Reference genome choice and filtering pipeline jointly impact phylogenetic analyses

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BACKGROUND

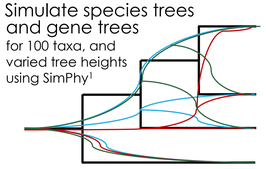
Although genomic datasets are increasingly used phylogenetics, we still have incomplete understanding of how decisions about the bioinformatic treatment of next-generation sequence data impact downstream phylogenetic results.

Analysis of genomic data requires researchers to make *a priori* decisions on **which data to retain and which to filter out**, which can introduce biases into the data used in analyses. Several recent studies have elucidated biases introduced by this data processing step, **which can substantially affect subsequent analyses** in both population genetics and phylogenetics.

Here, we investigated the effects of the average distance from the reference genome to the in-group taxa (i.e., whether the reference genome is an in- or outgroup individual) and the manner in which this interacts with bioinformatic filtering choices.

METHODS

1. Simulate species trees and gene trees
   for 100 taxa, and varied tree heights using SimPhy[1]

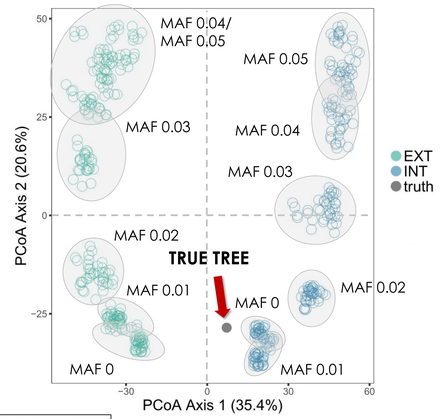


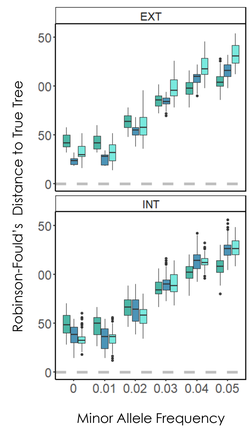
2. Simulate Illumina FASTQ reads for each taxon
   using TreeToReads[2]
3. Align reads to **outgroup** and one **random ingroup** reference, call and filter variants:
   - mapping quality
   - minor allele frequency
   - missing data per SNP
4. Build RAxML phylogeny, compare trees to simulated (known) species tree

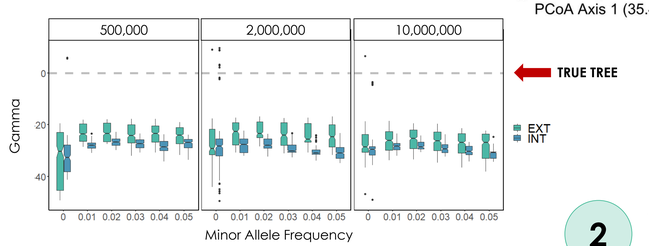
REFERENCES: [1] Mallo et al. 2015. Systematic Biology. [2] McTavish et al. 2017. BMC Bioinformatics.

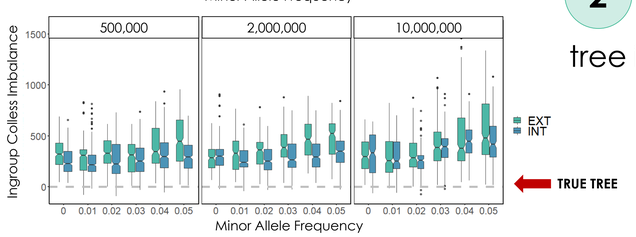
THE TAKEAWAYS

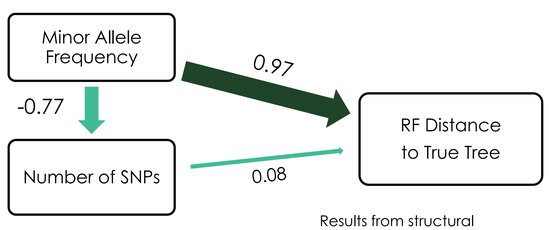
1 **Increasing** minor allele frequency cutoffs (MAF) leads to **more deviation** from the true tree, and this effect is greater in analyses using **ingroup reference genomes (INT)**.











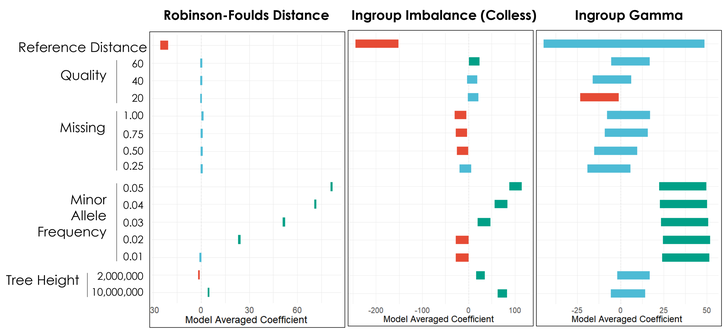
Results from structural equation model

2 This deviation from the true tree has a complex effect on tree **imbalance** and **gamma** statistics.

3 MAF remains a significant predictor of deviation from the true tree, even when **controlling for the number of SNPs retained** in the different data sets.

DETAILS AND DISCUSSION

Using linear mixed models with Robinson-Fould's distance (to the true tree), ingroup gamma, and ingroup Colless' imbalance statistic as the response variables, we found that **minor allele frequency** and **reference distance** impact all three of these measures, as shown below. In addition to these single variables, we found significant **pairwise interaction terms** between MAF, reference distance, and tree height for all three response variables. However, there remains much **unexplained variance** for the imbalance and gamma measures.



FUTURE DIRECTIONS for this project include using a **species tree method** (ASTRAL II) for inferring phylogenies to compare with the RAxML concatenation method. We also plan to **subsample the same number of SNPs** from all data sets, to further test whether minor allele frequency remains a significant consideration outside of its influence on data set size. We will also investigate how these filters alter the **mutation spectrum** of retained SNPs compared to the original set of SNPs.

ACKNOWLEDGEMENTS

Society of Systematic Biologists · ARCC · UWYO · Wyoming NASA Space Grant Consortium · Wyoming INBRE · PIE Program in Ecology