

Ancient tree genomes for old questions

Andrew Hipp¹ and Desanka Lazic²

¹The Morton Arboretum

²University of Göttingen

September 22, 2023

Abstract

Most foundational work on the evolution and migration of plant species relies on genomic data from contemporary samples. Ancient plant samples can give us access to allele sequences and distributions on the landscape dating back to the mid Holocene or earlier (Gugerli et al., 2005). Nuclear DNA from ancient wood, however, has been mostly inaccessible until now. In a From the Cover article in this issue of *Molecular Ecology*, Wagner et al. (2023) present the first nuclear genomes from ancient to subfossil oak wood, including two samples dated to the 15th century and one that dates to more than 3,500 years ago. These first assembled nuclear genomes from ancient trees open the possibility for investigating species adaptation, migration, divergence, and hybridization in the deep past. They pave the way for what we hope will be a new era in the use of paleogenomics to study Holocene tree histories.

Andrew L. Hipp^{1,2}

Desanka Lazic^{3,4}

¹ Herbarium and Center for Tree Science, The Morton Arboretum, Lisle, IL, U.S.A.

² Botany Department, The Field Museum, Chicago, IL, U.S.A.

³ Department of Forest Genetics and Forest Tree Breeding, University of Göttingen, Germany

⁴ Thünen Institute of Forest Genetics, Grosshansdorf, Germany

Correspondence

Andrew L. Hipp, Herbarium, The Morton Arboretum, Lisle, IL, U.S.A.

Email: ahipp@mortonarb.org

Desanka Lazic, Thünen Institute of Forest Genetics, Grosshansdorf, Germany

Email: desanka.lazic@thuenen.de

Most foundational work on the evolution and migration of plant species relies on genomic data from contemporary samples. Ancient plant samples can give us access to allele sequences and distributions on the landscape dating back to the mid Holocene or earlier (Gugerli et al., 2005). Nuclear DNA from ancient wood, however, has been mostly inaccessible until now. In a From the Cover article in this issue of *Molecular Ecology*, Wagner et al. (2023) present the first nuclear genomes from ancient to subfossil oak wood, including two samples dated to the 15th century and one that dates to more than 3,500 years ago. These first assembled nuclear genomes from ancient trees open the possibility for investigating species adaptation, migration, divergence, and hybridization in the deep past. They pave the way for what we hope will be a new era in the use of paleogenomics to study Holocene tree histories.

In an earlier paper, Wagner et al. (2018) assembled chloroplast genomes from archaeological and subfossil white oak logs collected from across France. That work demonstrated that expansion of European oaks from southern refugia may already have been established as we find them today by 6,000 years ago, providing important validation of our understanding of the rate of tree migration following the last glacial maximum. But while chloroplast genomes track gene flow and migration histories, they often obscure population divergence and speciation histories (Dumolin-Lapègue et al., 1997; Pham et al., 2017; Whittemore & Schaal, 1991). Moreover, paleobotanical samples in oaks are often limited to pollen and wood, neither of which is identifiable beyond clade or species complex for oaks based on morphology and anatomy.

In their current study, Wagner et al. assemble nuclear genomes of three oak individuals to address three questions: (1) what species are represented in their sample, (2) how has genomic diversity in oak populations changed over time, and (3) what can nuclear genomic data tell us about the phenology of ancient populations? They address question number one with a set of 54 SNPs scattered across the genome. They demonstrate that this relatively small sample can discriminate between species and identify hybrids. Two of their samples are relatively clean *Q. robur*, one from the 15th century is an early-generation hybrid between *Q. robur* and *Q. petraea*. This finding begs for a sequence-capture kit targeting these loci, perhaps tested on the DNAs already worked through carefully for Wagner et al.'s (2018) study. Such a tool could enable relatively economical species identification of ancient wood samples, contributing to our understanding of species distributions over time as well as archaeological questions that require identification of ancient samples.

This smattering of near-diagnostic SNPs does not suffice for the second and third goals of the present study, as sparse loci can be misleading about which genes are involved in introgression and adaptation (e.g., Lowry et al., 2017). To address diversity changes over time, Wagner et al. identified windows of their assembled genomes that exhibited higher nucleotide diversity in ancient genomes than in contemporary genomes, focusing just on the *Q. robur* samples. While they found no obvious systematic differences over time, their results echo Saleh et al.'s (2022) genomic investigation of selection across the Little Ice Age, which used genome resequencing data from 330 year-old trees across three sites. By contrast, Wagner et al. investigated genomic diversity on ca. 30- to 50-year-old individuals preserved in ancient sediments. Wagner et al.'s work suggests the possibility to directly estimate shifting selection pressures over the course of thousands of years. Combining population-level sampling at several time points with migration histories inferred from the plastome would enable a more direct test of the relative importance of migration and adaptation to past climate changes.

Finally, Wagner et al. investigated SNPs previously identified as associated with flowering time along elevational and latitudinal gradients in *Q. petraea* (Leroy et al., 2020) to estimate flowering time of the ancient populations. Their samples clustered with selected SNPs in northern populations, which might suggest only that the population structure observed today was largely in place by 3,500 years ago. However, they also clustered with high altitude, later-flowering populations from the south, providing stronger evidence that the ancient populations may in fact have been later-flowering. Additional validation with a wider range of contemporary populations (including some early flowering populations associated with low-elevation SNP frequencies) would have made this argument stronger, but the results still highlight the potential for ancient wood to provide a biotic view into historical environments.

The study left us with some questions about the assembled genomes. For example, is genomic coverage sufficient to quantify the distribution, and lengths of admixed blocks of the genome? These statistics could help refine our understanding of introgression's timing (cf. Fu et al., 2015) and adaptive significance (cf. Zhou et al., 2022). We were also left wondering how past introgression and species cohesion might appear from inferences drawn from the entire genome, instead of with preselected SNPs. The SNPs used may yield a biased estimate of introgression rates, even if they have high power to detect species and early-generation hybrids (cf. Hipp et al., 2019). However, our questions were balanced by our excitement about the possibilities. This work is particularly timely, given recent interest and progress in creating pan-genomes for tree species. Aligning sequencing reads from ancient samples against a pan-genome of the European white oak syngameon could improve mapping accuracy, capturing indels and structural rearrangements that may not be encompassed

by a single reference genome.

We are particularly enthused about the study’s potential impacts for two sets of questions:

Hybridization and introgression . The study shows that oak hybridization may have been not uncommon even in the 15th Century. Ancient tree genomes in landscape contexts, in combination with paleoclimate and paleoenvironmental reconstructions, could provide important tests of how introgression across the genome and at selected genes has changed over time, with shifting climates, and in human-altered landscapes (Anderson, 1948; Muller, 1952). We are only beginning to learn how important ancient gene flow has been to the ecology and distribution of oak species (O’Donnell et al., 2021). Ancient genomes could aid in detecting extinct species or populations and revealing the importance of adaptive introgression for the evolution of species ranges.

Tree responses to climate change . Genomic studies of contemporary tree cohorts established during discrete time periods can elucidate what alleles were selected for by past climates (e.g., Saleh et al., 2022). Ancient genomes could complement such approaches, more directly documenting the strength and direction of selection in the past. Combined with paleoclimate reconstructions, they could also be used to help validate genomic offset methods, directly testing whether allele frequencies have tracked climates as predicted (Rellstab et al., 2021).

Wagner has been working on these submerged oak logs for nearly a decade. The data are hard-earned, deriving from chainsaw work in the early days of the project, extracting samples from logs dating to near the beginning of the Holocene, and substantial sequencing and informatic effort on each sample. The work is paying off. Wagner’s study is a harbinger of an exciting turn in paleogenomics, releasing the mass of potential tree data locked up in saturated soils and drowned at the bottoms of lakes.

Acknowledgments

This work is partially supported by NSF Award #2129281 to ALH. Alan Whittemore provided feedback on a draft of this commentary.

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