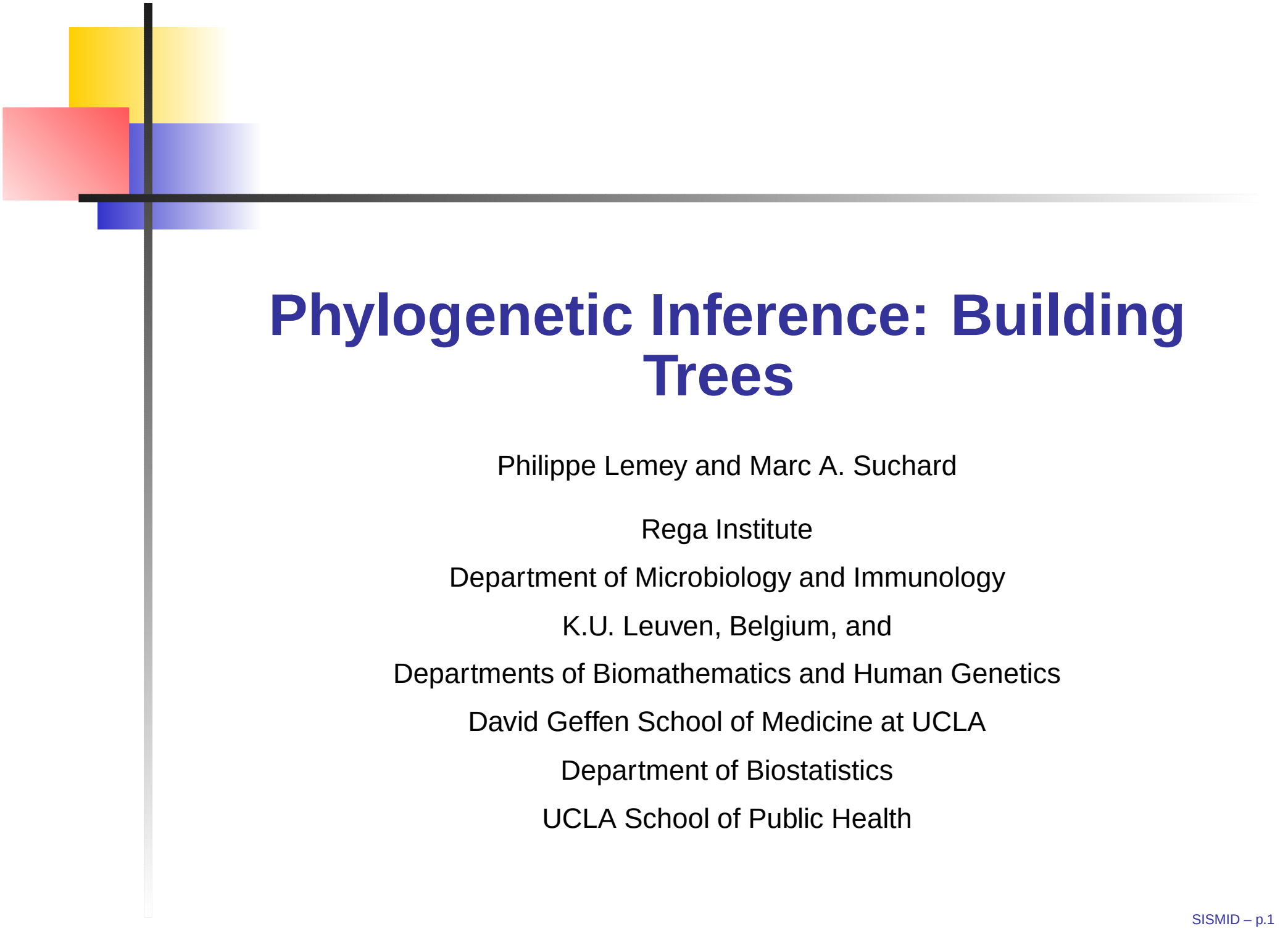

Phylogenetic Inference: Building Trees

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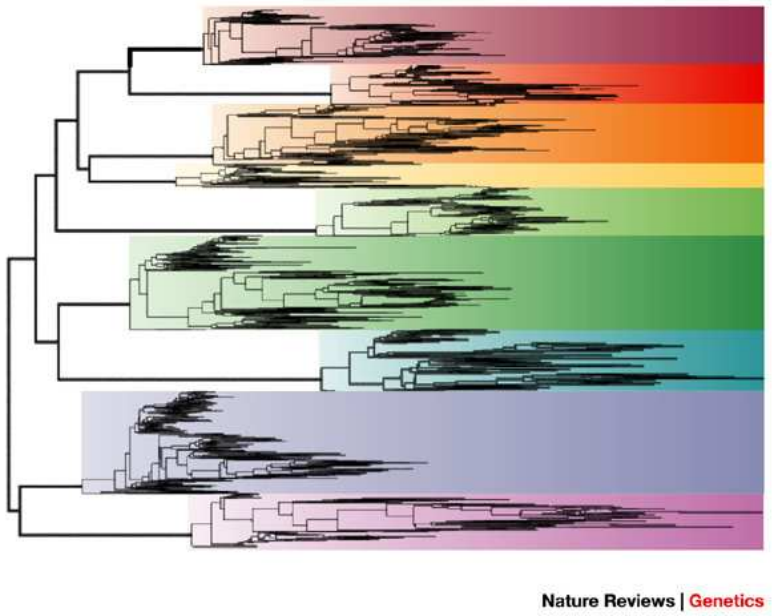
Intra-Host Viral Evolution



1195 *env* sequences from 9 HIV+ patients [taken from Rambaut et al. (2004)]

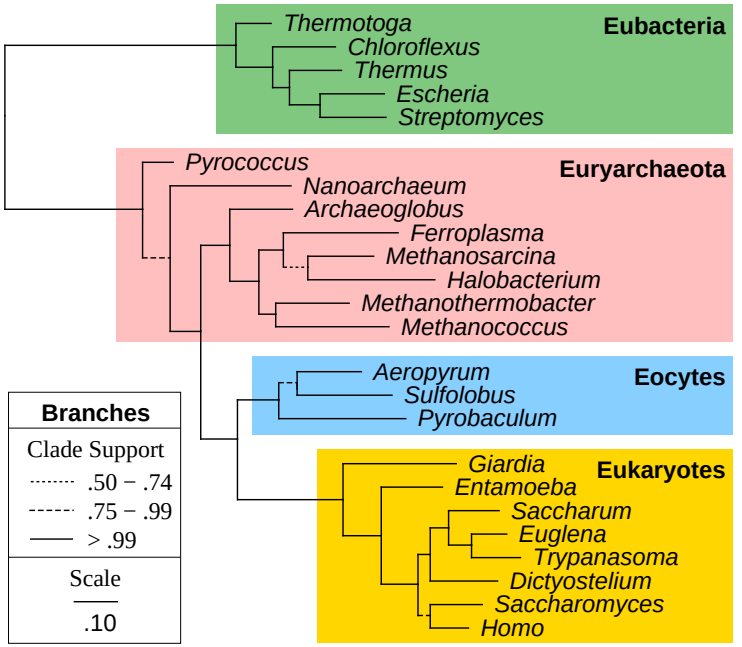
Retroviruses (and HBV) exist as a **quasi-species** within infected patients:

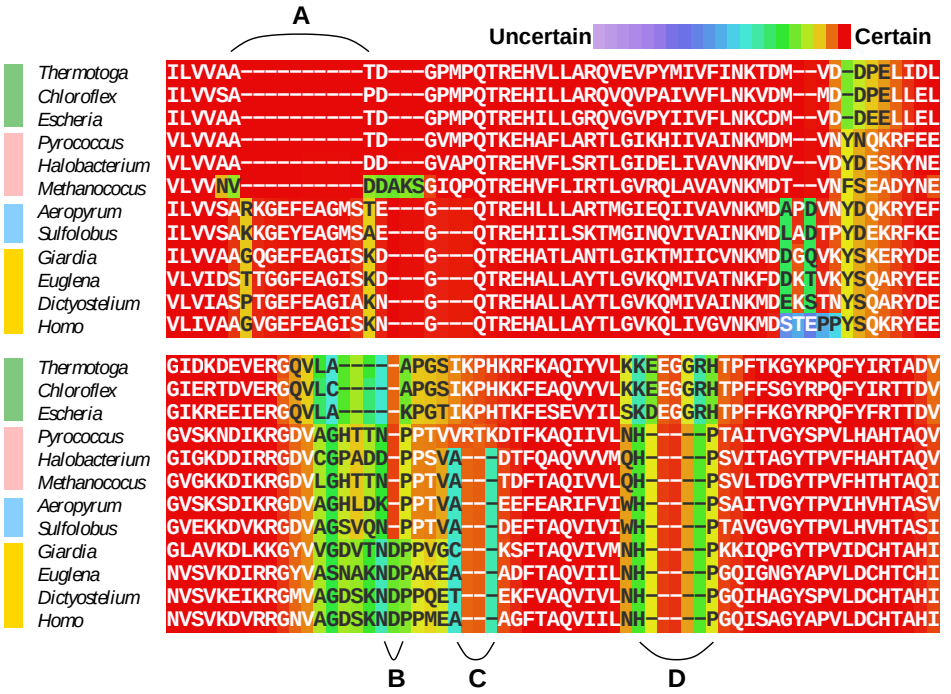
- Shared substitutions may be insufficient to resolve intra-host phylogenies

Improve resolution using joint model:

- Indel rates $\geq$ substitution rates
- Opportunity to detect intra-host recombination

Reconstructing the Tree of Life: Are Humans Just Big Slime Molds?



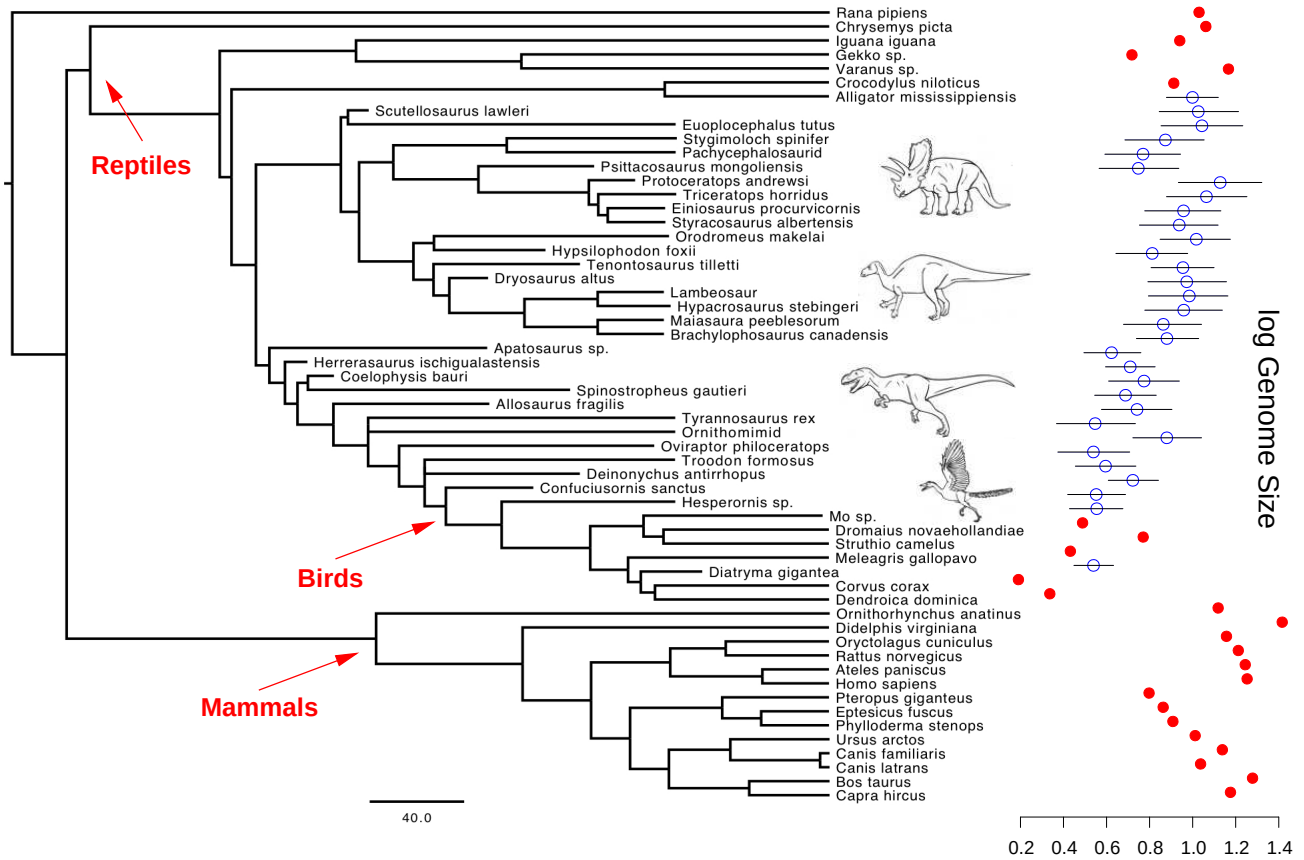


MAP Tree

Partial Au Plot

- Contentious issue among paleobiologists: Do **Archaea** (Euryarchaeota/Eocytes) form one or two domains? *Weekly World News* calls humans slime molds.

The Chicken or the (Small) Genome: Which Came First?



Evolutionary History and Genome Sizes of Reptiles, Dinosaurs, Birds and Mammals

Issue: Bird genomes are markedly smaller than those from other vertebrates.

Question: Did small genomes precede flight or co-evolve?

Maximum Parsimony (MP)

Most often used $\neq$ "best", not even statistically consistent, but **fast, fast, fast** . . . if you know the tree

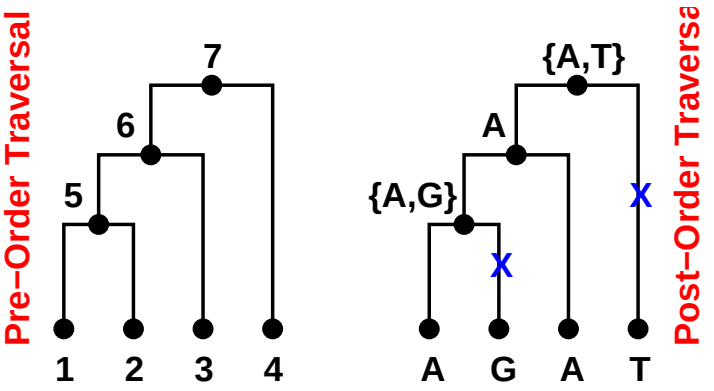
Key: Find tree with minimal # of "suspected" substitutions (internal states are not observed, 0/1 model process)

- Counting minimum # of substitutions is **easy**
- Enumerating (searching through) all possible trees is **hard**

Human	T	T	C	C	T	G	G	A	A	T
Chimp	T	A	C	C	T	G	G	A	A	T
Mouse	A	A	C	C	T	–	–	T	A	T
Fly	A	A	G	A	T	C	G	T	A	T
Site:	1	2	3	4	5	6	7	8	9	10

Along Molecular Sequence

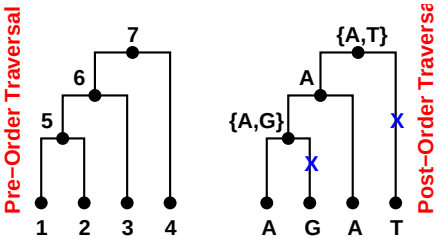
Sites are **independent**



Maximum Parsimony (MP)

A little history:

- Anthony Edwards/Luca Cavalli-Sforza (1963,1964)
  - Both students of R.A Fisher
  - Introduced both **parsimony** and **likelihood** methods (for continuous quantities, e.g. gene frequencies) in one paper
- Camin and Sokal (1965) provide first program for molecular sequences
- Fitch and Margoliash (1967) provide efficient algorithm

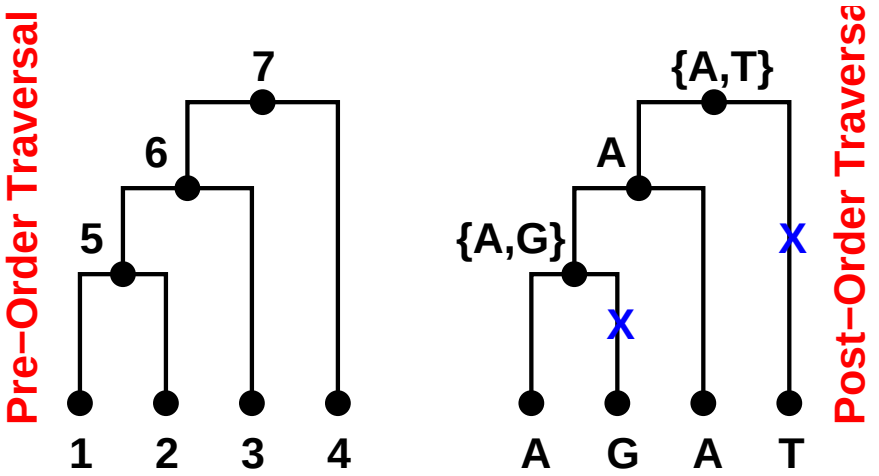


Maximum Parsimony Algorithm

procedure Fitch and Margoliash (1967) Algorithm

cost $C \leftarrow 0$ {**Initialization**}
pointer $k \leftarrow 2N - 1$ {at the root node}
To obtain the set R_k of possible states at node k {**Recursion**}
if k is leaf **then**
  $R_k \leftarrow$ observed character for taxon k
else
  Compute R_i, R_j for daughters i, j of k
  if $R_i \cap R_j \neq 0$ **then**
    $R_k \leftarrow R_i \cap R_j$
  else
    $R_k \leftarrow R_i \cup R_j$
    $C \leftarrow C + 1$
  end if
end if

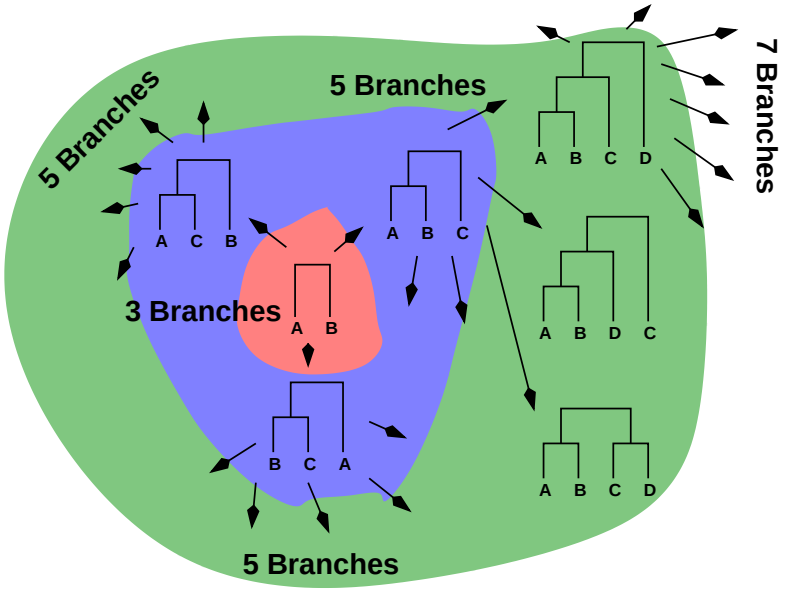
minimum cost is C {**Termination**}



Searching for the MP Tree

Complexity:

- Find MP score is **NP-complete**
- Find MP tree is **NP-hard**



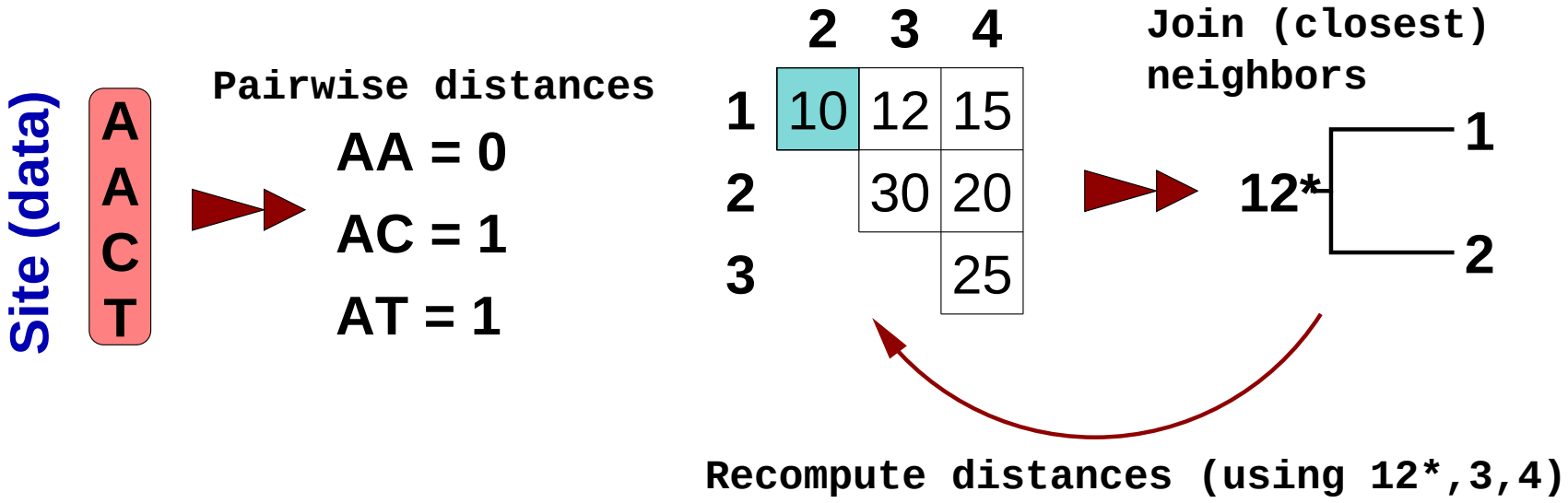
Recall that # of N-taxon rooted trees is $3 \times 5 \times \cdots \times 2N - 3$

Attack exponential-order space **Branch-and-Bound**:

- Monotonic order: min $\text{PS}_2 \leq$ min $\text{PS}_3 \leq \ldots$
- Bound if min $\text{PS}_k >$ best n-taxon PS found so far.

Neighbor-Joining (Saitou and Nei, 1987)

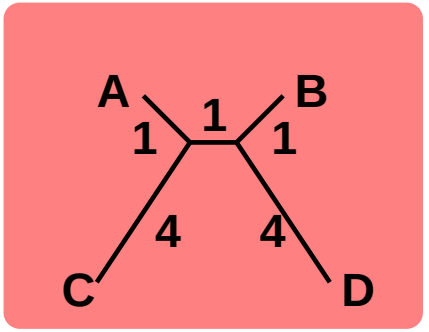
Computational algorithm: **alignment** → **single tree**



- **Advantages**: very fast, great for 1000s of sequences
- **Disadvantages**: no site-to-site rate variation, no **natural** ways to compare trees/measure data support

Neighbor-Joining

Caveat: Pairs i, j with min d_{ij} are **not** necessarily nearest neighbors. E.g., $d_{\text{AB}} = 3 < d_{\text{AC}} = 5$



Solution: Subtract off the average distances to all other leaves via

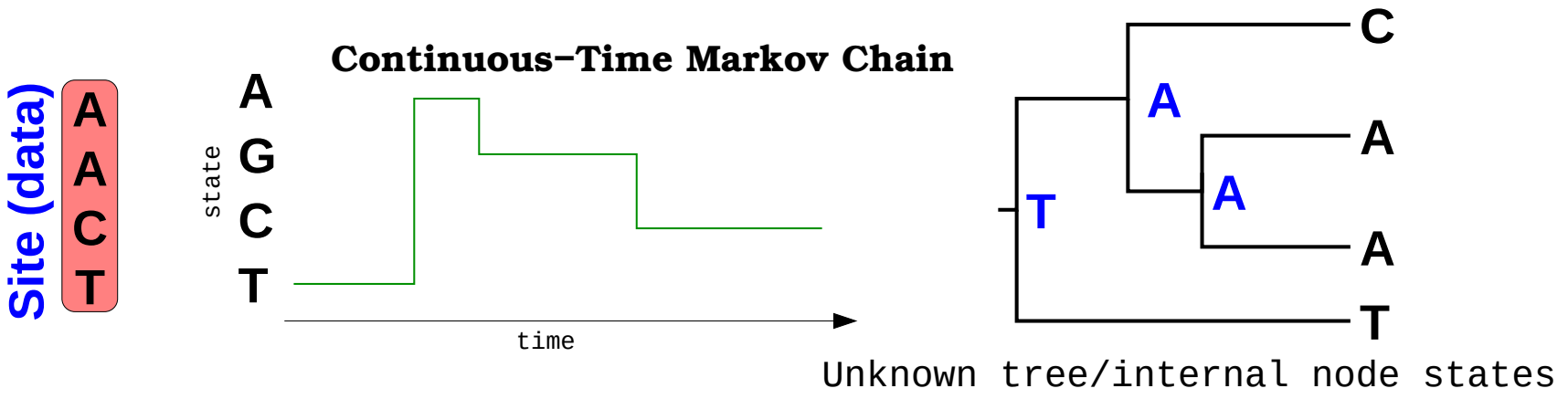
$$D_{ij} = d_{ij} - (r_i + r_j), \quad r_i = \frac{1}{|L| - 2} \sum_{k \in L} d_{ik},$$

where L is the current set of leaves. Proof in Studier and Keppler (1988).

Computational: $O(N^3)$

Likelihood-based Methods (Felsenstein, 1973)

Statistical technique: assumes an **unknown** tree and a stochastic model for character change along the tree

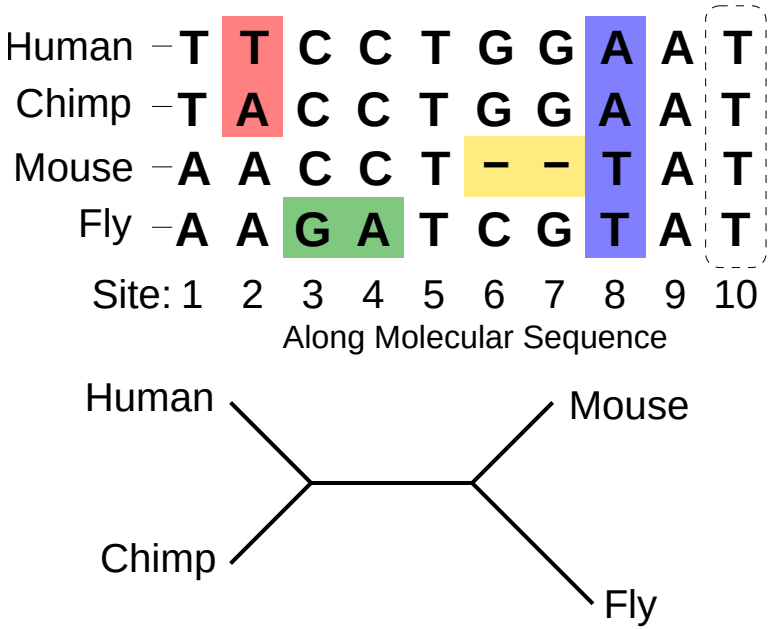


- **Advantages**: site-to-site rate/tree variation is easy, can formulate probability statements
- **Disadvantages**: must "search" tree-space $\rightarrow$ slow

Foundation of Bayesian Phylogenetics

Traditional Phylogenetic Reconstruction

Reconstruction Example



- **Substitution**: single residue replaces another
- **Insertion/deletion**: residues are inserted or deleted

Statistical Model

Assume: Homologous sites are iid and site patterns (e.g. dotted box)

$$XY \ldots Z \sim \text{Multinomial}(p_{XY \ldots Z})$$

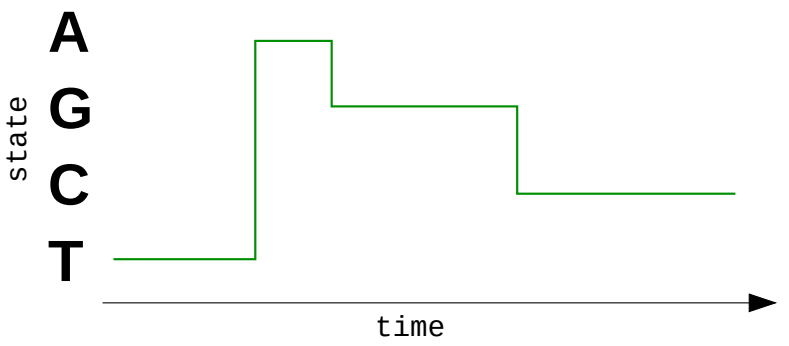
where $p_{XY \ldots Z}$ is determined by an unknown tree τ, branch lengths $t \in \mathbf{T}$ and continuous-time Markov chain model (for residue substitution) given by infinitesimal rate matrix $\mathbf{Q}$

$$\text{P}(X \to Y \text{ in time } t) = e^{t\mathbf{Q}}$$

CTMC(Q) = $\epsilon \sim$ Normal(μ, σ^2) of Phylogenetics

Continuous in elapsed time t, discrete in starting/ending state!

Memory-less process in which the probability that state b replaces state a during $(t, t+s) = sq_{ab} + o(s)$



- Infinitesimal generator matrix $\boldsymbol{Q}$ has off-diagonal entries q_{ab} and row sums $= 0$

Think: Exponential waiting time with rate $R_a = \sum_b q_{ab}$ until chain leaves a. Then the new state b is independently chosen with probabilities q_{ab}/R_a

From Infinitesimal to Finite Time

Let $p_{ab}(t) =$ the finite-time probability of the chain moving from state a at time 0 to state b at time t, then matrix $\boldsymbol{P}(t) = \{p_{ab}(t)\}$ satisfies

$$\frac{\mathrm{d}}{\mathrm{d}t}\boldsymbol{P}(t) = \boldsymbol{P}(t)\boldsymbol{Q} \text{ where } \boldsymbol{P}(0) = \boldsymbol{I}$$

with solution

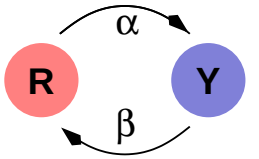
$$\boldsymbol{P}(t) = e^{t\boldsymbol{Q}} = \boldsymbol{I} + t\boldsymbol{Q} + \frac{1}{2}(t\boldsymbol{Q})^2 + \cdots = \sum_{k=0}^{\infty} \frac{1}{k!}(t\boldsymbol{Q})^k$$

as

$$\frac{\mathrm{d}}{\mathrm{d}t}e^{t\boldsymbol{Q}} = \boldsymbol{Q}e^{t\boldsymbol{Q}} = e^{t\boldsymbol{Q}}\boldsymbol{Q} \text{ for } t \text{ real}$$

Example: Two-State Model

Consider purines (R) $\leftrightarrow$ pyrimidines (Y). Kolmogorov forward equation:



$$p_{\mathsf{RY}}(t+s) = p_{\mathsf{RR}}(t)\alpha s + p_{\mathsf{RY}}(t)(1-\beta s) + o(s)$$

yielding

$$\frac{\mathrm{d}}{\mathrm{d}t} p_{\mathsf{RY}}(t) = \alpha p_{\mathsf{RR}}(t) - \beta p_{\mathsf{RY}}(t)$$

$$\boldsymbol{Q} = \begin{pmatrix} -\alpha & \alpha \\ \beta & -\beta \end{pmatrix}$$

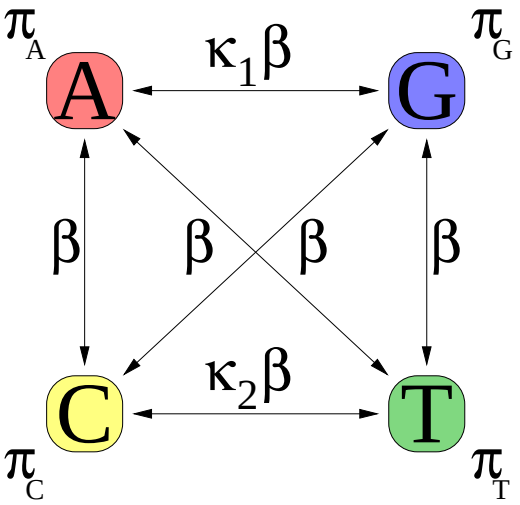
Solutions of $\boldsymbol{P}(t) = e^{t\boldsymbol{Q}}$ have the form

$$c + de^{-(\alpha+\beta)t}$$

with eigenvalues 0 and $-(\alpha+\beta)$

Standard CTMCs for Phylogenetics

- Jukes and Cantor (JC69), $\pi_a = \frac{1}{4}, \kappa_1 = \kappa_2 = 1$
- Kimura (K80), $\pi_a = \frac{1}{4}, \kappa_1 = \kappa_2$
- Hasegawa, Kishino and Yano (HKY85), $\kappa_1 = \kappa_2$ (most common)
- Tamura and Nei (TN93), right
- General Time Reversible (GTR)



Note identifiability concern in e^{tQ}. Common solution is to fix 1 d.f. such that

$$\sum_a q_{aa}\pi_a = -1$$

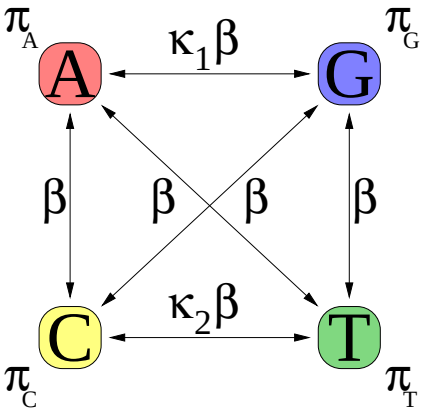
Scaling: $t = 1 \Rightarrow 1$ expected substitution per site

Explicit Parameterization of TN93

Nucleotides mutate according to a **Markovian** process

$$\Pr(X \to Y \text{ in time } t) = e^{tQ_{\text{Nuc}}}$$

where Q_{Nuc} is a 4x4 infinitesimal rate matrix and t is a branch length.



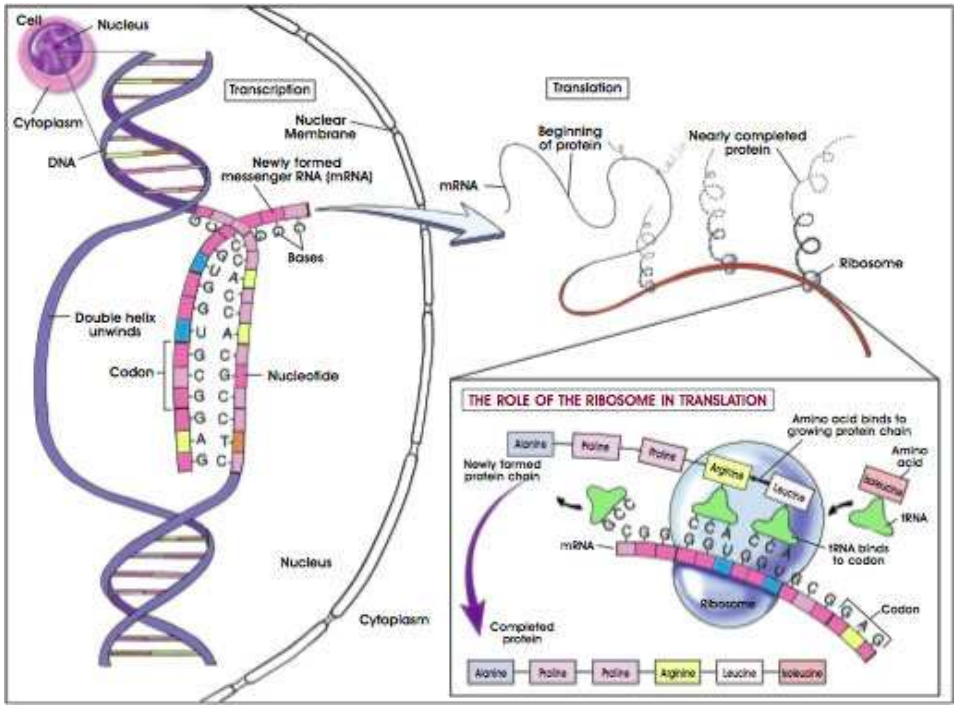
$$Q_{\text{Nuc}} = \beta \times \begin{pmatrix} - & \kappa_1\pi_G & \pi_C & \pi_T \\ \kappa_1\pi_A & - & \pi_C & \pi_T \\ \pi_A & \pi_G & - & \kappa_2\pi_T \\ \pi_A & \pi_G & \kappa_2\pi_C & - \end{pmatrix}, \text{ where}$$

κ_1, κ_2 are transition:transversion rate ratios and π is the stationary distribution of {A,G,C,T}. β controls the overall rate and can vary from site-to-site.

Site-to-Site Rate Variation

Variation occurs quite naturally and is also **an important inference**

- short range: codon phase (slow-slow-fast)
- long range: enzymatic active sites, protein folding, immunological pressures/selection



Assume: infinitesimal rates for site k are $r_k \times t \times q_{ab}$. Various priors on r_k with $\mathrm{E}(r_k) = 1$. Implicitly Bayesian

- Yang (1994) – discretized Gamma distribution

General Time Reversible CTMC

Let

$$\boldsymbol{Q} = \boldsymbol{R}\boldsymbol{D}_{\pi}$$

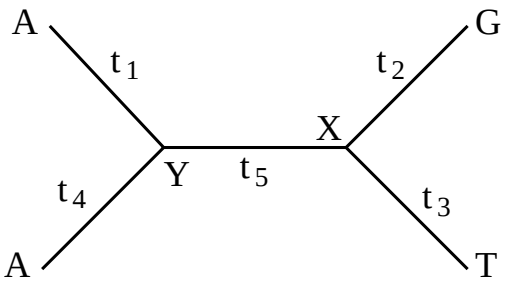
where $\boldsymbol{R}$ is symmetric and $\boldsymbol{D}_{\boldsymbol{\pi}}$ is a diagonal matrix composed of the stationary distribution $\boldsymbol{\pi}$.

- Detailed balance $\Leftrightarrow \pi_a q_{ab} = \pi_b q_{ba}$. Balance + irreducibility $\Leftrightarrow$ reversible
- **Note** $\boldsymbol{Q}$ is similar to $\boldsymbol{R}$, as $\boldsymbol{D}^{1/2}\boldsymbol{Q}\boldsymbol{D}^{-1/2} = \boldsymbol{R}$
- Hence, $\boldsymbol{Q}$ must have real eigenvalues and real eigenvectors

The properties speed up computation of the finite-time transition matrix $\boldsymbol{P}(t) = e^{t\boldsymbol{Q}}$

Calculating the Probability of a Single Site Pattern Y_i

Given the tree and **unobserved** internal node states, the probability is the product of the finite time mutation probabilities over all branches:



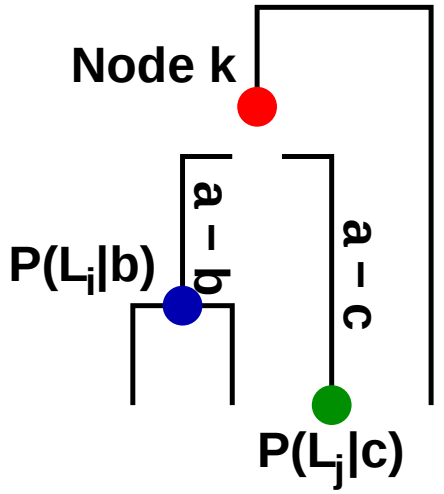
$$L(\boldsymbol{Y}_i) \propto p_{\mathsf{AAGT}} = \sum_X \sum_Y \Pr(Y \to \mathsf{A}, t_1) \Pr(X \to \mathsf{G}, t_2) * \Pr(X \to \mathsf{T}, t_3) \Pr(Y \to \mathsf{A}, t_4) \Pr(X \to Y, t_5) \pi_X \quad (1)$$

- Number of sumants grow rapidly in $N \to$ sum-product/peeling algorithm to distribute sums across the product

Pruning Algorithm Felsenstein (1981)

Let $P(L_k|a)$ = likelihood of leaves below node k given k is in state a. Then, recursively compute $P(L_k|a)$ given $P(L_i|b)$ and $P(L_j|c)$ for daughters i, j of k:

Set pointer $k \leftarrow 2N - 1$ {the root, **initialization**}
Compute $P(L_k|a)\ \forall\ a$ as follows: {**recursion**}
if k is a leaf node **then**
  if a is observed **then**
    $P(L_k|a) = 1$
  else
    $P(L_k|a) = 0$
  end if
else
  Compute $P(L_i|a)$ and $P(L_j|a)\ \forall a$ for daughters i, j of k {**post-order traversal**}
  $P(L_k|a) = \sum_b \sum_c \Pr(a \rightarrow b, t_i) P(L_i|b) \times \Pr(a \rightarrow c, t_j) P(L_j|c)$
end if
$L(\mathbf{Y}_i) \leftarrow \sum_a P(L_{2n-1}|a)\pi_a$ {**termination**}



ML Tree or MAP Tree?

Reporting uncertainty on tree estimates:

- The Bootstrap
  - Most common
  - Assumes evolutionary events are reproducible. “If I went back out to the field and recollected exchangeable data . . . ”
- Bayesian inference
  - Returns the probability of a tree given the observed data and model
  - Requires MCMC (e.g., **MrBayes** or **BEAST**)
  - **Advantages**
    - Does not rely on asymptotics (hypothesis testing)
    - Naturally incorporates uncertainty in all parameters (including discrete quantities: trees, site-classifications, etc.)
    - Arguably faster algorithms
  - **Disadvantages**
    - Must specify (justifiable) prior distributions