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, 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, paleo-lake drilling projects, archeological and geological outcrops, cave deposits, and hominin-bearing sediments 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. 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 ($\delta^2$H) 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. Despite their potential for...
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. 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 and paleoanthropological studies (Figure 3), lies in key advantages this technique holds over other proxies. Plant wax δ¹³C (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. Furthermore, pedogenic carbonates cannot capture short-term climatic and environmental variability like that recorded in plant wax δ¹³C because of slow formation processes. While phytoliths track the boundary between woodlands and grasslands, they cannot reliably identify photosynthetic pathways like plant wax δ¹³C (Figure 4). The production and dispersal of pollen varies widely between different plant families and genera, and preservation is contingent on anoxic conditions and other parameters. 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 paleo-climatic research, 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. 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. 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.

Generally, long-chains (C_{25}-C_{35} n-alkanes, C_{26}-C_{34} n-alkanols/acid) are most abundant in terrestrial plants, while submerged and floating aquatic macrophytes contain more mid-chain compounds (C_{21}-C_{25} n-alkanes, C_{20}-C_{24} n-alkanols/acid), and short-chains (C_{17}-C_{21} n-alkanes, C_{14}-C_{18} n-alkanols/acid) are dominant in algae.

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. 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. 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, but can also correlate with higher growing season temperature and aridity.

The Submerged/Terrestrial Ratio (STR) differentiates the fatty acids of submerged and terrestrial plants in lake sediments, while the P_{aq} Ratio, the relative proportion of mid-chain to long-chain length n-alkanes, compares the input of submerged/float 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. 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.

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

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

“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, 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.

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nonmaritime, specifically arid or semi-arid, regions. In contrast, water insoluble plant waxes are chemically inert and resistant to biodegradation in sediments over deep time following deposition and initial diagenesis. Because covalently bonded carbon, hydrogen, and oxygen atoms are difficult to degrade without subjecting them to high temperatures (≥150 °C) or catalytic enzyme and chemical activity, 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...
are often dominated by specific plant types due to differences among plant lifeforms and physiography. Lipid biomarkers ablated from leaf surfaces can also be transported long distances before deposition, and the size of a catchment area determines the amount of soil-derived waxes that settle in marine or lacustrine sediments. Yet, this allows for both “on-site” and “off-site” plant wax records to be compared and contrasted as regional and local 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, δ^{13}C efficiently detects C3/C4 vegetation shifts. Moreover, plant wax δ^{13}C allows for higher taxonomic discrimination among the grasses, as pollen cannot establish Poaceae photosynthetic subtypes. 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 δ^{13}C at least in duplicate and δD in triplicate.

3 | TAPHONOMIC NORMALIZATION

Like any organic material, the preservation of plant waxes is dependent on burial dynamics and resistance to biological decay. Diagenesis is the biological, physical, or chemical alteration of organic matter in sediments prior to significant changes caused by heat or pressure. 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 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 δ^{13}C. There is often a +4.0 ‰ to +6.0 ‰ increase in soil n-alkane and n-alkanoic acid δ^{13}C, attributed to a combination of the Suess Effect (≤ +2.0 ‰) and microbial δ^{13}C contribution to soil isotope archives (+2.0–4.0 ‰). 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. 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. Organic matter is preferentially deposited in fine-grained sediments as they exclude oxygen-rich waters and inhibit aerobic microbial biodegradation and detritivore bioturbation. 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. 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, while paleo-lake drill cores from West Turkana, which targeted ancient lacustrine sediments specifically, identified poor organic matter preservation in well-oxidized deltaic deposits.
Box 2  Interpreting δ13C and δD of plant wax biomarkers

The δ13C of all higher plants is a function of (1) the specific photosynthetic pathway (C3, C4, or Crassulacean Acid Metabolism); (2) the carbon isotopic composition of atmospheric CO2, and; (3) the ratio of CO2 partial pressure inside leaves relative to atmospheric partial pressure CO2.73,74 Photosynthetic pathway plays a substantial role in the fractionation of carbon because the observed differences in δ13C between C3 and C4 plants is a function of 13C discrimination, with C3 vegetation being more depleted by about −15 % on average compared to C4 plants.73 The source of this discrimination is the RuBisCo enzyme in C3 photosynthesis, which discriminates strongly against 13C, whereas the phosphoenolpyruvate (PEP) molecule used in C4 photosynthesis does not discriminate against 13C to such a large degree.75 As a result, C4 plants have a selective advantage over C3 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 CO2 concentrations.73 This results in bulk leaf δ13C between −20 to −35 % and −10 to −14 % for C3 and C4 plants, respectively. Biosynthesis of plant wax lipids results in further fractionation, with C3 and C4 plants being −5.0 to −7.0 % and −8.0 to −10 % lighter than bulk δ13C, respectively. Therefore, at many key African hominin fossil localities, δ13C is primarily used to interpret changes in C3 and C4 vegetation abundance and distribution in response to differences in aridity, water availability, atmospheric CO2, and other climatic factors.9,14,17,18,22

At higher latitudes and altitudes, or in well-watered environments where C3 plants typically outcompete C4, the intensity and duration of sunlight,76 canopy structure and wax production,77 plant taxonomy,58 and mean annual precipitation78 all contribute to changes in sedimentary δ13C. This can make interpreting the environmental effects on n-alkane δ13C 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 C29 and C31 relative to other n-alkanes.37 Additionally, gymnosperms are enriched in 13C 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 13C relative to their well-watered counterparts.72 WUE can be further explored using δD to investigate whether 13C enrichment/depletion correlates with the enrichment/depletion of plant wax 2H.

Meteoric water79,80 is the primary source of hydrogen recorded in the δD of terrestrial plant waxes. As water evaporates from the ocean (δD = 0 %) and travels inland to higher altitudes81 or latitudes82 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 Africa79 and Europe87 show that long-chain n-alkane (C29 and C31) δD is an overall excellent proxy for source water δD. Changes between moisture source (i.e., Indian vs. Atlantic Ocean80,88) 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,89,90 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. δD = −156 ‰) relative to trees and shrubs growing in the same environment (Avg. δD = −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 δ13C 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

4  ESTABLISHING THE PLANT WAX ECOLOGY OF MODERN AFRICAN SOILS

To interpret plant wax proxies for archaeology 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 biomes58–60 have aided in isotopically characterizing biomarker δ13C and δD synthesized under specific environmental stimuli. In Africa, field studies37,39,61,62 have shown that the C31
homolog is often the most abundant compound in African wooded grassland vegetation, with C₃ trees and shrubs and C₄ grasses having C₂₉ and C₃₃ as their second most prevailing compounds, respectively. The δ¹³C of the C₃₁ n-alkane is a reliable proxy for C₃/C₄ 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 C₂₉, so distinguishing between different habitat types can be challenging with n-alkane homologs alone.³⁷ The C₃₃ and C₃₅ 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 models¹¹,¹⁴,¹⁸ 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 C₃ or C₄ plants on the landscape. However, some plants are under-represented in sedimentary δ¹³C 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 C₃ or C₄ habitats, and the predicted percentage of each plant type can change significantly depending on the value applied. Mixing model end-members are frequently established using n-alkane δ¹³C of living plants from global datasets even though local or biome-specific C₃ and C₄ δ¹³C end-members¹⁸,⁶²,⁷¹ 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 δ¹³C values that overlap with those of terrestrial C₄ plants.¹⁰⁷ Caution must therefore be taken when analyzing n-alkane δ¹³C from paleo-lake sediments as the positive shift induced by some aquatic or wetland plants can confuse interpretations of relative C₃/C₄ abundance. Because of the ambiguity in δ¹³C from different combinations of vegetation types and habitats, it is best to report δ¹³C values instead of just percentage C₄.

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 δ¹³C and
δD. Changes in northeastern African flora have been attributed to variability in precession. This may have influenced human evolution and adaptability by controlling local water availability, and contributing to biome diversification and key speciation and dispersal events in eastern Africa. Environmental variability may also increase the adaptive versatility of organisms and their capacity to adjust to new habitats or geographic regions. Plant wax δD and δ13C have been used to investigate the timing and nature of changes in hydrology and vegetation cover, and the relationship to hominin species turnovers, the appearance of new stone tool technologies, the ability to control fire, and hominin dispersals out of Africa.

In southeastern Africa, C31 n-alkane δD and δ13C were analyzed alongside other proxies to investigate the hydrological context of the environment of Paranthropus robustus and to identify long-term regional climate. 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. 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, C4-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. 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, and H. sapiens inhabited numerous environments since the late Pleistocene if not before, alternations between C3 and C4-dominated plant landscapes may have prompted human dispersals. 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. 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.

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, 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 C33 and C35 alkanes), and n-alkanes and n-alkanoic acids δ13C from marine cores in the Gulf of Aden and the Somali Basin show that C4 grasslands had expanded in eastern Africa by 10 Ma, or about 4 million years before the earliest known evidence of bipedality and 7 million years
before the earliest stone tools.\textsuperscript{122,123} Then from 10 Ma onwards, a steady increase in \(n\)-alkane \(\delta^{13}C\) reflects an increase in C\(_4\) grasses\textsuperscript{10,120} or xeric shrublands\textsuperscript{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.\textsuperscript{7,10,110} Although the “Savannah Hypothesis” has been criticized and is no longer satisfying as a unifying theory (c.f.\textsuperscript{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.\textsuperscript{9,14,15} Precession-based fluctuations in rainfall influenced plant ecosystem changes, with drier conditions (Max \(\delta D_{C31} = -105 \, \%\); Avg. \(\delta D_{C31} = -129 \, \%\)) corresponding to C\(_4\) ecosystems (Avg. \(\delta^{13}C_{C31} = -21 \, \%\)). On the other hand, wetter conditions (Min \(\delta D_{C31} = -165 \, \%\); Avg. \(\delta D_{C31} = -140 \, \%\)) were associated with increased C\(_3\) woody coverage (Avg. \(\delta^{13}C_{C31} = -36 \, \%\)). Thus, as eastern Africa became drier and C\(_4\) plants were expanding around 2.0 Ma,\textsuperscript{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.\textsuperscript{125}

The \(\delta^{13}C\) 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.\textsuperscript{18} The carbon isotope record shows that throughout Nachukui deposition, mixed C\(_3\) and C\(_4\), and C\(_3\)-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 \(\delta^{13}C\) 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 \(\delta^{13}C\) data also indicate a dynamic plant landscape and available food-resources for \textit{Paranthropus} and \textit{Homo} in this part of the Turkana Basin. Tooth enamel and plant wax \(\delta^{13}C\) show that the increase in C\(_4\) plants coincided with \textit{Paranthropus} becoming a C\(_4\) specialist, while the diet of \textit{Homo} remained diverse, a defining feature of the genus.\textsuperscript{18,126}

\subsection*{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.\textsuperscript{124} 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.\textsuperscript{16,17} Across the \(\sim 1.84\) 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.\textsuperscript{16} Through these specific biomarkers, abrupt changes from wetland vegetation, to dense C\(_3\) woody coverage (Min \(\delta^{13}C_{C31} = -36 \, \%\)), to open C\(_4\) grassland (Max \(\delta^{13}C_{C31} = -21 \, \%\)) 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.\textsuperscript{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,\textsuperscript{17} including the Oldowan-bearing FLK North site. Measured \(n\)-acid \(\delta^{13}C\) highlight a mosaic environment that included dense woodlands (Min \(\delta^{13}C_{C30} = -35 \, \%\)), open grasslands (Max \(\delta^{13}C_{C30} = -19 \, \%\)), and mixed-vegetation ecotones. Lower \(n\)-alkanoic acid \(\delta^{13}C\) at FLK North suggests it was a dense woodland dominated by C\(_3\) plants (Avg \(\delta^{13}C_{C30} = -34 \, \%\)). The archeology and paleontology of each site indicate that animals were butchered at FLK Zinj\textsuperscript{127} while hard-shelled nuts and fruits were processed at FLK N.\textsuperscript{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 \textit{Homo erectus}\textsuperscript{129} or to hominin ecological settings.\textsuperscript{130} At the 1.7 Ma FLK West site, where the earliest Acheulean at Oldupai Gorge is found,\textsuperscript{131} \(n\)-alkane \(\delta^{13}C\) and phytolith\textsuperscript{132} data show that hominin activity took place in a riparian woodland (Avg \(\delta^{13}C_{C31} = -30 \, \%\)), and that when tree coverage started decreasing (Avg \(\delta^{13}C_{C31} = -24 \, \%\)), so too did stone tool use and the number of human-modified bones (Figure 7).\textsuperscript{17} Woodlands at FLK West likely offered an
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), but conceivably used for bone breaking or digging underground storage organs/tubers. Therefore, the site-specific n-alkane δ13C 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. At the Middle Stone Age Diepkloof Rock Shelter in South Africa, n-alkane concentrations and δ13C 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, C31 n-alkane δ13C only varies slightly (−30‰ to −32‰). Considering the large range of soil n-alkane δ13C exhibited in the local Fynbos biome, 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., C3) for use within the rock shelter regardless of hydroclimate, as recorded in δD.

At the Middle Paleolithic site of Lusakert Cave in Armenia, 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 δ13C 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 13C (δ13C = −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. 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 δ13C 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 δ13C show that the shelter sediments were dominated by C3 vegetation through its occupation (δ13C = −38‰ to −32‰), 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. Nevertheles, δD and δ13C co-vary throughout the archeological sequence, suggesting that fluctuations in temperature and evaporative stress act as dominant controls on plant wax δ13C in the region.

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 refuge near the rock shelter, providing plant food-resources which could be acquired by Neandertal groups.

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 δ13C and δD directly with archeological assemblages. Environmental variability is a catalyst for human evolution, but it can be difficult to assess hominin adaptations 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, δ13C 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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REFERENCES

[1] Cerling T, Wynn J, Andanje S, et al. Woody cover and hominin environments in the past 6 million years. Nature. 2011;476:51-56.
[2] deMenocal PB. Climate and human evolution. Science. 2011;331:540-542.
[3] Potts R. Environmental hypotheses of hominin evolution. Yearbook Phys Anthropol. 1998;41:93-136.
[4] Trauth MH, Maslin MA, Deino A. Human evolution in a variable environment: the amplifier lakes of eastern Africa. Quat Sci Rev 2010;29:2981-2988.
[5] Ibáñez E. On the connection between paleoclimate and evolution. In: Ibáñez E, Denton G, Partridge L, Burckle L, eds. Paleoclimate and Evolution, with Emphasis on Human Origins. Yale University Press; 1995:24-45.
[6] Castañeda IS, Milotti S, Schefuß E, Lopes dos Santos RA, Sinninghe Damste JS, Schouten S. Wet phases in the Sahara-Sahel region and human migration patterns in North Africa. Proc Natl Acad Sci. 2009;106(48):20159-20163.
[7] Feakins SJ, deMenocal PB, Eglington TI. Biomarker records of late Neogene changes in northeast African vegetation. Geology. 2005;33:977-980.
[8] Tierney JE, deMenocal PB, D. Z. A climatic context for the out-of-Africa migration. Geology. 2017;45(11):1023-1026.
[9] Colcord DE, Shilling AM, Sauer P, et al. Sub-Milankovitch paleoclimatic and paleoenvironmental variability in East Africa recorded by Pleistocene lacustrine sediments from Olduvai Gorge, Tanzania. Palaeogeogr, Palaeoclimatol, Palaeoecol. 2018;495:284-291.
[10] Feakins SJ, Eglington TI, deMenocal PB. A comparison of biomarker records of northeast African vegetation from lacustrine and marine sediments (ca. 3.40 ma). Org Geochem. 2007;38:1607-1624.
[11] Schuh A, Cohen AS. Vegetation change in the Baringo Basin, East Africa across the onset of Northern Hemisphere glaciation 3.3-2.6Ma. Palaeogeography, Palaeoclimatology, Palaeoecology. 2021;570:109426. https://doi.org/10.1016/j.palaeo.2019.109426.
[12] Lepin RL, Russell JM, Feibel C, et al. A leaf wax biomarker record of early Pleistocene hydroclimate from West Turkana, Kenya. Quat Sci Rev. 2018;186:225-235.
[13] Lepin RL, Russell JM, Yost CL, Kingston JD, Deino AL, Logan J, Schuh A. Cohen AS. Vegetation change in the Baringo Basin, East Africa across the onset of Northern Hemisphere glaciation 3.3-2.6Ma. Palaeogeography, Palaeoclimatology, Palaeoecology. 2021;570:109426. https://doi.org/10.1016/j.palaeo.2019.109426.
during the early Pleistocene in the Turkana Basin, Kenya. Quat Sci Rev. 2020;245:106531.

[14] Magill CR, Ashley GM, Freeman KH. Ecosystem variability and early human habitats in eastern Africa. Proc Natl Acad Sci. 2013;110(4):1167-1174.

[15] Magill CR, Ashley GM, Freeman KH. Water, plants, and early human habitats in eastern Africa. Proc Natl Acad Sci. 2013;110(4):1175-1180.

[16] Magill CR, Ashley GM, Dominguez-Rodrigo M, Freeman K. Dietary options and behavior suggested by plant biomarker evidence in an early human habitat. Proc Natl Acad Sci. 2015;113(11):2874-2879.

[17] Patalano R. The Environmental Context of the Earliest Acheulean at Olduvai Gorge, Tanzania. Department of Anthropology and Archaeology, University of Calgary; 2019.

[18] Uno KT, Pollissar PJ, Kahle E, et al. A Pleistocene palaeovegetation record from plant wax biomarkers from the Nachukui formation, West Turkana, Kenya. Philos Trans R Soc B. 2016;371(20150235):1-10.

[19] Brittingham A, Hren MT, Hartman G, et al. Geochemical evidence for the control of fire by middle Palaeolithic hominins. Nat Sci Rep. 2019;9(15368):1-7.

[20] Collins JA, Carr AS, Schefuß E, Boom A, Sealy J. Investigation of organic matter and biomarkers from Diepkloof rock shelter, South Africa: insights into middle age site usage and palaeoclimate. J Archaeol Sci. 2017;85:51-65.

[21] Connolly R, Jambrina-Enríquez M, Herrera-Herrera AV, et al. A multiplex record of palaeoenvironmental conditions at the middle Palaeolithic site of Abric del pastor (eastern Iberia). Quat Sci Rev. 2019;225:106023.

[22] Saylor BZ, Gibert L, Deino A, et al. Age and context of mid-Pliocene plant-derived δ13C in plant remains. Geology. 2012;40:221-249.

[23] Eglinton TI, Eglinton G. Molecular proxies for paleoclimatology. Nature. 2008;455(7212):284-288.

[24] Patalano R, Zech J, Roberts P. Leaf wax lipid extraction for archaeological applications. Curr Protoc Plant Biol. 2020;5(3):e20114.

[25] Edwards RA, Dinenno AF, Freimuth EJ. Extracting the most from terrestrial plant- and algal-derived δ13C and δ18O in algae and submerged plants from the northeastern Tibetan plateau. Org Geochem. 2017;113:17-26.

[26] Liu H, Yang H, Cao Y, Leng Q, Liu W. Inter-molecular variations of fatty acid δ13C in algae and submerged plants from the north-eastern Tibetan plateau. Org Geochem. 2018;122:17-28.

[27] Bush RT, McInerney FA. Leaf wax n-alkane distributions in and across modern plants: implications for paleoecology and chemotaxonomy. Geochim Cosmochim Acta. 2013;117:161-179.

[28] Liu H, Yang H, Cao Y, Leng Q, Liu W. Inter-molecular variations of fatty acid δD in algae and submerged plants from the north-eastern Tibetan plateau. Org Geochem. 2018;122:17-28.

[29] Duan Y, Xu L. Distributions of n-alkanes and their hydrogen isotopic composition in plants from Lake Qinghai (China) and the surrounding area. Appl Geochem. 2012;27:806-814.

[30] Rommerskirchen F, Plader A, Eglinton G, Chikarashii Y, Rullkötter J. Chemotaxonomic significance of distribution and stable carbon isotopic composition of long-chain alkanes and alkan-1-ols in C4 grasses. Org Geochem. 2006;37:1303-1332.

[31] Bush RT, McInerney FA. Influence of temperature and C4 abundance on n-alkane chain length distributions across the Central USA. Org Geochem. 2015;79:65-73.

[32] Bush B. Isotope notation and measurement. In: Fry B, ed. Stable Isootope Ecology. Springer Science+Business Media; 2006:308.

[33] Patalano R, Wang Z, Leng Q, et al. Hydrological changes facilitated by the control of fire by middle Palaeolithic hominins. Nat Sci Rep. 2012;50:36-43.

[34] Liu H, Liu W. Concentration and distributions of fatty acids in algae, submerged plants and terrestrial plants from the northeastern Tibetan plateau. Org Geochem. 2017;113:17-26.

[35] Liu H, Yang H, Cao Y, Leng Q, Liu W. Inter-molecular variations of fatty acid δD in algae and submerged plants from the north-eastern Tibetan plateau. Org Geochem. 2018;122:17-28.

[36] Bush RT, McInerney FA. Leaf wax n-alkane distributions in and across modern plants: implications for paleoecology and chemotaxonomy. Geochim Cosmochim Acta. 2013;117:161-179.

[37] Duan Y, Xu L. Distributions of n-alkanes and their hydrogen isotopic composition in plants from Lake Qinghai (China) and the surrounding area. Appl Geochem. 2012;27:806-814.

[38] Rommerskirchen F, Plader A, Eglinton G, Chikarashii Y, Rullkötter J. Chemotaxonomic significance of distribution and stable carbon isotopic composition of long-chain alkanes and alkan-1-ols in C4 grasses. Org Geochem. 2006;37:1303-1332.

[39] Bush RT, McInerney FA. Influence of temperature and C4 abundance on n-alkane chain length distributions across the Central USA. Org Geochem. 2015;79:65-73.

[40] Fry B. Isotope notation and measurement. In: Fry B, ed. Stable Isotope Ecology. Springer Science+Business Media; 2006:308.

[41] Bond AL, Holbow KA. Reporting stable-isotope ratios in ecology: recommended terminology guidelines and best practices. Waterbirds. 2012;35(2):324-331. 328.

[42] Coplen TB. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Commun Mass Spectrom. 2011;25(17):2538-2560.

[43] Patalano R, Zech J, Roberts P. Leaf wax lipid extraction for archaeological applications. Curr Protoc Plant Biol. 2020;5(3):e20114.

[44] Grice K, de Mesmay R, Glucina A, Wang S. An improved and rapid 5A molecular sieve method for gas chromatography isotope ratio mass spectrometry of n-alkanes (C15-C19). Org Geochem. 2008;39:284-288.

[45] Hilkert AW, Douthitt CB, Schuler HJ. Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. Commun Mass Spectrom. 1999;13:1226-1230.

[46] Nikolova-Damyanova B. Retention of lipids in silver ion high-performance liquid chromatography: facts and assumptions. J Chromatogr A. 2009;1216:1815-1824.

[47] Xu S, Sun Y. An improved method for the micro-separation of straight chain and branched/ cyclic alkanes: urea inclusion layer chromatography. Org Geochem. 2005;36:1334-1338.

[48] Egüez N, Makarewicz CA. Carbon isotope ratios of plant n-alkanes and microstratigraphy analyses of dung accumulations in a pastoral nomadic winter campsite (eastern Mongolia). Ethnarchaeology. 2018;10(2):141-158.

[49] Garín Y, Deschamps P, Ménot G, et al. Early anthropogenic impact on Western central African rainforests 2,600 y ago. Proc Natl Acad Sci. 2018;115(13):3261-3266.

[50] Lane CS, Horn SP, Kerr MT. Beyond the Mayan lowlands: impacts of the terminal classic drought in the Caribbean Antilles. Quat Sci Rev. 2018;10(2):141-158.

[51] Lee KS, Lee SW, Lee JY. Molecular signals of rice cultivation and rice fields. Geochim Cosmochim Acta. 2013;117:161-179.

[52] Ponton C. Aridification of the Indian Subcontinent during the Holocene: Implications for Landscape Evolution, Sedimentation, Carbon Cycle, and Human Civilizations. Joint Program in Oceanography/Applied Ocean Science and Engineering.
Bi X, Sheng G, Liu X, Li C, Fu J. Molecular and carbon and hydrogen isotope fractionation in plants. Phytochemistry. 1981;20(4):553-567.

Ehleringer JR. Carbon isotope ratios and physiological processes in Aridland plants. In: Rundel PW, Ehleringer JR, Nagy KA, eds. Stable Isotopes in Ecological Research. Vol 68. Springer-Verlag; 1989:525.

Yang H, Pagani M, Briggs DEG, et al. Carbon and hydrogen isotope fractionation under continuous light: implications for paleoenvironmental interpretations of the high Arctic during Paleogene warming. Oecologia. 2009;160:461-470.

Suh YJ, Diefendorf AF. Seasonal and canopy height variation in n-alkanes and their carbon isotopes in a temperate forest. Org Geochim. 2018;116:23-34.

Diefendorf AF, Mueller KE, Wing SL, Koch PL, Freeman KH. Global patterns in leaf 13C discrimination and implications for studies of past and future climate. Proc Natl Acad Sci. 2010;107(13):5738-5743.

Garcin Y, Schwab VF, Gleixner G, et al. Hydrogen isotope ratios of lacustrine sedimentary n-alkanes as proxies of tropical African hydrology: insights from a calibration transect across Cameroon. Geochim Cosmochim Acta. 2012;79:106-126.

Levin NE, Zipser EJ, Cerling TE. Isotopic composition of waters from Ethiopia and Kenya: insights into moisture sources for eastern Africa. J Geophys Res. 2009;114(D23306):1-13.

Jaeschke A, Rethemeyer J, Lappé M, Schouten S, Boeckx P, Schefuß E. Influence of land use on distribution of soil n-alkane δD and brGDGTs along an altitudinal transect in Ethiopia: implications for (paleo)environmental studies. Org Geochim. 2018;124:77-87.

Niedermeyer EM, Schefuß E, Sessions AL, et al. Orbital- and millennial-scale changes in the hydrologic cycle and vegetation in the western African Sahel: insights from individual plant wax δD and δ13C. Quat Sci Rev. 2010;29:2996-3005.

Alley RB, Cuffey KM. Oxygen- and hydrogen-isotopic ratios of water in precipitation: beyond Paleothermometry. In: Valley JW, Cole D, eds. Stable Isotope Geochemistry. Vol 43. Mineralogical Society of America; 2001:527-553.

Dansgaard W. Stable isotopes in precipitation. Tellus. 1964;16:436-468.

Ziegler H. Hydrogen isotope fractionation in plant tissues. In: Rundel PW, Ehleringer JR, Nagy KA, eds. Stable Isotopes in Ecological Research. Springer-Verlag; 1989:68-105-122.

Risi C, Bony S, Vimeux F. Influence of convective processes on the isotopic composition (δ18O and δD) of precipitation and water vapor in the tropics: 2. Physical interpretation of the amount effect. J Geophys Res Atm. 2008;113(D19):1-12.

Sachse D, Radke J, Gleixner G. Hydrogen isotope ratios of recent lacustrine sedimentary n-alkanes record modern climate variability. Geochim Cosmochim Acta. 2004;68(23):4877-4889.

Konecky BL, Russell JM, Johnson TC, et al. Atmospheric circulation patterns during late Pleistocene climate changes at Lake Malawi, Africa. Earth Planet Sci Lett. 2011;312:318-326.

Schefuß E, Schouten S, Schneider RR. Climatic controls on central African hydrology during the past 20,000 years. Nature. 2005;437(7061):1003-1006.

Liu W, Wang H, Leng Q, et al. Hydrogen isotopic compositions along a precipitation gradient of Chinese loess plateau: critical roles of precipitation/evaporation and vegetation change as controls for leaf wax δD. Chem Geol. 2019;528(119278):1-10.

Yapp CJ, Epstein S. A re-examination of cellulose carbon bound hydrogen δD measurement and some factors affecting plant-water δD/relationships. Geochim Cosmochim Acta. 1982-49:955-965.

Hou J, D’Andrea WJ, MacDonald D, Huang Y. Hydrogen isotopic variability in leaf waxes among terrestrial and aquatic plants around blood pond, Massachusetts (USA). Org Geochem. 2007;38:977-984.
Liu W, Yang H. Multiple controls for the variability of hydrogen isotopic compositions in higher plant n-alkanes from modern ecosystems. Glob Chang Biol. 2008;14:2166-2177.

Liu W, Yang H, Li L. Hydrogen isotopic compositions of n-alkanes from terrestrial plants correlate with their ecological life forms. Oecologia. 2006;150:330-338.

Collins JA, Scheu E, Mulitza S, et al. Estimating the hydrogen isotopic composition of past precipitation using leaf-waxes from western Africa. Quat Sci Rev. 2013;65:88-101.

Nguyen Tu TT, Derenne S, Largeau C, Bardoux G, Mariotti A. Diagenesis effects on specific carbon isotope composition of plant n-alkanes. Org Geochem. 2004;35:317-329.

Wang N, Zong Y, Brodie CR, Zheng Z. An examination of the fidelity of n-alkanes as a paleoclimate proxy from sediments of Palaeolake Tianyang, South China. Quat Int. 2014;333:100-109.

Zech M, Pedentchouk N, Buggie B, et al. Effect of leaf litter degradation and seasonality on D/H isotope ratios of n-alkane biomarkers. Geochim Cosmochim Acta. 2011;75(17):4917-4928.

Chikaraishi Y, Naraoka H. Carbon and hydrogen isotope variation of plant biomarkers. Geochim Cosmochim Acta. 2011;75(17):4917-4928.

Liu W, Yang H, Li L. Hydrogen isotopic compositions of n-alkanes in soils: a litterbag experiment using 13C labelled leaves. Soil Biol Biochem. 2011;43(11):2222-2228.

Brittingham A, Hren MT, Hartman G. Microbial alteration of the soil system. Chem Geol. 2006;231(3):190-202.

Grimalt JO, Torras E, Albaigés J. Bacterial reworking of sedimentary n-alkanes as a dual indicator of paleoenvironment in the Qinghai-Tibet plateau. Chem Geol. 2006;231(3):264-276.

Barboni D. Vegetation of northern Tanzania during the Plio-Pleistocene: a synthesis of the paleobotanical evidences from Laetoli, Olduvai, and Peninj hominin sites. Quat Int. 2014;322-323:264-276.

Copeland SR. Vegetation and plant food reconstruction of lowermost bed II, Olduvai Gorge, using modern analogs. J Hum Evol. 2007;53:146-175.

Peters CR. Blumenshine RJ. Landscape perspectives on possible land use patterns for early Pleistocene hominids in the Olduvai Basin, Tanzania. J Hum Evol. 1995;29:321-362.

Liu W, Yang H, Wang H, An Z, Wang Z, Leng Q. Carbon isotope composition of long chain leaf wax n-alkanes in lake sediments: a dual indicator of paleoclimate in the Qinghai-Tibet plateau. Org Geochem. 2015;83-84:190-201.

Cominelli M. Dihydrogenase activities in the Acheulean: the 1.7 million-year-old site of FLK West, Olduvai, and associated bones from OGS-6 and OGS-7, Gona, Afar, Ethiopia. J Hum Evol. 2003;45:169-177.

Blumenshine R. Percussion marks, tooth marks, and experimental determinations of the timing of hominid and carnivore access to long bones at FLK Zinjanthropus, Olduvai Gorge, Tanzania. J Hum Evol. 1995;29:21-51.

Dominguez-Rodrigo M. Is the “savanna hypothesis” a dead concept for explaining the emergence of the earliest hominins? Curr Anthropol. 2014;55(1):59-81.

Sponheimer M, Dufour DL. Increased dietary breadth in early hominin evolution: revisiting arguments and evidence with a focus on biogeochemical contributions. In: Helmin J-J, Richards MP, eds. The Evolution of Hominin Diets: Integrating Approaches to the Study of Paleoanthropological Subsistence. Springer; 2009:229-240.

Cerling TE, Manithi FK, Mbua EN, et al. Stable isotope-based diet reconstructions of Turkana Basin hominins. Proc Natl Acad Sci. 2013;110(26):10501-10506.

Osin U, Marchand P, Robineau A, et al. Importance of the Acheulean: the 1.7 million-year-old site of FLK West, Olduvai Gorge (Tanzania): a new early Acheulean site with evidence for human exploitation of fauna. Boreas. 2017;46(4):741-746.

Leakey MD. Olduvai Gorge Vol. 3: Excavations in Beds I and II, 1960–1963. Cambridge University Press; 1971.

Isaac GL. The diet of early man: aspects of archaeological evidence for explaining the emergence of hominids and carnivores. Adv Org Geochem. 1988;13(4-6):741-746.

Peter CR, Blumenshine RJ. Landscape perspectives on possible land use patterns for early Pleistocene hominids in the Olduvai Basin, Tanzania. J Hum Evol. 1995;29:321-362.

Carr AS, Boom A, Grimes HL, Chase BM, Meadows ME, Harris A. Leaf wax n-alkane distributions in arid zone south African flora: environmental controls, chemotaxonomy and palaeoecological implications. Org Geochem. 2014;67:72-84.

Council NR. Understanding Climate’s Influence on Human Evolution. The National Academies Press; 2010.

Feakins SJ, Levin NE, Liddy HM, Sieracki A, Eglington TI, Bonnefille R. Northeast African vegetation change over 12 m.y. Geology. 2013;41:295-298.

Potts R. Hominin evolution in settings of strong environmental variability. Quat Sci Rev. 2013;73:1-13.

Maslin MA, Trauth MH. Plio-Pleistocene east African pulsed climate variability and its influence on early human evolution. In: Grine FE, Fleagle JG, Leakey RE, eds. The First Humans - Origin and Early Evolution of the Genus Homo. Springer Netherlands; 2009:151-158.

Lepre CJ, Roche H, Kent DV, et al. An earlier origin for the Acheulean. Nature. 2011;477(7362):85-85.
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