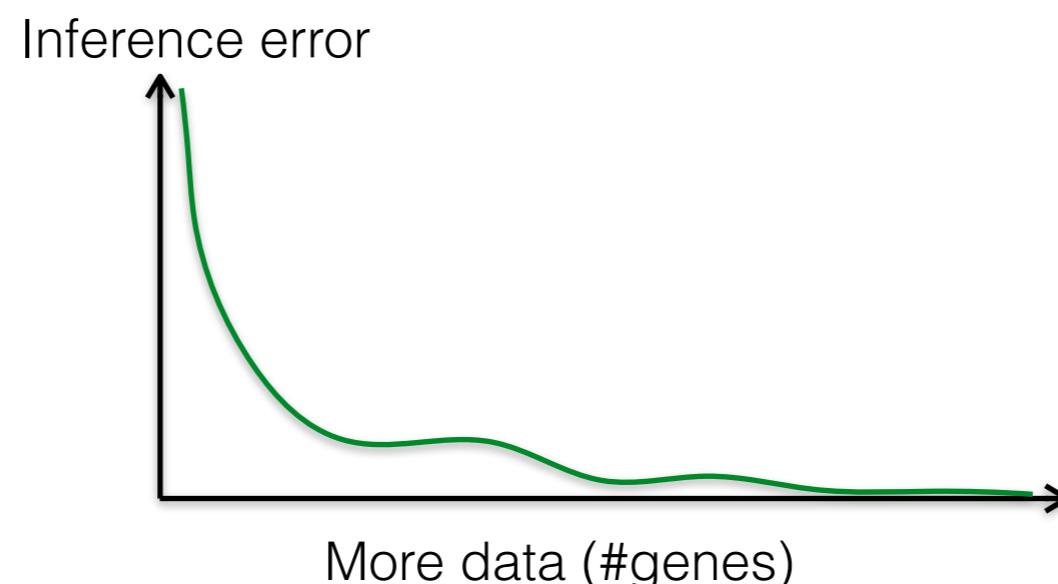
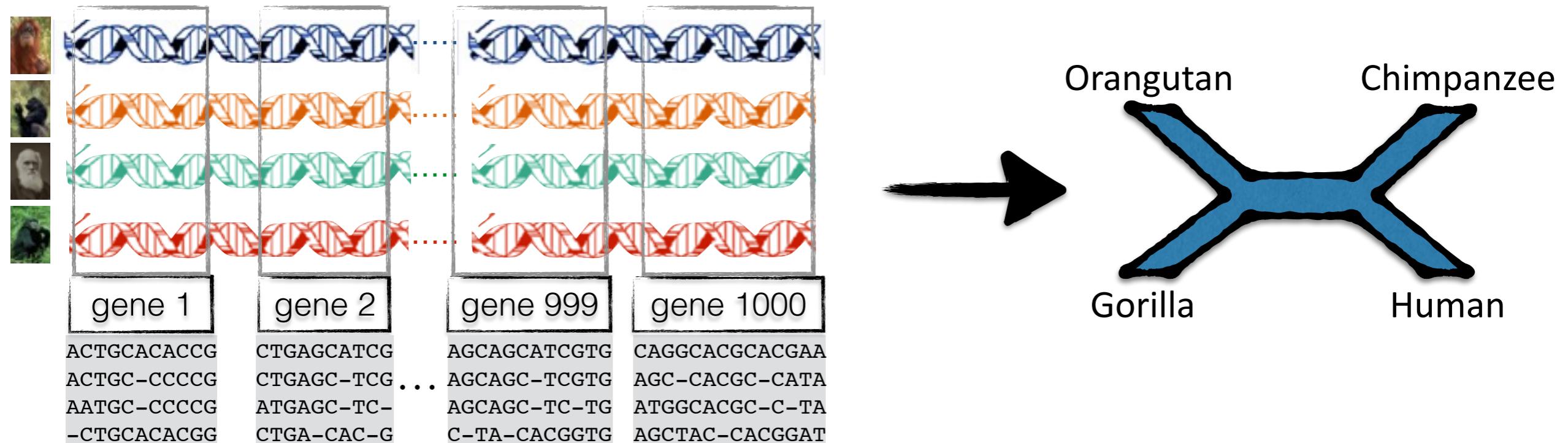


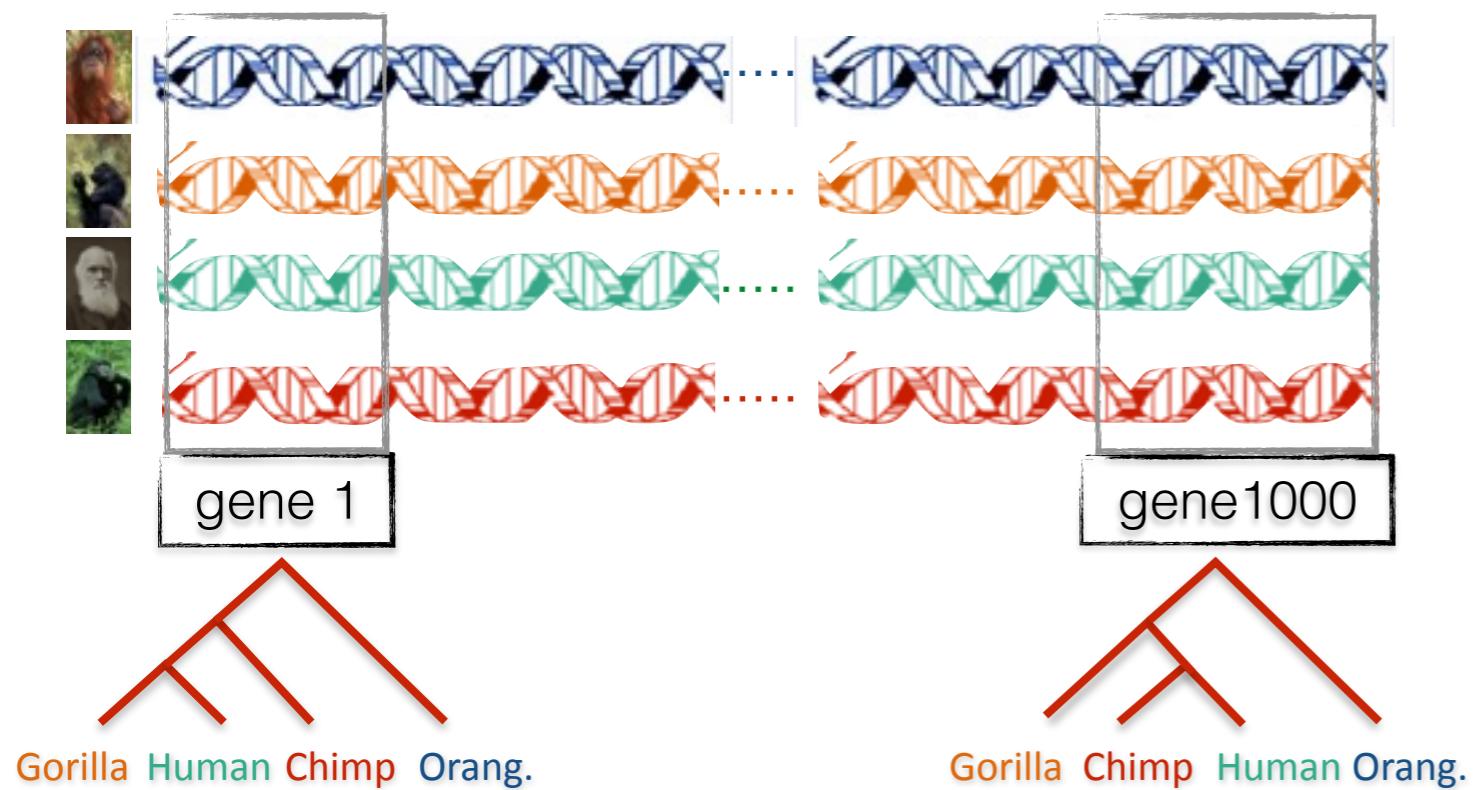
# Reconstruction of species trees from gene trees using ASTRAL

Siavash Mirarab  
University of California, San Diego (ECE)

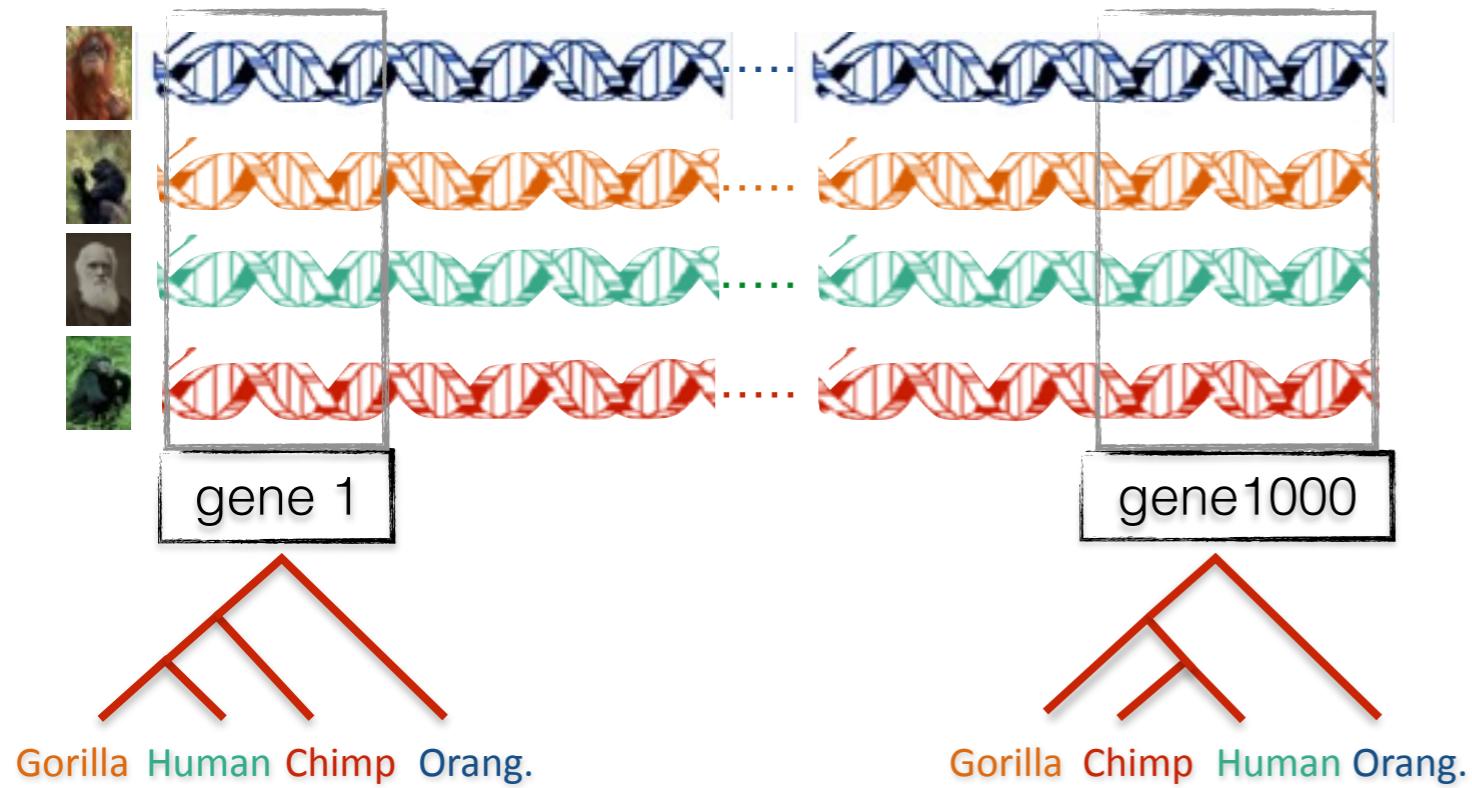
# Phylogenomics



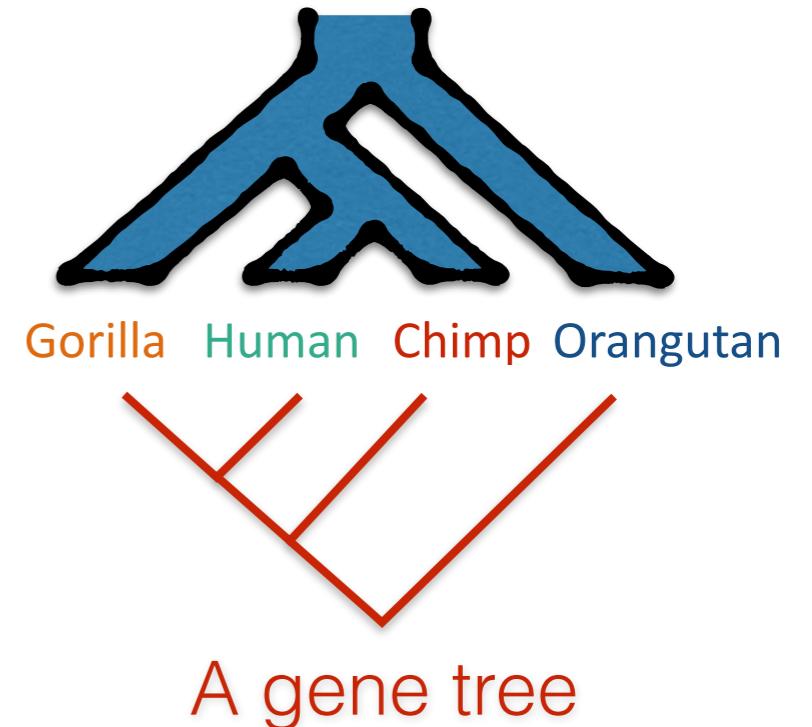
# Gene tree discordance



# Gene tree discordance

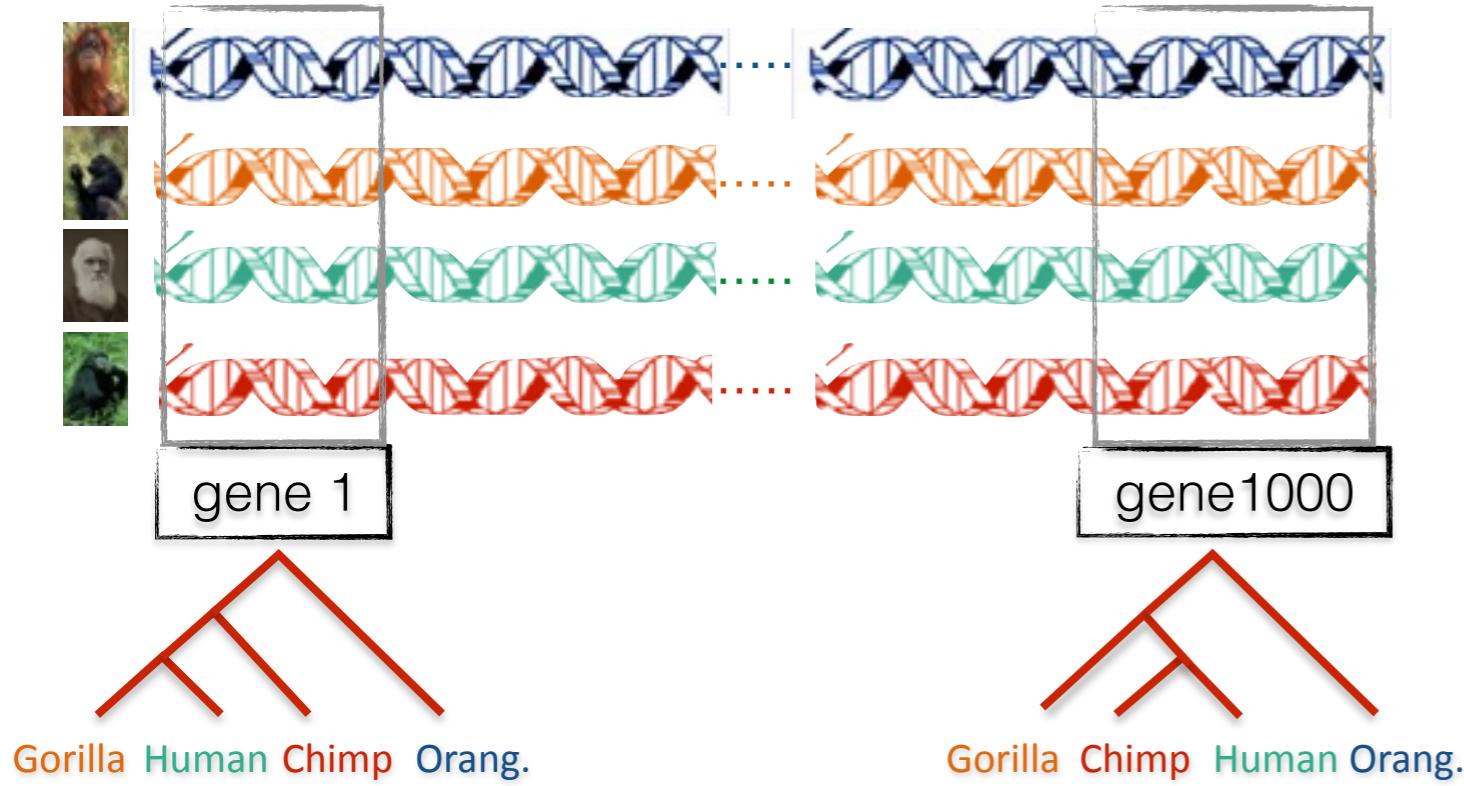


The species tree

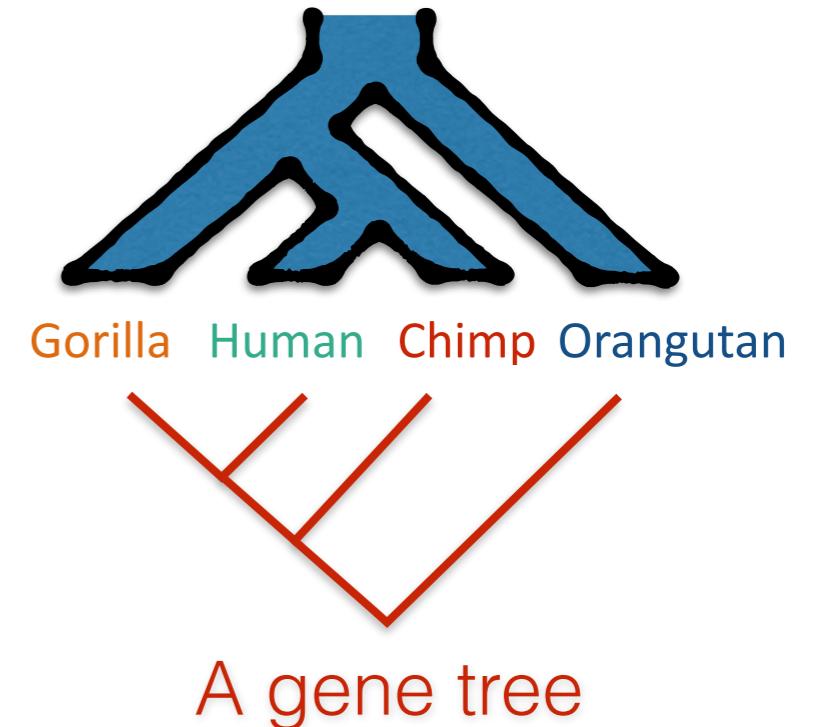


A gene tree

# Gene tree discordance



The species tree

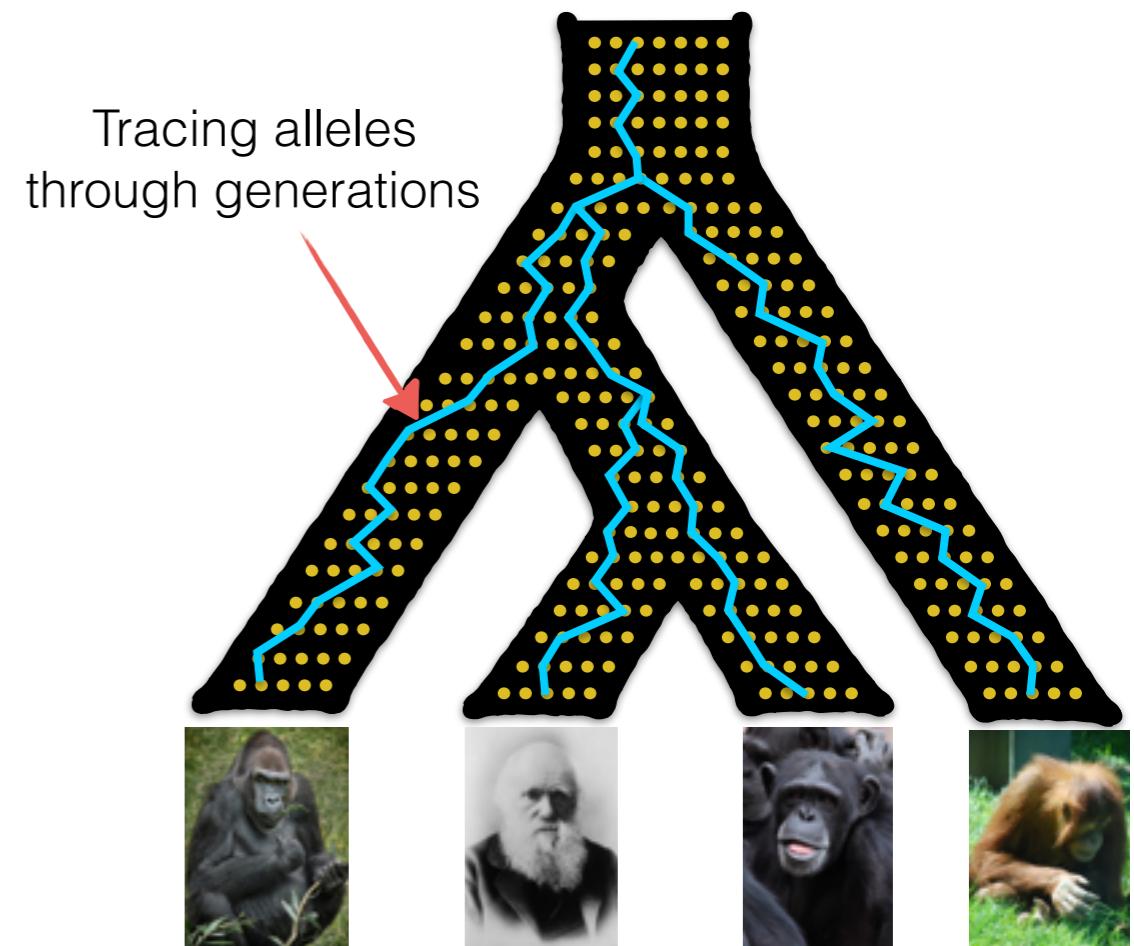


**Causes of gene tree discordance include:**

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

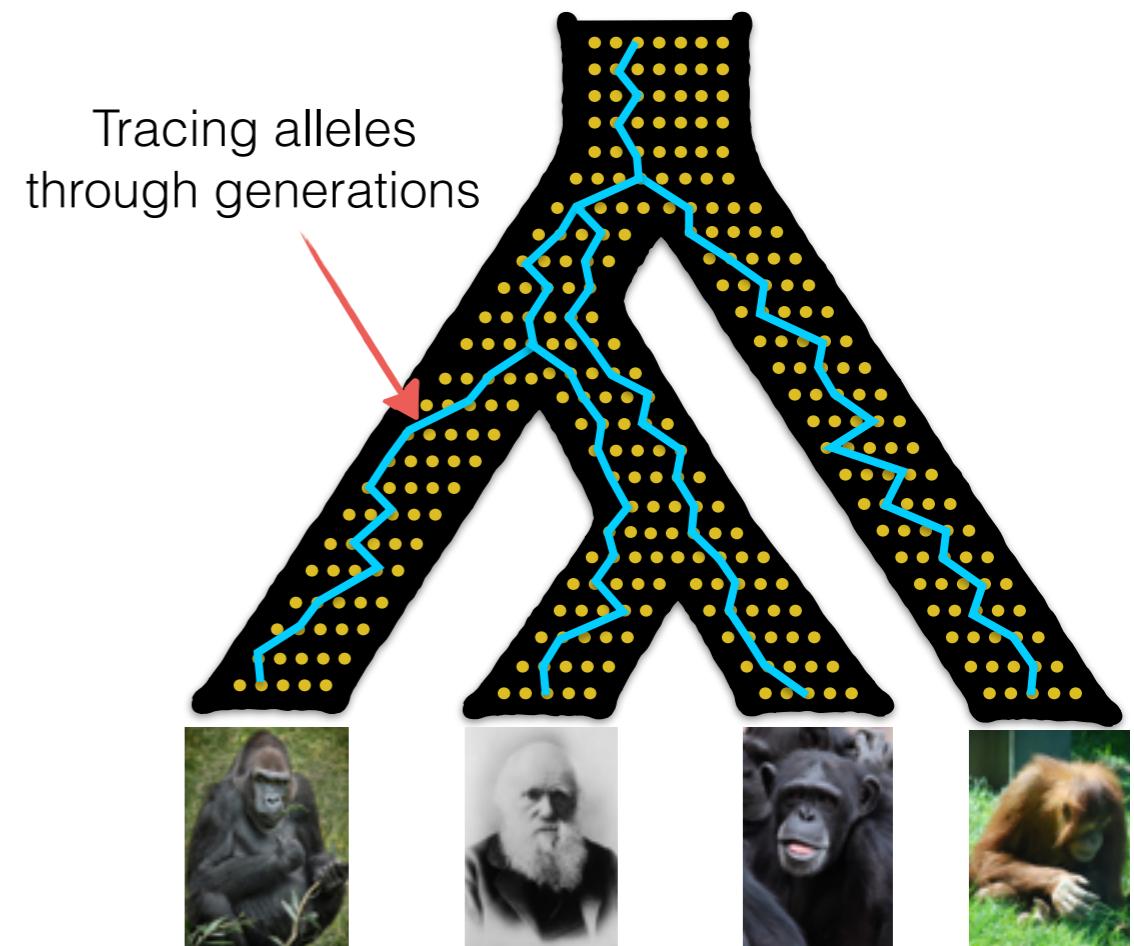
# Incomplete Lineage Sorting (ILS)

- A **random** process related to the coalescence of alleles across various populations



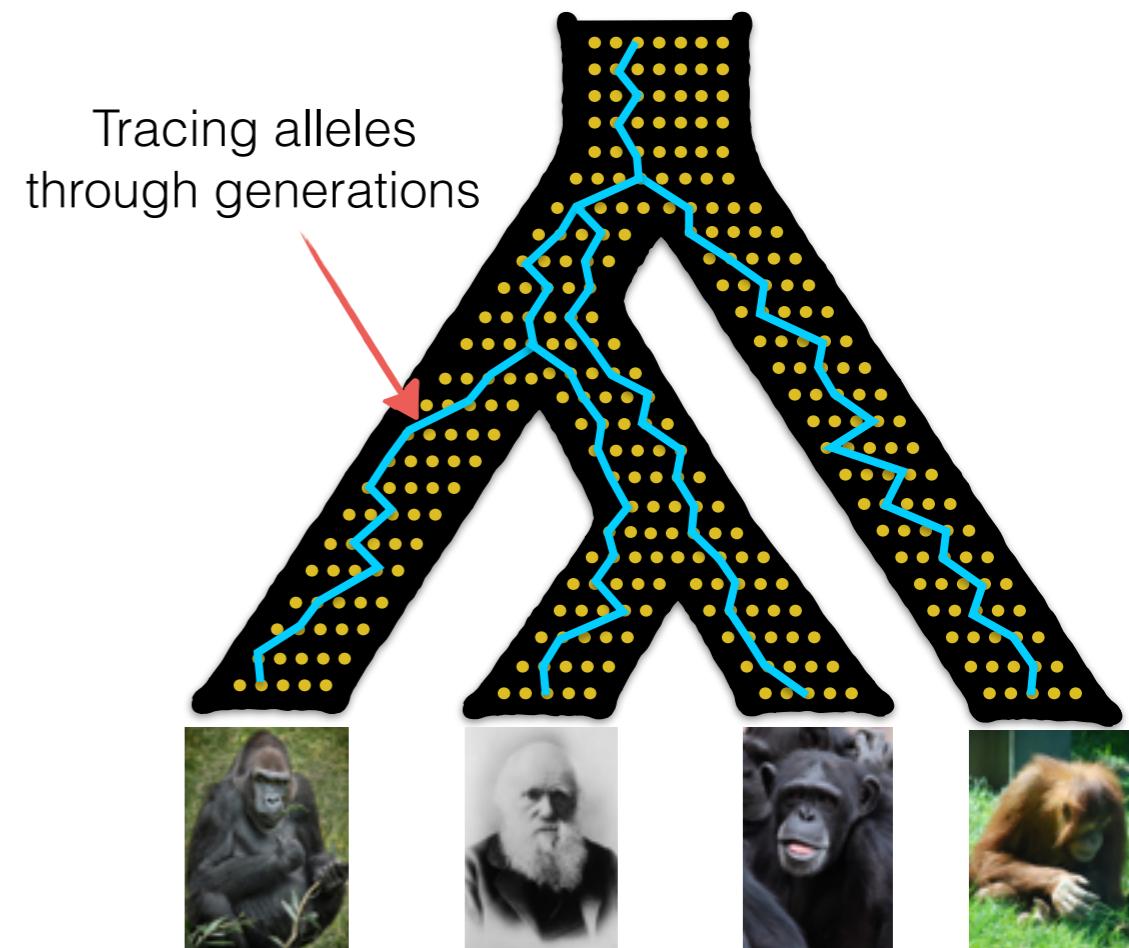
# Incomplete Lineage Sorting (ILS)

- A **random** process related to the coalescence of alleles across various populations



# Incomplete Lineage Sorting (ILS)

- A **random** process related to the coalescence of alleles across various populations
- Omnipresent: possible for every tree
  - Likely for short branches or large population sizes



# MSC and Identifiability

- The statistical process [multi-species coalescent](#) (MSC) is used to model ILS.

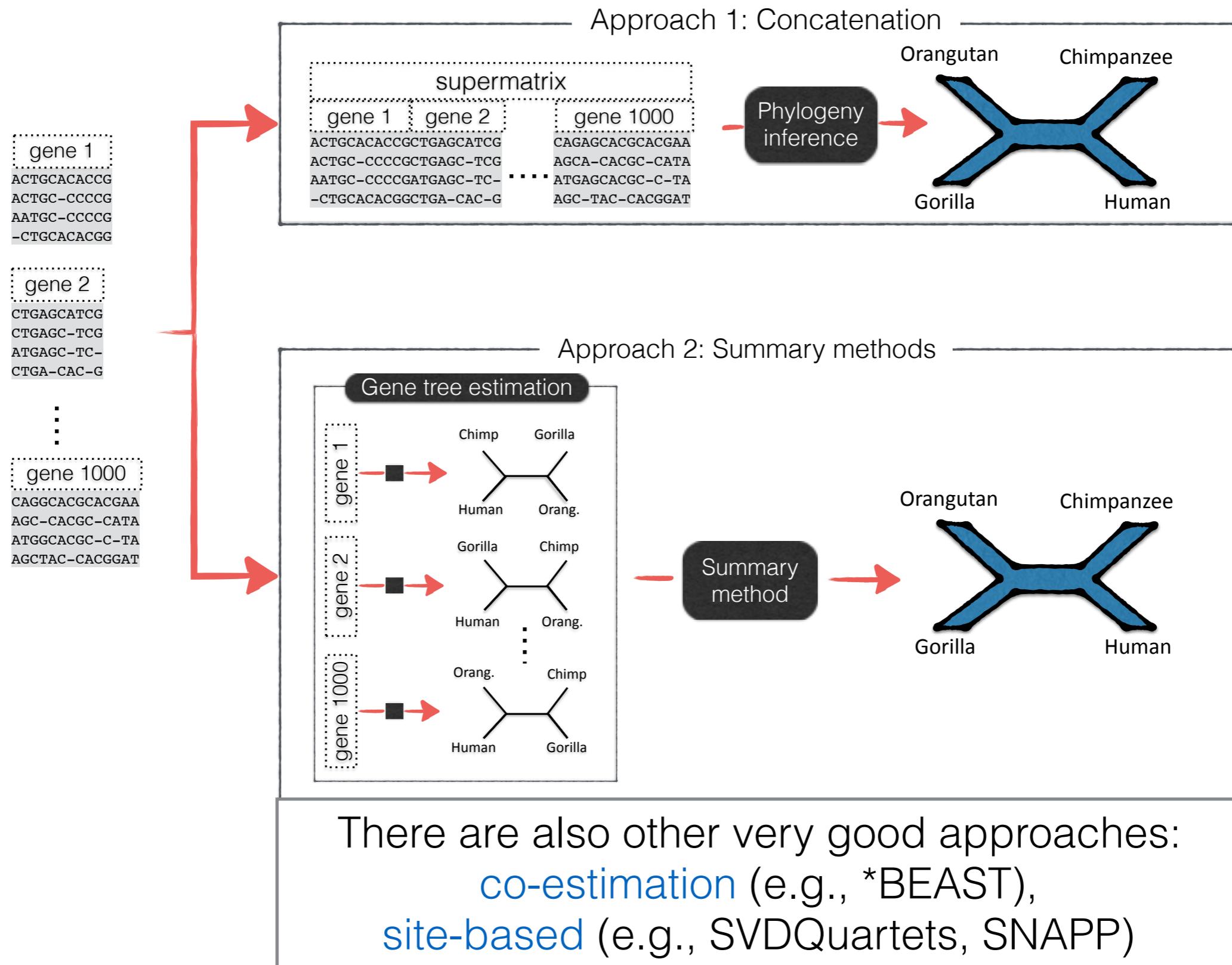
# MSC and Identifiability

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- Any species tree defines a [unique distribution](#) on the set of all possible gene trees

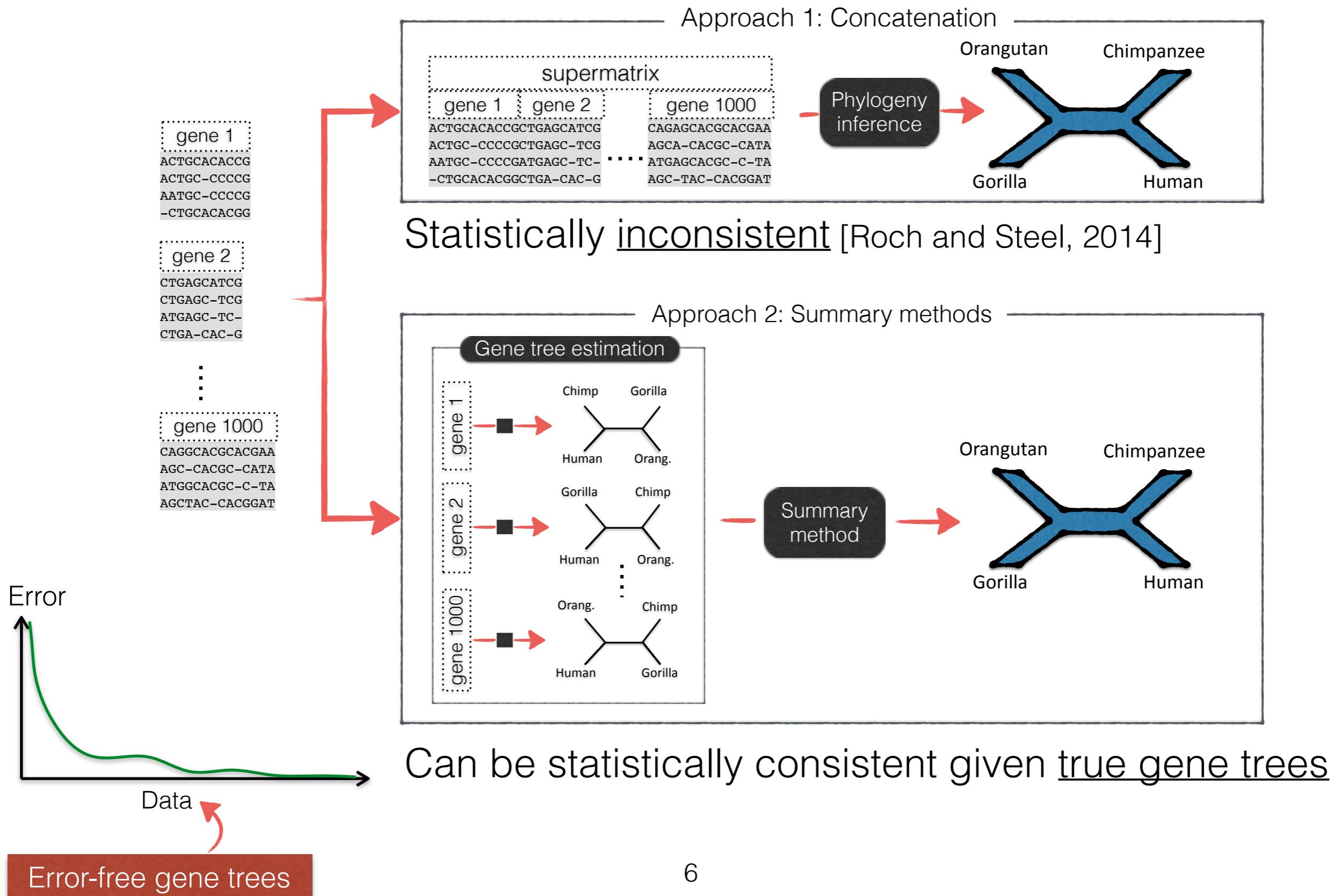
# MSC and Identifiability

- The statistical process [multi-species coalescent](#) (MSC) is used to model ILS.
- Any species tree defines a [unique distribution](#) on the set of all possible gene trees
- In principle, the species tree can be [identified despite high discordance](#) from the gene tree distribution

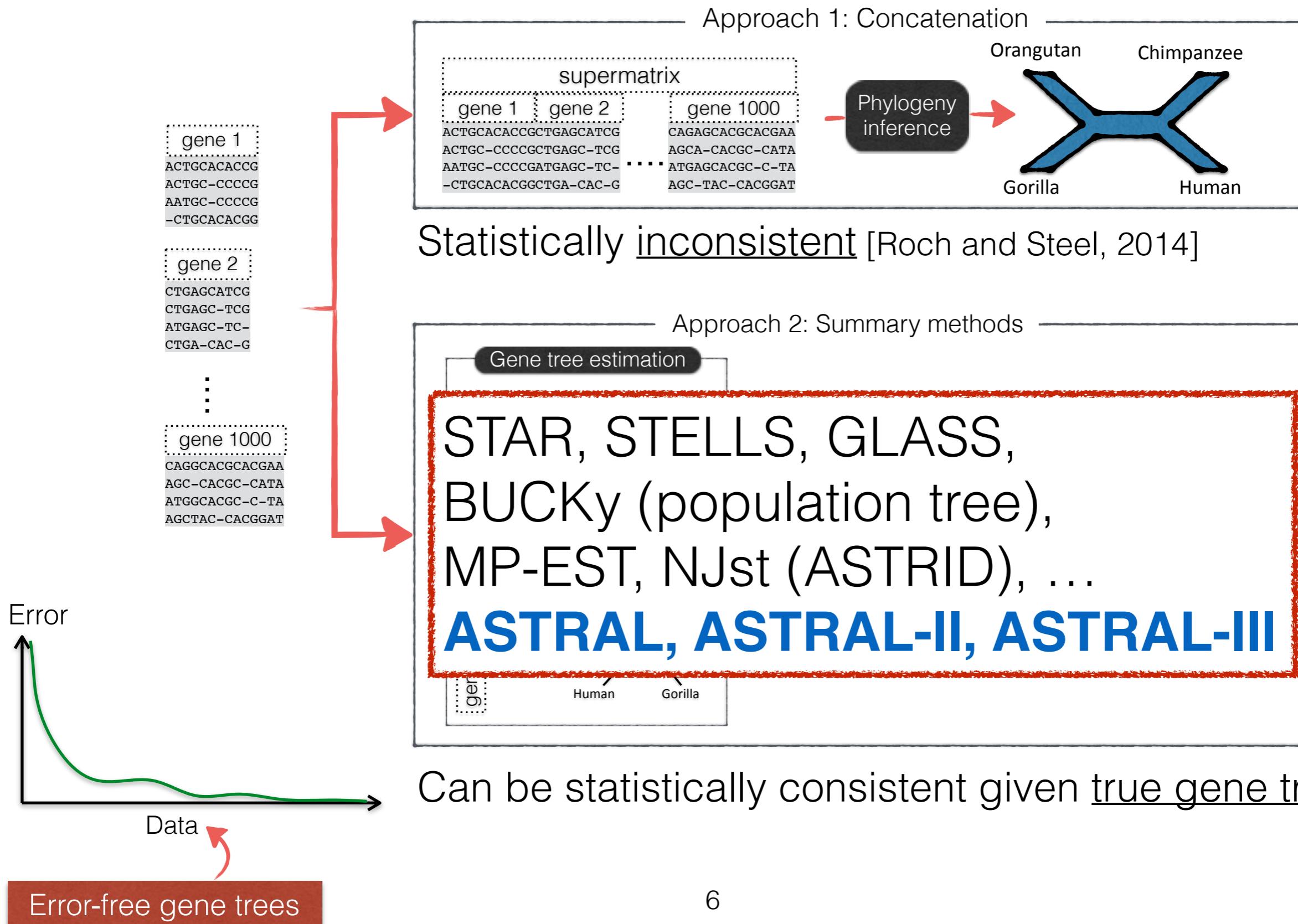
# Multi-gene tree estimation pipelines



# Multi-gene tree estimation pipelines

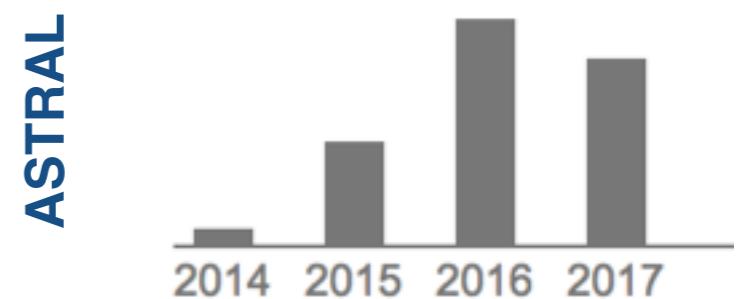


# Multi-gene tree estimation pipelines



# ASTRAL used by the biologists

- 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

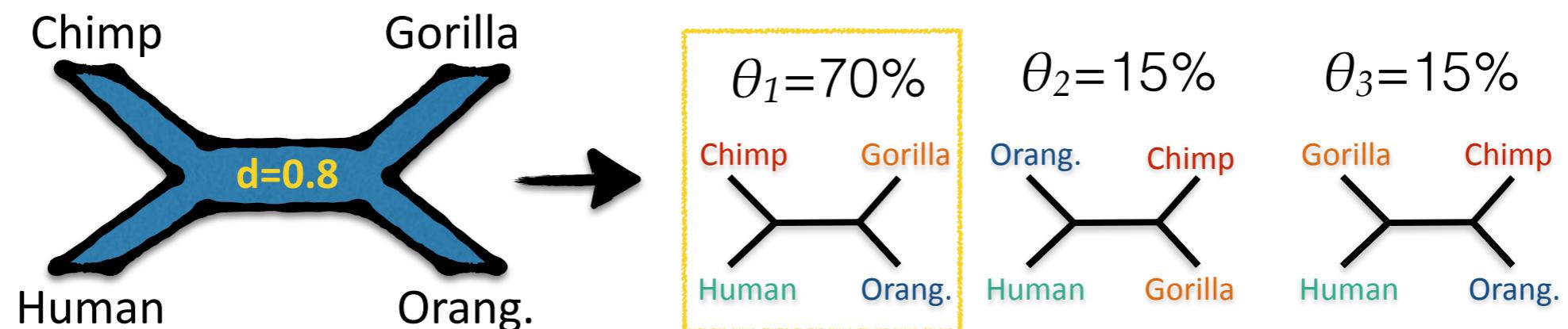


# This talk: ASTRAL

- Outlines of the method
- Accuracy in simulation studies
  - With strong model violations
- The impact of
  - Fragmentary sequence data
  - Sampling multiple individuals
- Visualization

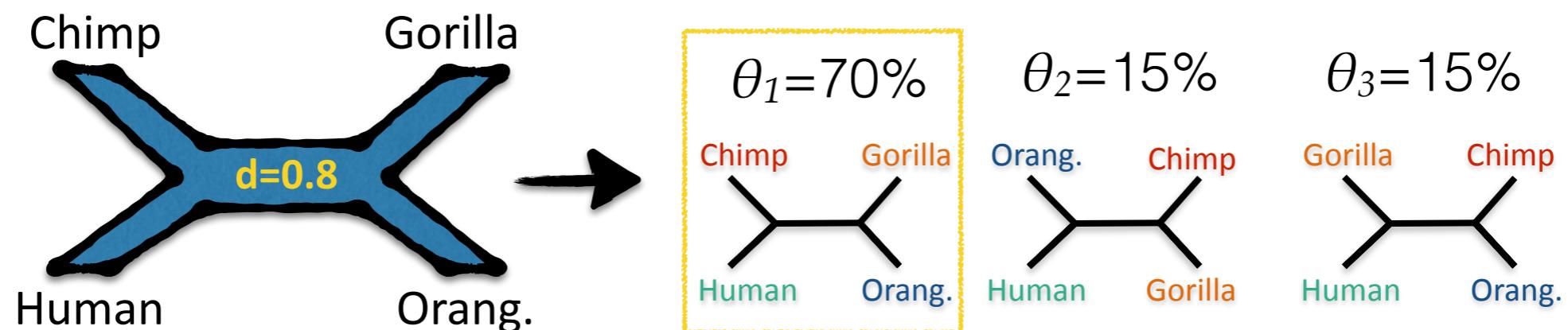
# Unrooted quartets under MSC model

For a quartet (4 species), the unrooted species tree topology has at least 1/3 probability in gene trees (Allman, et al. 2010)



# Unrooted quartets under MSC model

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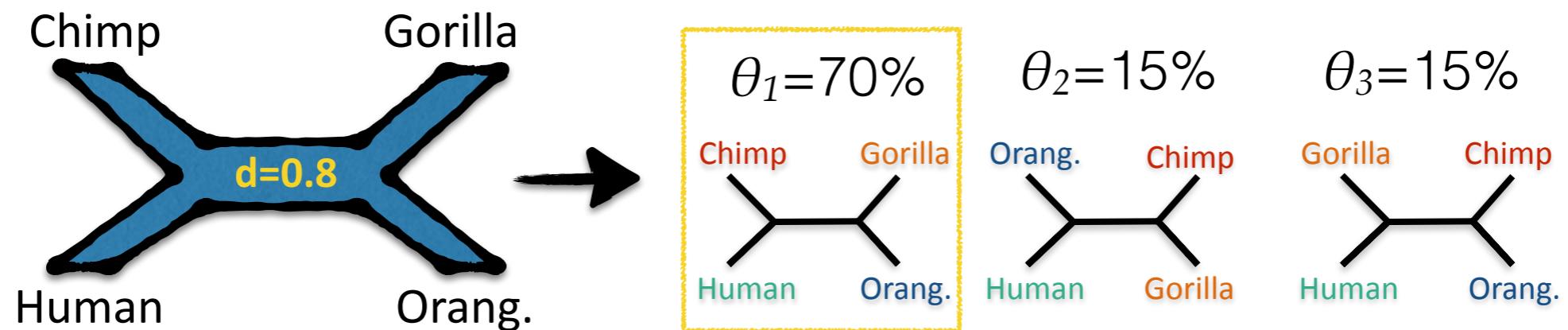
The most frequent gene tree

=

The most likely species tree

# Unrooted quartets under MSC model

For a quartet (4 species), the unrooted species tree topology has at least 1/3 probability in gene trees (Allman, et al. 2010)



The most frequent gene tree  
=  
The most likely species tree

shorter branches  $\Rightarrow$   
more discordance  $\Rightarrow$   
a harder species tree  
reconstruction problem

# More than 4 species

For  $>4$  species, the species tree topology can be different from the most like gene tree (called anomaly zone) (Degnan, 2013)



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For  $>4$  species, the species tree topology can be different from the most like gene tree (called anomaly zone) (Degnan, 2013)



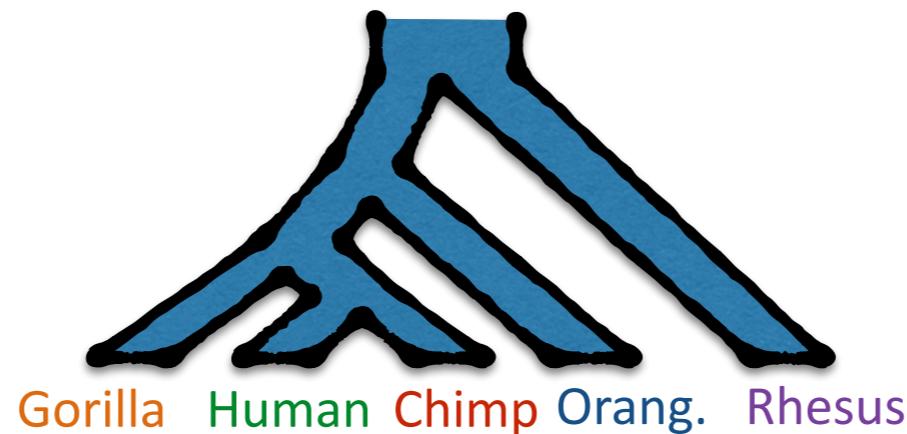
1. Break gene trees into  $\binom{n}{4}$  quartets of species
2. Find the dominant tree for all quartets of taxa
3. Combine quartet trees

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

(probabilities are made-up just as an example)			
Gorilla	Human	Orangutan	Chimp
Chimp	Gorilla	Orang.	Chimp
50%	25%	25%	
Gorilla	Human	Rhesus	Chimp
Chimp	Gorilla	Rhesus	Chimp
55%	21%	24%	
Gorilla	Human	Orangutan	Rhesus
dog	Gorilla	Orang.	dog
7%	87%	6%	
Gorilla	Rhesus	Orangutan	Chimp
Chimp	Gorilla	Orang.	Chimp
6%	88%	6%	
Rhesus	Human	Orangutan	Chimp
Chimp	Rhesus	Orang.	Chimp
95%	2%	3%	

# More than 4 species

For  $>4$  species, the species tree topology can be different from the most like gene tree (called anomaly zone) (Degnan, 2013)



**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	Chimp	Gorilla
Orangutan	Chimp	Human	Orang.
50%			25%
Gorilla	Human	Chimp	Rhesus
Rhesus	Chimp	Human	Gorilla
55%			19%
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Orangutan	Rhesus	Human	Gorilla
7%			87%
Gorilla	Rhesus	Chimp	Gorilla
Orangutan	Chimp	Rhesus	Gorilla
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95%			2%
			3%

# Maximum Quartet Support Species Tree

- Optimization problem:

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

$$Score(T) = \sum_1^m |Q(T) \cap Q(t_i)|$$

the set of quartet trees induced by T  
a gene tree

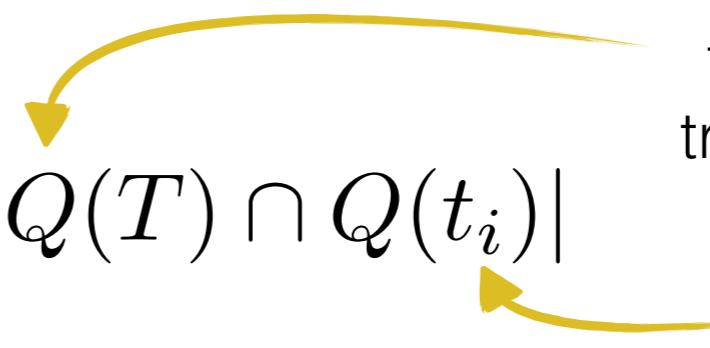
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the set of quartet trees induced by T  
a gene tree



- Theorem:** Statistically consistent under the multi-species coalescent model when solved exactly

# ASTRAL-I and ASTRAL-II

[Mirarab, et al., Bioinformatics, 2014] [Mirarab and Warnow, Bioinformatics, 2015]

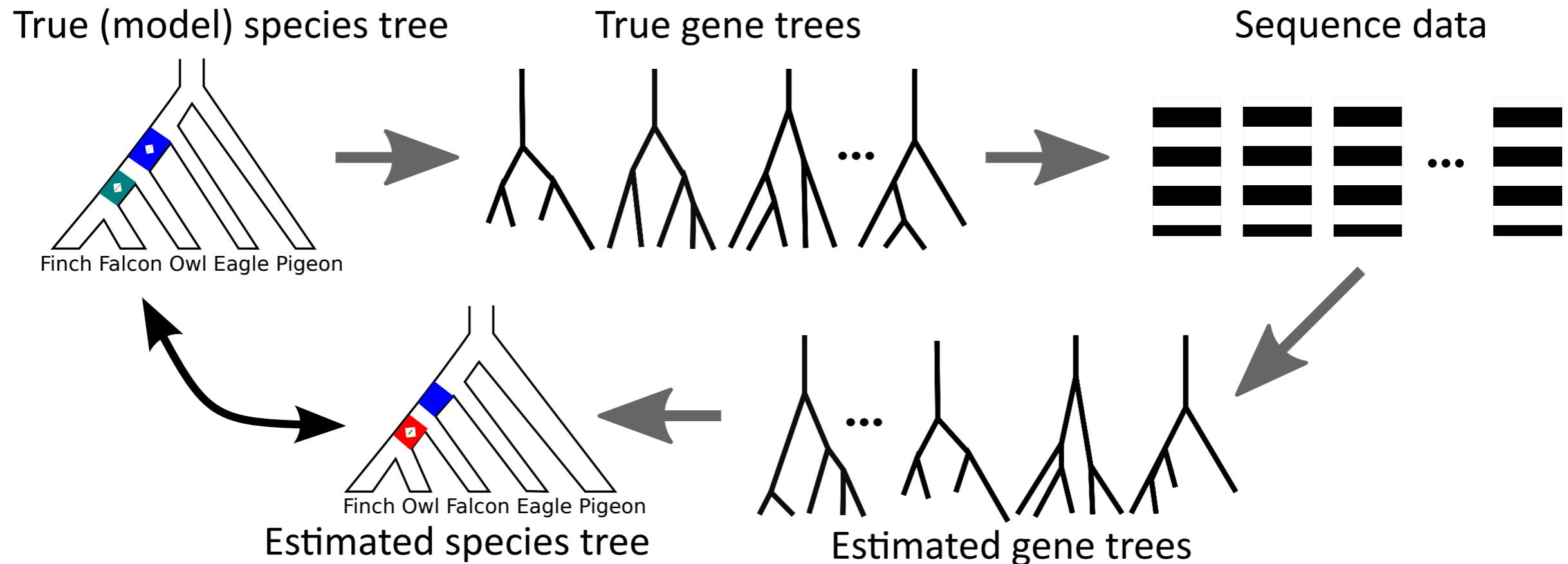
- Solve the problem exactly using [dynamic programming](#)
  - [Constrains](#) the search space to make large datasets feasible
  - The constrained version remains [statistically consistent](#)
  - Running time: polynomially increases with the number of genes and the number of species

# ASTRAL-I and ASTRAL-II

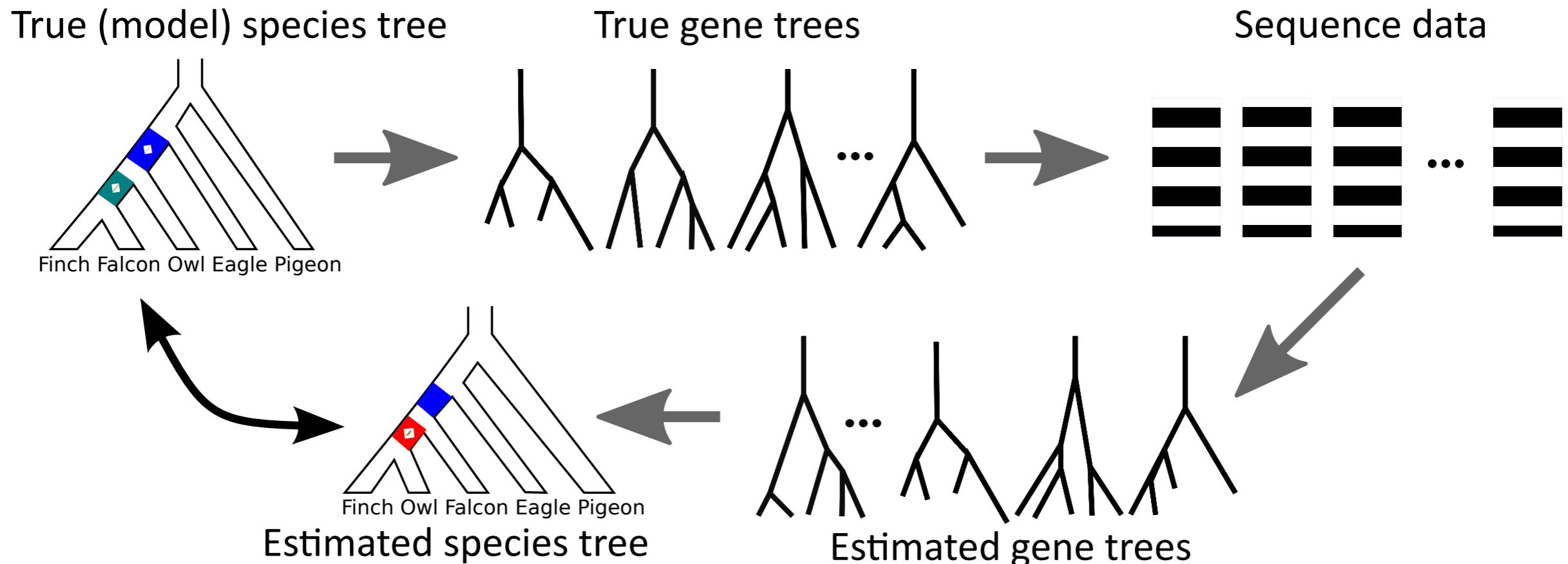
[Mirarab, et al., Bioinformatics, 2014] [Mirarab and Warnow, Bioinformatics, 2015]

- Solve the problem exactly using [dynamic programming](#)
  - [Constrains](#) the search space to make large datasets feasible
  - The constrained version remains [statistically consistent](#)
  - Running time: polynomially increases with the number of genes and the number of species
- ASTRAL-II:
  - Increased the search space
  - Improved the running time
  - Can handle polytomies (lack of resolution) in input gene trees

# Simulation study

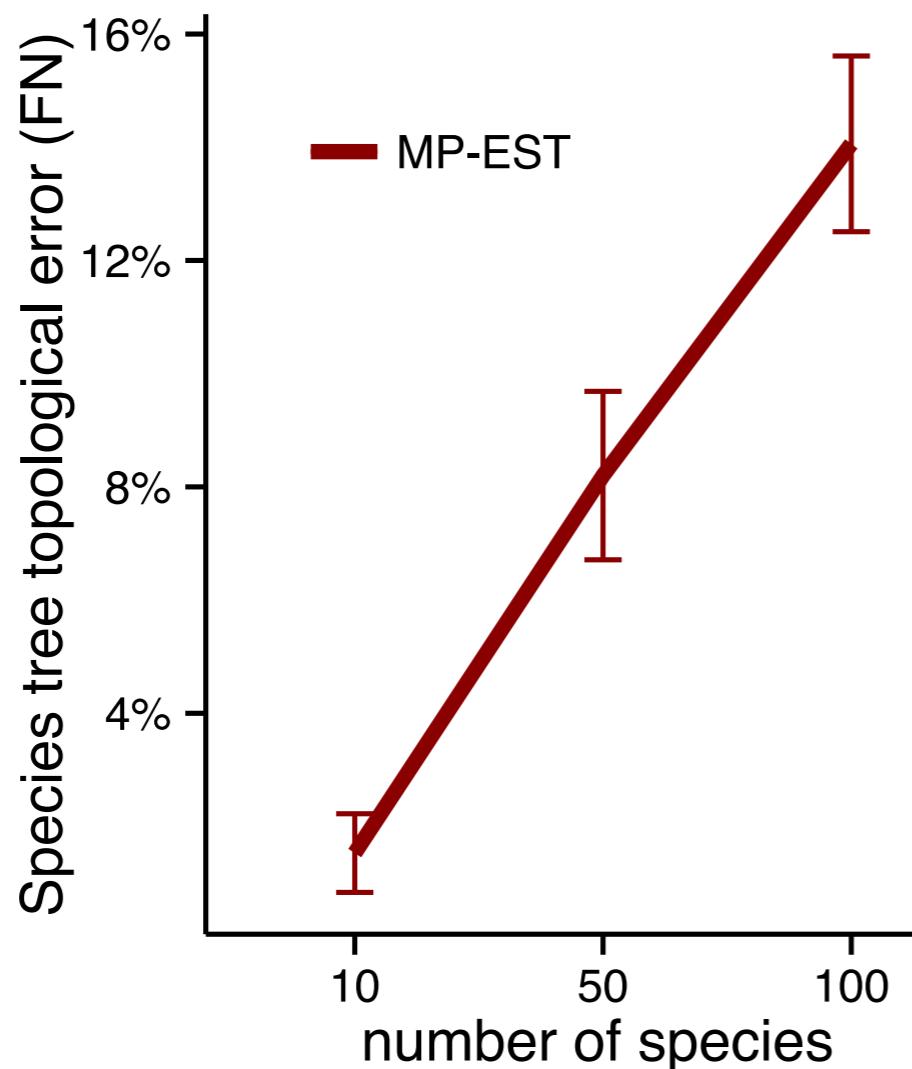


# Simulation study



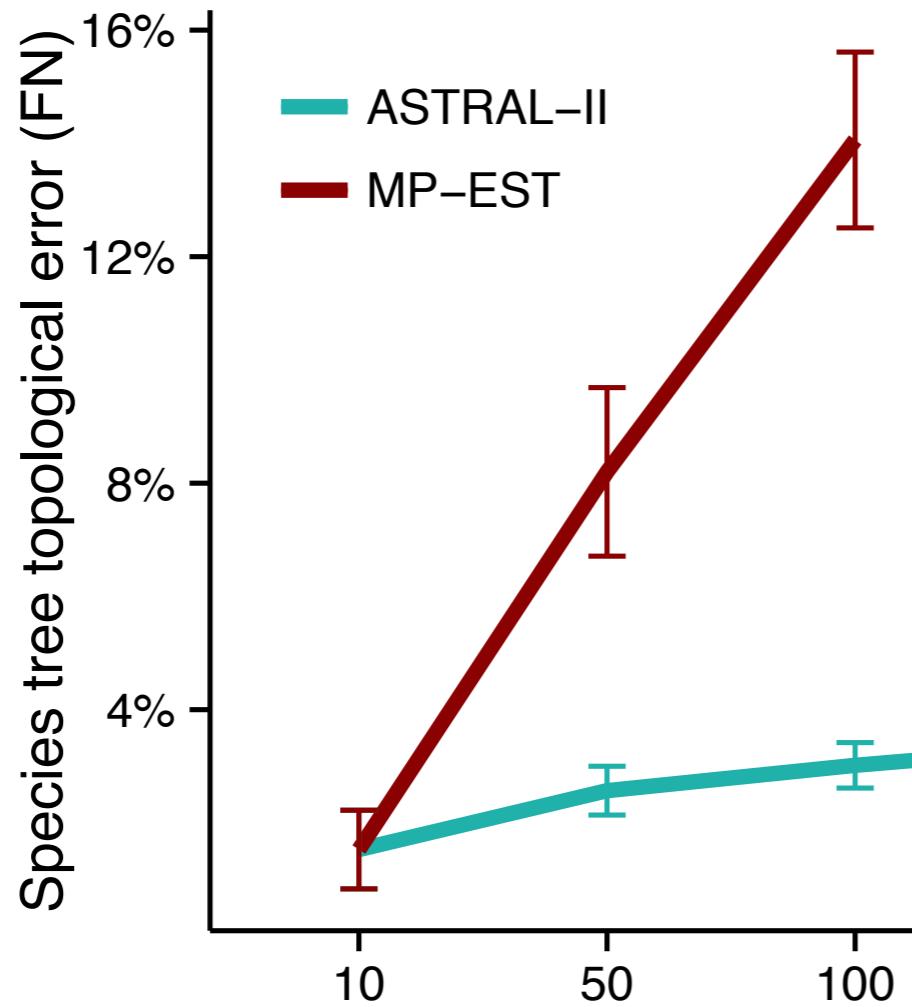
- Evaluate using the **FN rate**: the percentage of branches in the true tree that are missing from the estimated tree

# Number of species impacts estimation error in the species tree



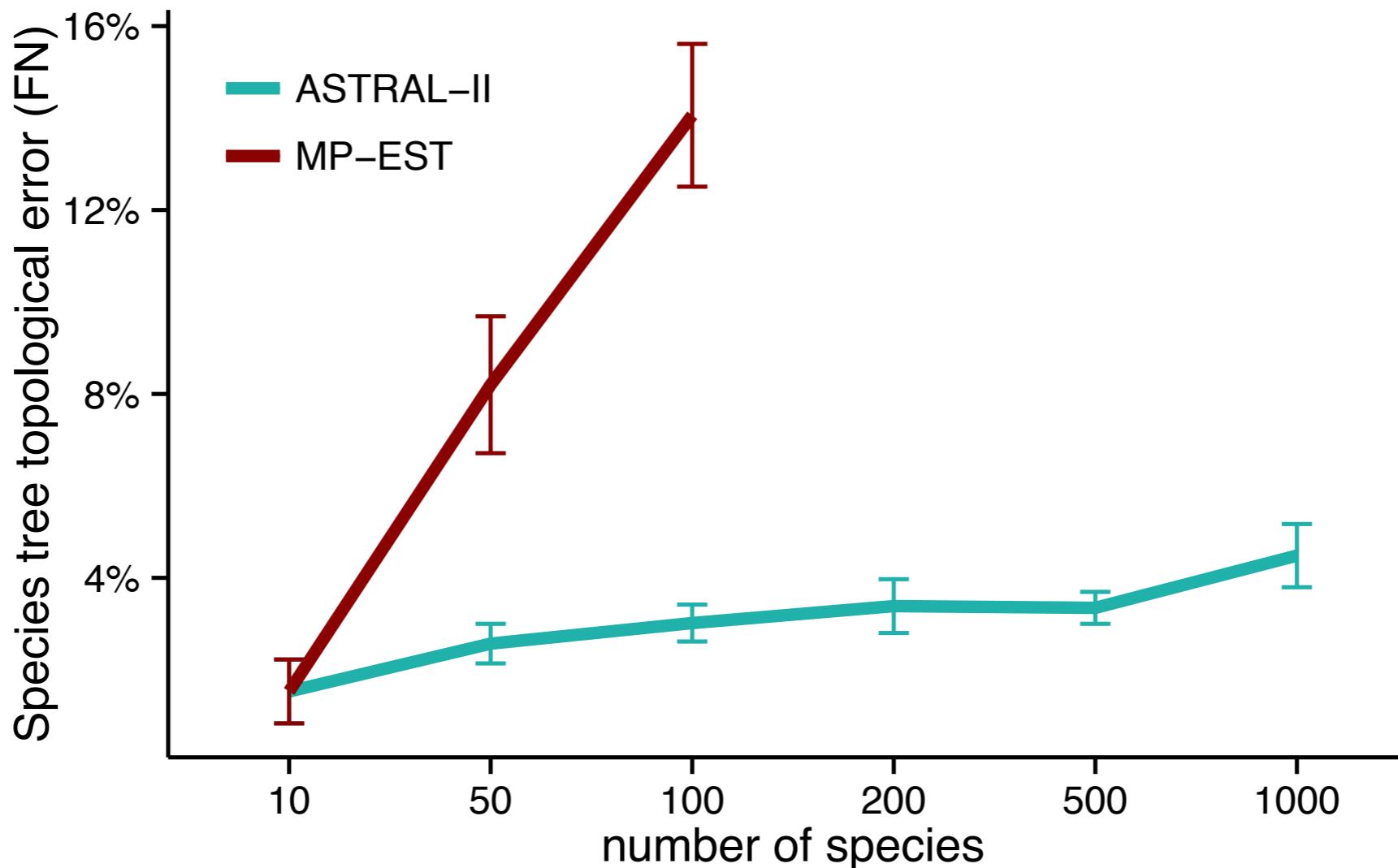
1000 genes, “medium” levels of ILS, simulated species trees  
[S. Mirarab, T. Warnow, 2015]

# ASTRAL: accurate and scalable



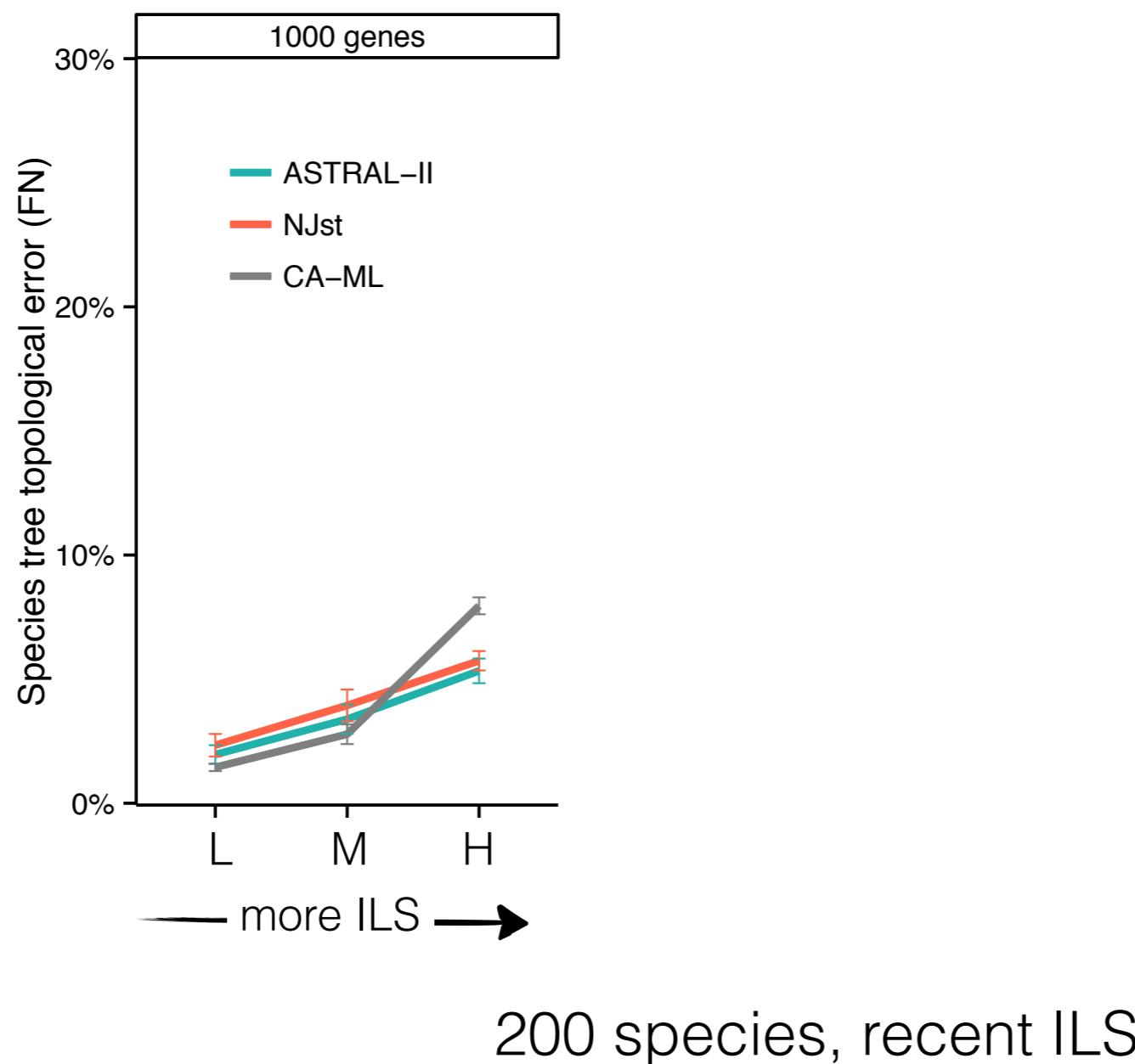
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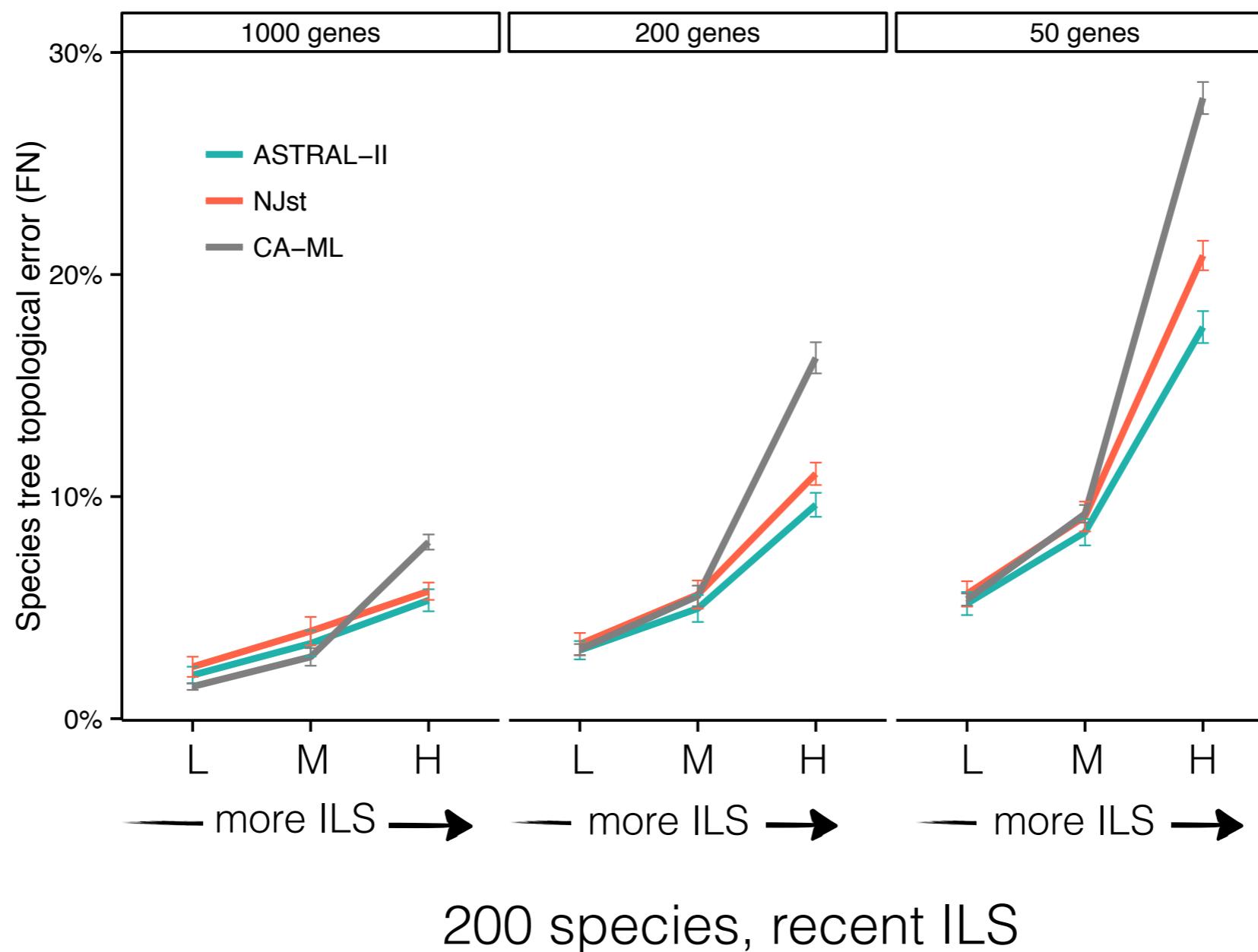


1000 genes, “medium” levels of ILS, simulated species trees  
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# Comparison to concatenation: depends on the ILS level



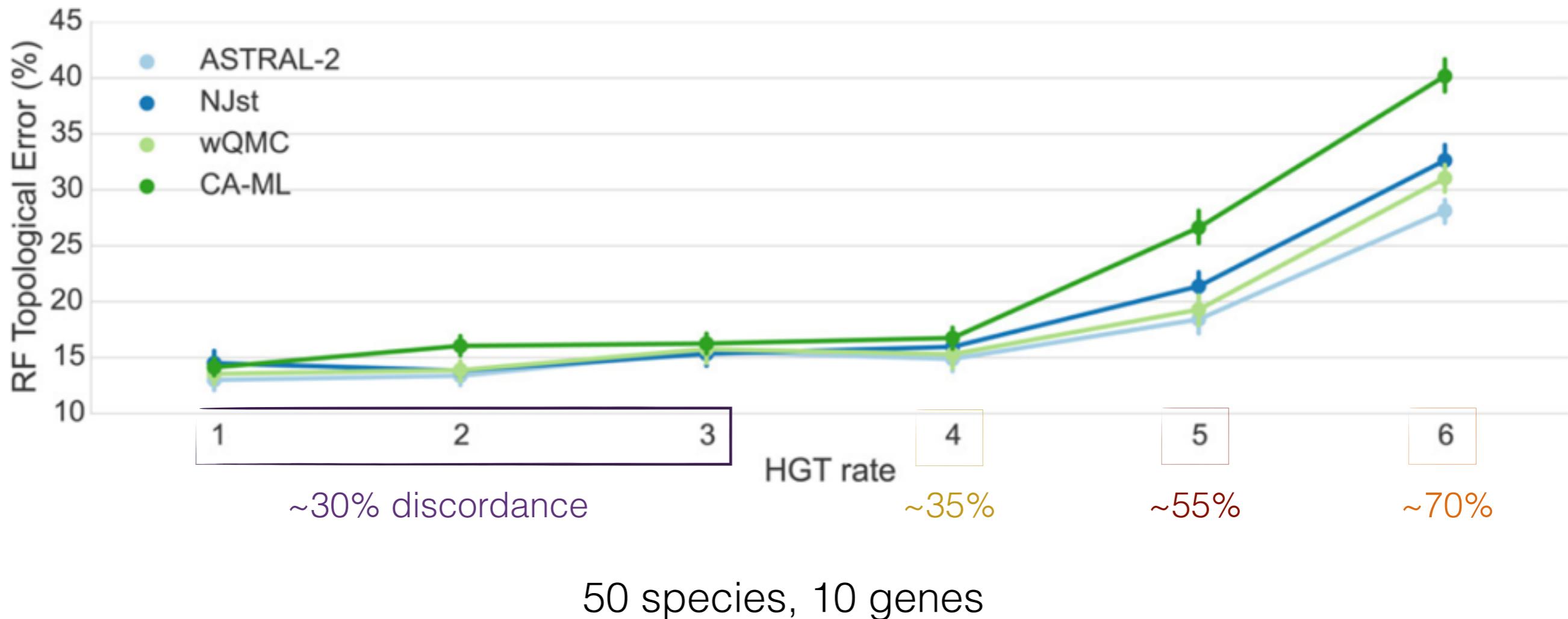
# Comparison to concatenation: depends on the ILS level



# Horizontal Gene Transfer (HGT)

[R. Davidson et al., BMC Genomics. 16 (2015)]

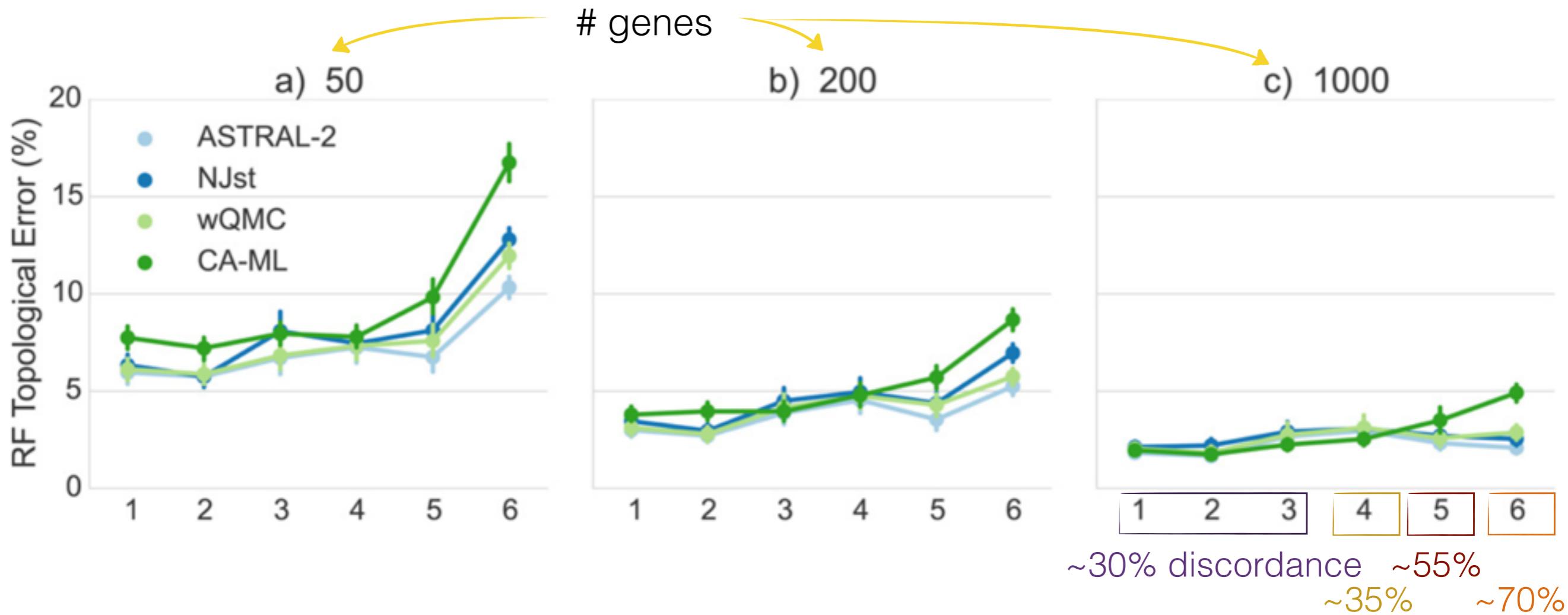
Model violation: the simulated discordance is due to  
both ILS and randomly distributed HGT



# Horizontal Gene Transfer (HGT)

[R. Davidson et al., BMC Genomics. 16 (2015)]

Randomly distributed HGT is tolerated with enough genes



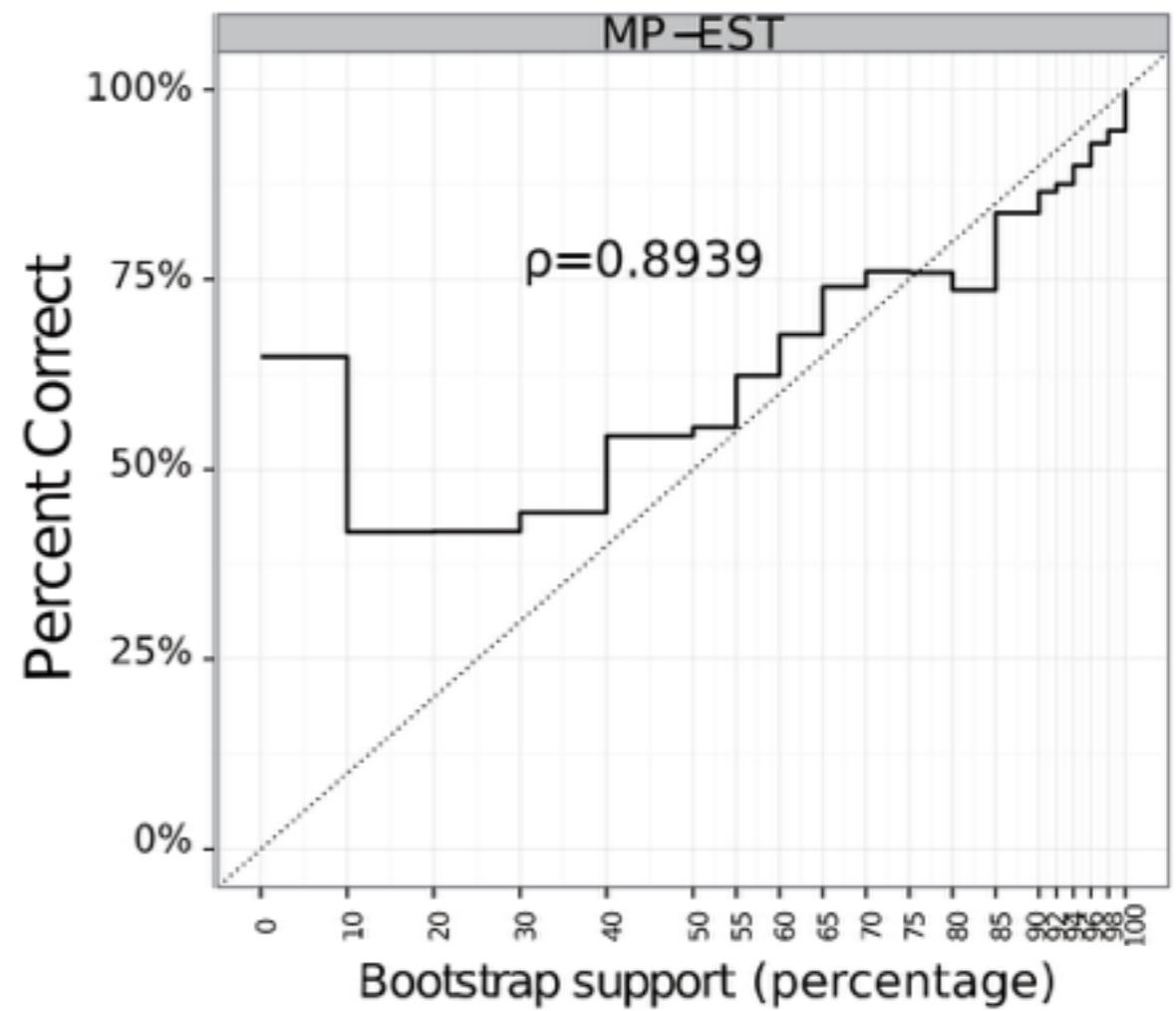
50 species, varying # genes

# UCSD Work

1. Statistical support
2. ASTRAL-III
  - Better running time
  - GPU and CPU Parallelism
  - Multi-Individual datasets
3. Impact of fragmentary data

# Branch support

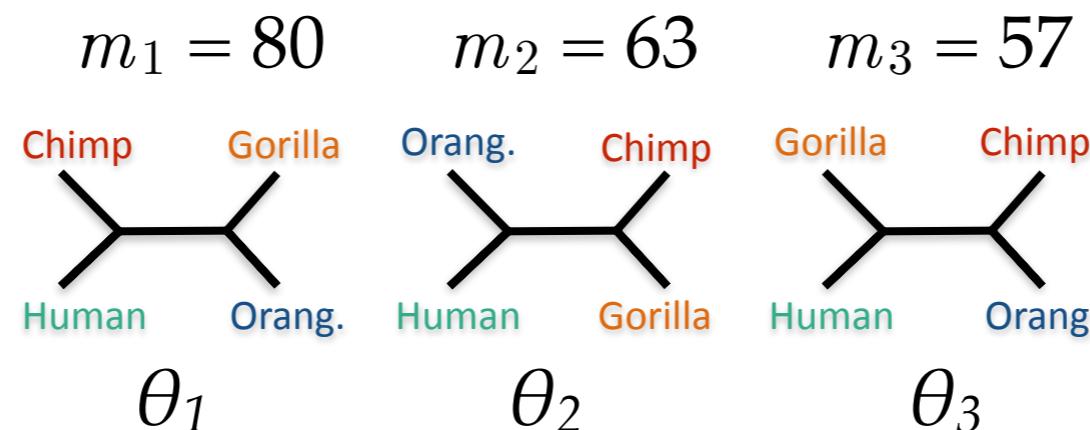
- Traditional approach:  
Multi-locus bootstrapping (MLBS)
  - Slow: requires bootstrapping all genes (e.g.,  $100 \times m$  ML trees)
  - Inaccurate and hard to interpret  
[Mirarab et al., Sys bio, 2014;  
Bayzid et al., PLoS One, 2015]
- We can do better!



[Mirarab et al., Sys bio, 2014]

# Local posterior probability

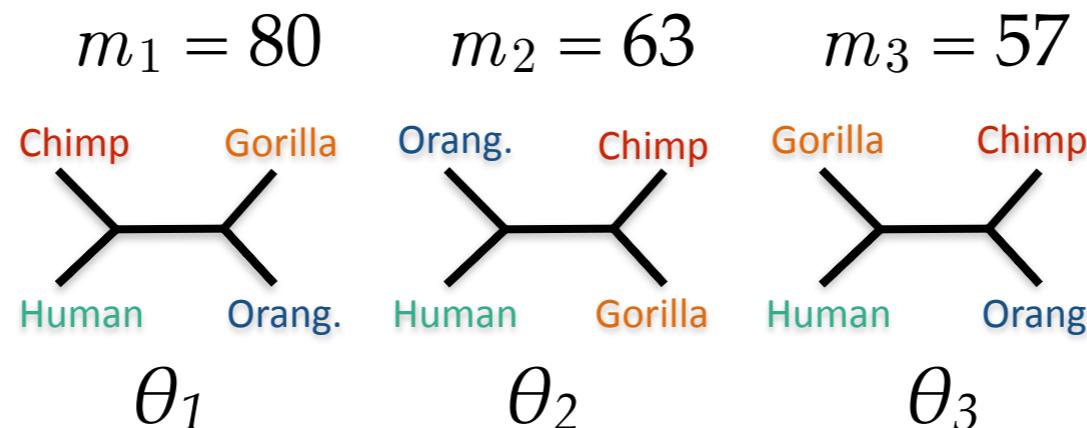
- Recall quartet frequencies follow a multinomial distribution



- $P(\text{topology seen in } m_1 / m \text{ gene trees is the species tree}) = P(\theta_1 > 1/3)$

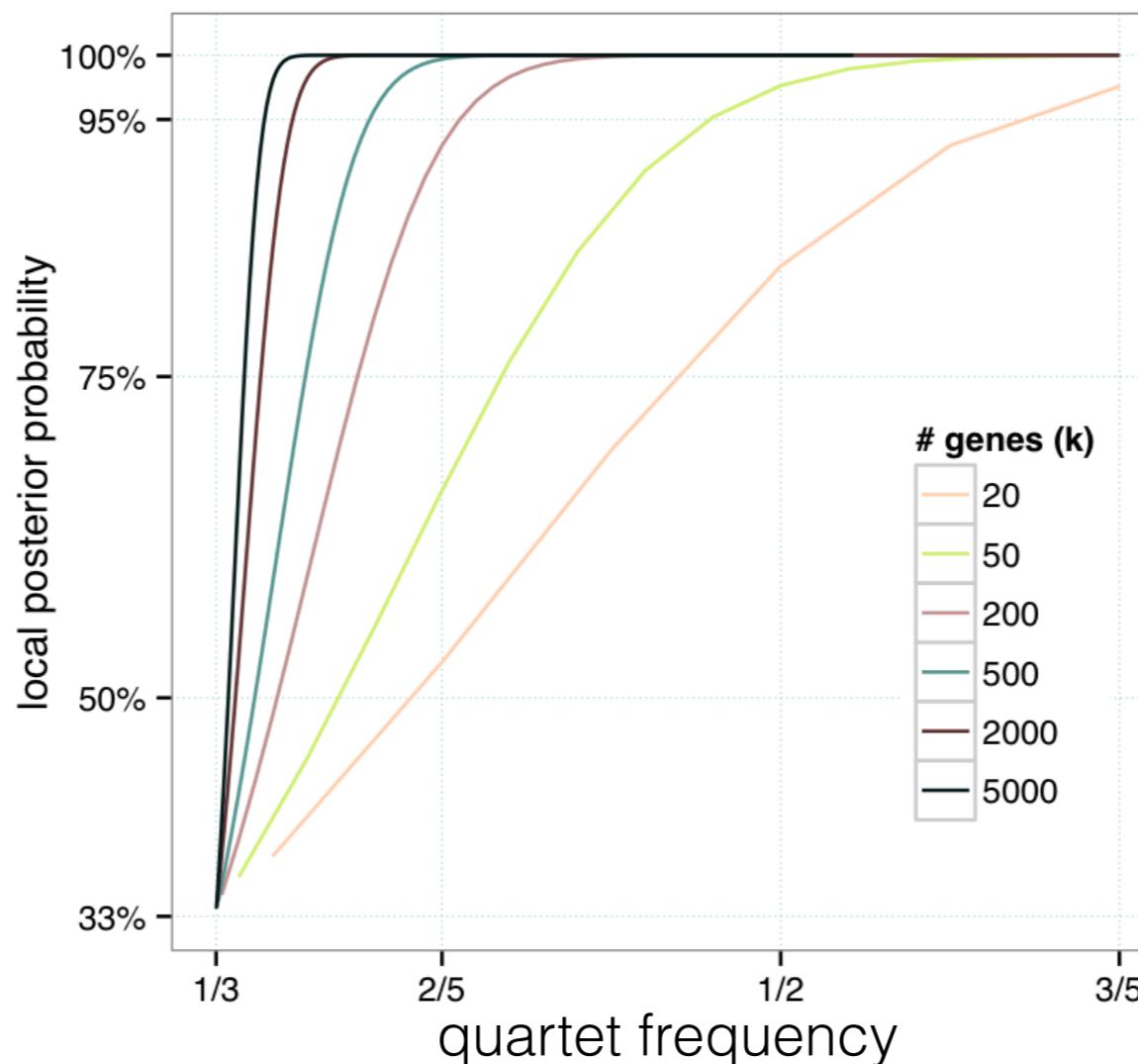
# Local posterior probability

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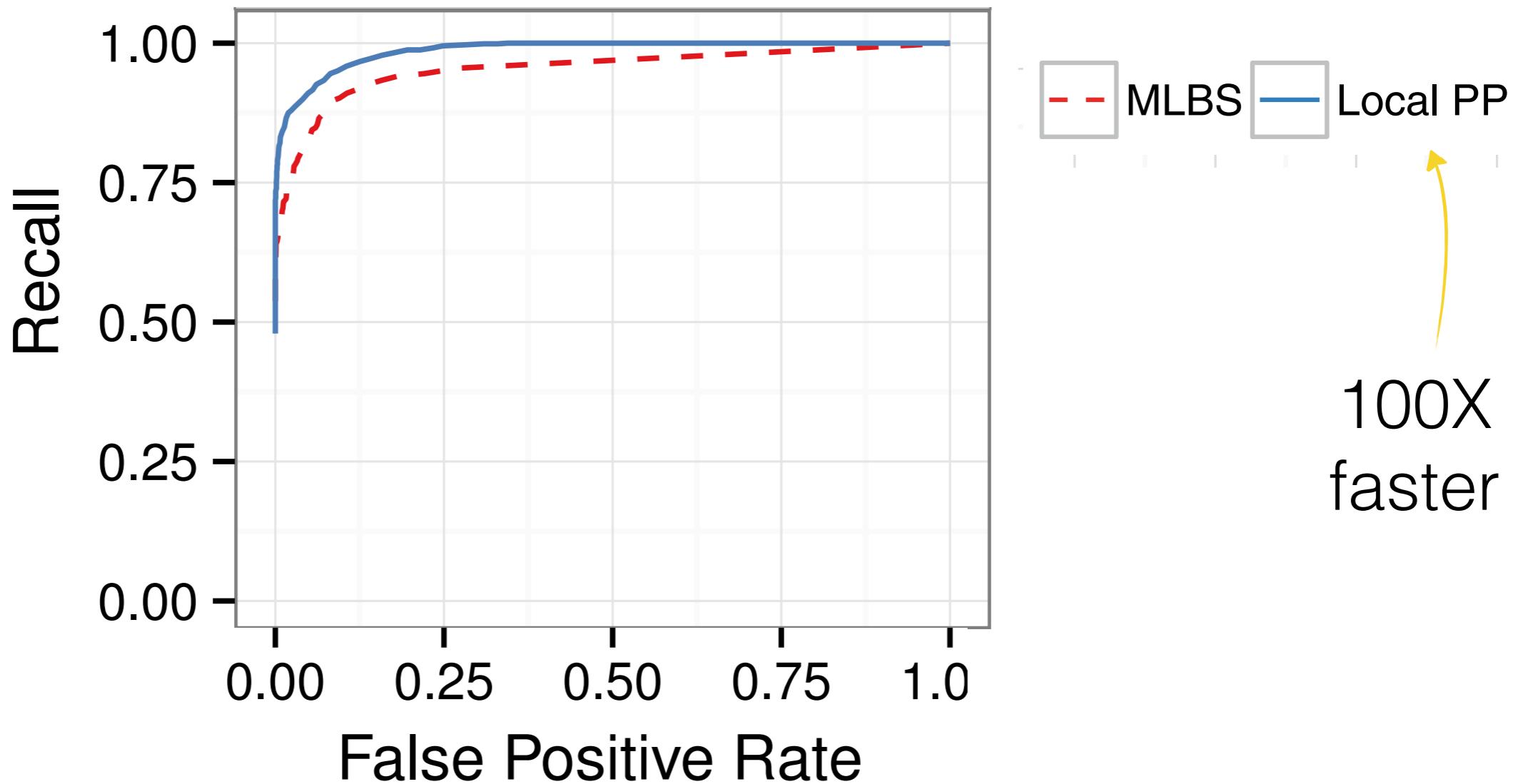
- $P$  ( topology seen in  $m_1 / m$  gene trees is the species tree ) =  $P(\theta_1 > 1/3)$
- Can be analytically solved
  - We implemented this idea in astral and called the measure “the local posterior probability” (localPP)

# Quartet support v.s. localPP



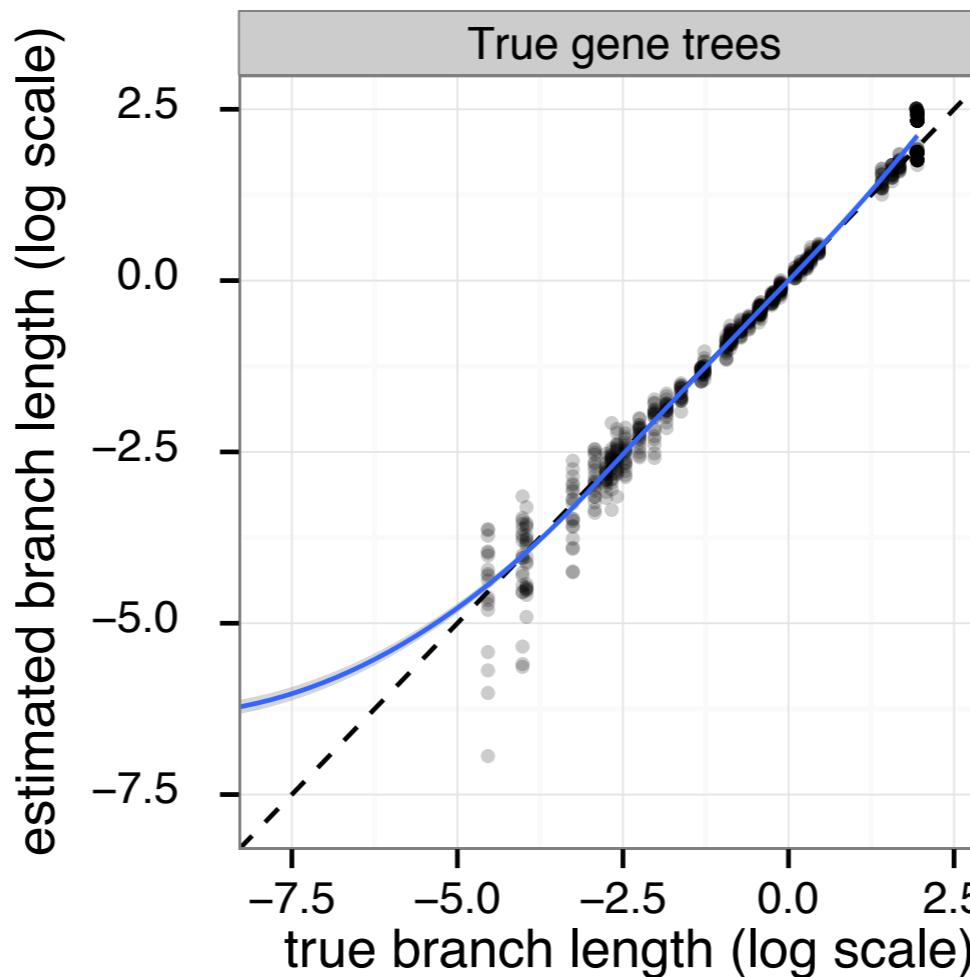
Increased number of genes ( $m$ )  $\Rightarrow$  increased support  
Decreased discordance  $\Rightarrow$  increased support

# localPP is more accurate than bootstrapping



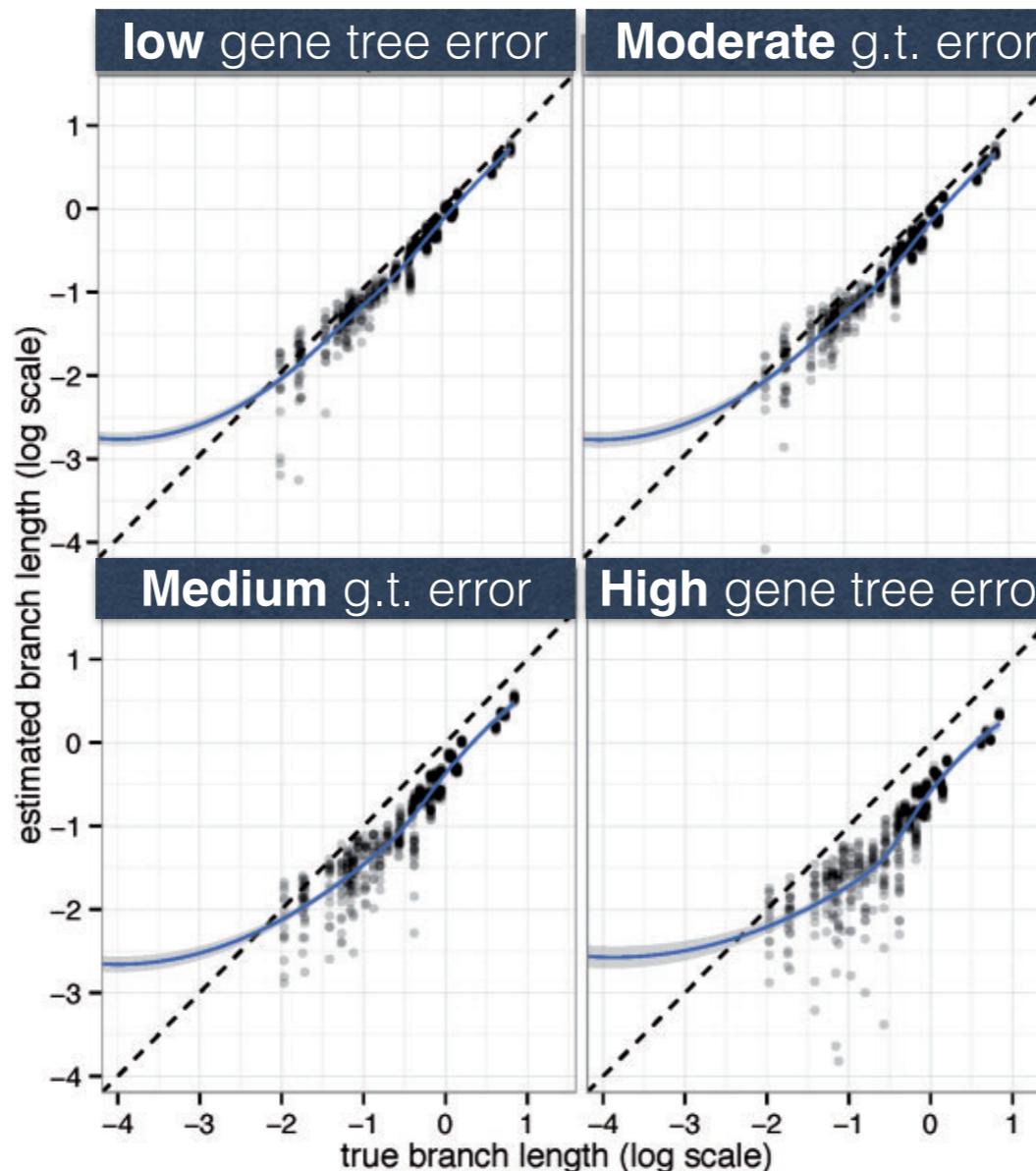
Avian simulated dataset (48 taxa, 1000 genes)  
[Sayyari and Mirarab, MBE, 2016]

# ASTRAL can also estimate internal branch lengths



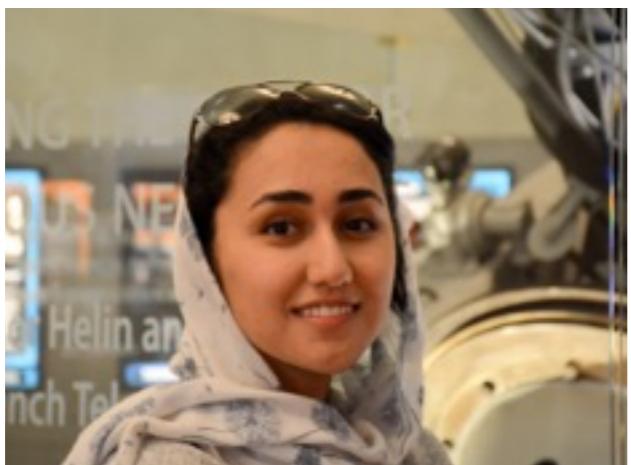
With **true** gene trees, ASTRAL **correctly estimates** BL

# ASTRAL can also estimate internal branch lengths

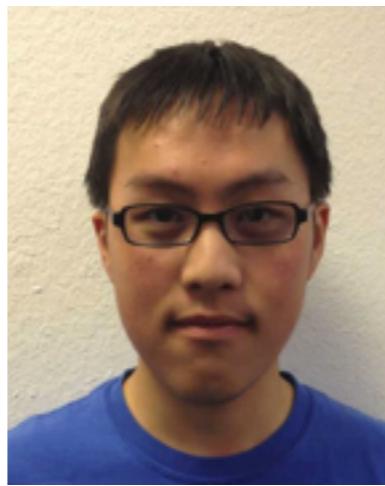


With error-prone **estimated** gene trees, ASTRAL **underestimates** BL

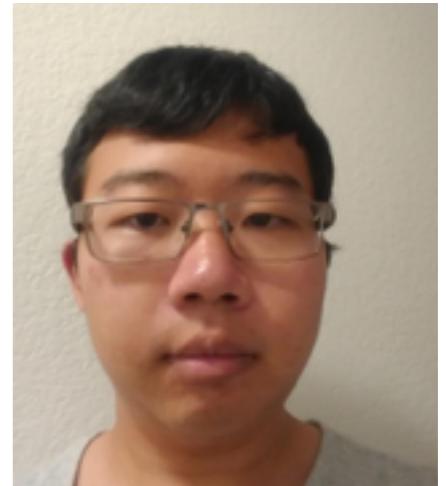
# ASTRAL-III and beyond (unpublished work)



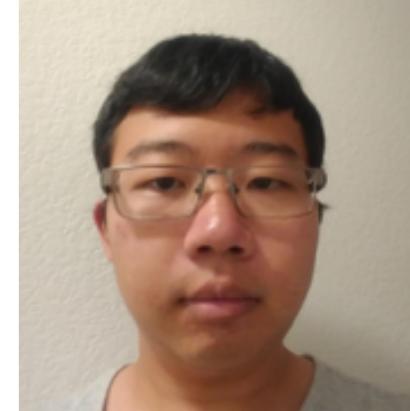
Maryam Rabiee Hashemi



John Yin



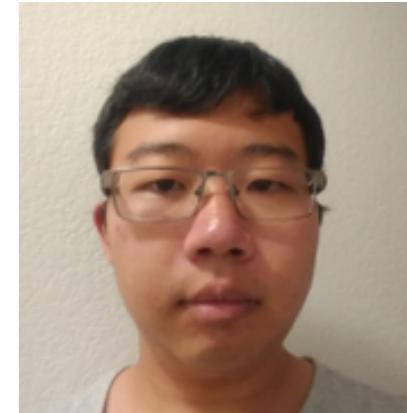
Chao Zhang



# ASTRAL-III

Chao Zhang

- Improved running time (to be released in July)
  - Can handle **polytomies** without slowing down
  - Can exploit similarities between gene trees to reduce running time



# ASTRAL-III

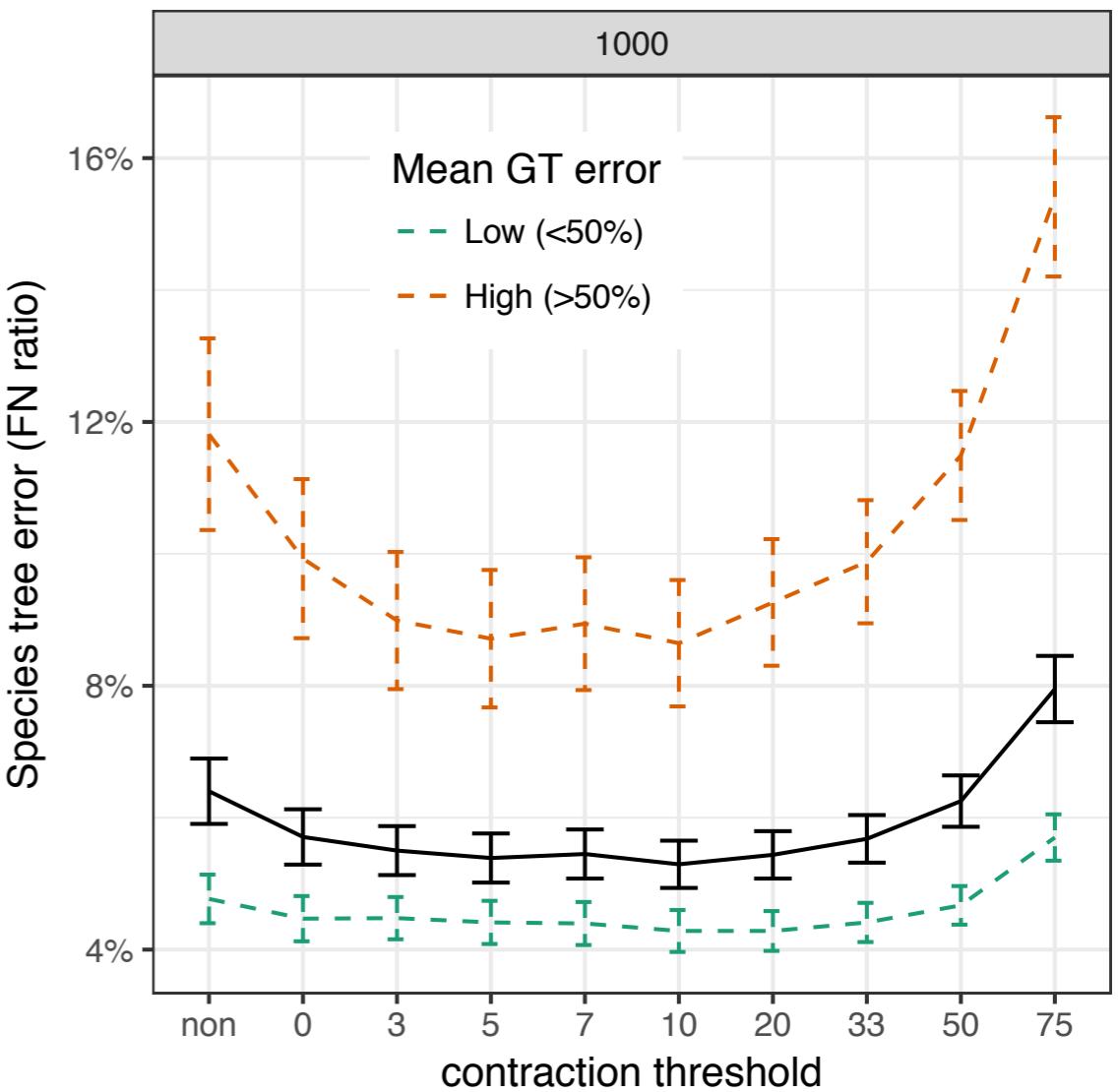
Chao Zhang

- Improved running time (to be released in July)
  - Can handle **polytomies** without slowing down
  - Can exploit similarities between gene trees to reduce running time
- To be released in August
  - Handling datasets with **multiple individuals** per species
  - GPU and CPU **parallelism**

# Low support branches

- Does it help to **contract** branches with low support?
- Yes, but **only for very low supports**
- Theoretical justifications not clear

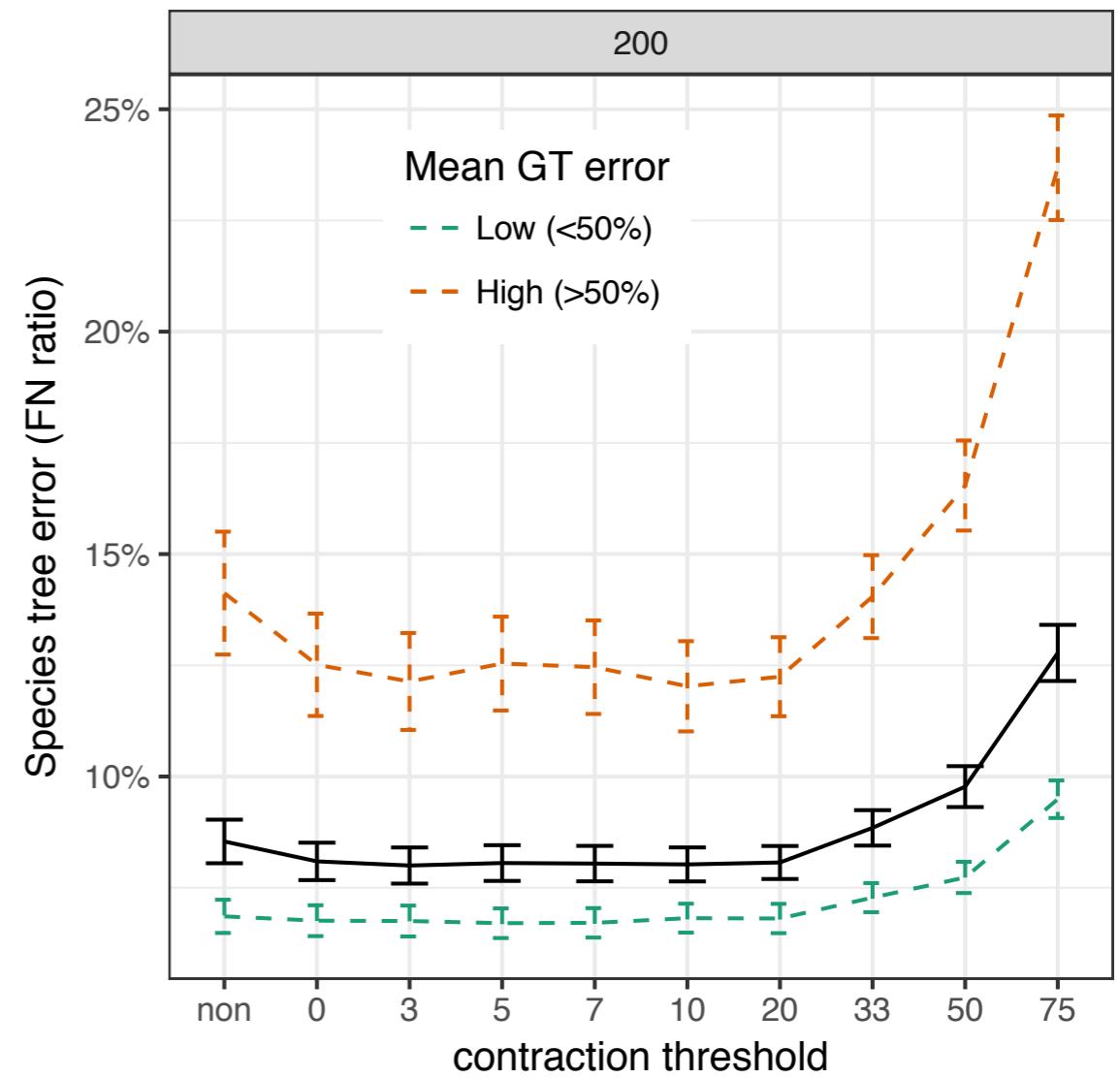
Simulations: 100 taxa, simphy,  
ILS: around 46% true discordance  
FastTree, support from bootstrapping



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# Multiple individuals

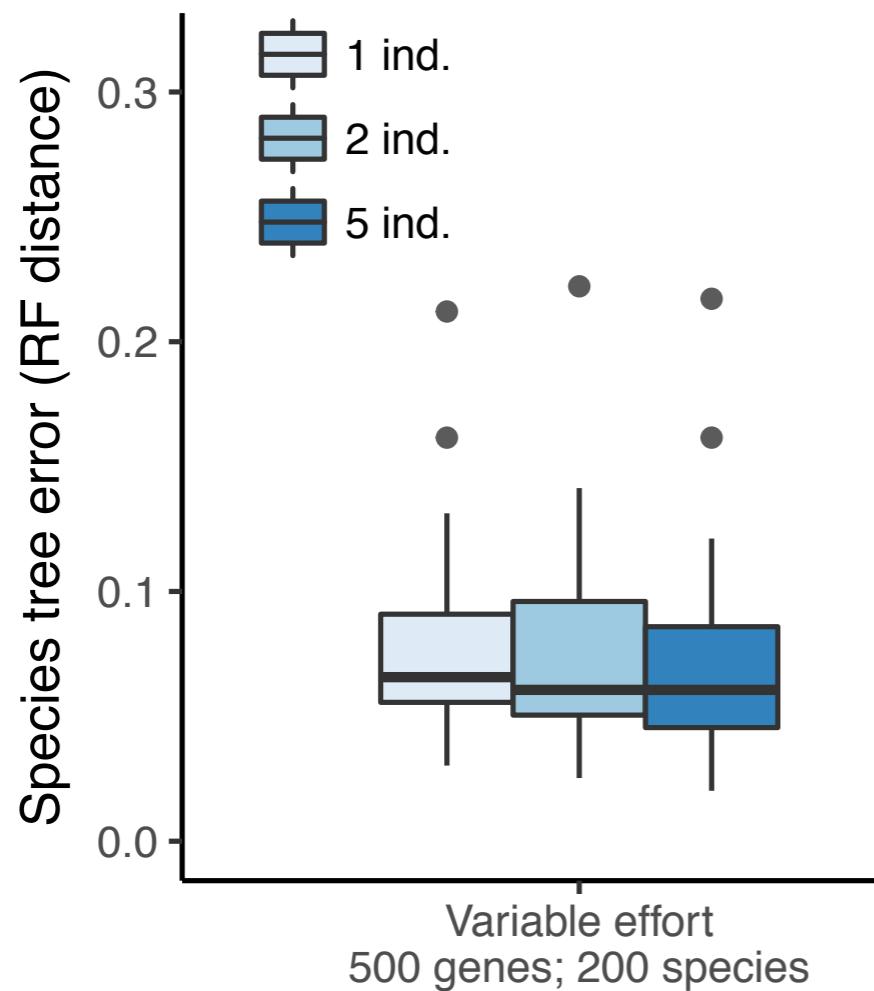


Maryam Rabiee  
Hashemi

- What if we sample **multiple** individuals from each species?
- In **recently diverged** species individuals ***may*** have different trees for each gene
- Sampling multiple individuals may provide **extra signal**

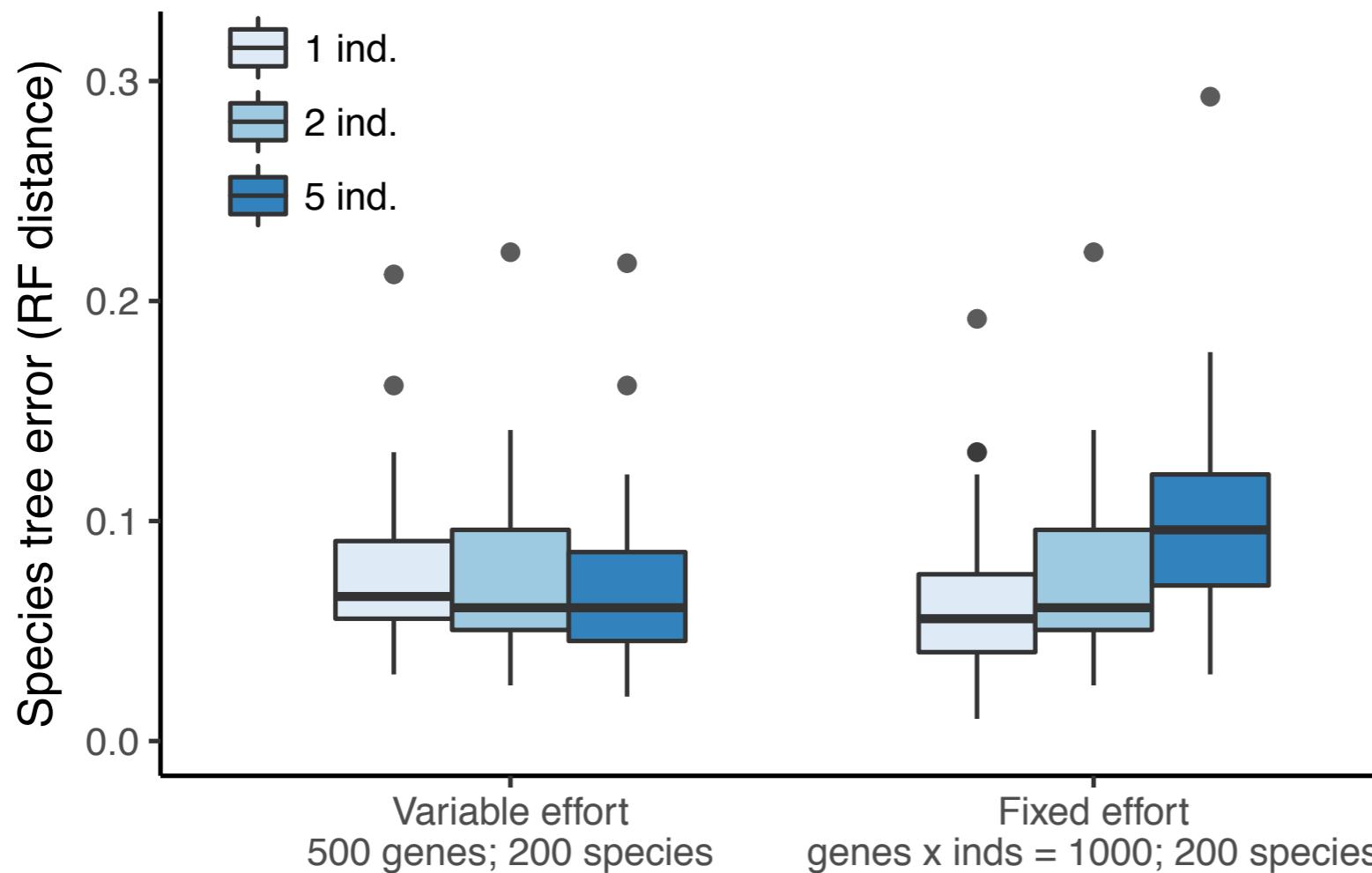


# Multiple individuals helpful?



Yes, it marginally helps accuracy

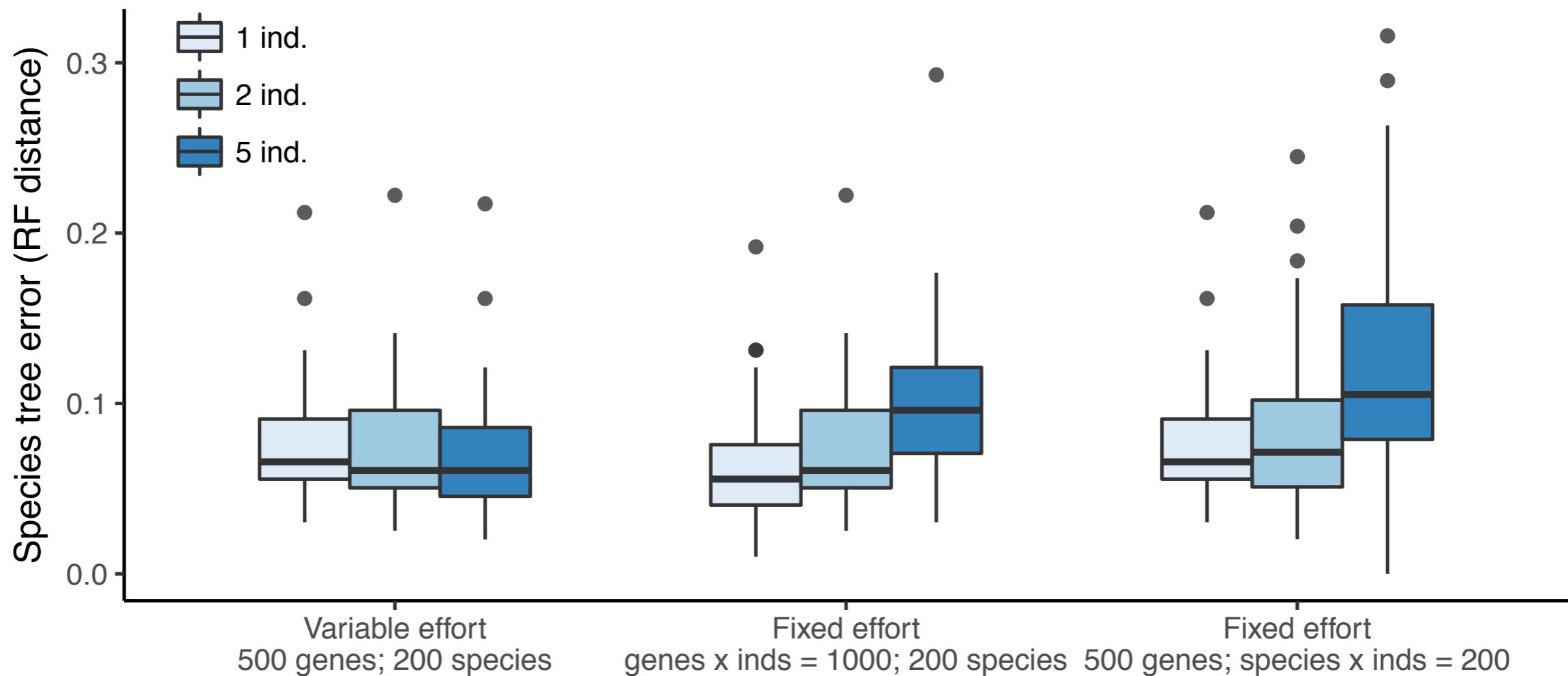
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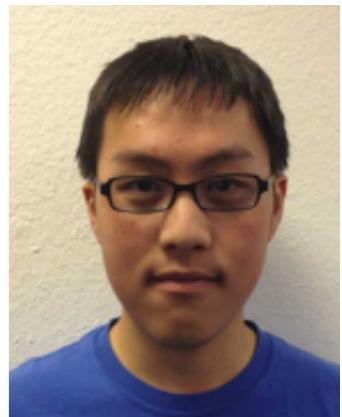
But **not** if sequencing **effort** is kept **fixed**

# Multiple individuals helpful?



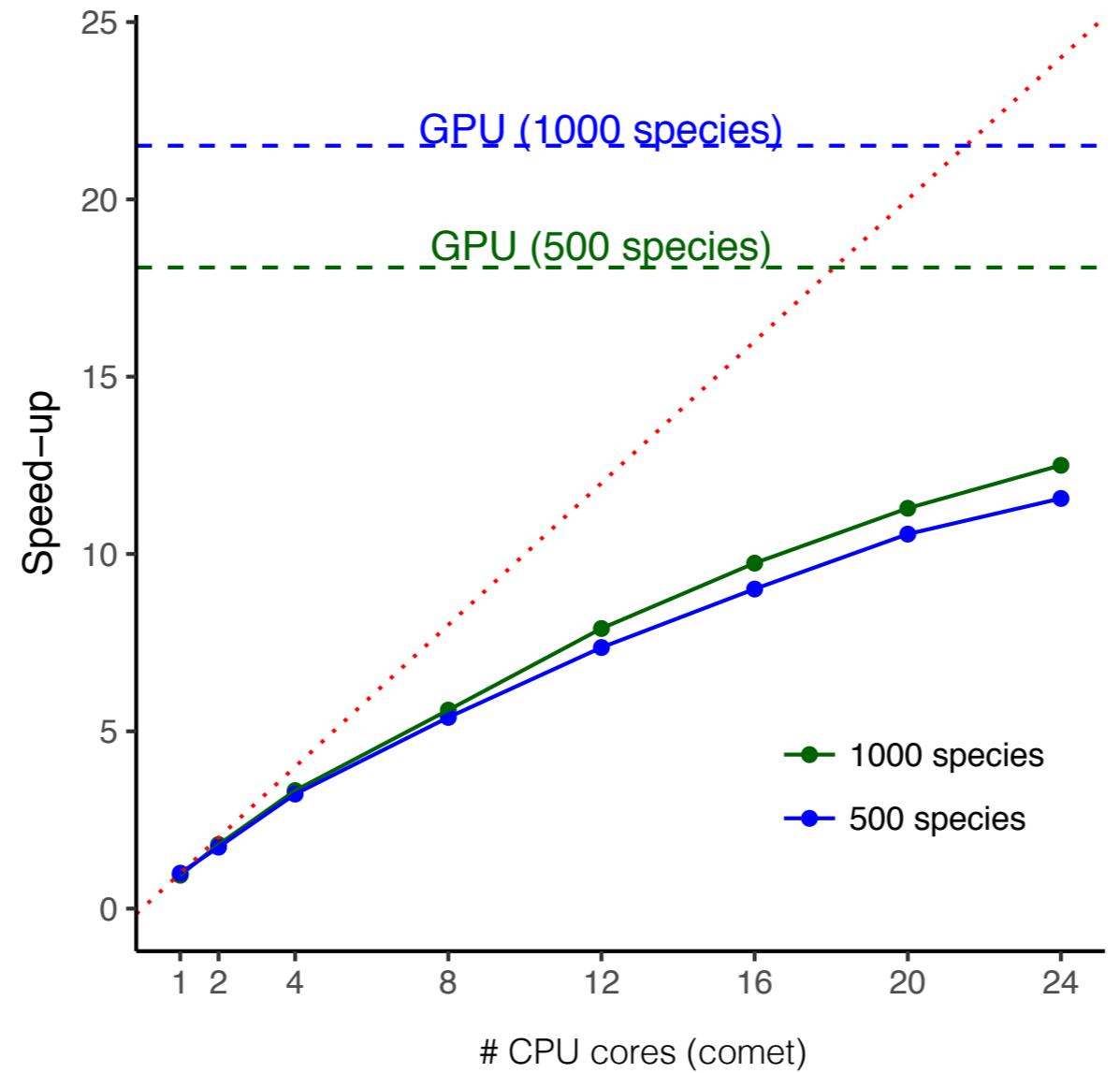
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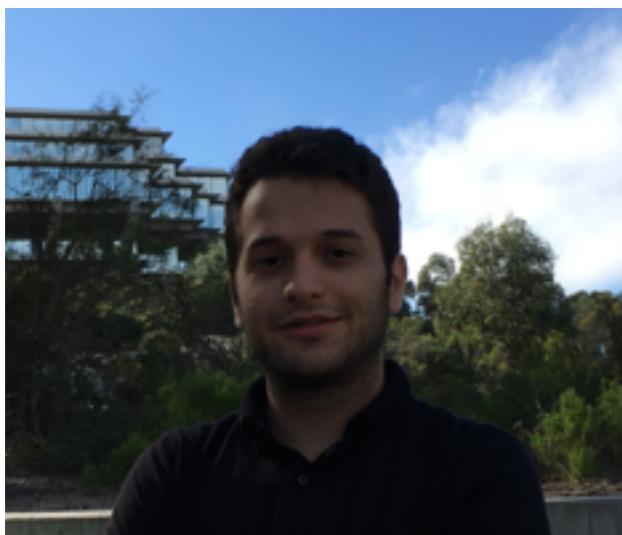
# Parallelism

John Yin



Can infer trees with 10,000 species & 400 genes in a day

# Impact of fragmentary data



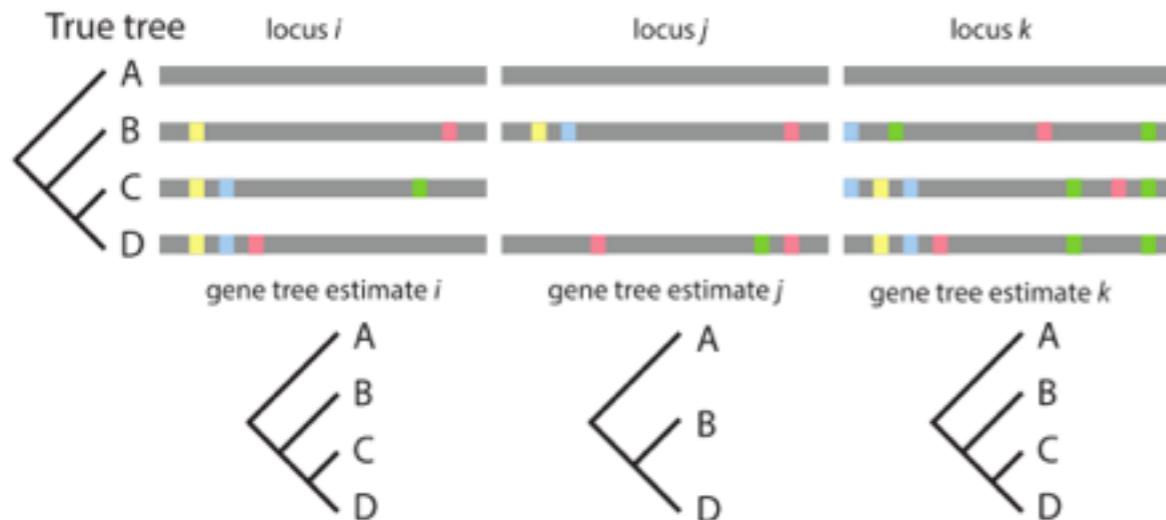
Erfan Sayyari



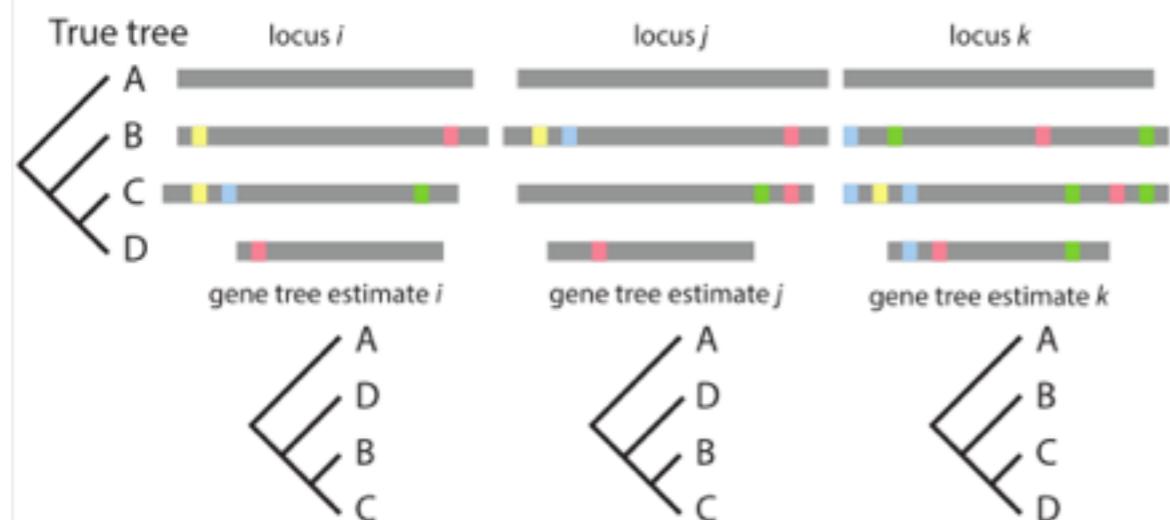
James B. Whitfield



# Two types of missing data



- **Missing genes:** a taxon misses the gene fully (taxon occupancy)



- **Fragmentary data:** the species is partially sequenced for some genes

Figure from Hosner et al, 2016

# Does missing data matter?

- Missing genes:
  - Evidence suggests summary methods are often robust

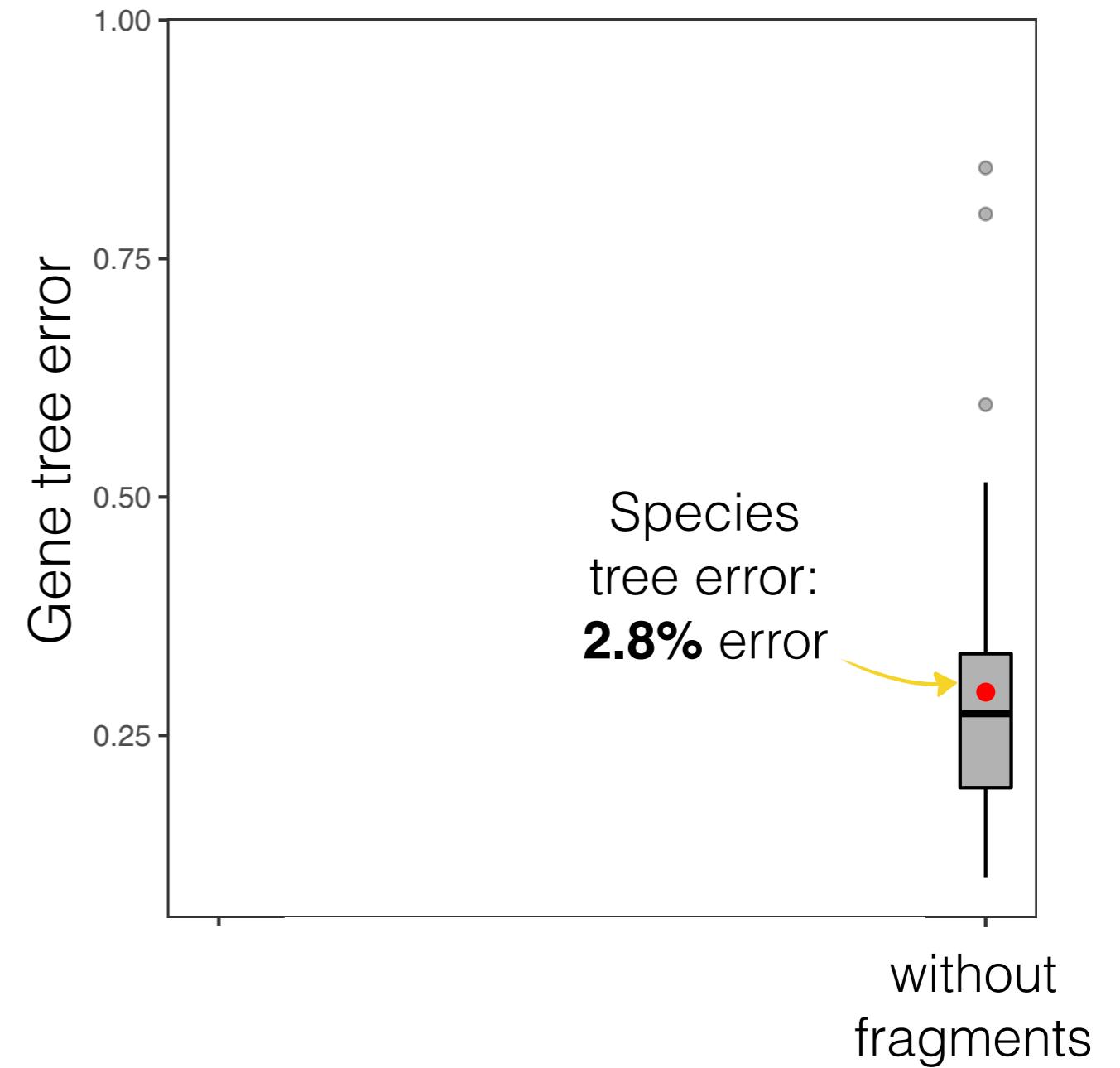
# Does missing data matter?

- Missing genes:
  - Evidence suggests summary methods are often robust
- Fragmentary data:
  - Less extensively studied
  - Hosner et al. indicated:
    - Fragments matter
    - They suggest removing genes with many fragments

# Our study

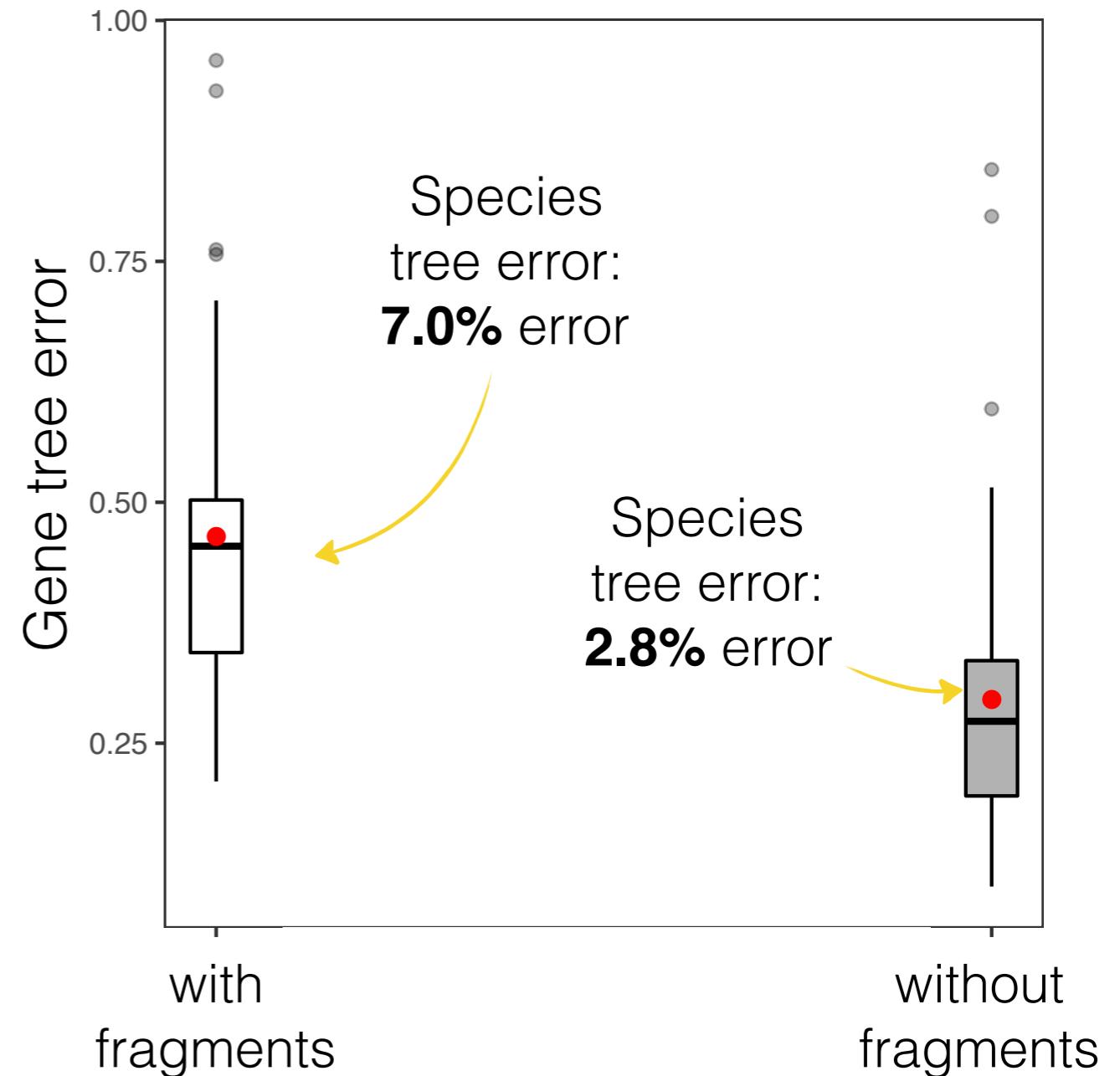
- Empirical data:
  - An insect dataset of 144 species and 1478 genes
  - 600 million years of evolution
  - Transcriptomics, so plenty of missing data of both types
- Simulated data:
  - 100 taxon, medium ILS, 1000 genes
  - Added fragmentation with patterns similar to the insect data

# Fragmentary data hurt gene trees and species trees



# Fragmentary data hurt gene trees and species trees

Fragmentary data dramatically increase gene tree and the species tree error



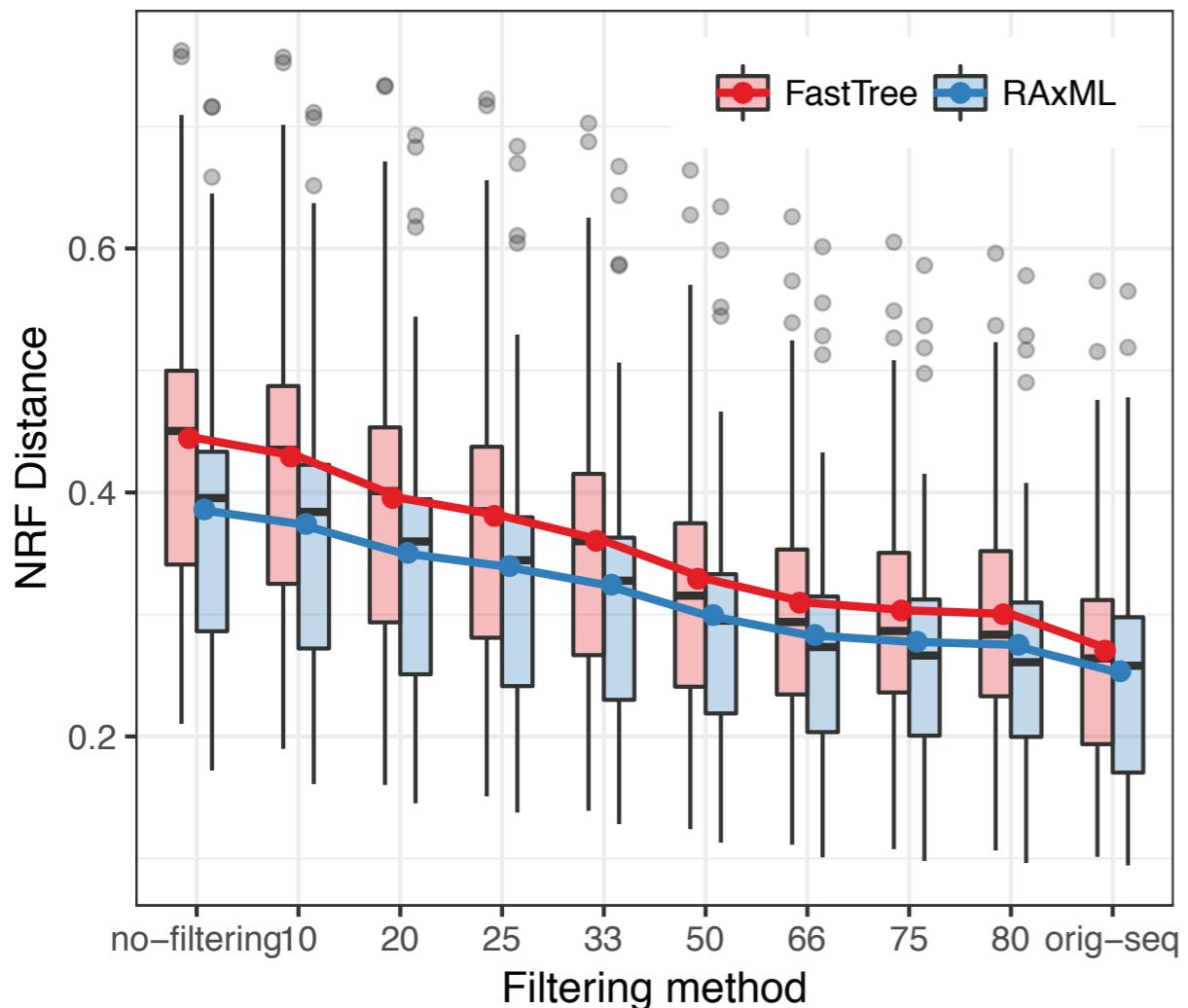
# How do we solve this?

- Hosner et al. suggested removing entire genes.
  - This is too conservative in our opinion.

# How do we solve this?

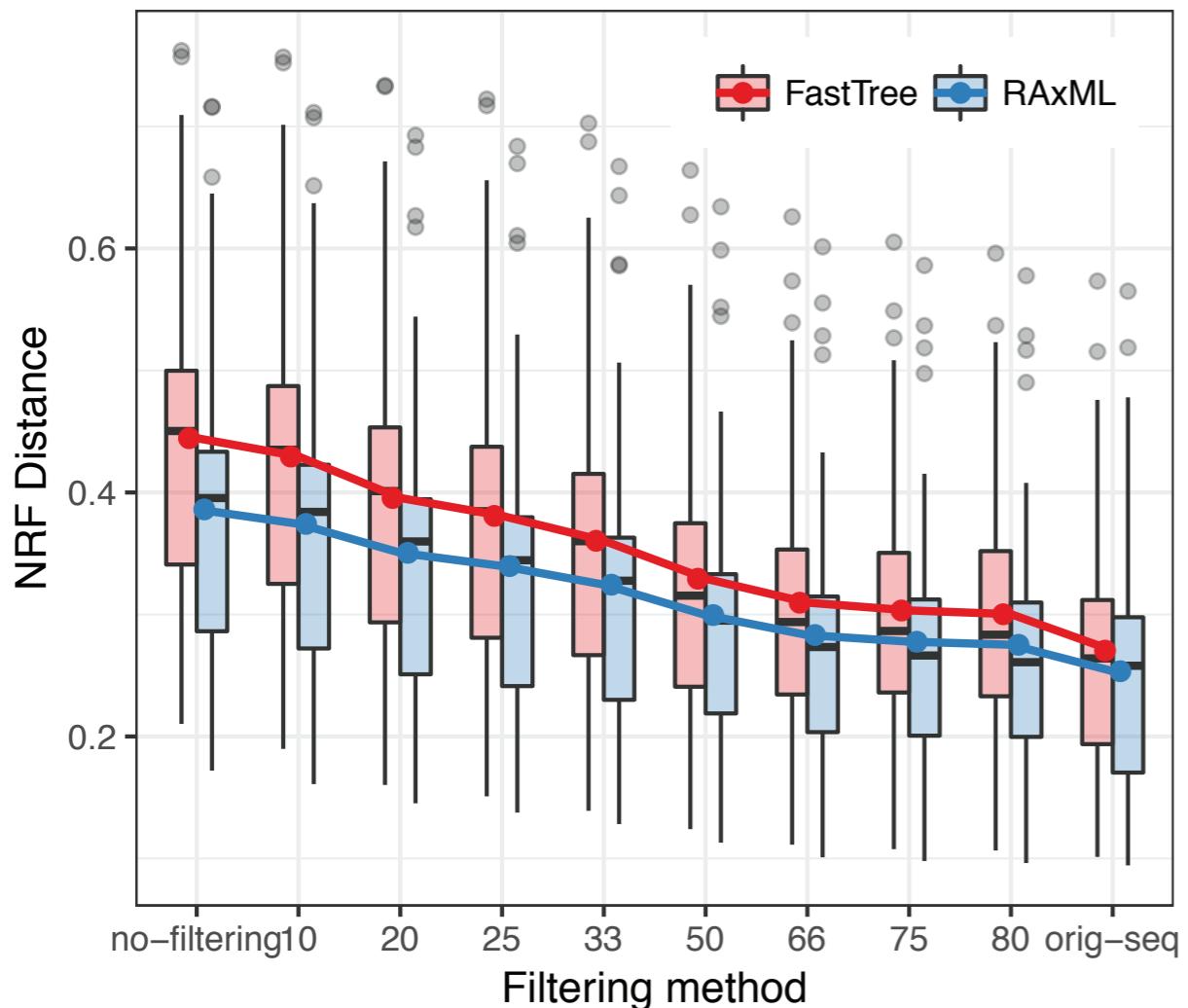
- Hosner et al. suggested removing entire genes.
  - This is too conservative in our opinion.
- We simply remove the fragmentary sequence from the gene and keep the rest of the gene
  - What is fragmentary?
    - Anything that has at most X% of sites
    - We study different X values

# Filtering helps to some level (simulations)

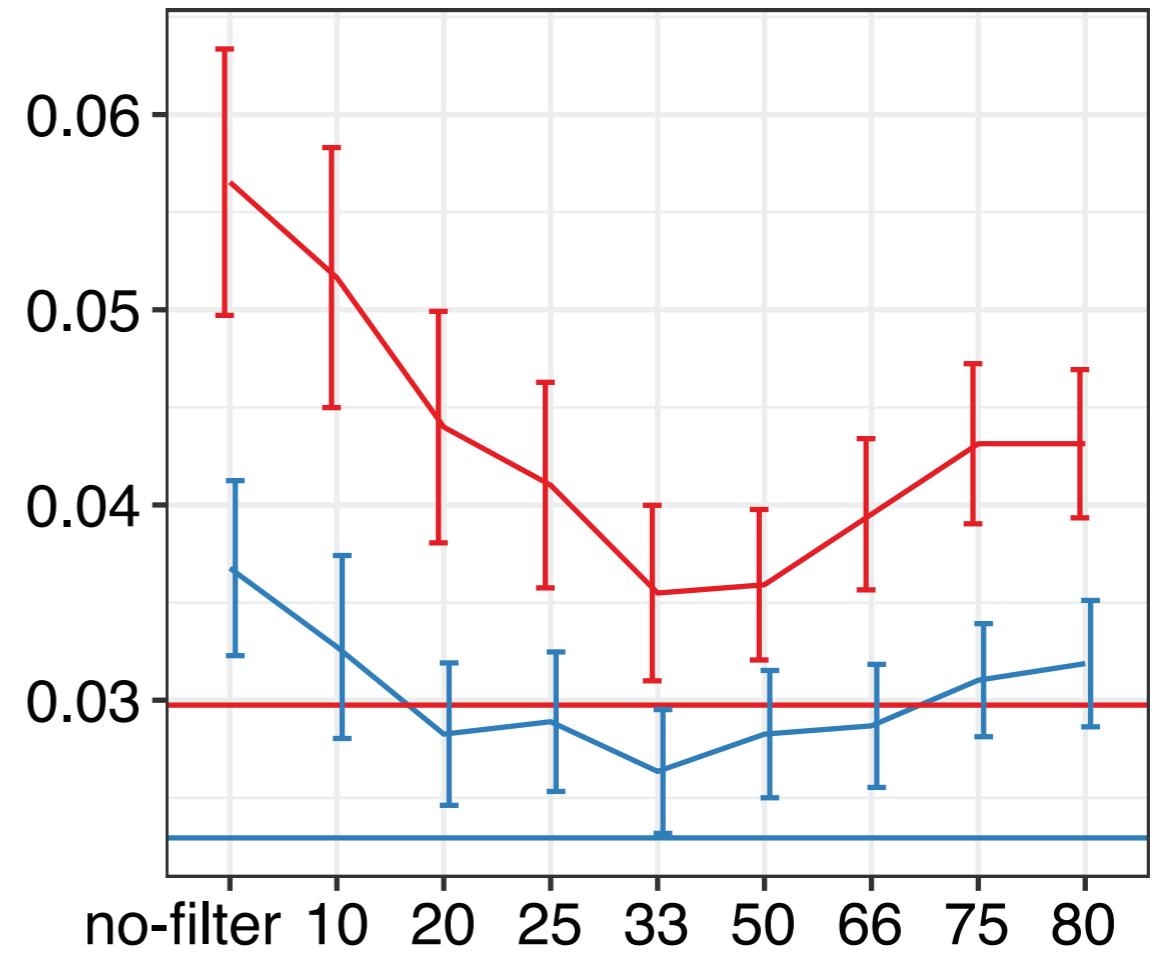


Gene tree error

# Filtering helps to some level (simulations)

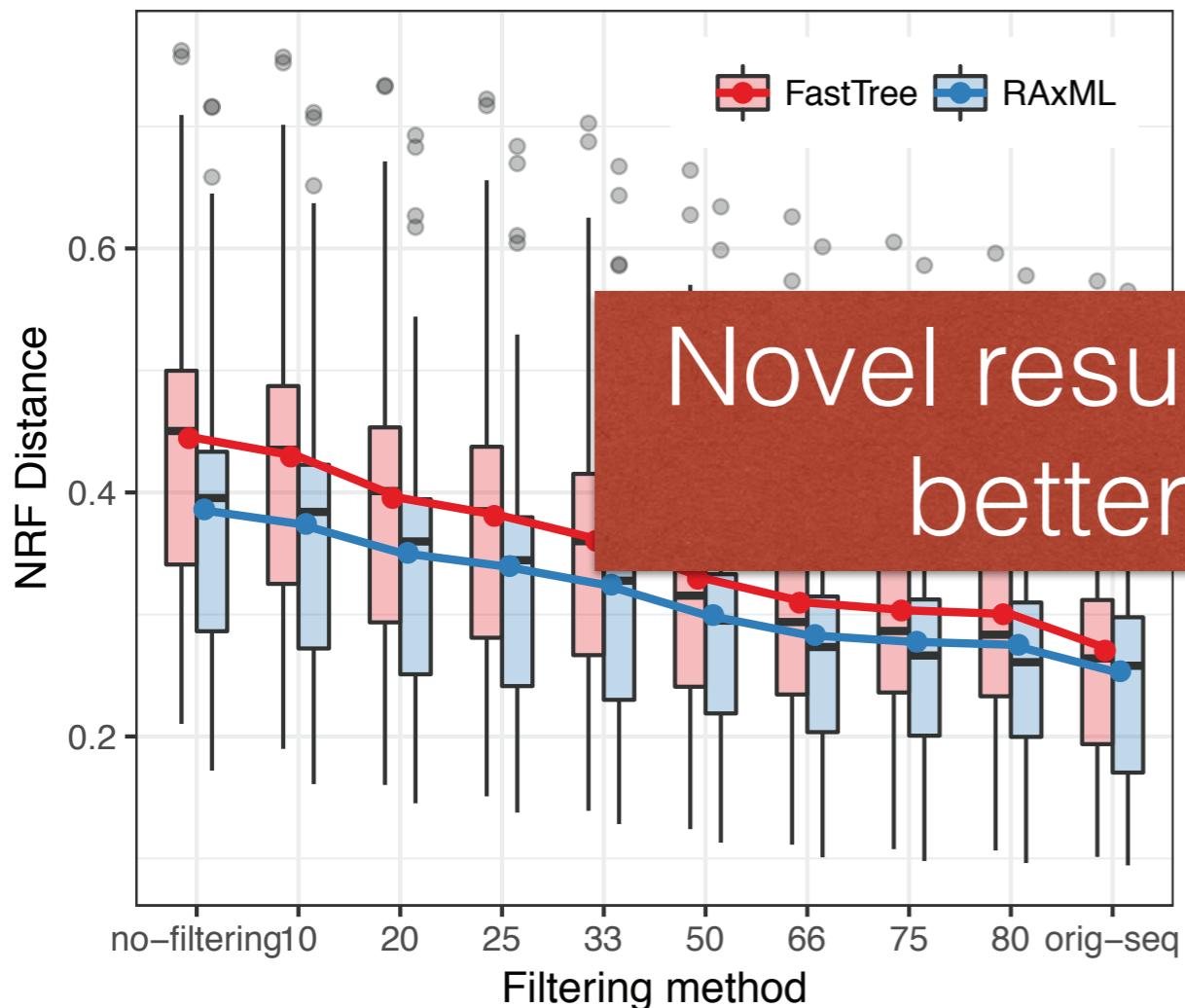


Gene tree error

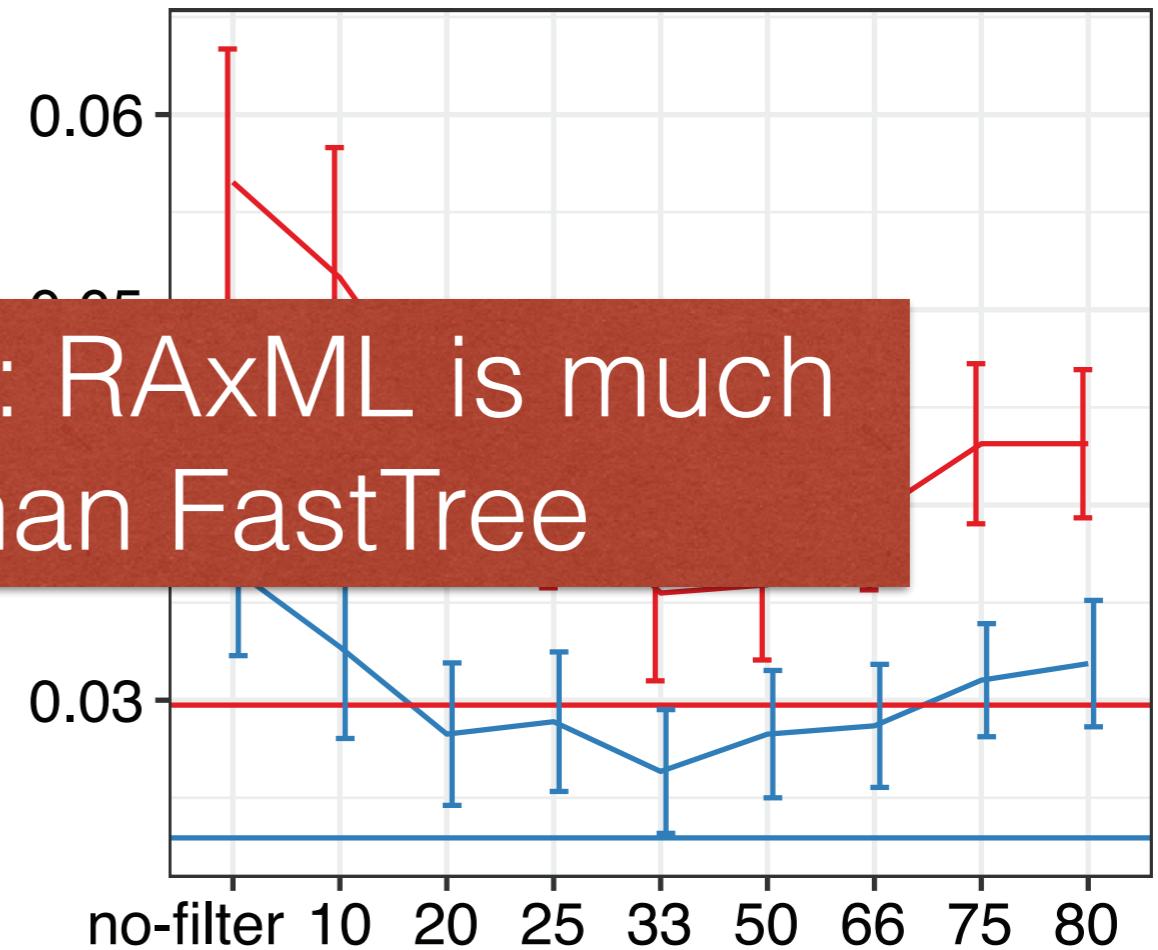


Species tree error

# Filtering helps to some level (simulations)



Novel results: RAxML is much better than FastTree

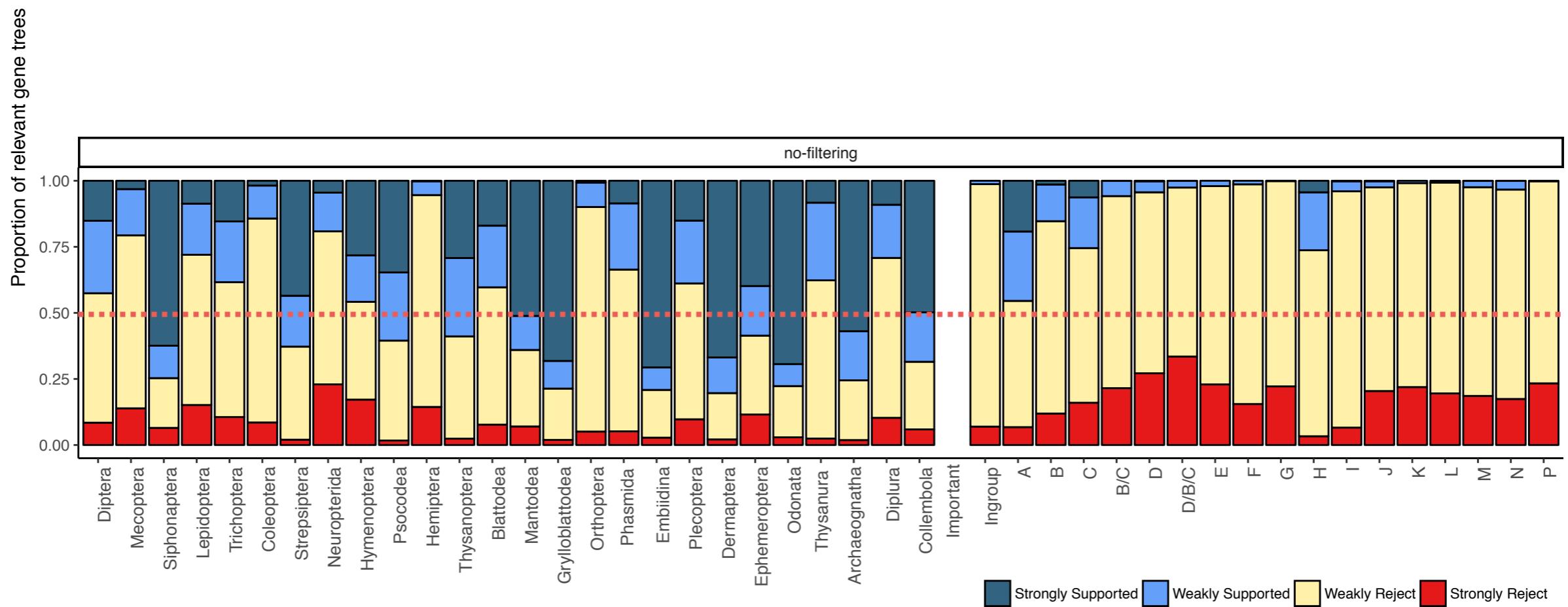


Gene tree error

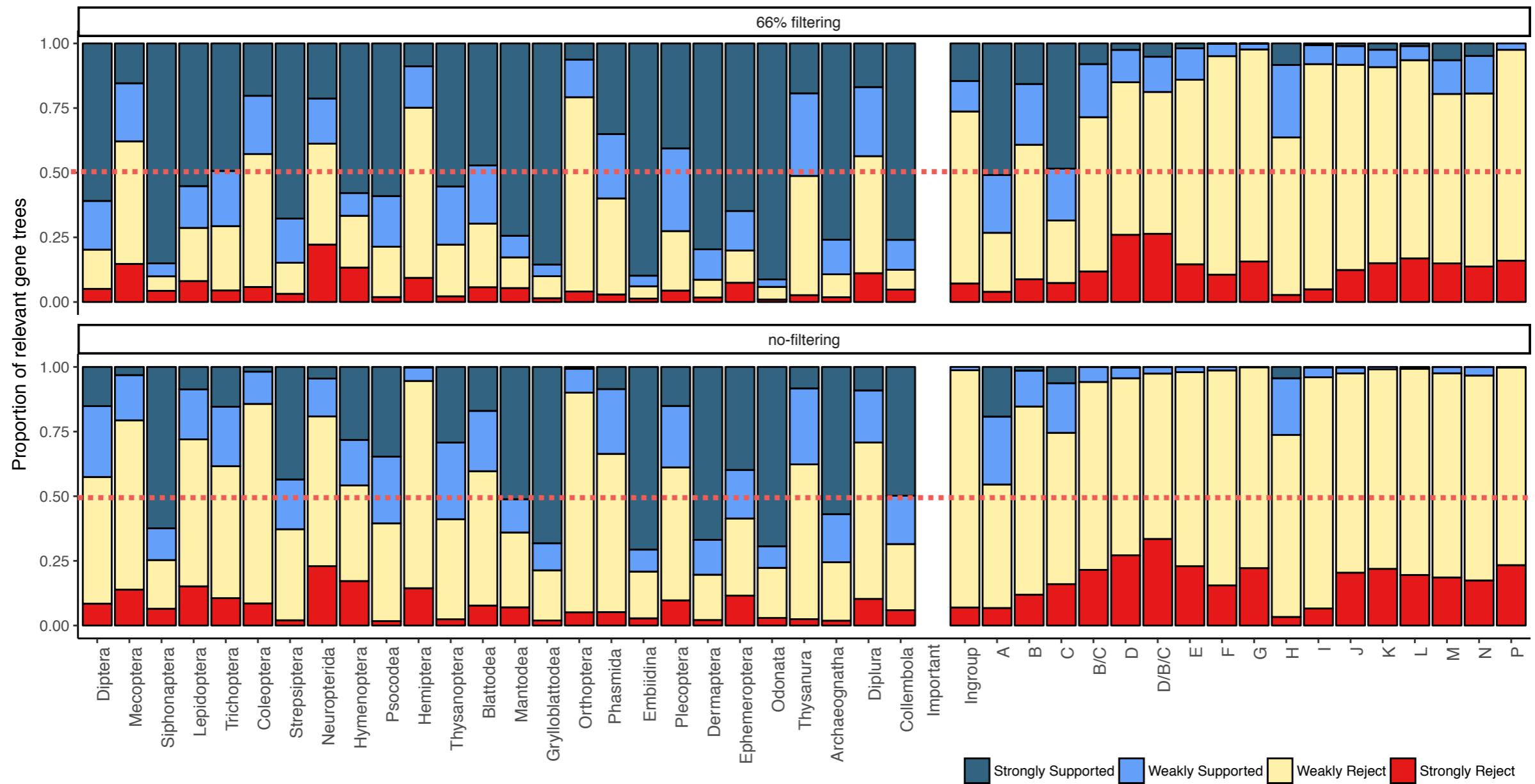
Species tree error

# How about real data?

# Gene trees seem to improve (less gene tree discordance)

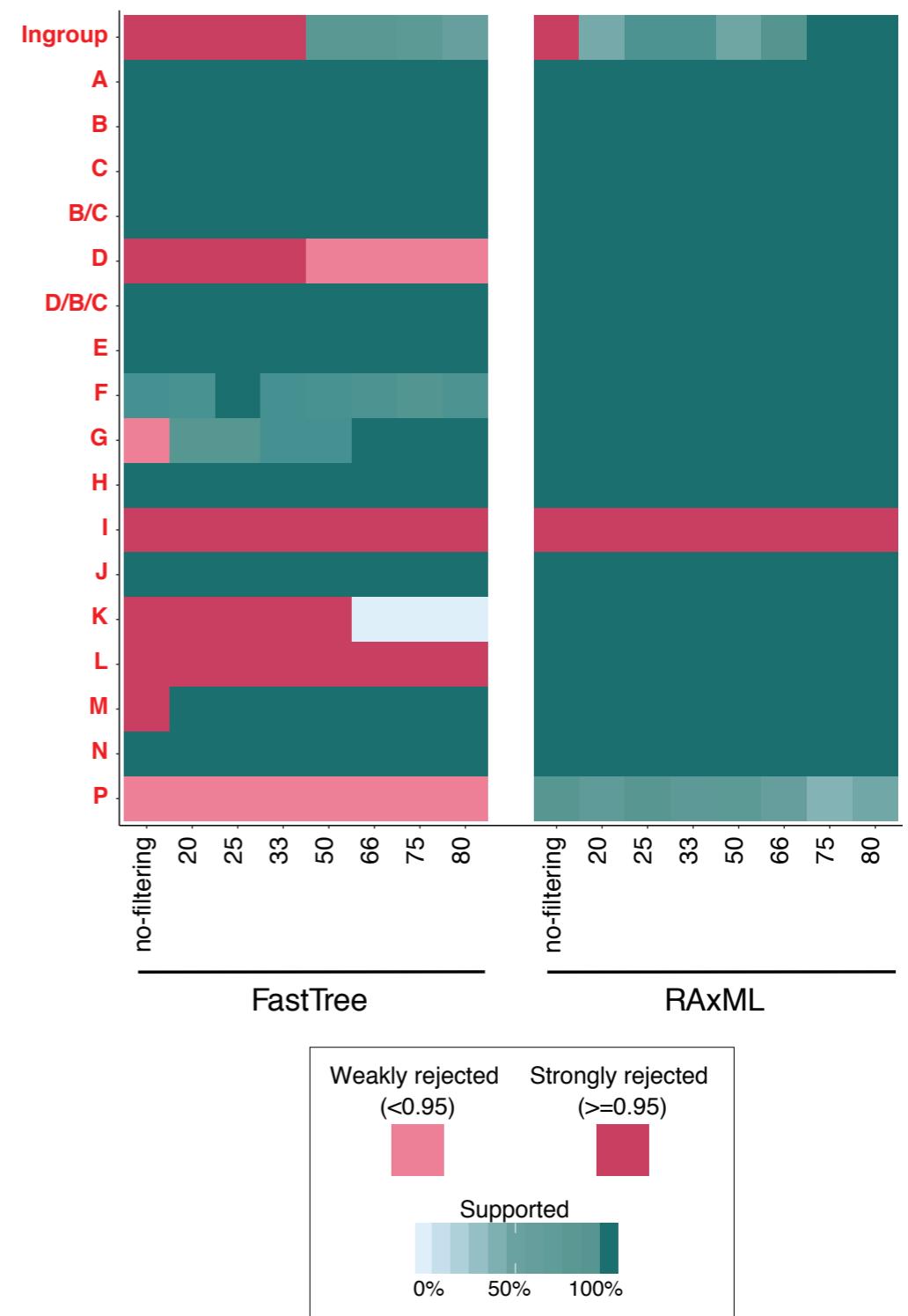


# Gene trees seem to improve (less gene tree discordance)

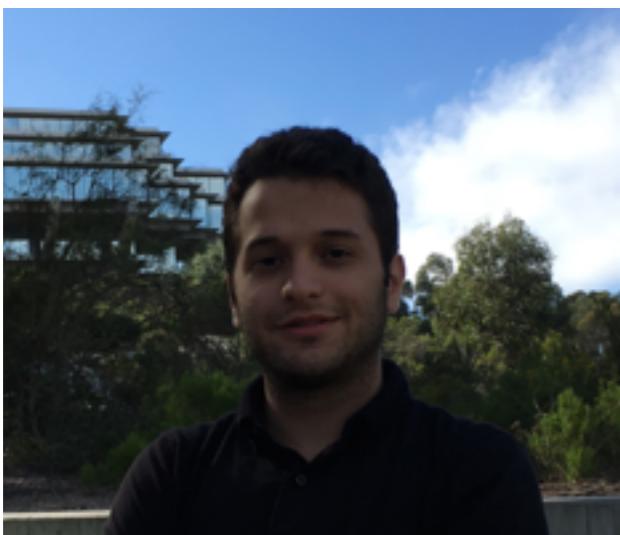


# The species tree also improves

- ASTRAL trees differed with concatenation initially
- After filtering, ASTRAL and concatenation were very similar
  - Differences on controversial nodes
- RAxML+ASTRAL is more robust than FastTree+ASTRAL



# Visualizing discordance: DiscoVista



Erfan Sayyari



James B. Whitfield

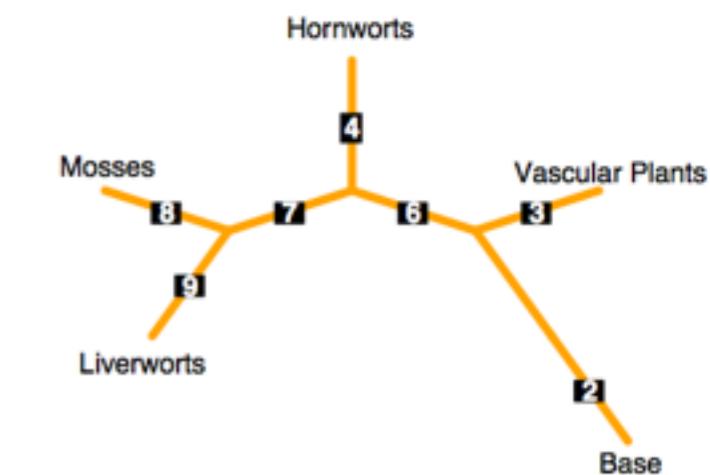
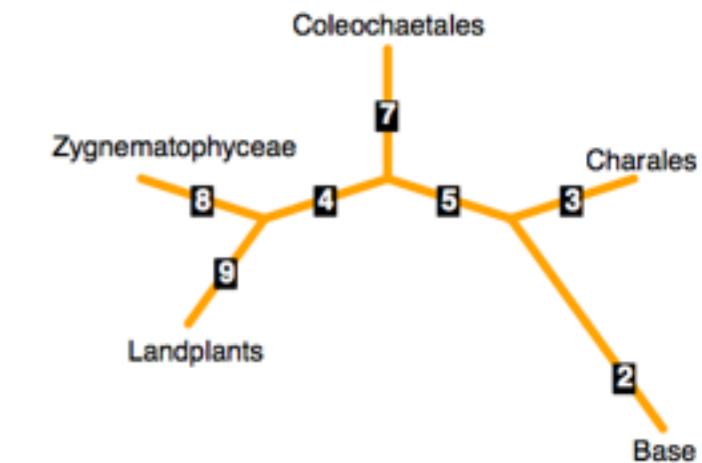
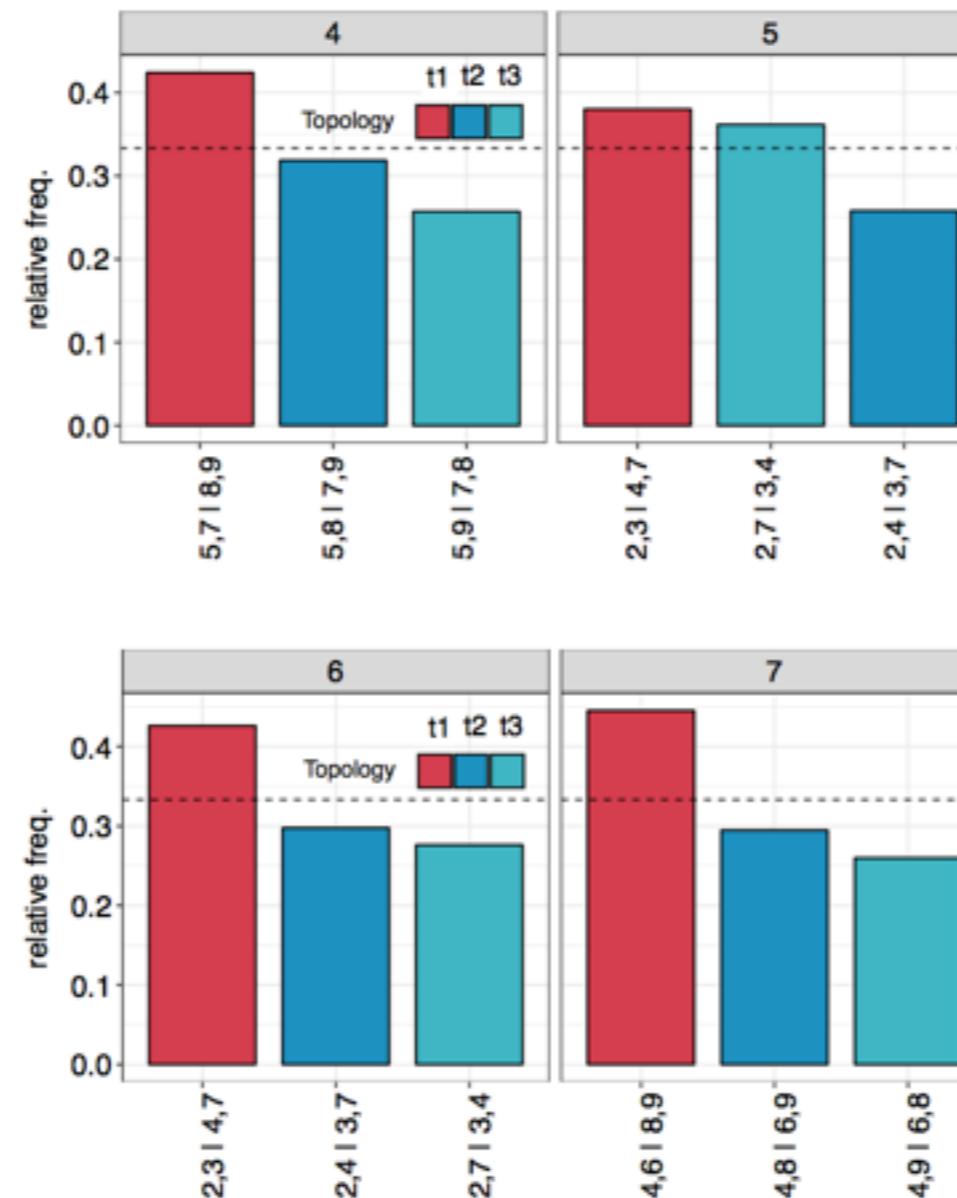


# DiscoVista

- Simple and interpretable
- Easy to run
- Focus on important branches

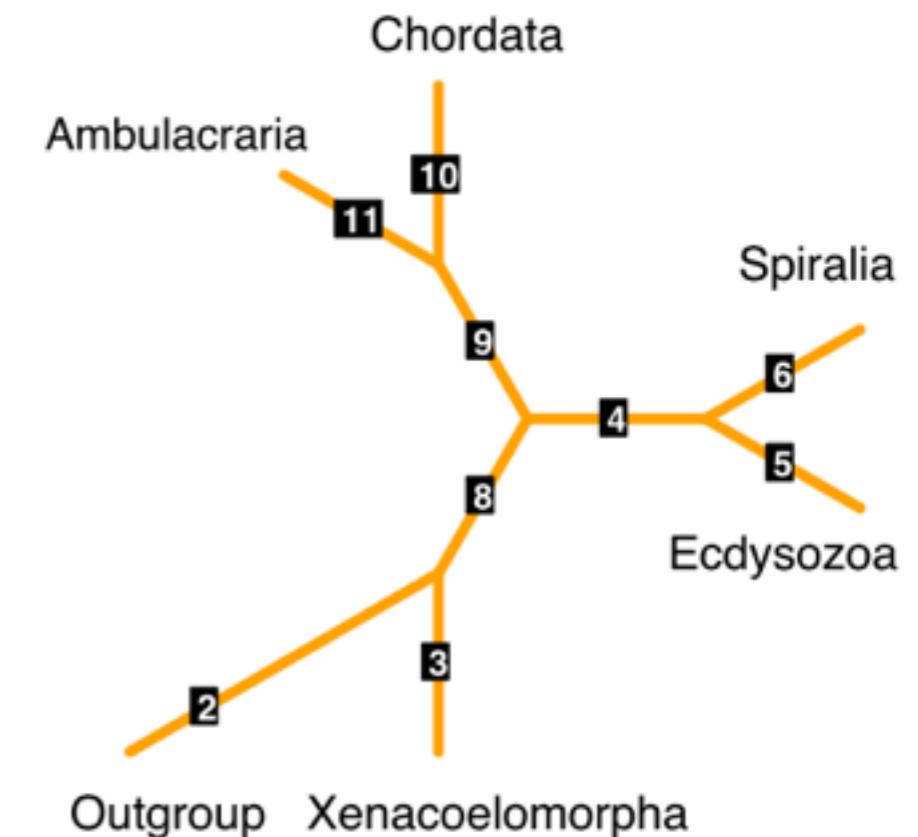
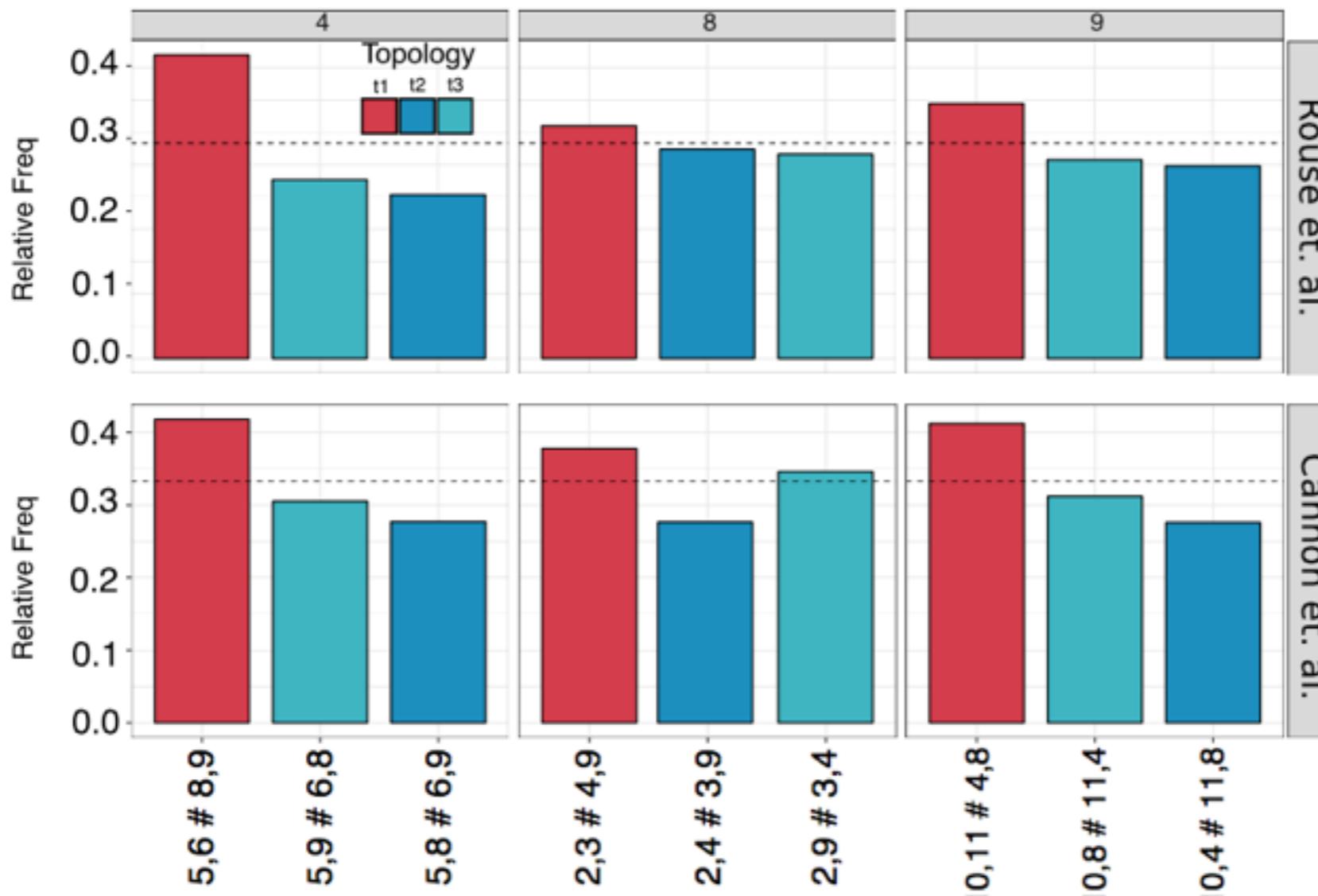
# Quartet-based gene tree discordance

- Input:
  - A species tree
  - A set of gene trees
  - Mapping of species to groups of interest
- Output:
  - Quartet frequency per interesting branches



Dataset (plants): Wicket et al, PNAS, 2014

# Compare different datasets



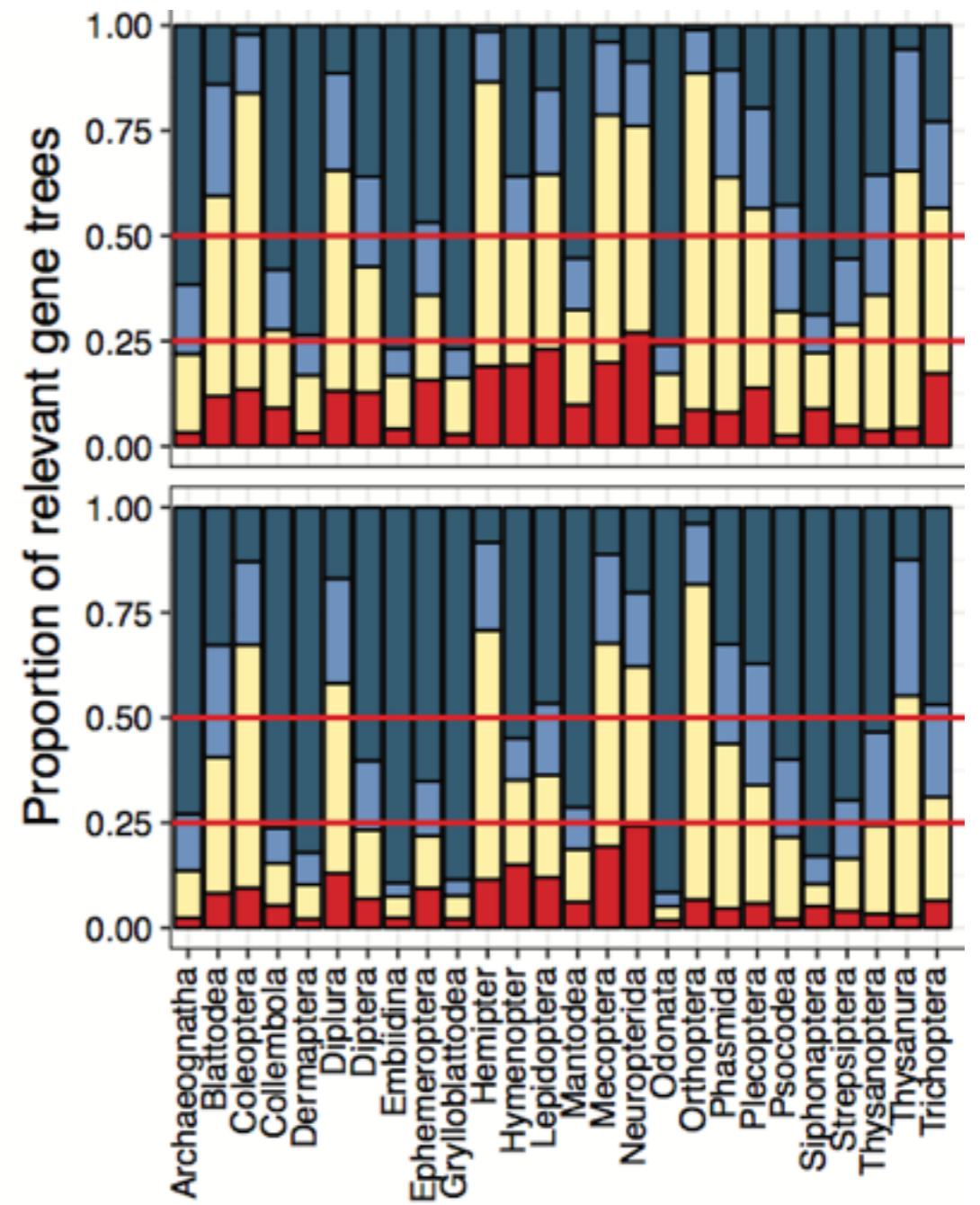
Datasets (metazoa): Rouse et al, Nature, 2016, Cannon et al, Nature, 2016

# More traditional gene tree discordance measures

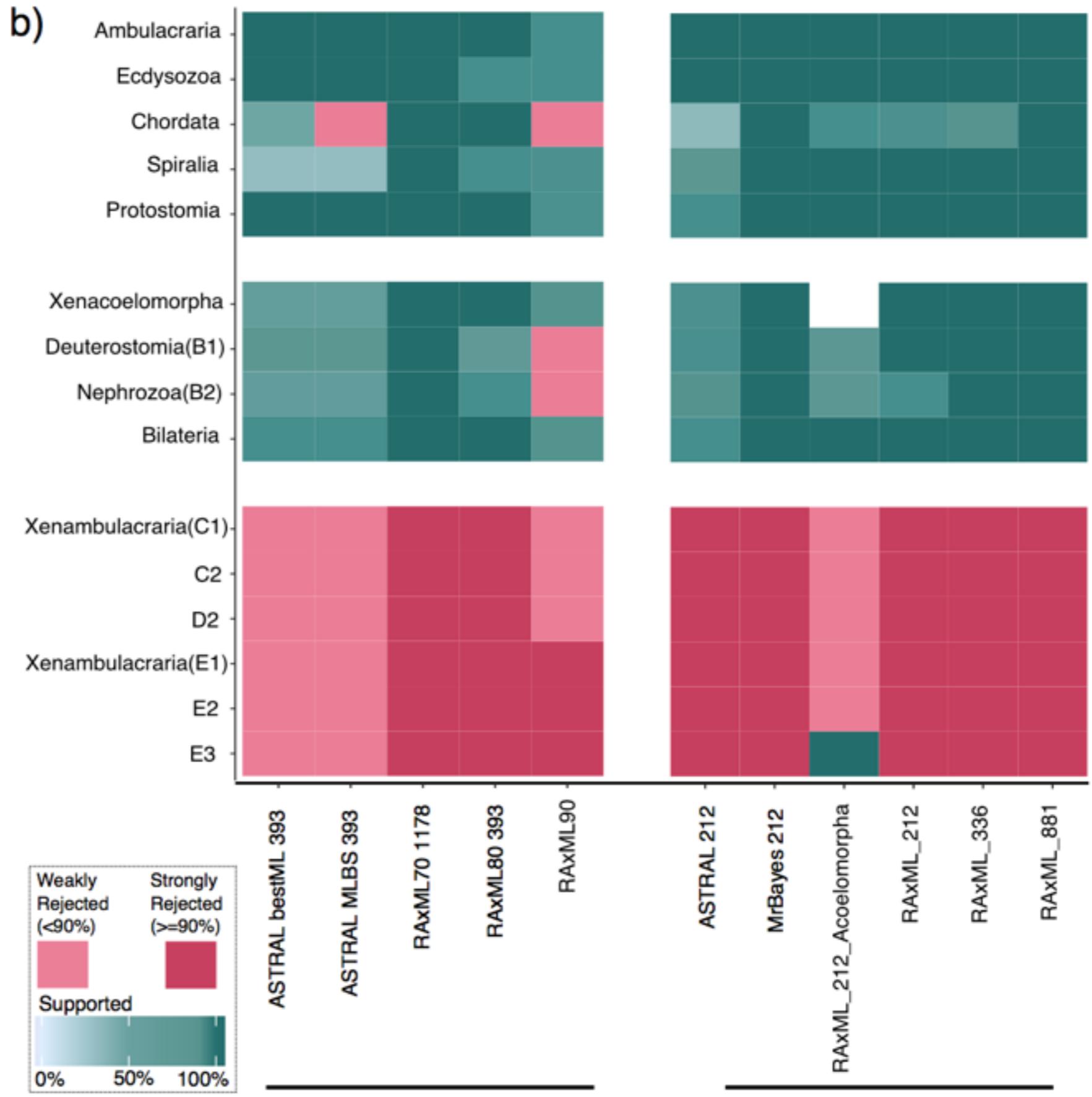
- Input:
  - Gene trees
  - Hypothetical clades
- Output:
  - What percent of gene trees are compatible with those clades

Dataset (insects): Misof et al, Science, 2014

Strongly Supported Weakly Supported Weakly Reject Strongly Reject



# Species tree comparison



# Summarize

- ASTRAL reconstructs species trees from gene trees with both
  - high accuracy
  - scalability to large datasets
  - robust to *some* levels of model violations
- Like any other summary method, ASTRAL remains sensitive to gene tree estimation error
  - try best to obtain highly accurate gene trees
  - removing fragmentary data seems to help
- Visualizing discordance may prove illuminating

# Future of ASTRAL

- Can it be changed to use characters directly?
  - More broadly, can alternative scalable methods be developed for better gene tree estimation?

# Future of ASTRAL

- Can it be changed to use characters directly?
  - More broadly, can alternative scalable methods be developed for better gene tree estimation?
- ASTRAL scales to 10K leaves. We have 90K bacterial genomes.
  - Can we **scale further**?



**Tandy Warnow**



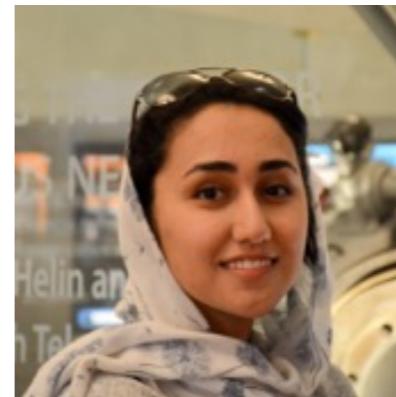
**James B. Whitfield**



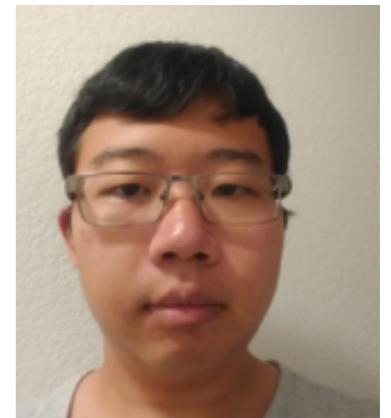
**S.M. Bayzid**



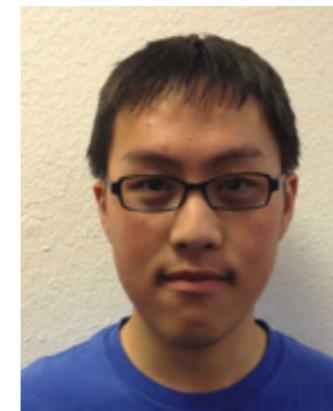
**Théo  
Zimmermann**



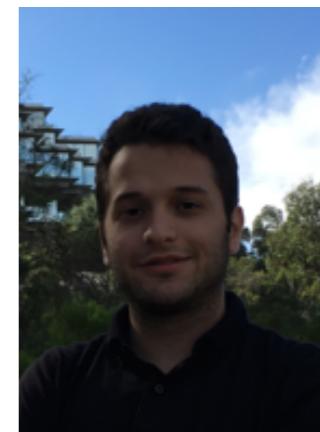
**Maryam Rabiee  
Hashemi**



**Chao Zhang**



**John Yin**



**Erfan Sayyari**



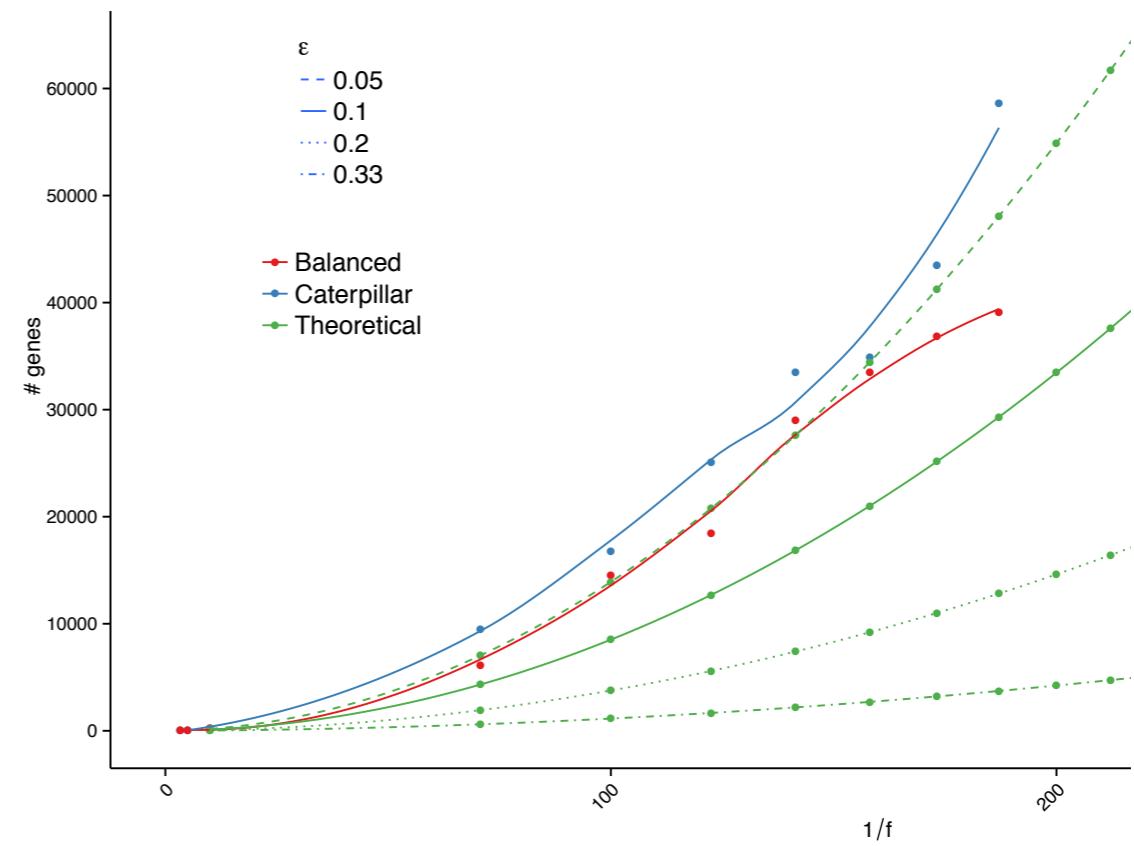
**Shubhanshu  
Shekhar**



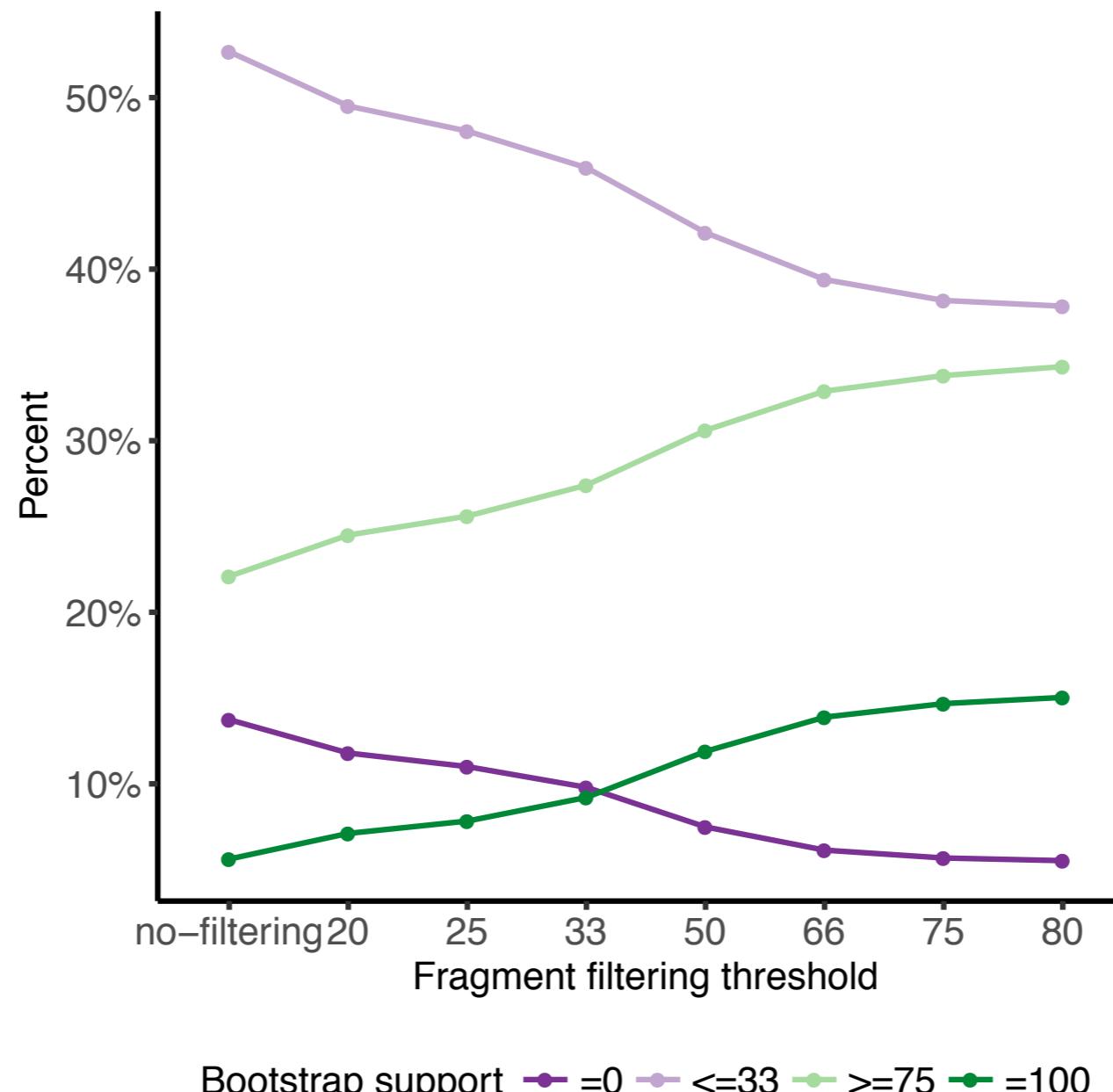
# Theoretical sample complexity results

How many genes are enough to reconstruct the tree?

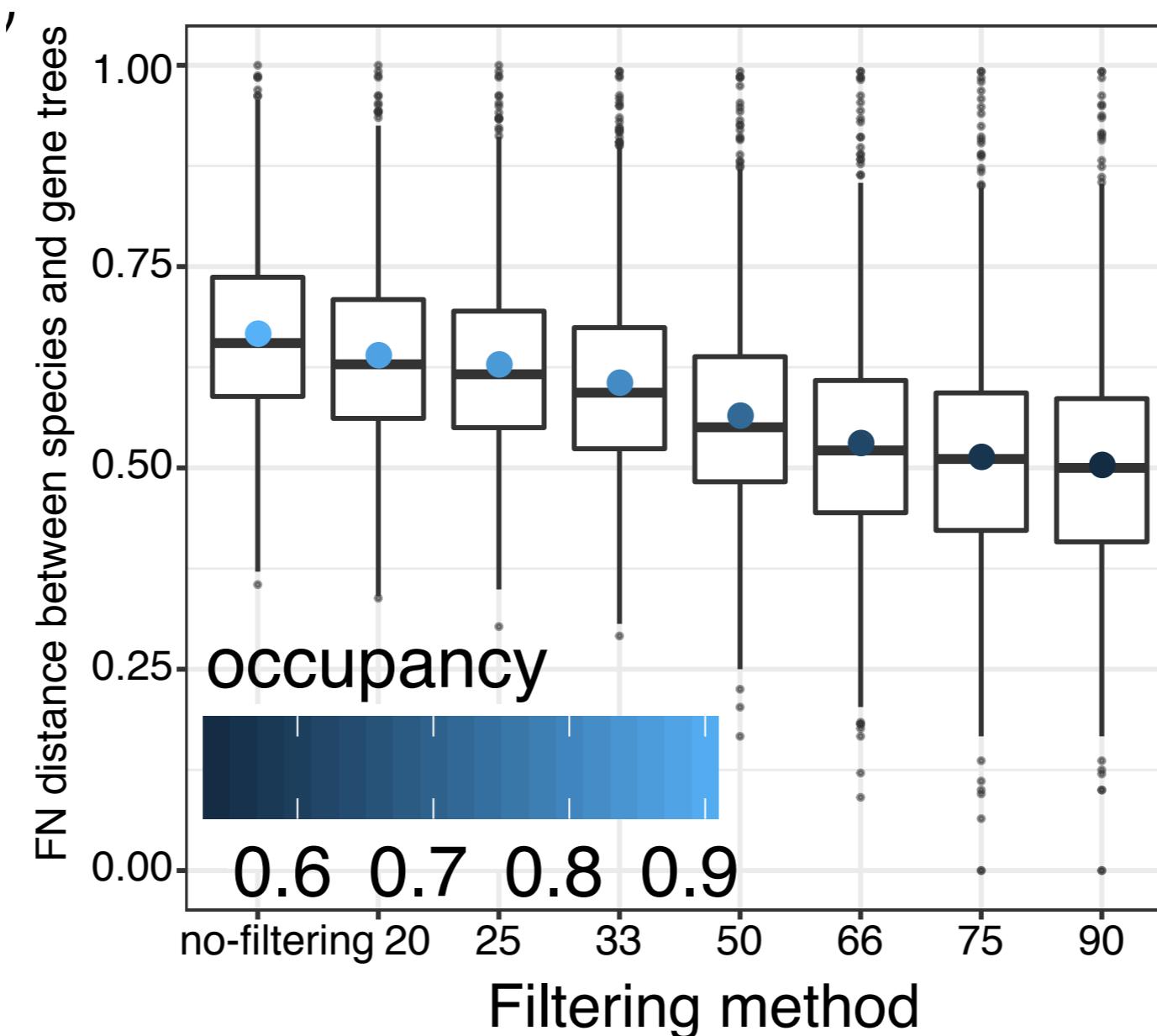
$$m \geq \frac{9}{2} \log \left( \frac{4 \binom{n}{4}}{\epsilon} \right) \frac{c}{\alpha^2 f^2}$$



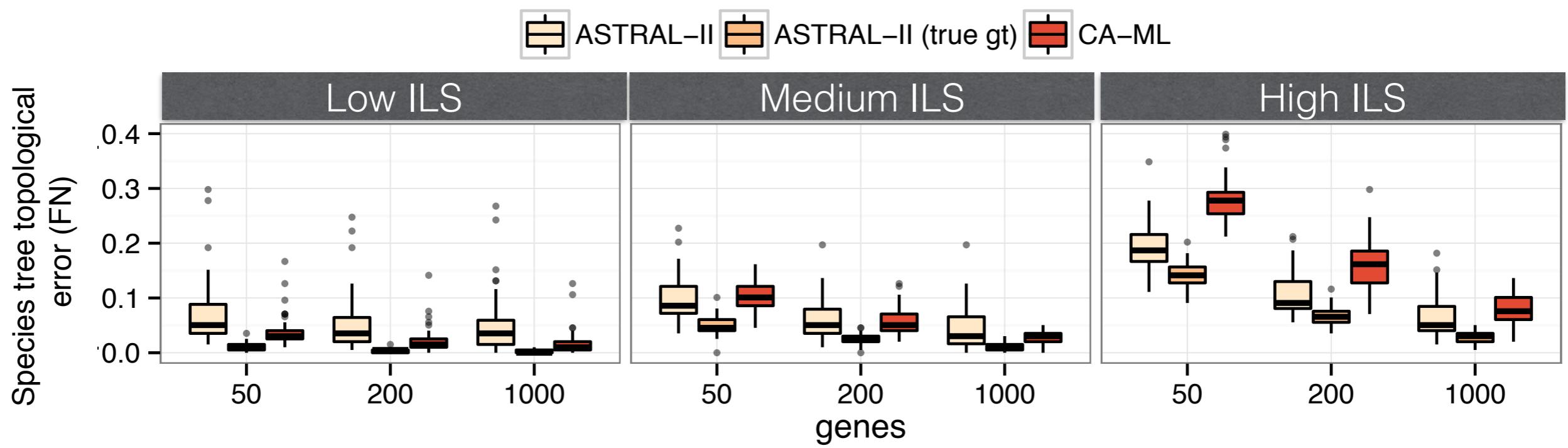
# Gene trees seem to improve (bootstrap)



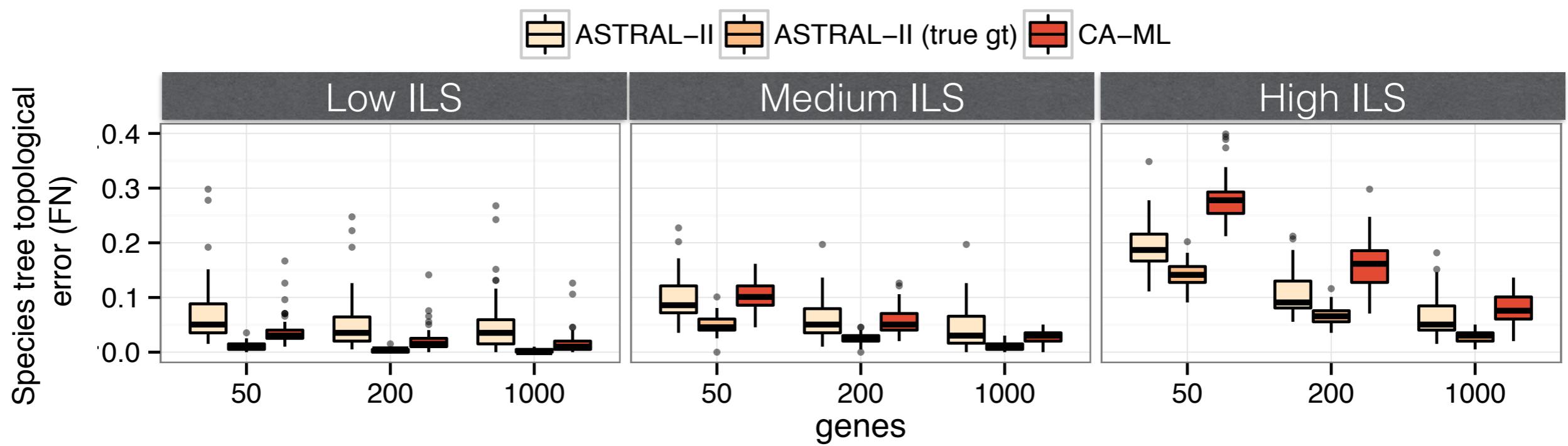
# Gene trees seem to improve (less gene tree discordance)



# Impact of gene tree error (using true gene trees)

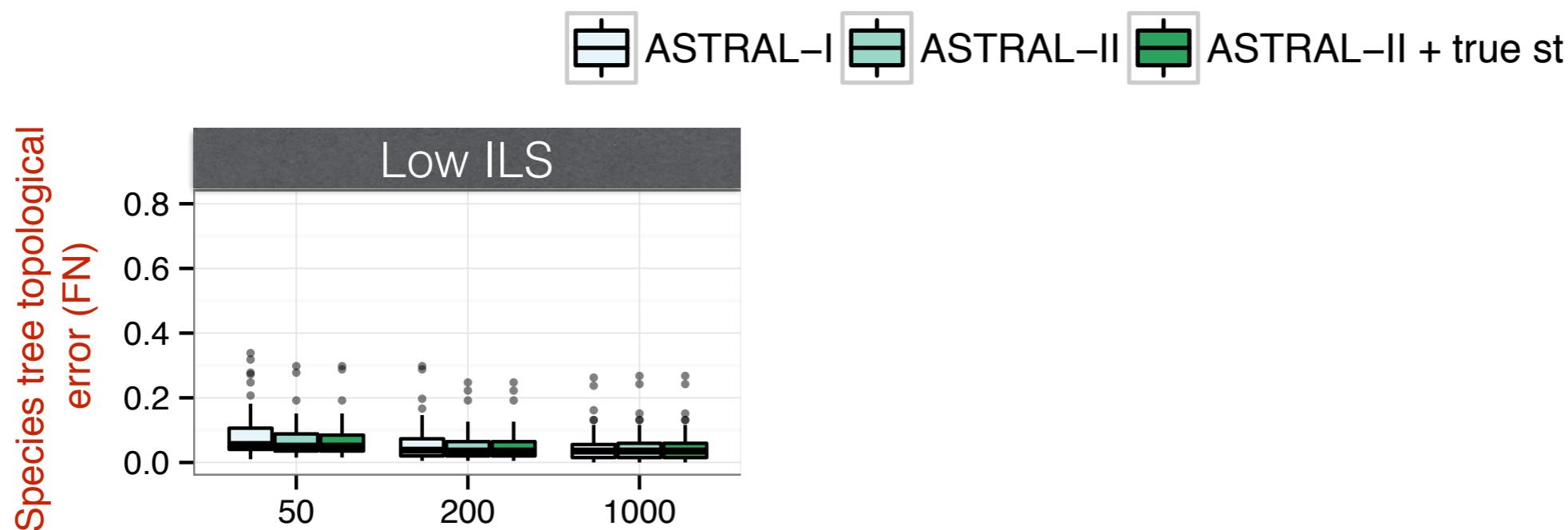


# Impact of gene tree error (using true gene trees)



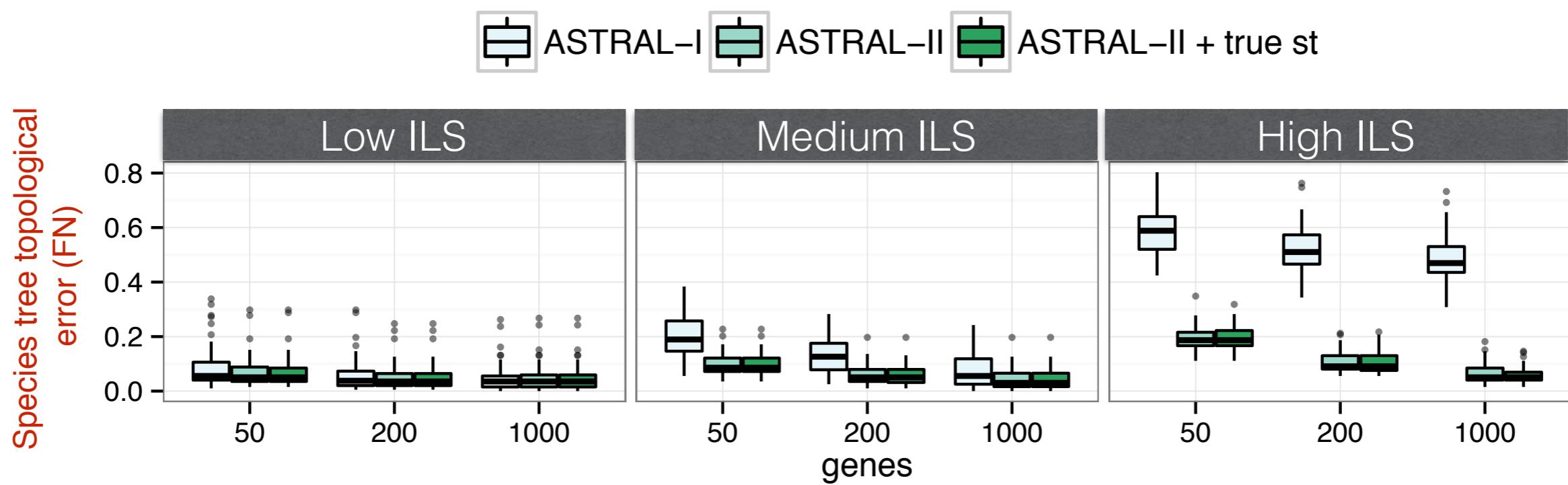
- When we divide our 50 replicates into low, medium, or high gene tree estimation error, ASTRAL tends to be better with low error

# ASTRAL-I versus ASTRAL-II



200 species, deep ILS

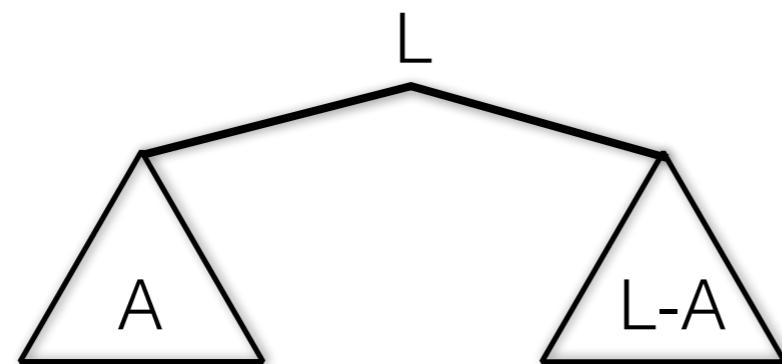
# ASTRAL-I versus ASTRAL-II



200 species, deep ILS

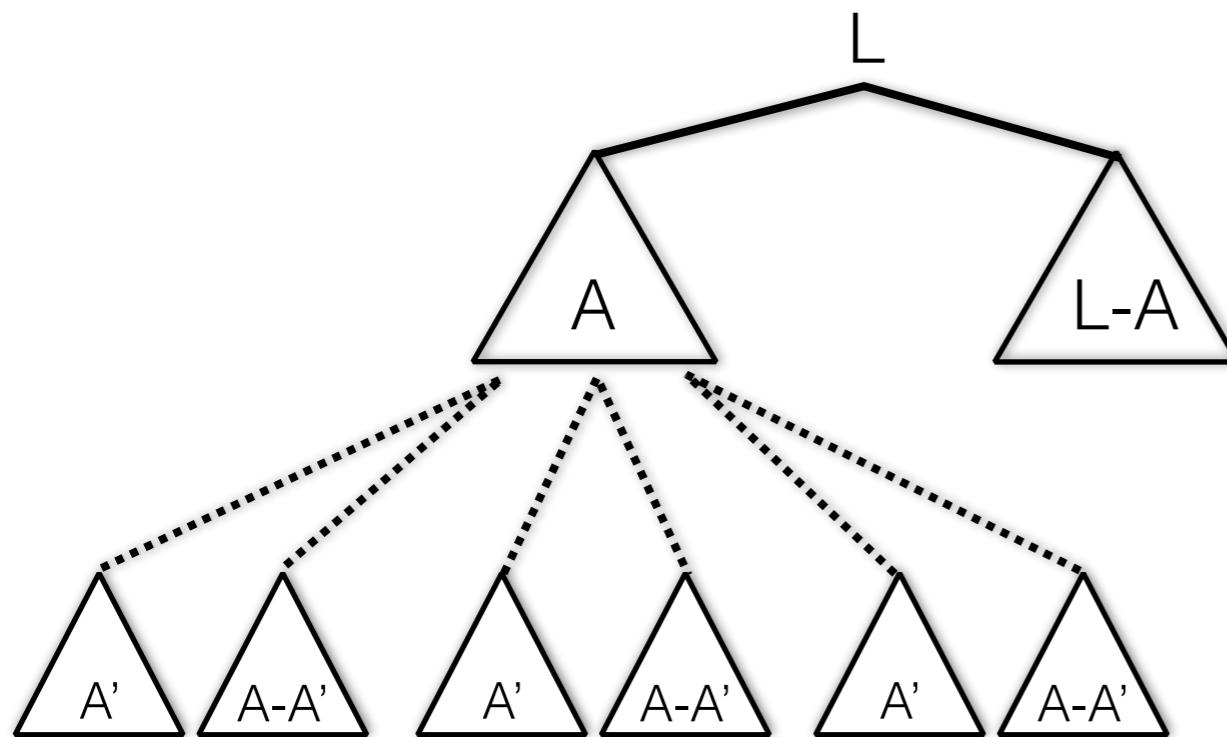
# Dynamic programming

$$S(\mathcal{A}) = \max_{\mathcal{A}' \subset \mathcal{A}} \{ S(\mathcal{A}') + S(\mathcal{A} - \mathcal{A}') + w(\mathcal{A}'|\mathcal{A} - \mathcal{A}'|\mathcal{L} - \mathcal{A}) \}$$



# Dynamic programming

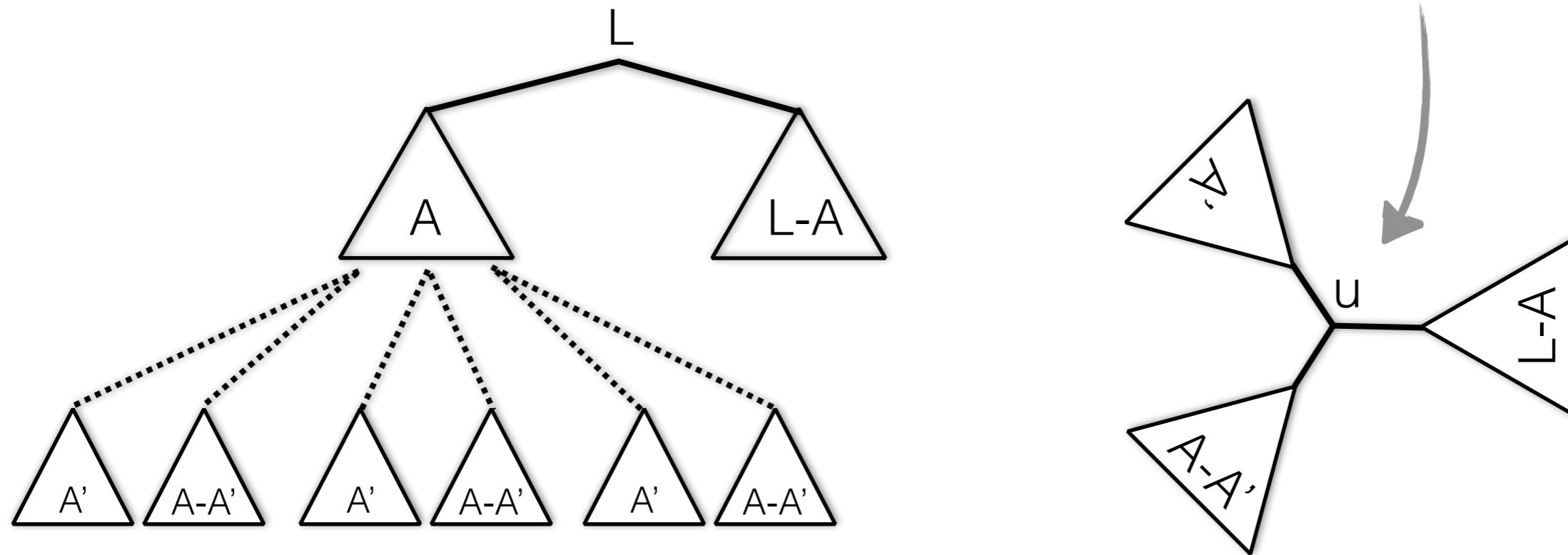
$$S(\mathcal{A}) = \max_{\mathcal{A}' \subset \mathcal{A}} \{ S(\mathcal{A}') + S(\mathcal{A} - \mathcal{A}') + w(\mathcal{A}'|\mathcal{A} - \mathcal{A}'|\mathcal{L} - \mathcal{A}) \}$$



- Recursively break subsets of species into smaller subsets

# Dynamic programming

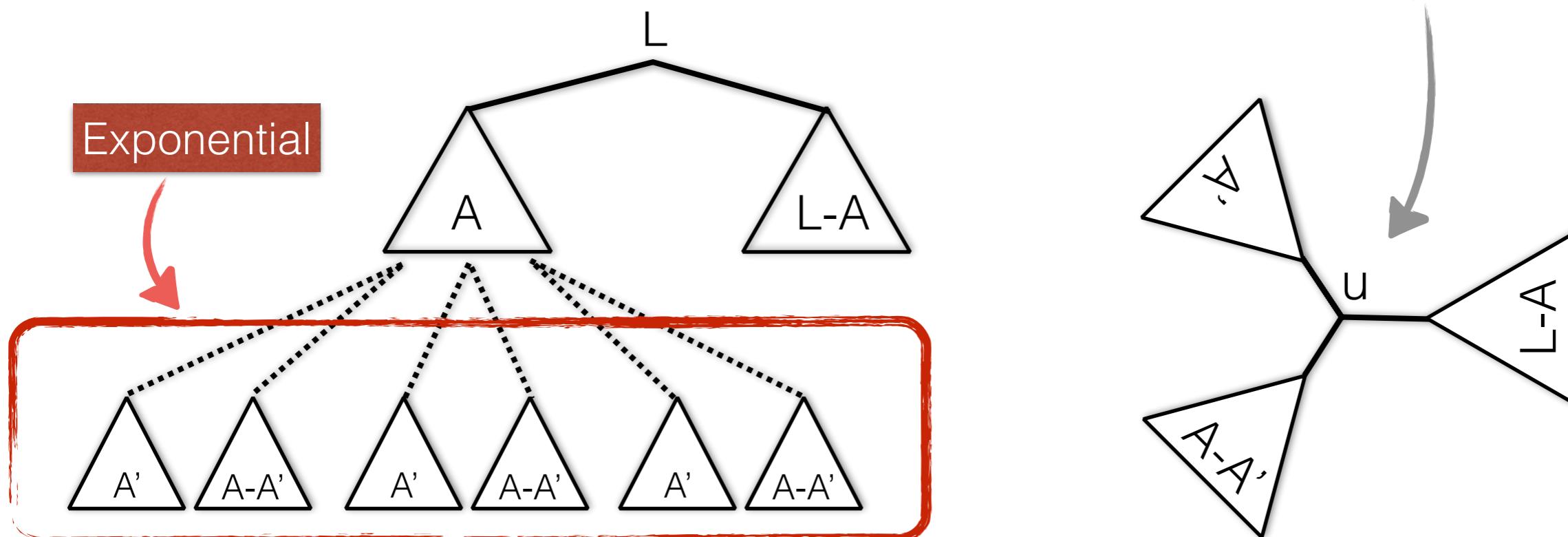
$$S(\mathcal{A}) = \max_{\mathcal{A}' \subset \mathcal{A}} \{S(\mathcal{A}') + S(\mathcal{A} - \mathcal{A}') + w(\mathcal{A}'|\mathcal{A} - \mathcal{A}'|\mathcal{L} - \mathcal{A})\}$$



- Recursively break subsets of species into smaller subsets
- $w(u)$ : Compare  $u$  against input gene trees and compute quartets from gene trees satisfied by  $u$

# Dynamic programming

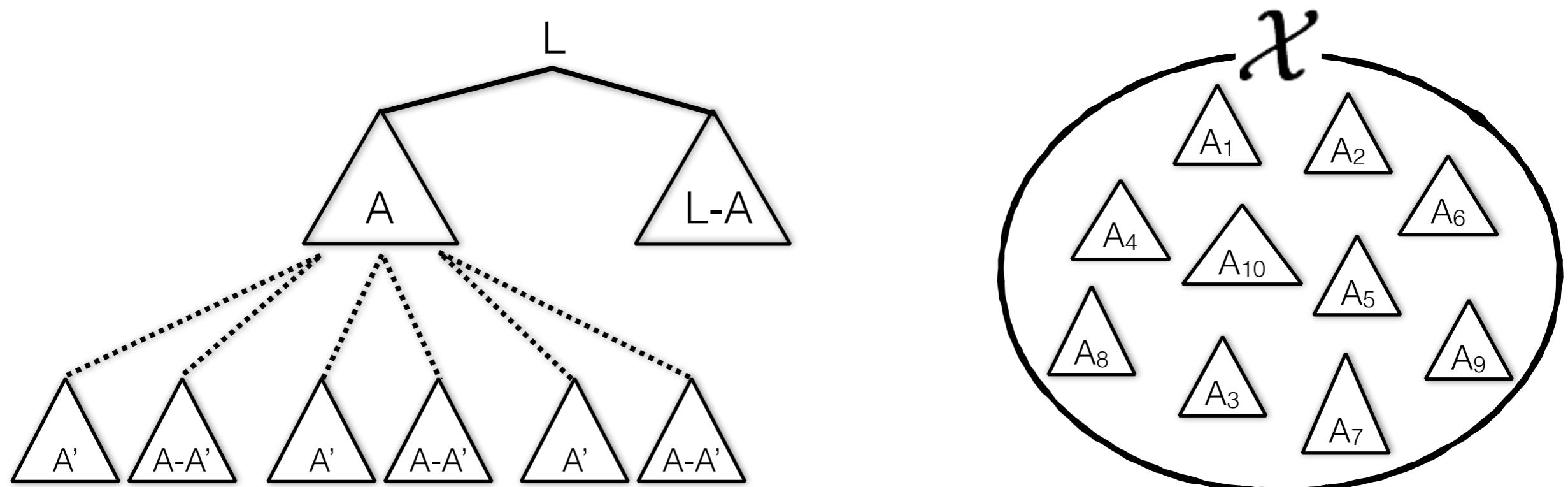
$$S(\mathcal{A}) = \max_{\mathcal{A}' \subset \mathcal{A}} \{ S(\mathcal{A}') + S(\mathcal{A} - \mathcal{A}') + w(\mathcal{A}'|\mathcal{A} - \mathcal{A}'|\mathcal{L} - \mathcal{A}) \}$$



- Recursively break subsets of species into smaller subsets
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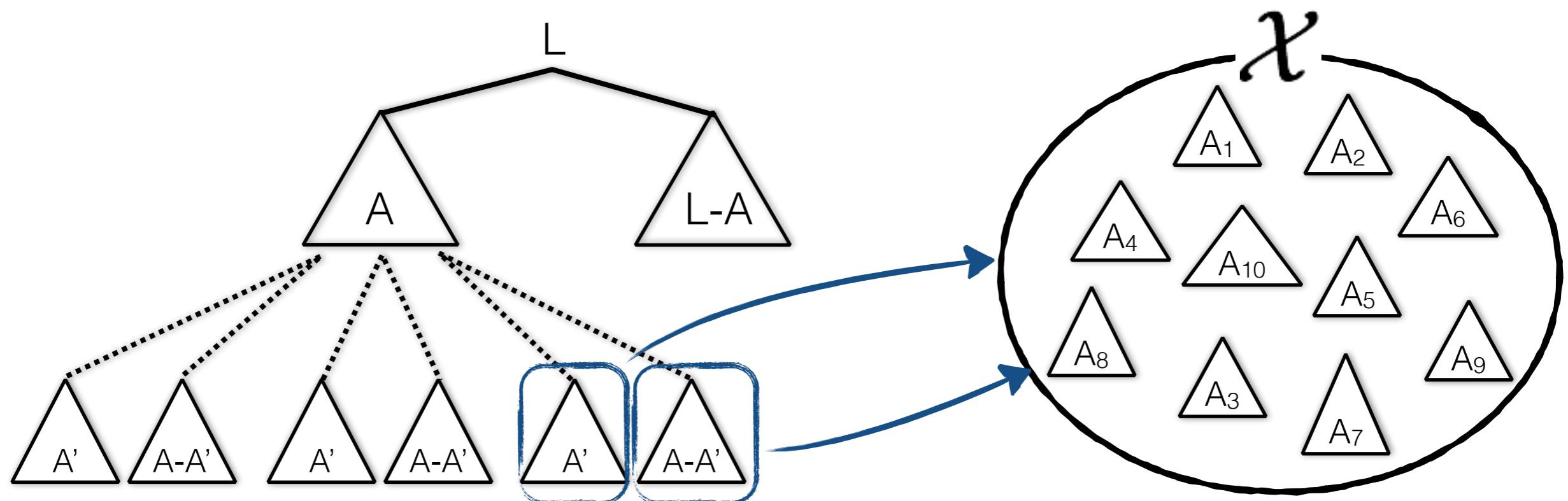
# Constrained version

$$S(\mathcal{A}) = \max_{\{\mathcal{A}', \mathcal{A} - \mathcal{A}'\} \subset \mathcal{X}} \{S(\mathcal{A}') + S(\mathcal{A} - \mathcal{A}') + w(\mathcal{A}'|\mathcal{A} - \mathcal{A}'|\mathcal{L} - \mathcal{A})\}$$



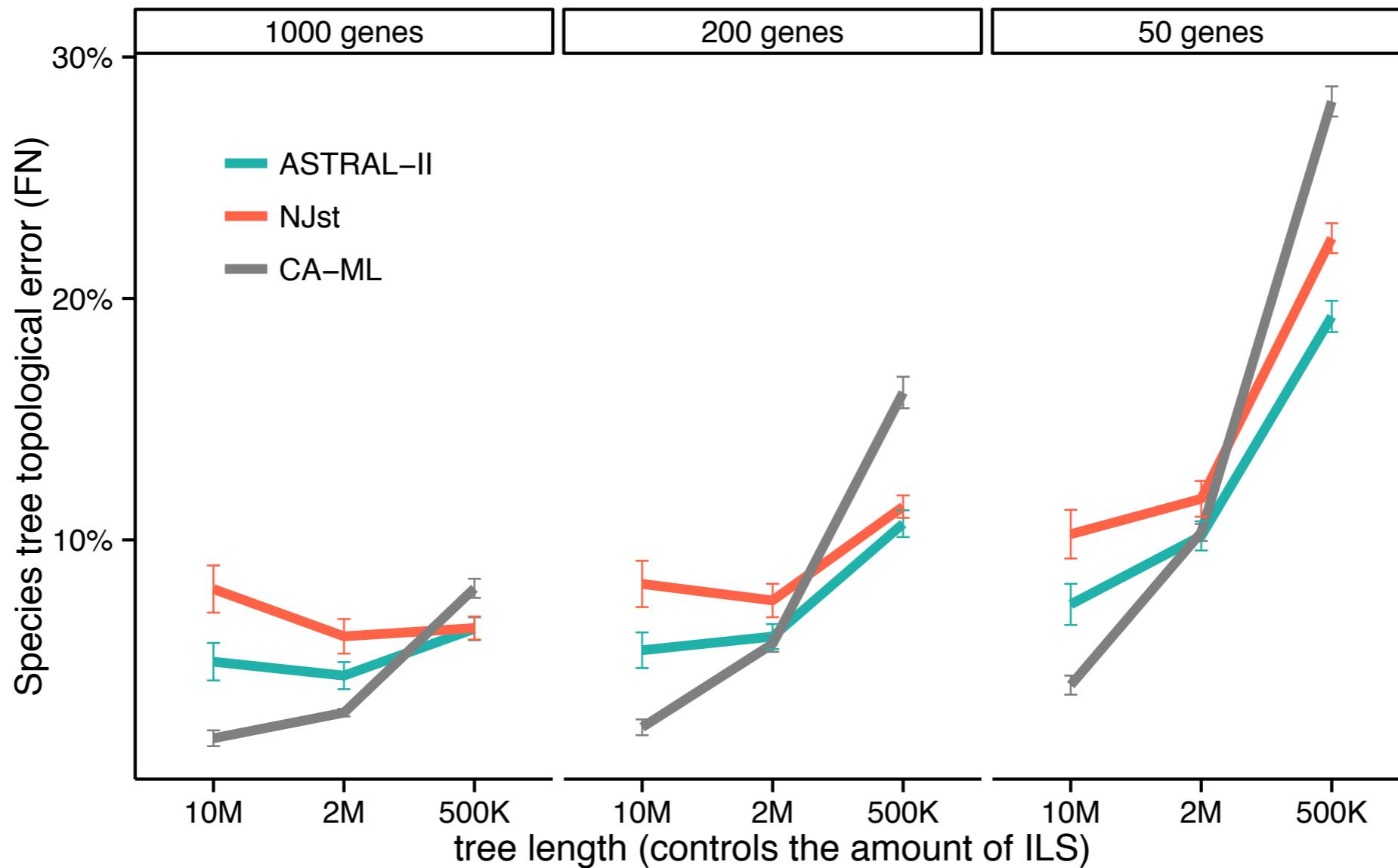
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- Restrict “branches” in the species tree to a given constraint set  $\mathcal{X}$ .

# Deep ILS



200 species, simulated species trees, deep ILS  
[Mirarab and Warnow, ISMB, 2015]