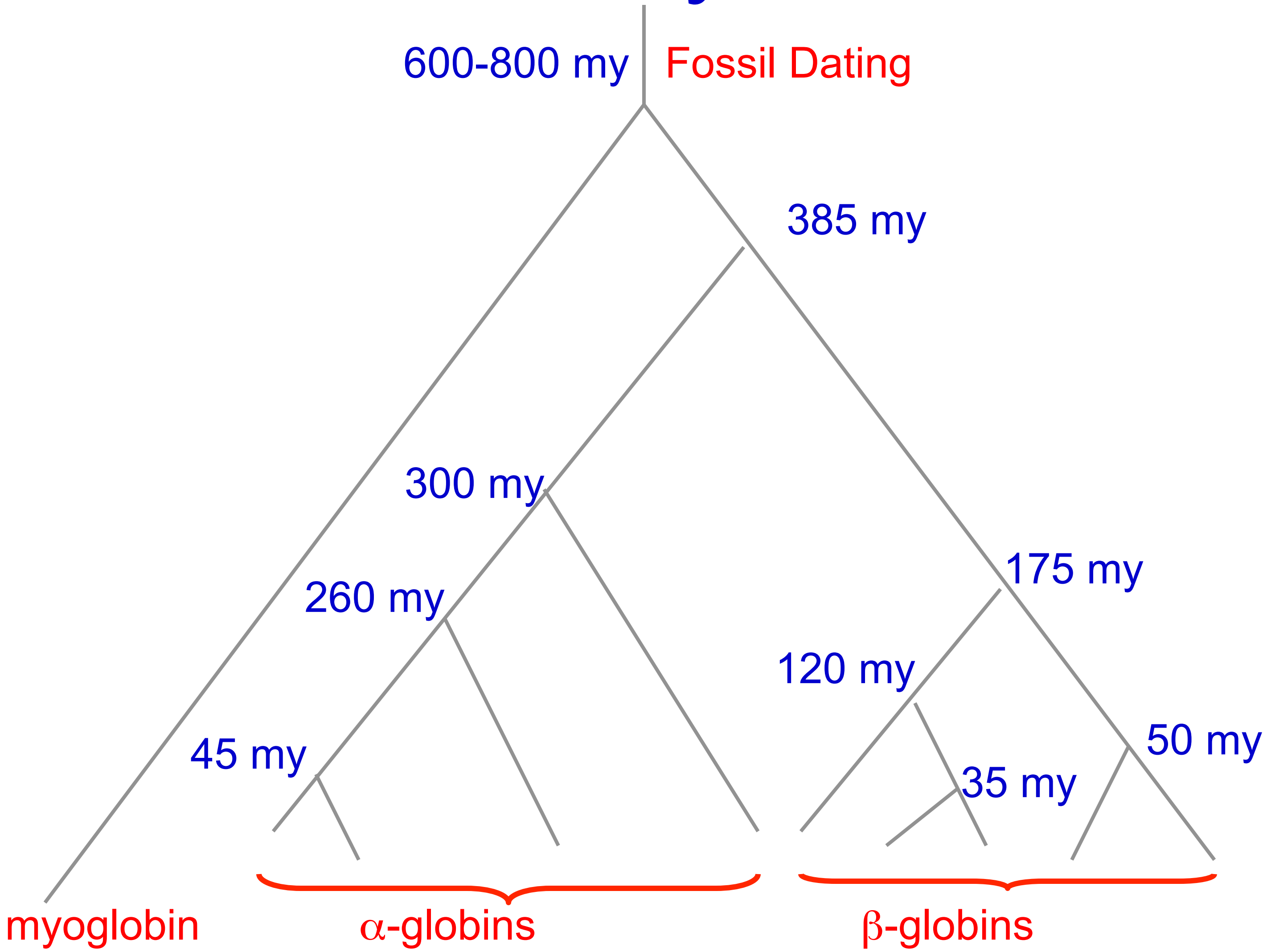
Phylogenetic Trees

- Phylogenetic tree: diagram showing evolutionary paths of species/genes.
- Why do we construct phylogenetic trees?
  - To understand the path (lineage) of various species.
  - To understand how various functions evolved.
  - To perform multiple alignment.

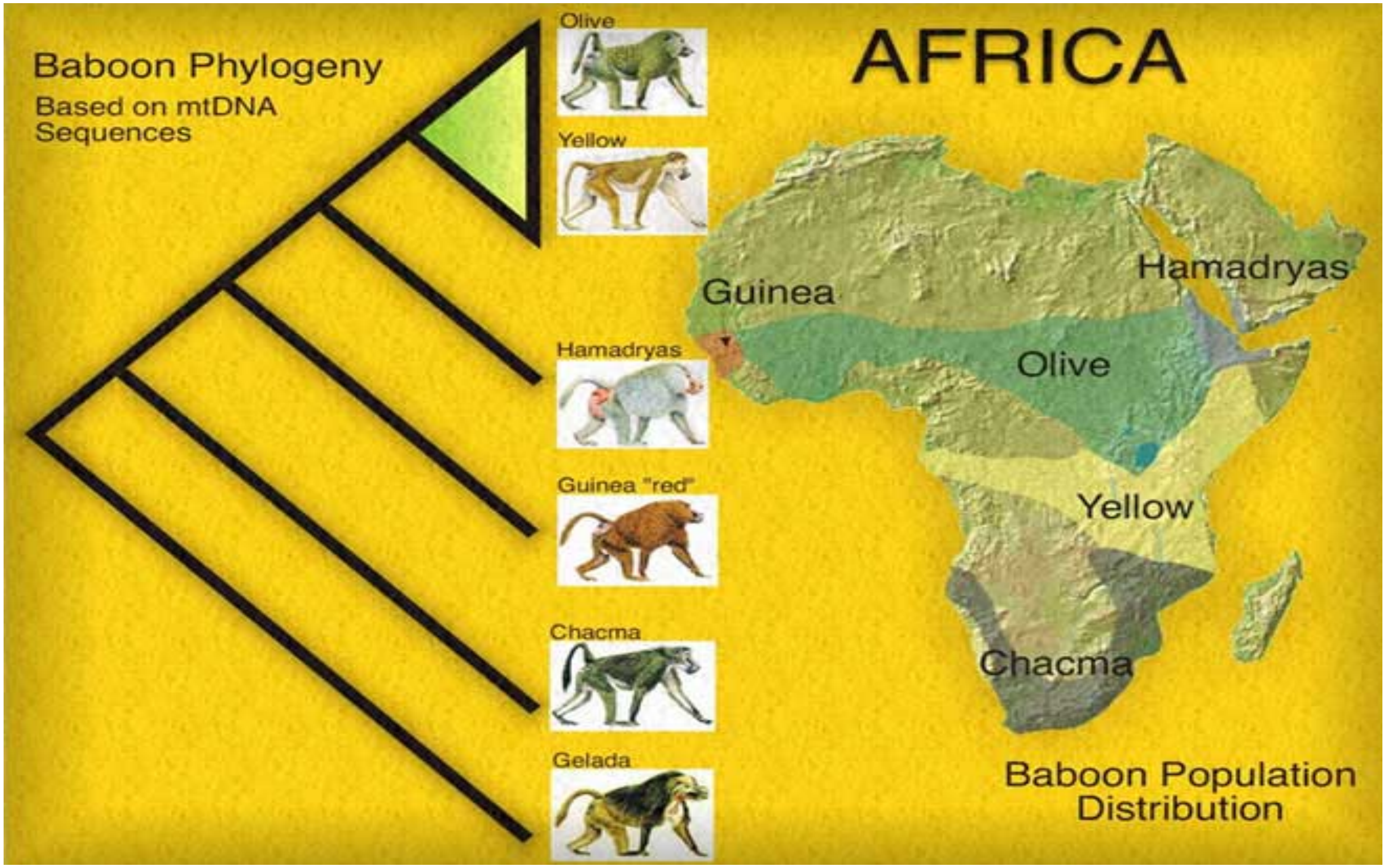
Additional Uses of Phylogenetic Trees

- To study the evolutionary relationships of different species and to understand how species relate to one another.
- To predict the unknown gene’s function according to its phylogenetic relationship to other genes.

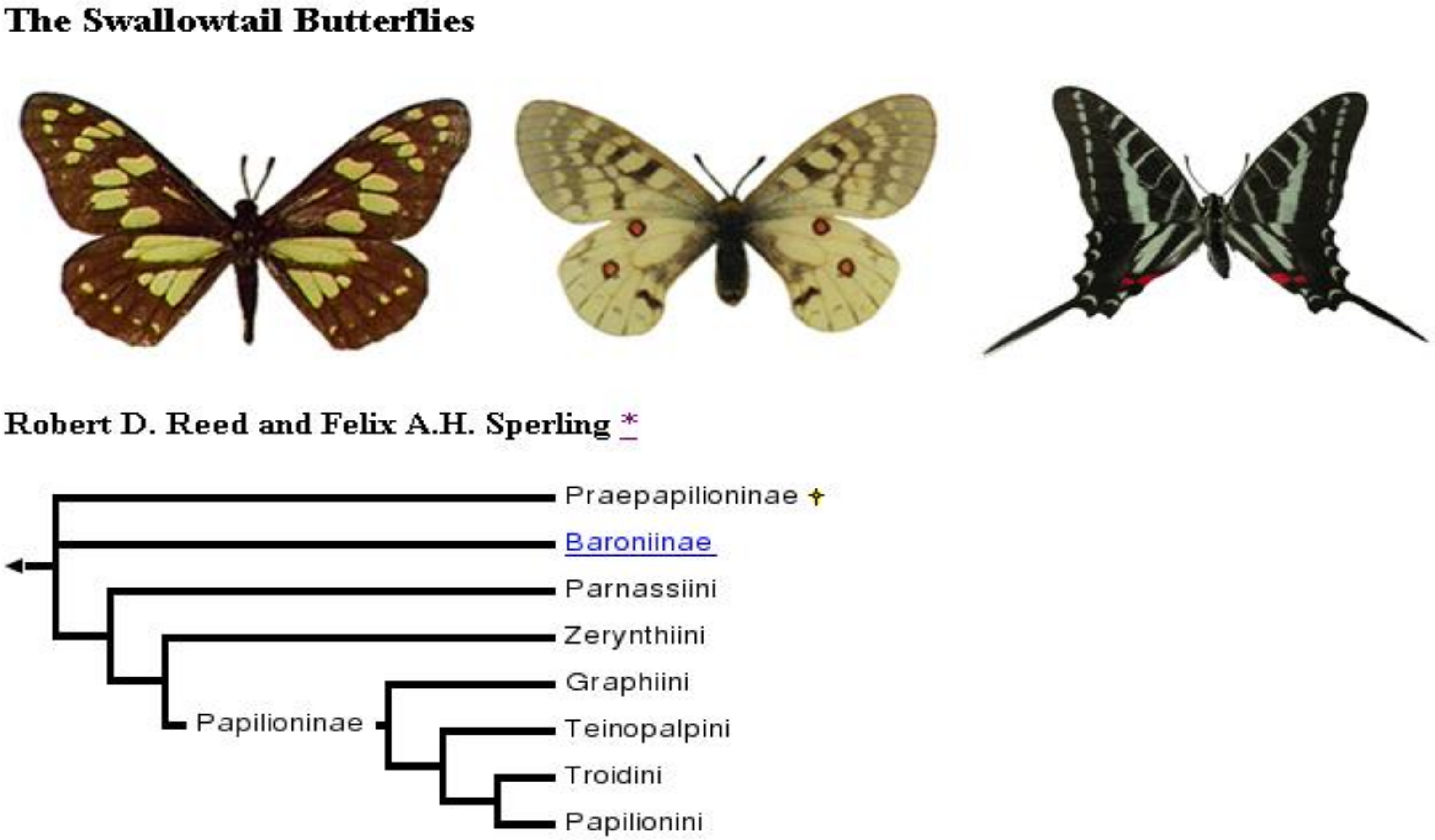
Globin Family Evolution



Baboon Phylogeny



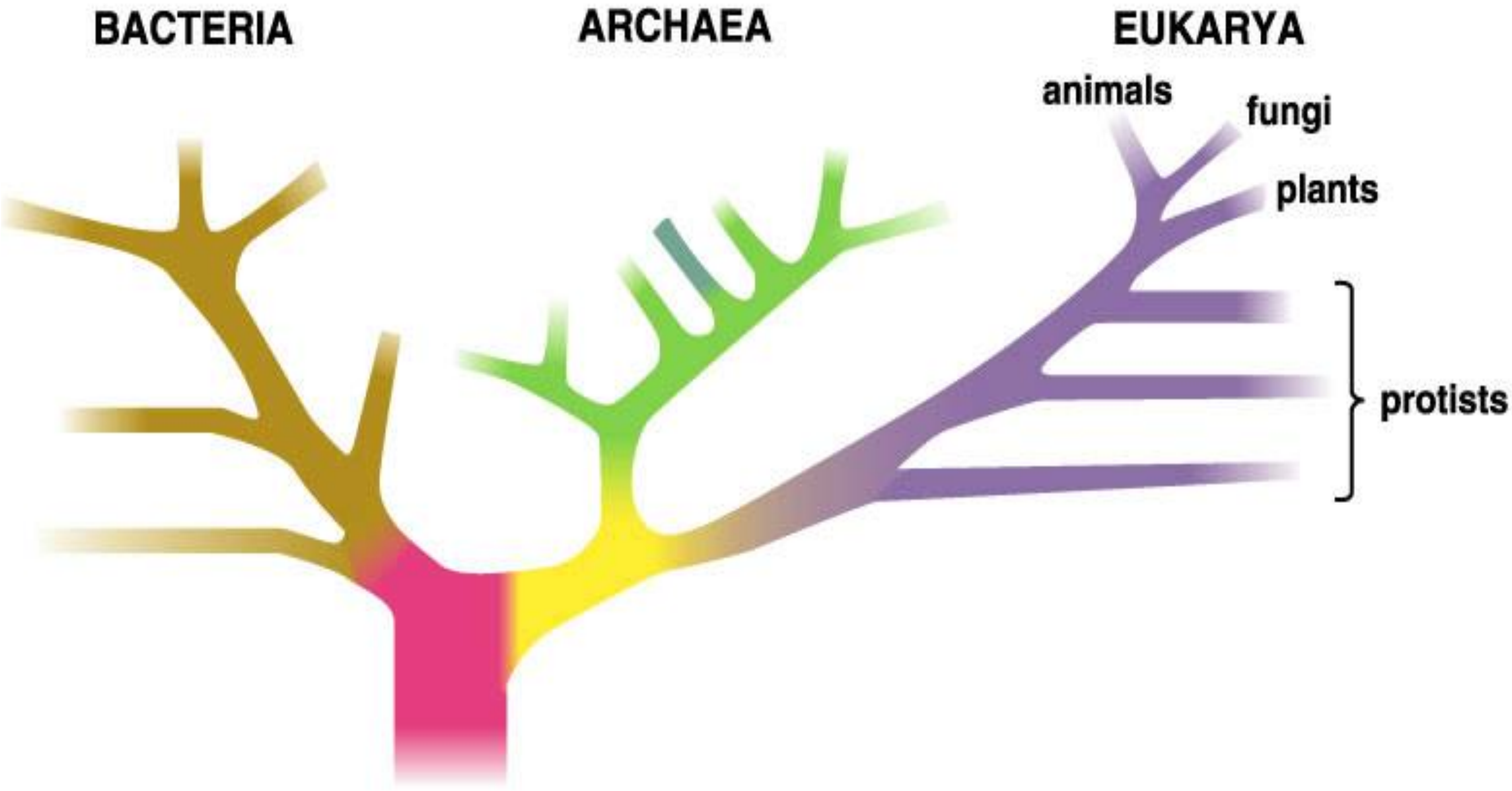
Swallowtail Butterflies



The Swallowtail Butterflies

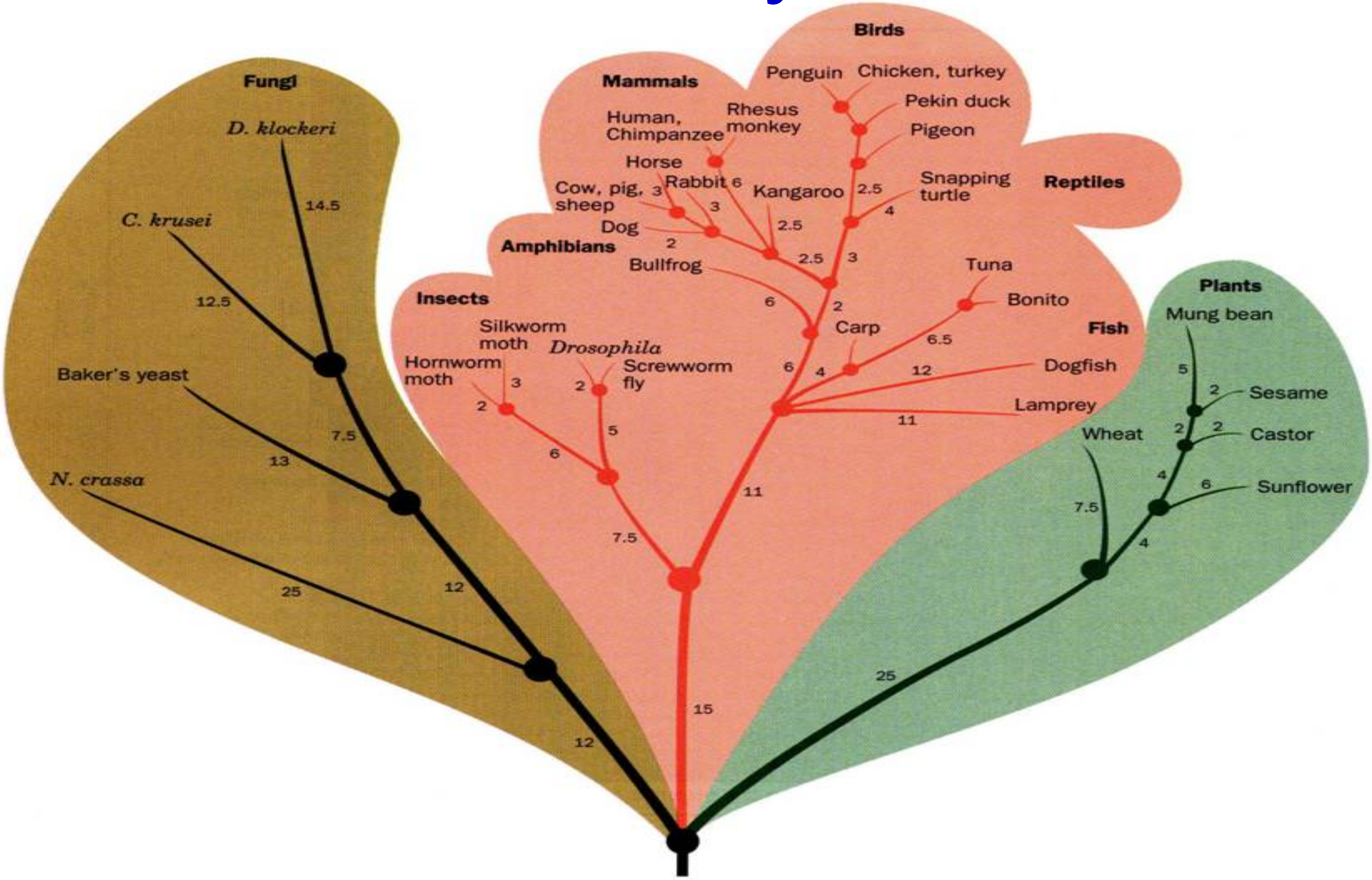
Robert D. Reed and Felix A.H. Sperling *

Major Divisions of Living Things



by C. Woese on the basis of 15S RNA sequences

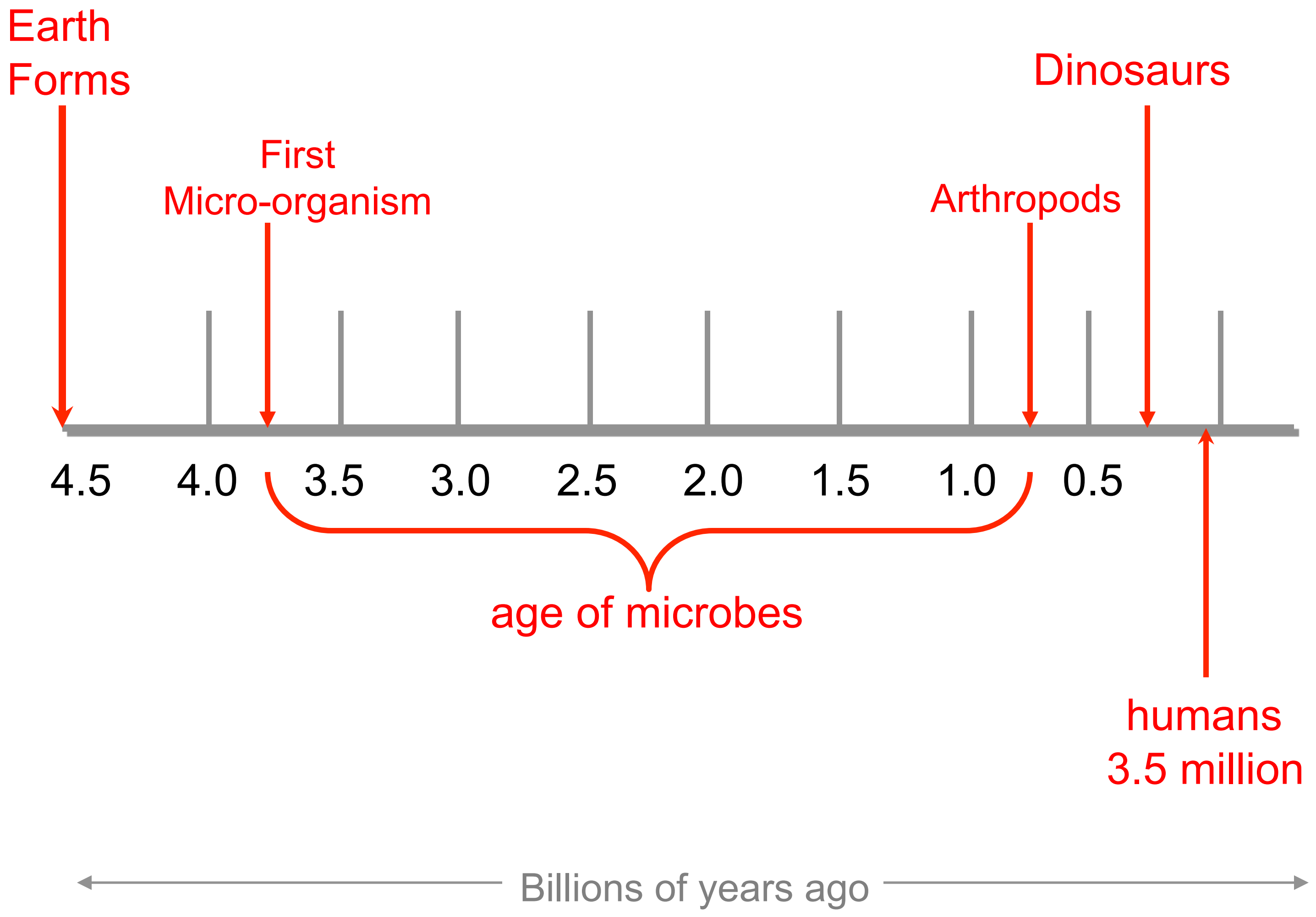
Evolutionary Tree



Advantages of Molecular Traits

- They directly reflect the underlying process of evolution- changes in the hereditary material
- There are a vast number of potential traits
- They can detect differences between very closely related organisms (even those that show no phenotypic difference)
- They are not affected by the environment (unlike some morphological traits)
- Since mutations generally occur as random events with specific probabilities, the number of mutations can be used to calibrate evolutionary time (molecular clocks)

Time Line For Life



Earth Forms

First Micro-organism

Arthropods

Dinosaurs

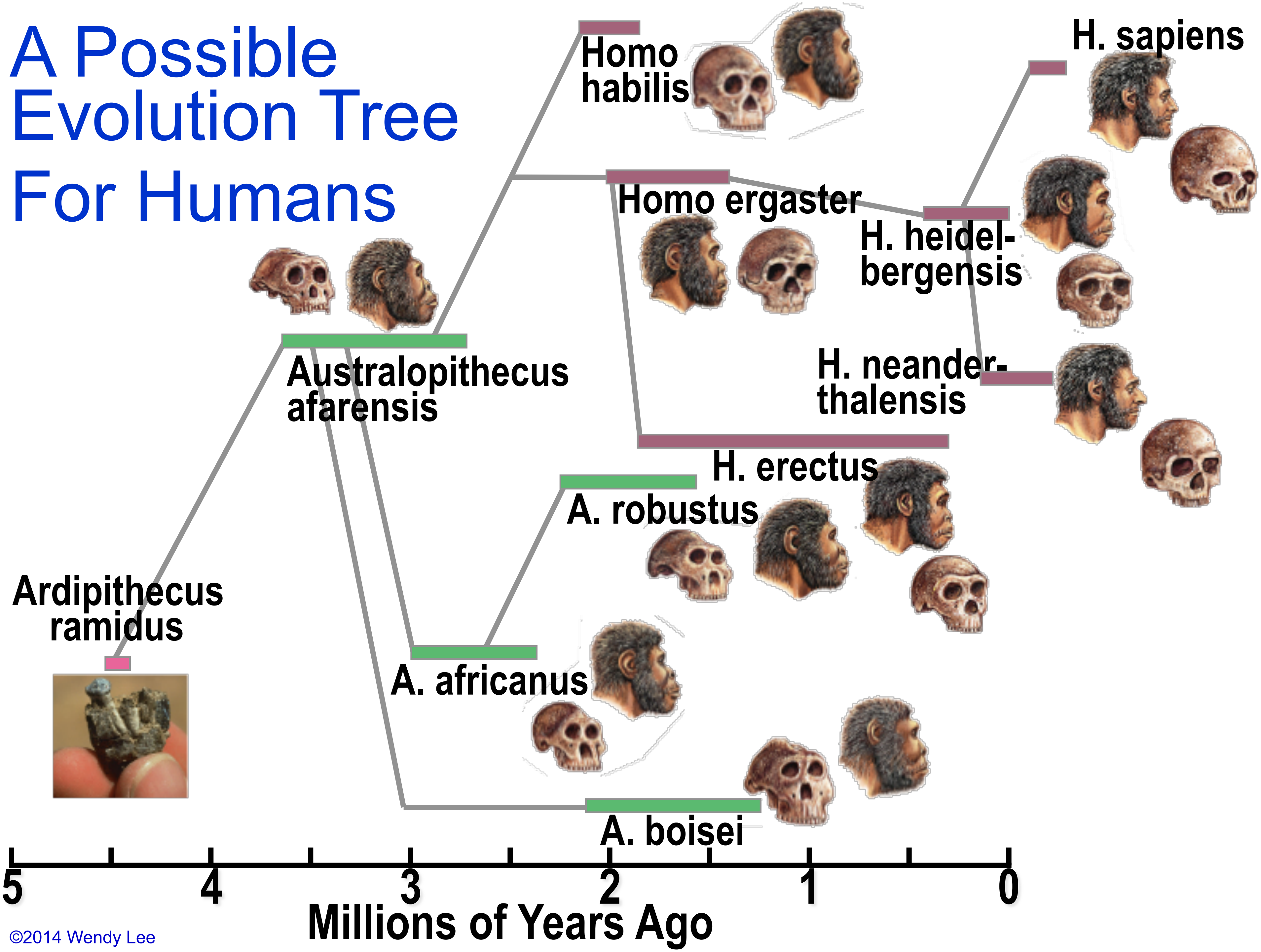
4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

age of microbes

humans
3.5 million

Billions of years ago

A Possible Evolution Tree For Humans



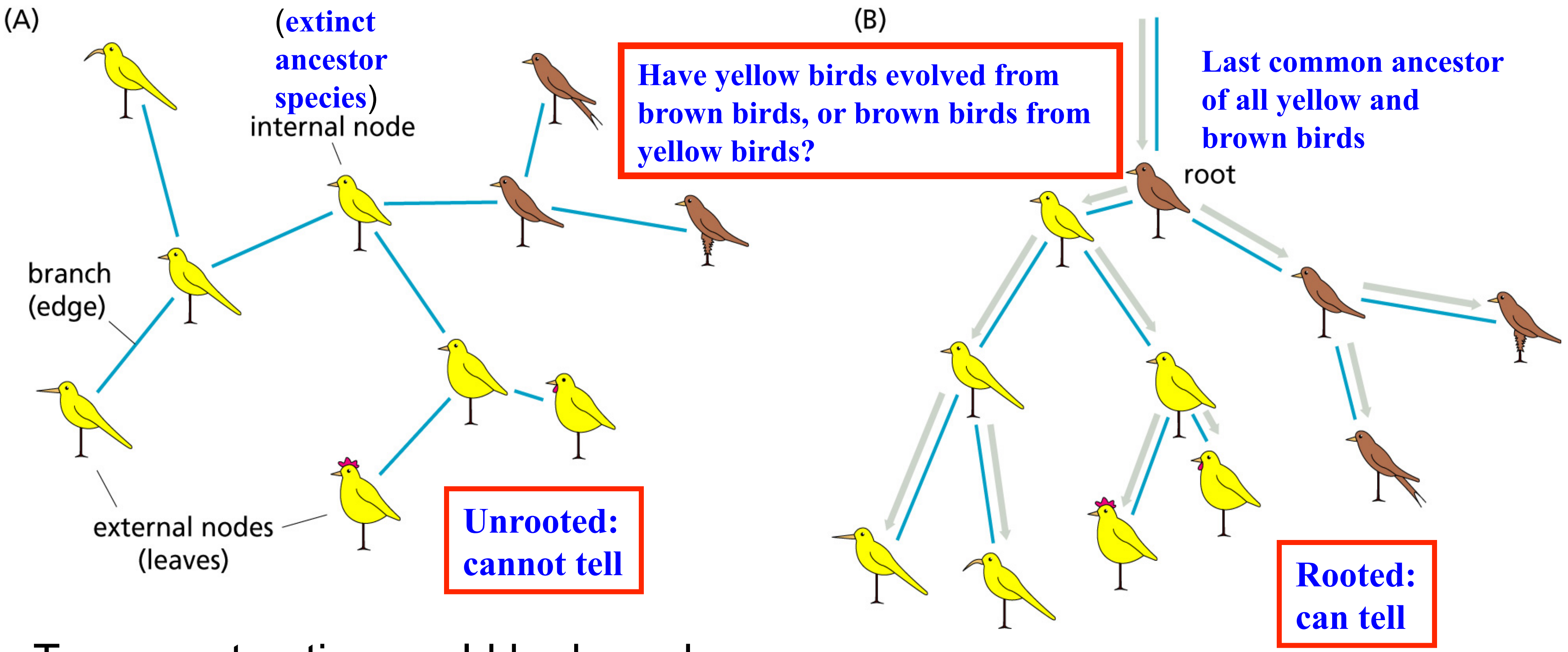
More Terminology

- Leaves represent objects (genes, species) being compared
  - Taxon refers to the leaves when they represent species and broader classifications of organisms.
- Internal nodes are hypothetical ancestral units
- In a rooted tree, the path from root to a node represents an evolutionary path.
- An unrooted tree specifies relationships among objects, but not evolutionary paths.

Rooted and Unrooted Trees

- All objects in a rooted tree have a single common ancestor.
  - In general, rooted trees require more information to construct than unrooted ones.
- Objects are leaves in an unrooted tree and internal nodes are common ancestors.
  - In general, given any two leaves, we cannot tell if they have a common ancestor.

Unrooted and Rooted Trees



(extinct ancestor species)

Have yellow birds evolved from brown birds, or brown birds from yellow birds?

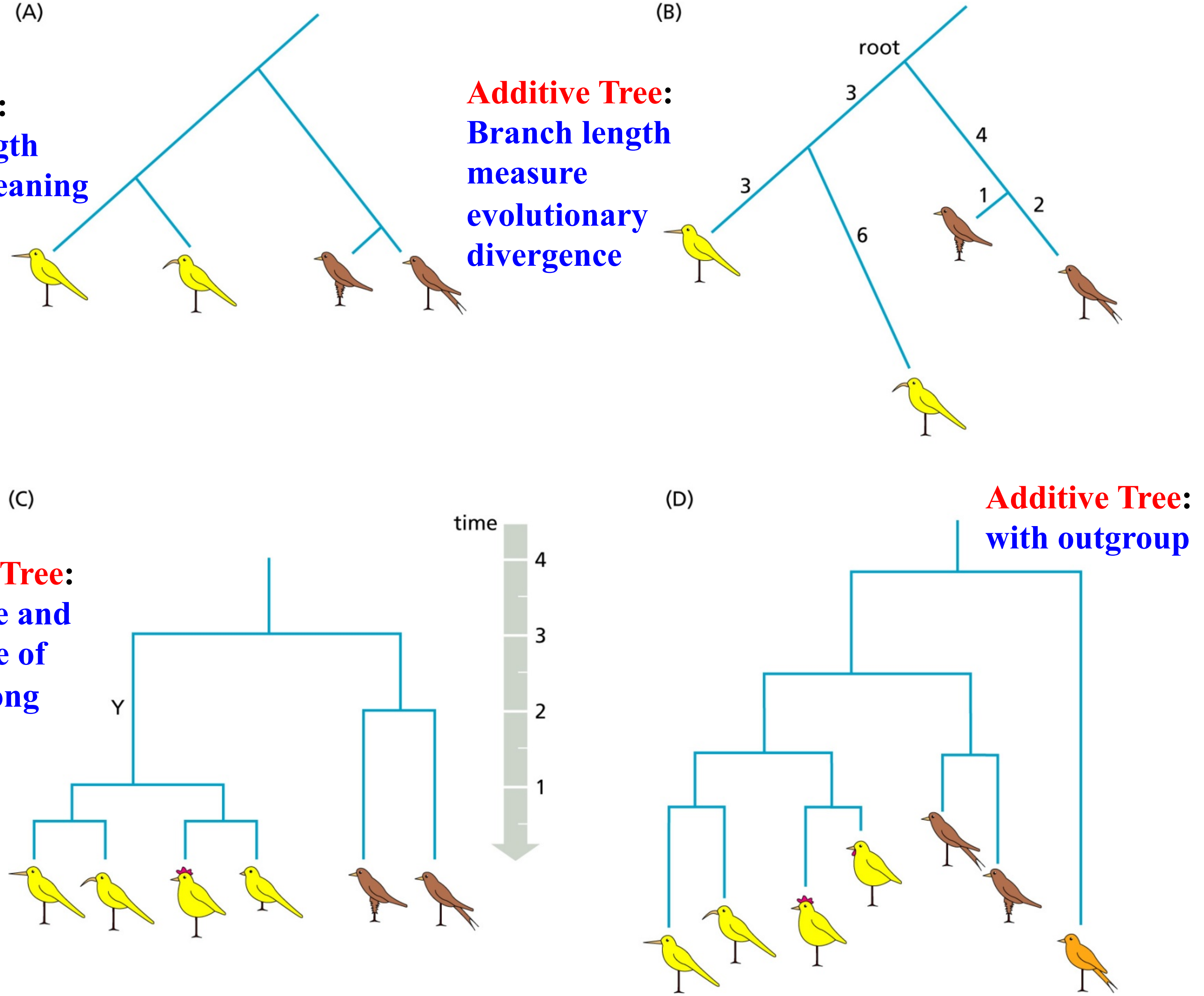
Last common ancestor of all yellow and brown birds

Unrooted: cannot tell

Rooted: can tell

Tree construction could be based on:

- morphological features, or
- sequence data



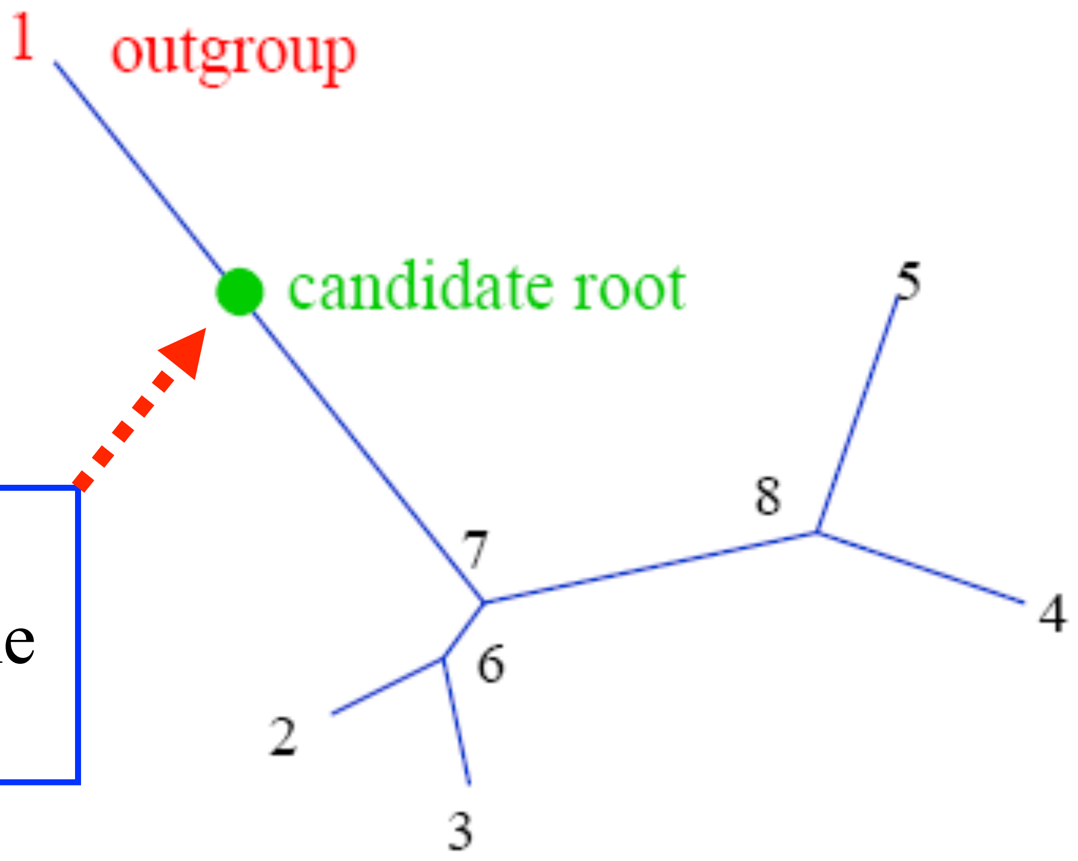
Cladogram: Branch length carry no meaning

Additive Tree: Branch length measure evolutionary divergence

Ultrametric Tree: Additive tree and constant rate of mutation along branches

Additive Tree: with outgroup

Rooting a Tree



The best place for having the root of the phylogenetic tree

Convergent and Parallel Evolution

- Convergent evolution

  independent evolution of similar traits due to similar selection pressure

  Example: wings in birds and bats

- Parallel evolution- independent evolution of common traits in organisms sharing distant relatives

  Example: patterns of butterfly wings.

Building of a Phylogenetic Tree

- Sequence Selection:
  - Identify a DNA or protein sequence.
  - Obtain related sequences by performing a database search.
- Perform multiple alignment.
- Build a phylogenetic tree.
- Check the robustness of the tree.

Distance and Character Based Trees

The construction of the tree is:

- distance-based: measures the distance between species/ genes (eg. mutations, time, distance metric).
  - First calculate the overall distance between all pairs of sequences, then construct a tree based on the distances.
- character-based: morphological features (eg. number of legs), DNA/protein sequences.
  - Use the individual substitutions among sequences to determine the most likely ancestral relationships.

    The tree is constructed based on the gain or loss of traits.

Methods for Constructing Phylogenetic Trees

- Distance-Based Methods:
  - Unweighted Pair Group Method Using Arithmetic Averages (UPGMA)
  - Fitch Margoliash (FM)
  - Neighbor Joining (NJ)
- Character-Based Methods:
  - Maximum Parsimony (MP)
  - Maximum Likelihood (ML)

Other Methods for Constructing Trees

Clustering Methods

- Follow a set of steps (an algorithm) and arrive at a tree.
- Use distance data.
- Produce a single tree.
- Do not use objective functions to compare the current tree to other trees.

Optimality Criterion

- Use objective functions to compare different trees.
- First define an optimality criterion, i.e. minimum branch length, and then find the tree with the best value for the objective function.

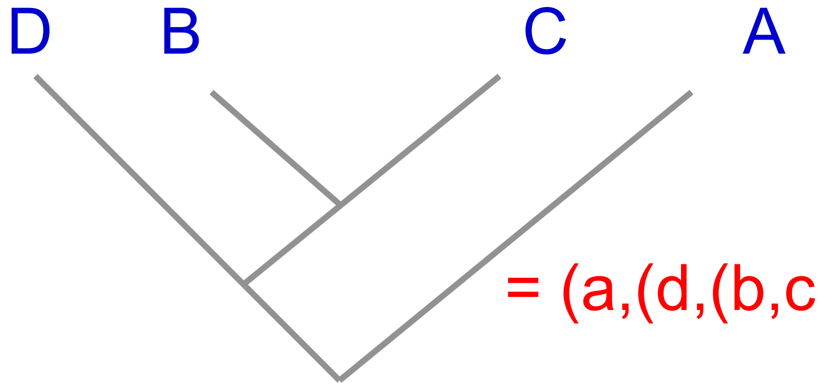
Clustering Algorithms

- The strength of clusterting algorithms is:
  - Their speed
  - Their robustenss
  - Their ability to reconstruct trees for very large numbers (thousands) of sequences.
  - Most clustering methods reconstruct phylogenetic trees for a set of sequences on the basis of their pairwise evolutionary distances.

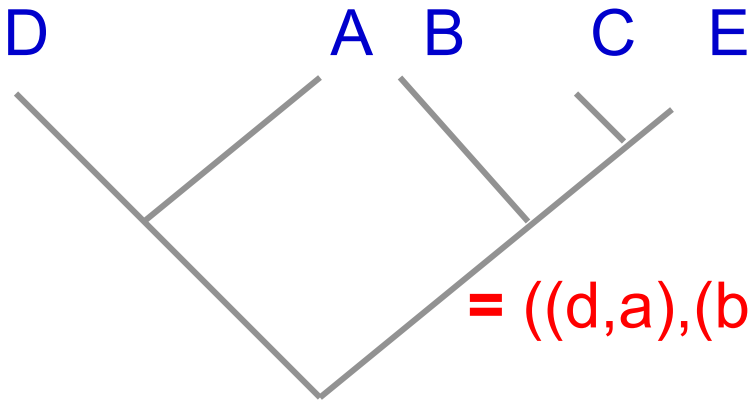
Classification of Tree Building Methods

		Tree Building Methods	
		Clustering Algorithm	Optimality Criterion
Type of Data	Distance-Based	UPGMA Neighbor Joining	Fitch-Margoliash
	Character-Based		Maximum Parsimony Maximum Likelihood

Non-graphical Representation of Trees



= (a,(d,(b,c))) or, equivalently (a,(d,(c,b)))

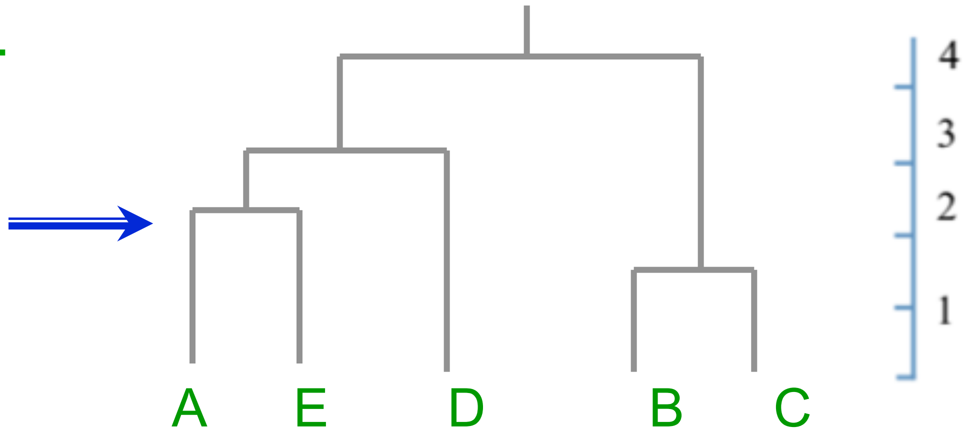


= ((d,a),(b,(c,e))) or ((b,(e,c),(a,d))

Distance-Based Method

- Given: an *n*× *n* matrix *M*, where *M(i,j)* is the distance between objects *i* and *j*
- Build an edge-weighted tree such that the distances between leaves *i* and *j* correspond to *M(i,j)*

	A	B	C	D	E
A	0	8	8	5	3
B		0	3	8	8
C			0	8	8
D				0	5
E					0



UPGMA

- UPGMA is a sequential clustering algorithm.
  - It works by clustering the sequences, at each stage amalgamating two operational taxonomic units (OTUs) and at the same time creating a new node in the tree.
  - The edge lengths are determined by the difference in the heights of the nodes at the top and bottom of an edge.

The Molecular Clock

- UPGMA assumes that:

  - the gene substitution rate is constant, in other words: divergence of sequences is assumed to occur at the same rate at all points in the tree.
    - Known as the Molecular Clock.
  - the distance is linear with evolutionary time.

Rates of Evolutionary Change

- Different rates throughout genomic DNA base-pair sequence, based mainly on coding.
- ORFs: codon position 3 changes faster than positions 1 and 2.
- Introns change faster than exons.
- Intergenic DNA (especially repeats) changes faster than intragenic (ORF) DNA.
- DNA overall: transition mutations more frequent than transversion mutations.

UPGMA Algorithm

- The algorithm iteratively picks two clusters and merges them, thus creating a new node in the tree.
- The average distance between two clusters is determined by:

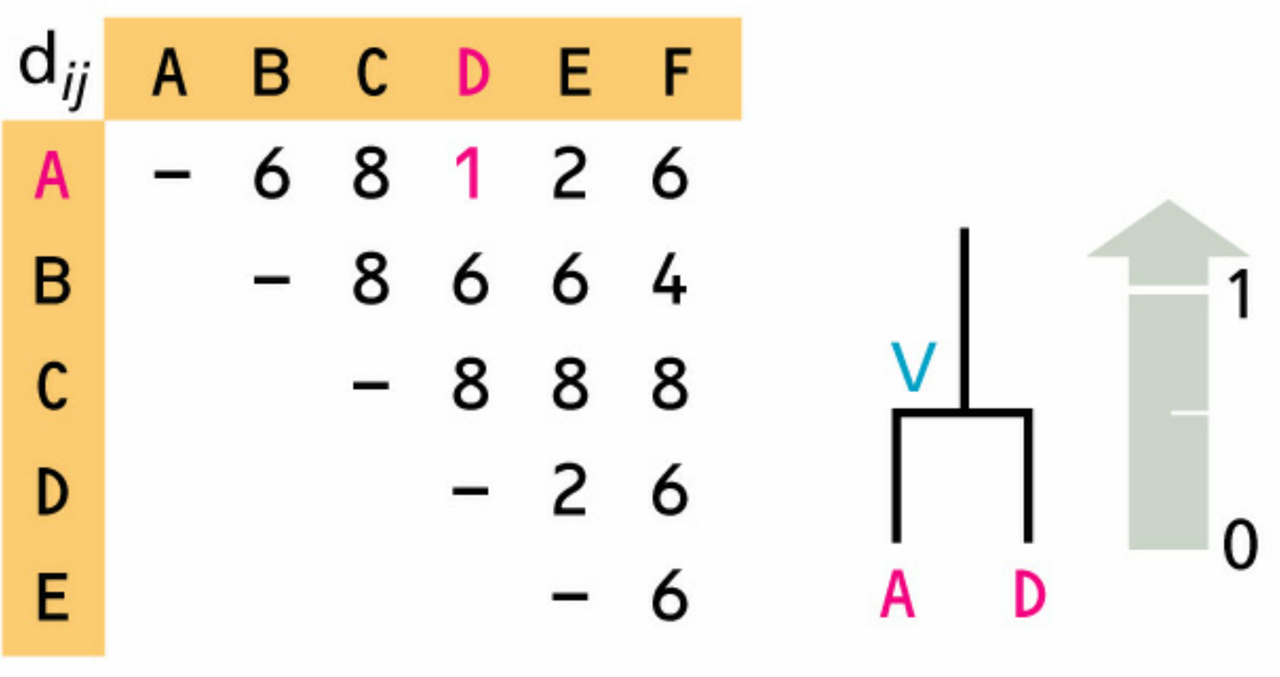
$$d_{ij} = \frac{1}{|C_i||C_j|} \sum_{p \in C_i,\, q \in C_j} d_{pq}, \text{ where } C_i \text{ and } C_j \text{ are clusters.}$$

The UPGMA Algorithm

- **Initialization**
  - Assign each sequence i to its own cluster C_i,
  - Define one leaf of T for each sequence; place at height zero.
- **Iteration** while more than two clusters, do
  - Determine the two clusters C_i, C_j for which d_{ij} is minimal.
  - Define a new cluster $C_k = C_i \cup C_j$; compute d_{kl} for all l.
  - Define a node k with children i and j; place it at height $d_{ij}/2$.
  - Replace clusters C_i and C_j with C_k.
- **Termination**
  - Join last two clusters, C_i and C_j; place the root at height $d_{ij}/2$.

UPGMA: Example (1st Iteration)

Sequences A and D are the closest and are combined to create a new cluster V of height ½ in T.



d_{ij}	A	B	C	D	E	F
A	–	6	8	1	2	6
B		–	8	6	6	4
C			–	8	8	8
D				–	2	6
E					–	6

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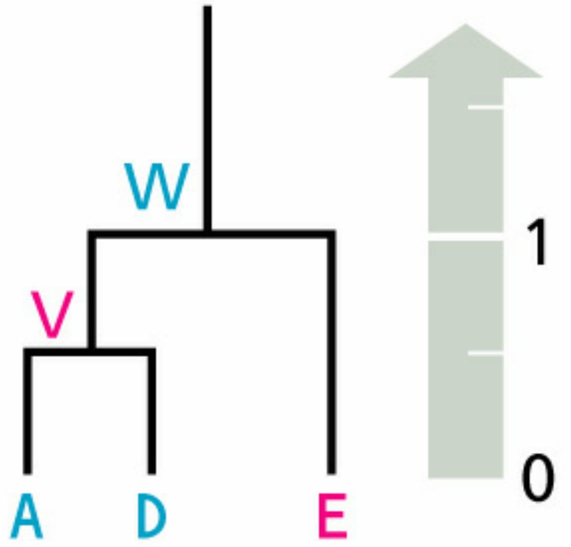
UPGMA: Example (2nd Iteration)

The table of distances is updated to reflect the average distances from V to the other sequences.

V and E are the closest and are combined to create a new cluster W of height 1 in T.

d_{ij}	B	C	E	F	V
B	–	8	6	4	6
C		–	8	8	8
E			–	6	2
F				–	6



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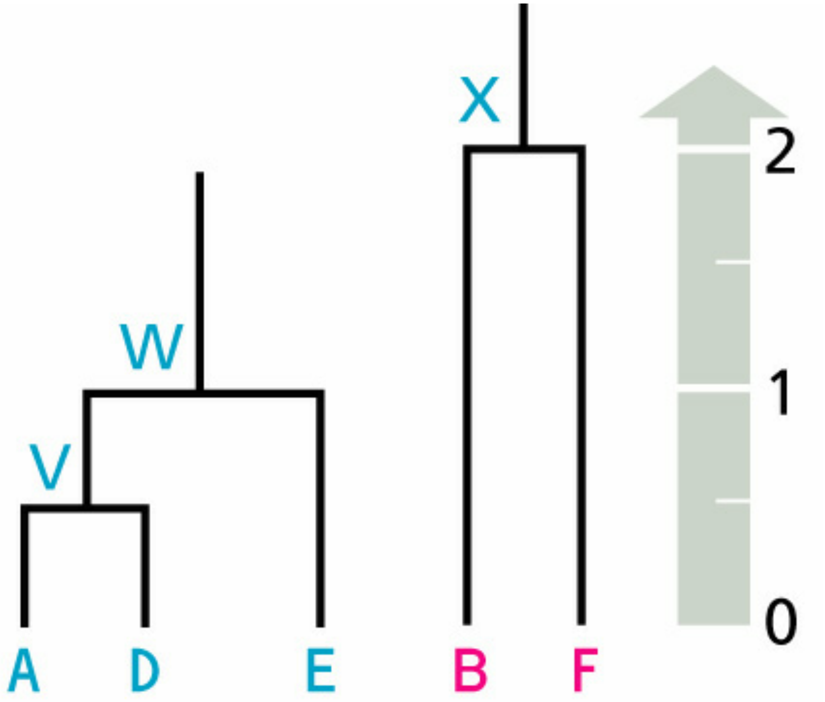
UPGMA: Example (3rd Iteration)

After updating the table of distances, B and F are the closest sequences and are combined to create a new cluster X of height 2 in T.

d_{ij}	B	C	F	W
B	–	8	4	6
C		–	8	8
F			–	6



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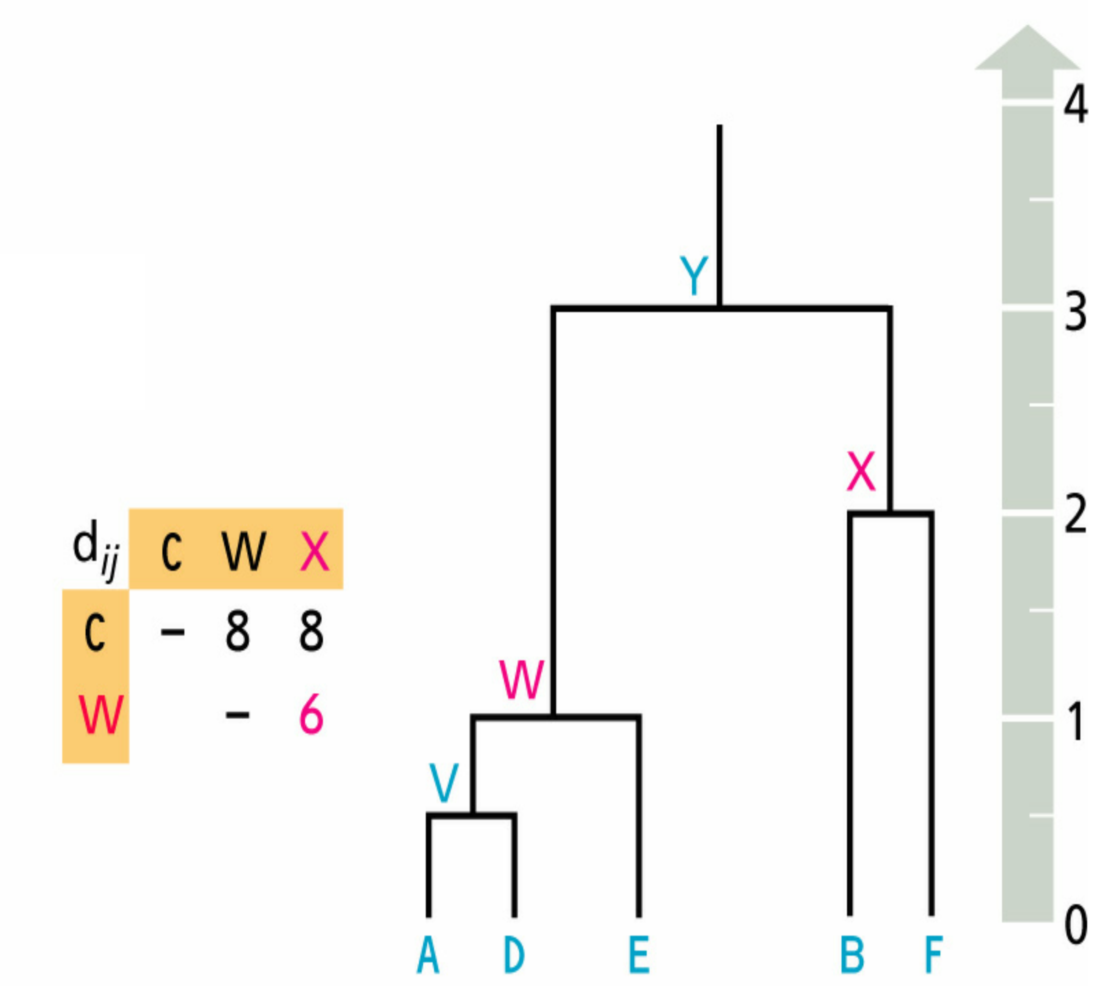
UPGMA: Example (4th Iteration)

Once more the table is updated. W and X are the closest sequences and are combined to create a new cluster Y of height 3 in T.



d_{ij}	C	W	X
C	–	8	8
W		–	6

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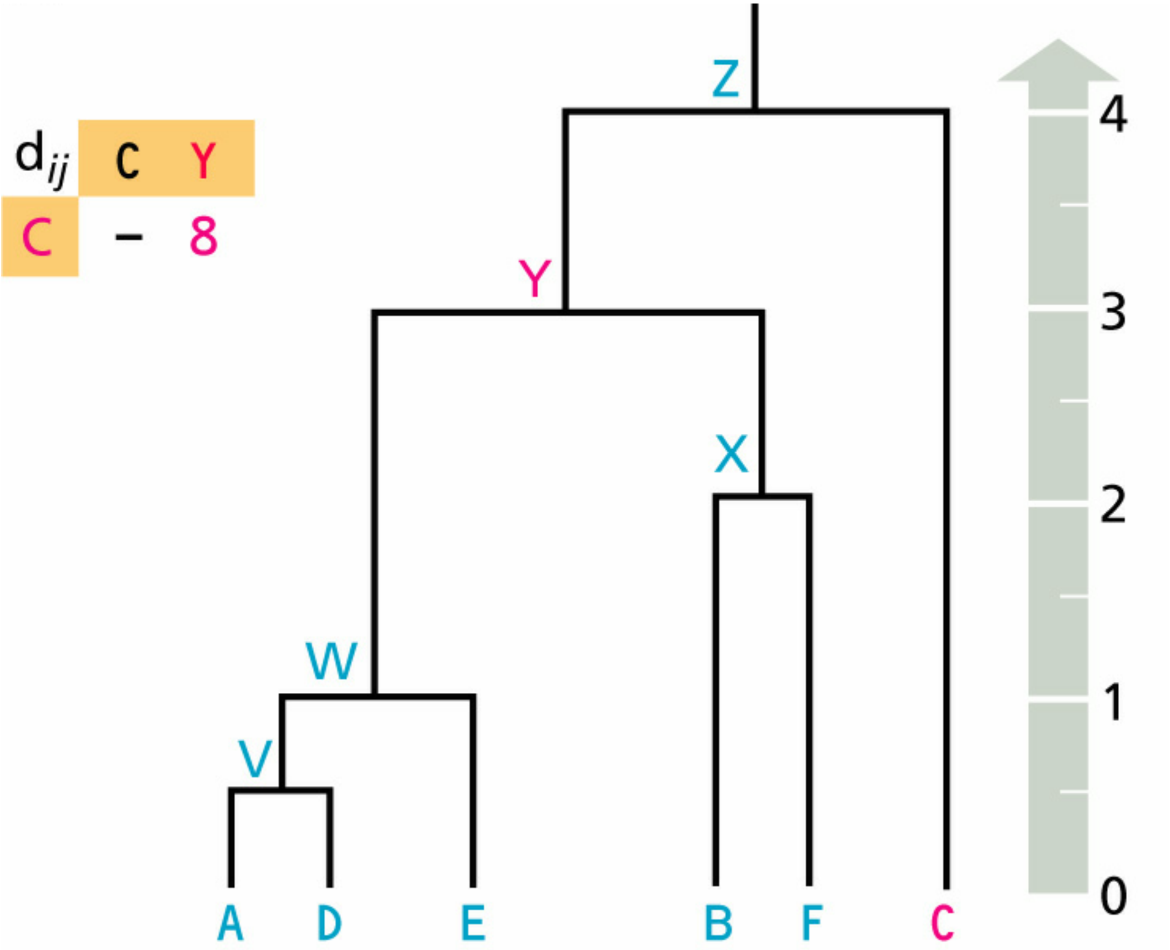
UPGMA: Example (Termination)



d_{ij}	C	Y
C	–	8

The remaining 2 sequences, C and Y of distance 8 are combined to create a new cluster Z of height 4 in T.

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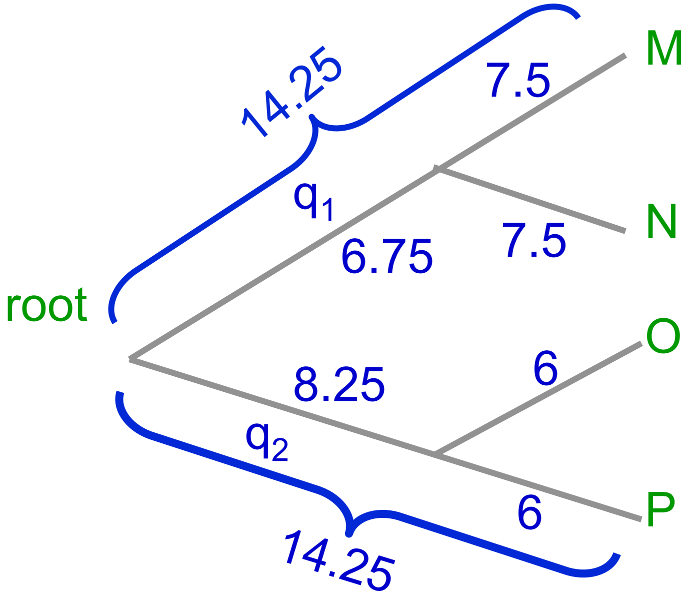
UPGMA Tree: Second Example

	M	N	O	P
M	-	15	26	28
N	-	-	29	31
O	-	-	-	12
P	-	-	-	-



UPGMA assumes a uniform rate of mutation in the tree branches. At any given time, the two sequences should have the same number of changes separating them from the common ancestor.

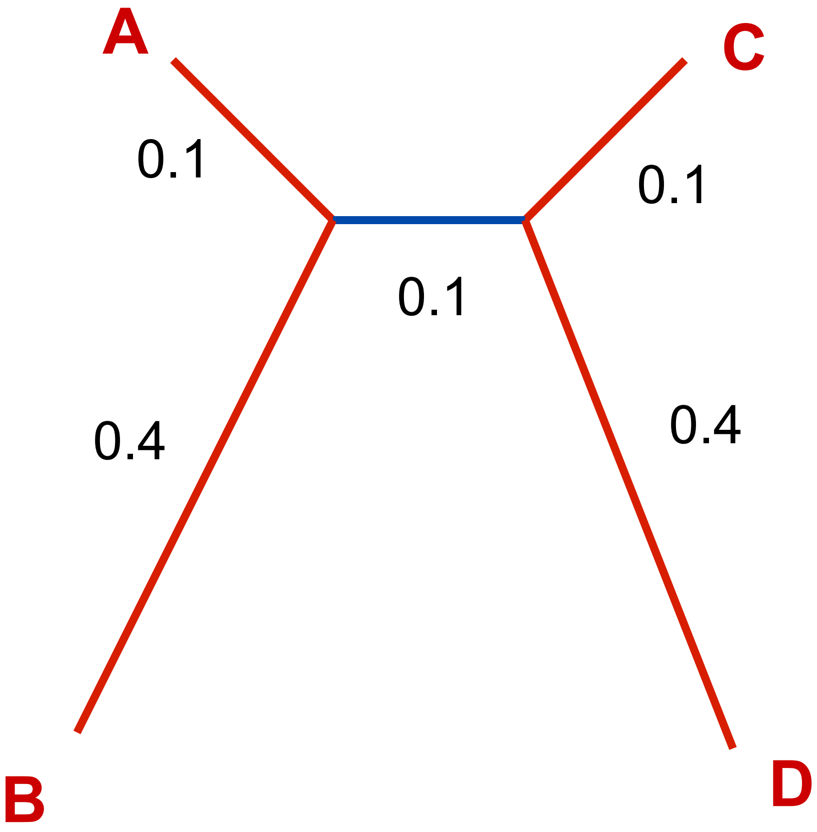
Bioinformatics by David Mount

UPGMA’s Shortcoming

A tree where closest pair of leaves are not neighboring leaves.

d(A,C) = 0.3
d(A,B) = 0.5

So the neighboring pair A and B are further apart than the non-neighboring pair A and C.



Fitch-Margoliash Method

- Fitch-Margoliash does not assume a constant mutation rate.
- With the Fitch-Margoliash Method, the sequences are combined in threes to define the branches of the predicted tree and to calculate the branch lengths of the tree.
- This method of averaging distances is most accurate for trees with short branches.

Introduction to Neighbor-Joining

- Neighbor –Joining does not assume a constant rate of evolution.
- The algorithm is based on the concept of minimum evolution; the true tree is the one for which the total branch length is minimum.
- The resulting tree is not rooted and is additive.

Limitations of Distance-Based Phylogenetic Trees

The distance-based phylogenetic tree is derived from the pairwise distance of aligned sequences and not from the original sequence data.

The distance information may not contain all the sequence information.

Observable Features

- Sometimes we do not have a distance metric between the species we are interested in.
- What we have instead, are observable features.
- We then use the observable features to build the tree. These trees are called Character-Based trees.

Character-Based Trees

- The building of the tree is based on morphological features and not on distances.
- Examples of morphological features:
  - has feathers
  - has a backbone
  - has a certain amino acid at a certain position in the sequence
  - whether or not a certain protein regulates another protein.

Maximum Parsimony Method

- The maximum parsimony method predicts the evolutionary tree that minimizes the number of steps required to generate the observed variation in the sequences
  - This method is also known as the minimum evolution method.
- The maximum parsimony method is used
  - for sequences that are quite similar, and
  - for small number of sequences.

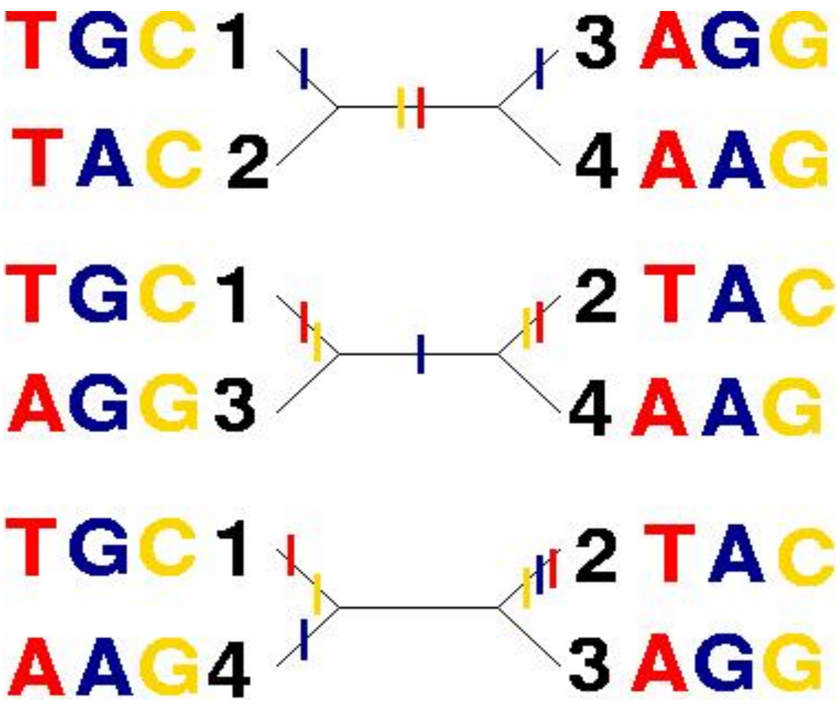
Maximum Parsimony

- Maximum parsimony means fewest evolutionary changes necessary to explain observed taxonomic relationships.
- Fewest postulated steps in evolutionary process.
- Leads to predictions for common ancestor and branch-point ancestors.
- Exhaustive search of trees is possible only for small number of species.

Parsimony: An Example

- Given four sequences:
  - Sequence 1: TGC
  - Sequence 2: TAC
  - Sequence 3: AGG
  - Sequence 4: AAG
- We want to find the tree with the smallest number of changes that explains the observed data.
- Draw all possible trees with 4 taxa.

Parsimony Example



The shortest tree is ((1,2),(3,4))

Position 1:
Only one change is introduced if seq1 and seq2 are grouped; and 2 changes if seq1 and seq3 or seq1 and seq4 are grouped.

Position 2:
Only one change is introduced if seq1 and seq3 are grouped; and 2 changes if seq1 and seq2 or seq1 and seq4 are grouped.

Position 3:
Only one change is introduced if seq1 and seq2 are grouped; and 2 changes if seq1 and seq3 or seq1 and seq4 are grouped.

Informative Sites

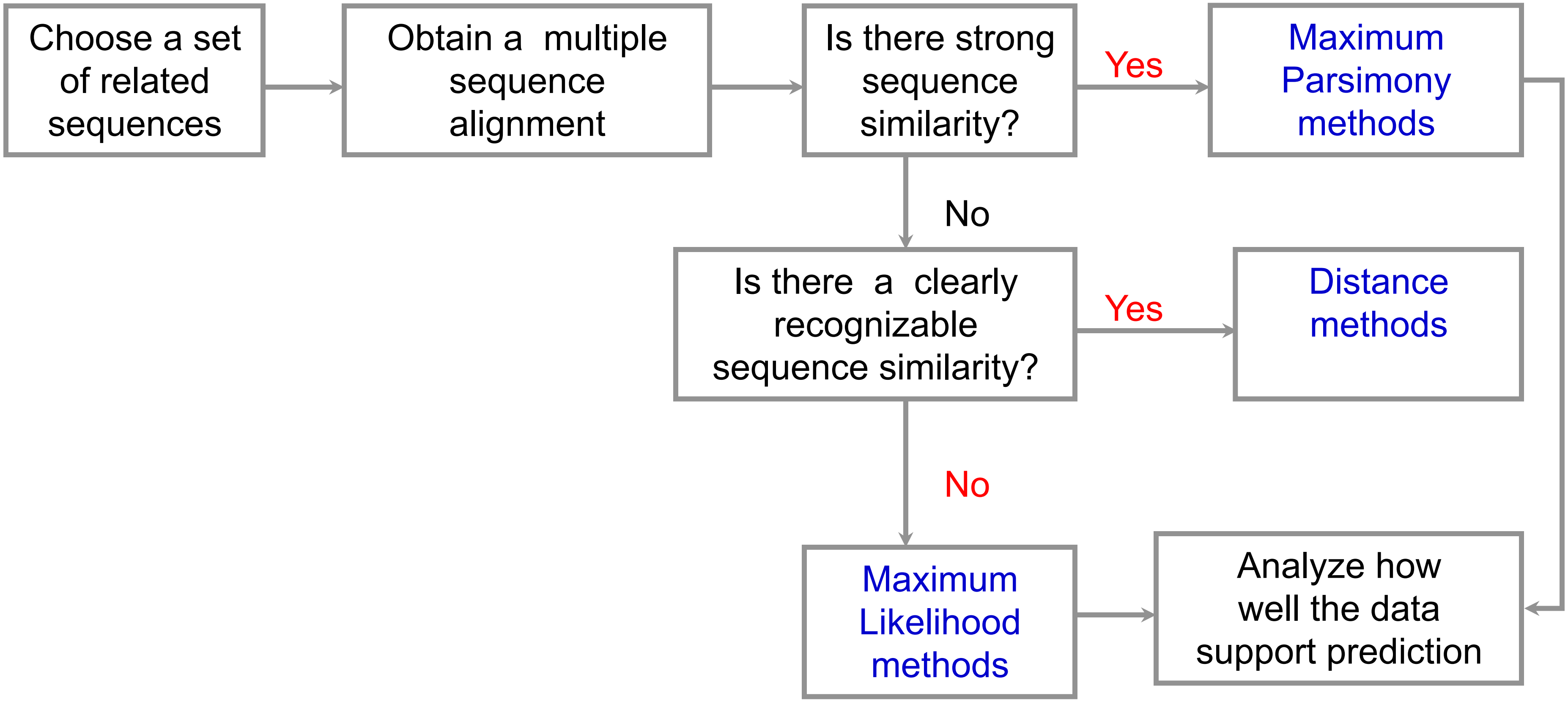
- A site that provides information for distinguishing between different topologies is said to be an **informative site**.
- Only **informative sites** need to be analyzed.
- A site is phylogenetically informative only when there are at least two different kinds of characters, each represented at least two times.

Informative Sites: An Example

Taxa	1	2	3	4	5	6	7	8	9
1	A	A	G	A	G	T	G	C	A
2	A	G	C	C	G	T	G	C	G
3	A	G	A	T	A	T	C	C	A
4	A	G	A	G	A	T	C	C	G

Only sites at columns 5, 7, and 9 are informatives sites

Steps of Tree Reconstruction



Choose a set of related sequences

Obtain a multiple sequence alignment

Is there strong sequence similarity?

Yes

Maximum Parsimony methods

No

Is there a clearly recognizable sequence similarity?

Yes

Distance methods

No

Maximum Likelihood methods

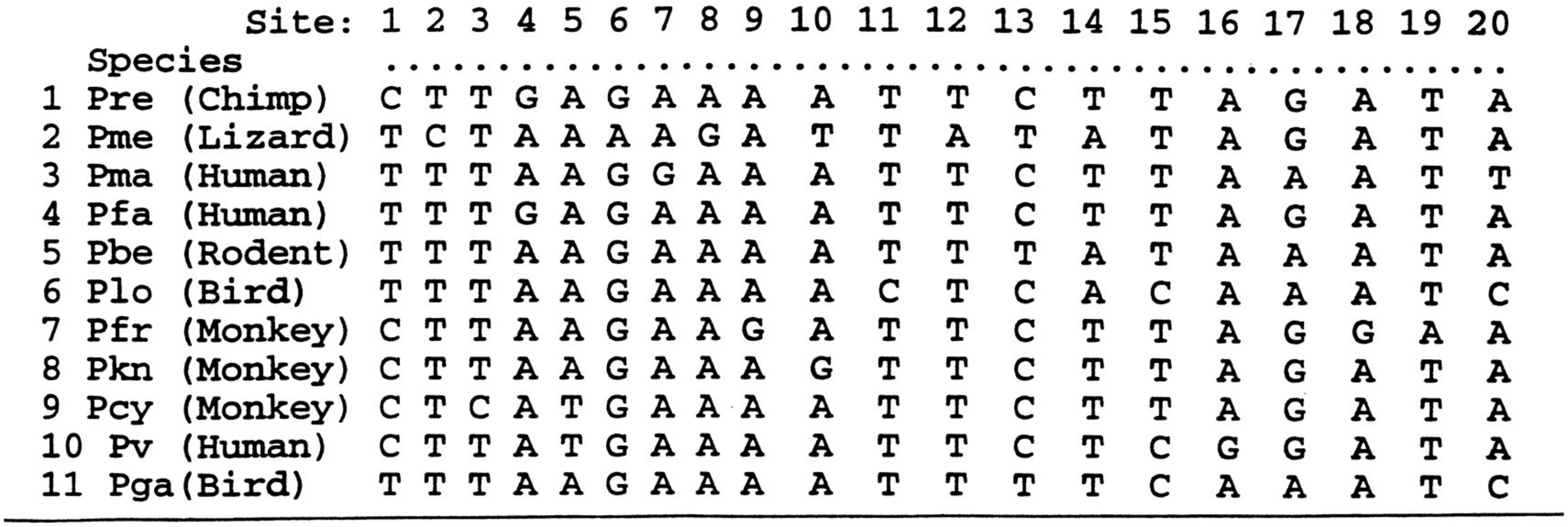
Analyze how well the data support prediction

David Mount

Bootstrapping

Bootstrapping is a statistical technique that uses computer intensive random resampling of data to determine sampling error or confidence intervals for some estimated parameter.

Bootstrap: An Example (I)

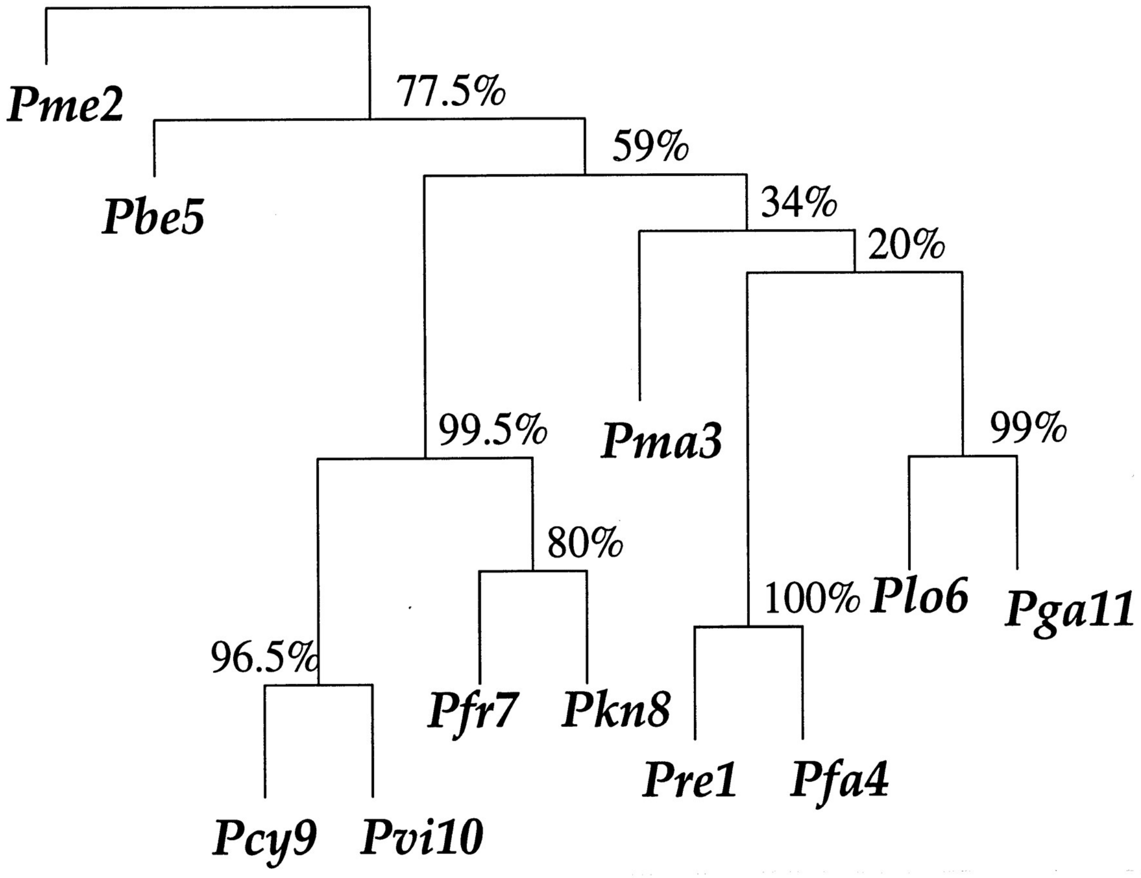


Site:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Species																				
1 Pre (Chimp)	C	T	T	G	A	G	A	A	A	A	T	T	C	T	T	A	G	A	T	A
2 Pme (Lizard)	T	C	T	A	A	A	A	G	A	T	T	A	T	A	T	A	G	A	T	A
3 Pma (Human)	T	T	T	A	A	G	G	A	A	A	T	T	C	T	T	A	A	A	T	T
4 Pfa (Human)	T	T	T	G	A	G	A	A	A	A	T	T	C	T	T	A	G	A	T	A
5 Pbe (Rodent)	T	T	T	A	A	G	A	A	A	A	T	T	T	A	T	A	A	A	T	A
6 Plo (Bird)	T	T	T	A	A	G	A	A	A	A	C	T	C	A	C	A	A	A	T	C
7 Pfr (Monkey)	C	T	T	A	A	G	A	A	G	A	T	T	C	T	T	A	G	G	A	A
8 Pkn (Monkey)	C	T	T	A	A	G	A	A	A	G	T	T	C	T	T	A	G	A	T	A
9 Pcy (Monkey)	C	T	C	A	T	G	A	A	A	A	T	T	C	T	T	A	G	A	T	A
10 Pv (Human)	C	T	T	A	T	G	A	A	A	A	T	T	C	T	C	G	G	A	T	A
11 Pga (Bird)	T	T	T	A	A	G	A	A	A	A	T	T	T	T	C	A	A	A	T	C

Part of the data matrix of aligned nucleotide sequences for the malaria parasite Plasmodium.
Only the first 20 columns of the 11 × 221 matrix are shown.

Efron, Bradley et al. (1996) Proc. Natl. Acad. Sci. USA 93, 13429

Bootstrap: An Example (II)



Randomly select, with replacement, 221 columns from the original matrix. Tree-building algorithm is applied to give a bootstrap tree. Repeat the process 200 times.

The numbers at the branches are confidence values based on Felsenstein's bootstrap method.

Efron, Bradley et al. (1996) Proc. Natl. Acad. Sci. USA 93, 13429

Software Tools

- PHYLIP
  - Phylogeny Inference Package.
  - http://evolution.genetics.washington.edu/phylip.html
  - Free.
  - Developed by Dr. Joe Felsenstein from the Department of Genome Sciences at the University of Washington.
  - Source code is written in ANSI C.
  - Executables are available for different platforms:
    - Windows, UNIX and Macintosh.
- PAUP*
  - Phylogenetic Analysis Using Parsimony.
  - http://paup.csit.fsu.edu/about.html
  - Developed by Dr. David Swofford of the Laboratory of Molecular Systematics, National Museum of Natural History
  - The most sophisticated parsimony program.