# Molecular phylogenetics

## Genetic distance

Define genetic distance between a pair of ‘homologous’ sequences *x* and *y* as the number of substitutions that have occurred (per alignment site) since *x* and *y* diverged from their common ancestor

## Genetic distance

Given the following sequence alignment, infer the genetic distance

```
A C G T T C A T T - - T G
A G - T C C C T G G G G G
```

Simplification: Ignore alignment positions with gaps

## Model of evolution

Continuous-time process over {A,C,G,T}

$$p_{ij}(t) = \left(e^{-Gt}\right)_{ij}$$

$$L(A \mid t) = \prod_i p_{A_i^1 A_i^2}(t)$$

$$\hat{t} = \arg\max_t L(A \mid t)$$

## Some standard models of nucleotide evolution: Jukes and Cantor

$$
\begin{array}{cccccc}
 & & A & T & C & G \\
 & A & -3\alpha & \alpha & \alpha & \alpha \\
 & T & \alpha & -3\alpha & \alpha & \alpha \\
 & C & \alpha & \alpha & -3\alpha & \alpha \\
 & G & \alpha & \alpha & \alpha & -3\alpha
\end{array}
$$

## Kimura two-parameter model (1980)

Models a difference in the rate of transitions and transversions

$$\begin{array}{cccccc} & A & T & C & G \\ A & - & \beta & \beta & \alpha \\ T & \beta & - & \alpha & \beta \\ C & \beta & \alpha & - & \beta \\ G & \alpha & \beta & \beta & - \end{array}$$

*With* $q_{ii} = -\sum_{i \neq j} q_{ij}$

Recall:

$$\pi_i p_{ij} = \pi_j p_{ji} \quad \text{for all } i,j$$

$$\sum \pi_i = 1$$

imply $\pi_i$ are the limiting probabilities of the chain and the chain is reversible

Analogous result applies to the $g_{ij}$ of a continuous-time chain

## Kishino-Hasegawa-Yano (1985)

Includes parameters $g_k$ for the equilibrium nucleotide frequencies

| | $A$ | $T$ | $C$ | $G$ |
|---|---|---|---|---|
| $A$ | – | $\beta\pi_T$ | $\beta\pi_C$ | $\alpha\pi_G$ |
| $T$ | $\beta\pi_A$ | – | $\alpha\pi_C$ | $\beta\pi_G$ |
| $C$ | $\beta\pi_A$ | $\alpha\pi_T$ | – | $\beta\pi_G$ |
| $G$ | $\alpha\pi_A$ | $\beta\pi_T$ | $\beta\pi_C$ | – |

## General Time-Reversible Model (Simon Tavaré 1986)

| | $A$ | $T$ | $C$ | $G$ |
|---|---|---|---|---|
| $A$ | – | $\alpha\pi_T$ | $\beta\pi_C$ | $\delta\pi_G$ |
| $T$ | $\alpha\pi_A$ | – | $\gamma\pi_C$ | $\varepsilon\pi_G$ |
| $C$ | $\beta\pi_A$ | $\gamma\pi_T$ | – | $\eta\pi_G$ |
| $G$ | $\delta\pi_A$ | $\varepsilon\pi_T$ | $\eta\pi_C$ | – |

Generator matrix (or equivalently time) scaled so that one substitution expected in one unit of time

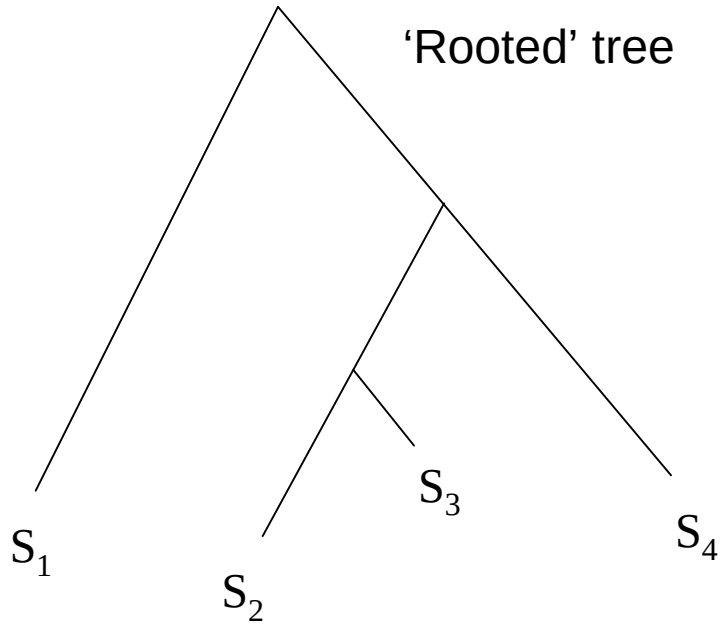
$$\sum_{i \neq j} \pi_i g_{ij} = 1$$

Commonly used evolutionary models also allow heterogeneity in rate of evolution across alignment sites (typically modeled with discretized gamma distribution)

In general, simpler nucleotide substitution models are nested within successively more complex models – standard model comparison techniques can be used to select an appropriate model.

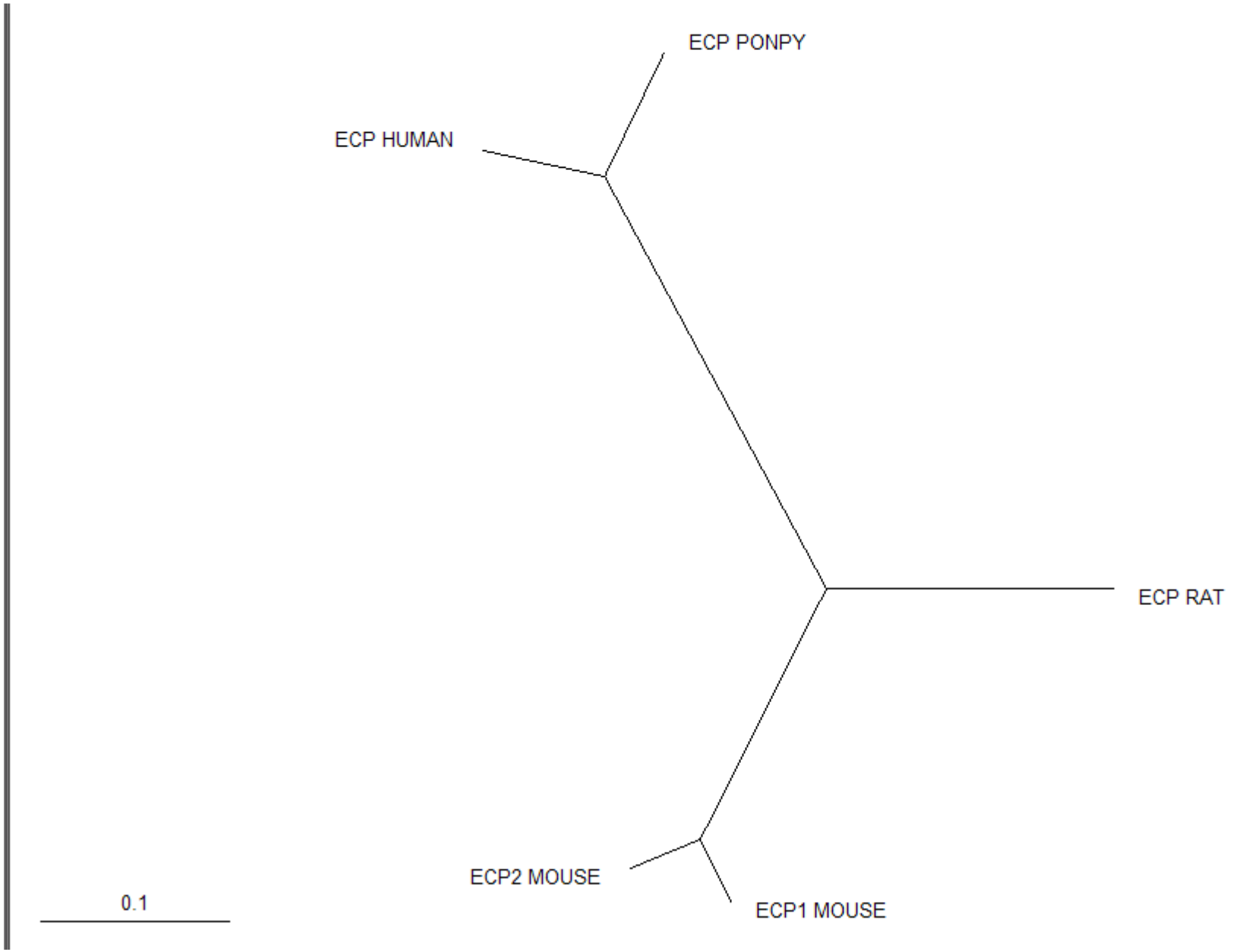
## Phylogenetic tree: binary tree with edges representing genetic distance

An evolving sequence can bifurcate (e.g. speciation), giving rise to two daughter sequences



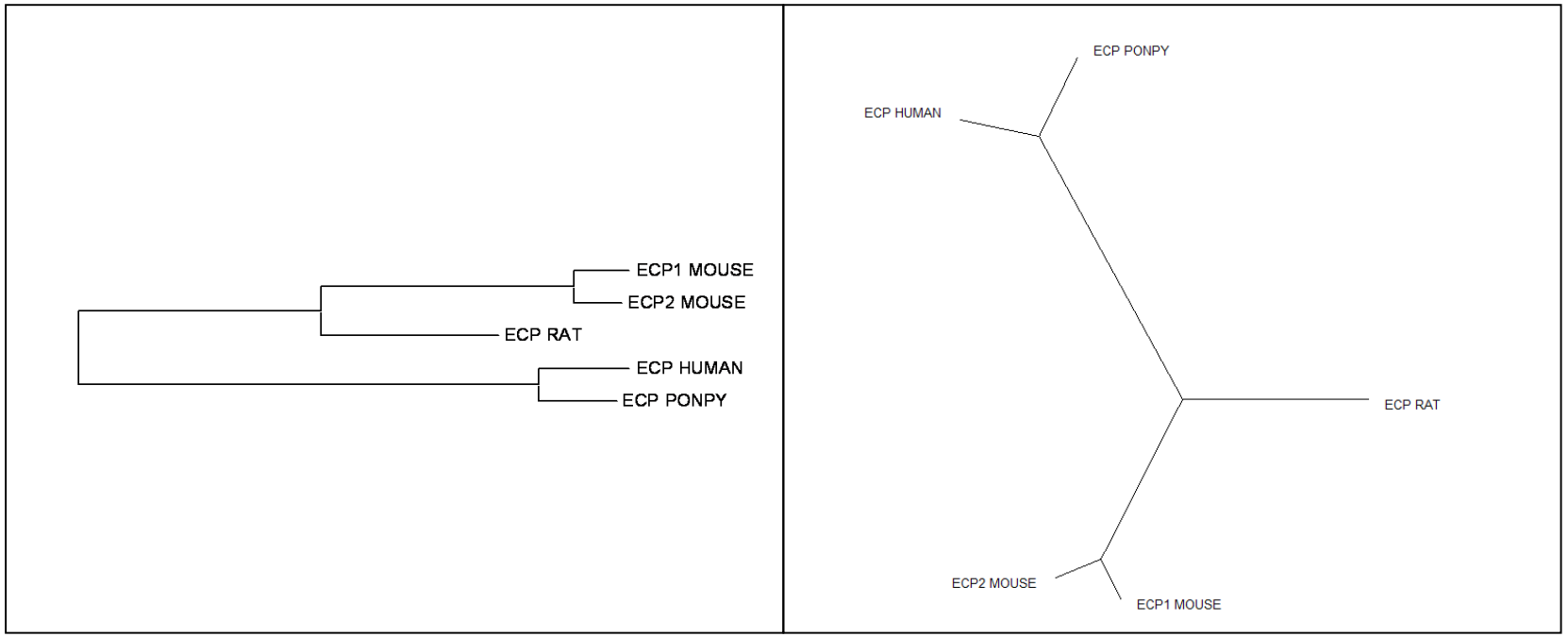

Branch length represents genetic distance between sequences (or hypothetisized sequences) at the nodes

‘Unrooted’ tree

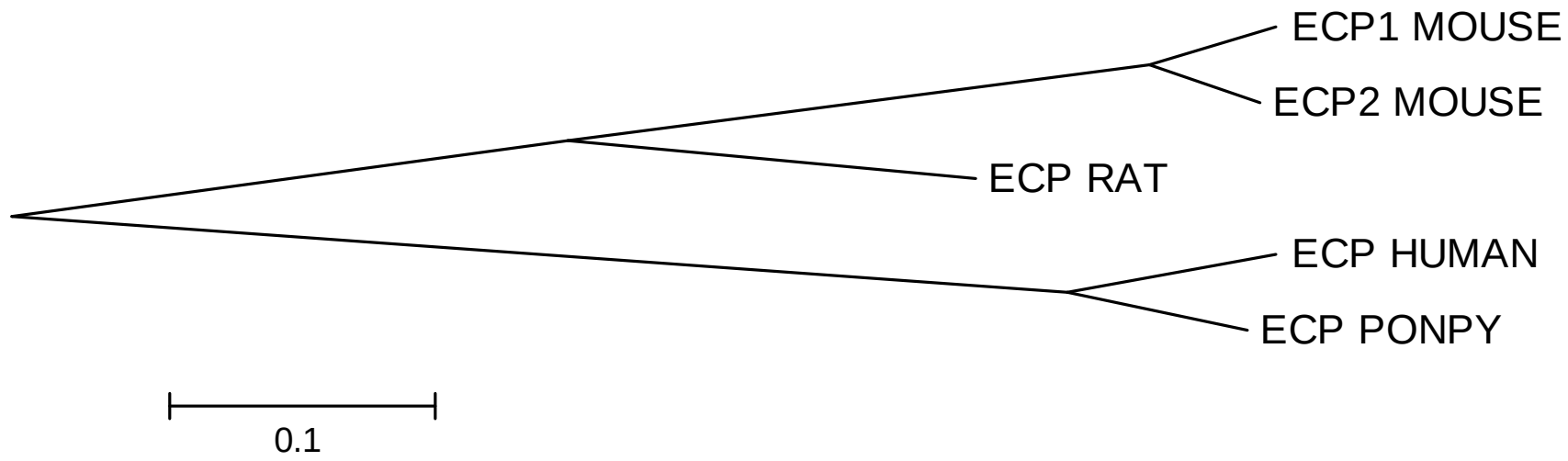


# Rooted versus unrooted tree

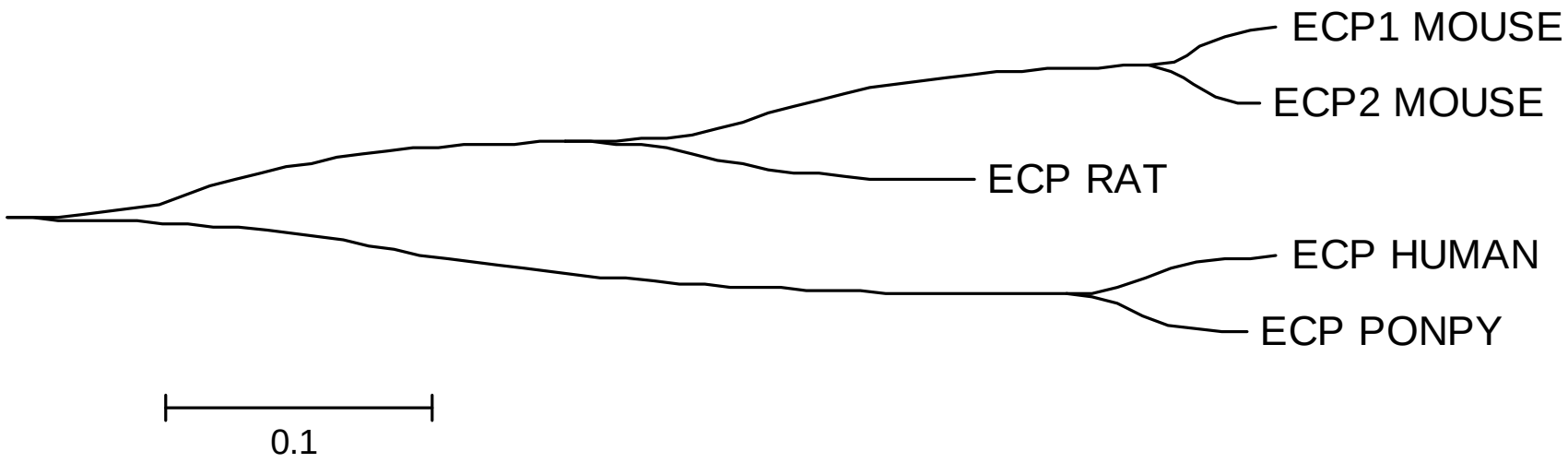
Most substitution models are reversible (see previous slides). Therefore the models cannot distinguish the time-direction of evolution. External information is usually incorporated to decide the position of the root (hypothetical ancestor of all of the sequences represented in the alignment)



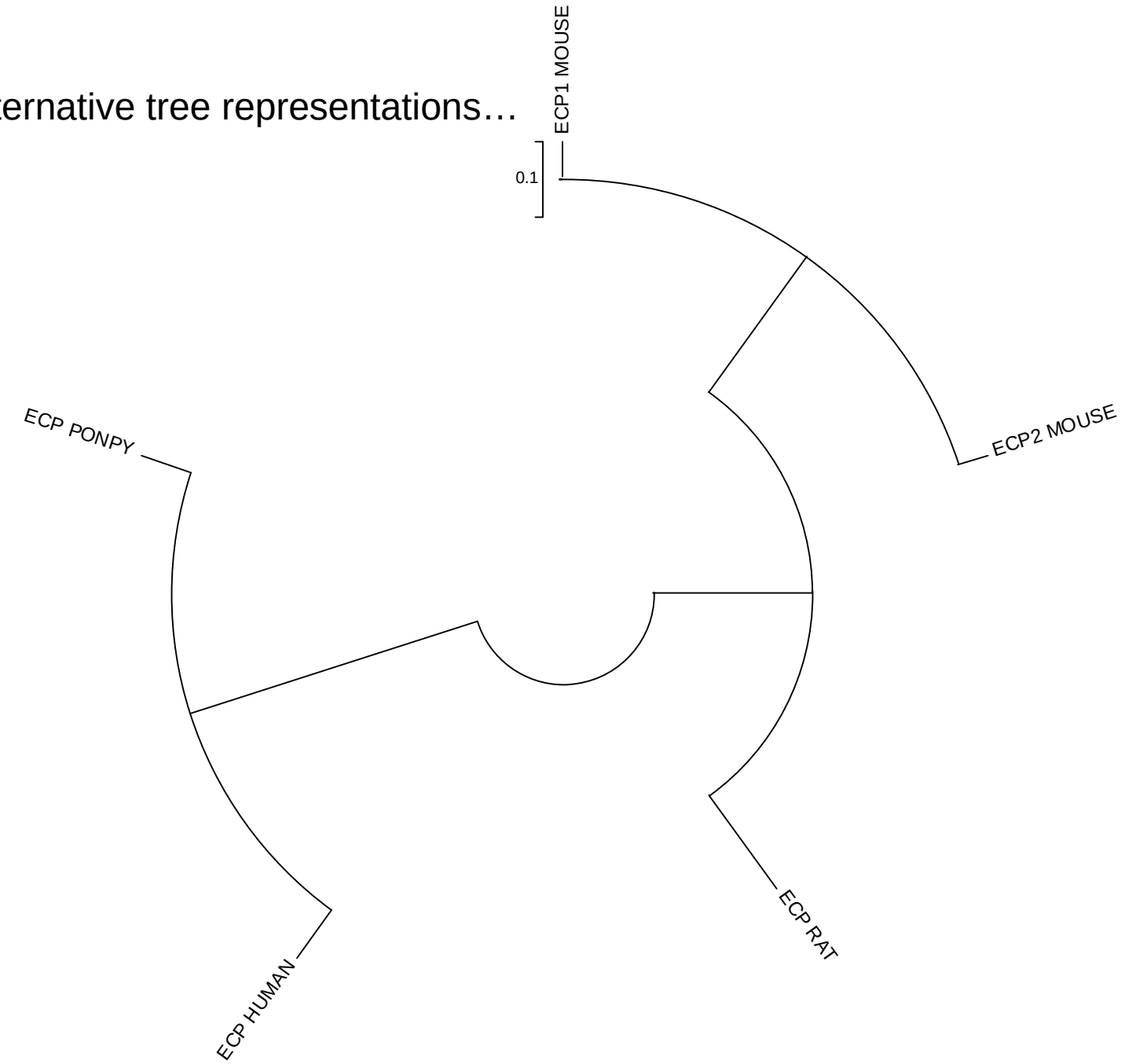
Alternative tree representations…



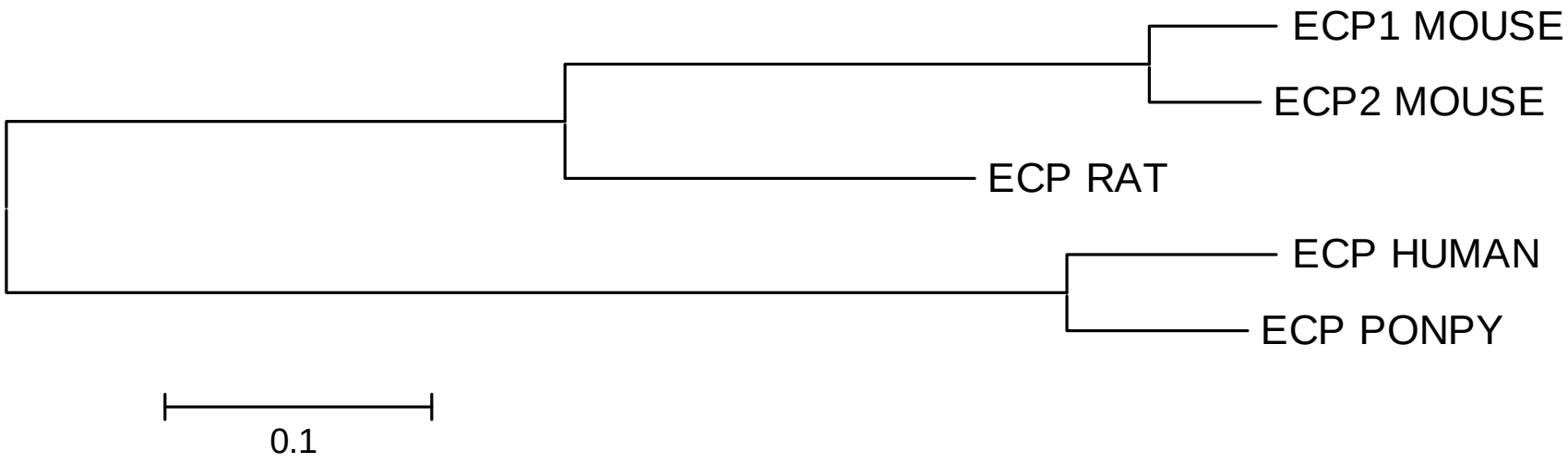
Alternative tree representations…



Alternative tree representations…

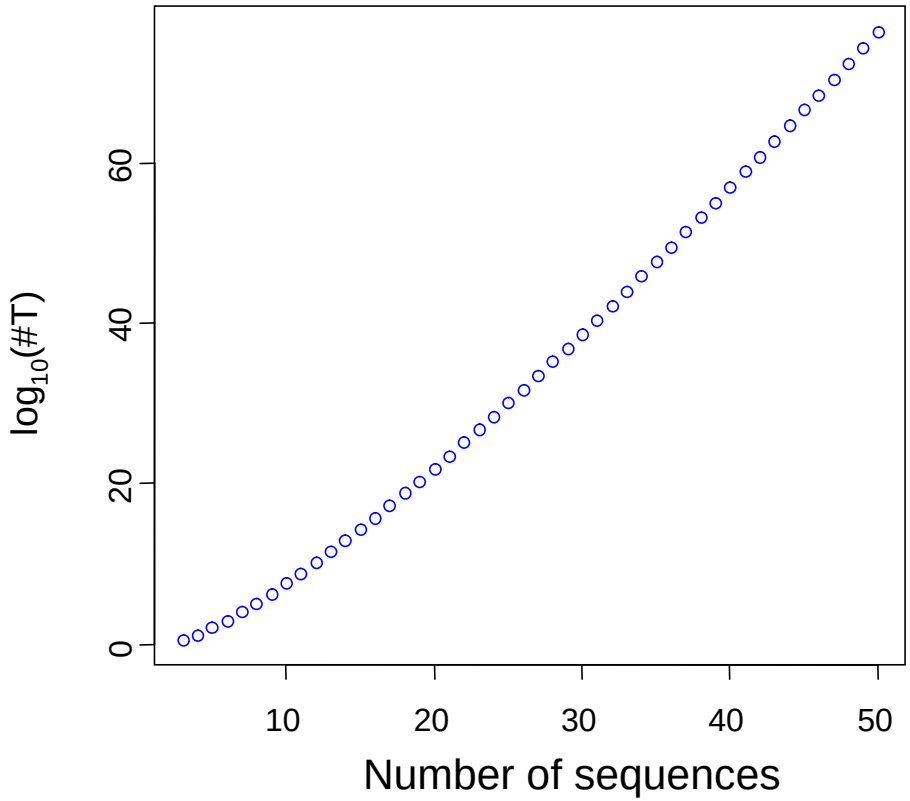


Most conventional representation of a ‘rooted’ phylogenetic tree



## How many trees?

$$(2n - 3)!/(2^{n-2}\,(n - 2)!)$$



## The phylogeny problem

Given a set of aligned DNA or amino acid sequences, infer the phylogenetic tree representing the evolution of the set of taxa

Requires:

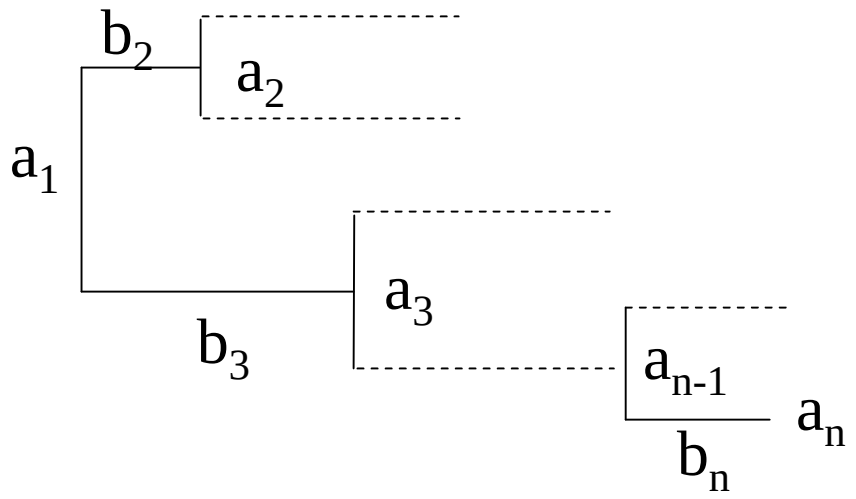
- An optimality criterion (what constitutes the ‘best’ tree)
- Search algorithm

Commonly applied optimality criteria are

- Minimum evolution (tree with shortest sum of branch lengths)
- Maximum parsimony (tree requiring smallest number of steps to explain the data)
- Maximum Likelihood
- Maximum *a posteriori* Probability (MAP)

The likelihood of a tree:

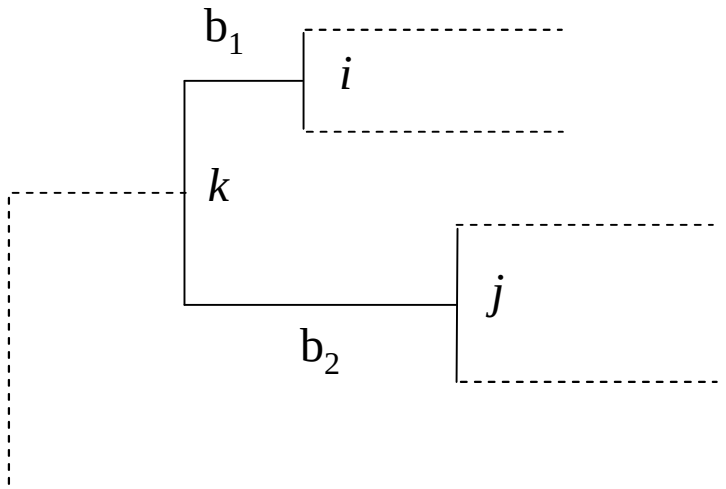
$$P(D|T) = \sum_{a_1}\sum_{a_2}\sum_{a_3}\ldots\sum_{a_n} P(a_1)P(a_2|b_2,a_1)P(a_3|b_3,a_1)\ldots P(a_{n-1}|b_n,a_n)$$



A recursive algorithm is used to avoid doing all the summations (Felsenstein's Pruning Algorithm)

Let $L_m^k$ be the likelihood of the *subtree* decended from node *k*, given that the nucleotide present at node *k*, is *m* then

$$L_m^k = \left( \sum_s L_s^i P(s \mid m, b_1) \right) \left( \sum_s L_s^j P(s \mid m, b_2) \right)$$



The *L'*s can be worked out easily for the leaf nodes:

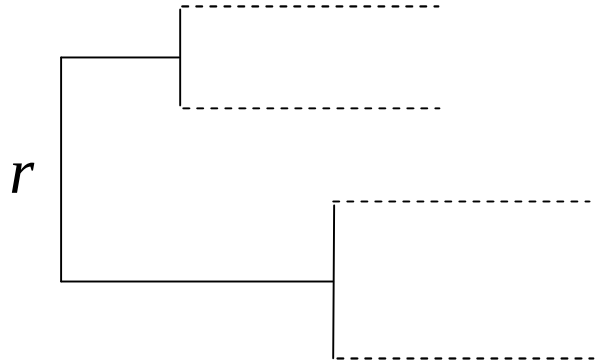
Consider position *i* in sequence *X*

If *b* is a leaf node, then

$L_a^{\,b} = 1$ if $X_i = a$

Missing information can be handled easily (using intermediate values at terminal nodes)

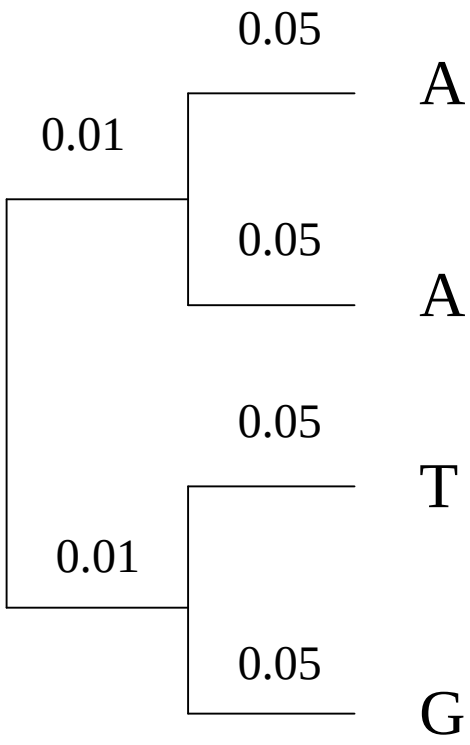
$$P(T \mid D) = \sum_{s} L_s^r \, p(s)$$




Complexity: O($n$ . $m$ . $k^2$)

($n$ = # sequences; $m$ = sequence length; $k$ = alphabet size)

Exercise: Given the instantaneous transition rate matrix and tree shown calculate the likelihood of the single alignment column shown at the tips of the tree.




| | *A* | *T* | *C* | *G* |
|---|---|---|---|---|
| *A* | – | 1 | 2 | 3 |
| *T* | 1 | – | 5 | 4 |
| *C* | 2 | 5 | – | 6 |
| *G* | 3 | 4 | 6 | – |

## Optimizing branch lengths

- If all branch lengths are known except one then the likelihood of the tree can be expressed as a function of the unknown branch length
- Standard problem of maximization in 1D for a single branch (e.g. Newton-Raphson)
- Although branches are not independent branch maximizations tend not to interfere to a great extent
- A small number of successive maximizations normally succeeds in achieving the maximum likelihood set of branch lengths

## Searching for optimal trees

Branch & Bound

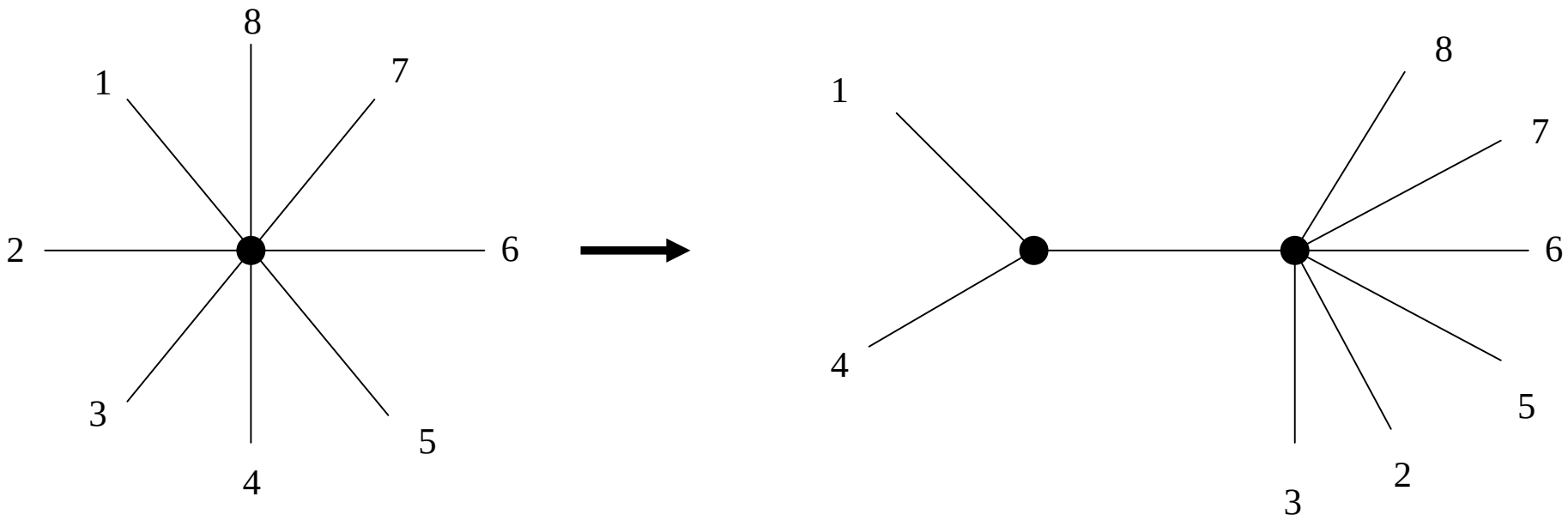
Heuristics – usually local perturbations with hill-climbing

Markov-Chain Monte Carlo ($MC^3$)

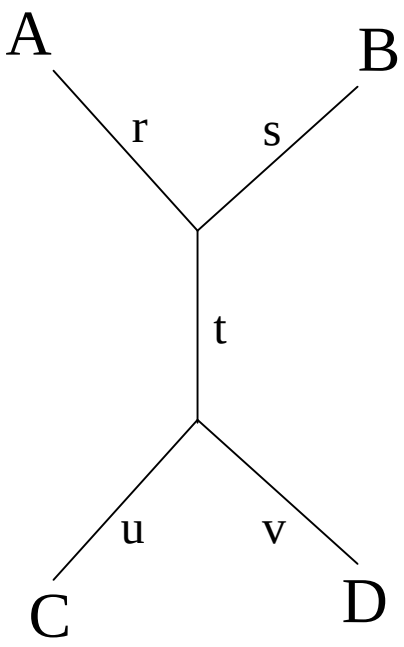
Genetic algorithms

etc.

# Common heuristic algorithm: Neighbor-Joining, an approximation to the minimum evolution tree




Choose the pair that minimizes the length of the resulting tree




$d_{AB} \sim r + s$
$d_{CD} \sim u + v$
$d_{AD} \sim r + t + v$
$d_{BC} \sim s + t + u$

(r, s, u, v, t are estimated using the least squares method)

Tree length = u + v + t + r + s

## Branch & Bound

Exact

Can be used with several different optimality criteria

**Algorithm**:

Traverse the search tree in some order

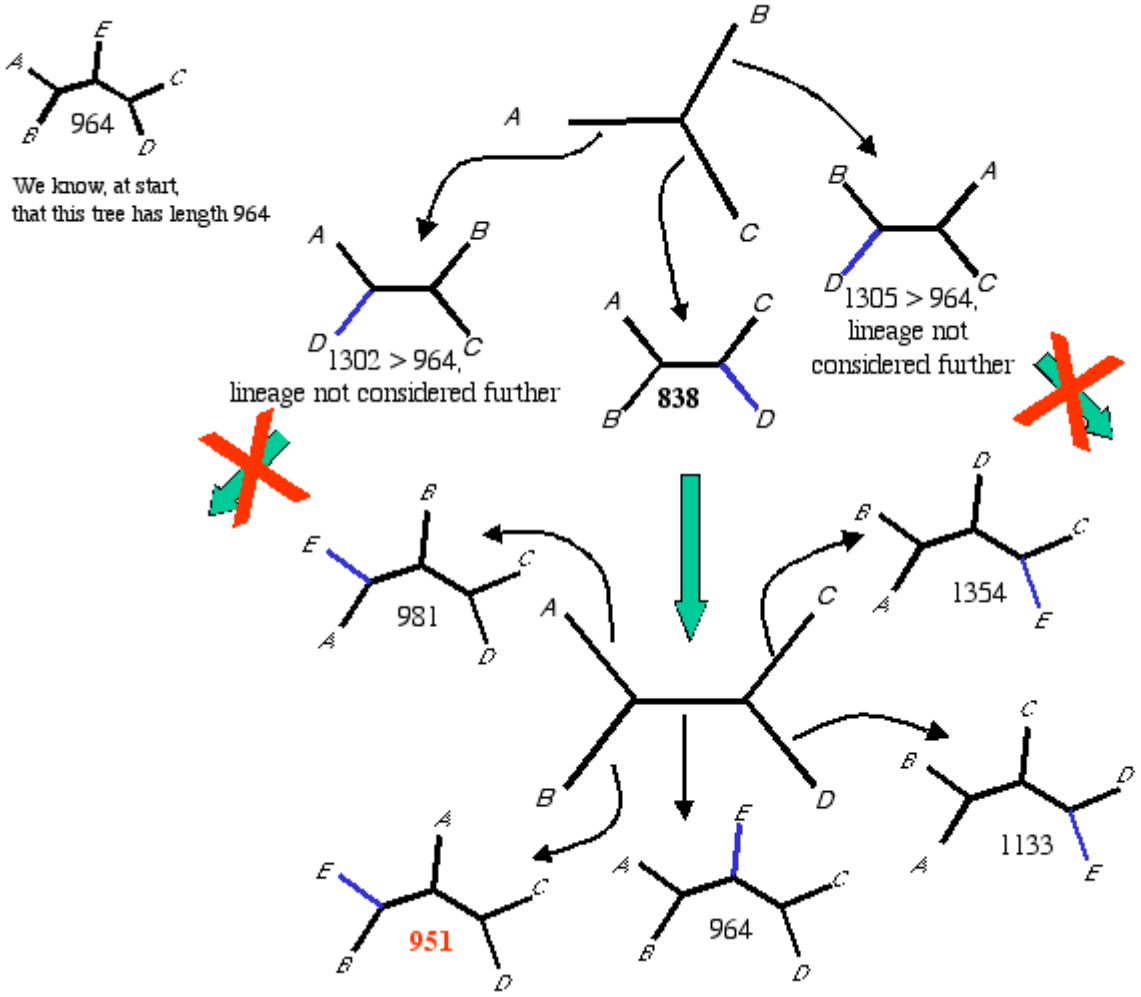
Exclude a subtree from the search if the score on the root node of the subtree is less than the best score achieved so far

Can improve speed by starting with a tree inferred using a different method

Works because the score only gets worse as you proceed towards the tips of the tree

Complexity:
At worst equal to the complexity of the exhaustive search

# Branch and bound




*http://artedi.ebc.uu.se/course/X3-2004/Phylogeny/Phylogeny-TreeSearch/Phylogeny-Search.html*

## Heuristic search algorithms

Greedy algorithms - Hill climbing approach

- NNI (Nearest Neighbour Interchange): break an interior branch and replace with one of the two alternative branches
- SPR (Subtree Pruning and Regrafting): remove a subtree from the tree and reinsert elsewhere
- TBR (Tree Bisection and Reconnection): break the tree to form two subtrees. Reconnect the two subtrees with a new branch between two existing branches in the two subtrees

Genetic Algorithms (e.g. MetaPig; Garli)

## Heuristic search algorithms

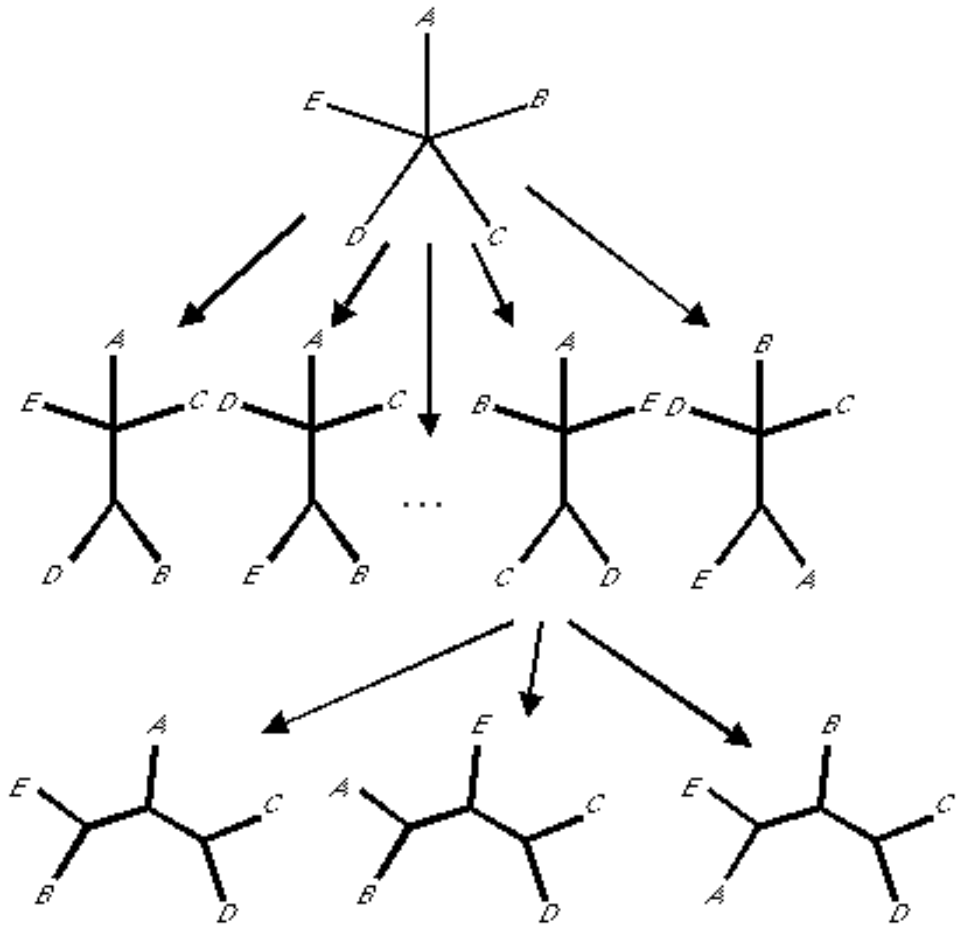
Can be sped up by starting with a reasonable tree (e.g. tree inferred with NJ algorithm).

Speed up also by estimating other parameters using an approximate tree prior to inferring the final tree topology (iterate if necessary).

Start tree can be from

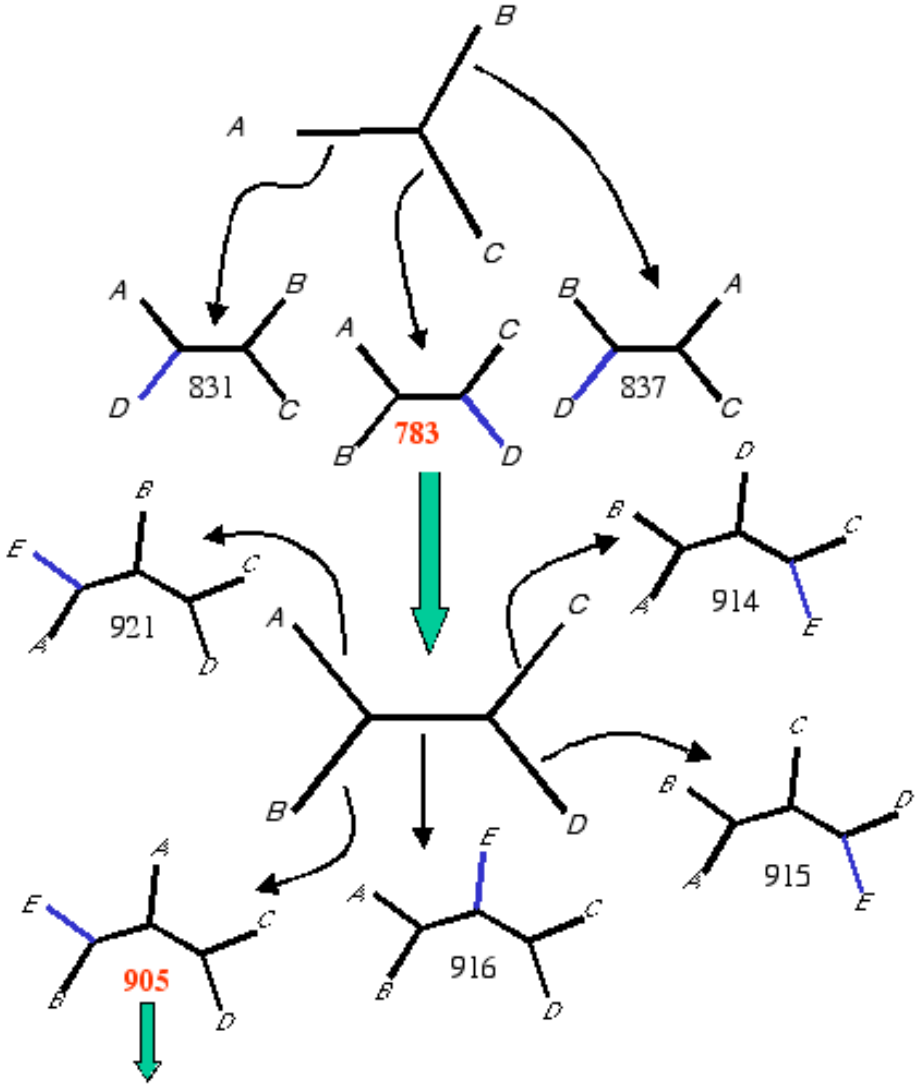
- an tree inferred from another method (e.g. NJ)
- Stepwise addition
- Star decomposition

# Star decomposition




*http://artedi.ebc.uu.se/course/X3-2004/Phylogeny/Phylogeny-TreeSearch/Phylogeny-Search.html*

# Stepwise addition




*http://artedi.ebc.uu.se/course/X3-2004/Phylogeny/Phylogeny-TreeSearch/Phylogeny-Search.html*

# Traversing a tree

Tree traversals:

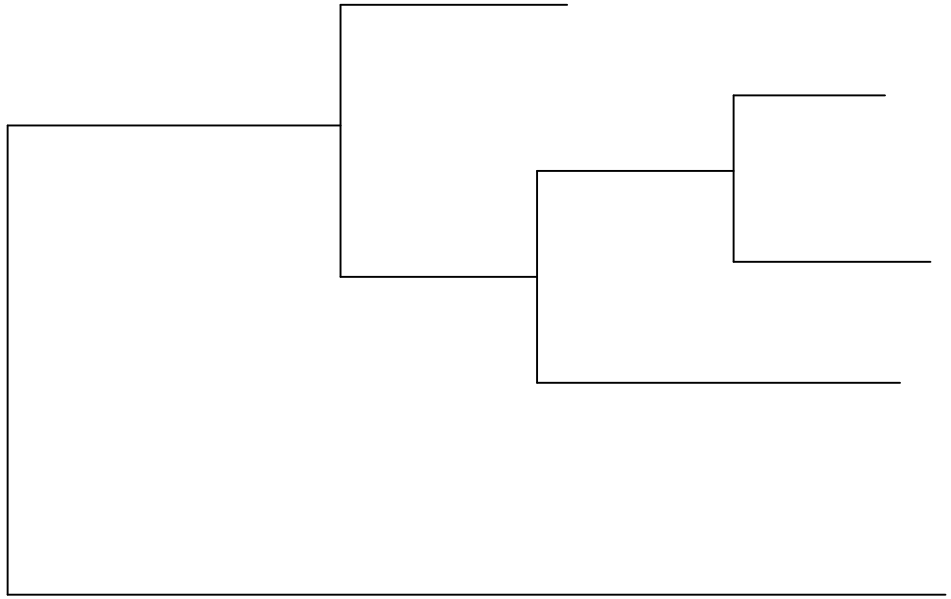
Preorder: node; left subtree; right subtree

Inorder: left subtree; node; right subtree

Postorder: left subtree; right subtree; node

All of these can be implemented using recursive functions

Exercise: Sketch this tree and label its nodes in the order in which they would be visited on preorder, inorder and postorder traversal, starting the algorithm at the root node



## Bayesian MCMC in phylogenetics

Prior over trees (often flat)

Starting tree (star decomposition, step-wise addition, NJ)

Proposals: tree perturbations

Acceptance depends on ratio of posterior probabilities ( = ratio of likelihoods)

Determine burn-in and convergence

In molecular phylogenetics the prior is usually ‘flat’

Why bother?

1. We get the answer as a probability

2. We get to use MCMC to sample over trees/search for ‘best’ tree

3. Allows us to integrate over nuisance parameters rather than using their optimal values

## Markov Chain Monte Carlo

Recall Markov Chain Monte Carlo: a Markov chain is specified with the desired limiting probabilities. Only ratios of limiting probabilities were required

$$p_{ij} = q_{ij} \min\left(1, \frac{\pi_j q_{ji}}{\pi_i q_{ij}}\right) \quad \text{for } i \neq j; \quad (p_{ii} \text{ s.t. } \sum_j p_{ij} = 1)$$

$$P(T, \Theta|D) = \frac{P(D|T, \Theta)P(T, \Theta)}{P(D)}$$

$P(D)$ is difficult to calculate, except for very small numbers of taxa, but cancels in the context of MCMC.

## Running a phylogenetic MCMC

Generate long chain of trees/parameters sampled according to their joint posterior probability

The number of times the chain visits tree X is proportional to the probability of tree X

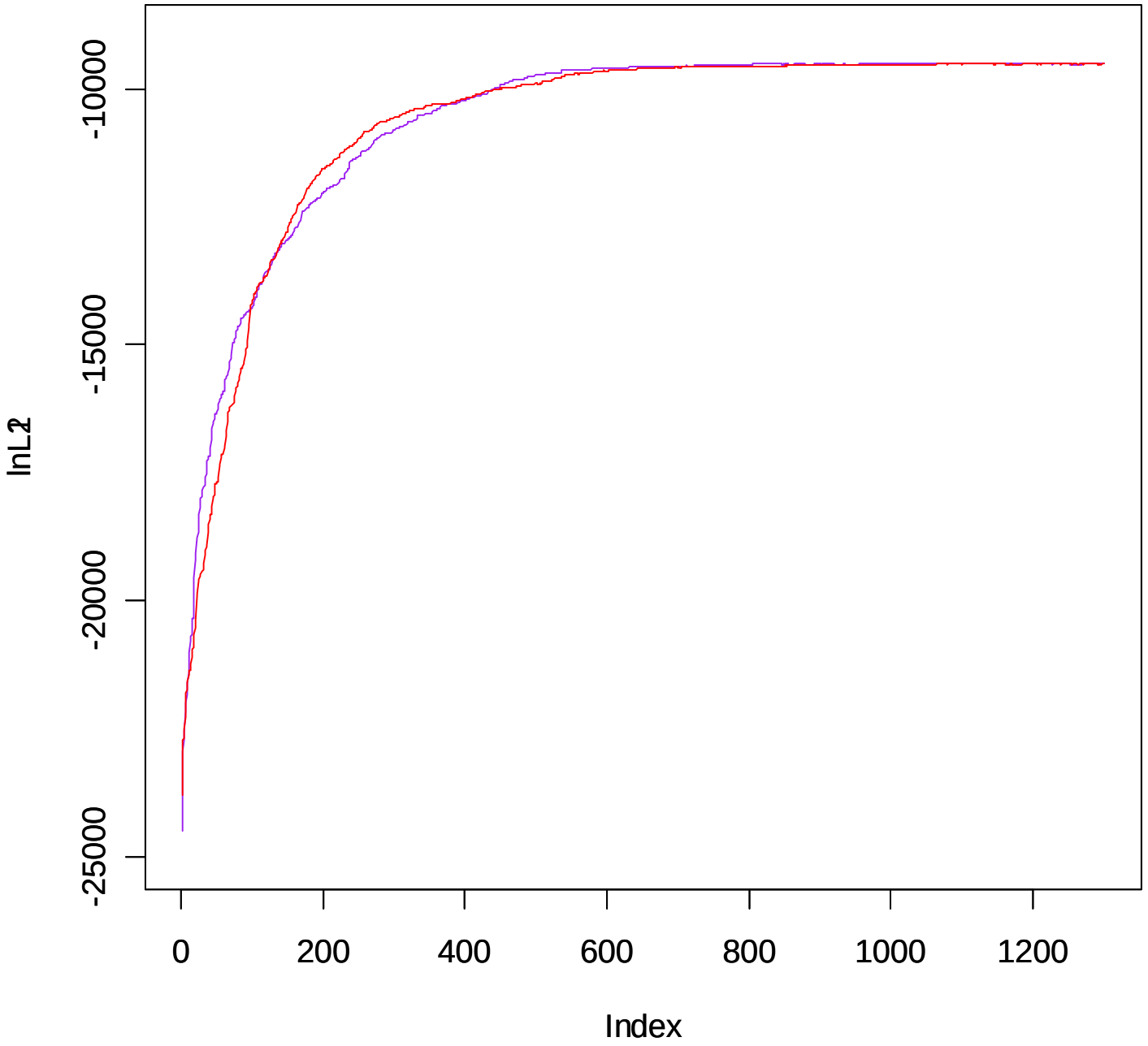
The number of times a specific branch is sampled can be used to estimate the posterior probability that the ‘clade’ of taxa specified by the branch is correct

Multiple chains may be used (Metropolis coupled MCMC = $MC^3$)

- Only one chain is sampled
- The other chains are heated (i.e. they can take bigger steps)
- Chains can swap states
- Allows crossing of valleys

# Burnin

- From an arbitrary starting point the chain can take some time to equilibriate
- Consequently, the chain takes some time before samples are obtaiend according to their posterior probabilites
- Initially probability of trees increases with time
- Programmes allowed to run until the probabilities are fluctuating randomly about a constant mean
- Data generated before the chain equilibriates are discarded


lnL2
-10000
-15000
-20000
-25000
0
200
400
600
800
1000
1200
Index

# Proposals

- Topology (e.g. NNI) or ‘coalescence time’ perturbations have been used
- Choice of proposal significant
  - Too aggressive results in rejection of most proposals
  - Too conservative takes too long to provide adequate sampling of parameter space

Advantages of Bayesian methods

- relatively fast
- easily interpretable
- often very accurate

Disadvantages of Bayesian methods

- can be difficult to be sure of convergence (this has improved with availability of better diagnostics)
- still controversial in molecular phylogenetics – choice of prior can be difficult to justify
- thought by some to exaggerate confidence

Software: e.g. MrBayes

## Further reading (molecular phylogenetics)

Inferring Phylogenies

Joseph Felsenstein, 2004

*Sinauer*

# Models of codon sequence evolution and inference of positive Darwinian selection

‘Universal’ genetic code is degenerate => natural classification of mutations as:

Nonsynonymous: amino acid changing (rate - dN)

Synonymous: no amino acid change (rate - dS)

ω: dN/dS

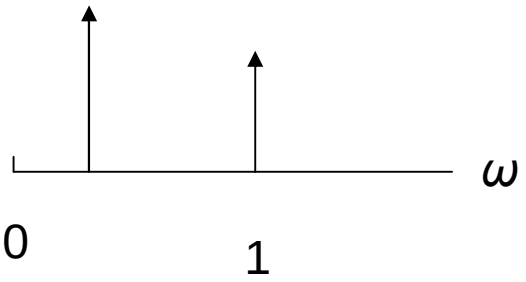
$\omega > 1$ => adaptive evolution (actually ‘diversifying selection’)

## Codon Substitution model

$$p_{ij} = \begin{cases} 0, & \text{codons differing at more than one position} \\ \pi_j, & \text{synonymous transversion} \\ \kappa\pi_j, & \text{synonymous transition} \\ \omega\pi_j, & \text{nonsynonymous transversion} \\ \omega\kappa\pi_j, & \text{nonsynonymous transition} \end{cases}$$

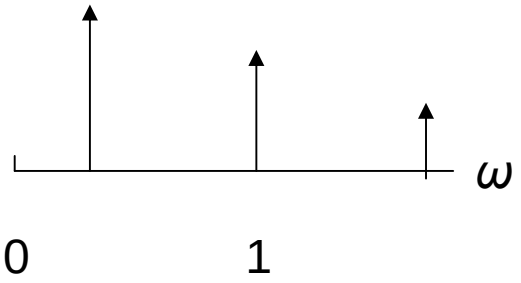
## Example: Analysis of selection using simple discrete $\omega$ distribution

Neutral model:




Free parameters: $\omega_{-} < 1$; $p_{\omega-}$

Selection model:




Free parameters: $\omega_{-} < 1$; $p_{\omega-}$; $\omega_{+} > 1$; $p_{\omega+}$

## Questions of interest

Is there a subset of sites with $\omega > 1$

- model comparison techniques

Which sites are evolving adaptively (empirical Bayes method)

- fix all parameter values to their ML estimates

- using ML estimates as priors, calculate posterior probabilities of belonging to selection site class

$$P(\omega_i > 1) \propto L(D \mid \omega_i > 1) P(\omega_i > 1)$$

## Analysis of selection

- Infer a phylogenetic tree

- Obtain ML estimates of all parameters

- Use LRT (or other model comparison method) to evaluate evidence for selection

- Use empirical Bayes method (or a variant) to estimate posterior probabilities of belonging to the selection site class

$$P(\omega_i = \omega_+ \mid D) = \frac{P(D \mid \omega_i = \omega_+)\, p_{\omega_+}}{\sum_k P(\omega_i = \omega_+)\, p_{\omega_k}}$$