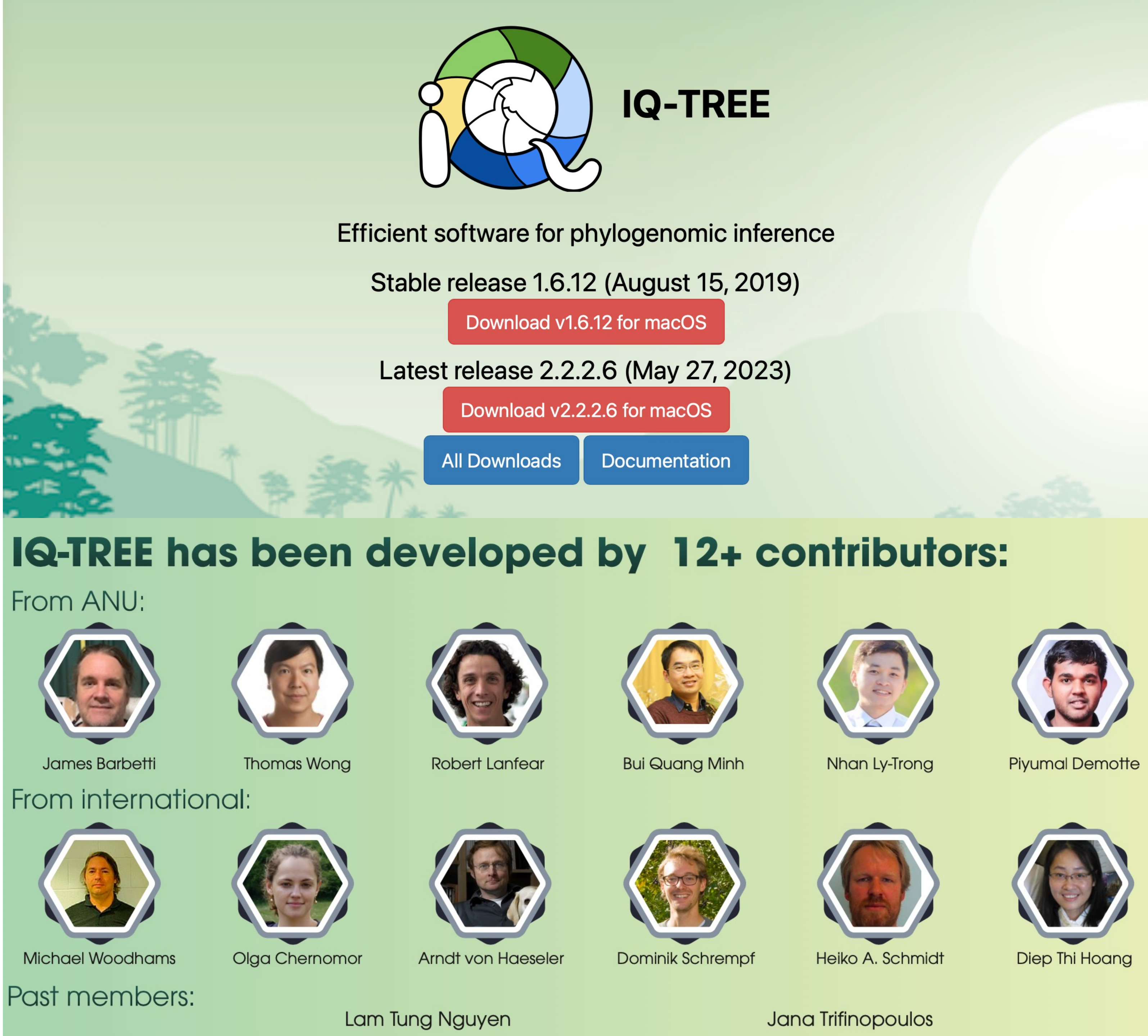


IQ-TREE

Efficient software for phylogenomic inference

Stable release 1.6.12 (August 15, 2019)

Latest release 2.2.2.6 (May 27, 2023)

IQ-TREE has been developed by 12+ contributors:

From ANU:

James Barbetti, Thomas Wong, Robert Lanfear, Bui Quang Minh, Nhan Ly-Trong, Piyumal Demotte

From international:

Michael Woodhams, Olga Chernomor, Arndt von Haeseler, Dominik Schrempf, Heiko A. Schmidt, Diep Thi Hoang

Past members:

Lam Tung Nguyen, Jana Trifinopoulos

IQ-TREE enables to infer phylogenetic trees of "SARS-CoV-2" virus

For identifying new variants and key mutations for vaccine design

Sequencing

DNA Sequence Alignment

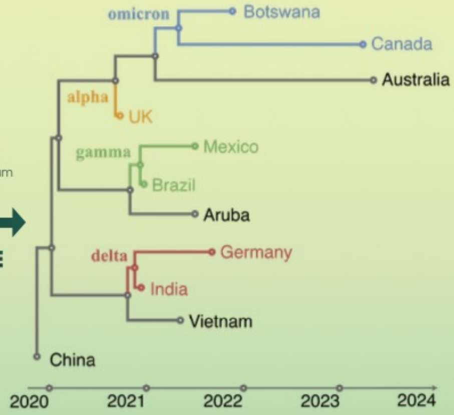
Canada	ATGTTTGTTTTTC....TCT....CGTTC....CATA....TAA
Botswana	ATGTTTGTTTTTC....TCT....AGTTC....CATA....TAA
Australia	ATGTTTGTTTTTC....TCT....AGTTC....CATC....TAA
UK	ATGTTTGTTTTTC....TCT....AGTTC....CACC....TAA
Mexico	ATGTTTGTTTTTC....TTT....AGTTC....TGCC....TAA
Brazil	ATGTTTGTTTTTC....TTT....AGTTC....TACC....TAA
Aruba	ATGTTTGTTTTTC....TCT....AGTTC....TACC....TAA
Germany	ATGTTTGTTTTTC....TCA....GAGTT....TACC....TAA
India	ATGTTTGTTTTTC....TCT....GAGTT....TACC....TAA
Vietnam	ATGTTTGTTTTTC....TCT....GATTT....TACC....TAA
China	ATGTTTGTTTTTC....TCT....AGTTT....TACC....TAA

IQ-TREE

Small & Medium alignments

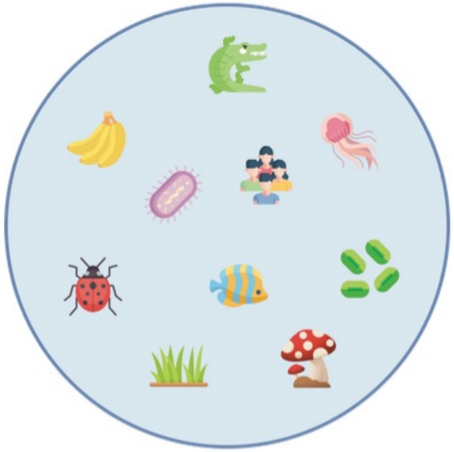
CMAPLE

Very large alignments



Source: nextstrain.org

IQ-TREE enables to infer the origins of life on earth

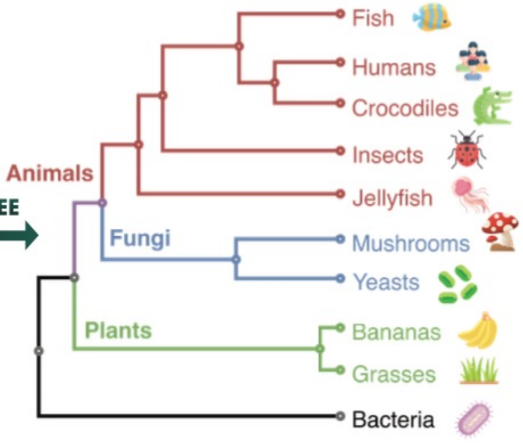


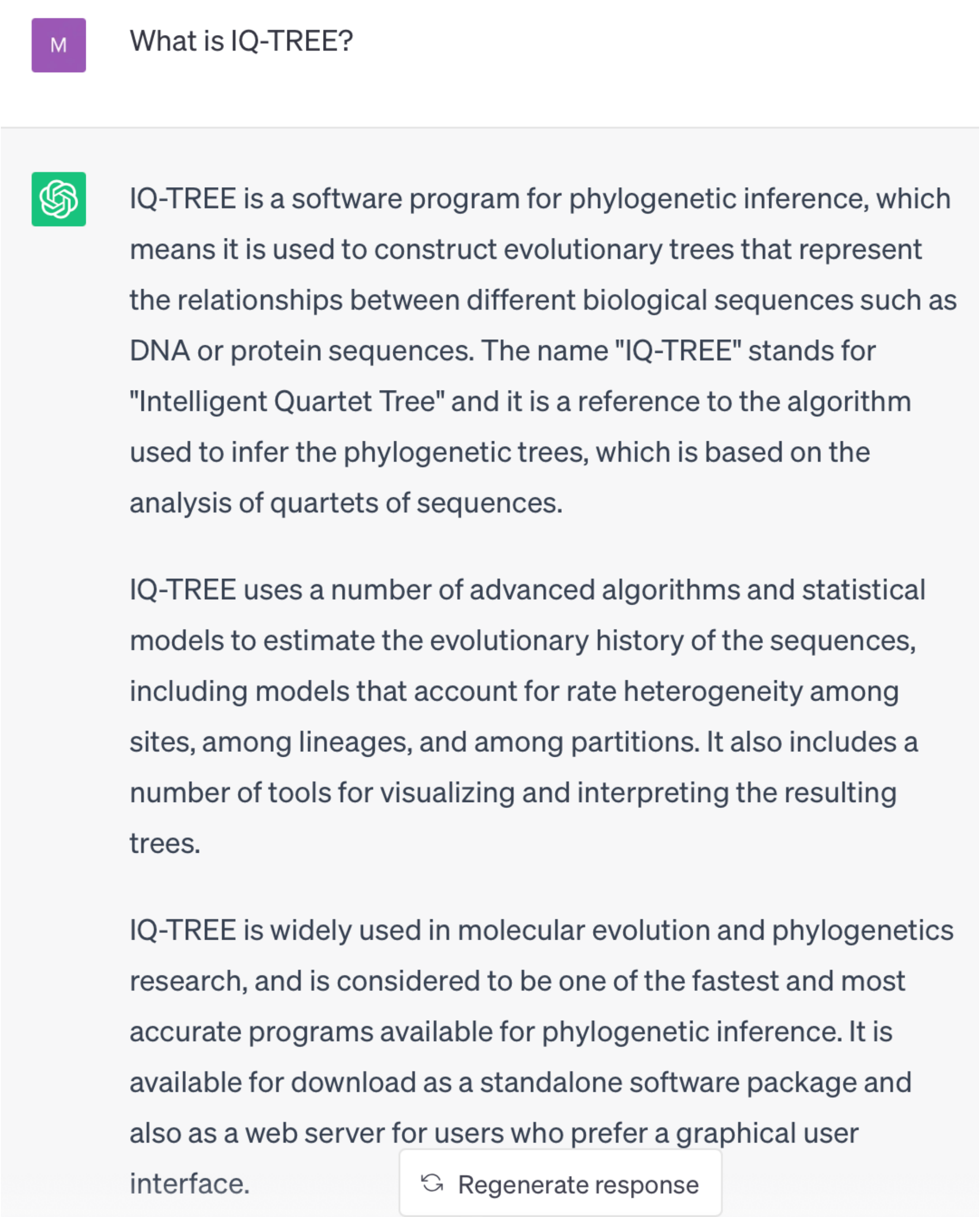
Sequencing

Protein Sequence Alignment

Humans	RMHYD....LIHTS....LARVV....FTLEYGYS....EYRS
Crocodiles	RMHYD....LIHTS....LARRV....FTLEYGYS....EYRS
Fish	RMHYD....LIHTS....LARRV....FTQEYGTS....EYRS
Insects	RMHYD....LIHTS....LARRV....FTLEYGTS....EYYS
Jellyfish	RMHYD....LIHTS....LARRV....FTLEYGTS....EYRS
Mushrooms	RMHYD....LLNRS....LARRV....FTLEYGTS....EYRS
Yeasts	RMHYD....LINRS....LARRV....FTLEYGTS....EYRS
Bananas	RMHYD....LIHTS....LASRV....FTLRYGTS....EYRS
Grasses	RMHYD....LIHTS....LASRV....FTLEYGTS....EYRS
Bacteria	RMHHD....LIHTS....LARRV....FTLEYGTS....EYRS

IQ-TREE





What is IQ-TREE?

IQ-TREE is a software program for phylogenetic inference, which means it is used to construct evolutionary trees that represent the relationships between different biological sequences such as DNA or protein sequences. The name "IQ-TREE" stands for "Intelligent Quartet Tree" and it is a reference to the algorithm used to infer the phylogenetic trees, which is based on the analysis of quartets of sequences.

IQ-TREE uses a number of advanced algorithms and statistical models to estimate the evolutionary history of the sequences, including models that account for rate heterogeneity among sites, among lineages, and among partitions. It also includes a number of tools for visualizing and interpreting the resulting trees.

IQ-TREE is widely used in molecular evolution and phylogenetics research, and is considered to be one of the fastest and most accurate programs available for phylogenetic inference. It is available for download as a standalone software package and also as a web server for users who prefer a graphical user interface.

Typical phylogenetic analysis under maximum likelihood

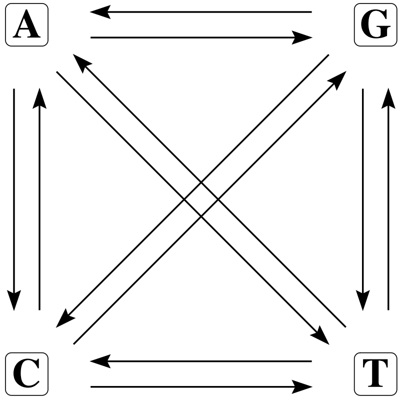
Multiple sequence alignment

```
ACGGGAT--C--C----CATTAC
ACGGGAT--C--C----CACTAC
CCGGGATAGCTTC----CATTAC
ACCCCCTATC--CACTGGATTAC
ACGACATATC--CACTGGATTCC
```

Model selection

ModelFinder (2017)

Substitution model

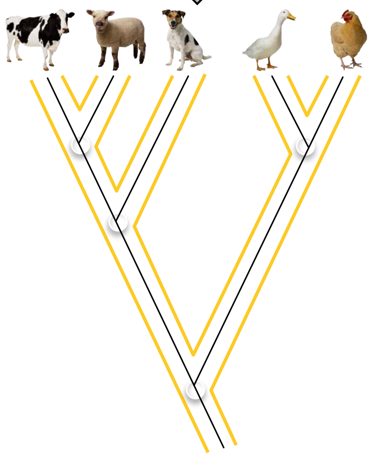


We focused on improving all three steps for large datasets!

IQ-TREE (2015, 2020)

Tree reconstruction

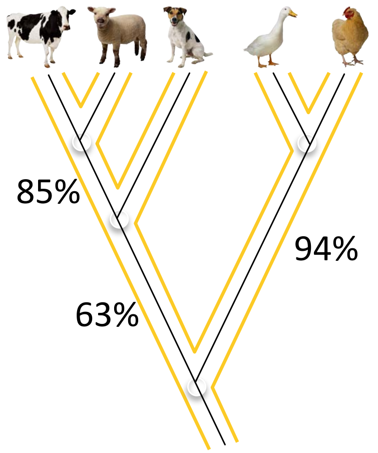
```
iqtree2 -s ALN_FILE -B 1000
```



Phylogenetic tree

Ultrafast bootstrap (2013, 2018)

Assessment of branch supports



Tree with branch supports

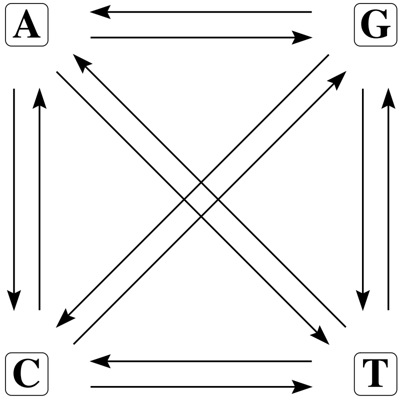
IQ-TREE tree search algorithm

Multiple sequence alignment

```
ACGGGAT--C--C----CATTAC
ACGGGAT--C--C----CACTAC
CCGGGATAGCTTC----CATTAC
ACCCCCTATC--CACTGGATTAC
ACGACATATC--CACTGGATTCC
```

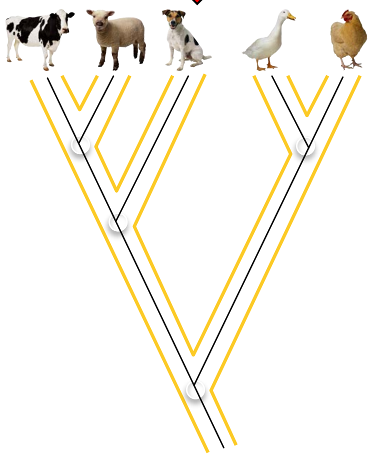
Model selection

Substitution model



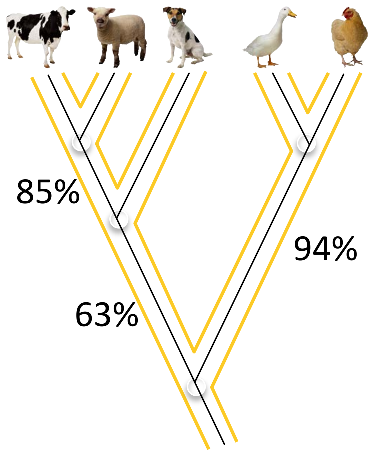
IQ-TREE (2015, 2020)

Tree reconstruction



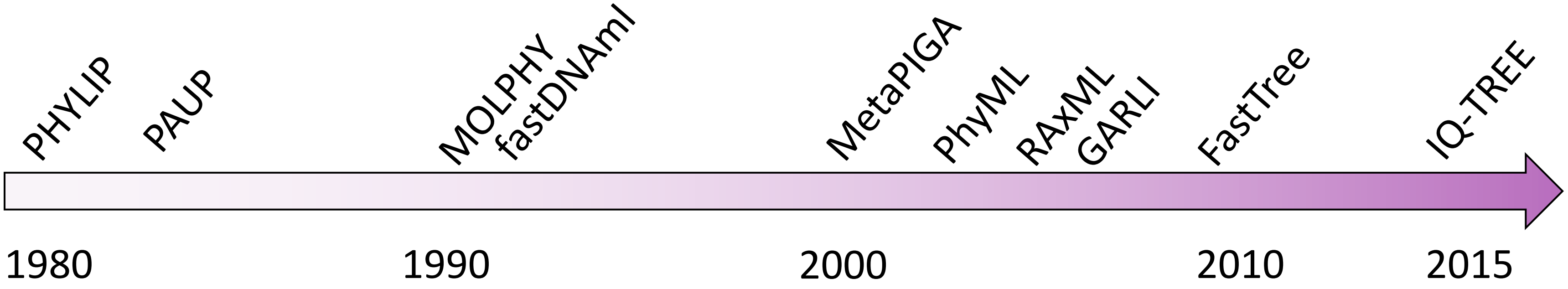
Phylogenetic tree

Assessment of branch supports

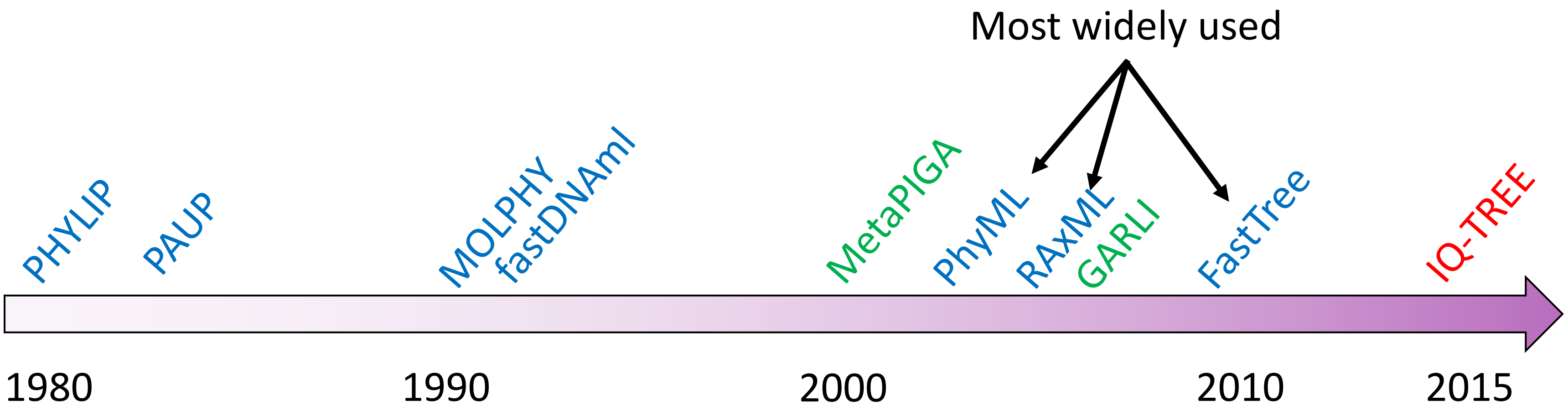


Tree with branch supports

Search heuristics for finding maximum likelihood trees



Search heuristics for finding maximum likelihood trees



Most widely used

PHYLIP

PAUP

MOLPHY

fastDNAml

MetaPIGA

PhyML

RAxML

GARLI

FastTree

IQ-TREE

1980

1990

2000

2010

2015

1. Hill-climbing / greedy algorithms:

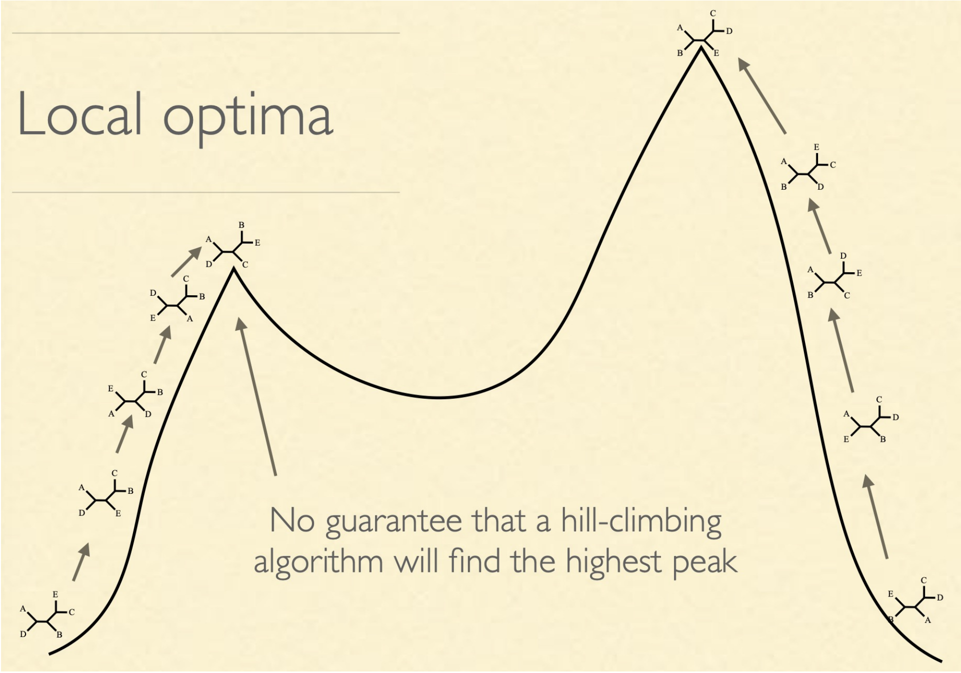
   Fast but local optimum

2. Genetic algorithm:

   Slow but escaping local optima

3. IQ-TREE:

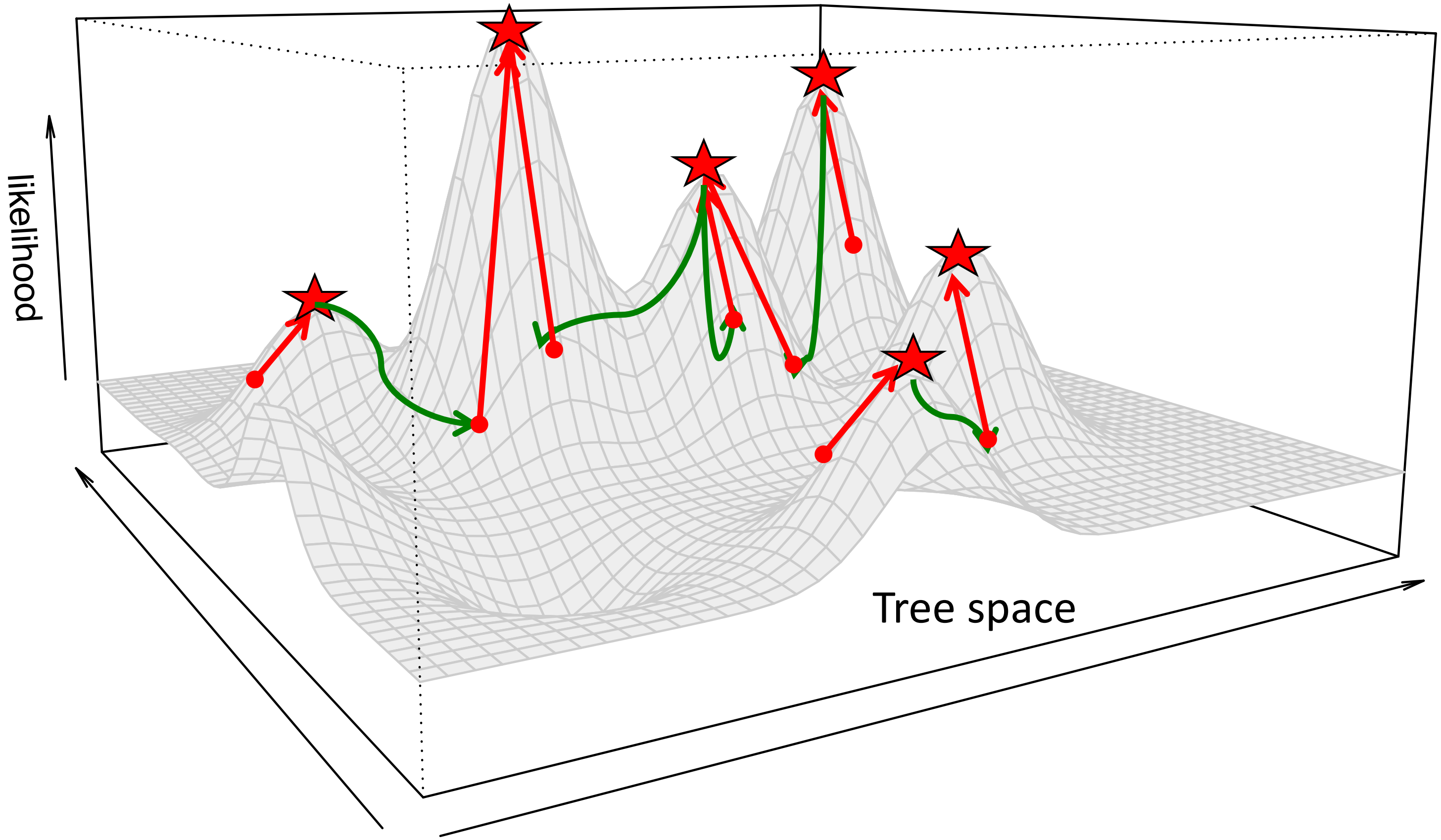
   Fast and escaping local optima

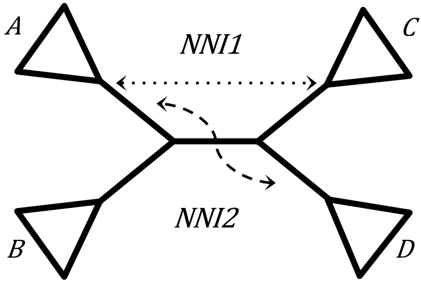


Local optima

No guarantee that a hill-climbing algorithm will find the highest peak

IQ-TREE: A new stochastic algorithm





Nearest neighbor interchange

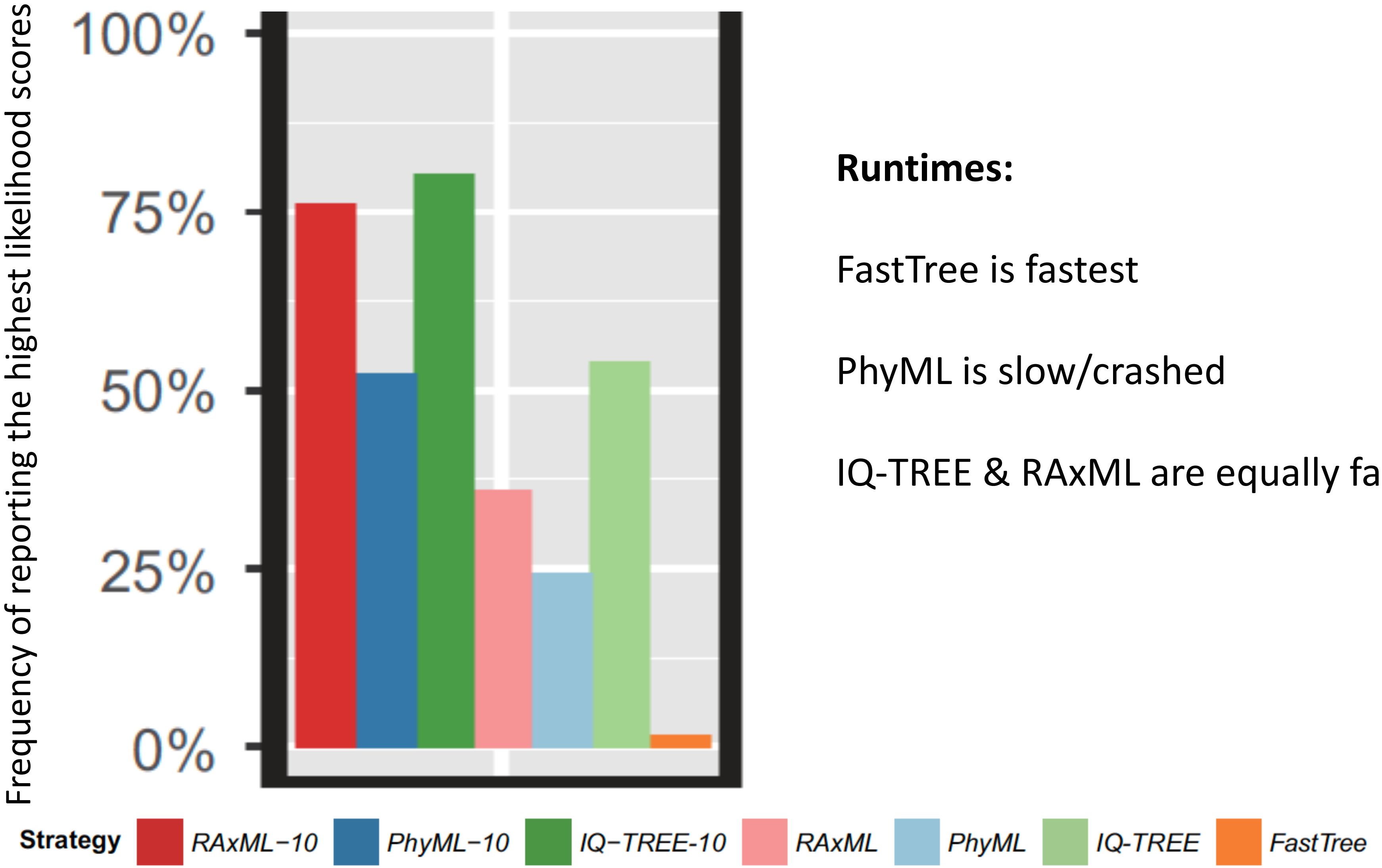
* 100 starting trees (99 parsimony, 1 NJ)
* Keeping a “population” of 20 best trees
* Stop if unsuccessful for 100 consecutive down-hill + up-hill moves

Lam-Tung Nguyen

Heiko Schmidt

Arndt von Haeseler

An independent benchmark by Zhou et al. (2018)



Runtimes:

FastTree is fastest

PhyML is slow/crashed

IQ-TREE & RAxML are equally fast

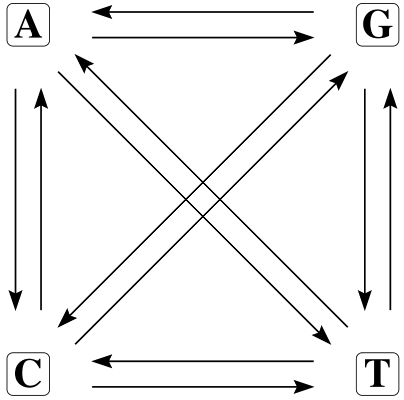
IQ-TREE tree search algorithm

Multiple sequence alignment

```
ACGGGAT--C--C----CATTAC
ACGGGAT--C--C----CACTAC
CCGGGATAGCTTC----CATTAC
ACCCCTATC--CACTGGATTAC
ACGACATATC--CACTGGATTCC
```

Model selection

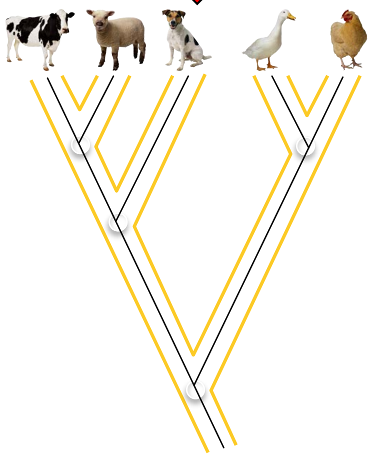
Substitution model



IQ-TREE algorithm efficiently explores tree space

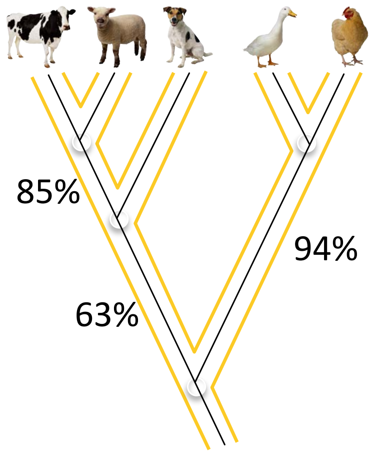
IQ-TREE (2015, 2020)

Tree reconstruction



Phylogenetic tree

Assessment of branch supports

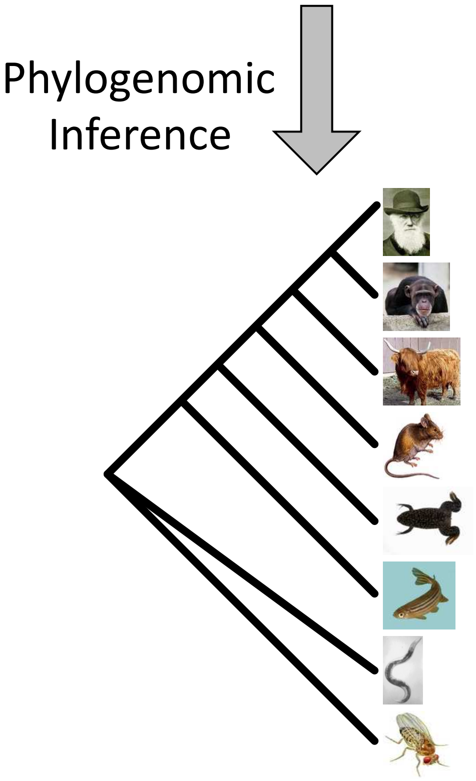


Tree with branch supports

Genome-scale data: Concatenation methods

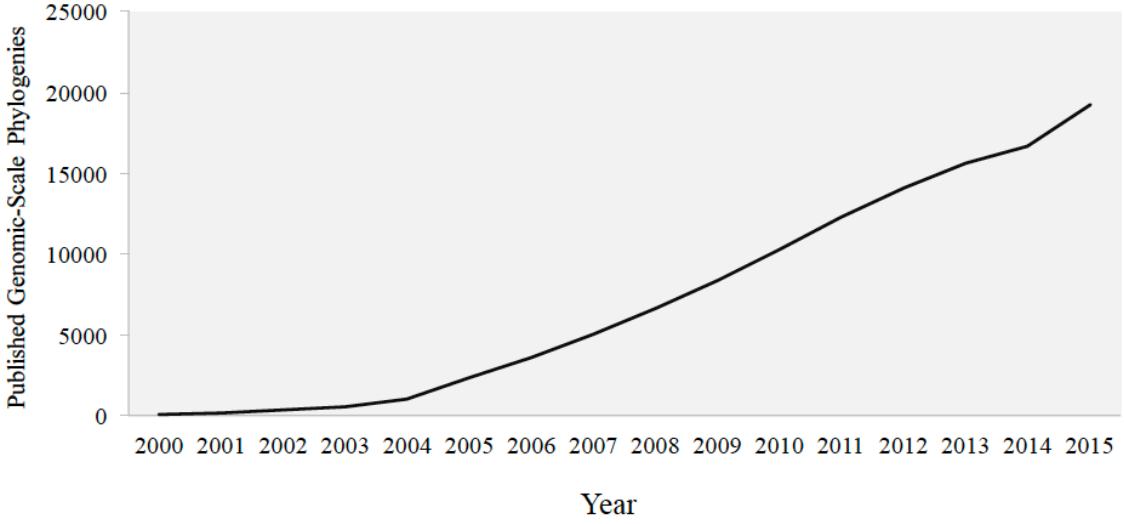
Supermatrix

Gene 1	Gene 2	……	Gene 1,000
CACCTGTCGT	----------	----------	TCTGGTGCAG
CAGCTGTCGT	GCTCTTTCTG	TTGAGCCTGG	TCTGGTGCAG
CAGCTGCCGT	GTTTTCTCTG	TTGAGCCTGG	TCTGGTACAG
CAGCTGCCGC	GTTCTCTCCG	----------	TCTGGTGCAA
CTCCTGCCGG	GTGCTCTCAG	----------	----------
CTCCTGCCGG	----------	CTGAGCCGGG	TCTGGTGCAG
CTCTTGCCGG	----------	CTGAGCCTTG	----------



Phylogenomic Inference

Species tree of life

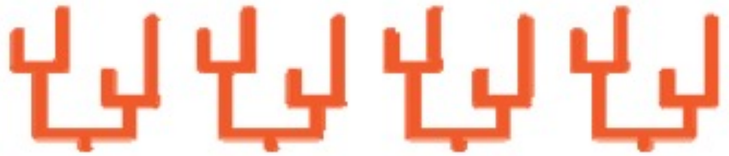
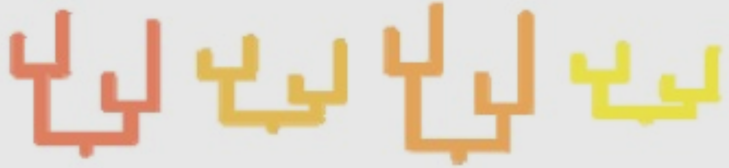
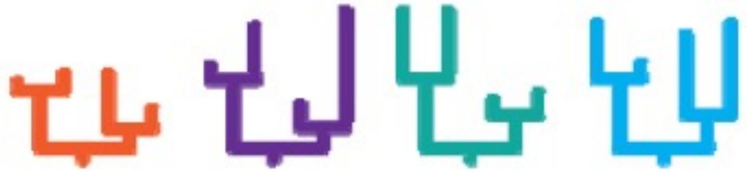


30 days of computation and 280 GB RAM for an insect data set!

Partition model

Supermatrix

	Gene 1	Gene 2	……	Gene 1,000
	CACCTGTCGT	----------	----------	TCTGGTGCAG
	CAGCTGTCGT	GCTCTTTCTG	TTGAGCCTGG	TCTGGTGCAG
	CAGCTGCCGT	GTTTTCTCTG	TTGAGCCTGG	TCTGGTACAG
	CAGCTGCCGC	GTTCTCTCCG	----------	TCTGGTGCAA
	CTCCTGCCGG	GTGCTCTCAG	----------	----------
	CTCCTGCCGG	----------	CTGAGCCGGG	TCTGGTGCAG
	CTCTTGCCGG	----------	CTGAGCCTTG	----------
Substitution models:	JC	HKY+G	……	GTR+G

Model of branch lengths	Gene trees
Universally shared	
Proportionally linked	
Unlinked	

```
iqtree2 -s ALN_FILE
  -q PARTITION_FILE
```

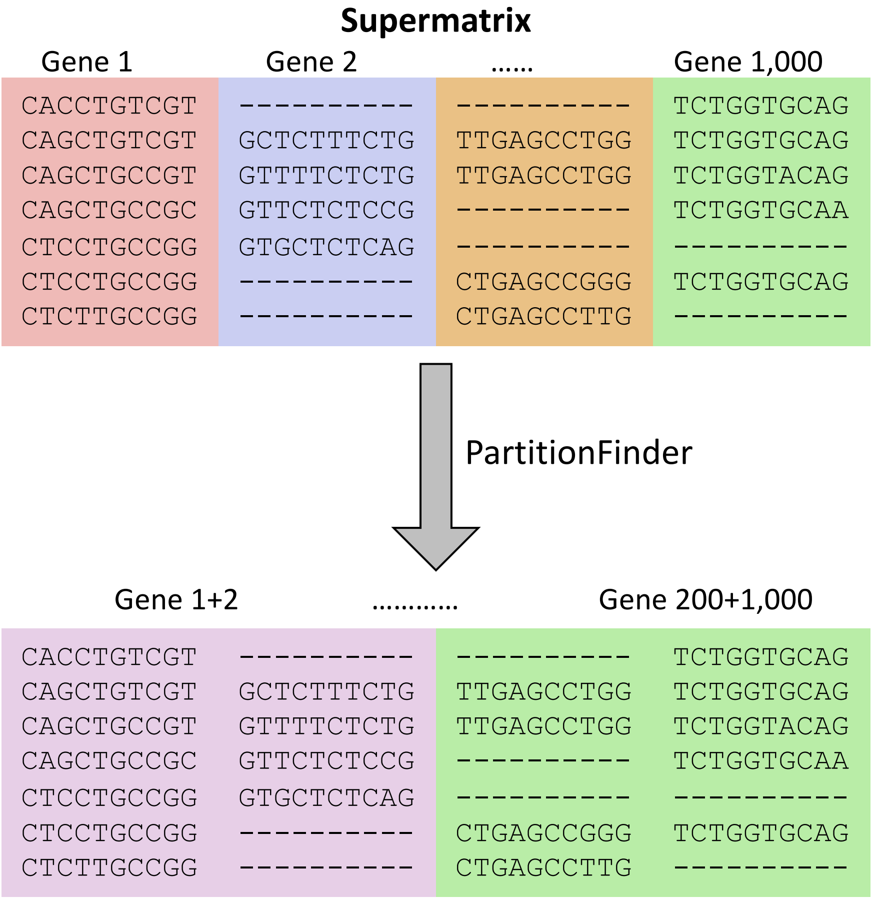
```
iqtree2 -s ALN_FILE
  -p PARTITION_FILE
```

```
iqtree2 -s ALN_FILE
  -Q PARTITION_FILE
```

Example partition file (turtle.nex)

```
#nexus
begin sets;
  charset ENSGALG00000000223.macse_DNA_gb = 1-846;
  charset ENSGALG00000001529.macse_DNA_gb = 847-1368;
  charset ENSGALG00000002002.macse_DNA_gb = 1369-2040;
  charset ENSGALG00000002514.macse_DNA_gb = 2041-2772;
  charset ENSGALG00000003337.macse_DNA_gb = 2773-3738;
  charset ENSGALG00000003700.macse_DNA_gb = 3739-4623;
  charset ENSGALG00000003702.macse_DNA_gb = 4624-6168;
  charset ENSGALG00000003907.macse_DNA_gb = 6169-6648;
  charset ENSGALG00000005820.macse_DNA_gb = 6649-7224;
  charset ENSGALG00000005834.macse_DNA_gb = 7225-7920;
  charset ENSGALG00000005902.macse_DNA_gb = 7921-8490;
  charset ENSGALG00000008338.macse_DNA_gb = 8491-9282;
  charset ENSGALG00000008517.macse_DNA_gb = 9283-9822;
  charset ENSGALG00000008916.macse_DNA_gb = 9823-10368;
  charset ENSGALG00000009085.macse_DNA_gb = 10369-11298;
  charset ENSGALG00000009879.macse_DNA_gb = 11299-11895;
  charset ENSGALG00000011323.macse_DNA_gb = 11896-12795;
  charset ENSGALG00000011434.macse_DNA_gb = 12796-13242;
  charset ENSGALG00000011917.macse_DNA_gb = 13243-14223;
  charset ENSGALG00000011966.macse_DNA_gb = 14224-14691;
  charset ENSGALG00000012244.macse_DNA_gb = 14692-15444;
  charset ENSGALG00000012379.macse_DNA_gb = 15445-15963;
  charset ENSGALG00000012568.macse_DNA_gb = 15964-16593;
  charset ENSGALG00000013227.macse_DNA_gb = 16594-17895;
  charset ENSGALG00000014038.macse_DNA_gb = 17896-18456;
  charset ENSGALG00000014648.macse_DNA_gb = 18457-18954;
  charset ENSGALG00000015326.macse_DNA_gb = 18955-19551;
  charset ENSGALG00000015397.macse_DNA_gb = 19552-20145;
  charset ENSGALG00000016241.macse_DNA_gb = 20146-20820;
end;
```

How to reduce potential model overfitting?



Supermatrix

Gene 1	Gene 2	......	Gene 1,000
CACCTGTCGT	----------	----------	TCTGGTGCAG
CAGCTGTCGT	GCTCTTTCTG	TTGAGCCTGG	TCTGGTGCAG
CAGCTGCCGT	GTTTTCTCTG	TTGAGCCTGG	TCTGGTACAG
CAGCTGCCGC	GTTCTCTCCG	----------	TCTGGTGCAA
CTCCTGCCGG	GTGCTCTCAG	----------	----------
CTCCTGCCGG	----------	CTGAGCCGGG	TCTGGTGCAG
CTCTTGCCGG	----------	CTGAGCCTTG	----------

Gene 1+2		....	Gene 200+1,000
CACCTGTCGT	----------	----------	TCTGGTGCAG
CAGCTGTCGT	GCTCTTTCTG	TTGAGCCTGG	TCTGGTGCAG
CAGCTGCCGT	GTTTTCTCTG	TTGAGCCTGG	TCTGGTACAG
CAGCTGCCGC	GTTCTCTCCG	----------	TCTGGTGCAA
CTCCTGCCGG	GTGCTCTCAG	----------	----------
CTCCTGCCGG	----------	CTGAGCCGGG	TCTGGTGCAG
CTCTTGCCGG	----------	CTGAGCCTTG	----------

Substitution models: HKY ...... GTR+G

PartitionFinder algorithm (Lanfear et al. 2012):

1. Evaluate all pairs of genes.
2. Find the pair with best score.
3. If score improves, merge two genes and repeat steps 1-3.
4. Otherwise, stop.

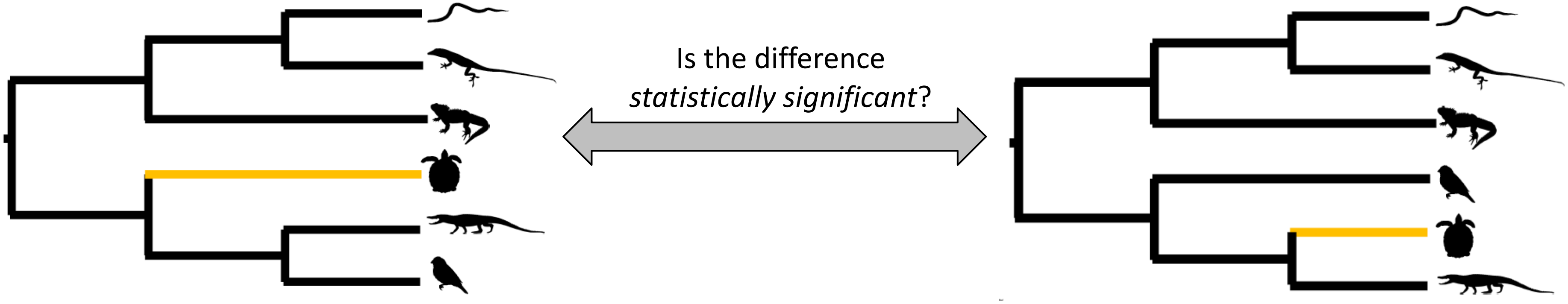
```
iqtree2 … -m MFP+MERGE
```

Relaxed clustering algorithm (Lanfear et al. 2014):

In step 1: only examine the top k% of most “promising” pairs.

```
iqtree2 … -rcluster 10
```

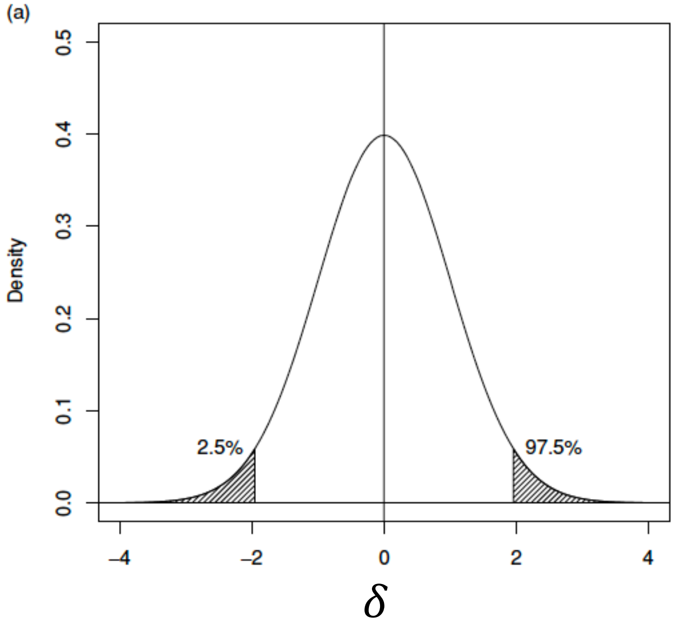
Tree topology tests



Is the difference *statistically significant*?

Testing two trees (Kishino & Hasegawa, 1989):

1. Statistic: $\delta = \log\left(likelihood(T_1)\right) - \log\left(likelihood(T_0)\right)$.
2. Generate distribution of δ from many “random” data (e.g. by 10,000 bootstrap resampling).
3. Compare the statistic between original and random data to obtain *p-value*.
4. If p-value < 0.05: YES! two trees are significantly different.
5. If p-value >= 0.05: NO! they are not.



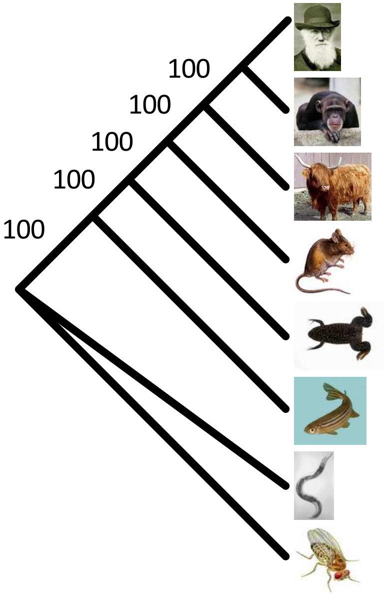
```
iqtree2 –s ALN_FILE –p PARTITION_FILE
  –z TREES_FILE –zb 10000 –au –n 0
```

Concatenation methods: Limitation

Supermatrix

Gene 1	Gene 2	……	Gene 1,000
CACCTGTCGT	----------	----------	TCTGGTGCAG
CAGCTGTCGT	GCTCTTTCTG	TTGAGCCTGG	TCTGGTGCAG
CAGCTGCCGT	GTTTTCTCTG	TTGAGCCTGG	TCTGGTACAG
CAGCTGCCGC	GTTCTCTCCG	----------	TCTGGTGCAA
CTCCTGCCGG	GTGCTCTCAG	----------	----------
CTCCTGCCGG	----------	CTGAGCCGGG	TCTGGTGCAG
CTCTTGCCGG	----------	CTGAGCCTTG	----------

Phylogenomic Inference



Species tree of life

Bootstrap supports and Bayesian posteriors tend to 100% as #genes increases!

Concatenation assumes a single tree across all loci

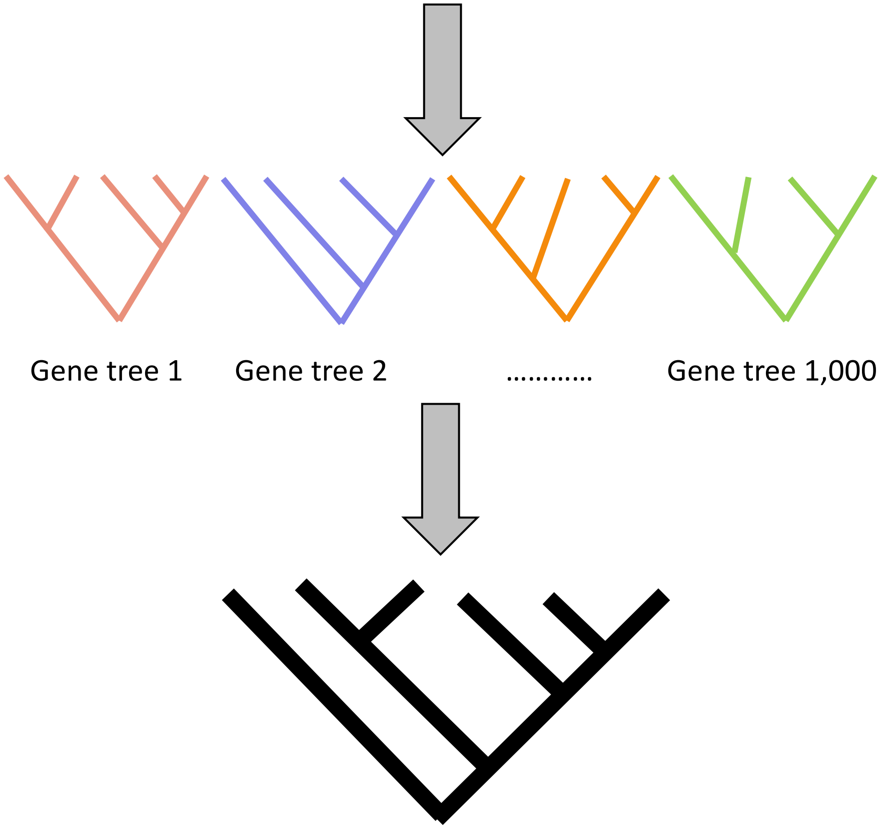
Potential *systematic bias*

"When the method of inferring phylogenies is one with undesirable statistical properties such as inconsistency, the bootstrap does not correct for these" (Felsenstein, 1985)

Coalescent methods

Supermatrix

Gene 1	Gene 2	......	Gene 1,000
CACCTGTCGT	----------	----------	TCTGGTGCAG
CAGCTGTCGT	GCTCTTTCTG	TTGAGCCTGG	TCTGGTGCAG
CAGCTGCCGT	GTTTTCTCTG	TTGAGCCTGG	TCTGGTACAG
CAGCTGCCGC	GTTCTCTCCG	----------	TCTGGTGCAA
CTCCTGCCGG	GTGCTCTCAG	----------	----------
CTCCTGCCGG	----------	CTGAGCCGGG	TCTGGTGCAG
CTCTTGCCGG	----------	CTGAGCCTTG	----------



Species tree

Gene Concordance Factor (gCF): How often a branch in species tree is found among gene trees?
0% ≤ gCF ≤ 100%

Implementation in IQ-TREE fully accounts for missing data

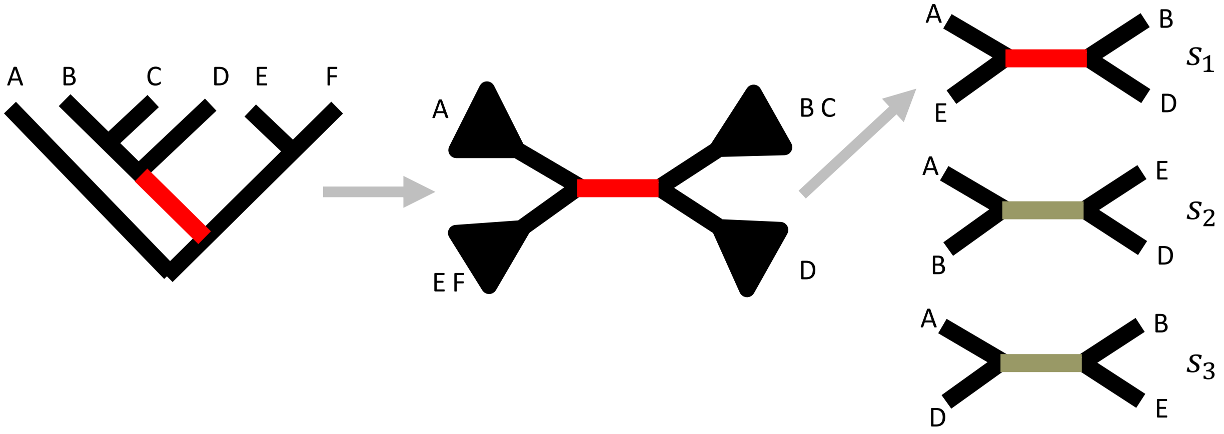
Problem: Uncertainties in gene trees!

Site Concordance Factor (sCF)

Supermatrix

Gene 1	Gene 2	……	Gene 1,000
CACCTGTCGT	----------	----------	TCTGGTGCAG
CAGCTGTCGT	GCTCTTTCTG	TTGAGCCTGG	TCTGGTGCAG
CAGCTGCCGT	GTTTTCTCTG	TTGAGCCTGG	TCTGGTACAG
CAGCTGCCGC	GTTCTCTCCG	----------	TCTGGTGCAA
CTCCTGCCGG	GTGCTCTCAG	----------	----------
CTCCTGCCGG	----------	CTGAGCCGGG	TCTGGTGCAG
CTCTTGCCGG	----------	CTGAGCCTTG	----------

Site Concordance Factor (sCF):
How often a branch is
“supported” by alignment sites?
33.3% ≲ sCF ≤ 100%



$$qCF(quartet) = \frac{s_1}{s_1 + s_2 + s_3}$$

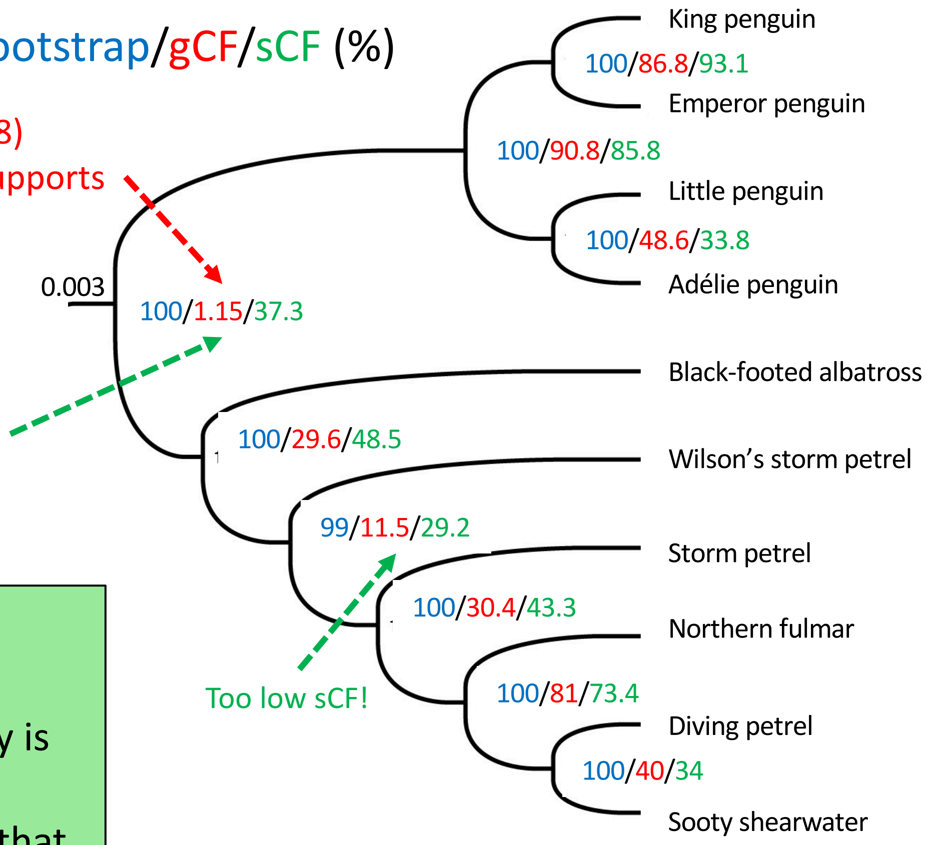
$$sCF = \overline{qCF(100\ quartets)}$$

An example birds data set (Reddy et al., 2017)

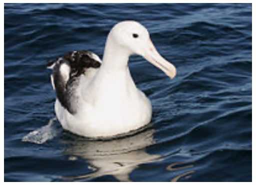
Bootstrap/gCF/sCF (%)

Only 1 (of 88) gene tree supports this branch!

- 131 sites support this branch
- 105 sites support NNI branch 1
- 114 sites support NNI branch 2



Penguins



Tubenoses

Felsenstein (1985): a difference of 20 sites favouring one topology is enough to give 100% bootstrap support for that one topology!

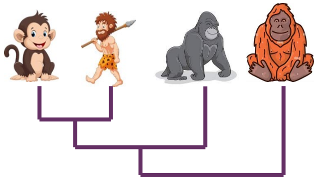
- gCF and sCF are useful when bootstrap supports reach 100%.
- CAUTION when gCF ~ 0% or sCF ~ 33%, even if BS ~ 100%.
- GREAT when gCF and sCF > 50%.

Mixture Across Sites and Trees (MAST) model

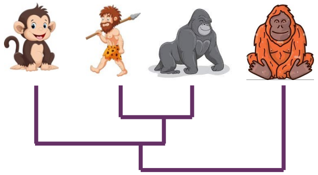
Concatenated alignment

S1:	A	A	-	T	A	A	A	T
S2:	T	A	A	C	C	T	T	T
S3:	T	A	T	A	A	G	T	T
S4:	A	C	-	A	C	A	A	A

L_1^1 L_2^1 L_3^1 L_4^1 L_5^1 L_6^1 L_7^1 L_8^1



L_1^2 L_2^2 L_3^2 L_4^2 L_5^2 L_6^2 L_7^2 L_8^2

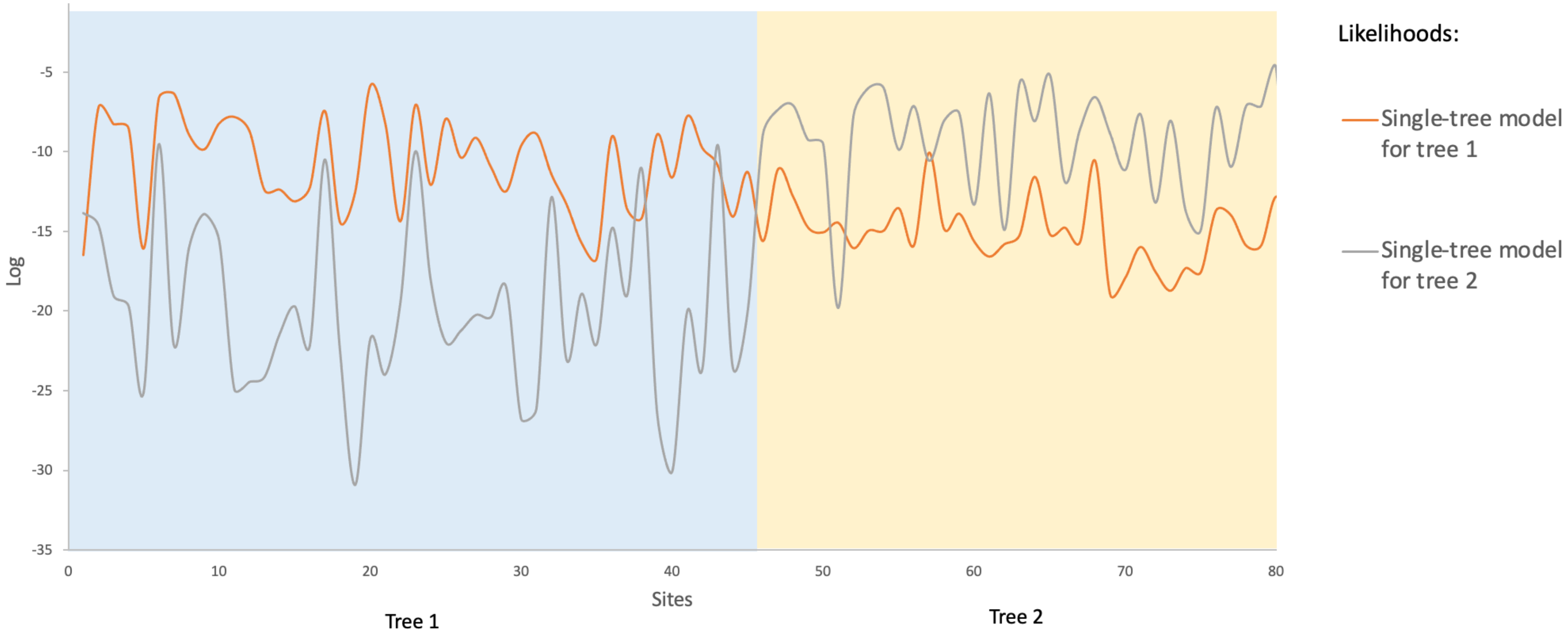


Likelihood for site *i*: $L_i = w_1 L_i^1 + w_1 L_i^2$
where w_j represents the portion of sites belonging to tree *j*

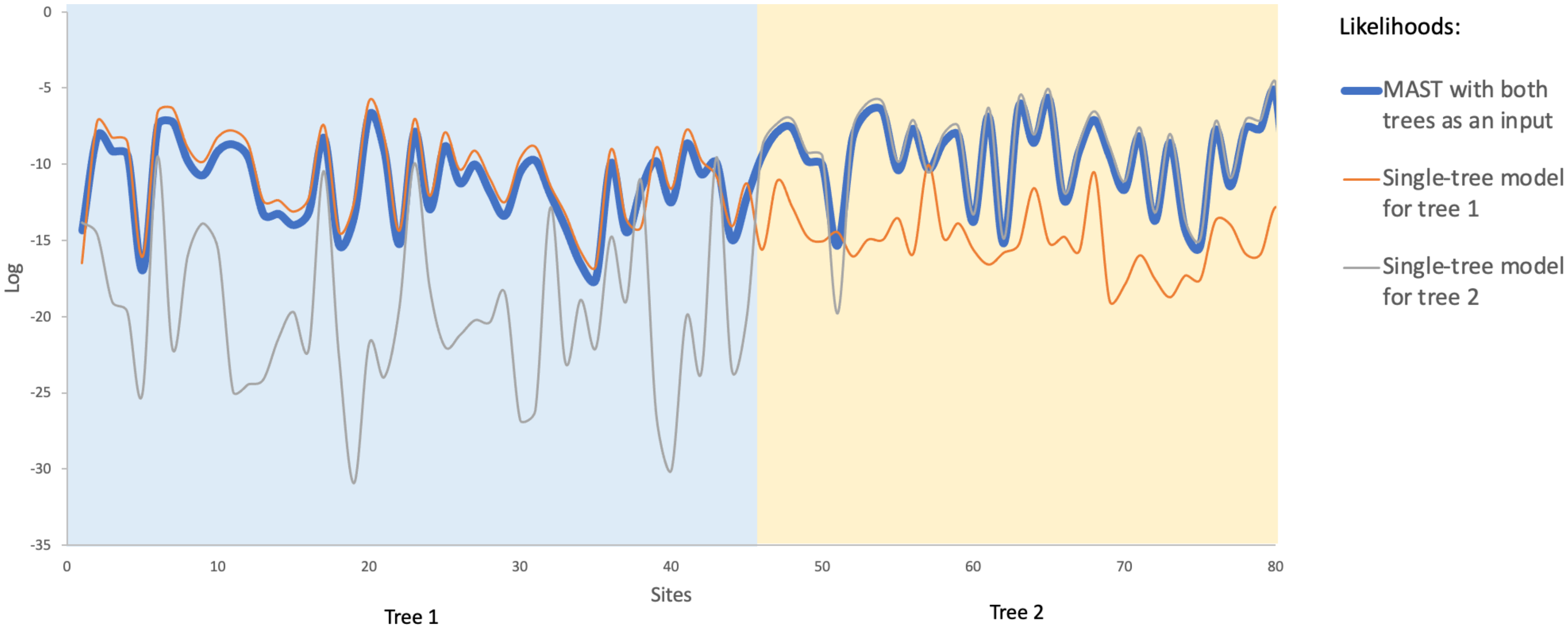
Log-likelihood of the trees: $\sum_i \log(L_i)$

```
iqtree2 –s ALN_FILE –te TREES_FILE –m GTR+G+T
```

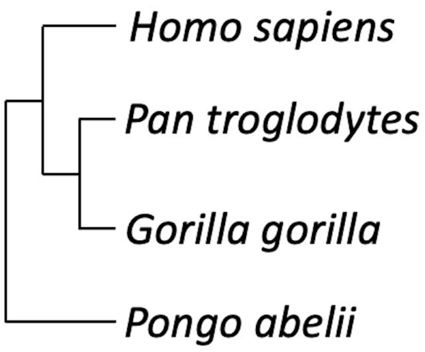
Toy example: Site log-likelihood



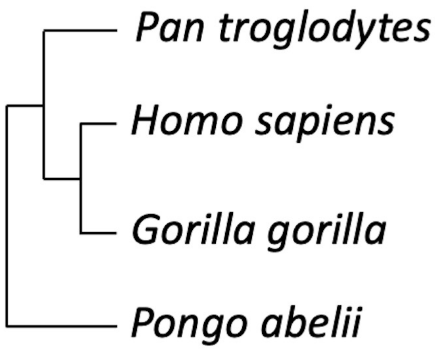
Toy example: Site log-likelihood



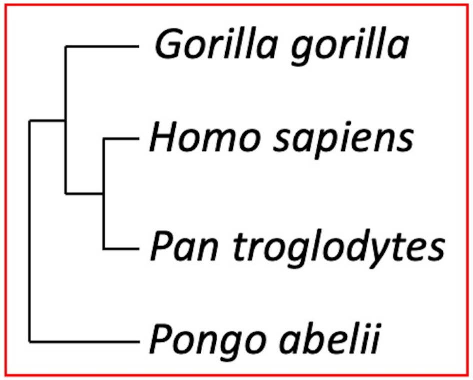
The classical example of Human, Chimp, Gorilla



T_{A1}



T_{A2}

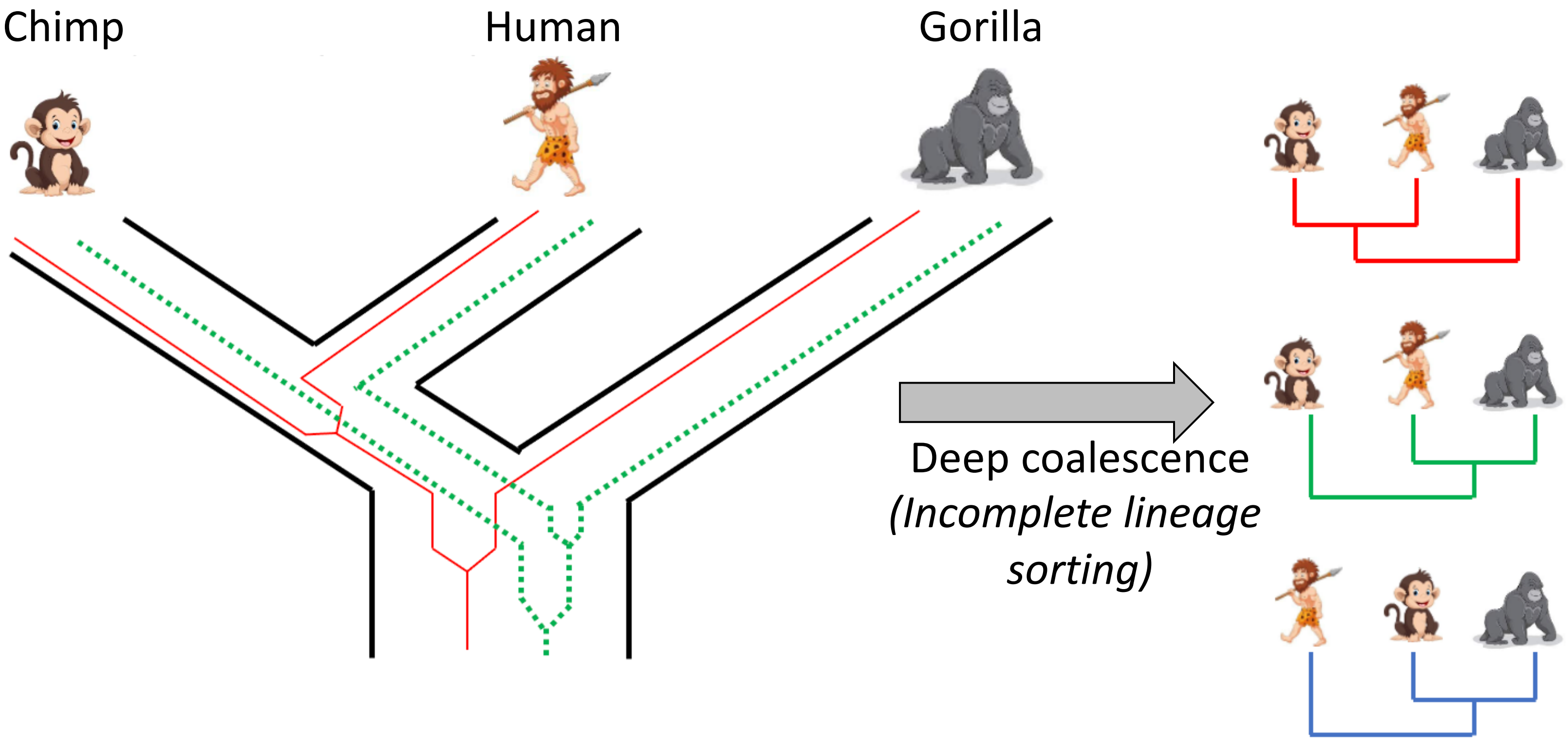


T_{A3}

Gene tree frequencies:	19.8%	20.1%	60.1%
MAST model weights:	**17.9%**	**17.4%**	**64.7%**

Data: 1,595 genes; 1,618,506 bp (Vanderpool et al. 2020)

Gene trees discordance due to deep coalescence



Chimp

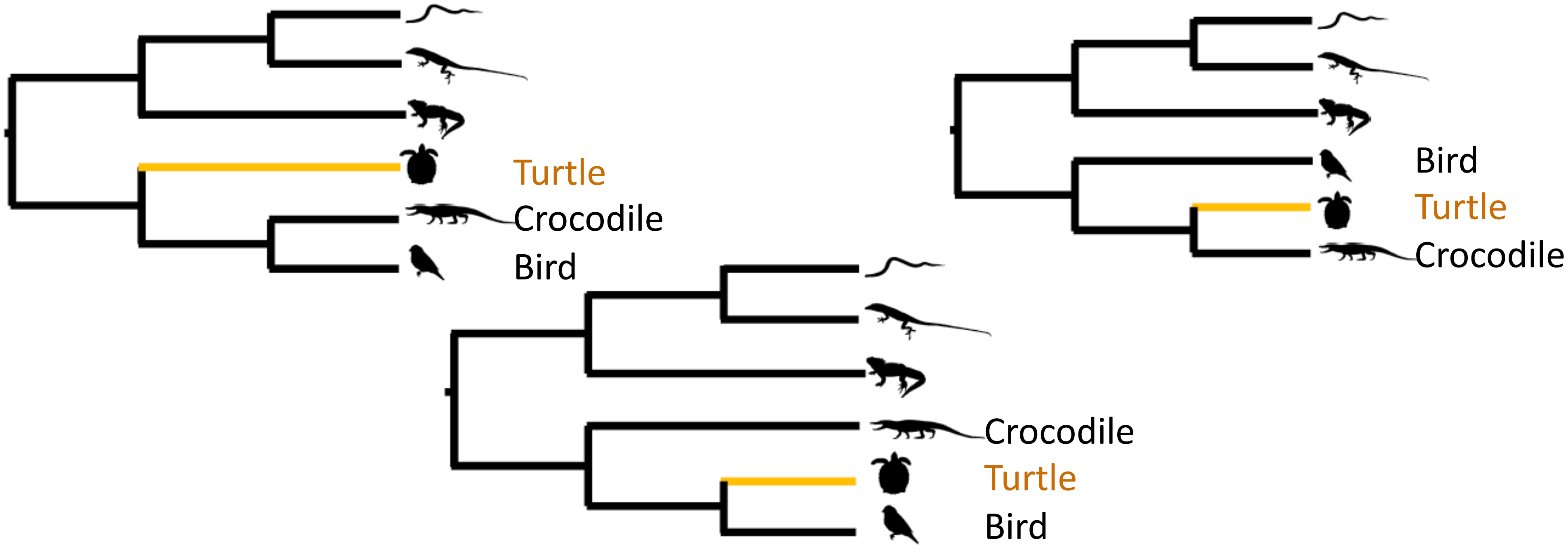
Human

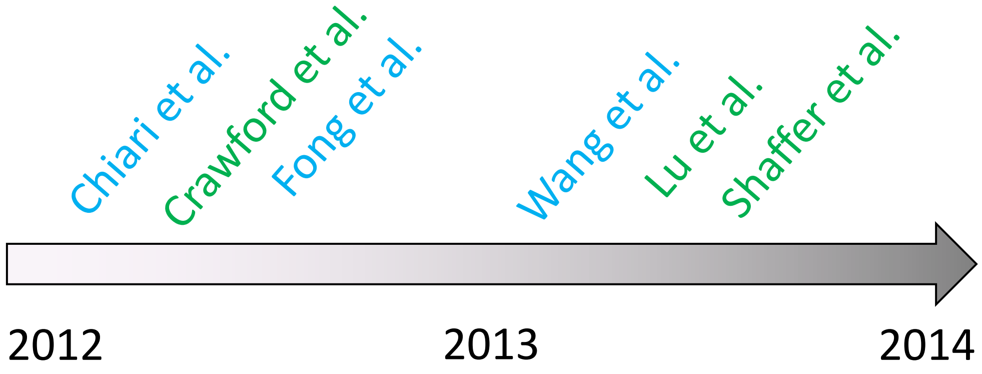
Gorilla

Deep coalescence

(Incomplete lineage sorting)

Dataset for IQ-TREE lab: Where is Turtle in the tree?





Different studies led to different trees!

Dataset: 16 species, 29 genes, 20,820 bp
(a subset of Chiari et al. 2012)

Thanks Jeremy Brown

IQ-TREE lab

1. Input data
2. Inferring the first phylogeny
3. Applying partition model
4. Choosing the best partitioning scheme
5. Tree topology tests
6. Tree mixture model (**NEW**)
7. Identifying most influential genes
8. Removing influential genes
9. Concordance factors (*advanced)

http://www.iqtree.org/workshop/molevol2023

Fill out your answers in a Google form (shared via Slack)