EE550
Computational Biology

Week 8 Course Notes

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Topics

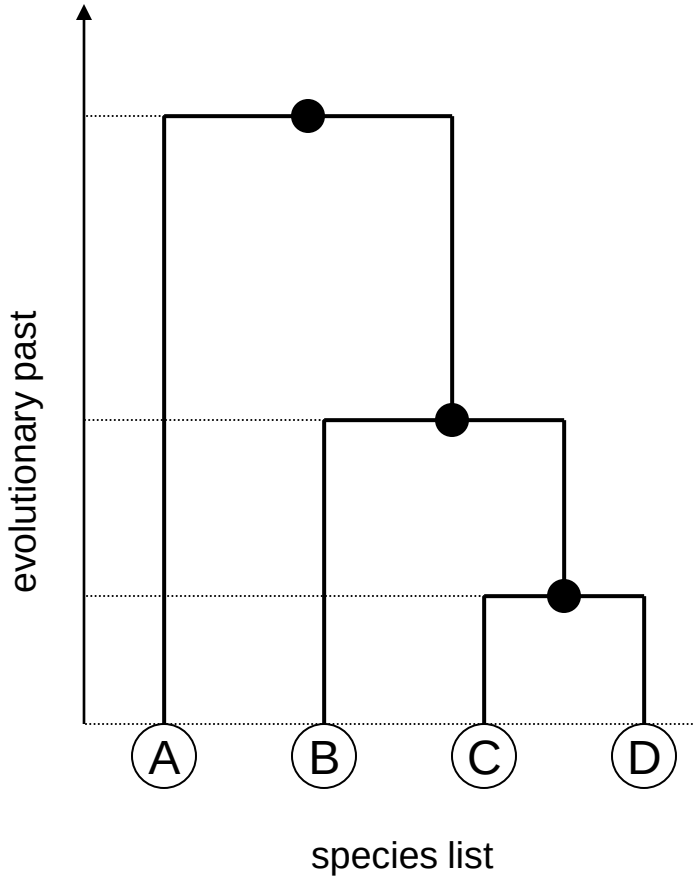
- Inter-species evolutionary relationships via phylogenetic trees
  - Trees
    - Rooted trees
    - Unrooted trees
    - Tree topology
  - Sequences
  - Distance metrics
  - Clustering schemes

Phylogenetic Trees

- From molecular sequences to evolutionary relationships
  - Molecular sequences express how close or far apart different species (taxa) are in terms of accumulated differences
    - Mutations in terms of substitutions and indels
  - Organisms with more similar sequences can be thought of descending from a more recent common ancestor
  - The evaluation of the ancestral history between different species is studied by molecular phylogenetics
    - Shared common ancestors: how far ago and between which species (taxa)?

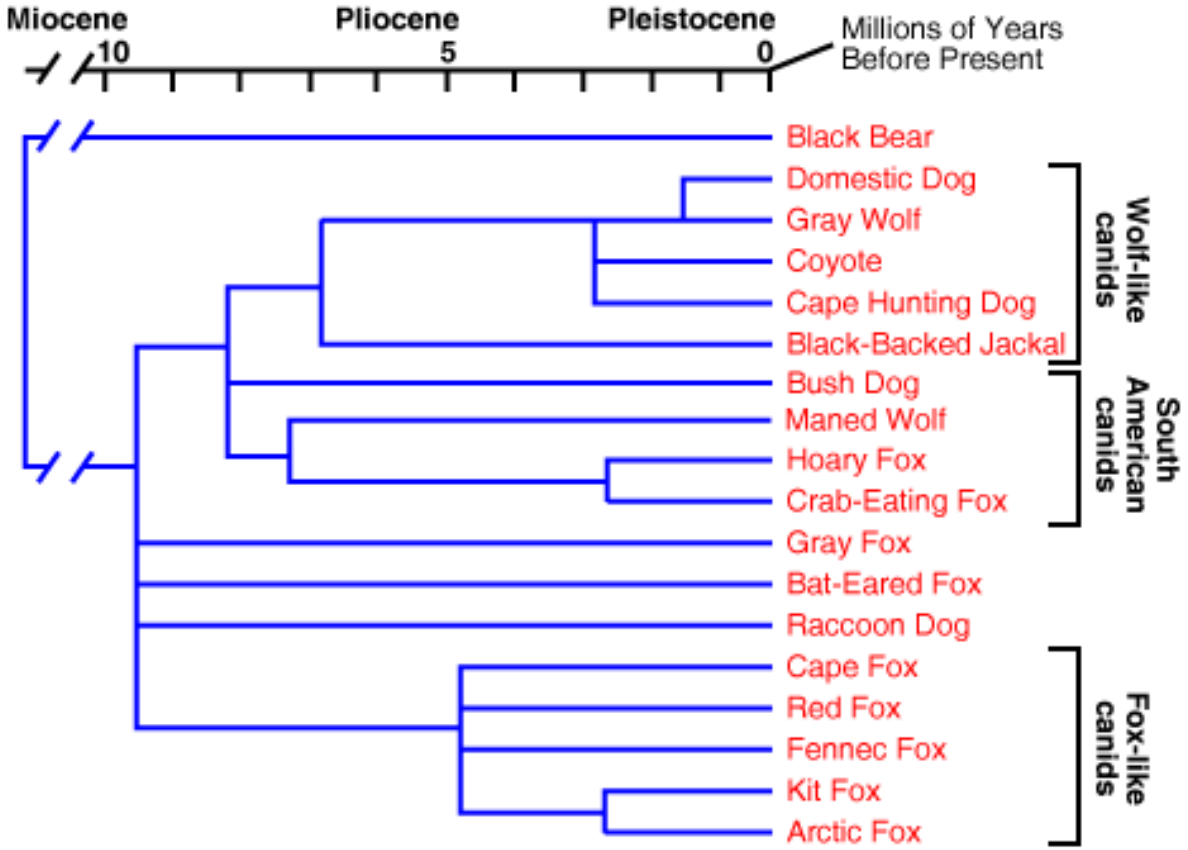
Phylogenetic Trees

- Trees provide the ideal scheme for representing the evolutionary relationships between different species
  - The species (taxa) at the present time start out as the leaves/nodes
  - In the time past, leaves are merged into branches/nodes indicating common ancestry
    - Leaves with more similar sequences are merged first
  - The branches/nodes are merged into more ancient species (taxa)
  - …



Example

- Phylogenetic tree of dogs (by Wayne et al., University of California)
  - Several parameters taken into account
    - characteristics of skulls, skeletons and chromosomes,
    - genetic analysis of mitochondrial DNA,
    - non-coding and coding nuclear DNA,
    - protein analysis
  - Corroboration with fossil evidence was also sought for validation

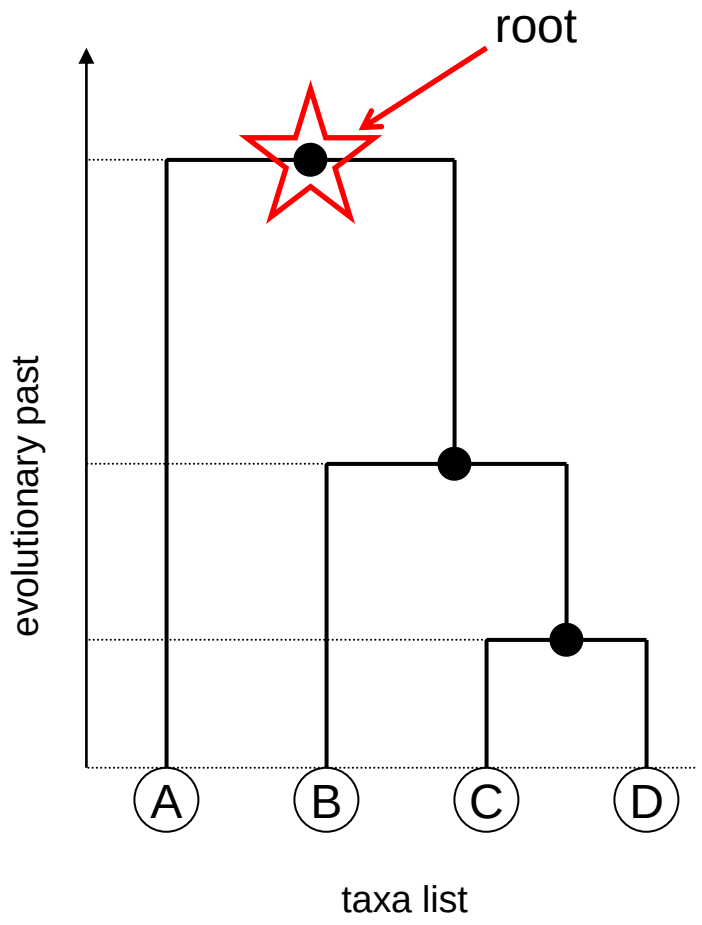


Source:
http://www.nbii.gov/portal/community/Communities/Ecological_Topics/Genetic_Diversity/Taxonomy,_Phylogenetics_&_Systematics/

Rooted Phylogenetic Trees

- The root of a phylogenetic tree (if it exists) indicates the original species that is ancestral to all
- Rooted phylogenetic trees thus have:
  - A **root** where all studied species have descended from
  - A **graded time axis** indicating the evolutionary time it took for each species to differentiate from the common origin
- Rooted phylogenetic trees provide information on
  - when a given pair of species had a common ancestor
  - which pair of species diverged earlier or later than another pair
  - how much evolutionary time has passed between the bifurcations

Rooted Phylogenetic Trees



- Notes:
  - Most tree construction algorithms produce rooted trees by default
  - Just because a tree is shown like it has a root does not make it a rooted tree
    - The fine print under the figure must be read carefully!!

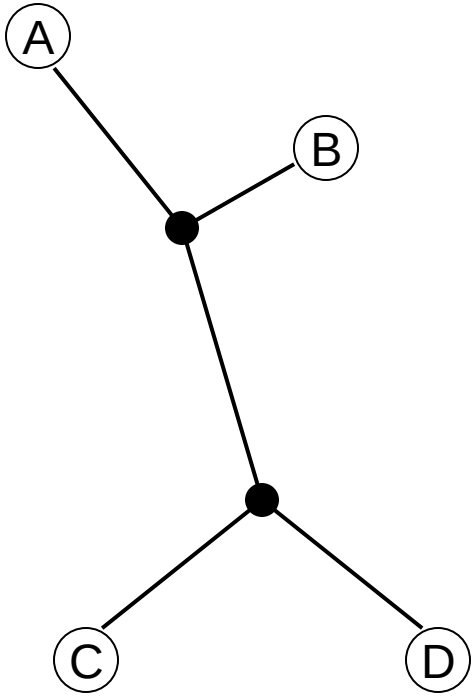
Unrooted Phylogenetic Trees

- It is not always possible to deduce a temporal order of events from the molecular data
  - The mutation rates may vary from species to species
- In such cases, the direction of changes are questionable among ancestral species
- Without a clear understanding of which species are descending from which ancestor, it is not possible/feasible/realistic to establish a common ancestral species

➔ Unrooted trees

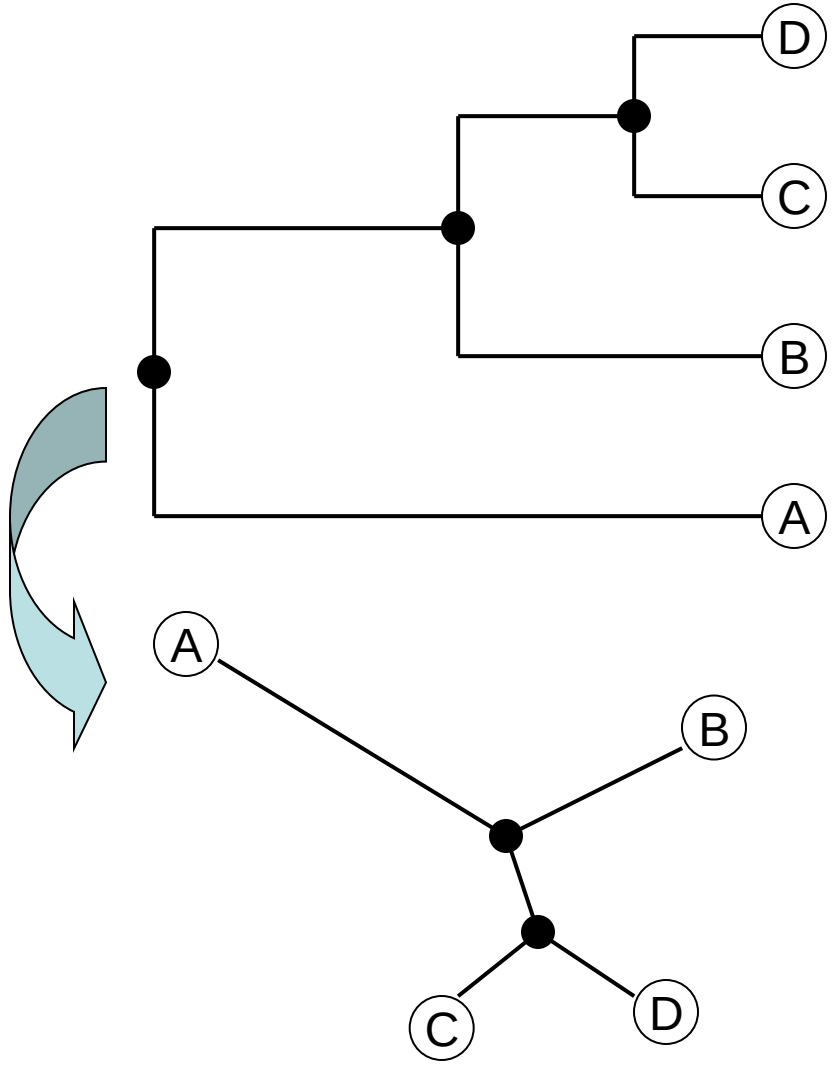
Unrooted Phylogenetic Trees

- The species (taxa) at the present time are at the outer rims of the tree
  - Leaves
- The ancestral species (taxa) are located inwards
- The hypothetical root is possibly somewhere around the ancestral species
  - Usually on a link between a pair of ancestral species (taxa)
    - outgroups
  - But the exact location is unknown

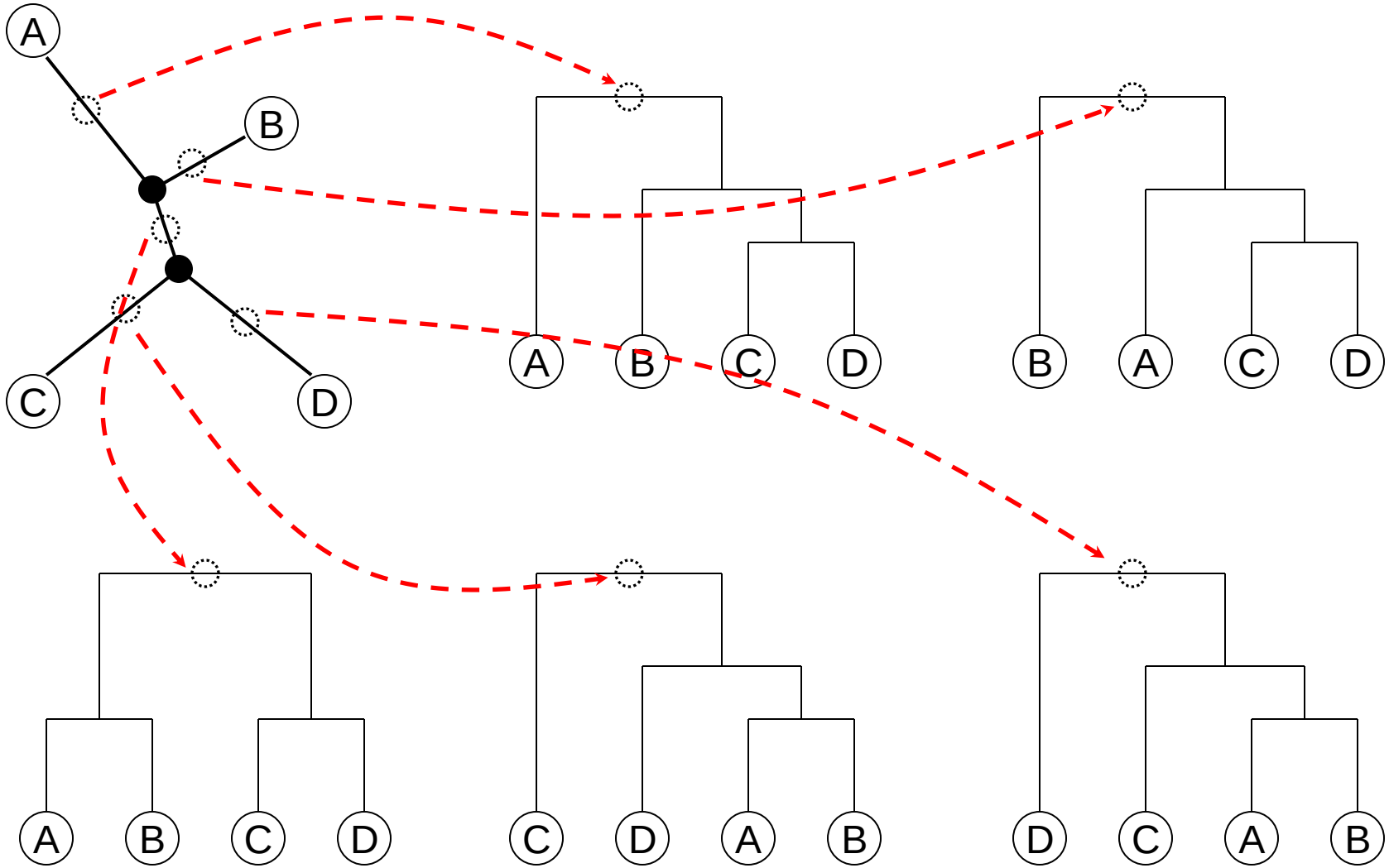


Unrooted Phylogenetic Trees

- Rooted trees can easily be converted into unrooted trees
  - Simply ignore the root and spread out the leaves
- Unrooted trees cannot be converted to rooted trees so easily
  - One would have to identify the root
  - Identifying the root of an unrooted tree requires a priori information
  - This can be addressed by including an **outgroup**
    - **sufficiently close** to the species of interest
    - **sufficiently far** so that the root would be on its link

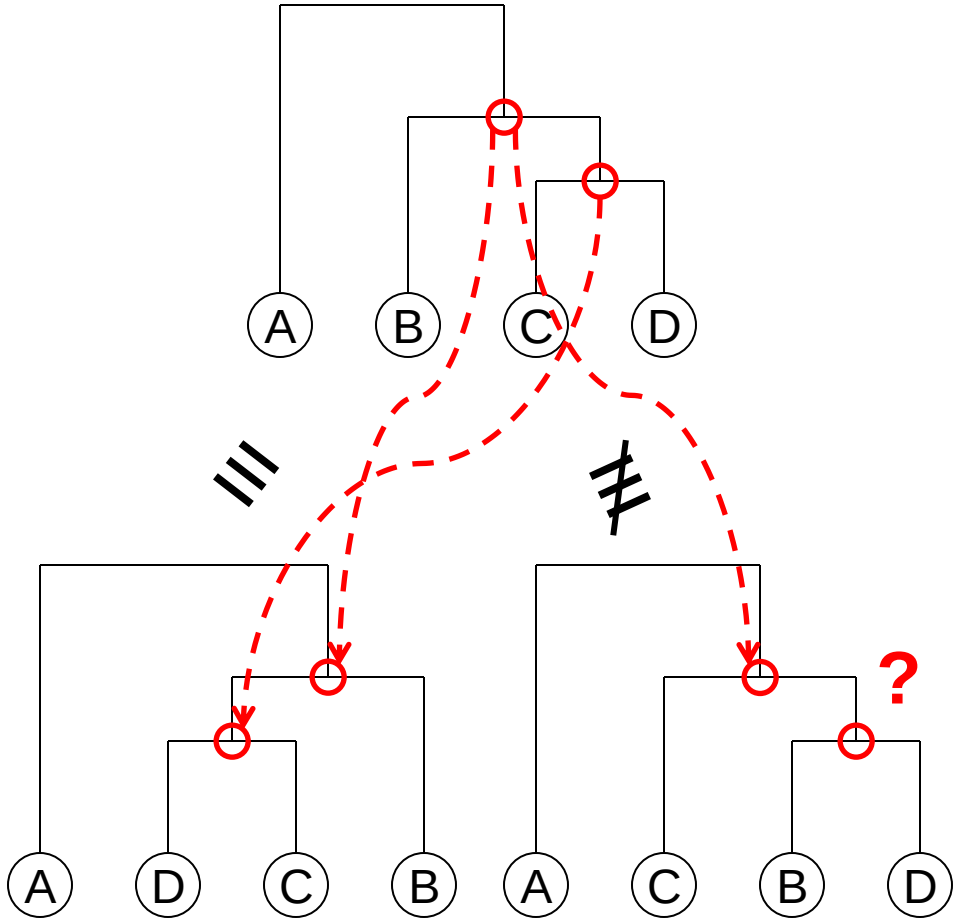


Unrooted Phylogenetic Trees



Tree Topology

- The topology of a phylogenetic tree indicates the set bifurcations observed among ancestral species (taxa)
  - Roughly the “shape” of a tree
- When one or more ancestral relationships change, one obtains a different phylogenetic tree
  - The topology of the tree is different
- Note:
  - The actual drawing of the tree may change without affecting the topology
  - One obtains **a different tree only when the ancestral relationships are altered**



Sequences

- Evolutionary distances are inferred from sequence differences
  - Nucleic acid sequences
  - Amino acid sequences
- Constructing phylogenetic trees over a set of species (taxa) requires sets of homologue sequences – sequences with common ancestry
  - Homologue genes
  - Homologue proteins
- Phylogenetic trees can be constructed on any homologue set
  - The results obtained on different homologue sets can vary!!
- A selection must be made with regard to the biological question at hand
  - Studying the evolution of a particular gene of interest
  - Studying the evolution of gene families
    - With lots of sequences available from many species (taxa)

Sequences – Selection

- Selection of sequences are also important for practical purposes
  - **If the sequences are too similar**, the study will lack reliable evolutionary differentiation information
    - High similarity between sequences is an indication that the evolutionary process has not had adequate time to induce significant sequence alterations
      - A slow mutation rate and/or a short time period
    - This is contrary to the goal of producing a phylogeny
  - **If the sequences are too distinct**, the errors in evolutionary distances will take over
    - The standard deviation of the error in evolutionary distance estimation increases with the extent of sequence differences
      - This will cause dramatic changes in the resulting tree topology
    - Large differences between sequences are also suggestive of the lack of necessary homology among the sequences

Sequences – Alignment

- Phylogenetic tree construction is guided by multiple sequence alignment
  - Multiple sequence alignment algorithms have a set of innate choices
    - Similarity scores between aligned nucleotides or amino acids
    - Gap penalties
    - Sequence to cluster alignment procedures
  - Variations in these choices produces different multiple alignments
  - It is imperative that the sequence alignments obtained at the beginning of tree construction are valid and reasonable
    - Manual verification likely to be worth the effort, if at all feasible
  - Choice of the set of sequences upon which to construct a phylogeny directly determines how reliable the alignments will be
    - Highly variable sequence segments are not suitable choices
      - High variability implies high noise and little phylogeny information

Distance Metrics

- Multiple sequence alignment determines
  - the locations and extents of gaps to be inserted into each sequence in the set
  - so that all sequences are jointly aligned
- The resulting alignment must then be used to compute the evolutionary distances between the sequences
  - Multiple sequence alignment algorithms make use of a substitution model to determine the rates at which they will evaluate the matches and the mismatches
    - Evolutionary model in nucleic acid sequences
      - Jukes-Cantor
      - Kimura's two-parameter model
    - Substitution matrices in amino acid sequences
      - PAM
      - BLOSUM
  - This substitution model determines the relationship between sequence differences and evolutionary distances

Distance Matrices

- Let S be a set of K homologous sequences, aligned to serve as the basis for phylogenetic tree construction

$$S = \{\boldsymbol{S}_1, \boldsymbol{S}_2, \dots, \boldsymbol{S}_K\}$$

  - $\boldsymbol{S}_k(i) \in \{\mathrm{A}, \mathrm{G}, \mathrm{T}, \mathrm{C}, \_\}$ for $k = 1,2, \dots, K$ and $i = 1,2, \dots, N$ (for nucleic acid sequences)
  - $\boldsymbol{S}_k(i) \in \{\mathrm{A}, \mathrm{R}, \mathrm{N}, \dots, \mathrm{C}, \_\}$ for $k = 1,2, \dots, K$ and $i = 1,2, \dots, N$ (for amino acid sequences)
    - Multiple sequence alignment produces same length aligned sequences, where the common length (after the insertion of gaps) is denoted by N
- Let $d_{k,m}$ denote the evolutionary distance between the k'th and the m'th sequence
  - The computation of $d_{k,m}$ depends on the underlying substitution model
    - J-K model: $d_{k,m} = -\frac{3}{4}\log\left(1 - \frac{3}{4}D_{k,m}\right)$, where $D_{k,m}$ denotes the substitution ratio
  - Evolutionary distances are computed between the sequences typically by ignoring the gaps
- The resulting distance matrix $\boldsymbol{d} = \left[d_{k,m}\right]$ for $k, m = 1,2, \dots, K$ is then to be used to derive the phylogenetic tree

Clustering Schemes

- Constructing a phylogenetic tree requires grouping the most similar species together into clusters earlier than the others

→ **hierarchical clustering**

  - Grouping species is easy:
    - Find the ones with the smallest evolutionary distance
    - Make a group containing the two closest species
  - Grouping clusters is not so easy:
    - Requires defining a distance for clusters

- Once a cluster distance is decided upon, a general strategy goes as follows:
  - Start with each species in a distinct cluster
  - Find the most similar pair of clusters among the available set
  - Merge the two clusters into a larger cluster
  - Update the distance matrix by

  (i) replacing the original two clusters with the newly formed cluster and

  (ii) updating the cluster-to-cluster distances involving the new cluster
    - The size of the distance matrix is reduced by one
  - Repeat until only one cluster remains

Cluster Distances

- Clusters are collections of sequences
- Distances between clusters can therefore be defined using the pairwise distances between their elements
- Let C_i and C_j be two clusters of size m and n respectively
  - $C_i = \{\boldsymbol{A}_1, \boldsymbol{A}_2, \dots, \boldsymbol{A}_m\}$
  - $C_j = \{\boldsymbol{B}_1, \boldsymbol{B}_2, \dots, \boldsymbol{B}_n\}$
- Let also $d(\boldsymbol{A}, \boldsymbol{B})$ denote the inferred evolutionary distance between any two aligned sequences $\boldsymbol{A}$ and $\boldsymbol{B}$
- Consider the following quantities:
  - $\rho_{\min}(C_i, C_j) = \min_{k,\ell} d(\boldsymbol{A}_k, \boldsymbol{B}_\ell)$
  - $\rho_{\max}(C_i, C_j) = \max_{k,\ell} d(\boldsymbol{A}_k, \boldsymbol{B}_\ell)$
  - $\rho_{\text{mean}}(C_i, C_j) = \frac{1}{mn} \sum_{k,\ell} d(\boldsymbol{A}_k, \boldsymbol{B}_\ell)$ (UPGMA)
- These quantities all satisfy the distance properties and can therefore be used to determine the distances between clusters

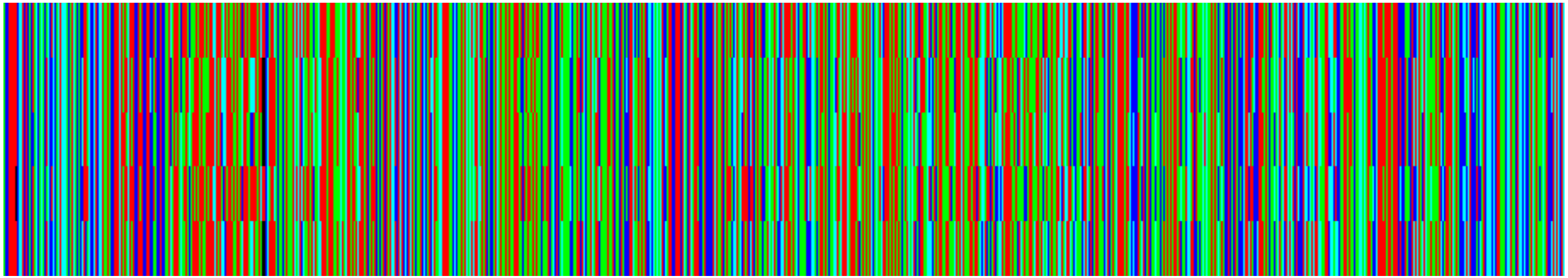
Example

- Phylogeny of blue tongue viruses
  - A set of 5 sequences obtained from the NCBI Nucleotide database
    - Bluetongue virus isolate BTV-9/RSAvvv1/09 VP6 protein gene, complete cds
    - Bluetongue virus isolate BTV-9/BOS2002/02 VP6 protein gene, complete cds
    - Bluetongue virus isolate BTV-4/RSAvvv3/04 VP6 protein gene, complete cds
    - Bluetongue virus isolate BTV-4/ARG2002/01 VP6 protein gene, complete cds
    - Bluetongue virus isolate BTV-1/GRE2001/01 VP6 protein gene, complete cds
  - Multiple alignment carried out using the Clustal Omega software package
    - Clustal Omega webserver at the EMBL website
  - Evolutionary distances between sequences identified using the Kimura two-parameter model
  - Phylogenetic tree constructed using the minimum distance metric

Example

- Multiple sequence alignment

SeqA	Name	Len(nt)	SeqB	Name	Len(nt)	Score
1	Gene_34	1052	2	Gene_35	1049	79
1	Gene_34	1052	3	Gene_36	1049	78
1	Gene_34	1052	4	Gene_37	1051	94
1	Gene_34	1052	5	Gene_38	1049	78
2	Gene_35	1049	3	Gene_36	1049	91
2	Gene_35	1049	4	Gene_37	1051	78
2	Gene_35	1049	5	Gene_38	1049	92
3	Gene_36	1049	4	Gene_37	1051	78
3	Gene_36	1049	5	Gene_38	1049	94
4	Gene_37	1051	5	Gene_38	1049	77



Example

- Pairwise distances:

	$\boldsymbol{S}_1$	$\boldsymbol{S}_2$	$\boldsymbol{S}_3$	$\boldsymbol{S}_4$	$\boldsymbol{S}_5$
$\boldsymbol{S}_1$	0	0.2749	0.2828	0.0593	0.2824
$\boldsymbol{S}_2$	0.2749	0	0.1015	0.2783	0.0901
$\boldsymbol{S}_3$	0.2828	0.1015	0	0.2679	0.0615
$\boldsymbol{S}_4$	0.0593	0.2783	0.2679	0	0.2766
$\boldsymbol{S}_5$	0.2824	0.0901	0.0615	0.2766	0

- These distances have been computed using Kimura's two-parameter model with

$$d = -\frac{1}{2}\log(1 - 2S - V) - \frac{1}{4}\log(1 - 2V)$$

where

S is the average substitutions between (A-G) or (T-C)

V is the average substitutions between purines and pyrimidines

Example

- Clustering:
  - Hierarchical clustering using the minimum distance definition for cluster distances
- Step 1:
  - The minimum distance is between $\boldsymbol{S}_1$ and $\boldsymbol{S}_4$
  - $\boldsymbol{S}_1$ and $\boldsymbol{S}_4$ are merged into a new cluster $\boldsymbol{S}_{1,4}$
  - Its distances to the remaining clusters ($\boldsymbol{S}_2$, $\boldsymbol{S}_3$, and $\boldsymbol{S}_5$) are computed using the minimum distance definition
    - $\rho(S_{1,4}, S_2) = \min(0.2749, 0.2783) = 0.2749$
    - $\rho(S_{1,4}, S_3) = \min(0.2828, 0.2679) = 0.2679$
    - $\rho(S_{1,4}, S_5) = \min(0.2824, 0.2766) = 0.2766$

Example

- Resulting distance matrix at the end of Step 1:

	$S_{1,4}$	S_2	S_3	S_5
$S_{1,4}$	0	0.2749	0.2679	0.2766
S_2	0.2749	0	0.1015	0.0901
S_3	0.2679	0.1015	0	0.0615
S_5	0.2766	0.0901	0.0615	0

Example

- Step 2:
  - The minimum distance is between $\boldsymbol{S}_3$ and $\boldsymbol{S}_5$
  - $\boldsymbol{S}_3$ and $\boldsymbol{S}_5$ are merged into a new cluster $\boldsymbol{S}_{3,5}$
  - Its distances to the remaining clusters ($\boldsymbol{S}_{1,4}$, $\boldsymbol{S}_2$) are computed using the minimum distance definition
    - $\rho\left(\boldsymbol{S}_{3,5}, \boldsymbol{S}_{1,4}\right) = \min(0.2679, 0.2766) = 0.2679$
    - $\rho\left(\boldsymbol{S}_{3,5}, \boldsymbol{S}_{2}\right) = \min(0.1015, 0.0901) = 0.0901$

Example

- Resulting distance matrix at the end of Step 2:

	$\boldsymbol{S}_{1,4}$	$\boldsymbol{S}_{2}$	$\boldsymbol{S}_{3,5}$
$\boldsymbol{S}_{1,4}$	0	0.2749	0.2679
$\boldsymbol{S}_{2}$	0.2749	0	0.0901
$\boldsymbol{S}_{3,5}$	0.2679	0.0901	0

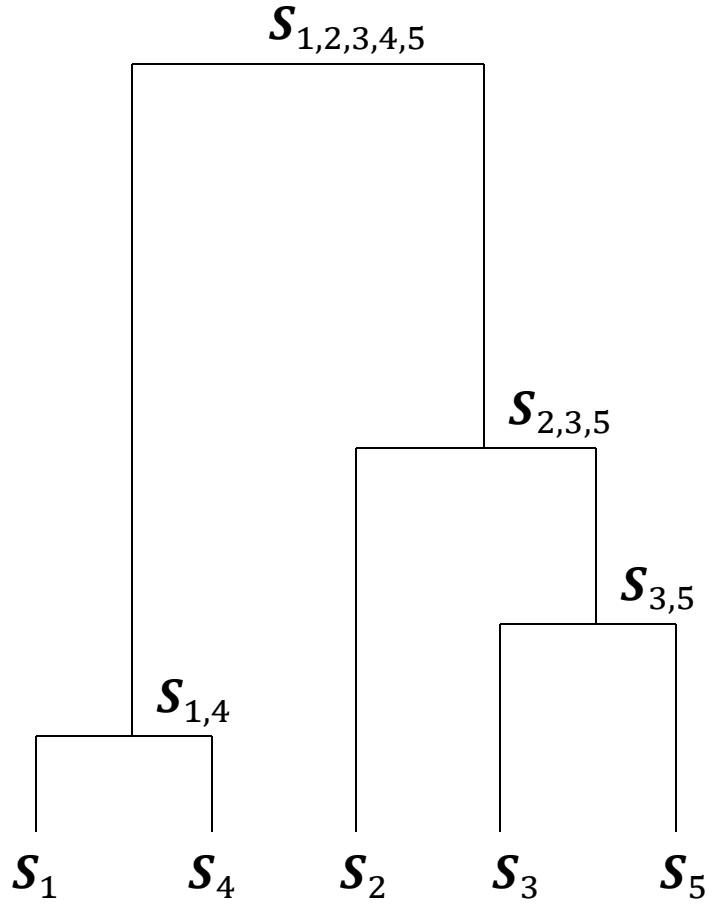
- Step 3:
  - The minimum distance is between $\boldsymbol{S}_{2}$ and $\boldsymbol{S}_{3,5}$
  - $\boldsymbol{S}_{2}$ and $\boldsymbol{S}_{3,5}$ are merged into a new cluster $\boldsymbol{S}_{2,3,5}$
  - Its distance to the remaining cluster ($\boldsymbol{S}_{1,4}$) is computed using the minimum distance definition
    - $\rho(\boldsymbol{S}_{2,3,5}, \boldsymbol{S}_{1,4}) = \min(0.2749, 0.2679) = 0.2679$

Example

- Resulting distance matrix at the end of Step 3:

	$S_{1,4}$	$S_{2,3,5}$
$S_{1,4}$	0	0.2679
$S_{2,3,5}$	0.2679	0

- The merger of the remaining two clusters are inevitable
  - and forms the common ancestor of all five species'
- Note that this is a rooted tree!!



Remarks

- The tree construction procedure determines the order in which similar species and clusters are merged together
- However, the evolutionary time between successive mergers are not well defined
  - The distances are not necessarily **additive**
    - At the time of estimation from the sequence distances using a substitution model
  - The minimum distance method does not seek the **additive nature of evolutionary distances**
- Clearly, different measures of cluster distance are likely to produce different trees
  - Which one is best for a particular application is subject for debate

Summary

- Phylogenetic trees express the evolutionary relationships between a set of species (taxa)
- The relationship is inferred from the similarities and differences between homologue sequences
- The inferred relationships are subject to influences from
  - The choice of homologue sequences
  - The multiple alignment method used
  - The method for converting sequences differences into evolutionary distances
  - The cluster distance definitions for assessing the similarities between clusters