Gene Tree Conflict and Reticulate Evolution in the *Protea* Radiation: Testing the Role of Pollinator-Mediated Gene Flow

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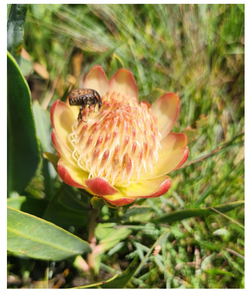
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INTRODUCTION

Protea (Proteaceae) is a diverse genus of South African flowering plants comprising over 110 species, representing one of the most fascinating rapid radiations in the plant kingdom. Rapid diversification events like this often result in conflicting phylogenetic signals, where different genes tell different evolutionary stories, through two distinct processes: incomplete lineage sorting (ILS) and hybridization (Xu et al., 2024). Distinguishing between these processes is critical because they have fundamentally different evolutionary implications. *Protea* species can be pollinated by birds, insects, or mammals, and pollinator identity acts as a reproductive barrier between species (Kuhn et al., 2017). We hypothesize that pollinator syndrome shifts may facilitate hybridization by bringing previously isolated species into contact with shared pollinators, enabling gene flow across species boundaries and driving further diversification.



P. aristata (bird pollinated)



P. simplex (insect pollinated)



P. laevis (mammal pollinated)

METHODS

245 unique DNA samples were collected from fresh leaf tissue in the field across South Africa, totaling 96 species, 6 outgroup species of the genera *Serruria* and *Faurea*, and 90 Protea species. Molecular work was done at the Center for Anchored Phylogenomics at Florida State University, and 548 genes were obtained in the final dataset.

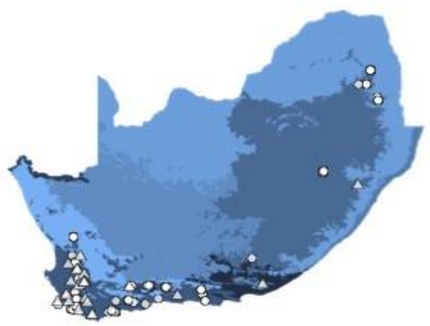


Figure 1. Sampling map for wild-collected *Protea* species across South Africa.

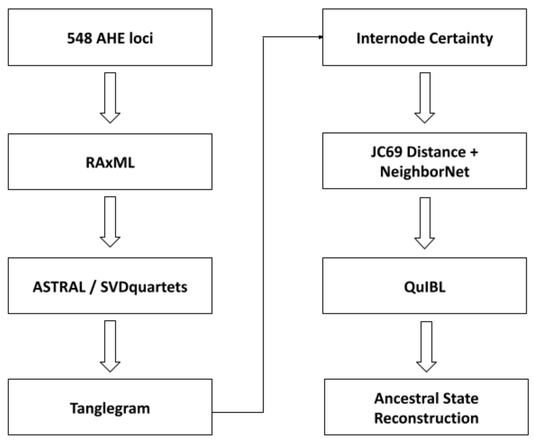


Figure 2. Computational workflow for phylogenomic analysis.

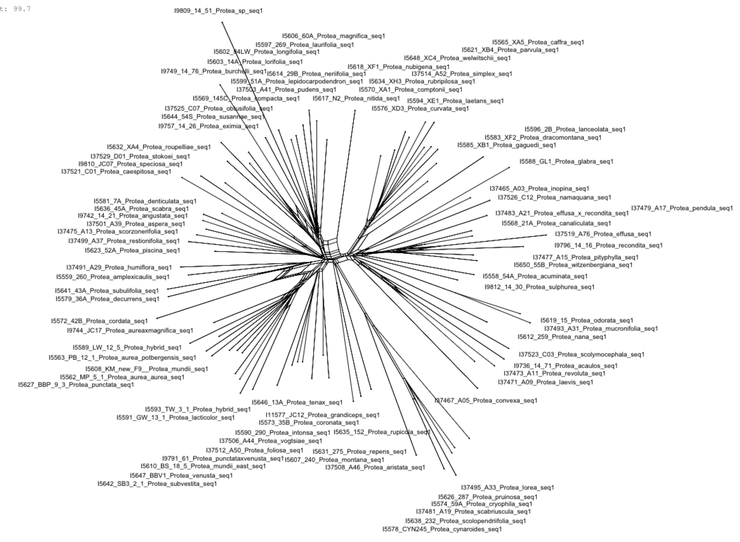


Figure 3. *Protea* NeighborNet phylogenetic network (fit=99.7%) inferred from JC distances across 548 loci, 90 ingroup taxa.

RESULTS

- ASTRAL and SVDquartets show high concordance (~75% shared bipartitions, normalized RF=0.247); discordance localized to specific nodes
- NeighborNet shows star-like topology with reticulation concentrated in high-conflict zones
- QuIBL identified 7/15 triplets with strong introgression signal (ΔBIC < 10); strongest in *P. scabra* (-80.8) and *P. inopina* (-62.4)
- Syndrome-shift triplets showed a statistically significant lower median ΔBIC than same-syndrome triplets (-19.5 vs. -7.5) (permutation test, p=0.007)

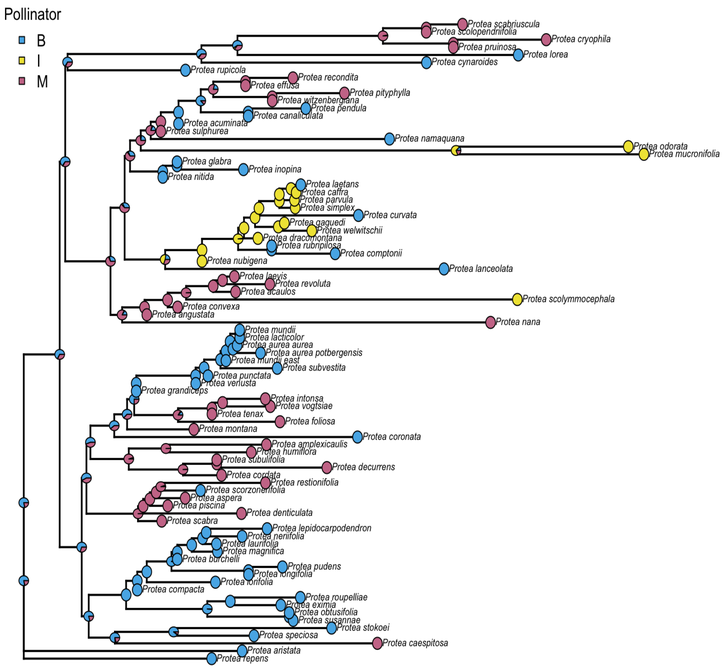


Figure 4. Pollination states mapped onto ASTRAL species tree via maximum likelihood ancestral state reconstruction (ER model, 100 stochastic mapping replicates). B=bird, I=insect, M=mammal.

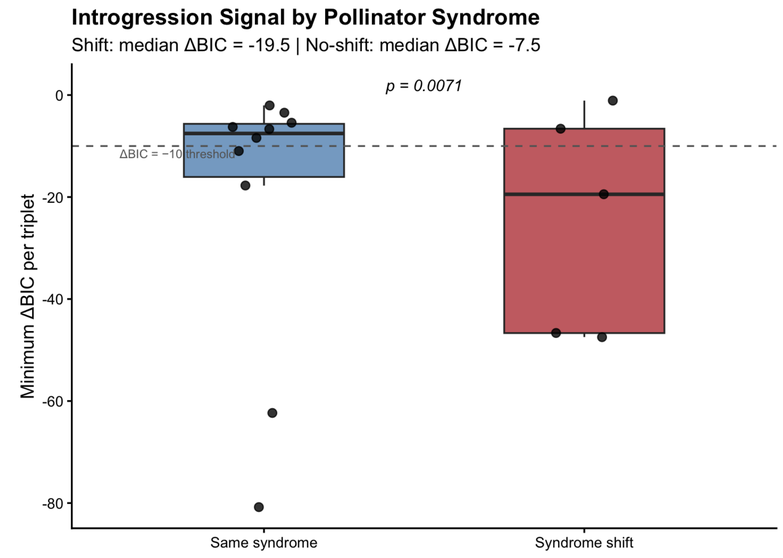


Figure 5. Minimum ΔBIC per triplet by pollinator syndrome classification (n=10 same-syndrome, n=5 syndrome-shift; permutation test p=0.007). More negative ΔBIC indicates stronger support for introgression over ILS.

DISCUSSION

- Similarity between ASTRAL and SVDquartets trees suggest that species tree methods resolve ILS in similar ways
- Conflict between species trees, as well as branches with low statistical support, may reflect ILS or hybridization
- Star-like network topology confirms *Protea* diversification was not strictly tree-like
- ASR reveals that bird pollination was the ancestral state, but more recent *Protea* species have evolved adaptations to insect and mammal pollinators
- Syndrome-shift triplets trended toward stronger introgression signal, but this preliminary result is based on a small dataset -- the shift group median was determined by just the 3rd-ranked value of only 5 triplets
- **Limitations:** triplet sampling targeted only high-conflict zones rather than genome-wide sampling, so results may not generalize; small and unbalanced group sizes limit confidence in effect size
- **Future directions:** expand triplet sampling across additional syndrome-boundary clades

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