Molecular Evolution and Phylogenetics

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Introduction
Tree Terminology
Homology
Molecular Evolution
Evolutionary Models
Distance Methods
Maximum Parsimony
Searching Trees
Statistical Methods
Tree Confidence
Phylogenetic Links
Credits

1. Objectives

- This short, but intensive course, has the purpose to introduce students to **the main concepts of molecular evolution and phylogenetics analysis**:
  - Homology
  - Models of Sequence Evolution
  - Cladograms & Phylograms
  - Outgroups & Ingroups
  - Rooted & Unrooted trees
  - Phylogenetic Methods: MP, ML, Distances
- The course consists of a series of **lectures, PC. Lab. sessions and manuscript discussion** that will familiarize the student with the statistical problem of phylogenetic reconstruction and its multiple uses in biology.



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2. Introduction

2.1. Three basic questions

- Why use phylogenies?
  - Like astronomy, biology is an **historical** science!
  - The knowledge of the past is important to solve many questions related to biological patterns and processes.
- Can we know the past?
  - We can postulate alternative evolutionary scenarios **(hypothesis)**
  - Obtain the proper dataset and get statistical confidence
- What means to know **"...the phylogeny"**?
  - The ancestral-descendant relationships **(tree topology)**
  - The distances between them **(tree branch lengths)**

Phylogenies are working hypotheses!!!



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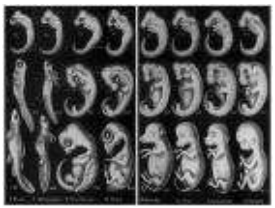
2.2. What are the roots of modern phylogenetics?

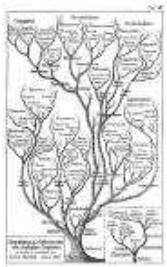
Phylogenies have been inferred by systematics ever since they were discussed by Darwin and Haeckel,











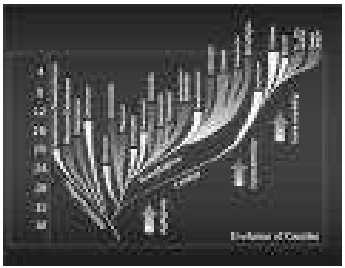


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However, since 1950s-60s classifications began to be more numerical, algorithmic and statistical. Principally due to progress in molecular biology, protein sequence data and computer development (initially, using punched card machines) [1].
Roughly, systematists divided in two:

1. Proponents of the **"Evolutionary Systematics"** classify organisms using different historical, ecological, numerical, and evolutionary arguments. It attemps to represent, not only the branching of phyletic lines (cladogenesis) but also its subsequent divergence (anagenesis) leading the invasion of a new adaptive zone by a particular class of organisms (a grade). Its representaties are Ernst Mayr[64] and George G. Simpson[89], among others.





[1] See: Chapter 5 of [65] and Chapter 10 of [26] for a detailed discussion on the issue.

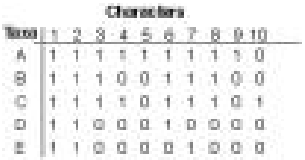


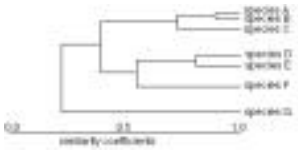
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2. Proponents who rejected the notion of theory-free method of classification, introduced **objectivity** by using explicit numerical approaches.

   (a) Numerical Taxonomy's school (**Phenetics**) originated by Michener[67], Sneath[92] and Sokal[93] in USA.







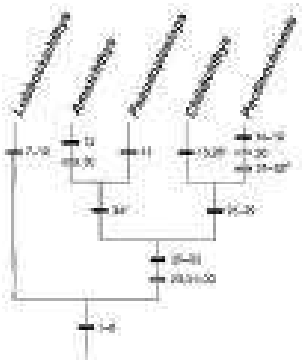
- **Main idea:**
  To score pairwise differences between OTU's (Operational Taxonomic Units) using as many characters as possible.
  Cluster by simmilarity using an algorithm that produces a single dendogram (**phenogram**)



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(b) Phylogenetic Systematic's school (**Cladistics**) originated by Hennig[44, 45] in Germany and followed by Wagner[99], Kluge[55] and Farris[21, 22] in USA.





- **Main idea:**
  To use recency of common ancestry to construct hierarchies of relationship, NOT similarity.
  Relationships depicted by phylogenetic tree, show sequence of speciation events (**cladogram**)[2].

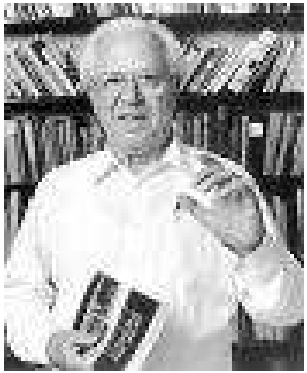
[2] Felsenstein[26] asserts that although Edwards and Cavalli-Sforza introduced parsimony, modern work on it springs from the paper of Camin and Sokal[8]



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(c) Statistical approaches developed around molecular data sets.

- Edwards and Cavalli-Sforza[9, 10] worked on the spatial representation of human gene frequencies differences, developed the **Minimum Evolution** and the **Least Square** distance methods, respectively. In order to reconcile results, they worked out an impractical **Maximum Likelihood** method and found that it was not equivalent to either of their two methods! Indeed, they discussed similarities between a **Maximum Parsimony** method and likelihood [9].

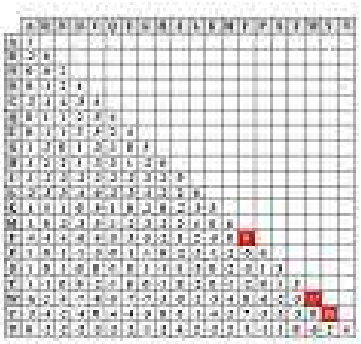




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- In the 1960s the molecular sequence data was mostly proteins. Margareth Dayhoff began to accumulate in **the first molecular database!** produced in a printed form [16]. In the second edition of the "Atlas..." they describe the **first molecular parsimony method**, based on a model in wich each of the 20 amino acids was allowed to change to any of the 19 others in a single step **(unordered method)**.



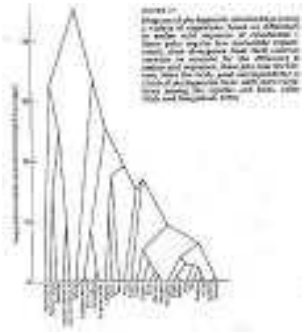




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- Although distance methods were first described by Edwards and Cavalli-Sforza [9, 10], Fitch and Margoliash [32] popularized distance matrix methods based on **least squares**. The distances were fractions of amino acids differences between a particular pair of sequences. The least squares was weighted with greather observed distance given less weight. **This introduces the concept that large distances would be more prone to random error owing to the stochasticity of evolution.**





- Explicit models of sequence evolution correcting the effects of **multiple replacement** was first implemented by Jukes and Cantor in 1969 [51].



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2.3. Applications of phylogenies

Phylogenetic information is used in different areas of biology. From population genetics to macroevolutionary studies, from epidemiology to animal behaviour, from forensic practice to conservation ecology [3]. In spite of this broad range of applications, **phylogenies are used by making inferences from**:

1. **Tree topology and branch lengths:**
    - Applications in **evolutionary genetics** deducing partial internal duplication of genes [30], recombination [28], reassortment [7], gene conversion [80], translocations [57] or, xenology [87, 78].
    - Applications in **population genetics** in order to quantify parameters and processes like gene flow [91], mutation rate, population size [25], natural selection [34] and speciation [46] [4]
    - Applications by **estimating rates and dates** in order to check clock-like behaviour of genes [31], to date events in epidemiological studies [105], or macroevolutionary events [56, 41, 40].
    - Applications by testing **evolutionary processes** like coevolution [37], cospeciation [72, 71], biogeography [95, 36], molecular adaptation, neutrality, convergence, tissue tropisms (HIV clones), the origin of geneteic code, stress effects in bacteria, etc.

[3] See [38] for a comprehensive revision on the issue
[4] See [20] for a review on these methods.

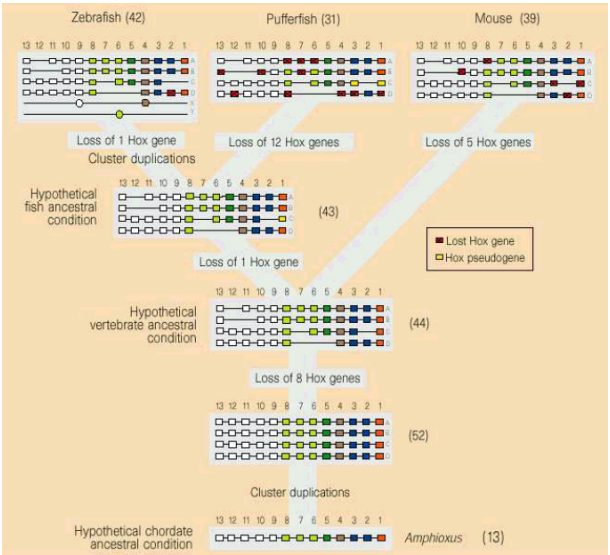


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- Applications in conservation biology [68], forensic or legal cases [47], *the list is far less than exhaustive!!!*

2. **Mapping character states on to the tree:**

   - Applications in comparative biology [39, 5, 72], in areas like animal behaviour [63, 5], development [66], speciation and adaptation [5]





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2.4. Bioinformatics uses

- **Phylogenomics**. Using genome scale phylogenetic analysis in:
  - **Systematic problems**. Testing the new animal phylogeny, **ecdysozoa** (arthropods + nematodes) vs **coelomata** (vertebrates + arthropods) [3, 103, 15]. Phylogenetic relationships among *H. sapiens, D.melanogaster* and *C. elegans* are unsolved. They are **model species** with their genomes almost full sequenced. Single gene and phylogenomics results **contadicts** each other.

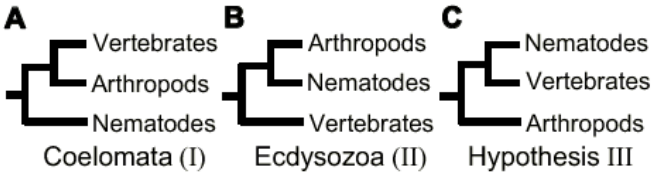
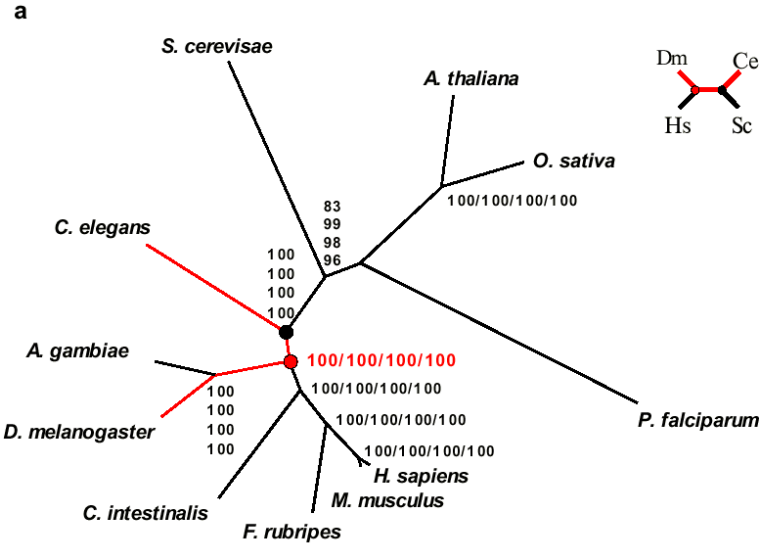


Figure 1
The three possible relationships of vertebrates, arthropods, and nematodes.



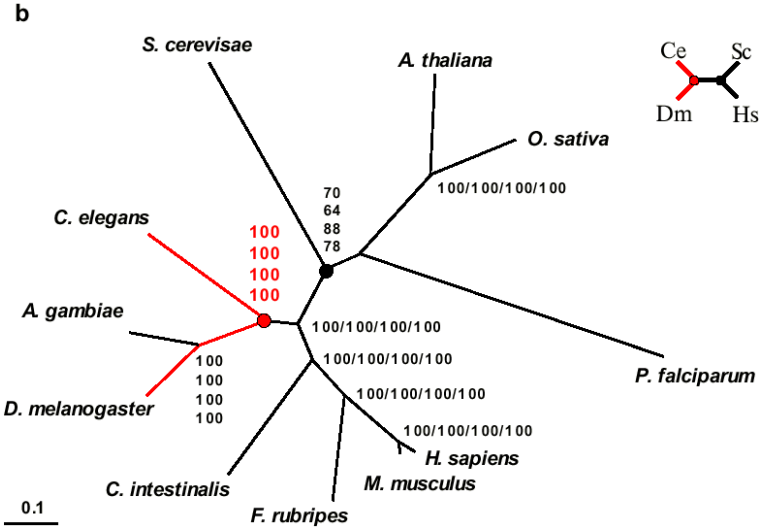
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Coelomata phylogeny using more than 1,000 sequences

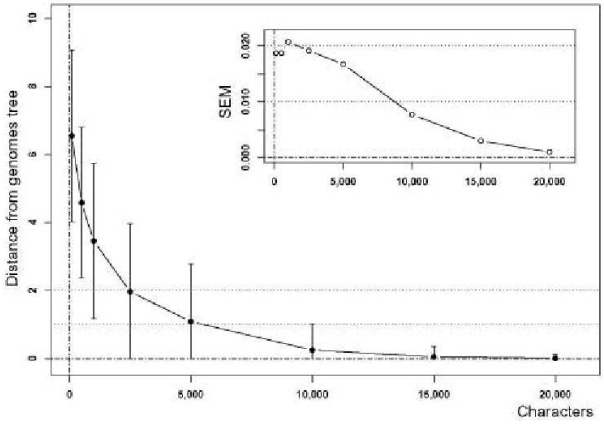


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Ecdysozoa phylogeny using more than 1,000 sequences



The use of a high number of characters give strong support on trees



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Long-branch attraction. Correction at genome scale[14]

Research

Open Access

Genome-scale evidence of the nematode-arthropod clade

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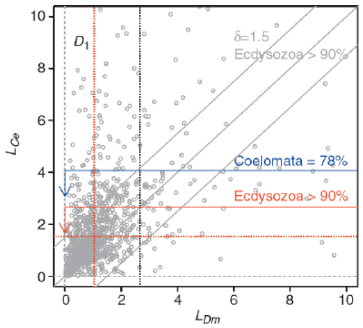
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Phylogenomics[13]

PHYLOGENOMICS AND THE RECONSTRUCTION OF THE TREE OF LIFE

Frédéric Delsuc, Henner Brinkmann and Hervé Philippe

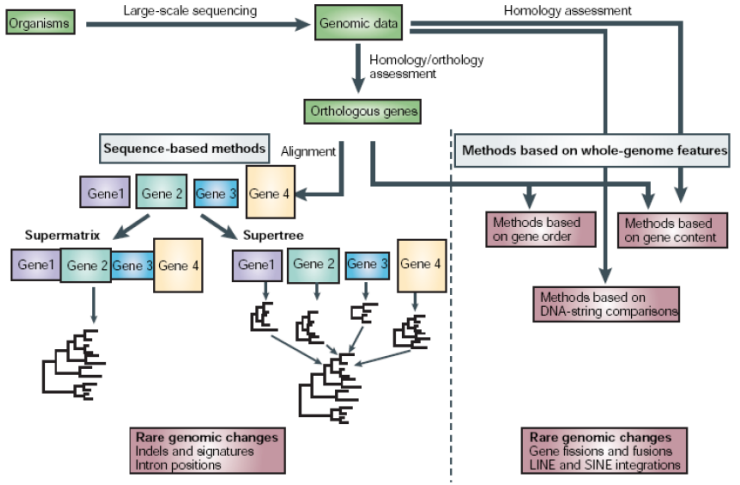
Abstract | As more complete genomes are sequenced, phylogenetic analysis is entering a new era — that of phylogenomics. One branch of this expanding field aims to reconstruct the evolutionary history of organisms on the basis of the analysis of their genomes. Recent studies have demonstrated the power of this approach, which has the potential to provide answers to several fundamental evolutionary questions. However, challenges for the future have also been revealed. The very nature of the evolutionary history of organisms and the limitations of current phylogenetic reconstruction methods mean that part of the tree of life might prove difficult, if not impossible, to resolve with confidence.



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Phylogenomics[13]

Box 2 | **Methods of phylogenomic inference**





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– **Gene function predictions**. Based principally in matching characters (functions) on to gene trees. [18, 19, 90]

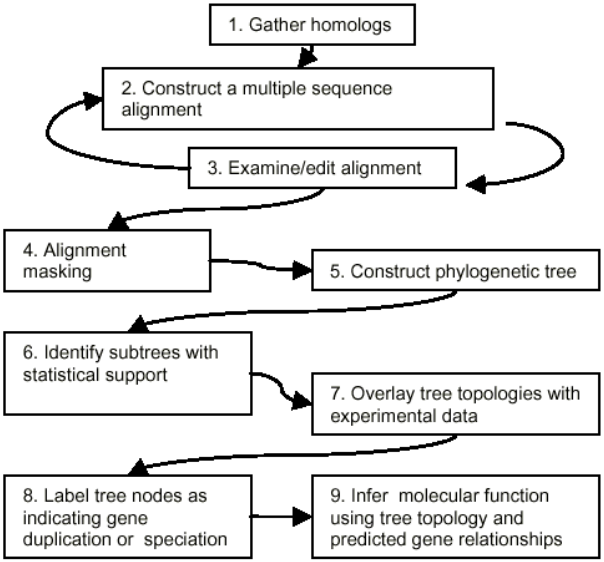
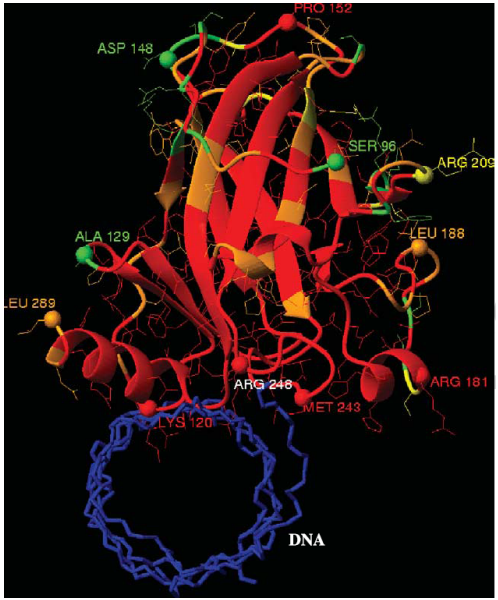


Fig. 2. Flowchart for phylogenomic analysis. Details on these steps are provided in the Methodology section.



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Selective constraints on protein codon sequences

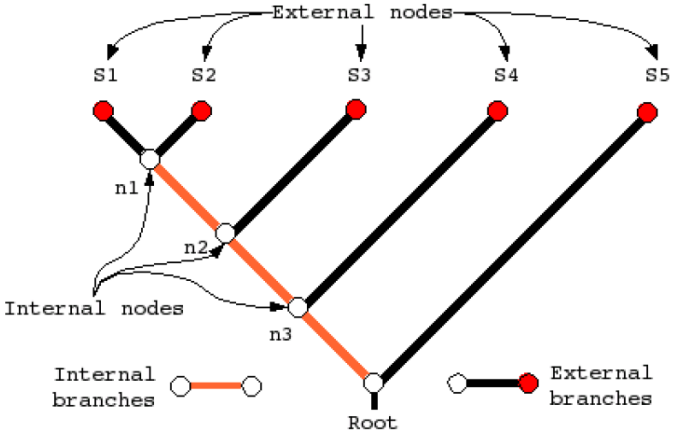


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3. Tree Terminology

3.1. Topology, branches, nodes & root

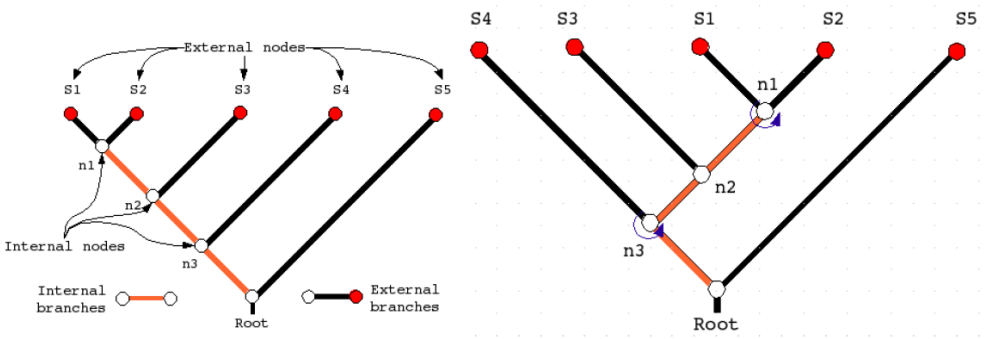
- **Nodes & branches.** Trees contain internal and external nodes and branches. In molecular phylogenetics, **external nodes** are sequences representing **genes, populations or species!**. Sometimes, **internal nodes** contain the ancestral information of the clustered species. A **branch** defines the relationship between sequences in terms of descent and ancestry.





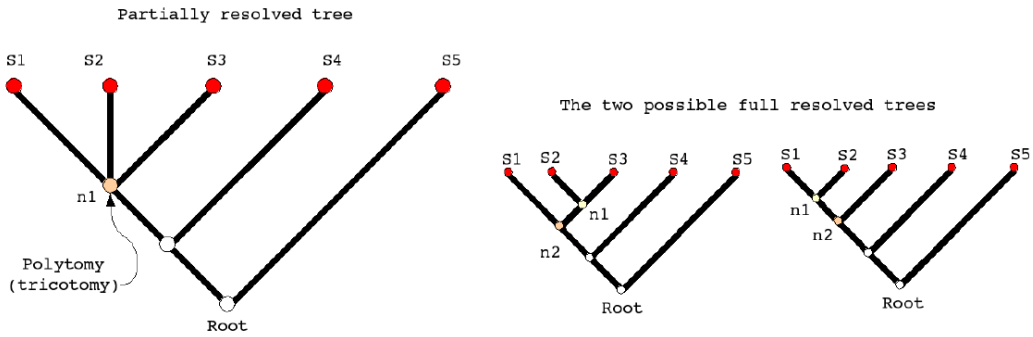
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- **Root** is the common ancestor of all the sequences.
- **Topology** represents the branching pattern. Branches **can rotate** on internal nodes. Instead of the singular aspect, the folowing trees represent a single phylogeny.



The topology is the same!!

- **Taxa**. (*plural of taxon or operaqtional taxonomic unit (OTU)*) Any group of organisms, populations or sequences considered to be sufficiently distinct from other of such groups to be treated as a separate unit.
- **Polytomies**. Sometimes trees does not show fully bifurcated (binary) topologies. In that cases, the tree is considered **not resolved**. Only the relationships of species 1-3, 4 and 5 are known.



Polytomies can be solved by using more sequences, more characters or both!!!

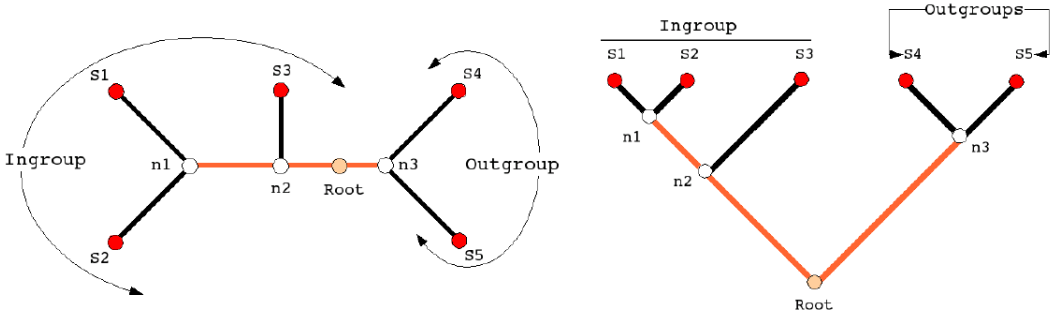


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3.2. Rooted & Unrooted trees

Trees can be **rooted** or **unrooted** depending on the explicit definition or not of **outgroup** sequence or taxa.

- **Outgroup** is any group of sequences used in the analysis that is not included in the sequences under study (**ingroup**).



- **Unrooted trees** show the topological relationships among sequences althoug it is impossible to deduce wether nodes (n_i) represent a primitive or derived evolutionary condition.
- **Rooted trees** show the evolutionary basal and derived evolutionary relationships among sequences.

Rooting by outgroup is frequent in molecular phylogenetics!!



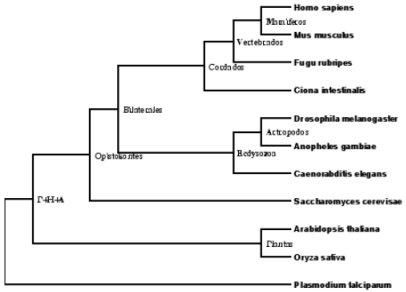
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3.3. Cladograms & Phylograms

Trees showing branching order exclusivelly (**cladogenesis**) are principally the interest of systematists[5] to make inferences on taxonomy[6]. Those interesting in the evolutionary processes emphasize on branch lengths information (**anagenesis**).



Unrooted dendogram showing branching order



Rooted cladogram (cladistic methods)

- **Dendrogram** is a branching diagram in the form of a tree used to depict degrees of relationship or resemblance.
- **Cladogram** is a branching diagram depicting the hierarchical arrangement of taxa defined by cladistic methods (the distribution of shared derived characters -synapomorphies-).

[5] The study of biological diversity.
[6] The theory and practice of describing, naming and classifying organisms

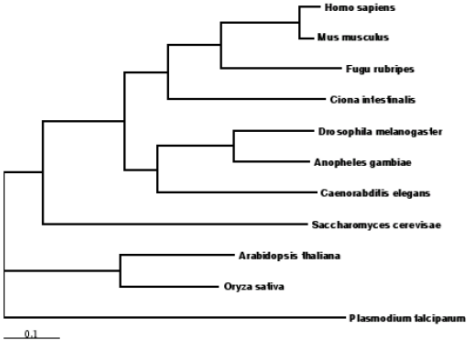


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- **Phylogram** is a phylogenetic tree that indicates the relationships between the taxa and also conveys a sense of time or rate of evolution. The temporal aspect of a phylogram is missing from a cladogram or a generalized dendogram.
- **Distance scale** represents the number of differences between sequences (e.g. 0.1 means 10 % differences between two sequences)



Unrooted phylogram showing branch lengths



Unrooted phylogram

Rooted and unrooted phylograms or cladograms are frequently used in molecular systematics!



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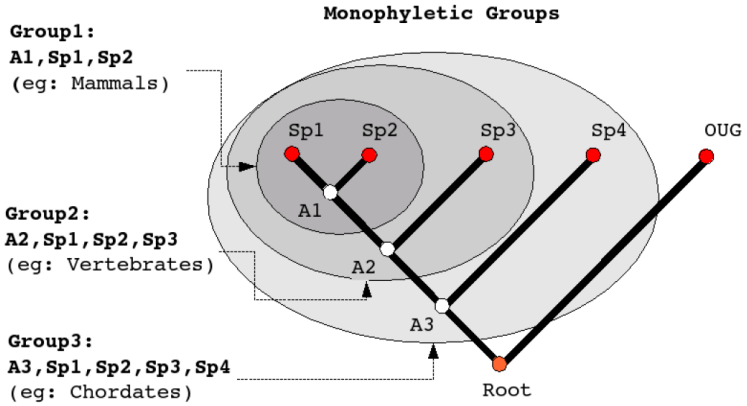
3.4. Monophyly, Paraphyly & Poliphyly

- Taxonomic groups, to be real, must represent **a community of organisms descending from a common ancestor**.
- This is the Darwinian legacy currently practised by **phylogenetic systematics.**
- A method of classification based on the study of evolutionary relationships between species in which the criterion of recency of common ancestry is fundamental and is assessed primarily by recognition of **shared derived character states** (synapomorphies).



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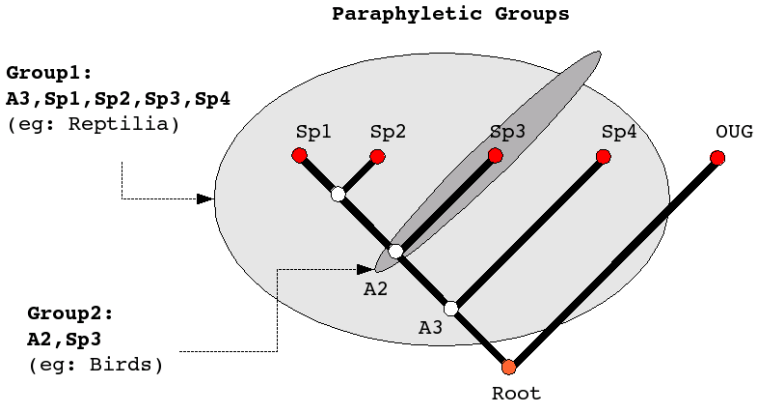
Monophyletic group represents a group of organisms with the same taxonomic title (say genus, family, phylum, etc.) that are shown phylogenetically to share a common ancestor that is exclusive to these organisms. They are, by definition, natural groups or **clades**[7].



[7] Monophyletic groups represent categories based on the common possession of apomorphic (derived) characters

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Paraphyletic group represents a group of organisms derived from a single ancestral taxon, but one which does not contain all the descendants of the most recent common ancestor[8].

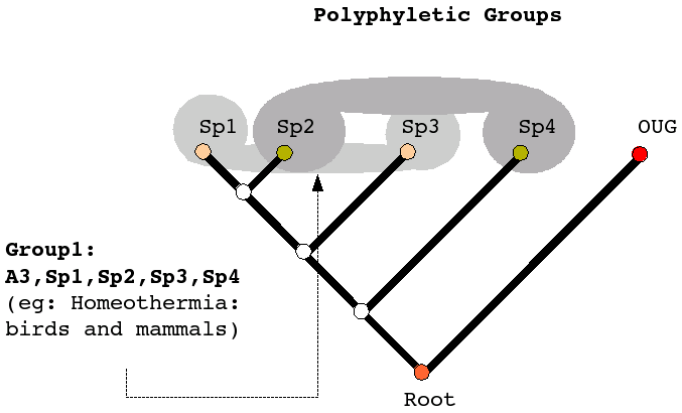


[8]Paraphyly derives from the evolutionary differentiation of some lineages, based on the accumulation of specific autapomorphies (eg: Birds)



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Polyphyletic group represents a group of organisms with the same taxonomic title derived from two or more distinct ancestral taxa[9]. Frequently, paraphyletic or polyphyletic groups are considered **grades**[10]



Sometimes is difficult to distinguish clearly between artificial groups.

The important contrast is between monophyletic and nonmonophyletic groups!!

[9] Polyphyly derives from convergence, paralelisms or reversion (homoplasy) rather than common ancestry (homology)

[10] It is an evolutionary concept supposed to represent a taxon with some level of evolutionary progress, level of organization or level of adaptation

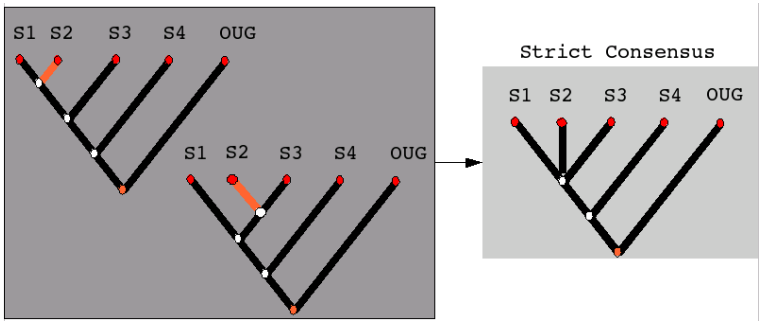


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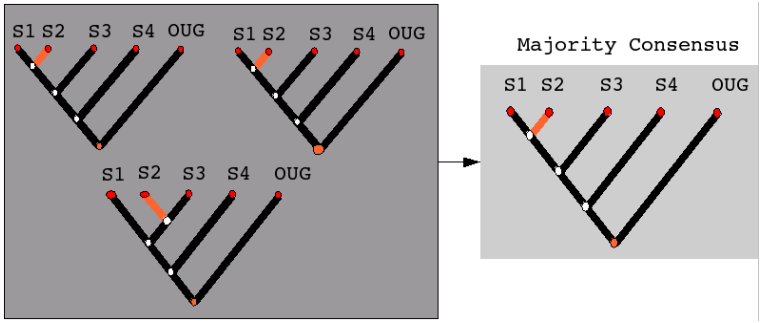
3.5. Consensus trees

It is frequent to obtain alternative phylogenetic hypothesis from a single data set. In such a case, it is usefull to summarize common or average relationships among the original set of trees. A number of different types of consensus trees have been proposed;

- The **strict consensus** tree includes only those monophyletic branches occurring in all the original trees. It is the most conservative consensus.



- The **majority rule consensus** tree uses a simple majority of relationships among the fundamental trees.



A consensus tree is a summary of how well the original trees agrees.

A consensus tree is NOT a phylogeny!!.[11]

A helpfull manual covering these and other concepts of the section can be obtained in [102, 73].

[11] Any consensus tree may be used as a phylogeny only if it is identical in topology to one of the original equally parsimonious trees.



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4. Homology

Richard Owen's (1847) most famous contributions to theorethical comparative anatomy were to distinguish between **homologous** and **analogous** features in organisms and to present the concept of **archetype**. The vertebrate archetype consists of a linear series of "vertebrae" and "apendages", little modified from a single basic plan. Each vertebra of the archetype is a **serial homologue** of every other vertebra of the archetype. Two corresponding vertebrae, each from different animal, are **special homologues** of one another, and **general homologues** of the corresponding vertebra of the archetype[12].





Homologue..."The same organ in different animals under every variety of form and function".
Analogue..."A part or organ in one animal which has the same function as another part or organ in a different animal".

[12] See [74] and chapters of the referenced book for a complete discussion of the term



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The Origin of Species. Charles Darwin. Chapter 14

> What can be more curious than that the hand of a man, formed for grasping, that of a mole for digging, the leg of the horse, the paddle of the porpoise, and the wing of the bat, should all be constructed on the same pattern, and should include similar bones, in the same relative positions?
>
> How inexplicable are the cases of serial homologies on the ordinary view of creation!
>
> Why should similar bones have been created to form the wing and the leg of a bat, used as they are for such totally different purposes, namely flying and walking?





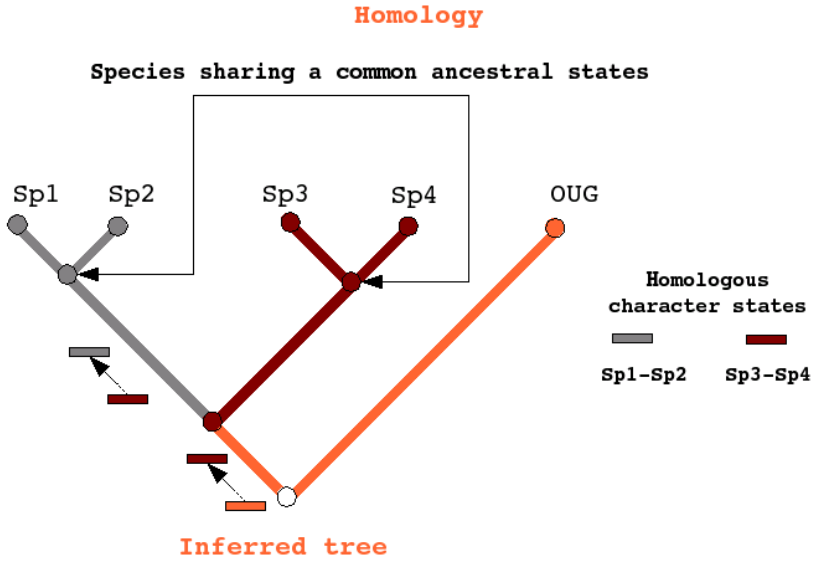
Since Darwin homology was the result of descent with modification from a common ancestor.



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4.1. Homoplasy

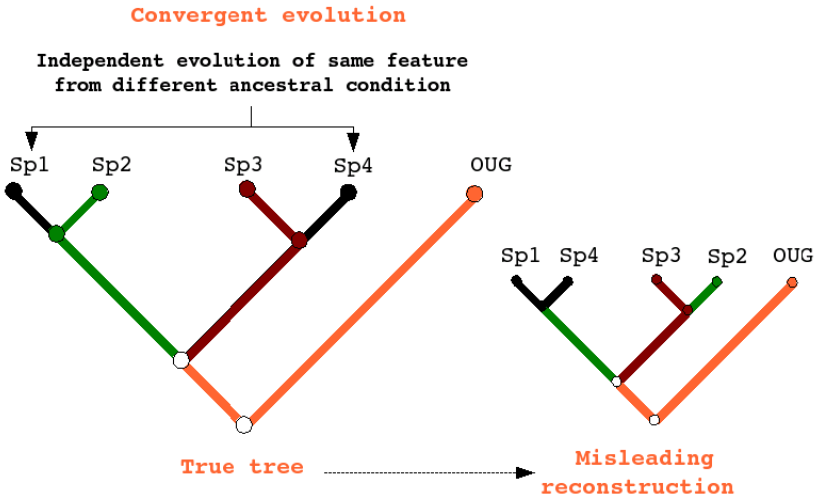
- Similarity among species could represent true homology (just by sharing the same ancestral state) or, **homoplastic** events like **convergence, parallelism** or **reversals**;
- **Homology** is *a posteriori* tree construction definition.





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- **Convergences** are ...

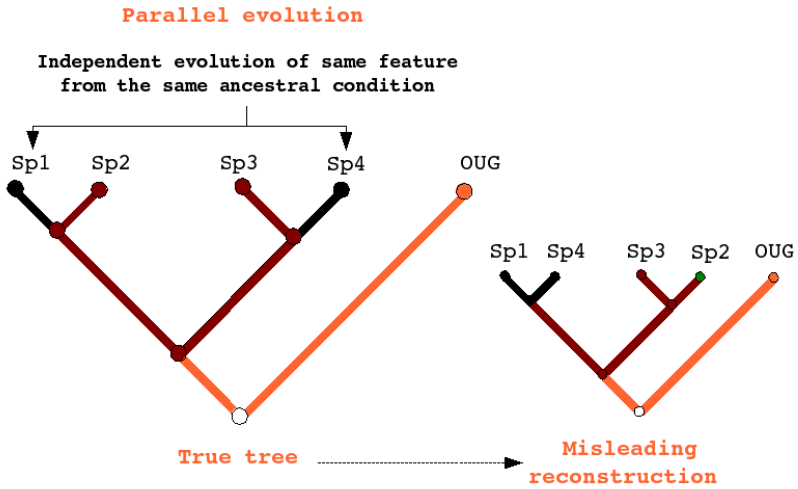


Homoplasy can provide misleading evidence of phylogenetic relationships!! (if mistakenly interpreted as homology).



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- **Parallels** are ...

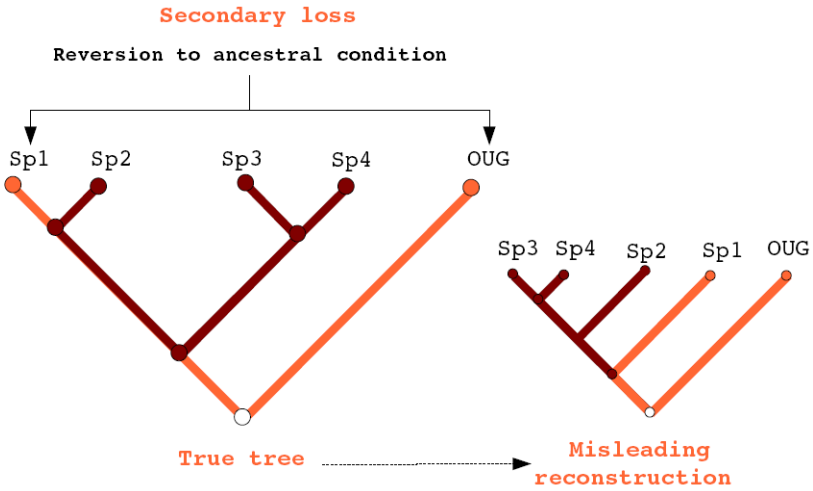


Homoplasy can provide misleading evidence of phylogenetic relationships!! (if mistakenly interpreted as homology).



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- **Reversions** are ...



Homoplasy can provide misleading evidence of phylogenetic relationships!! (if mistakenly interpreted as homology).



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4.2. Similarity

- For molecular sequence data, **homology** means that two sequences or even two characters within sequences are descended from a common ancestor.
- This term is frequently mis-used as a synonym of **similarity**.
- as in **two sequences were 70% homologous**.
- **This is totally incorrect!**
- Sequences show a certain amount of similarity.
- From this similarity value, we can probably infer that the sequences are homologous or not.
- Homology is like pregnancy. You are either pregnant or not.
- Two sequences are either homologous or they are not.

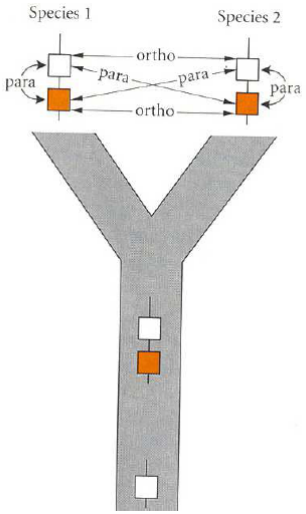


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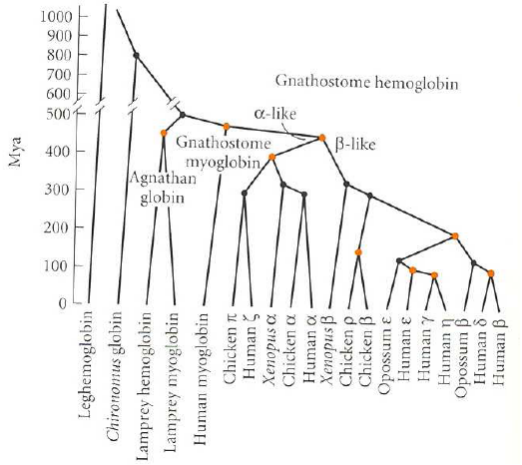
4.3. Sequence homology

In molecular studies it is important to distinguish among kinds of **homology**[33];

- **Ortholog**: Homologous genes that have diverged from each other after speciation events (e.g., human β- and chimp β-globin).
- **Paralog**: Homologous genes that have diverged from each other after gene duplication events (e.g., β- and γ-globin)



- **Xenolog**: Homologous genes that have diverged from each other after lateral gene transfer events (e.g., antibiotic resistance genes in bacteria).
- **Homolog**: Genes that are descended from a common ancestor (e.g., all globins).





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- **Positional homology**: Common ancestry of specific amino acid or nucleotide positions in different genes.

```
    11 50462
Homo.sapie VGLLGRTGSGKSTLLSAFLRLLNTEG-EIQI
Mus.muscul VGLLGRTGSGKSTLLSAFLRMLNIKG-DIEI
Fugu.rubri MGLLGRTGSGKSTLLSALLRLASTDG-EISI
Ciona.inte VGIVGRTGAGKSSLISTLFRLNEYSKGSVMI
Droso.mela VGIVGRTGAGKSSLIGALFRLAHIEG-EIFI
Anoph.gamb VGIVGRTGAGKSSLIGALFRLAQVEG-EIRL
Caeno.eleg VGIVGRTGAGKSSLTLALFRIIEADGGSIEI
Sacch.cere IGIVGRTGAGKSTIITALFRFLEPETGHIKI
Arabi.thal IGIVGRTGSGKTTLISALFRLVEPVGGKIVV
Oryza.sati IGVVGRTGSGKSTLVQALFRLVEPVEGHIIV
Plasm.falc IGIVGKSGAGKSTMILSILGLIGTTRGRITI
```


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4.4. Types of data

All of the experimental data gathered by molecular biologists fall into one of the two broad categories: **discrete characters** and similarities or **distances**.

- A discrete character provides data about an individual species or sequences.
- Character data are often transformed into distances.
- Discrete character data are those for which a data matrix X assigns a **character state** x_{ij} to each taxon i for each character j.
- Characters may be binary or multistate.
- Multistate characters may be ordered or unordered, depending on whether an ordering relationship is imposed upon the possible states

- The concepts of **character order** and **character polarity** should not be confused. The former defines the allowed character-states transformations, whereas the later refers to the **direction** of evolution.
- Nucleotide sequence data are generally treated as unordered multistate characters, since there is no *a priori* reasons to assume, for example, that state C is intermediate between A and G.



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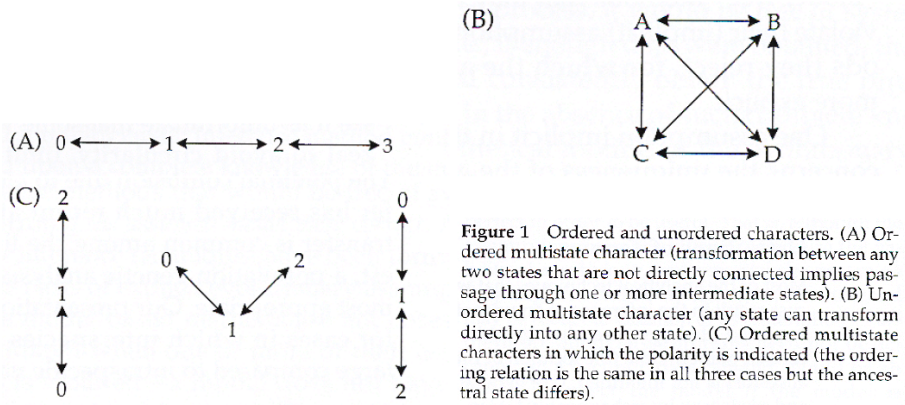


Figure 1 Ordered and unordered characters. (A) Ordered multistate character (transformation between any two states that are not directly connected implies passage through one or more intermediate states). (B) Unordered multistate character (any state can transform directly into any other state). (C) Ordered multistate characters in which the polarity is indicated (the ordering relation is the same in all three cases but the ancestral state differs).



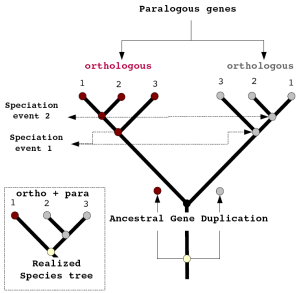
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5. Molecular Evolution

5.1. Species & Genes trees

It is obvious that all phylogenetic reconstruction of sequences are **genes trees**. The naive expectation of molecular systematics is that phylogenies for genes match those of the organisms or species (**species trees**). *There are many reasons why this needs not be so!!.*

1. If there were **duplications**, (gene family) only the phylogenetic reconstruction of **orthologous** sequences could guarantize the expected[13] or true **species tree**.



[13] The expected tree is the tree that can be constructed by using infinitely long sequences

2. In presence of **polymorphic alleles** at a locus, the time of gene splitting (producing polimorphisms) is usually earlier than population or species splitting.

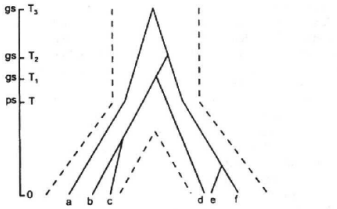


FIGURE 5.2. Diagram showing that the time of gene splitting (gs) is usually earlier than the time of population splitting (ps) when polymorphism exists. From Takahata and Nei (1985).

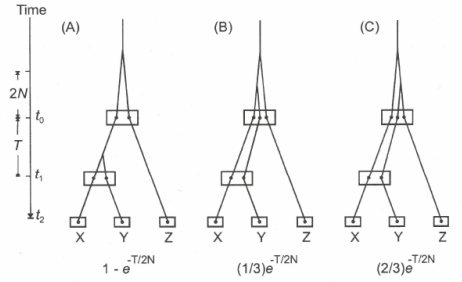
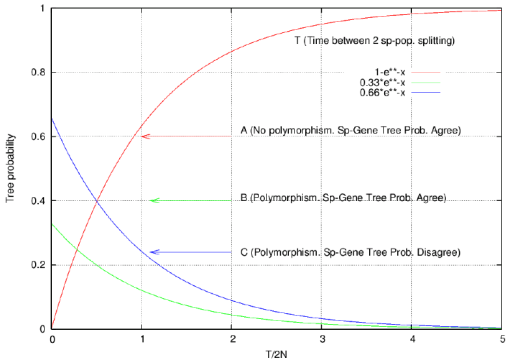


FIGURE 5.3. Three possible relationships between the species and gene trees for the case of three species in the presence of polymorphism. The times of the first and second species splitting are t_0 and t_1, respectively. The probability of occurrence of each tree is given underneath the tree. $T = t_1 - t_0$, and N is the effective population size. From Nei (1987).

The probability to obtain the expected species tree depends on T & N and random processes like lineage sorting [73].



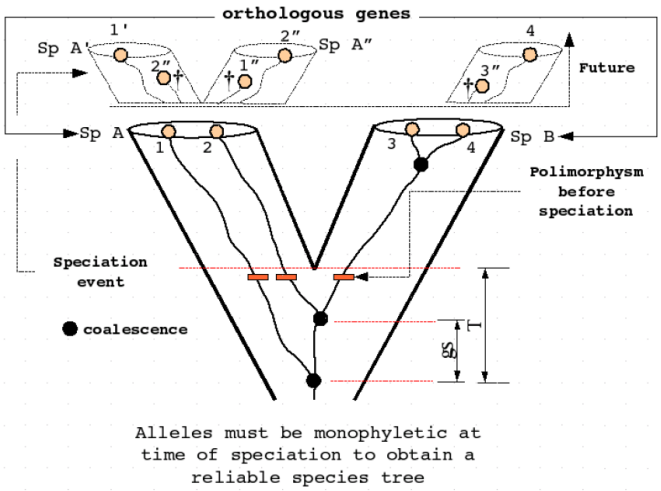
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- If alleles are monophyletic before population or species splitting, at time T/2N increase (longer times or low pop. numbers-*mammals*-), the probability to agree between trees increases (red, A tree pattern).
- This probability decreases if polymorphic alleles are present before the pop. splitting. For a constant T value, increasing population size reduces the probability of random processes reducing polymorphism (green, B tree pattern).
- In such conditions the probability of disagreement between trees is higher (blue, C tree pattern).
- Indeed future sorting events could prevent the correct tree gene.



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To obtain **a reliable tree of intraspecific populations or closely related species**, a large number of unlinked genes need to be used.

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5.2. Molecular clock

The **molecular clock hypothesis** postulates that for any given macromolecule (a protein or DNA sequence), the rate of evolution -*measured as the mean number of amino acids or nucleotide sequence change per site per year*- is approximately constant over time in all the evolutionary lineages [106].

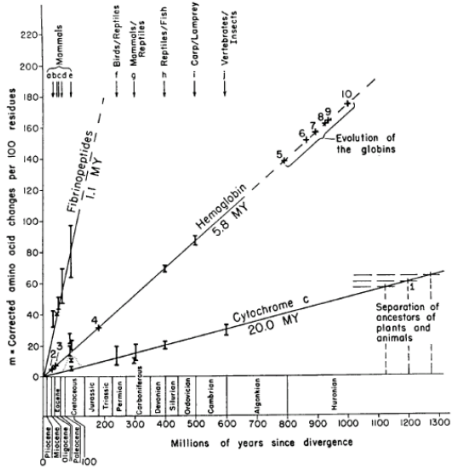


Fig. 8.3. Rates of amino acid substitution in the fibrinopeptides, hemoglobin, and cytochrome *c*. Comparisons for which no adequate time coordinate is available are indicated by numbered crosses. Point 1 represents a date of 1200 ± 75 MY (million years) for the separation of plants and animals, based on a linear extrapolation of the cytochrome *c* curve. Points 2–10 refer to events in the evolution of the globin family. The δ/β separation is at point 3, γ/β is at 4, and α/β is at 500 MY (carp/lamprey). From Dickerson (1971).

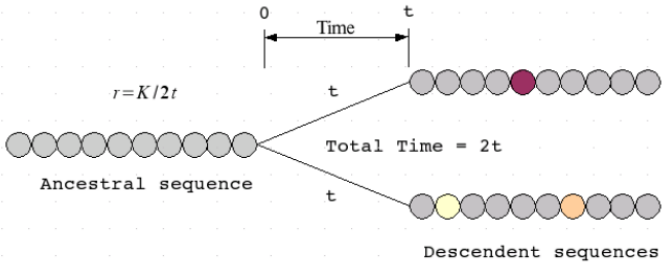
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This hypothesis has estimulated much interest in the use of macromolecules in evolutionay studies for two reasons:

- Sequences can be used as molecular markers to **date** evolutionary events.
- The degree of rate change among sequences and lineages can provide insights on **mechanisms** of molecular evolution. For example, a large increase in the rate of evolution in a protein in a particular lineage may indicate adaptive evolution.

Substitution rate estimation

It is based on the number of aa substitution (distance) and divergence time (fossil calibration),



There is no universal clock

It is known that **clock variation** exists for:

- different molecules, *depending on their functional constraints*,
- different regions in the same molecule,

Rates of amino acid substitution at the surface and heme pocket regions of the hemoglobin α- and β-chains (Kimura and Ohta, 1973b).

Region	α-chain	β-chain
Surface	1.4 (18)	2.7 (23)
Heme pocket	0.17 (19)	0.24 (21)

Note: The rate represents 'per amino acid site per year'. The values in the table should be multiplied by 10^{-9}. The figures in brackets are the number of amino acid sites involved.



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- different base position (synonimous-nonsynonimous),

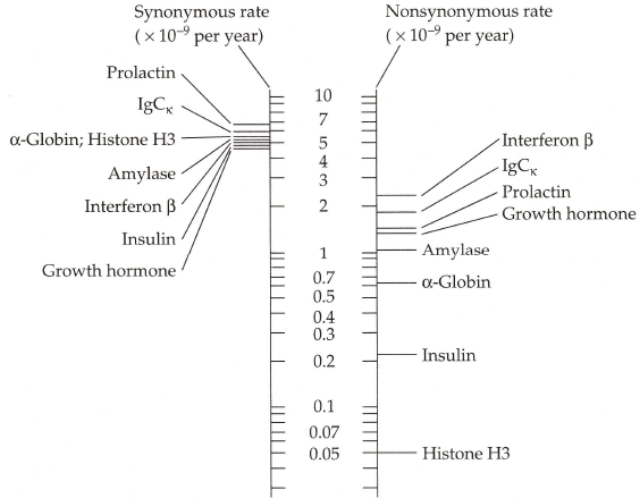
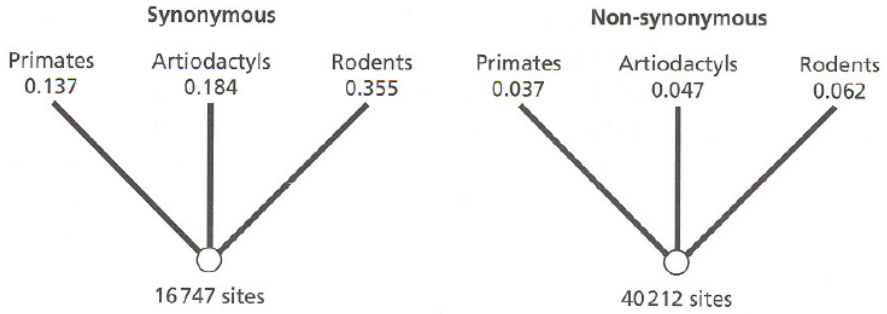


Figure 8.14 Comparison of rates of synonymous and nonsynonymous nucle otide substitutions. Synonymous rates are generally much faster and much more uniform than nonsynonymous rates. (From Kimura 1986.)



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- different genomes in the same cell,
- different regions of genomes,
- different taxonomic groups for the same gene (**lineage effects**)





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Sometimes there are local clocks

for example mouse and rat using (*hamster as outgroup*)[14]

TABLE 8.1 Numbers of nucleotide substitutions per 100 sites between species[a]

	Synonymous sites		Nonsynonymous sites	
Species pair	K_S	L^b	K_A	L^b
Mouse–rat	18.0 ± 0.7	4,229	1.8 ± 0.1	15,217
Mouse–hamster	30.3 ± 1.0	4,229	2.9 ± 0.1	15,217
Rat–hamster	31.3 ± 1.0	4,229	2.7 ± 0.1	15,217
Mouse–human	53.4 ± 1.5	4,229	5.2 ± 0.2	15,217
Rat–human	51.6 ± 1.5	4,229	5.0 ± 0.2	15,217
Hamster–human	52.3 ± 1.5	4,229	5.1 ± 0.1	15,217

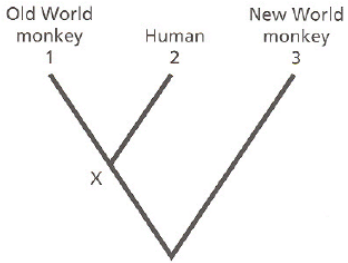
[14] See [4] for an actualized review.



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Relative Rate Test

How to test the molecular clock?[15]



Results

(a) Synonymous sites in nine nuclear genes (3520 bp)
$d_{12} = 6.7$
$d_{13} - d_{23} = 2.3 \pm 0.6$*

(b) ψη-globin pseudogene (1827 bp)
$d_{12} = 7.9$
$d_{13} - d_{23} = 1.5 \pm 0.4$*

(c) Three introns (3376 bp)
$d_{12} = 6.9$
$d_{13} - d_{23} = 1.0 \pm 0.5$

(d) Two flanking regions (936 bp)
$d_{12} = 7.9$
$d_{13} - d_{23} = 3.1 \pm 1.1$*

[15] See [79] and download RRtree!!

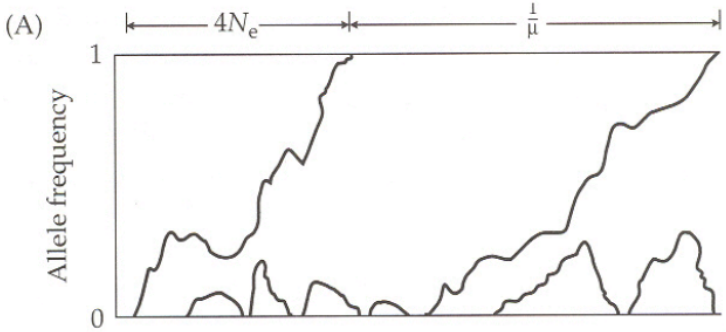


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5.3. Neutral theory of evolution

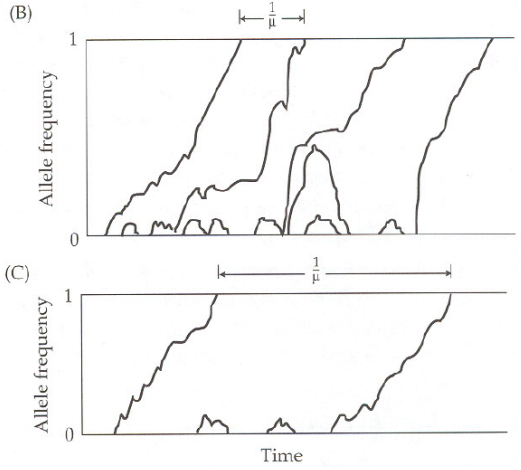
At molecular level, the most frequent changes are those involving fixation in populations of neutral selective variants [53].

- Allelic variants are functionaly equivalent
- Neutralism does not deny adaptive evolution
- Fixation of new allelic variants occurs at a constant rate μ.
- This rate does not depends on any other population parameter, then it's **like a clock!!** $2N\mu * 1/2N = \mu$



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Figure 8.1 Diagram showing the trajectory of neutral alleles in a population. New alleles enter the population by mutation and have an initial allele frequency of $1/2N$. Most alleles are lost, but those that go to fixation take an average of $4N$ generations. The time between successive fixations of neutral alleles is $1/\mu$ generations. (A) A moderate size population. (B) The same population size; a higher mutation rate gives the same time to fixation, but less time between fixations. (C) A smaller population has alleles that go to fixation more rapidly, but the time between fixations is still $1/\mu$. (After Kimura 1980.)



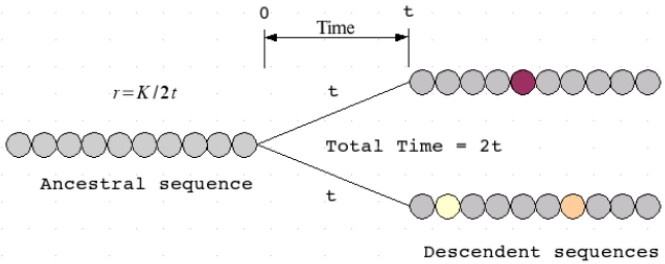


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6. Evolutionary Models

6.1. Multiple Hits

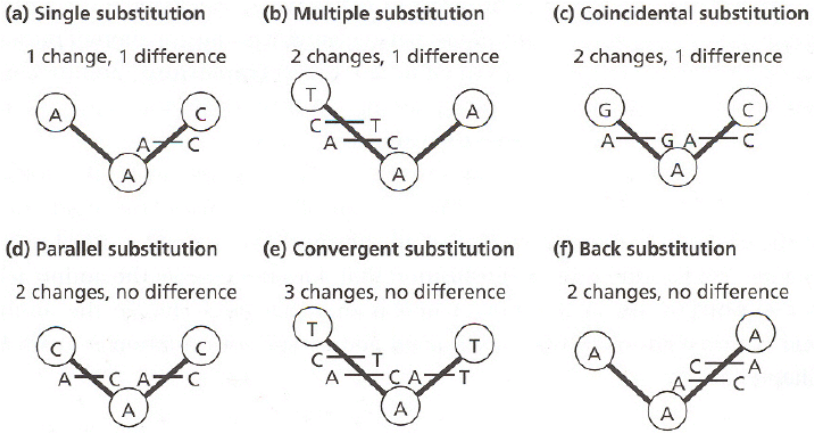
- The mutational change of DNA sequences varies with region. Even considering protein coding sequence alone, the patterns of nucleotide substitution at the first, second or third codon position are not the same.
- When two DNA sequences are derived from a common ancestral sequence, the descendant sequences gradually diverge by nucleotide substitution.
- A simple measure of sequence divergence is the proportion $p = N_d/N_t$ of nucleotide sites at which the two sequences are different.





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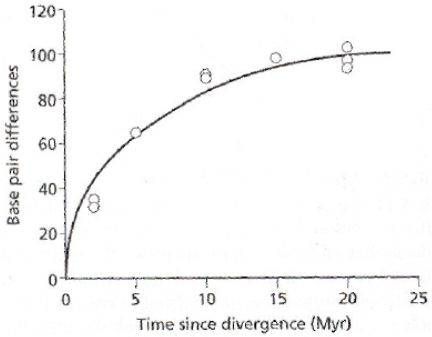
- When p is large, it gives an underestimate of the number of of substitutions, because it does not take into account **multiple substitutions**.

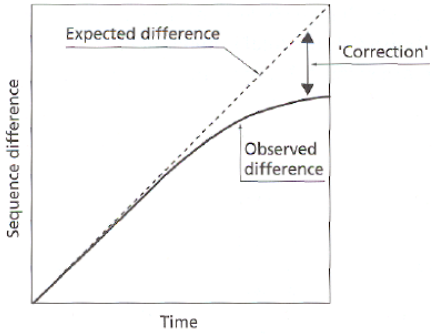




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- Sequences may saturate due to multiple changes (**hits**) at the same position after lineage splitting.
- In the worst case, data may become random and all the **phylogenetic information** about relationships can be lost!!!



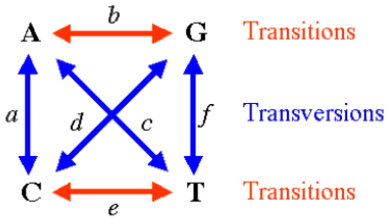




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6.2. Models of nucleotide substitution

- In order to estimate **the number of nucleotide substitutions ocurred** it is necessary to use a mathematical model of nucleotide substitution. The model would consider the nucleotide frequencies and the instantaneous rate's change among them.

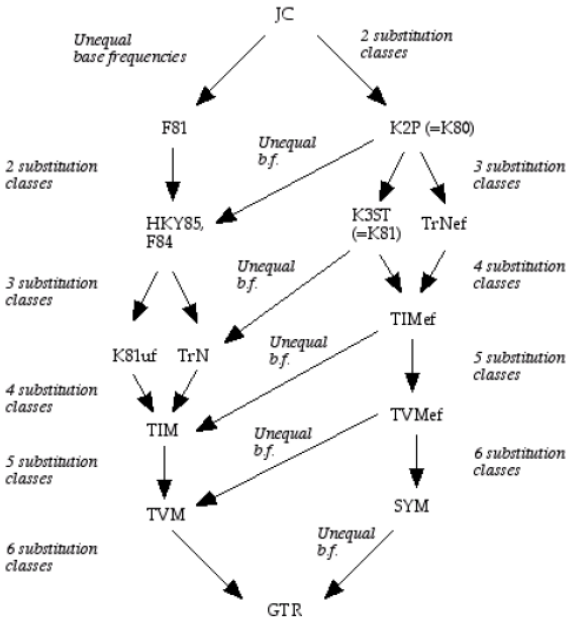


Designation	Rate params	Base frequencies	Number of free params
JC	a=b=c=d=e=f	$\pi_A = \pi_C = \pi_G = \pi_T$	1
K80, K2P	a=c=d=f, b=e	$\pi_A = \pi_C = \pi_G = \pi_T$	2
TrNef	a=c=d=f, b, e	$\pi_A = \pi_C = \pi_G = \pi_T$	3
K81, K3ST	a=f, b=e, c=d	$\pi_A = \pi_C = \pi_G = \pi_T$	3
TVMef	a, c, d, f, b=e	$\pi_A = \pi_C = \pi_G = \pi_T$	5
TIMef	a=f, c=d, b, e	$\pi_A = \pi_C = \pi_G = \pi_T$	4
SYM	a, b, c, d, e, f	$\pi_A = \pi_C = \pi_G = \pi_T$	6
F81	a=b=c=d=e	$\pi_A, \pi_C, \pi_G, \pi_T$	4
HKY	a=c=d=f, b=e	$\pi_A, \pi_C, \pi_G, \pi_T$	5
TrN	a=c=d=f, b, e	$\pi_A, \pi_C, \pi_G, \pi_T$	6
K81uf	a=f, b=e, c=d	$\pi_A, \pi_C, \pi_G, \pi_T$	6
TVM	a, c, d, f, b=e	$\pi_A, \pi_C, \pi_G, \pi_T$	8
TIM	a=f, c=d, b, e	$\pi_A, \pi_C, \pi_G, \pi_T$	7
GTR, REV	a, b, c, d, e, f	$\pi_A, \pi_C, \pi_G, \pi_T$	9



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- Interrrelationships among models for estimating the number of nucleotide substitutions among a pair of DNA sequences





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- For constructing phylogenetic trees from distance measures, sophisticated distances are not neccesary more efficient.

Table 3.3 Observed numbers of the 10 pairs of nucleotides between the DNA sequences for the human and Rhesus monkey mitochondrial cytochrome *b* genes.

	Transition		Transversion				Identical Pair					
Codon Position	TC	AG	TA	TG	CA	CG	TT	CC	AA	GG	n_d	Total (n)
First	21	22	5	1	5	4	68	93	100	56	58	375
Second	20	3	6	1	0	2	140	87	71	45	32	375
Third	60	16	6	5	49	2	11	122	102	2	138	375
All	101	41	17	7	54	8	219	302	273	103	228	1125

Note: The numbers at the first, second, and third codon positions are shown separately.

- Indeed, by using sophisticated models distances show higher variance values.

Table 3.4 Estimates ($\hat{d}$) of the number of nucleotide substitutions per site between the human and Rhesus monkey mitochondrial cytochrome *b* genes for the first, second, and third codon positions ($\hat{d} \times 100$).

Position in Codon	p	Jukes-Cantor	Kimura	Tajima-Nei	Tamura-Nei
First	15.5 ± 1.9	17.3 ± 2.4	17.8 ± 2.5	18.0 ± 2.6	17.9 ± 2.5
Second	8.5 ± 1.4	9.1 ± 1.6	9.2 ± 1.7	9.2 ± 1.7	9.3 ± 1.7
Third	36.8 ± 2.5	50.6 ± 4.9	52.3 ± 5.4	66.5 ± 9.4	87.9 ± 39.0



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- Of course, corrected distances are greather than the observed.

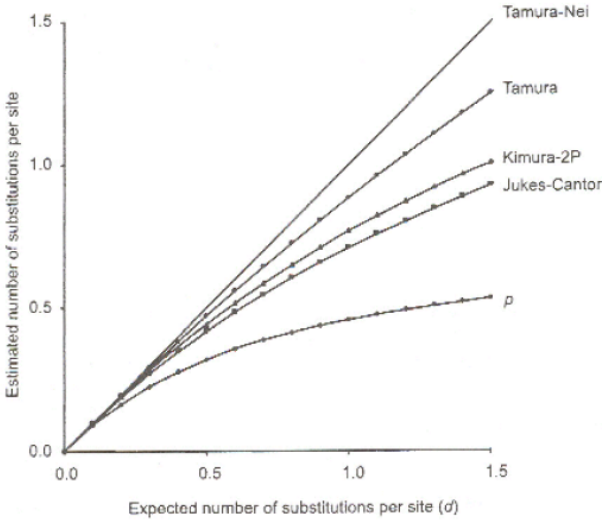


FIGURE 3.1. Estimates of the number of nucleotide substitutions obtained by different distance measures when actual nucleotide substitution follows the Tamura-Nei model. The nucleotide frequencies assumed are $g_A = 0.3$, $g_T = 0.4$, $g_C = 0.2$, and $g_G = 0.1$; and the two transition/transversion rate ratios assumed are $\alpha_1/\beta = 4$ and $\alpha_2/\beta = 8$.



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Distance correction methods share several assumptions:

- All nucleotide sites change independently.
- The substitution rate is constant over time and in different lineages
- The base composition is at equilibrium (all sequences have the same base frequencies)
- The conditional probabilities of nucleotide substitutions are the same for all sites and do not change over time.

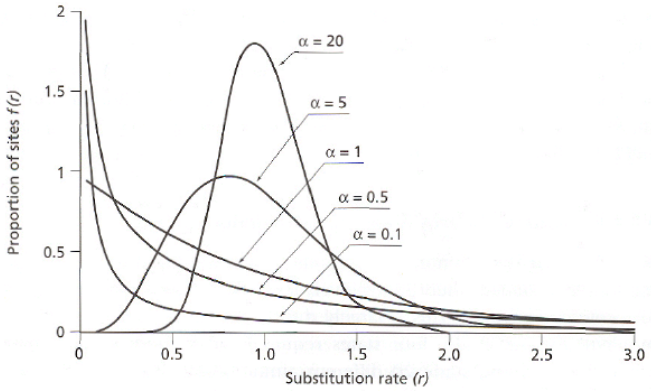
While these assumptions make the methods tractable, they are in many cases unrealistic.



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6.3. Rate heterogeneity correction

- In the evolutionary models considered, the rate of nucleotide substitution is assumed to be the same for all nucleotide. This rarely holds, and rates varies from site to site.
- In the case of protein coding genes this is obvious: 1, 2 and 3 positions.
- In the case of RNA coding genes, secondary structure consisting in loops and stems have different substitutions rates.



- Statistical analyses have suggested that the rate variation approximately follows the gamma (Γ) distribution



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- Rate variation on different genes,

Type of sequences	α
Nuclear genes	
Albumin genes	1.05
Insulin genes	0.40
c-myc genes	0.47
Prolactin genes	1.37
16S-like rRNAs, stem region	0.29
16S-like rRNAs, loop region	0.58
$\psi\eta$-globin pseudogenes	0.66
Viral genes	
Hepatitis B virus genomes	0.26
Mitochondrial genes	
12S rRNAs	0.16
Position 1 of four genes	0.18
Position 2 of four genes	0.08
Position 3 of four genes	1.58
D-loop region	0.17
Cytochrome *b*	0.44

- Low α values corresponds to large rate variation. As α gets larger the rate of variation diminishes, until as α approaches ∞ all sites have the same substitution rate [104].
- Models are labeled as **JC**+Γ, **K80**+Γ, **HKY**+Γ, *etc.*
- Indeed models can be corrected by considering the **proportion of invariable sites** (I) and the **nucleotide frequency** (F): (**JC**+Γ+$I+F$) ; (**K80**+$\Gamma+I+F$) ; (**HKY**+$\Gamma+I+F$); *etc.*



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6.4. Selecting models of evolution

The best-fit model of evolution for a particular data set can be selected through statistical testing. The fit to the data of different models can be contrasted through **likelihood ratio tests (LRTs)** , the **Akaike (AIC)** or the **Bayesian (BIC)** information criteria[77].

A natural way of comparing two models is to contrast their likelihood using the LRT statistic:

$$\Delta = 2(log_e L_1 - log_e L_0)$$

Where L_1 is the maximum likelihood under the more parameter-rich, complex model(i.e., alternative hypothesis) and L_0 is the maximum likelihood under the less parameter-rich, simple model (i.e., null hypothesis).

When model comparison is not nested, the **AIC** criteria, which measures the expected distance between the true model and the estimated model can be used.

$$AIC_i = -2(log_e L_i + 2N_i)$$

Where N_i is the number of free parameters in the ith model and L_i is the maximum likelihood value of the data under the ith model.[16]

When LRT is significant ($p \leq 0.05$, Chi-square comparison, degrees of freedom equal to the difference in number of free parameters between the two models), the more complex model is favored.

[16] See [75] for a clear theorethical and practical explanation on sequence model test's methods.



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Comparing 2 different **nested** models through an LRT means testing hypothesis about data. **MODELTEST** program [76] tests hierarchical LRTs in an ordered way and compute **AIC** values.

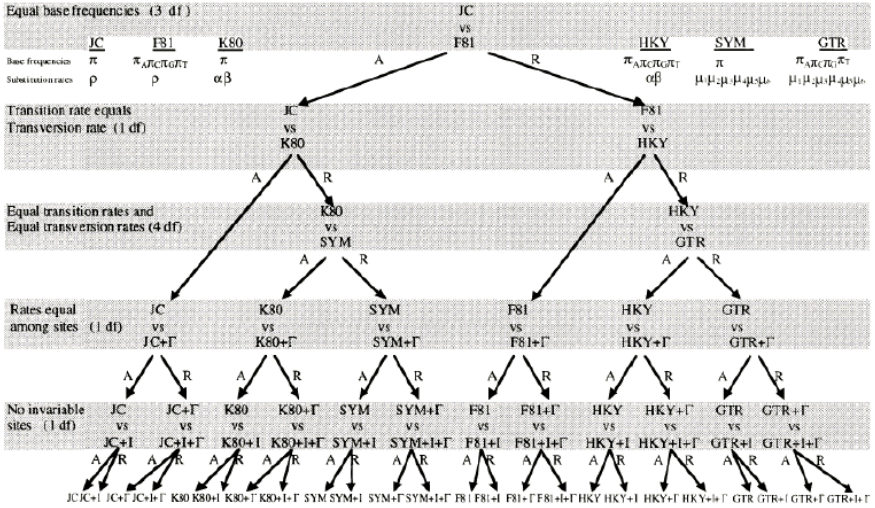


Fig. 1. Hierarchical hypothesis testing in MODELTEST. At each level the null hypothesis (upper model) is either accepted (A) or rejected (R). The models of DNA substitution are: JC (Jukes and Cantor, 1969), K80 (Kimura, 1980), SYM (Zharkikh, 1994), F81 (Felsenstein, 1981), HKY (Hasegawa *et al.*, 1985), and GTR (Rodríguez *et al.*, 1990). Γ: shape parameter of the gamma distribution; I: proportion of invariable sites. df: degrees of freedom. 1: equal base frequencies (0.25), π_A: frequency of adenine, π_C: frequency of cytosine, π_G: frequency of guanine, π_T: frequency of thymine. ρ: equal substitution rate, α: transition rate, β: transversion rate; μ_1: A⇒C rate, μ_2: A⇒G rate, μ_3: A⇒T rate, μ_4: C⇒G



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6.5. Amino acid models

In contrast to DNA, the modeling of amino acid replacement has concentrated on the **empirical approach**.
Dayhoff [11] developed a model of protein evolution that resulted in the development of a set of widely used replacement matrices. In the Dayhoff approach,

- Replacement rates are derived from alignments of protein sequences 85% identical,
- This ensures that the likelihood of a particular mutation (e.g., L $\mapsto$ V) being the result of a set of successive mutations (e.g., L $\mapsto$ x $\mapsto$ y $\mapsto$ V) is low.
- An implicit instantaneous rate matrix is estimated, and replacement probability matrices $\mathbf{P}(T)$ are generated at different values of T
- One of the main uses of the Dayhoff matrices has been in databases search methods, PAM50, PAM100, PAM250 corresponding to $\mathbf{P}(0.5)$, $\mathbf{P}(1)$ and $\mathbf{P}(2.5)$, respectivelly.
- The number 250 in PAM250 corresponds to an average of 250 amino acid replacements per 100 residues from a data set of 71 aligned sequences.



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A	B	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Z	
0.4	0.0	-0.4	0.0	0.0	-0.8	0.2	-0.2	-0.2	-0.2	-0.4	-0.2	0.0	0.2	0.0	-0.4	0.2	0.2	0.0	-1.2	-0.6	0.0	A
	0.5	-0.9	0.6	0.4	-1.0	0.1	0.3	-0.4	0.1	-0.7	-0.5	0.4	-0.2	0.3	-0.1	0.1	0.0	-0.4	-1.1	-0.6	0.4	B
		2.4	-1.0	-1.0	-0.8	-0.6	-0.6	-0.4	-1.0	-1.2	-1.0	-0.8	-0.6	-1.0	-0.8	0.0	-0.4	-0.4	-1.6	0.0	-1.0	C
			0.8	0.6	-1.2	0.2	0.2	-0.4	0.0	-0.8	-0.6	0.4	-0.2	0.4	-0.2	0.0	0.0	-0.4	-1.4	-0.8	0.5	D
				0.8	-1.0	0.0	0.2	-0.4	0.0	-0.6	-0.4	0.2	-0.2	0.4	-0.2	0.0	0.0	-0.4	-1.4	-0.8	0.6	E
					1.8	-1.0	-0.4	0.2	-1.0	0.4	0.0	-0.8	-1.0	-1.0	-0.8	-0.6	-0.6	-0.2	0.0	1.4	-1.0	F
						1.0	-0.4	-0.6	-0.4	-0.8	-0.6	0.0	-0.2	-0.2	-0.6	0.2	0.0	-0.2	-1.4	-1.0	-0.1	G
							1.2	-0.4	0.0	-0.4	-0.4	0.4	0.0	0.6	0.4	-0.2	-0.2	-0.4	-0.6	0.0	-0.4	H
								1.0	-0.4	0.4	0.4	-0.4	-0.4	-0.4	-0.4	-0.2	0.0	0.8	-1.0	-0.2	-0.4	I
									1.0	-0.6	0.0	0.2	-0.2	0.2	0.6	0.0	0.0	-0.4	-0.6	-0.8	0.1	K
										1.2	0.8	-0.6	-0.6	-0.4	-0.6	-0.6	-0.4	0.4	-0.4	-0.2	-0.5	L
											1.2	-0.4	-0.4	-0.2	0.0	-0.4	-0.2	0.4	-0.8	-0.4	-0.3	M
												0.4	-0.2	0.2	0.0	0.2	0.0	-0.4	-0.8	-0.4	0.2	N
													1.2	0.0	0.0	0.2	0.0	-0.2	-1.2	-1.0	-0.1	P
														0.8	0.2	-0.2	-0.2	-0.4	-1.0	-0.8	0.6	Q
															1.2	0.0	-0.2	-0.4	0.4	-0.8	0.6	R
																0.4	0.2	-0.2	-0.4	-0.6	-0.1	S
																	0.6	0.0	-1.0	-0.6	-0.1	T
																		0.8	-1.2	-0.4	-0.4	V
																			3.4	0.0	-1.2	W
																				2.0	-0.8	Y
																					0.6	Z

Several later groups have attempted to extend Dayhoff's methodology or re-apply her analysis using later databases with more examples.

- Jones, et al. [50] used the same methodology as Dayhoff but with modern databases and for membrane spanning proteins.

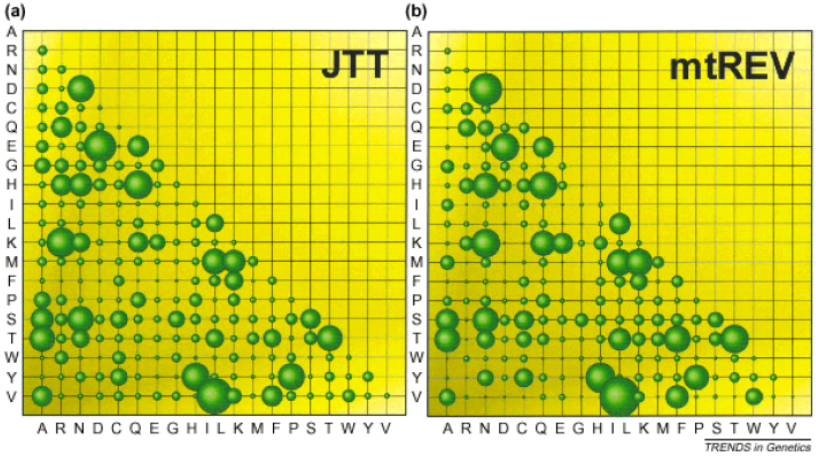
The BLOSUM series of matrices were created by Henikoff [43]. Features,

- Derived from local, ungapped alignments of distantly related sequences,
- All matrices are directly calculated; no extrapolations are used,
- The number of the matrix (BLOSUM62) refers to the minimum % identity of the blocks used to build the matrix; greater numbers, lesser distances,



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- The BLOSUM series of matrices generally perform better than PAM matrices for local similarity searches.
- Specific matrices modeling mitochondrial proteins exists [1, 62]
- Indeed, others approaches to have recently been done [61, 69, 100][17]



[17] See [60, 101] for a review of evolutionary sequence models

7. Distance Methods

Distance matrix methods is a major family of phylogenetic methods trying to fit a tree to a matrix of pairwise distance [10, 32]. Distance are generally corrected distances.

- The best way of thinking about distance matrix methods is to consider distances as estimates of the branch length separating that pair of species.
- Branch lengths are not simply a function of time, they reflect expected amounts of evolution in different branches of the tree.
- Two branches may reflect the same elapsed time (sister taxa), but they can have different expected amounts of evolution.
- The product $r_i * t_i$ is the branch length
- The main distance-based tree-building methods are **cluster analysis, least square** and **minimum evolution**.
- They rely on different assumptions, and their success or failure in retrieving the correct phylogenetic tree depends on how well any particular data set meet such assumptions.



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7.1. Ultrametric & Additive Trees

Distance to be represented in a tree diagram must be **metric** and **additive**. Let $d(a, b)$ the distance between 2 sequences, d is metric if:

1. $d(a, b) \geq 0 \mapsto$ (non-negative),
2. $d(a, b) = d(b, a) \mapsto$ (symmetry),
3. $d(a, c) \leq d(a, b) + d(b, c) \mapsto$ (**triangle inequality**),
4. $d(a, c) = 0$ if and only if $a = b \mapsto$ (distinctness)

   ♣ A metric is an ultrametric if it satisfies the additional criterion that:

5. $d(a, b) \geq maximum[d(a, c), d(b, c)] \mapsto$ (the two largest distance are equal),

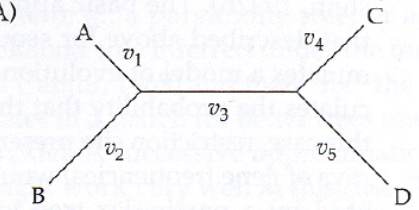
   ♣ Being metric (or ultrametric) is a necessary but not sufficient condition for being a valid measure of evolutionary change. A measure must also satisfy the **the four-point condition**:

6. $d(a, b) + d(c, d) \leq maximum[d(a, c) + d(b, d), d(a, d) + d(b, c)]$



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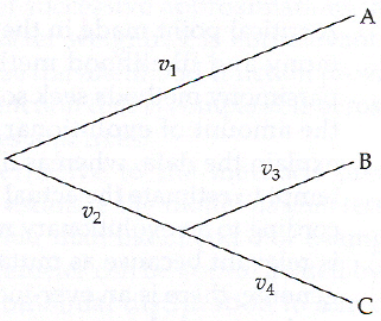
(A)



Additive properties:

$$d_{AB} = v_1 + v_2$$
$$d_{AC} = v_1 + v_3 + v_4$$
$$d_{AD} = v_1 + v_3 + v_5$$
$$d_{BC} = v_2 + v_3 + v_4$$
$$d_{BD} = v_2 + v_3 + v_5$$
$$d_{CD} = v_4 + v_5$$

(B)



Additive properties:

$$d_{AB} = v_1 + v_2 + v_3$$
$$d_{AC} = v_1 + v_2 + v_4$$
$$d_{BC} = v_3 + v_4$$

Ultrametric properties:

$$v_3 = v_4$$
$$v_1 = v_2 + v_3 = v_2 + v_4$$



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7.2. Cluster Analysis

Cluster analysis derived from clustering algorithms popularized by Sokal and Sneath[93]

7.2.1. UPGMA

One of the most popular distance approach is the **unweighted pair-group method with arithmetic mean (UPGMA)**, which is also the simplest method for tree reconstruction [67].

1. Given a matrix of pairwise distances, find the clusters (taxa) i and j such that d_{ij} is the minimum value in the table.
2. Define the depth of the branching between i and j (l_{ij}) to be $d_{ij}/2$
3. If i and j are the last 2 clusters, the tree is complete. Otherwise, create a new cluster called u.
4. Define the distance from u to each other cluster (k, with $k \neq i$ or j) to be an average of the distances d_{ki} and d_{kj}
5. Go back to step 1 with one less cluster; clusters i and j are eliminated, and cluster u is added.

   The variants of UPGMA are in the step 4. Weighted PGMA(WPGM::$d_{ku} = d_{ki} + d_{kj}/2$). Complete linkage ($d_{ku} = max(d_{ki}, d_{kj})$. Single linkage($d_{ku} = min(d_{ki}, d_{kj})$.



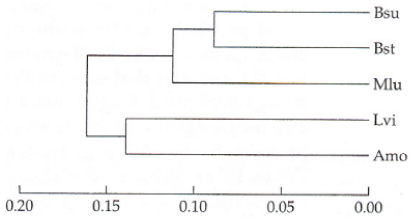
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	Bsu	Bst	Lvi	Amo	Mlu
Bsu	—	**0.1715**	0.2147	0.3091	0.2326
Bst		—	0.2991	0.3399	0.2058
Lvi			—	0.2795	0.3943
Amo				—	0.4289
Mlu					—

	Bsu-Bst	Lvi	Amo	Mlu
Bsu-Bst	—	0.2569	0.3245	**0.2192**
Lvi		—	0.2795	0.3943
Amo			—	0.4289
Mlu				—

	Bsu-Bst-Mlu	Lvi	Amo
Bsu-Bst-Mlu	—	0.3027	0.3593
Lvi		—	**0.2795**
Amo			—

	Bsu-Bst-Mlu	Lvi-Amo
Bsu-Bst-Mlu	—	**0.3310**
Lvi-Amo		—



The smallest distance in the first table is 0.1715 substitutions per sequence position separating *Bacillus subtilis* and *B. stearothermophilus*. The distance between Bsu-Bst to Lvi (*Lactobacillus viridescens*) is (0.2147+0.2991)/2=0.2569. In the second table, joins Bsu-Bst to Mlu(*Micrococcus luteus*) at the depth 0.1096(=0.2192/2). The distances Bsu-Bst-Mlu to Lvi is (2*0.2569+0.3943)/3=0.3027. Notice that this value is identical to (Bsu:Lvi+Bst:Lvi+Mlu:Lvi)/3. Each taxon in the original data table contributes equally to the averages, this is why the method called **unweighted**

UPGMA method supposes a cloclike behaviour of all the lineages, giving a rooted and ultrametric tree.



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7.2.2. NJ (Neighboor Joining)

A variety of methods related to cluster analysis have been proposed that will correctly reconstruct additive trees, whether the data are ultrametric or not. NJ removes the assumption that the data are ultrametric.

1. For each terminal node i calculate its net divergence (r_i) from all the other taxa using $\mapsto r_i = \sum_{k=1}^{N} d_{ik}$ [18].
2. Create a rate-corrected distance matrix $(\mathbf{M})$ in which the elements are defined by $\mapsto M_{ij} = d_{ij} - (r_i + r_j)/(N-2)$ [19].
3. Define a new node u whose three branches join nodes i, j and the rest of tree. Define the lengths of the tree branches from u to i and $j \mapsto v_{iu} = d_{ij}/2 + ((r_i - r_j)/[2(N-2)]; v_{ju} = d_{ij} - v_{iu}$
4. Define the distance from u to each other terminal node (for all $k \neq i$ or $j) \mapsto d_{ku} = (d_{ik} + d_{jk} - d_{ij})/2$
5. Remove distances to nodes i and j from the matrix, decrease N by 1
6. If more than2 nodes remain, go back to step 1. Otherwise, the tree is fully defined except for the length of the branch joining the two remaining nodes (i and j) $\mapsto v_{ij} = d_{ij}$

[18] N is the number of terminal nodes

[19] Only the values i and j for which M_{ij} is minimum need to be recorded, saving the entire matrix is unnecessary



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The main virtue of neighbor-joining is its efficiency. It can be used on very large data sets for which other phylogenetic analysis are computationally prohibitive.

	Bsu	Bst	Lvi	Amo	Mlu	R	R/3
Bsu	—	0.1715	0.2147	0.3091	0.2326	0.9279	0.3093
Bst	−0.4766	—	0.2991	0.3399	0.2058	1.0163	0.3388
Lvi	−0.4905	−0.4356	—	0.2795	0.3943	1.1876	0.3959
Amo	−0.4527	−0.4514	**−0.5689**	—	0.4289	1.3574	0.4525
Mlu	−0.4972	−0.5535	−0.4221	−0.4441	—	1.2616	0.4205

Lvi to node 1 distance = 0.2795/2 + (0.3959 − 0.4525)/2 = 0.1114
Amo to node 1 distance = 0.2795 − 0.1114 = 0.1681

	Bsu	Bst	Mlu	Node 1	R	R/2
Bsu	—	0.1715	0.2326	**0.1222**	0.5263	0.2631
Bst	−0.3701	—	0.2058	0.1798	0.5571	0.2785
Mlu	−0.3856	−0.4278	—	0.2719	0.7103	0.3551
Node 1	**−0.4278**	−0.3856	−0.3701	—	0.5739	0.2869

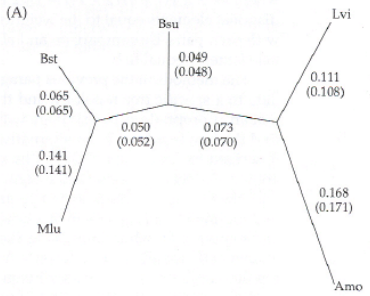
Bsu to node 2 distance = 0.1222/2 + (0.2631 − 0.2869)/2 = 0.0492
node 1 to node 2 distance = 0.1222 − 0.0492 = 0.0730

	Bst	Mlu	Node 2	R	R/1
Bst	—	0.2058	**0.1146**	0.3204	0.3204
Mlu	−0.5116	—	0.1912	0.3970	0.3970
Node 2	**−0.5116**	−0.5116	—	0.3058	0.3058

Bst to node 3 distance = 0.1146/2 + (0.3204 − 0.3058)/2 = 0.0646
node 2 to node 3 distance = 0.1146 − 0.0646 = 0.0500

	Mlu	Node 3
Mlu	—	0.1412
Node 3		—

Mlu to node 3 distance = 0.1412



Unlike the UPGMA, NJ does not assume that all lineages evolve at the same rate and produces an unrooted tree.



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7.3. Optimality Criteria

Inferring a phylogeny is an estimate procedure.

We are making **a "best estimate"** of an evolutionary history based **on the incomplete information** contained in the data.

Because we can postulate evolutionary scenarios by which any chosen phylogeny could have produced the observed data, **we must have some basis for selecting one or more preferred trees** among the set of possible phylogenies.

As we have seen, we can define a specific algorithm that leads to the determination of a tree, but also, we can define **a criterion for comparing alternative phylogenies to one another and decide which is better**.

Cluster analysis methods combine tree inference and the definition of the preferred tree **into a single** statement. In fact, UPGMA and NJ give a single tree.

Methods using optimality criterion has **two logical steps**.

The **first** is to define an objetive function to **score trees**, and the **second** is to **find alternative trees** to apply the criterion. The last problem will be covered below the title: **"searching trees"**.

This kind of procedure would produce **many alternative optimal solution**.



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7.3.1. Least squares family methods

We can now address the problem of choosing a tree from the following conceptual perspective: *We have uncertain data that we want to fit to a particular mathematical model* **(and additive tree)** *and find the optimal value for the adjustable parameters* **(the topology and the branch lengths)**.

Several methods depend on a definition of the disagreement between a tree and the data based on the following familiy of objective functions:

$$E = \sum_{i=1}^{T-1} \sum_{j=i+1}^{T} w_{ij} \mid d_{ij} - p_{ij} \mid^{\alpha}$$

Where E defines the error of fitting the distance estimates to the tree, T is the number of taxa, w_{ij} is the weight applied to the separation of taxa i and j, d_{ij} is the pairwise distance estimate (*matrix distances*), p_{ij} is the length of the path connecting i and j in the given tree[20], the vertical bars represent absolute values, and $\alpha = 1$ or 2.

Methods depend on the selection of specific α and the weighted scheme w_{ij}

- If $\alpha = 2$ and $w_{ij} = 1$, the unweighted squared deviations will be minimized, assuming that all the distance estimates are subject to the same magnitude of error (LS of C-S&E)[10].
- If $\alpha = 2$ and $w_{ij} = 1/d_{ij}^2$, the weighted squared deviations will be minimized, assuming that the estimates are uncertain by the same percentage (LS method of F&M)[32].

[20] p_{ij} is also called as **patristic distances**



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7.3.2. Minimum Evolution

The minimum evolution method [52, 81, 82, 83] uses a criterion:

the total branch length of the reconstructed tree.

$$S = \sum_{k=1}^{2T-3} \mid v_k \mid$$

That is, the optimality criterion is simply the sum of the branch lengths that minimize the sum of squared deviations between the observed (estimated) and path-length (patristic) distances.

Thus this method makes partial use of the LS (C-S&E) criterion.

Under the ME criterion, a tree is worse than another tree only if its S value is **significantly larger** than that of the other tree.

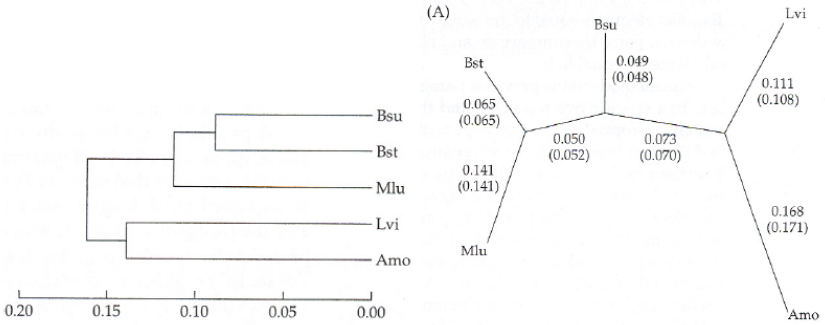
Thus, all trees whose S values are not significantly different from the minimum S value should be regarded as candidates for the true tree[21].

Rzhetsky & Nei [81] proposed a fast approximated search of the ME tree based on the observation that ME tree (*below*) is almost always identical to NJ tree.

[21] The statistical procedure for testing different trees will be discussed in "confidence on trees".



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7.4. Pros & Cons of Distance Methods

- **Pros**:
  - They are very fast,
  - There are a lot of models to correct for multiple,
  - LRT may be used to search for the best model.
- **Cons**:
  - Information about evolution of particular characters is lost



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8. Maximum Parsimony

Most biologists are familiar with the usual notion of **parsimony** in science, which essentially maintains that simpler hypotheses are prefereable to more complicated ones and that *ad hoc* hypotheses should be avoided whenever possible. The principle of *maximum parsimony* (MP) searches for a tree that requires **the smallest number of evolutionary changes** to explain differences observed among OTUs.

In general, parsimony methods operate by selecting trees that minimize the total tree length: **the number of evolutionary steps (transformation of one character state to another) require to explain a given set of data**.

In mathematical terms: from the set of possible trees, find all trees τ such that $L_{(\tau)}$ is **minimal**

$$L_{(\tau)} = \sum_{k=1}^{B} \sum_{j=1}^{N} w_j . diff(x_{k'j}, x_{k''j})$$

Where $L_{(\tau)}$ is the length of the tree, B is the number of branches, N is the number of characters, k' and k'' are the two nodes incident to each branch k, $x_{k'j}$ and $x_{k''j}$ represent either element of the input data matrix or optimal character-state assignments made to internal nodes, and diff(y, z) is a function specifying the cost of a transformation from state y to state z along any branch. The coefficient w_j assigns a weight to each character. Note also that diff(y, z) needs not to be equal diff(z, y).[22]

[22] For methods that yield unrooted trees diff(y, z) =diff(z, y).



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A common misconception regarding the use of parsimony methods is that they require *a priori* determination of character polarities.

In morphological studies, character polarity is commonly inferred using **outgroup comparison**, however, it is by no means a prerequisite to the use of parsimony methods.

Parsimony analysis actually compromises a group of related methods differing in their underlying evolutionary assumptions.

- **Wagner Parsimony** [55, 22] ordered, multistate characters with reversibility.
- **Fitch Parsimony** [29] unordered, multistate characters with reversibility.

	a	b	c	d
a	–	1	2	3
b	1	–	1	2
c	2	1	–	1
d	3	2	1	–

	a	b	c	d
a	–	1	1	1
b	1	–	1	1
c	1	1	–	1
d	1	1	1	–

- Since both Fitch and Wagner Parsimony allow reversibility, the tree may be rooted at any point without changing the tree length.



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- **Dollo Parsimony** [12], reversals allowed, but the derived state may arise only once [23]

	a	b	c	d
a	–	M	$2M$	$3M$
b	1	–	M	$2M$
c	2	1	–	M
d	3	2	1	–

- **Transversion Parsimony** [6], transition substitutions (Pu↦Pu; Py↦Py) occur more frequently than transversion (Pu↦Py; Py↦Pu) substitutions. Pu(A,G); Py(C,T).

	A	C	G	T
A	–	5	1	5
C	5	–	5	1
G	1	5	–	5
T	5	1	5	–

[23] Dollo Parsimony is suggested for restriction site data or for very complex characters that probably have only arisen once, such as legs in tetrapods or wings in insects. M is an arbitrary large number, guaranteeing that only one transformation to each derived state will be permitted.



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Determining the length of the tree is computed by algorithmic methods[29, 85]. However, we will show how to calculate the length of a particular tree topology ((W,Y),(X,Z))[24] for a specific site of a sequence, using Fitch (A) and transversion parsimony (B)[25]:

Seq. W ....ACA**G**GAT...
Seq. X ....ACA**C**GCT...
Seq .Y ....GTA**A**GGT...
Seq. Z ....GCA**C**GAC...

(A)

$$\text{equal} = \begin{bmatrix} 0 & 1 & 1 & 1 \\ 1 & 0 & 1 & 1 \\ 1 & 1 & 0 & 1 \\ 1 & 1 & 1 & 0 \end{bmatrix}$$

(B)

$$\text{tv4} = \begin{bmatrix} 0 & 4 & 1 & 4 \\ 4 & 0 & 4 & 1 \\ 1 & 4 & 0 & 4 \\ 4 & 1 & 4 & 0 \end{bmatrix}$$

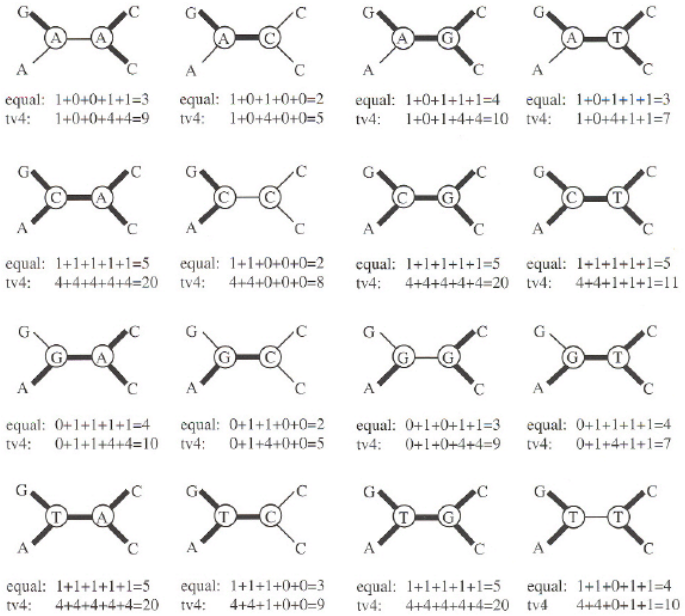
- With equal costs, the minimum is 2 steps, achieved by 3 ways (internal nodes "A-C", "C-C", "G-C"),
- The alternative trees ((W,X),(Y,Z)) and ((W,Z),(Y,X)) also have 2 steps,
- Therefore, the character is said to be **parsimony-uninformative**,[26]
- With 4:1 ts:tv weighting scheme, the minimum length is 5 steps, achived by two reconstructions (internal nodes "A-C" and"G-C"),

[24] Newick format
[25] Matrix character states: A,C,G,T
[26] A site is informative, only it favors one tree over the others



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- By evaluating the alternative topologies finds a minimum of 8 steps,
- Therefore, under unequal costs, the character **becomes informative**. The use of unequal costs may provide more information for phylogenetic reconstruction,





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8.1. Pros & Cons of MP

- **Pros**:
  - Does not depend on an explicit model of evolution,
  - At least gives both, a tree and the associated hypotheses of character evolution,
  - If homoplasy is rare, gives reliable results,
- **Cons**:
  - May give misleading results if homplasy is common (*Long branch attraction effect*)
  - Underestimate branch lengths
  - Parsimony is often justified by phylosophical, instead statistical grounds.



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9. Searching Trees

9.1. How many trees are there?

The obvious method for searching the most parsimonious tree is to consider all posible trees, one after another, and evaluate them. We will see that this procedure becomes impossible for more than a few number of taxa (∼11). Felsenstein [23] deduced that:

$$B(T) = \prod_{i=3}^{T} (2i - 5)$$

An unrooted, fully resolved tree has:

- T terminal nodes, $T - 2$ internal nodes,
- $2T - 3$ branches; $T - 3$ interior and T peripheral,
- $B(T)$ alternative topologies,
- Adding a **root**, adds one more **internal node** and one more **internal branch**,
- Since the root can be placed along any $2T - 3$ branches, the number of possible **rooted trees** becomes,

$$B(T) = (2T - 3) \prod_{i=3}^{T} (2i - 5)$$



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OTUs	Rooted trees	Unrooted trees
2	1	1
3	3	1
4	15	3
5	105	15
6	954	105
7	10,395	954
8	135,135	10,395
9	2,027,025	135,135
10	34,459,425	2,027,025
11	$> 654\text{x}10^{6}$	$> 34\text{x}10^{6}$
15	$> 213\text{x}10^{12}$	$> 7\text{x}10^{12}$
20	$> 8\text{x}10^{21}$	$> 2\text{x}10^{20}$
50	$> 6\text{x}10^{81}$	$> 2\text{x}10^{76}$

The observable universe has about $8.8\text{x}10^{77}$ atoms

There is not memory neither time to evaluate all the trees!!

For 11 or fewer taxa, a brute-force **exhaustive search** is feasible!!
For more than 11 taxa an **heuristic search** is the best solution!!



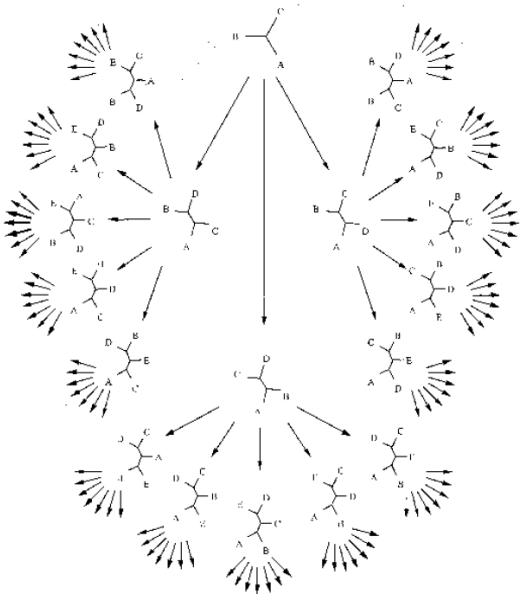
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9.2. Exhaustive search methods

- Every possible tree is examined; **the shortest tree will always be found**,
- Taxon addition sequence is important only in that **the algorithm needs to remember where it is**,
- Search will also generate **a list** of the lenths of all possible trees, which can be plotted as an histogram,



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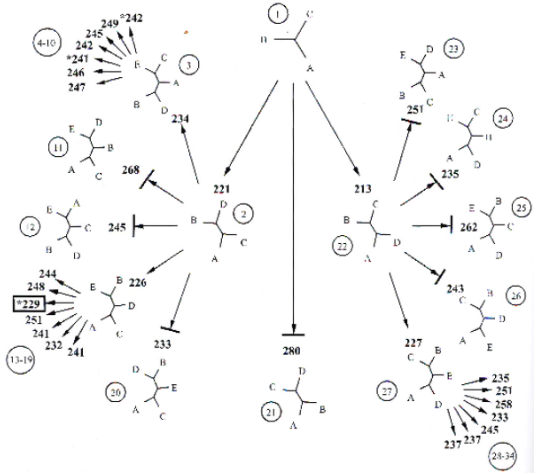
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Branch & Bound search[42]

- Much faster, but still **guaranteed to find the best tree**,
- Determine an **upper bound for the shortest tree**,
  - Use the length of **a random tree**, or the length of the shortest tree known
- Follow a predictable search path through possible tree topologies, **similar to an exhaustive search**,
- **Abandon** any fork of the search tree **when the upper bound is exceeded before the last taxon is added**,
- **Does not calculate the length of every tree, but always finds the best one**



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9.3. Heuristic search methods

When a data set is **too large to permit the use of exact methods**, optimal trees must be sought via heuristic approaches that **sacrifice the guarantee of optimality in favor of reduced computing time**

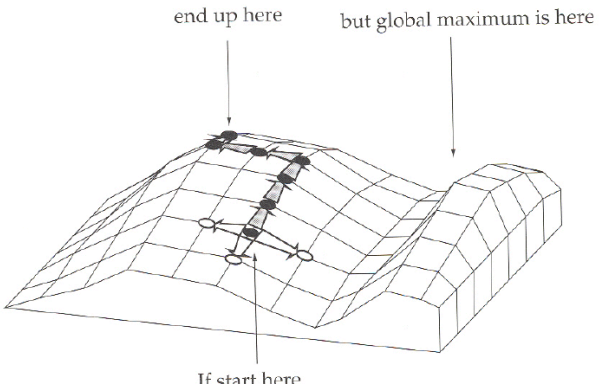
Two kind of algorithms can be used:

1. Greedy Algorithms
2. Branch Swapping Algorithms



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9.3.1. Greedy Algorithms



Strategies of this sort are often called *the greedy algorithm* because they seize the first improvement that they see. Two major algorithms exist:

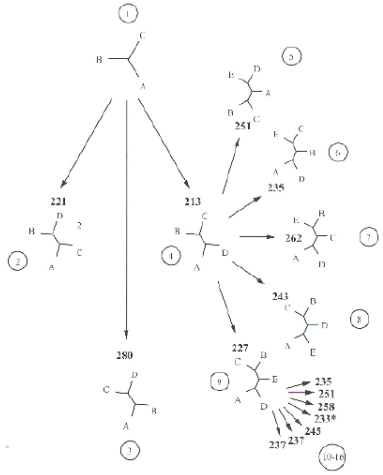
- Stepwise Addition,
- Star Decomposition[27]

Both algoritms are prone to entrapment in local optima

[27] The most common star decomposition method is the NJ algorithm

Stepwise Addition

- Use addition sequence similar to that for an exhaustive search, but at each addition, determines the shortest tree, and add the next taxon to that tree.
- Addition sequence will affect the tree topology that is found!





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Star Decomposition

- Start with all taxa in an unresolved (star) tree,
- Form pairs of taxa, and determine length of tree with paired taxa.

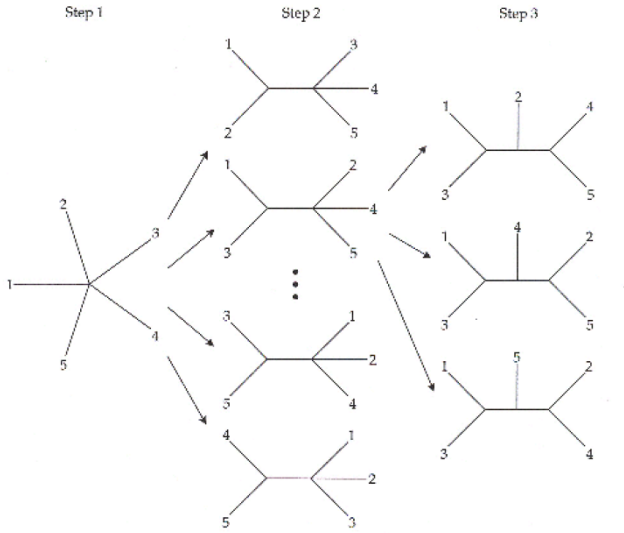


Figure 25 Heuristic tree selection using star decomposition method. At each step, the optimality criterion is evaluated for each possible joining of a pair of lineages leading away from the central node. The best tree found during each step becomes the starting point for the next step.



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9.3.2. Branch Swapping Algorithms

It may be possible to improve the *greedy* solutions by performing sets of predefined rearrangements, or branch swappings. Examples of branch swapping algorithms are:

- NNI - Nearest Neighbor Interchange,
- SPR - Subtree Pruning and Regrafting,
- TBR - Tree Bisection and Reconnection.



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Nearest Neighbor Interchange

- Identify an interior branch. It is flanked by four subtrees
- Swap two of the subtrees on opposite ends of the branch
- Two rearrangements are possible

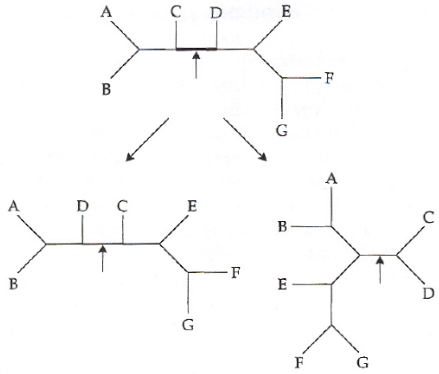


Figure 26 Branch swapping by nearest-neighbor interchanges (NNIs). Each interior branch of the tree defines a local region of four subtrees connected by the interior branch. Interchanging a subtree on one side of the branch with one from the other constitutes an NNI. Two such rearrangements are possible for each interior branch.



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Subtree Pruning & Regrafting

- Identify and remove a subtree
- Reattach to each possible branch of the remaining tree
- NNI is a subset of SPR

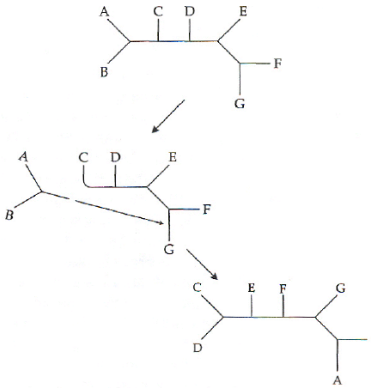


Figure 27 Branch swapping by subtree pruning ar regrafting. A subtree is pruned from the tree (e.g., tl subtree containing terminal nodes A and B as ind cated). The subtree is then regrafted to a different loc tion on the tree. All possible subtree removals and rea tachment points are evaluated.



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Tree Bisection & Reconnection

- Divide tree into two parts,
- Reconnect by a pair of branches, attempting every possible pair of branches to rejoin
- NNI and SPR are subsets of TBR

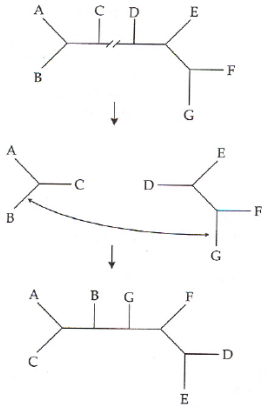


Figure 28 Branch swapping by tree bisection and reconnection. The tree is bisected along a branch, yielding two disjoint subtrees. The subtrees are then reconnected by joining a pair of branches, one from each subtree. All possible bisections and pairwise reconnections are evaluated.



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10. Statistical Methods

10.1. Maximum Likelihood

♣ The phylogenetic methods described **infered the history** (*or the set of histories*) that were **most consistent with a set of observed data**.
All the methods explained used **sequences as data** and give one or more **trees as phylogenetic hypotheses**. Then, they use the logic of:

$$P(H/D)$$

♠ **Maximum Likelihood** (*ML*)[28] methods (*or maximum probability*) computes **the probability of obtaining the data** (*the observed aligned sequences*) **given a defined hypothesis** (*the tree and the model of evolution*). That is:

$$P(D/H)$$

A coin example

The ML estimation of the heads probabilities of a coin that is tossed n times.

[28] ML was invented by Ronal A. Fisher [27]. Likelihood methods for phylogenies were introduced by Edwars and Cavalli-Sforza for gene frequency data [9]. Felsenstein showed how to compute ML for DNA sequences [24].



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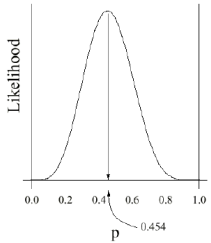
If tosses are all **independent**, and all have the same **unknown heads probability** p, then the observing sequence of tosses:

HHTTHTHHTTT

we can calculate the ML of these data as:

$L = Prob(D/p) = pp(1-p)(1-p)p(1-p)pp(1-p)(1-p)(1-p) = p^5(1-p)^6$

Ploting L against p, we observe the probabilities of the same data (D) for different values of p.



Thus the ML or the maximum probability to observe the above sequence of events is at $p = 0.4545$,

That is: $\frac{5}{11} \Rightarrow (\frac{heads}{heads+tails})$

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⋆ This **can be verified** by taking the derivative of L with respect to p:

$$\frac{dL}{dp} = 5p^4(1-p)^6 - 6p^5(1-p)^5$$

equating it to zero, and solving:

$$\frac{dL}{dp} = p^4(1-p)^5[5(1-p) - 6p] = 0 \longrightarrow \hat{p} = 5/11$$

⋆ More easily, likelihoods are often maximized **by maximizing their logarithms**:

$$lnL = 5lnp + 6ln(1-p)$$

whose derivative is:

$$\frac{d(lnL)}{dp} = \frac{5}{p} - \frac{6}{1-p} = 0 \longrightarrow \hat{p} = 5/11$$



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The likelihood of a sequence

Suppose we have:

- **Data**: a sequence of 10 nucleotides long, say **AAAAAAAATG**
- **Model**: Jukes-Cantor $\longrightarrow f_{(A,C,G,T)} = \frac{1}{4}$
- **Model**: $Model_1 \longrightarrow f_{(A,C,G,T)} = \frac{1}{2}; \frac{1}{5}; \frac{1}{5}; \frac{1}{10}$

$$L_{JC} = (\tfrac{1}{4})^8.(\tfrac{1}{4})^0.(\tfrac{1}{4}).(\tfrac{1}{4}) = (\tfrac{1}{4})^{10} = 9.53\text{x}10^{-07}$$

$$L_{M_1} = (\tfrac{1}{2})^8.(\tfrac{1}{5})^0.(\tfrac{1}{5}).(\tfrac{1}{10}) = 7.81\text{x}10^{-05}$$

L_{M_1} is almost 100 times higher than to L_{JC} model

Thus the JC model is not the best model to explain this data

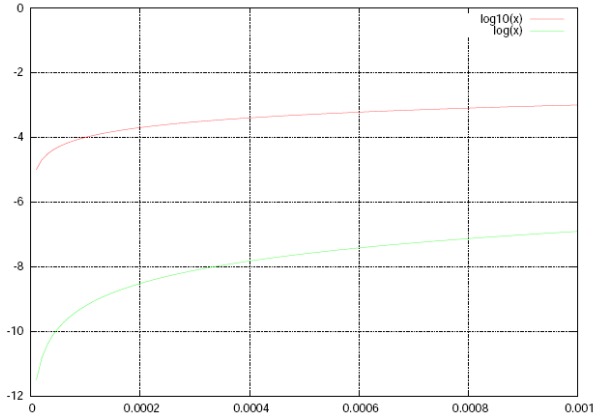


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Since likelihoods takes the form of:

$$\prod_{i=1}^{n} = x_i \text{ , where: } 0 \leq x_i \leq 1 \text{ and generally } n \text{ is large}$$

it is convenient **to report ML results as** lnL **or** $log_{(10)}L$



$$lnL_{(JC)} = -14.2711 \text{ ; } lnL_{(M_1)} = -9.4575$$

When the more positive (*less negative* lnL *values*) **the best likelihood**



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The likelihood of a one-branch tree

Suppose we have:

- **Data**:
  - Sequence 1 : 1 nucleotide long, say **A**
  - Sequence 2 : 1 nucleotide long, say **C**
  - Sequences are related by the simplest tree: **a single branch**
- **Model**:
  - Jukes-Cantor $\longrightarrow f_{(A,C,G,T)} = \frac{1}{4}$
  - $\mathbf{A} \overset{p}{\longleftrightarrow} \mathbf{C}$; $p = 0.4$

$$\text{So, } L_{tree} = \tfrac{1}{4}.(0.4) = 0.1$$

Since the model is **reversible**:

$$L_{tree:A \to C} = L_{tree:C \to A}$$



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Real Models

Suppose we have:

- **Data**:

Sequence 1 **C C A T**

Sequence 2 **C C G T**

- **Model**:[29]

$$\pi = [0.1, 0.4, 0.2, 0.3]$$

$$\boldsymbol{P} = \begin{bmatrix} 0.976 & 0.01 & 0.007 & 0.007 \\ 0.002 & 0.983 & 0.005 & 0.01 \\ 0.003 & 0.01 & 0.979 & 0.007 \\ 0.002 & 0.013 & 0.005 & 0.979 \end{bmatrix}$$

$$L_{(Seq._1 \rightarrow Seq._2)} = \pi_C P_{C \rightarrow C} \pi_C P_{C \rightarrow C} \pi_A P_{A \rightarrow G} \pi_T P_{T \rightarrow T}$$
$$0.4\text{x}0.983\text{x}0.4\text{x}0.983\text{x}0.1\text{x}0.007\text{x}0.3\text{x}0.979$$
$$= 0.0000300$$

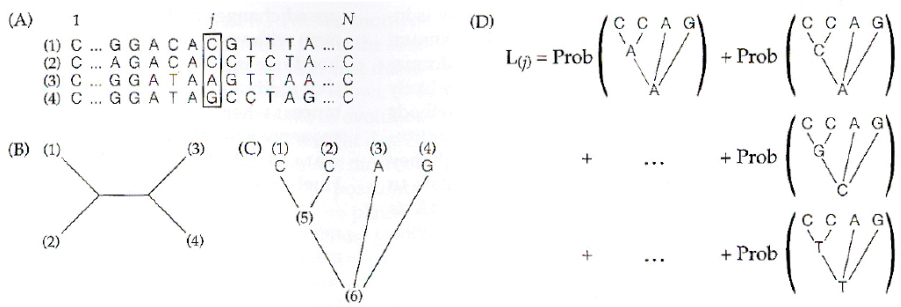
$$lnL_{tree:Seq_1 \rightarrow Seq_2} = -10.414$$

[29] Note that the base composition sum one, but indeed the the rows of substitution matrix sum one. Why?



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L computation in a real problem



- Tree after rooting in an arbitrary node (reversible model).
- The likelihood for a particular site is the sum of the probabilities of every possible reconstruction of ancestral states given some model of base substitution.
- The likelihood of the tree is the product of the likelihood at each site.

$$L = L_{(1)} \cdot L_{(2)} \cdot ... \cdot L_{(N)} = \prod_{j=1}^{N} L_{(j)}$$

- The likelihood is reported as the sum of the log likelihhod of the full tree.

$$lnL = lnL_{(1)} + lnL_{(2)} + ... + lnL_{(N)} = \sum_{j=1}^{N} lnL_{(j)}$$



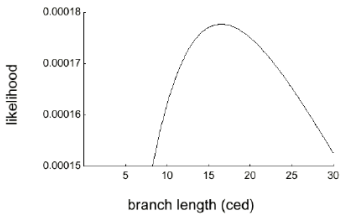
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Modifying branch lengths

At moment **for L computation** we do not take into acount the posibility of different branch lengths. However, we can infer that:

- For very short branches, the probability of characters staying the same is high and the probability of it changing is low.
- For longer branches, the probability of character change becomes higher and the probability of staying the same is low
- Previous calculations are based on a Certain Evolutionary Distance (**CED**)
- We can calculate the branch length being $2, 3, 4, ...n$ times larger (**nCED**) by multiplying the substitution matrix **P** by itself n times.[30]

branch length (ced units)	likelihood
1	0.0000300
2	0.0000559
3	0.0000782
10	0.000162
15	0.000177
20	0.000175
30	0.000152



[30] At time the branch length increases, the probability values on the diagonal going down at time the prob. off the diagonal going up. Why?

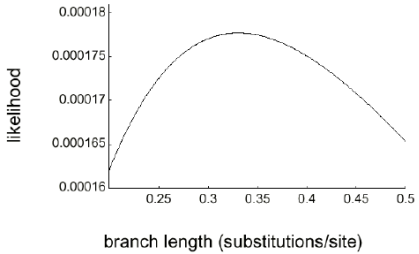


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Finally,

- The correct transformation of branch lengths (t) measured in *substitutions per site* is computed and maximized by:

$$P(t) = e^{Qt}$$



Where Q is the instantaneous rate matrix specifying the rate of change between pairs of nucleotides per instant of time dt.



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10.2. Pros & Cons of ML

- **Pros**:
  - Each site has a likelihood,
  - Accurate branch lengths,
  - There is no need to correct for "anything",
  - The model could include: instantaneous substitution rates, estimated frequencies, among site rate variation and invariable sites,
  - If the model is correct, the tree obtained is "correct",
  - All sites are informative,
- **Cons**:
  - If the model is correct, the tree obtained is "correct",
  - Very computational intensive,



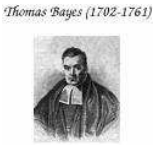
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10.3. Bayesian inference

♣ **Maximum Likelihood** will find the tree that is most likely to have produced the observed sequences, or formally $P(D/H)$ (the probability of seeing the data given the hypothesis).

♠ **A Bayesian approach** will give you the tree (or set of trees) that is most likely to be explained by the sequences, or formally $P(H/D)$ (the probability of the hypothesis being correct given the data).

♢ **Bayes Theorem** provides a way to calculate the probability of a model (*tree topology and evolutionary model*) from the results it produces (*the aligned sequences we have*), what we call a **posterior probability**[31].



$$P(\theta/D) = \frac{P(\theta) \cdot P(D/\theta)}{P(D)}$$

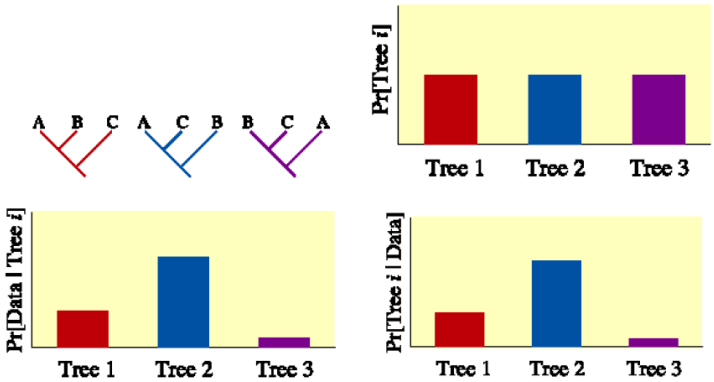
[31] See [58, 49, 48] for a clear explanation on bayesian phylogenetic method.



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The main components of Bayes analysis

- $P(\theta)$ The **prior probability** of a tree represents the probability of the tree before the observations have been made. Typically, all trees are considered equally probable.



- $P(D/\theta)$ The **likelihood** is proportional to the probability of the observations (data sets) conditional on the tree.
- $P(\theta/D)$ The **posterior probability** of a tree is the probability conditional on the observations. It is obtained combined the prior and the likelihood using the Bayes' formula

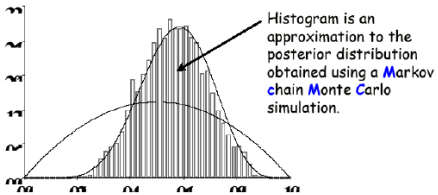


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How to find the solution

There's no analytical solution for a Bayesian system. However, giving:

- **Data**: Sequence data,
- **Model**: The evolutionary model, base frequencies, among site rate variation parameters, a tree topology, branch lengths
- **Priors** distribution on the model parameters, and
- **A method** for calculating posterior distribution from prior distribution and data: **MCMC** technique[32]



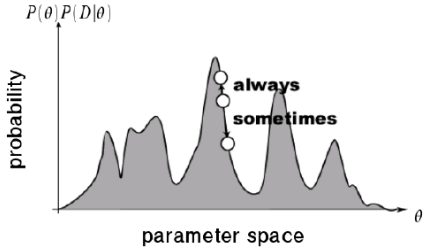
Posterior probabilities can be estimated!!!

[32] Markov Chain Monte Carlo or the Metropolis-Hastings algorithm. See [58] for an easy explanation of the techniques.



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- Each step in a Markov chain **a random modification** of the tree topology, a branch length or a parameter in the substitution model (e.g. substitution rate ratio) is assayed.
- If the **posterior computed is larger** than that of the current tree topology and parameter values, the proposed step **is taken**.
- Steps downhill are not authomatic accepted, depending on the magnitude of the decrease.



- Using these rules, the **Markov chain visits regions** of the tree space **in proportion of their posterior**.
- Suppose you sample 100,000 trees and a particular clade appears in 74,695 of the sampled trees. The probability (giving the observed data) that the group is monophyletic is 0.746, because **MC visits trees in proportion to their posterior probabilities**.



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10.4. Pros & Cons of BI

- **Pros**:
  - Faster than ML,
  - Accurate branch lengths,
  - There is no need to correct for "anything",
  - The model could include: instantaneous substitution rates, estimated frequencies, among site rate variation and invariable sites,
  - If the dataset is correct, the tree obtained is "correct",
  - All sites are informative,
  - There is no neccesary bootstrap interpretations
- **Cons**:
  - To what extent is the posterior distribution influenced by the prior?
  - How do we know that the chains have converged onto the stationary distribution?
  - **A solution**: Compare independent runs starting from different points in the parameter space

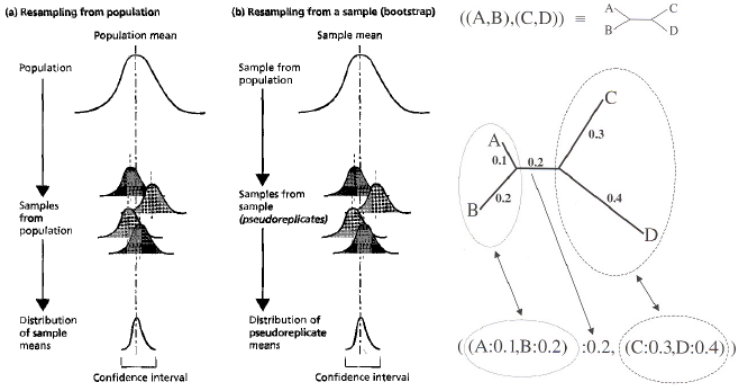


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11. Tree Confidence

11.1. Non-parametric bootstrapping

- For many simple distributions there are simple equations for calculating confidence intervals around an estimate (e.g., std error of the mean)
- Trees, however are rather complicated structures, and it is extremely difficult to develop equations for confidence intervals around a phylogeny.
- One way to measure the confidence on a phylogenetic tree is by means of the **bootstrap** non-parametric method of resampling the same sample many times.



- Each sample from the original sample is a **pseudoreplicate**. By generation many hundred or thousand pseudoreplicates, a *majority consensus rule tree* can be obtained.
- High bootstrap values $> 90\%$ is indicative of strong **phylogenetic signal**.
- Bootstrap can be viewed as a way of exploring the robustness of phylogenetic inferences to perturbations
- **Jackkniffe** is another non-parametric resampling method that differentiates from bootstrap in the way of sampling. Some proportion of the characters are randomly selected and deleted (withouth replacement).
- Another technique used exclusively for parsimony is by means of **Decay index** or **Bremmer support**. This is the length difference between the shortest tree including the group and the shortest tree excluding the group (The extra-steps required to overturn a group.[33]
- **DI** & **BPs** generally correlates!!

[33] See [98] for a practical example using PAUP*[96]

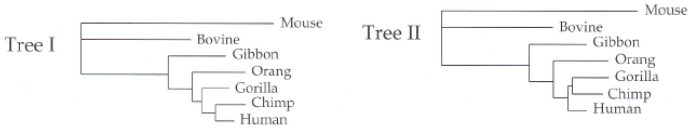


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11.2. Paired site tests

The basic idea of paired sites tests is that we can compare two trees for either parsimony or likelihood or likelihood scores.

- The expected log-likelihood of a tree is the average log-likelihood we would get per site as the number of sites grows withouth limit.
- If evolution is independent, then if 2 trees have equal expected log-likelihoods, differences must be zero.
- If we do a statistical test of whether the mean of these differences is zero, we are also testing whether there is significant statistical evidence that one tree is better than another.

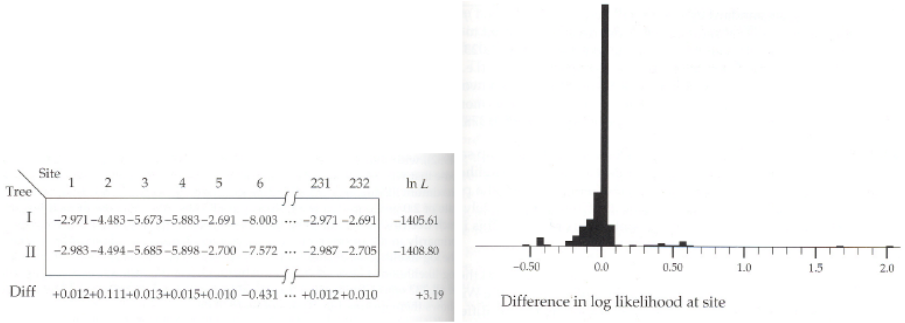


- The original **Kishino & Hasegawa test** (KHT) [54] calculates the z score; $z = \frac{D}{\sqrt{V_D}}$
- The z score is assumend to be normally distribuited. If z-score > 1.96, a topology is rejected at 0.05%.



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- The **RELL test** (*resampling-estimated log-likelihood*) where the variance of distance log-likelihood differences is obtained by bootstrap method.



Tree \ Site	1	2	3	4	5	6	∫∫	231	232	ln L
I	−2.971	−4.483	−5.673	−5.883	−2.691	−8.003	⋯	−2.971	−2.691	−1405.61
II	−2.983	−4.494	−5.685	−5.898	−2.700	−7.572	⋯	−2.987	−2.705	−1408.80
Diff	+0.012	+0.011	+0.013	+0.015	+0.010	−0.431	⋯	+0.012	+0.010	+3.19

- When more than two topologies are contrasted, a multiple topology testing must be performed. **Shimodaira & Hasegawa test** (SHT) [88], **Goldman, Anderson & Rodrigo test** (SOWH) [35] and **the expected likelihood weights** method (ELW) [94] are some of the most used methods to test many alternative topologies.[34]

[34] Tree-Puzzle [86] is one of the multiple programs containing many of the tests here discussed.



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12. Phylogenetic Links

- Software:
  - **The Felsenstein node** http://evolution.genetics.washington.edu/phylip/software.html
  - **The R. Page Lab.** http://taxonomy.zoology.gla.ac.uk/software/software.html
- Courses:
  - **Molecular Systematics and Evolution of Microorganisms.** http://www.dbbm.fiocruz.br/james/index.html
  - **Workshop on Molecular Evolution** http://workshop.molecularevolution.org/
  - **P. Lewis MCB/EEB Course** http://www.eeb.uconn.edu/Courses/EEB372/
- Tools:
  - **Clustalw at EBI** http://www.ebi.ac.uk/clustalw/
  - **Phyemon Web Server** http://phylemon.bioinfo.cipf.es





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This presentation is based on:[35]

- Major Book or Chapters References:
  - Swofford, D. L. *et al.* **1996**. Phylogenetic inference [97].
  - Harvey, P. H. *et al.* **1996**. New Uses for New Phylogenies [38].
  - Li, W. S. **1997** . Molecular Evolution [59].
  - Page, R. & Holmes, E. **1998**. Molecular evolution. A phylogenetic approach [38].
  - Nei, M. & Kumar, S. **1999** . Molecular evolution and phylogenetics [70].
  - Salemi, M. & Vandamme, A. (ed.) **2003**. The phylogenetic handbook [84].
  - Balding, Bishop & Cannings. (ed.) **2003**. Handbook of Statistical Genetics [2].
  - Felsenstein, J. **2004**. Inferring phylogenies [26].
  - Nielsen, R. (ed.) **2004**. Statistical Methods in Molecular Evolution [17].
- On Line Phylogenetic Resources:
  - http://www.dbbm.fiocruz.br/james/index.html .**Molecular Systematics and Evolution of Microorganisms**. The Natural History Museum, London and Instituto Oswaldo Cruz, FIOCRUZ.
  - Peter Foster's "The Idiot's Guide to the Zen of Likelihood in a Nutshell in Seven Days for Dummies" at http://filogeografia.dna.ac/PDFs/phylo/Foster_01_EasyIntro_MLPhylo.pdf
- Slides Production:
  - Latex and pdfscreen package.

[35] HJD take responsibility for innacuracies of this presentation.



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