REVIEW ARTICLE

Plant wax biomarkers in human evolutionary studies

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Abstract

Plant wax biomarkers are an innovative proxy for reconstructing vegetation composition and structure, rainfall intensity, temperature, and other climatic and environmental dynamics. Traditionally used in earth sciences and climate studies from "off-site" ocean and lake records, biomarker research is now incorporated in archeology and paleoanthropology to answer questions relating to past human-environment interactions and human evolution. Biomarker research is generating new and exciting information on the ecological context in which *Homo* and its closest relatives evolved, adapted, and invented stone tool technologies. In this review, we examine plant wax biomarkers and their use in reconstructing past plant landscapes and hydroclimates. We summarize the applications of plant wax molecular proxies in archeological research, assess challenges relating to taphonomy, consider the role of modern plant ecosystems in interpreting ancient habitats, and examine case studies conducted at key paleoanthropological locations in eastern and southern Africa and Europe.

1 | INTRODUCTION

While climatic and environmental variability have been prominently discussed in influencing the broad patterns of human evolution,[1-5] linking ecosystem changes to technological and behavioral responses remains challenging. To address such research challenges, the analysis of plant wax lipids from ocean and lake cores,[6-8] paleo-lake drilling projects,[9-13] archeological and geological outcrops,[14-18] cave deposits,[19-21] and hominin-bearing sediments[22] have the potential to provide well-integrated, high-resolution data on past plant ecology and environmental stresses in regions where hominins evolved.

The external surface of leaves and other plant parts with epidermal cells are coated with protective waxes (Figure 1) that help preserve the water balance of the plant and minimize damage to leaf cells from fungal and insect attack, wind abrasion, and excessive ultraviolet radiation.[23] As biologically-specific marker molecules, or *biomarkers*, plant wax lipids record environmental conditions in their compound distributions, abundances, and carbon ($\delta^{13}C$) and hydrogen (δD) isotope ratios (Box 1).

Plant wax lipids are readily dispersed through the environment and, due to their hydrophobic behavior, are well-preserved in a variety of soil, lake, and ocean sediments.[24] Despite their potential for
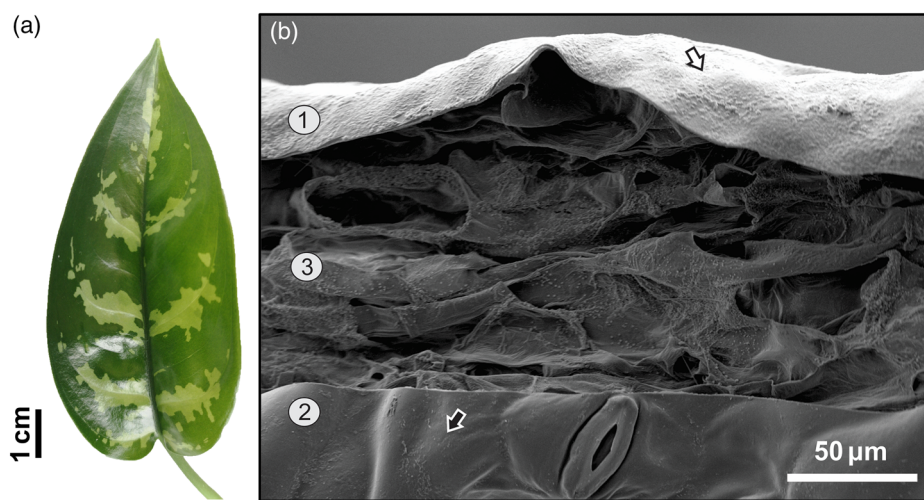
(a)

(b)

FIGURE 1   A schematic diagram of leaf anatomy highlighting the location of the plant wax lipids. (a) A dumb cane (*D. seguine*) leaf with a reflective waxy surface. (b) A scanning electron micrograph of the cross section of a leaf: (1) Upper epidermis covered by a cuticular membrane with epicuticular wax deposits on its outer surface visible (white arrow). (2) The lower epidermis covered with a cuticular membrane but with fewer epicuticular wax deposits (black arrow). A stoma is shown near center. (3) Mesophyll
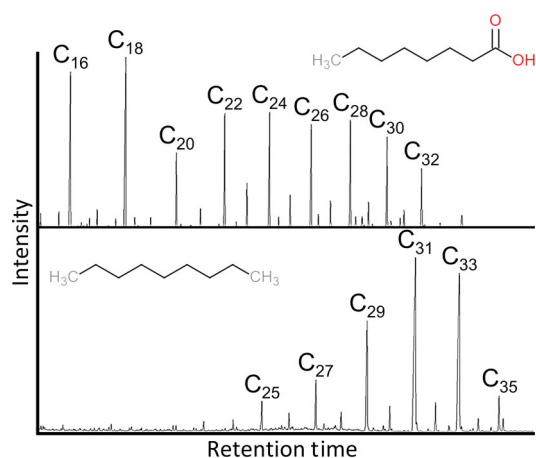

FIGURE 2   Typical GC–MS trace of *n*-alkanoic acid even-over-odd (top) and *n*-alkane odd-over-even (bottom) carbon chains, with hydrocarbon compounds labeled. The long-chain compounds (>C_{25}) are produced by terrestrial plants and are ideal for $\delta^{13}C$ and δD analyses

reconstructing human-environment interactions, the interpretation and evaluation of plant wax geochemistry has not been widely utilized by archeologists and paleoanthropologists. Here, we review the application of plant wax biomarkers used in human evolutionary studies. First, we discuss lipid biomarkers and the way in which they complement and diverge from other paleoenvironmental and climatic proxies. Although powerful on their own as a proxy for past vegetation change and paleohydrology, plant wax biomarkers are exceptional tools for reconstructing plant landscape variability at high taxonomic resolution, especially when combined with pollen or phytolith analyses.[25] Second, we assess biomarker taphonomy and preservation, and the ensuing challenges associated with this line of research. Plant waxes are ubiquitous and abundant in many soils, yet they are not preserved in all depositional settings and are susceptible to biodegradation. Marine and lacustrine sediments that act as high-resolution plant wax archives can even suffer from poor organic matter preservation if

oxidized, for example. Third, we examine how present-day vegetation formations may double as interpretive baselines for our use of plant waxes as an environmental proxy. Understanding the transport, accumulation, preservation, and isotopic variability of biomarkers in living plants and soils informs our reconstructions when using those recovered in paleosols or marine and lake cores. Fourth, we discuss how plant waxes can shed light on multiple aspects of human evolution through studies that have been conducted in eastern and southern Africa and Europe, the primary regions where biomarker research has thus far been deployed in paleoanthropology. We highlight work conducted at famous paleoanthropological locales, such as at Oldupai Gorge and the Turkana Basin in eastern Africa, and at cave and rock shelter sites occupied by Neanderthals in Europe and *H. sapiens* in South Africa. Finally, we consider the future of plant wax studies in paleoanthropology and the gaps that still need to be addressed when using biomarkers as archeological proxies.

2 | COMPLEMENTARY ADDITION TO MULTI-PROXY STUDIES

The interest in molecular fingerprinting plant biomarkers in archeological[49–55] and paleoanthropological[6–20,22,56] studies (Figure 3), lies in key advantages this technique holds over other proxies. Plant wax $\delta^{13}C$ (Box 2) is not biased toward herbivore feeding behavior like that of tooth enamel, and lipid biomarkers are more widespread than paleosol carbonates which only form in soils where annual rainfall is less than 1000 mm per year.[57] Furthermore, pedogenic carbonates cannot capture short-term climatic and environmental variability like that recorded in plant wax $\delta^{13}C$ because of slow formation processes. While phytoliths track the boundary between woodlands and grasslands, they cannot reliably identify photosynthetic pathways like plant wax $\delta^{13}C$ (Figure 4).[63] The production and dispersal of pollen varies widely between different plant families and genera, and preservation is contingent on anoxic conditions and other parameters.[64] Because of this, well-preserved pollen-bearing sediments are scarce within

Box 1 Plant wax biomarker analysis

While the environmental significance of plant waxes has been reviewed elsewhere within the context of paleoenvironmental and paleoclimatic research,[26-29] their potential for use within archeology remains understated. Biomarkers are complex organic compounds composed of carbon, hydrogen, and other elements that originate from formerly living organisms.[23] They provide information on the type and amount of organic content preserved within source rock or sediment, the organisms and environmental conditions during deposition and burial, and the degree of degradation of the original biological components.[30,31] The most common plant wax biomarkers assessed are long, straight-chain normal (n-) alkanes, n-alkanoic acids, and to a lesser extent, n-alkanols with between 24 and 36 carbon atoms (Figure 2). Plants and other autotrophs produce n-alkanes with a strong odd-over-even predominance, while the n-alkanols and n-alkanoic acids exhibit strong even-over-odd carbon chains.[23] Generally, long-chains (C_{27}–C_{35} n-alkanes, C_{26}–C_{34} n-alkanols/acids) are most abundant in terrestrial plants,[23,32] while submerged and floating aquatic macrophytes contain more mid-chain compounds (C_{21}–C_{25} n-alkanes, C_{20}-C_{24} n-alkanols/acids),[33,34] and short-chains (C_{17}-C_{21} n-alkanes, C_{14}-C_{18} n-alkanols/acids) are dominant in algae.[35,36]

Several metrics have been developed to analyze plant wax compound distribution, abundance, and degree of degradation. Carbon-number range (i.e. C_{25}–C_{35}) and abundance are often used to characterize particular plant groups, even though it is difficult to make chemotaxonomic distinctions between grasses and woody plants.[37] The carbon preference index (CPI) examines the odd-over-even carbon number predominance in n-alkanes or even-over-odd predominance in n-alkanoic acids and n-alkanols. It acts as a qualitative indicator of wax degradation, where a CPI <1 often indicates sample maturity or degradation.[37,38] Average chain length (ACL), or the weight-averaged number of carbon chains, is used (cautiously) as both a vegetation and climate proxy because it has been shown to be higher in C_4 grasses,[39] but can also correlate with higher growing season temperature and aridity.[40] The Submerged/Terrestrial Ratio (STR) differentiates the fatty acids of submerged and terrestrial plants in lake sediments,[35] while the P_{aq} Ratio,[33] the relative proportion of mid-chain to long-chain length n-alkanes, compares the input of submerged/floating aquatic macrophytes into lake sediments against that of emergent and terrestrial plants. The uncertainty associated with some of these qualitative indicators necessitates the application of stable isotope measurements on plant wax biomarkers.

Traditionally, geochemists have sought to recover plant waxes from terrestrial, ocean, and lake deposits to reconstruct past ecosystems and climates. This entails solvent extraction of biomarkers from ancient sediments or rocks followed by qualitative and quantitative profiling through a combination of chromatographic separation and mass spectrometry techniques. Gas Chromatography Mass Spectrometry (GC–MS) and GC Flame Ionization Detection (GC-FID) are the preferred methods for identifying and quantifying n-alkanes and n-alkanoic acids. Isotope ratio analysis (GC-IRMS) measures with high precision, small variations in the relative abundance of carbon, hydrogen, and other isotopes.[41] For carbon, the stable isotopic ratio between carbon-13 and carbon-12 is expressed as δ^{13}C relative to the Vienna PeeDee Belemnite (VPDB) international standard (Equation 1). For hydrogen, the stable isotopic ratio between deuterium (^{2}H) and protium (H) is expressed as δD relative to the Vienna Standard Mean Ocean Water (VSMOW) international standard (Equation 2). Isotope values are expressed using the delta (δ) notation in units of per mil (‰), which reports changes as deviations compared to designated standards.[41]

$$\delta^{13}C = \left[\left(\left(^{13}C/^{12}C \right)_{Sample} \div \left(^{13}C/^{12}C \right)_{Standard} \right) - 1 \right] \times 1000 \qquad (1)$$

$$\delta D = \left[\left(\left(^2H/^1H \right)_{Sample} \div \left(^2H/^1H \right)_{Standard} \right) - 1 \right] \times 1000 \qquad (2)$$

"Enrichment" is the term used to describe the process by which the relative abundance of the heavier isotope increases, making ‰ more positive. "Depletion" on the other hand, is the process by which the relative abundance of the heavier isotope decreases, making ‰ more negative. Please refer to the following publications for expressing and reporting stable isotope ratios,[42,43] and in-depth protocols and discussions on extracting and analyzing plant wax biomarkers and measuring δ^{13}C and δD from n-alkanes and n-alkanoic acids.[44-48]

nonmaritime, specifically arid or semi-arid, regions.[65] In contrast, water insoluble plant waxes are chemically inert and resistant to biodegradation in sediments over deep time following deposition and initial diagenesis.[24,66,67] Because covalently bonded carbon, hydrogen, and oxygen atoms are difficult to degrade without subjecting them to

high temperatures (≥150°C)[68] or catalytic enzyme and chemical activity,[26] the original isotopic content incorporated during biomarker biosynthesis generally remains intact.

Plant waxes do have their own interpretive challenges, however. Wax production varies by taxonomic group and sedimentary archives
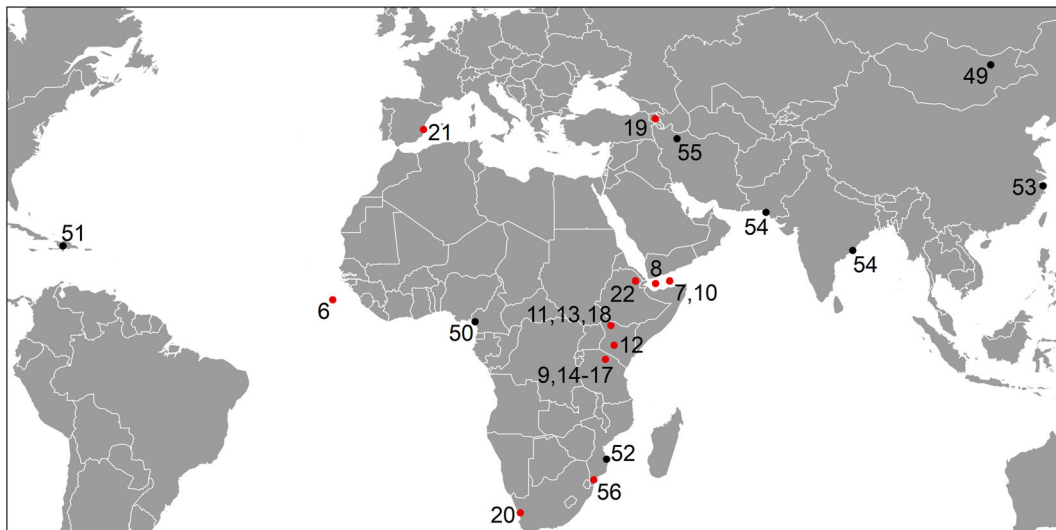
FIGURE 3 Location of plant wax isotope studies used at Holocene (black) and Pleistocene (red) archeological sites. Label at each location refers to reference citation number

are often dominated by specific plant types due to differences among plant lifeforms and physiography.[69] Lipid biomarkers ablated from leaf surfaces can also be transported long distances before deposition,[70] and the size of a catchment area determines the amount of soil-derived waxes that settle in marine or lacustrine sediments.[71] Yet, this allows for both "on-site" and "off-site" plant wax records to be compared and contrasted as regional[7] and local[10] vegetation proxies. Ideally, plant waxes should be studied along with pollen and phytoliths to calibrate interpretive biases stemming from differential wax production and transport histories.

When reported alongside grass pollen and phytoliths, $\delta^{13}C$ efficiently detects C_3/C_4 vegetation shifts. Moreover, plant wax $\delta^{13}C$ allows for higher taxonomic discrimination among the grasses, as pollen cannot establish Poaceae photosynthetic subtypes.[72] Compound-specific isotope analyses on individual n-alkanes (e.g., C_{31}) or n-alkanoic acids (e.g., C_{30}) also erases the ambiguity in isotope measurements inherent in bulk sediment isotope analysis. Lastly, from a sample processing perspective, biomarker analysis is less time consuming than pollen or phytolith extractions, which require extensive preparation and count time; though isotope measurements can become expensive due to the need to analyze $\delta^{13}C$ at least in duplicate and δD in triplicate.[44]

3 | TAPHONOMIC NORMALIZATION

Like any organic material, the preservation of plant waxes is dependent on burial dynamics and resistance to biological decay.[30] Diagenesis is the biological, physical, or chemical alteration of organic matter in sediments prior to significant changes caused by heat or pressure.[31] It is often assumed that diagenesis does not generate significant fractionation at the molecular level when using biomarker isotopes for paleo-reconstructions. Nevertheless, it is important to connect diagenesis to isotope change in any proxy record that relies on the stability of isotopes over geologic time. Studies[96–99] that have isotopically characterized diagenetic processes have had mixed results in identifying carbon and hydrogen alteration through chemical and microbial activity. As research compares leaf, leaf-litter, and soil isotopes, interesting patterns are appearing in post-depositional plant wax $\delta^{13}C$. There is often a + 4.0 ‰ to +6.0 ‰ increase in soil n-alkane and n-alkanoic acid $\delta^{13}C$, attributed to a combination of the Suess Effect ($\leq$+2.0 ‰) and microbial ^{13}C contribution to soil isotope archives (+2.0–4.0 ‰).[71,96]

Several factors can impact plant wax preservation in soils and sediments. There are complex interactions in depositional environments between lipid marker molecules, microbial (fungal and bacterial) communities, pH, alkalinity, temperature, and oxygen and moisture content.[98,100,101] Although the relative importance of these factors differs depending on depositional setting, the quantity and quality of organic matter preserved during burial is influenced greatly by sedimentation rate, grain size, and oxygen available to microbial metabolic processes.[31] Organic matter is preferentially deposited in fine-grained sediments as they exclude oxygen-rich waters and inhibit aerobic microbial biodegradation and detritovore bioturbation.[31] Under well-oxygenated conditions such as in soils however, aerobic microbes can readily degrade organic matter, with some bacteria and filamentous fungi containing n-alkane degrading enzymes that break down long-chain hydrocarbons.[102,103] In archeological research, plant wax preservation/degradation can have major implications on data collection and interpretation. For example, the variable sedimentological profiles exposed throughout Oldupai Gorge have differential plant wax preservation,[17] while paleo-lake drill cores from West Turkana, which targeted ancient lacustrine sediments specifically, identified poor organic matter preservation in well-oxidized deltaic deposits.[11]

Box 2 Interpreting $\delta^{13}C$ and δD of plant wax biomarkers

The $\delta^{13}C$ of all higher plants is a function of (1) the specific photosynthetic pathway (C_3, C_4, or Crassulacean Acid Metabolism); (2) the carbon isotopic composition of atmospheric CO_2, and; (3) the ratio of CO_2 partial pressure inside leaves relative to atmospheric partial pressure CO_2.[73,74] Photosynthetic pathway plays a substantial role in the fractionation of carbon because the observed differences in $\delta^{13}C$ between C_3 and C_4 plants is a function of ^{13}C discrimination, with C_3 vegetation being more depleted by about -15 ‰ on average compared to C_4 plants.[73] The source of this discrimination is the RuBisCo enzyme in C_3 photosynthesis, which discriminates strongly against ^{13}C, whereas the phosphoenolpyruvate (PEP) molecule used in C_4 photosynthesis does not discriminate against ^{13}C to such a large degree.[75] As a result, C_4 plants have a selective advantage over C_3 types in certain contexts given that they have a beneficial adaptation to hot, high-light, water-stressed conditions or during periods of low global atmospheric CO_2 concentrations.[73] This results in bulk leaf $\delta^{13}C$ between -20 to -35 ‰ and -10 to -14 ‰ for C_3 and C_4 plants, respectively. Biosynthesis of plant wax lipids results in further fractionation, with C_3 and C_4 plants being -5.0 to -7.0 ‰ and -8.0 to -10 ‰ lighter than bulk $\delta^{13}C$, respectively. Therefore, at many key African hominin fossil localities, $\delta^{13}C$ is primarily used to interpret changes in C_3 and C_4 vegetation abundance and distribution in response to differences in aridity, water availability, atmospheric CO_2, and other climatic factors.[9,14,17,18,22]

At higher latitudes and altitudes, or in well-watered environments where C_3 plants typically outcompete C_4, the intensity and duration of sunlight,[76] canopy structure and wax production,[77] plant taxonomy,[58] and mean annual precipitation[78] all contribute to changes in sedimentary $\delta^{13}C$. This can make interpreting the environmental effects on n-alkane $\delta^{13}C$ difficult. Chain length (Box 1) can help to constrain some of the variability resulting from taxonomy,[58] but both graminoids and woody angiosperms synthesize abundant C_{29} and C_{31} relative to other n-alkanes.[37] Additionally, gymnosperms are enriched in ^{13}C by $+2.0$–5.0 ‰ compared to angiosperms,[58,72] though the majority of soil and sediment n-alkanes will be dominated by angiosperms possibly due to differences in growth strategies and plant physiology.[69] Plants growing in arid environments have greater water-use efficiency (WUE) and are proportionally enriched in ^{13}C relative to their well-watered counterparts.[73] WUE can be further explored using δD to investigate whether ^{13}C enrichment/depletion correlates with the enrichment/depletion of plant wax ^2H.

Meteoric water[79,80] is the primary source of hydrogen recorded in the δD of terrestrial plant waxes. As water evaporates from the ocean ($\delta D = 0$ ‰) and travels inland to higher altitudes[81] or latitudes[82] with cooler temperatures,[83] deuterium is removed in each successive rainfall. This results in water vapor depleted in ^2H and lower δD rainfall values.[84] In tropical and subtropical regions with clear dry and wet seasons, the "amount effect"[84–86] dictates the isotopic composition of precipitation with depleted ^2H (i.e., more negative δD) corresponding to higher precipitation rates. Wet season precipitation is therefore depleted in ^2H compared to dry season rain. Latitudinal transects from western Africa[79] and Europe[87] show that long-chain n-alkane (C_{29} and C_{31}) δD is an overall excellent proxy for source water δD. Changes between moisture source (i.e., Indian vs. Atlantic Ocean[80,88]) and in ocean circulation and sea surface temperature patterns also modulate atmospheric moisture transport across tropical Africa.[89] In arid or semi-arid areas, an enrichment in sedimentary ^2H (i.e., higher δD) can indicate increases in evapotranspiration. Evapotranspiration is amplified by high temperatures, rainfall seasonality, or low mean annual precipitation,[90] which concentrate deuterium in plant leaf water.[91]

Isotopic discrimination during plant growth and leaf formation leads to additional changes in lipid δD. Plant lifeform (i.e., tree, shrub, or grass) has been linked to δD fractionation as leaf size and shape and water-use efficiency result in different degrees of evapotranspiration.[92,93] A global analysis of available plant wax data has shown that grasses possess lower δD values (Avg. $\delta D = -156$ ‰) relative to trees and shrubs growing in the same environment (Avg. $\delta D = -120$ ‰) because of differences in water absorption systems.[93,94] This is due to the hydrogen isotopic composition of plant leaf tissue being affected by two major fractionation processes; evapotranspiration in both soil- and leaf-water, which enriches leaf water ^2H, and enzymatic reactions during wax biosynthesis which depletes organic products through hydrogen exchange.[29] The sum of these chemical and physical fractionation processes can result in large declines in δD of up to -150 ‰ or more, depending on leaf size and shape and water source. Combining plant wax $\delta^{13}C$ and δD can help identify possible biases in interpreting δD in samples from archeological paleosols, specifically if δD variations derive from changes relating to vegetation type.[95]

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4 | ESTABLISHING THE PLANT WAX ECOLOGY OF MODERN AFRICAN SOILS

To interpret plant wax proxies for archeology and paleoanthropology, we need to understand wax preservation and distribution in modern ecosystems. Over the past 30 years, multiple studies have used different environmental proxies to identify extant ecosystems as ecological baselines for ancient hominin habitats in eastern Africa.[1,104–106] Additionally, plants grown in greenhouses and those from specific biomes[58–60] have aided in isotopically characterizing biomarker $\delta^{13}C$ and δD synthesized under specific environmental stimuli. In Africa, field studies[37,39,61,62] have shown that the C_{31}

(a)

(b)

C$_3$ dominates   Soil $\delta^{13}C_{n-alkanes}$ (‰ vs. VPDB)   C$_4$ dominates

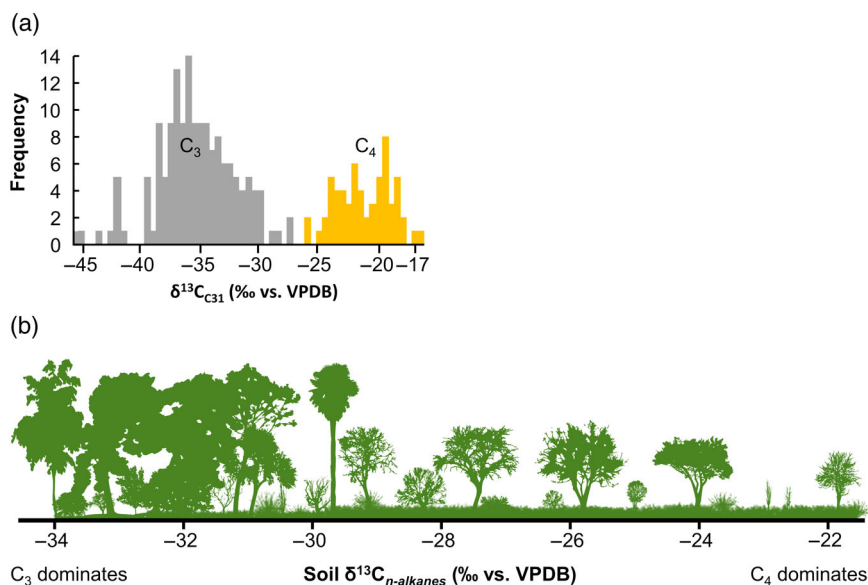**FIGURE 4** (a) The distribution of *n*-alkane C$_{31}$ δ^{13}C for C$_3$ and C$_4$ plants show that compound-specific isotope analyses can distinguish between the two photosynthetic pathways, a major advantage of plant wax biomarkers. 71% of the dataset comes from African plants.[39,58–62] (b) Schematic depiction of a reconstructed C$_3$-to-C$_4$ transitional landscape. Soil *n*-alkane δ^{13}C is largely influenced by plant communities (e.g., C$_3$ vs. C$_4$, open grassland vs. closed woodland), with C$_3$ habitats having lower δ^{13}C compared to C$_4$

homolog is often the most abundant compound in African wooded grassland vegetation, with C$_3$ trees and shrubs and C$_4$ grasses having C$_{29}$ and C$_{33}$ as their second most prevailing compounds, respectively. The δ^{13}C of the C$_{31}$ *n*-alkane is a reliable proxy for C$_3$/C$_4$ terrestrial vegetation composition as other autotrophs only produce it in relatively minor quantities.[107]

In some environments like the arid and semi-arid regions of southern Africa (i.e., Succulent Karoo and Fynbos), modern biomes may be distinguished through the yields and distributions of plant wax and soil *n*-alkane chain lengths.[108] On the other hand, woody angiosperms worldwide are typically characterized by an abundance of C$_{29}$, so distinguishing between different habitat types can be challenging with *n*-alkane homologs alone.[37] The C$_{33}$ and C$_{35}$ alkanes seem to differentiate graminoids from woody plants, but this too may reflect differences in biomes rather than specific plant type.[37] Therefore, more local or biome-specific biomarker studies are needed to guide context-specific interpretations of Pleistocene human ecology.

Paleo-reconstructions often employ percentage mixing models[1,14,18] to interpret ancient plant landscape changes. These models assume that the relative proportion of plant waxes in sedimentary archives correspond to the relative proportion of C$_3$ or C$_4$ plants on the landscape. However, some plants are over−/underrepresented in sedimentary δ^{13}C records due to differences in the total production of waxes between species (Box 1). It may also not be possible to know which species have disappeared in a specific study area due to recent climatic or human activity. There has also been a wide range of end-member values used to demarcate pure C$_3$ or C$_4$ habitats, and the predicted percentage of each plant type can change significantly depending on the value applied. Mixing model endmembers are frequently established using *n*-alkane δ^{13}C of living plants from global datasets even though local or biome-specific C$_3$ and C$_4$ δ^{13}C end-members[18,62,71] would generate a better estimate of vegetation composition. Yet, modern ecological studies are often foregone due to the drive to produce archeologically-significant isotope

data. Submerged and emergent plants can also produce *n*-alkanes with δ^{13}C values that overlap with those of terrestrial C$_4$ plants.[107] Caution must therefore be taken when analyzing *n*-alkane δ^{13}C from paleolake sediments as the positive shift induced by some aquatic or wetland plants can confuse interpretations of relative C$_3$/C$_4$ abundance. Because of the ambiguity in δ^{13}C from different combinations of vegetation types and habitats, it is best to report δ^{13}C values instead of just percentage C$_4$.

5 | PLANT WAXES SHED LIGHT ON MULTIPLE ASPECTS OF HUMAN EVOLUTION

Environmental change and hominin evolution are inextricably linked and there is an increasing focus on the degree to which the adaptability of *H. sapiens* to diverse environments compares to that of our ancestors and closest living relatives.[109] Fine-scale spatial and temporal plant wax data obtained from Plio-Pleistocene aged paleontological and archeological localities can help place human adaptive responses in a long-term climatic and environmental context. This is particularly useful when ecological variability is studied alongside stone tool assemblages, the presence or absence of hominin-modified bone, or instances of species turnover. In eastern Africa for example, relatively precise geochronology of archeological sequences and good preservation of wax biomarkers in paleo-lake sediments have allowed for studies on short- and long-term climate variability,[9] changes in response to aridity or humidity,[14] and plant landscape dynamics.[18]

5.1 | Orbital forcing and hominin ecology

The effects of orbital forcing (eccentricity, obliquity, precession) reorganized eastern and southern African plant landscapes, resulting in cyclical patterns of increasing and decreasing plant wax δ^{13}C and

δD. Changes in northeastern African flora have been attributed to variability in precession.[7,10,110] This may have influenced human evolution and adaptability by controlling local water availability[109,111] and contributing to biome diversification and key speciation and dispersal events in eastern Africa.[112] Environmental variability may also increase the adaptive versatility of organisms and their capacity to adjust to new habitats or geographic regions.[111] Plant wax δD and δ^{13}C have been used to investigate the timing and nature of changes in hydrology and vegetation cover, and the relationship to hominin species turnovers,[10,11] the appearance of new stone tool technologies,[13] the ability to control fire,[19,20] and hominin dispersals out of Africa.[6,8]

Carbon and hydrogen isotope data from East (3.45–3.40 Ma Wargolo Formation[10]) and West (1.9–1.4 Ma Nachukui Formation[11]) Turkana paleo-lake sediments suggest that precession-controlled summer insolation was the dominant driver of Pliocene and Pleistocene hydrology in the Turkana Basin. Fluctuations between very wet (lower δD) and very dry (higher δD) conditions advocate for cyclical changes in summer insolation in directing hydroclimate. Large variance in δ^{13}C and δD around 1.78 Ma coincides with high orbital eccentricity,[13] as well as the earliest known Acheulean tools at 1.76 Ma[113] and the last appearance dates for *Homo habilis* (Figure 5). These biomarker records highlight how dynamic ecosystems of mixed C$_3$ and C$_4$ species were an important aspect of northeastern African vegetation change during the past 4 million years and how such environmental complexity may have necessitated adaptive versatility in hominins.[111]

In southeastern Africa, C$_{31}$ *n*-alkane δD and δ^{13}C were analyzed alongside other proxies to investigate the hydrological context of the environment of *Paranthropus robustus* and to identify long-term regional climate.[56] Low-latitude insolation forcing and changes in ice volume at high latitudes likely drove southeastern African hydroclimate over the past 2.0 Ma, with sea surface temperatures having a secondary role on humidity-aridity cycles.[56] More-humid conditions observed between 2.0 and 1.75 Ma are characterized by the presence of both *Paranthropus* and *Australopithecus sediba* in the Limpopo River catchment, both of which lived in a wooded habitat within a mosaic, but generally open, C$_4$-dominated environment. Woodlands or wetlands in the catchment were replaced however as tropical rainfall contracted toward the equator in response to ice sheet expansion. These wooded habitats shrank during dry climatic periods, limiting both species' range and available food-resources. Thus, the long-term trend toward increased aridity driven by precession variability potentially contributed to the local extinction of *Paranthropus* in the Limpopo catchment due habitat marginalization.

Orbital-paced changes have also been implicated as a catalyst for "out-of-Africa" migrations throughout the Pleistocene, specifically when humid climates created vegetated, resource-rich avenues for dispersal out of the continent.[114,115] *H. sapiens* repeatedly dispersed within and beyond Africa due to their ability to expand their ranges, exploit new environments, and adapt to changing climatic conditions. Although simple out-of-Africa models are outdated,[116] and *H. sapiens* inhabited numerous environments since the late Pleistocene if not before,[117] alternations between C$_3$ and C$_4$-dominated plant landscapes may have prompted human dispersals.[6,8] Plant wax biomarker data show that human migrations occurred alongside climate deterioration during wet-to-dry transitions or even during periods of sustained aridity, not along vegetated corridors that formed during humid periods as expected.[8] Though the application of biomarkers to human migration research is relatively new, there is great potential to use plant waxes to further investigate the climatic conditions of out-of-Africa events, specifically when applied to the many Pleistocene *H. sapiens* archeological sites being discovered outside of Africa.[118]

5.2 | Plant landscape variability

It can be difficult to align orbital forcing and ecological change directly to human evolutionary processes, especially when using "off-site" sedimentary records. Thus, it is necessary to see the response of local environments to orbital scale changes and at timescales relevant to hominin populations. Large-scale ecological changes, such as the replacement of woodlands and forests with grasslands in eastern Africa, have been implicated in propelling certain developments in human evolution. One of the long-standing assumptions of climatically-driven grassland expansion was the evolution of bipedalism in hominins,[119] which had historically been considered a prerequisite for toolmaking. Wax biomarkers have been used to investigate this "Savannah Hypothesis," or the role of wooded grasslands in human evolution. Grass pollen, the molecular distribution (i.e., an increase in the relative abundance of C$_{33}$ and C$_{35}$ alkanes), and *n*-alkanes and *n*-alkanoic acids δ^{13}C from marine cores in the Gulf of Aden[110] and the Somali Basin[120] show that C$_3$ grasslands had expanded in eastern Africa by 10 Ma, or about 4 million years before the earliest known evidence of bipedality[65,121] and 7 million years

FIGURE 5 Eastern African relative hydroclimate record from plant wax δD. Large variance in δ^{13}C (not-shown) and δD attest to orbital-scale climate oscillations in the Turkana Basin during the Pleistocene from 1.9 to 1.4 Ma. Modified from Lupien et al.[11]
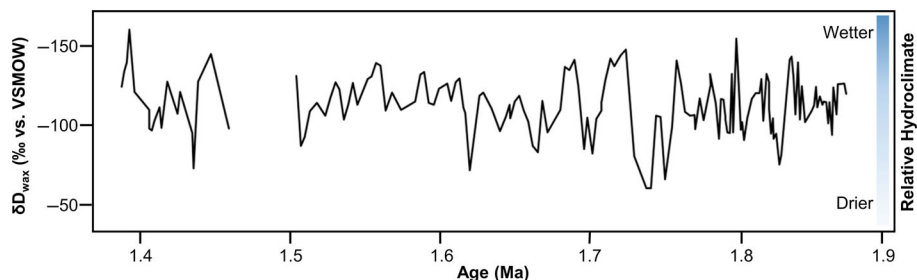
before the earliest stone tools.[122,123] Then from 10 Ma onwards, a steady increase in *n*-alkane δ^{13}C reflects an increase in C_4 grasses[10,120] or xeric shrublands[110] in eastern Africa (Figure 6). However, this was not a gradual process as ecosystem instability in this region included open landscapes being reorganized into closed woodlands and forests and then back to C_4-dominated terrains.[7,10,110] Although the "Savannah Hypothesis" has been criticized and is no longer satisfying as a unifying theory (c.f.[124]), plant waxes showed that grassland expansion was neither linear nor a causal mechanism for bipedalism or stone tool use.

Plant wax data recovered in a lake core and lacustrine outcrops from Oldupai Gorge show changes in regional ecosystems between closed C_3 woodlands and open C_4 grasslands from 1.9 to 1.8 Ma, coinciding with precession cycle summer insolation maxima and minima.[9,14,15] Precession-based fluctuations in rainfall influenced plant ecosystem changes, with drier conditions (Max $\delta D_{C31} = -105$ ‰; Avg. $\delta D_{C31} = -129$ ‰[9]) corresponding to C_4 ecosystems (Avg. $\delta^{13}C_{C31} = -21$ ‰[14]). On the other hand, wetter conditions (Min $\delta D_{C31} = -165$ ‰; Avg. $\delta D_{C31} = -140$ ‰[9]) were associated with increased C_3 woody coverage (Avg. $\delta^{13}C_{C31} = -36$ ‰[14]). Thus, as eastern Africa became drier and C_4 plants were expanding around 2.0 Ma,[110,120] increases in regional precipitation allowed resource-rich, C_3 microhabitats to form. Ecosystem reorganization from forests to grasslands during drier conditions likely enacted selective pressures on Oldupai hominins, such as the need to diversify diet to incorporate edible fruits, leaves, seeds, tubers, insects, meat, or eggs across multiple habitat types.[125]

The δ^{13}C measured on *n*-alkanes and *n*-alkanoic acids from the Nachukui Formation also demonstrate that changes in precipitation were a major driver in C_3 and C_4 plant extent.[18] The carbon isotope record shows that throughout Nachukui deposition, mixed C_3 and C_4, and C_4-dominated (*n*-Acid Max $\delta^{13}C_{C30} = -19$ ‰; *n*-Alkane Max $\delta^{13}C_{C31} = -20$ ‰) landscapes were common, specifically after 2.0 Ma. Higher δ^{13}C likely followed a decrease in mean annual precipitation for the Turkana Basin caused by changes in seasonality toward longer and more intense dry seasons. Yet, the wax biomarker δ^{13}C data also indicate a dynamic plant landscape and available food-resources for *Paranthropus* and *Homo* in this part of the Turkana Basin. Tooth enamel and plant wax δ^{13}C show that the increase in C_4 plants coincided with *Paranthropus* becoming a C_4 specialist, while the diet of *Homo* remained diverse, a defining feature of the genus.[18,126]

5.3 | Hominin targeted ecotones

In subtropical and tropical regions, perennial freshwater sources can foster the development of forests or woodlands as microhabitats even when dry regional climates do not support closed ecosystem types.[104] Elements of ecotonal selection on the part of our ancestors can also be identified using biomarker analyses. At Oldupai Gorge, hominin landscape use patterns were inferred from plant wax distributions at a spatial scale.[16,17] Across the ~1.84 Ma FLK *Zinj* paleo-landscape, a combination of *n*-alkanes, phenol derivatives of lignin which distinguishes woody from herbaceous plants, and fern and sedge biomarkers which demarcate wetlands, revealed the geographic distribution of different paleo-habitats.[16] Through these specific biomarkers, abrupt changes from wetland vegetation, to dense C_3 woody coverage (Min $\delta^{13}C_{C31} = -36$ ‰[16]), to open C_4 grassland (Max $\delta^{13}C_{C31} = -21$ ‰[16]) were identified at meter-level scales, showing that the FLK *Zinj* site was a forest microhabitat adjacent to a wetland situated within a greater grassland catchment.[16] Additionally, *n*-alkanoic acids recovered from clays directly in contact below marker Tuff IF (1.8 Ma) track plant landscape heterogeneity over a 2 km transect throughout Oldupai Gorge,[17] including the Oldowan-bearing FLK North site. Measured *n*-acid δ^{13}C highlight a mosaic environment that included dense woodlands (Min $\delta^{13}C_{C30} = -35$ ‰[17]), open grasslands (Max $\delta^{13}C_{C30} = -19$ ‰[17]), and mixed-vegetation ecotones. Lower *n*-alkanoic acid δ^{13}C at FLK North suggests it was a dense woodland dominated by C_3 plants (Avg $\delta^{13}C_{C30} = -34$ ‰[17]). The archeology and paleontology of each site indicate that animals were butchered at FLK *Zinj*[127] while hard-shelled nuts and fruits were processed at FLK N.[128] Though subsistence strategies may have differed, plant wax biomarkers from both locations show that dense woodlands acted as focal points of hominin activity on the Oldupai landscape.

The emergence of the Acheulean and other stone tool industries has been linked to certain evolutionary events like the appearance of *Homo erectus*[129] or to hominin ecological settings.[130] At the 1.7 Ma FLK West site, where the earliest Acheulean at Oldupai Gorge is found,[131] *n*-alkane δ^{13}C and phytolith[132] data show that hominin activity took place in a riparian woodland (Avg $\delta^{13}C_{C31} = -30$ ‰[17]), and that when tree coverage started decreasing (Avg $\delta^{13}C_{C31} = -24$ ‰[17]), so too did stone tool use and the number of human-modified bones (Figure 7).[17] Woodlands at FLK West likely offered an
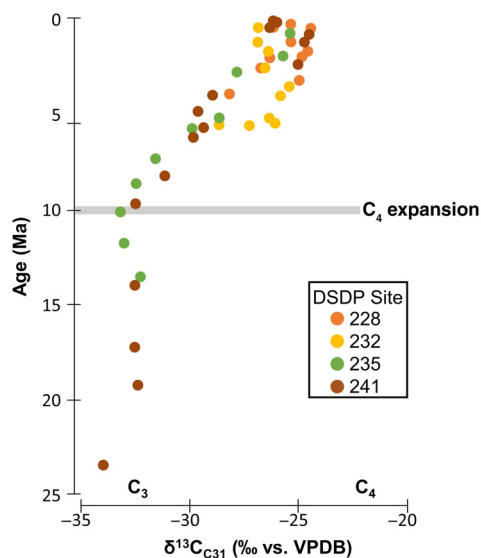

FIGURE 6 C_{31} *n*-alkane δ^{13}C indicate C_3 dominance from 24 to 10 Ma. After 10 Ma, the increase in δ^{13}C reflects the increasing proportion of C_4 vegetation on the landscape. DSDP (Deep Sea Drilling Project) sites suggest a clear expansion of C_4 vegetation in eastern Africa after 10 Ma. Modified from Figure 2, Uno et al.[120]
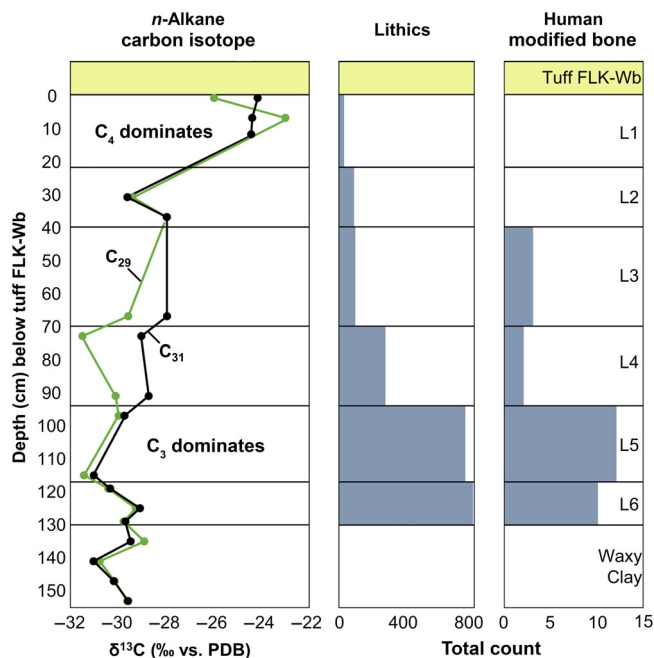
FIGURE 7   *n*-alkane $\delta^{13}C$ data from Oldupai Gorge FLK West plotted against total number of recovered lithics and bones with identifiable cut or percussion marks. The total number of lithics and bones correlate with woody vegetation cover as indicated by $\delta^{13}C$. As the landscape becomes more open, the number of lithics and bones drops significantly. Tuff FLK-Wb dated to 1.664 ± 0.019 Ma; Tuff FLK-Wa (not shown), underlines the waxy clay and is dated to 1.698 ± 0.015 Ma. Lithic data and dating from Diez-Martín et al.[131] Faunal data from Yravedra et al.[133] Modified from Patalano, [17]

advantage to Acheulean hominins in that they would have provided plant foodstuffs and protection from large carnivores or scavengers when butchering animal remains. Handaxes from the site were not used for defleshing and disarticulation of carcasses (this was most likely conducted using quartzite flakes[133]), but conceivably used for bone breaking or digging underground storage organs/tubers.[134] Therefore, the site-specific *n*-alkane $\delta^{13}C$ data from FLK West implies that closed vegetation attracted hominin activity, and that when the site's ecology changed from a closed woodland to a more-open grassland or wooded grassland, activity shifted away from the site as it no longer provided an ecological advantage.

5.4 | Activity areas in caves and rock shelters

In southern Africa and throughout Europe, many hominin fossil localities rich in material culture and organic records are found in caves and rock shelters. Plant waxes preserved in dry cave sediments can provide novel insights into site usage by different members of the genus *Homo*, specifically in response to broad environmental trends.[19–21] At the Middle Stone Age Diepkloof Rock Shelter in South Africa,[20] *n*-alkane concentrations and $\delta^{13}C$ and δD values were analyzed alongside indicators of burning, such as black carbon and polycyclic aromatic hydrocarbons (PAHs), to identify human activity within the rock

shelter. Burning activity indicators were found to be anti-phase to *n*-alkane abundance; that is, lower concentrations of *n*-alkanes were found in stratigraphic units containing evidence for increased burning. Thus, when occupants used fire, *n*-alkanes were preserved only in small quantities in cave sediments. Throughout site occupation, C_{31} *n*-alkane $\delta^{13}C$ only varies slightly (−30 ‰ to −32 ‰[20]). Considering the large range of soil *n*-alkane $\delta^{13}C$ exhibited in the local Fynbos biome,[108] the limited vegetation change in the rock shelter sequence is thought to indicate that specific plant types were being selected for and brought to the site by its occupants. This implies that humans targeted certain plant (e.g., C_3) for use within the rock shelter regardless of hydroclimate, as recorded in δD.

At the Middle Paleolithic site of Lusakert Cave 1 in Armenia,[19] local cave and regional wildfire frequency were distinguished using high (e.g., Benzo[a]pyrene) and low molecular weight PAHs (e.g., Anthracene), with particulate emissions from wood burned in hearths at temperatures ≥600°C corresponding to the high-weight PAHs. This was combined with plant wax $\delta^{13}C$ and δD for vegetation and temperature changes, respectively. Correlations between PAHs, plant waxes, and artifact density attests to an increase in site activity, with highest concentrations of high-weight PAHs coinciding with the densest archeological horizons. As the data show that artifact density is correlated with high weight-PAHs rather than wildfire frequency, Lusakert Cave hominins (possibly Neanderthals) were able to control fire independently of natural wildfires. Long-chain *n*-alkanes have high CPI (Box 1), demonstrating that they did not undergo significant thermal alteration, and are enriched in ^{13}C ($\delta^{13}C$ = −28 ‰), representing more open habitats, associated with the low-weight PAHs produced by wildfires. As there was no correlation between δD and PAHs, wildfire frequency was not determined by changes in temperature, but rather by changes in vegetation, with more-open environments brought on by aridity being prone to burning. These results suggest that pyrotechnology existed among Lusakert Cave occupants regardless of the regional occurrence of natural fires.

Carbon and hydrogen isotope ratios of *n*-alkanes were also utilized in the reconstruction of hydroclimate and vegetation at the Neanderthal Abric del Pastor rock shelter site in southeastern Spain.[21] Because the rock shelter has evidence of multiple, short-term Neanderthal occupation events, plant wax biomarkers attest to Neanderthal response to fluctuations in environmental conditions along the Iberian Peninsula during periods of global climate instability. Co-varying trends between $\delta^{13}C$ and δD were linked to the combined effects of changes in moisture source, precipitation amount, and changes in temperature and evaporative stress. Although *n*-alkane $\delta^{13}C$ show that the shelter sediments were dominated by C_3 vegetation through its occupation ($\delta^{13}C_{C29}$ = −38 ‰ to −32 ‰[21]), there is potential evidence for the burning and subsequent degradation of the *n*-alkanes leading to lower CPI values due to an increase in both short- and mid-chain homologs. Nevertheless, δD and $\delta^{13}C$ co-vary throughout the archeological sequence, suggesting that fluctuations in temperature and evaporative stress act as dominant controls on plant wax $\delta^{13}C$ in the region.[21] Dry and semi-arid conditions coupled with cooler temperatures are thought to have generally dominated the

Iberian Peninsula during the site's usage. Dry woodland formations extended throughout the region, with Mediterranean mixed forest and riverine taxa acting as a refugium near the rock shelter, providing plant food-resources which could be acquired by Neanderthal groups.

6 | WHERE DO WE GO FROM HERE?

The archeological applications of plant waxes for environmental reconstructions are promising given that they have already improved our understanding of hominin adaptive flexibility and responsiveness to environmental changes. The future of this research, however, depends on improving the taphonomic understanding of lipid molecule preservation within archeological sites and increasing the number of biome-specific ecological calibration studies. Additionally, there are several gaps that need to be addressed in plant wax research, such as differences in lipid production, transport and depositional histories, residence times in zones of bioturbation, and microbial diagenesis. Modern calibrations from extant plants and soils in proximity to archeological sites can help reconcile some of these challenges, but they are often omitted from research design.

There are also very few studies that have had the opportunity to correlate biomarker δ^{13}C and δD directly with archeological assemblages. Environmental variability is a catalyst for human evolution, but it can be difficult to assess hominin adaptions to changing physiography at timescales relevant to hominin populations with only off-site environmental records. Site-specific studies that compare plant wax abundances, distributions, and isotope composition to lithic or faunal remains are essential for reconstructing plant landscape variability at high taxonomic resolution, especially when trying to understand the implications of consistent or rapid ecological change on hominin dietary innovations, habitat diversification, or dispersals.

In this review, we focused on plant wax biomarkers in the study of human origins, the role they play in reframing old evolutionary questions, and how they have amended prominent environmental hypotheses for hominin evolution. Given that plant waxes have shown that broad, all-encompassing habitat-specific hypotheses are no longer viable for explaining human evolutionary processes, they are perhaps the best proxy for generating new evidence linking environmental variability with human technology and behavior. As a potential high-resolution proxy, δ^{13}C and δD provide insight into vegetation composition and environmental and climatic stresses and hominin adaptive responses. With the frequent, high-impact discoveries being made in paleoanthropology, specifically with advances in proteomics, ancient DNA, and new dating methods, now is the opportune time to apply plant wax isotope analyses to archeological sites around the world to expand our knowledge of human-environment evolutionary relationships.

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