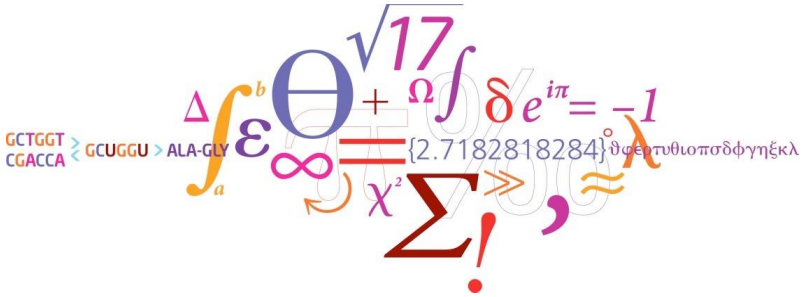
# Phylogenetic Reconstruction: Distance Matrix Methods

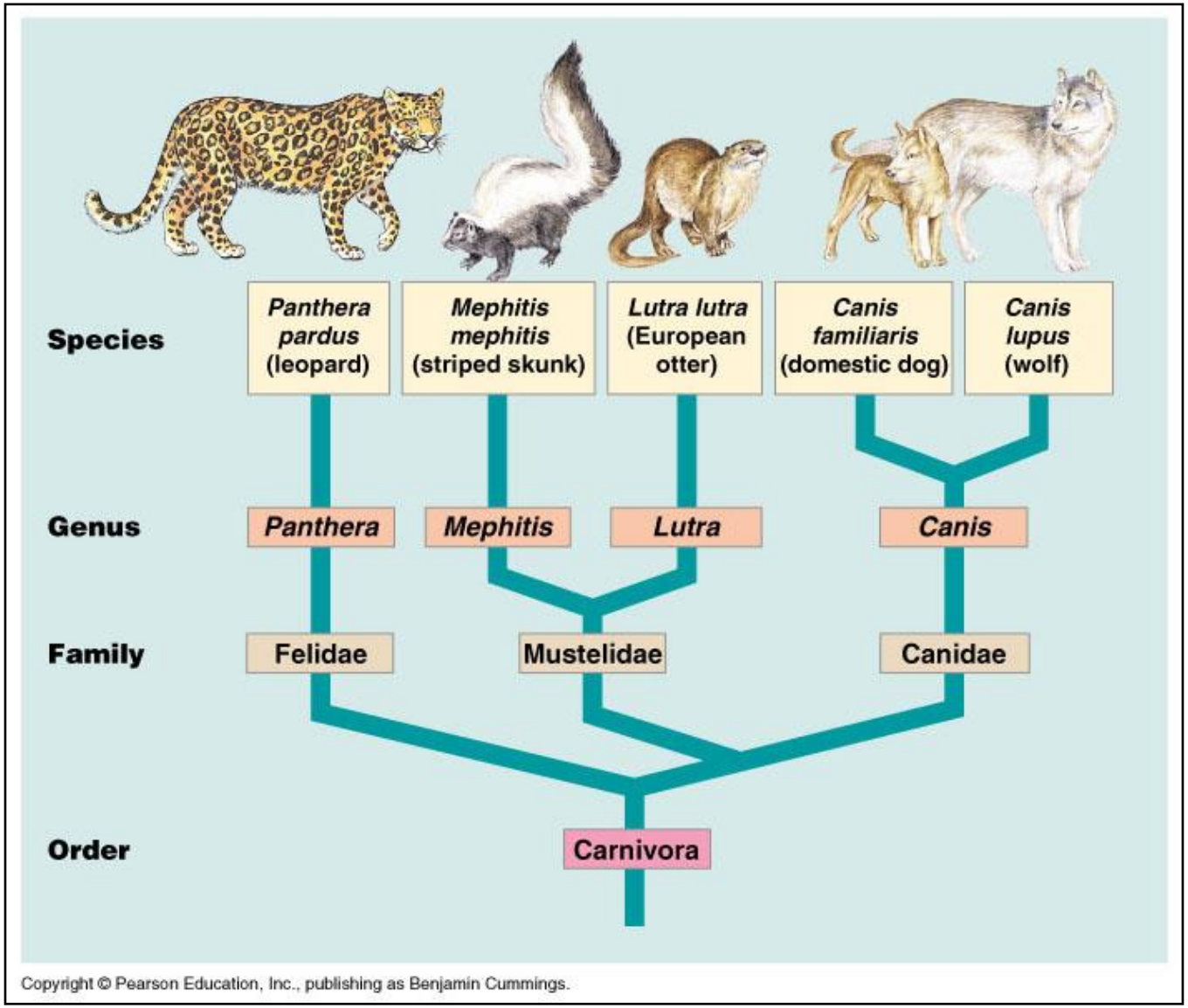
Henrik Nielsen

Slides by Anders Gorm Pedersen, with some additions by Rasmus Wernersson, Bent Petersen and Henrik Nielsen



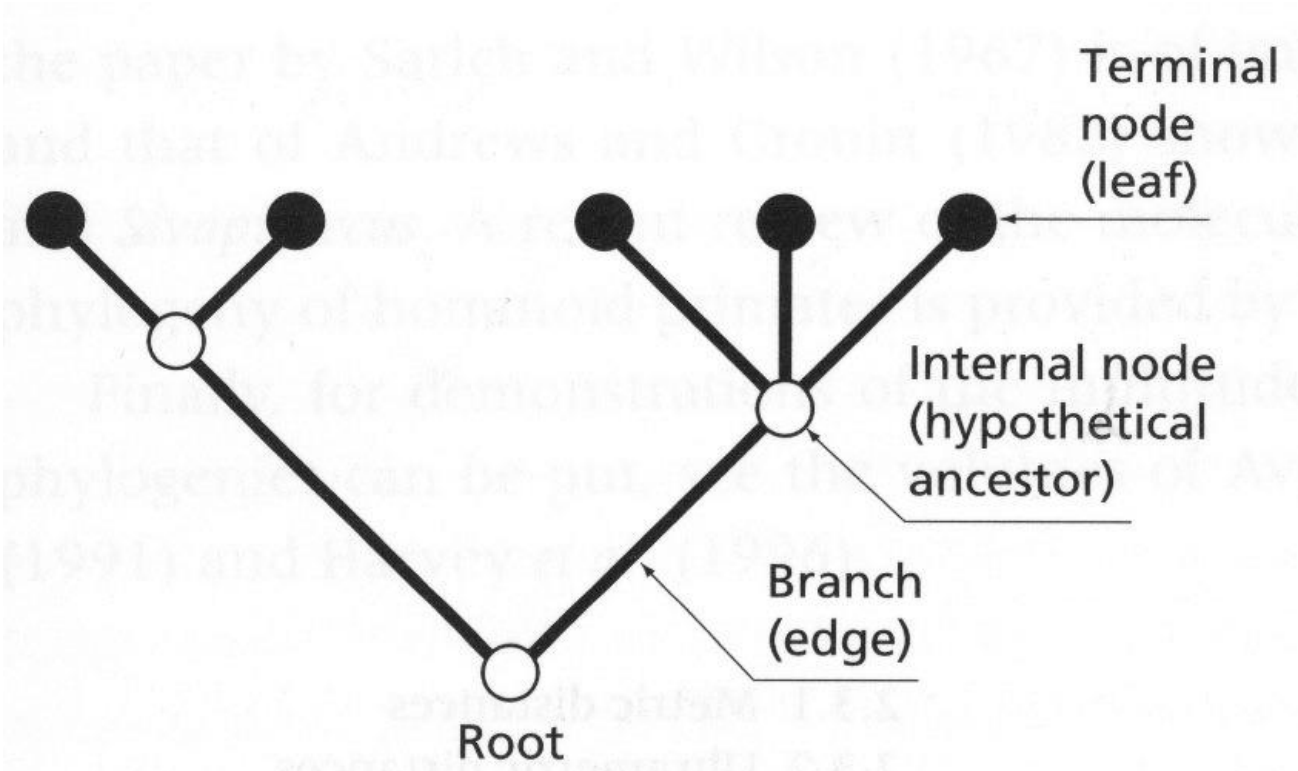
# Recap: From lecture #1





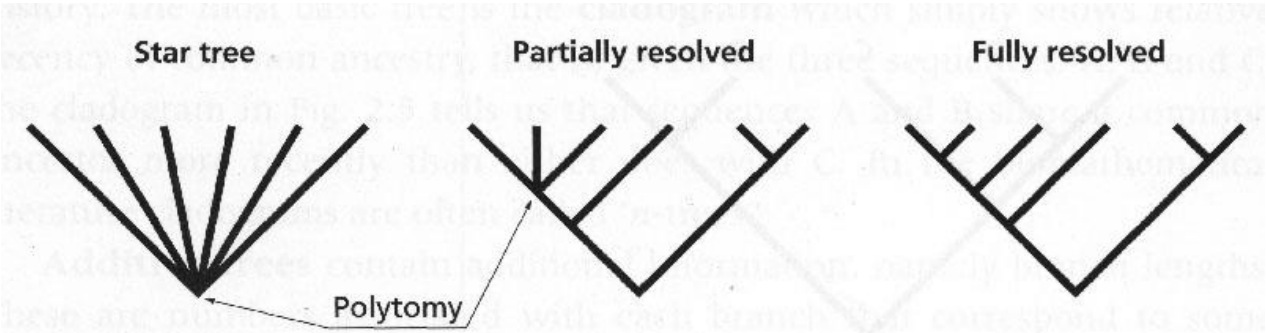
# Trees: terminology

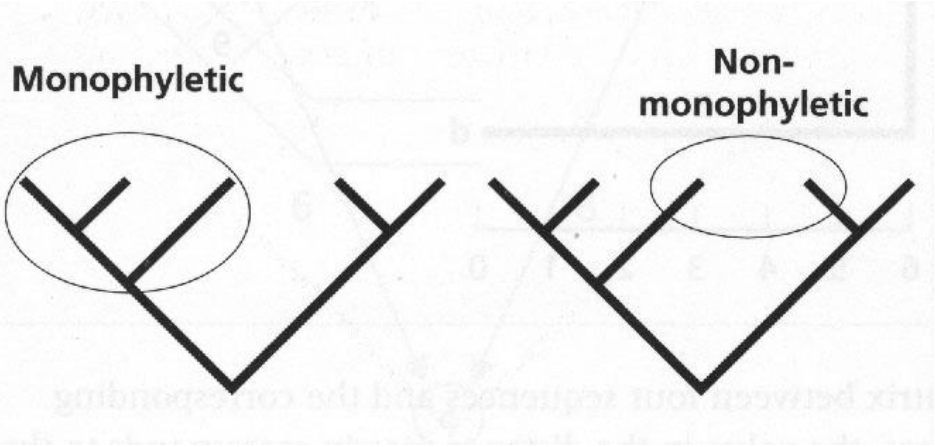




# Trees: terminology

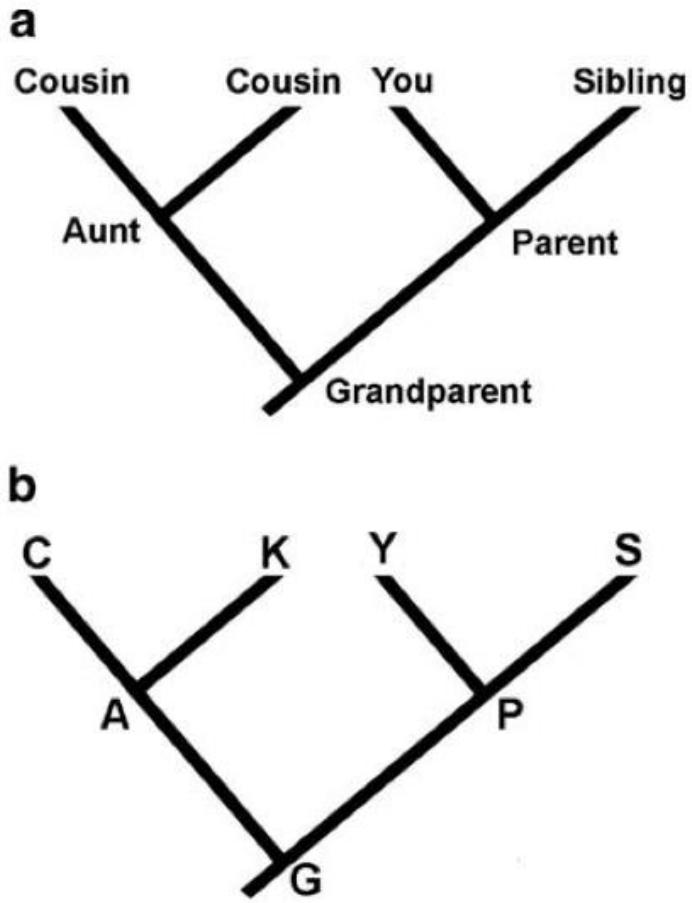




# Trees: meaning

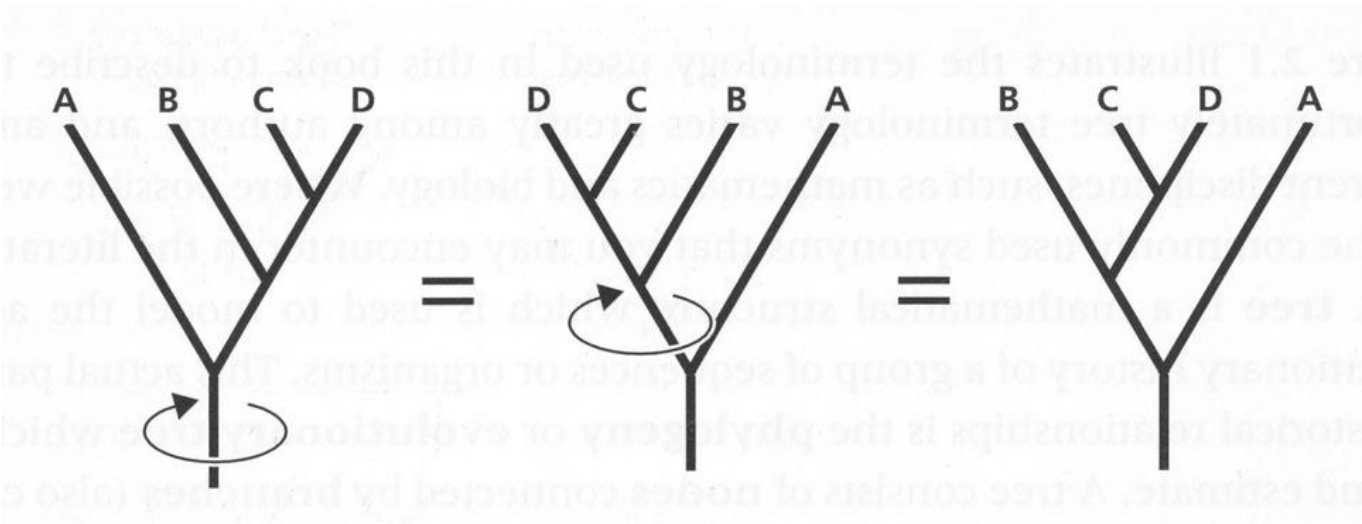





- Phylogenies indicate both relatedness and historical descent
- Y(ou) not descended from S(ister) (or vice versa) - both are contemporary and descended from P(arent)
- S and C are less closely related than S and Y: Their common ancestor is deeper in the tree

# Trees: representations

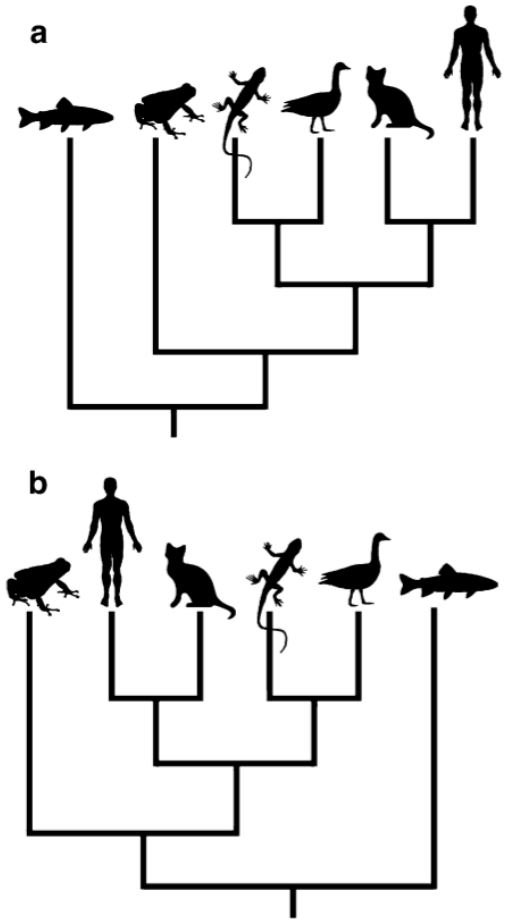





**Three different representations of the same tree**

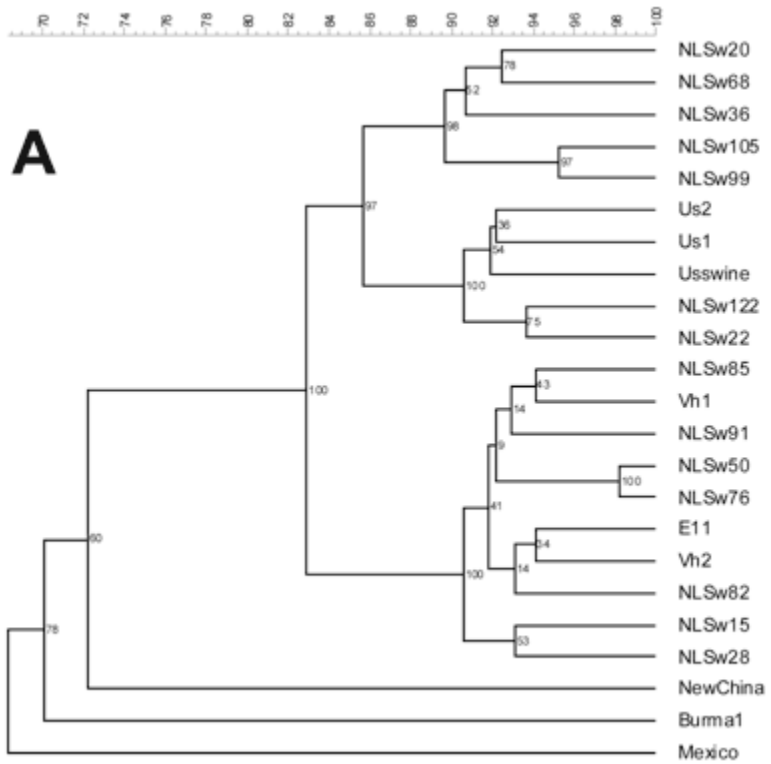
# Trees: Order of leafs is meaningless






- Order of terminal nodes contain no information about relatedness
- Frogs and humans are equally closely related to fishes

# Trees: rooted vs. unrooted



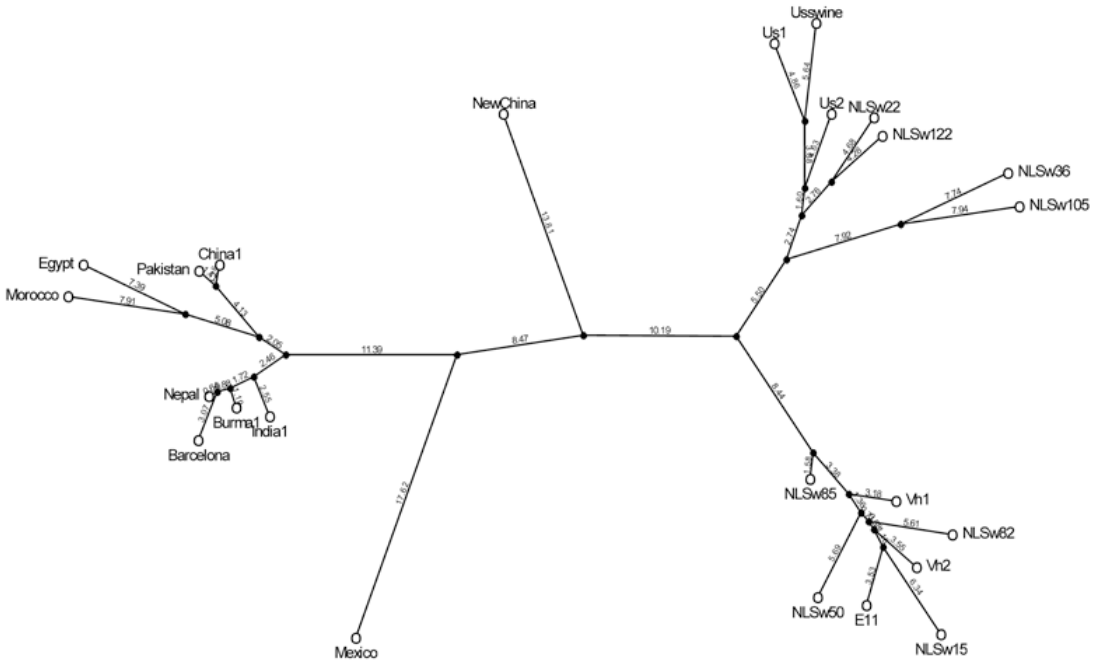

Early → Late

A rooted tree has a single node (the root) that represents a point in time that is earlier than any other node in the tree.

A rooted tree has directionality (nodes can be ordered in terms of “earlier” or “later”).

In the rooted tree, distance between two nodes is represented along the time-axis only (the second axis just helps spread out the leafs)

# Trees: rooted vs. unrooted



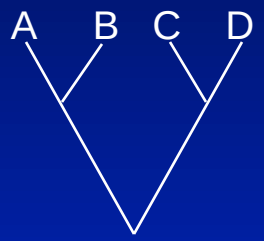

- In unrooted trees there is no directionality: we do not know if a node is earlier or later than another node
- Distance along branches directly represents node distance

Notice: All distance based trees are unrooted, unless an “outgroup” can be specified

# Newick format: named for seafood restaurant where standard was decided upon


# Trees: representation in computer files






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

Newick format:

- Leafs: represented by taxon name
- Internal nodes: represented by pair of matching parentheses
- Descendants of internal node given as comma-delimited list.
- Tree string terminated by semicolon

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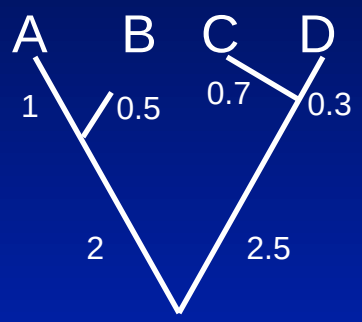


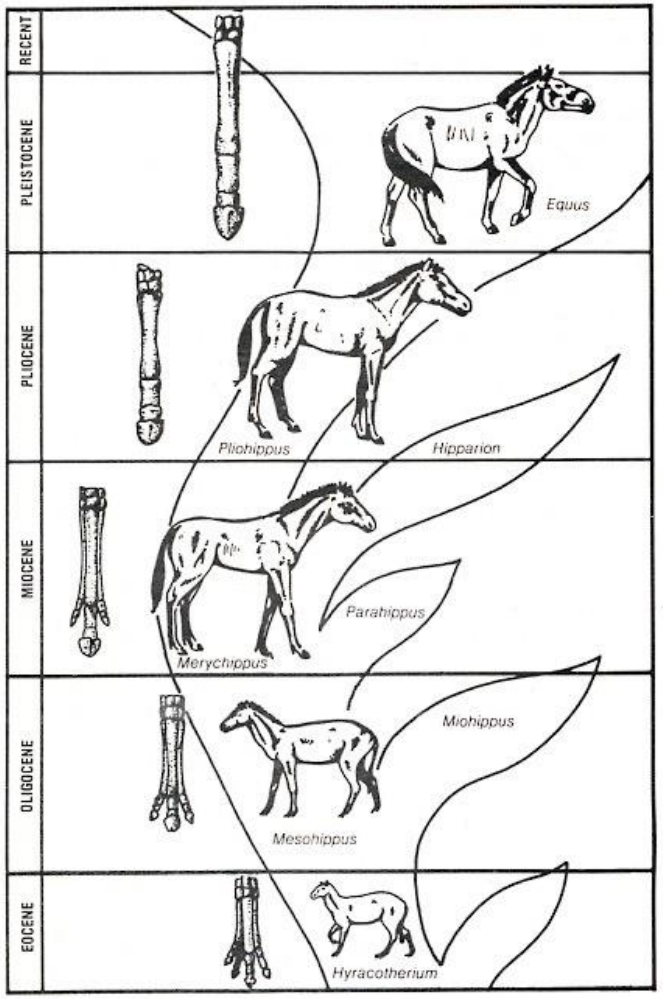

( (A:1 , B:0.5) :2 , (C:0.7 , D:0.3) :2.5);

Newick format:

- Branch length given as number following colon

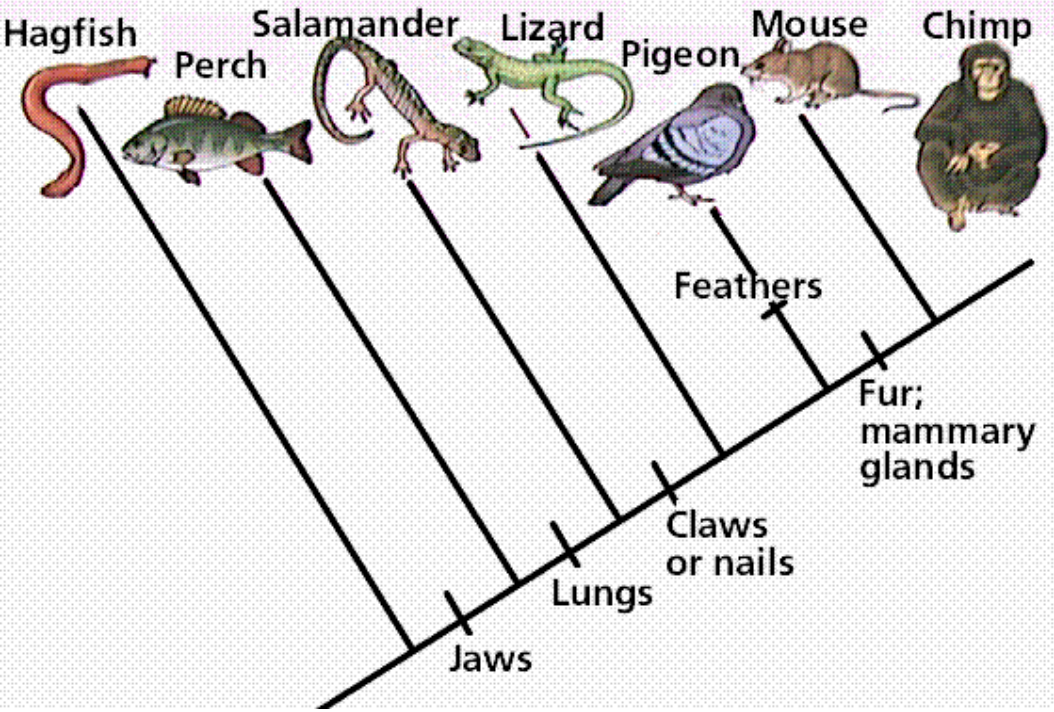
# Reconstructing a tree using non-contemporaneous data





# Reconstructing a tree using present-day data





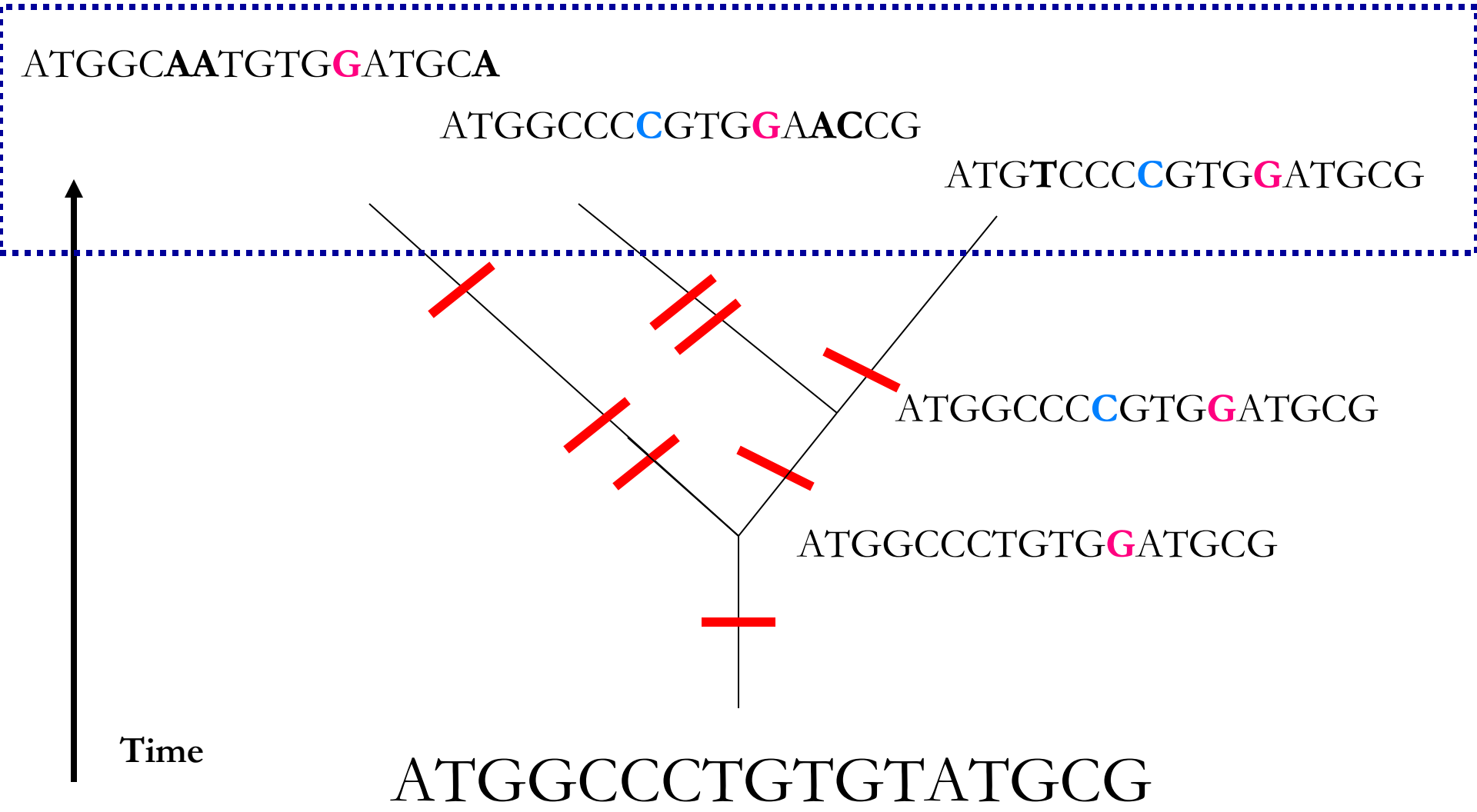
# Data: molecular phylogeny



- DNA sequences
  - genomic DNA
  - mitochondrial DNA
  - chloroplast DNA
- Protein sequences
- Restriction site polymorphisms
- DNA/DNA hybridization
- Immunological cross-reaction

# Recap: Example from Lecture #1

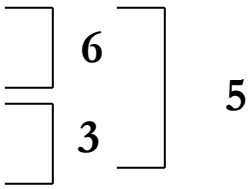




# Recap: Example from Lecture #1



- Species1: ATGGC**AA**TGTG**G**ATGC**A**
- Species2: ATGGCCC**C**GTG**G**A**AC**CG
- Species3: ATG**T**CCC**C**GTG**G**ATGCG



# Recap: Example from Lecture #1

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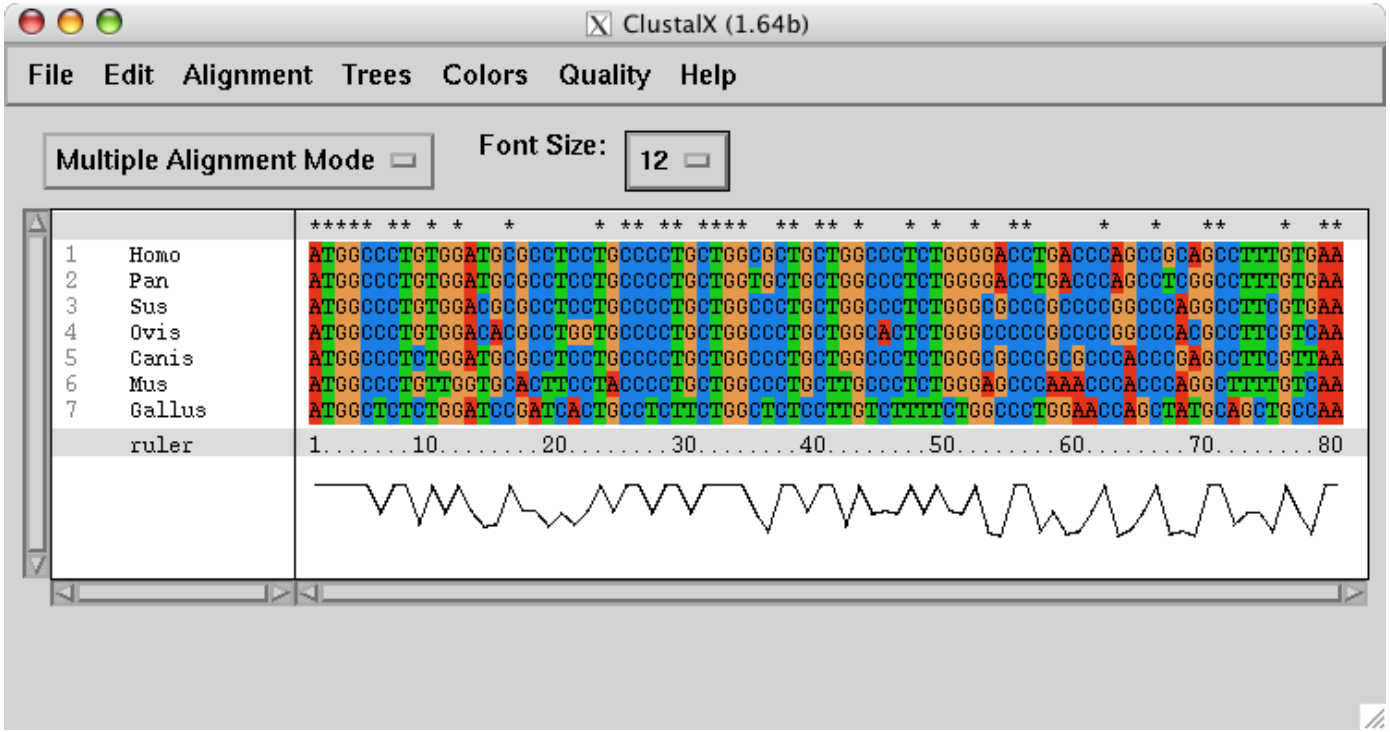
## Insulin from 7 different species

```
Homo:     ATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTGACCCAGCCGCAGCCTTTGTGAA
Pan:      ATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGTGCTGCTGGCCCTCTGGGGACCTGACCCAGCCTCGGCCTTTGTGAA
Sus:      ATGGCCCTGTGGACGCGCCTCCTGCCCCTGCTGGCCCTGCTGGCCCTCTGGGCGCCCGCCCCGGCCCAGGCCTTCGTGAA
Ovis:     ATGGCCCTGTGGACACGCCTGGTGCCCCTGCTGGCCCTGCTGGCACTCTGGGCCCCCGCCCCGGCCCACGCCTTCGTCAA
Canis:    ATGGCCCTCTGGATGCGCCTCCTGCCCCTGCTGGCCCTGCTGGCCCTCTGGGCGCCCGCGCCCACCCGAGCCTTCGTTAA
Mus:      ATGGCCCTGTTGGTGCACTTCCTACCCCTGCTGGCCCTGCTTGCCCTCTGGGAGCCCAAACCCACCCAGGCTTTTGTCAA
Gallus:   ATGGCTCTCTGGATCCGATCACTGCCTCTTCTGGCTCTCCTTGTCTTTTCTGGCCCTGGAACCAGCTATGCAGCTGCCAA
```



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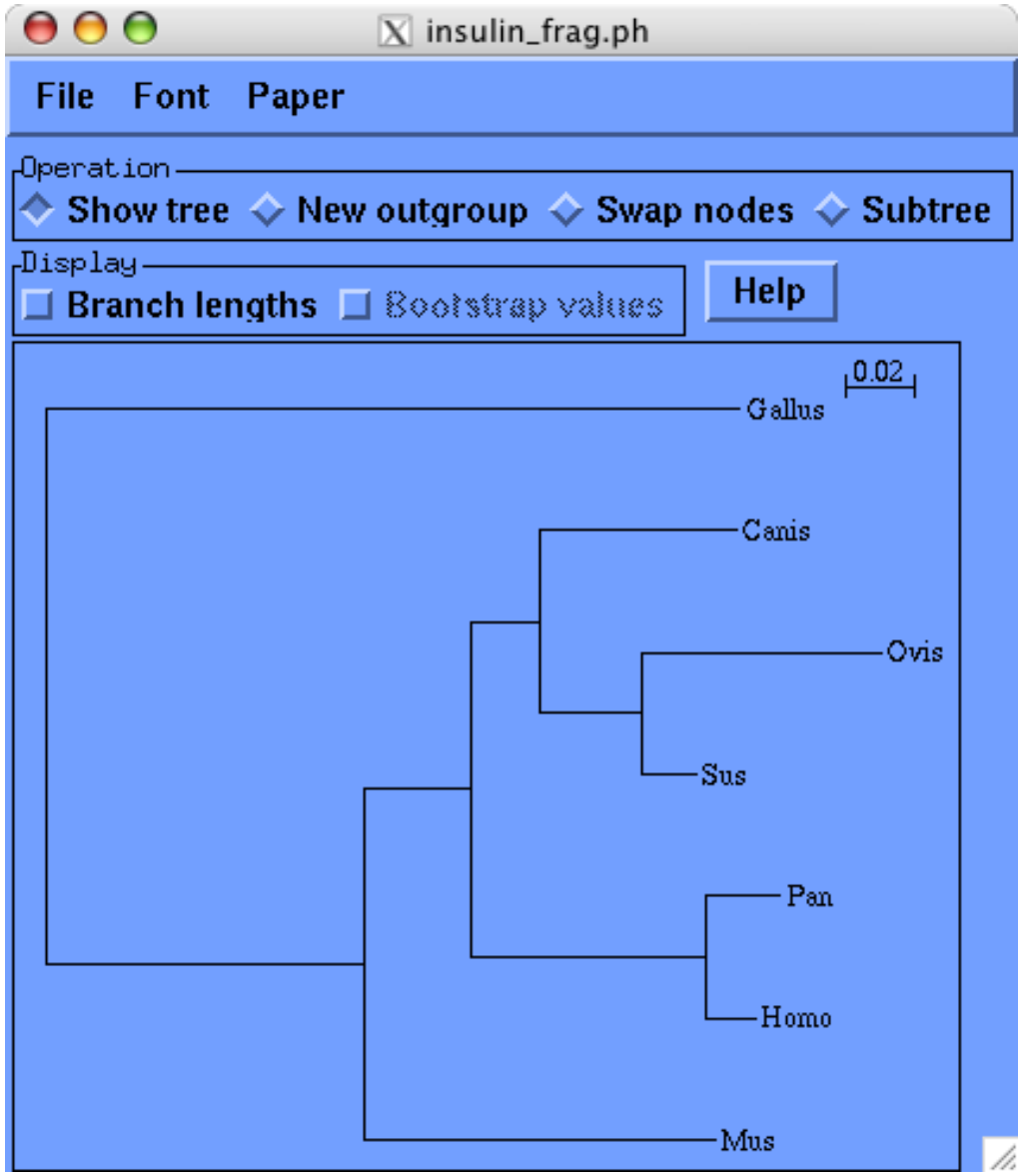


# Morphology vs. molecular data



African white-backed vulture
(old world vulture)

Andean condor
(new world vulture)

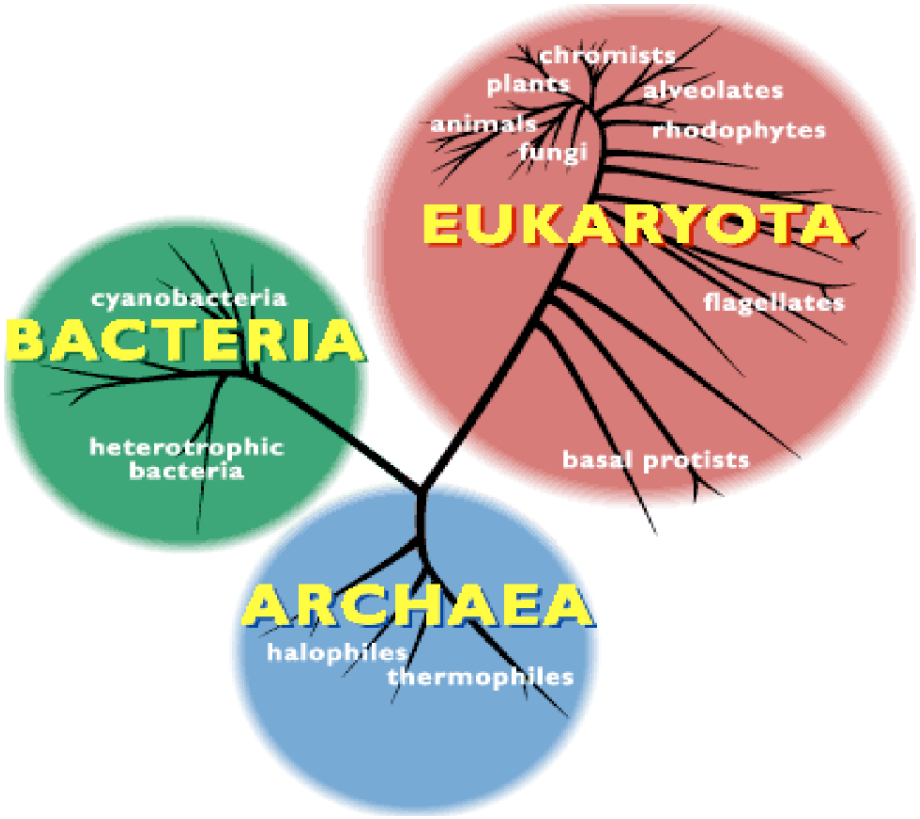
New and old world vultures seem to be closely related based on morphology.

Molecular data indicates that old world vultures are related to birds of prey (falcons, hawks, etc.) while new world vultures are more closely related to storks

Similar features presumably the result of convergent evolution

# Molecular data: single-celled organisms






Molecular data useful for analyzing single-celled organisms (which have only few prominent morphological features).

# Methods for Phylogenetic Reconstruction



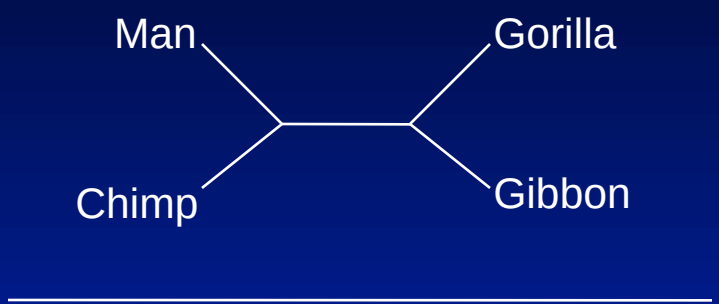
- Maximum Parsimony
  - Investigate all/many trees, best tree is the **shortest** one
- Maximum likelihood
  - Likelihood = Probability (Data | Model)
  - Investigate all/many trees, best tree is the one with the **highest likelihood**
- Bayesian
  - Find probability distribution over all possible trees
  - Also based on likelihoods (but different philosophical take on statistics...)
- Distance based methods
  - Genetic distance between sequences = number of mutations
  - Start by finding all pairwise distances between sequences
  - Discard original sequence data, find tree compatible with distance matrix
  - Investigate all/many trees, best tree is the **most compatible** one

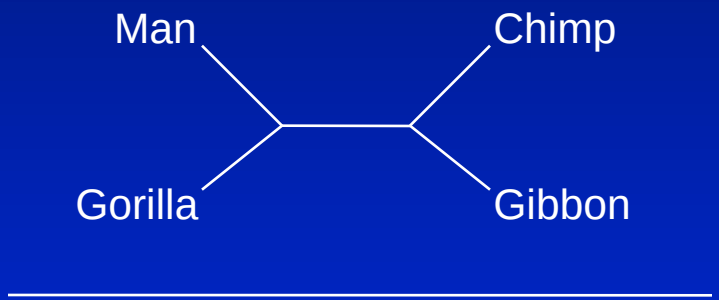
  OR
  - Use a **clustering** method

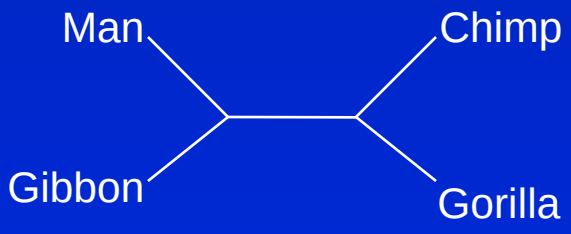
# Maximum Parsimony



| | |
|---|---|
| Man: | GGG |
| Chimp : | GGG |
| Gorilla: | GGT |
| Gibbon: | GTT |



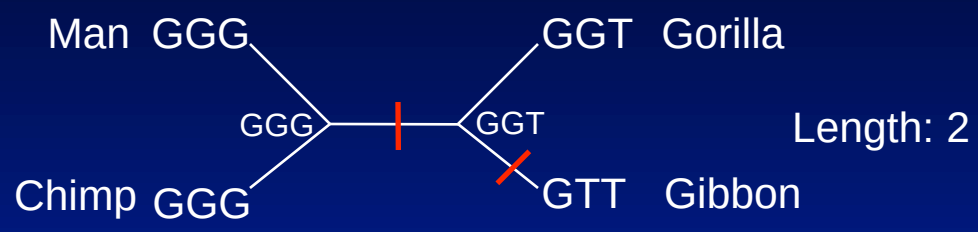



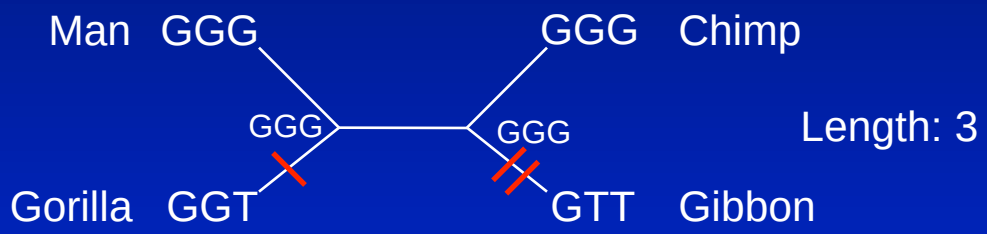



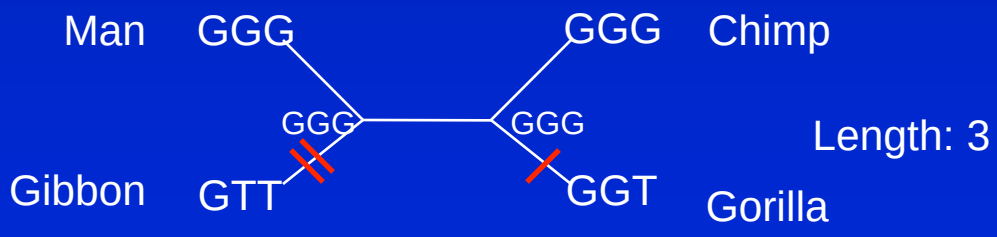
# Maximum Parsimony: best tree is shortest one



Man: GGG
Chimp : GGG
Gorilla: GGT
Gibbon: GTT



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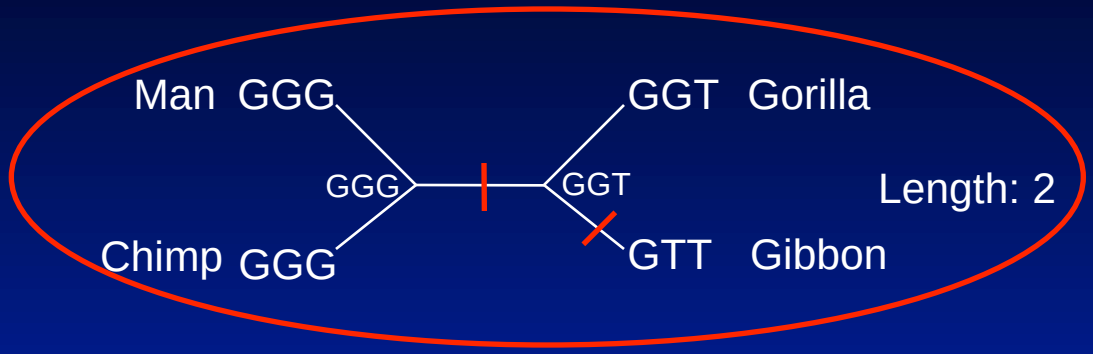


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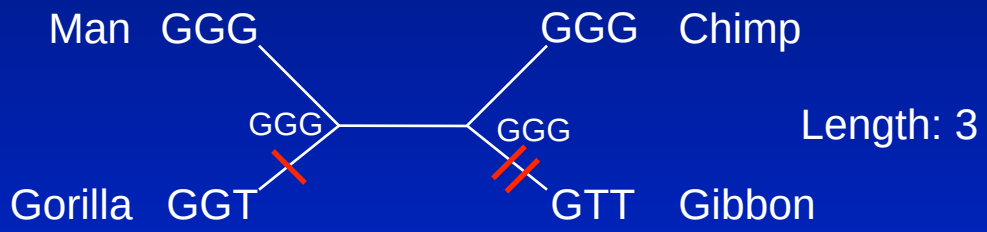
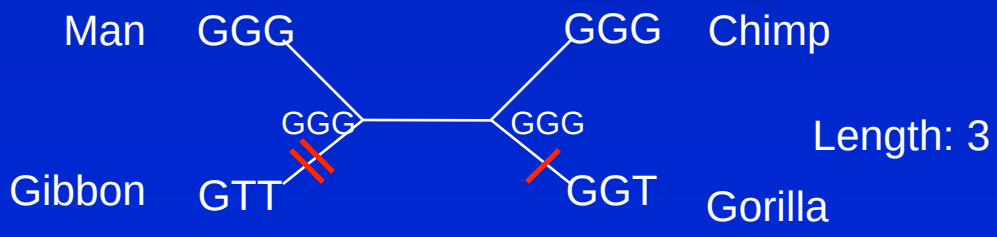
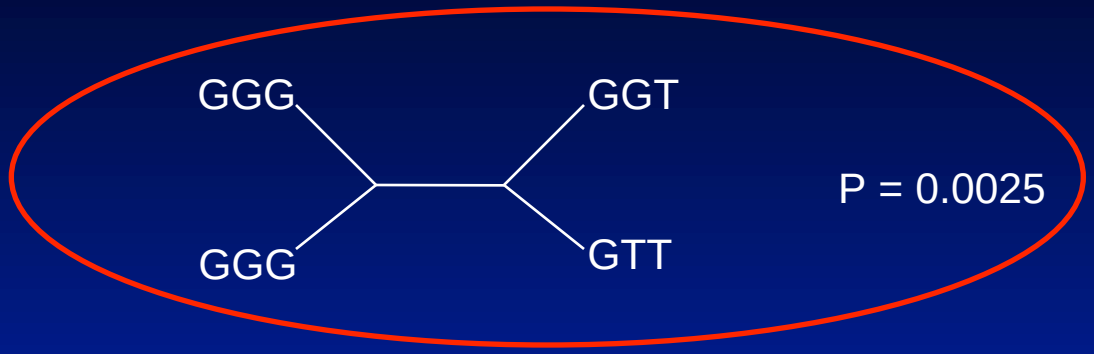


# Maximum Likelihood: best tree is the one giving the highest probability of the observed data

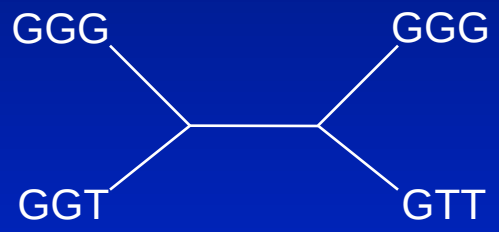


Man: GGG
Chimp : GGG
Gorilla: GGT
Gibbon: GTT




P = 0.0025

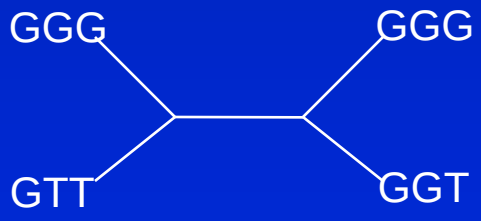
Likelihood = P(Data | Model)




P = 0.0002

$$P(t) = e^{Qt} = \begin{bmatrix} P_{AA} & P_{AC} & P_{AG} & P_{AT} \\ P_{CA} & P_{CC} & P_{CG} & P_{CT} \\ P_{GA} & P_{GC} & P_{GG} & P_{GT} \\ P_{TA} & P_{TC} & P_{TG} & P_{TT} \end{bmatrix}$$

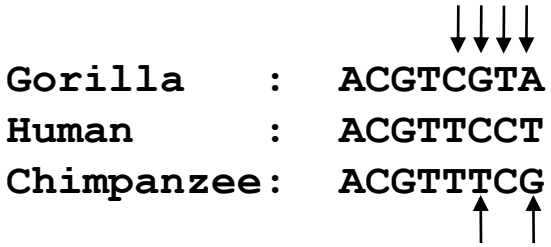
Probability matrix
(function of time t)




P = 0.0002

# Distance Matrix Methods

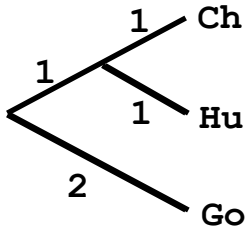





1. Construct multiple alignment of sequences

| | Go | Hu | Ch |
|---|---|---|---|
| Go | - | 4 | 4 |
| Hu | | - | 2 |
| Ch | | | - |

2. Construct table listing all pairwise differences (distance matrix)



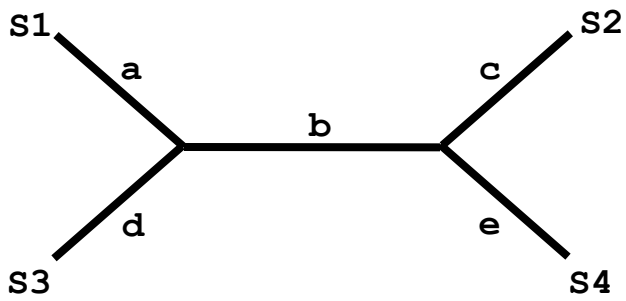

3. Construct tree from pairwise distances

# Finding Optimal Branch Lengths



| | $S_1$ | $S_2$ | $S_3$ | $S_4$ |
|---|---|---|---|---|
| $S_1$ | - | $D_{12}$ | $D_{13}$ | $D_{14}$ |
| $S_2$ | | - | $D_{23}$ | $D_{24}$ |
| $S_3$ | | | - | $D_{34}$ |
| $S_4$ | | | | - |

Observed distance




Distance along tree

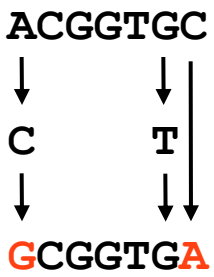
**Goal:**

$$D_{12} \approx d_{12} = a + b + c$$
$$D_{13} \approx d_{13} = a + d$$
$$D_{14} \approx d_{14} = a + b + e$$
$$D_{23} \approx d_{23} = d + b + c$$
$$D_{24} \approx d_{24} = c + e$$
$$D_{34} \approx d_{34} = d + b + e$$

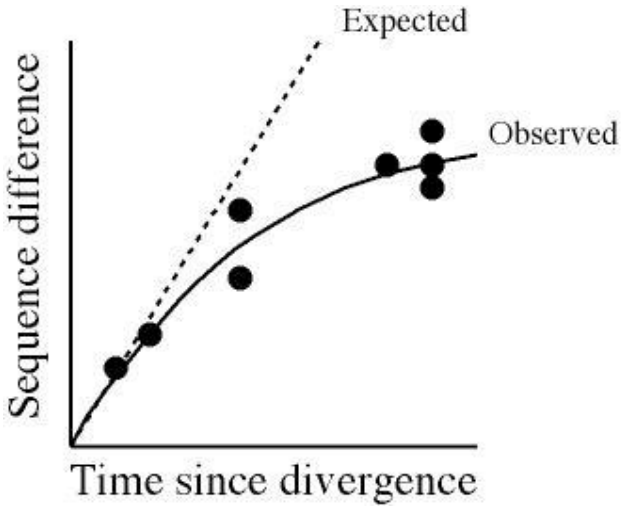
Handout exercise

# Superimposed Substitutions






- Actual number of evolutionary events: 5
- Observed number of differences: 2



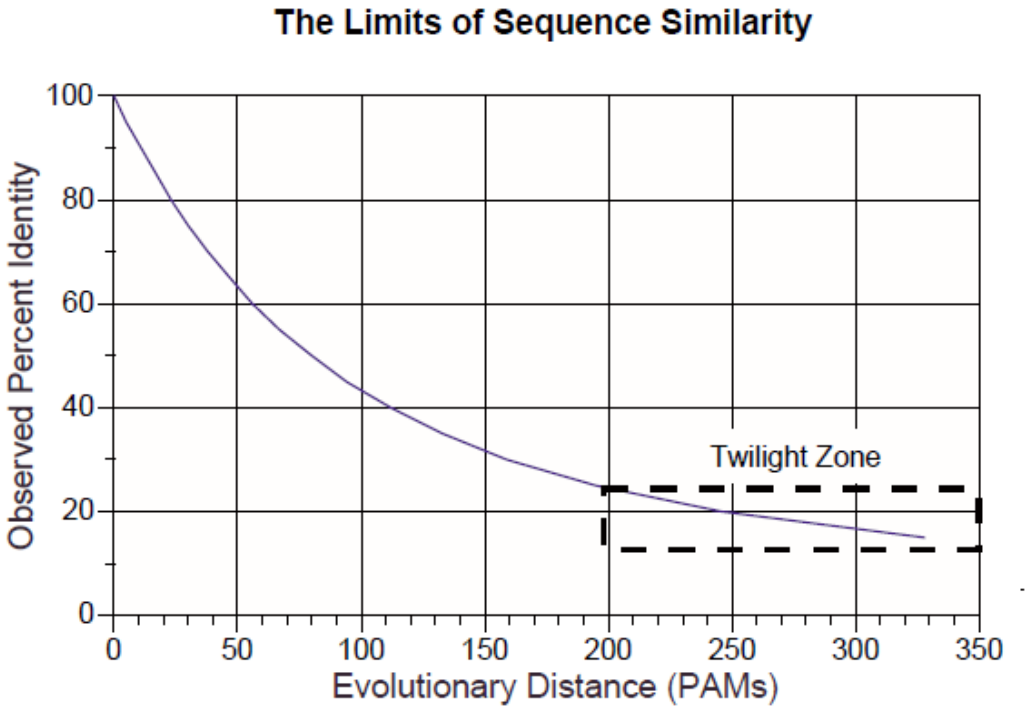

- Distance is (almost) always underestimated
- Real distance can be estimated from observed distance using models of how evolution occurs

# Percent Accepted Mutations (PAM)



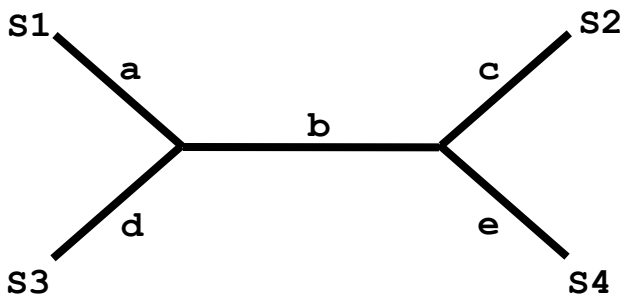
PAM (Percent Accepted Mutations) can be used as a measure of evolutionary distance.

**Note:** 100PAM does NOT mean that sequences are 100% different




In the “Twilight Zone”, it becomes difficult to see whether sequences are related

# Optimal Branch Lengths: Least Squares




Distance along tree

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$$D_{23} \approx d_{23} = d + b + c$$
$$D_{24} \approx d_{24} = c + e$$
$$D_{34} \approx d_{34} = d + b + e$$

- Fit between given tree and observed distances can be expressed as “sum of squared differences”:

$$Q = \sum_{j>i} (D_{ij} - d_{ij})^2$$

- Find branch lengths that minimize Q - this is the optimal set of branch lengths for this tree.

# Least Squares Optimality Criterion



- Search through all (or many) tree topologies
- For each investigated tree, find best branch lengths using least squares criterion
- Among all investigated trees, the best tree is the one with the smallest sum of squared errors (the most compatible tree)

# Exhaustive search impossible for large data sets

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| No. taxa | No. trees |
|---|---|
| 3 | 1 |
| 4 | 3 |
| 5 | 15 |
| 6 | 105 |
| 7 | 945 |
| 8 | 10,395 |
| 9 | 135,135 |
| 10 | 2,027,025 |
| 11 | 34,459,425 |
| 12 | 654,729,075 |
| 13 | 13,749,310,575 |
| 14 | 316,234,143,225 |
| 15 | 7,905,853,580,625 |

# Heuristic search



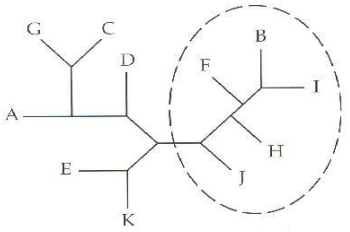
Construct random initial tree; determine tree goodness

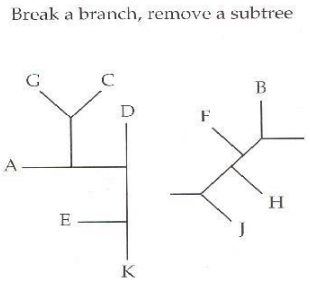
Construct set of “neighboring trees” by making small rearrangements of initial tree; determine goodness for each neighbor

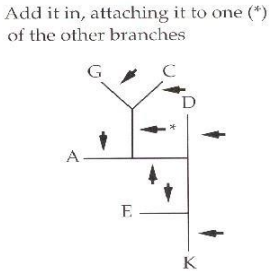
If any of the neighboring trees are better than the initial tree, then select it/them and use as starting point for new round of rearrangements. (Possibly several neighbors are equally good)

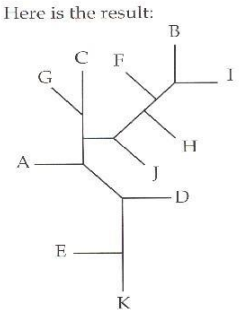
Repeat steps 2+3  until you have found a tree that is better than all of its neighbors.

This tree is a “local optimum” (not necessarily a global optimum!)



# Clustering Algorithms



- Starting point: Distance matrix
- Cluster most closely related pair of sequences
  - Tree: pair is connected to common ancestral node, compute branch lengths from ancestral node to both descendants
  - Distance matrix: combine two sequence entries into one. Compute new distance matrix, by finding distance from new node to all other nodes
- Repeat until all nodes are linked
- Results in only one tree, does not use any measure of “tree-goodness”.

# Neighbor Joining Algorithm



- For each tip compute $u_i = \Sigma_j D_{ij}/(n-2)$

  (this is essentially the average distance to all other tips, except the denominator is n-2 instead of n)

- Find the pair of tips, i and j, where $D_{ij}-u_i-u_j$ is smallest

- Connect the tips i and j, forming a new ancestral node. The branch lengths from the ancestral node to i and j are:

$$v_i = 0.5\ D_{ij} + 0.5\ (u_i-u_j)$$
$$v_j = 0.5\ D_{ij} + 0.5\ (u_j-u_i)$$

- Update the distance matrix: Compute distance between new node and each remaining tip as follows:

$$D_{ij,k} = (D_{ik}+D_{jk}-D_{ij})/2$$

- Replace tips i and j by the new node which is now treated as a tip

- Repeat until only two nodes remain.

# NJ visualized



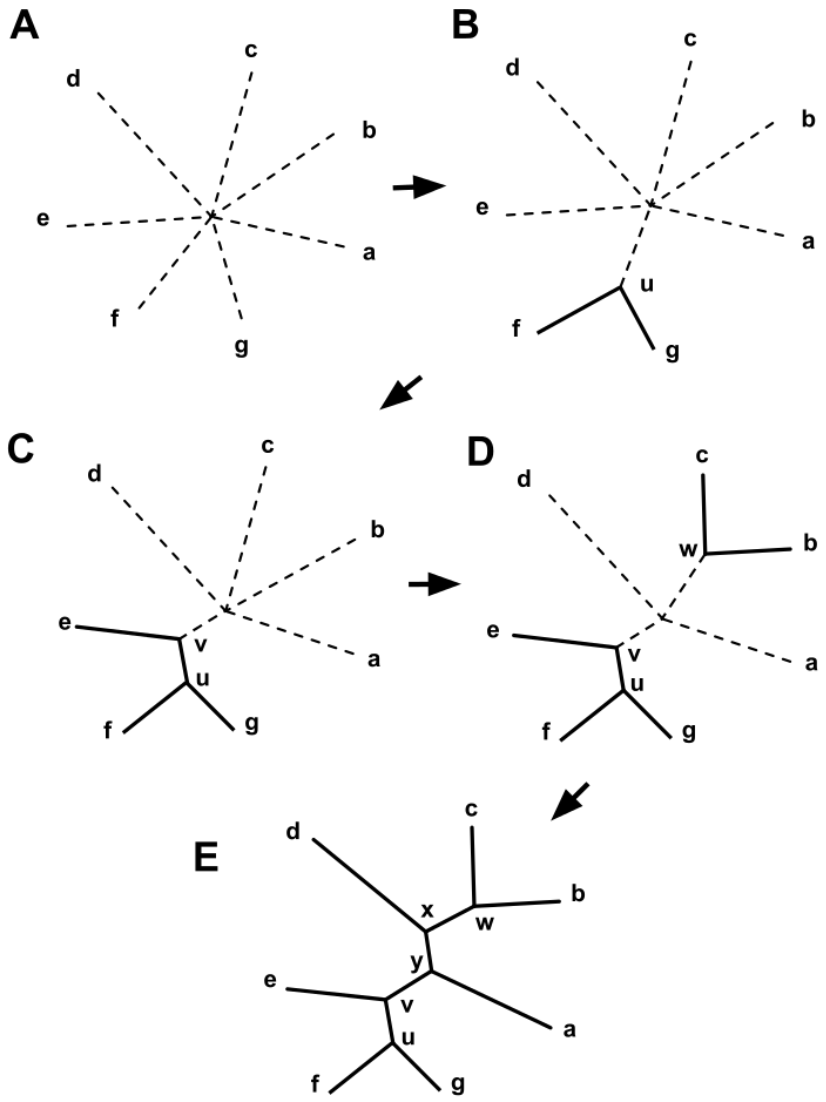


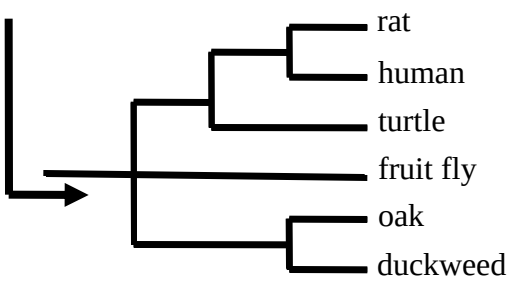

Image source: Wikipedia

# Bootstrapping



Original alignment

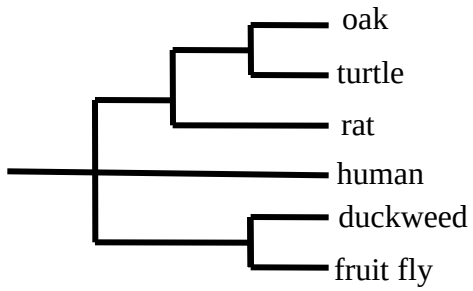
```
          0123456789
rat       GAGGCTTATC
human     GTGGCTTATC
turtle    GTGCCCTATG
fruitfly  CTCGCCTTTG
oak       ATCGCTCTTG
duckweed  ATCCCTCCGG
```




Inferred tree

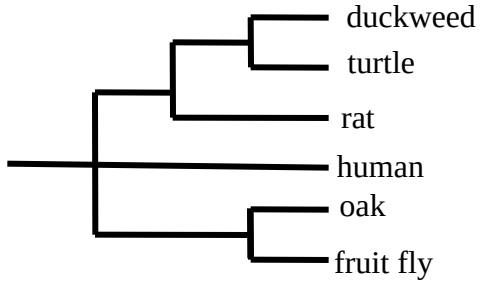
Bootstrapped alignment 1

```
          001122234556667
rat       GGAAGGGGCTTTTTA
human     GGTTGGGGCTTTTTA
turtle    GGTTGGGCCCCTTTA
fruitfly  CCTTCCCGCCCTTTT
oak       AATTCCCGCTTCCCT
duckweed  AATTCCCCCTTCCCC
```




Bootstrapped alignment 2

```
          445556777888899
rat       CCTTTTAAATTTTCC
human     CCTTTTAAATTTTCC
turtle    CCCCCTAAATTTTGG
fruitfly  CCCCCTTTTTTTTGG
oak       CCTTTCTTTTTTTGG
duckweed  CCTTTCCCCGGGGGG
```



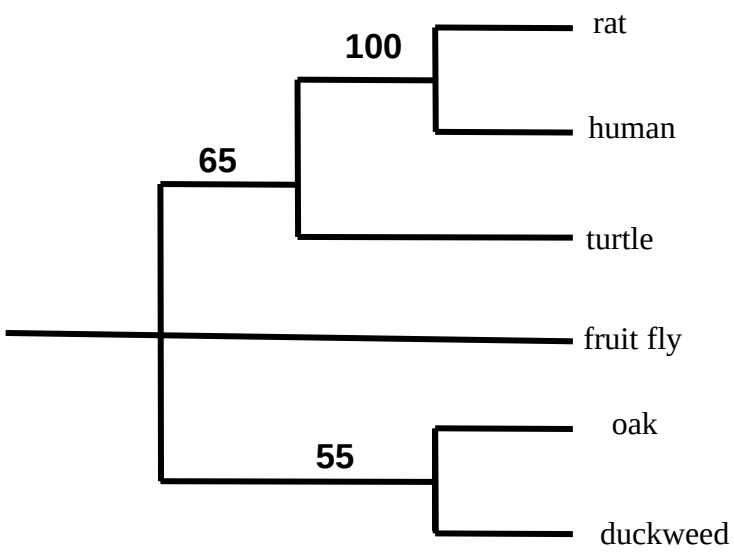

. . .

. . .

Many more replicates (between 100 - 1000)

# Bootstrapping values





# Key Takeaways

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- **Phylogenetic Relationships**: All living organisms ultimately descended from a single common ancestor (or a single common gene pool) ⇒ Present-day organisms are connected by a tree-like history.
- **Tree Structures**: Rooted and unrooted trees provide different perspectives on relatedness and evolutionary time.
- **Importance of Molecular Data**: Molecular sequences are crucial, especially for organisms with few distinguishing physical features.
- **Tree Building Methods:** There are four classes of methods for reconstructing phylogenies from molecular data. One of them is distance-based methods.
- **Distance-based Methods**: Either use least squares optimization and heuristic searches, or a clustering algorithm like Neighbor Joining.
- **Confidence in Results**: Bootstrapping provides a robust way to test and refine our phylogenetic trees.