Introduction to phylogenetic inference

Hélène Chiapello, INRA – Unité MaIAGE

24 octobre 2018

Hélène Chiapello
Helene.chiapello@inra.fr

Outline

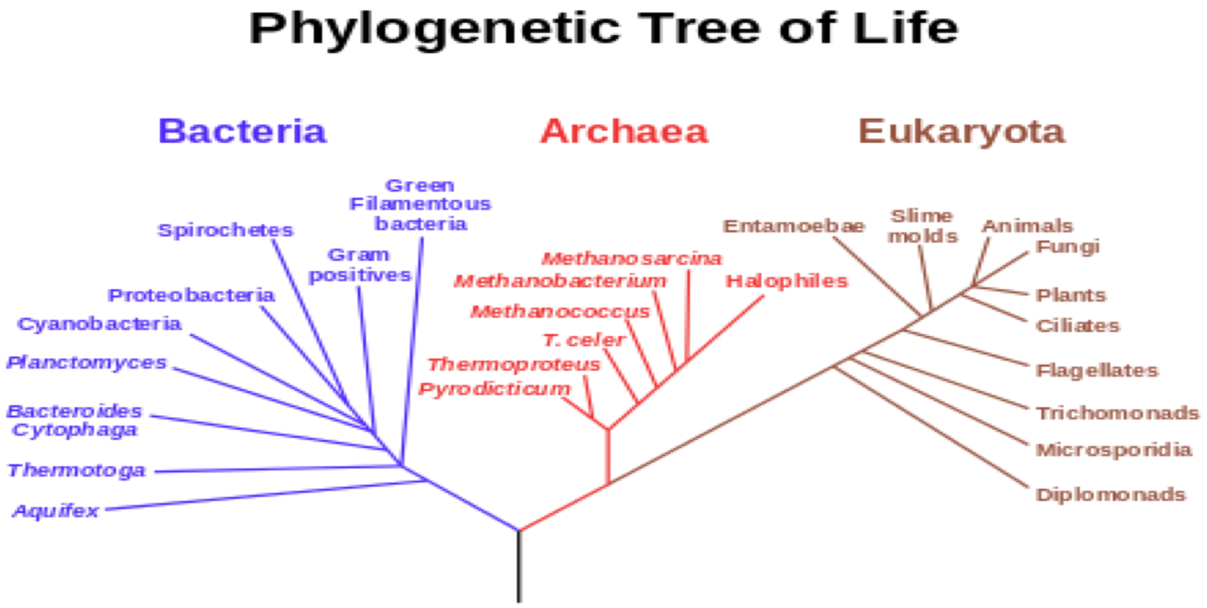
- Introduction and basic concepts in phylogeny
  - Trees
  - Alignements
  - Genetic distances and nucleotide substitution models
  - Alignment quality and filtering
- Phylogenetic inference methods
  - Distance methods
  - Parcimony methods
  - Maximum likehood methods
  - Bayesian methods
- Phylogeny 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

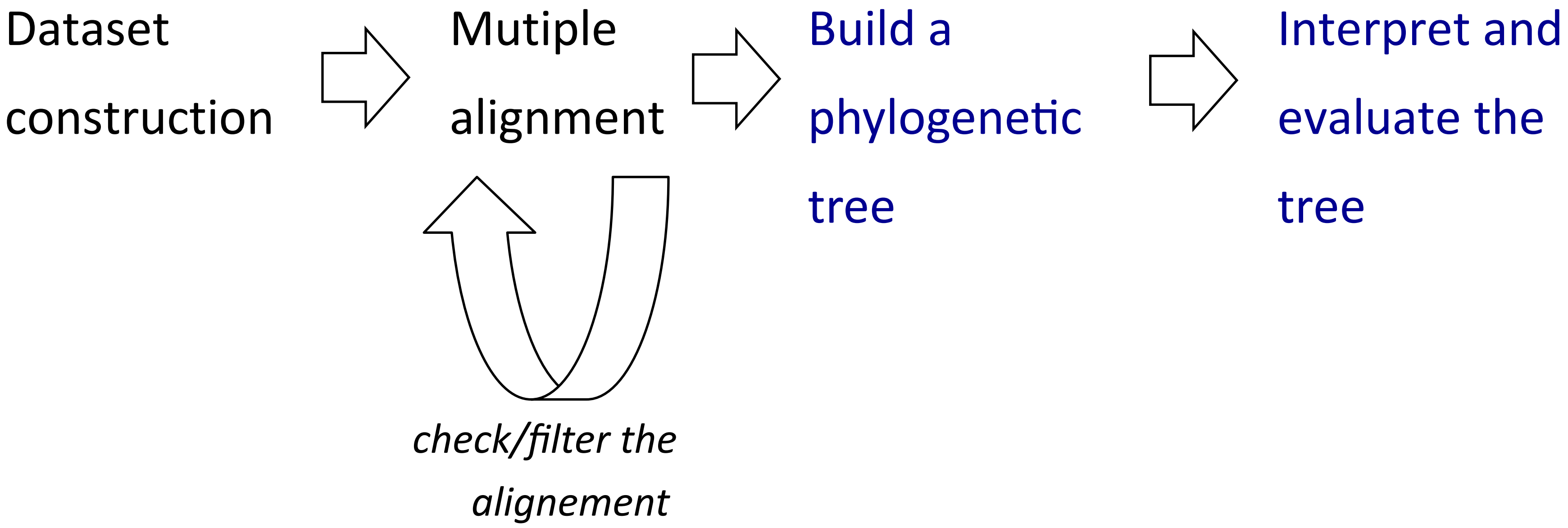
Here : we focus on **molecular phylogenetics**, based on different kind of **molecular sequence data**

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

check/filter the alignement

Build a phylogenetic tree

Interpret and evaluate the tree

Dataset construction

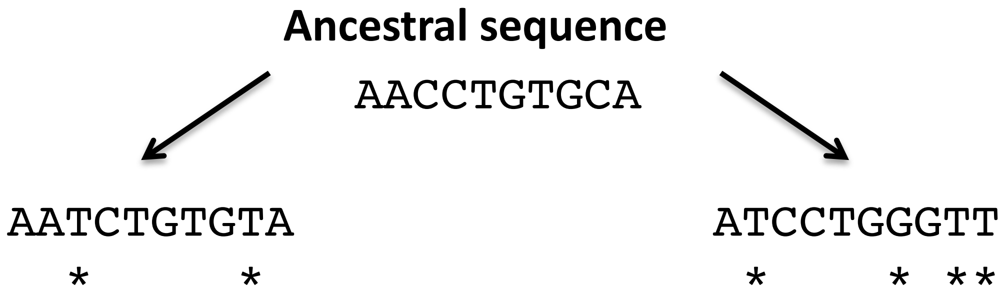
- **Criteria to choose good sequences dataset:** universality, conserved structure, no horizontal transfer, apropriate evolutionary rate.

- 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**



Ancestral sequence

```
AACCTGTGCA
```

```
AATCTGTGTA                    ATCCTGGGTT
  *     *                      *    * **
```

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

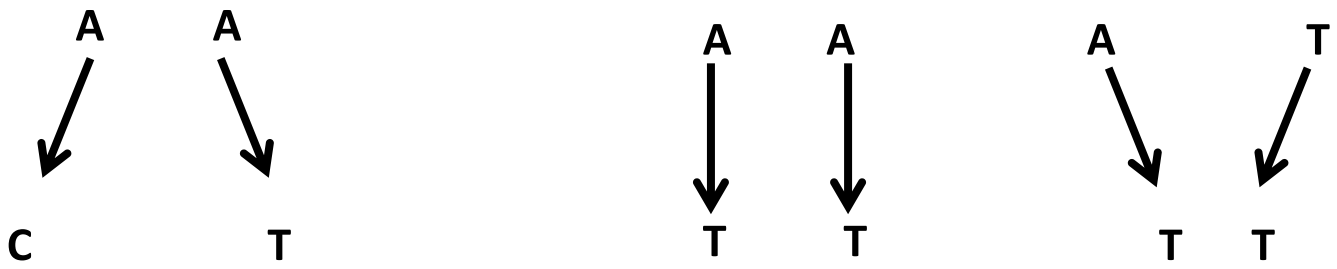
⇦ 1 site = 1 position in the alignment

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 quality and filtering

- **Why filtering ?**
  - Aligned regions with insertions/deletions are less reliable and often contain erroneous aligned residus
  - These positions can negatively affect the phylogentic tree inference

- **Removing unreliable columns before tree reconstruction** : a way to **increase the signal to noise ratio** of Multiple Sequence Alignments (MSAs)

*Tan *et al*. Systematic Biology 2015

Many filtering methods

Tool	Type of sites filtered	Account for tree structure	Use an evol model	Ref
Gblocks	Gap-rich and variable sites	no	non	Talavera and Castresana 2007
TrimAl	Gap-rich and variable sites	no	yes	Capella-Gutiérrez et al. 2009
Noisy	Homoplastic sites	In part	no	Dress et al. (2008)
Aliscore	Random-like sites	no	indirectly	Kück et al. (2010)
BMGE	High entropy sites	no	yes	Criscuolo and Gribaldo (2010)
Zorro	Sites with low posterior	yes	yes	Wu et al. (2012)
Guidance	Sites sensitive to the alignment guide tree	yes	indirectly	Penn et al. (2010)

Adapted from Tan *et al*. Systematic Biology 2015

Main filtering methods

Tool	Type of sites filtered	Account for tree structure	Use an evo model	Ref
Gblocks	Gap-rich and variable sites	no	non	Talavera and Castresana 2007
TrimAl	Gap-rich and variable sites	no	yes	Capella-Gutiérrez et al. 2009

Two conceptual similar methods based on sitewise summary statistics

- **Gblocks** identify and filter blocks of non-conserved positions and allow filtering if gappy (and adjacent) positions

- **TrimAl** affects a score to alignment positions based on gap content and residue similarity (using a susbtitution model) and allow filtering according to a threshold

Adapted from Tan *et al*. Systematic Biology 2015

Main filtering methods

Tool	Type of sites filtered	Account for tree structure	Use an evol model	Ref
Noisy	Homoplastic sites	In part	no	Dress et al. (2008)
Aliscore	Random-like sites	no	indirectly	Kück et al. (2010)

Mathematical based methods

- **Noisy**
  - Assess the degree of homoplastic sites compared to random columns
  - Computes a character compatibility score without assuming a particular tree topology
  - Needs at least 15 sequences aligned
- **Aliscore**
  - Assess the randomness of a MSA by considering all the pairwise alignment separately using a sliding window and same character frequencies
  - Score of a position should be better than the 95th percentile score for the majority of included pairwise alignments and windows of that residue

Adapted from Tan *et al*. Systematic Biology 2015

Main filtering methods

Tool	Type of sites filtered	Account for tree structure	Use an evol model	Ref
BMGE	High entropy sites	no	yes	Criscuolo and Gribaldo (2010)
Zorro	Sites with low posterior	yes	yes	Wu et al. (2012)

Mathematical based methods

- **BMGE (Block Mapping and Gene Entropy)**
  - Compute an entropy mesure on slidding windows and remove columns above a cutoff
  - The entropy measure take into account the expected similarity of DNA/AA
- **Zorro**
  - Estimates a confidence score for each column of the alignment and removes columns below a threshold
  - Uses posterior probabilities of a Hidden Markov Model computed on sequence pairs

Adapted from Tan *et al*. Systematic Biology 2015

Main filtering methods

Tool	Type of sites filtered	Account for tree structure	Use an evol model	Ref
Guidance	Sites sensitive to the alignment guide tree	yes	indirectly	Penn et al. (2010)

Mathematical based methods

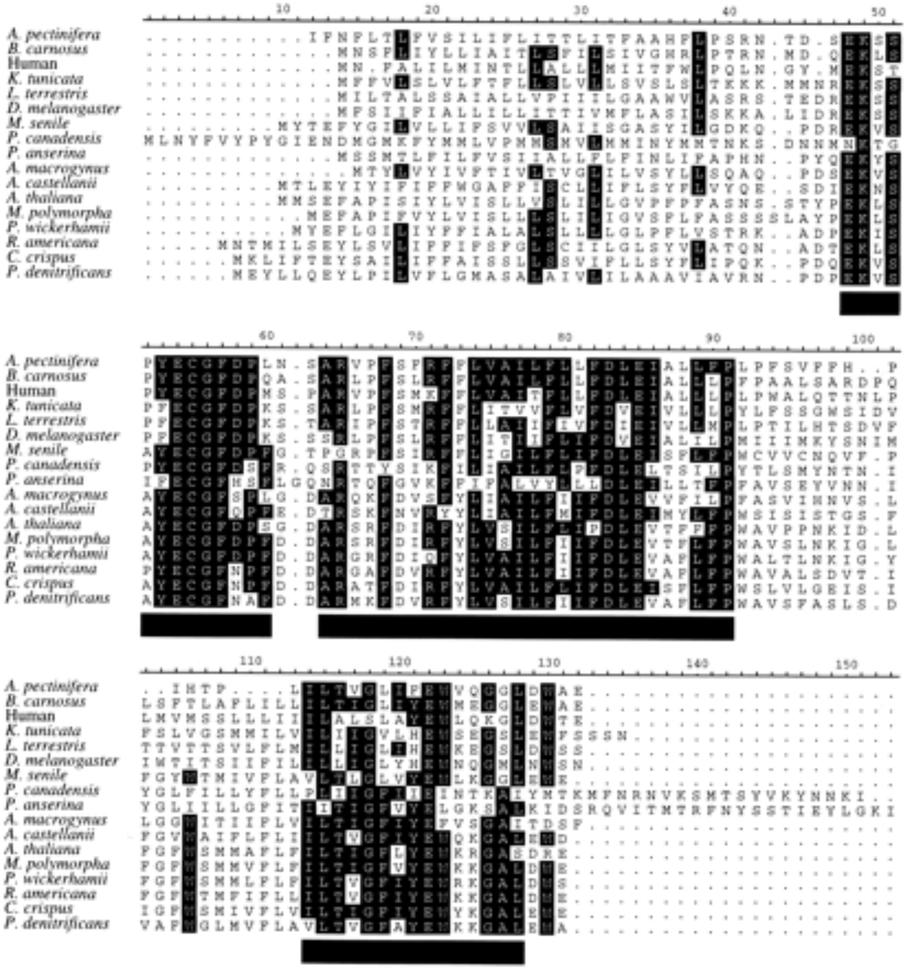
- **Guidance:**
  - Assumes that errors in the guide tree used by the aligner produces error in the alignment
  - Estimates errors in alignment positions by resampling MSA using bootstrap replicates
  - Guidance requires to know the aligner used to produce the new MSA for each guide tree bootstrap replicate
  - Guidance is computationaly very time-consuming

Adapted from 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

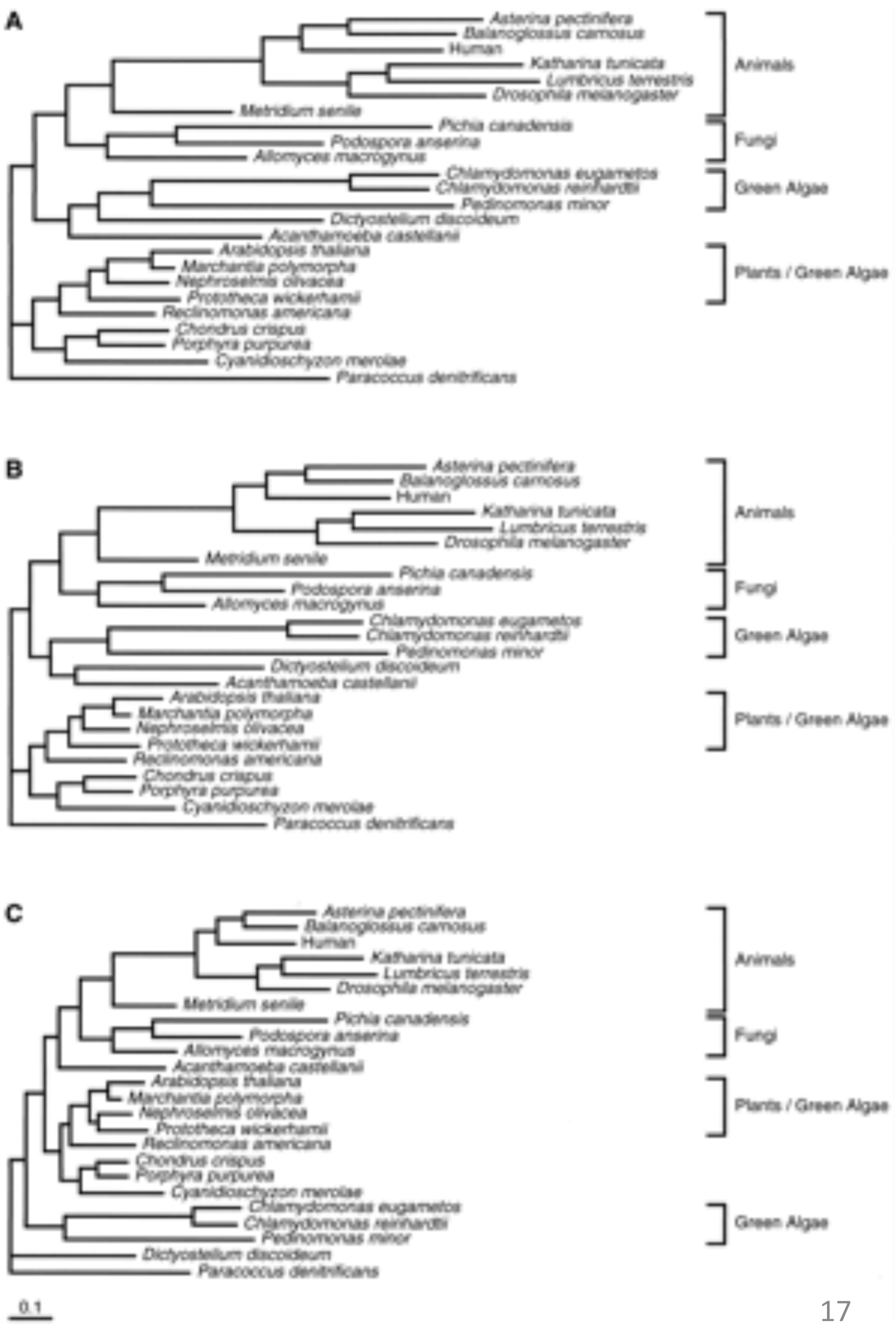
- Data : 5 mitochondial proteins aligned with clustalw

- Maximum Likehood Trees (mtRev models)

- A : original alignment
- B : gaps filtering
- C : Gblocks filtering

Filtering can change both branch lengths and tree topology !

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



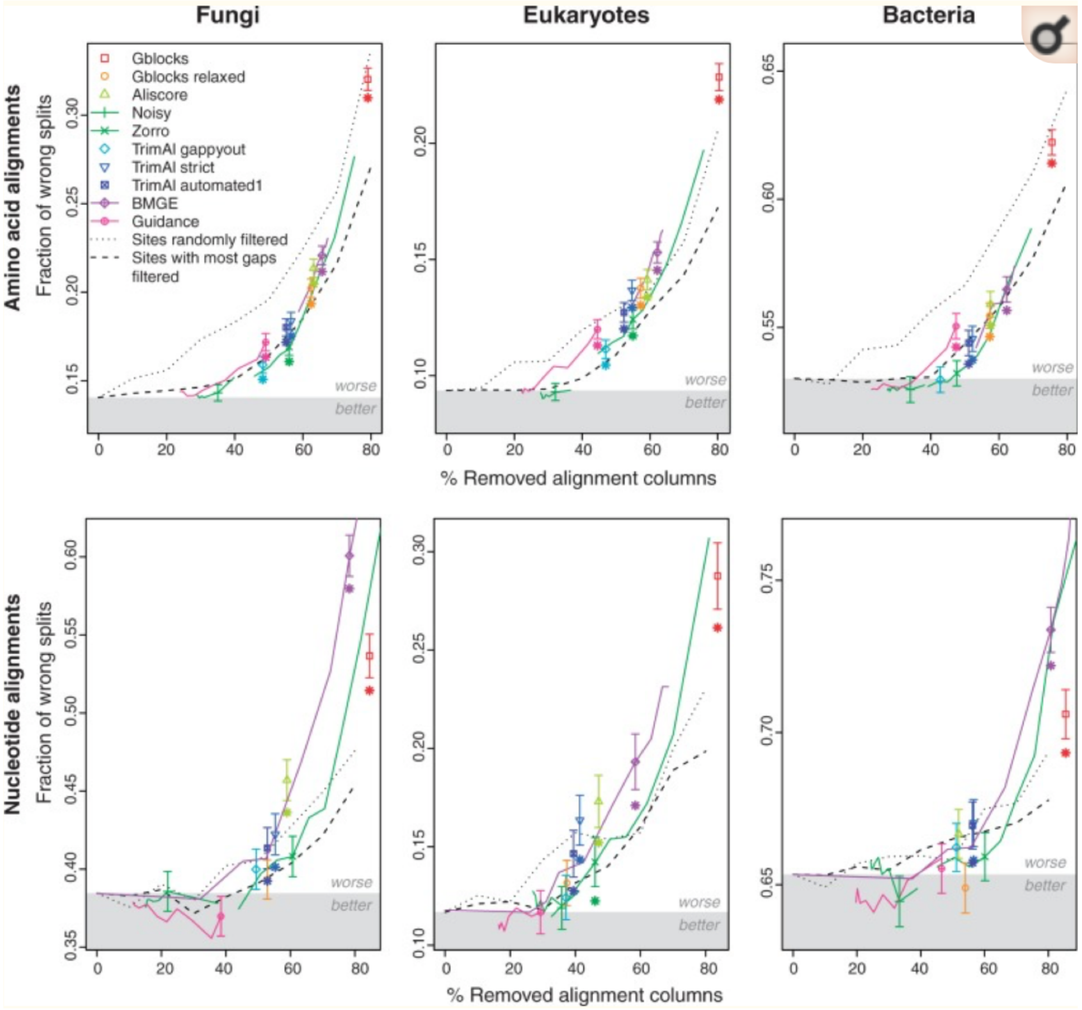
Be careful with filtering!

Dataset of 10,999 sets of six OMA orthologs with undisputed tree topology

PhyML inference

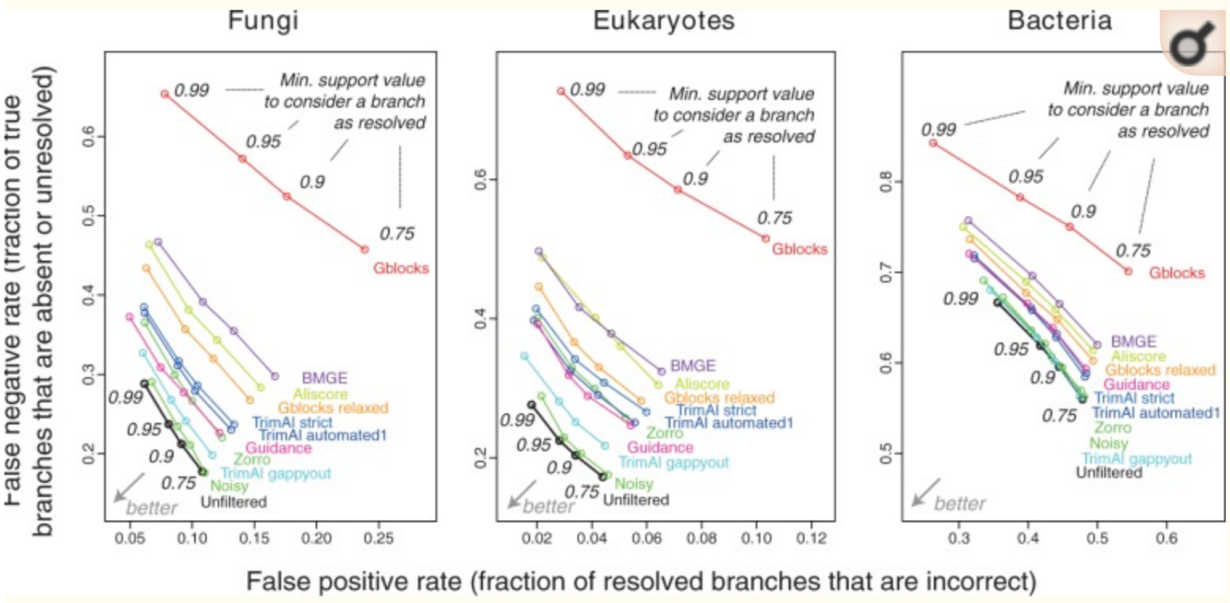
Tree inference does not generally improve after alignment filtering

Filtering fared slightly better on nucleotide alignments indeed



Adapted from Tan *et al*. Systematic Biology 2015

Be careful with filtering!



Filtering not only increases the fraction of branches that are unresolved, but also often increases the fraction of resolved branches that are incorrect.

Adapted from Tan *et al*. Systematic Biology 2015

So when do alignments need filtering ?

Difficulty: detection of unreliable columns without removing phylogentically informative sites

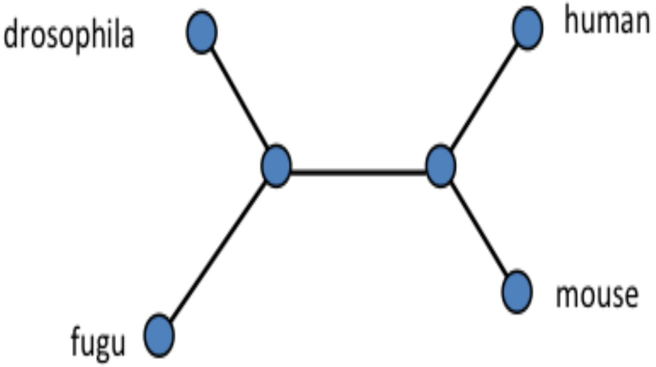
- **In the context of single-gene phylogeny do not filter**

- **In a phylogenomic context** it is highly recommanded to filter alignments !

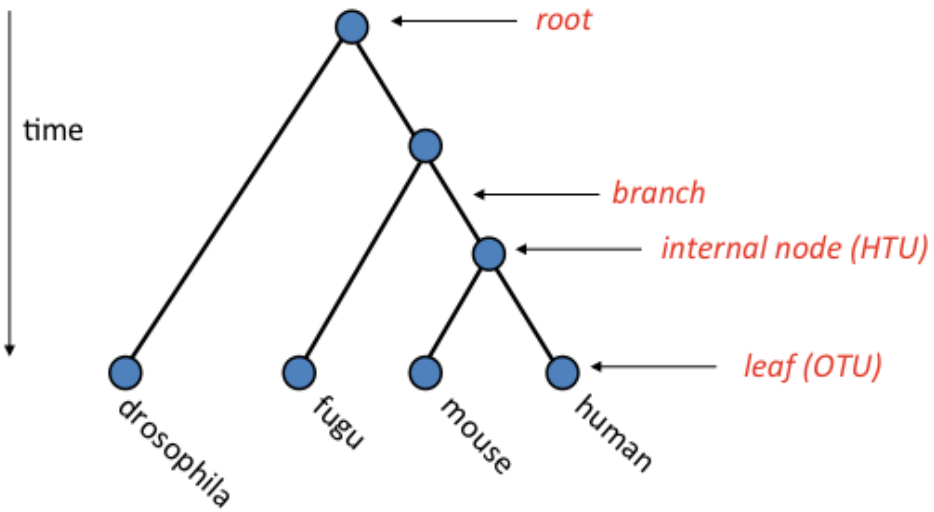
Phylogenetic tree: terminology

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

(a)



(b)



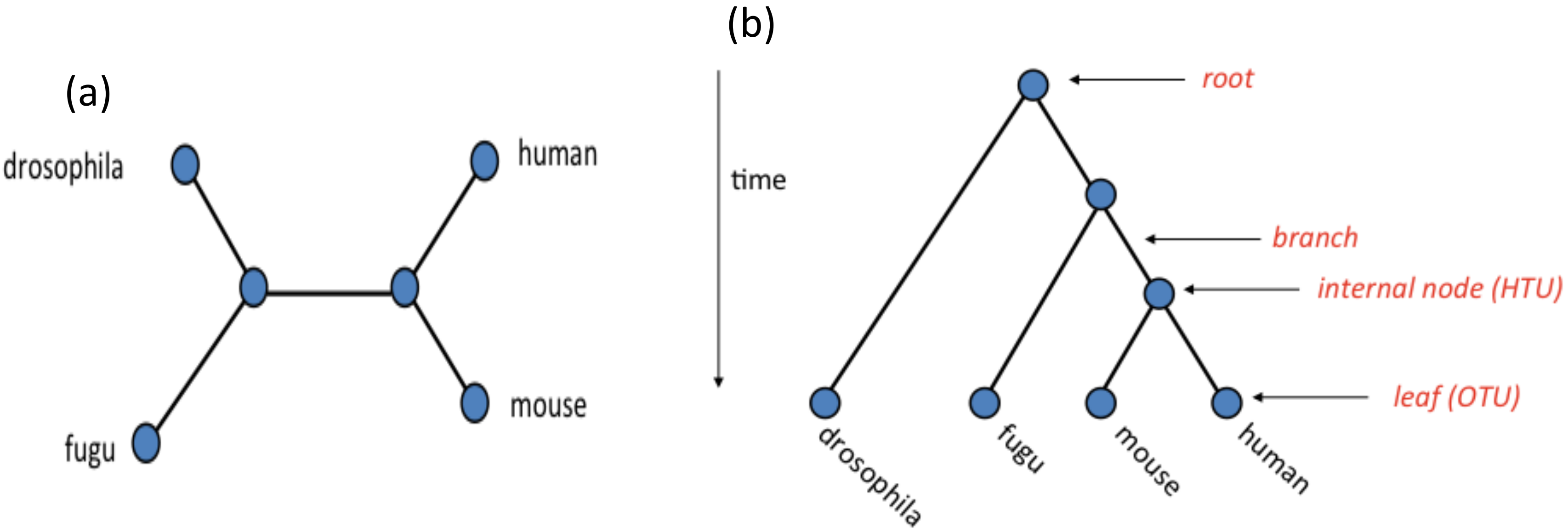
A tree is defined by its **topology** and its **branch lengths**.

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**

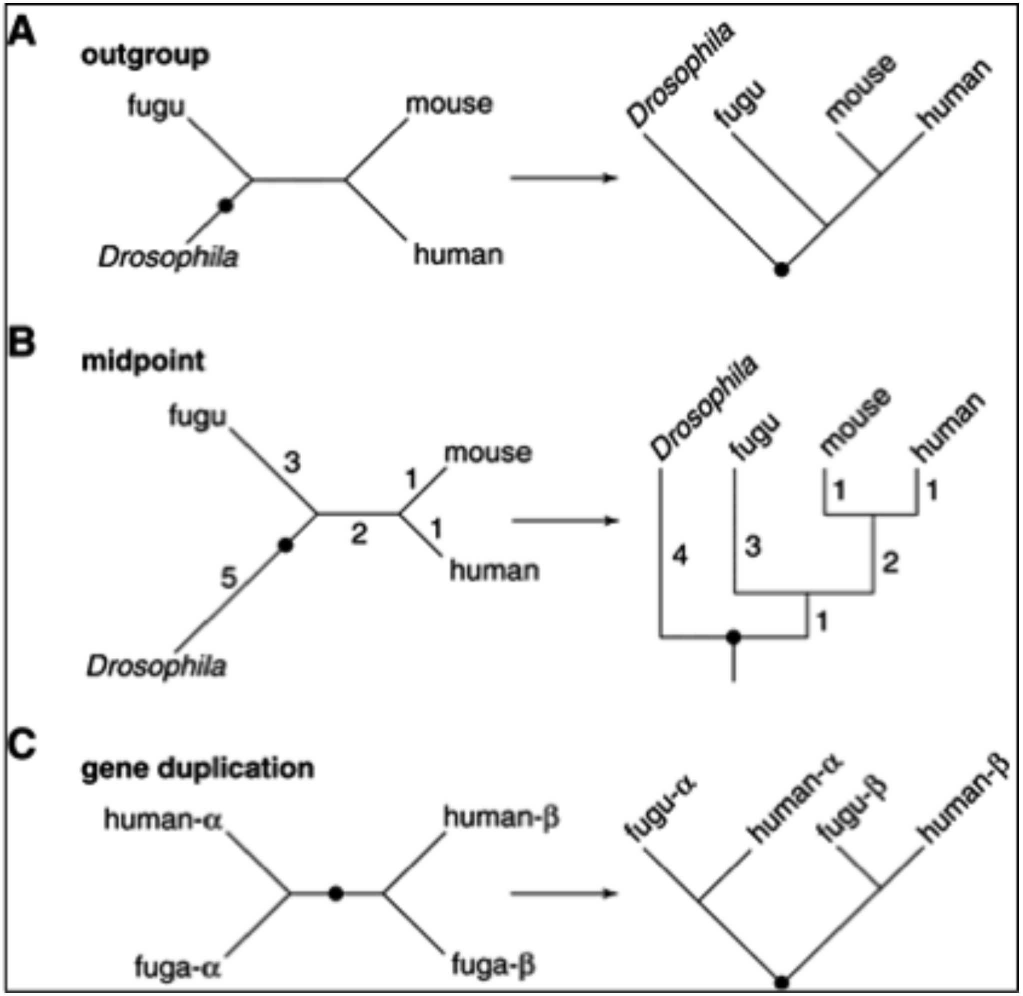
Introduction: how rooting a tree ?

- Three methods exist:

A. **Outgroup** rooting

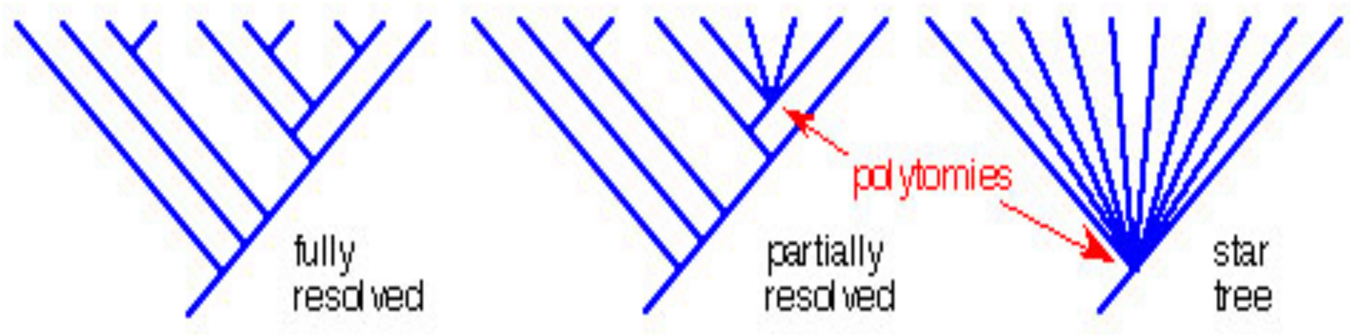
B. **Midpoint** rooting

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



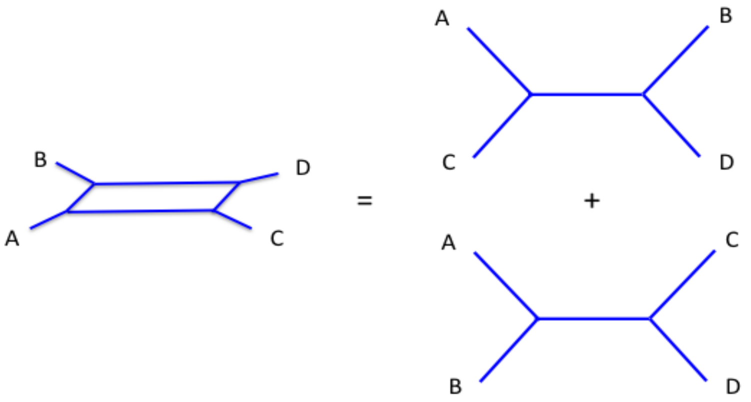
Is evolution always tree like ?

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



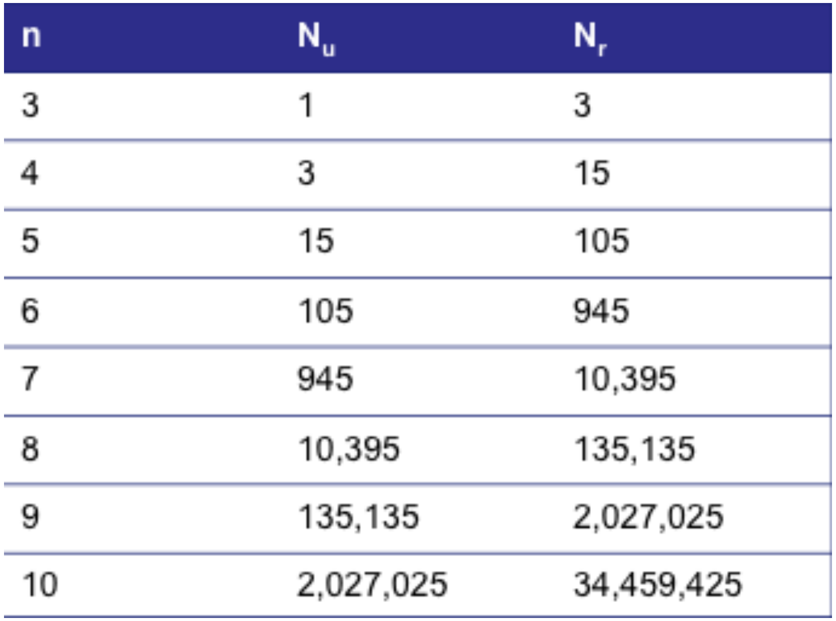
- Multifurcations on phylogenetic trees are konwn as **polytomies** an include trees with **internal polytonies** (partially unresolved tree) anc

- **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

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

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

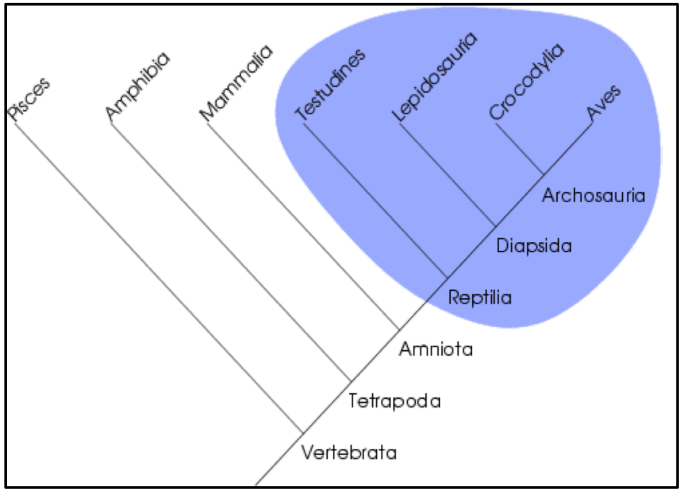
- Conclusion: an exhaustive search of all possible trees is usually impossible => **heuristic** strategies

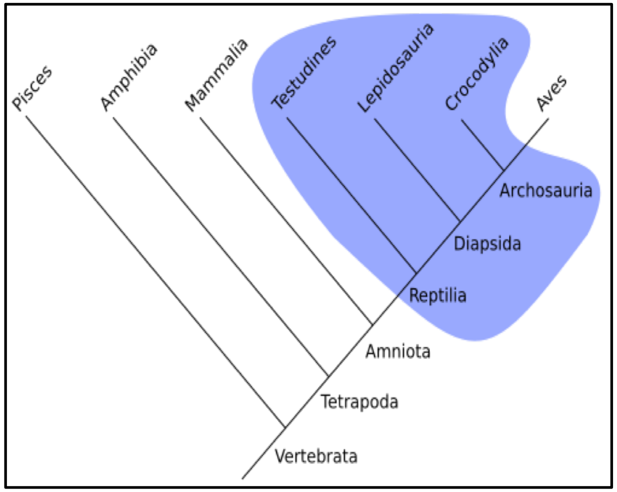
Terminology

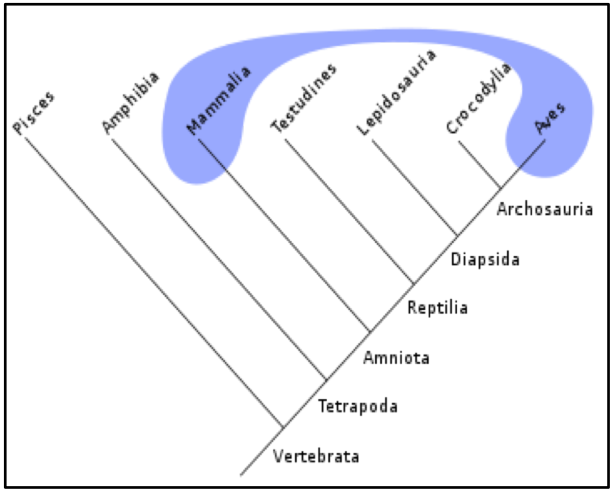
·**Monophyletic :** a group of taxa is monophyletic if it includes all descendants from its inferred common ancestor

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

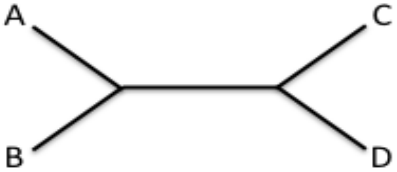


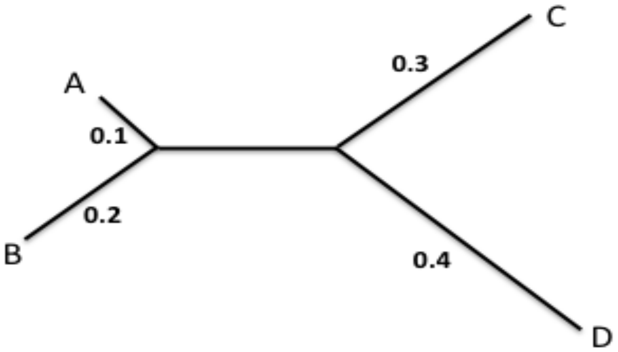




Formats for phylogenetic trees

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





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

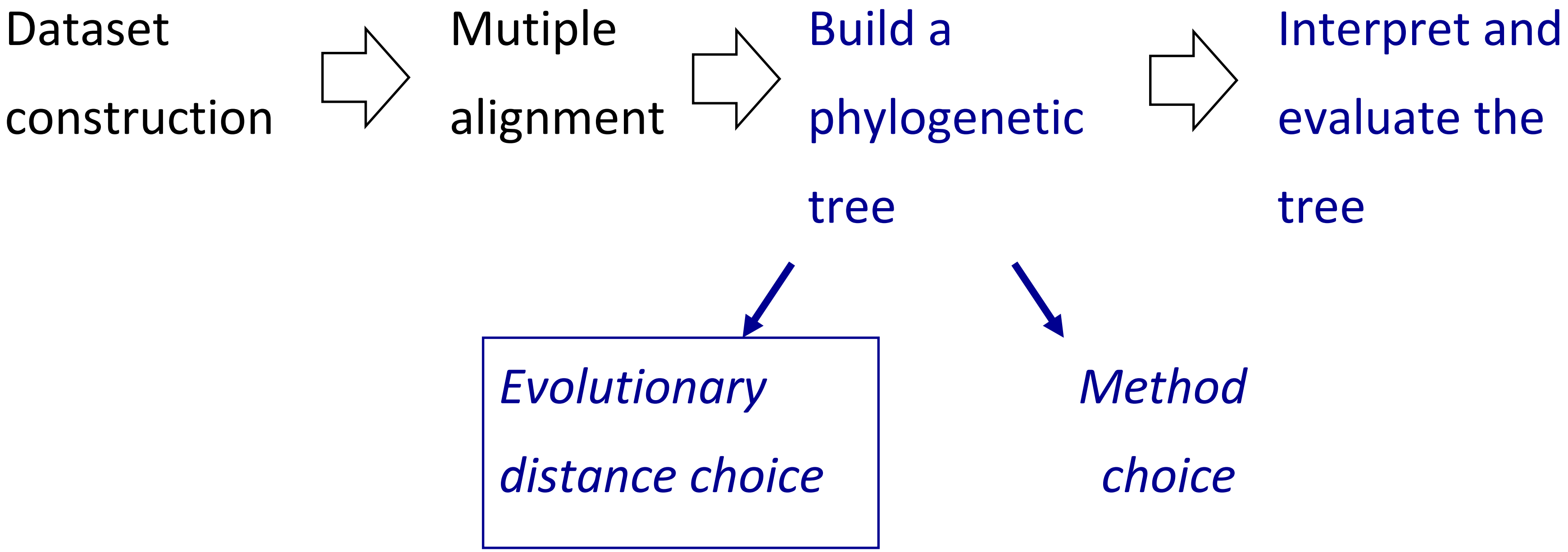
NEWICK: ((A:0.1,B:0.2):0.2, (C:0.3,D:0.4))

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

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

```
#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



Dataset construction

Mutiple alignment

Build a phylogenetic tree

Interpret and evaluate the tree

Evolutionary distance choice

Method choice

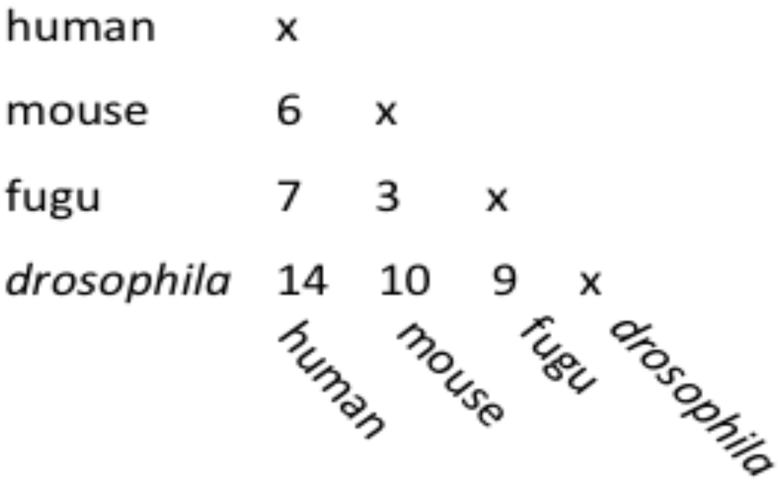
Genetic (evolutionary) distances

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

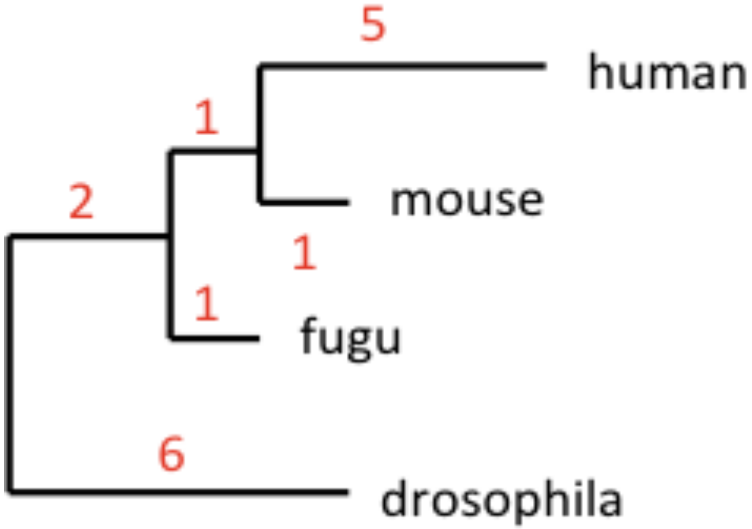
- Calculation of distance between two sequences is a central point on phylogenetic analysis
  - Pairwise distance calculation is the first step of **distance matrix methods** in phylogeny (UPGMA, NJ)
  - **Models of nucleotide/amino-acid sustitutions** used in distance-calculation form the basis of **likehood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

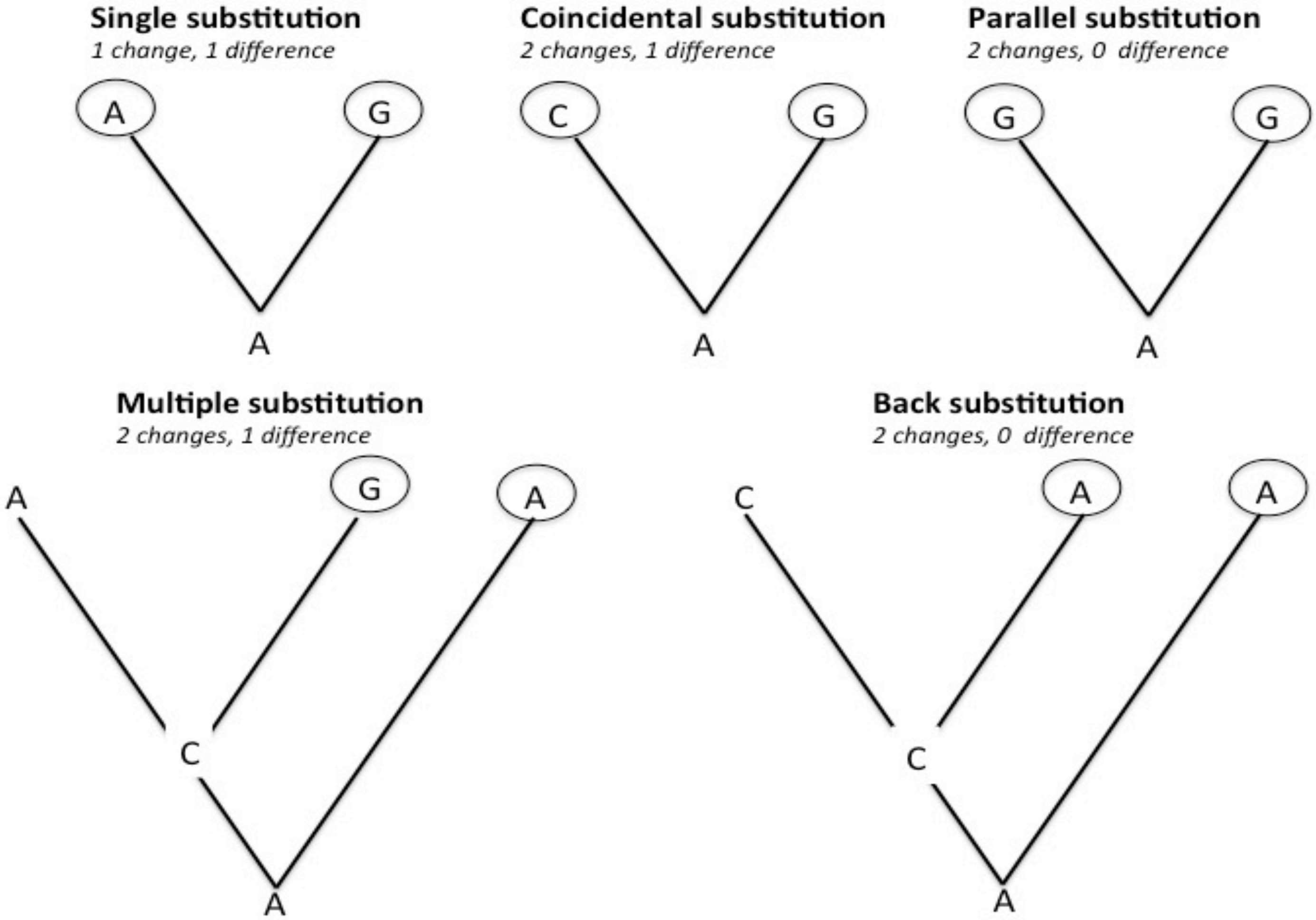


human	x			
mouse	6	x		
fugu	7	3	x	
drosophila	14	10	9	x
	human	mouse	fugu	*drosophila*



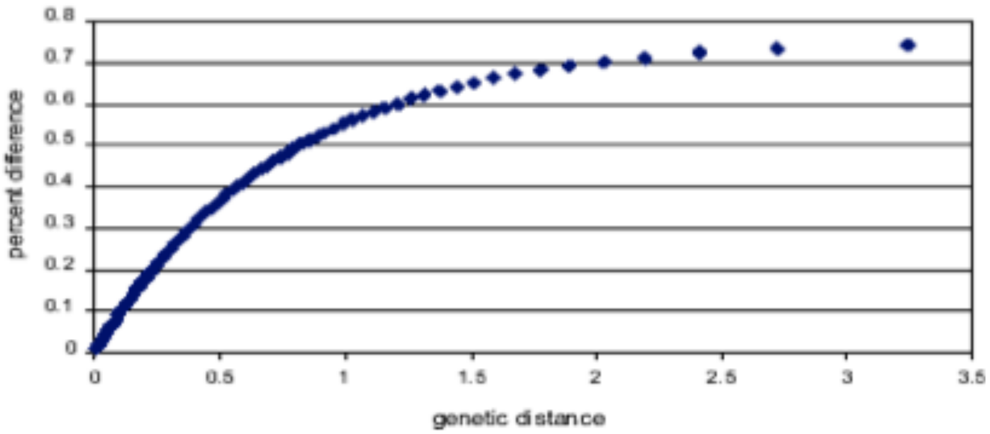
Observed and genetic distances

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 :

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

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

	Seq1	Seq2	Seq3
Seq2			
Seq3			

Observed distances

	Seq1	Seq2	Seq3
Seq2			
Seq3			

Evolutionary distances

Observed distance

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

J&C distance

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

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

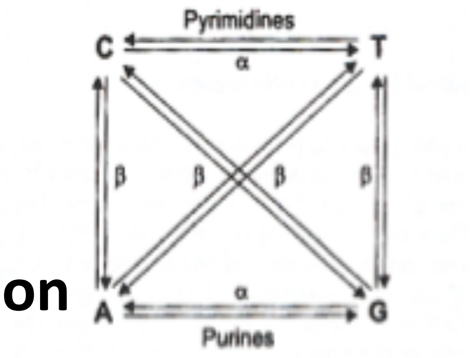
$$d_{JC}(seq1 - seq2) = -\frac{3}{4}(1 - \frac{4}{3}0.266) = 0.328$$

	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 transversion** substitutions **β**



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

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

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

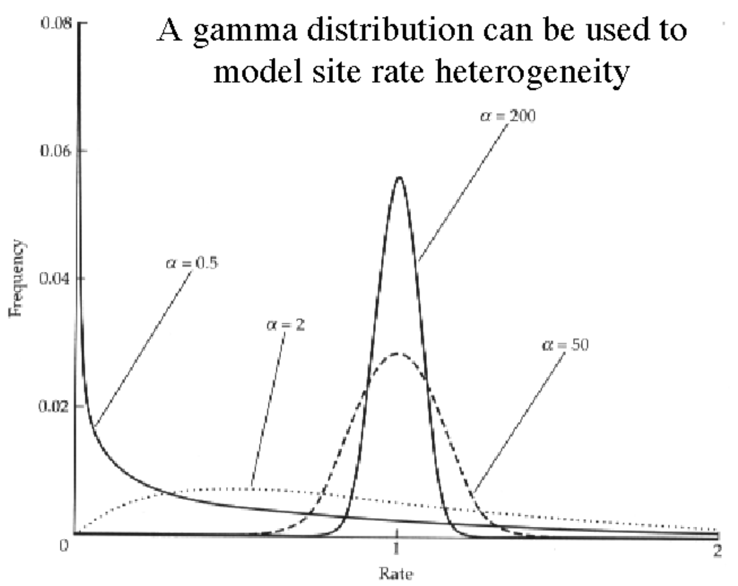
where p is the proportion of sites that show transitional differences and q is the proportion of sites that show transversional differences.

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

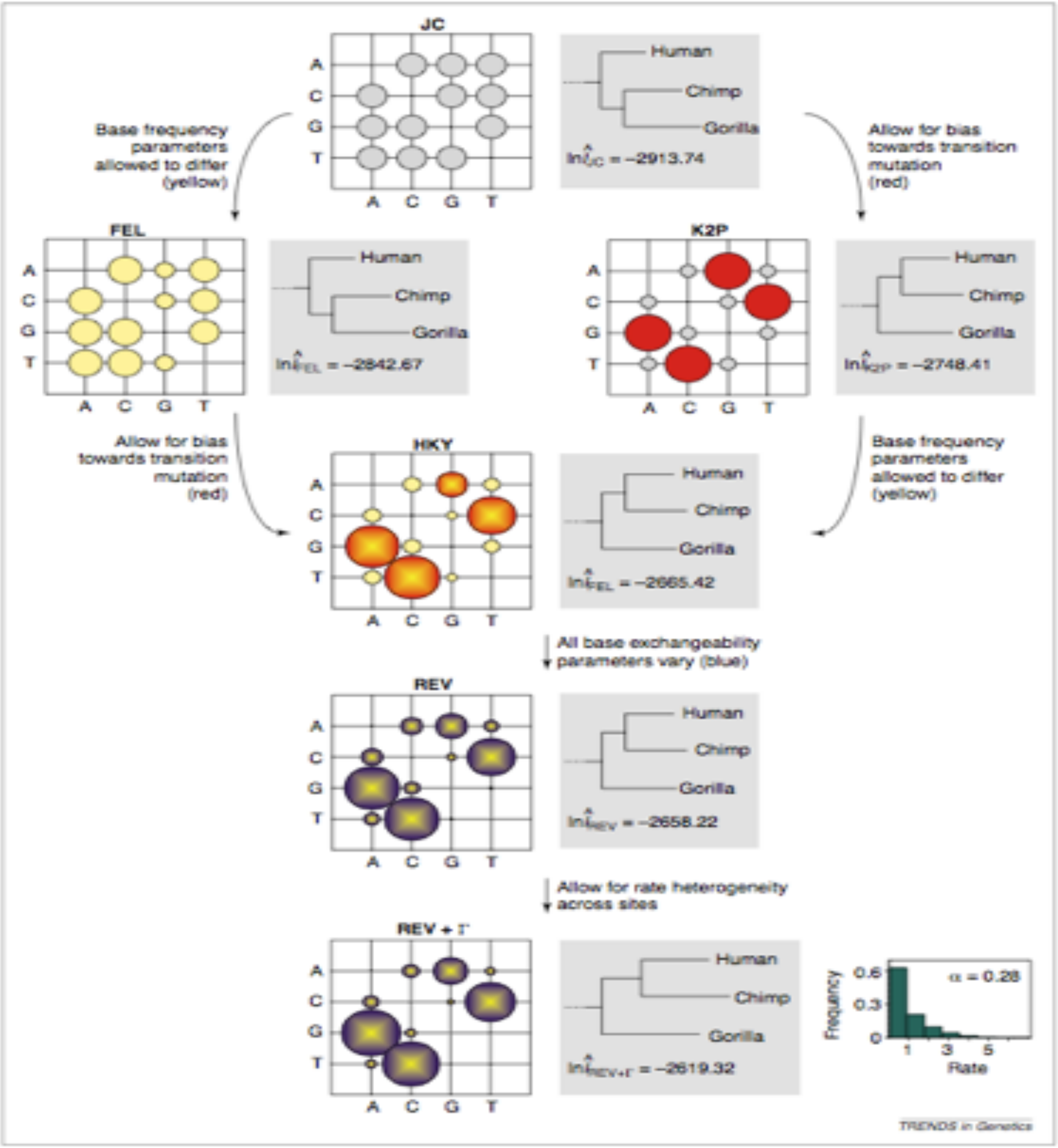
Rate heterogeneity among sites

- 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

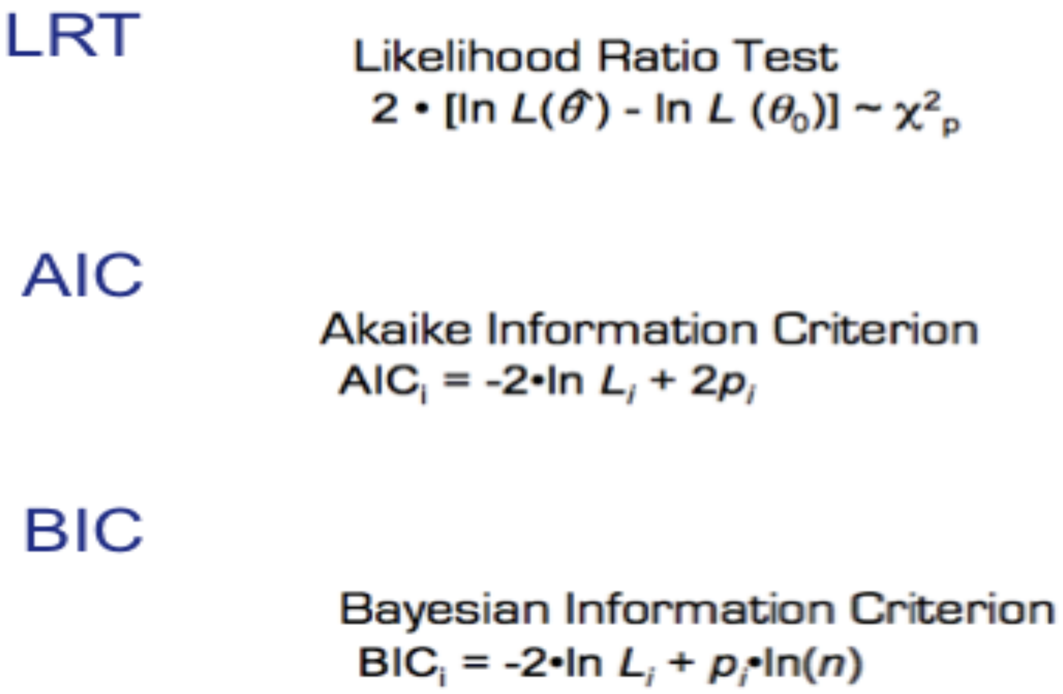

JC
A
C
G
T
A C G T
Human
Chimp
Gorilla
$\ln \hat{L}_{JC} = -2913.74$
Base frequency parameters allowed to differ (yellow)
Allow for bias towards transition mutation (red)
FEL
$\ln \hat{L}_{FEL} = -2842.67$
K2P
$\ln \hat{L}_{K2P} = -2748.41$
Allow for bias towards transition mutation (red)
Base frequency parameters allowed to differ (yellow)
HKY
$\ln \hat{L}_{FEL} = -2665.42$
All base exchangeability parameters vary (blue)
REV
$\ln \hat{L}_{REV} = -2658.22$
Allow for rate heterogeneity across sites
REV + Γ
$\ln \hat{L}_{REV+Γ} = -2619.32$
Frequency
0.6
0.3
0
α = 0.28
1 3 5
Rate
TRENDS in Genetics

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 necessarily 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

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

AIC

Akaike Information Criterion

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

BIC

Bayesian Information Criterion

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

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

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)

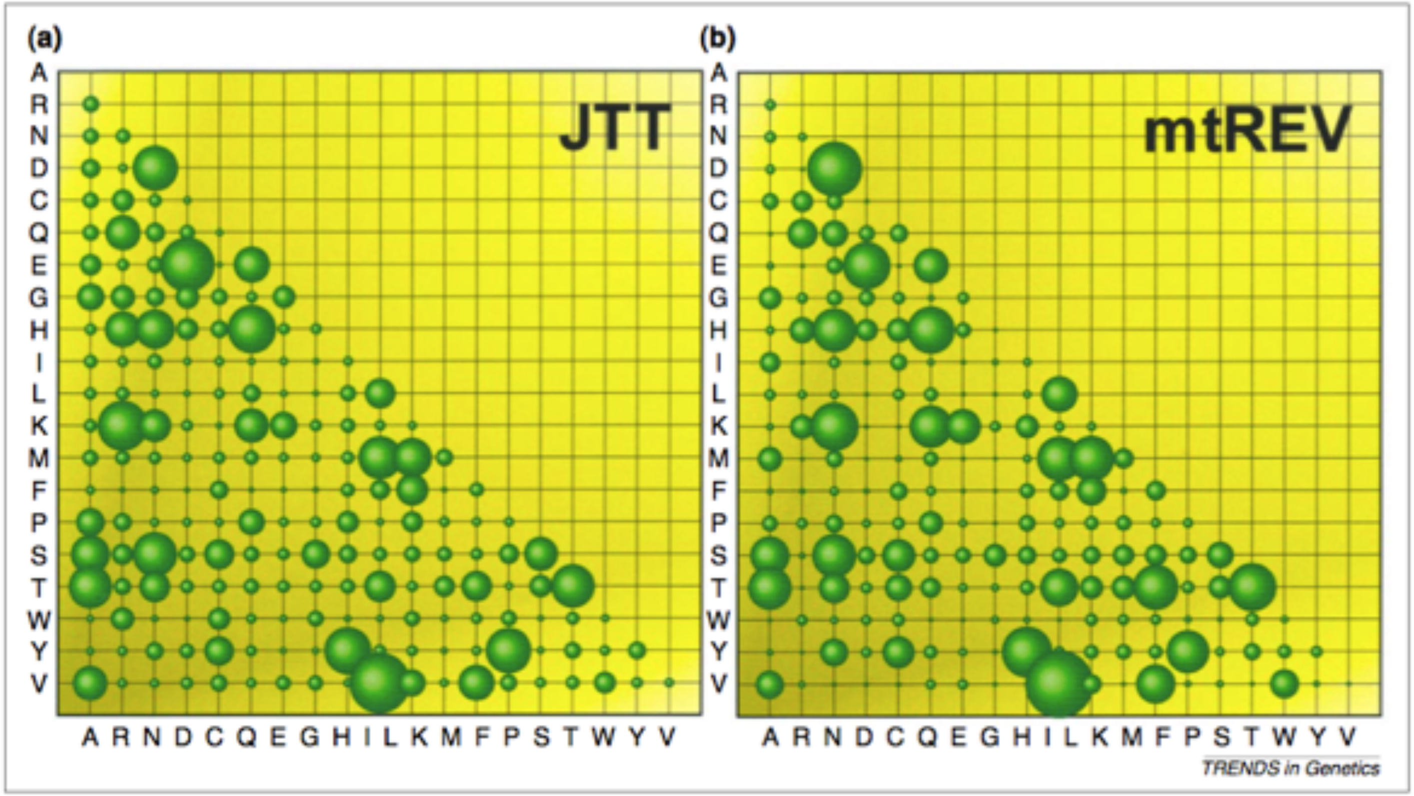
Main protein evolutionary models

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**

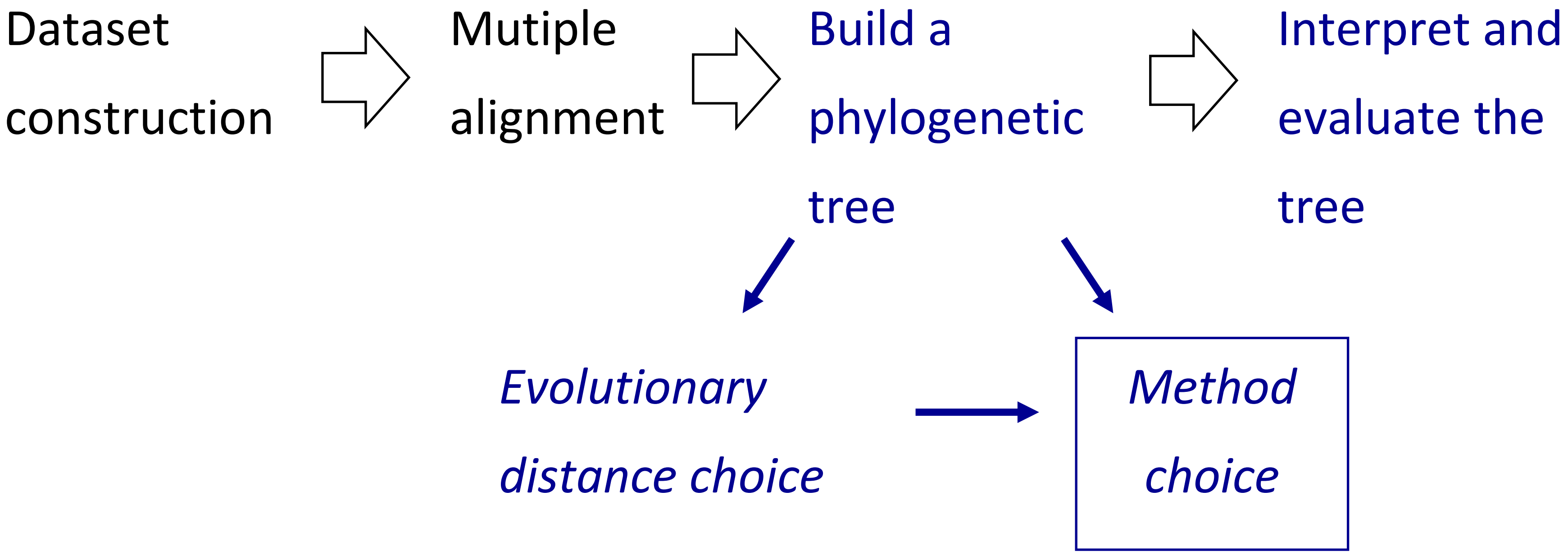
WAG and LG models are the more used models

Protein models

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



Usual workflow in phylogenetic analysis



Dataset construction

Mutiple alignment

Build a phylogenetic tree

Interpret and evaluate the tree

Evolutionary distance choice

Method choice

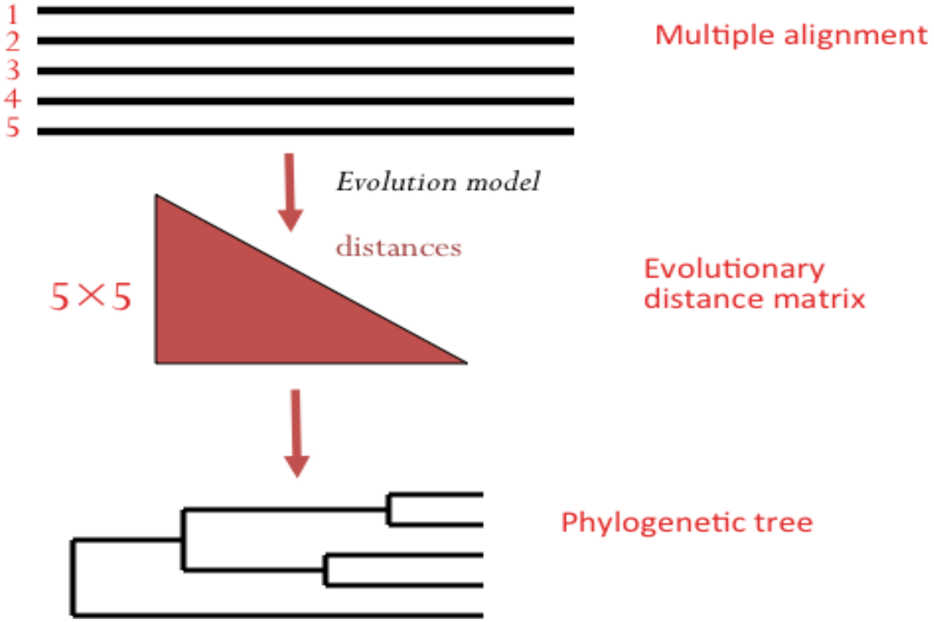
Method choice

- 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)

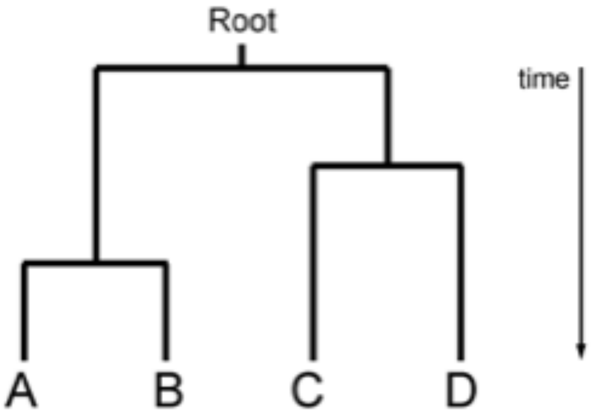
Distance methods for inferring a phylogenetic tree

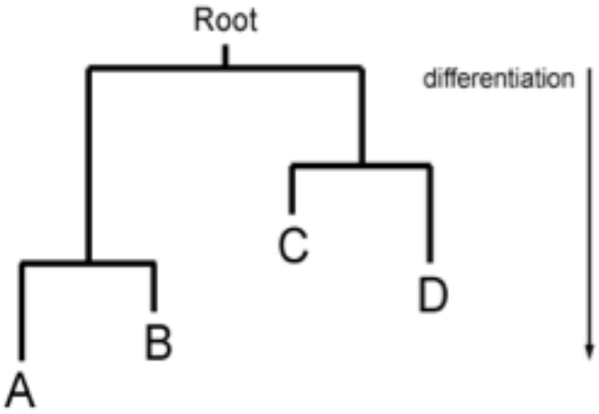
- Introduced in phylogeny in 1960
- Try to fit a tree **to a matrix of pairwise genetic distances**
- Need to choose an **evolutionary model**



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**

Neighbor-Joining (NJ)

- **Principle of the algorithm:**

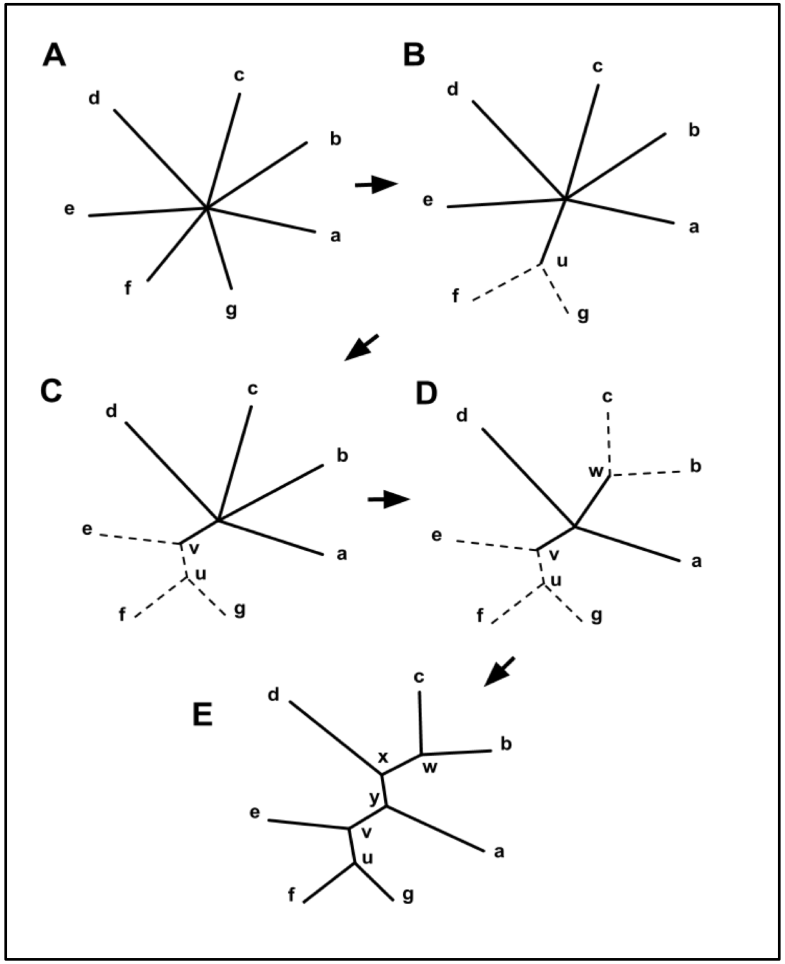
- Start with **a star tree** (A)

- 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**:

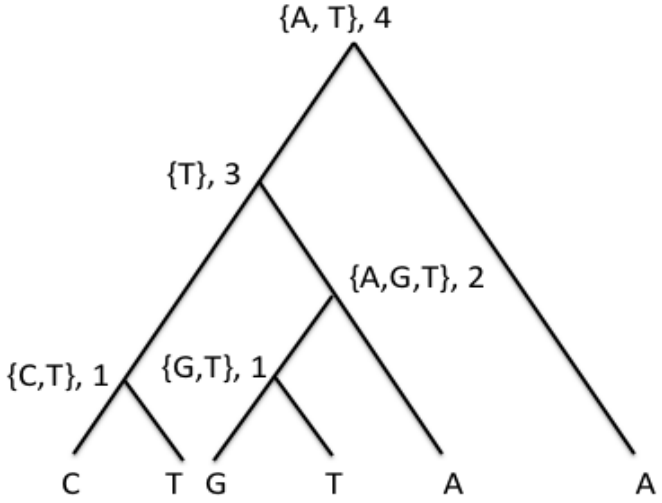
- **Step 1:** Compute the **minimal amount of character change** required in a given tree (compute changes for each character and sum up all characters)
- **Step 2:** Search for **all possible tree topologies**
- **Step 3:** Choose **the tree(s) that minimize this number of character changes**.

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...



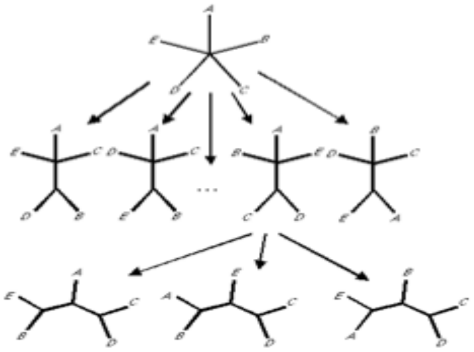
Parsimony score :
1+1+2+3+4 =11

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

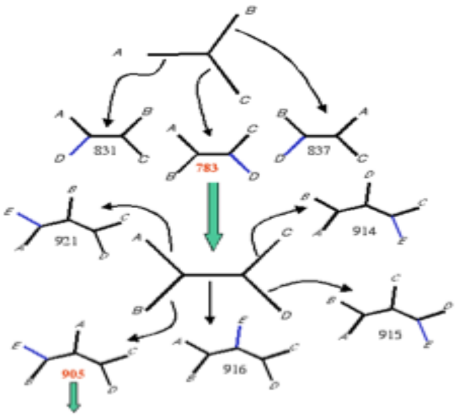
Parsimony

- **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

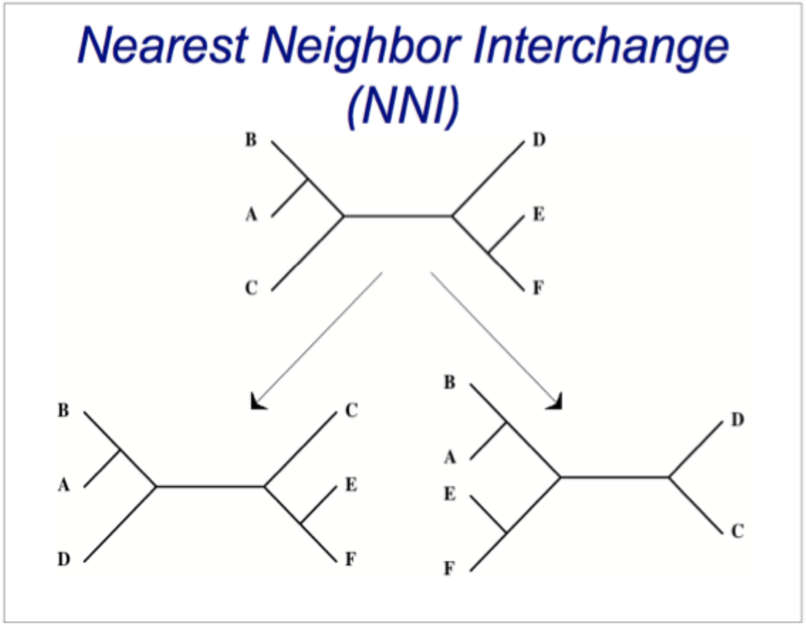


Stepwise addition

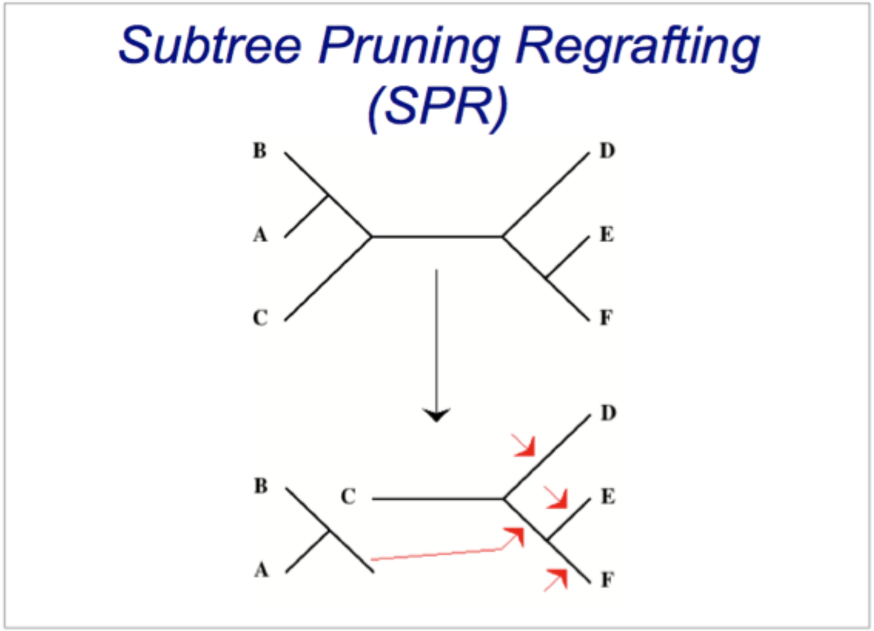


Tree rearrangments

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



Nearest Neighbor Interchange (NNI)



Subtree Pruning Regrafting (SPR)

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 ?

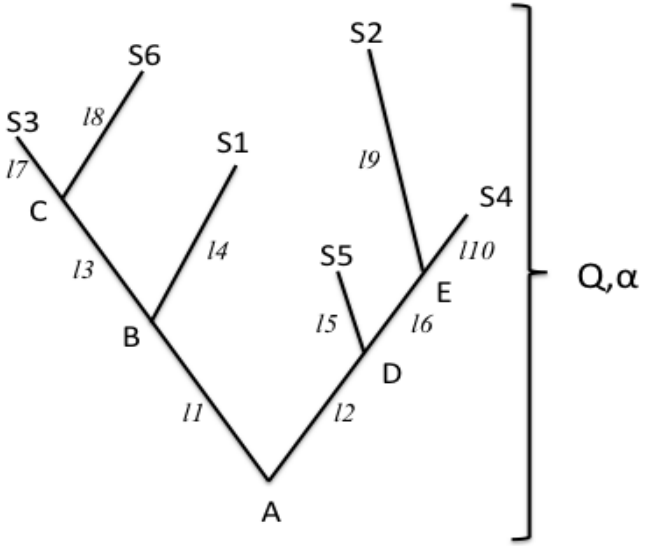
$$\Pr(D/T)$$

- **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 :

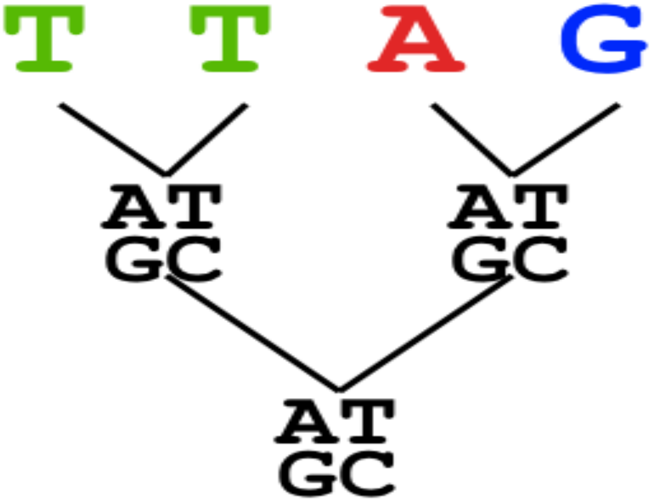
$$Log(L) = \sum_{sites} \log(L(site))$$

$$L(site) = \sum_A \sum_B \sum_C \sum_D \sum_E \Pr ob(S1,S2,S3,S4,S5,S6,A,B,C,D,E \mid T)$$

Maximum likehood: Example

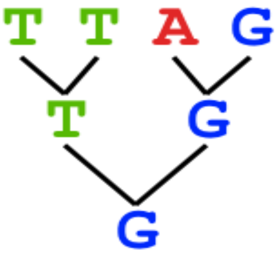
```
Sequence W: A C G C G T T G G G
Sequence X: A C G C G T T G G G
Sequence Y: A C G C A A T G A A
Sequence Z: A C A C A G G G A A
                      ▲
```

All possible evolutionary paths of a site



Likehood of a site

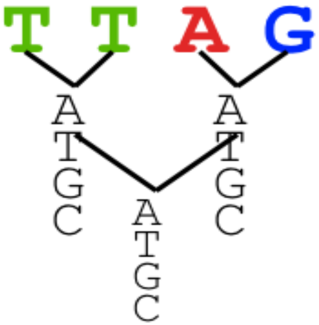
Likehood of
a path



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**

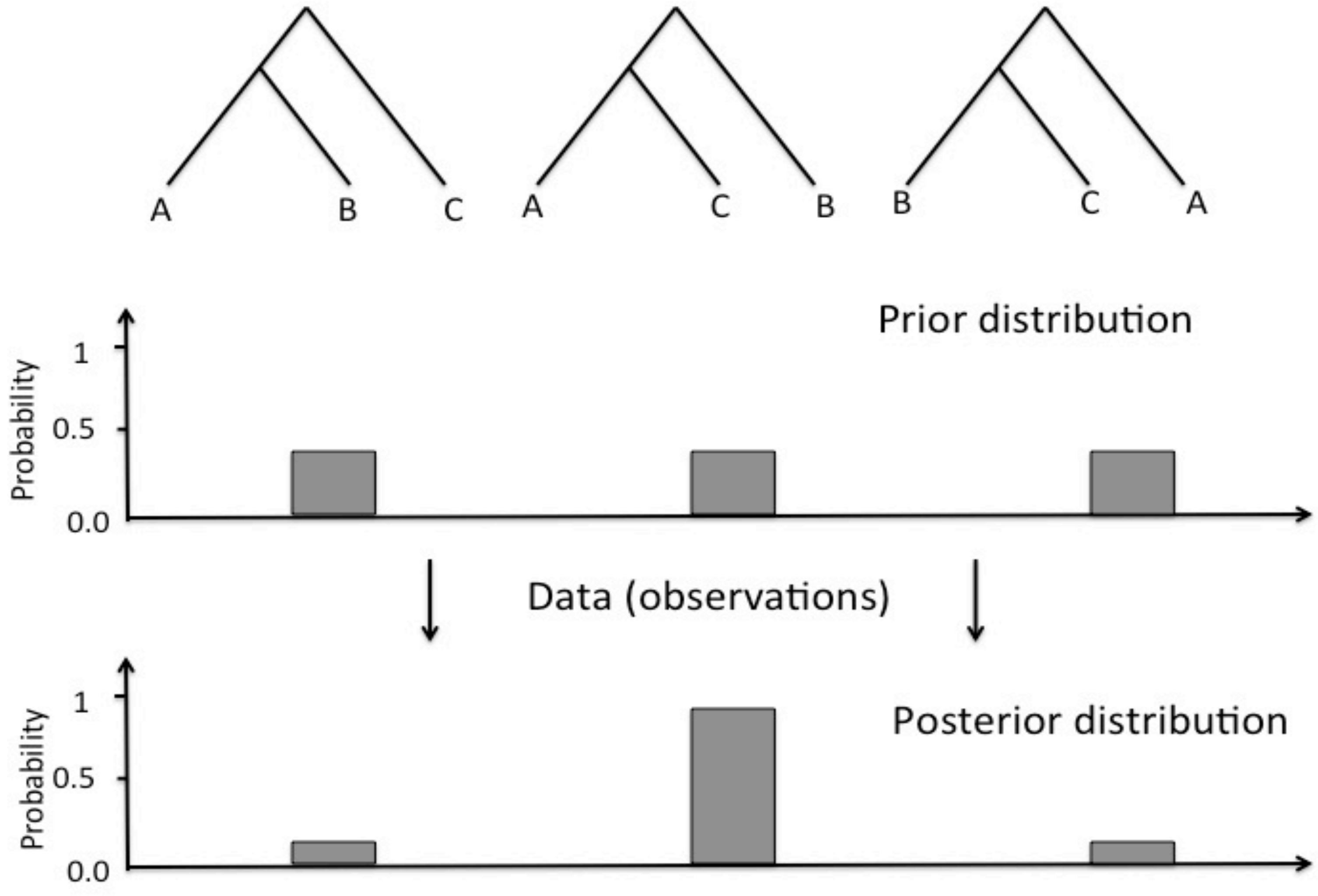
Bayesian phylogenetic inference

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**

$$\Pr(T/D)=\frac{\overset{\textbf{\textit{prior}}}{\Pr(T)}\,\overset{\textbf{\textit{likehood}}}{\Pr(D/T)}}{\underset{\textbf{\textit{data probability}}}{\Pr(D)}}$$

Bayesian phylogenetic inference



Prior distribution

Data (observations)

Posterior distribution

Bayesian phylogenetic inference

- Is is not possible to derive the posterior probability analytically
- The posterior probabilty is derived by using a simulation technique for sampling a probability distribution, the **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: key points

- **How do I choose the evolutionary model ?**

  - Ideally use programs such as *jModelTest* or *Modelgenerator* to choose the model

  - It is more problematic to under-specify than to over-specify the model in Bayesian phylogenetics

- **What are over- and under-parameterization?**

  - A to complex model (over-parameterization) can cause and inference difficulties (such as loss of power, strong correlations between parameters, large variance in the posterior, and extreme sensitivity to the prior and model assumptions) and computational problems

  - An overly simplistic model (under-parameterization) can result in systematically incorrect phy- logenetic trees and seriously biased estimates of branch lengths and substitution parameters

Bayesian phylogenetic inference: key points

- **How do I decide to concatenate or partition my data?**
  - Sites in the same partition should have similar evolutionary characteristics (substitution rates, base composition, branch lengths or even the tree topology)
  - Genes with different compositions (for ex. GC content) or evolutionary rates may be analysed as separate partitions in phylogeny reconstruction
- **How do I choose the prior for my Bayesian analysis?**
  - The prior should summarize the biologist's best knowledge about the model or parameters before the data are analysed
  - It is common to assign a uniform prior on the unrooted tree topologies
  - By evaluating the posteriors generated under different priors, the biologist can evaluate whether the posterior is robust to the prior

Bayesian phylogenetic inference: key points

- **How many iterations?**
  - Currently reliable automatic stopping rules do not exist
  - MCMC would be run long enough to obtain a reliable estimation of the posterior distribution, but not so long as to waste computational resources
  - the user has to specify the number of iterations, and then decide whether the chain is long enough or additional iterations are necessary using certain diagnosis tools
- **How many samples?**
  - MCMC algorithms tend to generate huge output files
  - To save disk space, samples are taken after a set number of iterations
  - Example: running an MCMC chain for 10^7 iterations and using a sample frequency of 10^3 iterations will produce 10^4 samples

Bayesian phylogenetic inference

- **Powerful** but **complex** method
- Can produce **either one or 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

Some popular Bayesian softwares

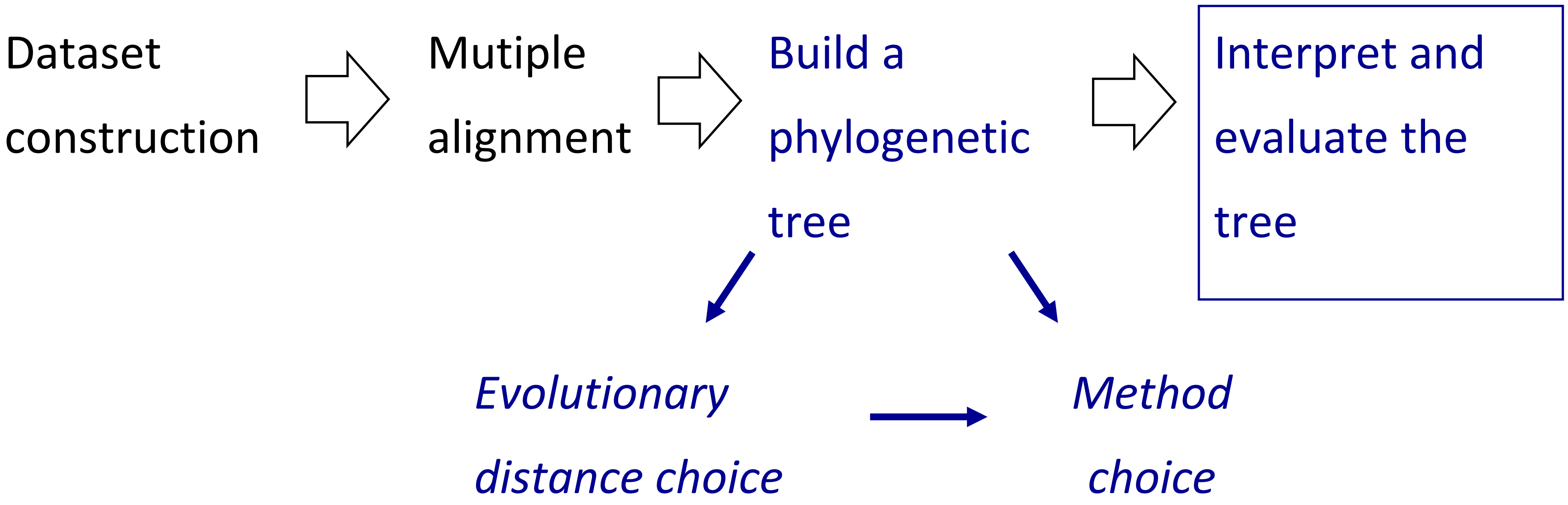
Program	Description	Reference
MrBayes	Implements a large number of models for analysis of nucleotide, amino acid and morphological data. Estimates species phylogenies and species divergence times.	Ronquist, F. et al. Syst. Biology 2012
BEAST	Implements a vast number of models. Examples are the simultaneous estimation of the tree topology and divergence times, phylodynamics, phylogeography, and species tree estimation under the MSC model.	Bouckaert et al. Plos Comp. Biol 2014
PhyloBayes	Reconstructs phylogenetic trees using infinite mixture models to account for among-site and among-lineage heterogeneity in nucleotide or amino acid compositions, which may be important for inferring deep phylogenies.	Lartillot et al. Bioinformatics 2009
Structure	Estimates population structure from multi-loci genotype data.	Pritchard, J. K et al. Genetics 2000

Adapted from **Nascimento *et al*. Nature Ecology and Evolution 2016**

Example : set 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



Dataset construction

Mutiple alignment

Build a phylogenetic tree

Interpret and evaluate the tree

Evolutionary distance choice

Method choice

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 ?

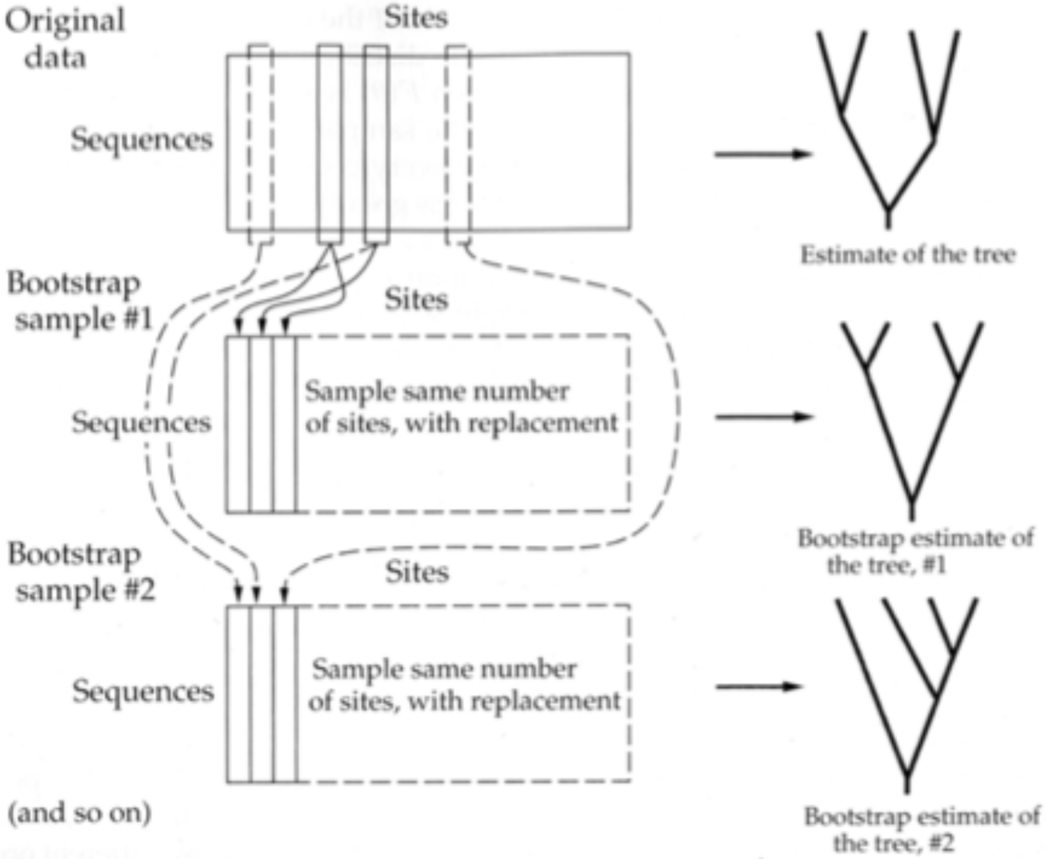
Problem: the true tree is unknown !

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;


Original data
Sites
Sequences
Estimate of the tree
Bootstrap sample #1
Sites
Sequences
Sample same number of sites, with replacement
Bootstrap estimate of the tree, #1
Bootstrap sample #2
Sites
Sequences
Sample same number of sites, with replacement
Bootstrap estimate of the tree, #2
(and so on)

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**

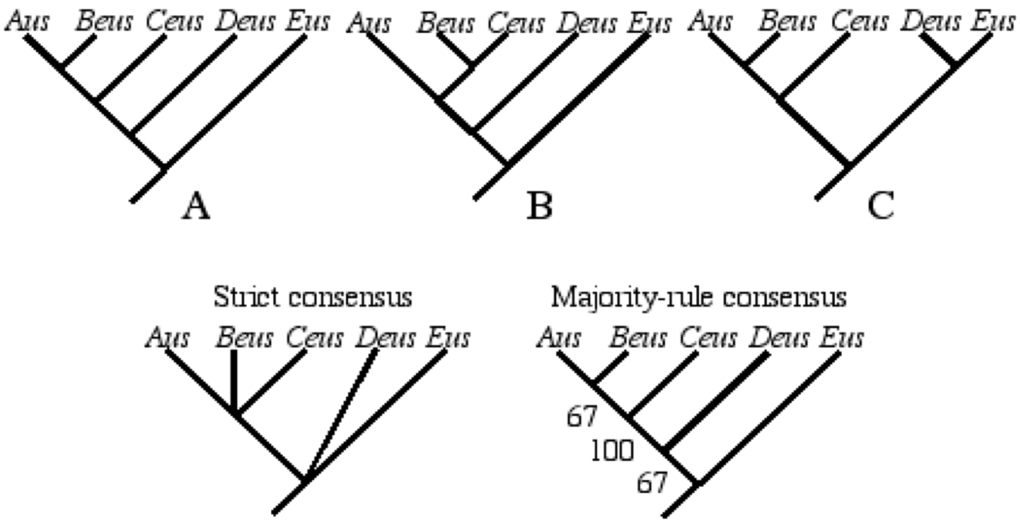
Consensus tree

- **.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;
- **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.



Boostrap values guidelines

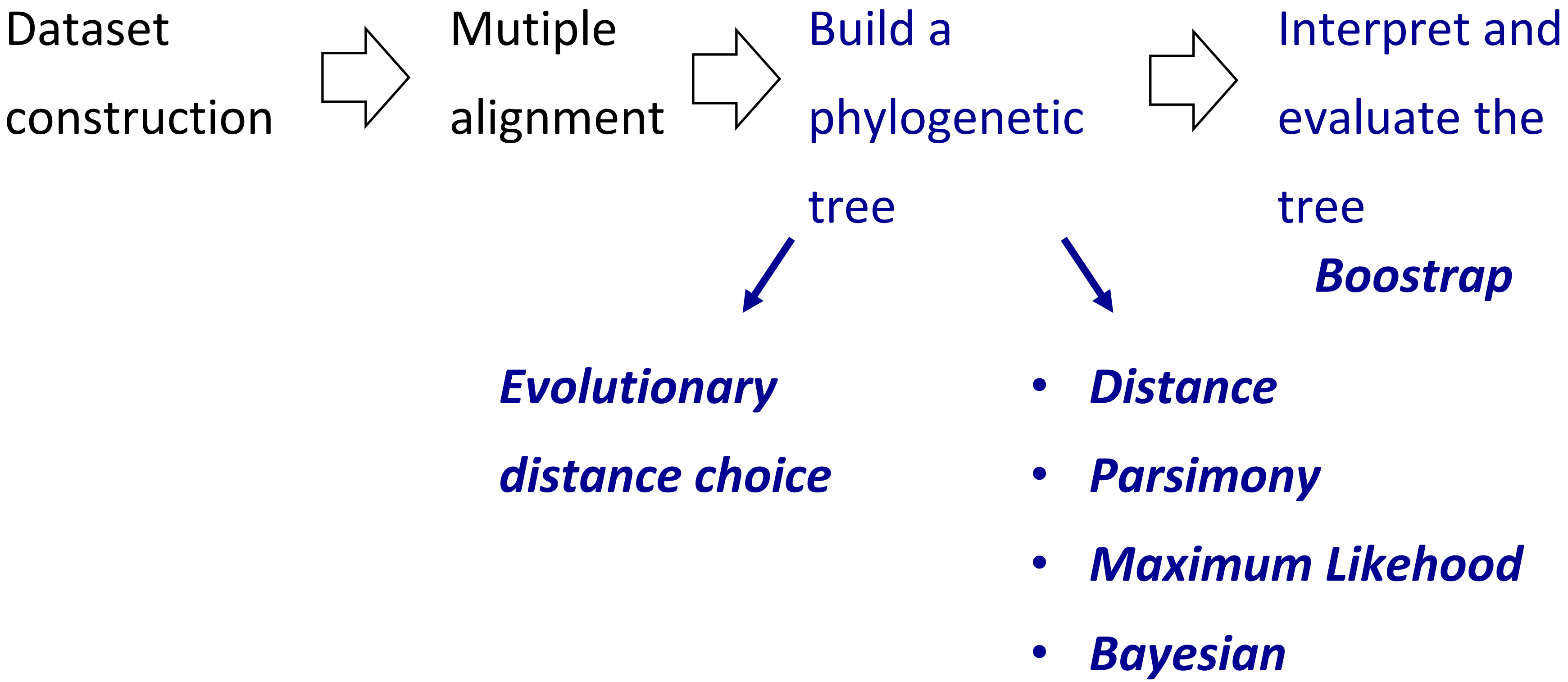
•**Be cautious with boostrap values interpretation**:

- 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



Dataset construction

Mutiple alignment

Build a phylogenetic tree

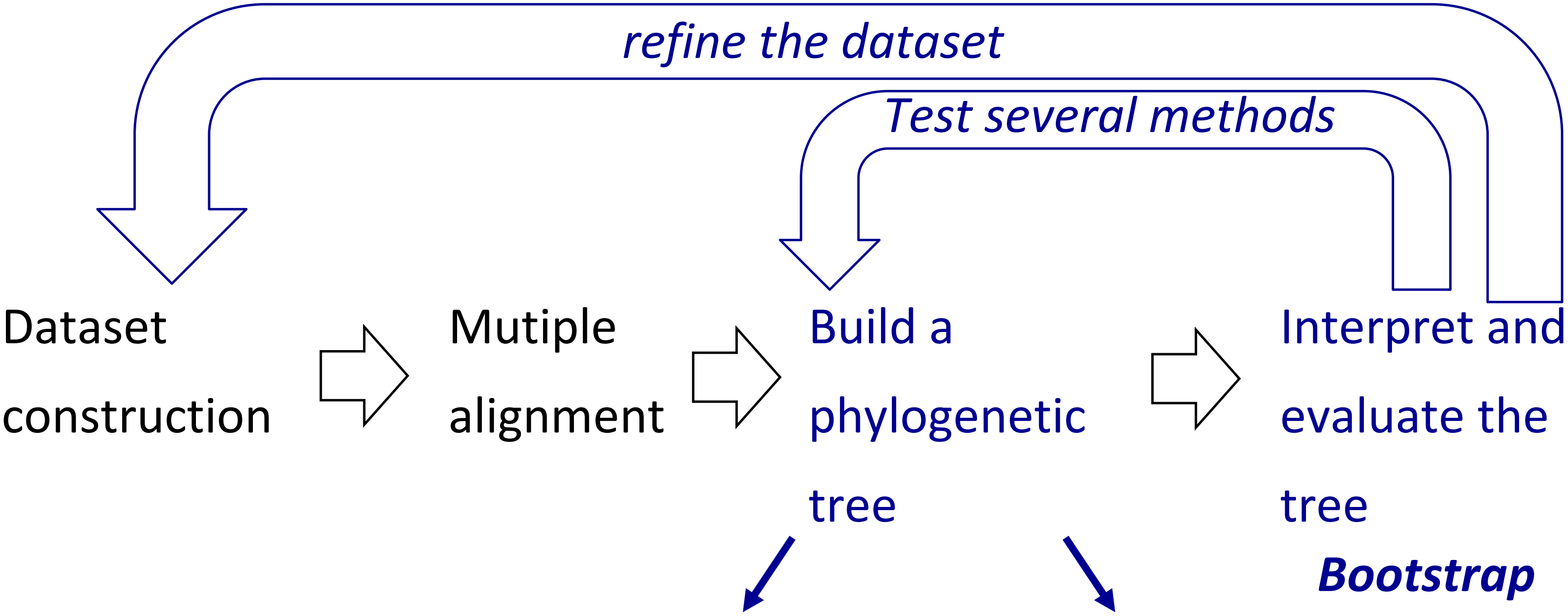
Interpret and evaluate the tree

Boostrap

Evolutionary distance choice

- ***Distance***
- ***Parsimony***
- ***Maximum Likehood***
- ***Bayesian***

Conclusion: overall view



refine the dataset

Test several methods

Dataset construction

Mutiple alignment

Build a phylogenetic tree

Interpret and evaluate the tree

Bootstrap

Evolutionary distance choice

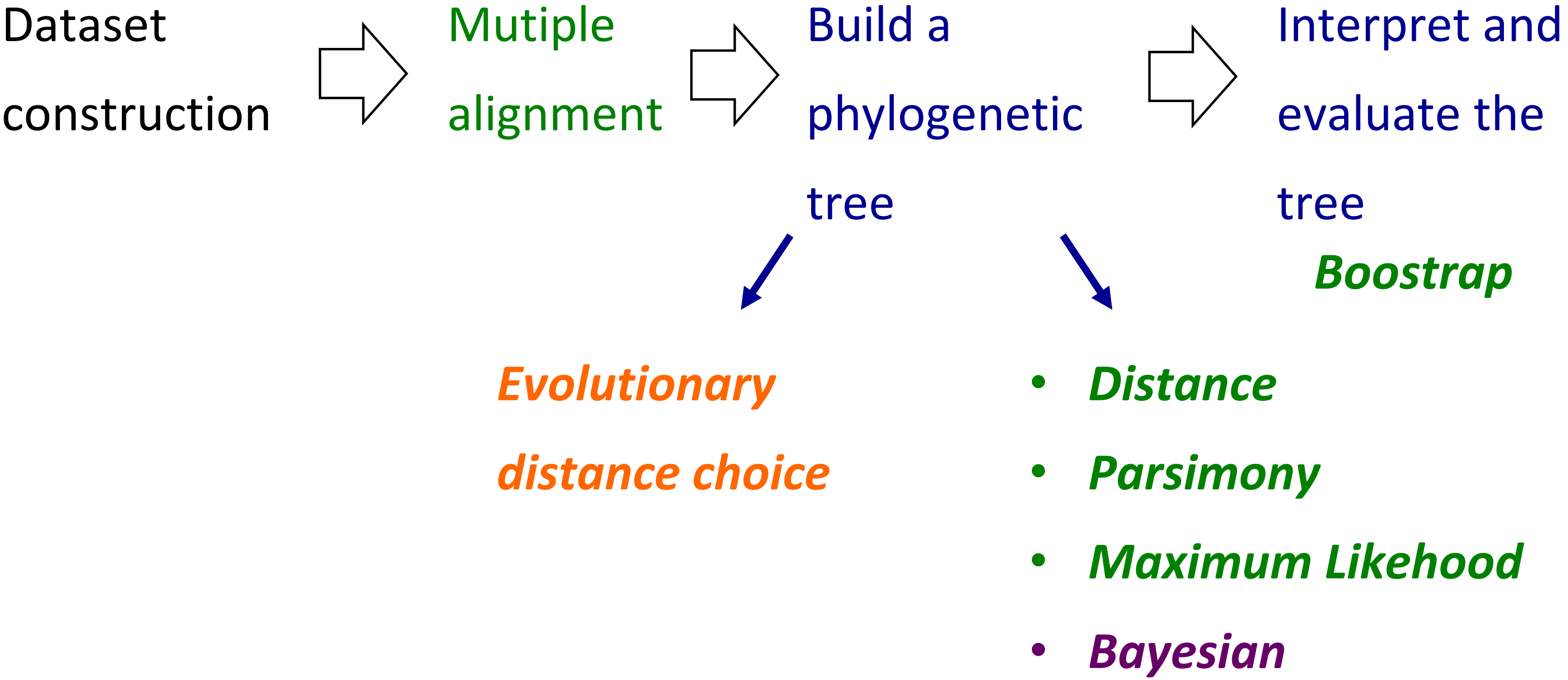
- ***Distance***
- ***Parsimony***
- ***Maximum Likehood***
- ***Bayesian***

Conclusion: overall view

Implemented in Seaview

Use modelgenerator.jar

Use Mr Bayes



Dataset construction

Mutiple alignment

Build a phylogenetic tree

Interpret and evaluate the tree

Boostrap

Evolutionary distance choice

- ***Distance***
- ***Parsimony***
- ***Maximum Likehood***
- ***Bayesian***

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

- **The Phylogenetic Handbook.** A Practical Approach to Phylogenetic Analysis and Hypothesis testing. 2009, 2nd edition. Edited by Philippe Lemey, Marco Salemi and Anne-Mieke Vandamme, eds. Cambridge University Press, Cambridge, U.K. 723 pp.

- **Concepts et méthodes en phylogénie moléculaire.** 2010. Guy Perrière and Céline Brochier-Armanet. Collection IRIS. Springer. 250 pp.

- **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 ?