Reconstruction of phylogenetic trees

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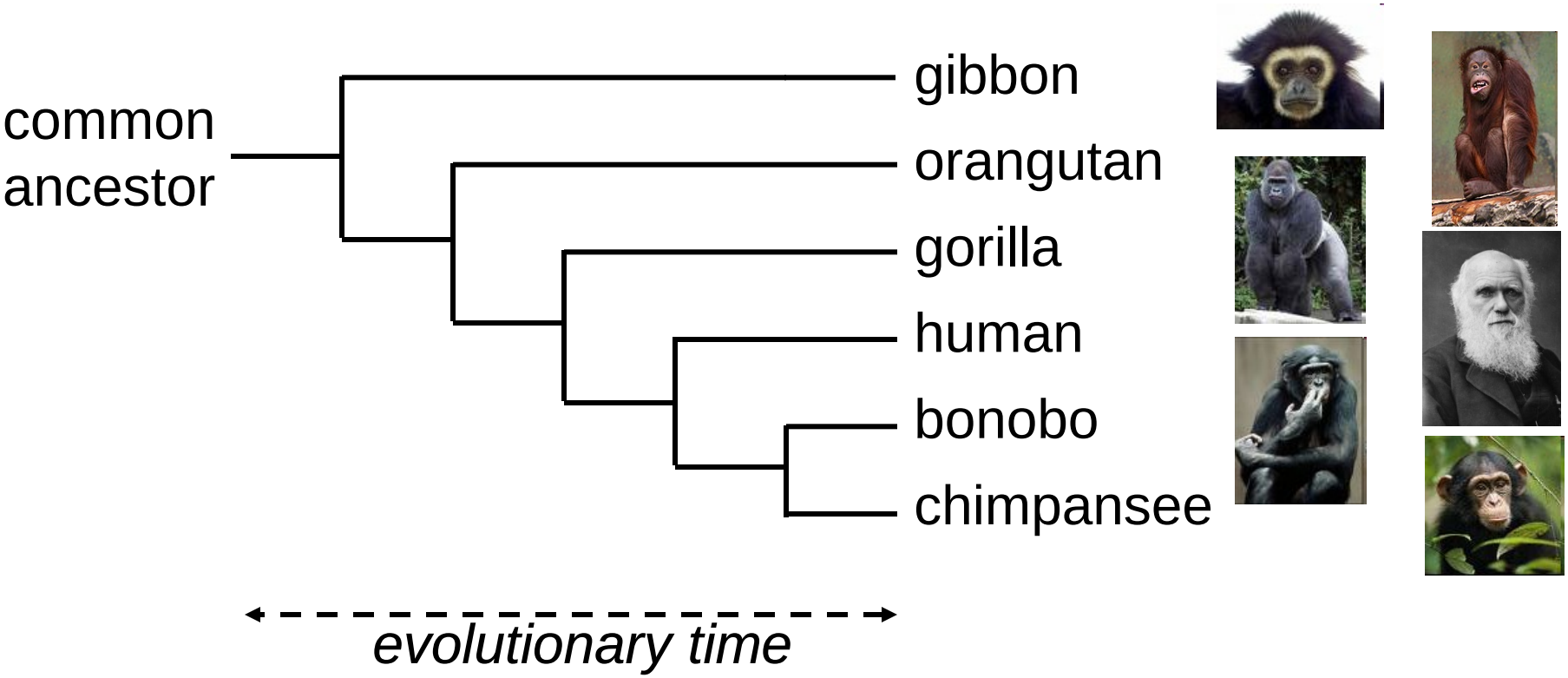
Phylogenetics

"Acceptance of the theory of evolution as the means of explaining observed similarities and differences among organisms invites the construction of trees of descent purporting to show evolutionary relationships"

-- Cavalli-Sforza, Edwards (1967)



Phylogenetics

Phylogenetics is the study of evolutionary relationships between organisms.

Goal

- Reconstruct correct genealogical ties among biogical entities.
- Estimate the time of divergence between organisms.
- Chronicle the sequence of events along evolutionary lineages.

Statistical operationalization: reconstruction of phylogenetic trees on the basis of DNA sequences.

This can also be done on the basis of other characteristics.

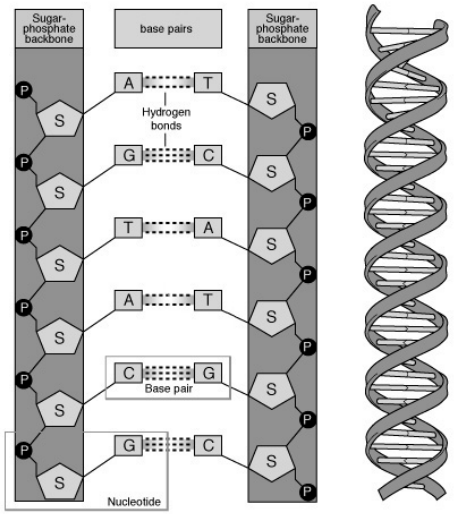
Phylogenetics

DNA of each individual is unique, but differences are small: 1 in 500 to 1000 nucleotides differ between two individuals.

Within a population each position in the DNA has a ‘pre-dominant’ nucleotide.

Over generations this ‘pre-dominant’-nucleotide of a position can change by evolution.

This process is called *substitution*, and takes place over 1000s of generations.



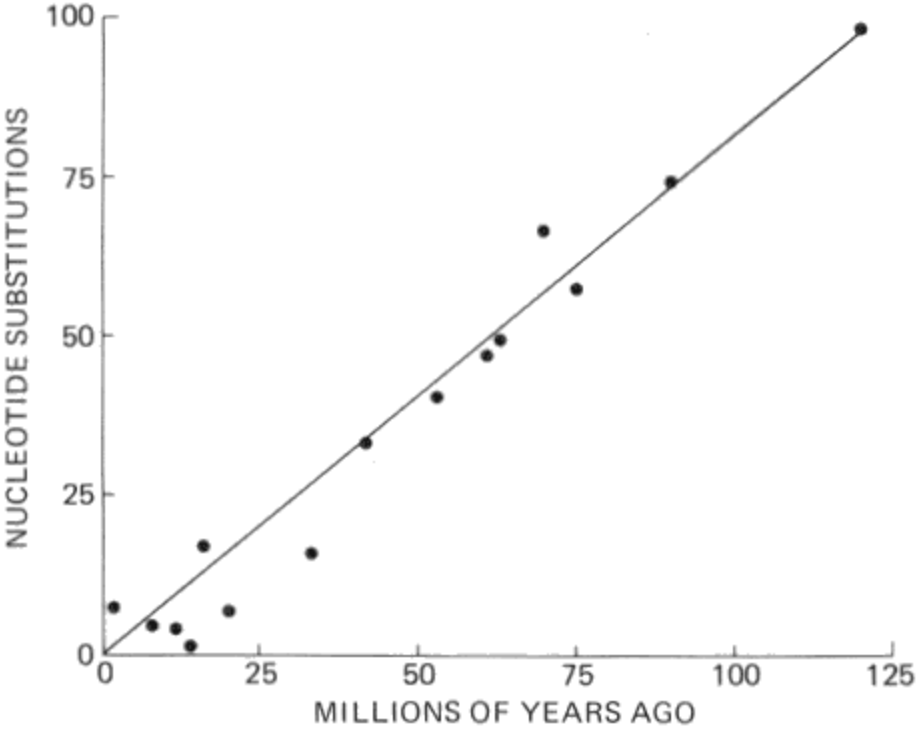
DNA is a double-stranded polymer comprising four basic molecular units, *nucleotides*, denoted by: **A**, **C**, **G** and **T**.

Phylogenetics

Molecular clock-hypothesis

Pair-wise DNA differences between 17 mammal species, plotted against their ‘time-of-divergence’, determined from fossil records.

The linear relation suggests that molecular differences between pairs of species are proportional to their ‘time-of-divergence’.



(Wilson *et al*., 1977)

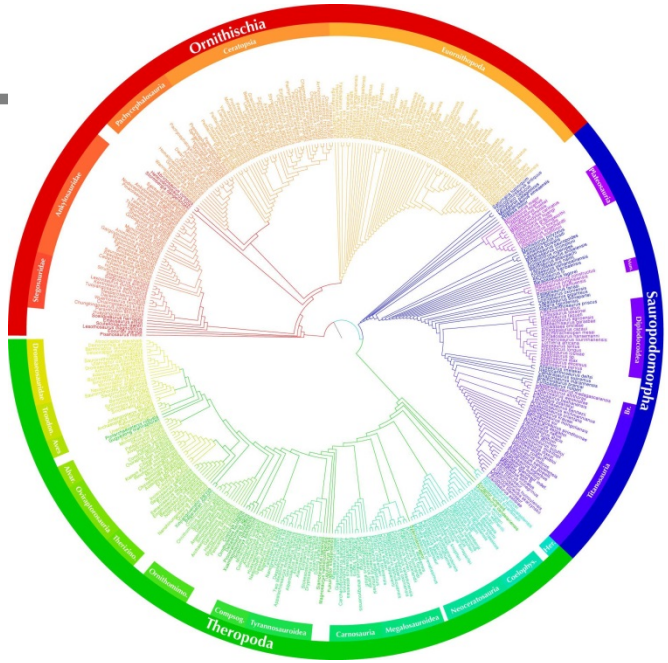
Phylogenetics

Reconstruction of molecular phylogenetic relations is a step-wise process:

1) Select sequences.
2) Build a model that describes evolution over time.
3) Find the tree that best describes the phylogenetic relations between the sequences.
4) Interpret the results.

this lecture

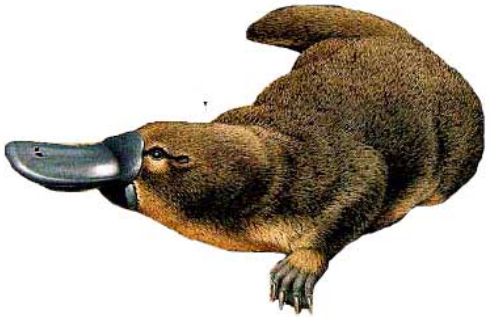
Phylogenetics

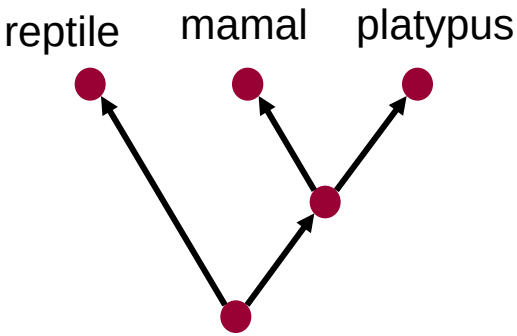


On-going effort, e.g.:

The platypus: reptile or mamal?
Recently, the genome of the platypus / duck bill has been sequenced.

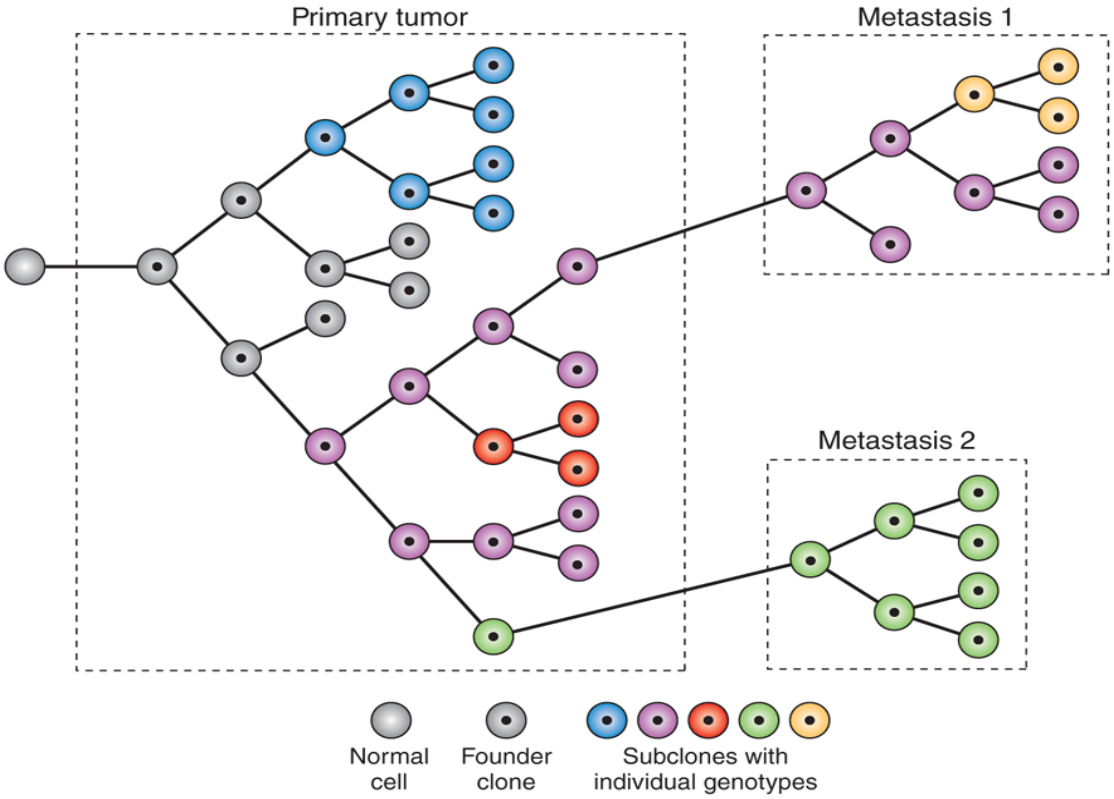
This revealed:
a) +/- 220 My ago separated from the reptiles,
b) +/- 170 My ago separated from the mamals,
and then evolved separately.





Phylogenetics

Cancer is an evolutionary process.



Substitution ≈ mutation.

Nowell (1976), *Science*.
Picture: Caldas (2012), *Nature Biotechnology*.

book Ewens, Grant (2005): Section 15.1.

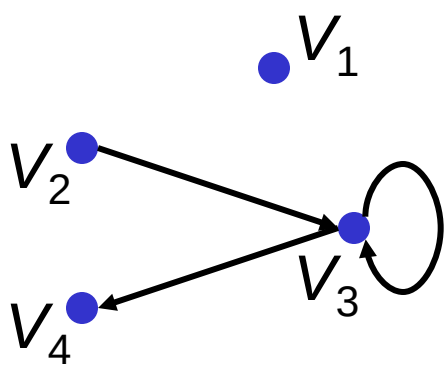
Intermezzo on graphs

Intermezzo on graphs

A *graph* is a system of connected components. The connections are called *edges*, and components *nodes*.

The *topology* of a graph is a pair (***V***, ***E***), where ***V*** the set of nodes and ***E*** a subset of ***V*** x ***V***.

A *path* in a graph is a set of connected edges. When the begin and end point of a path coincide, it is called a *cycle*.



$\boldsymbol{V} = \{ V_1, V_2, V_3, V_4 \}$

$\boldsymbol{E} = \{ (V_2, V_3), (V_3, V_4), (V_3, V_3) \}$

Path: $(V_2, V_3), (V_3, V_4)$

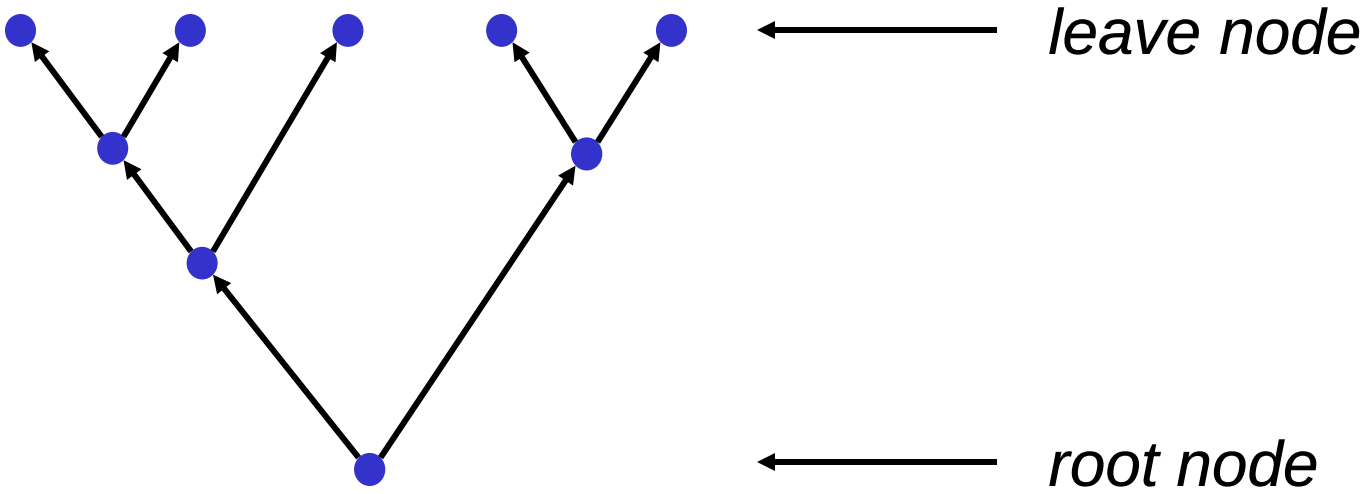
Cycle: (V_3, V_3)

Intermezzo on graphs

If all nodes of a graph are connected (i.e., there is a path between all nodes), the graph is called *connected*.

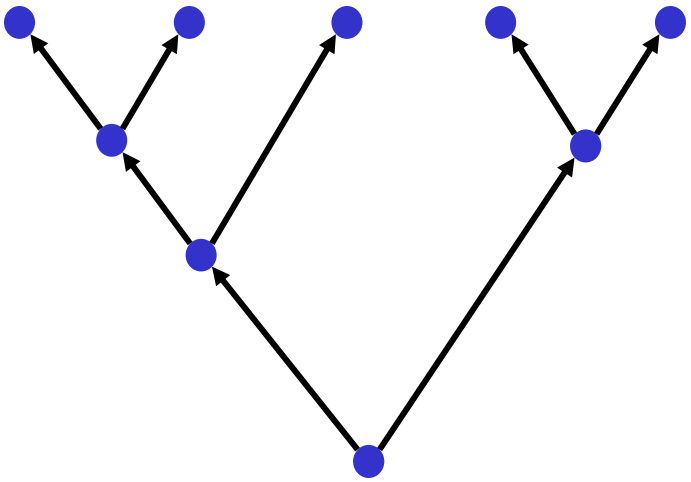
A connected graph that contains no cycles is called a *tree*.

In a *binary tree* every node has either one or three edges, except for the *root node*, if present, that has two edges.

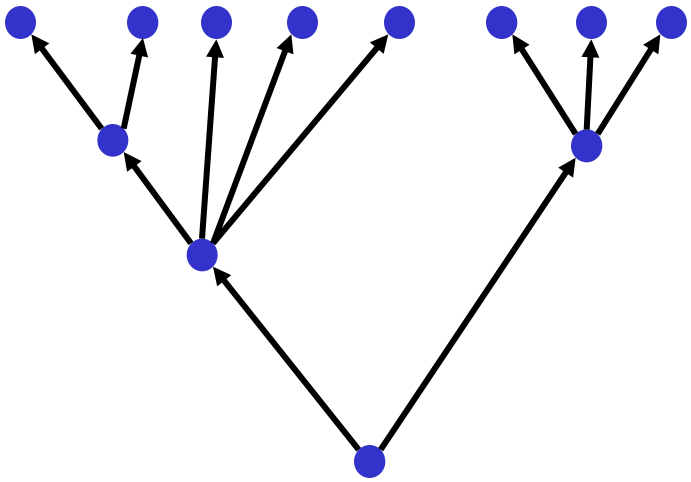


Intermezzo on graphs

This lecture: only consider binary trees. That rules out the possibility of one species evolving into three or more new species at a particular instance



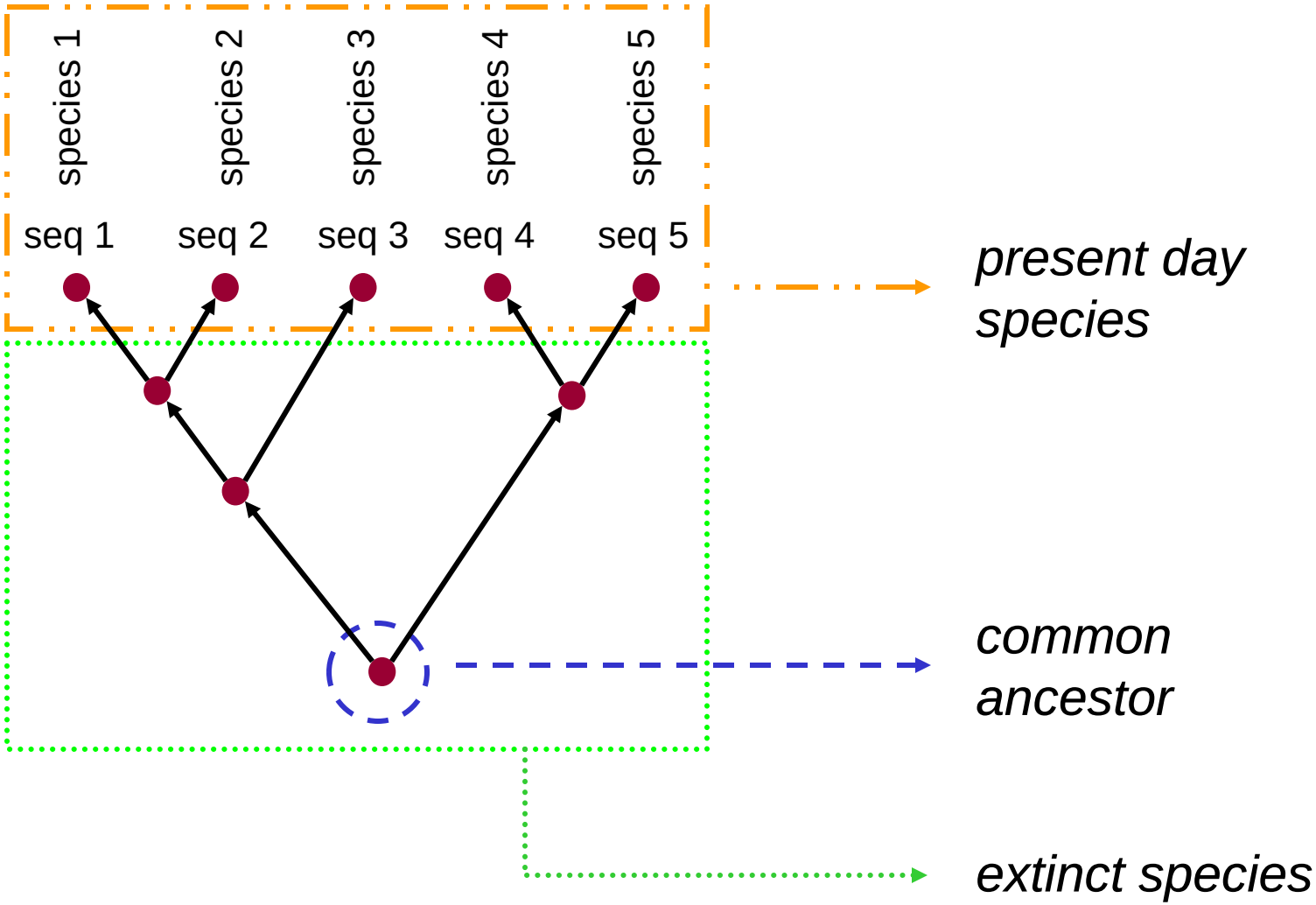
bifurcations



multifurcations

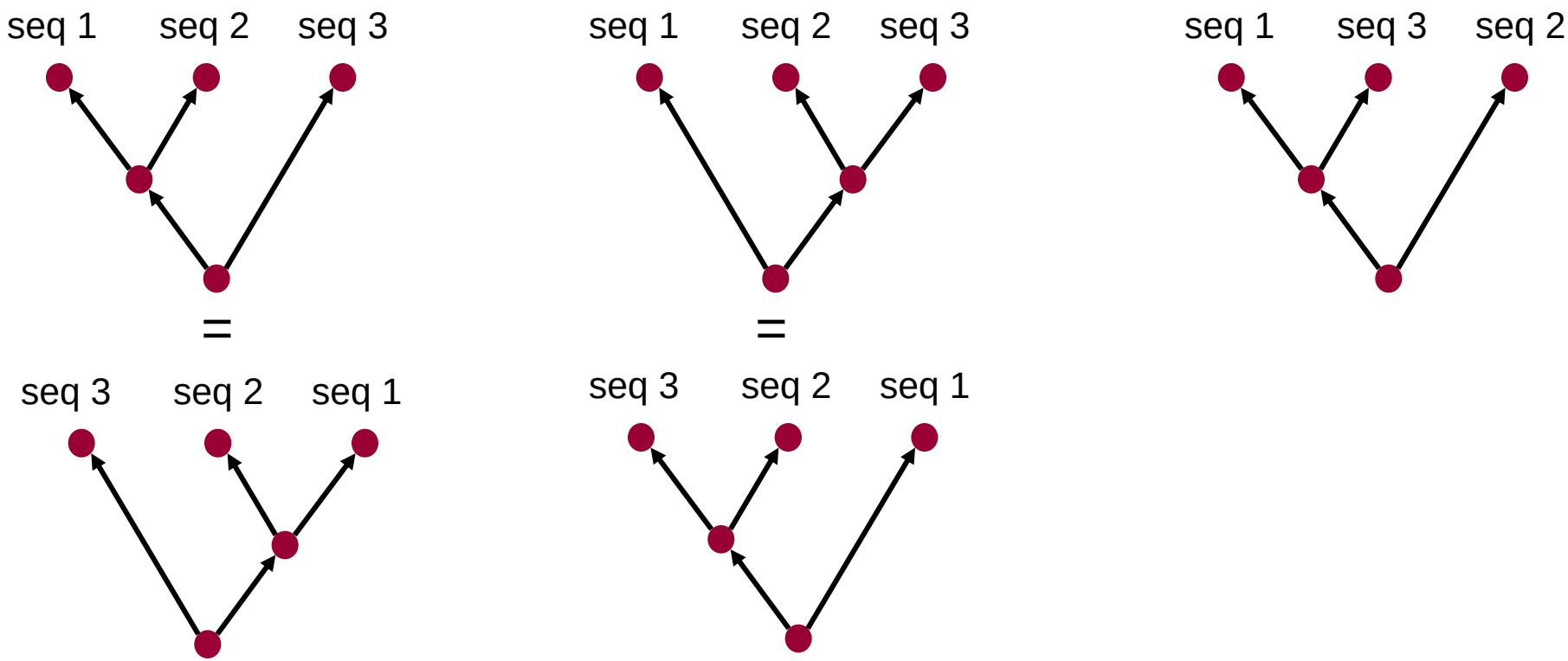
Intermezzo on graphs

In a phylogenetic tree:



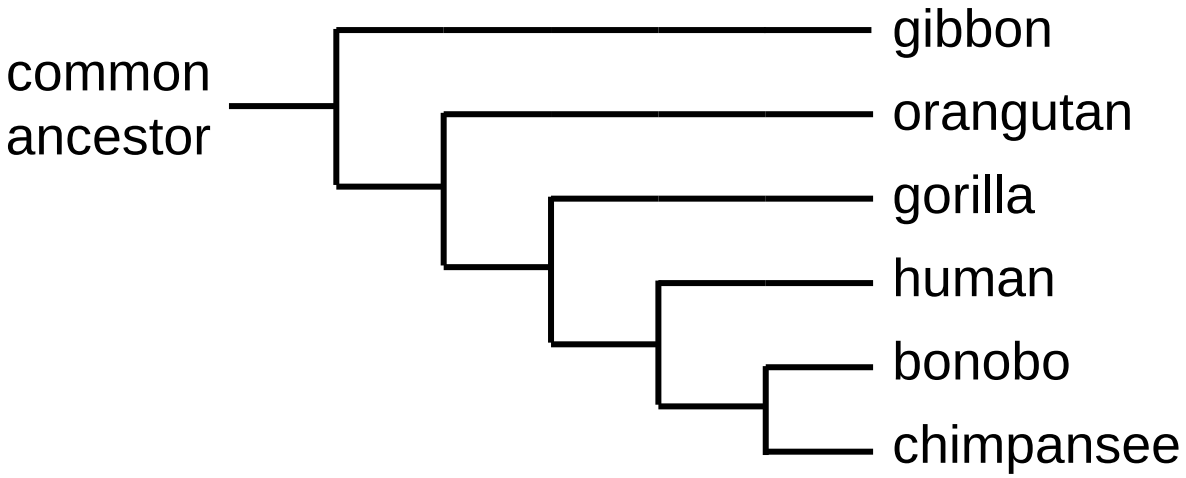
Intermezzo on graphs

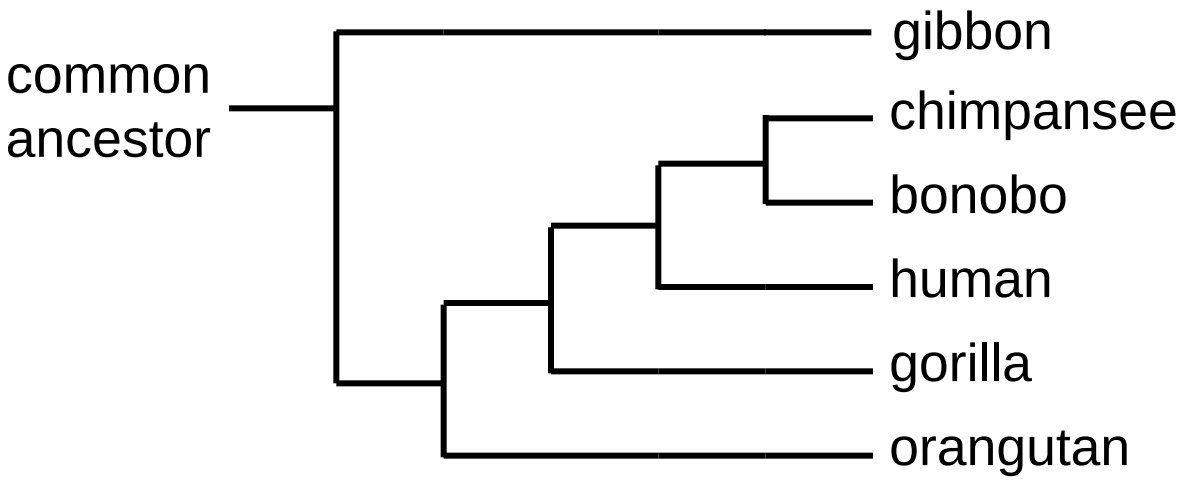
In case of three observed sequences, there are three different trees that connect the sequences:



Intermezzo on graphs

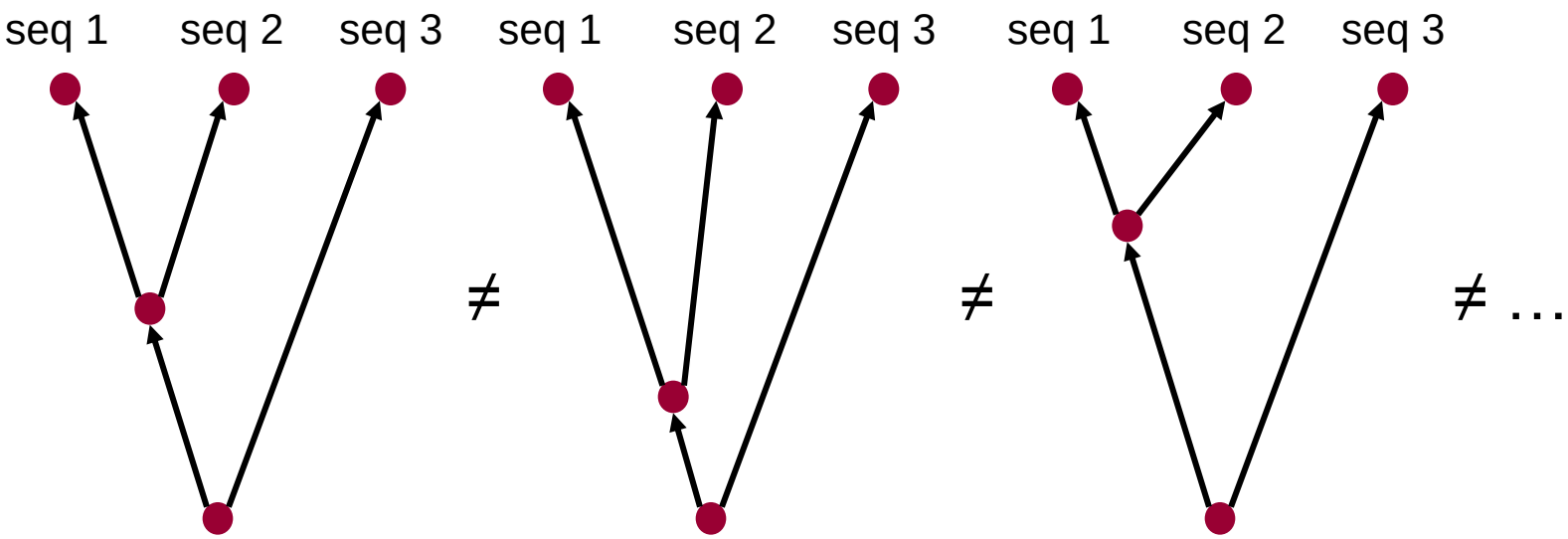
Hence, the following topologies are equivalent.





Intermezzo on graphs

Well … we have not taken into account the edge length. Then ….

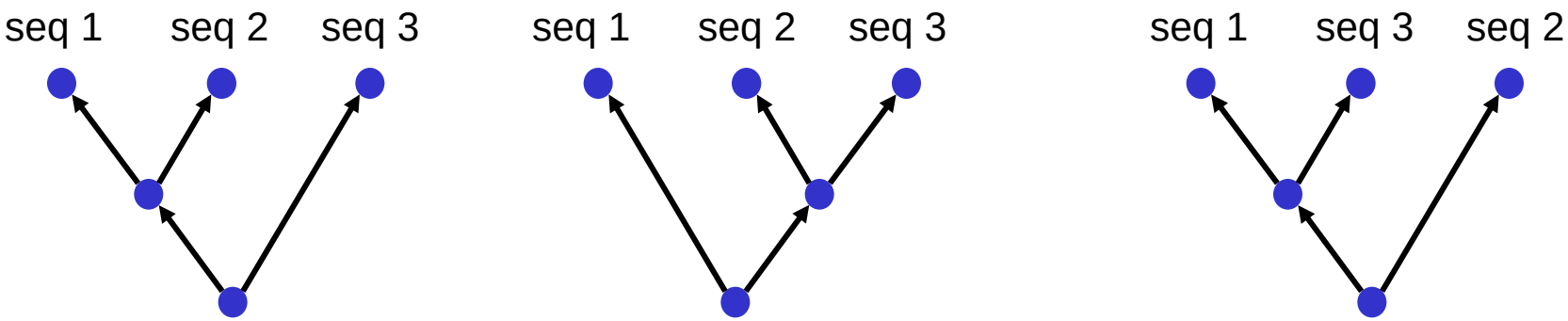


Question

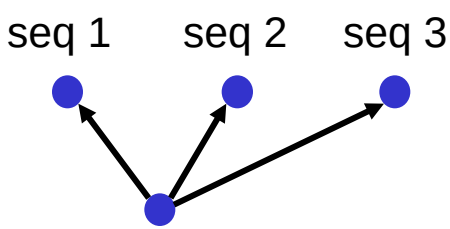
How many possible, different trees connect 3 organisms?

Intermezzo on graphs

If we have three observed sequences, we have three different *rooted* binary trees to connect the three sequences:



Unrooted binary trees: each node has either 1 or 3 neighbors.



Intermezzo on graphs

The number of possible topologies is enormous. If the number of observed sequences equals *n*, the number of different *rooted* or *unrooted* binary trees is:

$$(2n-3)! \,/\, 2^{n-2}\,(n-2)! \qquad\qquad (2n-5)! \,/\, 2^{n-3}\,(n-3)!$$

In case

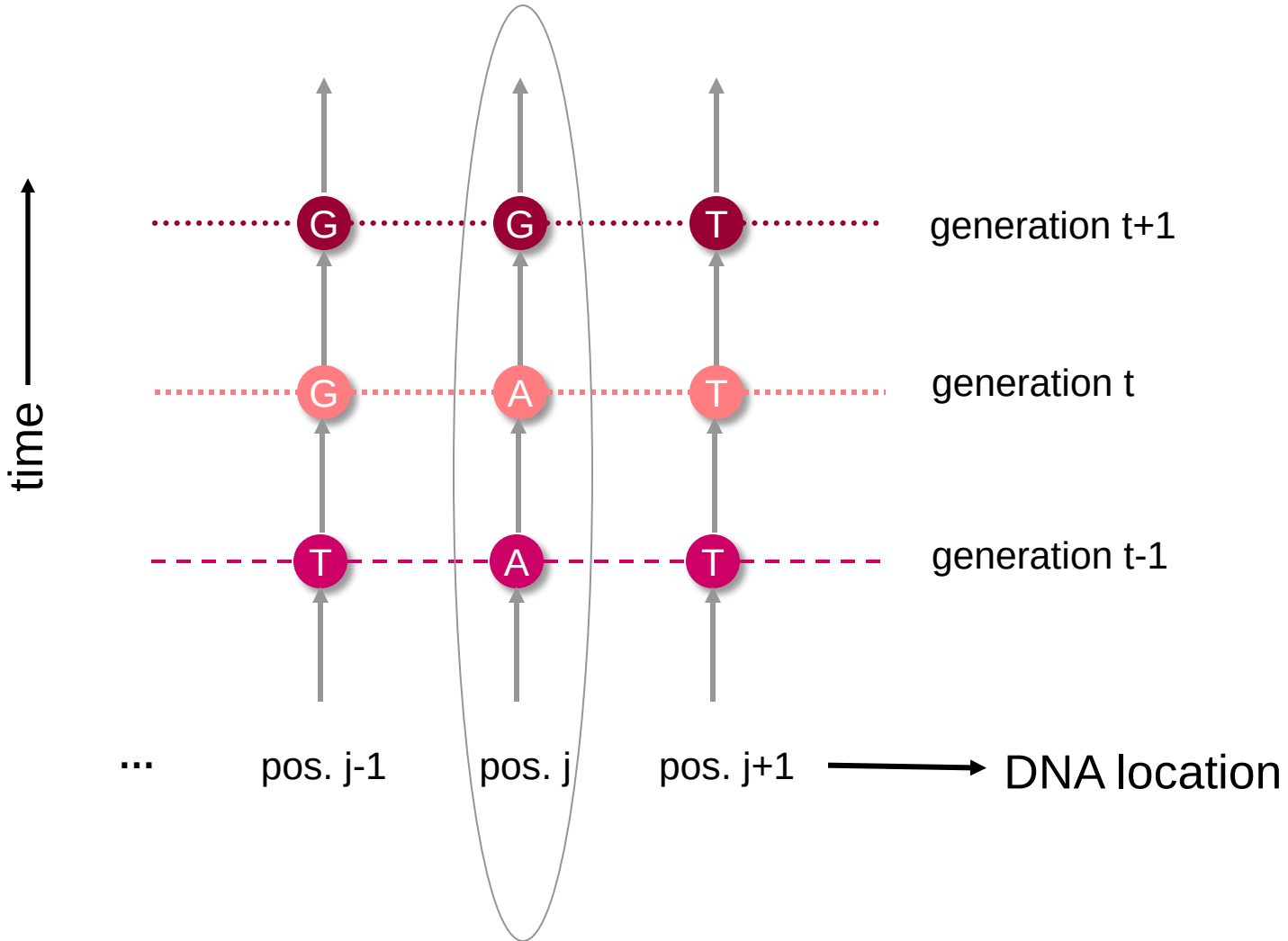
$n = 2$	: 1	$n = 2$	: 1
$n = 3$	: 3	$n = 3$	: 1
$n = 4$	: 15	$n = 4$	: 3
$n = 5$	: 105	$n = 5$	: 15
….	: ….	….	: ….
$n = 10$	: 34459425	$n = 10$	: 2027025

And we have not even considered the branch length!

A model for DNA evolution

Models for DNA evolution

DNA evolution (single species)



Models for DNA evolution

For an individual position the substitution process is modeled by a 1st order Markov process with the state space S={**A**, **G**, **C**, **T**}, now grouped by *purines* (**A** and **G**) and *pyrimidines* (**C** and **T**).

The considered models differ in their parametrization of **P**:

$$\mathbf{P} = \begin{pmatrix} p_{AA} & p_{AG} & p_{AC} & p_{AT} \\ p_{GA} & p_{GG} & p_{GC} & p_{GT} \\ p_{CA} & p_{CG} & p_{CC} & p_{CT} \\ p_{TA} & p_{TG} & p_{TC} & p_{TT} \end{pmatrix}$$

Certain position in the DNA **... AAAAAAGGGGGGGG ...** → *generations*

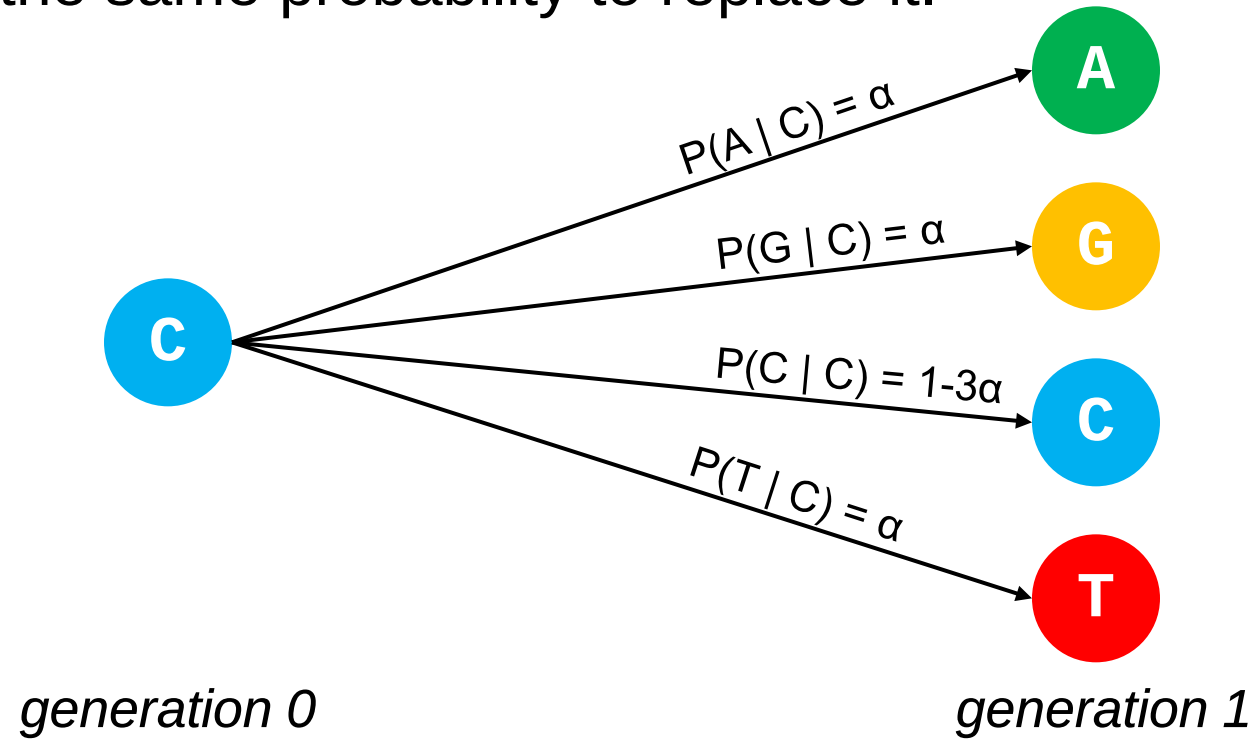
substitution

Models for DNA evolution (JC69)

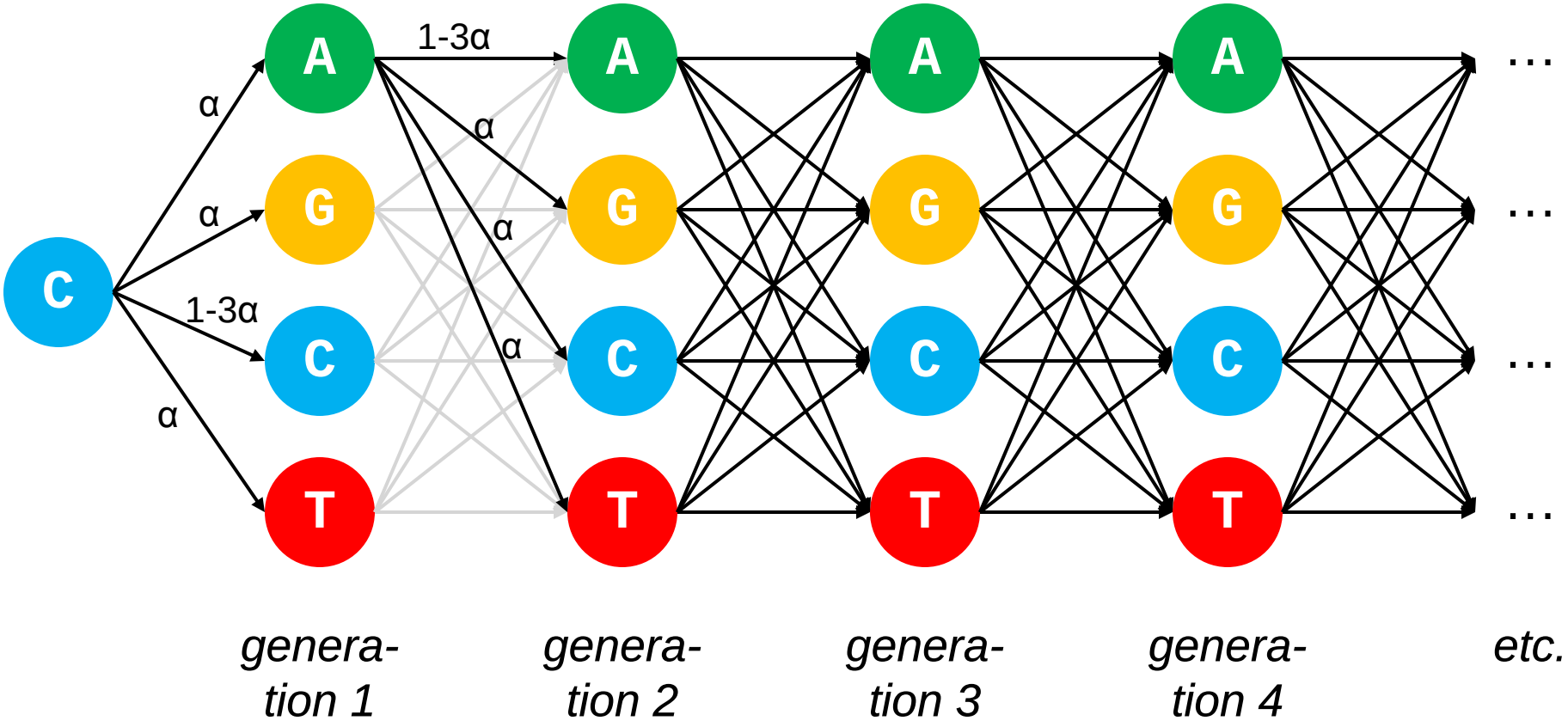
book Ewens, Grant (2005): Section 14.2.1.

The *Jukes-Cantor model* is a DNA substitution model which assumes that:

- each base in the sequence has an equal probability of being substituted.
- if a nucleotide substitution occurs, all other nucleotides have the same probability to replace it.

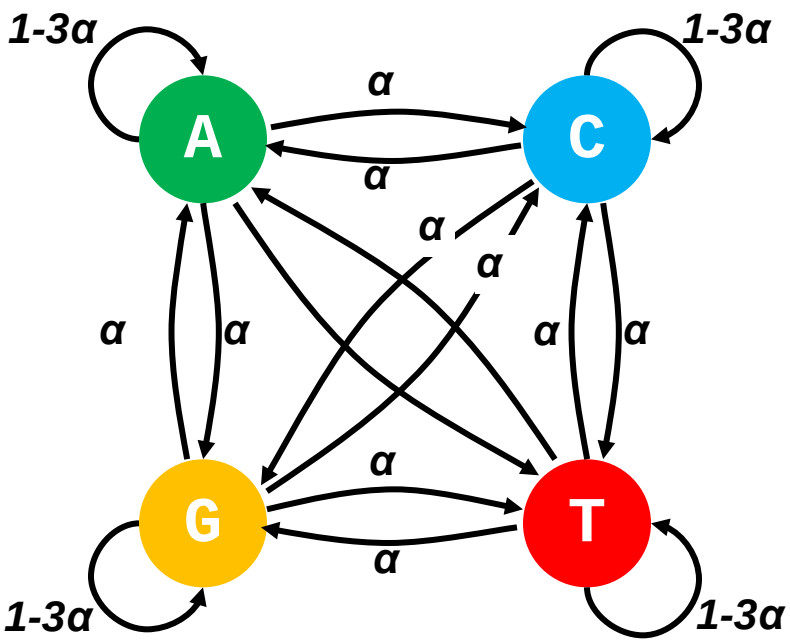


Models for DNA evolution (JC69)



Models for DNA evolution (JC69)

Over 1000s of generations (time homogeneity):



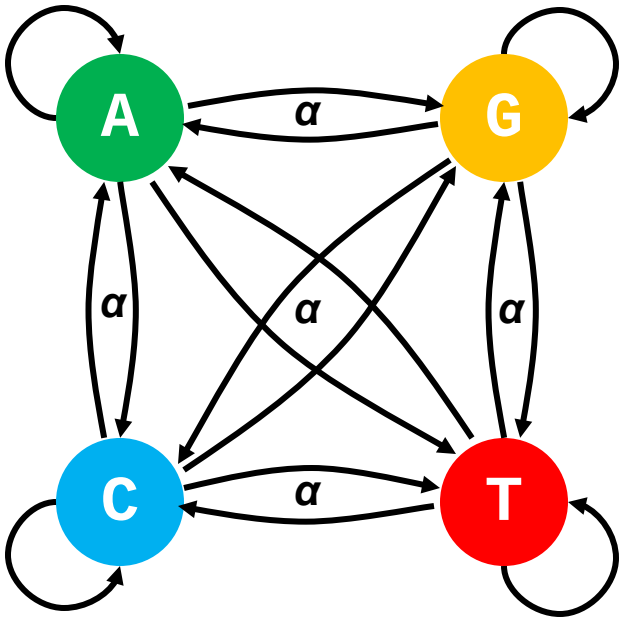
Models for DNA evolution (JC69)

book Ewens, Grant (2005): Section 14.2.1.

The Jukes-Cantor transition matrix:

$$\mathbf{P} = \begin{pmatrix} 1-3\alpha & \alpha & \alpha & \alpha \\ \alpha & 1-3\alpha & \alpha & \alpha \\ \alpha & \alpha & 1-3\alpha & \alpha \\ \alpha & \alpha & \alpha & 1-3\alpha \end{pmatrix}$$

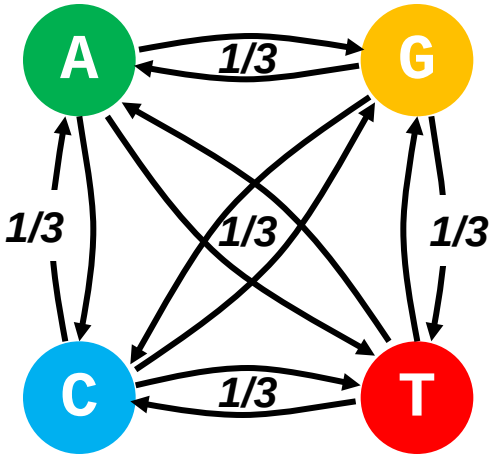
where
- $0 < \alpha < \frac{1}{3}$,
- α depends on the step size.



Models for DNA evolution (JC69)

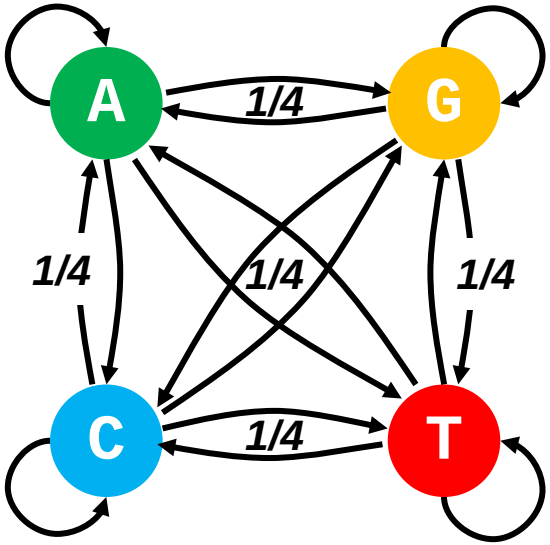
Always substitute if α=1/3:

$$\mathbf{P} = \begin{pmatrix} 0 & 1/3 & 1/3 & 1/3 \\ 1/3 & 0 & 1/3 & 1/3 \\ 1/3 & 1/3 & 0 & 1/3 \\ 1/3 & 1/3 & 1/3 & 0 \end{pmatrix}$$



No Markov property if α=1/4:

$$\mathbf{P} = \begin{pmatrix} 1/4 & 1/4 & 1/4 & 1/4 \\ 1/4 & 1/4 & 1/4 & 1/4 \\ 1/4 & 1/4 & 1/4 & 1/4 \\ 1/4 & 1/4 & 1/4 & 1/4 \end{pmatrix}$$



Models for DNA evolution (JC69)

book Ewens, Grant (2005): Section 14.2.1.

Properties

The eigenvalues of **P**:

$$\lambda = 1, 1\text{-}4\alpha, 1\text{-}4\alpha, 1\text{-}4\alpha.$$

The stationary distribution corresponding to $\lambda=1$:

$$\boldsymbol{\varphi} = (1/4, 1/4, 1/4, 1/4)^T$$

Indeed, after enough generations all four states are equally likely. That is, all four nucleotides are equally likely to be the predominant nucleotide at the position under consideration.

Models for DNA evolution (JC69)

book Ewens, Grant (2005): Section 14.2.1.

Properties

Its spectral decomposition:

$$\mathbf{P}^t = \frac{1}{4}\begin{pmatrix} 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \end{pmatrix} + \frac{1}{4}(1-4\alpha)^t \begin{pmatrix} 3 & -1 & -1 & -1 \\ -1 & 3 & -1 & -1 \\ -1 & -1 & 3 & -1 \\ -1 & -1 & -1 & 3 \end{pmatrix}$$

Question

What is $P(X_{t+2} = \mathbf{C} \mid X_t = \mathbf{T})$?

Models for DNA evolution (JC69)

Properties

Consider a stationary 1st order Markov chain with a Jukes-Cantor transition matrix. Probability of no substitution is:

$$P(X_t=\mathbf{A}, X_{t-1}=\mathbf{A}, \ldots, X_0=\mathbf{A}) = P(\mathbf{A} \mid \mathbf{A})^t P(\mathbf{A})$$
$$= (1-3\alpha)^t \varphi_A$$
$$= (1-3\alpha)^t / 4$$

Given that $X_0=\mathbf{A}$, the probability that **A** will be the pre-dominant nucleotide at time *t* is given by:

$$\tfrac{1}{4} + \tfrac{3}{4}(1-4\alpha)^t$$

Question

Explain the importance of the difference between the two probabilities above for phylogenetics.

Models for DNA evolution (JC69)

Properties

Now we know **P** and **φ**, and, hence, we can assess the reversibility of the Jukes-Cantor model by means of checking the detailed balance equations:

$$\varphi_i \, p_{ij} = \varphi_j \, p_{ji} \qquad \text{for all i and j.}$$

Recall

In order for the Jukes-Cantor model to link one species to another (via a common ancestor), the transition matrix **P** needs to be reversible.

Models for DNA evolution (JC69)

Properties

Consider two organisms with common ancestor.
Study proportion of site differences between their sequences.

In the long run this proportion convergences (under the JC69 model) to $P(X_t^{(1)} \neq X_t^{(2)}) = 1 - P(X_t^{(1)}=X_t^{(2)}) = 3/4$, as

$$
\begin{aligned}
P(X_t^{(1)}=X_t^{(2)}) &= P(X_t^{(1)}=\mathbf{A}, X_t^{(2)}=\mathbf{A}) + \ldots \\
&= P(X_t^{(1)}=\mathbf{A}, X_t^{(2)}=\mathbf{A} \mid X_0^{(ca)})\, P(X_0^{(ca)}) + \ldots \\
&= P(X_t^{(1)}=\mathbf{A} \mid X_0^{(ca)})\, P(X_t^{(2)}=\mathbf{A} \mid X_0^{(ca)})\, P(X_0^{(ca)}) + \ldots \\
&= \textstyle\sum_{x^{(ca)}} 1/4 * P(X_t^{(1)}=\mathbf{A} \mid X_0^{(ca)})\, P(X_t^{(2)}=\mathbf{A} \mid X_0^{(ca)}) + \ldots \\
&\approx 1/4 \textstyle\sum_{x(ca)} 1/4 * 1/4 + \ldots \\
&= 1/4 * 1.
\end{aligned}
$$

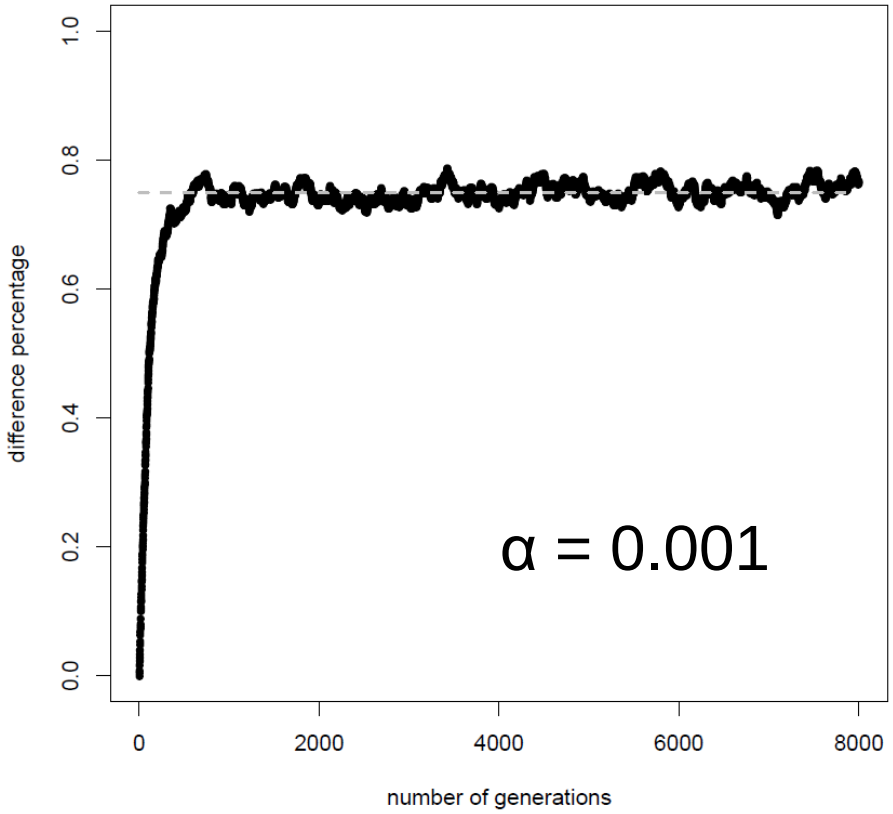
spectral decomposition and *t* large.

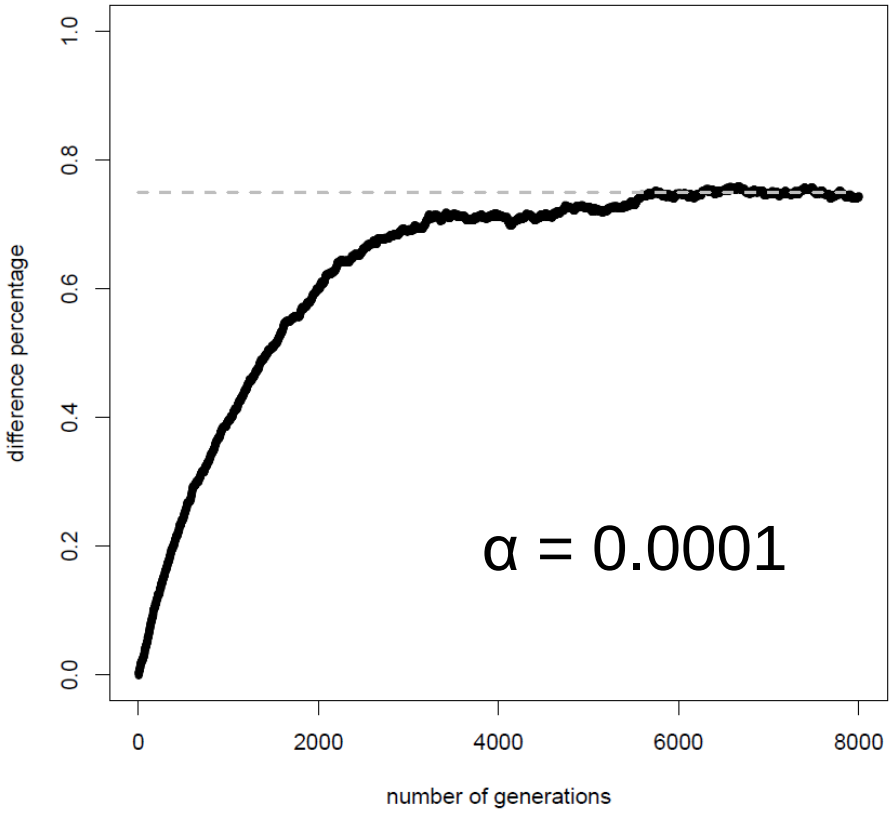
Note: probability accounts for substitutions, as long as at time *t* same nucleotide is observed.

Models for DNA evolution (JC69)

Properties

Proportion of site differences between two sequences in the JC69 model plotted against time from common ancestor.



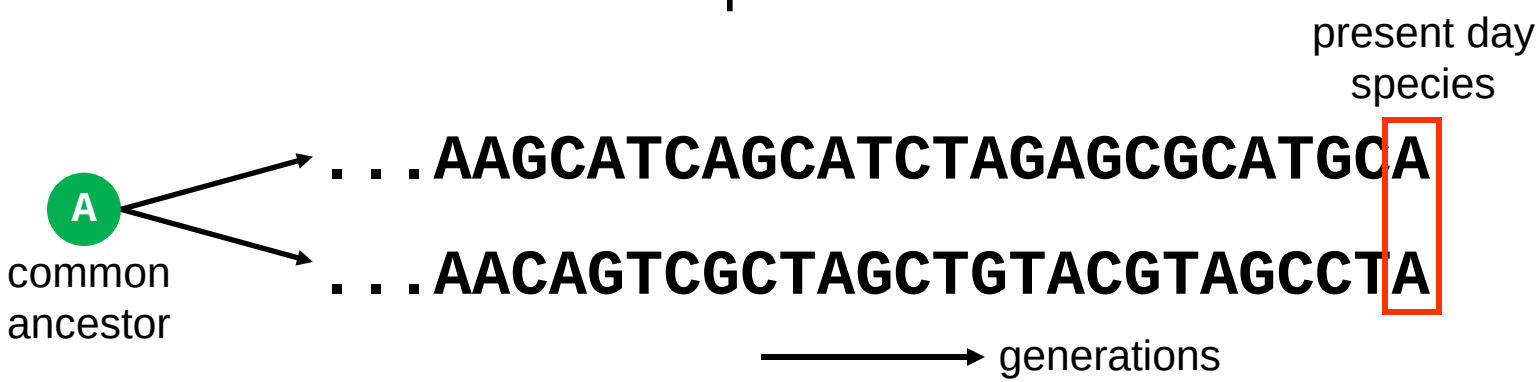


Models for DNA evolution (JC69)

Properties

Why care about $P(X_t^{(1)}=X_t^{(2)})$?

Consider evolution of two-species:



In both present day species the DNA position is occupied by the same nucleotide (an **A**).

Question

Conclusion: no divergence between species. Correct?

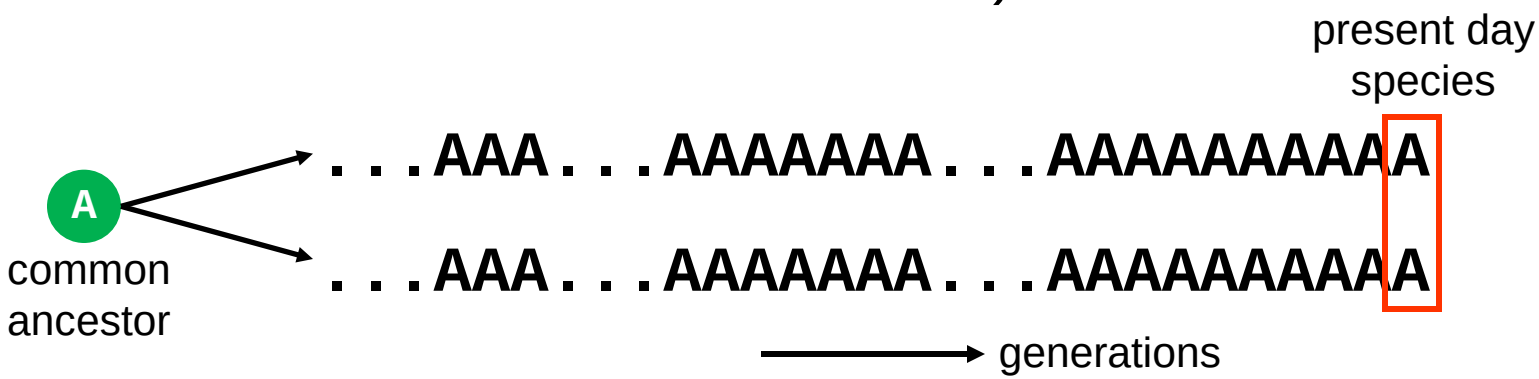
Models for DNA evolution (JC69)

Properties

Why care about $P(X_t^{(1)}=X_t^{(2)})$?

Selection of the DNA base pair position for the inference of a phylogenetic tree is crucial.

Typically, position under strong selection pressure (and with low substition rates are selected).



Conclusion

No divergence between species.

Models for DNA evolution (K80)

book Ewens, Grant (2005): Section 14.2.2.

The *Kimura model* is a generalization of the Jukes-Cantor model. It allows for different transition (pur -> pur, pyr. -> pyr) and transversion (pur -> pyr, pyr. -> pur) probabilities.

Similar to the Jukes-Cantor model, the Kimura is symmetrical. Therefore, after enough time it is equally likely for a base to be a purine or a pyrimidine.

Within the purine and pyrimidine categories there is complete symmetry between the nucleotides.

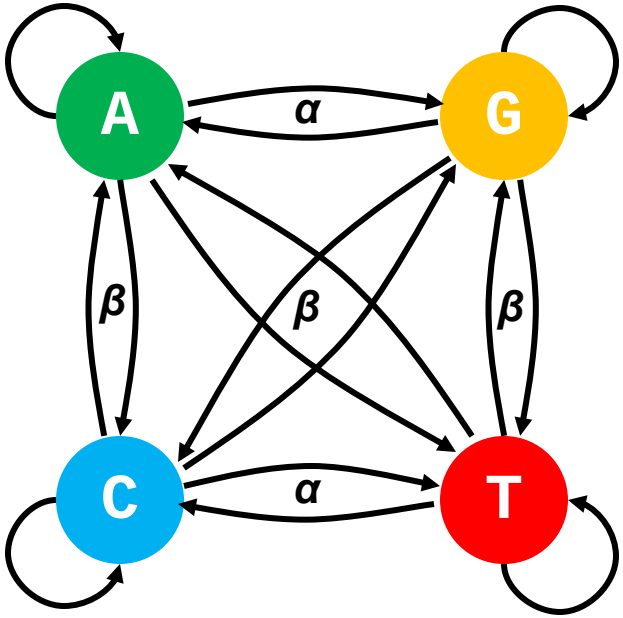
Models for DNA evolution (K80)

book Ewens, Grant (2005): Section 14.2.2.

The Kimura transition matrix:

$$\mathbf{P} = \begin{pmatrix} 1-\alpha-2\beta & \alpha & \beta & \beta \\ \alpha & 1-\alpha-2\beta & \beta & \beta \\ \beta & \beta & 1-\alpha-2\beta & \alpha \\ \beta & \beta & \alpha & 1-\alpha-2\beta \end{pmatrix}$$

where
- $\alpha + 2\beta < 1$, $\alpha > 0$, $\beta > 0$.
- α, β depend on the step size.



Question

For which α and β does Kimura reduce to Jukes-Cantor?

Models for DNA evolution (K80)

book Ewens, Grant (2005): Section 14.2.2.

Properties

The eigenvalues of **P**:

$$\lambda = 1,\ 1\text{-}4\beta,\ 1\text{-}2(\alpha+\beta),\ 1\text{-}2(\alpha+\beta).$$

The stationary distribution corresponding to $\lambda=1$:

$$\boldsymbol{\varphi} = (1/4,\ 1/4,\ 1/4,\ 1/4)^T$$

The Kimura model is reversible (**P** is symmetric and **φ** uniform).

Models for DNA evolution (K80)

Properties

Consider two organisms with common ancestor.
Study proportion of site differences between their sequences.

Question

Assume the Kimura model and that many generations have passed since the separation of the two organisms.

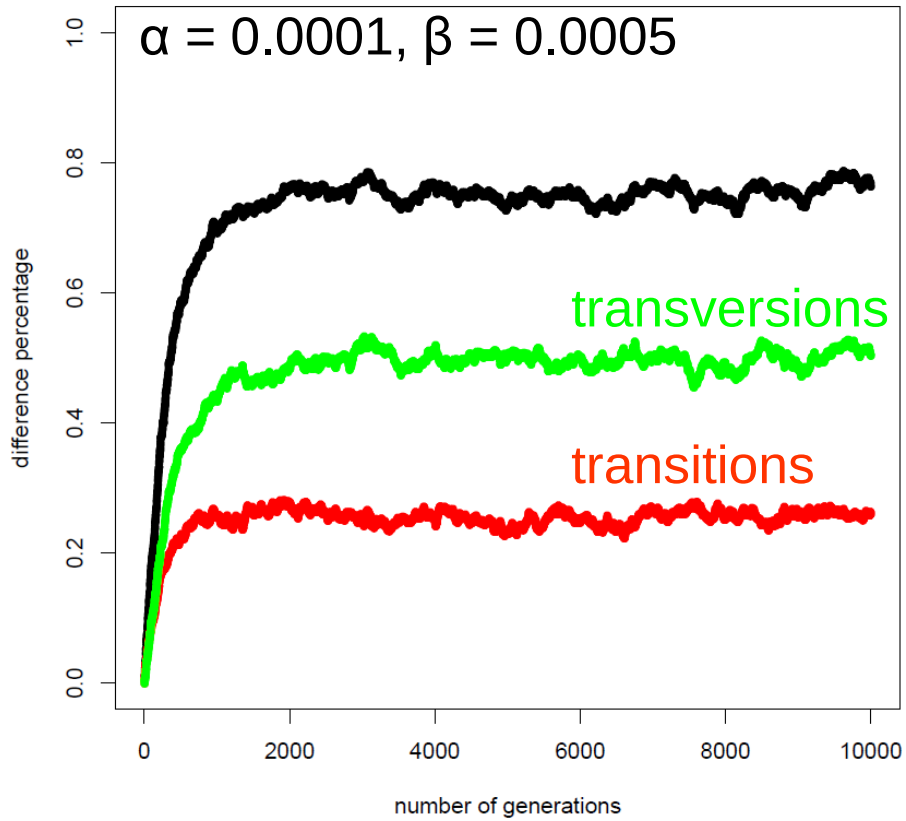
Which of these statements is true?

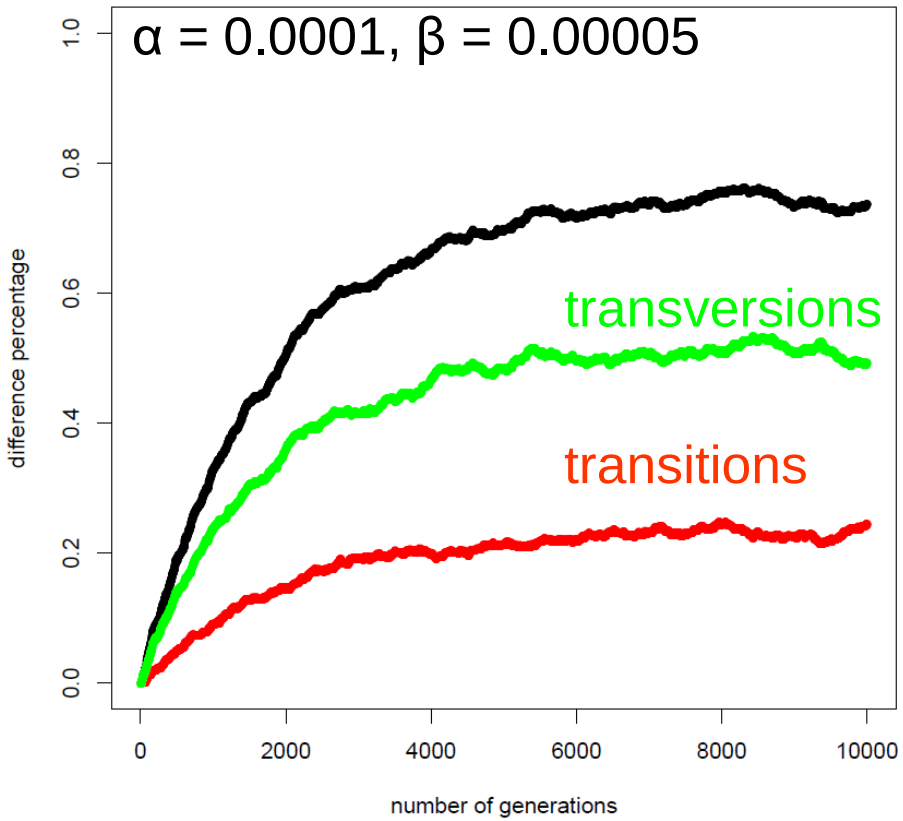
→ $P(X_t^{(1)}=X_t^{(2)}) = 1/4$.

→ $P(X_t^{(1)}$ is pur., $X_t^{(2)}$ is pur.) = $P(X_t^{(1)}$ is pyr., $X_t^{(2)}$ is pyr.)

Models for DNA evolution (K80)

Proportion of site differences between two sequences in the Kimura model plotted against time (# generations), starting from the common ancestor.



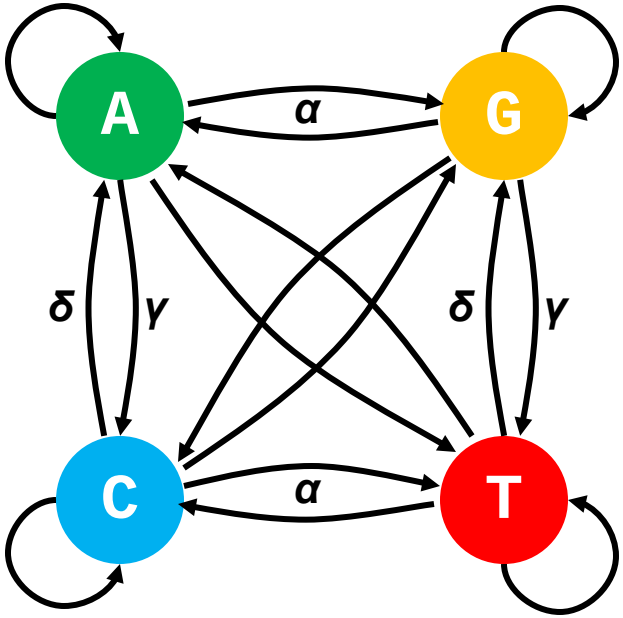


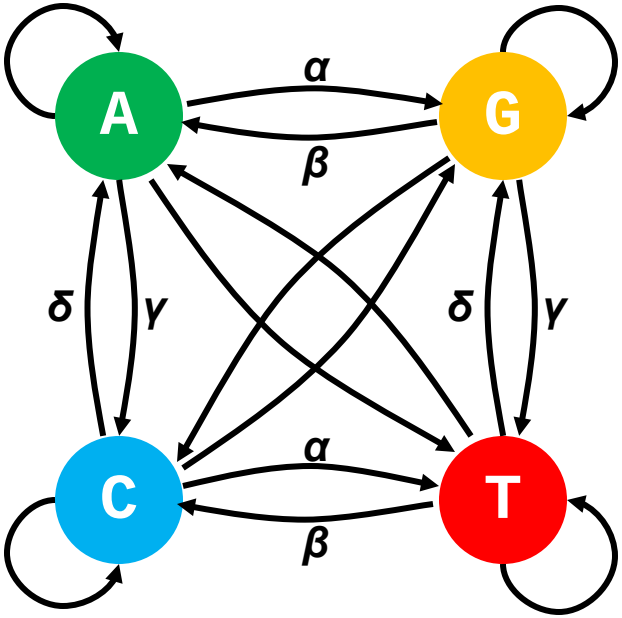
book Ewens, Grant (2005): Section 14.2.3.

Models for DNA evolution (K80)

The Kimura model has been generalized to allow, e.g.:

- The transition probability to differ from the transversion probability.
- Different within-transition and within-transversion substitution probabilities.





Models for DNA evolution (F81)

book Ewens, Grant (2005): Section 14.2.4.

The *Felsenstein model* is also a generalization of the Jukes-Cantor model. It relaxes the (implicit) assumption of the JC and Kimura model, both having a uniform stationary distribution.

In the Felsenstein model the probability of substitution of any nucleotide by another is proportional to the stationary probability of the substituting nucleotide.

The Felsenstein model does not distinguish between purines and pyrimidines.

Models for DNA evolution (F81)

book Ewens, Grant (2005): Section 14.2.4.

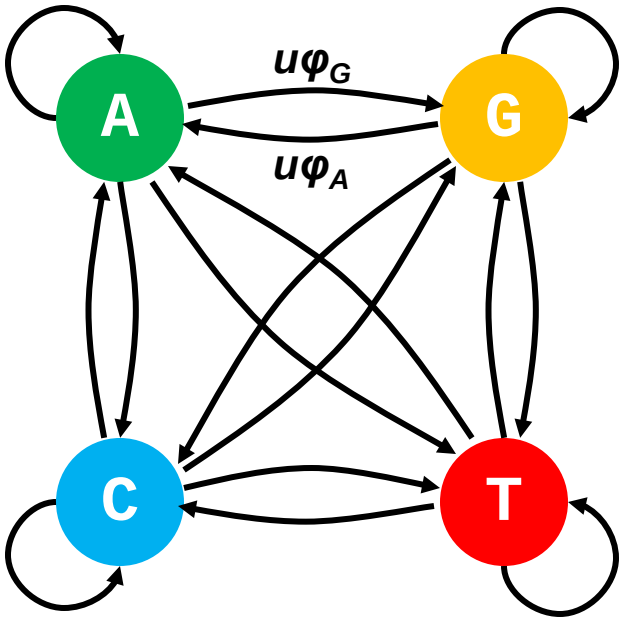
The Felsenstein transition matrix:

$$\mathbf{P} = \begin{pmatrix} 1-u+u\,\varphi_A & u\,\varphi_G & u\,\varphi_C & u\,\varphi_T \\ u\,\varphi_A & 1-u+u\,\varphi_G & u\,\varphi_C & u\,\varphi_T \\ u\,\varphi_A & u\,\varphi_G & 1-u+u\,\varphi_C & u\,\varphi_T \\ u\,\varphi_A & u\,\varphi_G & u\,\varphi_C & 1-u+u\,\varphi_T \end{pmatrix}$$

where

- $\varphi_A + \varphi_G + \varphi_C + \varphi_T = 1$.
- *u* a model parameter.

Take $\varphi_A=\varphi_G=\varphi_C=\varphi_T=1/4$: Jukes-Cantor.



Models for DNA evolution

book Ewens, Grant (2005): Section 14.2.7.

Question

→ Can you think of another substitution model?

→ What is the maximum number of “free” parameters of a substitution model?

$$\mathbf{P} = \begin{pmatrix} p_{AA} & p_{AG} & p_{AC} & p_{AT} \\ p_{GA} & p_{GG} & p_{GC} & p_{GT} \\ p_{CA} & p_{CG} & p_{CC} & p_{CT} \\ p_{TA} & p_{TG} & p_{TC} & p_{TT} \end{pmatrix}$$

The likelihood: a simple example

Why the likelihood approach?

Why use the likelihood approach when also the methodologically simpler *distance matrix* and *maximum parsimony methods* are available?

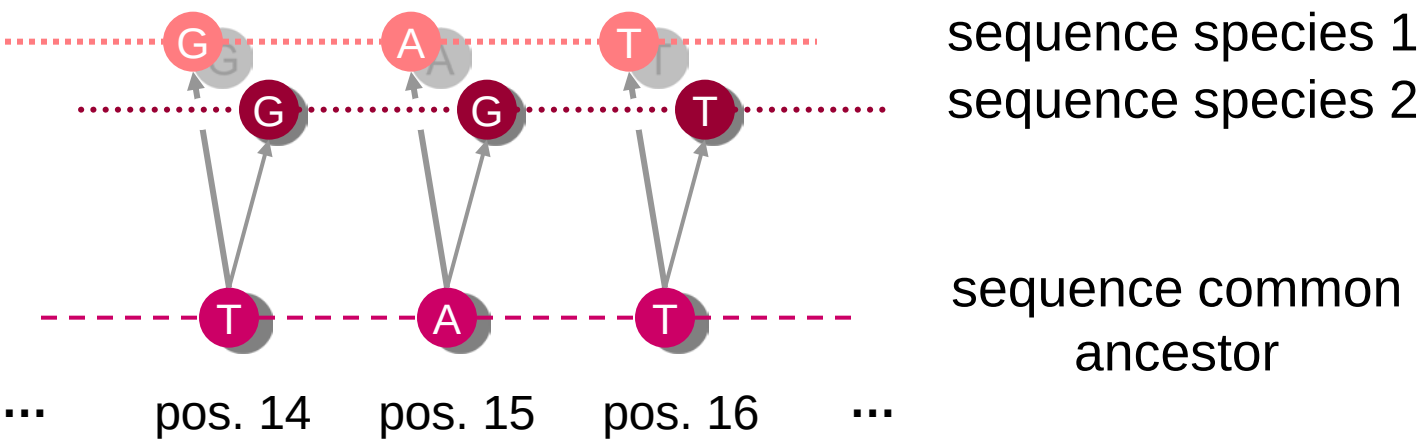
- The likelihood approach makes assumptions explicit. This enables us to assess their validity.
- Within the likelihood framework we may compare nested models using a likelihood ratio test.

The likelihood: an example

Consider two homologous sequences sampled from two different species (with a common ancestor):

```
species 1    : AATTGCGTAGCTAGATCGCTCGCTA
species 2    : AATTGCGTAGCTAGGTCGCTCGCTA
```

15th base



What is the likelihood of observing these two sequences?

The likelihood: an example

Let

X denote the sequence data of both species, and

X_{ij} denote the nucleotide at position *j*=1, …,25 of species *i*.

The likelihood for the Jukes-Cantor model is then:

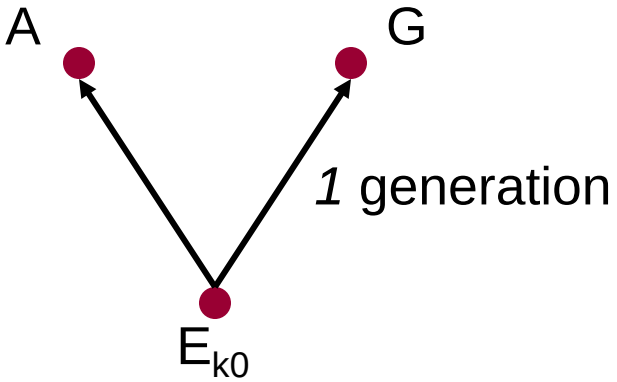
$$L(\mathbf{X}) = P(\mathbf{X})$$

which, assuming sites evolve independently, factorizes to

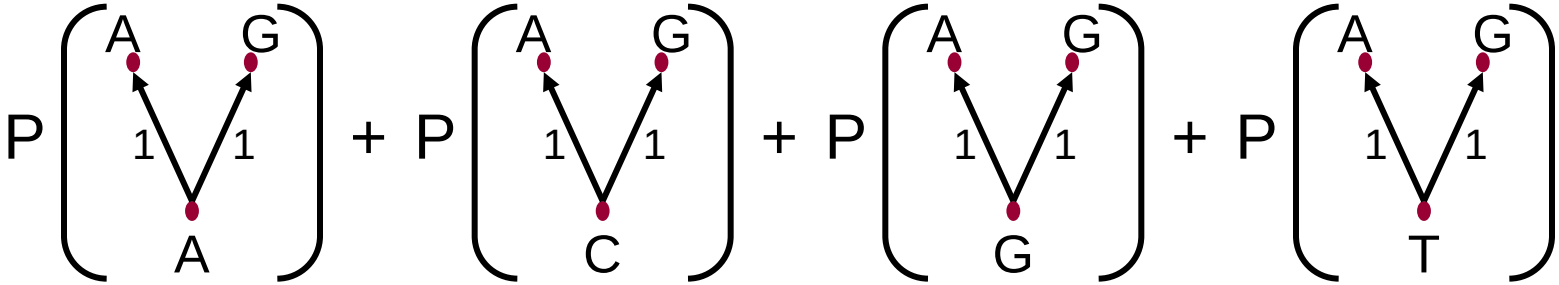
$$= \prod_{j=1}^{25} P((X_{1j}, X_{2j}))$$

The likelihood: an example

Assuming $(X_{1j}, X_{2j}) = (A, G)$ and that the species have evolved separately *one* generation since the common ancestor, then:



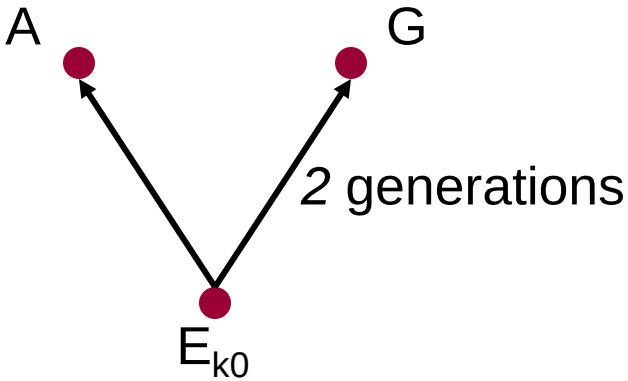
$P((X_{1j}, X_{2j}) = (A, G)) =$



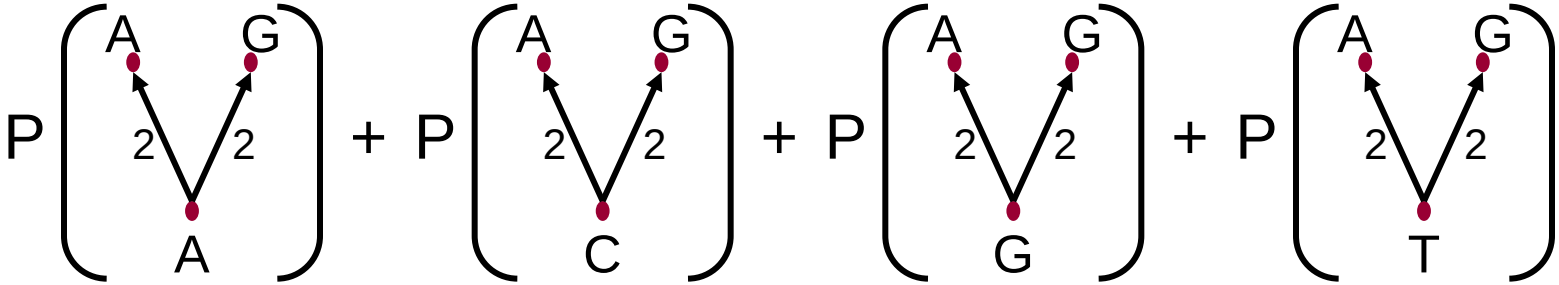
$$= \pi_A\, p_{AA}\, p_{AG} + \pi_C\, p_{CA}\, p_{CG} + \pi_G\, p_{GA}\, p_{GG} + \pi_T\, p_{TA}\, p_{TG}$$

The likelihood: an example

Assuming $(X_{1j}, X_{2j}) = (A, G)$ and that the species have evolved separately *two* generations since the common ancestor, then:

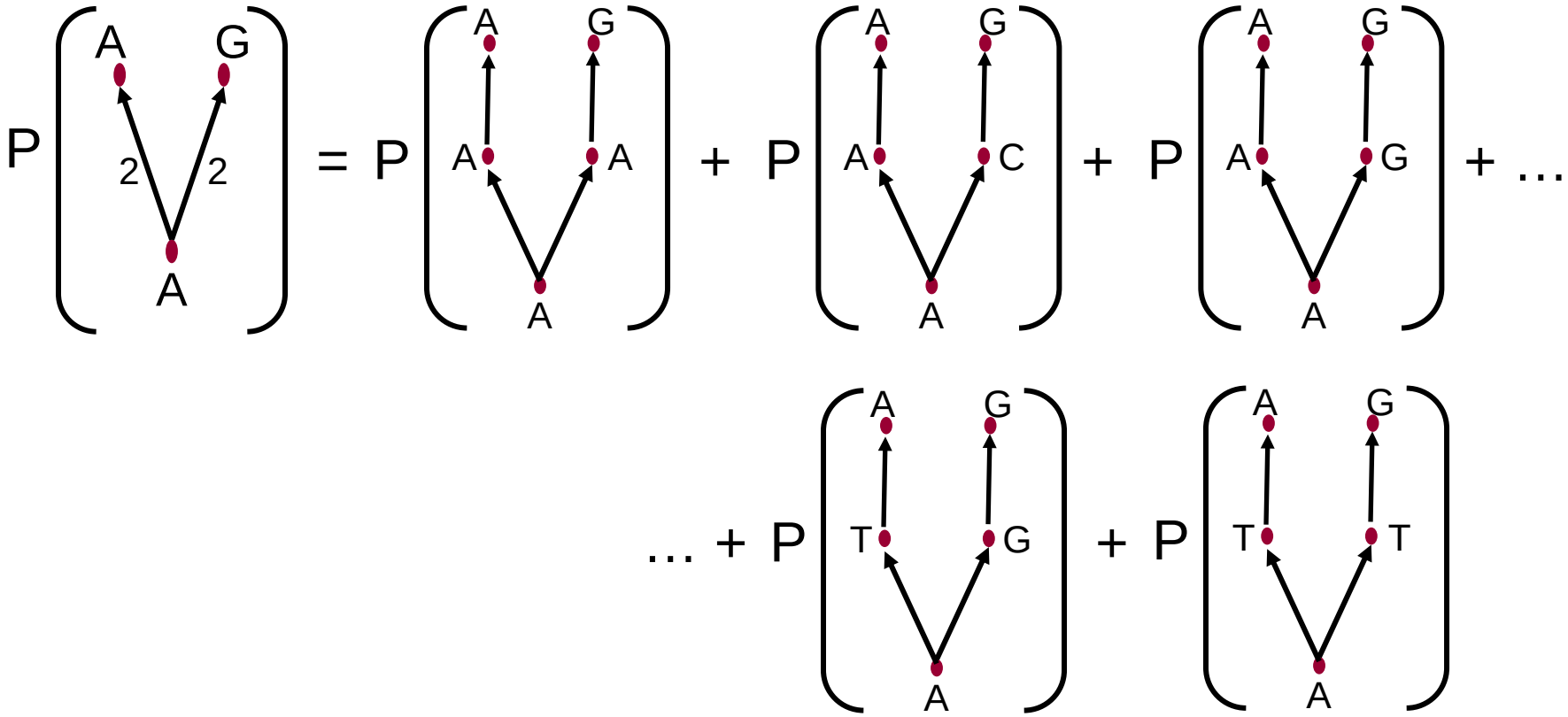


$P((X_{1j}, X_{2j}) = (A, G)) =$



The likelihood: an example

where:

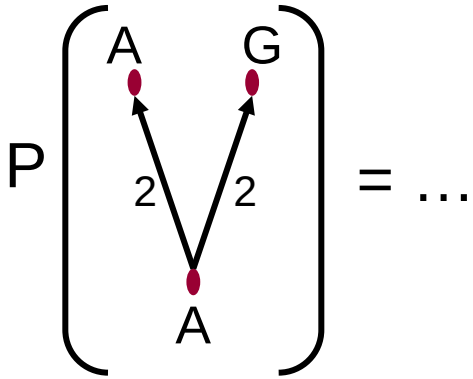


Sum over all possible choices for the intermediate generation.

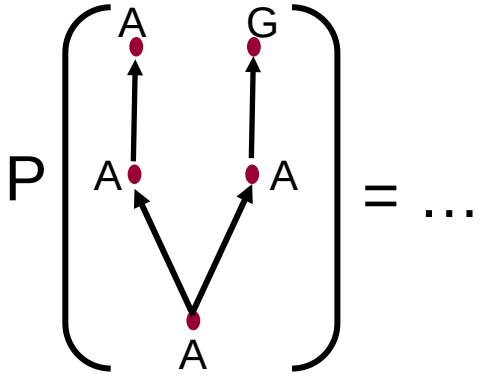
The likelihood: an example

Question

In formula:

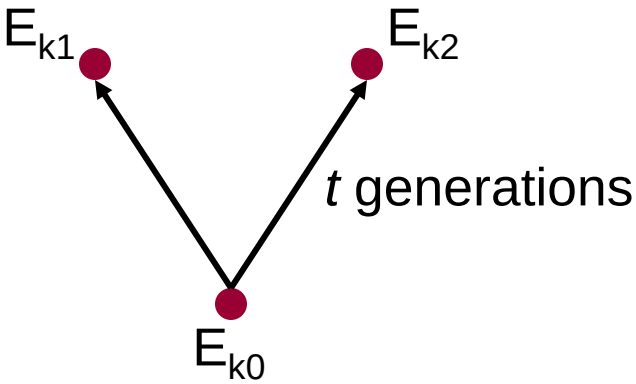


Start with:



The likelihood: an example

Assuming $(X_{1j}, X_{2j}) = (E_{k1}, E_{k2})$ and that the species have evolved separately *t* generations since the common ancestor, then:

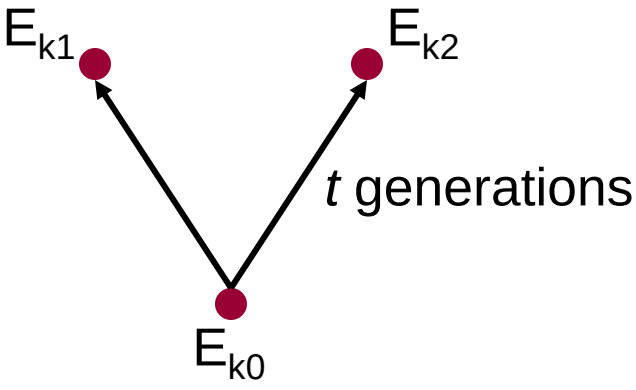


$$
\begin{aligned}
P((X_{1j}, X_{2j}) = (E_{k_1}, E_{k_2})) \\
&= \sum_{k_0 \in \mathcal{S}} \pi_{k_0} \left(\mathbf{P}^{(t)}\right)_{k_0,k_1} \left(\mathbf{P}^{(t)}\right)_{k_0,k_2} \\
&= \sum_{k_0 \in \mathcal{S}} \pi_{k_0} \left(\mathbf{P}^{t}\right)_{k_0,k_1} \left(\mathbf{P}^{t}\right)_{k_0,k_2}
\end{aligned}
$$

The likelihood: an example

Note

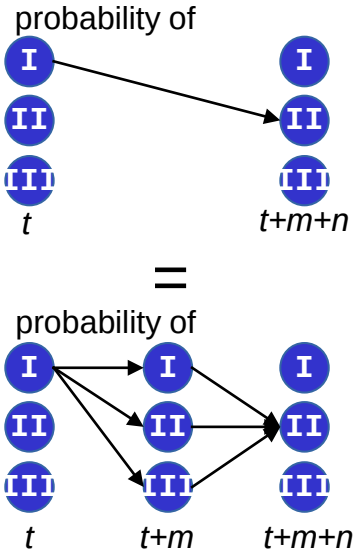
The life time of a generation may differ between the two present day organisms. In particular, if an evolutionary long time has passed since the common ancestor.



The solution is to use the actual time passed since the common ancestor. Modeling this requires continuous time Markov chains. Not treated here.

Many other assumptions need not hold: see later.

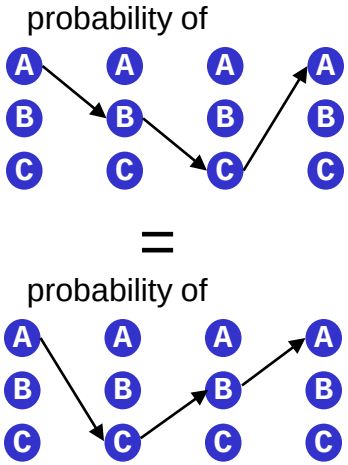
The likelihood: an example



To write down the likelihood, recall

- The Chapman-Kolmogorov equations:

$$p_{k_1 k_2}^{(t_1+t_2)} = \sum_{k_0 \in \mathcal{S}} p_{k_1 k_0}^{(t_1)} \, p_{k_0 k_2}^{(t_2)}$$



- Reversibility of Jukes-Cantor model:

$$\pi_{k_1} \, p_{k_1 k_2} = \pi_{k_2} \, p_{k_2 k_1}$$

- Symmetry of JC transition matrix **P**.

- Combining the last two yields:

$$\pi_{k_1} \, p_{k_1 k_2}^{(t)} = \pi_{k_2} \, p_{k_2 k_1}^{(t)}$$

The likelihood: an example

$$
\begin{aligned}
L(\mathbf{X}) &= \prod_{j=1}^{25} P((X_{1j}, X_{2j})) \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[P((X_{1j}, X_{2j}) = (E_{k_1}, E_{k_2}))\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}
\end{aligned}
$$

The likelihood: an example

$$
\begin{aligned}
L(\mathbf{X}) &= \prod_{j=1}^{25} P((X_{1j}, X_{2j})) \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[P((X_{1j}, X_{2j}) = (E_{k_1}, E_{k_2}))\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}} \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[\sum_{k_0 \in \mathcal{S}} \pi_{k_0} \left(\mathbf{P}^t\right)_{k_0,k_1} \left(\mathbf{P}^t\right)_{k_0,k_2}\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}
\end{aligned}
$$

1) substitute previously derived expression for probability of individual observation

2) substitution rates are the same for all sites

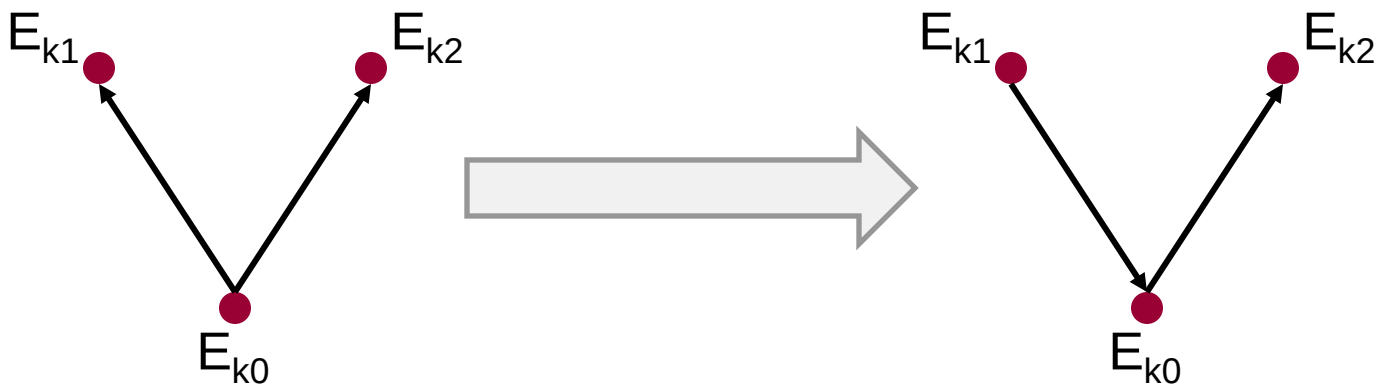
The likelihood: an example

$$
\begin{aligned}
L(\mathbf{X}) &= \prod_{j=1}^{25} P((X_{1j}, X_{2j})) \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[P((X_{1j}, X_{2j}) = (E_{k_1}, E_{k_2}))\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}} \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[\sum_{k_0 \in \mathcal{S}} \pi_{k_0} \left(\mathbf{P}^t\right)_{k_0,k_1} \left(\mathbf{P}^t\right)_{k_0,k_2}\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}} \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[\sum_{k_0 \in \mathcal{S}} \pi_{k_1} \left(\mathbf{P}^t\right)_{k_1,k_0} \left(\mathbf{P}^t\right)_{k_0,k_2}\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}
\end{aligned}
$$

use the time reversibility of the JC model

The likelihood: an example

By using the time reversibility of the JC model, we have reversed one arrow of the phylogenetic tree:



In the formulea:

$$= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \Big[\sum_{k_0 \in \mathcal{S}} \pi_{k_0} \left(\mathbf{P}^t\right)_{\boxed{k_0,k_1}} \left(\mathbf{P}^t\right)_{k_0,k_2} \Big]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}$$

$$= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \Big[\sum_{k_0 \in \mathcal{S}} \pi_{k_1} \left(\mathbf{P}^t\right)_{\boxed{k_1,k_0}} \left(\mathbf{P}^t\right)_{k_0,k_2} \Big]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}$$

The likelihood: an example

$$
\begin{aligned}
L(\mathbf{X}) &= \prod_{j=1}^{25} P((X_{1j}, X_{2j})) \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[P((X_{1j}, X_{2j}) = (E_{k_1}, E_{k_2}))\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}} \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[\sum_{k_0 \in \mathcal{S}} \pi_{k_0} \left(\mathbf{P}^t\right)_{k_0,k_1} \left(\mathbf{P}^t\right)_{k_0,k_2}\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}} \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[\sum_{k_0 \in \mathcal{S}} \pi_{k_1} \left(\mathbf{P}^t\right)_{k_1,k_0} \left(\mathbf{P}^t\right)_{k_0,k_2}\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}} \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[\pi_{k_1} \sum_{k_0 \in \mathcal{S}} \left(\mathbf{P}^t\right)_{k_1,k_0} \left(\mathbf{P}^t\right)_{k_0,k_2}\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}
\end{aligned}
$$

bringing π_{k1} outside the sum

The likelihood: an example

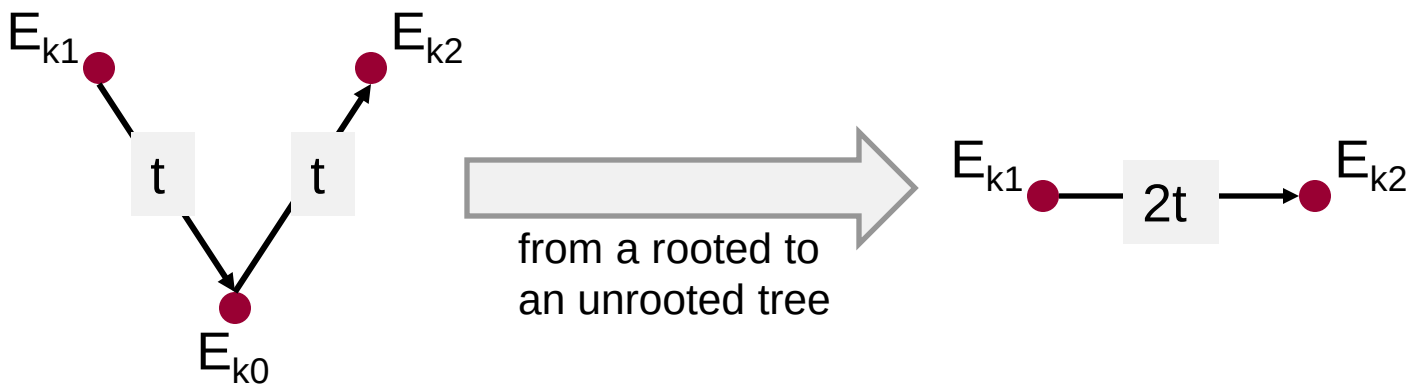
$$
\begin{aligned}
L(\mathbf{X}) &= \prod_{j=1}^{25} P((X_{1j}, X_{2j})) \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[P((X_{1j}, X_{2j}) = (E_{k_1}, E_{k_2}))\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}
\end{aligned}
$$

use the Chapman-Kolmogorov equations

$$
\begin{aligned}
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[\pi_{k_1} \sum_{k_0 \in \mathcal{S}} \left(\mathbf{P}^t\right)_{k_1,k_0} \left(\mathbf{P}^t\right)_{k_0,k_2}\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}} \\
&= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[\pi_{k_1} \left(\mathbf{P}^{2t}\right)_{k_1,k_2}\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}
\end{aligned}
$$

The likelihood: an example

By using the Chapman-Kolmogorov equations, we removed the common ancestor from the phylogenetic tree:



In the formulea:

$$= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \boxed{\left[\pi_{k_1} \sum_{k_0 \in \mathcal{S}} \left(\mathbf{P}^t\right)_{k_1,k_0} \left(\mathbf{P}^t\right)_{k_0,k_2}\right]}^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}$$

$$= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \boxed{\left[\pi_{k_1} \left(\mathbf{P}^{2t}\right)_{k_1,k_2}\right]}^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}}$$

The likelihood: an example

The likelihood can be further simplified, when exploiting the spectral decomposition the JC *t*-step transition matrix:

$$\mathbf{P}^t = \frac{1}{4}\begin{pmatrix} 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \end{pmatrix} + \frac{1}{4}(1-4\alpha)^t \begin{pmatrix} 3 & -1 & -1 & -1 \\ -1 & 3 & -1 & -1 \\ -1 & -1 & 3 & -1 \\ -1 & -1 & -1 & 3 \end{pmatrix}$$

The likelihood: an example

Finally, we have:

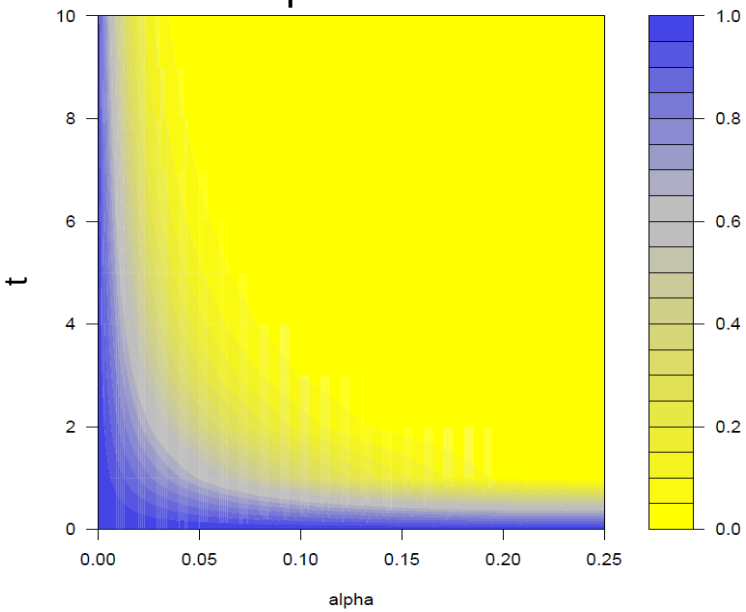
$$
\begin{aligned}
L(\mathbf{X}) &= \prod_{j=1}^{25} \prod_{k_1,k_2 \in \mathcal{S}} \left[\pi_{k_1} \left(\mathbf{P}^{2t}\right)_{k_1,k_2}\right]^{I_{\{(X_{1j},X_{2j})=(E_{k_1},E_{k_2})\}}} \\
&= \prod_{j=1}^{25} \frac{1}{4} \left[\frac{1}{4} + \frac{3}{4}(1-4\,\alpha)^{2t}\right]^{I_{\{X_{1j}=X_{2j}\}}} \\
&\qquad \times \left[\frac{1}{4} - \frac{1}{4}(1-4\,\alpha)^{2t}\right]^{I_{\{X_{1j}\neq X_{2j}\}}}
\end{aligned}
$$

where we have used that the stationary distribution of the JC model is uniform.

The likelihood: an example

From the likelihood, it is clear either α or *t* is identifiable not both. Many combinations (α, *t*) yield the same likelihood.

Contour plot of u vs *t* and α.



In the absence of external evidence of α, we replace:

$$u = (1 - 4\alpha)^{2t}$$

and obtain:

$$L(\mathbf{X}) = \prod_{j=1}^{25} \frac{1}{4}\left[\frac{1}{4} + \frac{3}{4}u\right]^{I\{x_{1j}=x_{2j}\}} \left[\frac{1}{4} - \frac{1}{4}u\right]^{I\{x_{1j}\neq x_{2j}\}}$$

The likelihood: an example

To estimate *u*, maximize the log-likehood:

$$\log(\frac{1}{4}+\frac{3}{4}u)\sum_{j=1}^{25} I_{\{X_{1j}=X_{2j}\}} + \log(\frac{1}{4}-\frac{1}{4}u)\sum_{j=1}^{25} I_{\{X_{1j}\neq X_{2j}\}}$$

This yields:

$$\hat{u} = \frac{3\sum_{j=1}^{25} I_{\{X_{1j}=X_{2j}\}} - \sum_{j=1}^{25} I_{\{X_{1j}\neq X_{2j}\}}}{3\sum_{j=1}^{25} I_{\{X_{1j}=X_{2j}\}} + 3\sum_{j=1}^{25} I_{\{X_{1j}\neq X_{2j}\}}}$$

Check that this is indeed a maximum.

The likelihood: an example

For our two-species example, with sequences:

```
species 1    : AATTGCGTAGCTAGATCGCTCGCTA
species 2    : AATTGCGTAGCTAGGTCGCTCGCTA
```

the ML estimate equals:

$$\hat{u} = \frac{3 \times 24 - 1}{3 \times 24 + 3 \times 1} = \frac{71}{75}$$

Are we there?

No. Only have estimate of *u*. How does this estimate translate to the evolution of the two species?

The likelihood: an example

Recall:

$$u = (1 - 4\alpha)^{2t}$$

Or:

$$\log(u) = 2\,t\log(1 - 4\alpha)$$

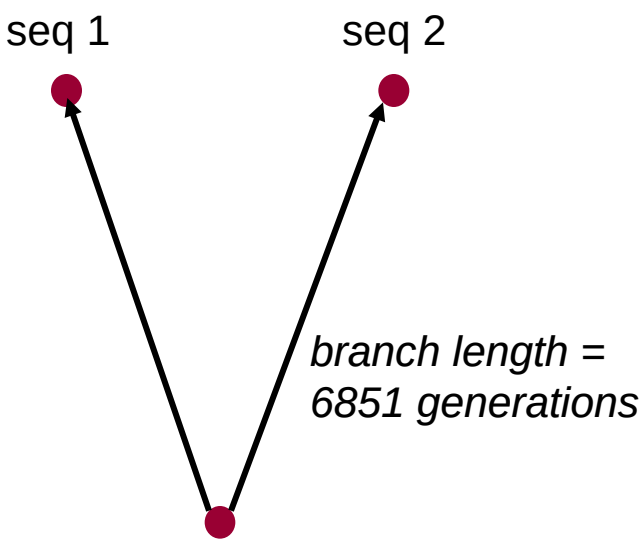
Assuming the substitution rate (α) is 1 in a million, we get:

$$\hat{t} = \frac{\log(\hat{u})}{2\log(1 - 4\alpha)} = \frac{\log(71/75)}{2\log(1 - 4\alpha)} \approx 6851$$

This estimate suggests that the two species shared a common ancestor 6851 generations ago.

The likelihood: an example

We obtain the following inferred phylogenetic tree:



But this inferred tree depends the assumption on α .

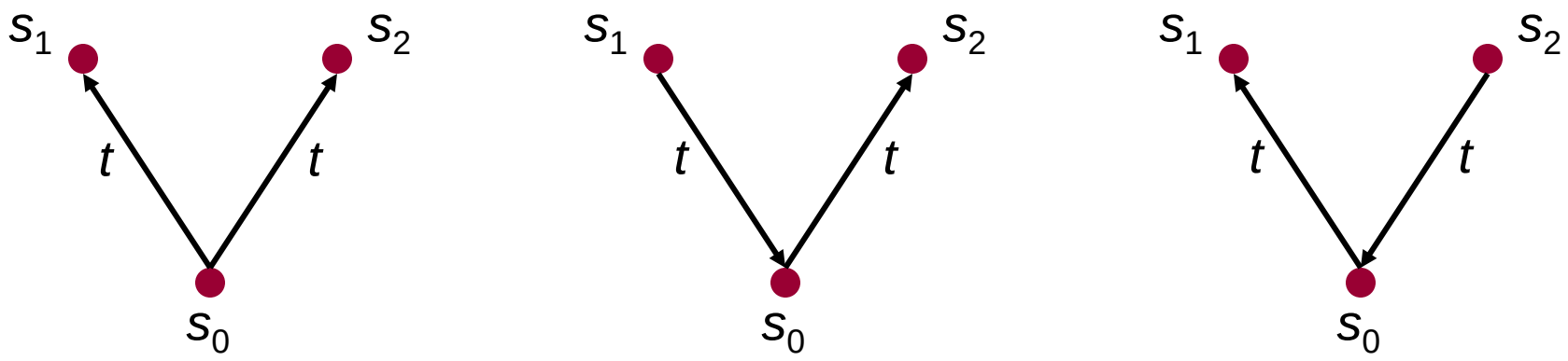
Question

How would the inferred tree look like when assuming a substitution rate (α) of 1 in a 1000?

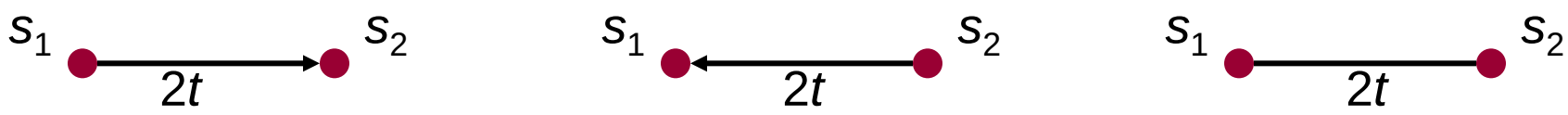
The pulley principle

The pulley principle

Due to reversibility, likelihood of trees below are equivalent:



But even to:

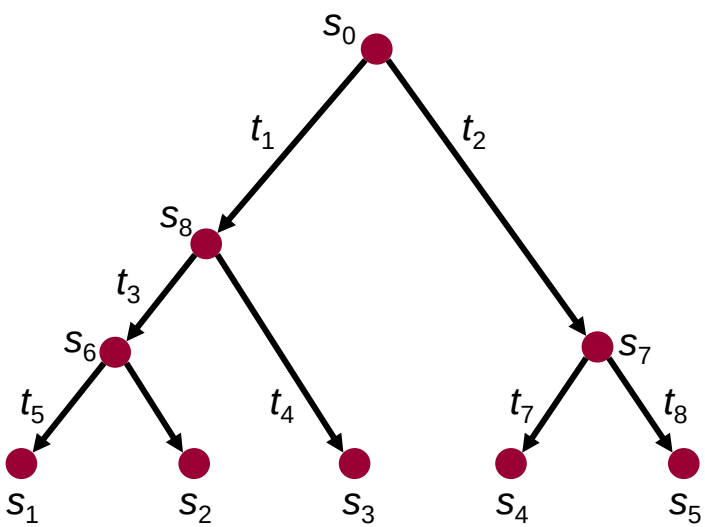


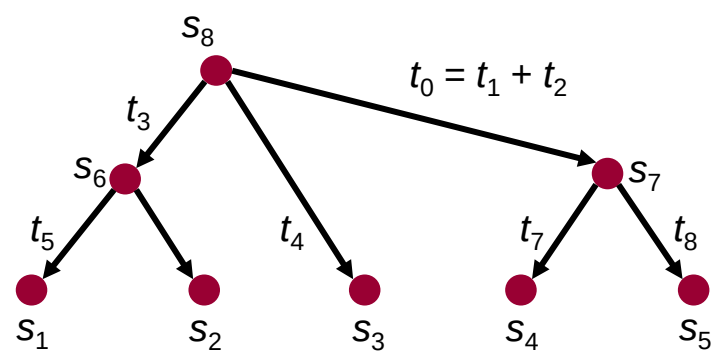
Pulley principle

The root node may be moved to any of the nodes without changing the likelihood.

The pulley principle

Due to the pulley principle, the likelihood of the following trees is equivalent:



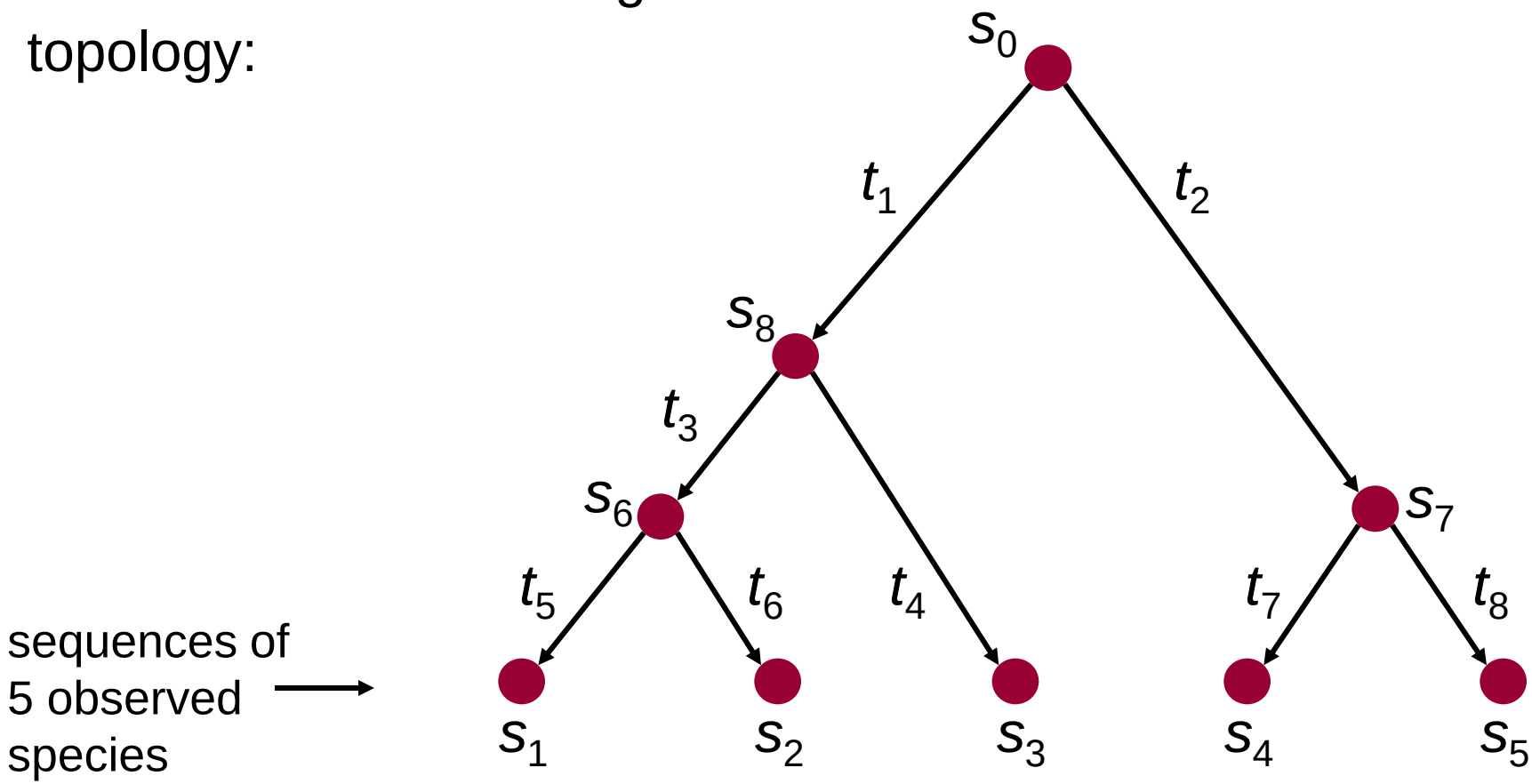


The likelihood: another example

The likelihood: another example

Consider the case where:

- DNA sequences from (say) 5 species are available.
- the sequences consist of (say) 25 bases.
- we assume the following topology:



The likelihood: another example

Step 1

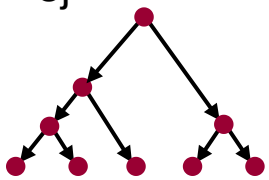
Assume the 25 sites evolve independently. The probability of evolution from (say) node / species s_7 to s_5 then becomes:

$$P(\mathbf{X}_7 \xrightarrow{t_8} \mathbf{X}_5) \quad = \quad \prod_{j=1}^{25} P(X_{7j} \xrightarrow{t_8} X_{5j})$$

where

$$P(X_{7j} \xrightarrow{t_8} X_{5j})$$

denotes the (conditional) probability of X_{7j} evolving to X_{5j} in t_8 generations.



The likelihood: another example

Step 1

Recall: the probability of the nucleotide at site *j* changing from X_{7j} in sequence 7 to X_{5j} in sequence 5 in t_8 generations, denoted by:

$$P(X_{7j} \xrightarrow{t_8} X_{5j})$$

is given by a multiple of the transition matrix of the evolutionary model of choice. Hence,

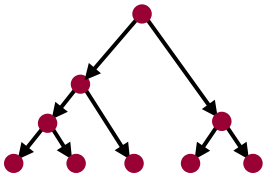
$$P(X_{7j} \xrightarrow{t_8} X_{5j}) \quad = \quad (\mathbf{P}^{t_8})_{X_{7j},X_{5j}}$$

The likelihood: another example

Step 2

If the sequence of all nodes / species (s_0, …, s_8) are known, the likelihood is given by:

$$
\begin{aligned}
L \quad = \quad & P(\mathbf{X}_0) \\
& P(\mathbf{X}_0 \xrightarrow{t_2} \mathbf{X}_7)\, P(\mathbf{X}_0 \xrightarrow{t_1} \mathbf{X}_8) \\
& P(\mathbf{X}_7 \xrightarrow{t_7} \mathbf{X}_4)\, P(\mathbf{X}_7 \xrightarrow{t_8} \mathbf{X}_5) \\
& P(\mathbf{X}_8 \xrightarrow{t_4} \mathbf{X}_3)\, P(\mathbf{X}_8 \xrightarrow{t_3} \mathbf{X}_6) \\
& P(\mathbf{X}_6 \xrightarrow{t_5} \mathbf{X}_1)\, P(\mathbf{X}_6 \xrightarrow{t_6} \mathbf{X}_2)
\end{aligned}
$$

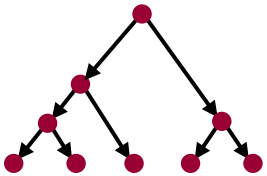


The likelihood: another example

Step 3

Since only the sequences of nodes $n_1, \ldots, n_5$ are observed, the likelihood has to be summed over all possible sequences for the unobserved nodes:

$$
\begin{aligned}
L \quad = \quad \sum_{\mathbf{X}_0} \sum_{\mathbf{X}_6} \sum_{\mathbf{X}_7} \sum_{\mathbf{X}_8} \big[P(\mathbf{X}_0) \\
& P(\mathbf{X}_0 \xrightarrow{t_2} \mathbf{X}_7)\, P(\mathbf{X}_0 \xrightarrow{t_1} \mathbf{X}_8) \\
& P(\mathbf{X}_7 \xrightarrow{t_7} \mathbf{X}_4)\, P(\mathbf{X}_7 \xrightarrow{t_8} \mathbf{X}_5) \\
& P(\mathbf{X}_8 \xrightarrow{t_4} \mathbf{X}_3)\, P(\mathbf{X}_8 \xrightarrow{t_3} \mathbf{X}_6) \\
& P(\mathbf{X}_6 \xrightarrow{t_5} \mathbf{X}_1)\, P(\mathbf{X}_6 \xrightarrow{t_6} \mathbf{X}_2) \big]
\end{aligned}
$$



The likelihood: another example

Step 3 (computational efficiency)

This likelihood can be calculated by exploiting the conditional likelihoods, e.g.:

$$L((X_{4j}, X_{5j}) \,|\, X_{7j}) \;=\; \Big[\sum_{X_{4j}} P(X_{7j} \xrightarrow{t_7} X_{4j})\, L(X_{4j})\Big] \Big[\sum_{X_{5j}} P(X_{7j} \xrightarrow{t_8} X_{5j})\, L(X_{5j})\Big]$$

which yields:

$$\begin{aligned} L \;=\; \sum_{\mathbf{X}_0} P(\mathbf{X}_0) \Bigg[\Big[\sum_{\mathbf{X}_7} P(\mathbf{X}_0 \xrightarrow{t_2} \mathbf{X}_7)\, P(\mathbf{X}_7 \xrightarrow{t_7} \mathbf{X}_4)\, P(\mathbf{X}_7 \xrightarrow{t_8} \mathbf{X}_5) \Big] \\ \times \Big[\sum_{\mathbf{X}_8} P(\mathbf{X}_0 \xrightarrow{t_1} \mathbf{X}_8)\, P(\mathbf{X}_8 \xrightarrow{t_4} \mathbf{X}_3)\, P(\mathbf{X}_8 \xrightarrow{t_3} \mathbf{X}_6) \\ \times \Big[\sum_{\mathbf{X}_6} P(\mathbf{X}_6 \xrightarrow{t_5} \mathbf{X}_1)\, P(\mathbf{X}_6 \xrightarrow{t_6} \mathbf{X}_2) \Big] \Big] \Bigg] \end{aligned}$$

The likelihood: another example

Step 3 (computational efficiency)

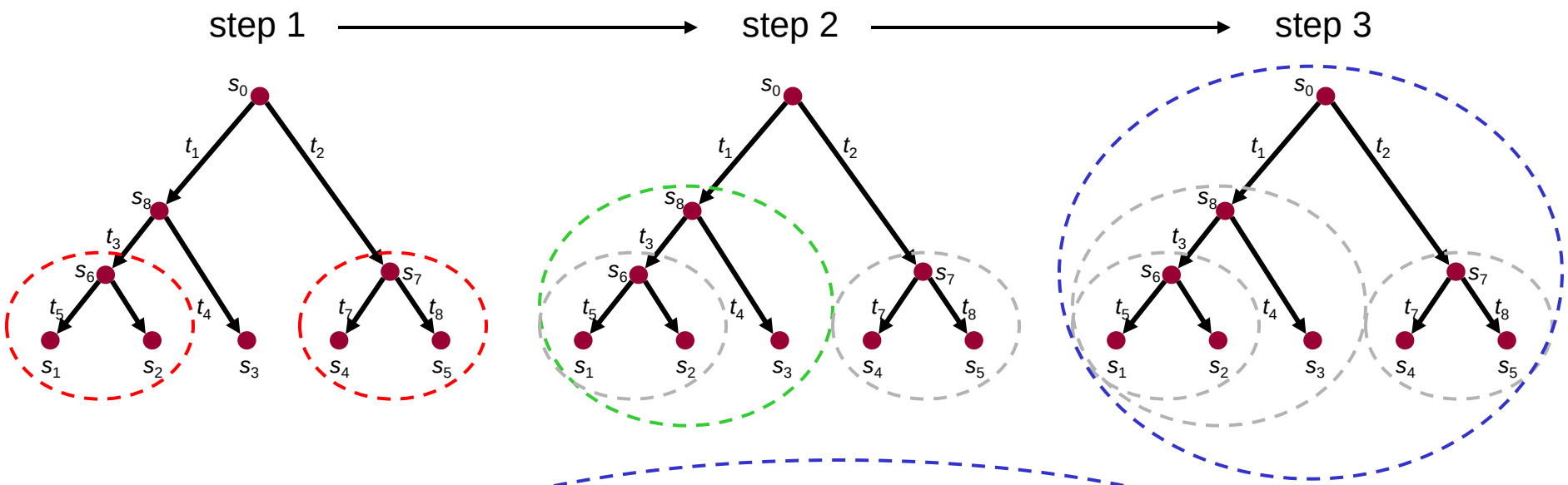
Without the exploitation of the conditional likelihood, calculation of the likelihood required the evaluation of 4^4=256 combinations (4 hidden nodes, 4 nucleotides).

In the reformulation on the previous slide, the likelihood is evaluated in for 4 * (4+4+4) = 48 steps.

This is (approximately) a factor 5!!!

The likelihood: another example

Pruning: calculate the likelihood by proceeding from the leaves towards the root.



$$
\begin{aligned}
L \;=\; & \sum_{\mathbf{X}_0} P(\mathbf{X}_0) \Bigg[\Bigg[\sum_{\mathbf{X}_7} P(\mathbf{X}_0 \xrightarrow{t_2} \mathbf{X}_7)\, P(\mathbf{X}_7 \xrightarrow{t_7} \mathbf{X}_4)\, P(\mathbf{X}_7 \xrightarrow{t_8} \mathbf{X}_5) \Bigg] \\
& \times \Bigg[\sum_{\mathbf{X}_8} P(\mathbf{X}_0 \xrightarrow{t_1} \mathbf{X}_8)\, P(\mathbf{X}_8 \xrightarrow{t_4} \mathbf{X}_3)\, P(\mathbf{X}_8 \xrightarrow{t_3} \mathbf{X}_6) \\
& \times \Bigg[\sum_{\mathbf{X}_6} P(\mathbf{X}_6 \xrightarrow{t_5} \mathbf{X}_1)\, P(\mathbf{X}_6 \xrightarrow{t_6} \mathbf{X}_2) \Bigg] \Bigg] \Bigg]
\end{aligned}
$$

The likelihood: another example

Step 4

As also the topology is in fact unobserved, we need to sum the likelihood from the previous step over all possible topologies.

The pulley principle comes to the rescue, partially.

- With 5 leave nodes, the number of possible rooted binary trees equals 105.
- The pulley principle tells us only to consider the unrooted binary trees, a total of 15.

Likelihood maximization

Likelihood maximization

To maximize the log-likelihood:

- *Step 1*: Select a tree topology.
- *Step 2*: Choose initial values for each edge.
- *Step 3*: Maximize edges individually, given the other edges.
- *Step 4*: Iterate step 3, until values no longer change.
- *Step 5*: Do this for all possible topologies.

The particular form of this algorithm described below may converge to local maxima!

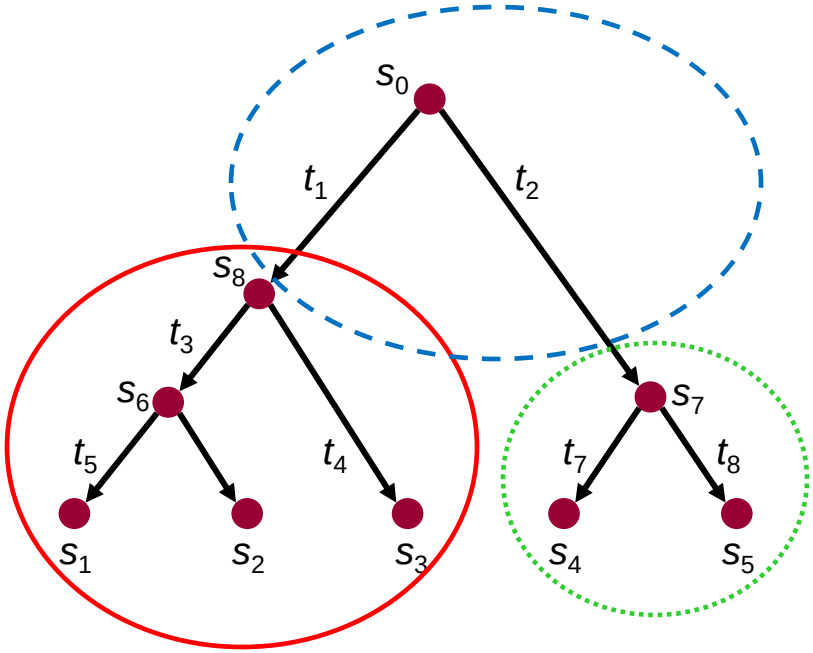
With respect to step 3

How to maximize the log-likelihood with respect to an edge?

The likelihood

Denote the conditional likelihood of subtree rooted at node *i* with nucleotide X_{ij} by $L_{i,X_{ij}}$.

The likelihood of site *j* for our tree, now assumed to be rooted at s_8, is given by:



$$L_j = \sum_{X_{8j}} \sum_{X_{7j}} P(X_{8j}) L_{8,X_{8j}} L_{7,X_{7j}} P(X_{8j} \xrightarrow{t_1+t_2} X_{7j})$$

Likelihood maximization

Using:

$$
\begin{aligned}
P(X_{8j} \xrightarrow{t} X_{7j}) &= \frac{1}{4}[1-(1-4\,u)^t] + \delta_{X_{8j},X_{7j}}\,(1-4\,u)^t \\
&= \frac{1}{4}(1-p) + \delta_{X_{8j},X_{7j}}\,p
\end{aligned}
$$

reformulate this to:

$$
\begin{aligned}
L_j &= p \sum_{X_{8j}} \sum_{X_{7j}} \delta_{X_{8j},X_{7j}}\, P(X_{8j})\, L_{8,X_{8j}}\, L_{7,X_{7j}} \\
&\quad + (1-p) \sum_{X_{8j}} \sum_{X_{7j}} \frac{1}{4}\, P(X_{8j})\, L_{8,X_{8j}}\, L_{7,X_{7j}} \\
&= A_j\, p + B_j\, (1-p)
\end{aligned}
$$

Likelihood maximization

This holds for all sites, thus:

$$L = \prod_{j=1}^{25} [A_j\, p + B_j\, (1-p)]$$

The log-likelihood and its derivative are given by:

$$\log(L) = \sum_{j=1}^{25} \log[A_j\, p + B_j\, (1-p)]$$

$$\frac{\partial \log(L)}{\partial p} = \sum_{j=1}^{25} \frac{A_j - B_j}{A_j\, p + B_j\, (1-p)} = 0$$

Likelihood maximization

The p maximizing the log-likelihood is found iteratively.

- Choose a step size *h* > 0.
- Let $p^{(k)}$ be the value of *p* from the *k*-th iteration.
- Then, define:

$$p^{(k+1)} = p^{(k)} + \frac{h}{m} \sum_{j=1}^{25} \left[\frac{A_j \, p^{(k)}}{A_j \, p^{(k)} + B_j \, (1 - p^{(k)})} - p^{(k)} \right]$$

This choice of $p^{(k+1)}$ implies the majorization:

$$\sum_{j=1}^{25} \log[A_j \, p^{(k+1)} + B_j \, (1 - p^{(k+1)})] \geq \sum_{j=1}^{25} \log[A_j \, p^{(k)} + B_j \, (1 - p^{(k)})]$$

Likelihood maximization

The majorization can be seen from:

$$
\begin{aligned}
p^{(k+1)} - p^{(k)} &= h\, p^{(k)} \left(\frac{1}{m} \sum_{j=1}^{25} \frac{A_j}{A_j\, p^{(k)} + B_j\, (1 - p^{(k)})} - 1 \right) \\
&= h\, p^{(k)} \left(1 - p^{(k)}\right) \frac{1}{m} \sum_{j=1}^{25} \frac{A_j - B_j}{A_j\, p + B_j\, (1 - p)} \\
&= h\, p^{(k)} \left(1 - p^{(k)}\right) \frac{1}{m} \left. \frac{\partial \log(L)}{\partial p} \right|_{p = p^{(k)}}
\end{aligned}
$$

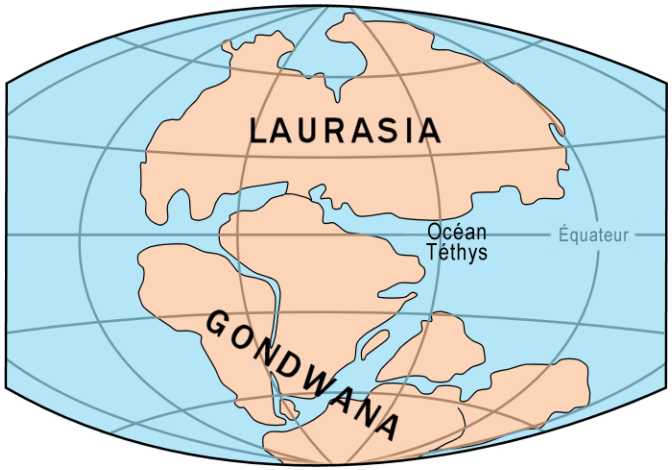
which has the same sign at the derivative of the log-likelihood, evaluated in the current estimate of *p*!

Example

Laurasiatherians

Example: Laurasiatherians

Laurasiatheria is a group of mammals originating from the former continent Laurasia.



The phylogenetic relationships between the Laurasiatherians are still uncertain.

Available:
- RNA sequence data of 47 Laurasiatherians.
- Sequence is 3179 bases long.

Reconstruct their phylogenetic tree.

Picture: Wikipedia

Example: Laurasiatherians

In R:

```
> # activate library
> library(phangorn)

> # load data
> data(Laurasiatherian)
```

```
Platypus  ttaaaggtttggtcctagccttactgttagatttgattagatttatacatgcagtatcc...
Wallaroo  ccaaaggtttggtcctggccttactgttaattgtagttagacctacacatgcagtttcc...
Possum    ccaaaggtttggtcctagccttactgttaattataattaaacctacacatgcagtttcc...
Bandicoot ccaaaggtttggtcctagcctttctattaattttaattaaacctacacatgcagtctcc...
Opposum   ccataggtttggtcctagccttattattagttctaattagacctacacatgcagtttcc...
Armadillo ccacaggtctggtcctagccttactattaattcataacaaaattacacatgcagtatca...
Elephant  ccaaaggtttggtcccggccttcttattggttactaggaaacttatacatgcagtatcc...
Aardvark  ttaaaggtttggtcctagcctttctattagttgacagtaaatttatacatgcagtatct...
Tenrec    ttaaaggtttggttctagcctttttattagttcttaataaaattatacatgcagtatcc...
Hedghog   aataaggtctggtcccagccttcctattttctattagtagaattacacatgcagtatca...
...       ...
```

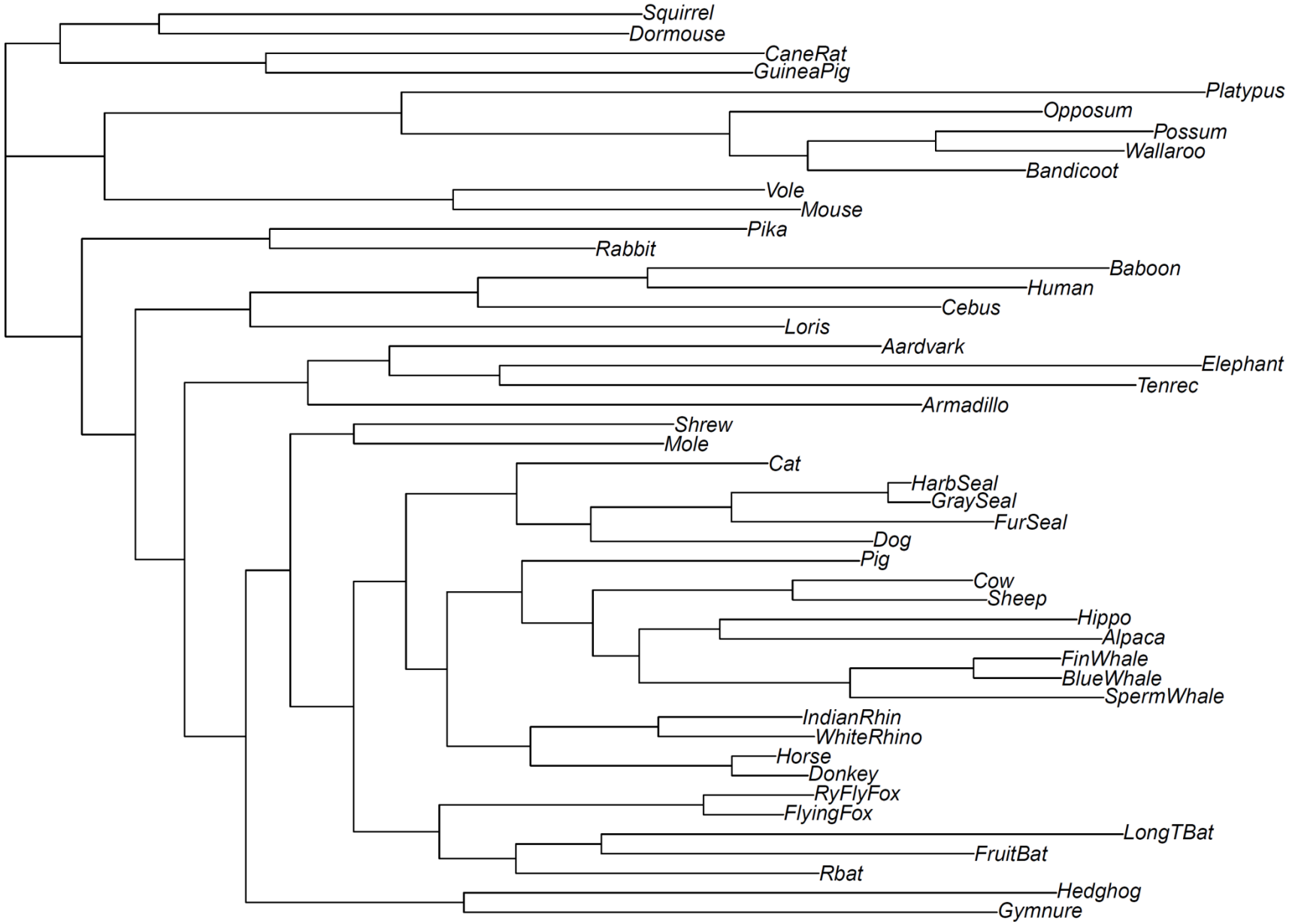
Example: Laurasiatherians

Now fit the model:

```
> # construct a starting tree
> distMat <- dist.logDet(Laurasiatherian)
> tree <- NJ(distMat)

> # fit Jukes-Cantor model
> fitJC <- pml(tree, Laurasiatherian, model="JC")
> fitJC <- optim.pml(fitJC, optNni=TRUE,
                     optEdge=TRUE, model="JC")
> plot(fitJC$tree)
```

Note: this fits a model with continuous time, instead of discrete time as treated in the lecture.

Example: Laurasiatherians



Example: Laurasiatherians

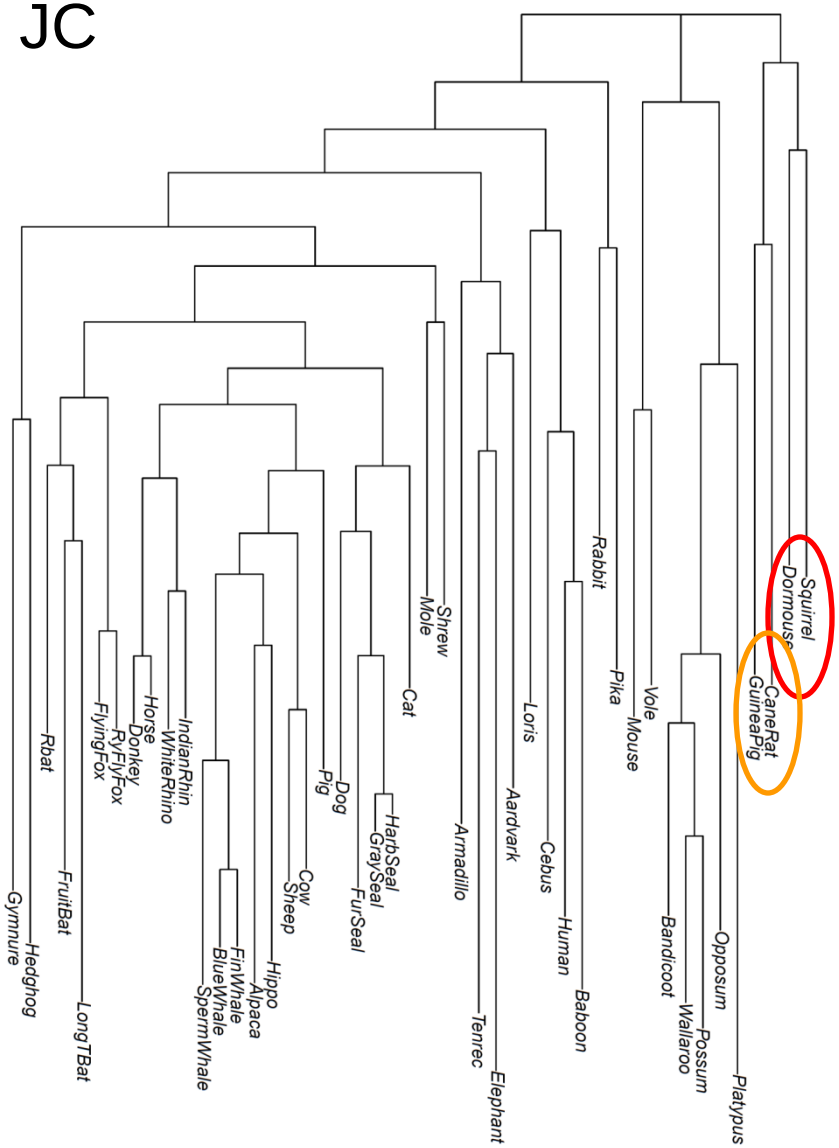
The Jukes-Cantor model is just one evolutionary model. Many more exist.

Fit different model:

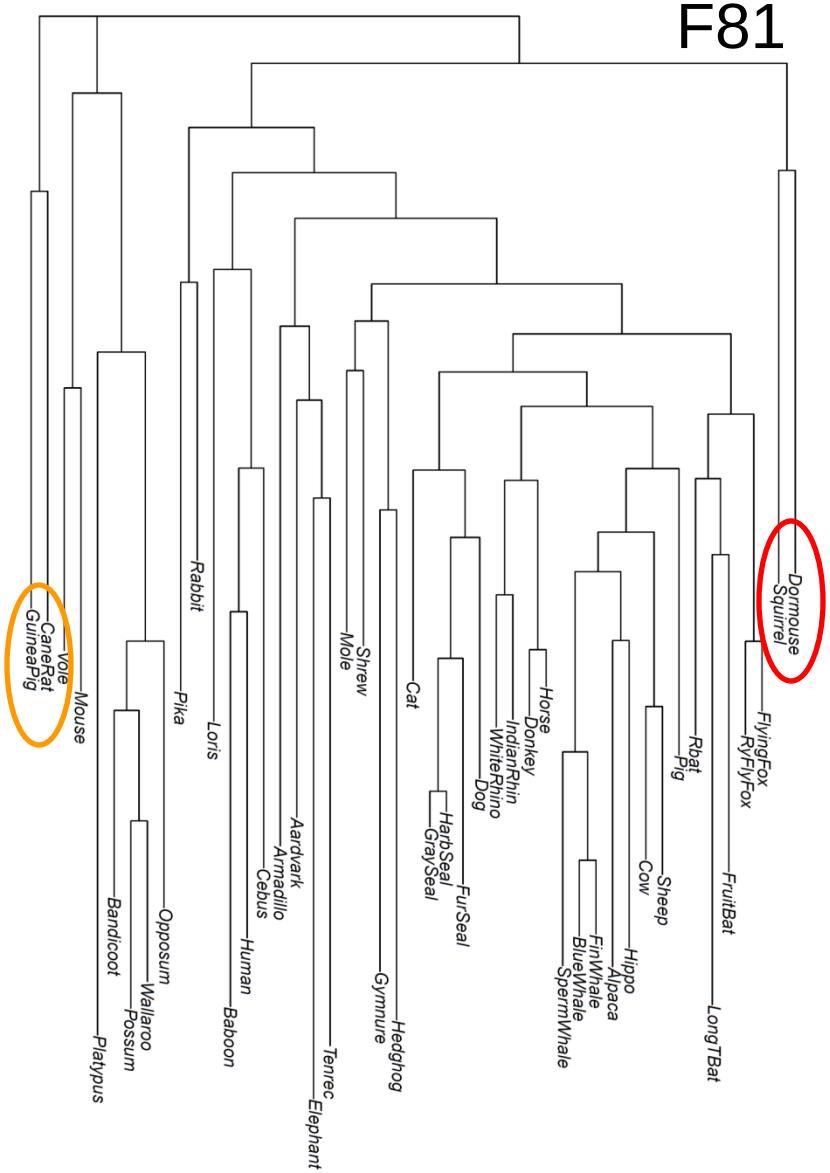
```
> # construct a starting tree
> distMat <- dist.logDet(Laurasiatherian)
> tree <- NJ(distMat)

> # fit Felsenstein model
> fitF81 <- pml(tree, Laurasiatherian, model="F81")
> fitF81 <- optim.pml(fitF81, optNni=TRUE,
                      optBf=TRUE, optEdge=TRUE,
                      model="F81")
> plot(fitF81$tree)
```

Example: Laurasiatherians

JC



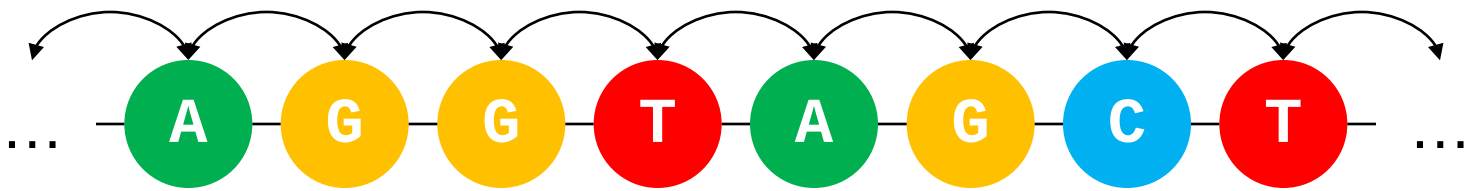
F81



Assumptions

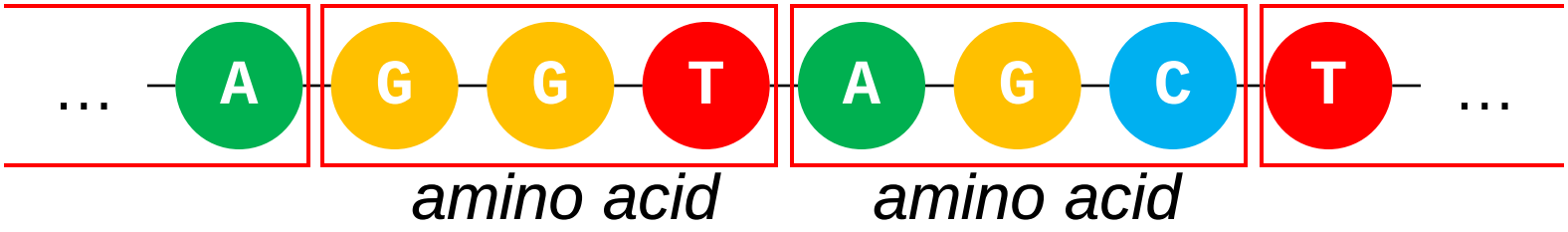
Assumptions

Positions do not evolve independently (*covarion*):



But also …

… three contiguous bases code for one amino acid:

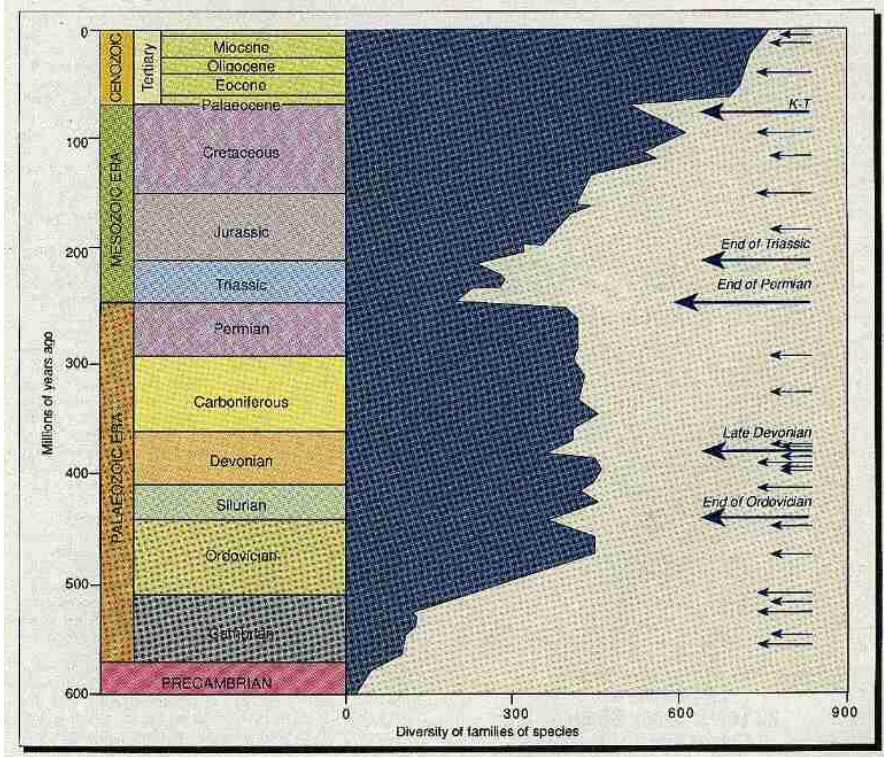


Assumptions

Heterotachy refers to within-site rate variation over time. Under heterotachy, evolutionary rates at different sites may vary in different ways over subtrees.

Hence, under heterotachy, the time-homogeneity assumption may be invalid. That is, the rate of nucleotide substitution (the transition probability) may not be constant over time.

The molecular hypothesis should be applied with care.



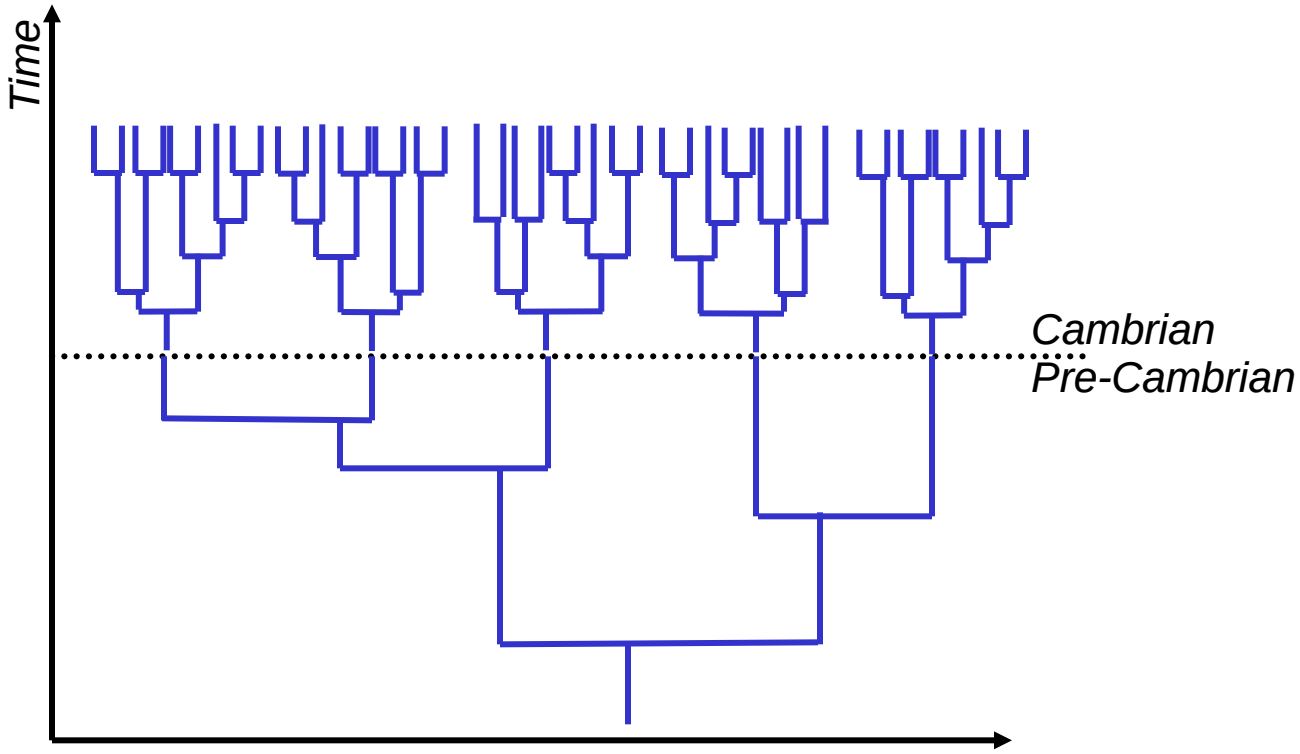
The principal extinction events (shown by arrows), indicating their effects on the diversity of species in the oceans over geological time. The "big five" mass extinctions are labelled, including the K-T extinction, which wiped out the dinosaurs and many forms of marine life at the end of the Cretaceous. The lengths of the arrows are roughly proportional to the intensity of extinction

Raup (1991), New Scientist

Assumptions

The *Cambrian explosion* refers to the period around 530 My ago in which the evolutionary pace seems accelarated.

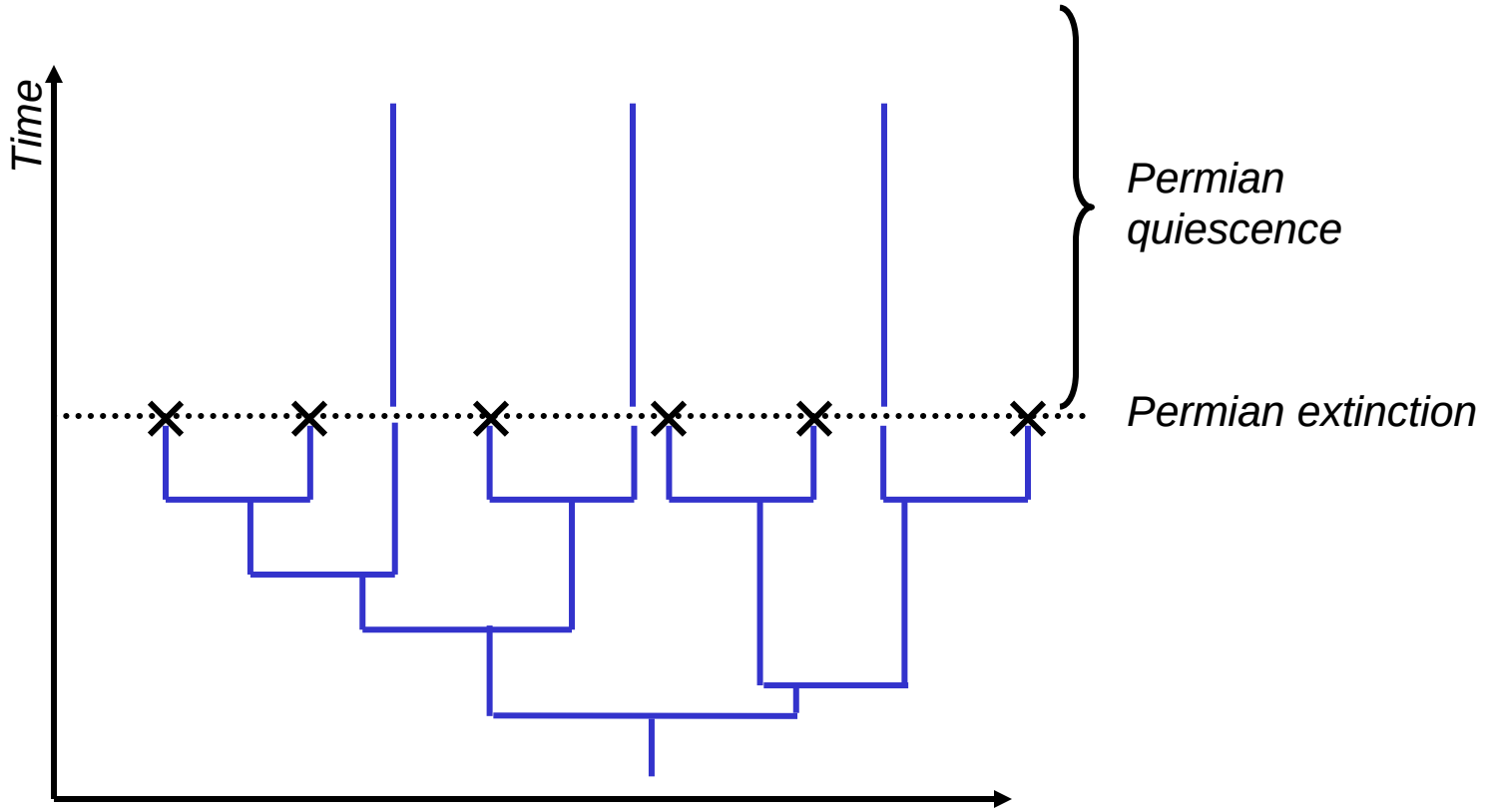
→ substitution-rate varies over time.



Assumptions

The *Permian quiescence* refers to the period after the Permian extinction (250 My ago), where the evolutionary pace seemed to have slowed down.

→ substitution-rate varies over time.



Assumptions

Implicitly, it has been assumed that organisms evolve independently.

However, often there is *co-evolution*:

References & further reading

References and further reading

Cavalli-Sforza, L., Edwards, A.W.F. (1967), “Phylogenetic analysis. Models and estimation procedures”, *Evolution*, **21**, 550-570.

Clote, P., Backoefen, R. (2000), *Computational Molecular Biology: An Introduction*, John Wiley, New York.

Ewens, W.J, Grant, G. (2006), *Statistical Methods for Bioinformatics*, Springer, New York.

Felsenstein, J. (1981), “Evolutionary trees from DNA sequences: a maximum likelihood approach”, *Journal of Molecular Evolution*, **17**, 368-376.

Felsenstein, J. (2004), *Inferring Phylogenies*, Sinauer Associates, Sunderland, Massachusetts.

Graur, D., Li, W.-H. (2000), *Fundamentals of Molecular Evolution*, 2nd Ed., Sinauer: Sunderland, Massachusetts.

Raup, D. (1991), ``Extinction: bad luck or bad genes?”, New Scientist, 1786.

Schliep, K.P. (2010), “phangorn: Phylogenetic analysis in R”, *Bioinformatics*, …

Wilson, A.C., Carlson, S.S., White, T.J. (1977), “Biochemical Evolution”, *Annual Review of Biochemistry*, **46**, 573-639.