Phylogenetics at the QIB 002

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Motivation for phylogenetics

Ancestral State Reconstruction

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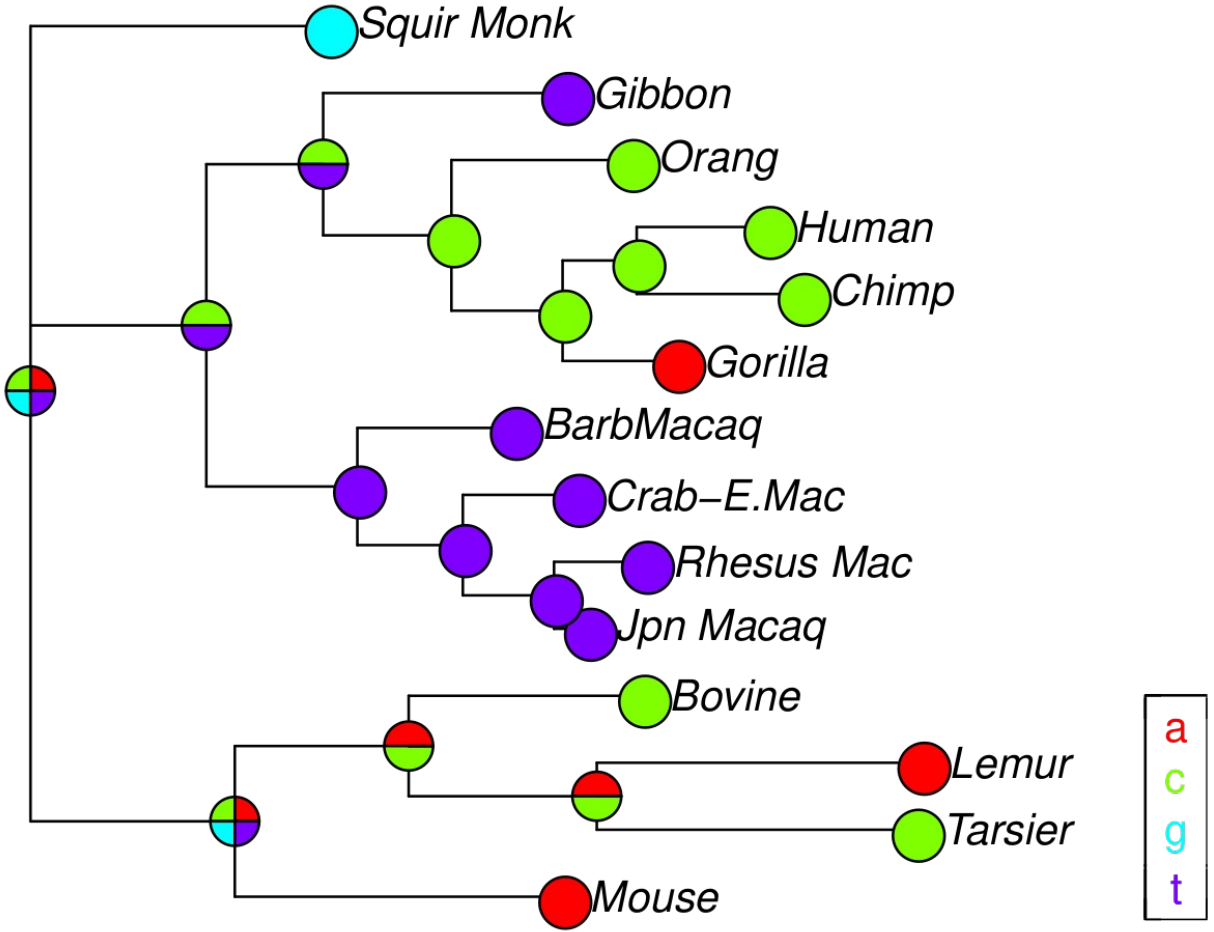
1. I know the “state” for these living organisms

2. I know the evolutionary tree

Can I estimate the states in the past?



tree from phangorn’s vignette “Ancestral sequence reconstruction with phangorn”

Alignment

Multiple sequence alignments

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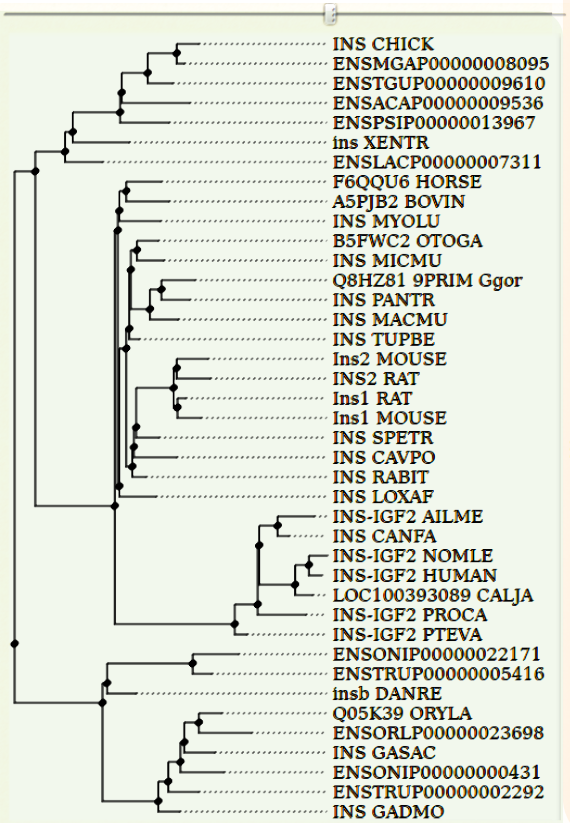
Alignment: homology (common ancestry) at every site



Phylogeny: evolution of the homologous sites along a tree

https://selectome.org/family/ENSGT00390000015440.1.Euteleostomi

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Composed of substitutions and indels (insertions/deletions)

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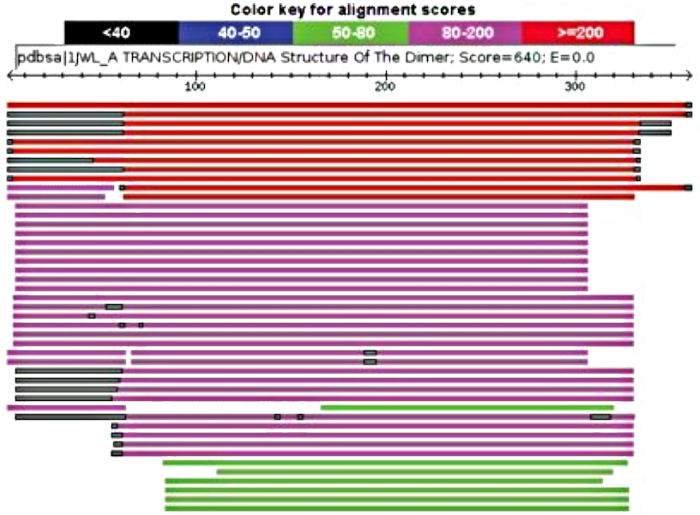
Alignment = multiple sequence alignment (MSA)

→ similar, but not the same as read mapping

Alignment = multiple sequence alignment (MSA)

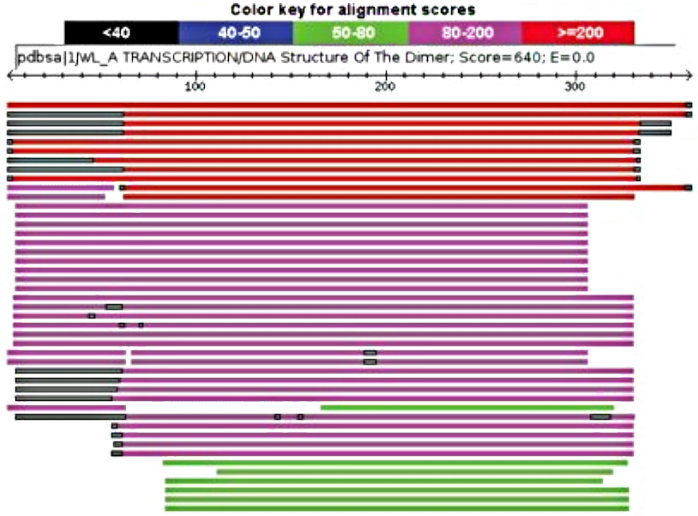


Sequences producing significant alignments:	Score (bits)	E value	Source DB	NCBI Entrez	Cath Prot/Chain
pdbsa|1LBG_A TRANSCRIPTION/DNA Lactose Operon Repressor Bo...	688	0	SDB	NCBI	CATH Prot
pdbsa|1LBH_A TRANSCRIPTION REGULATION Intact Lactose Opero...	688	0	SDB	NCBI	CATH Prot
pdbsa|1JYF_A TRANSCRIPTION Structure Of The Dimeric Lac Re...	669	0	SDB	NCBI	CATH Prot
pdbsa|1JYE_A TRANSCRIPTION Structure Of A Dimeric Lac Repr...	666	0	SDB	NCBI	CATH Prot
pdbsa|1JWL_A TRANSCRIPTION/DNA Structure Of The Dimeric La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1EFA_A TRANSCRIPTION/DNA Crystal Structure Of The La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1EFA_C TRANSCRIPTION/DNA Crystal Structure Of The La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1JWL_C TRANSCRIPTION/DNA Structure Of The Dimeric La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1EFA_B TRANSCRIPTION/DNA Crystal Structure Of The La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1TLF_A TRANSCRIPTION REGULATION Unprecedented Quater...	575	1e-164	SDB	NCBI	CATH Prot

→ similar, but not the same as database search (e.g. BLAST)

https://doi.org/10.1093/nar/gkn237

Alignment = multiple sequence alignment (MSA)



Sequences producing significant alignments:	Score (bits)	E value	Source DB	NCBI Entrez	Cath Prot/Chain
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pdbsa|1JYF_A TRANSCRIPTION Structure Of The Dimeric Lac Re...	669	0	SDB	NCBI	CATH Prot
pdbsa|1JYE_A TRANSCRIPTION Structure Of A Dimeric Lac Repr...	666	0	SDB	NCBI	CATH Prot
pdbsa|1JWL_A TRANSCRIPTION/DNA Structure Of The Dimeric La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1EFA_A TRANSCRIPTION/DNA Crystal Structure Of The La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1EFA_C TRANSCRIPTION/DNA Crystal Structure Of The La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1JWL_C TRANSCRIPTION/DNA Structure Of The Dimeric La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1EFA_B TRANSCRIPTION/DNA Crystal Structure Of The La...	640	0	SDB	NCBI	CATH Prot
pdbsa|1TLF_A TRANSCRIPTION REGULATION Unprecedented Quater...	575	1e-164	SDB	NCBI	CATH Prot

DB search → Smith-Waterman (local)

	(empty string)	A	AA	AAC	AACC	AACCT	AACCTG
(empty string)	0	1	2	3	4	5	6
C	1	1	2	2	3	4	5
CC	2	2	2	2	2	3	4
CCC	3	3	3	2	2	3	4
CCCG	4	4	4	3	3	3	3

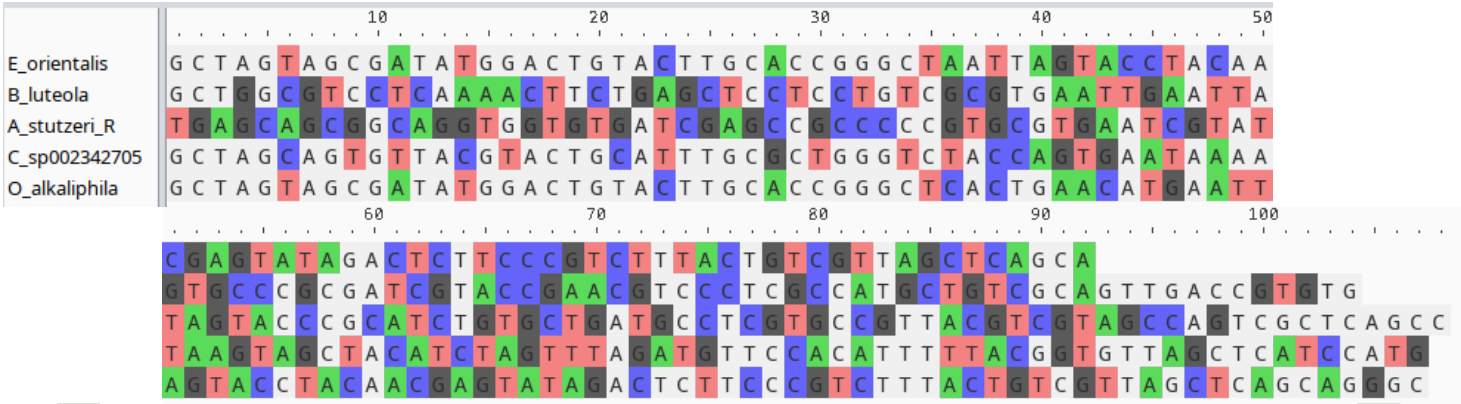
AACCTG
--CCCG

MSA → Needleman-Wunsch (global)

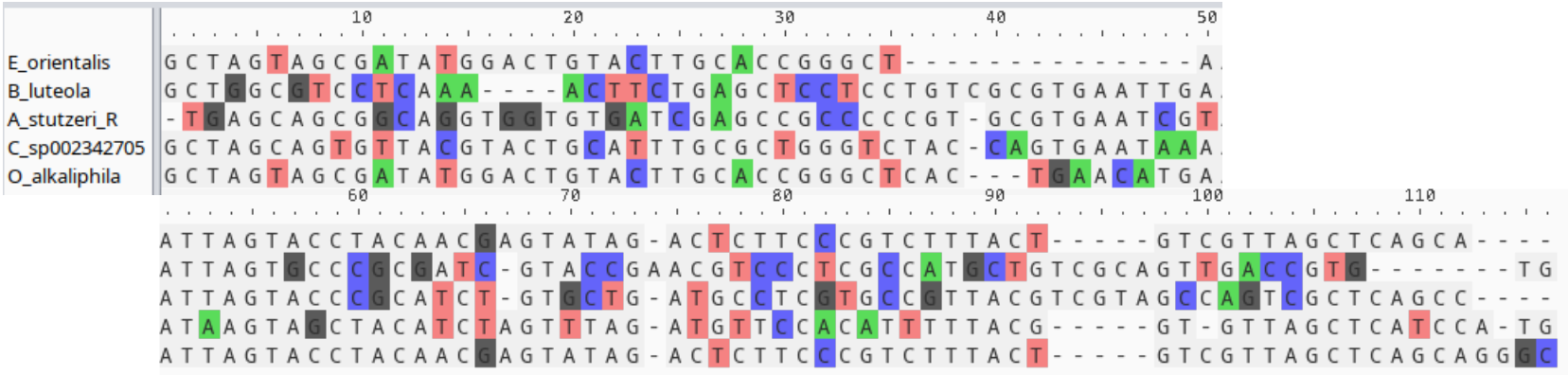
https://doi.org/10.1093/nar/gkn237

Alignment optimises homology by adding indels (–)

unaligned



aligned



grey represent strict consensus over rows

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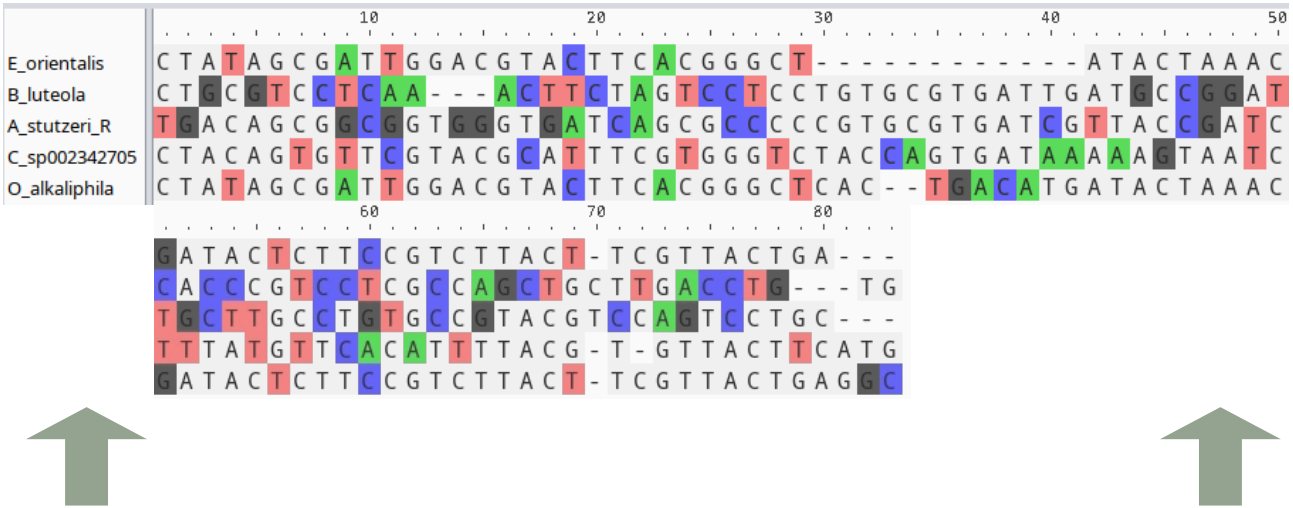
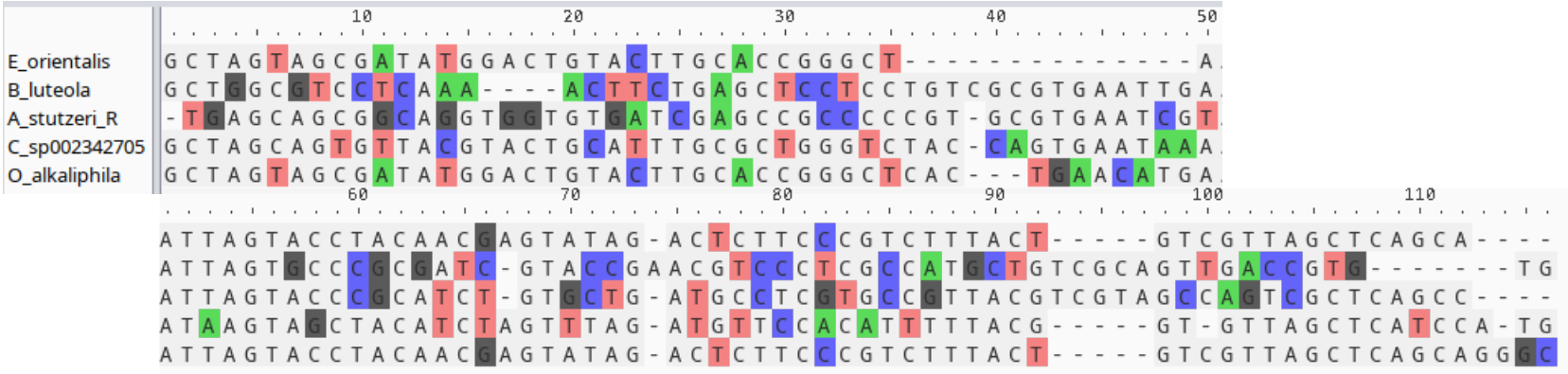
Sometimes we remove invariant sites

Only SNPs



aligned



grey represent strict consensus over rows

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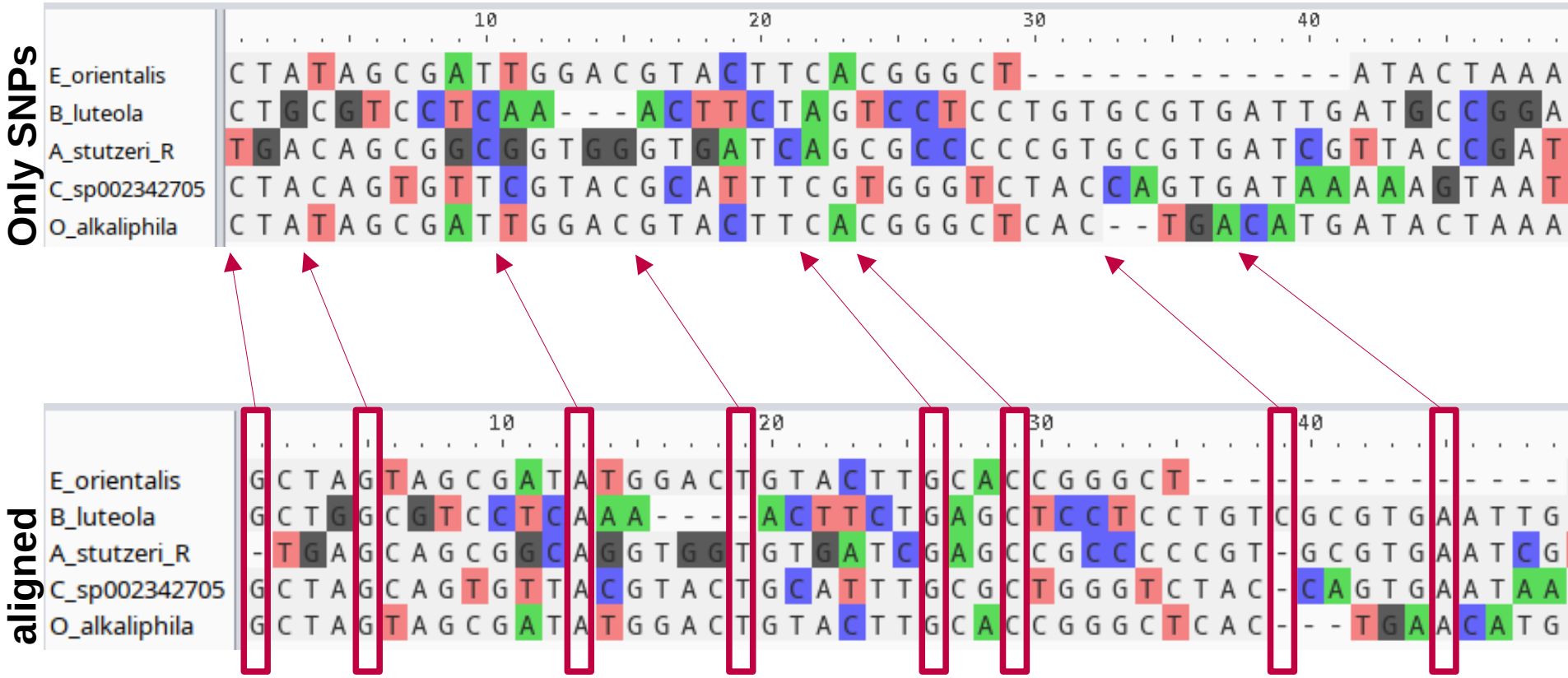
Sometimes we remove invariant sites



grey represent strict consensus over rows

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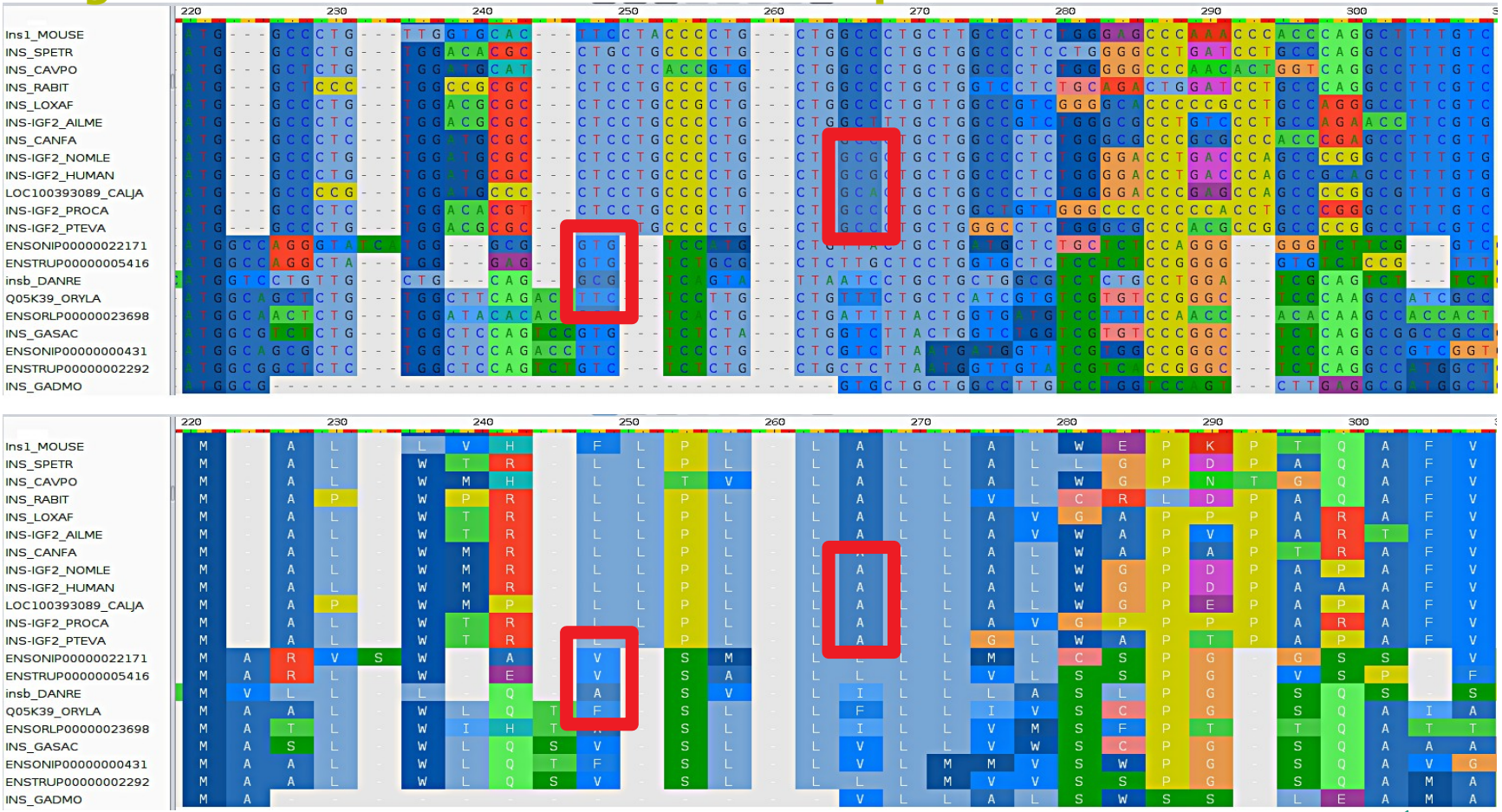
Coding DNA → codons or amino acid sequences



https://selectome.org/euteleostomi/family/ENSGT00390000015440.1.Euteleostomi

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Many sequence formats besides FASTA

```
 Known biosequence formats:
ID  Name              Read  Write  Int'leaf  Features  Sequence  Suffix  Content-type
 1  IG|Stanford       yes    yes       --        --       yes    .ig     biosequence/ig
 2  GenBank|gb        yes    yes       --       yes       yes    .gb     biosequence/genbank
 3  NBRF              yes    yes       --        --       yes    .nbrf   biosequence/nbrf
 4  EMBL|em           yes    yes       --       yes       yes    .embl   biosequence/embl
 5  GCG               yes    yes       --        --       yes    .gcg    biosequence/gcg
 6  DNAStrider        yes    yes       --        --       yes    .strider  biosequence/strider
 7  Fitch              --     --       --        --       yes    .fitch  biosequence/fitch
 8  Pearson|Fasta|fa   yes    yes       --        --       yes    .fasta  biosequence/fasta
 9  Zuker              --     --       --        --       yes    .zuker  biosequence/zuker
10  Olsen              --     --      yes        --       yes    .olsen  biosequence/olsen
11  Phylip3.2         yes    yes      yes        --       yes    .phylip2  biosequence/phylip2
12  Phylip|Phylip4    yes    yes      yes        --       yes    .phylip  biosequence/phylip
13  Plain|Raw         yes    yes       --        --       yes    .seq    biosequence/plain
14  PIR|CODATA        yes    yes       --        --       yes    .pir    biosequence/codata
15  MSF               yes    yes      yes        --       yes    .msf    biosequence/msf
16  ASN.1              --     --       --        --       yes    .asn    biosequence/asn1
17  PAUP|NEXUS        yes    yes      yes        --       yes    .nexus  biosequence/nexus
18  Pretty             --    yes      yes        --       yes    .pretty  biosequence/pretty
19  XML               yes    yes       --       yes       yes    .xml    biosequence/xml
20  BLAST             yes     --      yes        --       yes    .blast  biosequence/blast
21  SCF               yes     --       --        --       yes    .scf    biosequence/scf
22  Clustal           yes    yes      yes        --       yes    .aln    biosequence/clustal
23  FlatFeat|FFF      yes    yes       --       yes        --    .fff    biosequence/fff
24  GFF               yes    yes       --       yes        --    .gff    biosequence/gff
25  ACEDB             yes    yes       --        --       yes    .ace    biosequence/acedb
  (Int'leaf = interleaved format; Features = documentation/features are parsed)
```

Screenshot from *readseq* help page

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phylip format for aligned sequences

Here is a hypothetical example of interleaved format:

```
  5    42
Turkey    AAGCTNGGGC ATTTCAGGGT
Salmo gairAAGCCTTGGC AGTGCAGGGT
H. SapiensACCGGTTGGC CGTTCAGGGT
Chimp     AAACCCTTGC CGTTACGCTT
Gorilla   AAACCCTTGC CGGTACGCTT

GAGCCCGGGC AATACAGGGT AT
GAGCCGTGGC CGGGCACGGT AT
ACAGGTTGGC CGTTCAGGGT AA
AAACCGAGGC CGGGACACTC AT
AAACCATTGC CGGTACGCTT AA
```

while in sequential format the same sequences would be:

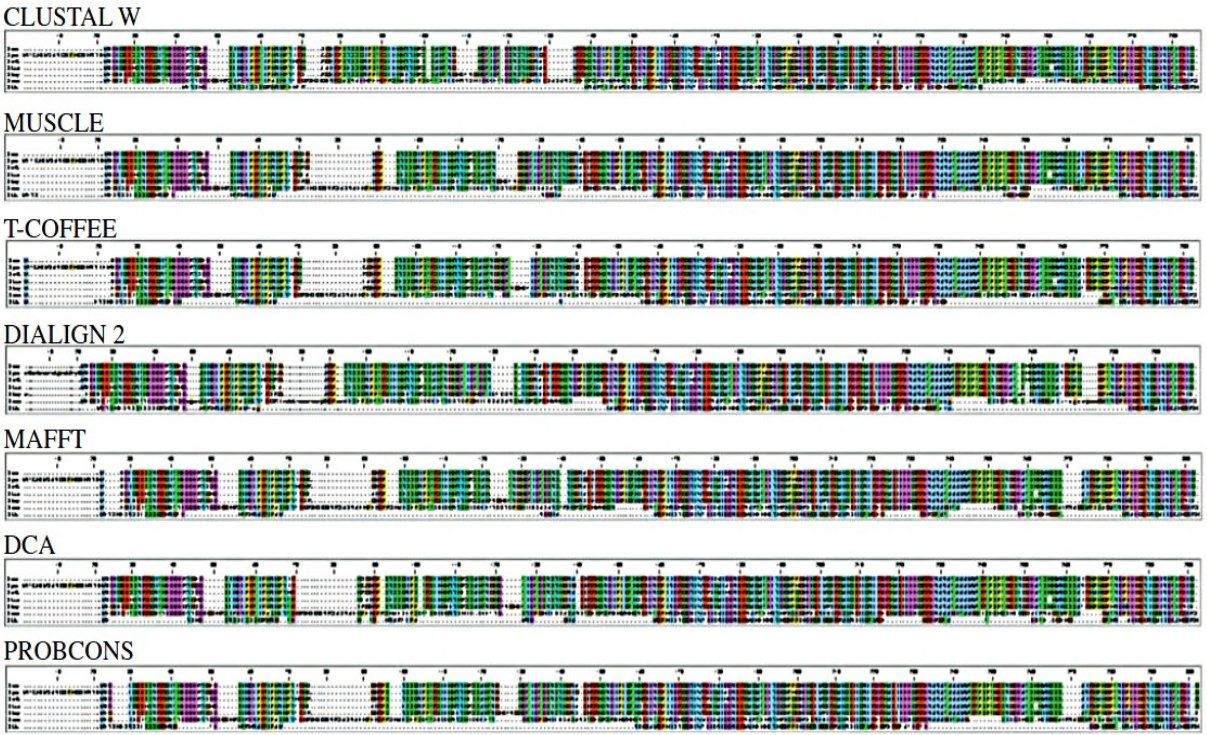
```
  5    42
Turkey    AAGCTNGGGC ATTTCAGGGT
GAGCCCGGGC AATACAGGGT AT
Salmo gairAAGCCTTGGC AGTGCAGGGT
GAGCCGTGGC CGGGCACGGT AT
H. SapiensACCGGTTGGC CGTTCAGGGT
ACAGGTTGGC CGTTCAGGGT AA
Chimp     AAACCCTTGC CGTTACGCTT
AAACCGAGGC CGGGACACTC AT
Gorilla   AAACCCTTGC CGGTACGCTT
AAACCATTGC CGGTACGCTT AA
```

Note, of course, that a portion of a sequence like this: **300 AAGCGTGAAC GTTGTACTAA TRCAG**

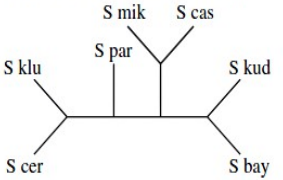
is perfectly legal, assuming that the species name has gone before, and is filled out to full length by blanks.

The above digits and blanks will be ignored,

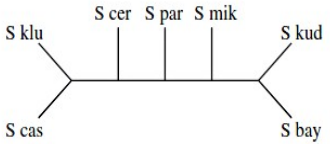
Caveat I: different programs may lead to distinct trees



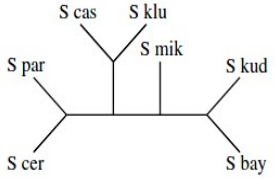
CLUSTAL/DIALIGN (0.24)



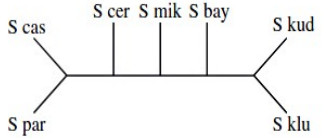
MUSCLE (0.25)



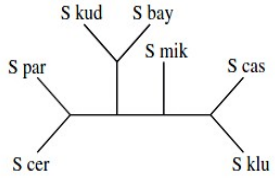
MAFFT (0.18)



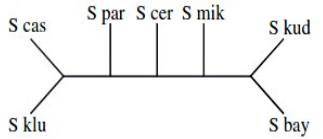
DCA (0.12)



T-COFFEE (0.30)



PROBCONS (0.05)



https://doi.org/10.1126/science.1151532

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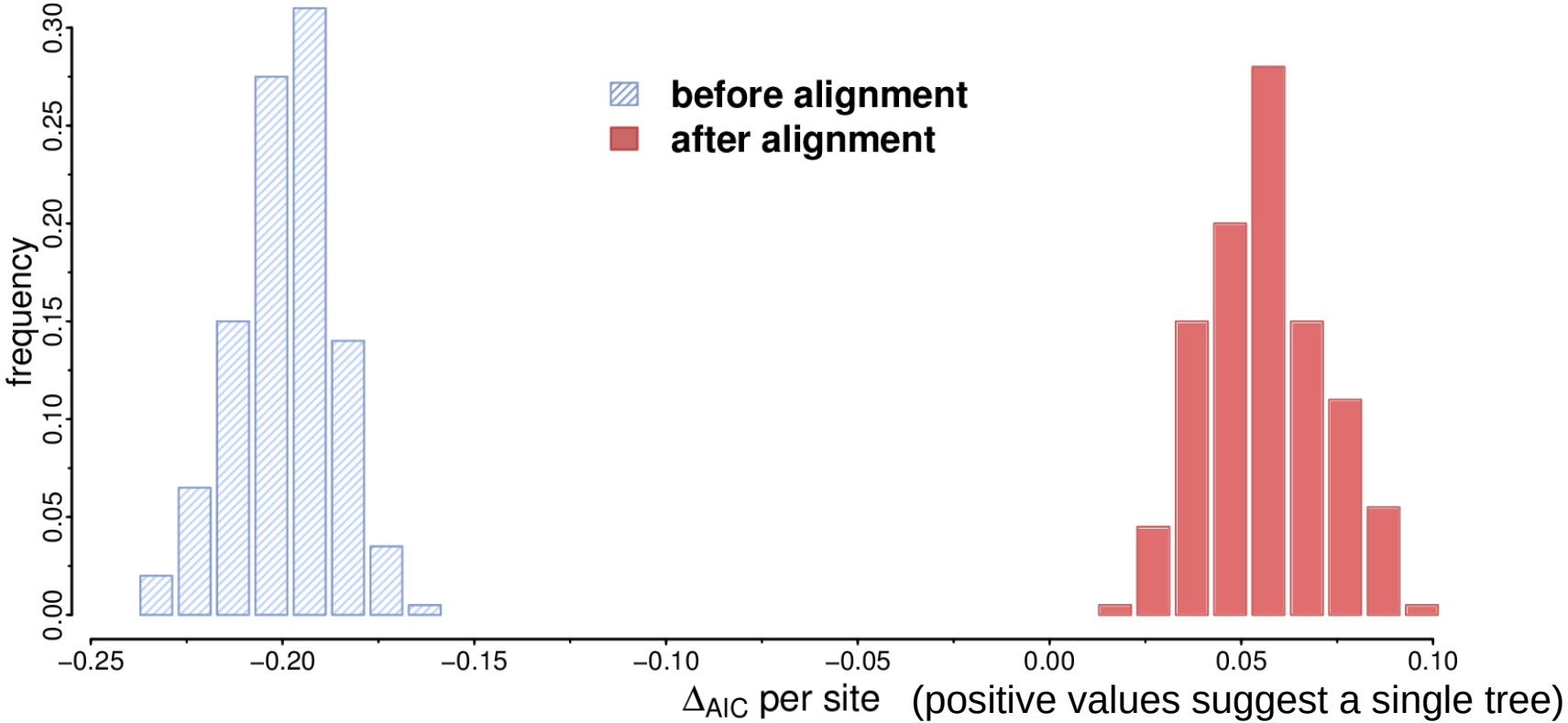
Caveat II: programs can align anything, even random sequences



doi: 10.1093/sysbio/syu041

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- Make sure that genes / contigs you want to align are homologous
- Alignment algorithms or sequences/regions can affect the tree
- Should you add more sequences before or after the alignment?
- coding regions can be translated or codon-aligned
- software: `trimal`, `guidance`

SNP-sites

for example

- snippy's "core SNP genome"
- gubbins output alignment

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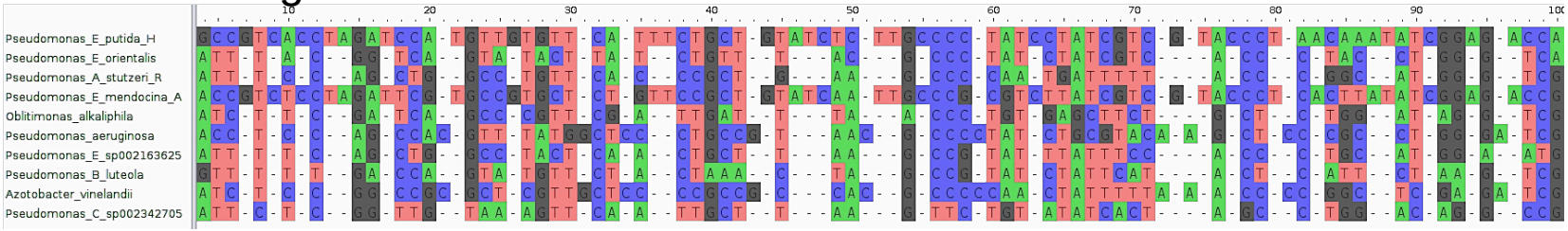
Did you try the github data sets?

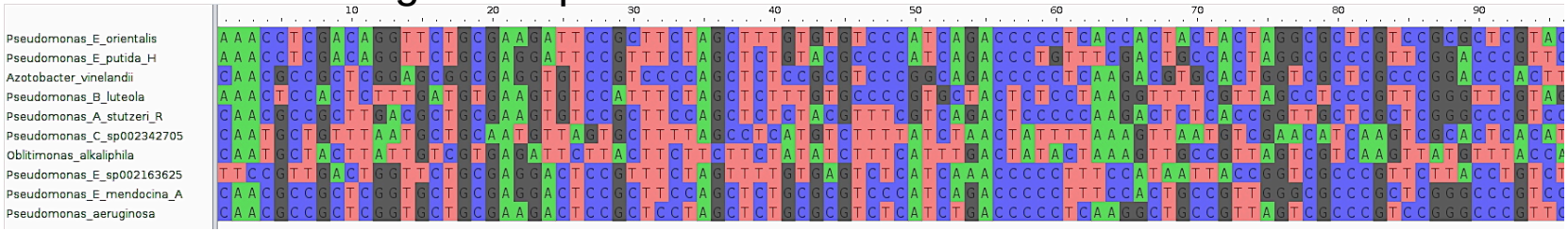
- Data set 1 (SNPs) only
- Data set 2 (concatenate alignments) only
- Both data sets
- I did not play with the data

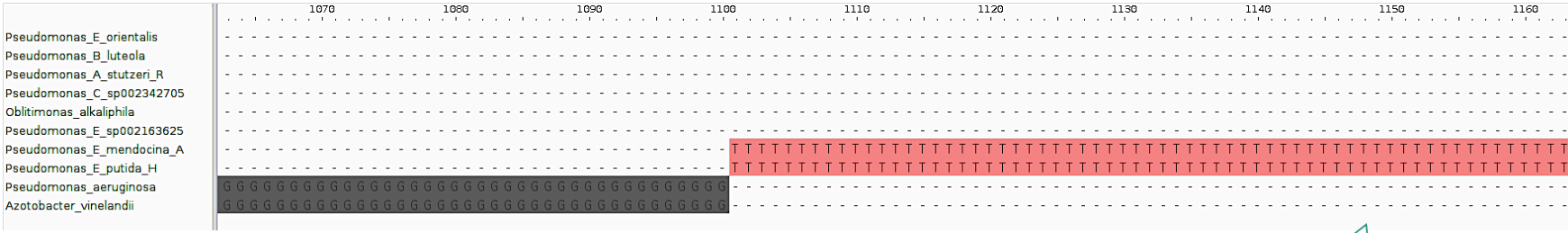
Data set 1 from github material

How it was shared on github: columns shuffled

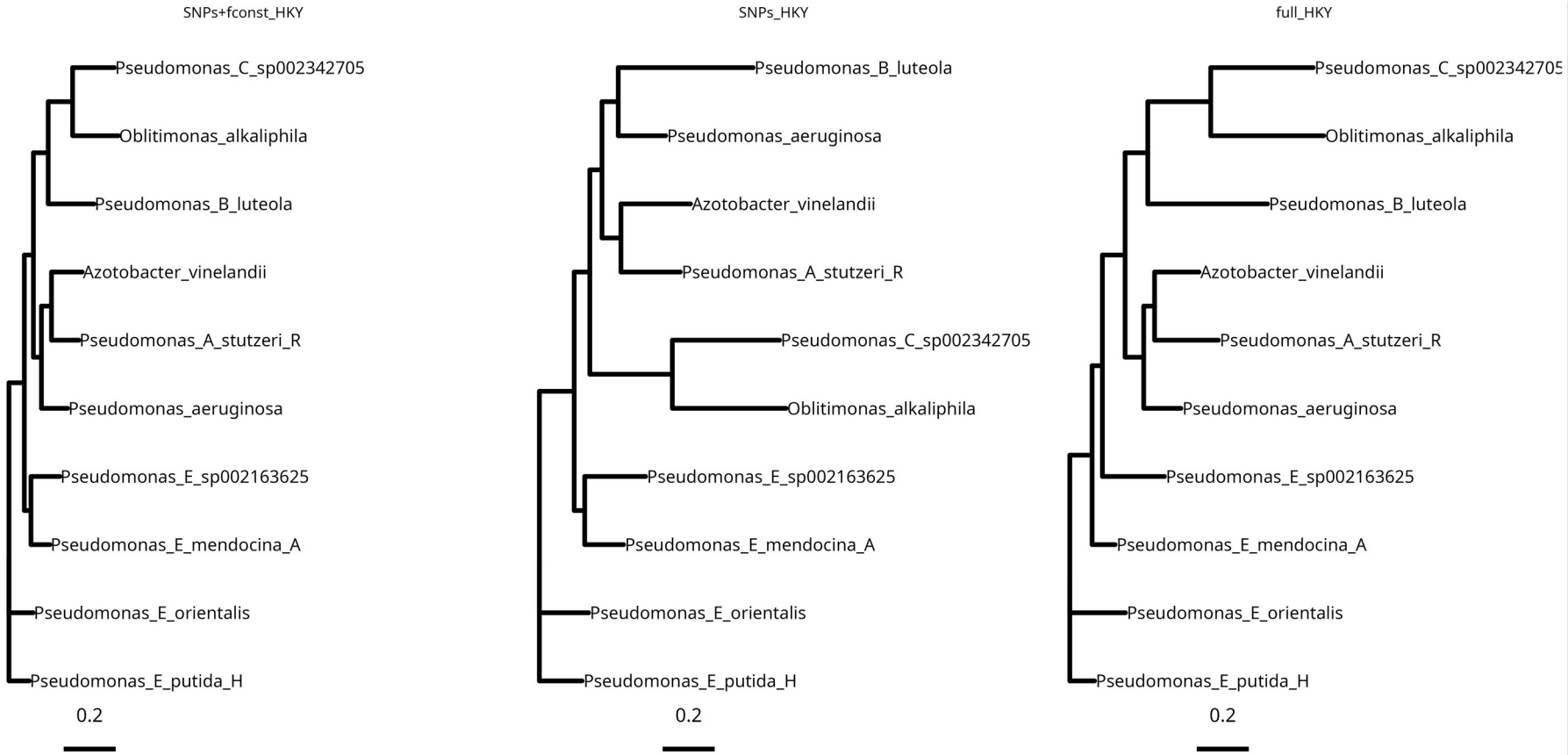


How it was created: real alignment plus “invariant” columns

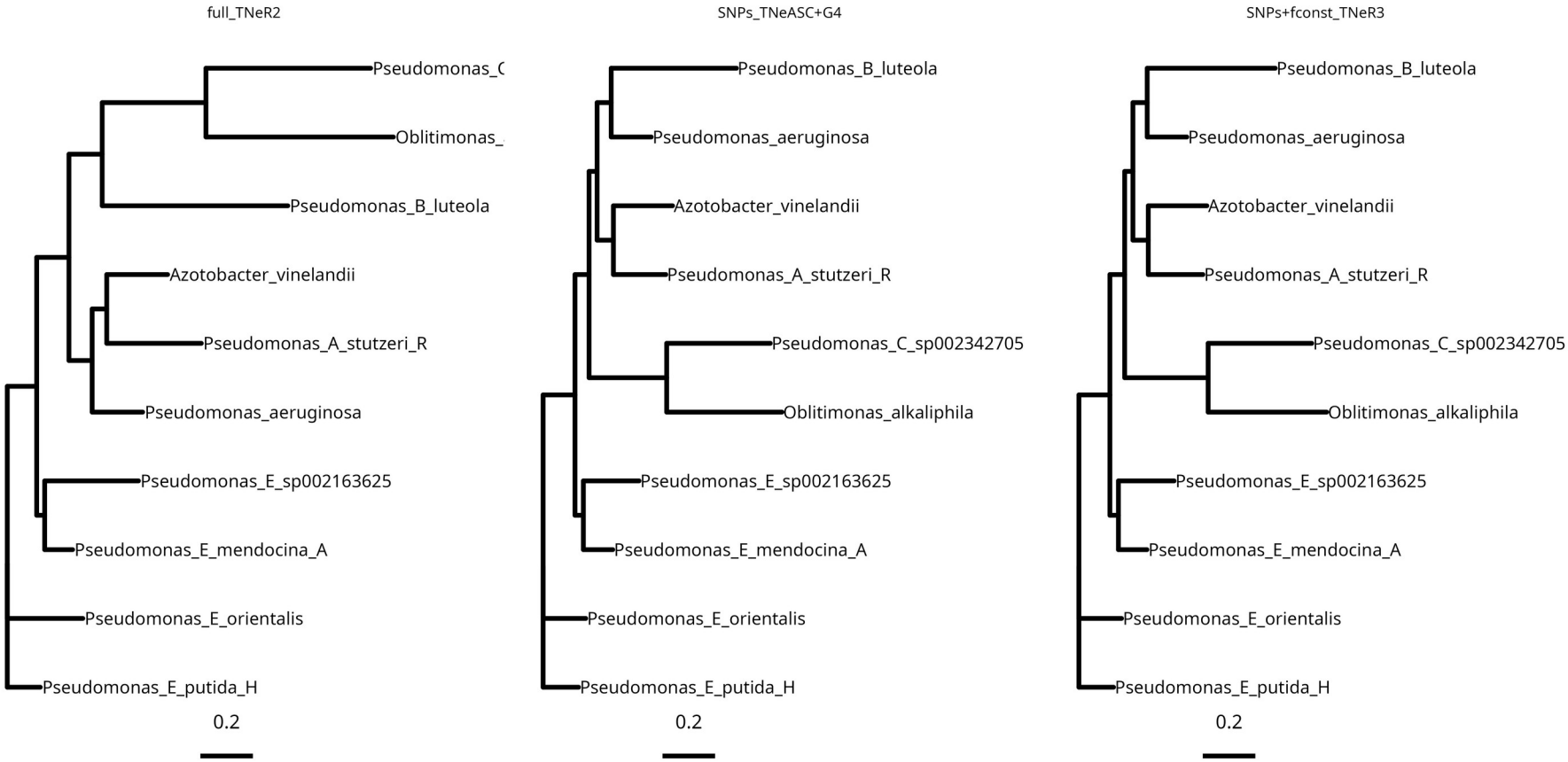




ML tree under fixed (HKY) model



ML tree under best model



Are the trees different?

	SNPs+fconst_HKY	SNPs_HKY	full_HKY	full_TNeR2	SNPs_TNeASC+G4
SNPs_HKY	1				
full_HKY	1	2			
full_TNeR2	0	1	1		
SNPs_TNeASC+G4	1	0	2	1	
SNPs+fconst_TNeR3	1	0	2	1	0

SPR

	SNPs+fconst_HKY	SNPs_HKY	full_HKY	full_TNeR2	SNPs_TNeASC+G4
SNPs_HKY	4				
full_HKY	2	6			
full_TNeR2	0	4	2		
SNPs_TNeASC+G4	4	0	6	4	
SNPs+fconst_TNeR3	4	0	6	4	0

RF

	SNPs+fconst_HKY	SNPs_HKY	full_HKY	full_TNeR2	SNPs_TNeASC+G4
SNPs_HKY	2.16060480				
full_HKY	1.65863479	0.92129548			
full_TNeR2	3.10375600	1.67082095	1.57537866		
SNPs_TNeASC+G4	1.79993846	0.36453405	0.72916240	1.71250523	
SNPs+fconst_TNeR3	1.84060299	0.34925395	0.74870724	1.64916454	0.07588229

wRF

file `08.snp_only_all_models.iqtree`

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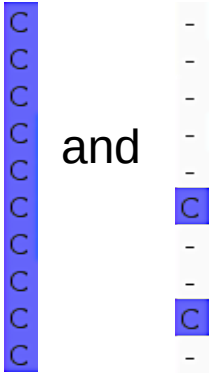
Are the trees significantly different? Based on data (=alignment)

```
See 08.snp_only_all_models.trees for trees with branch lengths.

Tree      logL    deltaL  bp-RELL    p-KH     p-SH    p-WKH    p-WSH       c-ELW       p-AU
--------------------------------------------------------------------------------------------
  1 -7566.973604  2.2582   0.162 +  0.377 +  0.589 +  0.377 +  0.804 +     0.173 +    0.492 +
  2  -7573.24419  8.5288  0.0564 +  0.169 +  0.195 +  0.128 +  0.201 +    0.0512 +    0.111 +
  3 -7566.973589  2.2582   0.172 +  0.377 +  0.589 +  0.377 +  0.716 +     0.175 +    0.491 +
  4 -7564.715398       0   0.304 +  0.501 +      1 +  0.501 +  0.756 +     0.301 +    0.564 +
  5 -7564.715444 4.6282e-05   0.306 +  0.499 +  0.854 +  0.499 +  0.852 +       0.3 +    0.578 +

deltaL  : logL difference from the maximal logl in the set.
bp-RELL : bootstrap proportion using RELL method (Kishino et al. 1990).
p-KH    : p-value of one sided Kishino-Hasegawa test (1989).
p-SH    : p-value of Shimodaira-Hasegawa test (2000).
p-WKH   : p-value of weighted KH test.
p-WSH   : p-value of weighted SH test.
c-ELW   : Expected Likelihood Weight (Strimmer & Rambaut 2002).
p-AU    : p-value of approximately unbiased (AU) test (Shimodaira, 2002).

Plus signs denote the 95% confidence sets.
Minus signs denote significant exclusion.
All tests performed 50000 resamplings using the RELL method.
```

RELL = resampling of estimated log-likelihoods

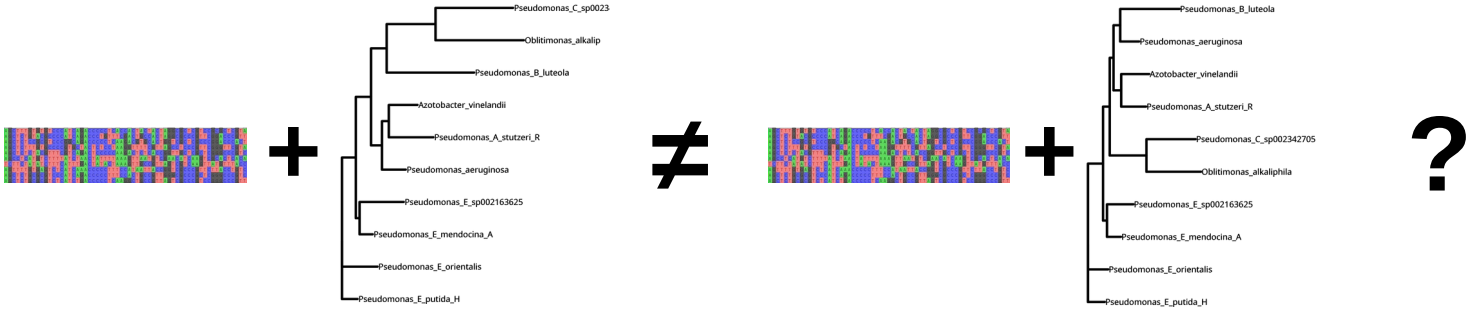
file `08.snp_only_all_models.iqtree`

- We hope that only SNPs have split (i.e. phylogenetic) information



- However likelihood models incorporate uncertainty (N or indel);i.e. and are different

- Tree likelihood tests: given the same alignment, are the trees significantly different?



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The molecular clock

strict and relaxed

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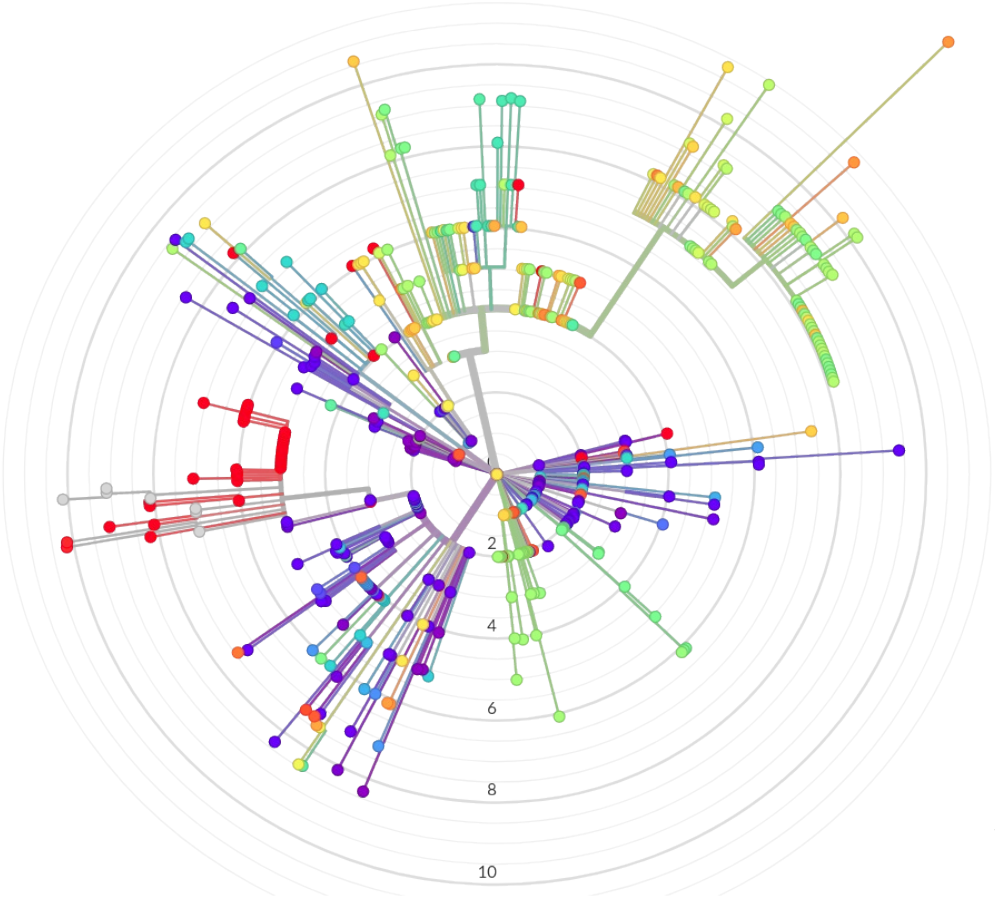
If sampling times are known, we can try to fit the tree tips

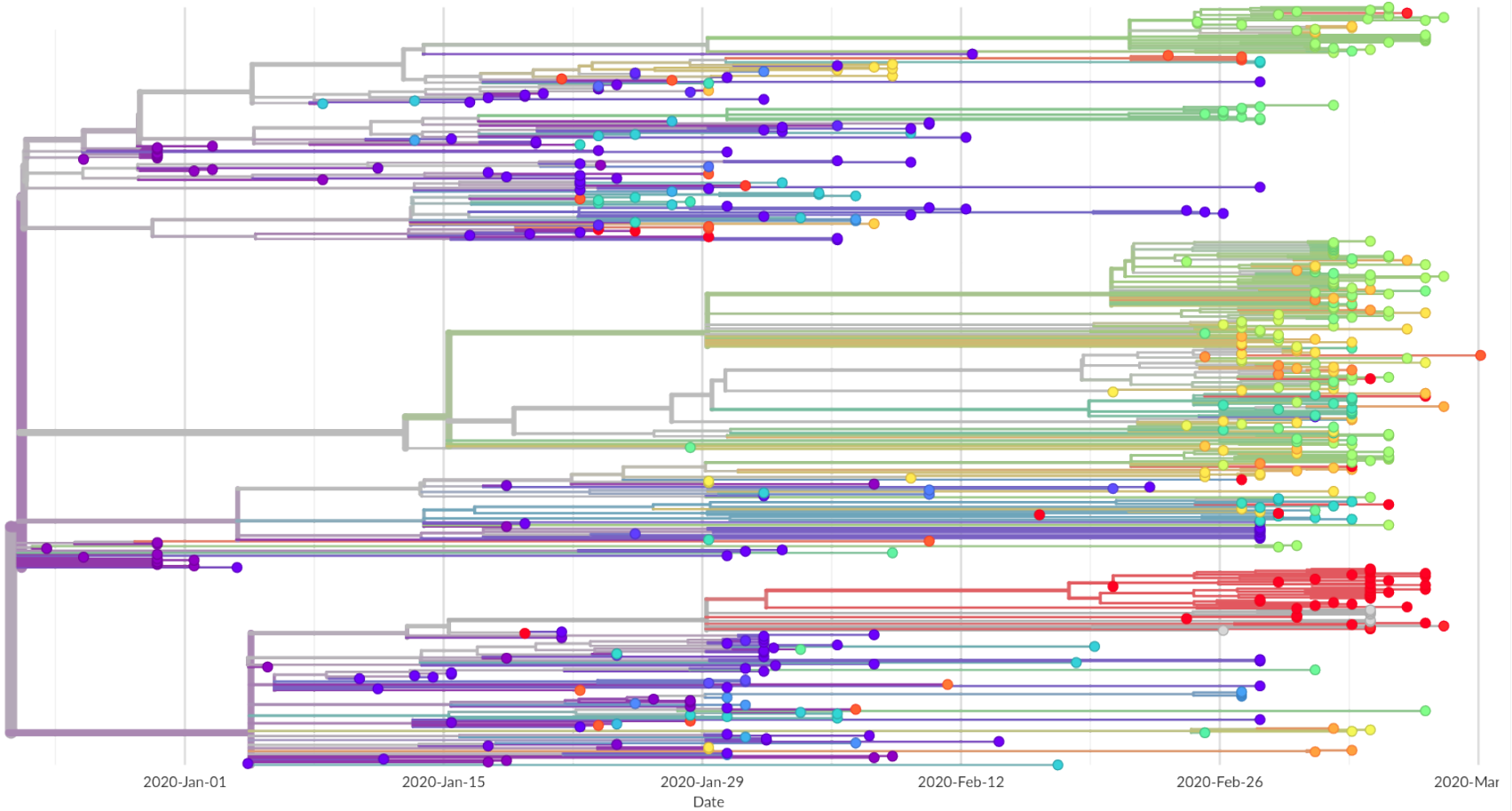


input data: ML (unrooted) tree

+

sampling times for each sequence

If sampling times are known, we can try to fit the tree tips



from https://nextstrain.org/

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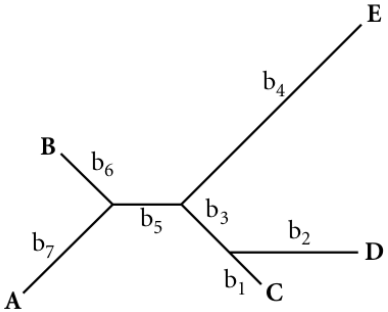
Curiosity: strict clock models used to assume ultrametricity

A

Nonclocklike phylogenetic tree
***n* taxa = 5**

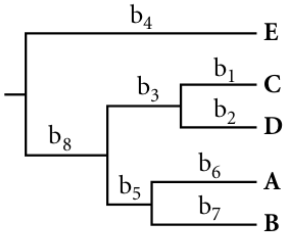


unrooted tree
$2n - 3$ independent branches

All b_1, b_2, b_3, b_4, b_5, b_6, and b_7 need to be estimated

B

Clocklike phylogenetic tree
***n* taxa = 5**



rooted tree
$n - 1$ independent branches

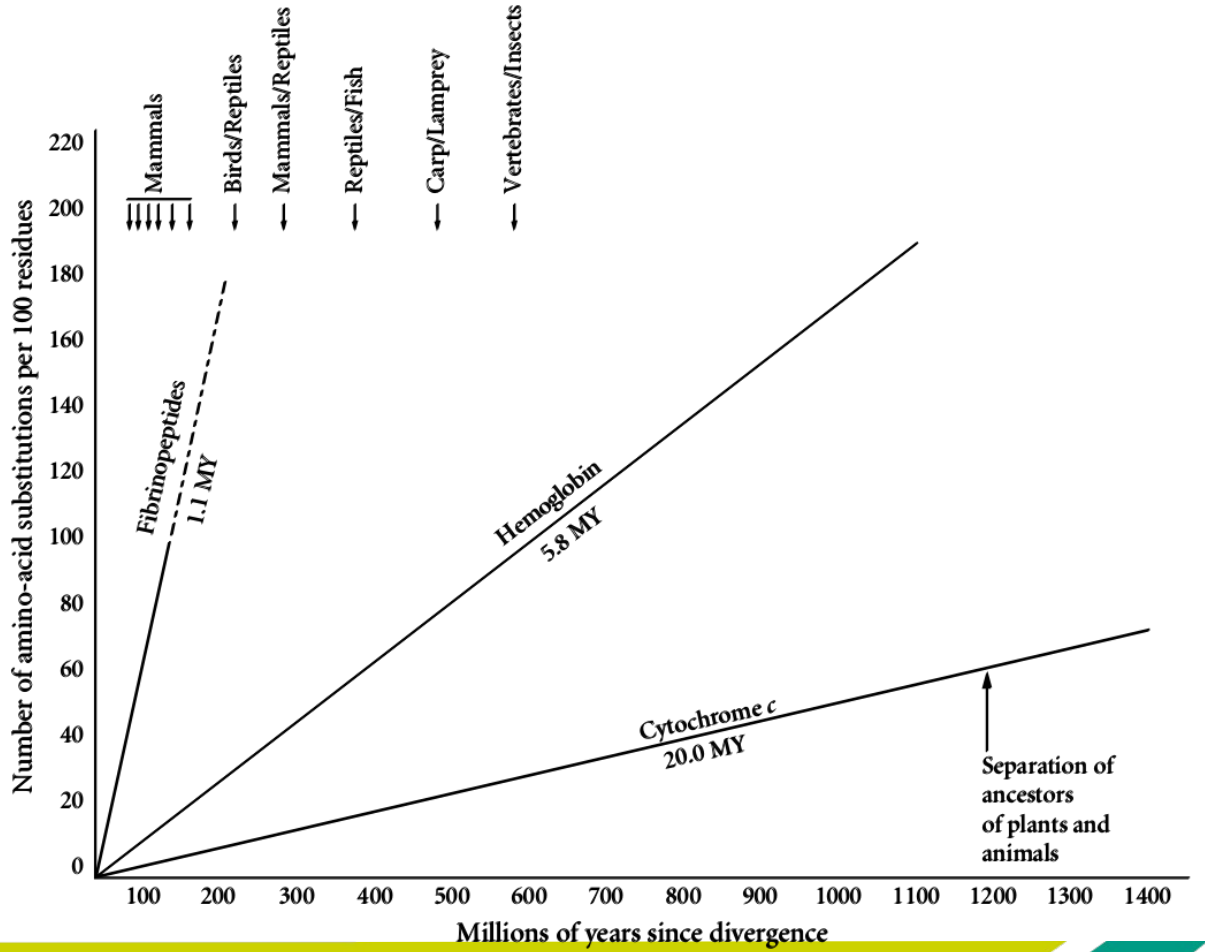
Only b_1, b_3, b_4, and b_6, for example, need to be estimated, because under the molecular clock:

$b_2 = b_1$
$b_5 = b_1 + b_3 - b_6$
$b_7 = b_6$
$b_8 = b_4 - b_5 - b_6$

Number of free parameters in clock and nonclock trees. Under the free rates model (= nonclock), all the branches need to be estimated ($2n - 3$). Under the molecular clock, only $n - 1$ branches have to be estimated. The difference in the number of parameters among a nonclock and a clock model is $n - 2$.

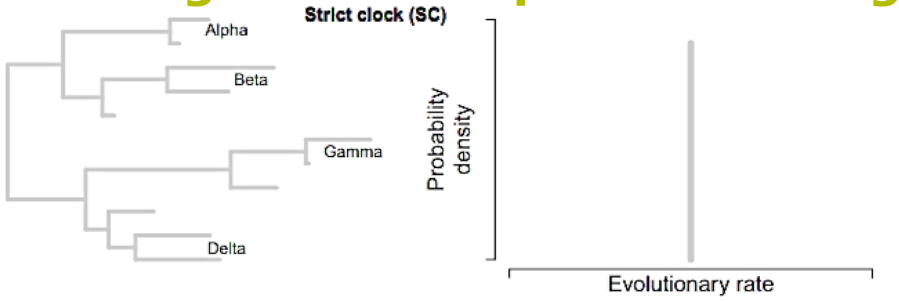
https://doi.org/10.1017/CBO9780511819049.012

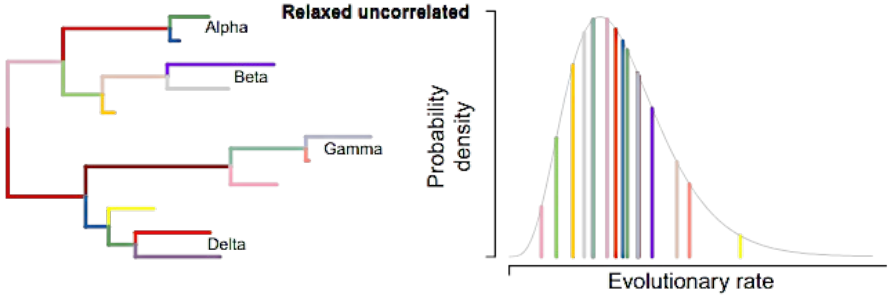
The (strict) molecular clock: each molecule appears to change at a constant rate

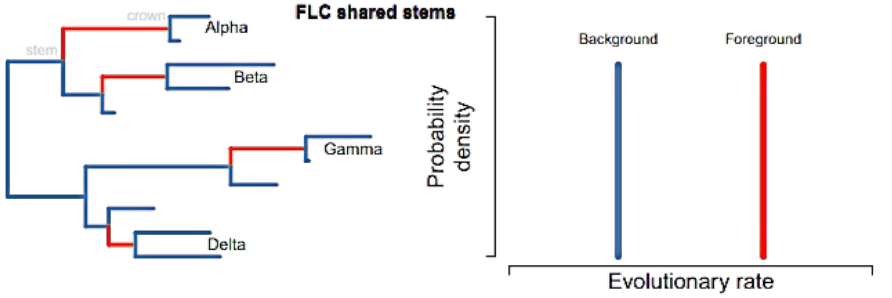


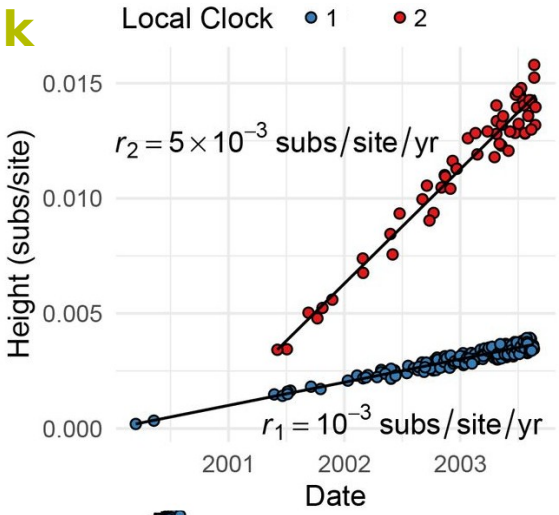
https://doi.org/10.1017/CBO9780511819049.012

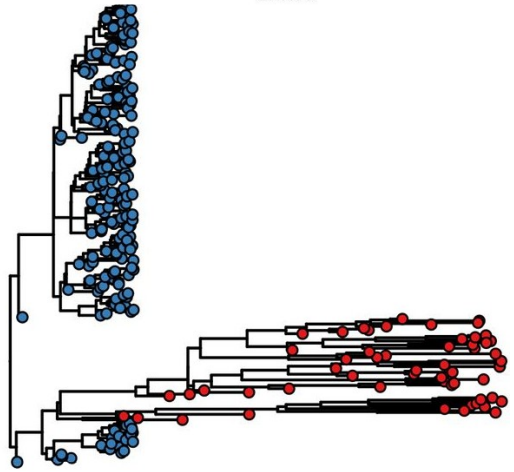
Relaxing the assumption of a single clock











doi:10.1093/molbev/msad212

doi:10.1101/2023.07.13.548947

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Which of these phylogenetic methods have you heard of?

- Distance
- NJ or UPGMA
- Bayesian
- Parsimony
- bootstrap

For which of these phylogenetic methods do you know a software implementing them?

- Distance
- NJ or UPGMA
- Bayesian
- Parsimony
- bootstrap

Tree inference — Distance

- Clustering and minimum evolution

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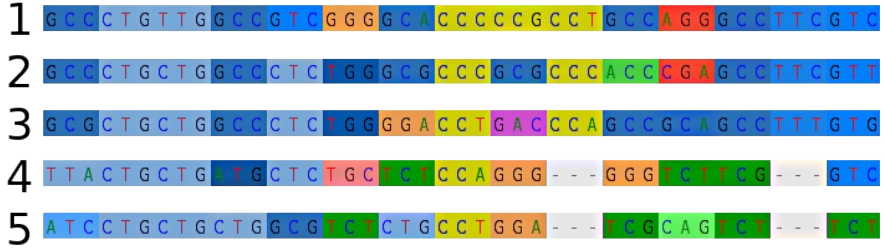
$$\begin{array}{c} \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{array} \begin{array}{c} \begin{array}{ccccc} 1 & 2 & 3 & 4 & 5 \end{array} \\ \begin{pmatrix} 0 & & & & \\ 2 & 0 & & & \\ 3 & 5 & 0 & & \\ 4 & 7 & 8 & 0 & \\ 7 & 8 & 7 & 6 & 0 \end{pmatrix} \end{array}$$



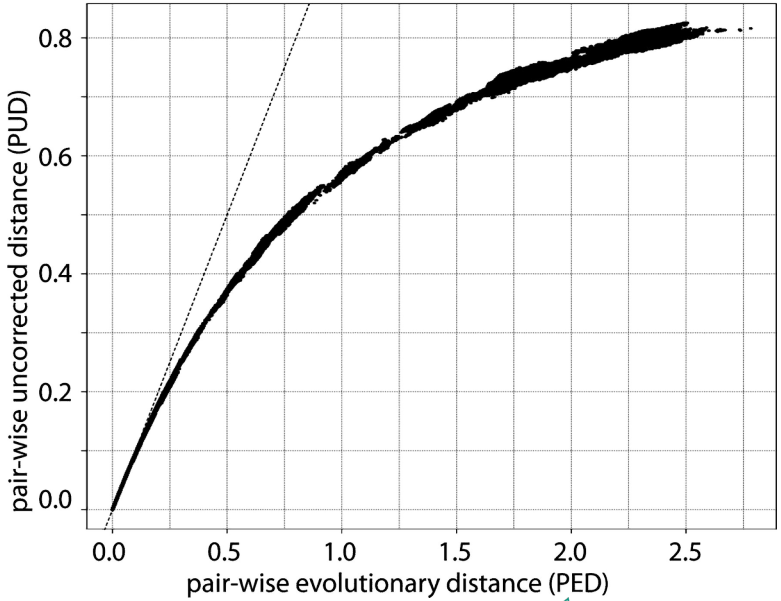
- based on pairwises similarity between sequences (distance matrix)

- clustering algorithms (*Neighbour Joining*, *UPGMA*) or "minimum evolution"

Caveats:

- assumes a homogeneous infinite sites model →

- single number representing differences



https://doi.org/10.1128/jvi.07173-11

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Hierarchical clustering:

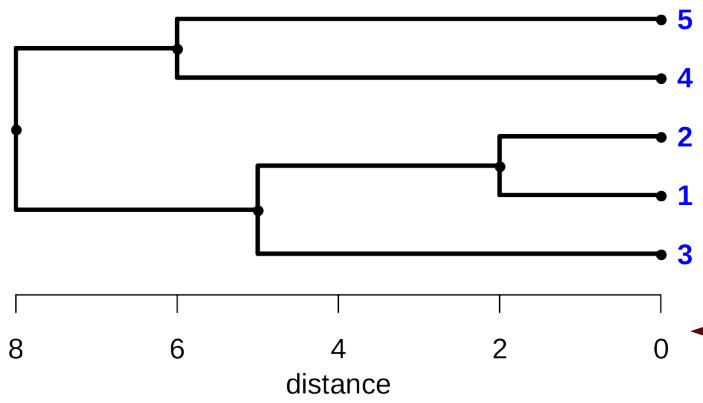
1. find smallest distance
2. merge groups
3. update distances

$$\begin{array}{c|ccccc} & 1 & 2 & 3 & 4 & 5 \\ 1 & 0 & & & & \\ 2 & \mathbf{2} & 0 & & & \\ 3 & 3 & 5 & 0 & & \\ 4 & 4 & 7 & 8 & 0 & \\ 5 & 7 & 8 & 7 & 6 & 0 \end{array} \Rightarrow (1,2)$$

$$\begin{array}{c|cccc} & (1,2) & 3 & 4 & 5 \\ (1,2) & 0 & & & \\ 3 & \mathbf{5} & 0 & & \\ 4 & 7 & 8 & 0 & \\ 5 & 8 & 7 & 6 & 0 \end{array} \Rightarrow ((1,2),3)$$

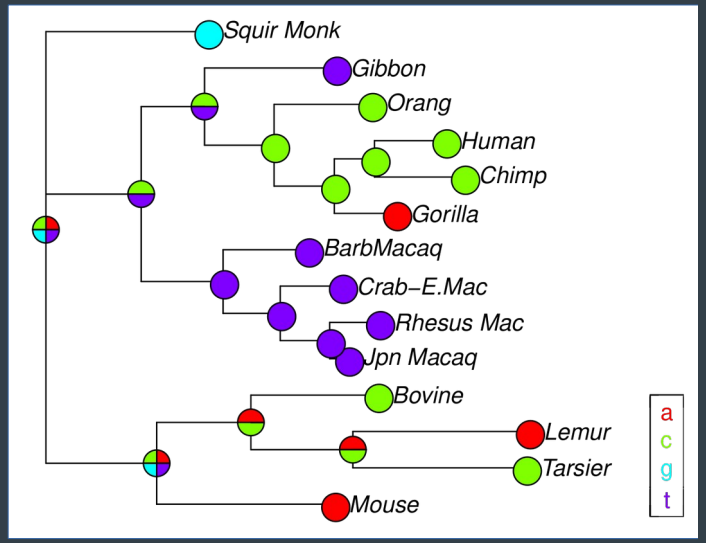
$$\begin{array}{c|ccc} & ((1,2),3) & 4 & 5 \\ ((1,2),3) & 0 & & \\ 4 & 8 & 0 & \\ 5 & 8 & \mathbf{6} & 0 \end{array} \Rightarrow (4,5)$$

$$\begin{array}{c|cc} & ((1,2),3) & (4,5) \\ ((1,2),3) & 0 & \\ (4,5) & 8 & 0 \end{array} \Rightarrow (((1,2),3),(4,5))$$



Tree inference — Parsimony

- Least amount of changes



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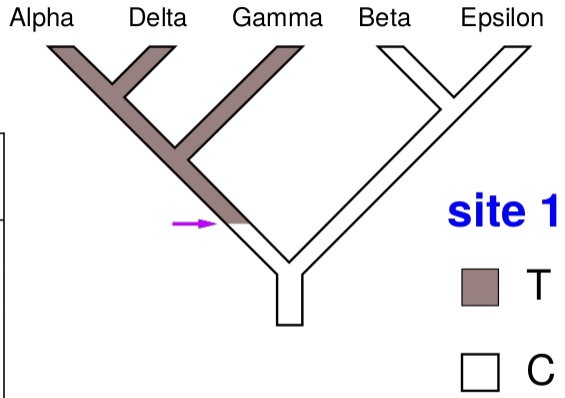
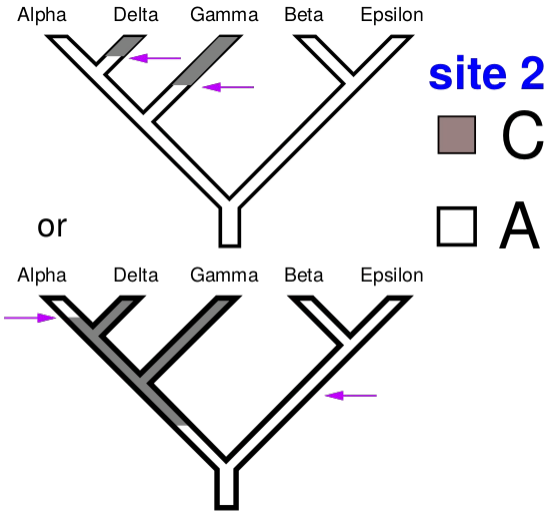
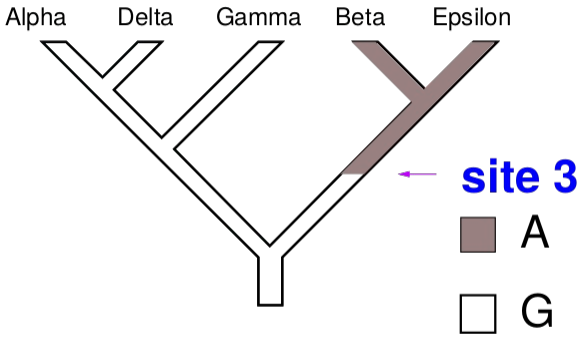
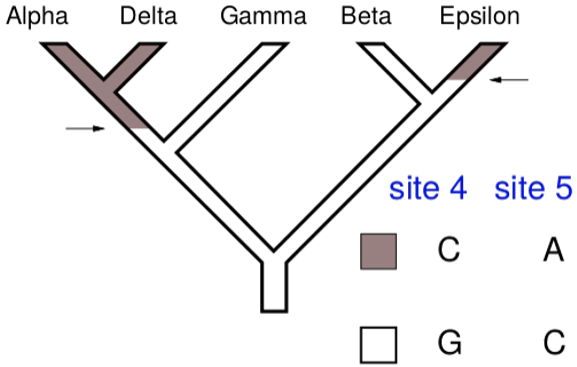
Parsimony score of each site

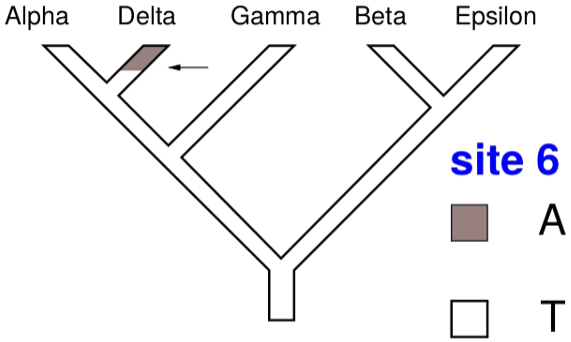
Species	Characters 1	2	3	4	5	6
Alpha	T	A	G	C	A	T
Beta	C	A	A	G	C	T
Gamma	T	C	G	G	C	T
Delta	T	C	G	C	A	A
Epsilon	C	A	A	C	A	T







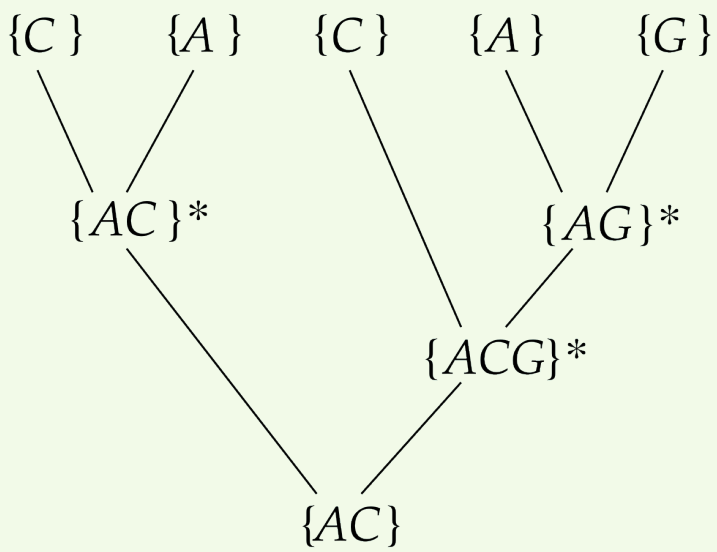




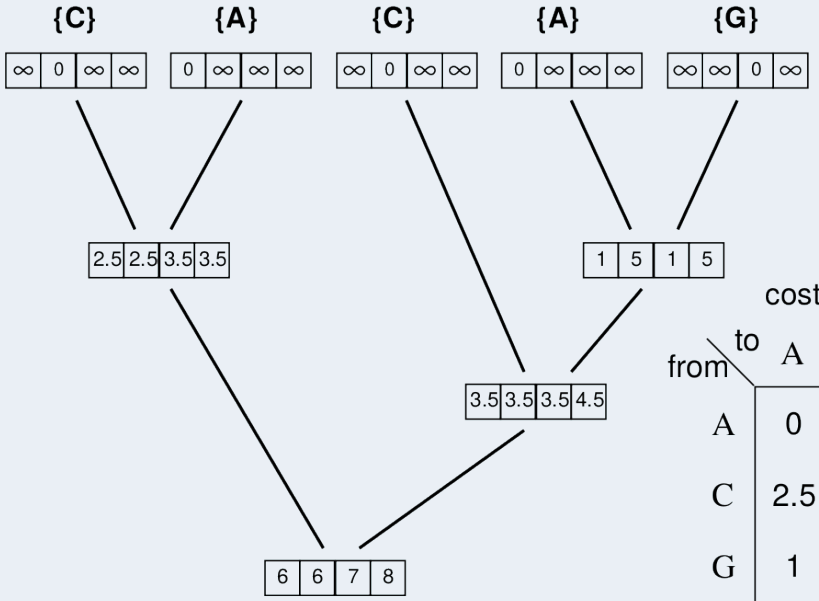
figures from https://evolution.gs.washington.edu/

Algorithms to calculate the parsimony score of a site

Fitch algorithm



Sankoff algorithm



cost matrix:

from \ to	A	C	G	T
A	0	2.5	1	2.5
C	2.5	0	2.5	1
G	1	2.5	0	2.5
T	2.5	1	2.5	0

figures from https://evolution.gs.washington.edu/

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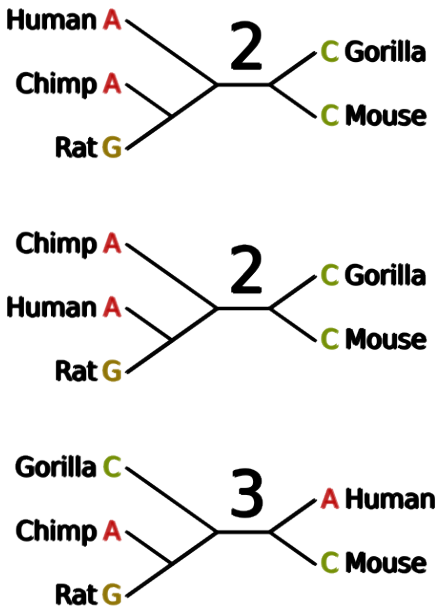
Finding tree with lowest parsimony score



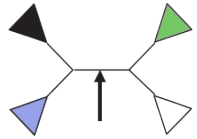
1. calculate parsimony score of tree

2. change tree

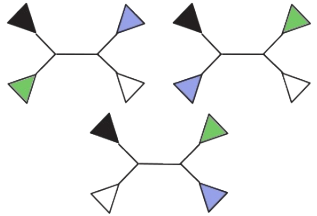
3. repeat

Nearest Neighbour Interchange

Step 1: Take original topology

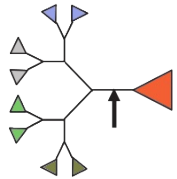


Step 2: choose one of three possible rearrangements

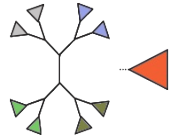


Subtree Pruning and Regrafting

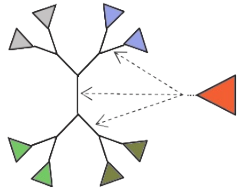
Step 1: Take original topology



Step 2: Break branch under consideration



Step 3: Attach subtree to any branch in other subtree



doi:10.1007/978-1-60327-159-2

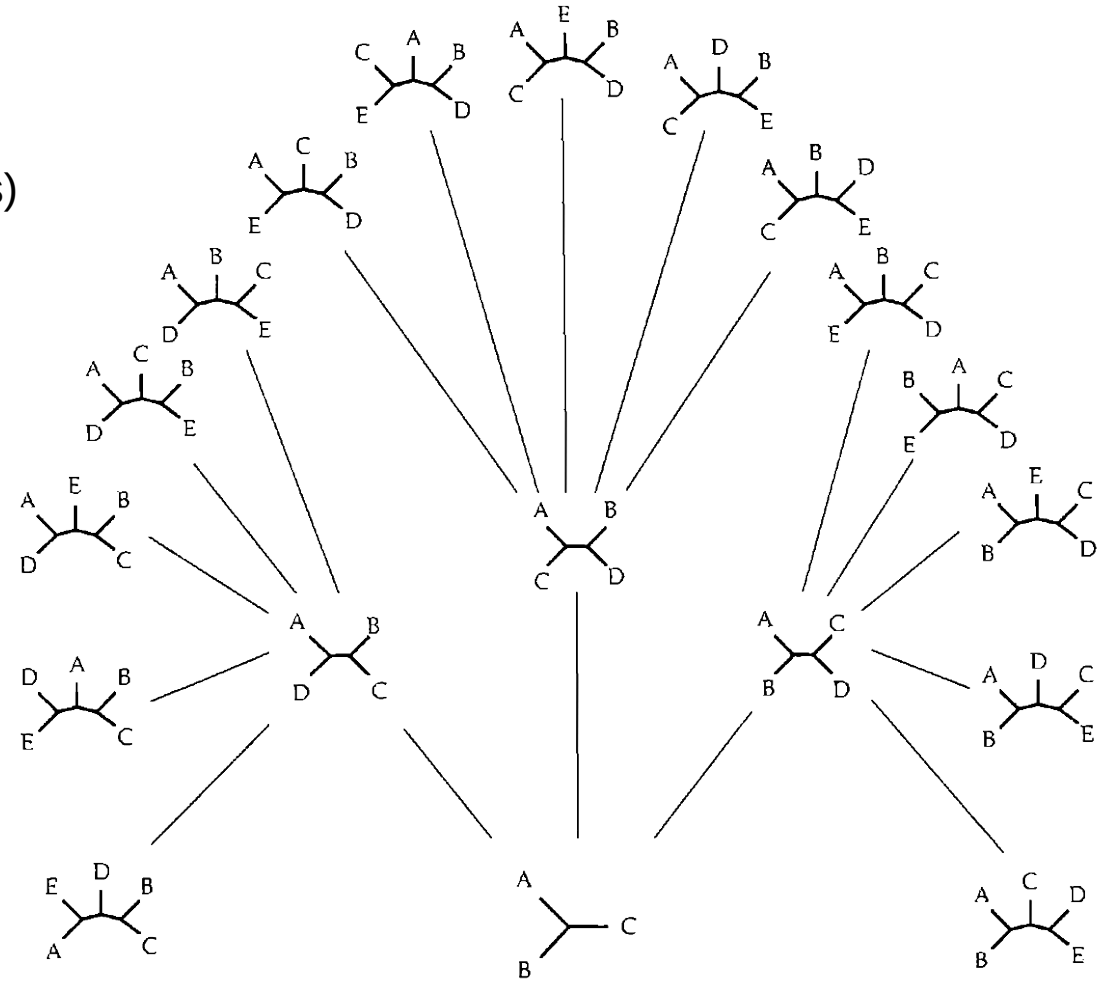
Caveats:

- heuristic (impossible to look at all trees)
- no branch lengths
- many equally good trees
- evolution is not parsimonious

Parsimony renaissance:

10.1186/s12862-018-1131-3 (MPboost)

10.1038/s41588-021-00862-7 (UShER)



from Inferring Phylogenies; Joseph Felsenstein 2004 ISBN: 9780878931774

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Tree inference — Likelihood

- statistical models of evolution
- main component of Bayesian phylogenetics

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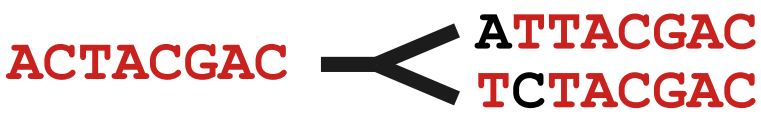
Why likelihood?

ancestral extant



https://montypython.fandom.com/

Only two differences are observed between the two present-day sequences, so that the proportion of different sites is $\hat{p} = 2/8 = 0.25$

Why likelihood?

ancestral

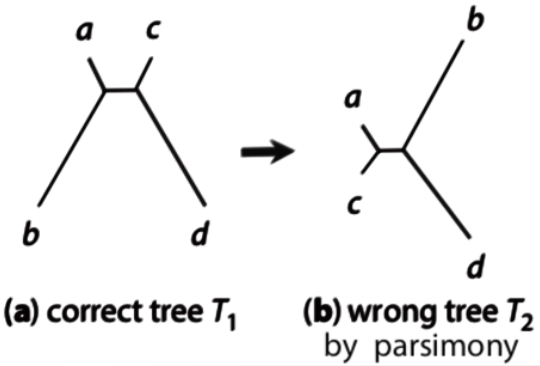
TTCAAGAC

extant

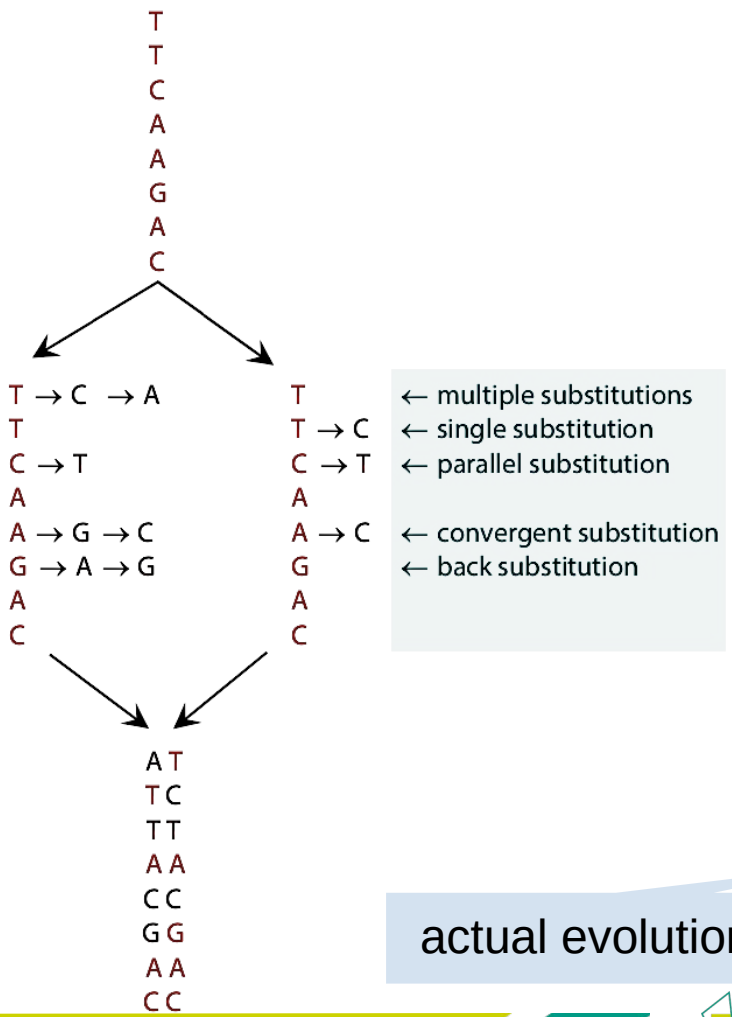
ATTACGAC

TCTACGAC

Only two differences are observed between the two present-day sequences, so that the proportion of different sites is $\hat{p} = 2/8 = 0.25$, while in fact as many as 10 substitutions (seven on the left lineage and three on the right lineage) occurred so that the true distance is 10/8 = 1.25 substitutions per site.



(a) correct tree T_1 **(b) wrong tree T_2** by parsimony



actual evolution

from http://abacus.gene.ucl.ac.uk/MESA/

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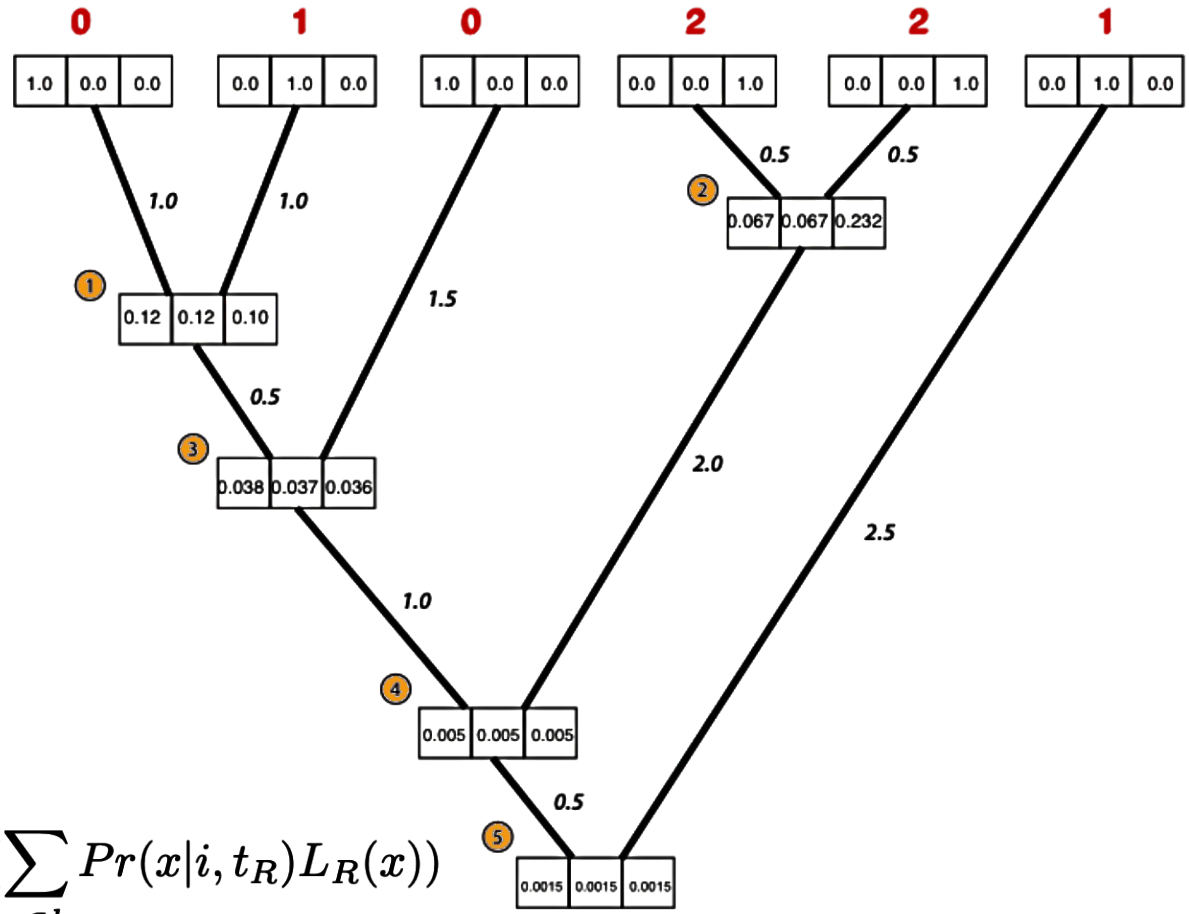
1. “probabilities” of all states (0,1,2) in the past

2. calculate these conditionals in specific order {1,2,3,4,5}

3. change tree and branch lengths

4. repeat



$$L_P(i) = (\sum_{x \in k} Pr(x|i, t_L) L_L(x)) \cdot (\sum_{x \in k} Pr(x|i, t_R) L_R(x))$$

Bootstrap and posterior

- tree uncertainty

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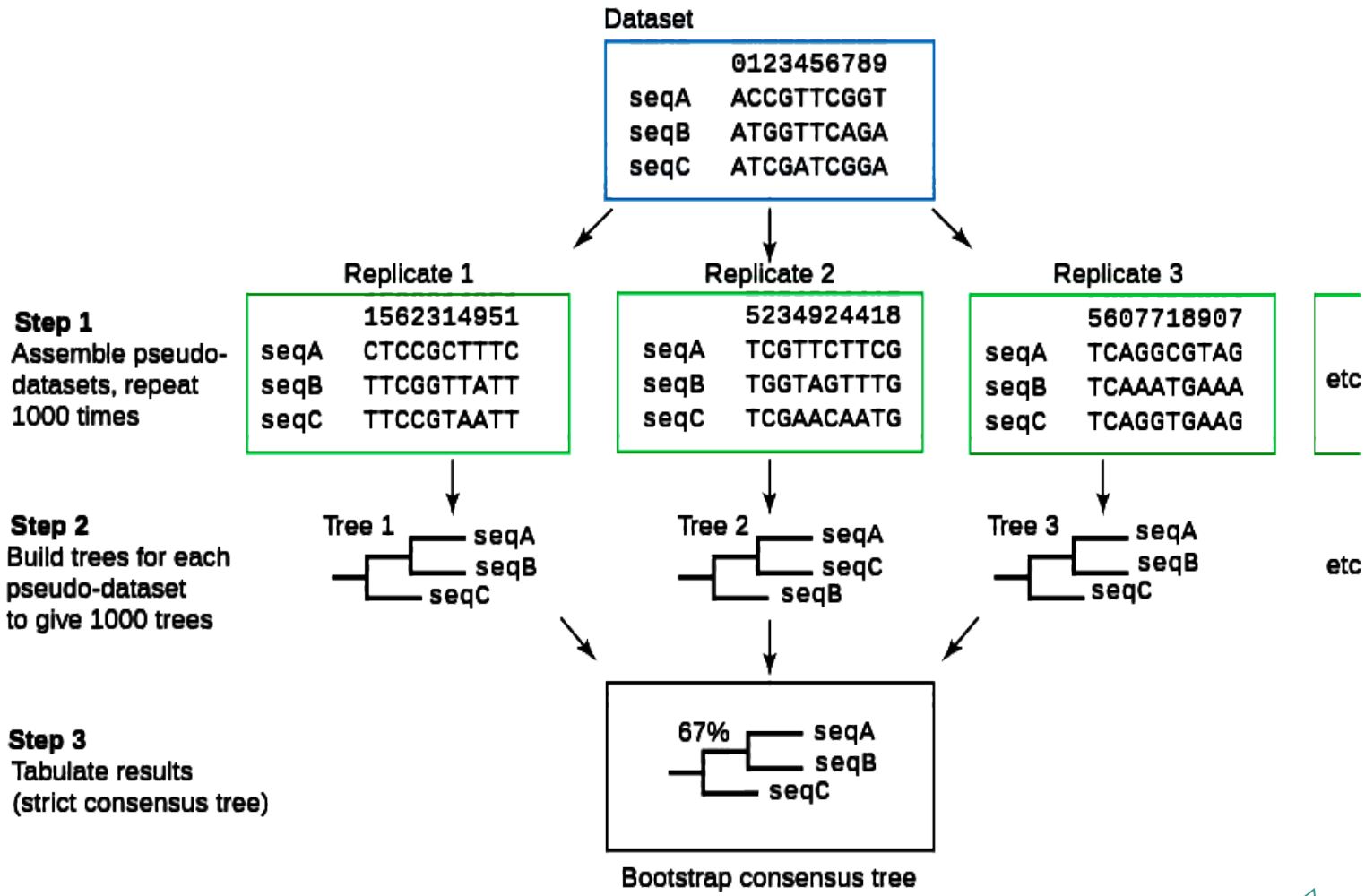
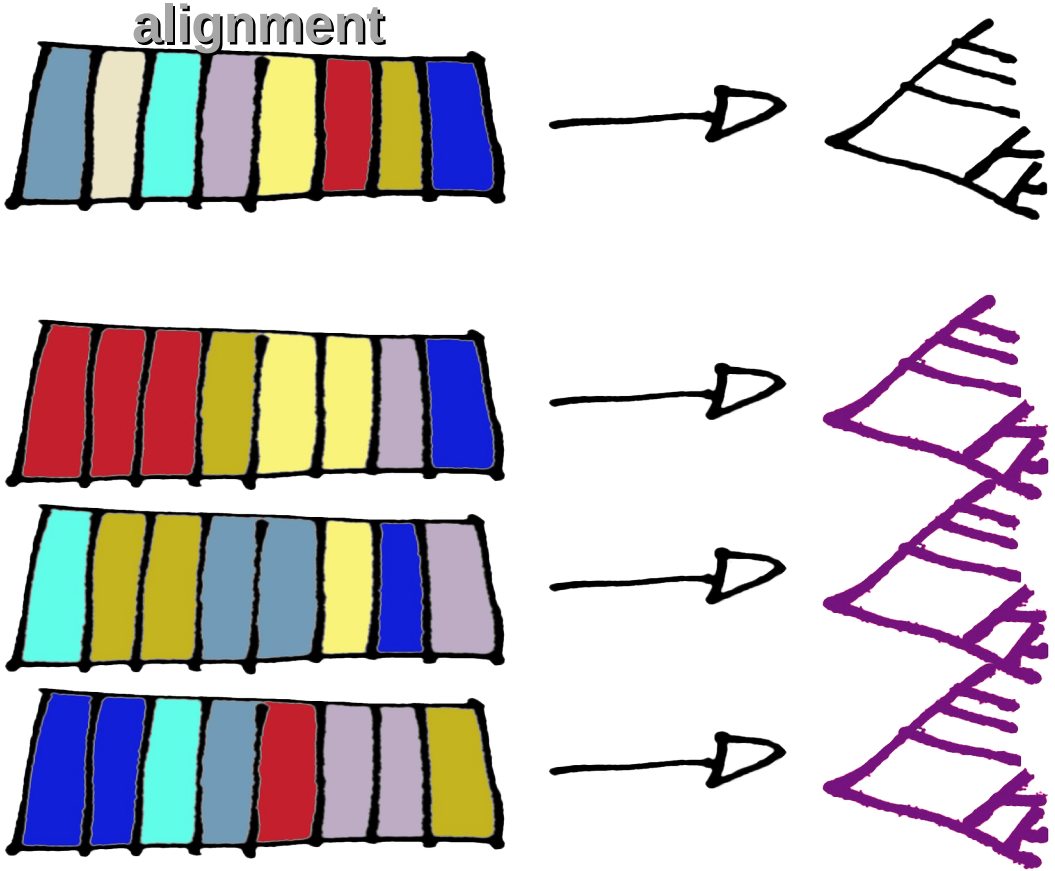


doi:10.1016/S0168-9525(03)00112-4

Phylogenetic Bootstraps (“Transfer” and “Felsenstein”)

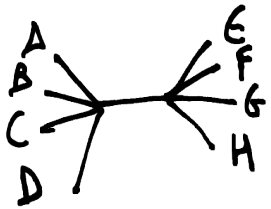
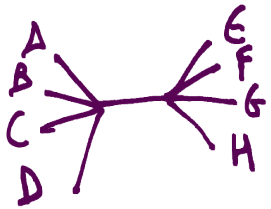
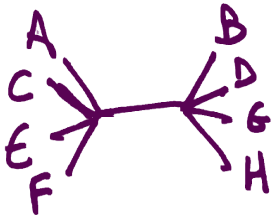
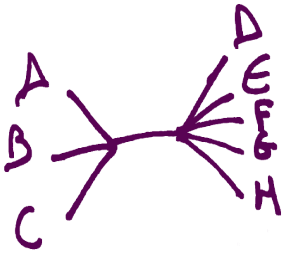
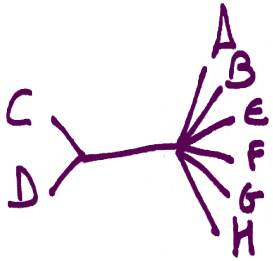


“reference tree” (estimated from original data)

bootstrap replicates (trees estimated from bootstrapped data)

with replacement → replicates have same size as original data

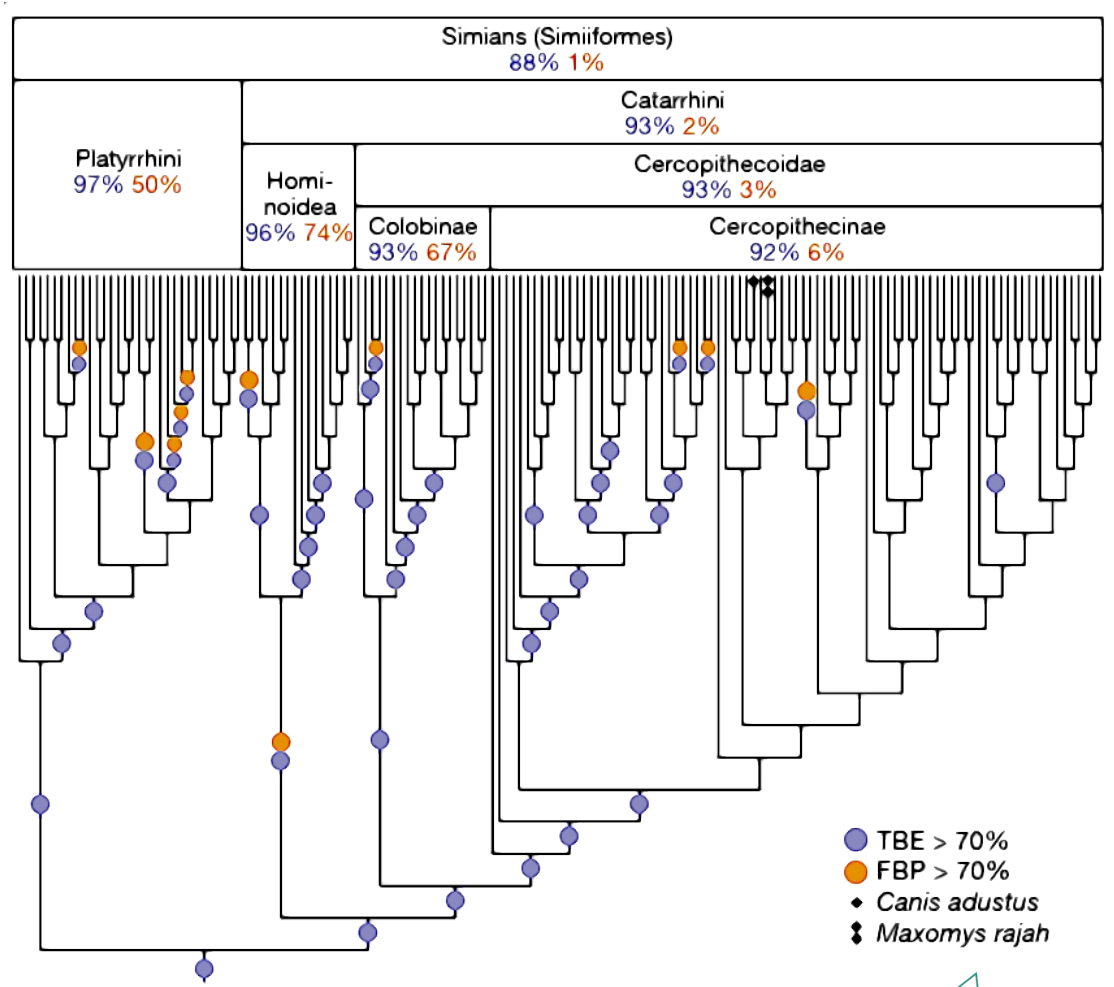
Transfer Bootstrap Expectation (TBE)

ref tree	replicate1	replicate2	replicate3	replicate4
				
FBP	1	0	0	0
transfer index	**0**	**3**	**1**	**2**

Interpretation

If TBE of branch is 95%, it means that on average 5% of leaves have to be removed from bootstrap samples.

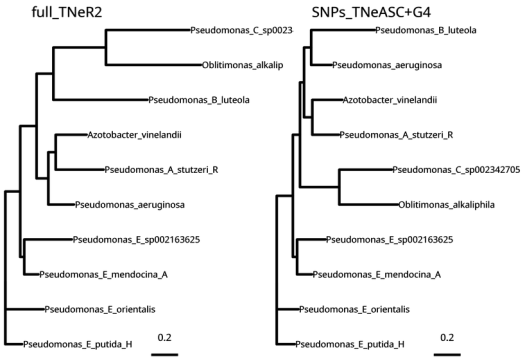
If FBP of branch is 95%, it means that 5% of the bootstrap replicate trees didn't have this split.



doi:10.1038/s41586-018-0043-0

Fig. 3 | FBP and TBE bootstrap supports on the simian clade.

Revisiting data set 1 (full alignment) with RAxML



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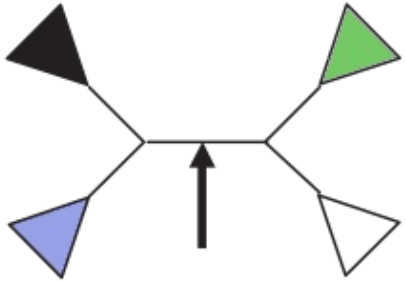
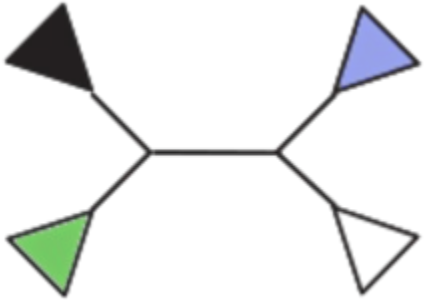
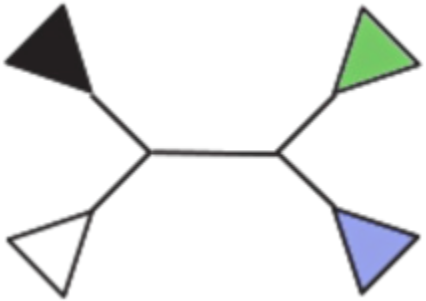
Alternative

UFBoot (ultra fast bootstrap): at every internal branch, bootstraps around the two other competing NNI trees.







see also “concordance factors”:
doi:10.1093/molbev/msaa106 (IQTREE)
doi:10.1093/molbev/msw040 (RAxML)

curiosity: MCMC convergence

Posterior samples of trees or parameters in Bayesian Phylogenetics

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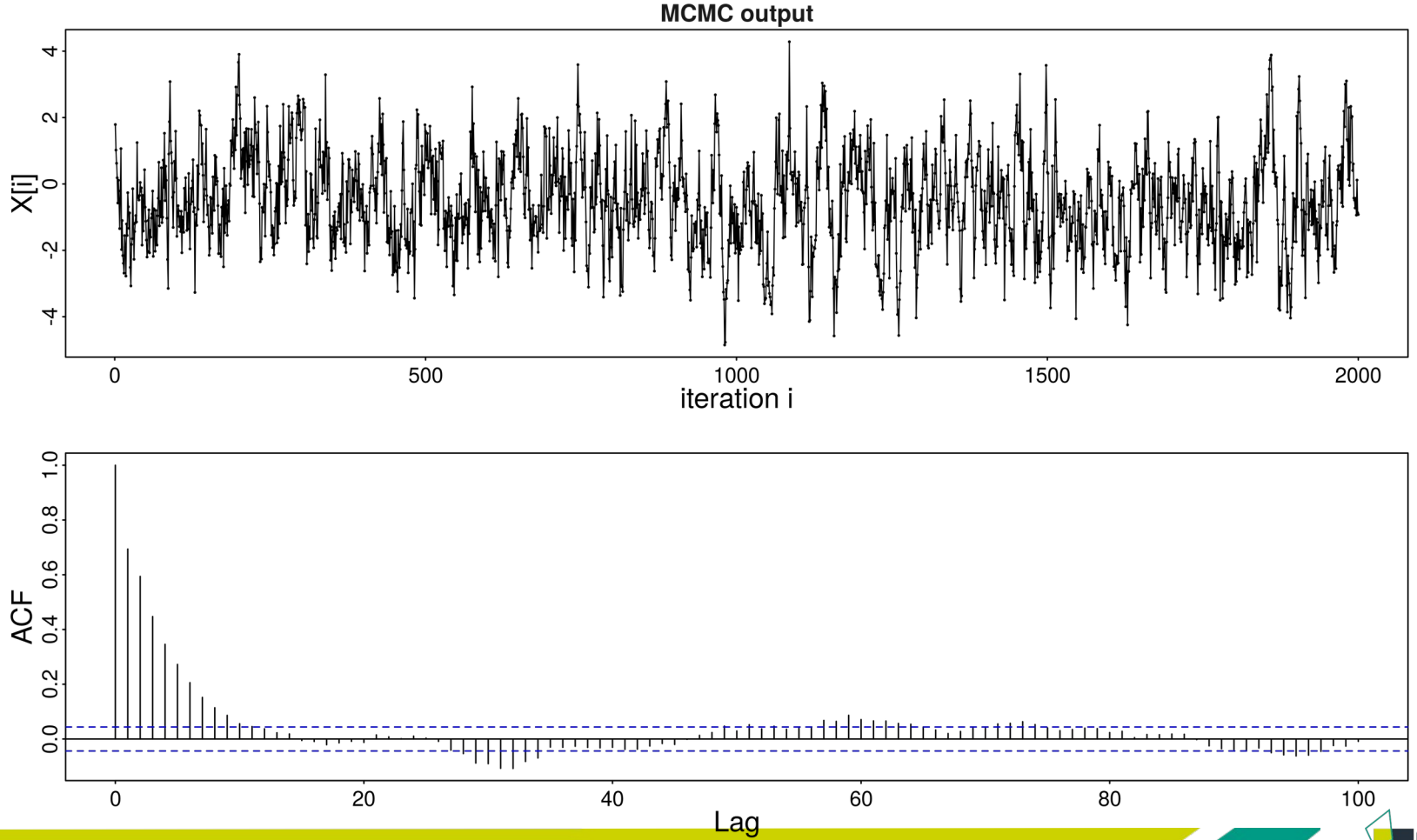
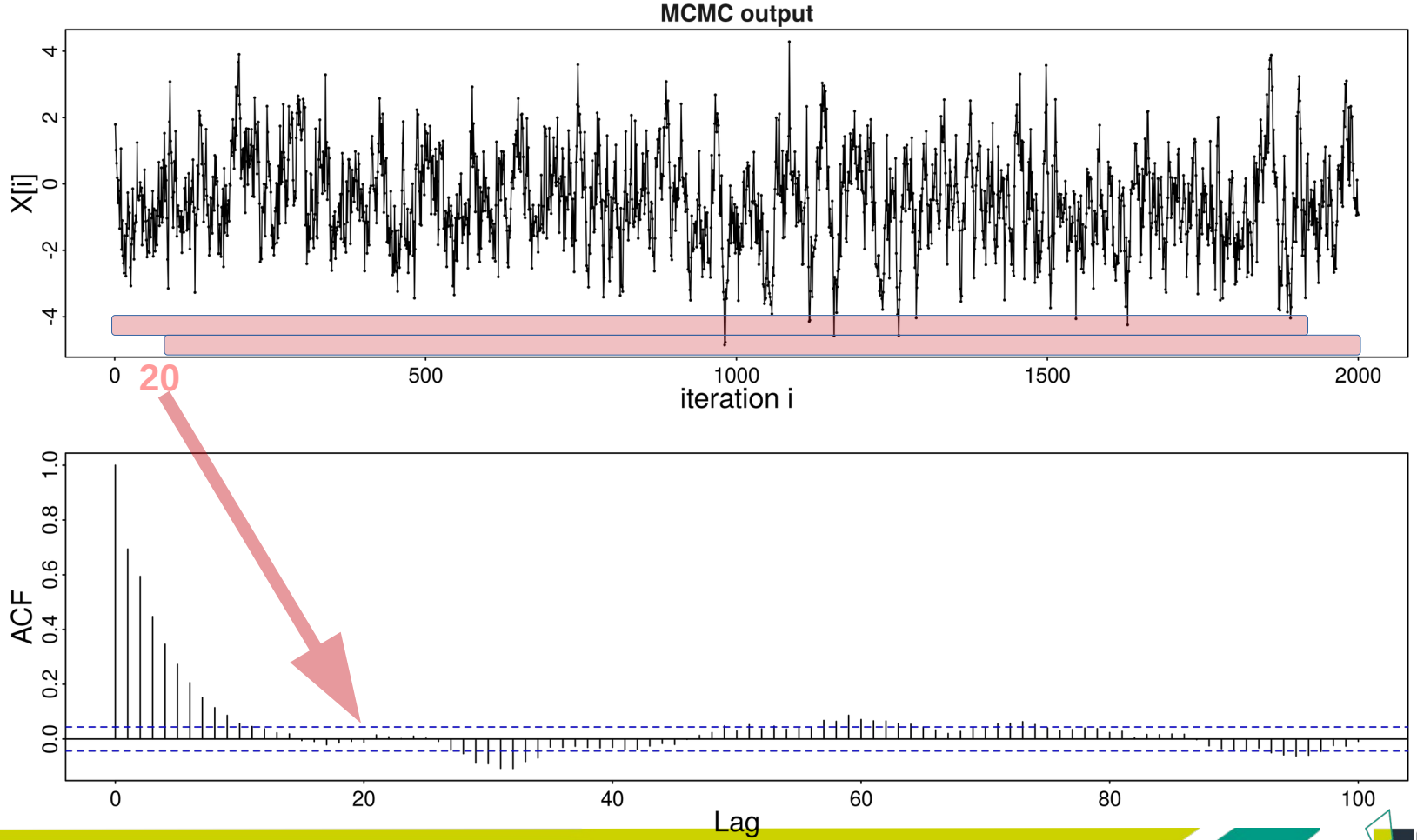
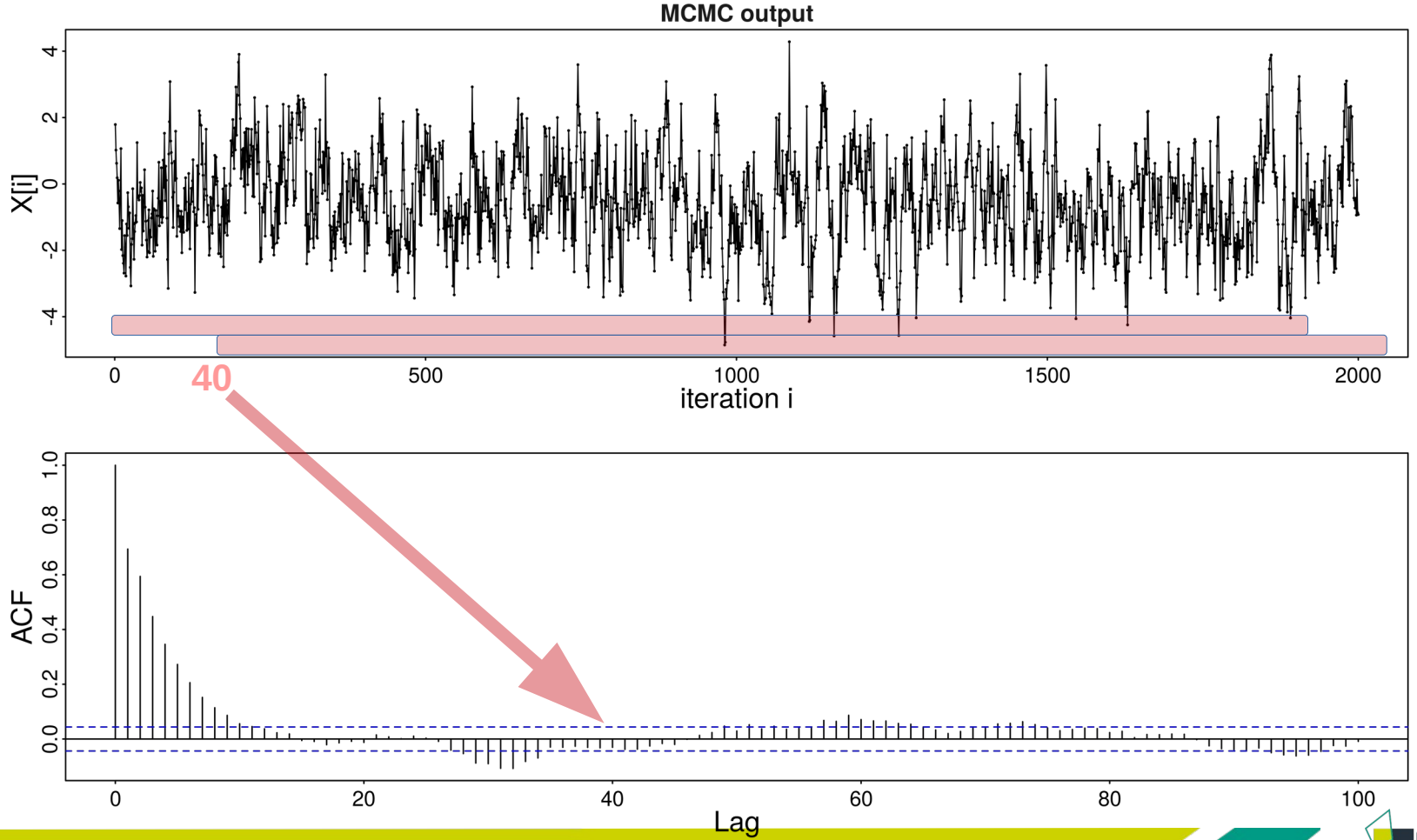
Markov chain Monte Carlo samples are correlated



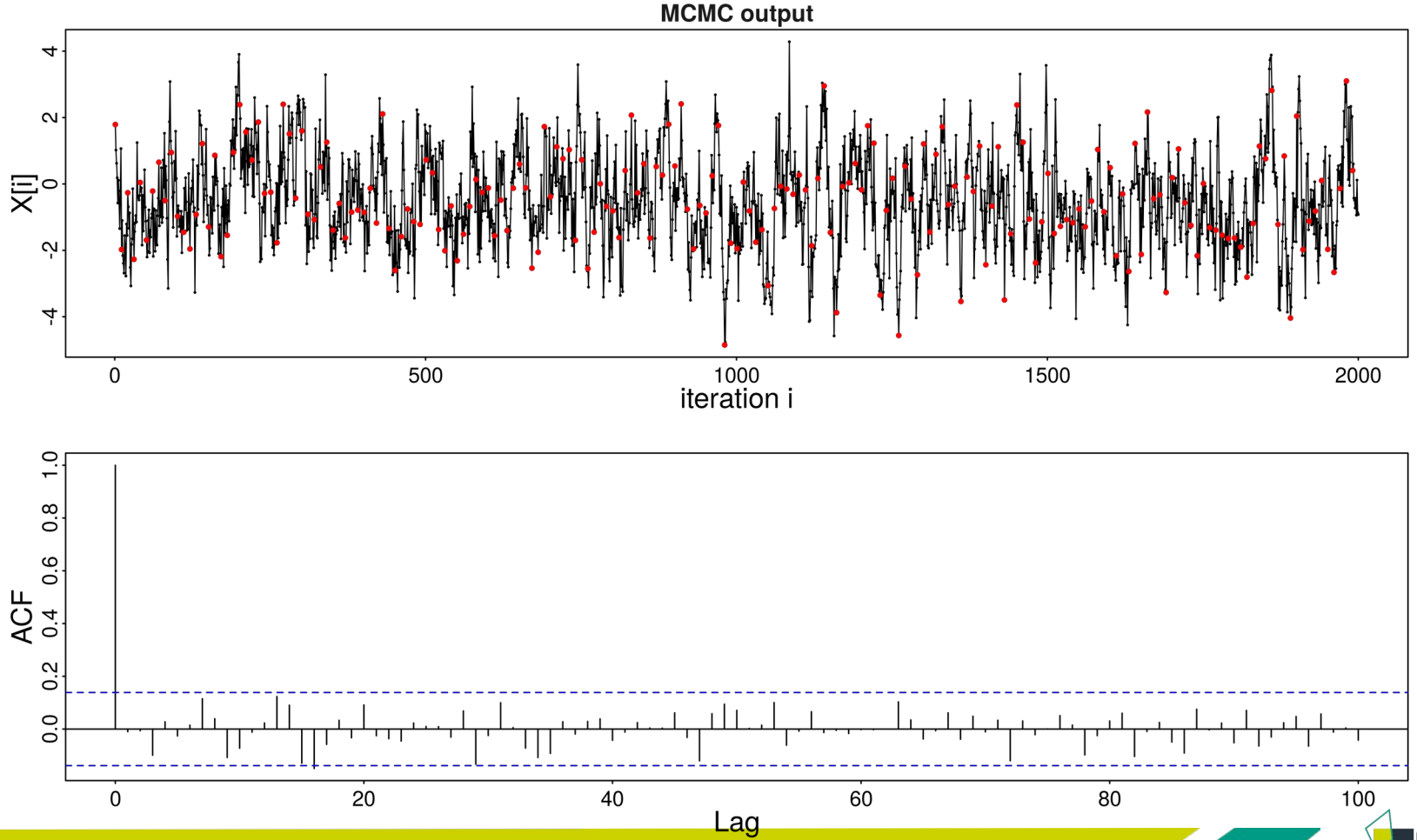
What is the autocorrelation function (ACF)?



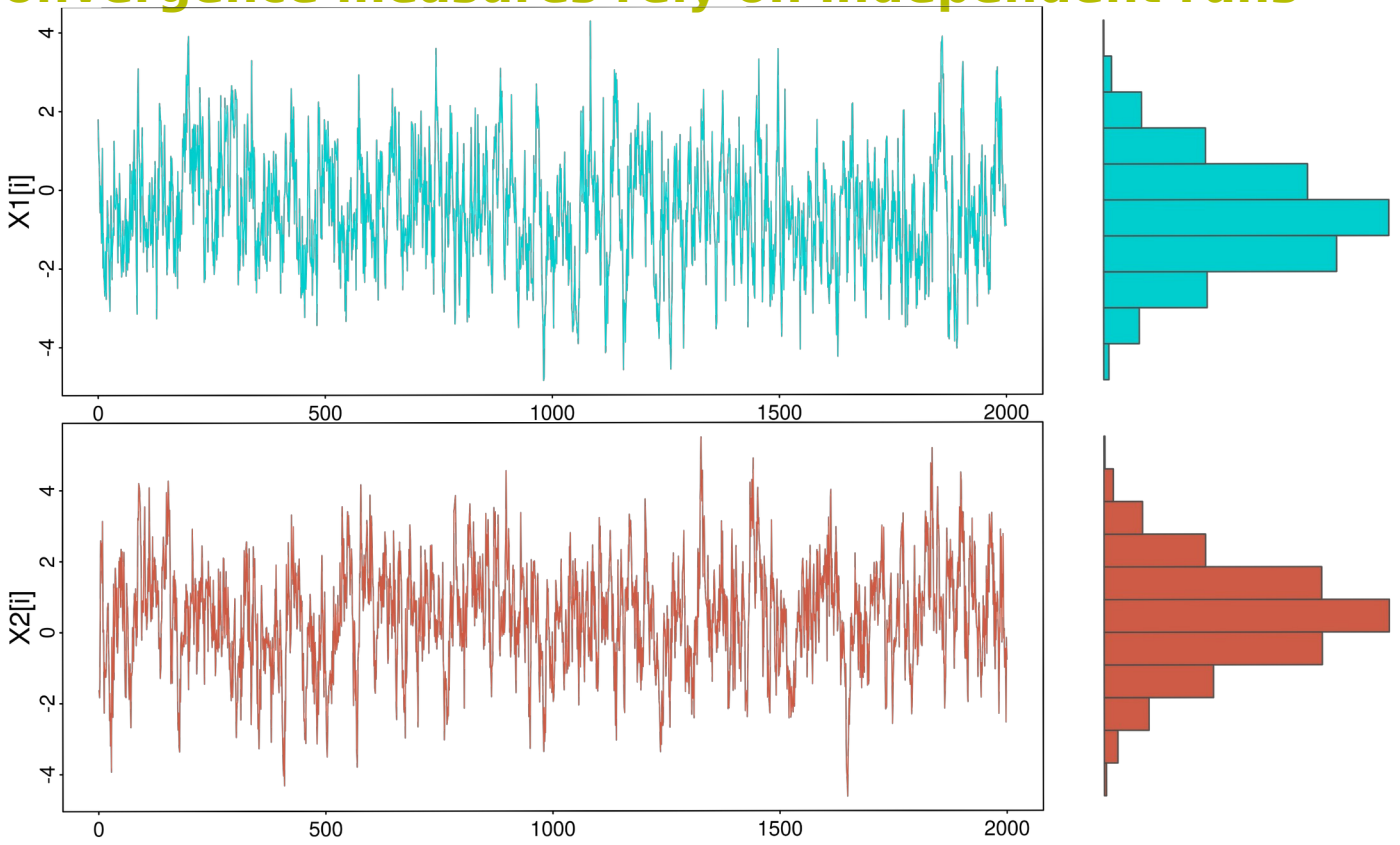
What is the autocorrelation function (ACF)?



Thinning (shown) and burn-in (not shown) reduce correlation



Other convergence measures rely on independent runs



ACF, scaled regeneration quantile plot, potential scale reduction factor (PSRF)

software: RWTY and AWTY

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Maximum Likelihood Inference

IQTREE2 line by line

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```
IQ-TREE multicore version 2.2.2.7 COVID-edition for Linux 64-bit built Jun  7 2023
Developed by Bui Quang Minh, James Barbetti, Nguyen Lam Tung,
Olga Chernomor, Heiko Schmidt, Dominik Schrempf, Michael Woodhams, Ly Trong Nhan.

Host:    N114312.nbi.ac.uk (AVX2, FMA3, 15 GB RAM)
Command: iqtree2 --ninit 1000 -t PARS -s 08.full_align.aln --prefix o
Seed:    994063 (Using SPRNG - Scalable Parallel Random Number Generator)
Time:    Wed Nov  8 13:51:34 2023
Kernel:  AVX+FMA - 1 threads (8 CPU cores detected)

HINT: Use -nt option to specify number of threads because your CPU has 8 cores!
HINT: -nt AUTO will automatically determine the best number of threads to use.

Reading alignment file 08.full_align.aln ... Fasta format detected
Reading fasta file: done in 0.000737228 secs using 62.67% CPU
Alignment most likely contains DNA/RNA sequences
Constructing alignment: done in 0.00205722 secs using 70% CPU
Alignment has 10 sequences with 1455 columns, 615 distinct patterns
564 parsimony-informative, 235 singleton sites, 656 constant sites
                         Gap/Ambiguity  Composition  p-value
Analyzing sequences: done in 3.0332e-05 secs
   1  Pseudomonas_E_putida_H      20.69%    passed     15.91%
   2  Pseudomonas_E_orientalis    45.09%    passed     60.31%
   3  Pseudomonas_A_stutzeri_R    45.09%    failed      0.01%
   4  Pseudomonas_E_mendocina_A   20.69%    passed     49.21%
   5  Oblitimonas_alkaliphila     45.09%    failed      0.00%
   6  Pseudomonas_aeruginosa      24.40%    failed      0.00%
   7  Pseudomonas_E_sp002163625   45.09%    passed     23.84%
   8  Pseudomonas_B_luteola       45.09%    failed      0.00%
   9  Azotobacter_vinelandii      24.40%    failed      0.00%
  10  Pseudomonas_C_sp002342705   45.09%    failed      0.00%
****  TOTAL                       36.07%  6 sequences failed composition chi2 test (p-value<5%; df=3)
Checking for duplicate sequences: done in 4.8551e-05 secs
```

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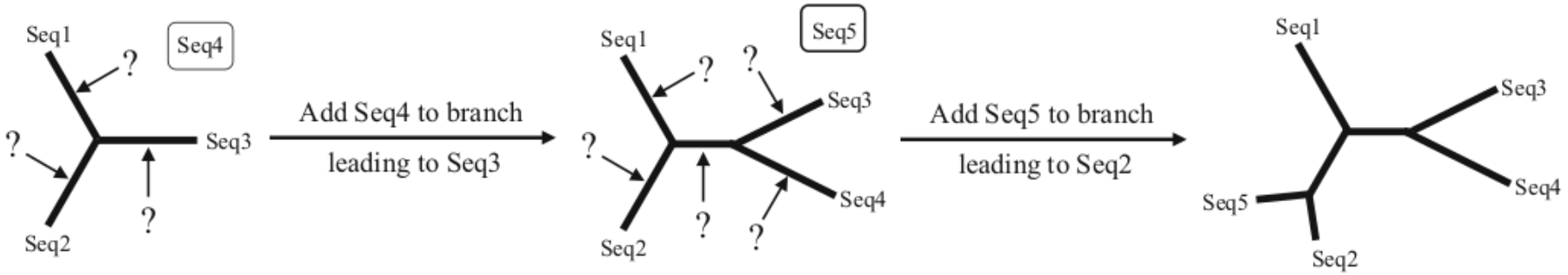
```
Creating fast initial parsimony tree by random order stepwise addition...
0.001 seconds, parsimony score: 1923 (based on 799 sites)
Perform fast likelihood tree search using GTR+I+G model...
Estimate model parameters (epsilon = 5.000)
Perform nearest neighbor interchange...
Optimizing NNI: done in 0.00406809 secs using 99.95% CPU
Estimate model parameters (epsilon = 1.000)
1. Initial log-likelihood: -8801.377
2. Current log-likelihood: -8799.214
3. Current log-likelihood: -8797.933
Optimal log-likelihood: -8797.173
Rate parameters:  A-C: 0.61481  A-G: 2.99951  A-T: 0.94860  C-G: 0.58165  C-T: 4.12668  G-T: 1.00000
Base frequencies:  A: 0.203  C: 0.310  G: 0.243  T: 0.244
Proportion of invariable sites: 0.002
Gamma shape alpha: 6.268
Parameters optimization took 3 rounds (0.014 sec)
Time for fast ML tree search: 0.102 seconds

NOTE: ModelFinder requires 2 MB RAM!
ModelFinder will test up to 484 DNA models (sample size: 1455) ...
 No. Model         -LnL         df  AIC          AICc         BIC
  1  GTR+F         8805.128     25  17660.256    17661.166    17792.325
  2  GTR+F+I       8805.126     26  17662.252    17663.235    17799.604
  3  GTR+F+G4      8796.062     26  17644.124    17645.107    17781.476
  4  GTR+F+I+G4    8796.199     27  17646.399    17647.458    17789.033
  5  GTR+F+R2      8767.610     27  17589.221    17590.280    17731.855
  6  GTR+F+R3      8767.525     29  17593.051    17594.272    17746.251
```

```
Creating fast initial parsimony tree by random order stepwise addition...
0.001 seconds, parsimony score: 1923 (based on 799 sites)
Perform fast likelihood tree search using GTR+I+G model...
Estimate model parameters (epsilon = 5.000)
```




```
Gamma shape alpha: 6.268
Parameters optimization took 3 rounds (0.014 sec)
Time for fast ML tree search: 0.102 seconds

NOTE: ModelFinder requires 2 MB RAM!
ModelFinder will test up to 484 DNA models (sample size: 1455) ...
 No. Model         -LnL         df  AIC          AICc         BIC
  1  GTR+F         8805.128     25  17660.256    17661.166    17792.325
  2  GTR+F+I       8805.126     26  17662.252    17663.235    17799.604
  3  GTR+F+G4      8796.062     26  17644.124    17645.107    17781.476
  4  GTR+F+I+G4    8796.199     27  17646.399    17647.458    17789.033
  5  GTR+F+R2      8767.610     27  17589.221    17590.280    17731.855
  6  GTR+F+R3      8767.525     29  17593.051    17594.272    17746.251
```

doi:10.1007/978-1-4939-6622-6_14

```
Creating fast initial parsimony tree by random o
0.001 seconds, parsimony score: 1923 (based on 7
Perform fast likelihood tree search using GTR+I+
Estimate model parameters (epsilon = 5.000)
Perform nearest neighbor interchange...
Optimizing NNI: done in 0.00406809 secs using 99
Estimate model parameters (epsilon = 1.000)
1. Initial log-likelihood: -8801.377
2. Current log-likelihood: -8799.214
3. Current log-likelihood: -8797.933
Optimal log-likelihood: -8797.173
Rate parameters:  A-C: 0.61481  A-G: 2.99951  A-
Base frequencies:  A: 0.203  C: 0.310  G: 0.243
Proportion of invariable sites: 0.002
Gamma shape alpha: 6.268
Parameters optimization took 3 rounds (0.014 sec
Time for fast ML tree search: 0.102 seconds

NOTE: ModelFinder requires 2 MB RAM!
ModelFinder will test up to 484 DNA models (samp
 No. Model         -LnL         df  AIC
  1  GTR+F         8805.128     25  17660.256
  2  GTR+F+I       8805.126     26  17662.252
  3  GTR+F+G4      8796.062     26  17644.124
  4  GTR+F+I+G4    8796.199     27  17646.399
  5  GTR+F+R2      8767.610     27  17589.221
  6  GTR+F+R3      8767.525     29  17593.051
```

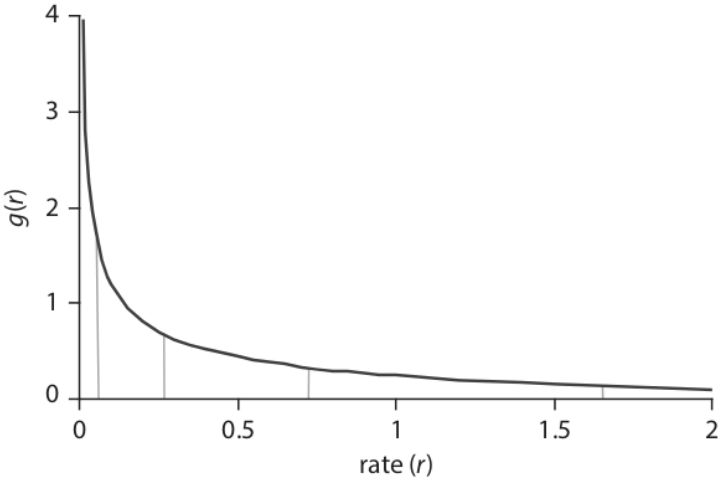


Fig. 4.9 The discrete gamma model of variable rates across sites uses K equal-probability categories to approximate the continuous gamma distribution, with the mean rate in each category used to represent all rates in that category. Shown here is the use of $K = 5$ categories to approximate the gamma density $g(r)$ with $\alpha = 0.5$ (and mean 1). The four vertical lines are at $r = 0.06418,\ 0.27500,\ 0.70833,$ and 1.64237. These are the 20%, 40%, 60%, and 80% percentiles of the distribution and cut the density into five categories, each of proportion 1/5. The mean rates in the five categories are 0.02121, 0.15549, 0.46708, 1.10712, and 3.24910.

1.00000

from http://abacus.gene.ucl.ac.uk/MESA/

```
Akaike Information Criterion:           TIM3e+R2
Corrected Akaike Information Criterion: TIM3e+R2
Bayesian Information Criterion:         TNe+R2
Best-fit model: TNe+R2 chosen according to BIC

All model information printed to o.model.gz
CPU time for ModelFinder: 4.589 seconds (0h:0m:4s)
Wall-clock time for ModelFinder: 4.685 seconds (0h:0m:4s)

NOTE: 0 MB RAM (0 GB) is required!
Estimate model parameters (epsilon = 0.100)
1. Initial log-likelihood: -8752.796
Optimal log-likelihood: -8752.794
Rate parameters:  A-C: 1.00000  A-G: 4.15899  A-T: 1.00000  C-G: 1.00000  C-T: 7.21407  G-T: 1.00000
Base frequencies:  A: 0.250  C: 0.250  G: 0.250  T: 0.250
Site proportion and rates:  (0.950,0.622) (0.050,8.129)
Parameters optimization took 1 rounds (0.004 sec)
Wrote distance file to...
Computing ML distances based on estimated model parameters...
Calculating distance matrix: done in 0.000293082 secs using 97.58% CPU
Computing ML distances took 0.000417 sec (of wall-clock time) 0.000413 sec (of CPU time)
Setting up auxiliary I and S matrices: done in 5.2725e-05 secs using 94.83% CPU
Constructing RapidNJ tree: done in 0.000119629 secs using 96.97% CPU
Computing RapidNJ tree took 0.000281 sec (of wall-clock time) 0.000276 sec (of CPU time)
Log-likelihood of RapidNJ tree: -8759.295
--------------------------------------------------------------------
|             INITIALIZING CANDIDATE TREE SET                      |
--------------------------------------------------------------------
```

```
Akaike Information Criterion:           TIM3e+R2
Corrected Akaike Information Criterion: TIM3e+R2
Bayesian Information Criterion:         TNe+R2
Best-fit model: TNe+R2

All model information p
CPU time for ModelFinde
Wall-clock time for Mod

NOTE: 0 MB RAM (0 GB) i
Estimate model paramete
1. Initial log-likeliho
Optimal log-likelihood:
Rate parameters:  A-C:
Base frequencies:  A: 0
Site proportion and rat
Parameters optimization
Wrote distance file to.
Computing ML distances
Calculating distance ma
Computing ML distances
Setting up auxiliary I
Constructing RapidNJ tr
Computing RapidNJ tree
Log-likelihood of Rapid
-----------------------
|             INITIALIZ
-----------------------
```

Model	df	Explanation	Code
F81	3	Equal rates but unequal base freq. (Felsenstein, 1981).	000000
K80 or K2P	1	Unequal transition/transversion rates and equal base freq. (Kimura, 1980).	010010
TN or TN93	5	Like `HKY` but unequal purine/pyrimidine rates (Tamura and Nei, 1993).	010020
TNe	2	Like `TN` but equal base freq.	010020
K81 or K3P	2	Three substitution types model and equal base freq. (Kimura, 1981).	012210
K81u	5	Like `K81` but unequal base freq.	012210
TPM2	2	AC=AT, AG=CT, CG=GT and equal base freq.	010212
TPM2u	5	Like `TPM2` but unequal base freq.	010212
TPM3	2	AC=CG, AG=CT, AT=GT and equal base freq.	012012
TPM3u	5	Like `TPM3` but unequal base freq.	012012
TIM	6	Transition model, AC=GT, AT=CG and unequal base freq.	012230
TIMe	3	Like `TIM` but equal base freq.	012230
TIM2	6	AC=AT, CG=GT and unequal base freq.	010232
TIM2e	3	Like `TIM2` but equal base freq.	010232
TIM3	6	AC=CG, AT=GT and unequal base freq.	012032
TIM3e	3	Like `TIM3` but equal base freq.	012032
GTR	8	General time reversible model with unequal rates and unequal base freq. (Tavare, 1986).	012345

The last column `Code` is a 6-digit code defining the equality constraints for 6 *relative* substitution rates: A-C, A-G, A-T, C-G, C-T and G-T.

```
Akaike Information Criterion:           TIM3e+R2
Corrected Akaike Information Criterion: TIM3e+R2
Bayesian Information Criterion:         TNe+R2
Best-fit
All mode
CPU time
Wall-clo
NOTE: 0
Estimate
1. Initi
Optimal
Rate par
Base fre
Site pro
Paramete
Wrote di
Computin
Calculat
Computin
Setting
Construc
Computin
Log-like
--------
|
--------
```

Table 1.1 Substitution rate matrices for commonly used Markov models of nucleotide substitution

	p	From	To T	To C	To A	To G
JC69 (Jukes and Cantor 1969)	1	T	·	λ	λ	λ
		C	λ	·	λ	λ
		A	λ	λ	·	λ
		G	λ	λ	λ	·
TN93 (Tamura and Nei 1993)	6	T	·	$\alpha_1\pi_C$	$\beta\pi_A$	$\beta\pi_G$
		C	$\alpha_1\pi_T$	·	$\beta\pi_A$	$\beta\pi_G$
		A	$\beta\pi_T$	$\beta\pi_C$	·	$\alpha_2\pi_G$
		G	$\beta\pi_T$	$\beta\pi_C$	$\alpha_2\pi_A$	·
GTR (REV) (Tavaré 1986; Yang 1994b; Zharkikh 1994)	9	T	·	$a\pi_C$	$b\pi_A$	$c\pi_G$
		C	$a\pi_T$	·	$d\pi_A$	$e\pi_G$
		A	$b\pi_T$	$d\pi_C$	·	$f\pi_G$
		G	$c\pi_T$	$e\pi_C$	$f\pi_A$	·

from http://abacus.gene.ucl.ac.uk/MESA/

```
Akaike Information Criterion:           TIM3e+R2
Corrected Akaike Information Criterion: TIM3e+R2
Bayesian Information Criterion:         TNe+R2
Best-fit model: TNe+R2 chosen according to BIC

All model information printed to o.model.gz
CPU time for ModelFinder: 4.589 seconds (0h:0m:4s)
Wall-clock time for ModelFinder: 4.685 seconds (0h:0m:4s)

NOTE: 0 MB RAM (0 GB) is required!
Estimate model parameters (epsilon = 0.100)
1. Initial log-likelihood: -8752.796
Optimal log-likelihood: -8752.794
Rate parameters:  A-C: 1.00000  A-G: 4.15899  A-T: 1.00000  C-G: 1.00000  C-T: 7.21407  G-T: 1.00000
Base frequencies:  A: 0.250  C: 0.250  G: 0.250  T: 0.250
Site proportion and rates:  (0.950,0.622) (0.050,8.129)
Parameters optimization took 1 rounds (0.004 sec)
```

Model	df	Explanation	Code
TNe	2	Like `TN` but equal base freq.	010020

The last column `Code` is a 6-digit code defining the equality constraints for 6 *relative* substitution rates: A-C, A-G, A-T, C-G, C-T and G-T.

```
Log-likelihood of RapidNJ tree: -8759.295
--------------------------------------------------------------------
|             INITIALIZING CANDIDATE TREE SET                      |
--------------------------------------------------------------------
```

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```
--------------------------------------------------------------------
|             INITIALIZING CANDIDATE TREE SET                      |
--------------------------------------------------------------------
Generating 998 parsimony trees... 0.777 second
Computing log-likelihood of 198 initial trees ... 0.169 seconds
Current best score: -8752.794

Do NNI search on 20 best initial trees
Optimizing NNI: done in 0.00232697 secs using 95.32% CPU
Estimate model parameters (epsilon = 0.100)
BETTER TREE FOUND at iteration 1: -8752.794
Optimizing NNI: done in 0.00581815 secs using 99.96% CPU
Optimizing NNI: done in 0.0056042 secs using 99.98% CPU
Optimizing NNI: done in 0.00809975 secs using 99.93% CPU
Optimizing NNI: done in 0.00765216 secs using 99.96% CPU
Optimizing NNI: done in 0.0058694 secs using 40.98% CPU
```

```
Iteration 10 / LogL: -8753.261 / Time: 0h:0m:1s
```

```
Optimizing NNI: done in 0.00756817 secs using 99.98% CPU
Optimizing NNI: done in 0.00564065 secs using 99.97% CPU
Iteration 20 / LogL: -8754.235 / Time: 0h:0m:1s
Finish initializing candidate tree set (2)
Current best tree score: -8752.794 / CPU time: 1.101
Number of iterations: 20
--------------------------------------------------------------------
|               OPTIMIZING CANDIDATE TREE SET                      |
--------------------------------------------------------------------
Optimizing NNI: done in 0.0116323 secs using 99.99% CPU
Optimizing NNI: done in 0.00910824 secs using 99.99% CPU
```

```
Iteration 100 / LogL: -8753.231 / Time: 0h:0m:1s (0h:0m:0s left)
Optimizing NNI: done in 0.0105482 secs using 62.7% CPU
Optimizing NNI: done in 0.0139313 secs using 99.82% CPU
TREE SEARCH COMPLETED AFTER 102 ITERATIONS / Time: 0h:0m:1s

--------------------------------------------------------------------
|                    FINALIZING TREE SEARCH                        |
--------------------------------------------------------------------
Performs final model parameters optimization
Estimate model parameters (epsilon = 0.010)
1. Initial log-likelihood: -8752.794
Optimal log-likelihood: -8752.794
Rate parameters:  A-C: 1.00000  A-G: 4.16143  A-T: 1.00000  C-G: 1.00000  C-T: 7.22268  G-T: 1.00000
Base frequencies:  A: 0.250  C: 0.250  G: 0.250  T: 0.250
Site proportion and rates:  (0.950,0.621) (0.050,8.155)
Parameters optimization took 1 rounds (0.004 sec)
BEST SCORE FOUND : -8752.794
Total tree length: 4.635

Total number of iterations: 102
CPU time used for tree search: 1.673 sec (0h:0m:1s)
Wall-clock time used for tree search: 1.900 sec (0h:0m:1s)
Total CPU time used: 1.700 sec (0h:0m:1s)
Total wall-clock time used: 1.925 sec (0h:0m:1s)

Analysis results written to:
  IQ-TREE report:                o.iqtree
  Maximum-likelihood tree:       o.treefile
  Likelihood distances:          o.mldist
  Screen log file:               o.log
```

Identical sequences are identified and sometimes removed during inference

```
NOTE: NZ_CP041754.1 is identical to NC_005773.3 but kept for subsequent analysis
NOTE: NZ_CP026676.1 is identical to NC_022738.1 but kept for subsequent analysis
NOTE: NZ_CP014343.1 is identical to NZ_CP023299.1 but kept for subsequent analysis
NOTE: NZ_CP008863.1 is identical to NZ_LT608330.1 but kept for subsequent analysis
NOTE: NZ_CP061848.1 is identical to NC_019905.1 but kept for subsequent analysis
NOTE: NZ_CP045359.1 is identical to NZ_CP045349.1 but kept for subsequent analysis
NOTE: NZ_CP030750.1 is identical to NZ_CP011789.1 but kept for subsequent analysis
NOTE: NZ_CP011110.1 is identical to NZ_CP010892.1 but kept for subsequent analysis
NOTE: NZ_LR130527.1 is identical to NC_017548.1 but kept for subsequent analysis
NOTE: NZ_CP033439.1 is identical to NZ_CP070467.1 but kept for subsequent analysis
```

```
Checking for duplicate sequences: done in 0.00459436 secs using 89.39% CPU
Identifying sites to remove: done in 0.00152092 secs using 99.87% CPU
NOTE: 327 identical sequences (see below) will be ignored for subsequent analysis
NOTE: NC_017911.1 (identical to NZ_CP017296.1) is ignored but added at the end
```

```
NOTE: NZ_CP027721.1 (identical to NZ_CP027722.1) is ignored but added at the end
NOTE: NZ_CP031659.1 (identical to NZ_CP013989.1) is ignored but added at the end
NOTE: NZ_CP029088.1 (identical to NZ_CP013989.1) is ignored but added at the end
NOTE: NZ_CP010555.1 (identical to NZ_CP013989.1) is ignored but added at the end
NOTE: NZ_CP050323.1 (identical to NZ_CP013989.1) is ignored but added at the end
NOTE: NZ_CP065866.1 (identical to NZ_CP013989.1) is ignored but added at the end
NOTE: NZ_CP058332.1 (identical to NZ_CP013989.1) is ignored but added at the end
NOTE: NZ_LR739068.1 (identical to NZ_CP013989.1) is ignored but added at the end
NOTE: NZ_CP046069.1 (identical to NZ_CP013989.1) is ignored but added at the end
NOTE: NZ_CP025053.1 (identical to NZ_CP013989.1) is ignored but added at the end
```

File {prefix}.iqtree has some more information and important notices

```
Input data: 700 sequences with 445 nucleotide sites
Number of constant sites: 188 (= 42.2472% of all sites)
Number of invariant (constant or ambiguous constant) sites: 188 (= 42.2472% of all sites)
Number of parsimony informative sites: 246
Number of distinct site patterns: 266
```

```
Total tree length (sum of branch lengths): 11.2056
Sum of internal branch lengths: 6.7741 (60.4527% of tree length)

WARNING: 117 near-zero internal branches (<0.0022) should be treated with caution
         Such branches are denoted by '**' in the figure below

NOTE: Tree is UNROOTED although outgroup taxon 'NZ_CP017296.1' is drawn at root
```

Thank you

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