

# Phylogenetic Inference: Building Trees

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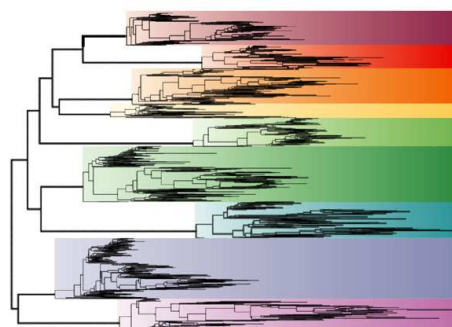
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# Intra-Host Viral Evolution



Nature Reviews | Genetics

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

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

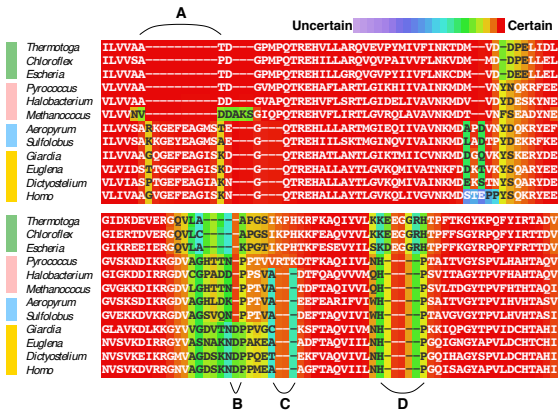
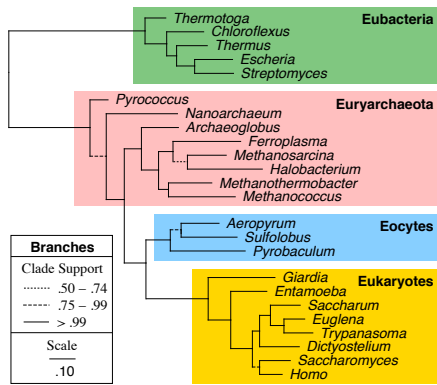
- Shared substitutions may be insufficient to resolve intra-host phylogenies

Improve resolution using joint model:

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

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



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

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



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

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

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

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# Maximum Parsimony (MP)

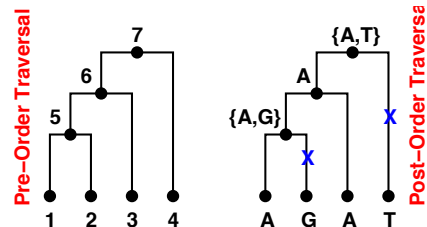
**Most often used**  $\neq$  “best”, not even statistically consistent, but **fast, fast, fast** . . . if you know the tree

**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	C	C	T	G	G	A	A	T
Chimp	-	A	C	C	T	G	G	A	A	T
Mouse	-	A	A	C	T	-	-	T	A	T
Fly	-	A	A	G	A	T	C	G	T	A
Site:	1	2	3	4	5	6	7	8	9	10
	Along Molecular Sequence									

Sites are **independent**

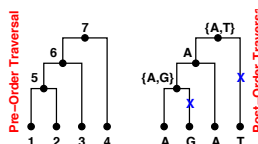


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# Maximum Parsimony (MP)

**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



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# 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**  $R_i \cap R_j \neq \emptyset$  **then**

$R_k \leftarrow R_i \cap R_j$

**else**

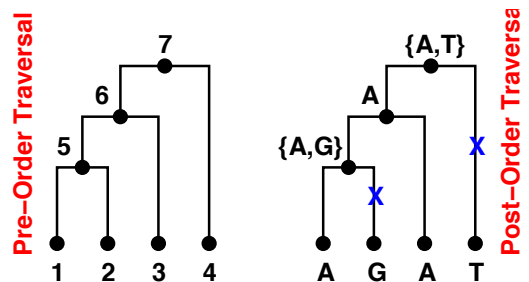
$R_k \leftarrow R_i \cup R_j$

$C \leftarrow C + 1$

**end if**

**end if**

minimum cost is  $C$  {Termination}

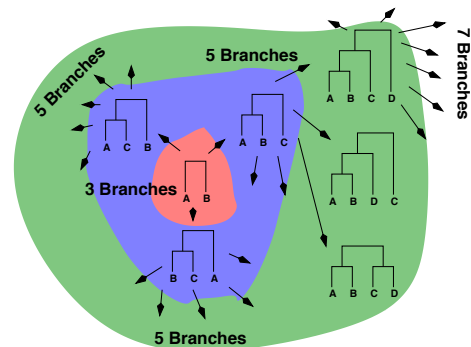


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# Searching for the MP Tree

**Complexity:**

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



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

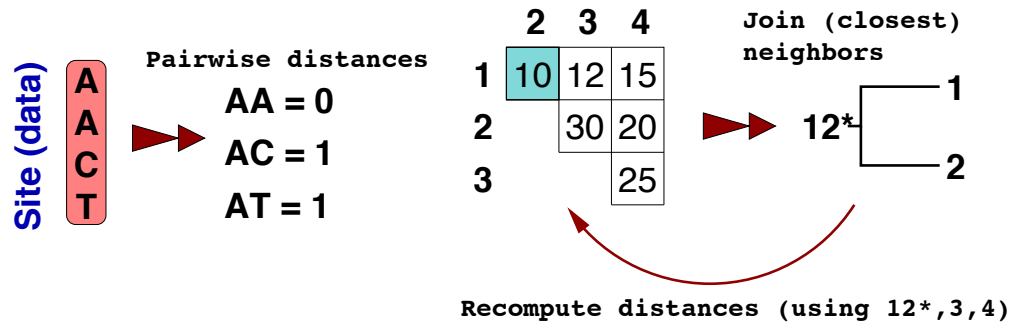
Attack exponential-order space **Branch-and-Bound**:

- Monotonic order:  $\min PS_2 \leq \min PS_3 \leq \dots$
- Bound if  $\min PS_k >$  best  $n$ -taxon PS found so far.

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# Neighbor-Joining (Saitou and Nei, 1987)

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

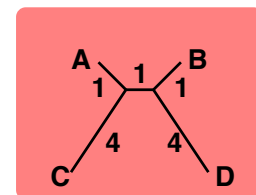


- **Advantages:** very fast, great for 1000s of sequences
- **Disadvantages:** no site-to-site rate variation, no **natural** ways to compare trees/measure data support

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# Neighbor-Joining

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



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

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

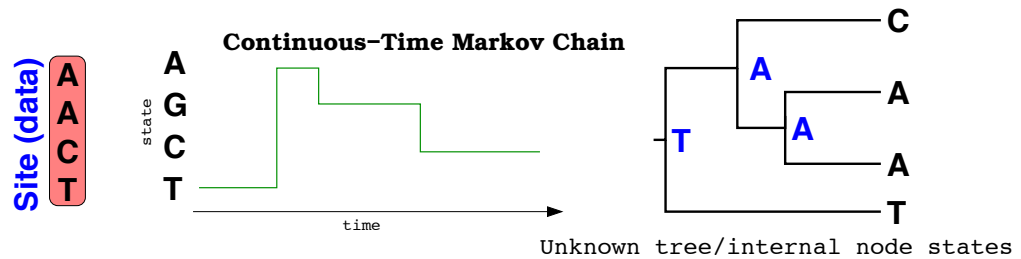
where  $L$  is the current set of leaves. Proof in Studier and Keppler (1988).

**Computational:**  $O(N^3)$

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# 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 → slow

## Foundation of Bayesian Phylogenetics

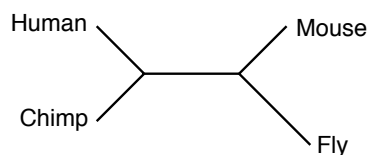
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# Traditional Phylogenetic Reconstruction

## Reconstruction Example

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



- **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)

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

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

$$P(X \rightarrow Y \text{ in time } t) = e^{tQ}$$

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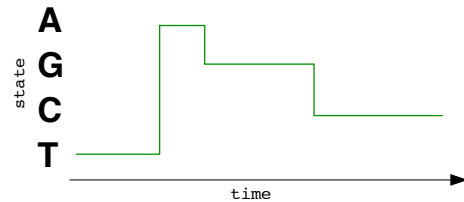
# CTMC( $Q$ ) = $\epsilon \sim \text{Normal}(\mu, \sigma^2)$ of Phylogenetics

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  $Q$  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  $q_{ab}/R_a$



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## 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  $P(t) = \{p_{ab}(t)\}$  satisfies

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

with solution

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

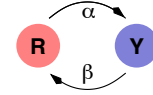
as

$$\frac{d}{dt}e^{tQ} = Qe^{tQ} = e^{tQ}Q \text{ for } t \text{ real}$$

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## Example: Two-State Model

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



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

yielding

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

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

Solutions of  $P(t) = e^{tQ}$  have the form

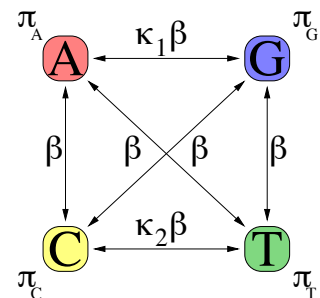
with eigenvalues 0 and  $-(\alpha + \beta)$

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

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## 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

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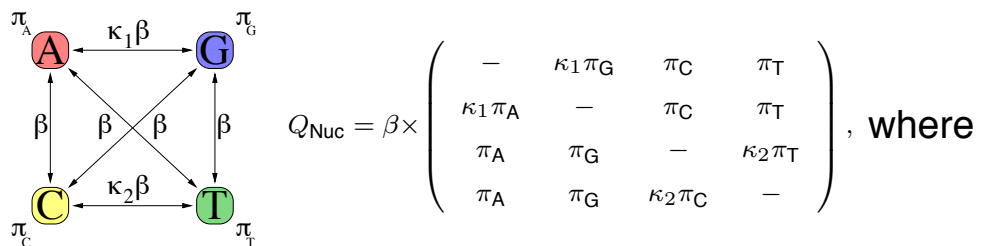


# Explicit Parameterization of TN93

Nucleotides mutate according to a **Markovian** process

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

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

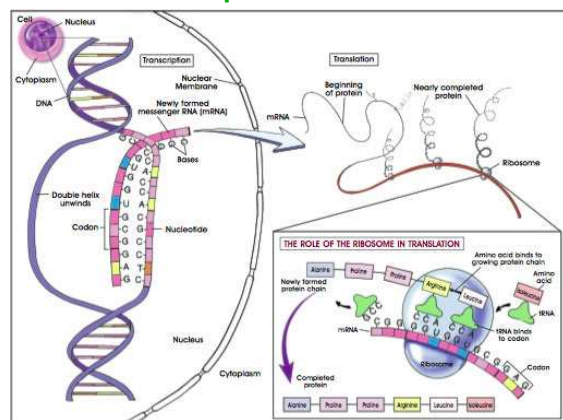


$k_1, k_2$  are transition:transversion rate ratios and  $\pi$  is the stationary distribution of  $\{A, G, C, T\}$ .  $\beta$  controls the overall rate and can vary from site-to-site.

# 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  $E(r_k) = 1$ . Implicitly Bayesian

- Yang (1994) – discretized Gamma distribution

## General Time Reversible CTMC

Let

$$Q = RD_{\pi}$$

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

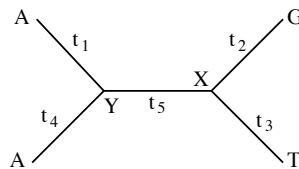
- Detailed balance  $\Leftrightarrow \pi_a q_{ab} = \pi_b q_{ba}$ . Balance + irreducibility  $\Leftrightarrow$  reversible
- **Note**  $Q$  is similar to  $R$ , as  $D^{1/2} Q D^{-1/2} = R$
- Hence,  $Q$  must have real eigenvalues and real eigenvectors

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

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## 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:



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

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

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# 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**

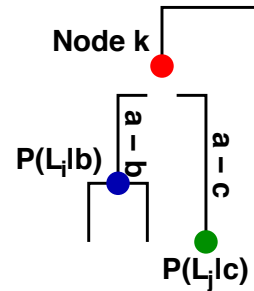
**else**

  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**}



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# 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

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