International Oaks

The Journal of the International Oak Society

…new oaks and new galls, conserving the Fagaceae of Taiwan, urban oaks in France, ecological specialization and evolutionary patterns, ancient DNA, oak adventures around the world…

Issue No. 35 / 2024 / ISSN 1941-2061

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Issue No. 35 / 2024 / ISSN 1941-2061

International Oaks Issue No. 35

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Ancient DNA, Oaks, Forests, and Humans

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ABSTRACT

Ancient DNA (aDNA) analysis involves studying DNA extracted from ancient biological remains, primarily wood in the case of trees. Abundant subfossil oak wood logs and archaeological timber from temperate Europe, including specimens with exceptionally well-preserved morphological features, offer unique insights into the evolutionary history of oaks, forests, and humans. This review highlights the enhanced value that aDNA brings to evolutionary inferences and the results obtained from genetic analyses conducted on modern populations. First addressing the technical challenges, constraints, and limitations associated with aDNA analysis of oaks, it then underlines the questions than can be addressed with this approach and offers some preliminary answers obtained from recent studies on European White Oaks. Species diagnostic marker analysis can be applied to aDNA for the taxonomic classification of fossil or archaeological remains, overcoming limitations associated with wood anatomical features. Organelle aDNA can serve as a unique tool to elucidate the origin of founder populations and refine the picture of the temporal dynamics of past colonization. Nuclear genomes can retrace genetic exchanges between species, providing historical support for admixture in most modern oak forests, as well as for succession scenarios. Coupled with current genome-wide association studies in modern experimental populations, ancient genome analysis enables the reconstruction of ancient phenotypes and the assessment of temporal evolutionary changes in fitness-related traits. Origins of silviculture can also be addressed by exploring footprints in population-scale data of aDNA. Finally, genome-wide data retrieved from aDNA can provide unique insights into the spatio-temporal variation of genetic diversity during and after the establishment of populations. This comprehensive overview of feasible applications of aDNA analysis in the evolutionary biology of oaks opens new perspectives in broader research fields, including history, archaeology, dendroecology, and dendroclimatology.

Keywords: microevolution, *Quercus*, taxonomic assignment, admixture, colonization, genetic diversity, archaeology

Introduction

Ancient DNA (aDNA) refers to DNA extracted from ancient biological remains, such as bones, teeth, hair, plant tissue, or other preserved organic material. This field of study enables researchers to investigate the genetic makeup of past animals and plants, providing insights into genetic diversity, evolutionary relationships, and population dynamics of extinct and ancient organisms (Brunson and Reich 2019; Kistler et al. 2020; Orlando et al. 2015). Additionally, historical DNA, including DNA recovered from herbarium vouchers, can offer retrospective insights into the evolutionary history of trees and other plants. Ancient DNA analysis has become a valuable tool for understanding the genetic history and evolutionary processes across various taxa.

Oak trees alive today that are between 400 to 1,500 years old (Parker and Lewington 2012) are known. But such very old trees, like the King Offa's oak,[1] estimated to be 1,300 to 1,500 years old, or populations older than 300 years (Saleh et al. 2022) are rare and geographically restricted. DNA from ancient oak timber, toghether with the DNA of living old-growing oaks, allows the study of tree populations from very recent to very ancient times. Subfossil (non-fossilized) wood logs and archaeological timber from temperate Europe, including some with exceptionally well-preserved morphological features, are abundantly available and have been extensively studied across various disciplines, including dendroarchaeology (Tegel et al. 2022), dendrochronology (Haneca et al. 2009), dendrochemistry (Canning et al. 2023), and dendroclimatology (Esper et al. 2018). Archaeological wood samples, often excavated from waterlogged environments such as groundwater-saturated wetlands and submerged archaeological layers within lakes and oceans, have provided unique insights into the past of both humans and forests. Notably, the prehistoric pile dwellings around the Alps,[2] over 800 of which have earned UNESCO World Heritage status, serve as a unique archive for studying forest changes and timber usage. Such sites house a vast array of trees that span multiple generations since the Neolithic period (Hafner et al. 2020).

Delving into the intricate realm of oak population history, modern DNA has proven to be an invaluable tool for unravelling both macro- and micro-evolutionary changes (Kremer and Hipp 2020; Hipp et al. 2020). Present-day distributions of chloroplast DNA, transmitted through acorns, have been scrutinized in tandem with temporal sequences of fossil pollen distributions. These investigations have revealed postglacial refuges, migration pathways, and glimpses into the interspecific gene flow that has led to the sharing of chloroplast variants among distinct European White Oak species (Petit et al. 2002). Nuclear DNA markers have confirmed the existence of extensive gene flow and scrutinized imprints of inter- and intraspecific matings in natural oak populations, shedding light on their biological significance (Curtu et al. 2007; Gerber et al. 2014). The availability of the first whole-genome sequence generated for *Quercus robur* (pedunculate oak) (Plomion et al. 2018) marked a major shift leading from the characterization of a few nuclear markers to whole-genome characterizations. This advance facilitated screening for species barriers and signatures of selection and adaptation (Saleh et al. 2022; Leroy et al. 2020a; Leroy et al. 2020b).

In this review, I will emphasize the value that ancient DNA contributes to other time-sequential analyses, such as dendroarchaeology, dendroecology, and dendroclimatology,

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[1] A pollarded *Quercus robur* growing in Windsor Great Park (Berkshire, UK).

[2] See https://palafittes.org.

specifically focused on oak-wood remains. Additionally, I underscore its significance in drawing evolutionary inferences complementing results that can be obtained based on genetic analyses on extant material. First, I will recall the technical challenges inherent in studying ancient oak-wood DNA based on the experience gained from a large-scale exploration of aDNA in European oaks (Wagner et al. 2018, 2024). By elucidating the potential of aDNA and related techniques, I highlight their pivotal role in addressing longstanding questions concerning oak history, oak forests, and past oak-human interactions.

Photo 1/ Remains of pile dwellings dating back 2,800 years, submerged in the Lake of Bourget near the city of Aix Les Bains in the French Alps. Multiple piles are regrouped in submerged villages and can be excavated during underwater diving campaigns.

Challenges in ancient oak-wood DNA analysis

Ancient DNA, characterized by its preservation in minute quantities and often accompanied by degradation and chemical modifications (DNA damage), poses challenges such as a high risk of contamination and false positives from modern contaminants. Moreover, ancient samples, typically found embedded in sediment, may contain varying proportions of DNA from associated microorganisms, which, when sequenced, often predominate the sequencing data (Der Sarkissian et al. 2015; Palmer et al. 2012). Modern sequencing techniques have revolutionized aDNA analysis enabling whole genome sequencing of ancient specimens (Brunson and Reich 2019; Orlando et al. 2015; Kistler et al. 2020; Orlando et al. 2021). These improvements have allowed the authentification of ancient DNA fragments belonging to the target taxon based on the absence of postmortem damages in modern DNA. However, extraction of DNA from wood tissue presents additional challenges: the number of living cells containing DNA is limited, and wood harbours many secondary metabolities that complicate DNA extraction (Liepelt et al. 2006; Rachmayanti et al. 2009).

Nevertheless, steady advances in DNA sequencing technology have helped design new protocols for extraction, enabling an aDNA sequencing project based on a large collection of wood remains in temperate Europe. Starting in 2014, 167 samples of subfossil and archaeological oak-wood samples (*Q. petraea* and *Q. robur*) were collected from 26 sites covering temporal points between 500 and 9,800 years ago. Of the 167 collected samples, 140 could be sequenced (Wagner et al. 2018). In 2018, the first authenticated complete ancient chloroplast genomes dating back to the Neolithic (around 5,500 years), the Bronze Age (around 3,500 years), and the Middle Ages (around 500 years) were generated from oak excavated at various European locations (Fig. 1) and compared to modern DNA variants from the same regions (Wagner et al. 2018). A total of 21 chloroplast genomes from across the Holocene were generated in the same study. Subsequently, a follow-up study produced the first ancient wood nuclear genomes based on medieval wood from a northern and a southern German site, along with a Bronze Age (3,700 years) site from northern France, providing valuable insights into previously unanswered questions (Wagner et al. 2024).

There are several lessons learned from these experiments that may guide future explorations of aDNA in European oaks (Wagner et al. 2018, 2024). Oak-wood resources that may serve for aDNA analysis are numerous and widespread in the form of either subfossil or archaeological remains. Waterlogged wood remains emerge as the most promising source for reconstructing ancient individual nuclear and plastid genomes of oaks from recent centuries and millennia. As an alternative to waterlogged material, remains preserved in calcareous, anoxic, and neutral organic sediments are as well suited for aDNA and provide better results than those extracted from acidic, oxygen-rich deposits. Successful DNA extraction is highly variable within excavation sites, making extensive DNA screening prior to full genome sequencing mandatory in order to select promising candidates. Oak authenticated aDNA preserved in waterlogged wood remains represents only a minor quantity of the DNA pool extracted from the remains: in a recent study only 10% of a random sample contained more than 1% of oak DNA (Wagner et al. 2018). The remaining DNA originated from the diverse microorganisms present in waterlogged wood. As found in earlier studies, chloroplast genomes are easier to sequence than nuclear genomes due to their higher cell content. Chloroplasts are present in multiple copies in each cell, whereas there is only one copy of the nuclear genome. Wood remains that contain sapwood (outer wood) offer higher success of aDNA extraction, as they contain higher proportions of living cells than heartwood (innerwood). For the time being, next-generation sequencing enables recovery of whole oak genome sequences from wood remains dating back to the Bronze Age.

Pending questions from aDNA analysis in European White Oaks

Ancient DNA analysis raises high expectations for answers to old questions about oaks, forests, and human history. Immediate responses to origins and movements of wood material used for various purposes can be obtained by discovering genetic footprints in wood remains. Access to aDNA signatures is therefore a breakthrough in solving past traceability issues of historical timber. Beyond providing one-off answers to historical

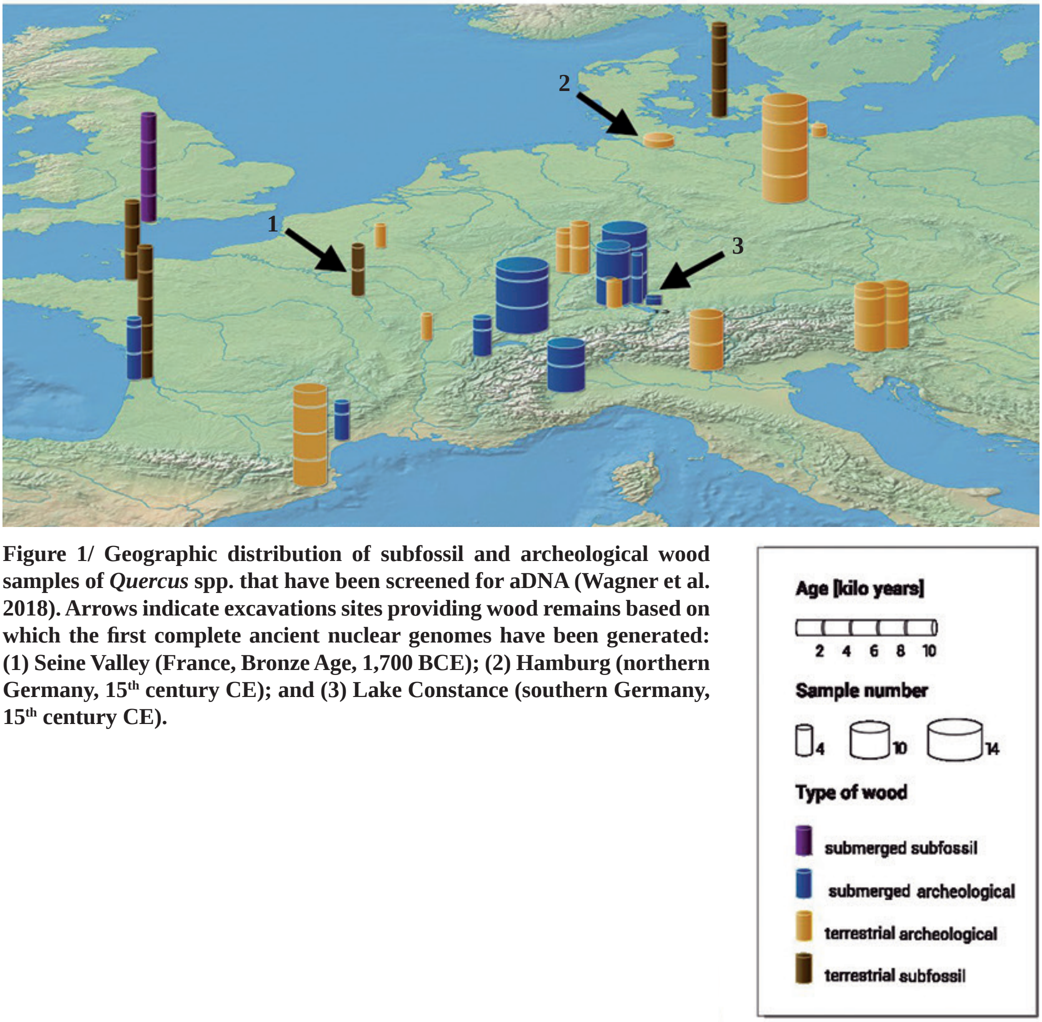

Figure 1/ Geographic distribution of subfossil and archeological wood samples of *Quercus* spp. that have been screened for aDNA (Wagner et al. 2018). Arrows indicate excavations sites providing wood remains based on which the first complete ancient nuclear genomes have been generated: (1) Seine Valley (France, Bronze Age, 1,700 BCE); (2) Hamburg (northern Germany, 15th century CE); and (3) Lake Constance (southern Germany, 15th century CE).

puzzles, aDNA opens new avenues on broader subjects regarding microevolution of oaks and past oak-human interactions that I will address in this section. Some of them have been tackled in the aforementioned studies (Wagner et al. 2018, 2024) and have received partial answers, but others remain untouched. Although successful sequencing of oak aDNA could only be carried out, in the current state of technology, on remains dating back to the Bronze Age, I will address expectations covering longer periods going back to the beginning of postglacial warming.

Taxonomic assignment

Accurate taxonomic assignment of fossil or archaeological remains is a pivotal concern in evolutionary and dendroarcheological studies. Even in modern tissue, wood anatomical traits can, at best, discriminate between botanical sections (such as section *Lobatae* and section *Quercus* in American oaks) (Tillson and Muller 1942). Recognizing species within sections based on wood anatomical characteristics is deemed impossible (Wheeler et al. 2022). For example, the three European temperate White Oak species (*Q. petraea*, *Q. robur*, and *Q. pubescens*) cannot be differentiated on the basis of their wood anatomy (Schoch et al. 2004). Concerning pollen grains, exine sculptures of pollen grains exhibit micromorphological differences between the eight recognized sections of the genus (Denk and Grimm 2009; Tekleva et al. 2023). These microstructures allow reliable taxonomic assessments at the sectional level for modern specimens and comparisons between modern and fossil pollen grains (Denk and Bouchal 2021). However, intrasectional species distinctions remain ambiguous as shown by a detailed quantitative study conducted between *Q. petraea, Q. robur,* and *Q. pubescens* and their hybrids (Wronska-Pilarek et al. 2016). Leaf morphology has received considerable attention in oak taxonomic studies, where species recognition can be achieved even between closely related species. However, the assignment procedure is usually based on quantitative analysis of multiple traits including comparing the specimen's trait values to the distribution of a reference population of samples (Kremer et al. 2002; Viscosi and Cardini 2011). These requirements severely constrain taxonomic resolutions of leaf remains, where samples can be limited in number and integrity. In summary, achieving taxonomic assignment based on morphological features of the most common fossil or archaeological remains (wood, pollen, or leaf) is challenging and rarely possible below the sectional level in oaks.

In cases where aDNA can be successfully extracted from the remains, molecular fingerprinting presents a potential solution to species assignment challenges. Genetic surveys conducted in natural populations, employing various genetic marker systems, have revealed that specific genomic regions with narrow but robust species barriers persist in closely related species, contributing to the maintenance of species integrity (Gailing and Curtu 2014; Scotti-Saintagne et al. 2004). Recent whole-genome analyses at the populational level have further refined these genomic regions, leading to the identification of nearly species-diagnostic single nucleotide polymorphisms (SNPs) in European White Oaks (Kremer et al. 2024; Leroy et al. 2020b; Schroeder and Kersten 2023) and in American White Oaks (Fitzek et al. 2018; Hipp et al. 2019). In the context of European oaks, the selected markers exhibited high diagnosticity. This suggests that a small number of markers are sufficient for confidently assigning a species name to subfossil or archaeological remains of unknown origin. Consequently, even in cases of highly degraded aDNA, these SNPs can be instrumental in capturing species-informative genomic sites and calculating probabilities of belonging to one of the species *Q. robur*, *Q. petraea*, *Q. pubescens*, or *Q. pyrenaica*. The expanding repository of whole genome sequences from oaks (Abbott et al. 2024) will facilitate the development and refinement of molecular fingerprinting techniques for enhanced species identification in modern, archaeological, and paleogenomic studies.

Wagner et al. (2024) utilised the diagnostic markers to ascertain the taxonomic signature of three oak specimens dating to the Bronze Age and the Middle Ages, for which nuclear genomes had been generated. Significantly, in this example, the authors demonstrated the unequivocal classification of a hybrid, and this classification was confirmed by whole genome data.

As a pressing expected application of taxonomic assignment, aDNA will help to answer a long-standing question regarding the evolutionary trajectories of distinct species across Europe. What were their separate migration pathways during postglacial warming? When and where did the first contact zones in central Europe build up? Were there any local or widespread species extinctions?

Photos 2/ A 1,400-year-old fishery pile excavated in the Bay of the Mont-Saint-Michel (near Champeaux, Normandy, France): (a) longitudinal view illustrating the well-preserved wood in the buried part of the pile, while the exposed part (dark brown) is more degraded; (b) cross-sectional view of the pile (Wagner et al. 2018).

Colonization dynamics

Phylogeographic studies, focusing on the analysis of chloroplast DNA in oaks, have brought to light Holocene migration pathways and identified potential refugial areas during the last glacial period (Petit et al. 2002). Organelle DNA is transmitted through seeds, and the existing geographic distribution of haplotypes (genetic variants of organelle DNA) represents genomic imprints of past migration events (Avise 2009; Emerson and Hewitt 2005). Furthermore, the presence of shared haplotypes between species cohabiting the same forests provides evidence of earlier hybridization and introgression that occurred during colonization processes (Petit et al. 1997; Whittemore and Schaal 1991).

What additional insights does organelle aDNA analysis contribute to the historical inferences already derived from modern chloroplast DNA studies? It provides a temporal framework for understanding past colonization dynamics. Firstly, by enabling the examination of whether present-day maternal lineages have resulted from repeated phases of colonization or if the initial lineages have endured over time (lineage replacement hypothesis). This inquiry involves comparing aDNA and modern DNA haplotypes at the same locations. Such genetic comparisons between ancient and contemporary imprints also help verify whether migration routes inferred from modern organelle DNA have altered the original historical routes. Secondly, in addition to genomic imprints in nuclear aDNA, the detection of aDNA haplotypes shared between coexisting species will provide the timeline of genetic exchanges between species. Thirdly, reconstruction of fine-scale spatial organelle diversity in oaks and humans (chloroplast in oaks, mitochondria in humans) based on ancient remains may elucidate if humans facilitated the rapid spread of oaks during the postglacial warming (Kremer 2015).

The lineage replacement hypothesis was tested in European temperate White Oaks, by comparing ancient haplotypes (from the Neolithic, the Bronze Age, and medieval times) with modern haplotypes at 21 sites across Europe (Wagner et al. 2018). Ancient haplotypes matched modern haplotypes in all cases. This is not a surprise, as oak stands renew themselves not only by sexual but also by vegetative (sprouting) reproduction, thus reinforcing the maintenance of the original maternal structure through time.

Admixture

The contemporary composition of gene pools in Central European White Oak species is shaped by two introgression dynamics: the merging of lineages originating from different refugial areas and the interbreeding between different species (Kremer et al. 2010; Kremer 2016; Petit et al. 2003a). This insight has been gleaned from phylogeographic investigations and nuclear genetic surveys based on extant populations (Gailing et al. 2021; Petit et al. 2002). While secondary contacts between oak species likely occurred at the onset of the postglacial period (Leroy et al. 2017), the later temporal trends in introgression dynamics remain largely unknown. The only certainty is that modern oak populations are admixed, and introgression has, in some instances, contributed to local adaptation (Fu et al. 2022; Leroy et al. 2020a), though the timing and mechanisms of introgression are yet to be fully understood.

After assessing maternal lineages, both modern and ancient nuclear genomes were sequenced to explore historical hybridization. For the first time, historical admixture between *Q. robur* and *Q. petraea* was detected dating back to the Middle Ages and originating from southern Germany (Wagner et al. 2024). Two other ancient individuals (from central France and northern Germany; Fig. 1) were identified as *Q. robur*, showing no signs of introgression from *Q. petraea* or any other temperate species. While these results are fragmentary and relatively recent, they pave the way for future investigations into the temporal genetic exchanges between species during colonization and after population establishment. Access to aDNA in Southern Europe from the Early Holocene would enable the inventorying of gene pools of refugial populations and species that persisted in Southern Europe during the last glacial period. Building on this discovery, the level of introgression between lineages and species could be explored in subsequent remains extracted in Central or Northern Europe, contributing to reconstruct the shaping of the modern oak gene pool. A limitation to this is the availability of suitable old oak macrofossils from southern range areas from which can be extracted sufficient DNA for sequencing nuclear genomes or parts of them.

Succession

Introgression was instrumental in the succession of the pioneer species *Q. robur* by the late successional species *Q. petraea*, as well as in the migration of the latter (Petit et al. 2003b). In ecological succession scenarios, microenvironmental changes generated by the installation of a pioneer species facilitate the establishment of a late successional species. In the case of these two oak species, the succession process is enhanced and boosted by introgression, resulting ultimately in the “replacement by pollen” of *Q. robur* by *Q. petraea* (Kremer 2016; Petit et al. 2003b). These succession dynamics are most visible today at the northern or eastern margins of their natutal distributions where colonization is ongoing (Beatty et al. 2016; Degen et al. 2023). What remains largely unknown are the spatio-temporal dynamics of succession. Where and how fast did succession operate during the colonization? Beyond the example of *Q. robur* and *Q. petraea*, how widespread was succession and “replacement by pollen” in other interfertile White Oaks (or in other sections), and how did it contribute to the overall distribution of the genus in the Northern Hemisphere?

“Replacement by pollen” may have also operated at the intraspecific level, although the maternal footprint of the initial founder population remained unchanged. Was the nuclear gene pool of installed populations replaced after the merging of different lineages during colonization, and subsequent pollen swamping? Similar questions can also be raised when abrupt climate changes (for example the Little Ice Age) exert strong new selection pressures (Caignard et al. 2023; Schnitzler 2020). Did northern populations – better adapted to cold conditions – replace southern populations by long distance pollen flow? With the development of silviculture, when and where was succession reinforced by human intervention? Answers from the past to these questions are of the utmost importance in understanding future adaptive responses of tree populations to climate change. "Replacement by pollen", between or within species, has not yet been investigated using aDNA.

Pace of microevolution

Extant natural populations of oak species display geographic patterns of genetic variation that align with geographical and climatic gradients (Kleinschmit 1993; Kriebel 1993). These patterns, observable in genetic markers and phenotypic traits through common garden experiments, have been interpreted as evidence of local adaptation that developed after the establishment of populations (Leroy et al. 2020a; Sáenz-Romero et al. 2017). However, what remains unknown is the rate at which local adaptation occurred. How quickly did microevolution take place during the Holocene? Was it a gradual process during the postglacial warming, or did it fluctuate during the climatic oscillations characterising the Holocene (Peteet 2000)? Understanding the temporal adaptive responses to past climate changes is crucial, especially since today there are growing concerns about the ability of long-lived species to adapt to ongoing climate change (Mitchell and Whitney 2018).

A recent retrospective study on multicentennial living oak trees revealed how microevolutionary changes fluctuated during the Little Ice Age-Anthropocene transition (Caignard et al. 2024; Saleh et al. 2022). Given the increasing availability of past climatic reconstructions (Pfister and Wanner 2021; Rull 2020), aDNA provides an invaluable tool to trace temporal genetic changes. In modern oak populations, genomic and phenotypic assessments conducted in natural populations raised in common garden plantations have identified genetic associations between fitness-related traits (height, phenology, reproduction) and genomic variations (Leroy et al. 2020a). These statistical associations provide the baseline for the reconstruction of past phenotypes based on allelic profiles at phenotype-informative loci in ancient samples, providing a means to estimate the rate of evolutionary responses of fitness-related traits to past climatic changes.

In the investigation of nuclear aDNA of European oaks (Wagner et al. 2024), this method was used to reconstruct the timing of bud phenology for three ancient trees. Due to the limited sample size, the methodology did not allow the estimation of evolutionary changes, which typically requires comprehensive samples representing the entire population. However, with the abundance of subfossil and archaeological remains dating back to different time periods since the Neolithic (Tegel et al. 2022), population genetic surveys based on aDNA have the potential to track temporal evolutionary changes over known climatic oscillations, for example during transitions between the Medieval Warm Period and the Little Ice Age and finally the Anthropocene (Le Roy Ladurie 2004, 2006).

Protosylviculture

Since *Homo sapiens* invaded Europe they have used oaks extensively for food, fodder, dwellings, and art. In turn, this implies human intervention in forests, including specific sylvicultural practices that have lead to some sort of phenotypic selection. Selection of oak trees for sweet acorns has been widely used in the Northern Hemisphere for centuries

Photos 3/ A 3,700-year-old water well excavated from limy sediments in Alsace (Erstein, near the city of Strasbourg): (a) overall view of the well; (b) detailed view of a wooden plank, in which the heartwood is dark brown and the sapwood, used for DNA sequencing, is light brown.

(McCorriston 1995; Mason 2000), pollarding has been a recurrent method for generating fodder for cattle and sheep (Petit and Watkins 2003), and the artificial bending of trees, or phenotypic selection of naturally bent trees, was implemented for shipbuilding (Gautier-Bérubé and Thakar 2023). These examples illustrate how humans started to manage oak forests for their own needs and how sylvicultural practices are rooted in ancient people-tree interactions (Warren et al. 2014).

Insights to understanding the development of protosylviculture may be provided by aDNA analyses that can assess the level of selection implemented by the mobilization of wood resources for human use. For example, large-scale constructions (such as pile dwellings (Fig. 1), historical buildings, cathedrals, and ships) necessitated bringing together great quantities of logs which, today, can be used for demographic and population genetics investigations. Forensic analysis conducted with aDNA will allow us to estimate the number and relatedness of the genotypes mobilized, while the taxonomic assignment methods based on aDNA will tell us if species selection was also carried out. These investigations will ultimately yield an estimation of the level of genetic variation of the samples taken from these ancient constructions, which can then be compared to the level of genetic variation of extant populations, thus providing a measure of the human selection processes involved. Given the number of analyses needed, such investigations of aDNA on a population scale have not yet been performed.

Genetic diversity

The level of genetic diversity within oak populations is remarkably consistent across Europe (Zanetto and Kremer 1995). At a continental scale, surveys indicate minimal geographic differences in nuclear diversity, a result of extensive pollen-mediated gene flow since the establishment of oak forests dated to around 8,000-6,000 years ago (Buschbom et al. 2011; Gerber et al. 2014; Giesecke et al. 2017). However, this overall pattern may obscure the temporal changes in diversity stemming from colonization dynamics. Both experimental and theoretical evidence suggest that rare, long-distance dispersal events played a significant role in the rapid postglacial migration of oaks (Clark 1998; Powell and Zimmermann 2004). Such movements would have resulted in a patchy distribution of small populations originating from foundation events, which coalesced later to form the extant distribution of oaks. Theoretically, foundation effects (the creation of populations by a very few founder trees) leave genetic drift signatures indicating a reduction of the level of genetic diversity (Hedrick 2000). In general, abrupt demographic changes, whether in population reduction or expansion, should induce corresponding changes in the level of diversity. These predictions have not yet received any experimental support when recent living oak populations of different ages were compared. This lack of support may stem from minimal demographic changes or the limited time span of diversity assessments (Saleh et al. 2022).

The availability of the whole genome sequence from unique individuals offers reliable estimates of genetic diversity at the population level. This opportunity was used to estimate the level of overall genetic diversity of individuals dating back to the Bronze Age and the Middle Ages and did not reveal any difference between these periods, most likely for the same reasons (minimal demographic changes or limited time span of diversity assessments) (Wagner et al. 2024). However, temporal dynamics of diversity could manifest itself at genomic subscales and should become more apparent with increased sample sizes and over extended periods.

Conclusion

This overview of feasible applications of aDNA analysis in the evolutionary biology of oaks opens new perspectives in broader research fields such as history, archaeology, dendroecology, and dendroclimatology. Leveraging ancient DNA for species assignment and maternal lineage identification will improve the capacity to trace the historical origins of wood products employed in human constructions. Ancient DNA analyses of archaeological and subfossil oak-wood remains provides a unique opportunity to explore the origins and trajectory of modern sylviculture. This approach allows for the examination of genomic changes in oaks shaped by local adaptation and selection over time, and those resulting from human activities necessitating the use of wood such as the construction of houses, ships, and other vehicles essential to human mobility, trade, and warfare. Ancient DNA studies provide valuable insights into the historical changes in forests and sylvicultural practices, shedding light on unresolved questions regarding human contributions to the expansion and local evolution of oaks during the Holocene.

Other research fields utilising ancient oak-wood remains stand to gain significant benefits from the enhanced taxonomic and geographical resolution offered by ancient genomics. Dendrochronological, dendroclimatological, and dendroecological studies in Europe, which mainly rely on tree-ring sequences from oaks, with annual resolution, currently assume similar growth behavior across the different temperate deciduous White Oak species and lineages. However, genetic variations between species and geographical origins exist, and these differences may inflate noise in dendrochronological or dendroecological studies. Tools allowing accurate geographic and taxonomic assignment would be a valuable complement to improve precision in these studies.

In summary, the analysis of ancient oak genomes, or the investigation of selected target regions has the potential to augment insights provided by other proxies studied for wood. The study of ancient DNA is emerging as an invaluable tool for understanding the long-term interplay between humans and oak forests and its consequences on the genomic makeup of modern-day forests.

Acknowledgements

I would like to express my deep gratitude to Stefanie Wagner for her assistance during the writing of the manuscript. The research on ancient oak DNA presented in this manuscript was conducted in the frame of a cooperation between INRAE Bordeaux (UMR Biodiversity Genes & Communities), the University of Copenhagen (Centre for Geogenetics) and CNRS Toulouse (Centre for Anthropobiology and Genomics of Toulouse (CAGT)). I acknowledge Prof. L. Orlando (Director of CAGT) for the supervision of the aDNA analysis, and Stefanie Wagner and the entire team of CAGT for their invaluable contributions throughout the project that produced the results reviewed in this article. The research work on ancient DNA of European oaks was supported by an ERC (European Research Council) Avanced Grant (Project TREEPEACE (#FP7-339728).

Photographers. Title page: Alexis Ducousso (*Quercus petraea*). Photo 1: Yves Billaud. Photo 2: Antoine Kremer. Photo 3: Stefanie Wagner.

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