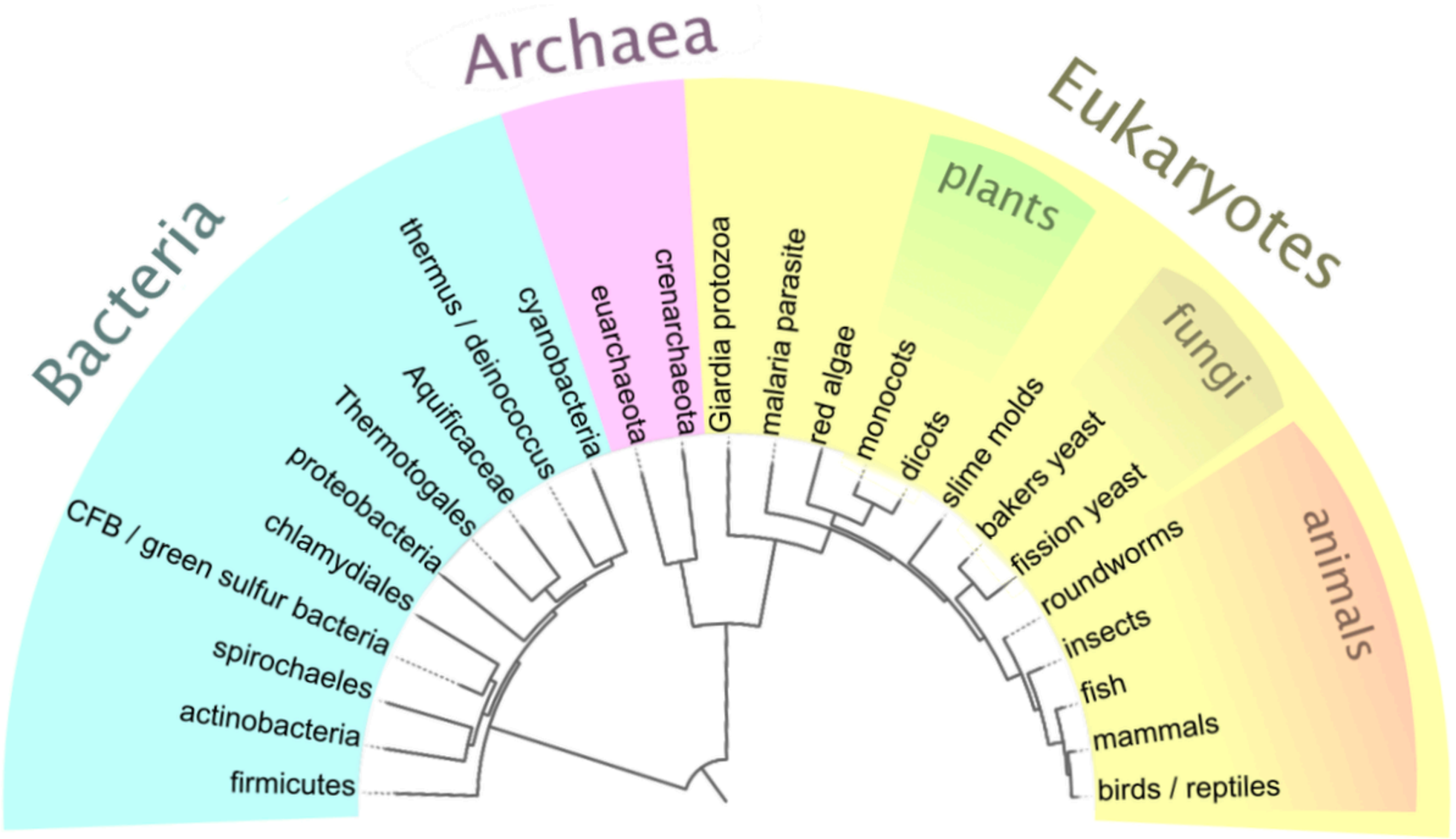
Phylogenomics

UNIVERSITY OF MARYLAND

slides marked with * by Carl Kingsford

Tree of Life



https://simple.wikipedia.org/wiki/Tree_of_life_(biology)

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TREE OF LIFE web project

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Agaricales
(a group of fungi)

image info

The Agaricales, or euagarics clade, is a monophyletic group of approximately 8500 mushroom species...

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The Tree of Life Web Project (ToL) is a collaborative effort of biologists and nature enthusiasts from around the world. On more than 10,000 World Wide Web pages, the project provides information about biodiversity, the characteristics of different groups of organisms, and their evolutionary history (phylogeny).

Each page contains information about a particular group, e.g., salamanders, segmented worms, phlox flowers, tyrannosaurs, euglenids, *Heliconius* butterflies, club fungi, or the vampire squid. ToL pages are linked one to another hierarchically, in the form of the evolutionary tree of life. Starting with the root of all Life on Earth and moving out along diverging branches to individual species, the structure of the ToL project thus illustrates the genetic connections between all living things.

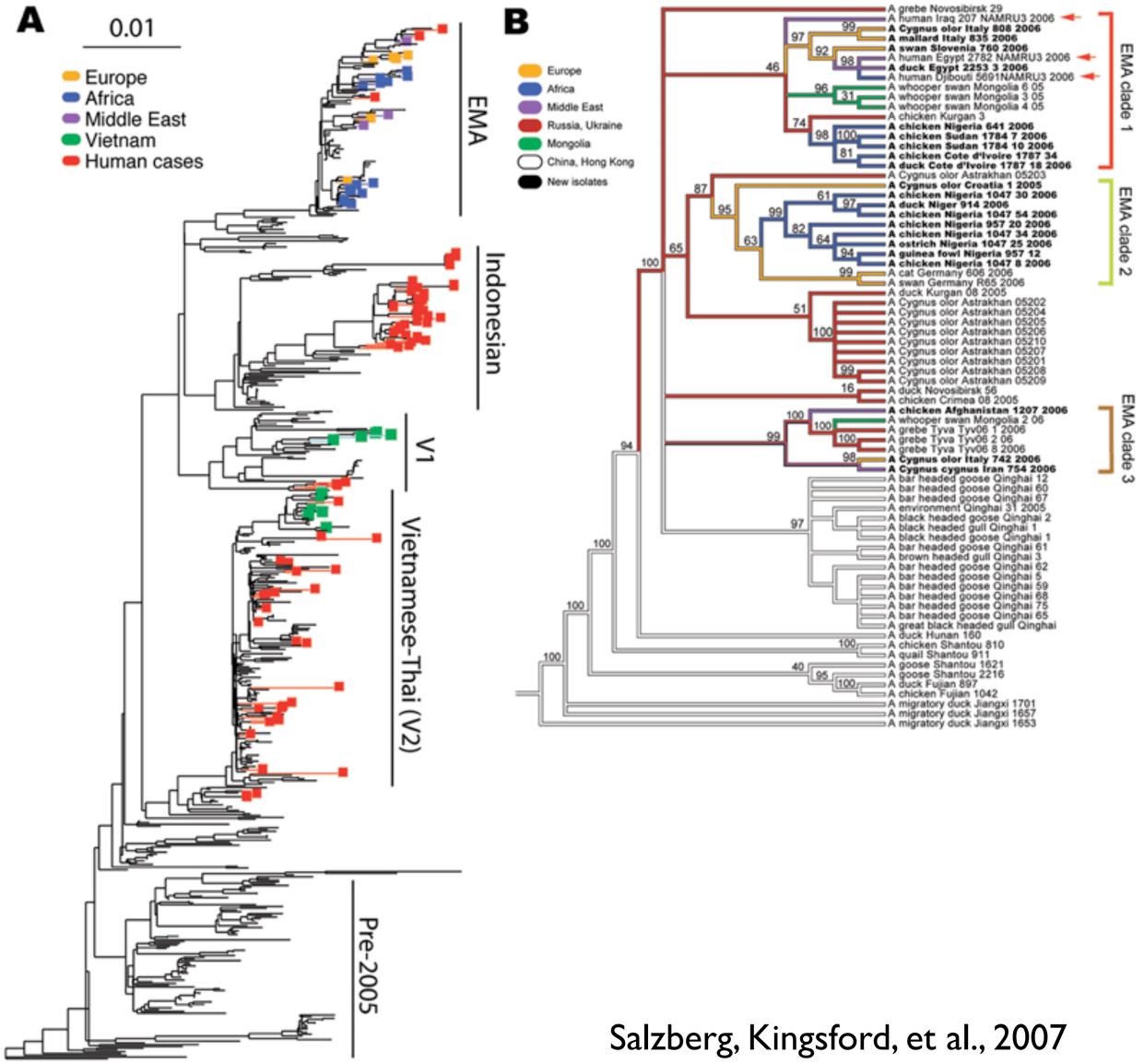
read more about the Tree of Life Web Project...

"The affinities of all the beings of the same class have sometimes been represented by a great tree... As buds give rise by growth to fresh buds, and these if vigorous, branch out and overtop on all sides many a feebler branch, so by generation I believe it has been with the great Tree of Life, which fills with its dead and broken branches the crust of the earth, and covers the surface with its ever branching and beautiful ramifications."

Charles Darwin, 1859

http://tolweb.org/tree/

H5N1 Influenza Strains



Salzberg, Kingsford, et al., 2007

*

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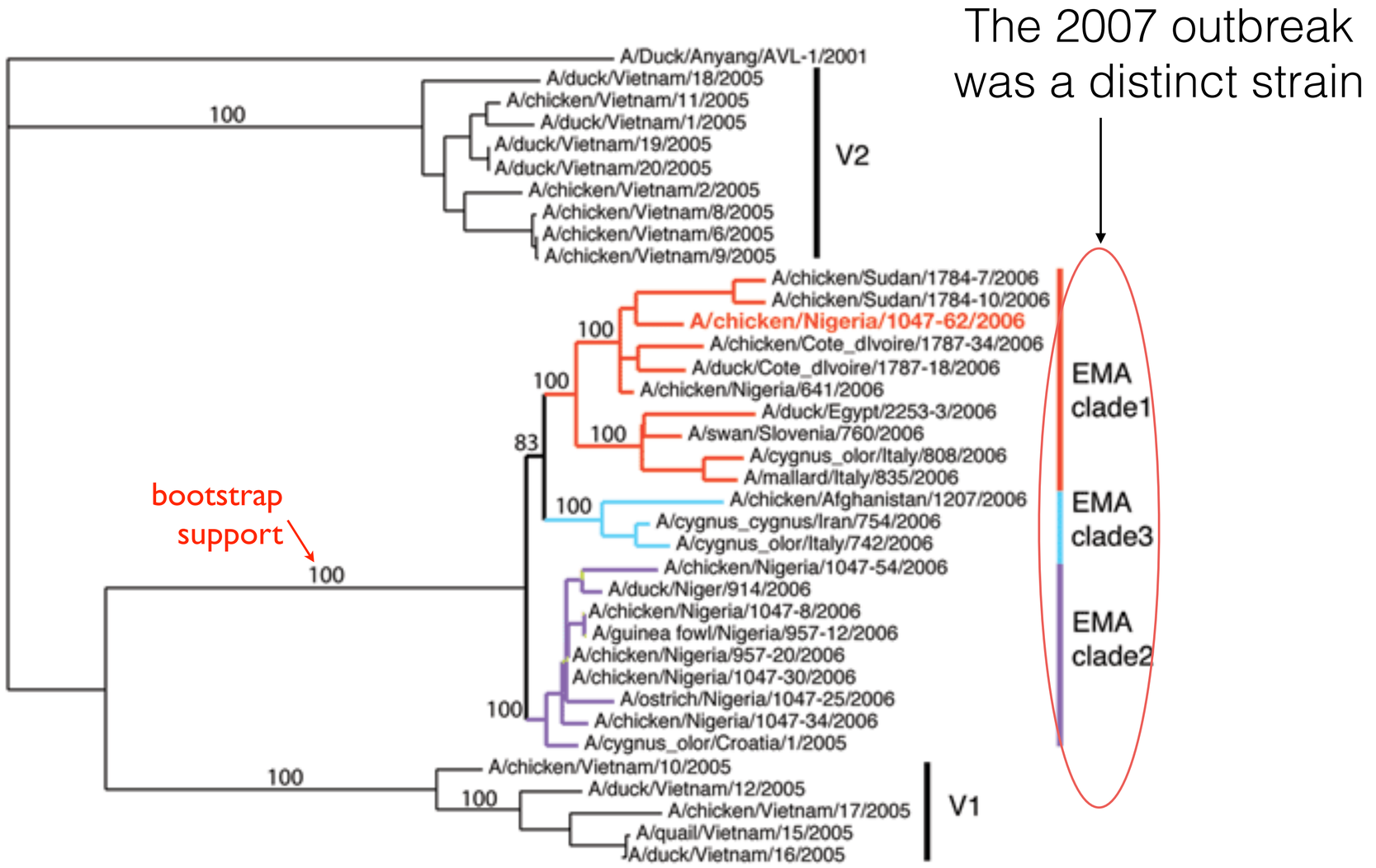

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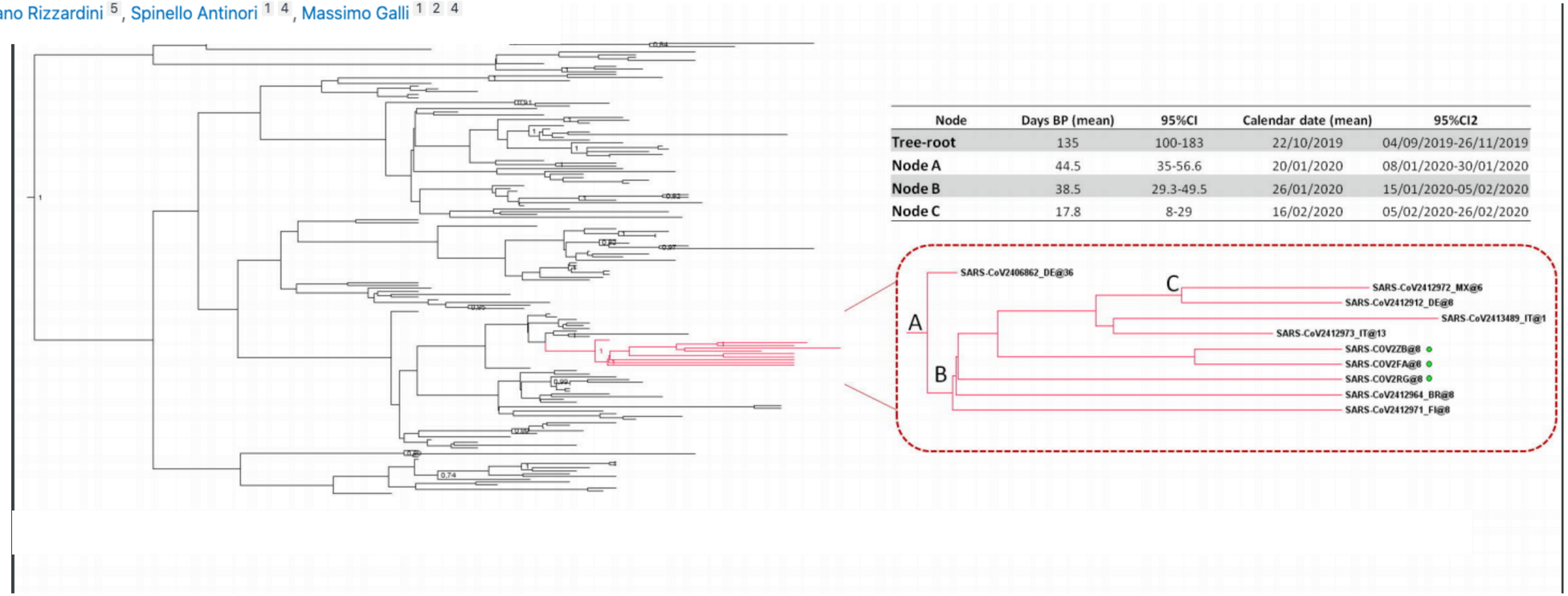
J Med Virol. 2020 Sep;92(9):1637-1640. doi: 10.1002/jmv.25794. Epub 2020 Apr 10.

Genomic characterization and phylogenetic analysis of SARS-COV-2 in Italy

Gianguglielmo Zehender [1 2 3], Alessia Lai [1 2], Annalisa Bergna [1], Luca Meroni [4], Agostino Riva [4], Claudia Balotta [1], Maciej Tarkowski [1], Arianna Gabrieli [1], Dario Bernacchia [4], Stefano Rusconi [1 4], Giuliano Rizzardini [5], Spinello Antinori [1 4], Massimo Galli [1 2 4]



Node	Days BP (mean)	95%CI	Calendar date (mean)	95%CI2
Tree-root	135	100-183	22/10/2019	04/09/2019-26/11/2019
Node A	44.5	35-56.6	20/01/2020	08/01/2020-30/01/2020
Node B	38.5	29.3-49.5	26/01/2020	15/01/2020-05/02/2020
Node C	17.8	8-29	16/02/2020	05/02/2020-26/02/2020

Our tMRCA estimate showed that the root of clade A was in the month of January 2020, a period compatible with this event.

Our data suggest that SARS-CoV-2 virus entered northern Italy between the second half of January and early February 2020, which is weeks before the first Italian case of COVID-19 was identified and therefore long before the current containment measures were taken.

Questions Addressable by Phylogeny

- How many times has a feature arisen? been lost?
- How is a disease evolving to avoid immune system?
- What is the sequence of ancestral proteins?
- What are the most similar species?
- What is the rate of speciation?
- Is there a correlation between gain/loss of traits and environment? with geographical events?
- Which features are ancestral to a clade, which are derived?
- What structures are homologous, which are analogous?

*

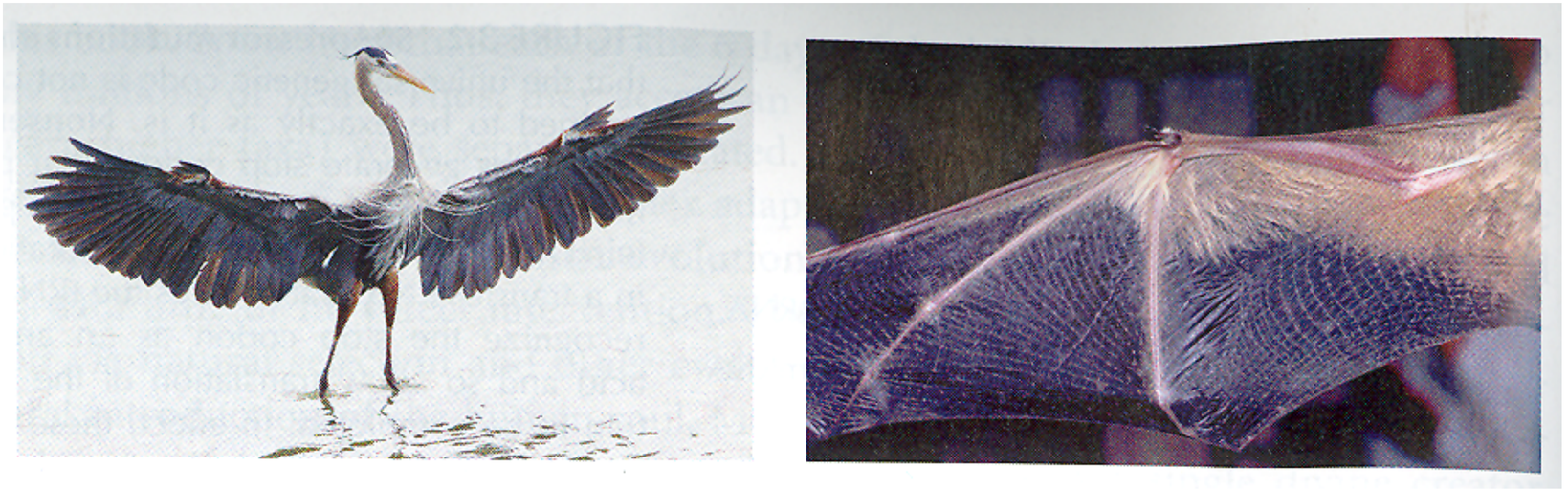
Study Design Considerations

- **Taxon sampling:**
  - how many individuals are used to represent a species?
  - how is the "outgroup" chosen?
  - Can individuals be collected or cultured?

- **Marker selection: Sequence features should:**
  - be Representative of evolutionary history (unrecombined)
  - have a single copy
  - be able to be amplified using PCR
  - able to be sequenced
  - change enough to distinguish species, similar enough to perform MSA

*

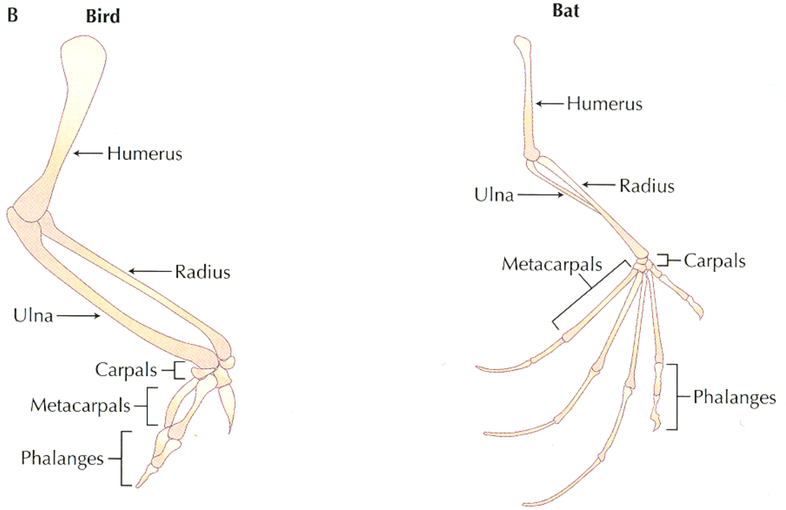
Convergent Evolution



Bird & bat wings arose independently (analogous)

"Has wings" is thus a "bad" trait for phylogenetic inference

Bone structure has common ancestor (homologous)



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"Divergent" Evolution

"Obvious" phenotypic traits are not necessarily good markers

These are all one species!

FIGURE 3.7. Diverse varieties of *Brassica oleracea* include (*A*) cabbage; (*B*) broccoli; (*C*) cauliflower; (*D*) brussels sprouts; and (*E*) flowering kale.

*

Two phylogeny "problems"

*Note: "*Character" below is not a letter (e.g. A,C,G,T), but a particular characteristic under which we consider the phylogeny (e.g. column of a MSA). Each character takes on a *state* (e.g. A,C,G,T).

The **small** phylogeny problem

Given: a set of characters at the leaves (extant species), a set of states for each character, the cost of transition from each state to every other, and the *topology* of the phylogenetic tree

Find: a labeling for each internal node that minimizes the *overall* cost of transitions.

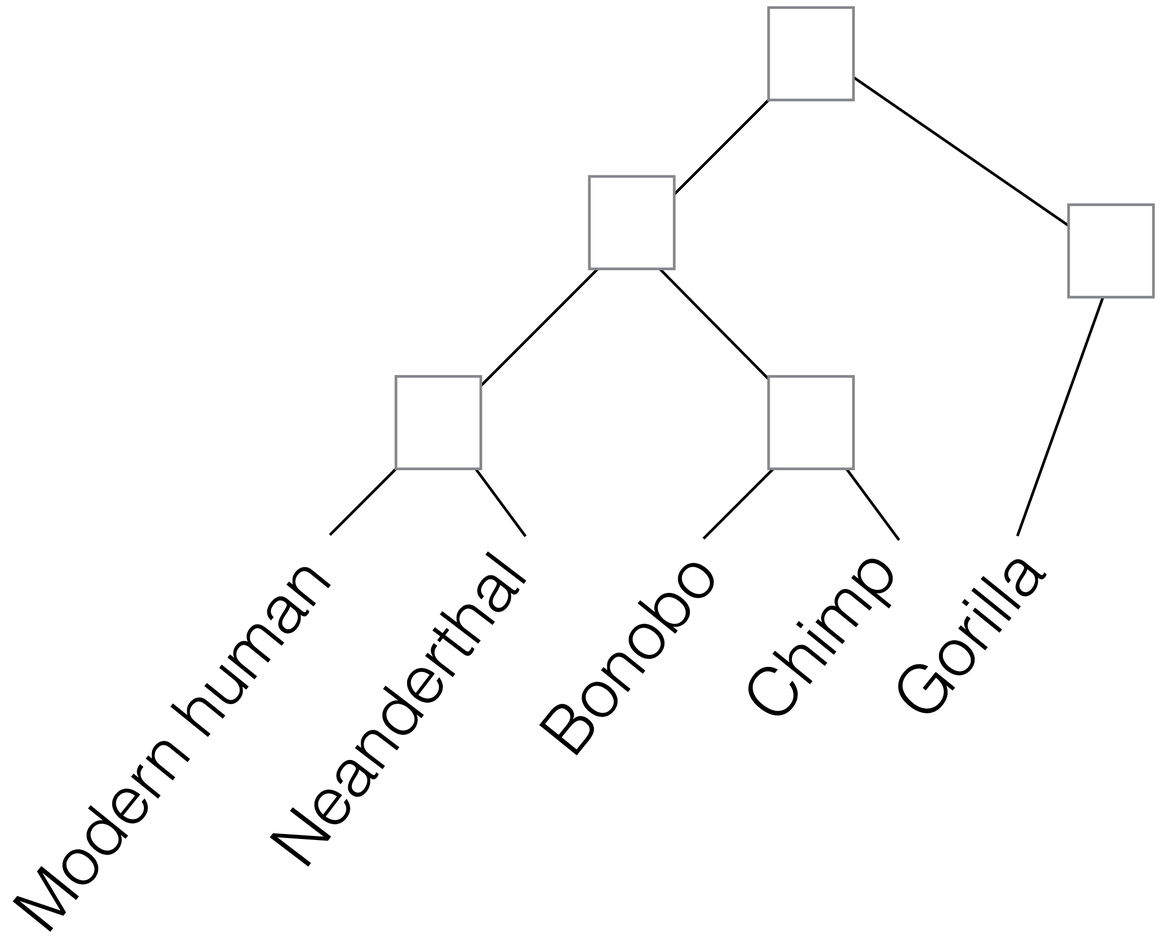
The **large** phylogeny problem

Given: a set of characters at the leaves (extant species), a set of states for each character, the cost of transition from each state to every other

Find: a tree topology and labeling for each internal node that minimizes the *overall* cost (over all trees and internal states)

Small phylogeny problem

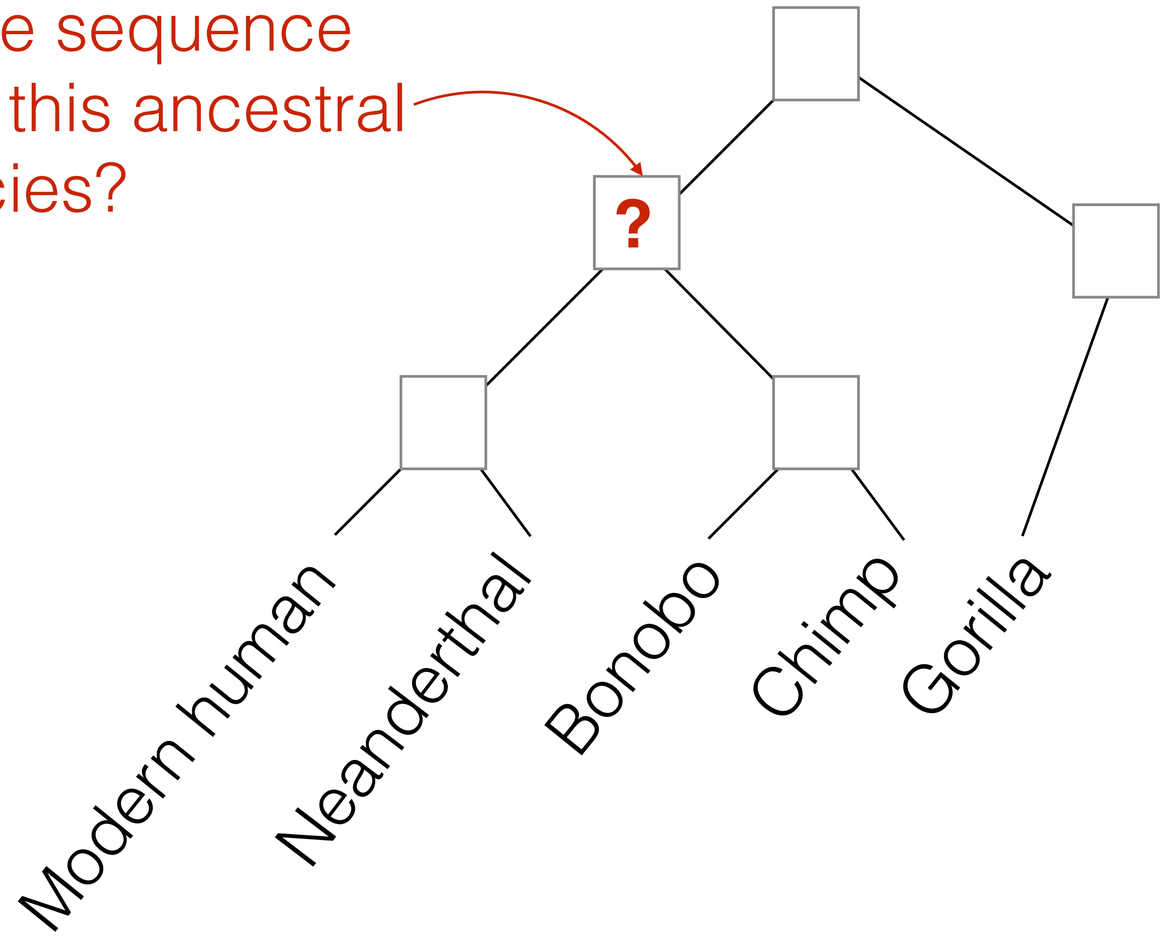
Marker gene



Small phylogeny problem

What was the sequence of the gene in this ancestral species?

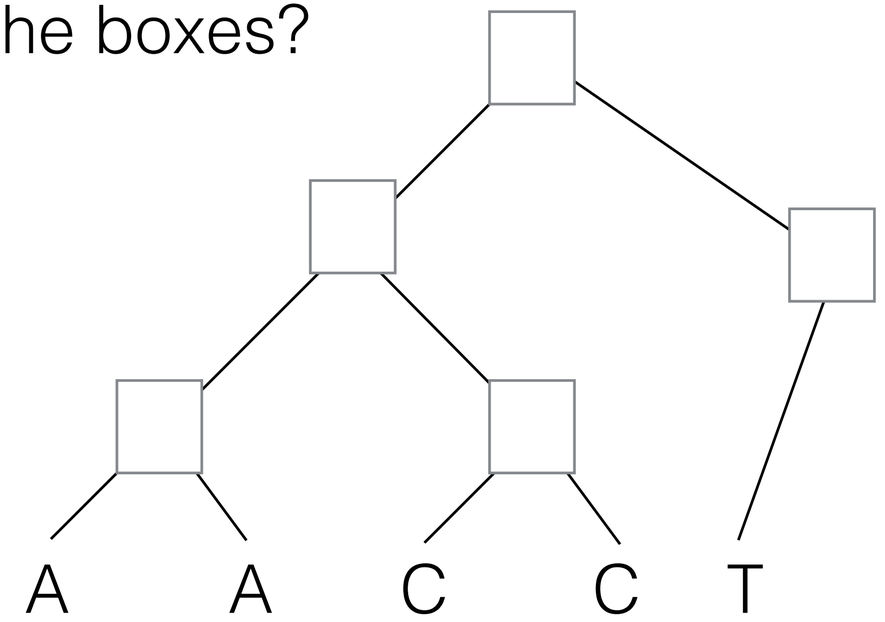
Marker gene



Small phylogeny problem — parsimony

One way to define the lowest *cost* set of transitions is to maximize *parsimony*. That is, posit as few transitions as necessary to produce the observed result.

What characters should appear in the boxes?



Note: We'll consider this "independent site" model; to determine the sequence of a gene we'll optimize each nucleotide *individually.*

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Assume transitions all have unit cost:

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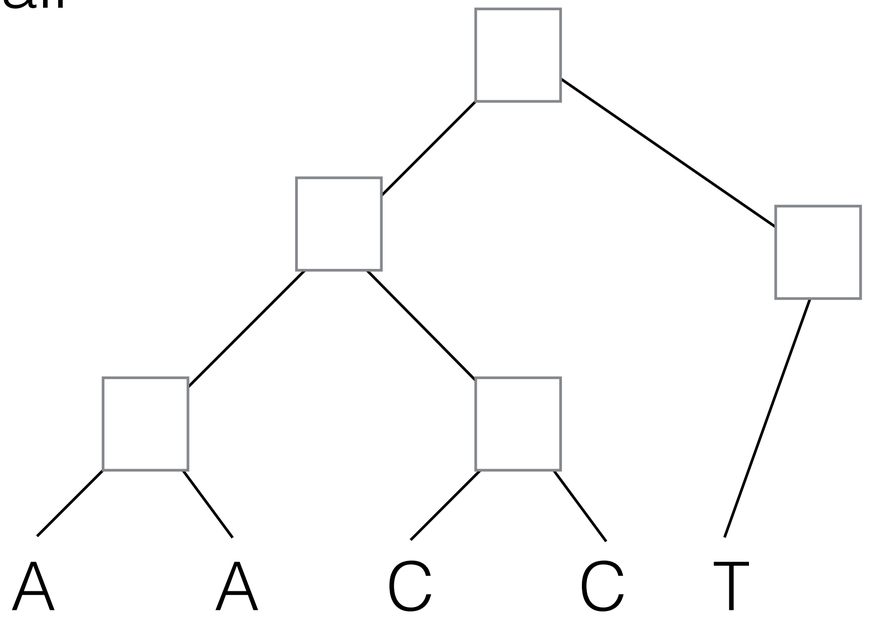
A	0	1	1	1
C	1	0	1	1
G	1	1	0	1
T	1	1	1	0



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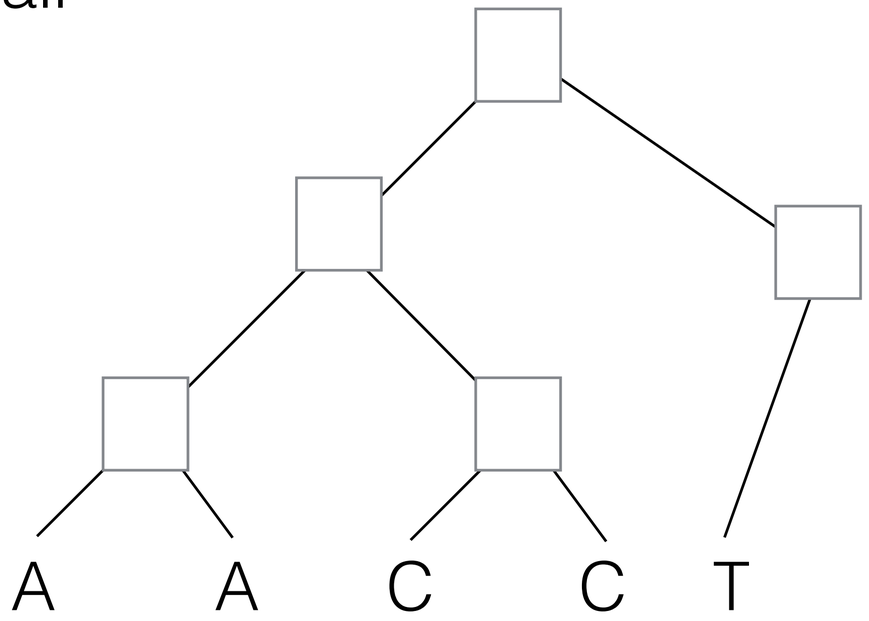
Fitch's algorithm provides a solution.



Small phylogeny problem — parsimony

Fitch's algorithm (2-pass):

Visit nodes in *post-order* traversal:

store a *set* of characters at each node

take the intersection of child's set if not empty; else take the union

Visit nodes in *pre-order* traversal:

If a child's character set has its parent's label, choose it.

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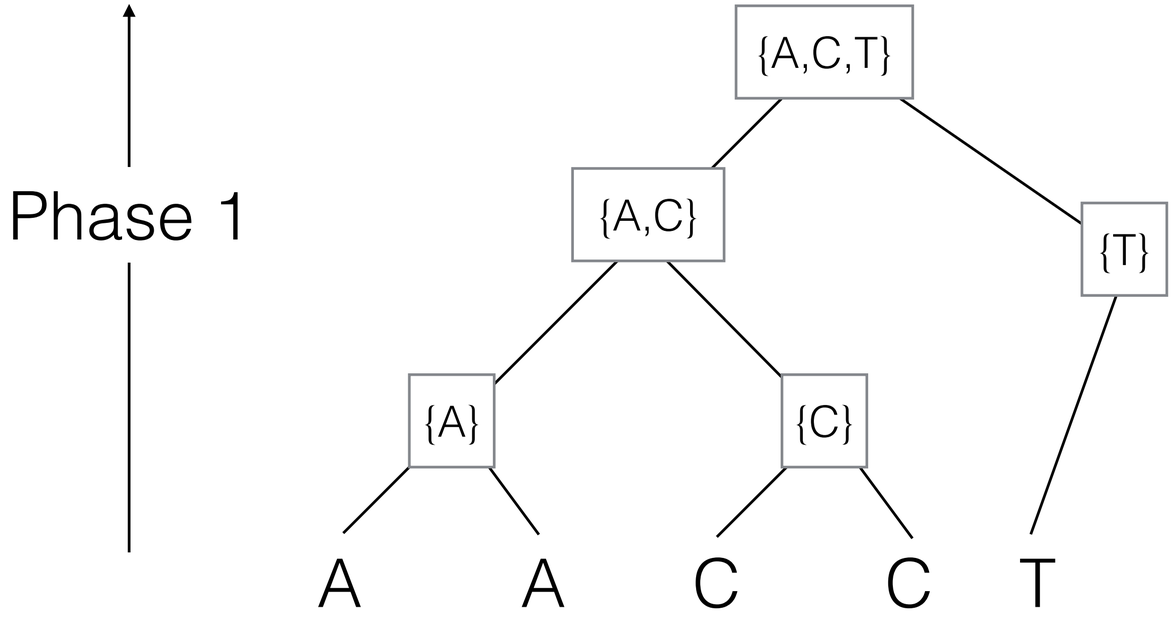

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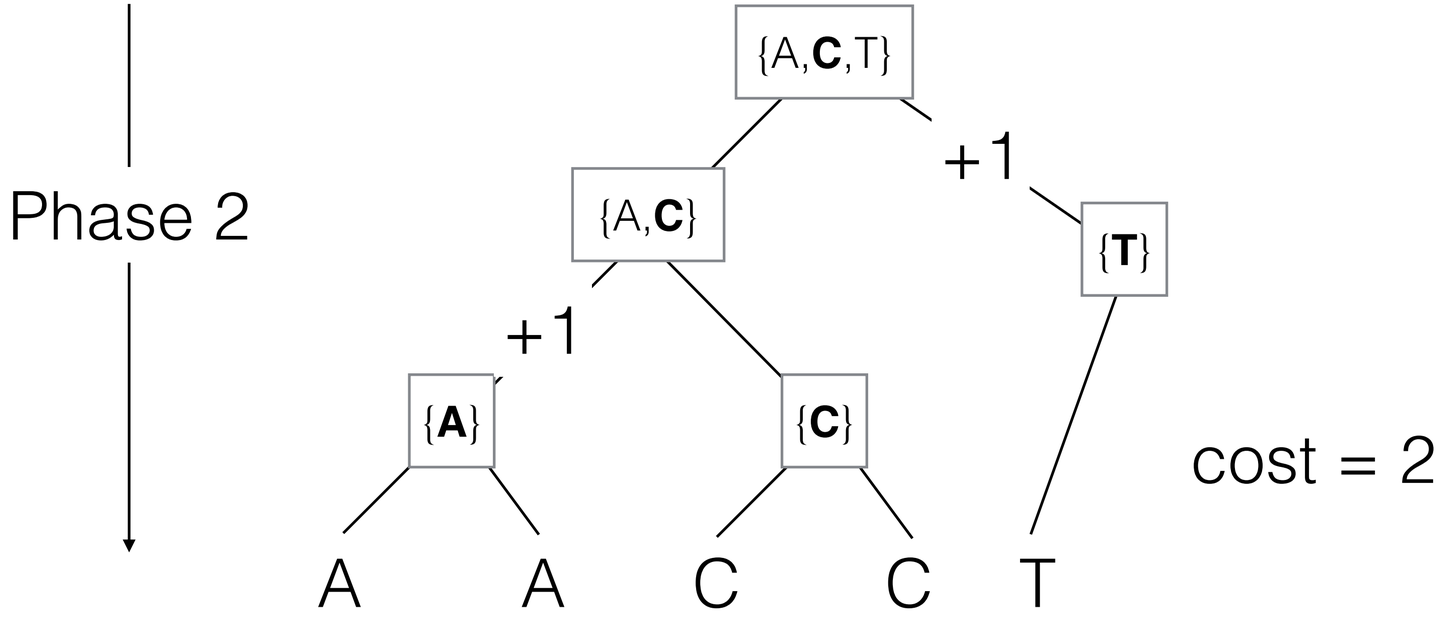

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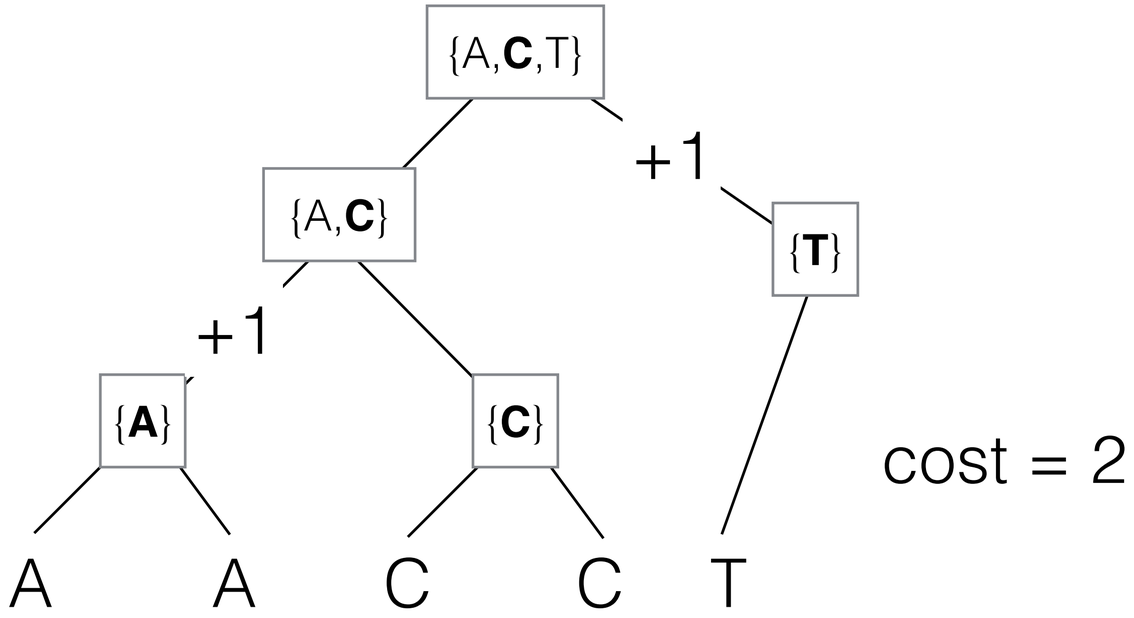
Note: There are generally *many* solutions of optimal cost.



cost = 2

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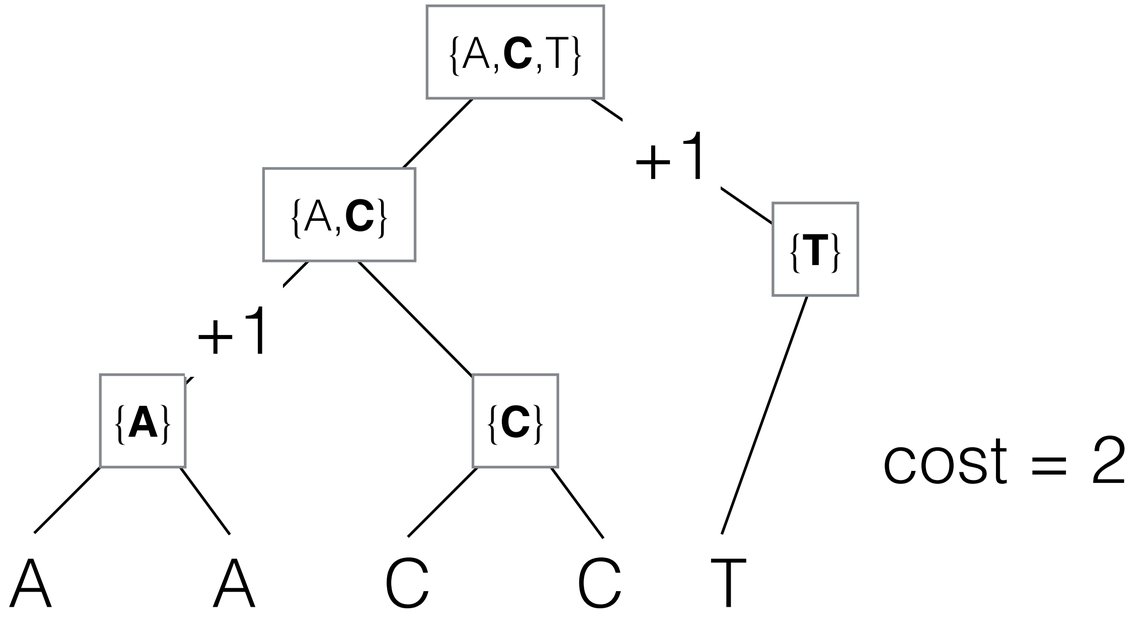
How might you count them?



cost = 2

Small phylogeny problem — parsimony

What if there are different costs for each transition?
Sankoff* provides a dynamic program to solve this case.

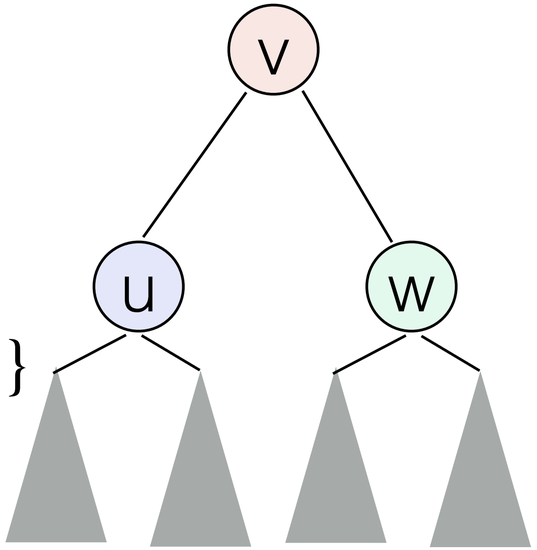
For simplicity, consider only a single character, c

Phase 1 (post-order):

For each leaf v, state t, let $S_t(v) = \begin{cases} 0 & \text{if } v_c = t \\ \infty & \text{otherwise} \end{cases}$

For each internal v, state t, let $S_t(v) = \min_i \{C_{t \to i} + S_i(u)\} + \min_j \{C_{t \to j} + S_j(w)\}$

Choose the best parent states.



Phase 2 (pre-order):

Let the root take state $r_c = \arg\min_t S_t(r)$

For all other v with parent u, let: $v_c = \arg\min_t \left(C_{u_c \to t} + S_t(v) \right)$

Choose the best child states given the parent states chosen above

*Sankoff & Cedergren (1983)

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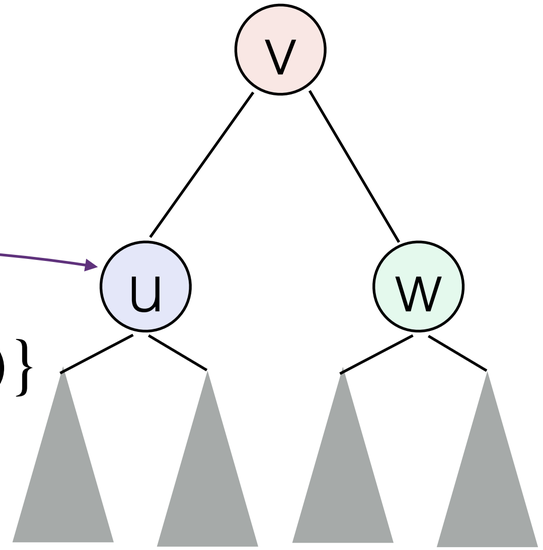
Cost of getting subtree of u in state i

Choose the best parent states.



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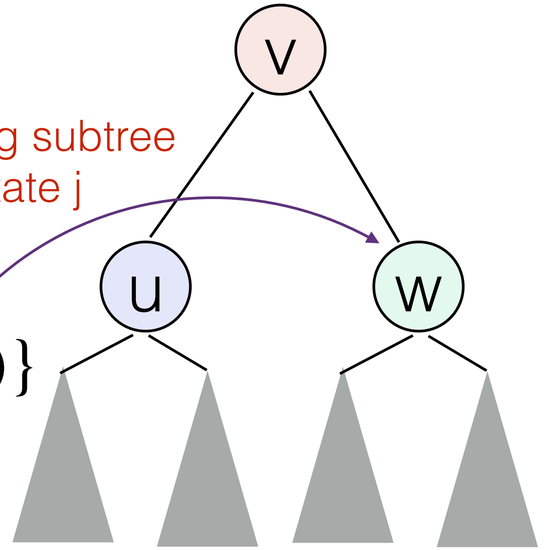

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Best cost of getting to v in state t

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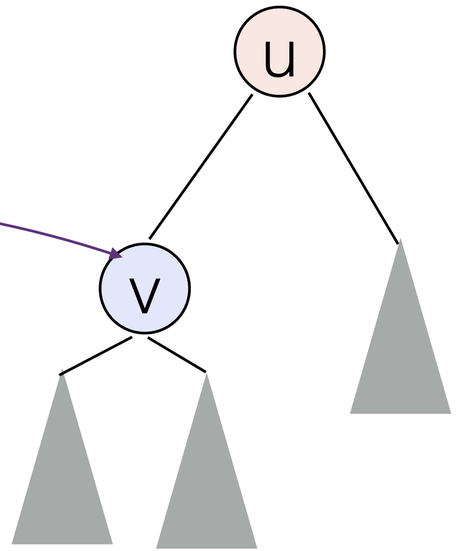

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Small phylogeny problem — parsimony

Consider the following tree and transition matrix:

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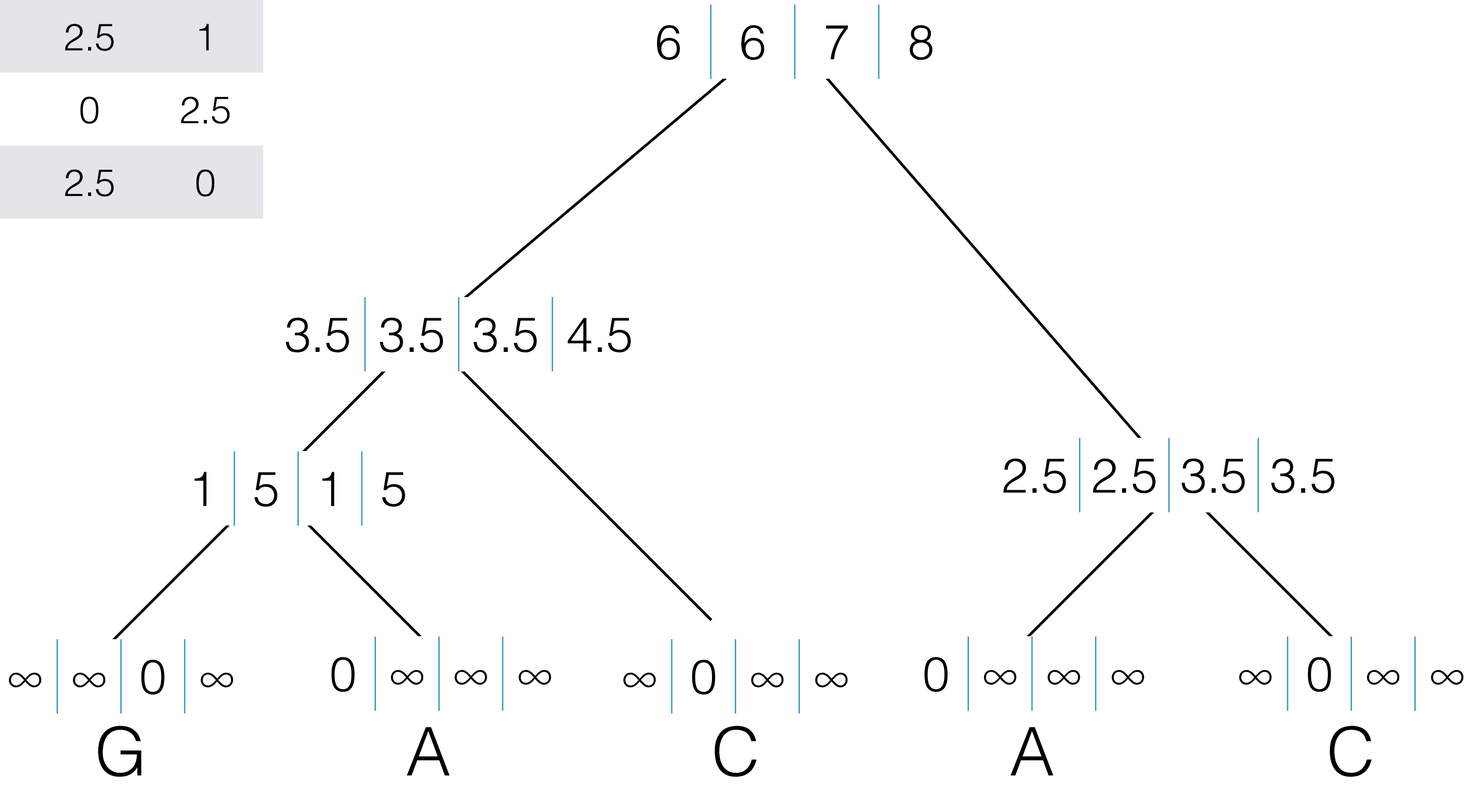

example:http://evolution.gs.washington.edu/gs541/2010/lecture1.pdf

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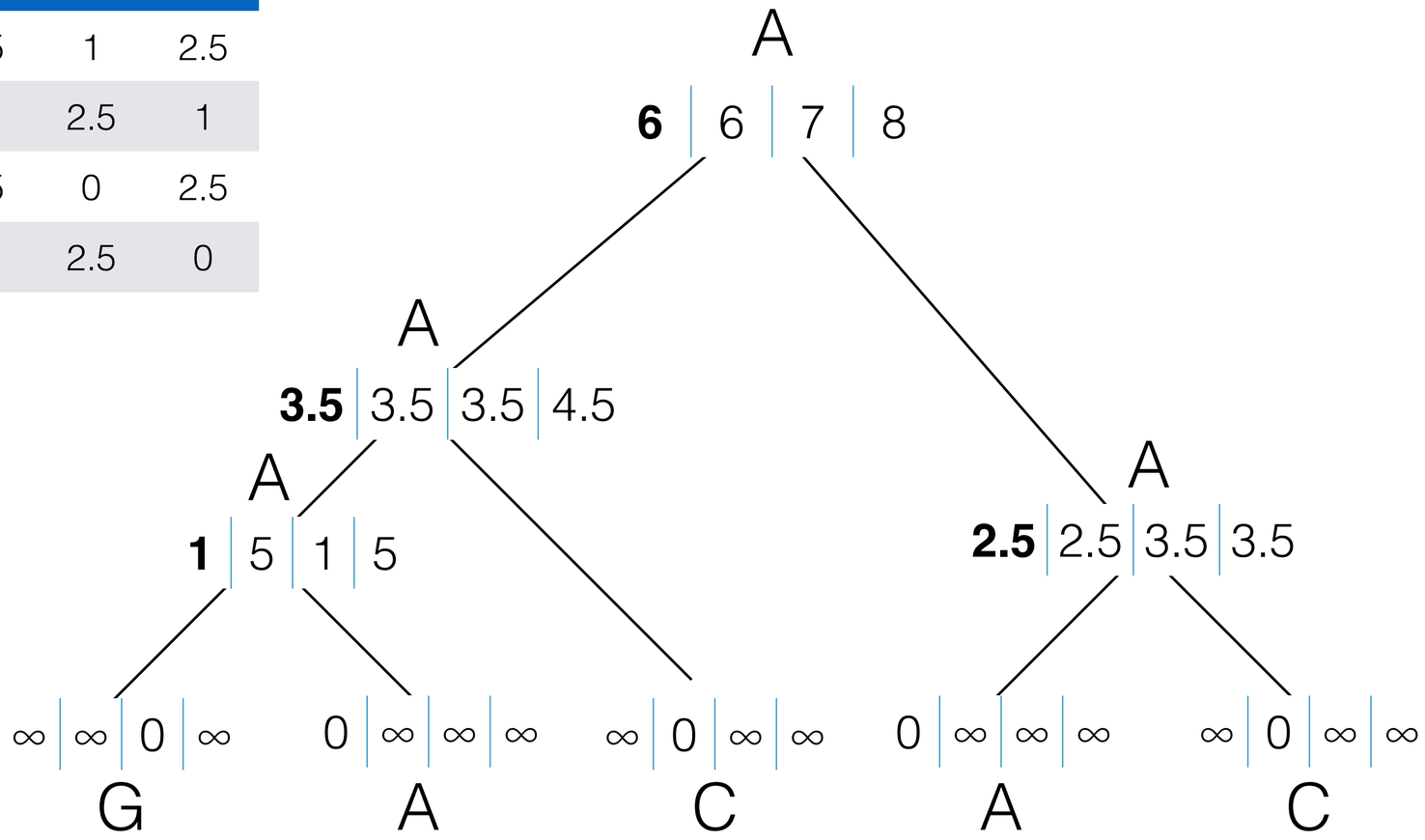

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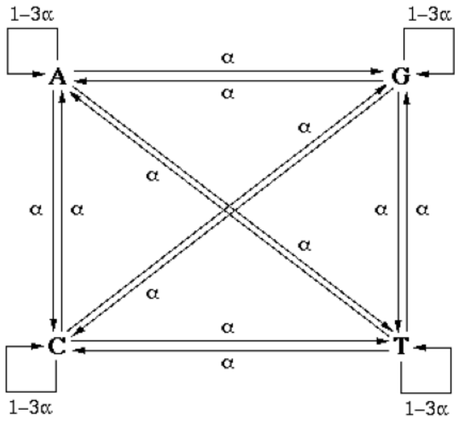
Small phylogeny problem — Maximum Likelihood

Imagine we assume a specific, probabilistic model of sequence evolution. For example:

Jukes-cantor



α is the probability to mutate (per-unit time)

https://en.wikipedia.org/wiki/Models_of_DNA_evolution

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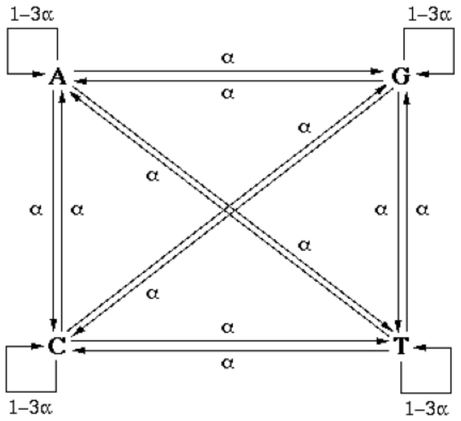


α is the probability to mutate (per-unit time)

or General Time Reversible

Time reversible:

$$\pi_i Q_{ij} = \pi_j Q_{ji}$$

Base frequencies:

$$\mathbf{\Pi} = (\pi_T, \pi_C, \pi_A, \pi_G)$$

Rate matrix (per unit time):

$$Q = \begin{pmatrix} -(\alpha\pi_C + \beta\pi_A + \gamma\pi_G) & \alpha\pi_C & \beta\pi_A & \gamma\pi_G \\ \alpha\pi_T & -(\alpha\pi_T + \delta\pi_A + \epsilon\pi_G) & \delta\pi_A & \epsilon\pi_G \\ \beta\pi_T & \delta\pi_C & -(\beta\pi_T + \delta\pi_C + \eta\pi_G) & \eta\pi_G \\ \gamma\pi_T & \epsilon\pi_C & \eta\pi_A & -(\gamma\pi_T + \epsilon\pi_C + \eta\pi_A) \end{pmatrix}$$

Transition matrix at time t:

$$P(t) = e^{Qt} = \sum_{n=0}^{\infty} Q^n \frac{t^n}{n!}$$

$$\begin{aligned} \alpha &= r(T \to C) = r(C \to T) \\ \beta &= r(T \to A) = r(A \to T) \\ \gamma &= r(T \to G) = r(G \to T) \\ \delta &= r(C \to A) = r(A \to C) \\ \epsilon &= r(C \to G) = r(G \to C) \\ \eta &= r(A \to G) = r(G \to A) \end{aligned}$$

https://en.wikipedia.org/wiki/Models_of_DNA_evolution

Small phylogeny problem — Maximum Likelihood

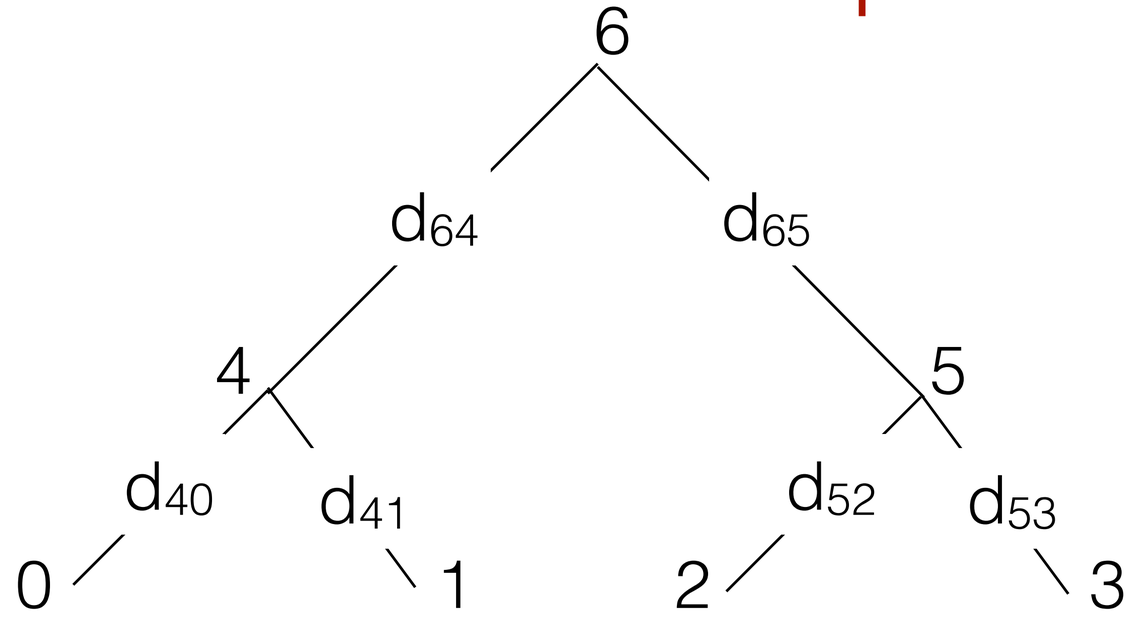
Imagine we assume a specific, probabilistic model of sequence evolution.

Given a tree topology (with branch lengths), a set of states for each character, and the assumed model of state evolution

Find the states at each internal node that *maximizes* the likelihood of the observed data (i.e. states at the leaves)

Rather than choosing the *best* state at each site, we are summing over the possibility of *all* states (phylogenetic histories)

Consider the simple tree



For particular ancestral states s_6, s_4 and s_5, we can score their likelihood as:

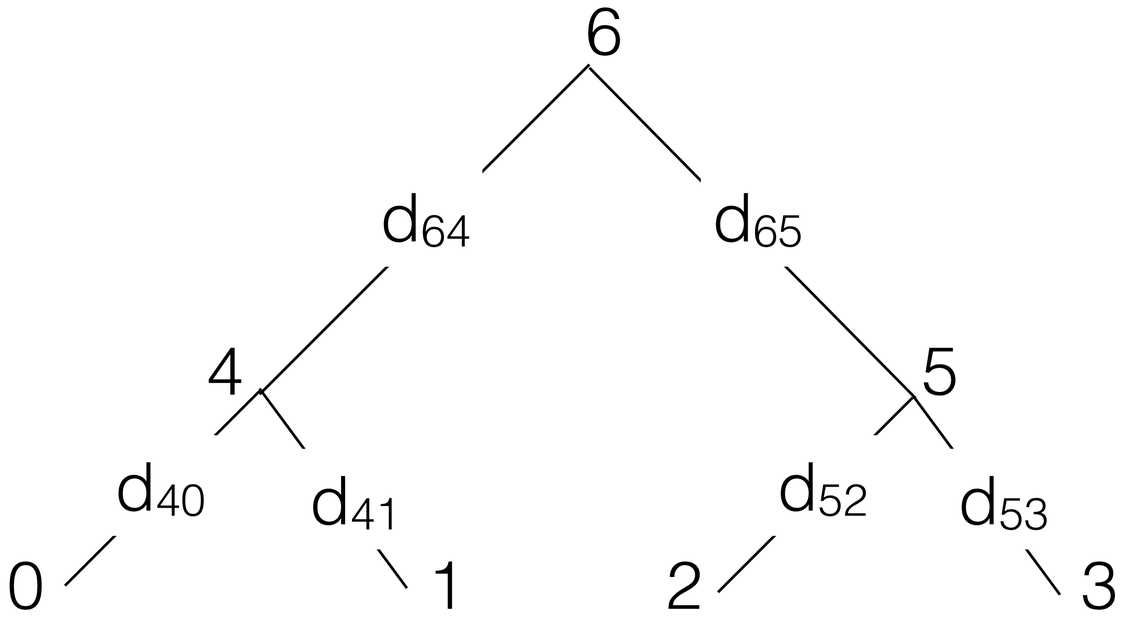
$$\tilde{\mathcal{L}}(s_6, s_4, s_5) = p_{s_6 \to s_4}(d_{64}) \cdot p_{s_6 \to s_5}(d_{65}) \cdot p_{s_4 \to s_0}(d_{40}) \cdot p_{s_4 \to s_1}(d_{41}) \cdot p_{s_5 \to s_2}(d_{52}) \cdot p_{s_5 \to s_3}(d_{53})$$

Since we don't know these states, we must *sum over* them:

$$\mathcal{L} = \sum_{s_6} \sum_{s_4} \sum_{s_5} \pi_{s_6} \tilde{\mathcal{L}}(s_6, s_4, s_5)$$

Example from: http://www.bioinf.uni-freiburg.de/Lehre/Courses/2013_SS/V_Bioinformatik_1/phylo-maximum-likelihood.pdf

Small phylogeny problem — Maximum Likelihood



It turns out that this objective (maximum likelihood) can also be optimized in polynomial time.

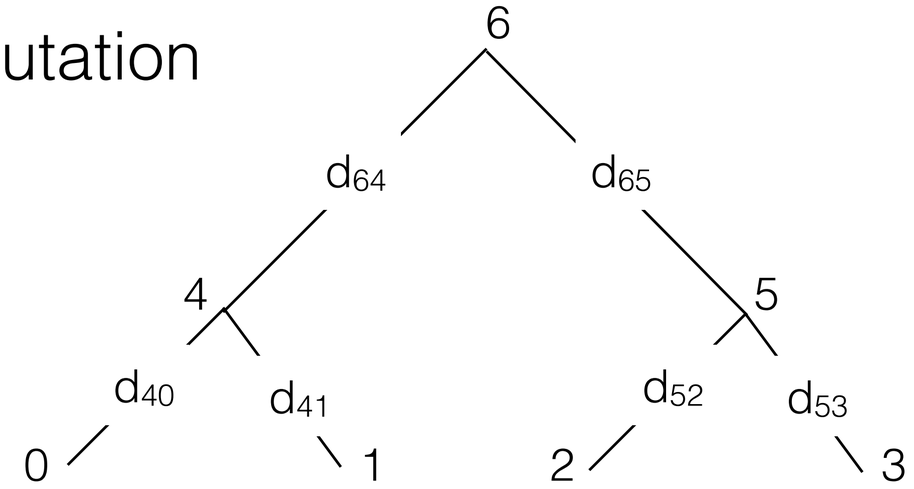
This is done by re-arranging the terms and expressing them as conditional probabilities.

The algorithm is due to Felsenstein* — again, it is kind of a dynamic program

Felsenstein, Joseph. "Evolutionary trees from DNA sequences: a maximum likelihood approach." Journal of molecular evolution 17.6 (1981): 368-376.

Small phylogeny problem — Maximum Likelihood

Idea 1: Re-arrange the computation to be more favorable

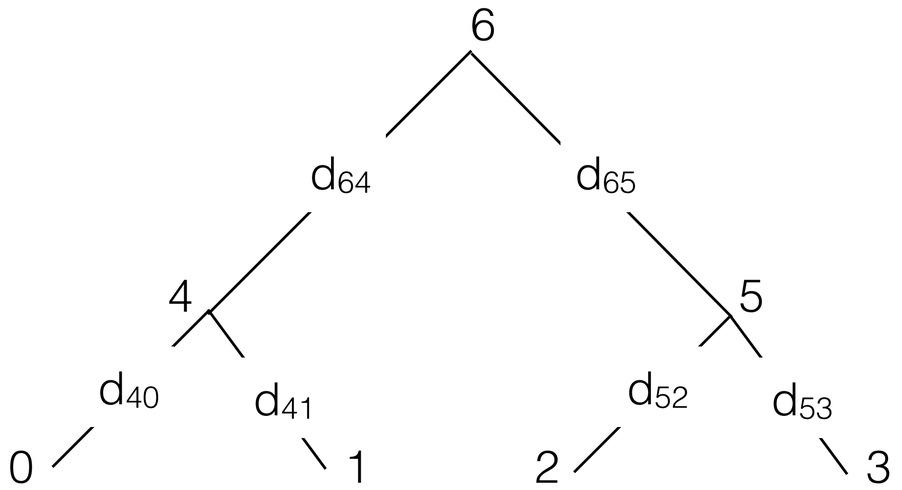


$$\mathcal{L} = \sum_{s_6} \sum_{s_4} \sum_{s_5} \pi_{s_6} \tilde{\mathcal{L}}(s_6, s_4, s_5)$$

via. Horner's method (push summations to the right)

$$= \sum_{s_6} \pi_{s_6} \times \left\{ \begin{array}{c} \sum_{s_4} p_{s_6 \to s_4} d(s_{64}) \left(p_{s_4 \to s_0} d(s_{40}) p_{s_4 \to s_1} d(s_{41}) \right) \\ \times \\ \sum_{s_5} p_{s_6 \to s_5} d(s_{65}) \left(p_{s_5 \to s_2} d(s_{52}) p_{s_5 \to s_3} d(s_{53}) \right) \end{array} \right\}$$

Small phylogeny problem — Maximum Likelihood

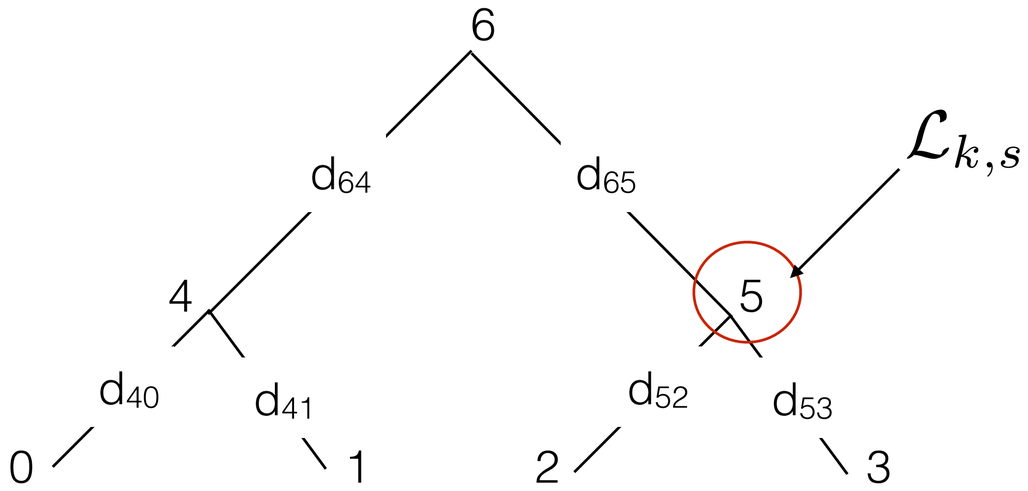


$$\sum_{s_6} \pi_{s_6} \times \left\{ \begin{array}{c} \sum_{s_4} p_{s_6 \to s_4} d(s_{64}) \left(p_{s_4 \to s_0} d(s_{40}) p_{s_4 \to s_1} d(s_{41}) \right) \\ \times \\ \sum_{s_5} p_{s_6 \to s_5} d(s_{65}) \left(p_{s_5 \to s_2} d(s_{52}) p_{s_5 \to s_3} d(s_{53}) \right) \end{array} \right\}$$

The structure of the equations here *matches* the structure of the tree ((.,.)(.,.)) — see e.g. nested parenthesis encoding of trees.

Small phylogeny problem — Maximum Likelihood

Idea 2: define the total likelihood in terms of *conditional* likelihoods.



Conditional likelihood of the *subtree rooted at k*, assuming *k takes on states s.*

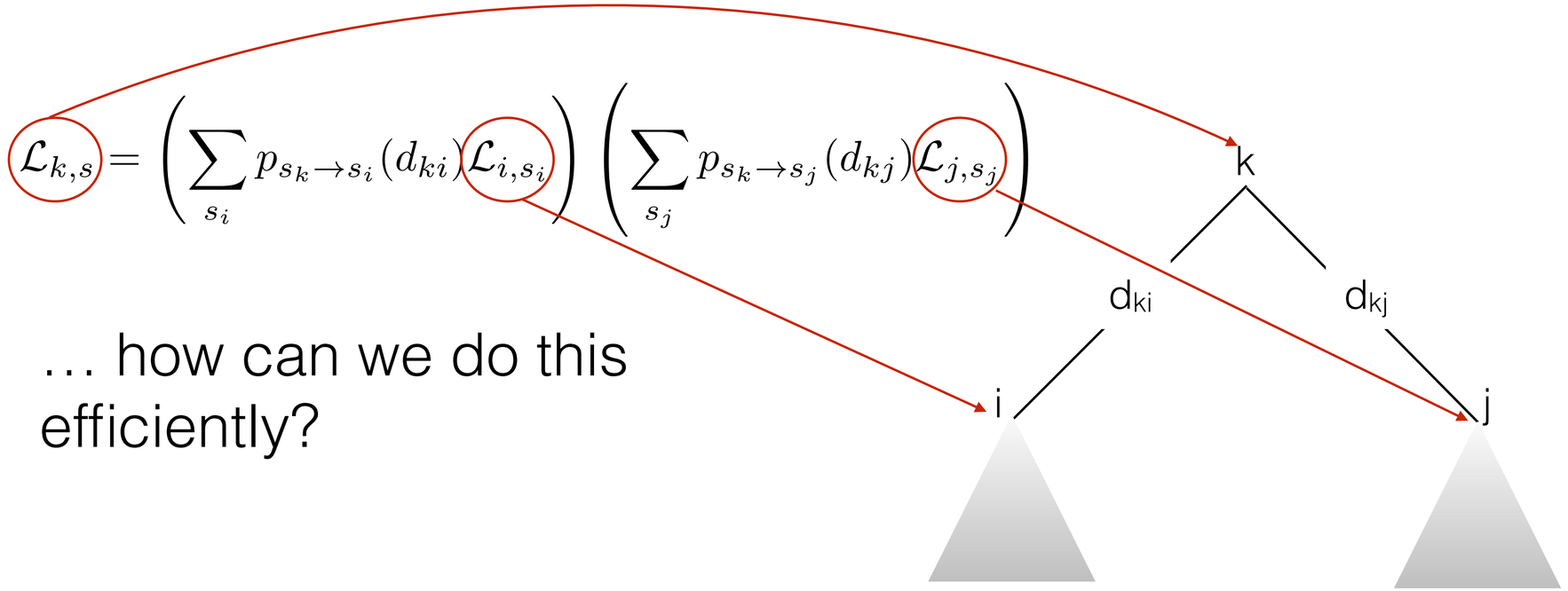
Small phylogeny problem — Maximum Likelihood

Now, we can define likelihood recursively!

$\mathcal{L}_{k,s} = \Pr(s_k = s)$ if k is a leaf

$$\mathcal{L}_{k,s} = \left(\sum_{s_i} p_{s_k \to s_i}(d_{ki}) \mathcal{L}_{i,s_i} \right) \left(\sum_{s_j} p_{s_k \to s_j}(d_{kj}) \mathcal{L}_{j,s_j} \right)$$

… how can we do this efficiently?



Small phylogeny problem — Maximum Likelihood

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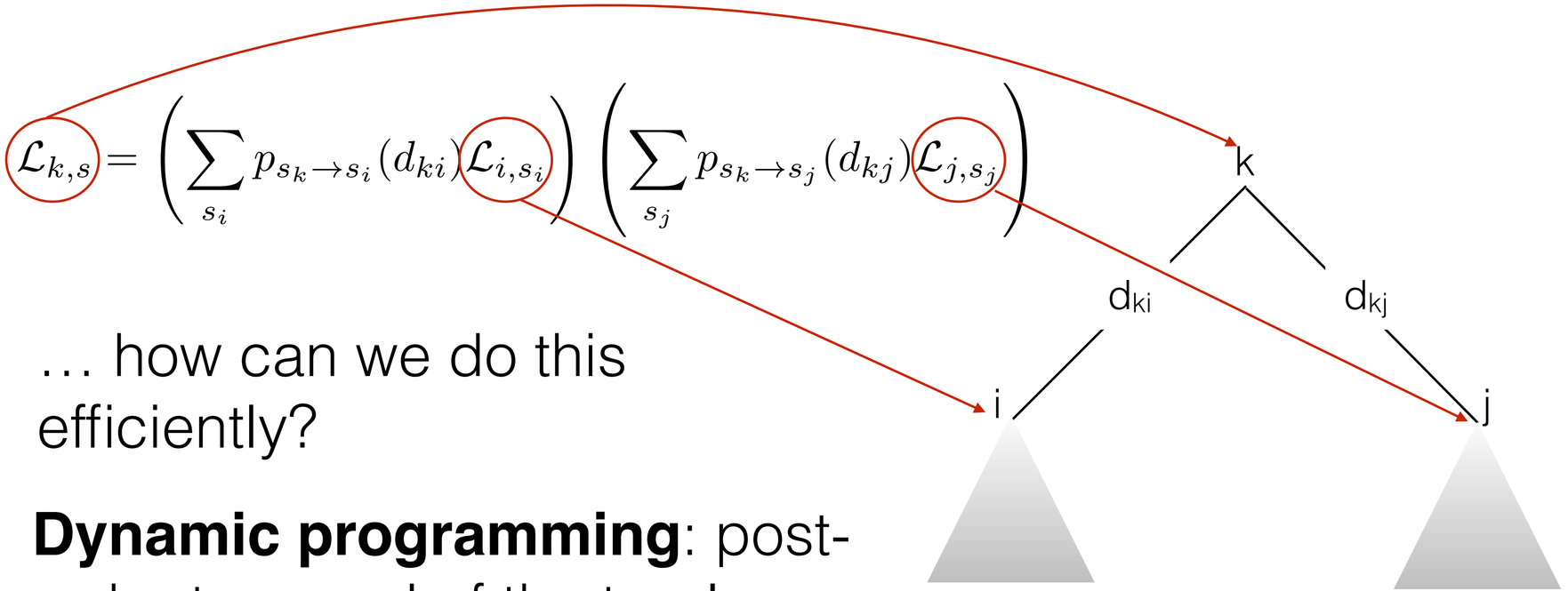


… how can we do this efficiently?

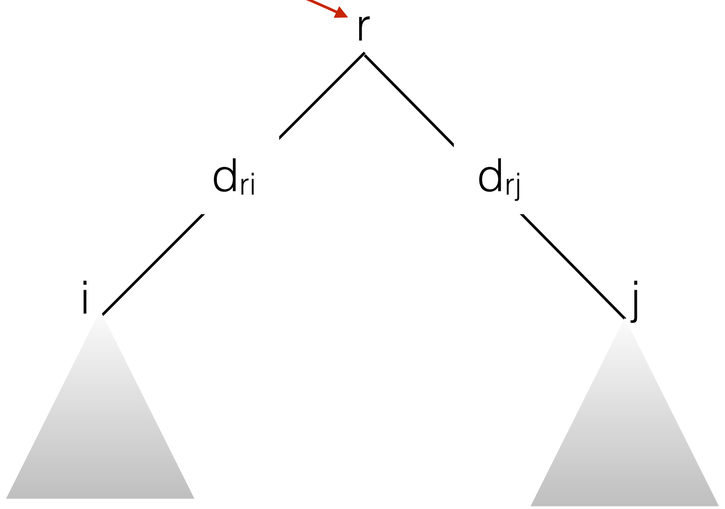
Dynamic programming: post-order traversal of the tree!

Small phylogeny problem — Maximum Likelihood

At the root, we simply sum over all possible states to get the likelihood for the entire tree:

$$\mathcal{L} = \sum_{s_r} \pi_{s_r} \mathcal{L}_{r,s_r}$$

Using these likelihoods, we can ask questions like:



What is the probability that node k had state ‘A’?

What is the probability that node k didn’t have state ‘C’?

At node k, how likely was state ‘A’ compared to state ‘C’?

Small phylogeny problem — Maximum Likelihood

This maximum likelihood framework is very powerful.

It allows us to consider *all* evolutionary histories, weighted by their probabilities.

Also lets us evaluate other tree parameters like branch-length.

But we there can be many assumptions baked into our *model* (and such a model is part of our ML framework)

- What if our parameters are wrong?
- What if our assumptions about “Markovian” mutation are wrong?
- What if the *structure* of our model is wrong (correlated evolution)?

Small phylogeny problem — Maximum Likelihood

Two ways to think about ancestral state reconstruction:

1) Marginal; what state at each node is the most likely when summing over all possible states in the rest of the tree?

2) Joint; what is the highest likelihood *consistent* setting of all internal states?

How are these different?
Is one “better” than the other?
Why is the maximum marginal state != state in best joint reconstruction?

Small phylogeny problem — Maximum Likelihood

Two ways to think about ancestral state reconstruction:

1) Marginal; what state at each node is the most likely when summing over all possible states in the rest of the tree (essentially greedy)?

$$P(x = s_x \,|\, \mathbf{x}, T, \theta) = \frac{\pi_{s_x} L_{n,s_x}}{\sum_{s_y} \pi_{s_y} L_{n,s_y}}$$

2) Joint; what is the highest likelihood *consistent* setting of all states?

More complicated, but multiple (fast) algorithms :

A Fast Algorithm for Joint Reconstruction of Ancestral Amino Acid Sequences FREE

Tal Pupko, Itsik Pe, Ron Shamir, Dan Graur

Molecular Biology and Evolution, Volume 17, Issue 6, June 2000, Pages 890–896,
https://doi.org/10.1093/oxfordjournals.molbev.a026369
Published: 01 June 2000 **Article history** ▾

Large phylogeny problem — searching for trees

- Distance-based methods:
  - Sequences -> Distance Matrix -> Tree
  - Neighbor joining, UPGMA
- Maximum Likelihood:
  - Sequences + Model -> Tree + parameters
- Bayesian MCMC:
  - Markov Chain Monte Carlo: random sampling of trees by random walk

*

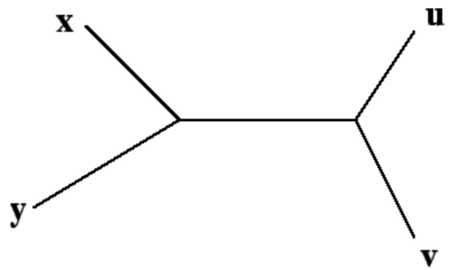
Additivity (for distance-based methods)

- A distance matrix M is additive if a tree can be constructed such that $d_T(i,j)$ = path length from i to j = M_{ij}.

- Such a tree faithfully represents all the distances

- 4-point condition: A metric space is additive if, given any 4 points, we can label them so that

$$M_{xy} + M_{uv} \leq M_{xu} + M_{yv} = M_{xv} + M_{yu}$$



- If our metric is additive, there is exactly one tree realizing it, and it can be found by successive insertion#

lectures.molgen.mpg.de/Phylogeny/Reconstruction

*

What if our distances aren't so nice?

UPGMA

- Find two most similar taxa (ie. such that M_{ij} is smallest)
- Merge into new "OTU" (operational taxonomic unit)
  - distance from k to to new OTU = average distance from k to each of OTUs members
- Repeat.
- **Even if there is perfect tree, it may not find it.**

*

Maximum Parsimony

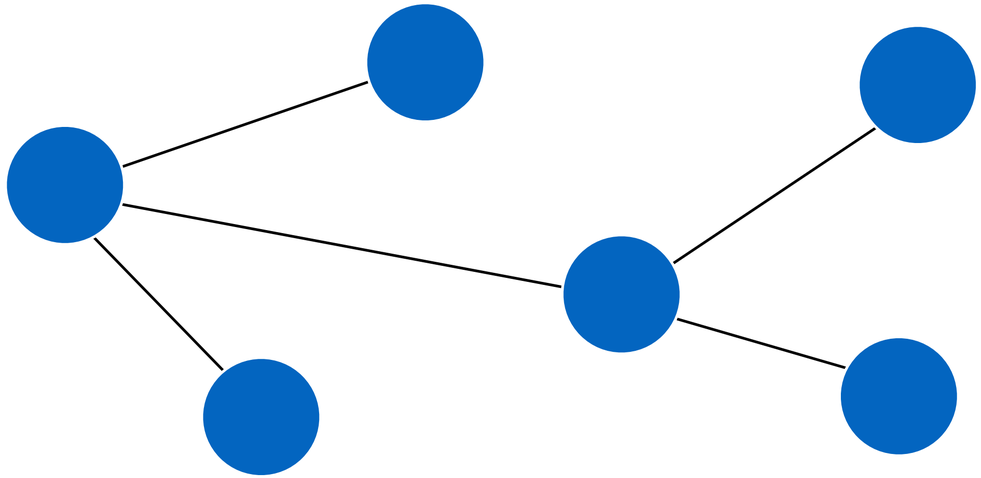
- **Input:** n sequences of length k
- **Output:** A tree T = (V, E) and a sequence s_u of length k for each node u to minimize:

$$\sum_{(u,v)\in E} \text{Hamming}(s_u, s_v)$$

NP-hard (reduction from Hamming distance Steiner tree)
Can score a given tree in time O(|∑|nk).

*

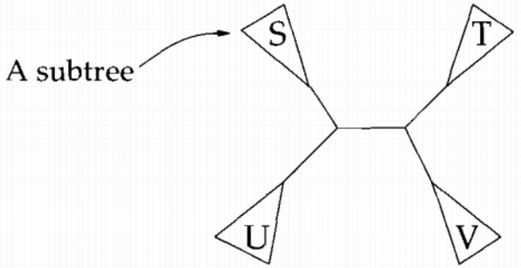
Heuristic: Nearest Neighbor Interchange



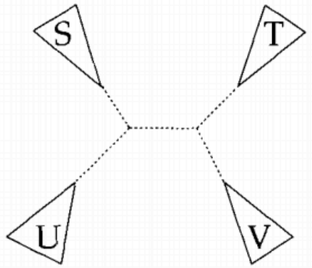
Walk from tree T to its neighbors, choosing best neighbor at each step.

*

Heuristic: Nearest Neighbor Interchange



is rearranged by dissolving the connections to an interior branch



and reforming them in one of the two possible alternative ways:

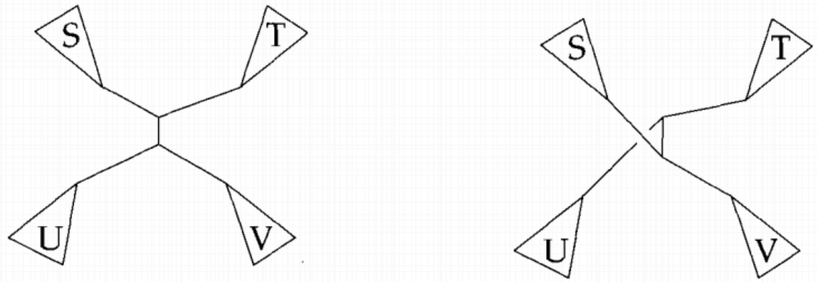


Figure 4.2: The process of nearest-neighbor interchange. An interior branch is dissolved and the four subtrees connected to it are isolated. These then can be reconnected in two other ways.

http://www.cs.cmu.edu/~durand/03-711/2010/37-44.pdf

Maximum Likelihood

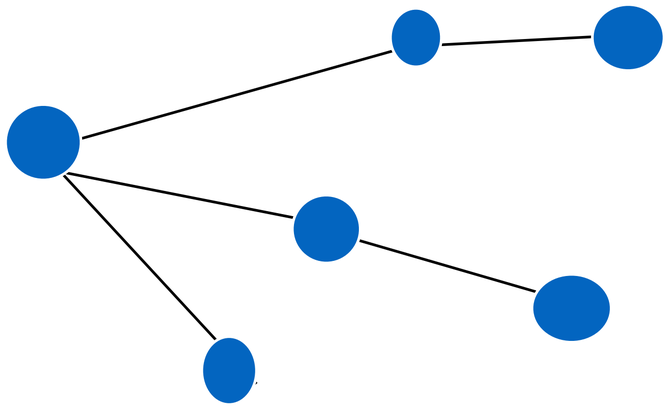
- **Input:** n sequences $S_1,\ldots,S_n$ of length k; choice of model
- **Output:** Tree T and parameters p_e for each edge to maximize:

$$\Pr[S_1,\ldots,S_n \mid T, p]$$

NP-hard if model is Jukes-Cantor; probably NP-hard for other models.

*

Bayesian MCMC



Walk from tree T to its neighbors, choosing a particular neighbor at each step with probability related to its improvement in likelihood. This walk in the space of trees is a Markov chain.

Under “mild” assumptions, and after taking many samples, trees are visited proportional to their true probabilities.

- # of times you visit a tree (after “burn in”)= probability of that topology
- Outputs a distribution of trees, not a single tree.

*

Bootstrapping

- How confident are we in a given edge?

- Bootstrapping:

1. Create (e.g.) 1,000 data sets of same size as input by sampling markers (MSA columns) with replacement.
2. Repeat phylogenetic inference on each set.
3. Support for edge is the % of trees containing this edge (bipartition).

- **Interpretation**: probability that edge would be inferred on a random data set drawn from the same distribution as the input set.

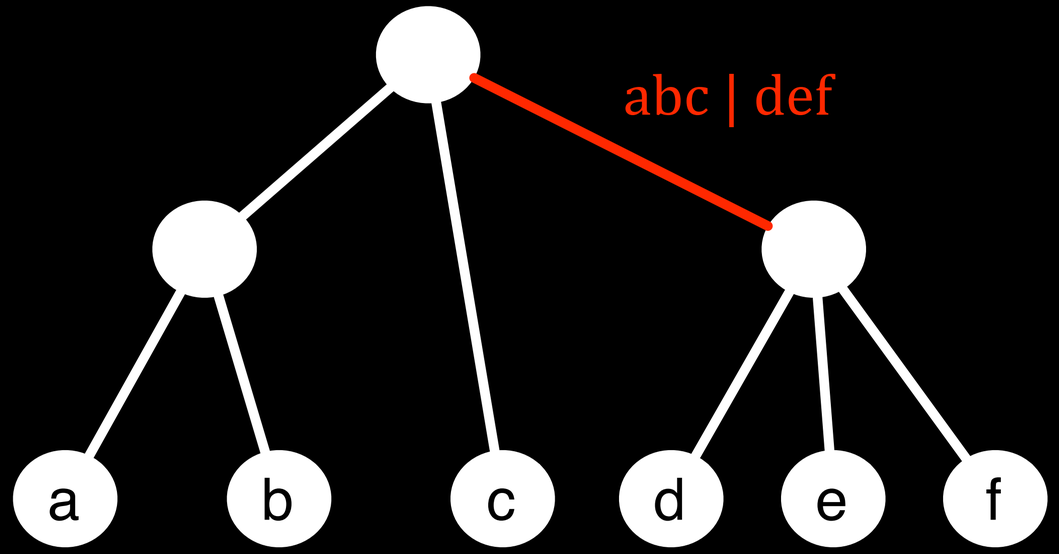
*

Going from an “ensemble” to a single tree

Even if we can generate such an ensemble (e.g. a collection of trees where each is proportional to its true probability).

How can we “extract” a single, meaningful, tree from this ensemble?

Splits



Every edge ⇒ a split, a bipartition of the taxa

- taxa within a clade leading from the edge
- taxa outside the clade leading from the edge

Example: this tree = {abc|def, ab|cdef + 'trivial' splits}

*

Consensus

- Multiple trees: from bootstrap, from Bayesian MCMC, trees with sufficient likelihood, same parsimony:

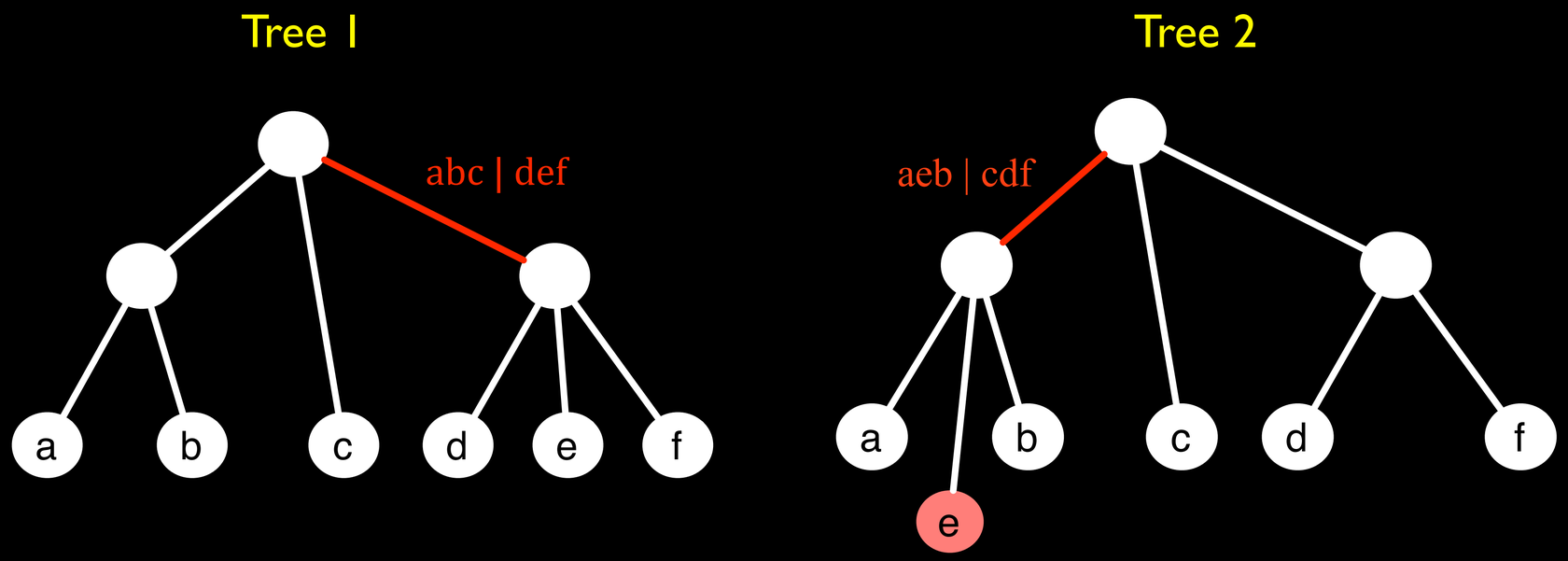
$$T = \{T_1, \ldots, T_n\}$$

- Splits of $T_i := C(T_i) = \{ b(e) : e \in T_i \}$
  $b(e)$ is the split (bipartition) for edge e.

- **Majority consensus:** tree given by splits which occur in > half inferred trees.

*

Incompatibility



Two splits are incompatible if they cannot be in the same tree.

*

Majority Consensus Always Exists

- **Proof**:

1. Let $\{s_k\}$ be the splits in > half the trees.

2. Pigeonhole: for each s_i, s_j in $\{s_k\}$ there must be a tree containing both s_i and s_j.

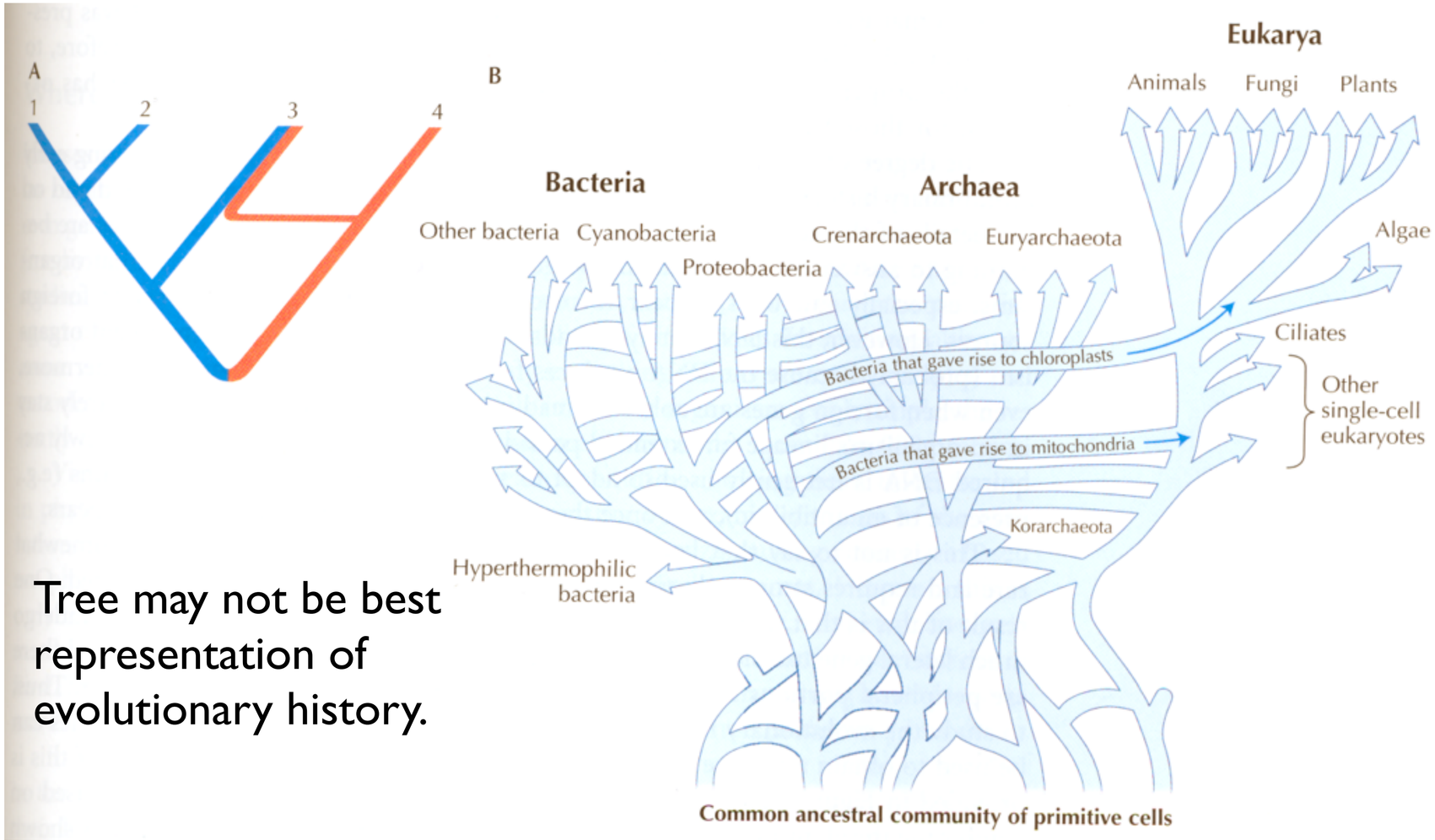
3. If s_i and s_j are in same tree they are compatible.

4. Any set of compatible splits forms a tree.

$\Rightarrow$ The $\{s_i\}$ are pairwise compatible and form a tree.

*

Horizontal Gene Transfer



Tree may not be best representation of evolutionary history.

DNA uptake; retroviruses

*