Introduction to phylogenetic inference

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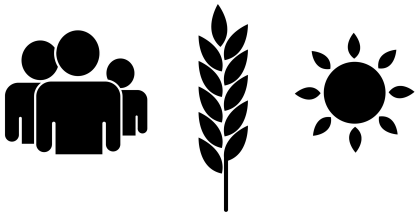

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Outline

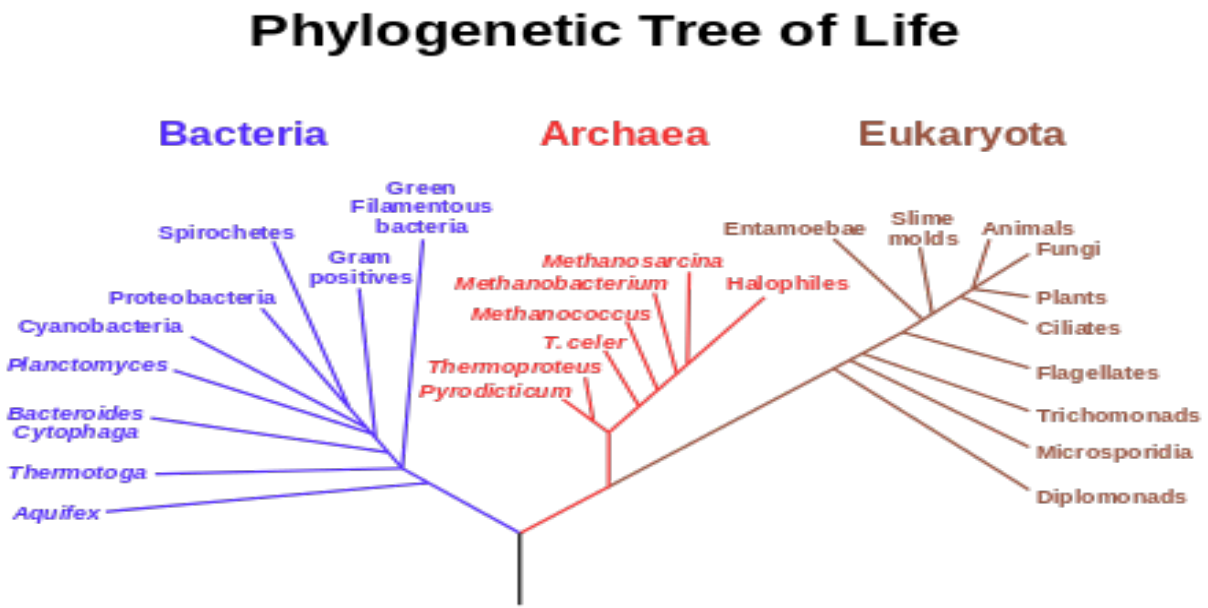
- Introduction and basic concepts in phylogeny
  - Trees
  - Alignements
  - Genetic distances and nucleotide substitution models
- Phylogenetic inference methods
  - Distance methods
  - Parcimony methods
  - Maximum likehood methods
  - Bayesian methods
- Phylogeny in practice
  - Testing tree topologies (bootstrap)
  - How to choose a method ?

Introduction

- **Phylogenetics** is the study of evolutionary relationships among groups of organisms (e.g. species, populations)
- The result of phylogenetic studies is a hypothesis about the evolutionary history of taxonomic groups: their **phylogeny**
- **Phylogenetic methods** aims at representing similarities and differences between taxa using a **phylogenetic tree**
- Underlying asumption : taxa joined together in the tree are implied to have descended from a common ancestor **through different speciation events**

What is a phylogenetic tree ?

- In **biology**, a phylogenetic tree is a **branching diagram** for representing the inferred evolutionary relationships among various biological entities
- In **mathematics**, a tree is an **undirected graph** in which any two vertices are connected by exactly **one simple path**. In other words, any connected graph without simple cycles is a tree.



Molecular phylogenetics

Here : we focus on **molecular phylogenetics**, based on different kind of **molecular sequence data**

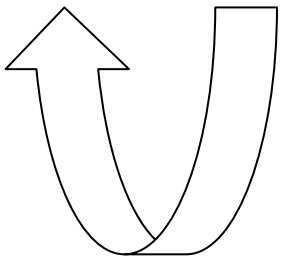
Trees are infered from **heritable characters** like:

- Binary patterns : presence/absence, 0/1
- Microsattelites data, SNPs, Insertions, Deletions
- **Aligned genetic sequences** (ADN, ARN, proteins) **in most cases**

In molecular phylogenetics, we infered the evolutionary history of sequences: it is not always the same of the one of the corresponding species !!!

Usual workflow in phylogenetic analysis

Dataset construction → Mutiple alignment → Build a phylogenetic tree → Interpret and evaluate the tree



check/filter the alignement

Dataset construction

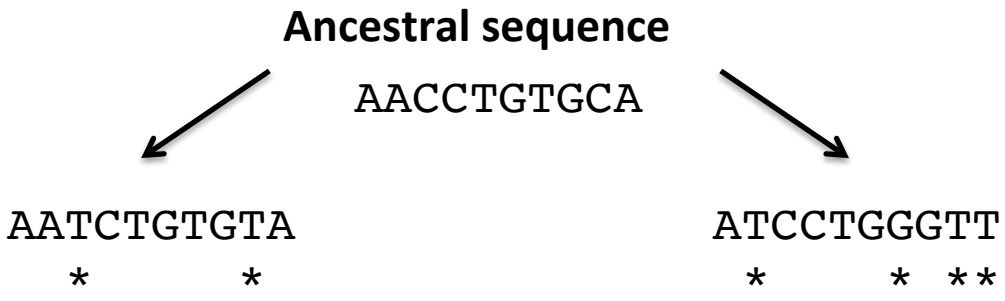
- **Criteria to choose good sequences dataset:** universality, conserved structure, no horizontal transfer, apropriate evolutionary rate.

- Some **popular genes** used in molecular phylogenetics
  - **Procaryotes**: ribosomal RNA (rRNA) 16S, betaglucosidase,…
  - **Eukaryotes**: rRNA 18S, actin, EF1, RPB1, mitochondrial genes,…

- **Protein coding genes:** nucleic alignments (if closed sequences) or proteic alignements (if distant sequences) of homolog sequences

Mutiple alignment as dataset

Hypothese: aligned sequences are **homologous, *i.e.* vertically derived from an ancestral sequence of common ancestor**




```
Seq1 AATCTGTGTA
Seq2 ATCCTGGGTT
      **   *  *
```

⇦ 1 site = 1 position in the alignment

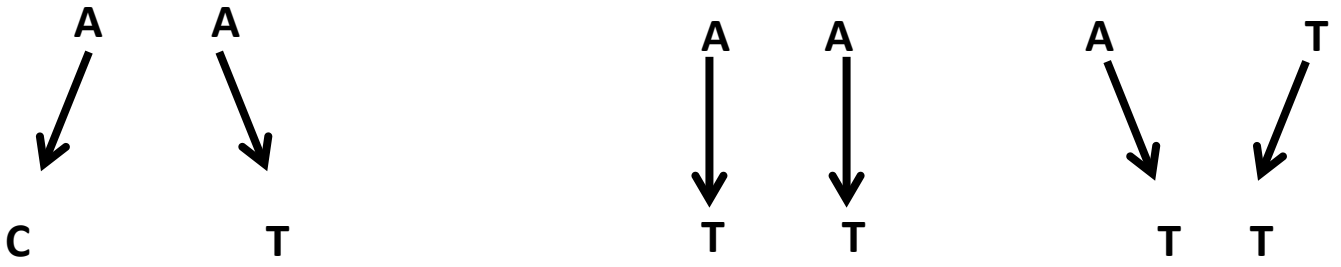
In phylogeny we will focus on **sites** of the alignment, either directly or indirectly via computation of a distance**.**

Homology vs Homoplasy

- **Homology** is any similarity between shared characters that is due to their shared ancestry

- **Homoplasy** occurs when characters are similars, but are not derived from a common ancestor

Homoplasies often result from **parallel** or **convergent** evolution



Phylogenetic inference should distinguish homoplasies from real phylogenetic signal

Quality of the genetic dataset is essential !

Alignment filtering

- **Filtering—removing unreliable columns before tree reconstruction** : a way to **increase the signal to noise ratio** of Multiple Sequence Alignments (MSAs)
- **Numerous filtering methods published:** Gblocks (Talavera and Castresana 2007) , TrimAl (Capella-Gutiérrez et al. 2009) , Noisy (Dress et al. 2008), BMGE (Criscuolo and Gribaldo 2010),…
- **In the context of single-gene phylogeny** a recent study* shows that the trees obtained from filtered MSAs are on average worse than those obtained from unfiltered MSA
- **In a phylogenomic context** it is highly recommanded to filter alignments !

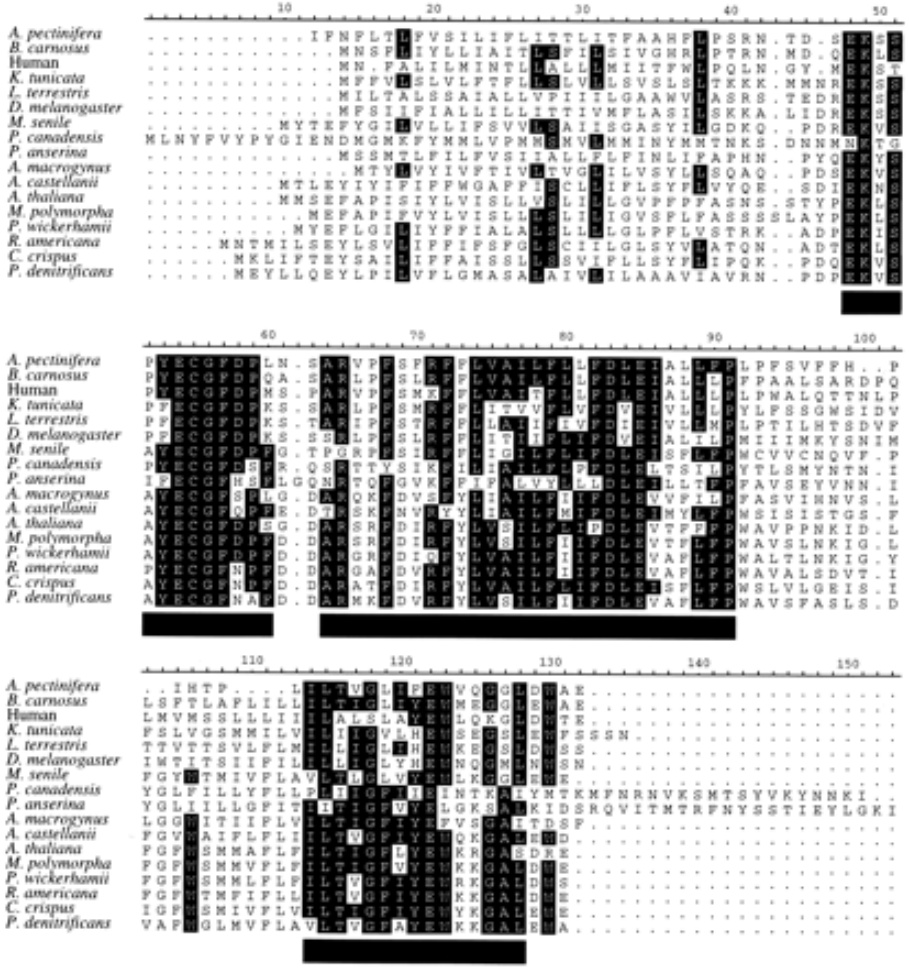
*Tan *et al*. Systematic Biology 2015

Filtering alignments: example

- Principle: selection of blocks of positions that fulfill a simple set of requirements with respect to the **number of contiguous conserved positions**, **lack of gaps**, and **high conservation of flanking positions**

Example of Gblocks filtering:

Alignment of ND3 sequences from several eukaryotes and a bacterial outgroup with the blocks selected Gblocks (default parameters) underlined.
Positions at which more than 50% of the residues are identical and have no gaps are shaded.



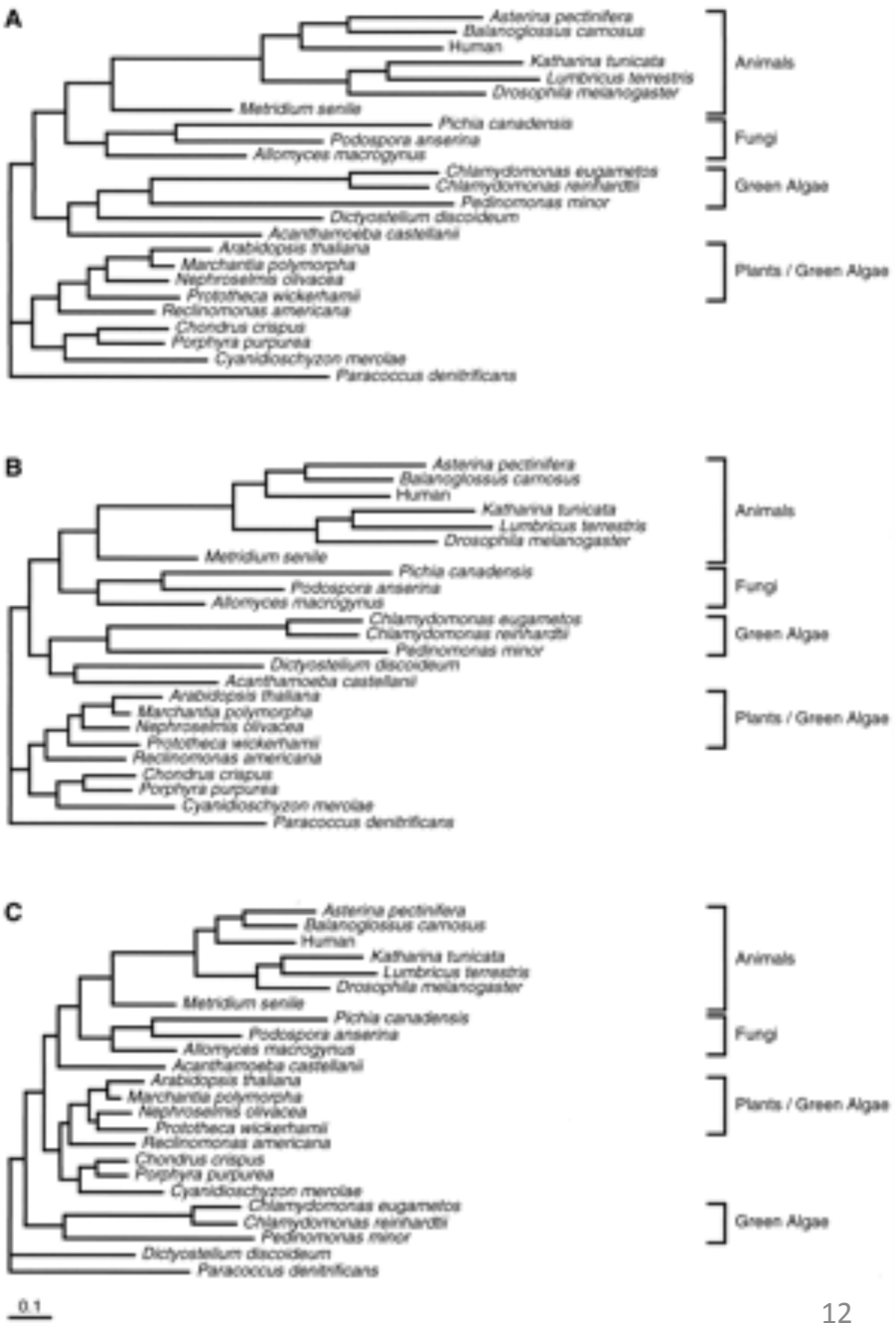
Castresana J Mol Biol Evol 2000;17:540-552

Influence of filtering on results

- Data : 5 mitochondial proteins aligned with clustalw

- Maximum Likehood Trees (mtRev models)
  - A : original alignment
  - B : gaps filtering
  - C : Gblocks filtering

Filtering can change both branch lengths and tree topology !



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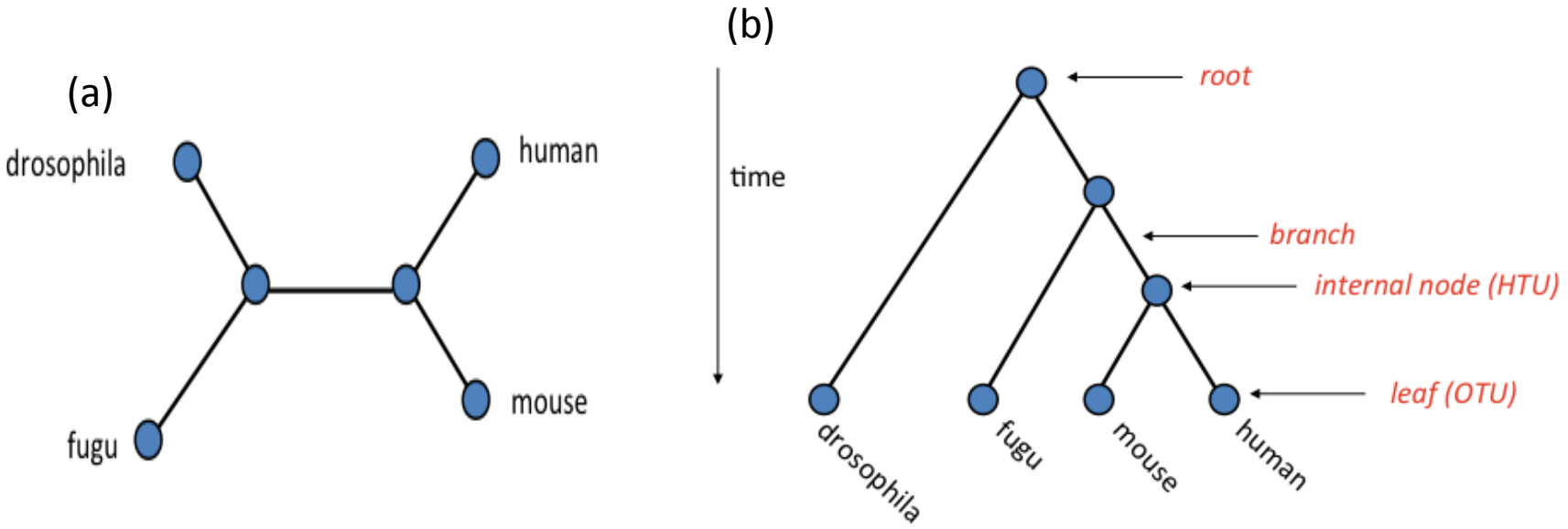
Phylogenetic tree: terminology

- Structure of an **unrooted** (a) and a **rooted phylogenetic tree** (b)



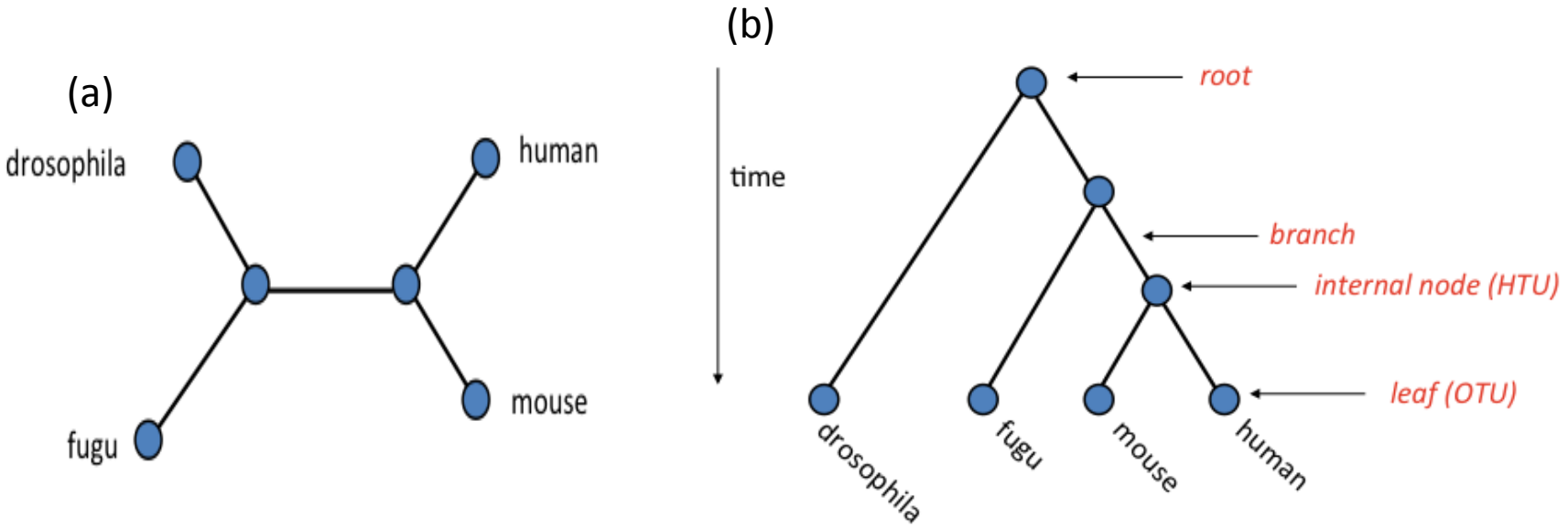
A tree is defined by its **topology** and its **branch lengths**.

Taxa are often named

- OTU: Operational Taxonomic Units
- HTU: Hypothetical Taxonomic Units

Phylogenetic tree: terminology

•Structure of an **unrooted** (a) and a **rooted phylogenetic tree** (b)



- Phylogeny focus on **bifurcating trees** : each internal node is of degree 3
- Most phylogenetical methods produce **unrooted trees**

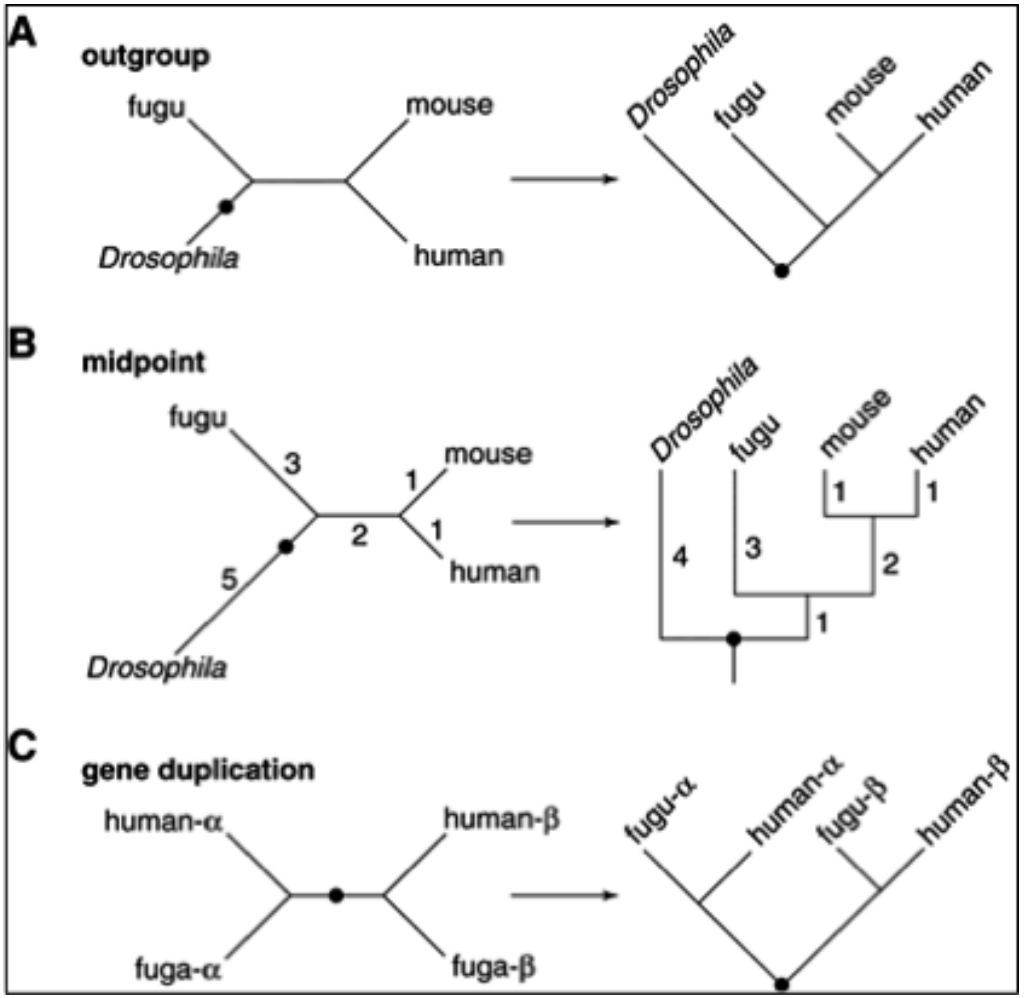
Introduction: how rooting a tree ?

•Three methods exist:

A. **Outgroup** rooting

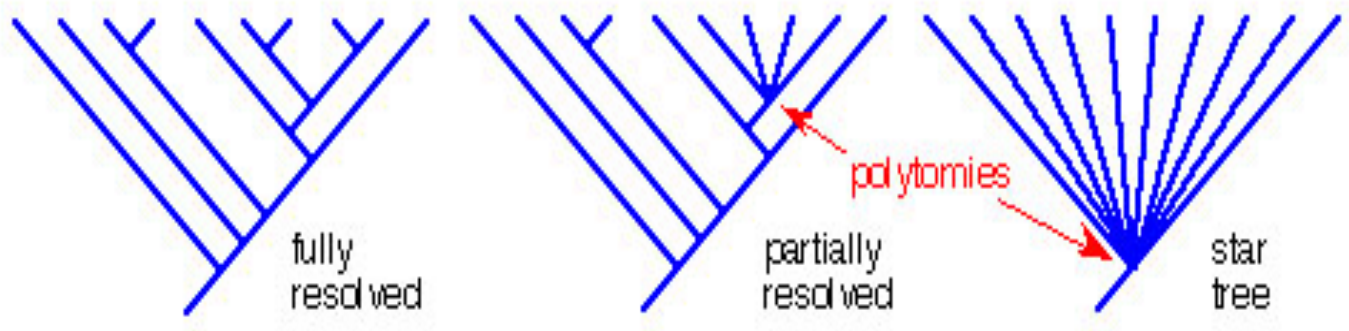
B. **Midpoint** rooting

C. Usage of external knowledge (ex. ancestral **gene duplication**)



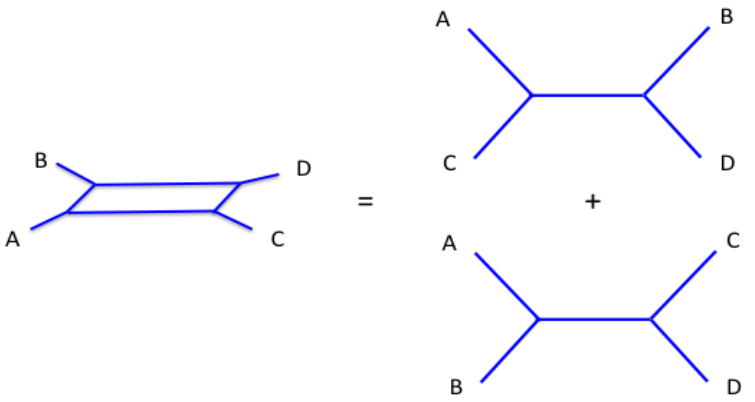
Is evolution always tree like ?

- Some processes lead to **non-bifurcating trees** :



- Multifurcations on phylogenetic trees are konwn as **polytomies** an include trees with **internal polytonies** (partially unresolved tree) and **star-like**

- **Networks** are a way of representing two conflicting tree topologies



Number of tree topologies

- Number of possible unrooted (N_U) and rooted (N_T) trees for n=1 to 10 OTUs

n	N_u	N_r
3	1	3
4	3	15
5	15	105
6	105	945
7	945	10,395
8	10,395	135,135
9	135,135	2,027,025
10	2,027,025	34,459,425

$$N_u = 3x5x7x\ldots(2n-5) = \frac{(2n-5)!}{2^{n-3}(n-3)!}$$

$$N_r = \frac{(2n-3)!}{2^{n-2}(n-2)!}$$

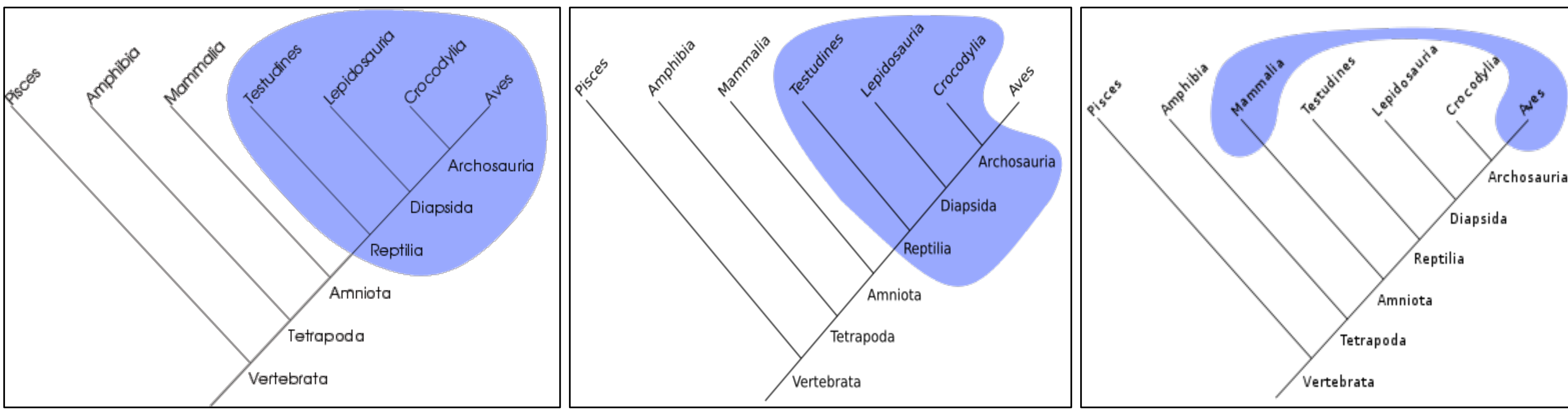
- Conclusion: an exhaustive search of all possible trees is usually impossible => **heuristic** strategies

Terminology

·**Monophyletic :** a group of taxa is monophyletic if it includes all descendants from its inferred common ancestor

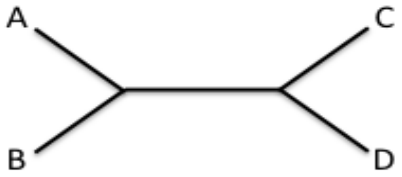
Paraphyletic : a group of taxa is paraphyletic if it does not include all descendants from its inferred common ancestor

Polyphyletic : a group of taxa is poliphyletic if it includes some descendants but not the inferred common ancestor



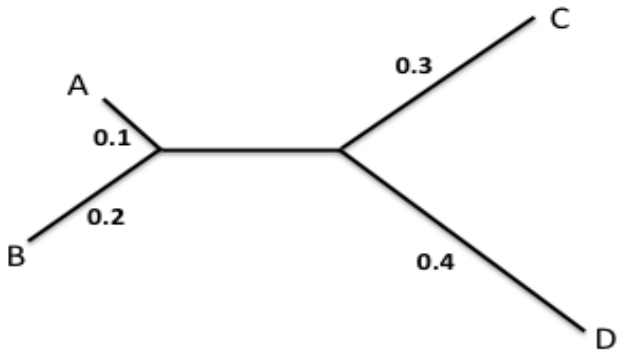
Formats for phylogenetic trees

- **Two main formats: NEWICK and NEXUS**



NEWICK: ((A,B), (C,D))

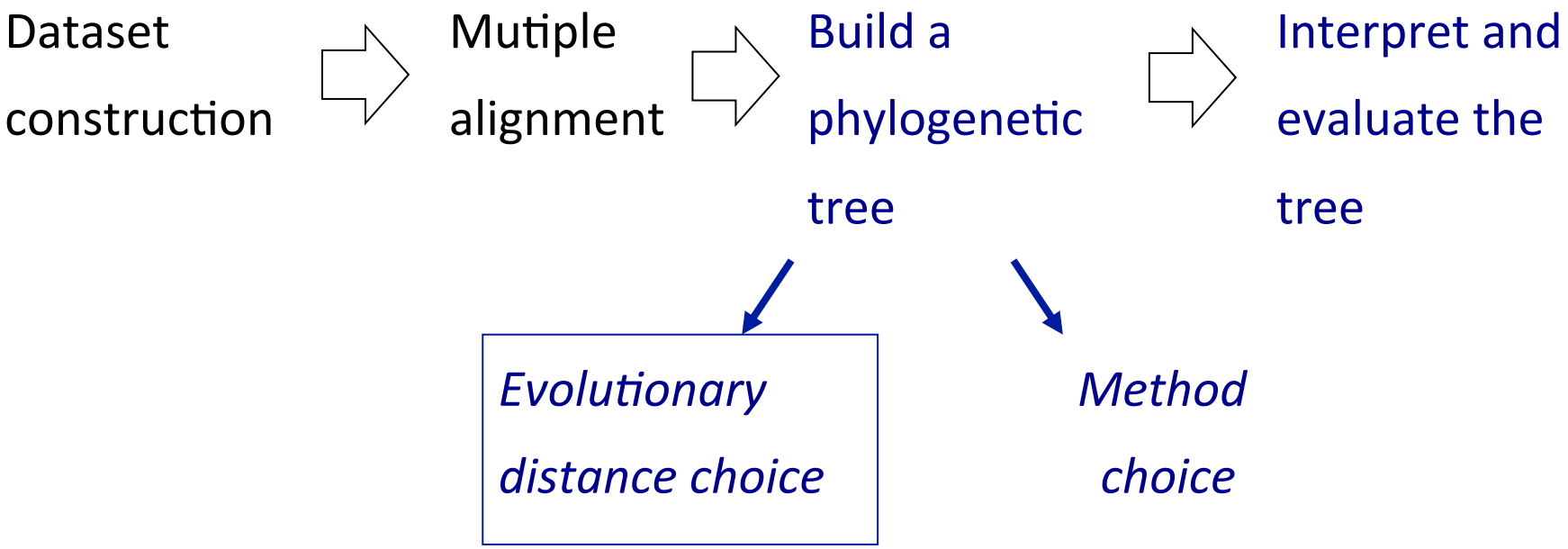
```
#NEXUS
BEGIN TAXA;
  TAXLABELS A B C D;
END;

BEGIN TREES;
  TREE tree1 = ((A,B),(C,D));
END;
```



NEWICK: ((A:0.1,B:0.2):0.2, (C:0.3,D:0.4))

```
#NEXUS:
Begin trees;
Translate
1 A,
2 B,
3 C,
4 D,
;
Tree tree2= [&U] ((1:0.1,2:0.2):0.2, (3:0.3,3:0.4));
End;
```

Usual workflow in phylogenetic analysis



Genetic (evolutionary) distances

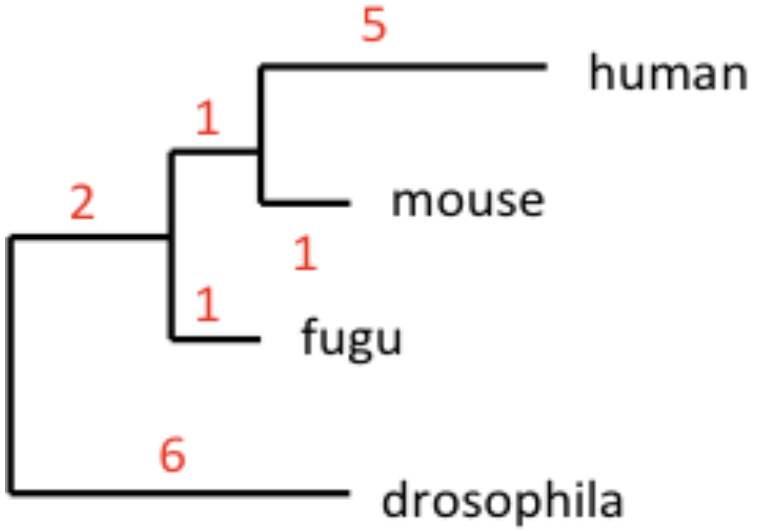
A **genetic (evolutionary) distance** is a measure of the divergence between two genetic sequences

- Calculation of distance between two sequences is a central point on phylogenetic analysis
  - Pairwise distance calculation is the first step of **distance matrix methods** in phylogeny (UPGMA, NJ)
  - **Models of nucleotide/amino-acid sustitutions** used in distance-calculation form the basis of **likehood and Bayesian analysis methods**

Distances and trees

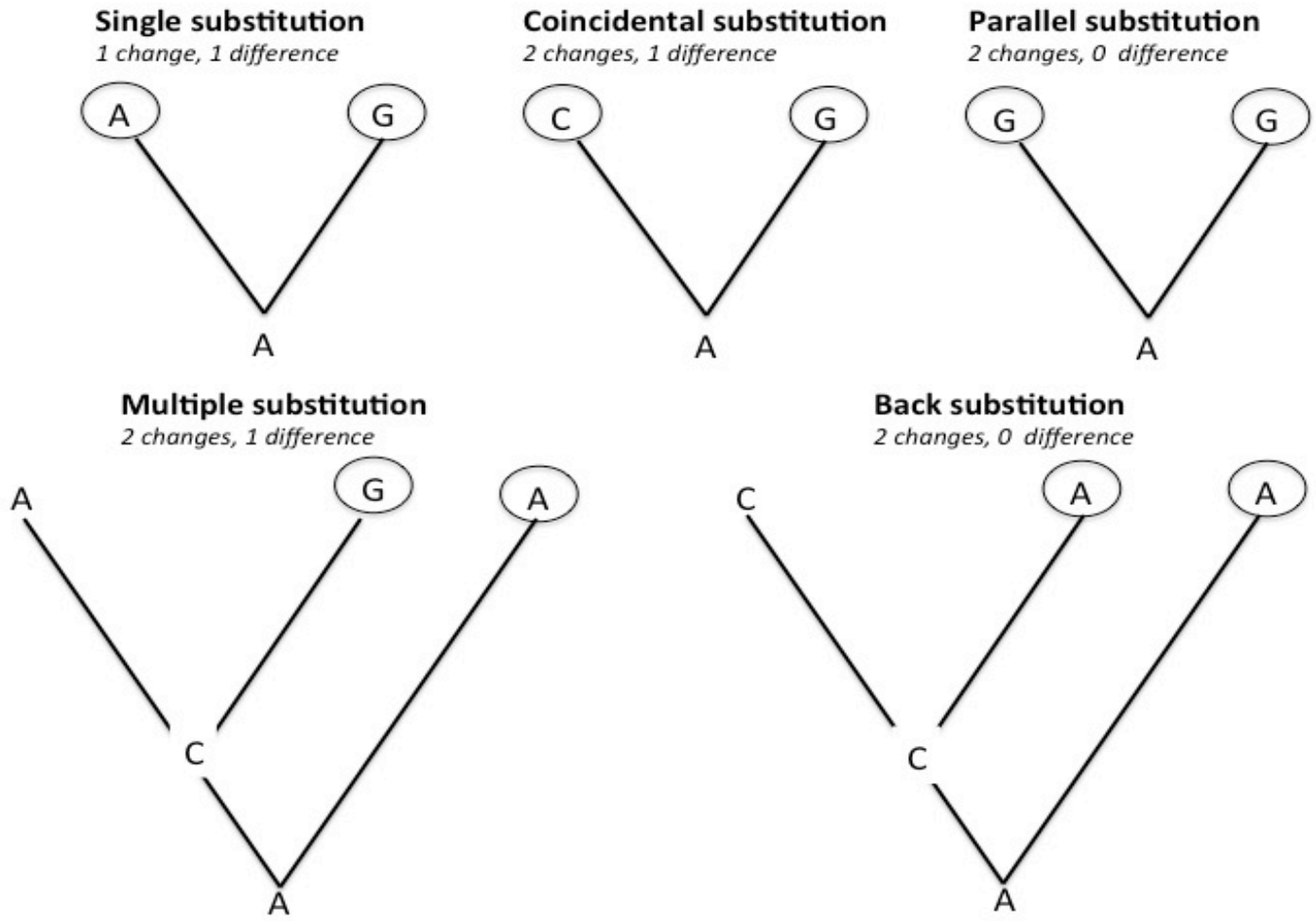
- For sequences related by an evolutionary tree, the **branch lengths** represent the distance between the nodes (sequences) in the tree
- If a **molecular clock hypothesis** is assumed then the genetic distance is linearly proportional to the time elapsed

human	x			
mouse	6	x		
fugu	7	3	x	
drosophila	14	10	9	x
	human	*mouse*	*fugu*	*drosophila*



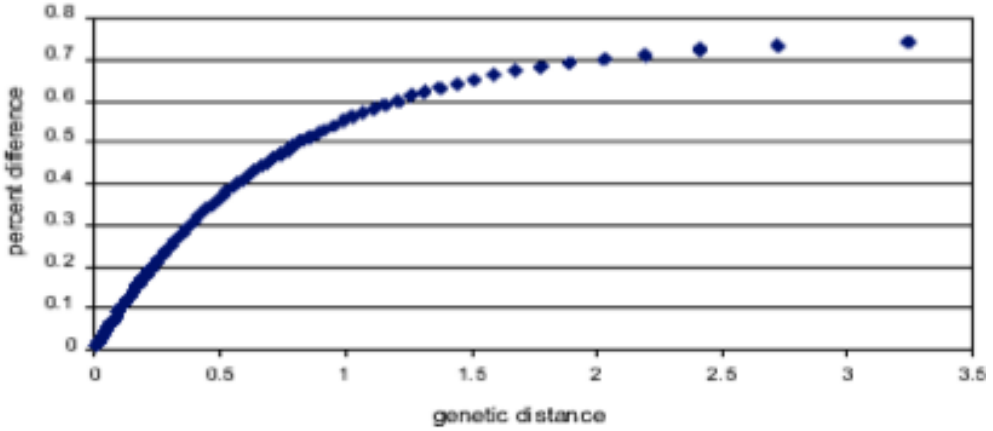
Observed and genetic distances

Observed nucleotide differences are not very informative !



Observed and genetic distances

- **The observed distance** can be computed by counting the number of sites where two sequences differ : it is expressed as **the number of nucleotide differences per site (p-distance) ;**
- The observed distance is an under-estimation of the genetic distance due to multiple substitutions per site and saturation : **substitution models** are used.



Nucleotide substitution models

- Nucleotide substitution rate can be modeled as a stochastic process using **time continuous stationary Markov models ;**
- Underlying asumptions :
  - At any given site, the rate of change from base i to j is independant from the base that occupied that site prior i (**Markov property**) ;
  - Substitution rates do not change over time (**homogenity**) ;
  - The relative frequencies of A, C, G, and T are at equilibrium (**stationarity**)

Instantaneous rate matrix Q :

$$Q = \begin{array}{c} \\ A \\ T \\ C \\ G \end{array} \begin{array}{c} \begin{array}{cccc} A & T & C & G \end{array} \\ \begin{bmatrix} -\mu_A & \mu_{AT} & \mu_{AC} & \mu_{AG} \\ \mu_{TA} & -\mu_T & \mu_{TC} & \mu_{TG} \\ \mu_{CA} & \mu_{CT} & -\mu_C & \mu_{CG} \\ \mu_{GA} & \mu_{GT} & \mu_{GC} & -\mu_G \end{bmatrix} \end{array}$$

Probability of from base i to base j :

$$P_{ij}(t) = e^{Q(t)}$$

The Jukes & Cantor model (JC, 1969)

- The simplest possible nucleotide substitution model :
  - All base frequencies are equal (0.25)
  - Only **one parameter** = the **susbtitution rate μ**
- Given the **proportion p of sites** that differ between the two sequences the Jukes-Cantor estimate of the **evolutionary distance d** is given by :

$$Q = \begin{bmatrix} * & \frac{\mu}{4} & \frac{\mu}{4} & \frac{\mu}{4} \\ \frac{\mu}{4} & * & \frac{\mu}{4} & \frac{\mu}{4} \\ \frac{\mu}{4} & \frac{\mu}{4} & * & \frac{\mu}{4} \\ \frac{\mu}{4} & \frac{\mu}{4} & \frac{\mu}{4} & * \end{bmatrix}$$

$$d = -\frac{3}{4}\ln\left(1 - \frac{4}{3}p\right)$$

where p is the proportion of sites that show differences.

The JC model - exercise

- Seq1 TCAAGTCAGGTTCGA
- Seq2 TCCAGTTAGACTCGA
- Seq3 TTCAATCAGGCCCGA

	Seq1Seq2Seq3
Seq2	
Seq3	

Observed distances

Observed distance

$$d_{obs}(seq1 - seq2) = ?$$

J&C distance

$$d_{JC}(seq1 - seq2) = ?$$

	Seq1Seq2Seq3
Seq2	
Seq3	

Evolutionary distances

The JC model - solution

- Seq1 TCAAGTCAGGTTCGA
- Seq2 TCCAGTTAGACTCGA
- Seq3 TTCAATCAGGCCCGA

	Seq1	Seq2	Seq3
Seq2	0.266		
Seq3	0.333	0.333	

Observed distances

Observed distance

$$d_{obs}(seq1 - seq2) = \frac{4}{15} = 0.266$$

J&C distance

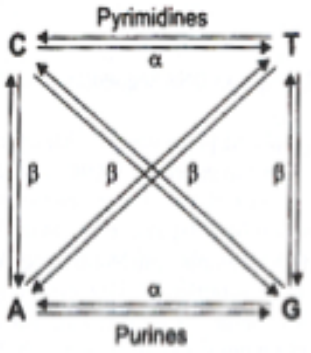
$$d_{JC}(seq1 - seq2) = -\frac{3}{4}(1 - \frac{4}{3}0.266) = 0.328$$

	Seq1	Seq2	Seq3
Seq2	0.328		
Seq3	0.441	0.441	

Evolutionary distances

The Kimura model (1980)

- The model is defined by **2 parameters**
  - all base frequencies are equal (0.25)
  - It distinguishes **the rate of transition** substitutions **α** and **the rate of tr** substitutions **β**



- The **Kimura two-parameter distance d** is given by:

$$Q = \begin{array}{c} \\ A \\ T \\ C \\ G \end{array} \begin{array}{c} \begin{array}{cccc} A & T & C & G \end{array} \\ \begin{bmatrix} -\mu_A & \beta & \beta & \alpha \\ \beta & -\mu_T & \alpha & \beta \\ \beta & \alpha & -\mu_C & \beta \\ \alpha & \beta & \beta & -\mu_G \end{bmatrix} \end{array}$$

$$d = -\frac{1}{2}\ln(1-2p-q) - \frac{1}{4}\ln(1-2q)$$

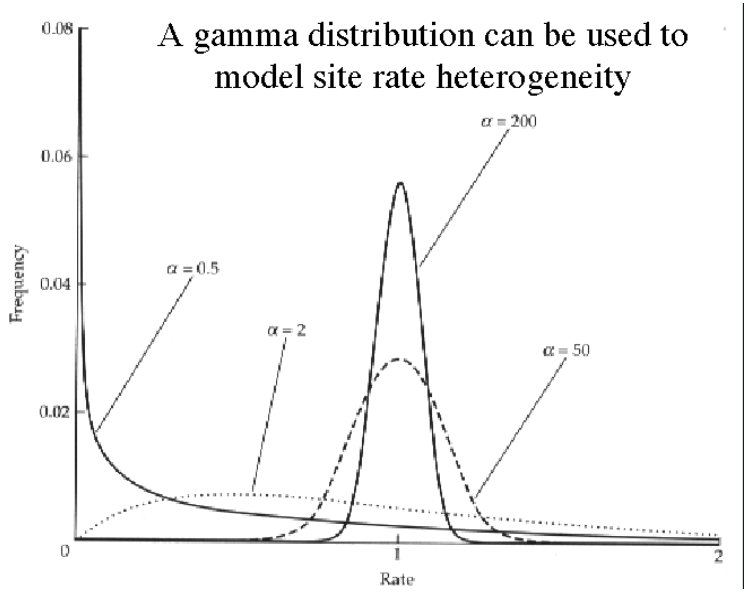
where p is the proportion of sites that show transitional differences and q is the proportion of sites that show transversional differences.

Other models

- The **Felsenstein's 1981** model is an extension of the JC69 model in which base frequencies are allowed to vary from 0.25
- The **HKY85 mode**l can be thought of as combining the extensions made in the Kimura80 and Felsenstein81 models: it distinguishes between the rate of transitions and transversions and it allows unequal base frequencies.
- The **GTR (Generalised time-reversible, Tavaré 1986)** model is the most general neutral, independent, finite-sites, time-reversible model possible :
    - All bases can have unequal frequencies
    - All type of mutations are distinghuished

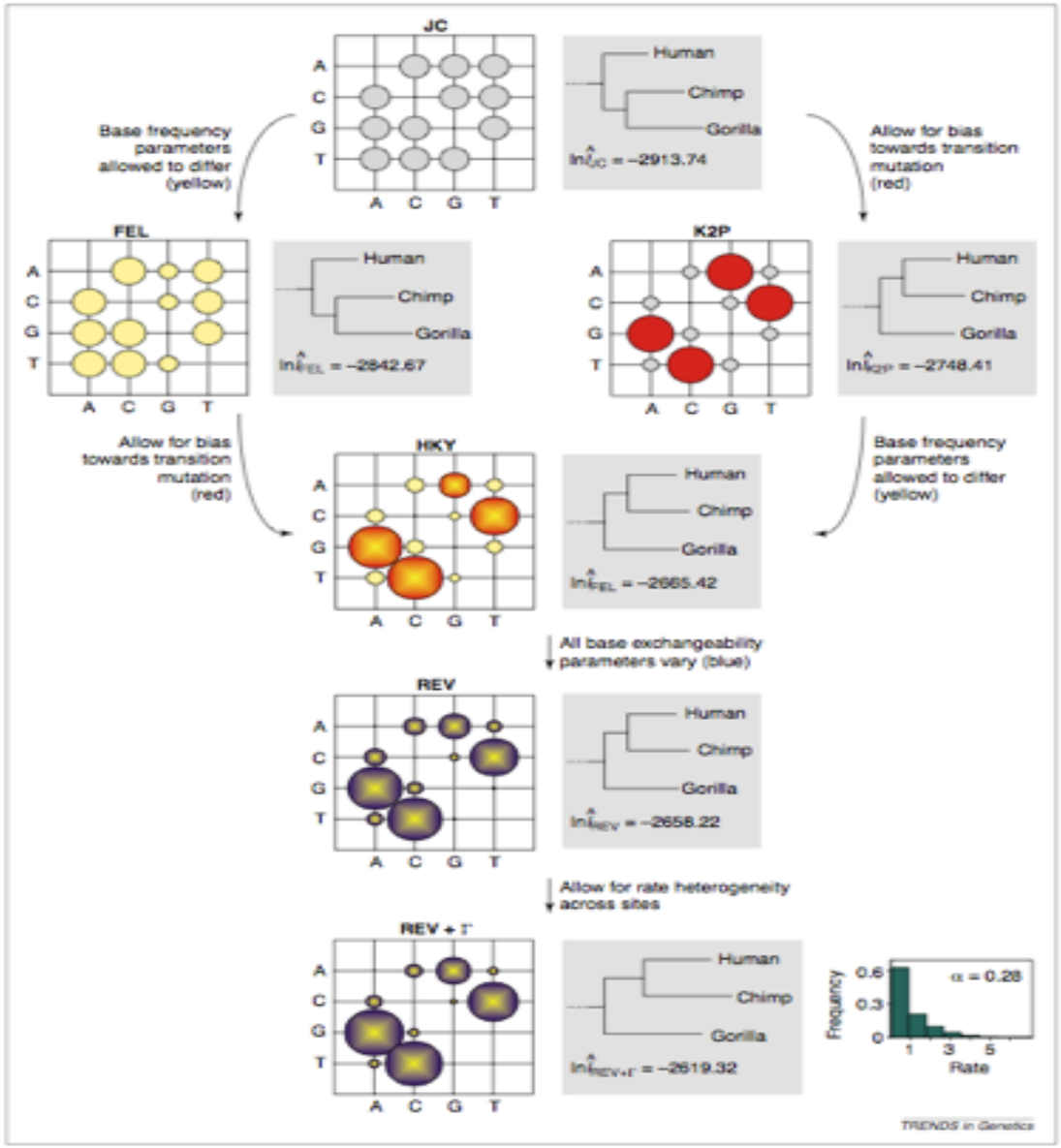
Rate heterogeneity among sites

- The **rate of substitution can vary substantially** for different position of an an alignment
- To account for the site-dependent rate variation, the common approach is to use a **Gamma distribution which model distribution rates between sites**



Usually, rather than using the continuous Gamma distribution, **discrete categories of equally probable substitution rates** are used to obtained an approximation of the function (4 to 8 site categories)

Nucleotide models : summary



Choosing among models

- **It is crucial step**
- Different evolutionary models can lead to different results : **inaccurate branch lengths**, even sometimes **wrong tree topology**
- The most complex model with the largest number of parameters is not necessarly the most appropriate, it **depends of the question and the data**
- The best-fit model of evolution for a particular dataset can be selected using **sound statistical techniques, for example :**
  - Hierarchical **Likehood Ratio Tests** (hLTRs)
  - **Information criteria** (ex : Akaike Information criterion=AIC)

Choosing among models

- In practice : **adjust the model to the analyzed dataset**
- **Use statistical methods to select the best fitted model* :**

LRT

Likelihood Ratio Test

$$2 \cdot [\ln L(\hat{\theta}) - \ln L(\theta_0)] \sim \chi^2_p$$

LRT criterion can be used to compare models which are subsets of each other

AIC

Akaike Information Criterion

$$AIC_i = -2 \cdot \ln L_i + 2p_i$$

BIC

Bayesian Information Criterion

$$BIC_i = -2 \cdot \ln L_i + p_i \cdot \ln(n)$$

AIC and BIC criteria compare all of the models simultaneously according to some measure of fitness

**Keane & al., BMC Evolutionary Biology 2006*

Selection of the best fitted model

- **Example: Hierarchical LRT of models of molecular evolution**

Ho	Models compared
Equal base frequencies	Ho: JC69 1 parameter H1 : F81 2 parameters
Equal ti/tv rates	Ho : F81 2 parameters H1 : HKY 5 parameters
Equal ti and equal tv rates	Ho : HKY 5 parameters H1 : GTR 9 parameters
Equal rates among sites	Ho : GTR 9 parameters H1 : GTR+ τ 9 parameters +n
Proportion of invariable sites	Ho : GTR+ τ 9 parameters +n H1 : GTR+ τ + I 9 parameters +n +1

where I means there is a significant proportion of invariable sites, and τ means a gamma distribution is being used to account for rate variation among sites

Protein models

- **Similar concept: multiple substitutions of amino acids** lead to underestimation of evolutionary distances between two homologous proteins.
- **Substitution frequency of amino acids depends of the AA :** it is higher between closed amino-acids in term of physical properties (polarity, hydrophibicity,...)
- **Too much (190) parameters to estimate parameters of probabilistic model** => **empirical models** are used
- Transition rate between amino acids are estimated once from **big reference alignments obtained by concatenation of several homologs proteins**

Main protein evolutionary models

Model	Dataset	Ref
Poisson	Poisson process	Zuckerkandl, 1965
PAM	1300 protein sequences from 71 homolog families	Dayhoff 1978
Blosum	Extension of PAM dataset	Henikoff 1992
JTT	16 300 sequences	Jones 1992
mtREV	Mitochondrial DNA	Adachi 1996
WAG & LG	**Likehood methods**	**Whelan 2001**

Model choice is based on the same tests as for nucleotide evolutionary models (LRT, AIC, BIC)

Main protein evolutionary models

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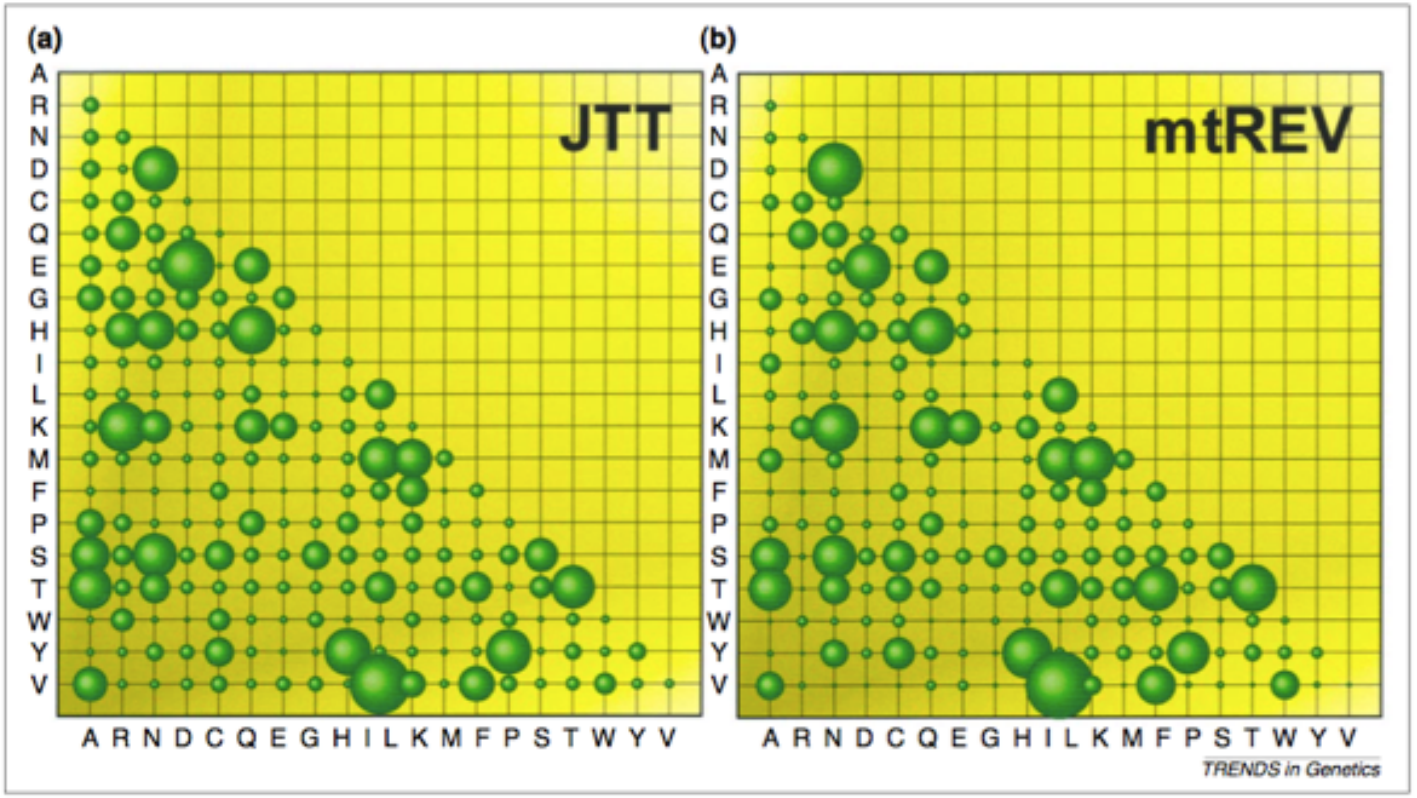
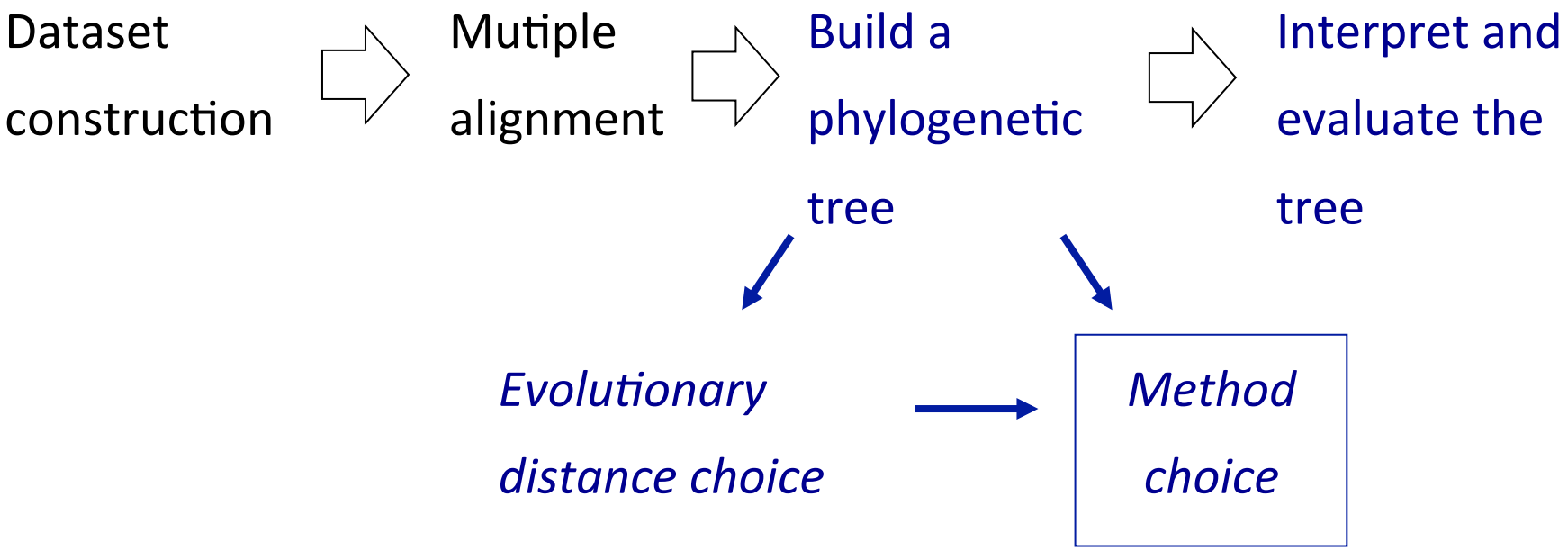
WAG and LG models are the more used models

Protein models

- Example: JTT (1992, 16 300 sequences) vs mtREV (for mitochondrial proteins)



Usual workflow in phylogenetic analysis



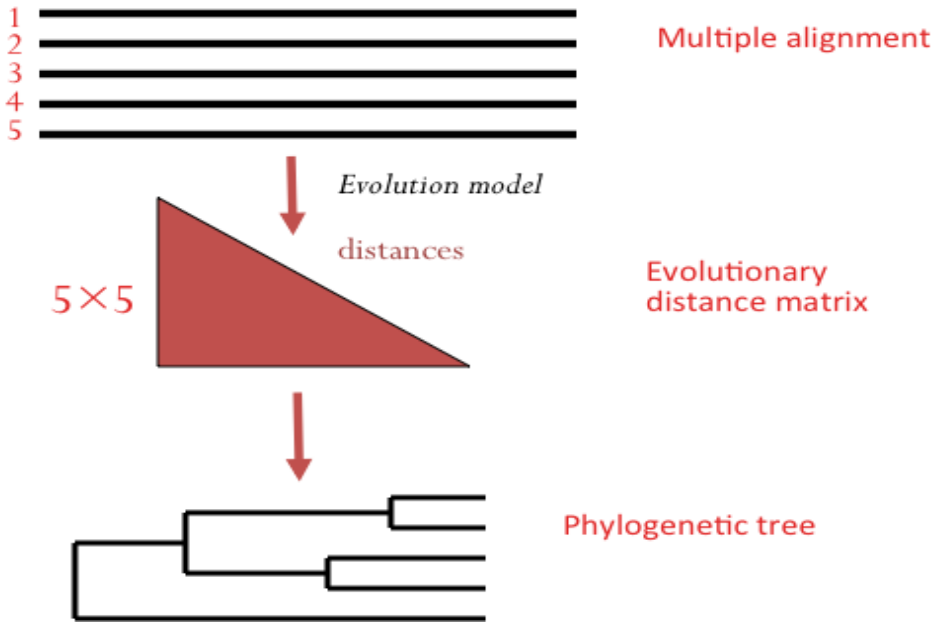
Method choice

- Main methods for inferring phylogenetic trees:

Input data	Method	Principle of the algorithms
Distance matrix	Unweighted Pair Group Method (UPGMA)	clustering
	Neighbor-Joining (NJ)	clustering
Character state	Maximum Parsimony (MP)	Search for the tree(s) of minimum character changes
	Maximum Likehood (ML)	Search for the tree(s) that maximizes the probability of observing the character states giving a tree topology and a model of evolution
	Bayesian Inference	Target a probability distribution of trees (set of possible trees for the data)

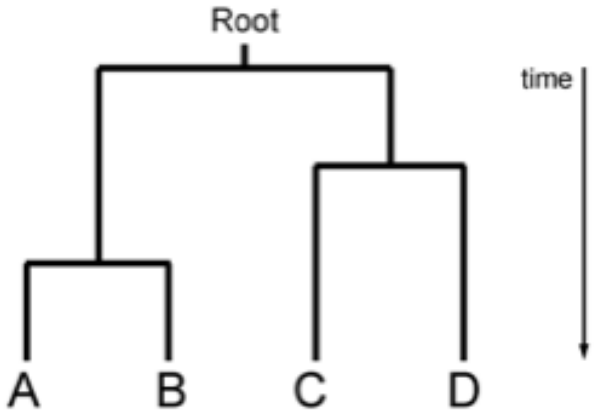
Distance methods for inferring a phylogenetic tree

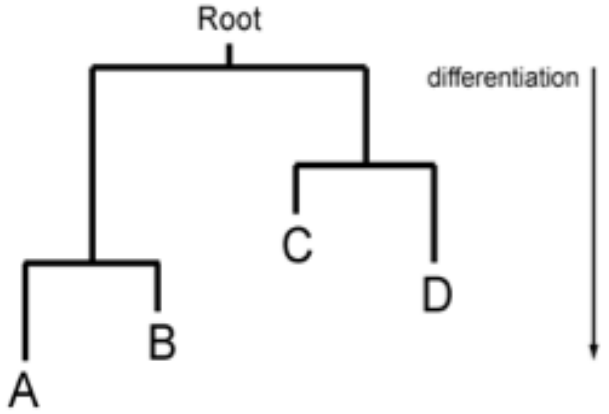
- Introduced in phylogeny in 1960
- Try to fit a tree **to a matrix of pairwise genetic distances**
- Need to choose an **evolutionary model**



Distance methods

- Two main methods
  - **UPGMA**: a clustering method that produced ultrametric trees
  - **Neighbor-Joining**: use a greedy algorithm to compute the Minimal Evolution tree *i.e.* the optimal topology is the one which minimizes the tree length

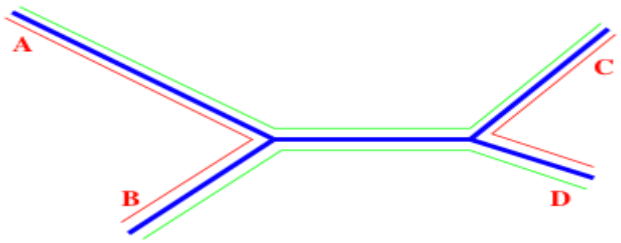




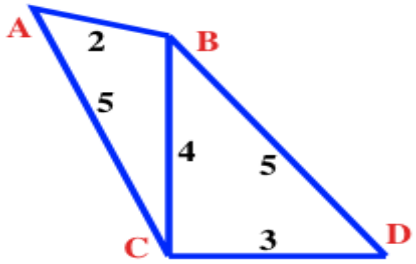
Neighbor-Joining

- First algorithm proposed by **Saitou & Nei** (1987)
- Very fast : polynomial-time algorithm
- Produces unrooted trees
- Produces the wright topology if matrix **distances are patristic**

Dist(A,B)+Dist(C,D) ≤ Dist(A,C)+Dist(B,D)=Dist(B,C)+Dist(A,D)



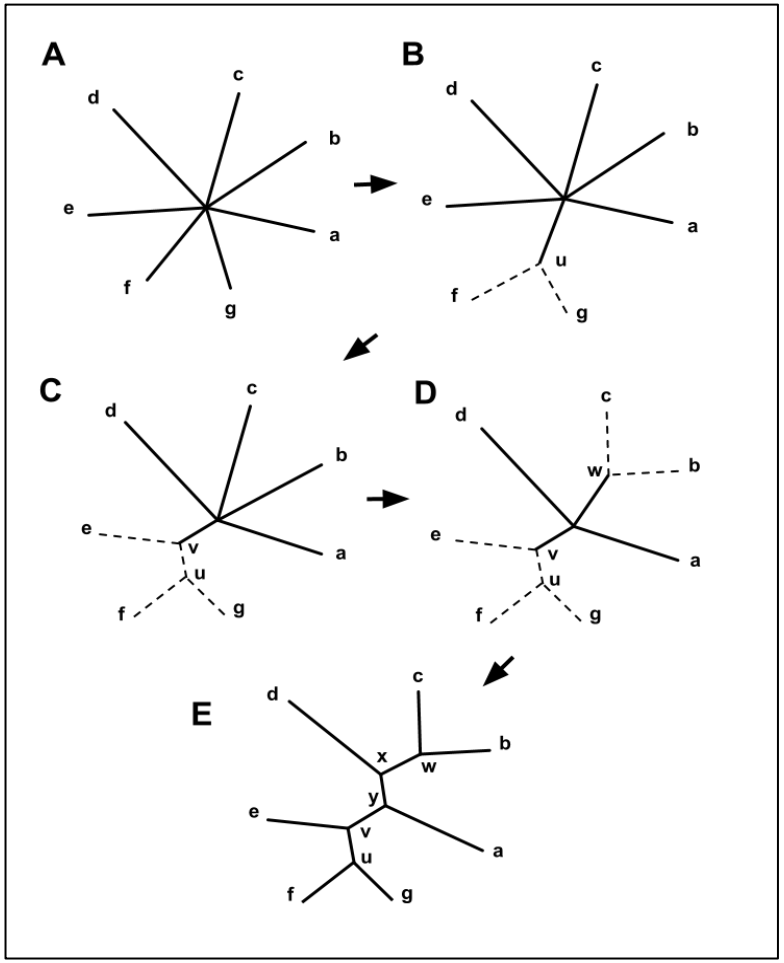
ok



no

Neighbor-Joining (NJ)

- **Principle of the algorithm:**
  - Start with **a star tree** (A)
  - Compute the **matrix Qij** and find the pair of taxa with lowest value (here f and g)
  - Join f and g and **create a new internal node, u**, as shown in (B)
  - Compute the distances from node u to the nodes a-e
  - **Repeat the process** :
    - u and e are joined to the newly created v, as shown in (C).
    -Two more iterations lead first to (D), and then to (E).



Neighbor-Joining in practice

- **NJ: Fast** but problems may occur for **very divergent sequences** or **heterogeneous** datasets
- **BioNJ* algorithm:**
  - A variant of NJ which improves its accuracy by making use of a simple first-order model of the variances and covariances of evolutionary distance estimates.
  - When the substitution rates are low (maximum pairwise divergence ~0.1 substitutions per site) or when they are constant among lineages, BIONJ is only slightly better than NJ.
  - When the substitution rates are higher and vary among lineages, BIONJ clearly has better topological accuracy*.

Gascuel Molecular Biology and Evolution 1997

Neighbor-Joining in practice

- Choose **an evolutionary model** and compute **a distance matrix (see next slide)**
- **NJ/BioNJ softwares:**
  - **Neighbor** (PHYLIP, NJ) http://evolution.genetics.washington.edu/phylip.html
  - **BioNJ** http://www.atgc-montpellier.fr/bionj/ or http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=bionj
  - **QuickTree (NJ)** http://www.sanger.ac.uk/resources/software/quicktree/
  - **Seaview** (NJ and BioNJ) http://pbil.univ-lyon1/fr/software/seaview

Evolutionary models in NJ

NJ softwares do not implement all models !

- **At small distances** (~10% of variable sites) the different evolutionary models produce very similar distance estimates => no problem
- **At intermediate distances** (20 to 30% of variable sites), different model asumptions become more important => It is recommanded to use realistic models for distance estimation, especially if the sequences are longs
- **At large distances** (40% of variable sites), the different model produces very different distance estimates. Sometimes the distance estimates become infinite. => The solution is to use realistic models for distance estimation AND to add sequences to break down the long distances

Parsimony

- Main concept (adapted from Fitch, 1971):

  Seek the tree(s) that **minimizes the net amount of evolutionary change (in term of character change) required to explain the data**
- Very used on **morphological data** (presence/absence of characters) but also relevant for **biological sequences** (a character = a site with 4 states=A,T,C,G or molecular polymorphism data like SINE)
- Produces **unrooted tress**
- Does not require any evolutionary model
- Take into account explicitly ancestral states

Parsimony

The problem of finding the parsimony tree can be separated into **three steps**:

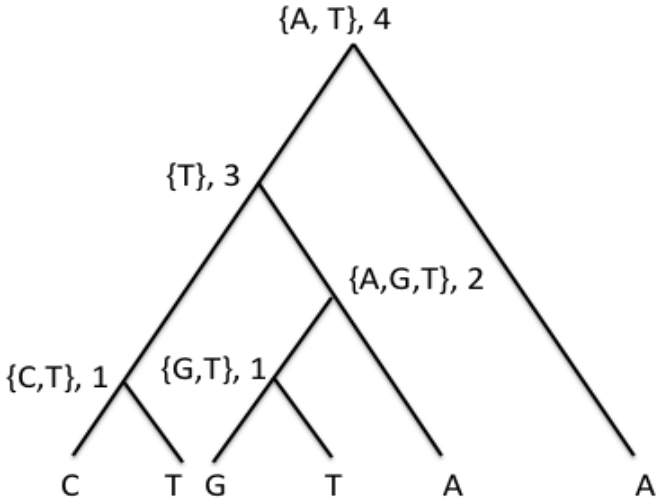
- **Step 1:** Compute the **minimal amount of character change** required in a given tree (compute changes for each character and sum up all characters)
- **Step 2:** Search for **all possible tree topologies**
- **Step 3:** Choose **the tree(s) that minimize this number of character changes**.

Parsimony

Step 1: Compute the minimal amount of character change required in a given tree (compute changes for each character and sum up all characters)

- Compute the minimum number of changes for a site in a tree (for instance with the ***Fitch algorithm***)

Seq1. …C…
Seq2. …T…
Seq3. …G…
Seq4. …T…
Seq5. …A…
Seq6. …A…



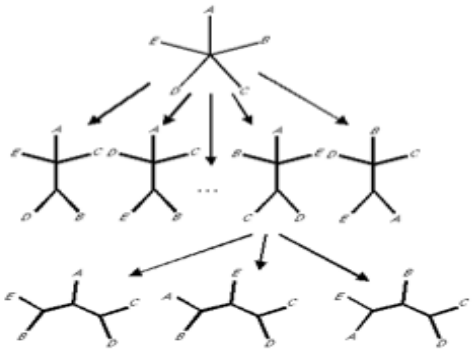
Parsimony score :
1+1+2+3+4 =11

- Sum over the number of sites to obtain the **parsimony score** of a tree

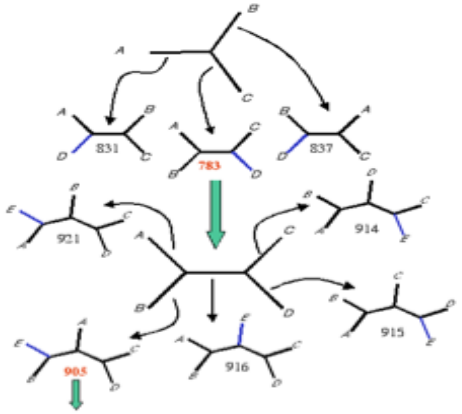
Parsimony

- **Step 2 : generate all possible tree topologies**
  - **Exact methods** (max. 20 taxa) : example=*Branch and Bound* algorithm
  - **Heuristic methods** : choose an **intitial tree topology** (star decomposition, stepwise addition, random choice) and perform **tree-rearrangement perturbations** like *Nearest Neighbor Interchange (NNI)* or *Subtree Pruning and Regrafting (SPR)*

Star decomposition

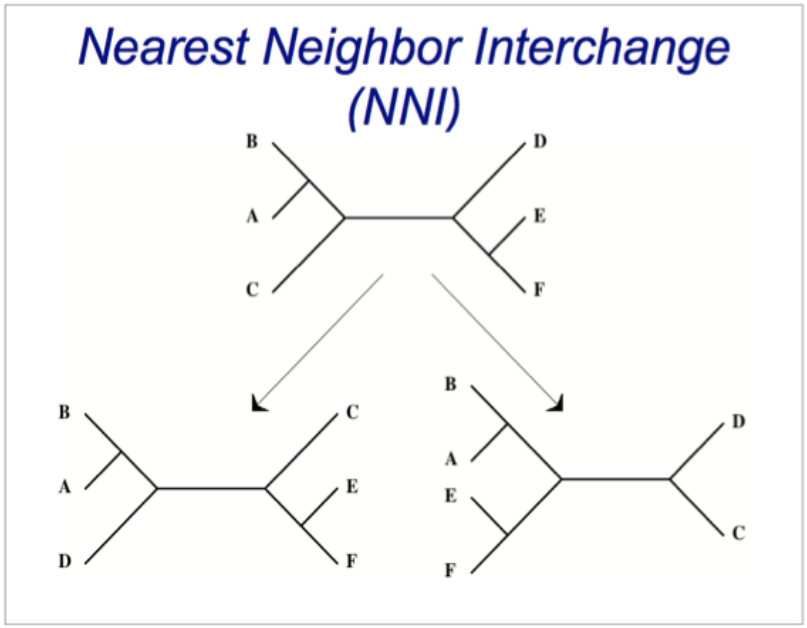


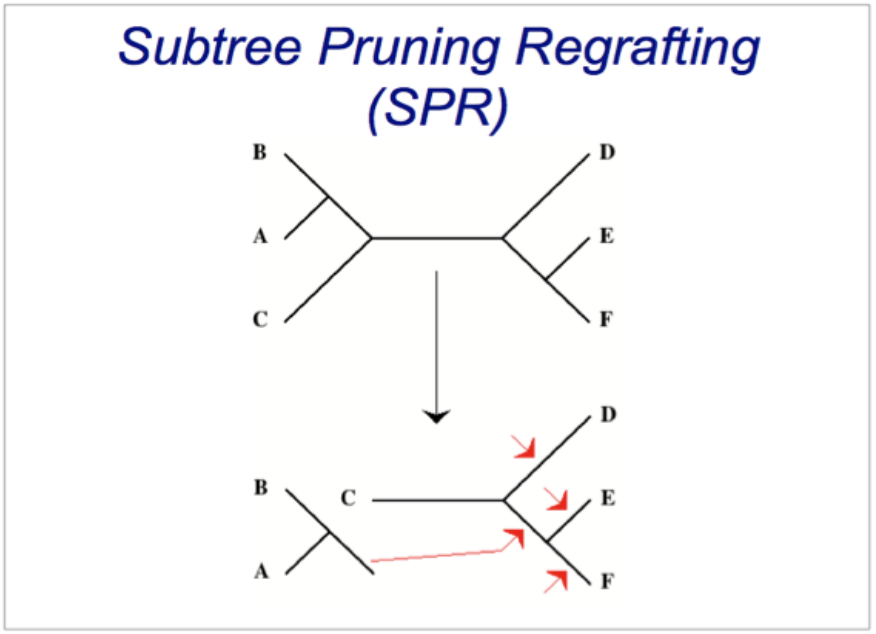
Stepwise addition



Tree rearrangments

- Exploration of tree topologies using different kind of local rearrangments:





Small changes => local space exploration

Medium changes => best space exploratio

- Iterate and keep always the best (more parsimonious) tree
- Stop after n iterations if the swapping process do not produce a better tree

Parsimony in practice

- + : can be applied to any kind of characters, good performances if substitution events are rare
- - : no statistical justification and some sites are excluded i.e. **non informative sites** = invariant sites (AAAA) and two-states sites with one character in one occurrence (AAAT) (all the tree are equal for theses sites)
- **Sotwares for parsimony:**
  - PHYLIP (dnapars, protpars)
  - Seaview
  - MacClade http://macclade.org/macclade.html
  - (PAUP)

Maximum Likehood methods

- **The most frequently used methods**
- **Sound mathematical and statistical foundation**s
- The **evolution model is central**, the method is only possible for aligned sequences
- In statistics, **maximum-likelihood estimation (MLE) i**s a general method of estimating the parameters of a statistical model. When applied to a data set and given a statistical model, maximum-likelihood estimation provides estimates for the model's parameters.

Maximum Likehood methods

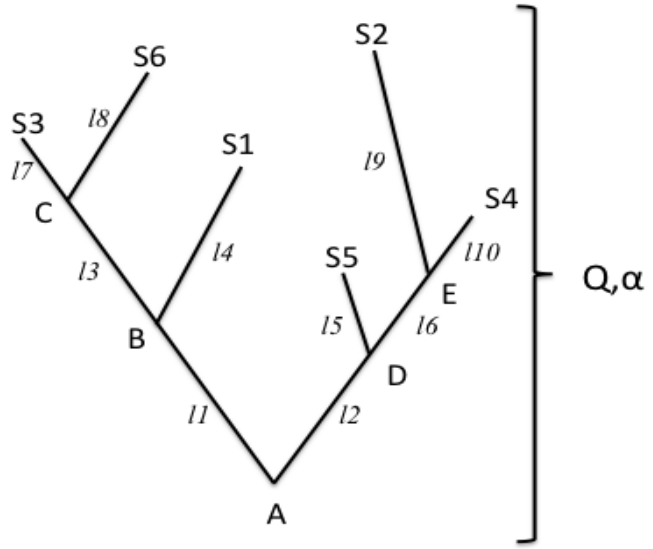
- Adressed question: what is the probabilty to observe the data by considering an evolutionary model with its parameters and a tree topology ?

$$\Pr(D / T)$$

- **Input:** A set of observed sequences and an underlying evolutionary model.
- **Desired Output:** The weighted tree that maximizes the likelihood of the data

Maximum Likehood methods

- **Parameters of the probabilistic model:**
  - A phylogenetic tree T, with an arbitrary root and valuated branch lengths
  - A normalized Q-matrix, common to all tree branches
  - An α parameter which determines the variation of the evolutionary rates between sites using the Gamma distribution



- l_i are branch lengths (#subst/site)
- A, B, C, D, E are the unknown ancestral states

- Likehood computation of observed data :

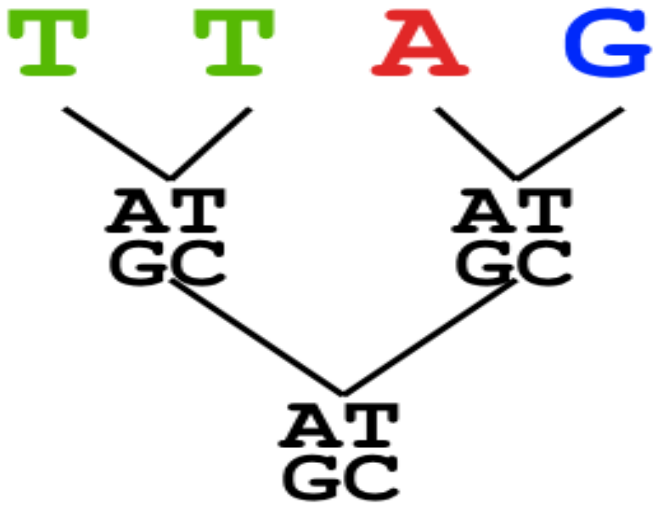
$$Log(L) = \sum_{sites} \log(L(site))$$

$$L(site) = \sum_{A}\sum_{B}\sum_{C}\sum_{D}\sum_{E} \Pr ob(S1,S2,S3,S4,S5,S6,A,B,C,D,E \mid T)$$

Maximum likehood: Example

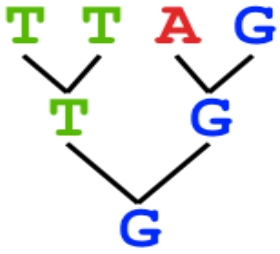
```
Sequence W: A C G C G T T G G G
Sequence X: A C G C G T T G G G
Sequence Y: A C G C A A T G A A
Sequence Z: A C A C A G G G A A
                      ▲
```

All possible evolutionary paths of a site



Likehood of a site

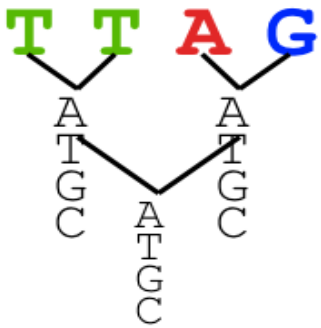
Likehood of a path



L(path) = L(root) x Π L(branches)

=P(G→T)P(G→G) P(G→A)P(G→G) P(T→T)P(T→T)

Sum over all paths



L(Column Cluster 1) = Σ L(all possible Evolutionary Paths)

= L(path1) + L(path2) + L(path3) + … + L(path64)

Felsenstein algorithm

- 5 internal nodes => 5^4 = 1024 possible combinations

- **Pruning Felsenstein algorithm** :
  progressive computation of **the likehood of a site to have nucleotide i** (with tree T and model M fixed) **from leaves to root** by using a **recursive** strategy

- **Calculate tree Likelihood by multiplying the likehood for each position**

Maximum Likehood features

- **Branch length l are estimated using the Q matrix (of an evolutionary model).**

  l=expected number of subtitutions per site = μt (mutation rate x time) $P_{(l)} = e^{Q_l}$

- **Reversibility of the process** (symetry of Q matrix) : it is possible to show that if the base substitution model is reversible
- **Root position** : Likelihood remains the same regardless of where the root is. So search for the best tree only needs to be carried out on unrooted trees
- **Can take into account variation of the evolutionary rates** between sites using K possible categories of sites

Maximum likehood algorithm in practice

- **Pick an evolutionary model** (result of modelgenrator can help)
- For each site, generate **all possible tree structures** (same methods as in MP)
- Based on the e**volutionary model**, calculate **likelihood** of these trees.
- **Choose the tree with the Maximum Likelihood**

Maximum likehood in practice

- **+:** Works well for distantly related sequences and under different molecular clock theory ; Can incorporate any desirable evolutionary model ; Sound mathematical foundations

- **-:** Bad Approx. under Bad Evolutionary Models ; Computationally Intensive (=>slow)

- **Sotwares for Maximum likehood**

- PHYLIP (dnaml, protml)
- PhyML http://atgc.lirmm.fr/phyml/
- RaXML http://sco.h-its.org/exelixis/web/software/raxml/index.html

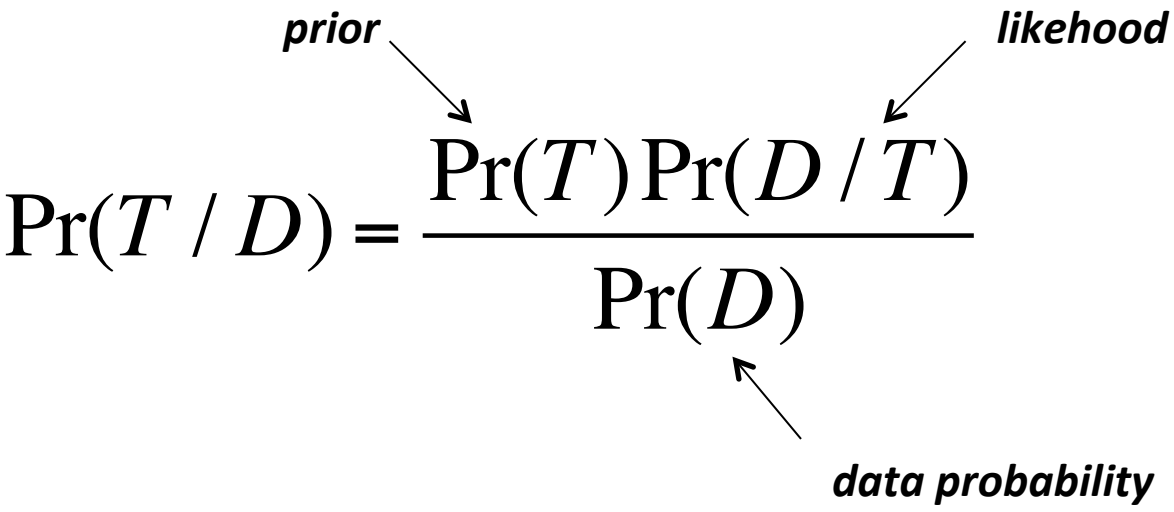
Bayesian phylogenetic inference

- **The most recent method, now** becomes very used
- **Use probabilistic evolutionary models** (the same as in maximum likehood methods)
- The central concept of the method is **posterior probability**; a Bayesian analysis produces a posterior probability distribution of trees
- If the data are informative, most of the posterior probabilities will focus on **one tree or a small subset of trees**

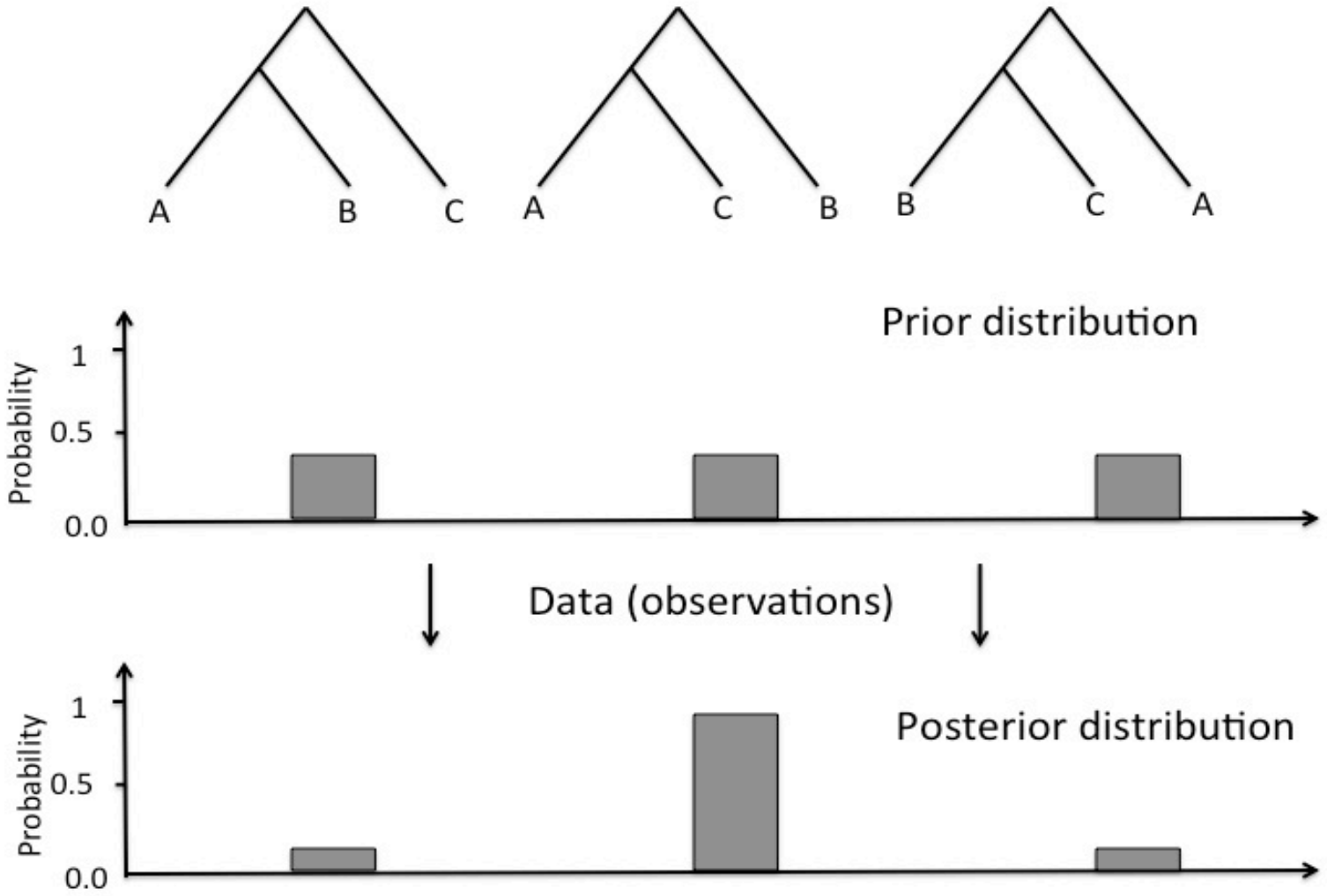
Bayesian phylogenetic inference

Central question: what is the probability of the model/tree taking into account the data D ?

- **Start with a prior belief about trees** (prior distribution of possible trees)
- Collect data and use **an evolutionary model** and **Bayes theorem** to obtain a **posterior probability distribution of trees**



Bayesian phylogenetic inference



Bayesian phylogenetic inference

- Is is not possible to derive the posterior probability analytically
- The posterior probabilty is derived by using a **Markov Chain Monte-Carlo sampling (MCMC)** strategy:

1- start from an arbitrary point

2- make small random changes to the current values of the model parameters

3- accept or reject these changes according to its posterior probability

- This process is repeated during **n generations** until **convergence**.

Bayesian phylogenetic inference

- **Input:**

  - A set of aligned sequences

  - A prior distribution about trees

  - An underlying evolutionary model.

- **Desired Output:**

  - One (or a few) valuated tree(s) with maximal posterior probabilities

Bayesian phylogenetic inference

- **Powerful** but **complex** method
- Can produce **either one either several tree topologies** with high posterior probabilities
- Use an **a priori distribution** for parameters
- Use **heuristic to explore tree spaces**
- **Convergence problems:** for some phylogenetic problems, difficult or impossible to achieve convergence within a reasonable number of generations

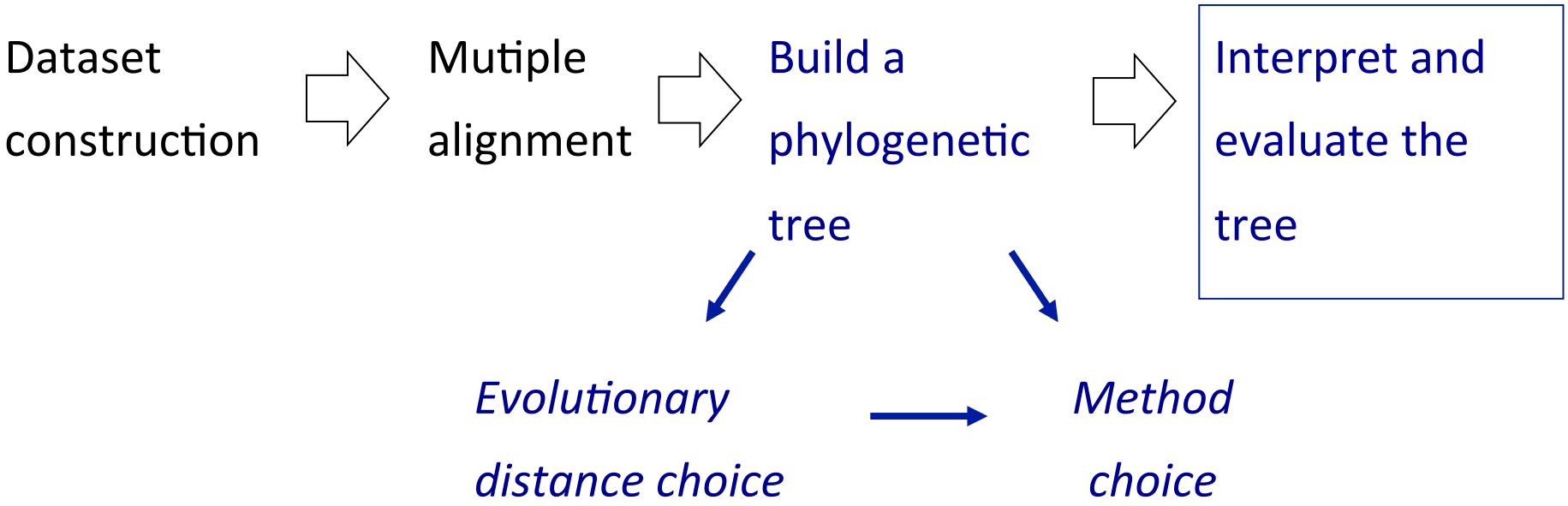
Sotware for Bayesian inference

- **MrBayes** : http://mrbayes.sourceforge.net

Mr Bayes parameters

- **Set the evolutionary model,** eventually with a discrete gamma-distributed rate variation across sites (N=4) and a proportion of invariable sites (I) (or let MrBayes choose)
- **Set the MCMC parameters:**
  - **Number of chains Nc:** by default Nc=2 and MrBayes will run two simultaneous, completely independent analyses starting from different random tree
  - **Number of generations Ngen : typically** Ngen≥10000
  - **Criterion for convergence diagnostic,** typically by comparing the variance among and within tree samples MrBayes will run diagnostic every **runfreq** generations and report clades ot at least **minfrequency.**

Usual workflow in phylogenetic analysis



Testing tree topologies

- **Confidence issue**
  - How confident are we on the inferred tree ?
  - Which parts of the tree are reliable/not reliable ?
  - How can we validate the tree ?

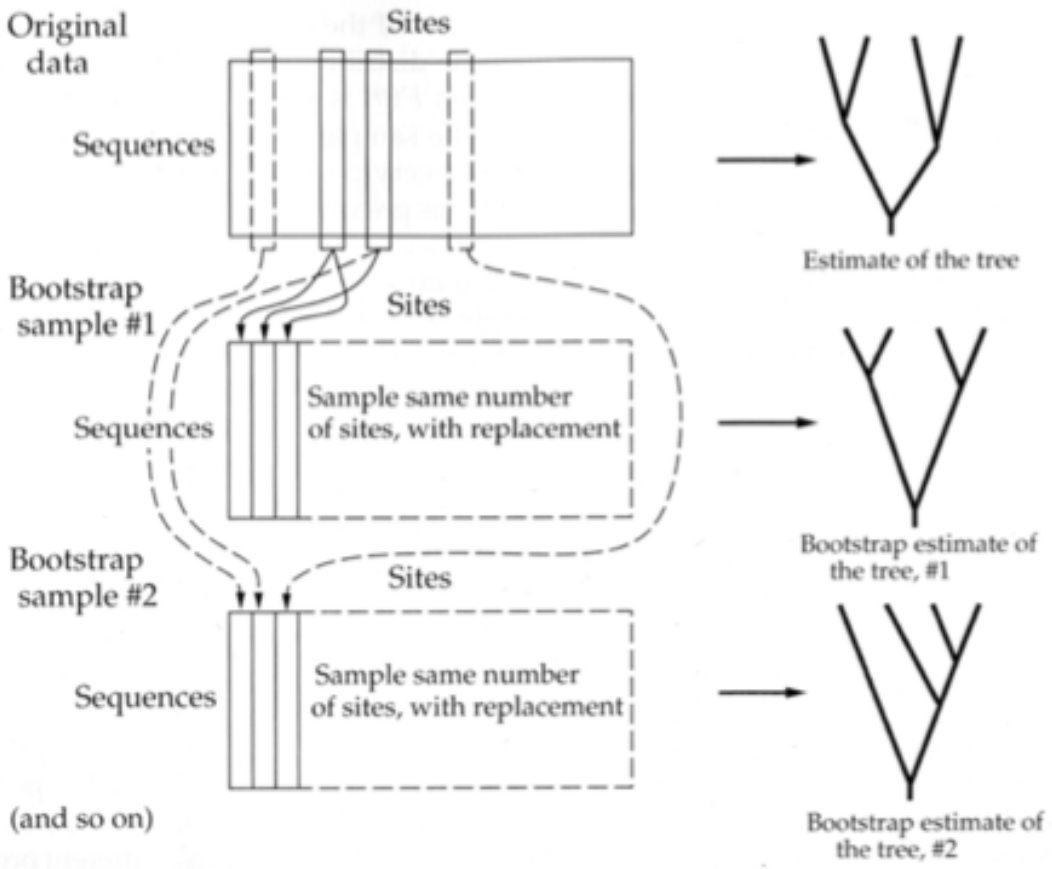
Problem: the true tree is unknown !

Solution :

- use **bootstrap (**or jacknife) to evaluate the **reliability** of the inferred tree and specific clades
- combine **subsampling** and **consensus trees** to get support values on branches

Testing tree topologies

- **Bootstrap**: resample "nucleotides" from the alignment;



Bootstrap process and consensus tree

- **Bootstrap process**
  - Infer **several trees** using **resampling techniques;**
  - Identify and conserve only the **core information contained and repeated in many trees ;**
  - Combine the several trees to produce a **consensus tree** which is compatible with all (or most) of the trees**.**
  - In general, the consensus tree has **no branch lengths** and **a lower resolution** than the original tree**.**
  - Superimpose boostrap values **on the original tree**

Consensus tree

•**.Consensus rules:**

- **Strict Consensus:** clades presents in all trees;
- **Majority Rule:** clades presents in at least half of the trees;
- **Extended Majority Rule:** clades presents in at least half of the trees and some more until the tree is resolved.

Consensus tree

- **.Consensus rules:**
  - **Strict Consensus:** clades presents in all trees;
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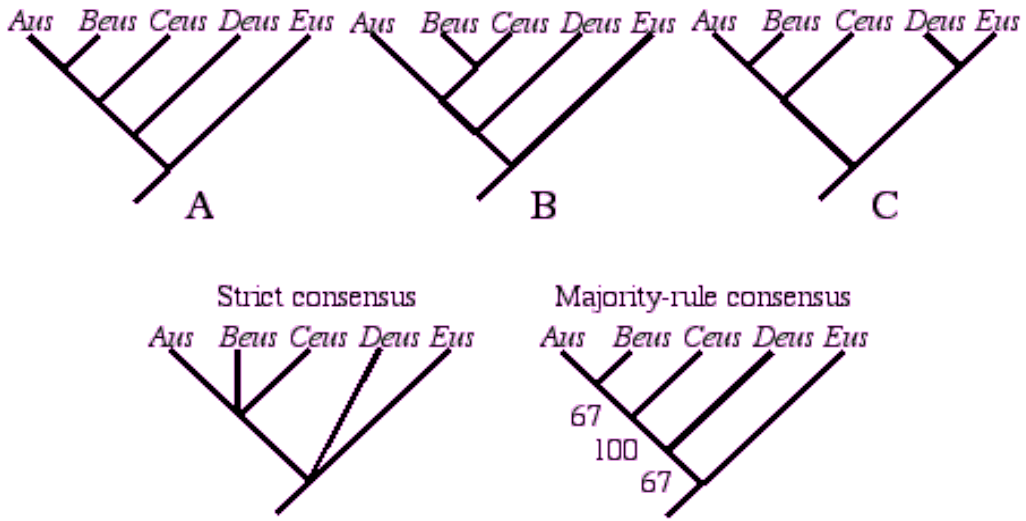


Boostrap values guidelines

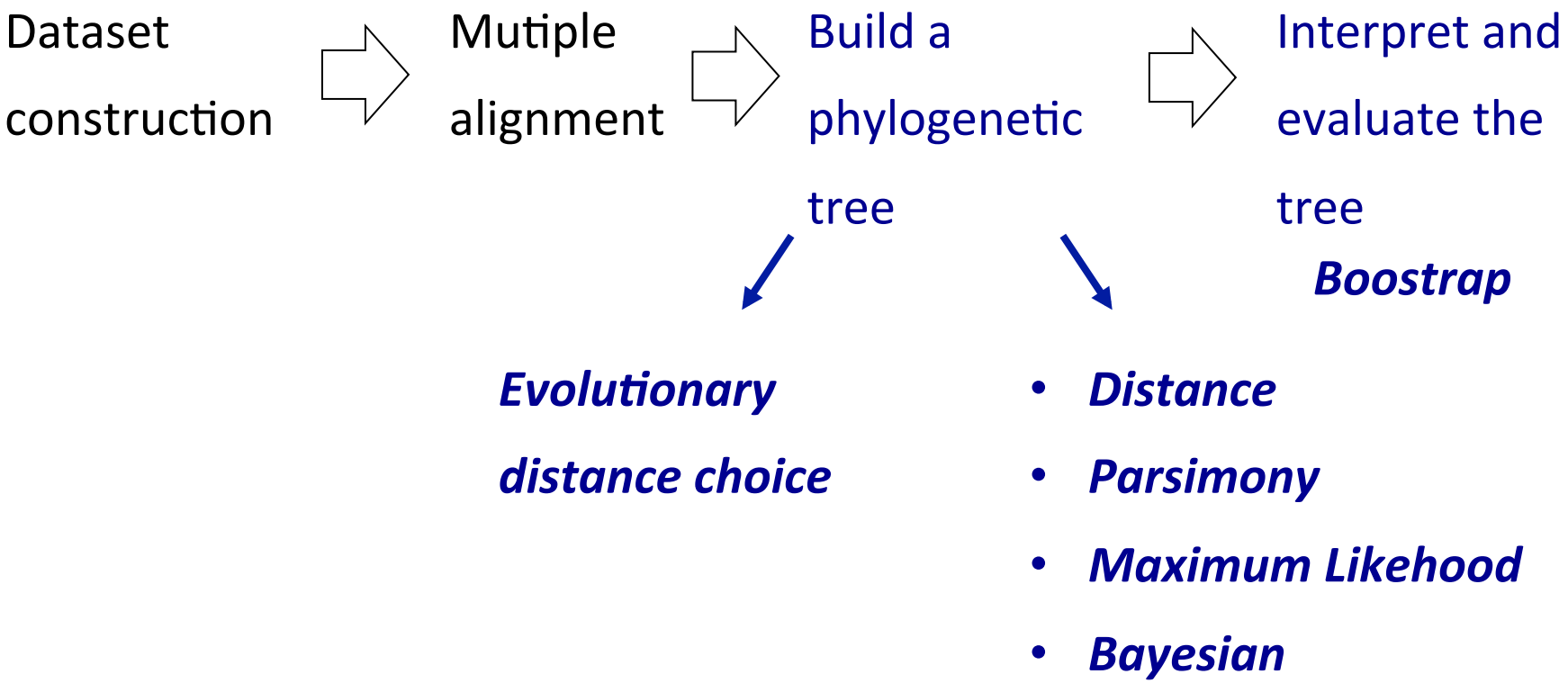
•**Be cautious with boostrap values interpretation**:

- Bootstrap values have no clear-cut statistical interpretation;
- A bootstrap value of 95% doesn't mean that the corresponding clade has 95% chance of being "true";
- Bootstrap values are **difficult to interpret quantitavely**.

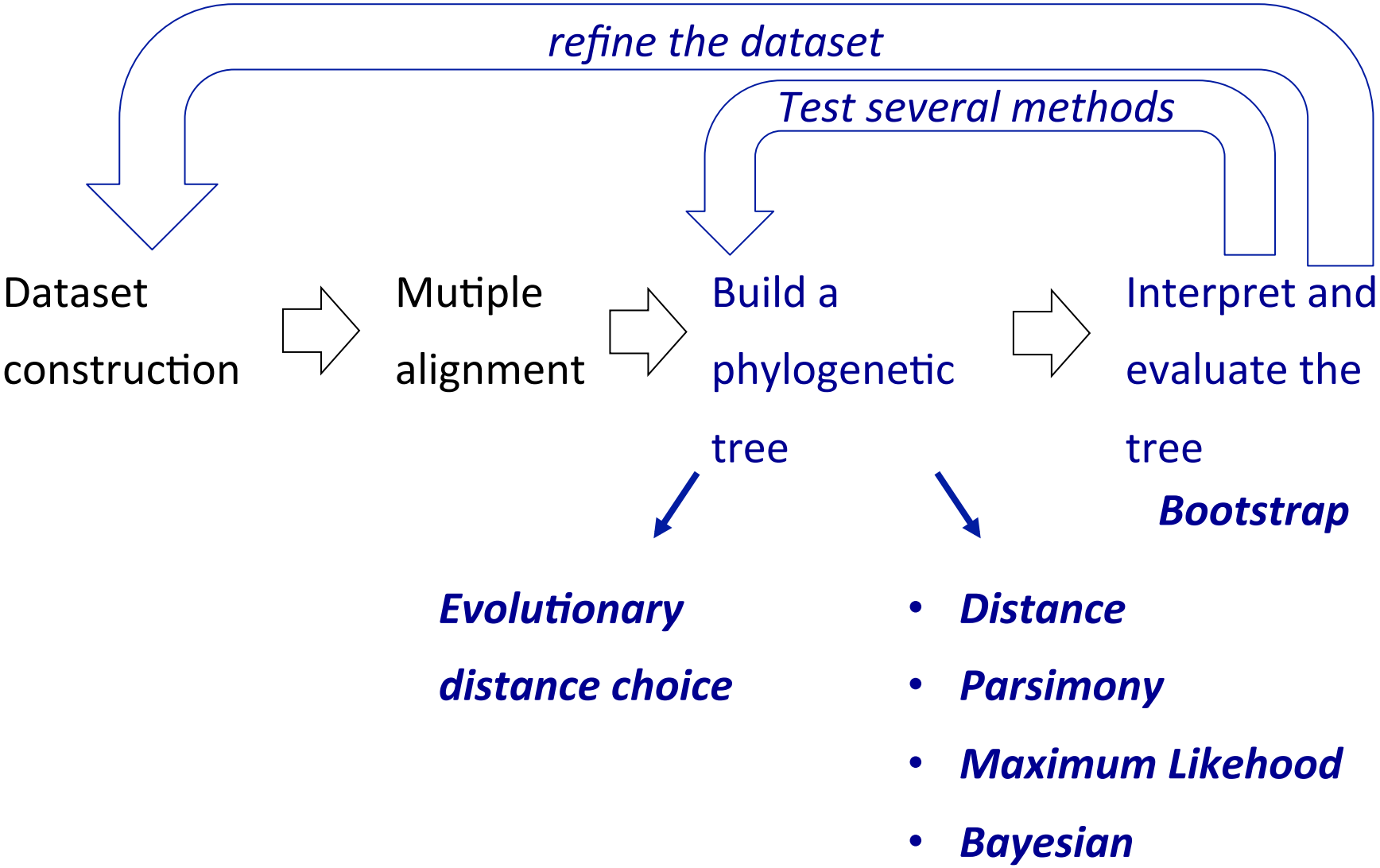
However Bootstrap values are (quite) easy to interpret **qualitatively**:

- The higher the bootstrap value, the more **confident** you can be in your clade;
- 95%, 90% and 66% consitute traditional threshold for being confident in a clade.

Conclusion: overall view



Conclusion: overall view

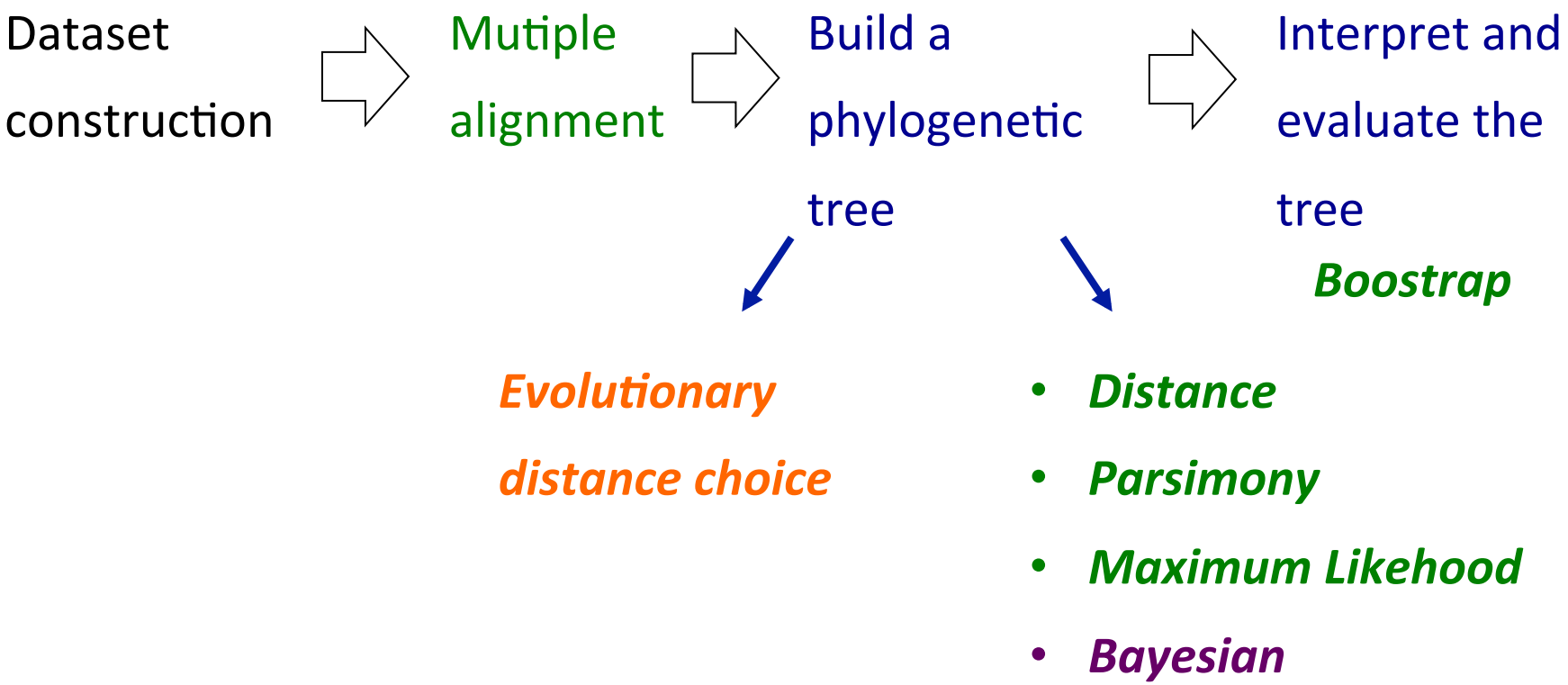


Conclusion: overall view

Implemented in Seaview

Use modelgenerator.jar

Use Mr Bayes



Conclusion: method comparison

- **Neighbor-joining** (fast)
  - Consistent: proven to construct the correct tree if distances are patristic.
  - Problems with long and divergent sequences

Parsimony (medium)

- good for closely related sequences
- can be used with any kind of data
- No clear interpretation of branch length

Conclusion: method comparison

Likelihood method (slow)

- Sound statistic foundations
- Works well for distantly related sequences
- Can incorporate any desirable evolutionary model

Bayesian method (very slow)

- Powerful but complex method

Frequent problems

- **Long Branch Attraction:** Long branches tend to cluster together in the tree:

Solution: "break down" long branches by adding some taxa to the analysis;

- **Saturation:** Characters have evolved for so long that they are almost random:

Solutions: Remove saturated sites and/or taxa; When available, use proteic sequences instead of nucleic sequences;

- **Missing Data:** Some characters are missing from the alignment:

Solutions: Use methods that can handle missing values, such as ML; Use as many characters as possible.

Bibliography

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- **Computational Molecular Evolution.** 2006. Ziheng Yang. Oxford Series in Ecology and Evolution, Oxford University Press. Oxford, U.K. 357 pp.
- **Inferring Phylogenies**. 2004. Joseph Felsenstein. Sinauer Associates, Inc. Sunderland, MA, U.S.A. 664 pp.

Useful web sites

- **LIRMM web site :**
- http://phylogeny.lirmm.fr

- **PHYLIP (Felsenstein lab, Univ. of Washington) web site :**

http://evolution.gs.washington.edu/phylip/software.html

The End !

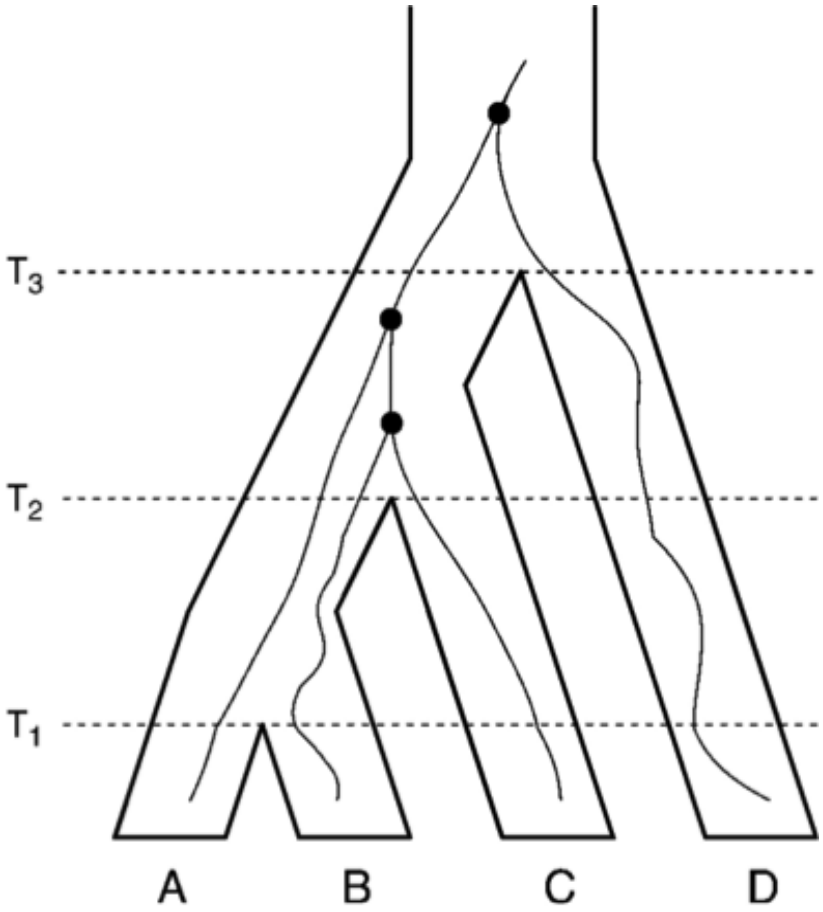
Questions ?

The PAM matrix (Dayhoff 1978)

- **Dataset = 1300 protein sequences** from 71 homolog families with at least 85% of identity (to minimize multiple subtitutions)
- Estimation of a transition matrix between all amino acids for a branch length of d=0.01 subsitution per site **= the PAM1 matrix** (1 Point Accepted Mutations per 100 amino acids in average)
- PAM matrix are computed for more divergent proteins by mutiplying PAM matrix k times : **PAM250** matrix correponds to **250 Point Accepted Mutations per 100 amino acids in average**
- **The PAM value** is proportional to the true evolutionary distance between two proteins.

Frequent problems

- **Incomplete Lineage Sorting (ILS)**: Species tree with embedded gene tree showing incongruence



Kubatko L S Syst Biol 2009;58:478-488

Consequence: gene tree topology that differs from the species tree