



# Molecular Archiving of Stoneville and Other Eastern Cottonwood Clones

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## ABSTRACT

The current high demand for eastern cottonwood (*Populus deltoides*) (Fig. 1) in carbon fixation and biofuel production applications has raised the necessity to genotype and archive the Stoneville (ST) clones, which are used worldwide. In Spring 2024, we will collect fresh leaves of 100 ST clones and 300 additional individuals from trials in Mississippi, Alabama, and Tennessee. DNA will be extracted from leaves and genotyped. We will perform fingerprinting and estimate genetic diversity for each clone with the most informative SNPs. Finally, we will upload the information to a public repository.



Fig 1. Eastern cottonwood (*P. deltoides*) tree.

## INTRODUCTION

Eastern cottonwood (*P. deltoides*) is a model platform for engineering carbon fixation and storage, phytoremediation, water and nutrient use efficiencies, biofuel quality, and urban site tolerance (Helton et al., 2015). Clones originally developed by the US Forest Service, Southern Research Station have seen a significant increase in national and international demand in the last decade (Creutzig et al., 2015). Unfortunately, molecular identities, material origins, and genetic structures of ST clones are not known.

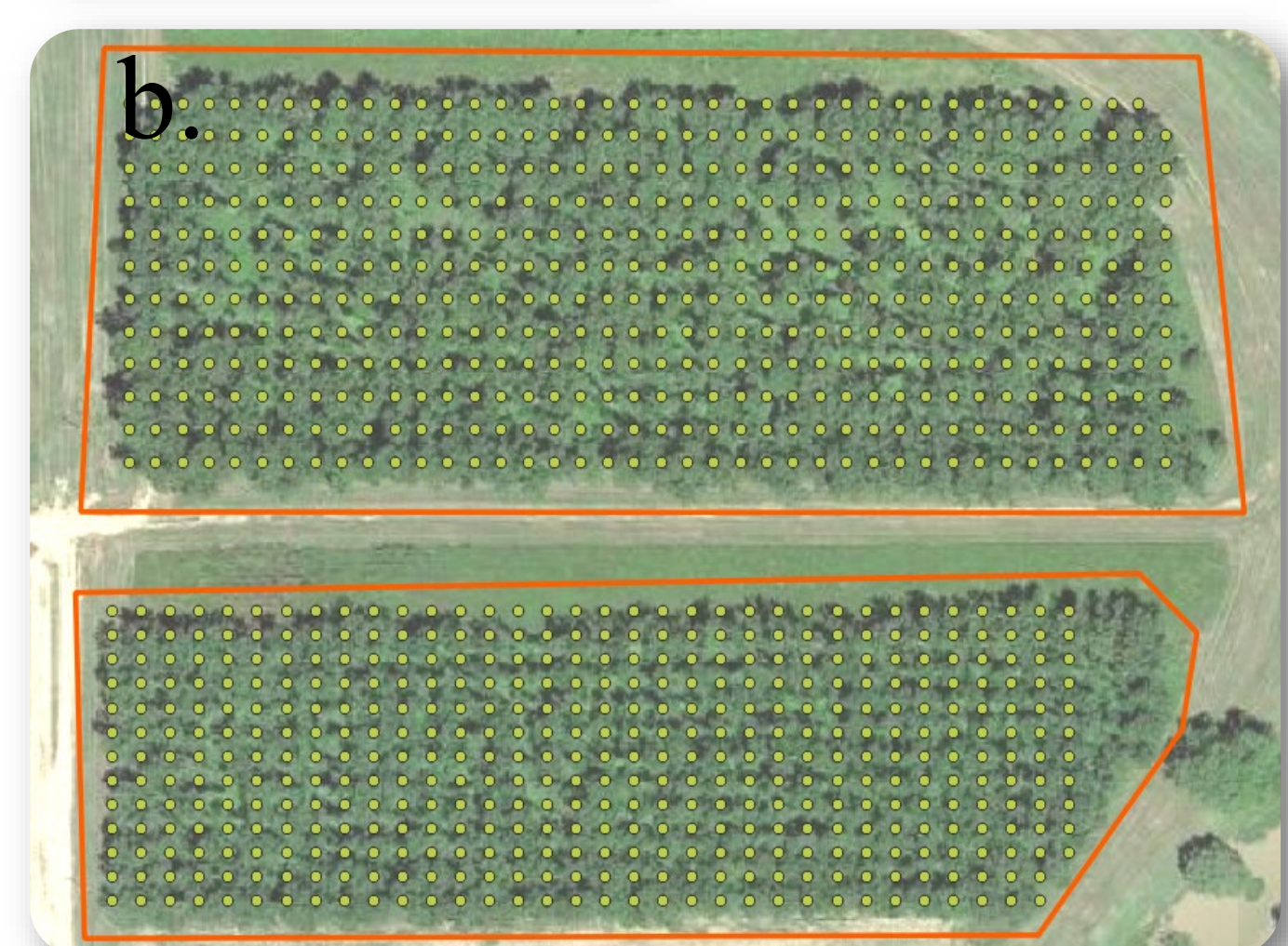
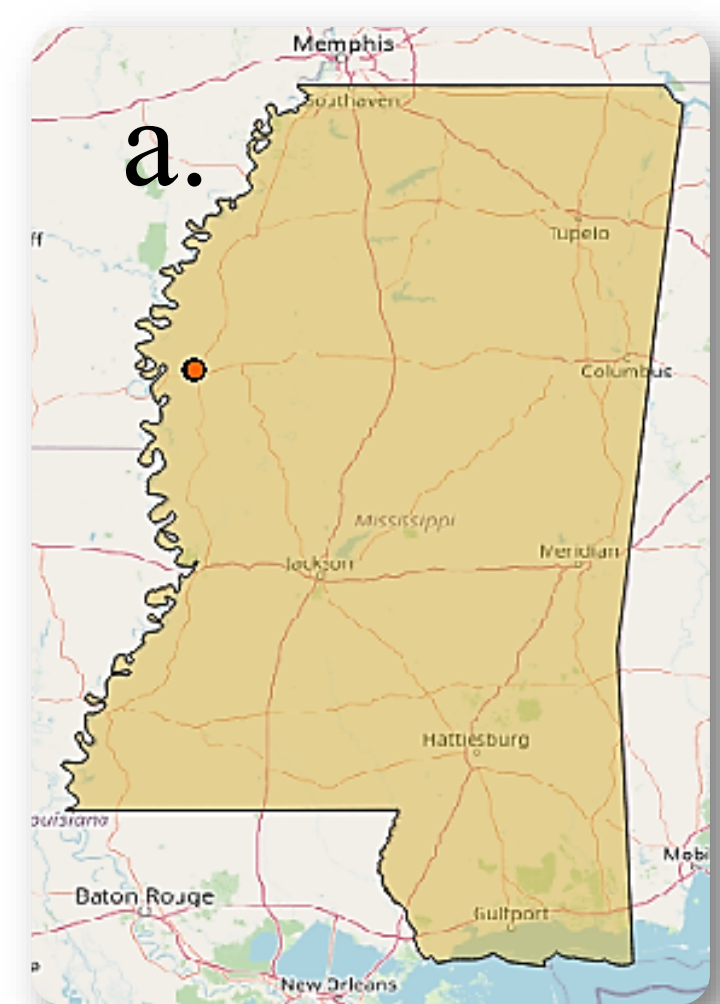


Fig 2. a) Location of Southern Research Station. b) Orchard arrangement.

## OBJECTIVE

The objective of this project is to produce and archive molecular identities and quantify the genetic diversity of the Stoneville *P. deltoides* clones.

## MATERIALS AND METHODS

**Plant material:** We will collect fresh leaves from 100 ST clones in Stoneville, MS (USDA Forest Service, Fig. 2a), from the Clone Bank and Breeding Orchard (Fig. 2b). Another 300 samples will be collected from appropriate sites in Mississippi, Alabama, and Tennessee.



Fig 3: DNA extraction and genotyping process

**DNA extraction and genotyping:** DNA will be extracted from leaves in the Galeano Lab with DNeasy® Plant Mini Kit (ThermoFisher Scientific Lab) (Fig. 3). Afterwards, DNA samples will be sent to NEOGEN for genotyping.

**Data analysis:** The analysis will include cleaning of SNPs (high calling rate and MAF); selection of most informative SNPs (expected 50 – 75 thousand); and estimation of genetic diversity and fingerprinting for each clone (Fig. 4).

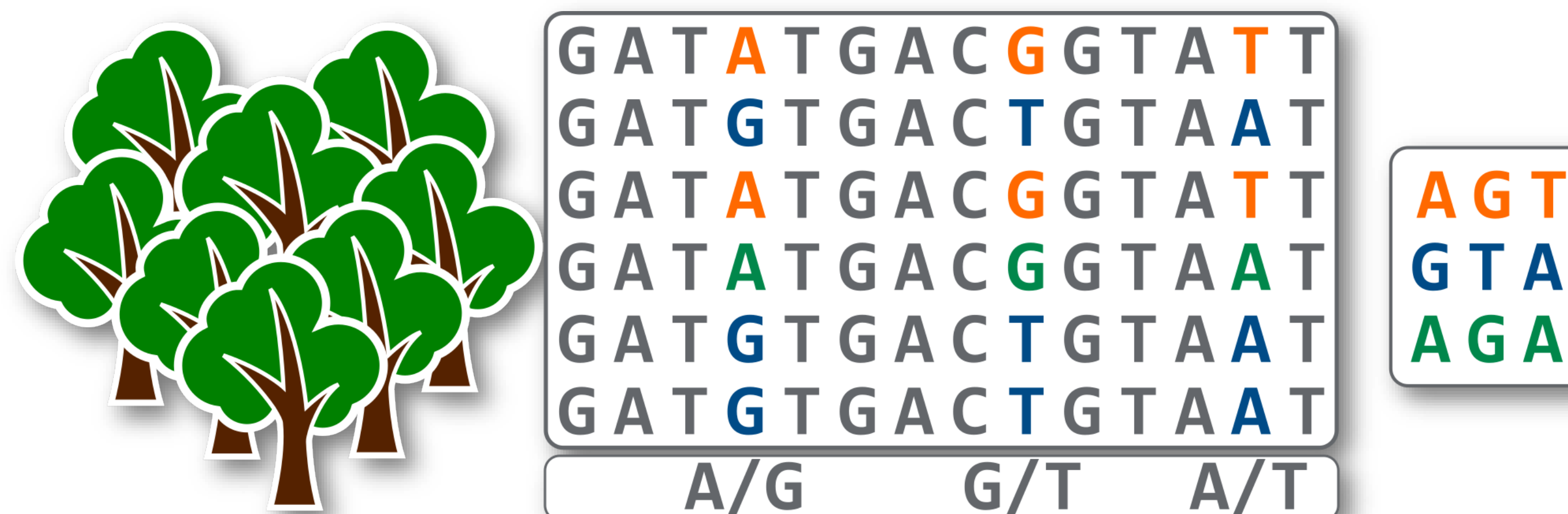


Fig 4: Example of SNPs structure and haplotypes

**Submission:** Sequences will be archived in an open-access genetic data repository.

## EXPECTED RESULTS

- Large number of SNPs.
- High genetic diversity.
- Haplotype construction.
- Population structure (Fig. 5).
- Public repository.

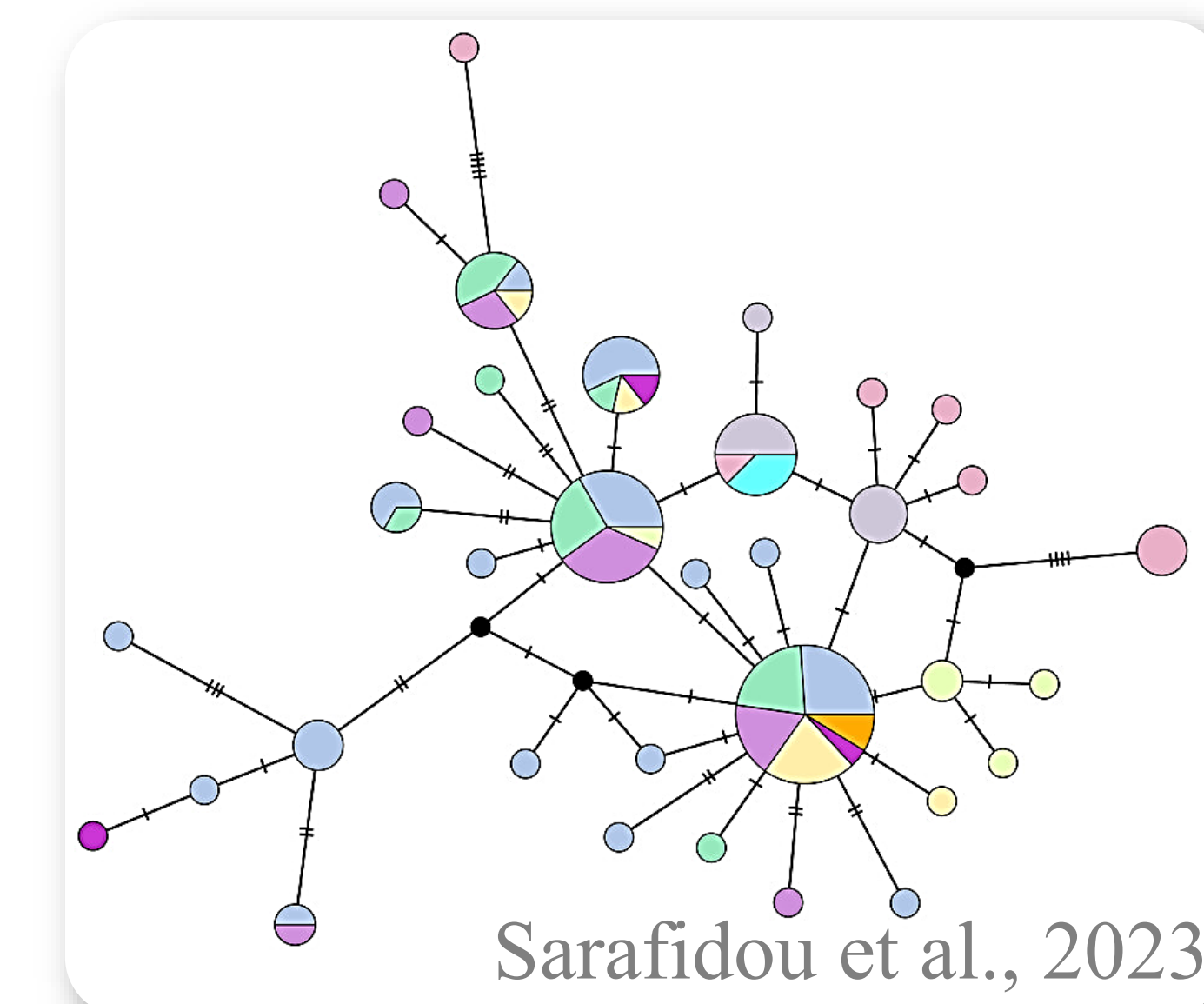


Fig 5: Example of a population structure diagram (Sarafidou et al., 2023)

## PERSPECTIVE

- This project aims to contribute fundamental genetic information necessary for further advancement and application of U.S. Forest Service produced *P. deltoides* clones.
- The generation of DNA fingerprints and population structures will modernize our understanding of this valuable germplasm.
- Our results will be publicly accessible and useful to universities, public and private institutes, and labs studying or developing *P. deltoides* molecular biology applications in the southern US and around the globe.

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## REFERENCES