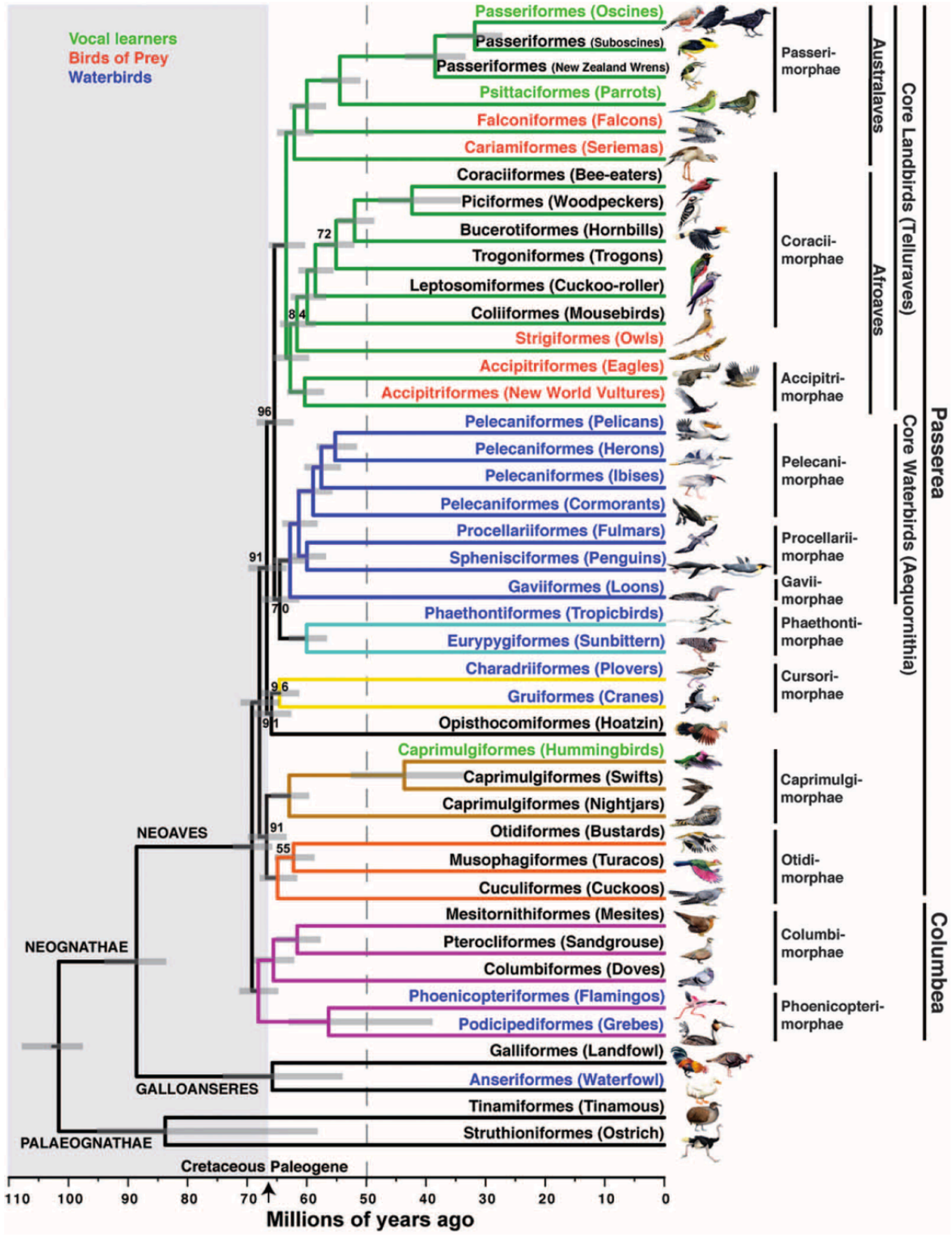
[some]

Methodological advances since the first avian phylogenomics project

[mostly our work]

Siavash Mirarab
University of California, San Diego



TENT tree from Jarvis et al, Science, 2014

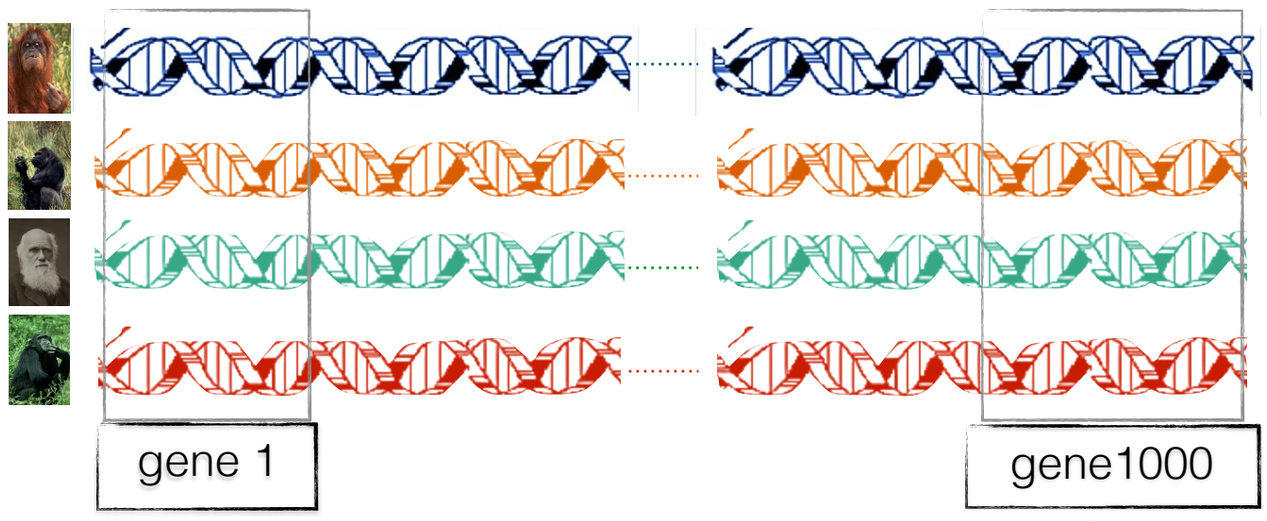
Challenges

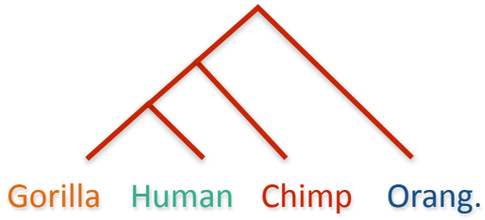
- Errors and incompleteness in data due to annotation, assembly, or other unknown origins
- Models of sequence evolution
- Gene tree discordance
  - True discordance
  - Spurious discordance
- Scalability

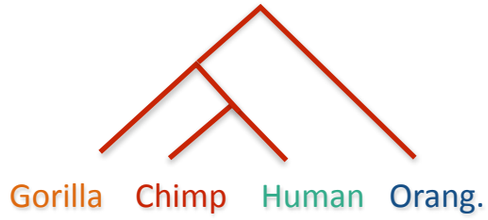
Challenges

- Errors and incompleteness in data due to annotation, assembly, or other unknown origins
- Models of sequence evolution
- Gene tree discordance
  - True discordance
  - Spurious discordance
- Scalability

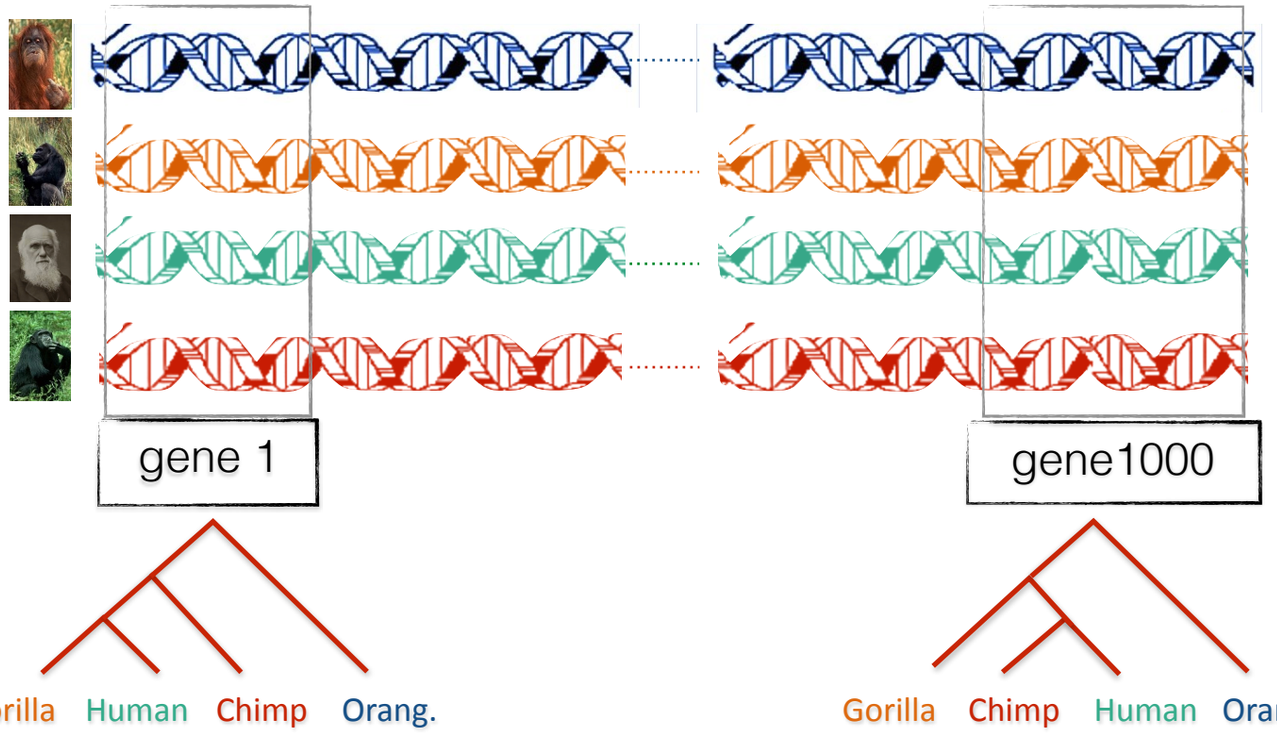
Gene tree discordance

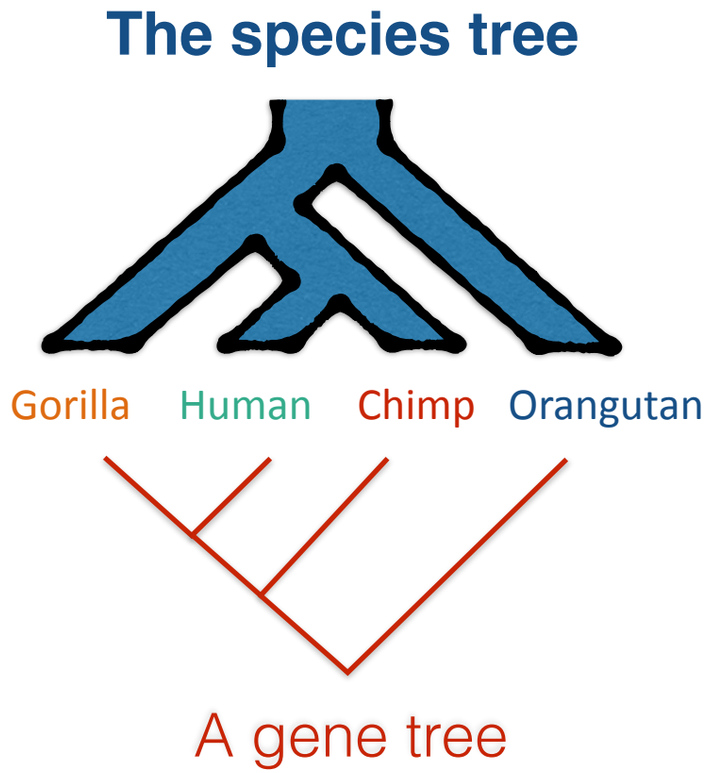




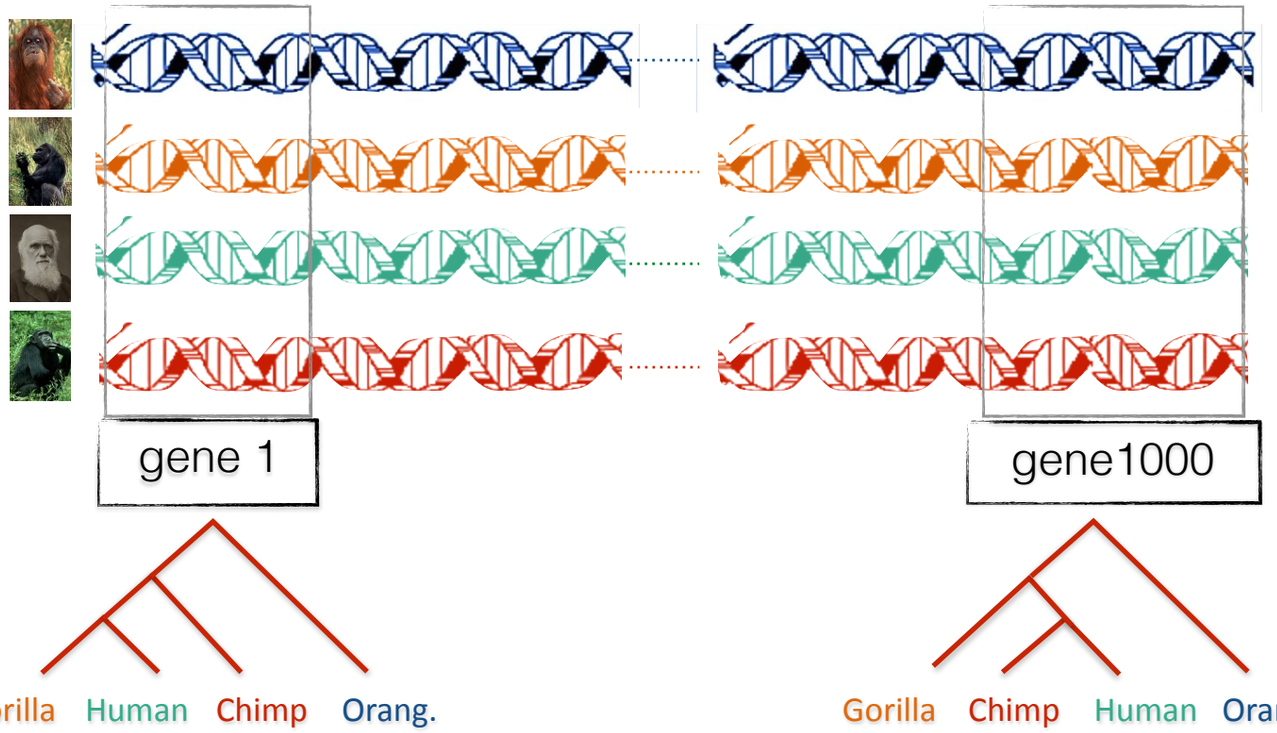


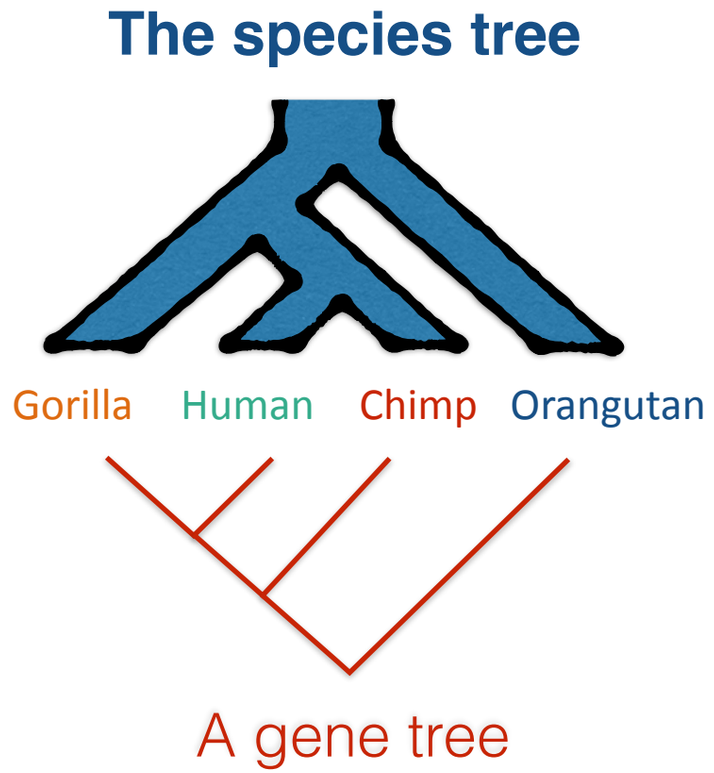
Gene tree discordance





Gene tree discordance





Causes of gene tree discordance include:

- Duplication and loss
- Horizontal Gene Transfer (HGT) and Hybridization
- **Incomplete Lineage Sorting (ILS)**

Incomplete Lineage Sorting (ILS)

- Can occur when multiple alleles of a gene persist (fail to coalesce) during the lifetime of an ancestral population

"gene" here simply refers to a recombination-free part of the genome

Incomplete Lineage Sorting (ILS)

- Can occur when multiple alleles of a gene persist (fail to coalesce) during the lifetime of an ancestral population

"gene" here simply refers to a recombination-free part of the genome

Incomplete Lineage Sorting (ILS)

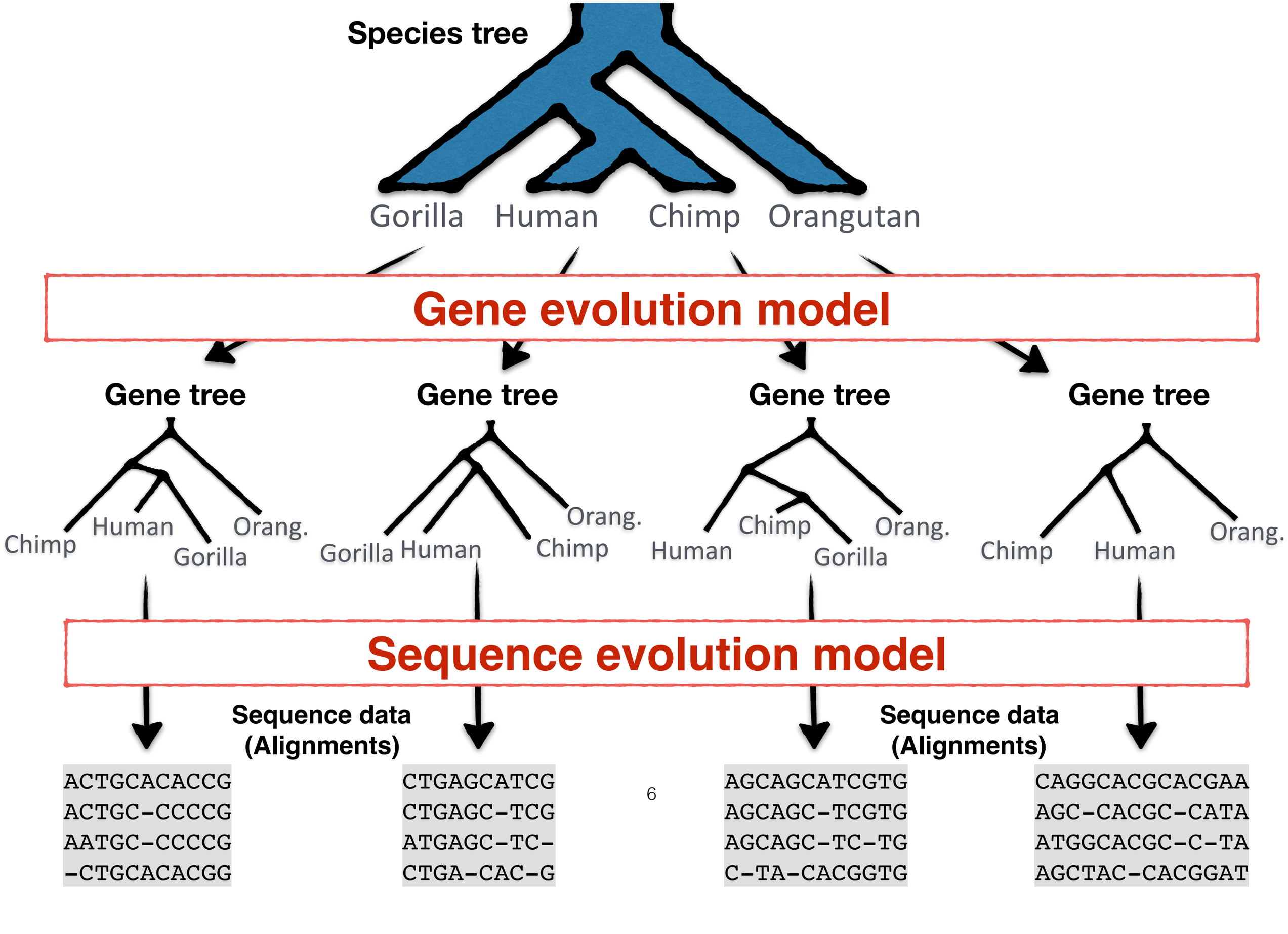
- Can occur when multiple alleles of a gene persist (fail to coalesce) during the lifetime of an ancestral population
- Always possible. Likely for:
  - Short branches (# generations)
  - Large populations

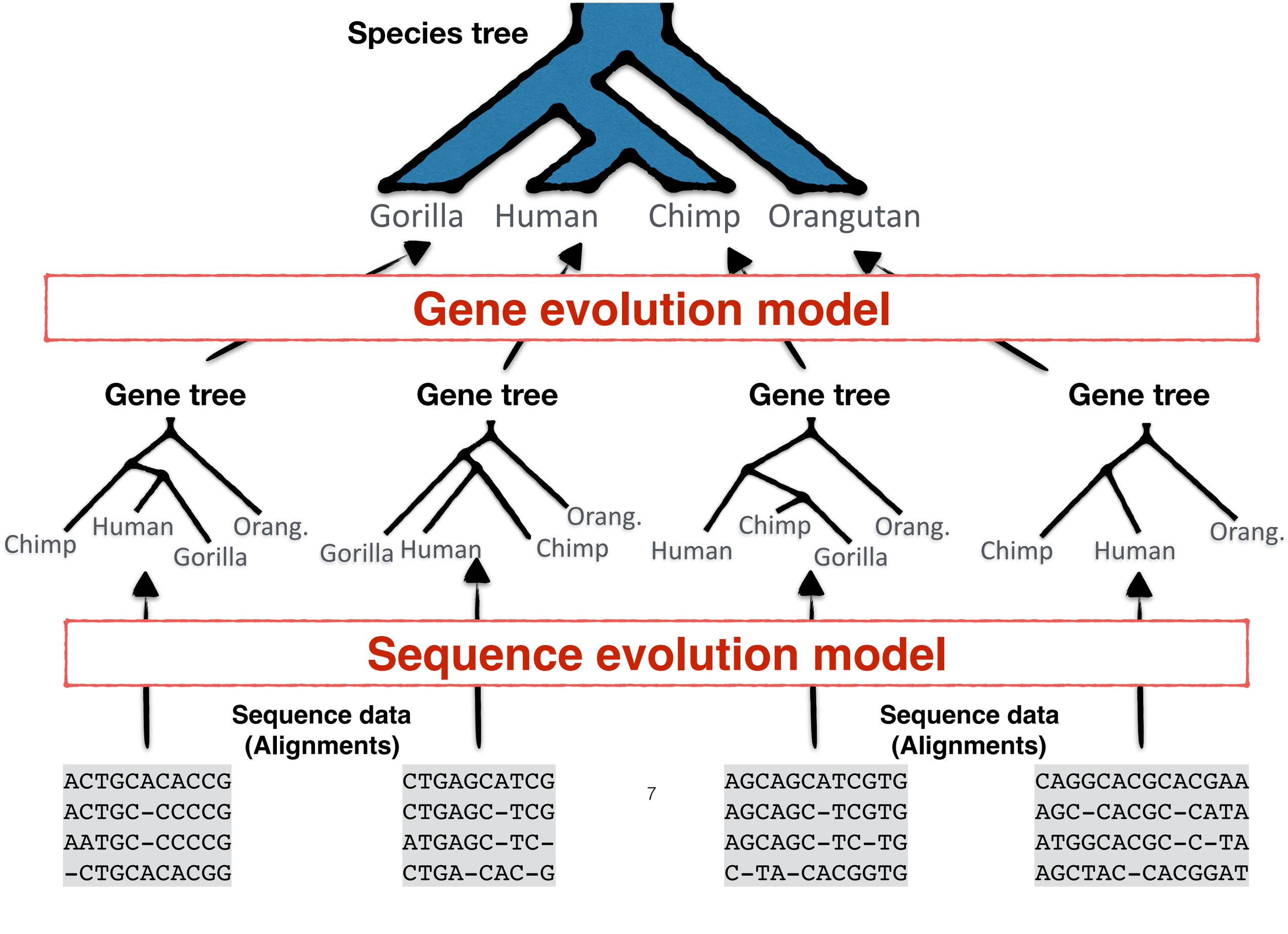
"gene" here simply refers to a recombination-free part of the genome

Incomplete Lineage Sorting (ILS)

- Can occur when multiple alleles of a gene persist (fail to coalesce) during the lifetime of an ancestral population
- Always possible. Likely for:
  - Short branches (# generations)
  - Large populations
- Both characterize rapid radiations

"gene" here simply refers to a recombination-free part of the genome


Species tree
Gorilla
Human
Chimp
Orangutan
Gene evolution model
Gene tree
Gene tree
Gene tree
Gene tree
Chimp
Human
Orang.
Gorilla
Gorilla
Human
Chimp
Orang.
Human
Chimp
Gorilla
Orang.
Chimp
Human
Orang.
Sequence evolution model
Sequence data (Alignments)
Sequence data (Alignments)
ACTGCACACCG
ACTGC-CCCCG
AATGC-CCCCG
-CTGCACACGG
CTGAGCATCG
CTGAGC-TCG
ATGAGC-TC-
CTGA-CAC-G
AGCAGCATCGTG
AGCAGC-TCGTG
AGCAGC-TC-TG
C-TA-CACGGTG
CAGGCACGCACGAA
AGC-CACGC-CATA
ATGGCACGC-C-TA
AGCTAC-CACGGAT


Species tree
Gorilla
Human
Chimp
Orangutan
Gene evolution model
Gene tree
Gene tree
Gene tree
Gene tree
Chimp
Human
Orang.
Gorilla
Gorilla
Human
Orang.
Chimp
Human
Chimp
Orang.
Gorilla
Chimp
Human
Orang.
Sequence evolution model
Sequence data
(Alignments)
Sequence data
(Alignments)
ACTGCACACCG
ACTGC-CCCCG
AATGC-CCCCG
-CTGCACACGG
CTGAGCATCG
CTGAGC-TCG
ATGAGC-TC-
CTGA-CAC-G
AGCAGCATCGTG
AGCAGC-TCGTG
AGCAGC-TC-TG
C-TA-CACGGTG
CAGGCACGCACGAA
AGC-CACGC-CATA
ATGGCACGC-C-TA
AGCTAC-CACGGAT

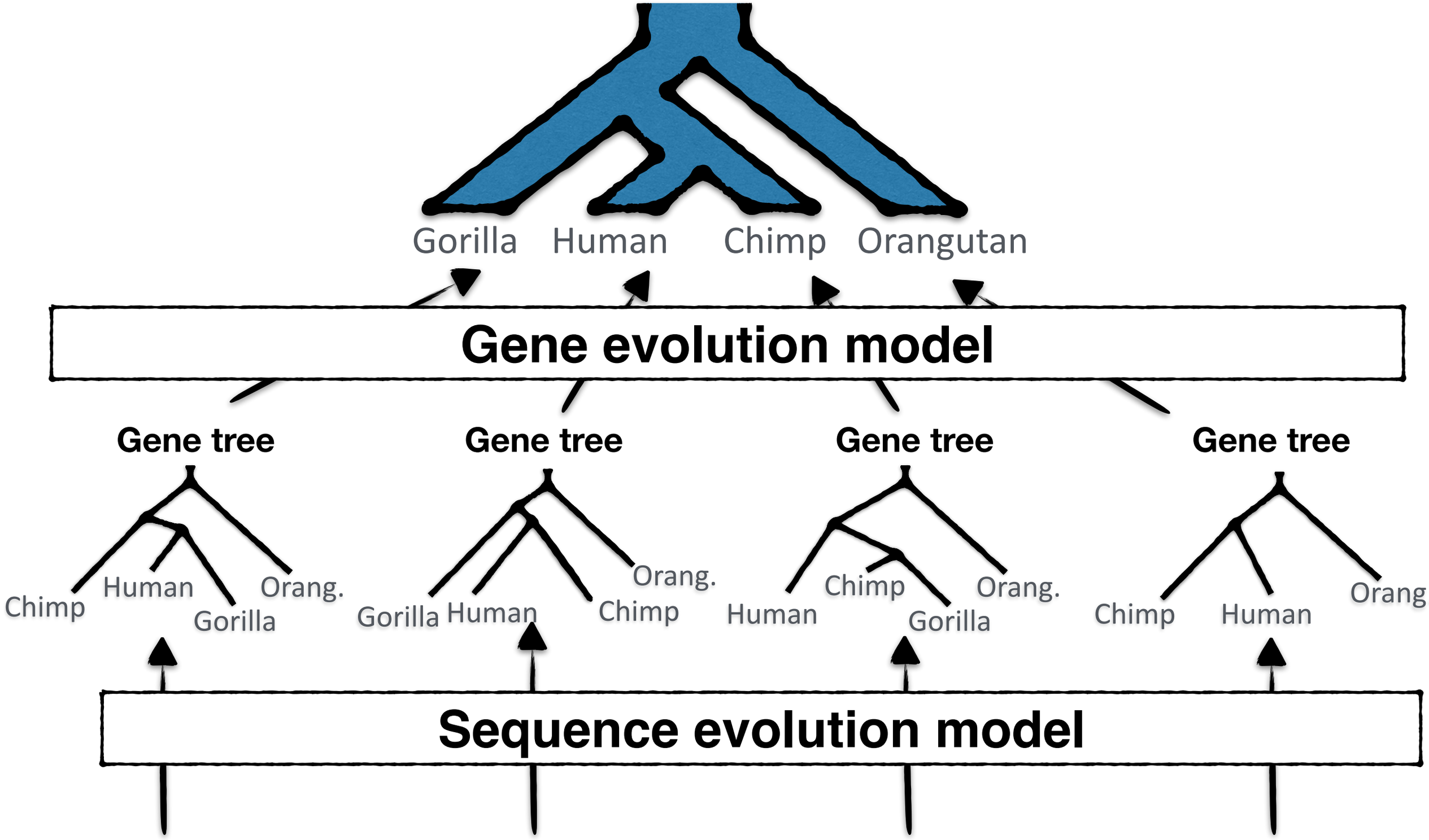
Multi-species coalescent (MSC) model

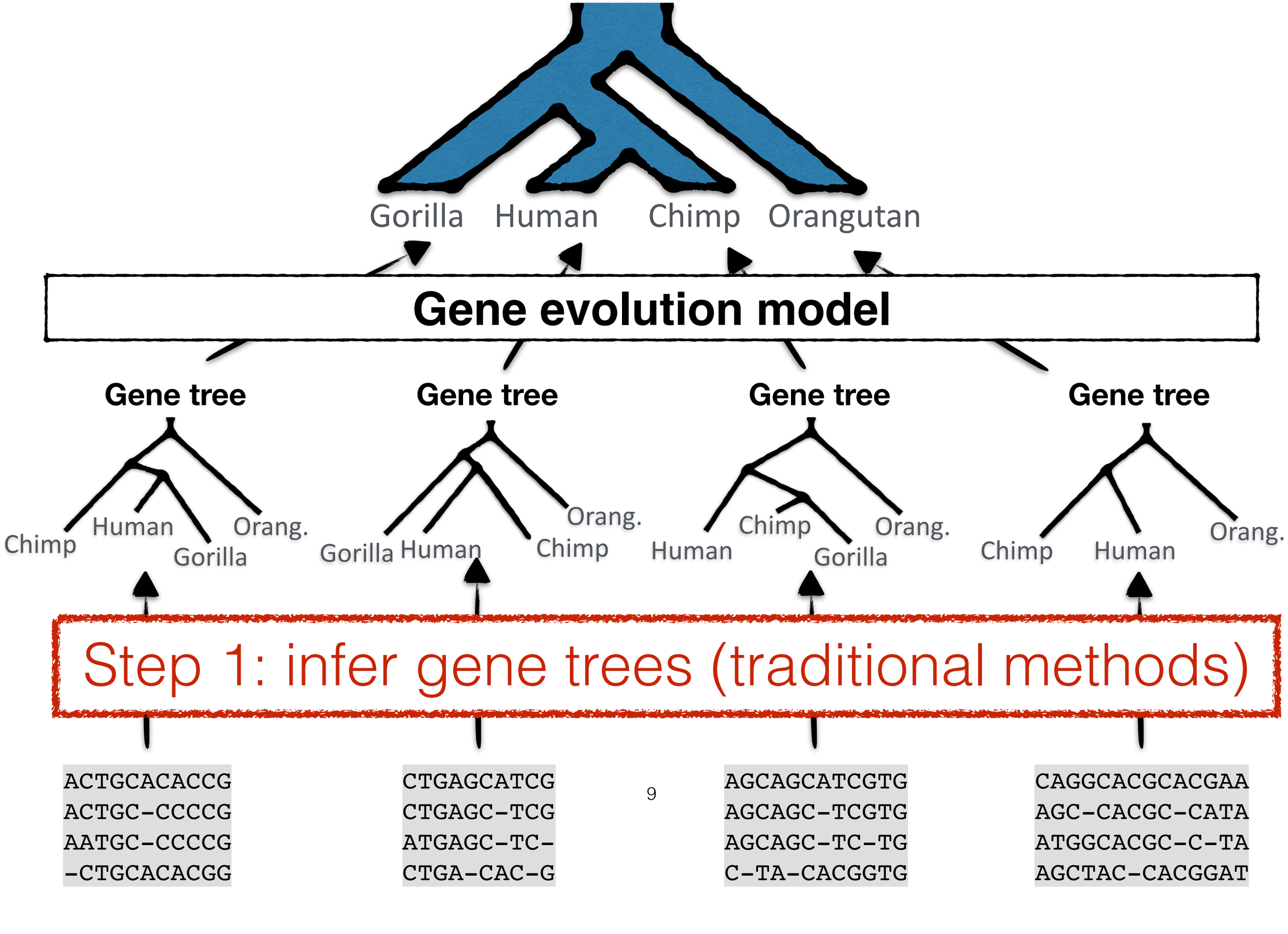
- A statistical gene tree evolution model for ILS
  [Pamilo and Nei, 1988] [Rannala and Yang, 2003]
- Does not model recombination within a gene

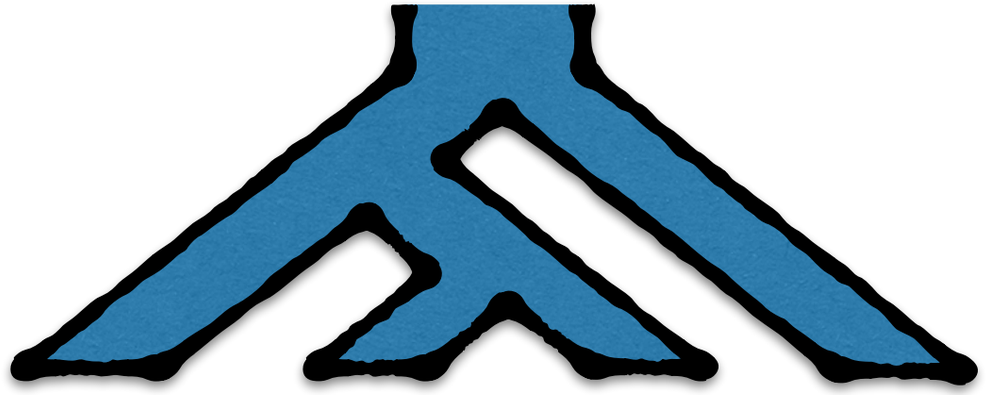
Multi-species coalescent (MSC) model

- A statistical gene tree evolution model for ILS
  [Pamilo and Nei, 1988] [Rannala and Yang, 2003]
  - Does not model recombination within a gene

- In theory, we can infer the species tree given a large **randomly distributed** sample of **recombination-free**, **reticulation-free, orthologous, error-free** gene trees

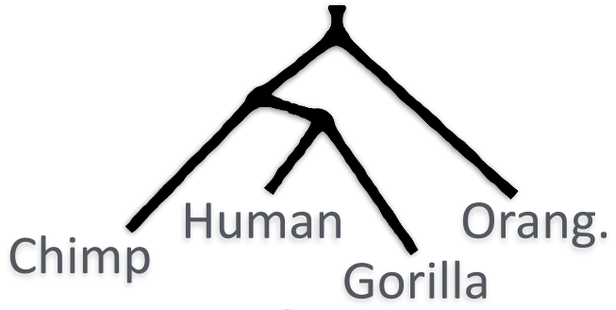

Gorilla
Human
Chimp
Orangutan
Gene evolution model
Gene tree
Gene tree
Gene tree
Gene tree
Chimp
Human
Orang.
Gorilla
Gorilla
Human
Orang.
Chimp
Human
Chimp
Orang.
Gorilla
Chimp
Human
Orang.
Sequence evolution model
ACTGCACACCG
ACTGC-CCCCG
AATGC-CCCCG
-CTGCACACGG
CTGAGCATCG
CTGAGC-TCG
ATGAGC-TC-
CTGA-CAC-G
AGCAGCATCGTG
AGCAGC-TCGTG
AGCAGC-TC-TG
C-TA-CACGGTG
CAGGCACGCACGAA
AGC-CACGC-CATA
ATGGCACGC-C-TA
AGCTAC-CACGGAT


Gorilla
Human
Chimp
Orangutan
Gene evolution model
Gene tree
Gene tree
Gene tree
Gene tree
Chimp
Human
Orang.
Gorilla
Gorilla
Human
Orang.
Chimp
Human
Chimp
Orang.
Gorilla
Chimp
Human
Orang.
Step 1: infer gene trees (traditional methods)
ACTGCACACCG
ACTGC-CCCCG
AATGC-CCCCG
-CTGCACACGG
CTGAGCATCG
CTGAGC-TCG
ATGAGC-TC-
CTGA-CAC-G
AGCAGCATCGTG
AGCAGC-TCGTG
AGCAGC-TC-TG
C-TA-CACGGTG
CAGGCACGCACGAA
AGC-CACGC-CATA
ATGGCACGC-C-TA
AGCTAC-CACGGAT

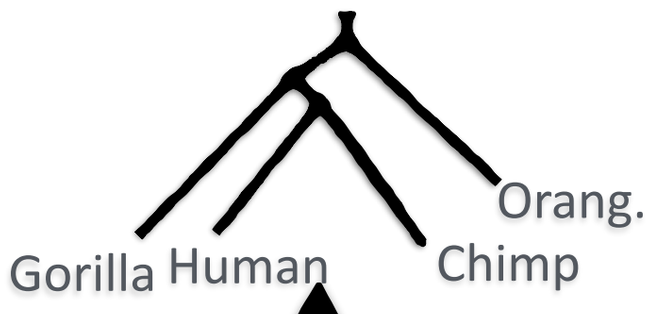


Step 2: infer species trees

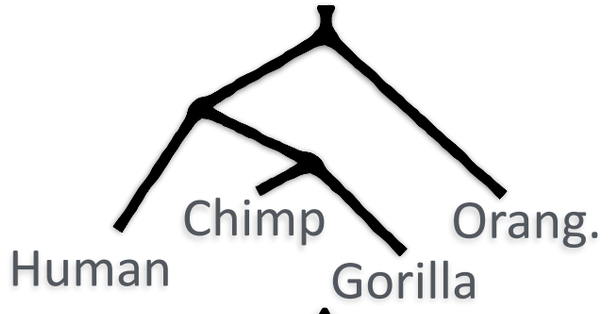
Gene tree



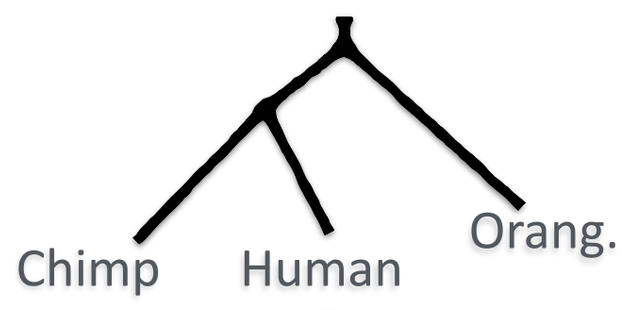
Gene tree



Gene tree



Gene tree



Step 1: infer gene trees (traditional methods)

```
ACTGCACACCG
ACTGC-CCCCG
AATGC-CCCCG
-CTGCACACGG
```

```
CTGAGCATCG
CTGAGC-TCG
ATGAGC-TC-
CTGA-CAC-G
```

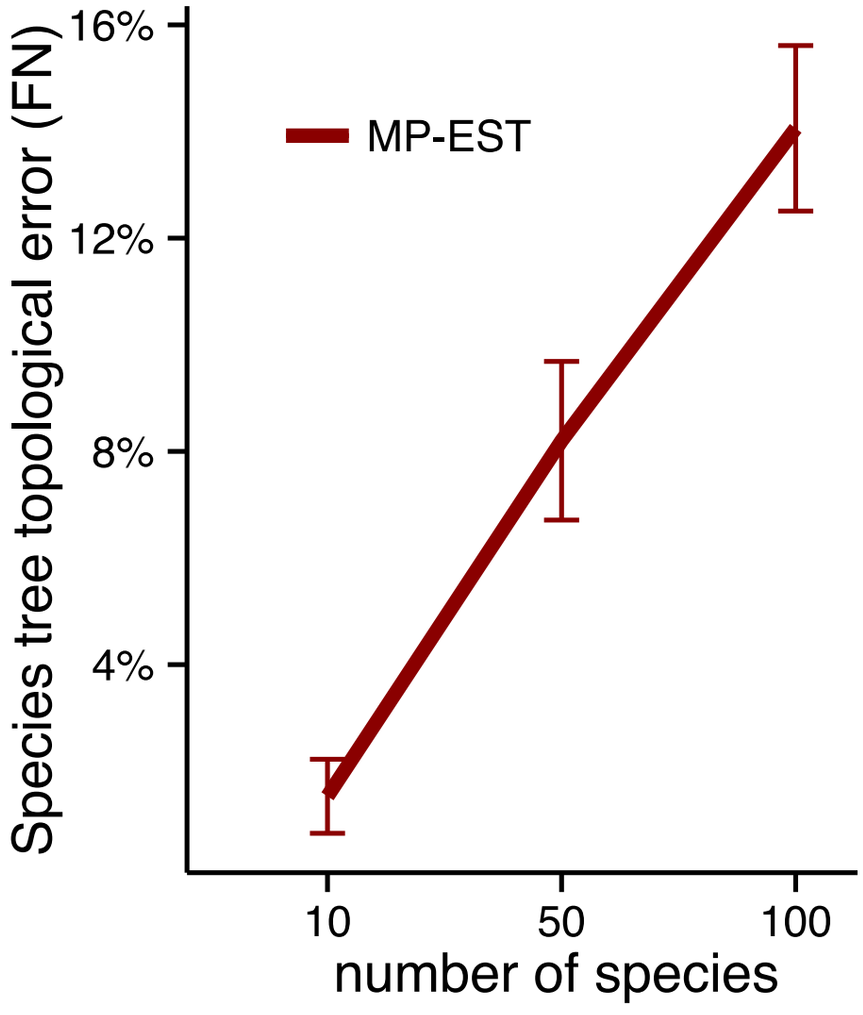
```
AGCAGCATCGTG
AGCAGC-TCGTG
AGCAGC-TC-TG
C-TA-CACGGTG
```

```
CAGGCACGCACGAA
AGC-CACGC-CATA
ATGGCACGC-C-TA
AGCTAC-CACGGAT
```

Challenge 1:

Inferring the species tree from a set of gene trees is difficult for large datasets

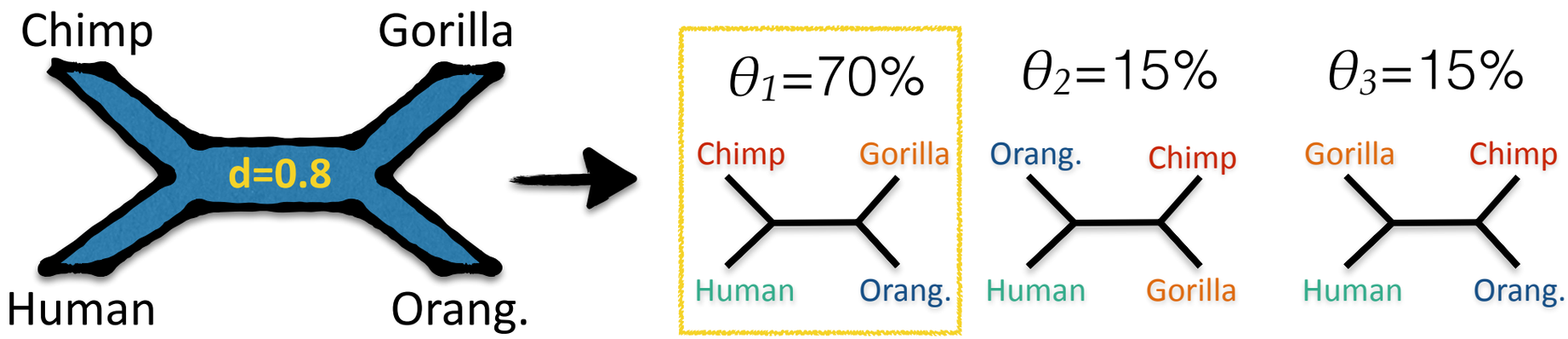
Number of species impacts estimation error in the species tree



1000 genes, “medium” levels of ILS, simulated species trees
[S. Mirarab, T. Warnow, 2015]

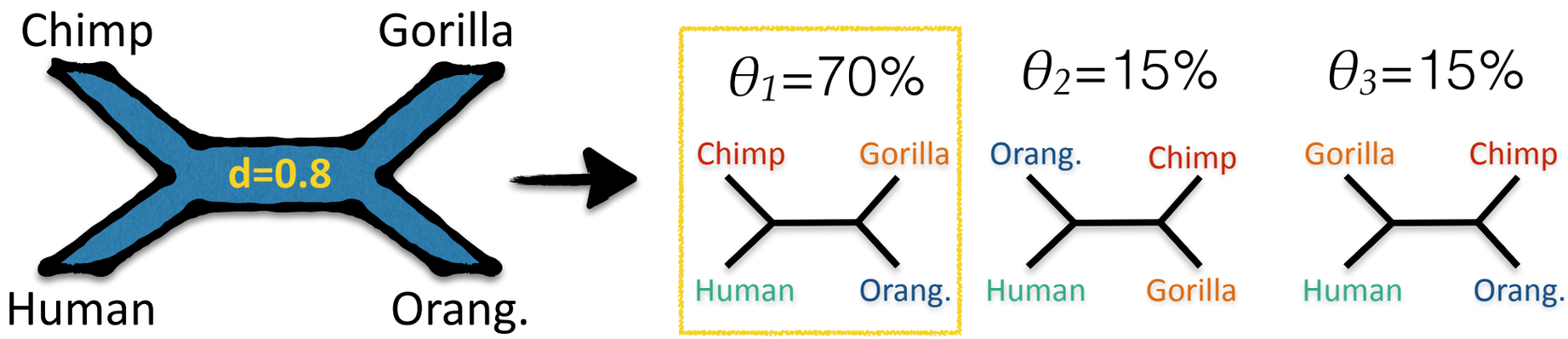
Unrooted quartets under MSC model

For a quartet (4 species), the most probable unrooted quartet tree (among the gene trees) is the unrooted species tree topology
(Allman, et al. 2010)



Unrooted quartets under MSC model

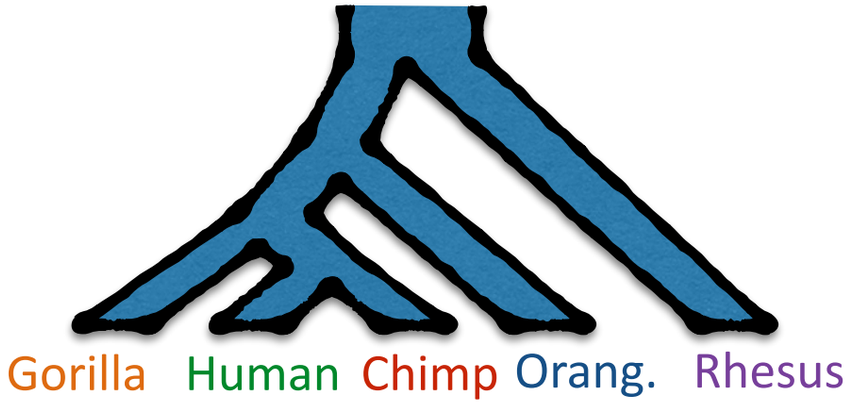
For a quartet (4 species), the most probable unrooted quartet tree (among the gene trees) is the unrooted species tree topology
(Allman, et al. 2010)



The most frequent gene tree
=
The most likely species tree

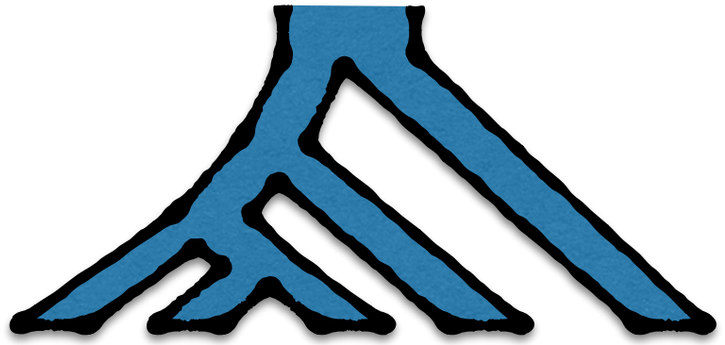
More than 4 species

For 5 or more species, the unrooted species tree topology can be different from the most probable gene tree (called “anomaly zone”)
(Degnan, 2013)



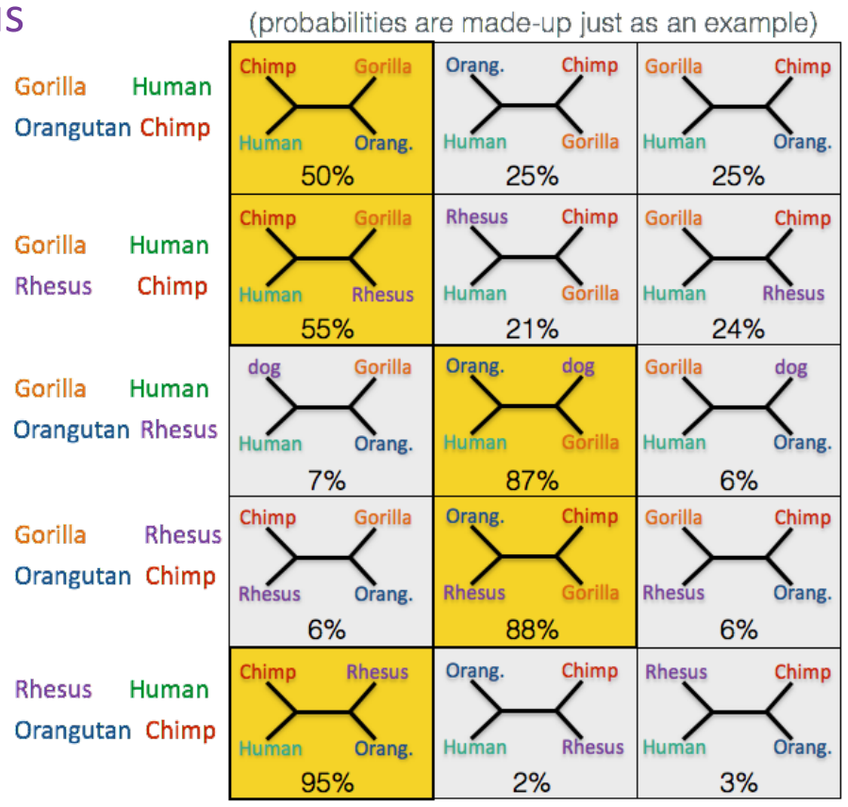
More than 4 species

For 5 or more species, the unrooted species tree topology can be different from the most probable gene tree (called “anomaly zone”)
(Degnan, 2013)



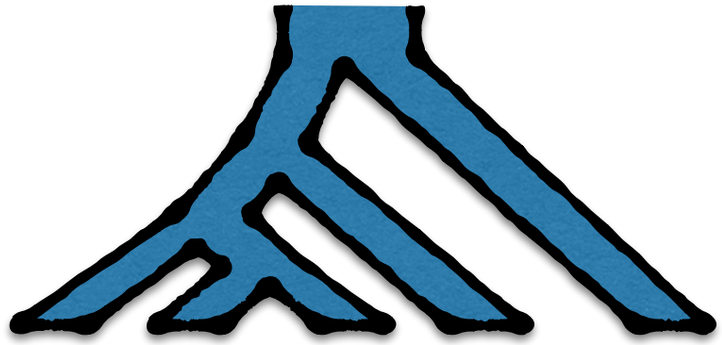
1. Break gene trees into $\binom{n}{4}$ quartets of species
2. Find the dominant tree for all quartets of taxa
3. Combine quartet trees

   Some tools (e.g.. BUCKy-p [Larget, et al., 2010])



More than 4 species

For 5 or more species, the unrooted species tree topology can be different from the most probable gene tree (called "anomaly zone")
(Degnan, 2013)



1. E
2. F
3. C
   S

Alternative:
weight all $3\binom{n}{4}$ quartet topologies
by their frequency
and find the optimal tree

(probabilities are made-up just as an example)

Gorilla Human / Orangutan Chimp	Chimp Gorilla / Human Orang. 50%	Orang. Chimp / Human Gorilla 25%	Gorilla Chimp / Human Orang. 25%
Gorilla Human / Rhesus Chimp	Chimp Gorilla / Human Rhesus 55%	Rhesus Chimp / Human Gorilla 19%	Gorilla Chimp / Human Rhesus 26%
Gorilla Human / Orangutan Rhesus	dog Gorilla / Human Orang. 7%	Orang. dog / Human Gorilla 87%	Gorilla dog / Human Orang. 6%
Gorilla Rhesus / Orangutan Chimp	Chimp Gorilla / Rhesus Orang. 6%	Orang. Chimp / Rhesus Gorilla 88%	Gorilla Chimp / Rhesus Orang. 6%
Rhesus Human / Orangutan Chimp	Chimp Rhesus / Human Orang. 95%	Orang. Chimp / Human Rhesus 2%	Rhesu Chimp / Human Orang. 3%

Maximum Quartet Support Species Tree

$$Score(T) = \sum_{1}^{k} |\, Q(T) \cup Q(t_i) \,|$$

the set of quartet trees induced by T

a gene tree

- Optimization problem:

Find the species tree with the maximum number of induced quartet trees shared with the collection of input gene trees

Maximum Quartet Support Species Tree

$$Score(T) = \sum_{1}^{k} |\, Q(T) \cup Q(t_i) \,|$$

the set of quartet trees induced by T

a gene tree

- Optimization problem:

  Find the species tree with the maximum number of induced quartet trees shared with the collection of input gene trees

- Statistically consistent under the multi-species coalescent model when solved exactly
  [Mirarab, et al, Bioinformatics 2014]

Maximum Quartet Support Species Tree

$$Score(T) = \sum_{1}^{k} |\, Q(T) \cup Q(t_i) \,|$$

the set of quartet trees induced by T

a gene tree

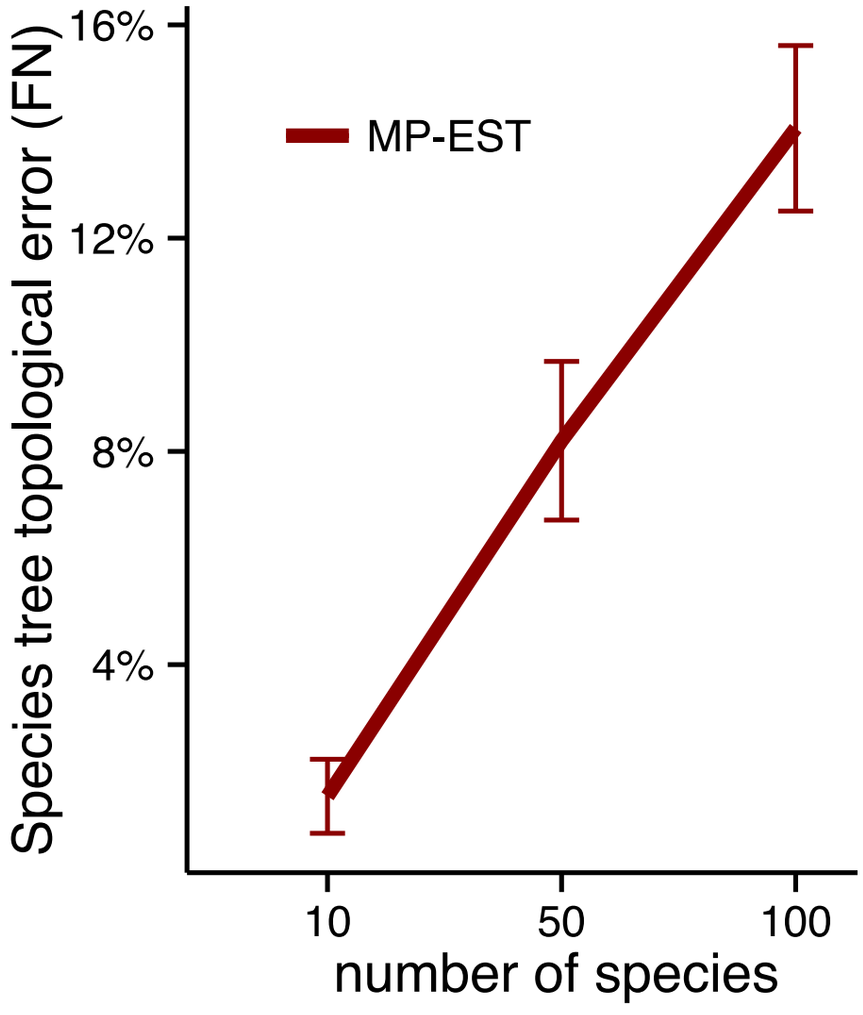
- Optimization problem:

  Find the species tree with the maximum number of induced quartet trees shared with the collection of input gene trees

- Statistically consistent under the multi-species coalescent model when solved exactly
  [Mirarab, et al, Bioinformatics 2014]

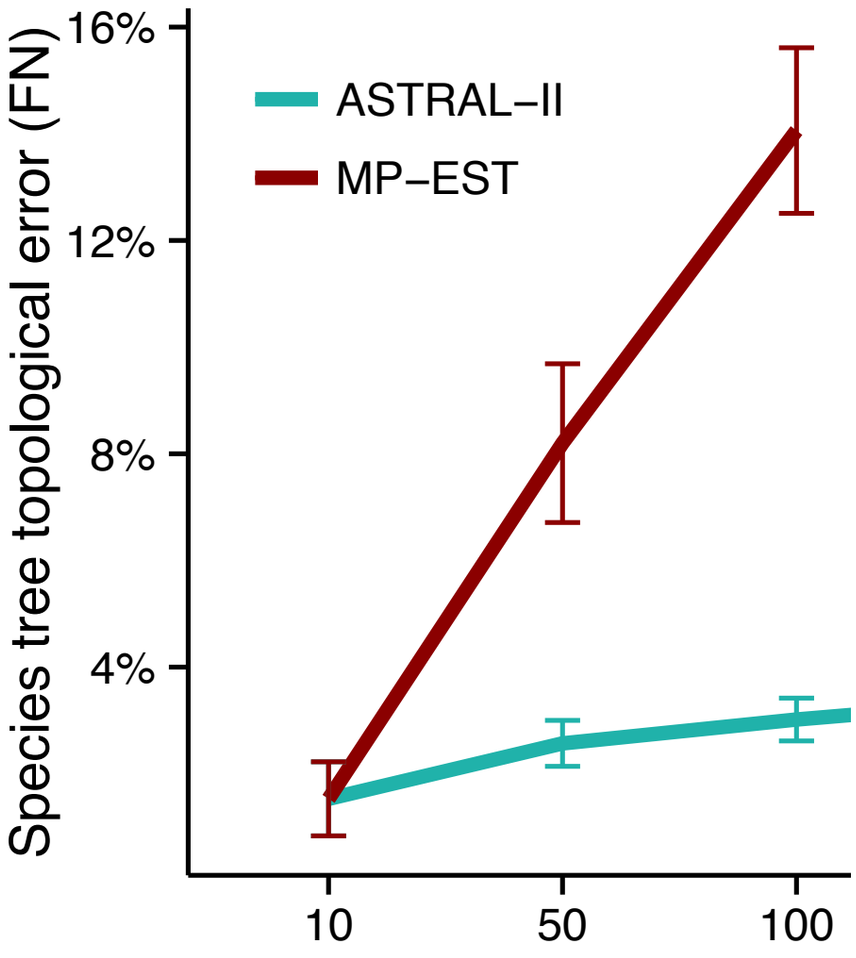
- ASTRAL: an exact solution using dynamic programming

Number of species impacts estimation error in the species tree



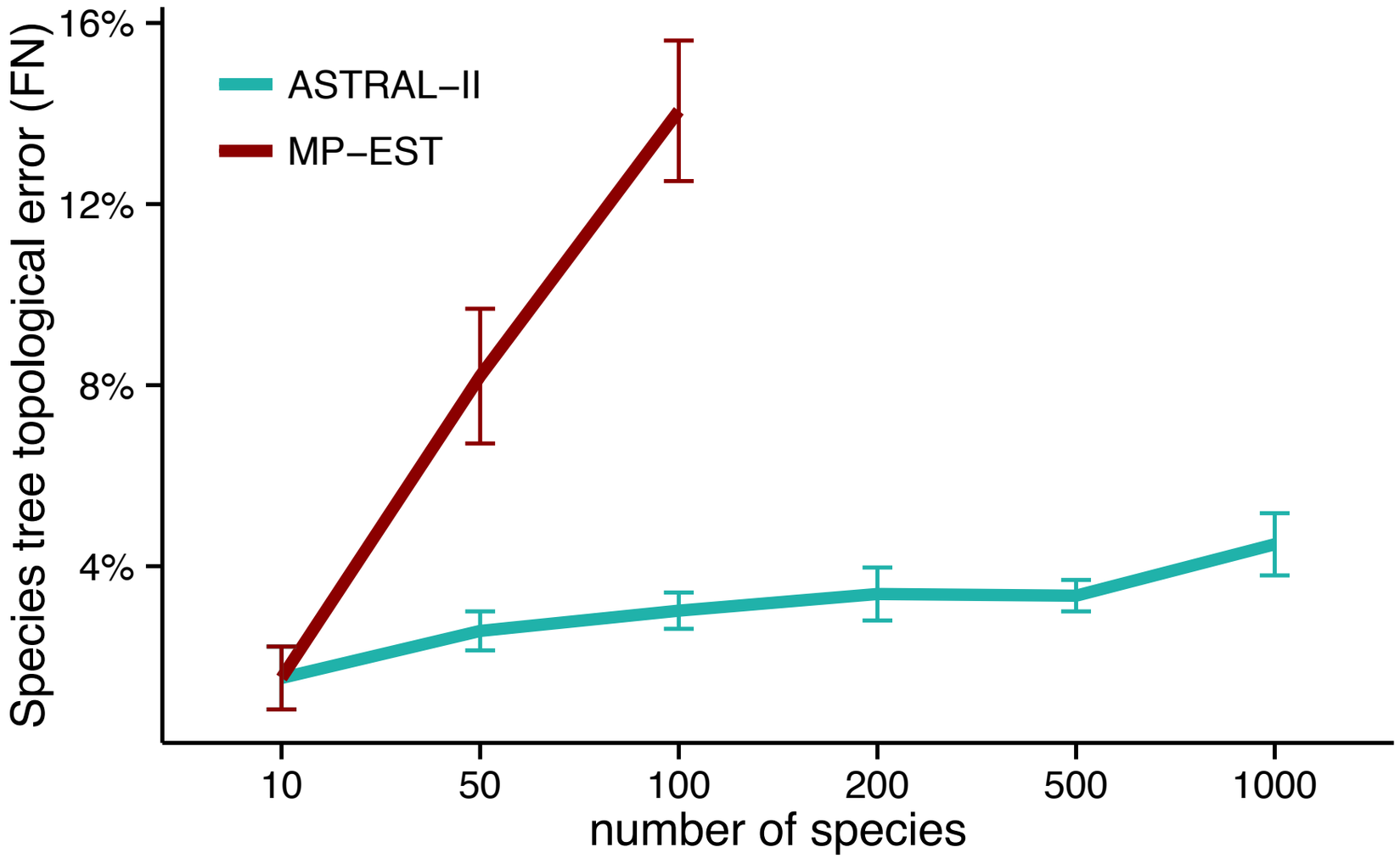
1000 genes, “medium” levels of ILS, simulated species trees
[S. Mirarab, T. Warnow, 2015]

ASTRAL: accurate and scalable



1000 genes, “medium” levels of ILS, simulated species trees
[S. Mirarab, T. Warnow, 2015]

ASTRAL: accurate and scalable



1000 genes, “medium” levels of ILS, simulated species trees
[S. Mirarab, T. Warnow, 2015]

ASTRAL versions

- ASTRAL-I (<v. 4.7.3): 2014 - 2015

ASTRAL versions

- ASTRAL-I (<v. 4.7.3): 2014 - 2015
- ASTRAL-II (<v. 5.1.0): 2015 - 2017
    - Increased the accuracy by expanding the search space and improved the scalability
    - Can handle polytomies in input gene trees

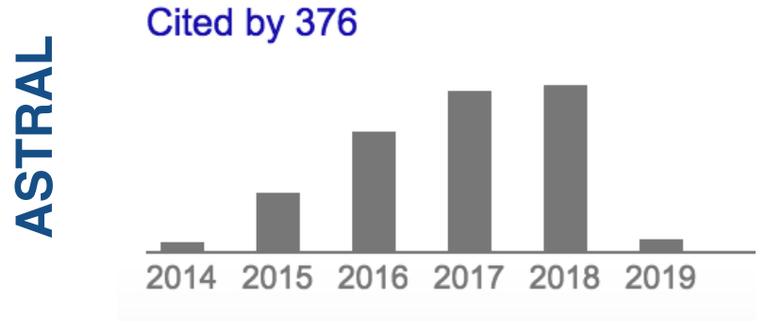
ASTRAL versions

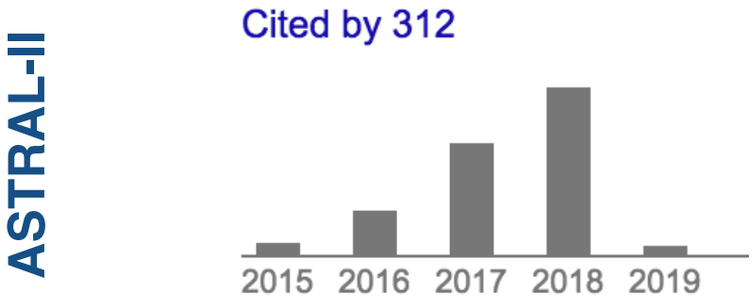
- ASTRAL-I (<v. 4.7.3): 2014 - 2015
- ASTRAL-II (<v. 5.1.0): 2015 - 2017
  - Increased the accuracy by expanding the search space and improved the scalability
  - Can handle polytomies in input gene trees
- ASTRAL-III (>v. 5.1.1): since 2017
  - Better running time, and better search space
  - Especially improved for unresolved trees, making it feasible to remove very low support branches

ASTRAL used widely

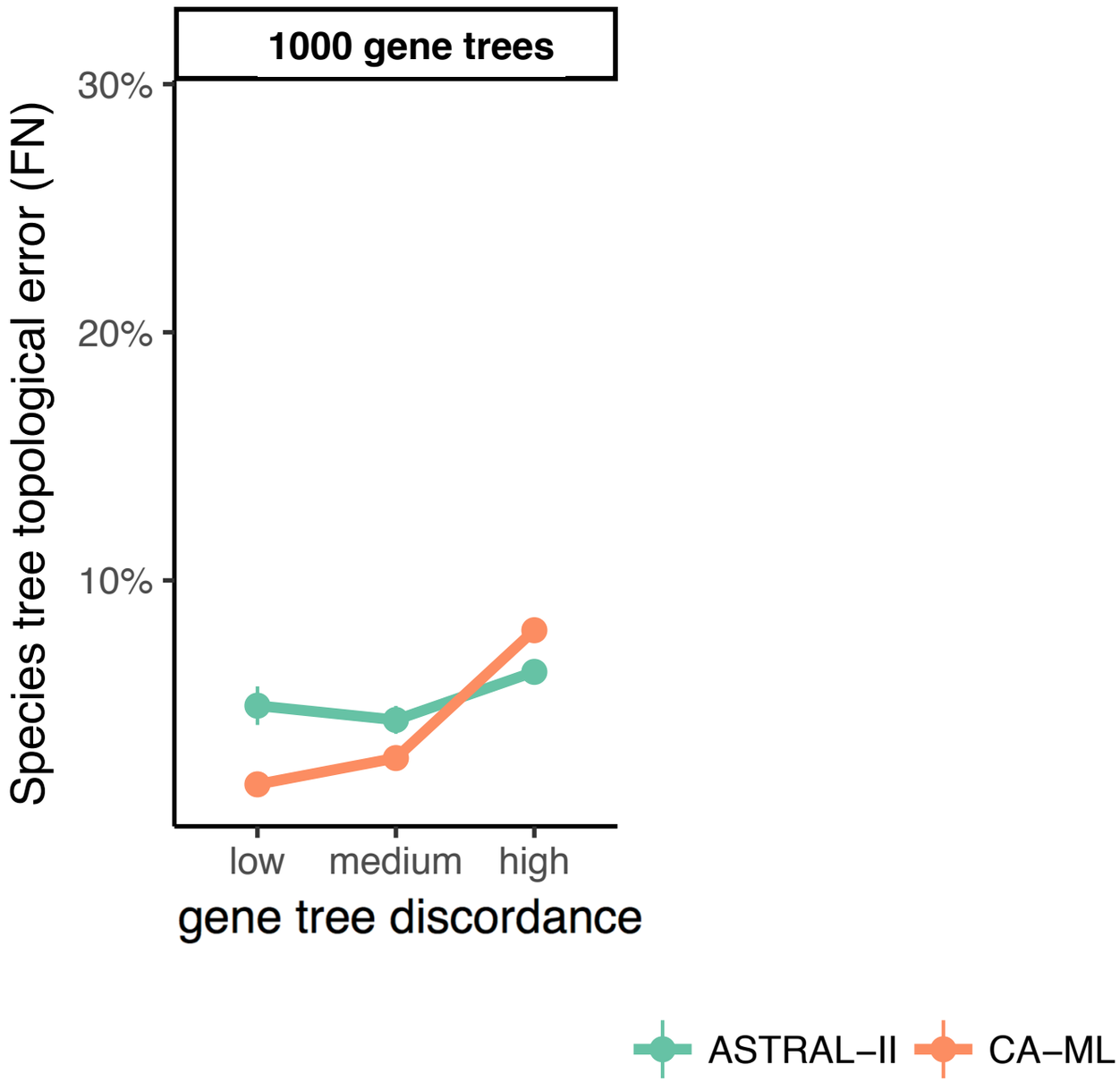
Early use:

- Plants: Wickett, et al., 2014, PNAS
- Birds: Prum, et al., 2015, Nature
- Xenoturbella, Cannon et al., 2016, Nature
- Xenoturbella, Rouse et al., 2016, Nature
- Flatworms: Laumer, et al., 2015, eLife
- Shrews: Giarla, et al., 2015, Syst. Bio.
- Frogs: Yuan et al., 2016, Syst. Bio.
- Tomatoes: Pease, et al., 2016, PLoS Bio.
- Angiosperms: Huang et al., 2016, MBE
- Worms: Andrade, et al., 2015, MBE



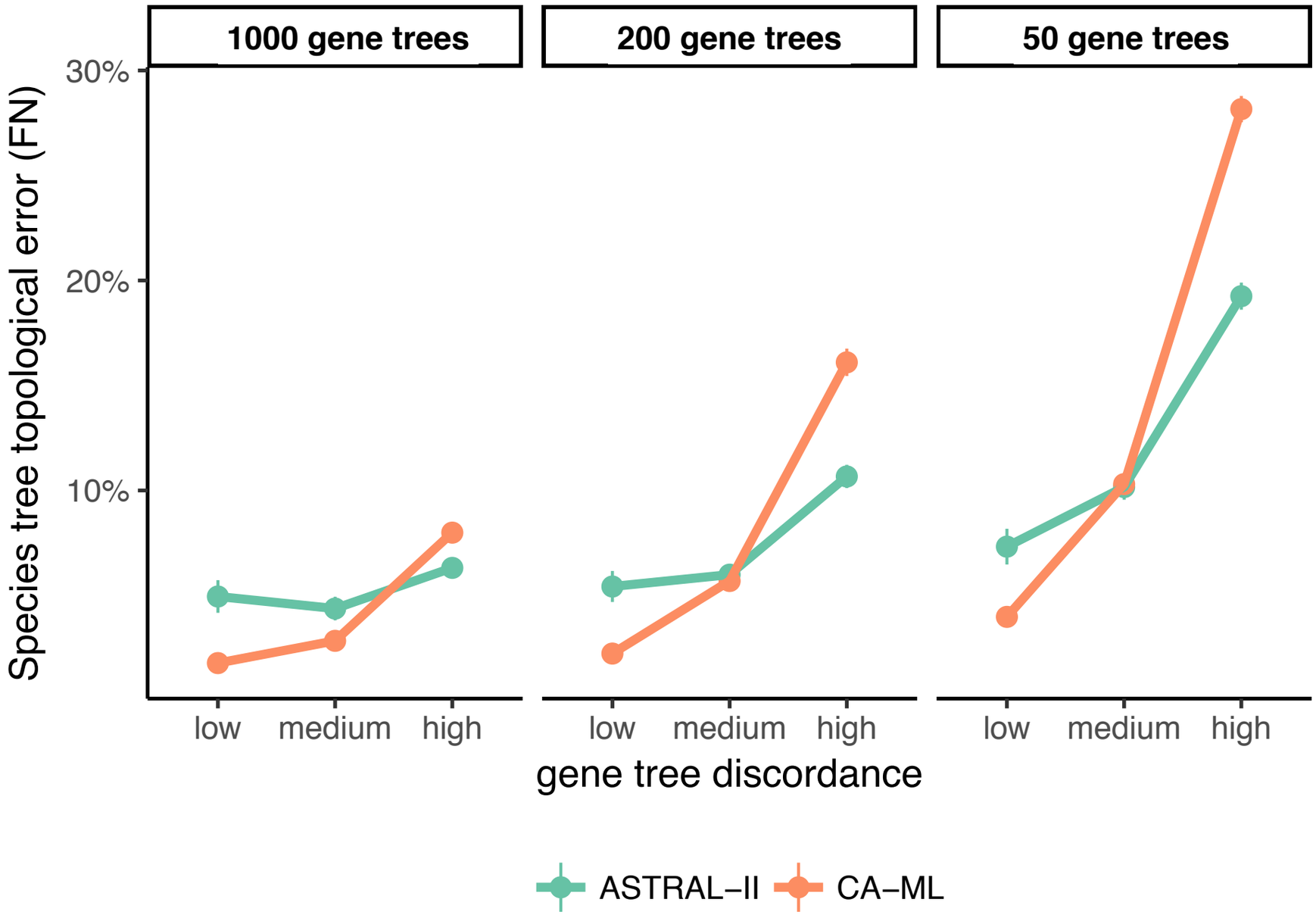


Comparison to concatenation: depends on the level of discordance

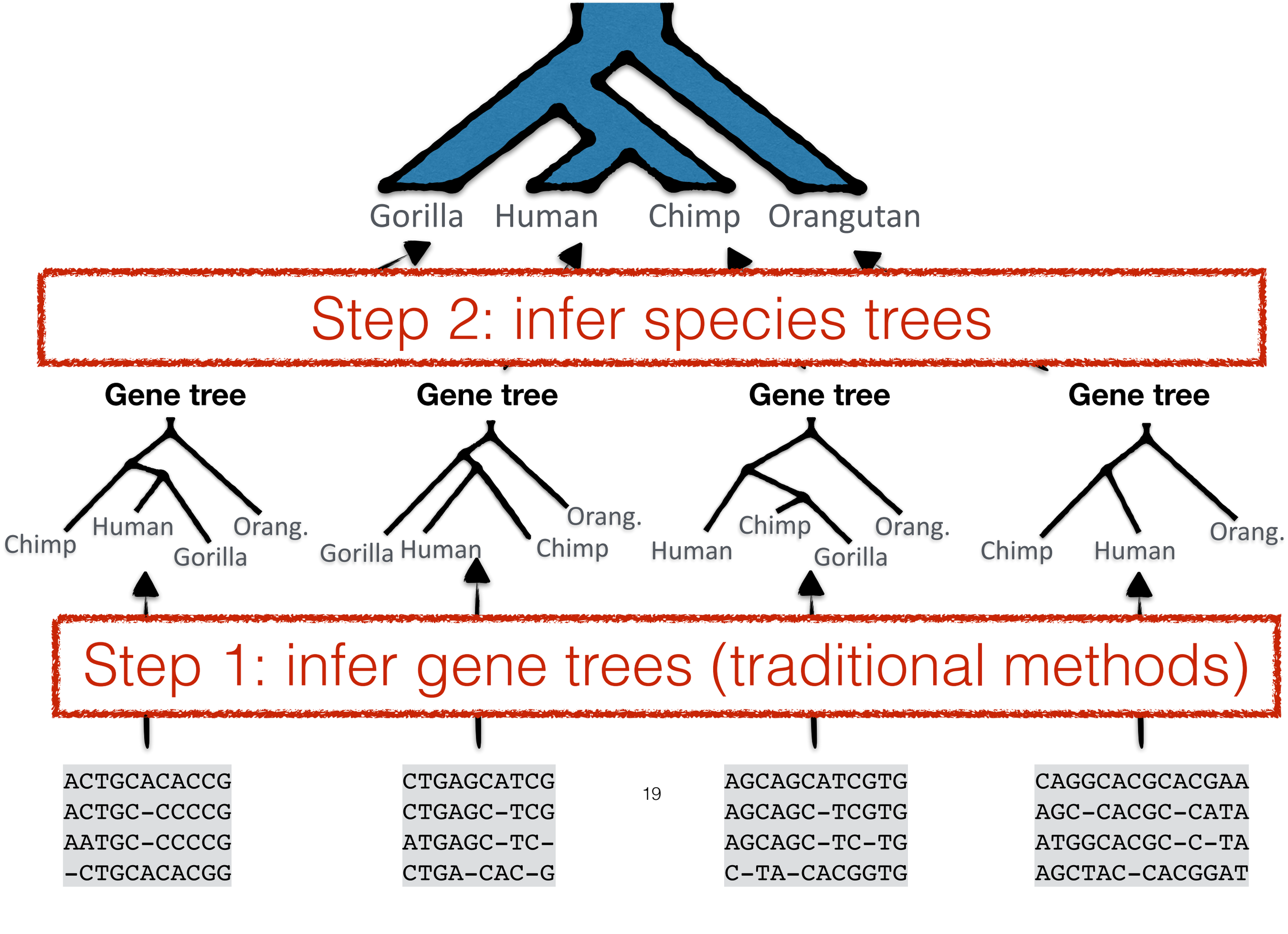


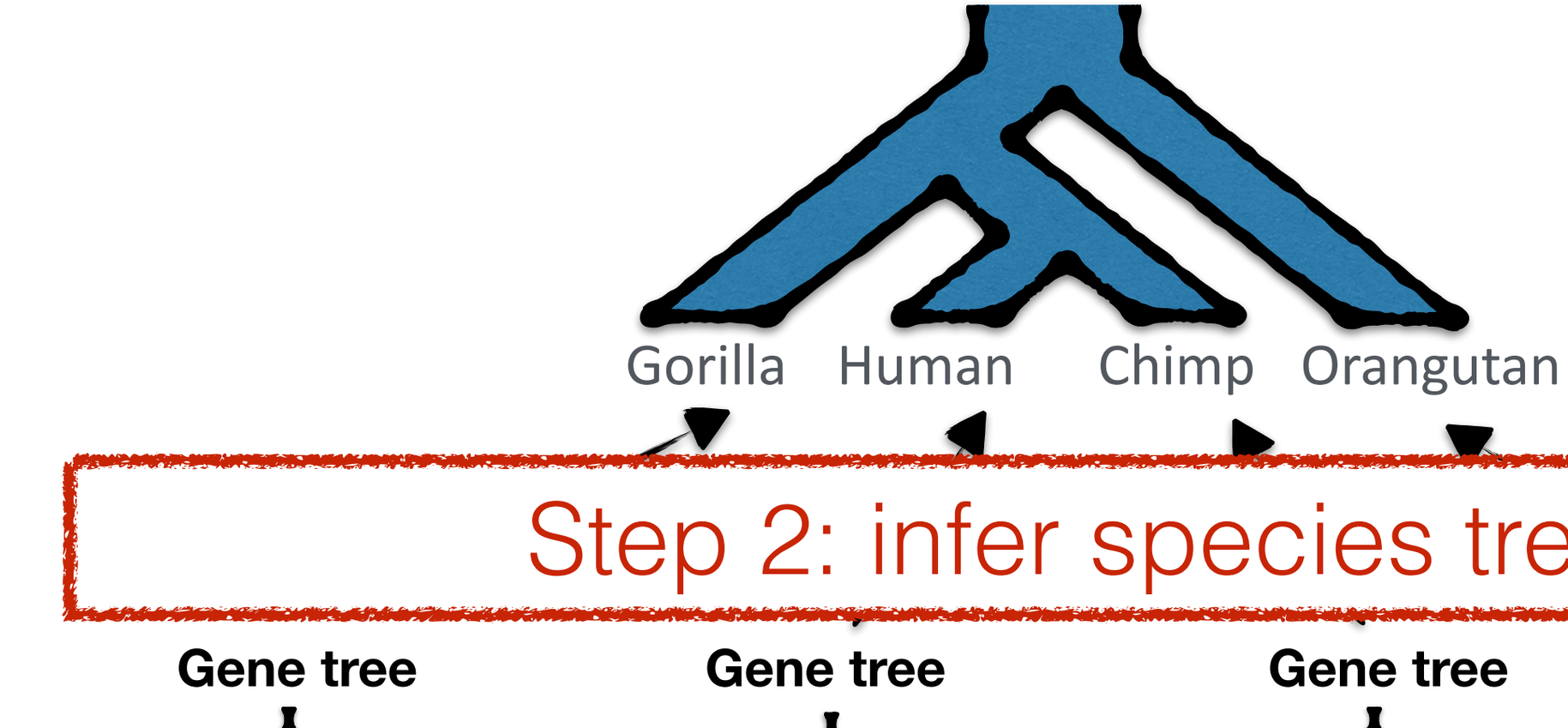
Simulations, 200 species, deep ILS [Mirarab and Warnow, 2016]

Comparison to concatenation: depends on the level of discordance

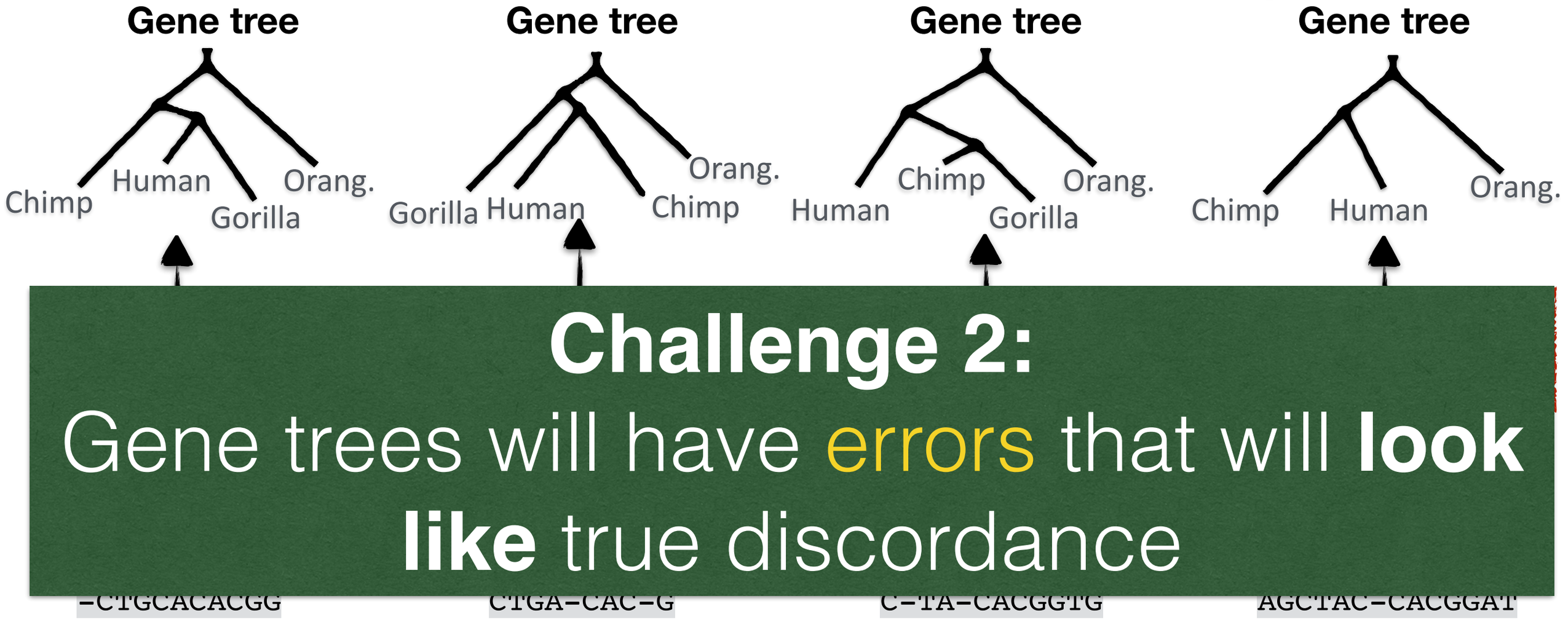


Simulations, 200 species, deep ILS [Mirarab and Warnow, 2016]


Gorilla
Human
Chimp
Orangutan
Step 2: infer species trees
Gene tree
Gene tree
Gene tree
Gene tree
Chimp
Human
Orang.
Gorilla
Gorilla
Human
Orang.
Chimp
Human
Chimp
Orang.
Gorilla
Chimp
Human
Orang.
Step 1: infer gene trees (traditional methods)
ACTGCACACCG
ACTGC-CCCCG
AATGC-CCCCG
-CTGCACACGG
CTGAGCATCG
CTGAGC-TCG
ATGAGC-TC-
CTGA-CAC-G
AGCAGCATCGTG
AGCAGC-TCGTG
AGCAGC-TC-TG
C-TA-CACGGTG
CAGGCACGCACGAA
AGC-CACGC-CATA
ATGGCACGC-C-TA
AGCTAC-CACGGAT



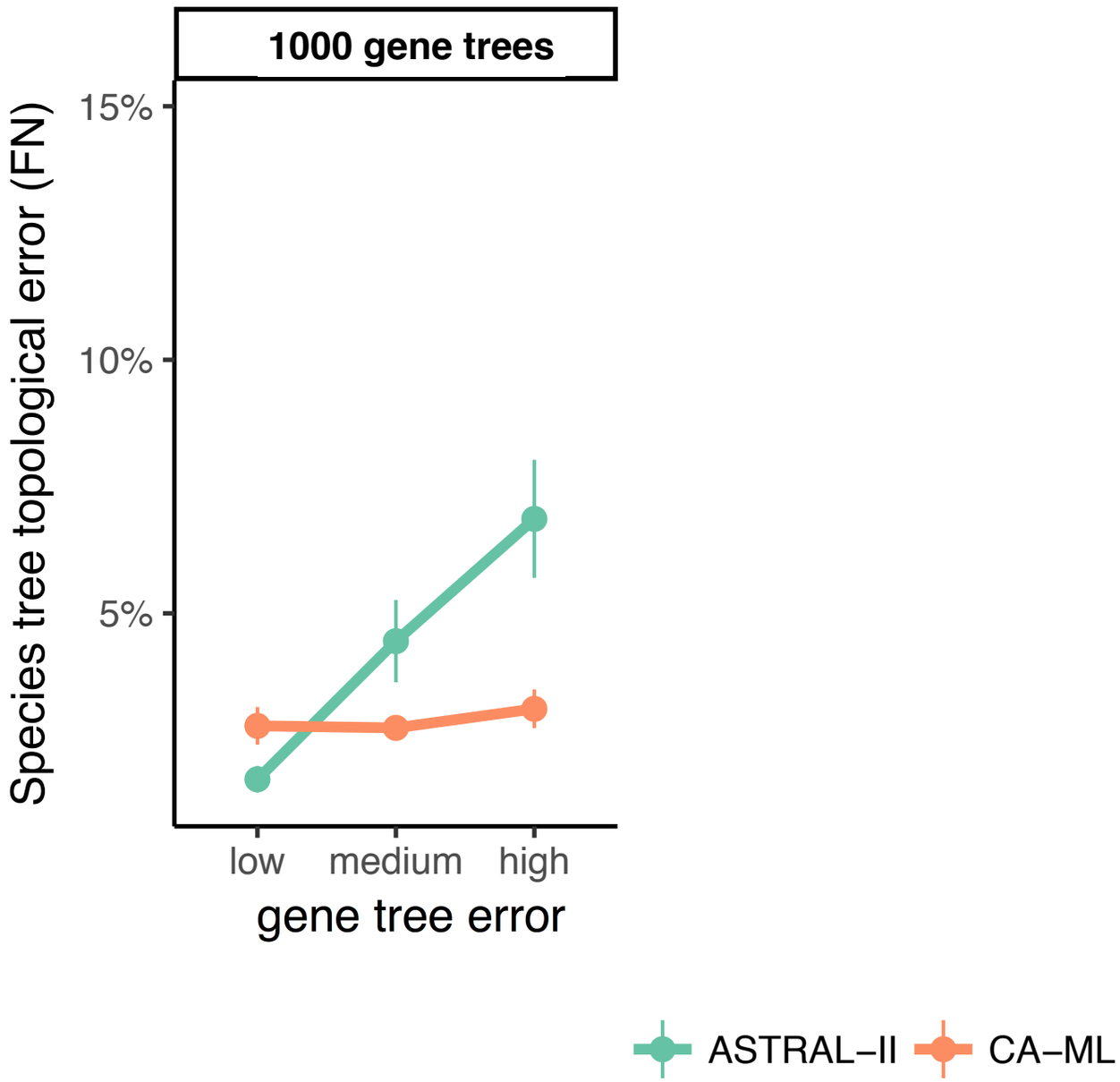
Step 2: infer species trees



Challenge 2:

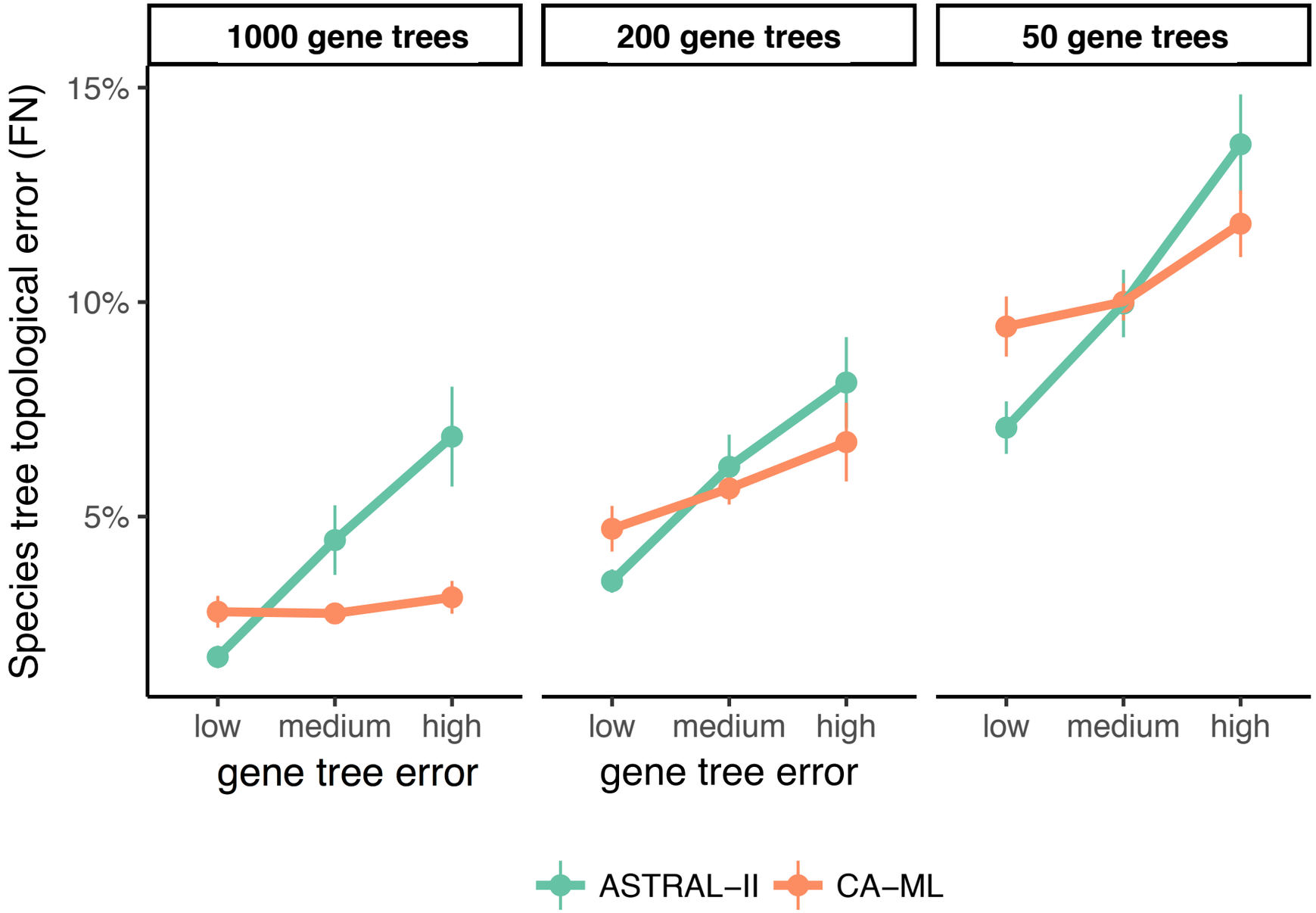
Gene trees will have errors that will **look like** true discordance

Comparison to concatenation: depends on the level of gene tree error



Simulations, 200 species, deep medium level ILS [Mirarab and Warnow, 2016]

Comparison to concatenation: depends on the level of gene tree error



Simulations, 200 species, deep medium level ILS [Mirarab and Warnow, 2016]

How to deal with gene tree error?

- Statistical binning (used in Jarvis *et al*.) [Mirarab et al, Science, 2014] and other forms of binning have emerged since [Bayzid, et al, PLOS One, 2015]

How to deal with gene tree error?

- Statistical binning (used in Jarvis *et al*.) [Mirarab et al, Science, 2014] and other forms of binning have emerged since [Bayzid, et al, PLOS One, 2015]

- There are new site-based methods, which avoid gene trees altogether
  - SVDQuartets [Chou, BMC Genomics, 2015]

How to deal with gene tree error?

- Statistical binning (used in Jarvis *et al.*) [Mirarab et al, Science, 2014] and other forms of binning have emerged since [Bayzid, et al, PLOS One, 2015]

- There are new site-based methods, which avoid gene trees altogether
  - SVDQuartets [Chou, BMC Genomics, 2015]

- *BEAST: co-estimate gene trees and species trees.
  - Its recent second version is more scalable

How to deal with gene tree error?

- Statistical binning (used in Jarvis *et al.*) [Mirarab et al, Science, 2014] and other forms of binning have emerged since [Bayzid, et al, PLOS One, 2015]

- There are new site-based methods, which avoid gene trees altogether
  - SVDQuartets [Chou, BMC Genomics, 2015]

- *BEAST: co-estimate gene trees and species trees.
  - Its recent second version is more scalable

- revPoMo: concatenation with ILS-aware sequence evolution models [Schrempf et al, J. Theor. Bio., 2016]

How to deal with gene tree error?

- Statistical binning (used in Jarvis *et al.*) [Mirarab et al, Science, 2014] and other forms of binning have emerged since [Bayzid, et al, PLOS One, 2015]

- There are new site-based methods, which avoid gene trees altogether
  - SVDQuartets [Chou, BMC Genomics, 2015]

- *BEAST: co-estimate gene trees and species trees.
  - Its recent second version is more scalable

- revPoMo: concatenation with ILS-aware sequence evolution models [Schrempf et al, J. Theor. Bio., 2016]

- ASTRAL-III allows a different solution …

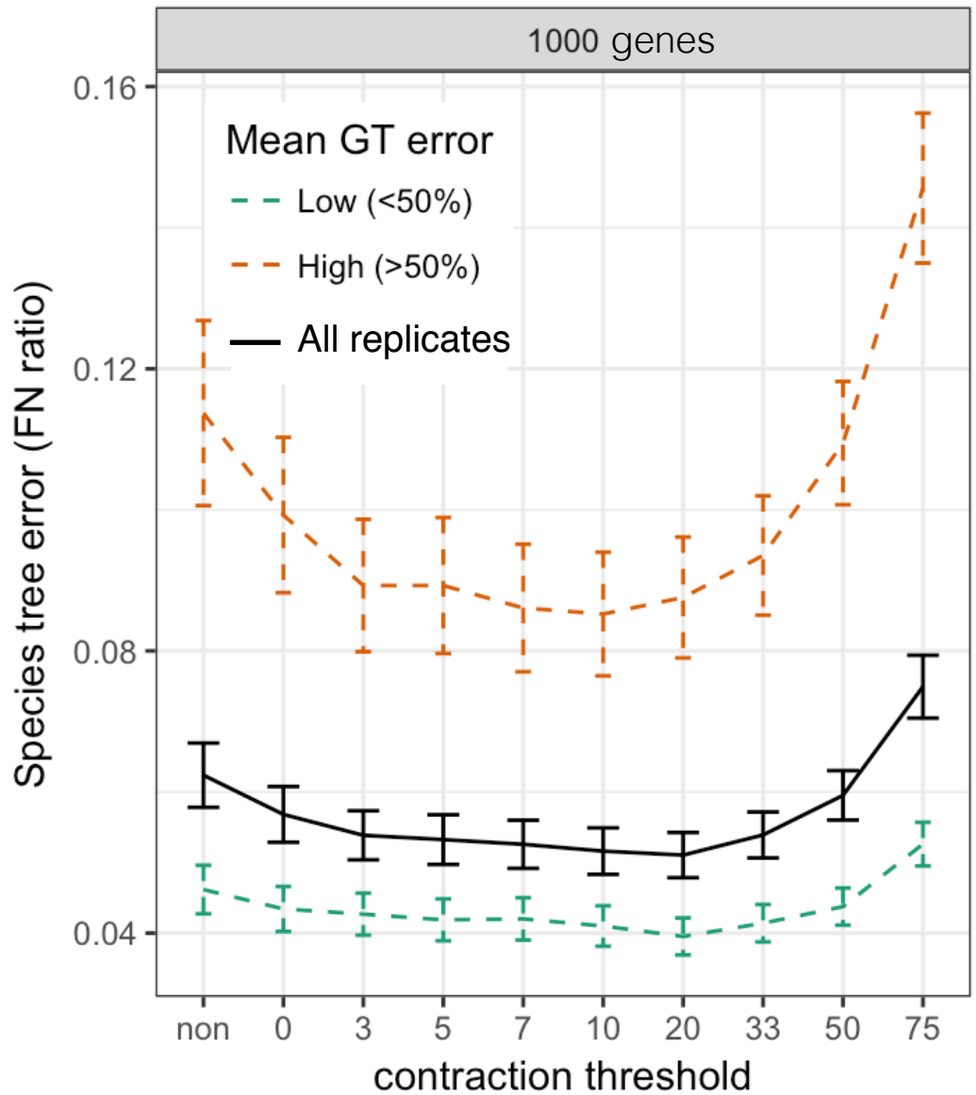
Contract low support branches

- It help to contract very low support branches

- Mostly helps in the presence of low support gene trees

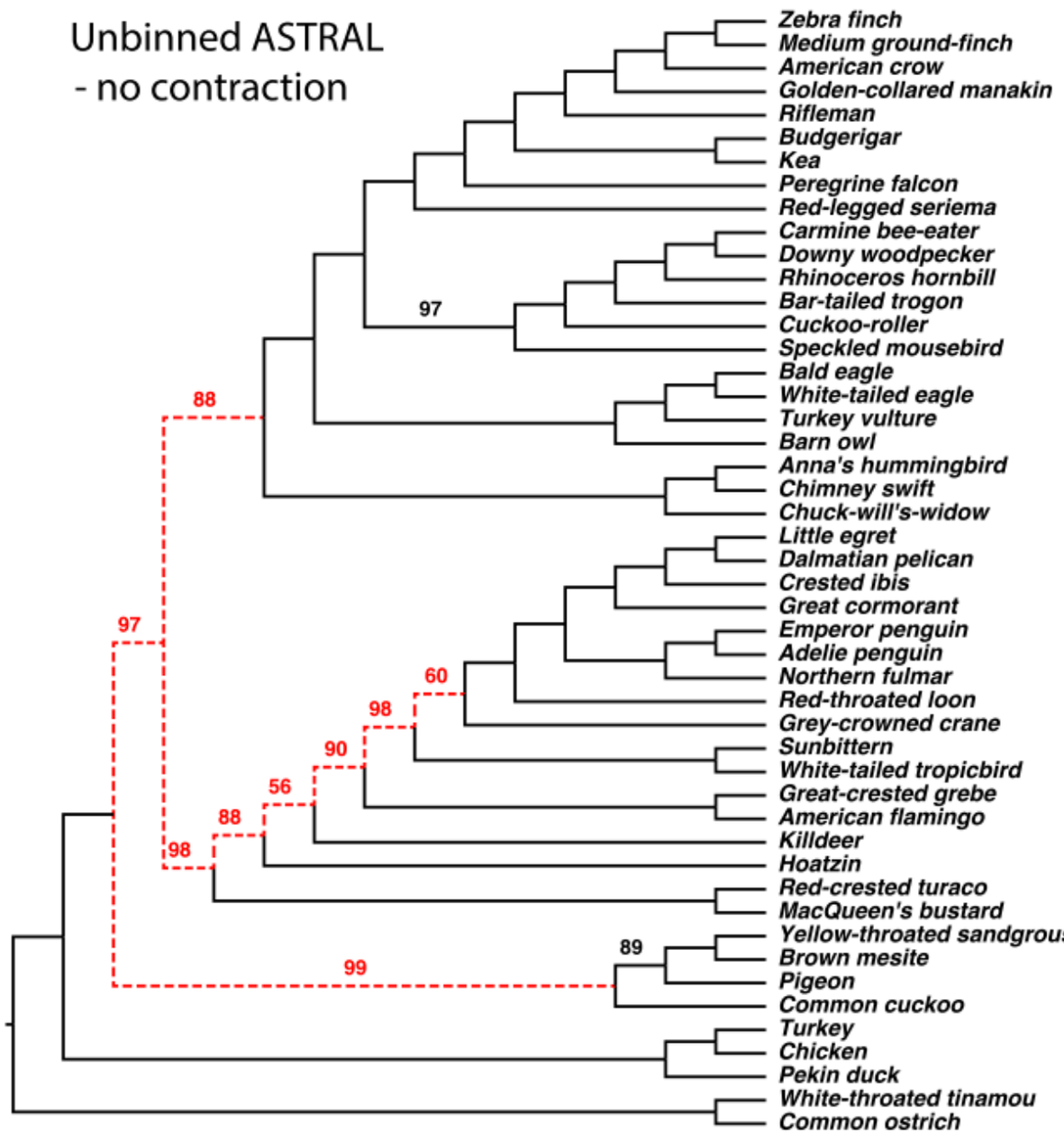
- Helps most for large numbers of gene trees

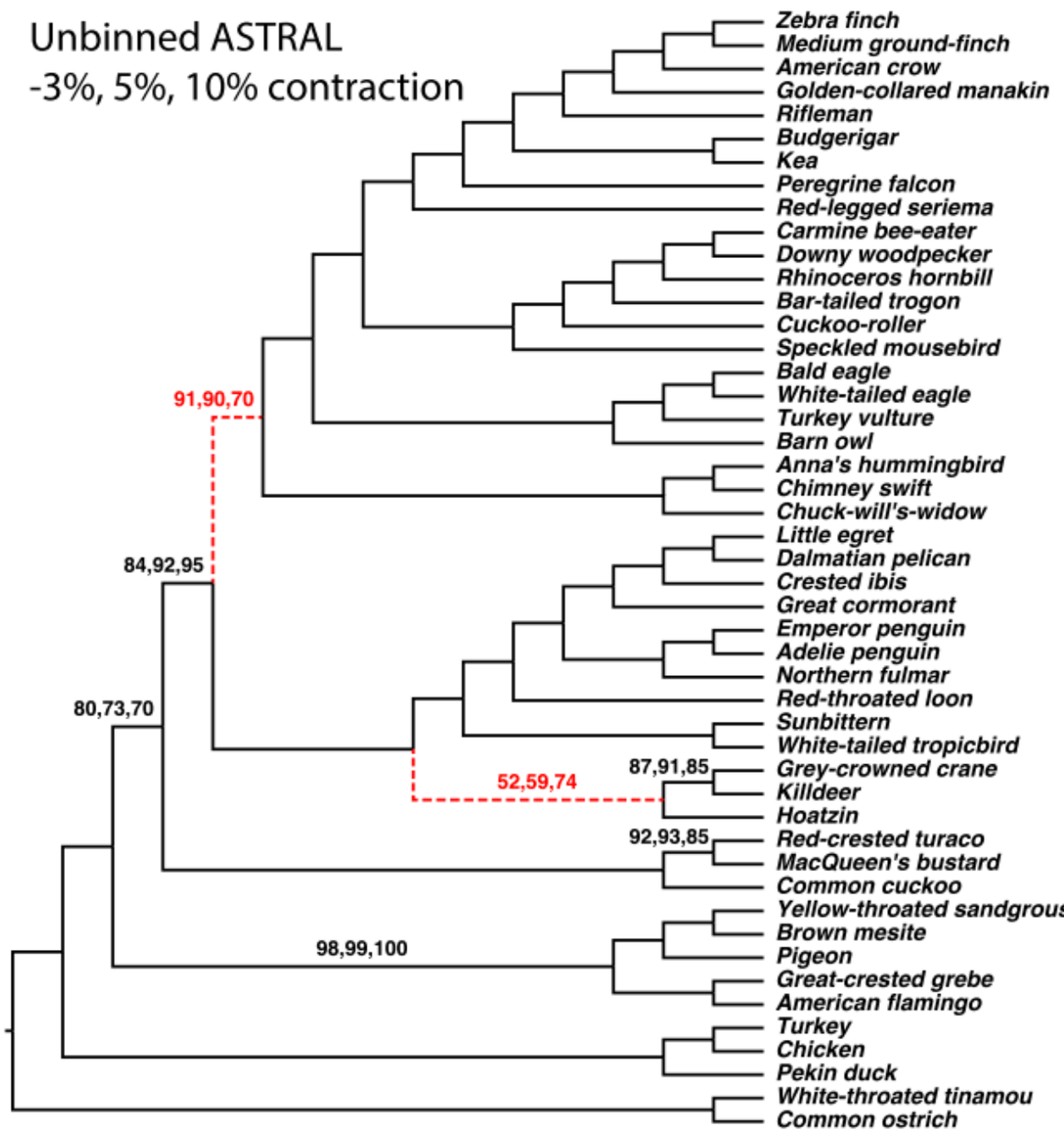
BMC Bioinformatics, 2018, Zhang et al.



Simulations: 100 taxa, simphy,
ILS: around 46% true discordance
FastTree, support from bootstrapping

ASTRAL-III on all 14,446 unbinned gene trees





Zhang, Chao, et al. BMC Bioinformatics 19, no. S6 (2018): 153. https://doi.org/10.1186/s12859-018-2129-y

Beyond topology, ASTRAL estimates …

- length of internal branches in coalescent units:
  # generations / population size

Beyond topology, ASTRAL estimates …

- length of internal branches in coalescent units:
  # generations / population size
- a measure of branch support called local posterior probability [Sayyari and Mirarab, MBE, 2016]

Beyond topology, ASTRAL estimates …

- length of internal branches in coalescent units:
  # generations / population size

- a measure of branch support called local posterior probability [Sayyari and Mirarab, MBE, 2016]

- P-values for a polytomy test
  [Sayyari and Mirarab, Genes, 2018]

Beyond topology, ASTRAL estimates …

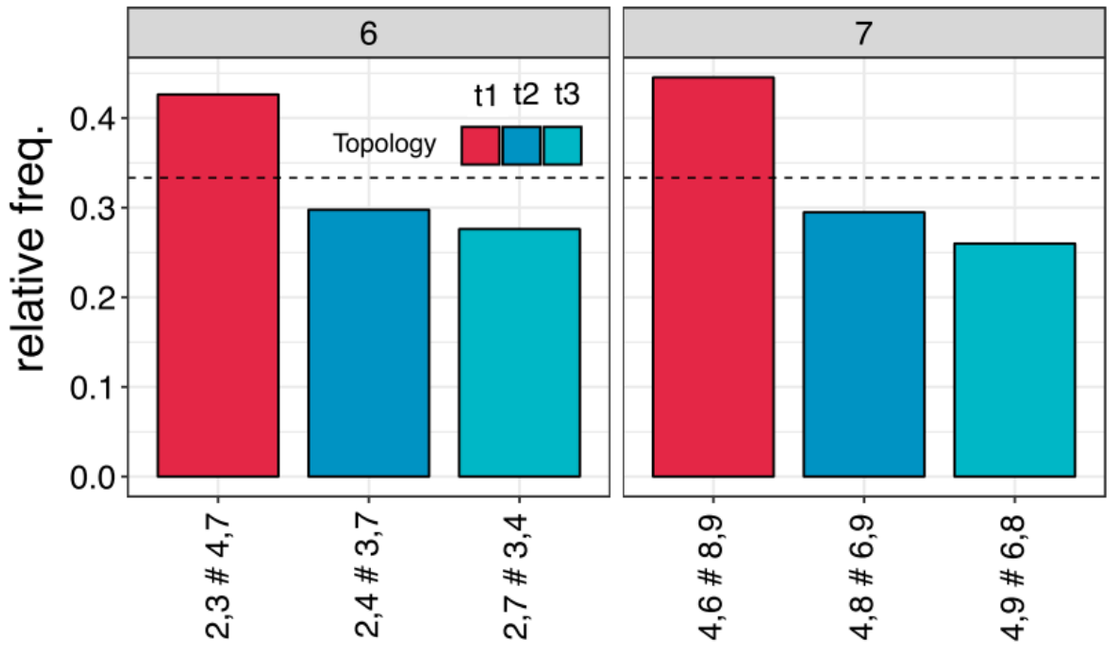
- length of internal branches in coalescent units:
  # generations / population size

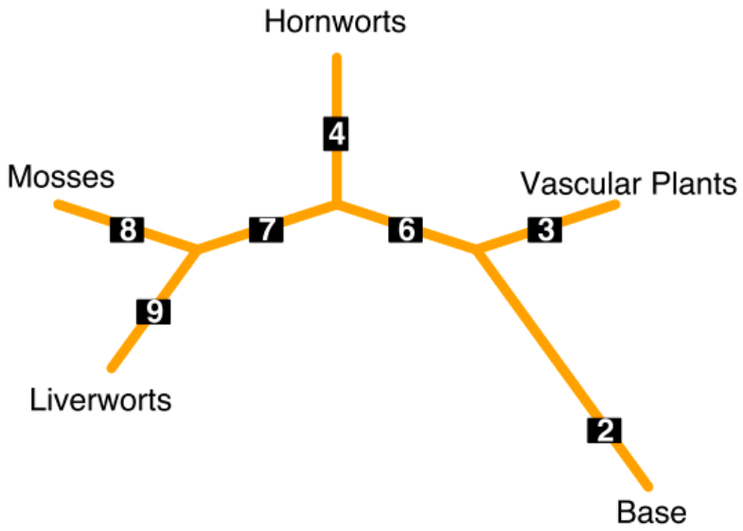
- a measure of branch support called local posterior probability [Sayyari and Mirarab, MBE, 2016]

- P-values for a polytomy test
  [Sayyari and Mirarab, Genes, 2018]

- quartet-based measures of gene tree discordance

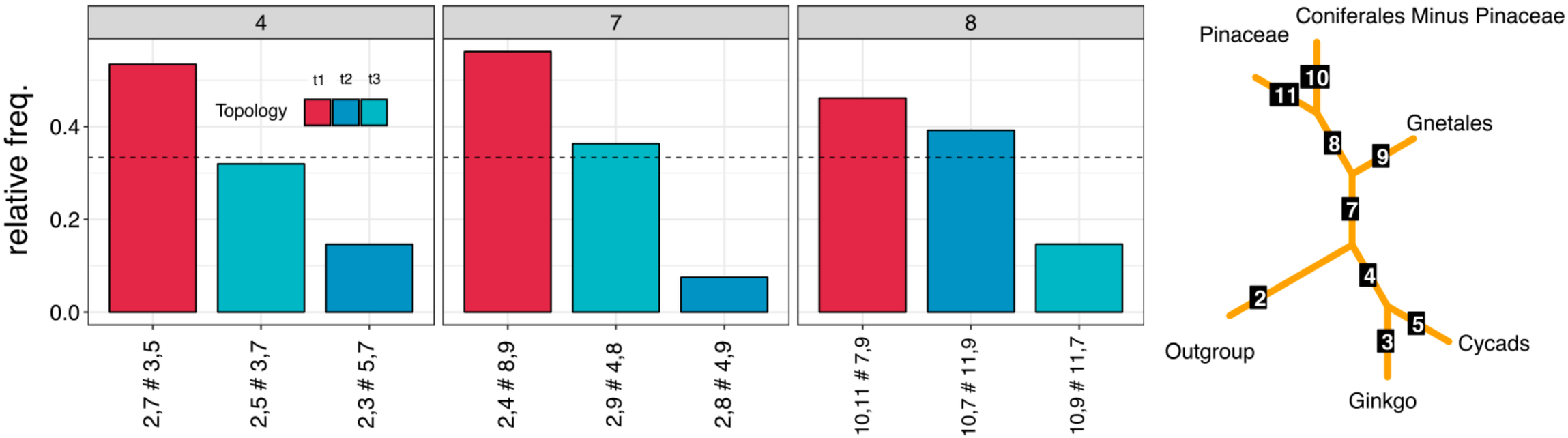
Discovista: visualizing discordance





- https://github.com/esayyari/DiscoVista
- Sayyari, et. al. “DiscoVista: Interpretable Visualizations of Gene Tree Discordance.” MPE 122 (2018): 110–15.

Discovista: visualizing discordance



- https://github.com/esayyari/DiscoVista
- Sayyari, et. al. “DiscoVista: Interpretable Visualizations of Gene Tree Discordance.” MPE 122 (2018): 110–15.

How about hybridization?

- Phylonet suit of tools make an effort to distinguish ILS and hybridization [e.g., Yu et al, 2014, PNAS]
  - Scalability remains to be tested
- PhyloNetworks takes a pseudo-likelihood approach [e.g., Solís-Lemus et al, 2016]

Other source of discordance?

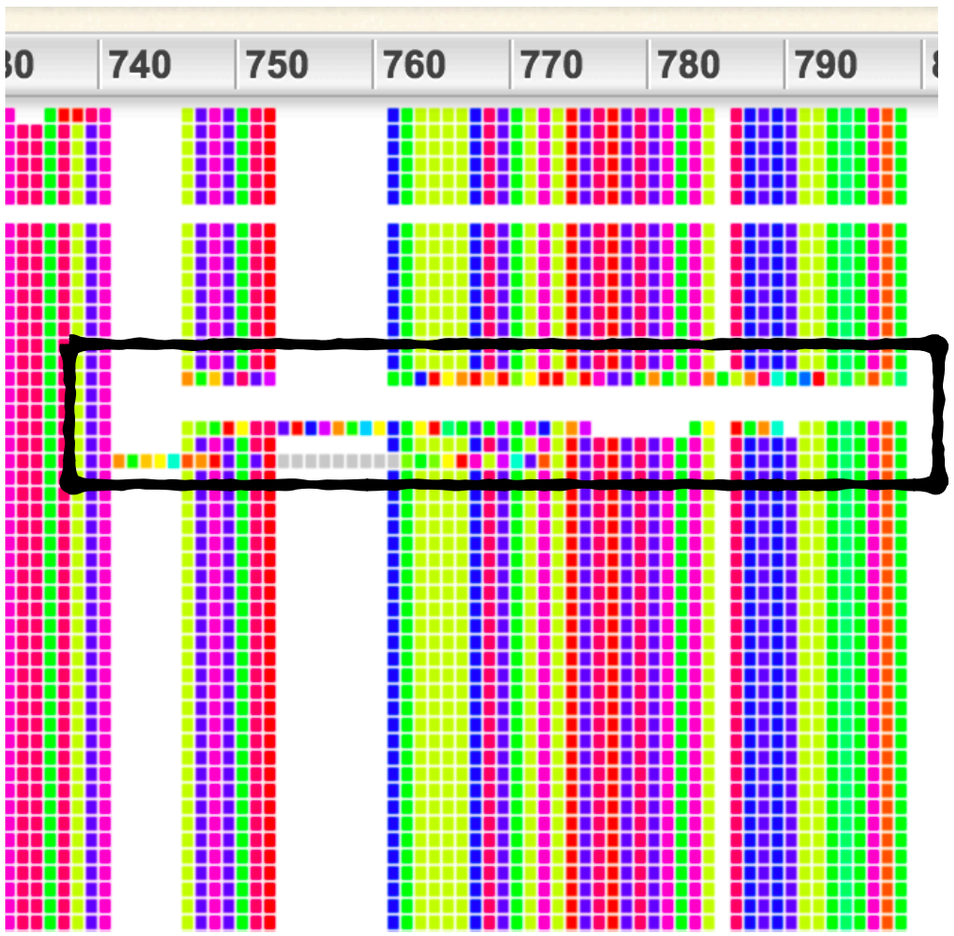
- There has been progress on duplication and loss, but perhaps less relevant to avian phylogenomics

Challenges

- Errors and incompleteness in data due to annotation, assembly, or other unknown origins
- Models of sequence evolution
- Gene tree discordance
  - True discordance
  - Spurious discordance
- Scalability

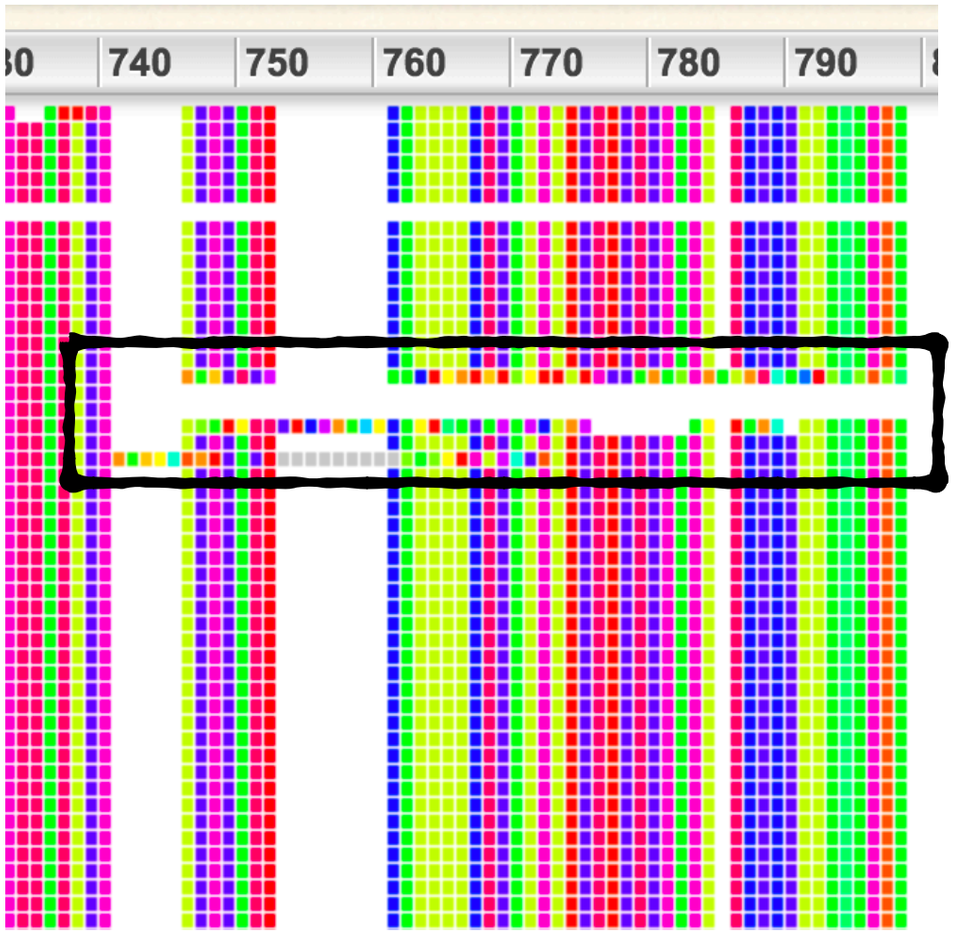
Challenges

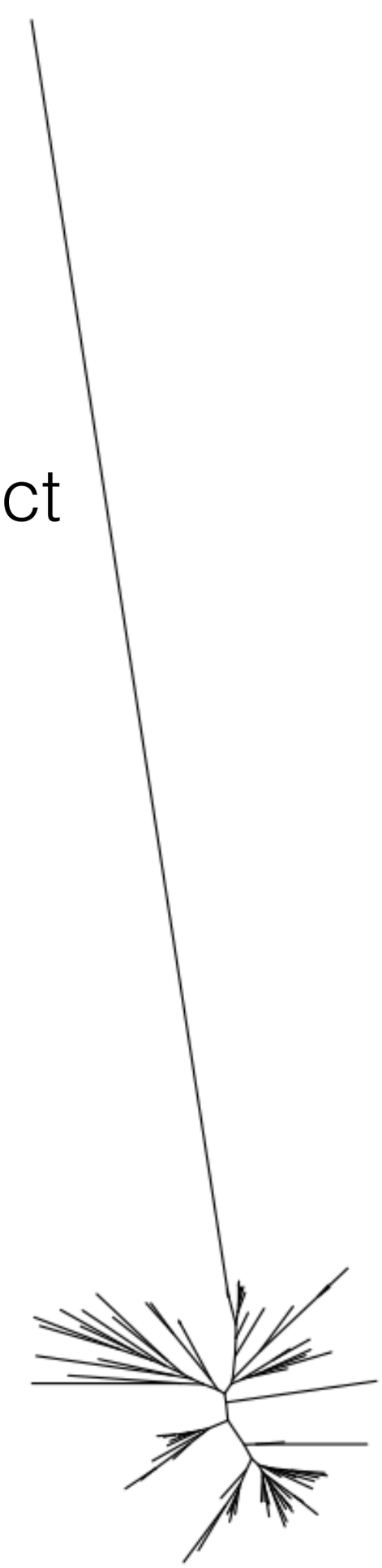
- Sequences that look like they may be incorrect
  - Perhaps wrong annotation



Challenges

- Sequences that look like they may be incorrect
  - Perhaps wrong annotation





TreeShrink

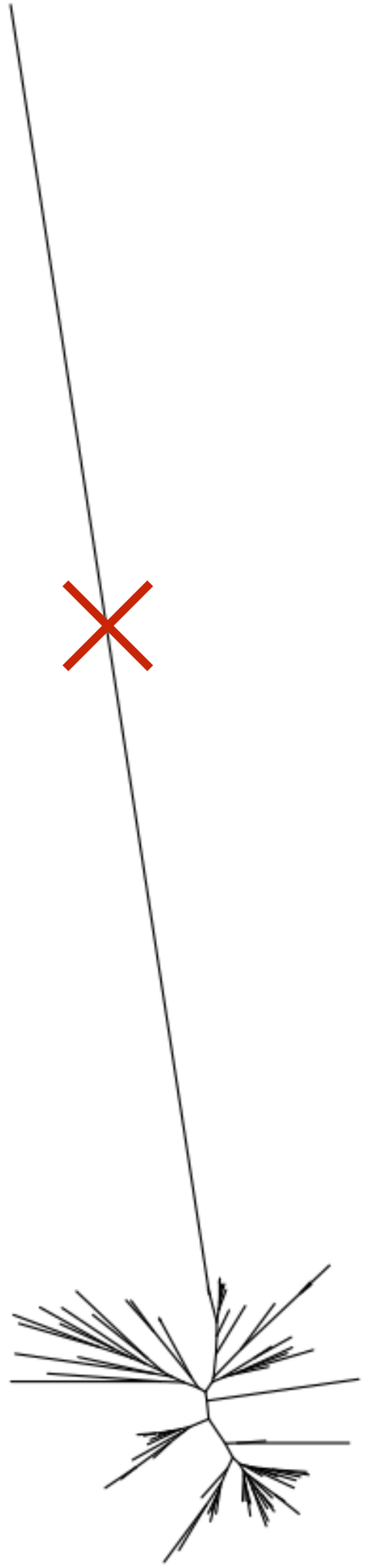
[Mai and Mirarab, BMC Genomics, 2018]

- Automatically detect long branches in gene trees

- It learns a distribution of branch length **per species** and looks for outliers

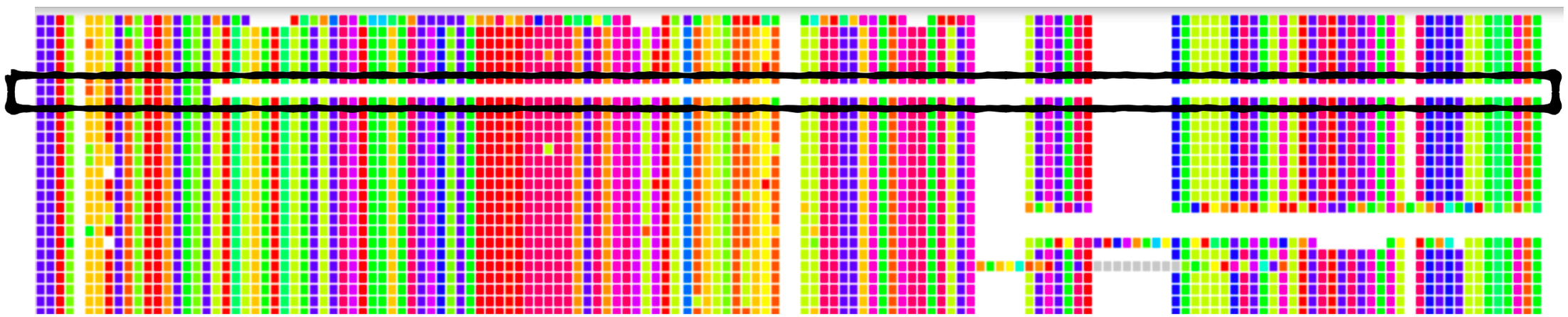
  - Avoids removing species that have long branches in all genes

  - Reduces discordance of gene trees



Fragmentary sequences

- Sequence of some species is present for some gene, but just a small portion of it

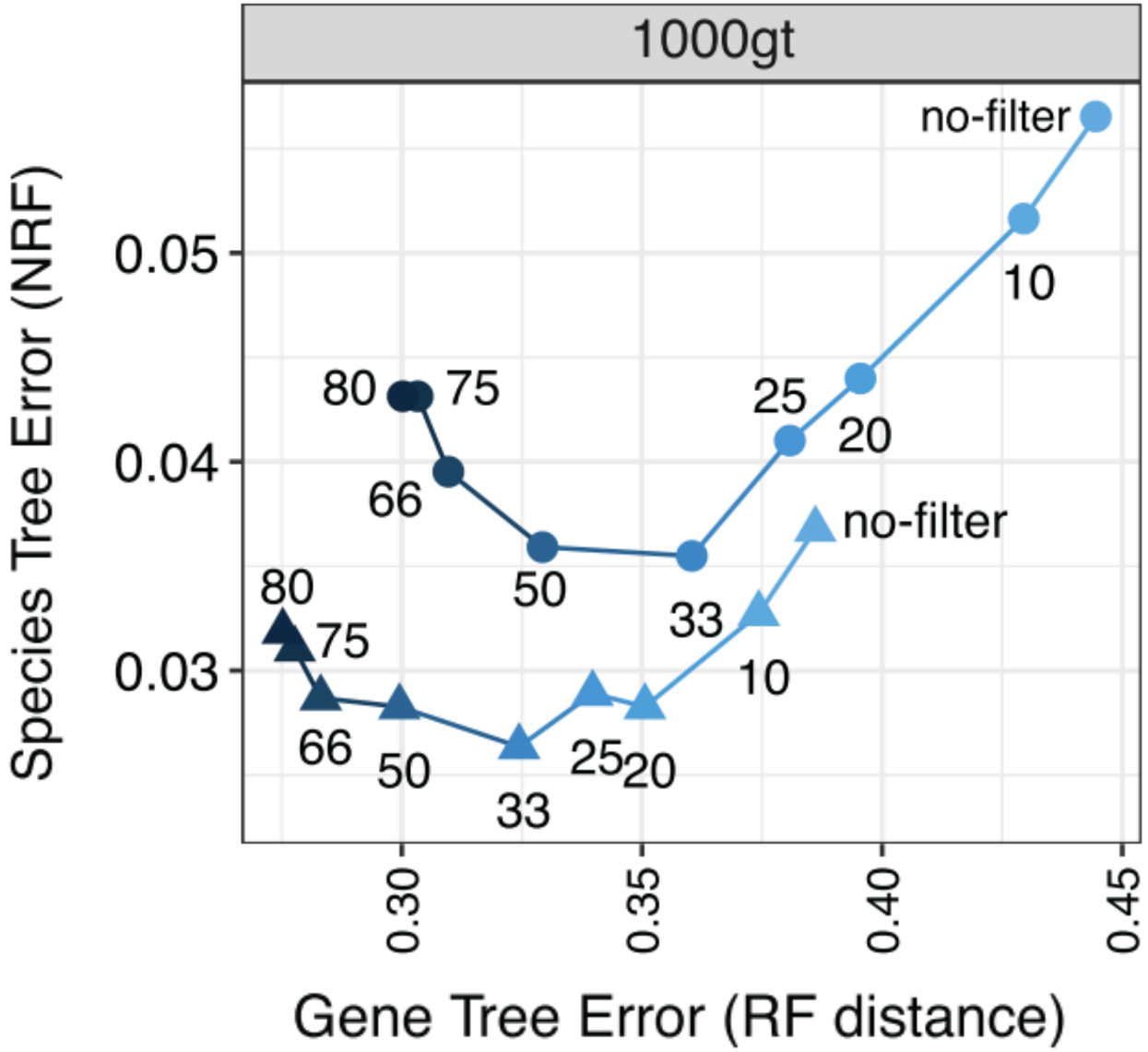


Filtering fragments

[Sayyari et al, MBE, 2017]

- Added fragmentation to simulated data with patterns similar to the Misof. et. al. insect (transcriptome data)

- Filtering simply removes fragmentary data from genes but keeps the gene



Should you remove whole genes?

- Filtering genes based on missing data?
  - Generally not beneficial [Molloy and Warnow, 2018]

Should you remove whole genes?

- Filtering genes based on missing data?
  - Generally not beneficial [Molloy and Warnow, 2018]

- Filtering genes based on gene tree estimation error?
  - Depends on conditions. Occasionally beneficial [Molloy and Warnow, 2018]

Challenges

- Errors and incompleteness in data due to annotation, assembly, or other unknown origins
- Models of sequence evolution
- Gene tree discordance
  - True discordance
  - Spurious discordance
- Scalability

Many tools have improved speed since 2014!

- ASTRAL-MP: super scalable ASTRAL using GPU and CPU multi-threading [under review]

- RAxML-ng+ParGenes: scalable gene tree estimation

- ASTRID, which is similar to NJst, and is quite good, but is super-fast

Challenges

- Errors and incompleteness in data due to annotation, assembly, or other unknown origins
- Models of sequence evolution
- Gene tree discordance
  - True discordance
  - Spurious discordance
- Scalability

Some new models …

- IQ-TREE:
  - PMFS: Wang et al., Systematic Biolog, 2018
  - Heterotachy (GHOST): Crotty et al.
  - Partition models: Chernomor et al., Systematic Biology, 2016
- AA-biochemical model: Braun, ISMB, 2018

Summary

- There are better methods of species tree estimation and data correction available
- Sequence evolution models have not changed dramatically
- Scalability has improved and is not an issue for some but not all analyses
- Many challenges remain!

Acknowledgments

Tandy Warnow

Siavash Mirarab

S.M. Bayzid

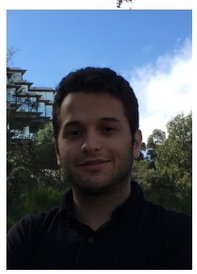
Nam Nguyen (now at UIUC)

Jim Leebens-mack (UGA)

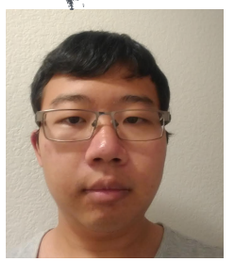
Norman Wickett (U Chicago)

Gane Wong (U of Alberta)

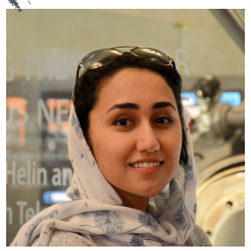
. . .



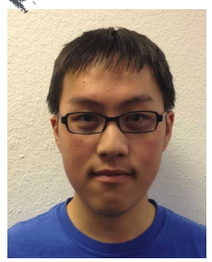
Erfan Sayyari



Chao Zhang



Maryam Hashemi



John Yin

Uyen Mai

Guojie Zhang (BGI, China)

Tom Gilbert (U Copenhagen)

Erich Jarvis (Duke, HMMI)

Bastien Boussau (Université Lyon)

Ed Braun (U Florida)

HHMI HOWARD HUGHES MEDICAL INSTITUTE

NSERC CRSNG

ALFRED P. SLOAN FOUNDATION

NSF

华大基因 BGI