

Species Tree Estimation Lab: SVDQuartets, qAge, and ASTRAL



May 25, 2024

Outline

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2 ASTRAL Method

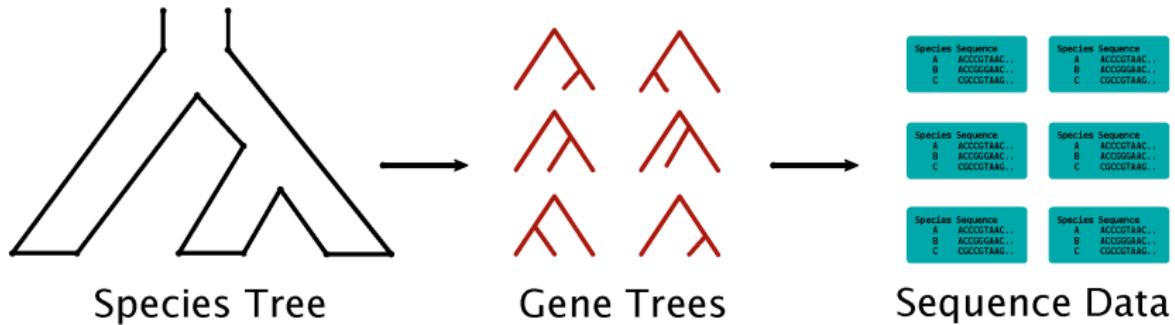
3 SVDQuartets Method

4 qAge

5 Tutorial

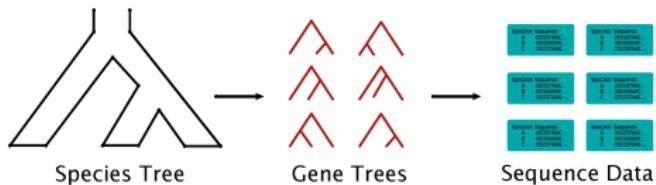
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Species tree inference



ASTRAL background

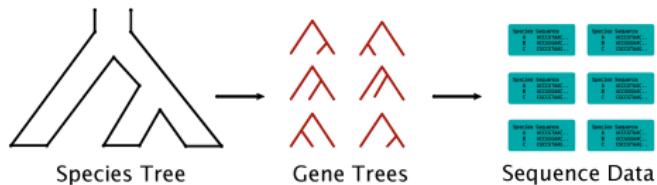
- Recall our ideas about inference under the phylogenetic coalescent model



- ASTRAL** is a summary statistic method for species tree estimation:
 - Step 1. Estimate gene trees for each locus
 - Step 2. Extract all quartet relationships from the estimated gene trees
 - Step 3. Find the species tree that “agrees” with as many quartets as possible

ASTRAL background

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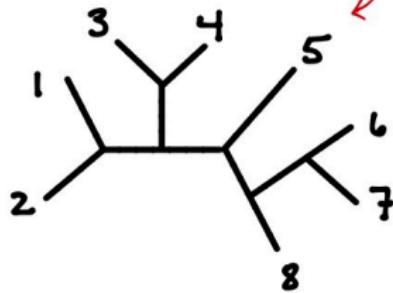
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ASTRAL

- Step 2. Extract all quartet relationships from the estimated gene trees

Example:

Input: {gene tree 1 , gene tree 2} = τ

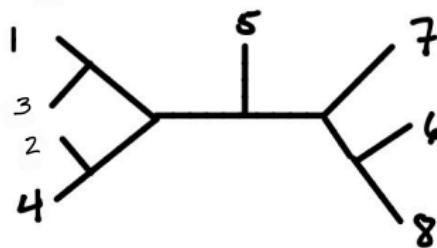


$$q_{11} = 12|34$$

$$q_{12} = 12|58$$

$$q_{13} = 13|58$$

⋮



$$q_{21} = 13|24$$

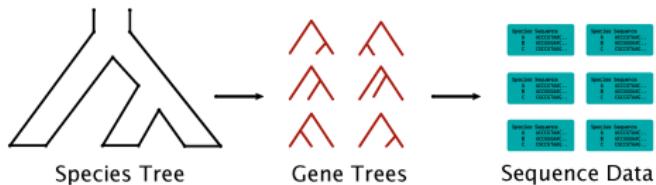
$$q_{22} = 12|58$$

$$q_{23} = 13|58$$

⋮

ASTRAL background

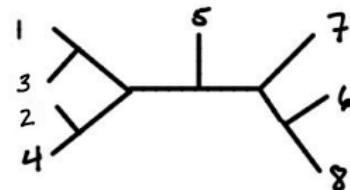
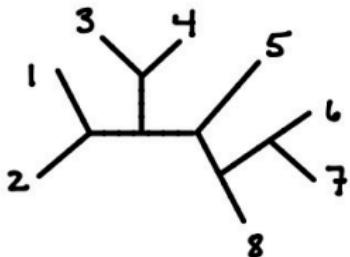
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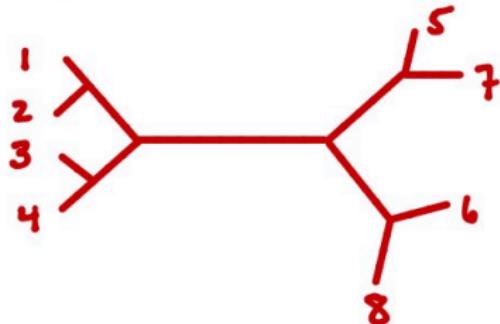
- ASTRAL** is a summary statistic method for species tree estimation:
 - Step 1. Estimate gene trees for each locus ✓
 - Step 2. Extract all quartet relationships from the estimated gene trees ✓
 - Step 3. Find the species tree that “agrees” with as many quartets as possible

- **Step 3.** Find the species tree that “agrees” with as many quartets as possible
 - ▶ This is a non-trivial problem recall that we expect substantial incongruence among trees
 - ▶ However, *unrooted* gene trees cannot be anomalous for four taxa in the absence of gene flow, so *if the gene trees are correct*, then this is easy
 - ▶ ASTRAL uses the **Weighted Quartet Score** of a candidate species tree – defined to be the number of quartets from the set of input gene trees that agree with the candidate species tree
 - ▶ Optimization problem – need to search for the species tree that maximizes the Weighted Quartet Score

- Example:



Consider the species tree $T_1 =$



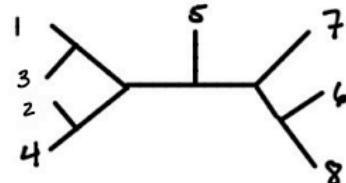
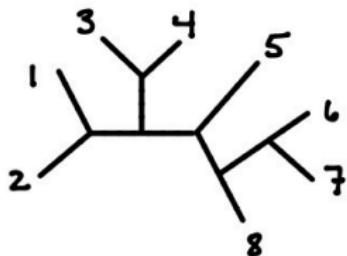
$$\text{Score}(T_1) = \sum_{\text{quartets in tree } T_1, q} w(q, T)$$

$$q_1 = 12|34 \rightarrow w(q_1, T) = 1 \quad (\text{appears in gene tree 1})$$

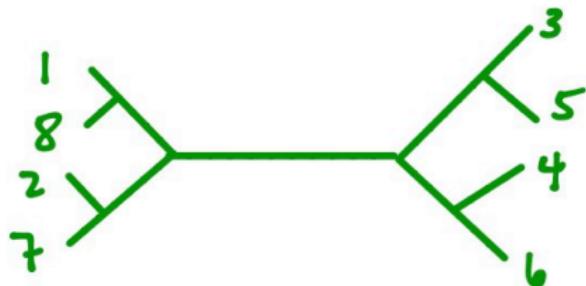
$$q_2 = 12|58 \rightarrow w(q_2, T) = 2 \quad (\text{appears in both input gene trees})$$

+ all quartets in T_1

- Example:



Consider the species tree $T_2 =$



$$\text{Score}(T_2) = \sum_{\substack{\text{quartets in} \\ \text{true } T_2, q}} w(q, \tau)$$

$q_1 = 18|27 \rightarrow w(q_1, \tau) = 0$ (doesn't appear in either input gene tree)

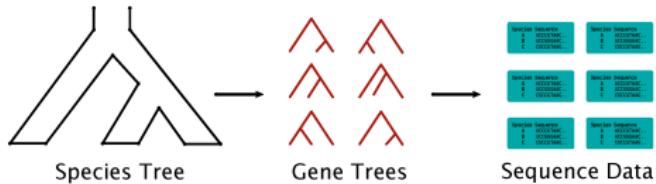
$q_2 = 12|34 \rightarrow w(q_2, \tau) = 1$ (appears in gene tree 1)

$q_3 = 18|23 \rightarrow w(q_3, \tau) = 0$ (doesn't appear in either input gene tree)

+ all quartets in T_2

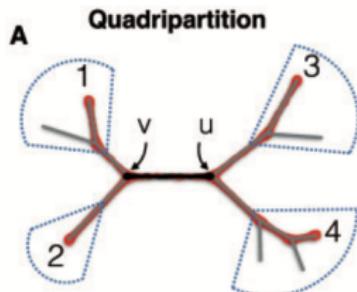
ASTRAL background

- Recall our ideas about inference under the phylogenetic coalescent model



- ASTRAL** is a summary statistic method for species tree estimation:
 - Step 1. Estimate gene trees for each locus ✓
 - Step 2. Extract all quartet relationships from the estimated gene trees ✓
 - Step 3. Find the species tree that “agrees” with as many quartets as possible ✓

- ASTRAL can also estimate branch lengths (in coalescent units)
- ASTRAL also provides a measure of uncertainty: *local posterior probability*



Sayyari and Mirarab, 2016

- ▶ Assume that the “clusters” on each edge of the branch under consideration are correct
- ▶ Use the gene trees to obtain quartet frequencies for the three possible arrangements of clusters
- ▶ Assume a prior distribution on the quartet trees (Yule prior with parameter λ)
- ▶ Compute the posterior probability that this branch appears in the true species tree, given the observed quartet frequencies

ASTRAL performance

- ASTRAL is **statistically consistent** *when the gene trees are known without error*
- ASTRAL will perform well when the gene trees can be estimated well
- **Computational efficiency:** the estimation of gene trees is the time-consuming step, but can be parallelized
- **Crucial assumption:** true unrooted quartets have higher probability than other quartet relationships
- **Assessment of uncertainty:** use the local posterior probability (now recommended over the bootstrap)

SVDQuartets (or just SVDQ)

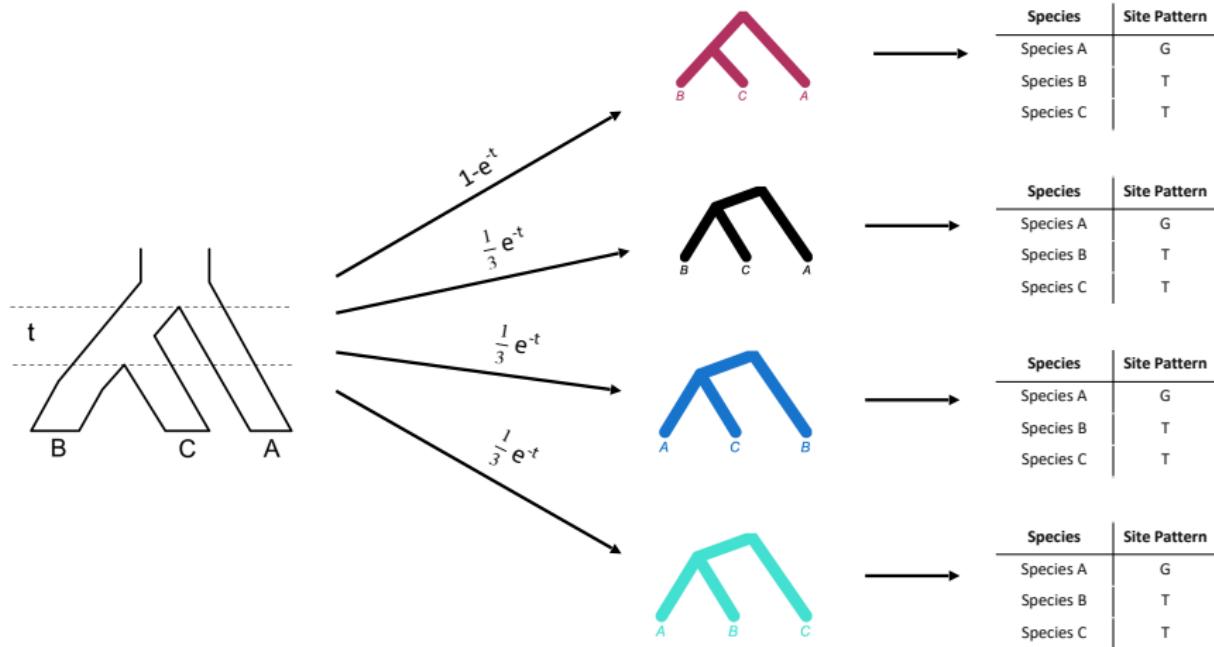
Goal of this work:

Develop a full data approach that is computationally feasible for large-scale data

How?

- Summarize data differently, so that model requires less computation
- Develop theory to infer relationships among quartets of taxa very accurately
- Use a quartet assembly method to build a large tree

Recall the phylogenetic coalescent model with mutation



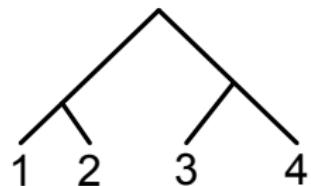
But there are a lot of histories!

TABLE 3. The number of valid coalescent histories when the gene tree and species tree have the same topology. The number of histories is also the number of terms in the outer sum in equation (12).

Taxa	Number of histories		
	Asymmetric trees	Symmetric trees	Number of topologies
4	5	4	15
5	14	10	105
6	42	25	945
7	132	65	10,395
8	429	169	135,135
9	1430	481	2,027,025
10	4862	1369	34,459,425
12	58,786	11,236	13,749,310,575
16	9,694,845	1,020,100	6.190×10^{15}
20	1,767,263,190	100,360,324	8.201×10^{21}

- This means that **calculating the likelihood** – and thus using likelihood-based methods for inference – will be difficult, especially for large-scale data
- **Alternative approach:** compute explicitly (i.e., write formulas for) the site pattern probabilities for 4-taxon trees, and look for “structure”

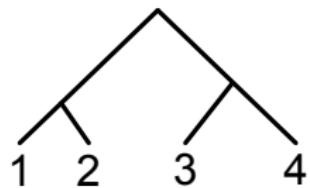
Looking for structure in site pattern probabilities



Taxon	Sequence
1	ACCAATGCCGATGCCAAA
2	ACCATTGCCGATGCCATA
3	ACGAAAGCGGAAGCGAAA
4	ATGAAAGCGGAAGCCAAA

$$Flat_{12|34}(P) = \begin{pmatrix} & [AA] & [AC] & [AG] & [AT] & [CA] & \dots \\ [AA] & p_{AAAA} & p_{AAAC} & p_{AAAG} & p_{AAAT} & p_{AAC} & \dots \\ [AC] & p_{ACAA} & p_{ACAC} & p_{ACAG} & p_{ACAT} & p_{ACCA} & \dots \\ [AG] & p_{AGAA} & p_{AGAC} & p_{AGAG} & p_{AGAT} & p_{AGCA} & \dots \\ [AT] & p_{ATAA} & p_{ATAC} & p_{ATAG} & p_{ATAT} & p_{ATCA} & \dots \\ [CA] & p_{CAAA} & p_{CAAC} & p_{CAAG} & p_{CAAT} & p_{CAC} & \dots \\ [\dots] & \dots & \dots & \dots & \dots & \dots & \dots \end{pmatrix}$$

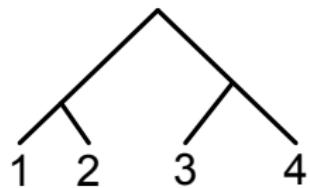
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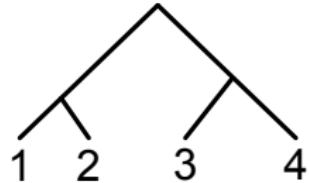
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Taxon	Sequence
1	ACCAATGCCGGAGCCCAA
2	ACCATTGACGGAGCCAATA
3	ACGAAAGACGGAAGCAAAA
4	ATGAAAGTCGGAAGCTAAA

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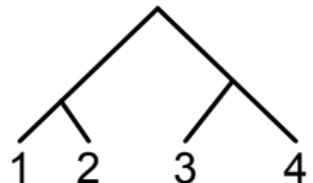
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$$Flat_{12|34}(P) = \begin{pmatrix} & [AA] & [AC] & [AG] & [AT] & [CA] & \dots \\ [AA] & 5 & PAAAC & PAAAG & PAAAT & PAACA & \dots \\ [AC] & PACAA & PACAC & PACAG & PACAT & PACCA & \dots \\ [AG] & PAGAA & PAGAC & PAGAG & PAGAT & PAGCA & \dots \\ [AT] & PATAA & PATAC & PATAG & PATAT & PATCA & \dots \\ [CA] & PCAAA & PCAAC & PCAAG & 2 & PCACA & \dots \\ [\dots] & \dots & \dots & \dots & \dots & \dots & \dots \end{pmatrix}$$

Looking for structure in site pattern probabilities



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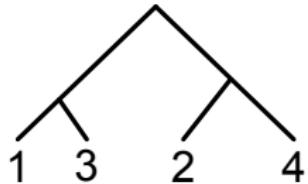
$$Flat_{12|34}(P) = \begin{pmatrix} & [AA] & [AC] & [AG] & [AT] & [CA] & \dots \\ [AA] & 5 & PAAAC & PAAAG & PAAAT & PAACA & \dots \\ [AC] & PACAA & PACAC & PACAG & PACAT & PACCA & \dots \\ [AG] & PAGAA & PAGAC & PAGAG & PAGAT & PAGCA & \dots \\ [AT] & PATAA & PATAc & PATAG & PATAT & PATCA & \dots \\ [CA] & PCAAA & PCAAC & PCAAG & 2 & PCACA & \dots \\ [\dots] & \dots & \dots & \dots & \dots & \dots & \dots \end{pmatrix}$$

These two columns are identical – matrix rank is reduced by one

Main Result:

- **Species tree inference:** For a flattening matrix constructed on the true four-taxon tree, **the matrix rank is 10** under the following model
 - ▶ species tree → gene tree :::: coalescent process
 - ▶ gene tree → data :::: nucleotide substitution models: GTR+I+Γ and submodels
- **This result still holds** when the species tree violates the molecular clock and/or when there is variation in effective population size across the branches and/or when there is gene flow between sister taxa

What about the incorrect tree?



Taxon	Sequence
1	ACCAATGCCGGAGCCCAA
2	ACCATTGACGGAGCCAATA
3	ACGAAAGACGGAAGCAAAA
4	ATGAAAGTCGGAAGCTAAA

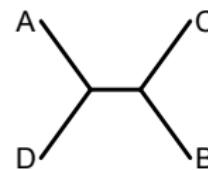
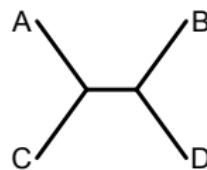
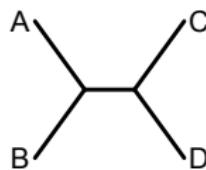
$$\text{Flat}_{12|34}(\mathbf{P}) = \begin{pmatrix} & [AA] & [AC] & [AG] & [AT] & [CA] & \dots \\ [AA] & 5 & \mathbf{PAAAC} & p_{AAAG} & p_{AAAT} & \mathbf{PAACA} & \dots \\ [AC] & p_{ACAA} & \mathbf{PACAC} & p_{ACAG} & p_{ACAT} & \mathbf{PACCA} & \dots \\ [AG] & p_{AGAA} & \mathbf{PAGAC} & p_{AGAG} & p_{AGAT} & \mathbf{PAGCA} & \dots \\ [AT] & p_{ATAA} & \mathbf{PATAC} & p_{ATAG} & p_{ATAT} & \mathbf{PATCA} & \dots \\ [CA] & p_{CAAA} & \mathbf{PCAAC} & p_{CAAG} & 2 & \mathbf{PCACA} & \dots \\ [\dots] & \dots & \dots & \dots & \dots & \dots & \dots \end{pmatrix}$$

These two columns are no longer identical – full rank matrix in both cases (rank = 16)

How can we use these facts to estimate the species tree?

- **Basic idea:**

- ▶ **Data:** aligned DNA sequences for **multiple loci** or for a collection of **SNPs**
- ▶ Estimate the **flattening matrix** for each of the following trees:

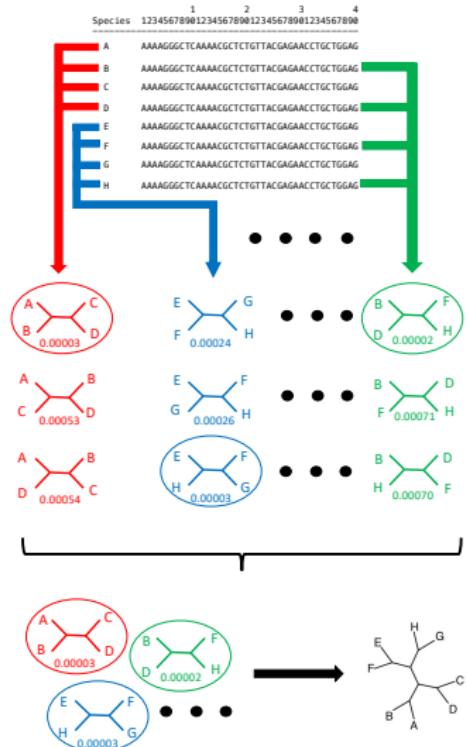


- ▶ Compute a measure of how close each of the three observed flattening matrices is to a matrix with rank 10 – we use the **SVDScore**
- ▶ Pick the tree relationship that gives the **smallest SVDScore**

How do we assess variability?

- How can we measure confidence in the inferred split?
- Use a **nonparametric bootstrap** procedure
 - ▶ Generate bootstrap data sets from the original data matrix
 - ▶ Compute split scores on all three splits for each bootstrap data matrix
 - ▶ Record the number of bootstrap data sets for which each split is inferred, and use the proportion of these as a bootstrap support measure

Extension to larger trees



Algorithm

- 1 Generate all quartets (small problems) or sample quartets (large problems)
- 2 Estimate the correct quartet relationship for each sampled quartet
- 3 Use a quartet assembly method to build the tree - PAUP* uses the method of Reaz-Bayzid-Rahman (2014), called QFM, to build the tree.

- Multiple lineages are handled as follows:
 - ① Sample four species
 - ② Select one lineage at random from each species
 - ③ Estimate the quartet relationships among the four sampled lineages
 - ④ Restore the species labels (but lineage quartets are saved, too)
- Quantify uncertainty using the bootstrap

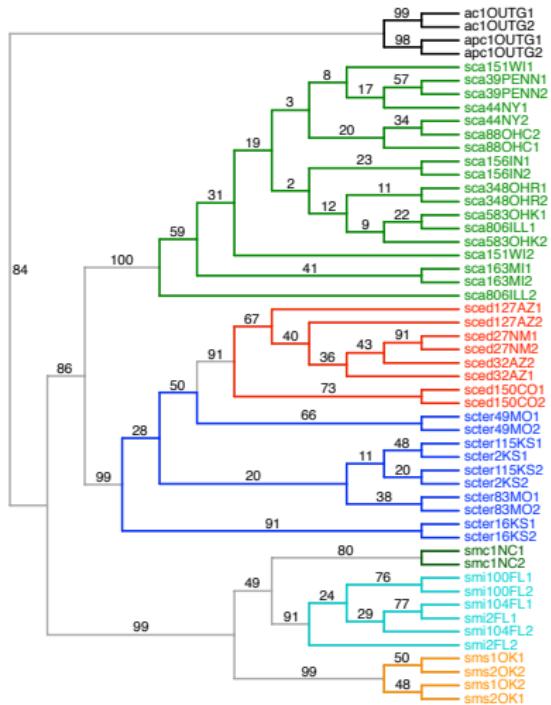


- Data: 7 (sub)species, 26 individuals (52 sequences), 19 genes

Species	Location	No. of individuals per gene
<i>S. catenatus catenatus</i>	Eastern U.S. and Canada	9
<i>S. c. edwardsii</i>	Western U.S.	4
<i>S. c. tergeminus</i>	Western and Central U.S.	5
<i>S. miliaris miliaris</i>	Southeastern U.S.	1
<i>S. m. barbouri</i>	Southeastern U.S.	3
<i>S. m. streckerii</i>	Southeastern U.S.	2
Agkistrodon sp. (outgroup)	U.S.	2

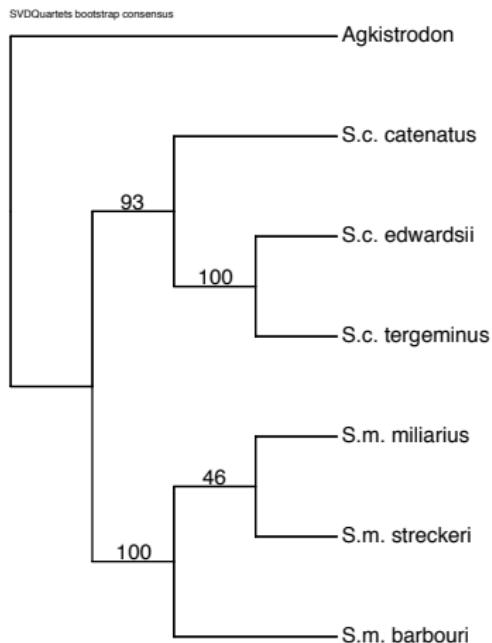
Empirical example: *Sistrurus* rattlesnakes

All quartets and 100 bootstrap replicates



Empirical example: *Sistrurus* rattlesnakes

All quartets and 100 bootstrap replicates



- SVDQuartets

- ▶ Statistically consistent for estimating the quartet trees
- ▶ Will perform well when there are a lot of data (multilocus or SNP) available
- ▶ More complex model \Rightarrow more data needed
- ▶ Valid when the molecular clock is violated
- ▶ Valid when there is gene flow between sister taxa
- ▶ Computationally efficient, including bootstrapping
- ▶ A related method (qAge) can estimate branch lengths

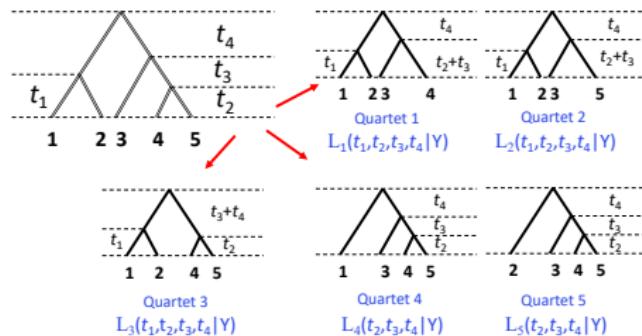
- ASTRAL

- ▶ Statistically consistent when gene trees are known without error
- ▶ Will perform well when gene trees can be estimated well
- ▶ Gene flow can cause the method to fail (because then quartets can be anomalous)
- ▶ Computationally efficient after individual gene trees have been estimated
- ▶ Can provide estimates of branch lengths
- ▶ Local posterior probabilities used to quantify uncertainty in the data

- How do these compare to Bayesian methods, such as STARBEAST2 and BPP?
 - ▶ STARBEAST2 and BPP carry out **estimation** under the model, including all model components
 - ▶ Estimation of the posterior distribution provides a natural way to quantify uncertainty
 - ▶ ASTRAL and SVDQ use features of the model to assess **fit of the data** to the model
 - ★ ASTRAL: gene trees
 - ★ SVDQ: site pattern probabilities
 - ▶ Trade-offs involved in choosing among methods: computational efficiency, robustness to the model, etc.

Estimating branch lengths with qAge

- Use a **composite likelihood** approach to estimate branch lengths along a fixed species tree, for either multilocus or SNP data
- Form the composite likelihood by taking the product of the species tree likelihoods for each quartet
- Use prior distributions on the node age and effective population size
- Find the parameters values that optimize the composite likelihood – supported by theory



Now on to the tutorial!

References

• ASTRAL

- ▶ Zhang, Chao, Maryam Rabiee, Erfan Sayyari, and Siavash Mirarab. 2018. "ASTRAL-III: Polynomial Time Species Tree Reconstruction from Partially Resolved Gene Trees." *BMC Bioinformatics* 19 (S6): 153. doi:10.1186/s12859-018-2129-y.
- ▶ Sayyari, Erfan, and Siavash Mirarab. 2016. "Fast Coalescent-Based Computation of Local Branch Support from Quartet Frequencies." *Molecular Biology and Evolution* 33 (7): 1654-68. doi:10.1093/molbev/msw079.
- ▶ Mirarab, Siavash, Rezwana Reaz, Md. Shamsuzzoha Bayzid, Theo Zimmermann, M. S. Swenson, and Tandy Warnow. 2014. "ASTRAL: Genome-Scale Coalescent-Based Species Tree Estimation." *Bioinformatics* 30 (17): i541-48. doi:10.1093/bioinformatics/btu462.
- ▶ Mirarab, Siavash, and Tandy Warnow. 2015. "ASTRAL-II: Coalescent-Based Species Tree Estimation with Many Hundreds of Taxa and Thousands of Genes." *Bioinformatics* 31 (12): i44-52. doi:10.1093/bioinformatics/btv234.

References

- SVDQuartets/qAge

- ▶ Chifman, J. and L. Kubatko. 2014. Quartet inference from SNP data under the coalescent model, *Bioinformatics* 30(23): 3317-3324.
- ▶ Chifman, J. and L. Kubatko. 2015. Identifiability of the unrooted species tree topology under the coalescent model with time-reversible substitution processes, site-specific rate variation, and invariable sites, *Journal of Theoretical Biology* 374: 35-47.
- ▶ Swofford, D. L. and L. S. Kubatko. Species tree estimation using site pattern frequencies, Chapter 4, pgs. 68-88 in *Species Tree Inference: A Guide to Methods and Applications*, edited by L. Kubatko and L. Knowles, 2023.
- ▶ Peng, J., D. Swofford, and L. Kubatko. 2022. Estimation of speciation times under the multispecies coalescent, *Bioinformatics* 38(23): 5182-5190
- ▶ Swofford, D. PAUP* (* Phylogenetic Analysis Using PAUP), Version 4.0a165, available at <https://paup.phylosolutions.com>.