

Supplementary Materials for

Ancient DNA Reveals a Multi-Step Spread of the First Herders into Sub-Saharan Africa

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Other Supplementary Materials for this manuscript include the following:

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Data S1 (.gz)

Materials and Methods

Permissions and sampling protocols

Permissions to conduct research and to export samples for destructive analysis

Permission to conduct research in Kenya was obtained from the National Commission for Science, Technology, and Innovation (NACOSTI permit P/17/34239/17088 issued to MEP), through affiliation with the National Museums of Kenya (NMK). Permission to export skeletal samples for destructive sampling was issued by the Cabinet Secretary, Ministry of Sports and Heritage.

Permission to conduct research in Tanzania was granted by the Commission for Science and Technology (COSTECH permits 2017-220/221/222-NA-2012-50 issued to MEP/EAS/JO), through affiliation with the National Museums of Tanzania (NMT). Permission to export skeletal samples for destructive sampling was granted by the Division of Antiquities, Ministry of Natural Resources and Tourism (Export License 03/2018/2019).

Permission to conduct research in the Livingstone Museum, Zambia was granted by the Director of the Museum. Permissions to loan and sample skeletal remains for destructive sampling were received from the Director and from the National Heritage Conservation Commission (Permit NHCC/8WR/004/17).

Skeletal sampling protocols

Samples were selected following protocols outlined in detail by (53). In brief:

- 1) Skeletons were chosen for sampling based upon their relevance to archaeological questions, preservation, available context information, and geographic location, among other factors.
- 2) No more than two skeletal tissue samples (bone and/or tooth) were selected per individual. In the vast majority of cases, the second sample, taken as a backup for aDNA or radiocarbon dating, was returned without having been sampled.
- 3) Samples were chosen to maximize aDNA success by preferentially choosing petrous or tooth, or where unavailable, dense postcranial bone; and by using appropriate protections to minimize contamination with modern DNA. Sample choice also sought to minimize negative impacts on osteological collections, for example by choosing samples with an antimere (opposite-side pair), samples that were already fragmentary, and/or samples that did not exhibit informative morphological or life history traits, and did not bear otherwise useful materials such as dental calculus.
- 4) All examined skeletal collections, and all sampling activity, are fully documented in digital and photographic databases, shared with the curating institutions.

All samples from sites that produced usable aDNA are listed in **Table S1**. This table includes samples for which we attempted to extract DNA but failed, as well as samples that were not tested and have since been returned to their collections fully intact. In addition, a number of other sites were sampled from which no aDNA was recoverable (see below). Records of all sampling activity, including details of sampled specimens and their results, have been deposited with their respective curatorial institutions, and can also be obtained by contacting E. Sawchuk or M. Prendergast.

Sample return and curation of derived products

Skeletal tissue samples exported from Kenya for aDNA analysis were repatriated to the National Museums of Kenya between October 2017 and July 2018, with remaining tissues from radiocarbon analyses to be repatriated by the end of 2019. While no intact tissue samples will remain outside the country, remaining powder, DNA extracts, and libraries remain under curation at the Reich Laboratory for Medical and Population Genetics at

Harvard University (Reich Lab), as agreed upon between the laboratory and the Head of Earth Sciences, National Museums of Kenya. All skeletal tissue samples exported from Tanzania will be repatriated to the National Museum of Tanzania in May 2019, in keeping with a Memorandum of Agreement (MOA) between the museum and Reich Lab; this MOA allows for curation of remaining powder, DNA extracts, and libraries in the lab. All skeletal tissue samples used in aDNA analysis from the Livingstone Museum were repatriated to that institution in June 2018, and remaining tissue samples from radiocarbon dating will be repatriated by the end of 2019.

Archaeological site summaries

We provide background on archaeological sites that produced usable ancient DNA samples presented in this paper. We also sampled a number of other sites relevant to questions addressed in this paper, but these failed to produce genome-wide data. These are the Galana Boi Formation sites, Lothagam area sites, Lothagam North, Lowasera, Ileret Stone Bowl Site, Il Lokeridede, Lobo, Aora Pundo, Homa Bay, Kanam Two, Kanam Shell Mound, Siror, Wadh Lang'o, and Gogo Falls. All are located in Kenya. Full details of sites and samples tested can be obtained from the NMK and/or relevant publications.

SASES (Standard African Site Enumeration System) refers to a site indexing system for Africa (49); some sites have not yet been indexed in this system. Latitudes and longitudes are approximate, and have been rounded to the nearest thousandth of a degree; more detailed site locations may be obtained from the curating institutions and/or relevant publications. Altitudes are expressed in meters above sea level (m asl), and are also approximate.

Dates are presented as uncalibrated years before present (BP), and as calibrated years before present (cal BP). Calibrated dates were modeled in OxCal version 4.3.2 (50), employing a uniform prior (U(0,100), as in (54), to model mixture of two curves: IntCal13 (51) and SHCal13 (52).

Cole's Burial

SASES GrJj5a. Lat: -0.442, Long: 36.267, ~1850 m asl. Nakuru County, Kenya.

Cole's Burial is located on the eastern side of Lake Elmenteita and has been classified as a Savanna Pastoral Neolithic site (12). There are two sites by this name: one found by Louis Leakey and another documented by Charles Nelson and Stanley Ambrose in 1976 and given the SASES GrJj5. In the later campaign, human remains were observed in one niche in the highest cliffs above the lake. The bodies were ostensibly broken down prior to interment in order to fit into the crevices, possibly prior to skeletonization, since a proximal femur was observed still in articulation with its pelvis. No diagnostic artifacts were found in association with the burials.

Schepartz (55) reported several fragmentary individuals from this site at the NMK, although only two were complete enough for study. A minimum of three individuals were recovered from the 1976 excavation, based on recovery of three sternal manubria. A tibia from one well-preserved individual (CB1) yielded a date of 2355±150 BP on apatite (2750-2013 cal BP; GX4714-A) and 2500±130 BP on gelatin (2854-2185 cal BP; GX4714-G) (12, p. 215); the other two skeletons were not dated. Stable isotope ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) values are presented for two individuals (56, 57); they are consistent with other PN individuals sampled.

We extracted powder from the left petrous of the complete skull of a well-preserved individual using the cranial base drilling method (CB1.01). A loose upper right central incisor that refit into the maxilla produced a date of 3070±20 BP (3351-3180 cal BP; PSUAMS-4723), considerably older than the previously reported date. This renders Cole's Burial the earliest directly-dated human remains from the PN era, with the exception of Prettejohn's Gully. The collagen $\delta^{13}\text{C}$ value of -4.5‰ for this individual (**Table S3**) and -4.8‰ and -5.0‰ for the previously analyzed sample from GrJj5a (56) are among the highest (least negative) values recorded in Africa and worldwide, indicating diets with high reliance on C₄ plant and

C₄ feeding (tropical grass-feeding, i.e. grazing) animal protein. These values are close to the mean of $-5.7 \pm 0.8\text{‰}$ for the SPN (57).

Deloraine Farm

SASES GqJh6. Lat. -0.183, Long. 35.809, 1998 m asl. Nakuru County, Kenya

Deloraine Farm lies on the southeastern footslopes of Mount Londiani, on the west side of the central Rift Valley. It is a large open-air site with a single thick occupation layer. It was excavated in several campaigns, starting with a single test trench by M. Cohen in 1969 (58), and followed by larger-scale excavations in 1978 by N. Chittick and S. Ambrose (32, 59). In 1987, additional excavations by J. Sutton further explored the Iron Age “Deloraine main site” (renamed Deloraine I & II (47)). Sutton also excavated the more recent Pastoral Iron Age (Sirikwa Hole) occupation of Deloraine III, located one kilometer northwest of sites I & II (47).

Collectively, these campaigns demonstrate that the Deloraine main site is essential to understanding the shift from Stone Age pastoralism to Iron Age mixed herding and farming in the Rift Valley. The main site deposits revealed abundant Iron Age habitation debris: remains of livestock, mainly cattle (notably large in size); relatively sparse obsidian tools, possibly used for a reduced set of specialized tasks; upper and lower grindstones; evidence of iron-working, including finished objects, slag, and tuyeres; and a large and diverse ceramic assemblage that has been the focus of multiple studies (32, 47, 58, 60). This distinctive ceramic assemblage has not been recovered in other occupation sites. While the excavated stone tools have been attributed to the Elmenteitan tradition (32), they are few in number, and merit further technological and geological source chemistry analysis. This sharp decline in lithic production has been linked to the appearance of iron-working. Resemblances between classic Elmenteitan and Deloraine ceramic assemblages are primarily seen in position and size of lug handles. This similarity was interpreted as evidence for continuity of the Elmenteitan PN into the Later Iron Age (32). However, several classes of Deloraine vessel forms, types, decoration techniques and motifs are unknown in Elmenteitan ceramic assemblages. Therefore, the interpretation that it marks population continuity of the Elmenteitan PN to the Middle/Later Iron Age remains open to alternatives. Importantly, flotation at Deloraine Farm produced a single grain of carbonized finger millet (*Eleusine corocana*), often cited as the earliest evidence for farming in the Rift Valley, though the grain has not been directly dated (14).

Ten radiocarbon dates were previously reported from Deloraine Farm, though two of these are thought to be unrelated to the occupation described above (32, 47, 58, 59). The other eight dates all coincide, placing the occupation around c. 1185 cal BP, at the chronological boundary between the PN and Iron Age eras in the central Rift Valley and adjacent savanna grasslands.

The first excavations at Deloraine Farm revealed the skeleton of a child at the base of the test trench (58). The child’s skeleton was found in a flexed, upright position, and was encountered in Level 11-12, below the main cultural deposits, without visible evidence of a burial pit. The 1978 excavations also produced human remains of a different individual, but these are only recorded in the faunal table (32). In the NMK, we found fragmentary cranial and postcranial remains of the child from the 1972 campaign, including a nearly complete maxilla and mandible that enabled us to estimate his age at death around six years. We collected a right petrous (DEL.01.01) and a lower left lateral incisor (DEL.01.02) from this individual, but the latter was not sampled. DEL.01.01 produced usable aDNA, and a direct date on this sample confirms the previously-obtained dates on charcoal and fauna (1160±15 BP; 1173-970 cal BP, PSUAMS-4625). We also located the mandible of the individual recovered in 1978, but did not sample, since the only tooth present was firmly in the jaw and sampling it would have caused damage. The collagen $\delta^{13}\text{C}$ value of -4.7‰ for this individual (**Table S3**) and -4.8‰ for the previously analyzed individual from Deloraine (56) indicate diets with high reliance on C₄ plant and C₄ feeding animal protein.

Egerton Cave

SASES GrJh10. Lat -0.375, Long: 35.933, ~2250 m asl. Nakuru County, Kenya.

Egerton Cave is one of several caves along the Njoro River, a few km from the large and well-documented cremation burials at Njoro River Cave (61), on the Mau Escarpment of the western Rift Valley highlands. Excavations by Faugust and Sutton (62) revealed deposits containing obsidian tools, six stone bowls, lower grindstones, and sparse ceramics.

Additional ground stone artifacts were found outside the cave. Faunal remains included a single possible antelope bone and a piece of burnt ivory. Human remains were found in a fragmentary state and were determined to represent at least four individuals: two complete mandibles and two right hemi-mandibles were found near to one another and at the back of the cave, and loose teeth were also recovered in the deposit. Egerton Cave is considered similar in terms of its lithic and ceramic traditions to Njoro River Cave. However, human remains are not cremated nor associated with ochre and elaborate grave goods in Egerton Cave, as is the case in Njoro River Cave (61). The excavators suggested that the burials in Egerton Cave may have been secondary and/or disturbed after deposition (62). In the NMK, we identified fragmentary cranial material including two petrous bones potentially belonging to the same individual, and we did not observe the dentition described by the excavators (62). We sampled the right petrous (EC1.01), and this sample produced usable aDNA.

Egerton Cave is classified as an Elmenteitan PN site on the basis of its location, its overall similarity to other better-documented Elmenteitan PN sites such as Njoro River Cave, and its obsidian and stone bowl artifacts (62, 63). The site was previously undated, though Faugust and Sutton (62) argued for either a date similar to that of Njoro River Cave, c. 3000 BP, or a date younger than 2000 BP on the basis of ceramic similarities to the Iron Age. Sample EC1.01 was dated in the present study to 1880±15 BP (1870-1738 cal BP; PSUAMS-4741), placing Egerton Cave at the later end of the documented timespan for Elmenteitan sites. The collagen $\delta^{13}\text{C}$ value of -5.2‰ for this individual (**Table S3**) is close to the mean of $-5.8 \pm 0.7\text{‰}$ for the Elmenteitan (57), indicating a diet with high reliance on C₄ plant and C₄-feeding animal protein.

Emurua Ole Polos

SASES GvJh122. Lat: -1.396, Long: 35.983, 1630 m asl. Narok County, Kenya.

This burial cairn was salvaged by S. Ambrose, M.D. Kyule, M.C. Kyule, M. Kiiti, J. Munyiri, A. Wangeci, N. Ole Simpai, and J.K. Ole Tomboya in 2010, after it had been disinterred by honey collectors during excavation to expose an underground beehive. The cairn sits downslope from an unexcavated prehistoric settlement, likely PN in age (S. Ambrose, personal observations); an intact cairn is located 20 m north of this cairn. The disturbed cairn is ~3x3 m in area. It had been excavated to a depth of 1 m and partially backfilled with cairn stones and sediments by the honey collectors. The pit and surrounding scatter of displaced boulders and cobbles contained the jumbled remains of teeth and fragmentary cranial and postcranial bones belonging to one individual. Bone preservation was poor, with substantial termite damage to many bones, highly variable weathering, and modern breakage. All exhumed deposits were sieved through 5 mm mesh screens. The only artifacts recovered from sieving of the backdirt pile are a lava side scraper and a possibly ochre-stained grindstone. The association of these artifacts with the skeleton cannot be ascertained. Detailed documentation of this burial can be found at the NMK.

In the NMK, we observed the fragmentary cranium, teeth, and postcrania of one individual, and we collected two samples: a right petrous (GvJh122.01) and a lower right first molar (GvJh122.02); the latter was not sampled. We obtained usable aDNA from the petrous and a direct date of 270±15 BP (421-159 cal BP; PSUAMS-4938). The collagen $\delta^{13}\text{C}$ value of -6.8‰ for this individual indicates a diet with heavy reliance on C₄ plants and C₄ feeding animal protein. The $\delta^{15}\text{N}$ value of +16.3‰ is among the highest reported for East African humans (56, 57), which indicates a diet with very high proportions of animal protein.

Gishimangeda Cave

Lat: -3.476, Long: 35.348, ~1030 m asl. Karatu District, Tanzania.

Gishimangeda Cave is located along the northeastern shores of Lake Eyasi in northern Tanzania, and was one of two sites excavated in 1967 as part of the Kyoto University African Scientific Excavation. According to the limited published report on this excavation (64), a 6x2 m trench revealed three stratigraphic layers: a ~60 cm upper layer with human and animal bones, stone saddle querns, a bowl awl, and pottery sherds (layer 1); a ~120 cm layer containing seven human burials (layer 2); and a ~15 cm cemented calcareous layer with no reported human remains, and with few artifacts, including a bone awl and a grindstone (layer 3). A radiocarbon date on burned animal bone from the upper part of layer 3 suggested an age of 610±260 BP (unclear if calibrated; no lab number reported). The burials were interpreted by the excavators as being just a few centuries old and belonging to local people of the Mang'ola village.

Of the seven primary burials described in the second layer (six adults and one infant, illustrated in the report as Nos. 1-7), five were placed on their left sides, one individual on the right, and one on their back. All of the bodies were flexed. Human remains in layer 1 were not described, despite the fact that many elements are relatively complete. Craniometrics were reported for eight adults and limb measurements for up to 10 adults (based on femora), suggesting that data were collected on remains from layers 1 and 2. Two different labeling conventions are used in the presentation of these data (G-1 and G-01, for example, are different burials). Unfortunately, the labeling conventions were not described, many bones were never labelled, and the skeletons have since become extensively commingled.

Years later, Mehlman (65) compared an unpublished fieldwork report and personal communications with the excavator with the original publication and found serious discrepancies among these records. Mehlman noted that in unpublished reports, layer 2 is in fact subdivided by a calcareous horizon: upper layer 2 contained pottery and an obsidian and quartz lithic assemblage, while lower layer 2 contained the seven burials illustrated (64) as well as a quartz assemblage. He also suggested that burials labeled G-01 through G-05 were from layer 1, while those labeled G-1 through G-7 were from layer 2, and that there may be multiple individuals in some burials. Finally, he argued the burials were likely older than 600 BP due to the depth of deposits and uncertain provenience of the dated animal bone.

While accession records for Gishimangeda Cave at the NMT contain partial layer information, accession numbers, and basic inventory descriptions, they do not include burial numbers and the remains are not physically separated by individual. Accession numbers associated with G1, G3-6, and G01-02 have layer notes in the accession register, however these contradict the above scheme wherein burial numbers containing a "0" are in layer 1 and those without in layer 2 (e.g., G-1 and G-01 are both associated with layer 1). At present, it is not possible to reconstruct the provenience for any of the burials with confidence.

Little research has been conducted on this collection. Ambrose (56, 57) sampled 15 individuals for stable isotope analysis, with results obtained from twelve. He sampled left calcanei to overcome problems with labeling and commingling, so it is not possible to link the isotopic results to specific burial numbers. However, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values suggest three separate clusters of based on dietary signatures. Six individuals cluster with those sampled from Kenyan SPN sites and are interpreted as being reliant upon C4-based plant and animal resources. Two individuals with very high $\delta^{15}\text{N}$ values are interpreted as having a diet dependent upon grazing animals, and possibly upon milk and/or blood. Finally, four individuals cluster near the isotopic ranges of modern-day pastoralists from northern Kenya, with intermediate values suggesting reliance on browsing and grazing animals and C3 plants.

Skeletal analysis at the NMT in 2014 and 2017 found at least 15 individuals present based on cranial and dental remains (66), with more individuals likely present within the extensive assemblage of postcranial fragments. Bone preservation is good, and many elements are relatively complete. Material culture collections from the site include decorated and undecorated pottery and stone tools, though the saddle quern described by (64) was not located. Ceramic sherds represent multiple vessels from very different technological

traditions, including one potentially recent pottery tradition. It is difficult to interpret the associations of this material culture with human skeletons because provenience labels are missing or incomplete.

We collected samples from 15 individuals for ancient DNA analysis, including all labelled individuals (G-1 to G-7 and G-01 to G-03) as well as a second individual from G-2 (G-2A) and two other individuals designated GISH-A and GISH-B by (66). An isolated petrous and a deciduous tooth without accession numbers were given sample ID numbers (e.g., Gishimangeda 2017.01). Powder drilled from the right petrous of G-1 (G1.01) and left petrous of G-5 (G5.01) produced usable aDNA. Usable aDNA was also recovered from isolated petrous bones from G-2, G2-A, G-01, G-02, G-4, G-6, and G2017.01. Teeth from G-3 and GISH-A also produced usable aDNA. This gives a total of eleven individuals with genome-wide data from this site. Similar to Ambrose's isotopic results (56), we observed distinct clusters in genetic data, although it is not possible to match individuals across the two studies.

The eight individuals (G-1, G-2, G-3, G-4, G-01, G-02, GISH-A, and G-2A) that were successfully radiocarbon dated all fall within the later third millennium to early second millennium BP (**Table S3, Figure S1**). For individual G-2A, with a petrous-based date of 2355 ± 20 BP (2402-2309 cal BP; PSUAMS-5653), we note that a high C:N ratio (3.53) may indicate a higher degree of uncertainty for this date; however it fits comfortably within the range of dates of other individuals. All of the dated individuals fall within or near the PN cluster genetically, except for G-3 which was excluded from genome-wide analysis due to low coverage. It is striking that the three individuals that cluster genetically with foragers (G-5, G-6, G2017.01) did not produce sufficient collagen for radiocarbon dating, and also produced low-coverage genetic data. Their poor preservation leads us to speculate that these individuals may represent earlier burial events, even though burials G1 through G7 are supposed to be in the same depositional layer.

It is difficult to evaluate this argument, however, given the limited and conflicting archaeological records discussed above. Individuals in the G-01 to G-05 series, ostensibly from layer 1 according to (65) and thus overlying those of the G1-G7 series, are not younger as would be expected if these stratigraphic positions are secure. Sometimes they are several centuries older than burials in layer 2. This is further supported by a date on a tooth fragment from G-03 previously reported by (66), of 2120 ± 20 BP (2142-2010 cal BP; ISGS-A3612); while not as ancient as the dates obtained in the present study on G-01 and G-02, that of G-03 is earlier than several dated individuals in the purportedly underlying layer.

Other newly reported dates can be compared to those previously reported by (66). Dates based on two different teeth from individual G-3 match almost exactly: 2005 ± 20 BP (1995-1890 cal BP; ISGS-A3614) and 2030 ± 20 BP (1995-1886 cal BP; PSUAMS-5650). The tooth-based date of 1870 ± 20 BP (1865-1725 cal BP; ISGS-A3613) previously reported by (66) as representing individual G-2A does not overlap with the date obtained for that individual in this study. However, further study suggests the unlabeled tooth belongs to a separate individual.

Taken together, the dates suggest several possible sources of error in chronological interpretations. Skeletons may not be labelled correctly; the ties between burial number and layers may have been reported incorrectly by (64); layers 1 and 2 may have been relatively contemporaneous; and/or site formation processes, including disturbance of burials, are more complex than reported. Nonetheless, all dates on human material are significantly older than the burned bone previously dated from the lowermost level, corroborating Mehlman's arguments that the site is much earlier than reported in (64).

Ilkek Mounds

SASES GsJj 66. Lat: -0.603, Long: 36.374, ~1950 m asl. Nakuru County, Kenya.

The Ilkek site is a group of three burial mounds (with a possible fourth) found at the base of the west side of a small rocky ridge beside the old Nairobi-Nakuru road, 600 m south of the abandoned Ilkek railway station. The mounds were designated A, B, and C and were

excavated between 1957 and 1963 (67). Mound A yielded a well-preserved adult male in a contracted position on his left side, facing north. Obsidian flakes and one small undecorated potsherd were found within the burial fill. Mound B was larger, about 7 m in diameter and >1 m above the old land surface, and contained a poorly preserved partial skull, and a cluster of two long bones, some skull fragments, and a partial mandible half a meter away, with teeth scattered in-between. Red ochre, a broken stone bowl and platter, and obsidian flakes were found within the cairn fill but no artifacts were found in direct association with the human bones. Mound C contained powdery traces of human remains within a rocky cavity in the northwest quadrant and two partial long bones. A broken stone platter, pestles, a stone hammer, an obsidian blade, and what may have been a gourd were found with the remains.

A capstone was placed above the human remains in each mound, but the mounds' architecture otherwise varied. Poor preservation of human remains in Mounds B and C, and the position of the bodies in B slightly away from the capstone, were used to argue that those individuals may have died elsewhere and were placed in the cairns later as secondary burials. A fragment of cattle bone (context not reported) produced dates of 2200 ± 130 BP on collagen (2682-1869 cal BP; GX4323-C) and 2040 ± 155 BP on apatite (2348-1618 cal BP; GX4323-A) (68, 69). The mounds were thus presumed to be Pastoral Neolithic based on these dates and the presence of stone bowls similar to those found at Hyrax Hill and Njoro River Cave (61, 70).

We sampled two individuals, Skull I and Skull II, from Mound B. A third individual, described only as a few odd teeth from an adult (67, p. 63), could not be located. Skull I consists of cranial fragments as well as mixed adult and deciduous teeth pressed into plasticine. Although the plasticine limited analysis of root development, this child was 8-10 years old at the time of death (67, Appendix). Skull II consists of a left hemi-mandible with the canine to third molar *in situ*, and five left maxillary teeth set in plasticine (a sixth maxillary tooth was missing) belonging to an adult of undetermined sex. We could not find the relatively complete skeleton of a young adult male from Mound A, nor the fragmentary remains from Mound C. Skull II has yielded a direct date of 1170 ± 15 (1174-981 cal BP; PSUAMS-4942), suggesting that at least Mound B is more consistent with the Pastoral Iron Age. This collagen dating sample has less than half the carbon and nitrogen concentrations of the best-preserved skeletons in **Table S3**, indicating poor protein preservation. This may impact the radiocarbon date, biasing it toward underestimating the true age. Its collagen $\delta^{13}\text{C}$ value of -5.6‰ indicate a diet with high reliance on C_4 plants and C_4 -feeding animal protein. Its $\delta^{15}\text{N}$ value of +13.1‰ is slightly higher than the average for 10 SPN humans (56, 57), which indicates a diet with moderately high proportions of protein of grazing herbivores.

Jawuoyo Rockshelter

Lat: -0.067, Long: 34.667, ~1200 m asl. Kisumu County, Kenya.

This rockshelter is one of six excavated by Gabel (71) near the northern shore of Lake Victoria's Winam Gulf. Deposits contain a microlithic LSA (formerly "Wilton") industry and sparse wild faunal remains, no domestic mammal fauna, and recent ceramics attributed to Luo. Although this site is sometimes linked to the Kansyore tradition of ceramic-using foragers (e.g., 72), this is mainly based upon the shelter's chronology and its proximity to better-documented Kansyore sites (73, 74).

Two individuals were buried together c. 100 cm below surface. They were found lying on their left sides in a semi-flexed position and were not associated with grave goods (71). The skeletons had been gnawed by rodents and crushed by the weight of overlying stones, leaving much of the postcrania fragmentary. The report describes a mostly intact skull and mandible from one individual, an older juvenile, and an intact mandible from a young adult, as the most complete remains. In the NMK, we observed an intact maxilla and mandible of a juvenile associated with cranial fragments (labeled Jawuoyo II), from which we collected a left petrous bone (sample JW2.01), which was sampled but did not produce readable aDNA; and a mostly complete maxilla and other cranial fragments of an adult

(labeled Jawuoyo I) from which we collected a right petrous (JW1.01) that produced readable aDNA, and a left upper lateral incisor (JW1.02) that was not sampled.

A single date of 2040 ± 85 BP (2303-1749 cal BP; GX-1096) was obtained from charcoal in the same stratum as the burial (71). This date agrees with the one obtained on sample JW1.01 in the present study (1895 ± 15 BP, 1878-1745 cal BP; PSUAMS-4783). The collagen $\delta^{13}\text{C}$ value of -14.3‰ for this individual is significantly more negative than other individuals analyzed, and indicates a diet with lower reliance on C_4 plants and C_4 feeding animals. The $\delta^{15}\text{N}$ value of $+10.3\text{‰}$ is also relatively low compared to that of SPN individuals (56, 57), which indicates a diet with relatively low proportions of animal protein.

Kasiolo 2

SASES GvJh54. Lat: -1.326, Long: 35.939, 1658 m asl. Narok County, Kenya.

This site was identified by Leslea Hlusko during paleontological survey in the Lemudong'o Formation on Kasiolo Hill and was excavated by Stanley Ambrose and colleagues at the NMK in 2002 in a rescue operation. It is located on the steep western slopes of the hill, above the Ntuka Valley. Cranial parts were found eroding downslope, and the skeleton was in danger of further erosion. Skeletal remains were found to be intentionally buried underneath boulders in a shallow depression in popcorn clays. There were no visible associated artifacts, nor a cairn. The skeleton was hyper-flexed on its right side, and was missing its feet. While preservation varied widely, much of the bone appeared unweathered and had a fresh appearance. Detailed documentation and an inventory of the human remains from this burial can be found at the NMK.

In the NMK, we observed fragmentary cranial and postcranial remains of one individual, and we collected an upper central incisor (GvJh54.01) and a metacarpal (GvJh54.02); the latter was not sampled. The incisor produced usable aDNA and a direct date of 1110 ± 15 BP (1056-937 cal BP; PSUAMS-4942), placing the burial at the beginning of the Pastoral Iron Age (PIA). The carbon and nitrogen isotope values for this individual are similar to those of other PN and PIA individuals, indicating high levels of protein from C_4 plants and/or C_4 -grazing herbivores.

Keringet Cave

SASES GrJg4. Lat: -0.358, Long: 35.699, ~2800 masl. Nakuru County, Kenya.

Keringet Cave is located near Molo in the western Rift Valley highlands. It was initially excavated by Richard Wright in approximately 1960 (67), and materials from this site are also curated under the name "Wright's Cave" at the NMK. It has also been previously reported as "Kean-Hammerson's Cave," after the landowner (75). Brown (67) reports that Wright uncovered both cremated and non-cremated burials, and artifacts similar to those of Njoro River Cave (61), including beads, pendants, and grindstone artifacts. While little is reported, Wright's excavation must have been extensive and/or the site must have been disturbed, since Cohen (63) reports that when he visited the site, he could only cut a trench 1.5 m wide in the intact deposit.

Cohen's excavation took place in the same month as his test excavation at Deloraine Farm (58). In the NMK, there are human remains in a bag with a tag reading "Wright's Cave" in a tray that is otherwise labeled Deloraine Farm, and there are unbagged, unlabeled, and relatively complete but fragmentary crania in the same tray. This led us to mislabel samples from these crania (DEL.02.01 and DEL.03.01) as Deloraine Farm, but further study reveals these are definitively not from Deloraine Farm, and are possibly from the 1969 Wright's Cave/Keringet Cave excavations. That the crania are not from Deloraine Farm is supported by publications of that site, and this has been confirmed by the excavators (M. Cohen and S. Ambrose). The identification of these crania with Wright's Cave/Keringet Cave is supported by the surface appearance and preservation of the bones and teeth, and by the direct radiocarbon date obtained from sample DEL.03.01 (see below).

Cohen (63) reported recovering the remains of six or seven individuals, all fragmentary, in a disturbed deposit that also contained small numbers of beads, a stone bowl,

a pestle rubber, and undecorated ceramics. Two charcoal samples from the disturbed deposit containing the burials provided dates of 2430±110 BP (2751-2180 cal BP; N-654) and 2050±110 BP (2310-1744 cal BP; N-655). A third charcoal sample was taken from the intact, undisturbed deposit into which the burials were cut, and this gave a date of 2910±115 BP (3343-2783 cal BP; N-653).

In the NMK, we located highly fragmentary skeletal material variously labeled “R. Wright’s Molo Cave Crematorium,” “R. Wright’s Excavation Molo-Keringet Caves,” “Wright’s Cave,” and “Keringet Cave.” We concluded that together these remains represent six unique individuals, and we suspect these all come from the same site. Due to problems with label legibility, however, we are not fully confident about this. Samples of petrous and tooth were collected following the naming conventions on each tray or bag (see **Table S1**). Two of these samples produced usable aDNA and direct dates: DEL.03.01, an adult right petrous (2465±20 BP, 2701-2361 cal BP, PSUAMS-4716); and KC.01.01, a heavily worn permanent upper right molar (1585±15 BP, 1529-1403 cal BP, PSUAMS-4943). These dates, while substantially different from one another, are reasonably in line with expectations for Keringet Cave based upon the archaeology, which suggests burial during the PN era. Given the reported disturbance of the burials, and the scanty context information for the sampled individuals, we cannot rule out the possibility that the burials span several centuries. The individual represented by sample KC.01.01 has high carbon and nitrogen isotope values indicating high levels of protein from C₄-grazing herbivores. The high δ¹³C value combined with a low δ¹⁵N value of +9.4‰ for the individual represented by sample DEL.03.01 indicates a mainly C₄ plant-based diet with small amounts of animal protein.

Kisima Farm, Site KFR-A5, Porcupine Cave

Lat: 0.458, Long: 36.709, ~1780 m asl. Laikipia County, Kenya.

KFR-A5, or Porcupine Cave, is a granite rockshelter excavated by A. Siiriäinen (76), who noted that the shelter had a long history of occupation up to recent times. An 8 m trench placed inside the cave and excavated to >140 cm yielded extensive stratified archaeological deposits and six (possibly seven) burials. Burial 1 was the most complete, representing an articulated, primary burial of an individual placed within a pit facing southwest. This adult male was found flexed on his left side/partly slumped onto his stomach, with his hands under his chest and his face pointing downwards. Beneath the body was a layer of red ochre. He may have died from being shot in the stomach—an obsidian microlith was lodged in one of his vertebral bodies, and the bone shows no sign of healing (76, p. 167, 77, Fig. 3). This is the only potential example of a violent death known from the PN. Human bone and charcoal from this burial place it between 2490±110 BP (2781-2320 cal BP; HEL-851) and 2830±120 BP (3329-2741 cal BP; HEL-871) respectively, although a bone sample run by a different lab produced a significantly younger date (76, p. 170).

Other skeletons within the shelter were incomplete. Burial 2 consisted of leg bones, a mandible, and other scattered remains (no skull) found underneath a stone ‘cist’ made of gabled rock slabs. Burials 3 through 6 were isolated skulls (one of an infant) found beneath similar cists. The presence of ash throughout the deposits and the stone cist *in situ* over Burial 2 were used to argue that bodies were either compressed to fit in these ‘ovens,’ or that defleshed bones were ‘baked’ in the cists as part of a secondary funeral rite. Burial 7 was identified as a possible burial beneath a large horizontal stone slab but was not excavated. Although Siiriäinen (76) compared the ‘baked’ burials to Elmenteitan cremations at Njoro River and Egerton Caves, the remains are not charred or burned. A radiocarbon date of 2320±160 BP (2749-1984 cal BP; HEL-852) from Burial 2 suggests they date to the PN. We sampled Burials 1 through 5; however, only Burials 1 and 5 yielded DNA. The sample from Burial 1 yielded a radiocarbon date of 2855±20 BP (3033-2864 cal BP; PSUAMS-4510), consistent with the previous dates on bone and charcoal. A date of 2675±20 BP (2842-2741 cal BP; PSUAMS-4717) on Burial 5 suggests the other burials are also PN.

Kisima Farm, Site KFR-C4

Lat: 0.458, Long: 36.709, ~1780 m asl. Laikipia County, Kenya.

Siiriäinen (76) also excavated this cairn located on the northern foot of a kopje about 300m southwest of KFR-A5 (see above). The feature was approximately 3.5m in diameter and 0.5m high and contained two burials. Burial 1 was an articulated skeleton in primary context within the center of the cairn within a shallow pit, laying on its right side with the head to the west. Stones within the pit had crushed the chest and upper torso and displaced the jaw. A radiocarbon date of 760 ± 90 BP (905-546 cal BP; HEL-853) is listed for this individual, ostensibly from human bone (76, p. 170). Burial 2 was reported as a secondary burial of “badly ant-eaten bones and scattered teeth” above Burial 1 between the stones of the cairn (76, p. 170). Unretouched obsidian, quartz, and chert flakes were also found within the cairn; however, no grave goods were in association with either skeleton.

We sampled material from both individuals at the NMK. The third molars of Burial 1 were in the process of developing and the second molar roots were incomplete, suggesting an age at death between 12-15 years. The lower central incisors were removed well before death; the alveoli are completely resorbed. We were not able to estimate sex from the human remains, but full genome data from a lower premolar suggests this individual’s genetic sex is male. The tooth was radiocarbon dated to 1110 ± 15 BP (PSUAMS-4743, 1056-937 cal BP), raising questions about which material was previously dated from this cairn. An isolated left petrous labelled KFR-C4, AB, No 7 is also male and may be from Burial 1, but did not yield whole genome data. Material from Burial 2 consists of isolated tooth crowns and two metacarpals; age and sex could not be estimated, and a single lower molar crown did not yield readable DNA. The high carbon and nitrogen isotope values of all individuals from KFR-A5 and KFR-C4 are similar to those of other PN and PIA individuals, indicating high levels of protein from C₄ plants and/or C₄-grazing herbivores.

Kokurmatakore

SASES GdJn1-6. Lat: 3.132, Long: 37.433, ~390 m asl. Marsabit County, Kenya.

Kokurmatakore consists of a series of stone cairns in the Chalbi Desert east of Lake Turkana. The cairns vary in form, but most are circular mounds 2-10+ m in diameter. Approximately 55 mound cairns, nine rings, and three platforms cairns were documented on a hill by Stiles and Munro-Hay (37). Six were selected for excavation and given SASES numbers GdJn 1 through 6. Bodies within the cairns were found in different positions, orientations, and states of preservation. Sex was undetermined for individuals in cairns 1 and 2, and estimated male for 3-6. All individuals were assessed to be adults. The burials were primary, with the exception of fragmentary human remains from at least one other individual found within cairn 4. Artifacts (mostly stone tools) were present in most cases, but none were interpreted as grave goods. Radiocarbon dates of human bone apatite range from 3460 ± 155 BP for cairn 4 (4149-3365 cal BP; GX-7421A) to 125 ± 120 BP for cairn 5 (434-0 BP; GX-7432A) (37, p. 162). Although apatite dates are considered unreliable (78), this suggests that cairns at Kokurmatakore were potentially constructed over thousands of years.

We sampled human remains from cairns 1, 2, 4, and 6, as well as two individuals labelled GdJn 10 and GdJn 7. Since there is no mention of additional excavations at this site, these may represent mislabeled remains from cairns 3 and 5. Only the individual from cairn 2 yielded readable DNA. Stiles and Munro-Hay (37, p. 154-5) describe the body as that of a tall (~185 cm) middle aged adult, flexed on the right side, oriented with the head to the north, facing west. Sex could not be determined morphologically, but genetic sex is male. Microlithic stone tools were found within the burial fill. Bone apatite from this individual was previously dated to 960 ± 190 BP (1261-562 cal BP; GX-7396A). New attempts to date the remains were not successful, but we interpret this cairn as broadly Pastoral Iron Age.

Laikipia District Burial (GoJI45)

SASES GoJI45. Lat: 0.380, Long: 36.893, ~1700 m asl. Laikipia County, Kenya.

This burial was excavated by Kathleen Ryan and colleagues as part of the NSF-funded project “The Arrival and Expansion of Pastoralist Economies on the Laikipia Plateau” (79). The unpublished site is located just at the northwestern edge of the Ol Jogi Wildlife Conservatory on the Laikipia Plateau.

In the NMK, we identified cranial fragments and isolated teeth of a single individual of unknown age and sex. A left petrous (LDBS.01.01) and right lower first molar (LDBS.01.02) were collected; the latter was not sampled. LDBS.01.01 produced usable aDNA and was radiocarbon dated to 635±15 BP (653-555 cal BP; PSUAMS-4939). This is in line with expectations for a Pastoral Iron Age burial cairn, of which there are many (in addition to Pastoral Neolithic burials) on the Laikipia Plateau (2, 80). The high carbon and nitrogen isotope values of this individual are similar to those of other PN and PIA individuals, indicating high levels of protein from C₄ plants and/or C₄-grazing herbivores.

Naishi Rockshelter

Lat: -0.458, Long: 36.081, ~1820 m asl. Nakuru County, Kenya.

In February 1928, Dr. A.S. Parkinson of the British Museum East African Expedition, along with Mr. A.M. Cooper of the Kenya Forestry Department, collected a series of skulls from a rockshelter along the Naishi River in Elmenteita. The account of their discovery is described by Louis Leakey in Kitson’s (81, p. 19) study of the skulls. The condition of the remains suggested they were a few hundred years old, although a child’s skull and some cattle bones appeared to be more recent. The remains were found in association with four stone bowls, as well as an ellipsoidal pebble interpreted as a pestle. The remains of at least fifteen individuals (based on skulls) were collected from the surface of the cave. Two skulls were presented to Leakey and are curated at the NMK; the rest were sent to England. Leakey himself visited the cave twice in 1928-29 and collected additional remains from the surface but wrote to Kitson that “detailed excavations had to be postponed” (81, p. 19). The remains were never dated but are attributed to the PN based on the presence of stone bowls.

We sampled one tooth from each of the two individuals curated at the NMK; both produced readable aDNA. Radiocarbon dates of 2550±15 (2745-2500 cal BP; PSUAMS-4715) and 2470±15 BP (2701-2365 cal BP, PSUAMS-4624) confirm previous associations with the PN. The high carbon and moderately low nitrogen isotope values of these individuals are similar to those of other Elmenteitan PN individuals from the Mau Escarpment in the central Rift Valley (57), indicating a diet with high amounts of protein from C₄ plants and moderate amounts from C₄-grazing herbivores.

Naivasha Burial Site

Lat: -0.663, Long: 36.410, ~1900 m asl. Nakuru County, Kenya.

Naivasha Burial Site (alternatively referred to as Naivasha Burial Ground) yielded the remains of at least 14 individuals associated with stone bowls. Little is known about the site as it was never formally published. Leakey (82, p. 144) mentioned it in an appendix as “certain burial mounds associated with the ‘Gumban A Culture’” (now subsumed into the PN). He described the human remains as “in such an unsatisfactory condition as to be of little scientific value” (82, p. 44).

Leakey may have been referring to the 1929 excavations by Mr. T. Powys Cobb and Mrs. Creasy of the East African Archaeological Expedition at Old Government Farm in Naivasha. Mary Leakey and colleagues (70, p. 331) described their excavations of a grave yielding a badly preserved female skeleton and stone platter. However, they also referenced excavations at ‘Naivasha Burial Site’ in 1940 that produced a stone platter and eight stone bowls. Brown (67, p. 69) noted the same artifacts from a 1940 excavation but provided no additional information. It is unclear if Mary Leakey returned to the same site as the 1929 expedition and excavated additional mounds, or if the remains come from a separate site.

At least 14 individuals are represented (based on dental remains) at the NMK, with commingled crania and postcrania. Although no published descriptions of the skeletons exist, the bones are numbered by individual burial (up to NV 31, suggesting more individuals may be present). Two individuals were sampled by Ambrose (56) for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$; the burials were described as SPN based on their interment in mounds/cairns. We sampled 8 individuals, with five producing readable aDNA (burials NV 4, 5, 6 and 7, with two discrete individuals, a male and a female, within NV 4). Dates for all individuals range from 2400 ± 20 BP (2483-2342 cal BP, PSUAMS-4784) and 2235 ± 20 BP (2324-2154 cal BP, PSUAMS-4744). These are the first radiocarbon dates generated from the site and confirm its chronology within the PN. The high carbon and nitrogen isotope values are similar to those of other SPN individuals (56, 57), indicating high levels of protein from C_4 plants and/or C_4 -grazing herbivores.

Njoro River Cave II

Lat: -0.389, Long: 35.917, ~2350 m asl. Nakuru County, Kenya.

These remains stem from an undated, unpublished excavation by J. Desmond Clark, and are curated at the Livingstone Museum in Zambia, where Clark served as director. The site is located ~400 m from the better-known Njoro River Cave (curated at the NMK) (61, 83). As at the original Njoro River Cave, artifacts at Njoro River Cave II are consistent with Elmenteitan cultural traditions, and the remains are partially cremated (personal observation). The human remains represent at least fourteen fragmentary individuals labelled according to presumed burial number and/or grid units. We sampled seven individuals, with one producing readable aDNA and a radiocarbon date of 2070 ± 15 BP (2105-1933 cal BP, PSUAMS-4758). This falls significantly later than charcoal dates obtained by Merrick and Monaghan (84) for the original Njoro River Cave (ranging from 3634-2851 cal BP), but is still comfortably within the Elmenteitan cultural period.

Ol Kalou

Lat: ~-0.250 to -0.275; Long: ~36.375; ~2300-2400 m asl. Nyandarua County, Kenya.

There is limited contextual information on this site in the NMK, and it appears not to have been excavated as an archaeological site. Rather, the accession register indicates that this calvarium was donated to the museum by W.F. Delap, Esquire, and was noted as of “Neolithic age?”; this may indicate an accidental find. The NMK was supportive of sampling this individual for aDNA and radiocarbon dating, in the absence of good contextual information, in order to increase the available information about the museum’s collections.

Colonial administrative records indicate that William Francis Delap, Esquire owned land in the area of Ol Kalou (e.g., *Kenya Gazette*, August 7, 1951), and a recent oral colonial history places the farm – Rayetta – along the River Wanjohi, in Nyahururu, approximately 25 km north of Ol Kalou and just northwest of Lake Ol Bolossat (85). A primary school, formerly Rayetta Estate Primary School, may mark its location today. This is one possibility for the burial location. A geological survey also lists W.F. Delap’s property as within the Ol Bolossat Plain, though it does not specify further (86). Conservatively, we suggest that the burial was located within an area bounded by the towns of Nyahururu, the lake, and Ol Kalou, assuming Delap encountered the burial on his own property. This area is just northeast of the Nakuru-Elmenteita Basins, where many PN burials are documented.

The remains curated at the NMK consist of a nearly complete, partially fragmented adult calvarium, without any associated artifacts. We sampled the left petrous of this individual (OK1.01), and it produced usable aDNA, and was dated to 1800 ± 20 BP (1812-1615 cal BP, PSUAMS-4940), confirming initial suspicions of its Neolithic age.

Prettejohn's Gully

SASES GsJi11. Lat: -0.545, Long: 36.106, ~1900 m asl. Nakuru County, Kenya.

Prettejohn’s Gully is a dry streambed incised into the lower footslopes of Mt. Eburu and the Mau Escarpment. It leads into the Nderit River, a seasonal tributary of Lake Nakuru, ~15 km to the north. It stands within a kilometer of the well-known Holocene site of

Gamble's Cave (87). A series of geological trenches were excavated in the gully in 1969 by the University of California Archaeological Research Group, under the leadership of Glynn Isaac. A goal was to establish relationships among lake levels and depositional events across the Nakuru-Elmenteita Basin, as well as to document archaeological sites (88-90). During excavation of geological witness sections in Prettejohn's Gully, a crevice burial was encountered, of which no details are published, though it is highly probable that the crevice was not part of any of the witness sections. Unfortunately, the associated documentation folder was reported missing in the NMK since 1990.

The fragmentary remains of two individuals were recovered along with ~50 lithic obsidian artifacts and some charcoal, according to accession records. Accession records indicate that both individuals were recovered in Unit B. Although the skeletal remains were highly fragmented and commingled (but not cremated), we were able to identify and choose samples from two individuals. Sample PJ.01.01 is a lower left second molar, which the excavators assigned Specimen #21 (Unit B, 115 cm in vertical provenience; it is not clear if this is depth below surface). At a similar depth, obsidian and additional human bone were found. Sample PJ.02.01 is an upper left canine, which the excavators assigned Specimen #82 (Unit C, no depth indicated; notes indicate it may come from the overburden).

The units indicated on bone bags may correspond to the broader Nakuru Basin geological units reported by Washbourn-Kamau (90), where sedimentary Unit B is correlated with the Gamble's Cave early Holocene shoreline, and Unit C is thought to date to the middle Holocene, with a single charcoal date of 3540 ± 210 BP ($4424-3355$ cal BP; N-821) reported from a few kilometers north of the gully (88, p. 1071, 89). However, accession registers suggest that in fact Unit B may overlie Unit C in the GsJi11 excavation. Regardless of their stratigraphic positions, the two individuals in fact produced closely overlapping date ranges, and potentially could have been buried in the same event, as indicated by the direct dates: PJ.01.01 dates to 3670 ± 20 BP ($4084-3891$ cal BP, PSUAMS-4982) and PJ.02.01 to 3640 ± 20 BP ($4063-3855$ cal BP, PSUAMS-4983). Both produced usable aDNA. Their high carbon and moderately low nitrogen isotope values are similar to those of Elmenteitan PN individuals from the Mau Escarpment in the central Rift Valley (57), indicating a high dietary component of C_4 plants and moderate component from C_4 -grazing herbivores.

Crevice burials are commonly found in the south-central Rift Valley, and have often been often associated with the SPN (13); however, in light of the early date of these burials, and in the absence of additional material culture, this seems an unlikely and insecure attribution. Given the early date and the distinctive genetic profiles of these two individuals, we have designated the individuals buried at Prettejohn's Gully as possible early pastoralists.

Rigo Cave

SASES GrJh3. Lat: -0.464, Long: 35.971, ~2300 m asl. Nakuru County, Kenya.

Rigo Cave, located along the Naishi River on the Mau Escarpment, was excavated in 1981 by Simiyu Wandibba and researchers from the National Museums of Kenya (91). Two trenches within the shelter and one on the slope revealed up to a meter of deposits containing Neolithic artifacts such as stone tools, stone bowls, ornaments, and diverse faunal remains including possible domestic caprine bones. The lithic assemblage is Elmenteitan. A well-preserved human skull was found on the surface of the site. The individual lost their teeth well before death, and all the alveoli had resorbed. Combined with evidence of obliterated cranial sutures, they were likely an older adult when they died. The other human remains were isolated, commingled fragments found in all three trenches. Multiple individuals are present among the commingled fragments, but Wandibba (91, p. 87) reports "no clear evidence of formal burial or complete interment." Four pieces of human bone and 56 other fragments of unidentifiable species were burned, but not to the extent as those reported from Elmenteitan cremation sites such as Njoro River Cave. Rodent tooth gnaw marks and carnivore puncture marks were also observed on human and animal bone, suggesting scavenger activity.

We sampled the complete surface cranium via cranial base drilling and selected 6 other isolated human elements for aDNA analysis. Two of the fragments were ultimately from the same individual, but four samples in total were successful. Dates for the complete cranium were based on a hand phalanx that was found in the same tray and had similar preservation traits; however genetic analysis of this poorly preserved sample (RC19.01) is not able to confirm whether it belongs to the same individual as the petrous (RC44.01). All dates from the site fall between 2570 ± 15 BP (2751 - 2509 cal BP, PSUAMS-4946) and 2400 ± 15 BP (2460 - 2345 cal BP, PSUAMS-4724), comfortably falling within the range of the PN. The high carbon and moderately low nitrogen isotope values of these individuals are similar to those of other Elmenteitan PN individuals from the Mau Escarpment in the central Rift Valley (57), indicating high amounts of protein from C₄ plants and moderate amounts from C₄-grazing herbivores.

White Rock Point

SASES GrJb2. Lat: -0.450, Long: 34.321, ~1140 m asl. Homa Bay County, Kenya.

White Rock Point is one of several shell middens excavated by Robertshaw et al. (92) along the southern shore of Lake Victoria's Winam Gulf. The midden contains evidence of a LSA fisher-forager tradition, including abundant fish and shellfish remains, wild mammalian fauna, numerous bone points, and a microlithic industry. Similarly to nearby middens, White Rock Point produced Kansyore ceramics, a widespread and long-lasting Holocene tradition linked to semi-sedentary fisher-foragers in the Victoria Basin, sometimes found coinciding with or underlying early evidence of herding (93, 94).

Fragmentary and commingled remains of multiple human skeletons, perhaps representing secondary burials, were encountered in a disturbed deposit that appeared to be cut into the main shell midden. At least four individuals were identified, and gnawing and/or cutting was observed on the bones. Robertshaw (92) noted that the burial fill contained both Kansyore and more recent (second millennium CE pottery, and suggested the burials might be associated with the latter. A date of 4015 ± 260 BP (5288 - 3728 cal BP; GX-8745) was obtained on mammal bone apatite from two strata below the overburden that contained the human remains.

Radiocarbon dating procedures

A total of 43 samples from 41 individuals producing aDNA were submitted to the Pennsylvania State University (PSU) Radiocarbon Laboratory for radiocarbon dating via accelerator mass spectrometry (AMS) (**Table S3**). As a precaution we removed possible contaminants (conservants/adhesives) by sonicating all bone samples in successive washes of ACS grade methanol, acetone, and dichloromethane for 30 minutes each at room temperature, followed by three washes in Nanopure water to rinse. Bone collagen for ¹⁴C and stable isotope analyses was extracted and purified using a modified Longin method with ultrafiltration (95). Samples (200–400 mg) were demineralized for 24–36 h in 0.5N HCl at 5 °C followed by a brief (<1 h) alkali bath in 0.1N NaOH at room temperature to remove humates. The residue was rinsed to neutrality in multiple changes of Nanopure H₂O, and then gelatinized for 12 h at 60 °C in 0.01N HCl. The resulting gelatin was lyophilized and weighed to determine percent yield as a first evaluation of the degree of bone collagen preservation. Rehydrated gelatin solution was pipetted into pre-cleaned Centriprep (96) ultrafilters (retaining 430 kDa molecular weight gelatin) and centrifuged 3 times for 20 min, diluted with Nanopure H₂O and centrifuged 3 more times for 20 min to desalt the solution.

In some instances, collagen samples were too poorly preserved and were pre-treated at PSU using a modified XAD process (97). Samples were demineralized in 0.5 N HCl for 2-3 days at 5°C. The demineralized collagen pseudomorph was gelatinized at 60°C in 1-2 mL 0.01 N HCl for eight to ten hours. Sample gelatin was pipetted into a pre-cleaned 10 mL disposable syringe with an attached 0.45 mm Millex Durapore PVDF filter (pre-cleaned with methanol and Nanopure H₂O) and driven into a thick-walled culture tube. The filtered solution was then lyophilized and percent gelatinization and yield determined by weight. The

sample gelatin was then hydrolyzed in 2 mL 6 N HCl for 22 h at 110°C. Supelco ENVI-Chrom® SPE (Solid Phase Extraction; SigmaAldrich) columns were prepped with 2 washes of methanol (2 mL) and rinsed with 10 mL DI H₂O. With a 0.45 mm Millex Durapore filter attached, the SPE Column was equilibrated with 50 mL 6 N HCl and the washings discarded. 2 mL collagen hydrolyzate as HCl was pipetted onto the SPE column and driven with an additional 10 mL 6 N HCl dropwise with the syringe into a 20 mm culture tube. The hydrolyzate was finally dried into a viscous syrup by passing UHP N₂ gas over the sample heated at 50°C for ~12 h.

For all bone samples that were subject to radiocarbon dating, carbon and nitrogen concentrations and stable isotope ratios of the XAD amino acid samples were measured at the Yale Analytical and Stable Isotope Center with a Costech elemental analyzer (ECS 4010) and Thermo DeltaPlus isotope ratio mass spectrometer. Sample quality was evaluated by % crude gelatin yield, %C, %N and C/N ratios before AMS ¹⁴C dating. C/N ratios for all samples fell between 2.9 and 3.6, indicating good collagen preservation (98). Samples (~2.1 mg) were then combusted for 3 h at 900°C in vacuum-sealed quartz tubes with CuO and Ag wires. Sample CO₂ was reduced to graphite at 550°C using H₂ and a Fe catalyst, with reaction water drawn off with Mg(ClO₄)₂ (99). Graphite samples were pressed into targets in Al boats and loaded on a target wheel with OX-1 (oxalic acid) standards, known-age bone secondaries, and a ¹⁴C-free Pleistocene whale blank. ¹⁴C measurements were made at PSU on a modified National Electronics Corporation compact spectrometer with a 0.5 MV accelerator (NEC 1.5SDH-1). The ¹⁴C ages were corrected for mass-dependent fractionation with measured δ¹³C values (100) and compared with samples of Pleistocene whale bone (backgrounds, 48,000 ¹⁴C BP), late Holocene bison bone (~1,850 ¹⁴C BP), late 1800s CE cow bone, and OX-2 oxalic acid standards for calibration.

Seven samples failed to produce sufficient collagen for a date. For the 36 successful samples (from 35 individuals), radiocarbon ages were calibrated using OxCal version 4.3.2 (50), employing a uniform prior (U(0,100)) that allows the program to model an unspecified mixture of two curves: IntCal13 (51) and SHCal13 (52). This approach may help to account for the effects of the Intertropical Convergence Zone (ITCZ). Alternative results were also obtained using either IntCal13 alone, or a 70:30 ratio of IntCal13:SHCal 13. Reservoir corrections were not applied based stable carbon and nitrogen isotopes and the absence of marine or aquatic resources in associated archaeological deposits. Results are shown in **Table S4**, and it was decided to use the uniform prior mix model since this fell intermediately between the other two sets of results. Calibrated dates using this model are plotted in **Figure S1**.

Results improve the chronology of the Pastoral Neolithic era. Chronological resolution of the PN has been poor until now, despite the numerous published dates, because many of these were obtained from unreliable materials such as bone apatite, or because the dated material had an unclear stratigraphic relationship to the archaeological phenomenon being dated, as discussed by (11, 101). Dates on human remains are further problematized by the fact that R. Protsch – who performed many direct date assessments of African Holocene human material at the UCLA and Frankfurt laboratories – is widely considered to have falsified his data (102). Dates published by Protsch (103-105) have therefore been discarded by archaeologists working in East Africa.

The dated samples show several important trends. The individuals buried at Prettejohn's Gully are the earliest directly dated Holocene human remains in the Rift Valley, and are contemporaneous with the earliest appearance of domesticates south of Lake Turkana, at the nearby forager site of Enkapune ya Muto (7). Individuals buried at sites with PN archaeological traditions generally cluster tightly within the middle to later third millennium BP and the early second millennium BP. This is in line with expectations from prior dating of the PN era in the south-central Rift, reviewed by (11).

There are some important exceptions to the PN chronological cluster. Cole's Burial and Porcupine Cave are relatively early compared to archaeologically similar sites. A pair of contrasting dates are seen at Keringet Cave, where one of the individuals has a relatively late

date of ~1450 cal BP, and the other individual argued to be from the same site (see site description above) has a median date of ~2530 cal BP, placing it firmly within the PN chronological cluster.

Another cluster is observable ~1000 cal BP among three sites that may be classified as Iron Age: the MIA/LIA site of Deloraine Farm, which as noted earlier is a key site for understanding the origins of iron-working communities in the Rift Valley, and the PIA cairn burials at Kasiole 2 and Kisima Farm. Laikipia District Burial Site, and Emurua Ole Polos Cairns date to several centuries later but can still be considered to fall within the IA era.

Ancient DNA laboratory procedures

We successfully generated genome-wide ancient DNA data from a total of 43 human skeletal elements: 31 petrous bones, 1 other bone, and 11 teeth (two teeth were later determined to be from the same individual based on DNA results). We also processed an additional 34 samples (13 petrous, 20 teeth, and 1 other bone) in the same way but did not obtain usable DNA. In dedicated clean rooms at Harvard Medical School, we used a dental sandblaster to isolate cochlear sections from 27 disarticulated petrous bones or a dental drill to drill directly into the root of 11 teeth following surface cleaning. In four cases of complete skulls, we obtained bone powder directly from the cochlea using cranial base drilling (CBD) (*106*) at the NMK and NMT.

We extracted DNA from between 61 and 87 mg of bone powder using one of two modifications of the same method, manually with silica columns or robotically with a liquid handler (**Table S2**).

The first method consisted of a manual silica-column-based extraction protocol optimized for ancient DNA extraction (*107*), which was modified by replacing the MinElute column assembly with a pre-assembled spin column device as in (*108*), to obtain a total volume of 90 μ l DNA extract. We first added 1.5 ml of extraction buffer (0.45 M EDTA, pH 8.0 (BioExpress), 0.25 mg/ml Proteinase K (Sigma Aldrich), 0.5% Tween 20) to the bone powder and incubated the reaction at 37°C overnight, with rotation. Following incubation, we centrifuged the samples at maximum speed for 2 minutes and added 15 ml of binding buffer (5 M guanidinium hydrochloride (Sigma Aldrich), 40% 2-propanol (Sigma Aldrich), 0.05% Tween 20) and 600 μ l 3 M sodium acetate pH 5.2 (Sigma Aldrich) to the supernatant. We transferred this mixture to a High Pure Extender from a Viral Nucleic Acid Large Volume Kit (Roche) and centrifuged at 2000xg until all liquid disappeared from the funnel. We detached the silica column from the funnel, placed it in a fresh 2mL collection tube (Qiagen), and spun for 1 minute at 8000xg. We performed two washes by adding 700 μ L PE buffer (Qiagen) to the columns, and spun at 8000xg for 30 seconds, replacing the collection tube after each wash. We then performed a dry spin at maximum speed for 1 minute. We eluted the DNA from the column by adding 45 μ l of TE with 0.05% Tween to the silica matrix, incubated for 5 minutes at room temperature, and then spun at maximum speed for 1 minute. We repeated this step until we obtained a total volume of 90 μ l DNA extract.

In the second modification of the silica extraction method (robotic), the lysis in extraction buffer was identical as described above, but we then only used 150 μ l of the supernatant and added it to silica coated magnetic beads in order to perform the washes in an automated way on a liquid handler (*109*). In detail, 150 μ l (instead of the entire 1.5 ml as above) of the lysis buffer was added to 1560 μ l of the same binding buffer (5M GuHCl, 40% 2-propanol, 0.12 M sodium acetate and 0.05% Tween 20) and 10 μ l of washed silica coated magnetic beads (G-Biosciences). After 15 min incubation at room temperature to bind the DNA to the silica coated magnetic beads, the beads were separated from the liquid on a 96-well ring-magnet, the supernatant removed and beads washed three times with PE buffer by full resuspension off the magnet. After air drying of the beads, DNA was eluted twice with 15 μ l TE and 0.05% Tween 20 by full resuspension off the magnet and incubated for 5 minutes at room temperature, and the eluate was collected after bead separation for a total of 30 μ l extract.

From the DNA extracts, we prepared either double-stranded Illumina sequencing libraries (43 total; see **Table S2**), affixing 7-base-pair sequences (barcodes) to either end of the DNA molecules to allow multiplex sequencing of the libraries and to greatly minimize subsequent infiltration of contaminant DNA, or single-stranded Illumina sequencing libraries to increase coverage (24 total). We built double-stranded libraries using uracil-DNA-glycosylase (UDG) to greatly reduce the presence of errors characteristic of ancient DNA at all sites except for the terminal nucleotides (*110*). All libraries were prepared using an automated partial UDG protocol according to (*110*) with the modification that silica coated magnetic beads (G-BioSciences) instead of MinElute columns were used for the cleanup steps. The following describes the details of the library preparation procedures. We combined 10 μl (for manual extractions) or 30 μl (for robotic extraction) of DNA extract with the USER treatment mixture (1x Buffer Tango (ThermoFisher), 100 μM dNTP Mix (ThermoFisher), 1 mM ATP (ThermoFisher), 0.06 U/ μl USER enzyme (NEB)), and incubated the reaction at 37°C for 30 minutes. We then inhibited the UDG by adding Uracil Glycosylase Inhibitor (0.12 U/ μl ; NEB) to the mix and incubating for a further 30 minutes at 37°C. We performed blunt-end repair on the samples by adding T4 PNK (0.5 U/ μl ; ThermoFisher) and T4 Polymerase (0.1 U/ μl ; ThermoFisher) to the mixture and incubating for 15 minutes at 25°C, followed by 5 minutes at 12°C. We cleaned up the reactions by adding 420 μl PB (Qiagen; 7x volume of enzymatic reaction) and 4.2 μl washed silica coated magnetic beads. After a 5-minute incubation for DNA binding and subsequent magnetic separation, supernatant was removed and beads were washed two times with 100 μl PE (Qiagen) each time while off the magnet. After air drying of the beads, DNA was eluted in 18 μl of 10 mM Tris-HCl and allowed to sit for 5 minutes, followed by magnetic separation and transfer of the cleaned DNA into the adapter mix. We ligated unique adapters to the molecules in each sample by incubating the sample mixture in a ligation reaction mixture (1x T4 DNA ligase buffer (ThermoFisher), 5% PEG-4000 (ThermoFisher), 0.25 μM P5-adapter, 0.25 μM P7-adapter (*110*), 0.125 U/ μl T4 DNA ligase (ThermoFisher)) for 30 minutes at room temperature. We cleaned up the ligation mixture using the clean-up procedure described above (7x volume of enzymatic reaction and 2.8 μl beads), eluting in 15 μl 10 mM Tris-HCl. We proceeded with adapter fill-in by adding the reaction mixture (1x Isothermal Amplification Buffer (NEB), 250 μM dNTP Mix (ThermoFisher), 0.4 U/ μl Bst Polymerase 2.0, large fragment (NEB)) to the cleaned ligation product in a total of 25 μl and incubating this mixture at 37°C for 20 minutes, and then at 80°C for 20 minutes. Finally, we amplified the libraries via PCR by adding 24.5 μl (0.5 μl were used for a qPCR) of the fill-in reaction product to 75.5 μl PCR reaction (1x Pfu Turbo Cx Reaction Buffer (Agilent Technologies), 1 μM PreHyb-F (5'-CTTCCCTACACGACGCTCTTC-3'), 1 μM PreHyb-R (5'-GTGACTGGAGTTCAGACGTGTGCTCTTCC*G*A*T*C*T-3'; * = phosphor thioate) replaced CL130. We performed initial denaturation of the samples for 2 minutes at 95°C, followed by 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute, and performed a final extension at 72°C for 10 minutes. The final cleanup of amplified libraries, but still with truncated adapters, was done using SPRI technology (*111*, *112*).

We built additional libraries from existing DNA extracts with the single-stranded library preparation 2.0 method (*113*) using optimized oligonucleotides: oligo TL181 {5'-AGATCGGAAGAAA[A][A][A][A][A][A][A]-3'; [A] = ortho methyl RNA} replaced CL78; TL159a {5'-[A][A][A]CTTCCGATCTNNNNNNNN[A]-3', [A] = ortho methyl RNA} replaced TL110; and CL128 {5'-GTGACTGGAGTTCAGACGTGTGCTCTTCC*G*A*T*C*T-3'; * = phosphor thioate} replaced CL130. Although data generated with the single-stranded libraries were usable, we caution that the preparation in these instances was not ideal (about 10x reduced efficiency based on positive oligo quantification) due to liquid handler problems.

For 21 out of the 67 libraries (**Table S2**), we initially screened for authentic DNA by enriching the libraries for sequences overlapping the mitochondrial genome (rsrs) plus 3000 positions in the nuclear genome using biotinylated baits immobilized on streptavidin beads

(114), and sequencing the enriched product (Illumina NextSeq500: 2x76 cycles + 2x7 cycles) after adding a pair of unique 7-base-pair indices to each library. Non-enriched libraries were also shotgun sequenced to low coverage. We then enriched libraries passing screening for sequences overlapping 1,233,013 genome-wide SNPs (35, 115-117) via two rounds of in-solution capture. Again we attached unique indices, before sequencing a multiplexed pool of samples. For the remaining 46 libraries, we did not carry out the screening step, and instead directly enriched for the set of 1,233,013 genome-wide SNPs and the mitochondrial genome in the same reaction and performed shallow shotgun sequencing in parallel.

Bioinformatic procedures

Initial processing of raw sequencing data was performed using in-house software tools, available at <https://github.com/DReichLab/ADNA-Tools>. We merged paired forward and reverse reads with a minimum of 15 base pairs overlap, allowing mismatching at either (a) one base with quality score at least 20, or (b) up to three bases with quality scores less than 20. With perfect matching, merged reads were assigned the higher quality score of the two reads at each base. In the mismatching case, merged reads were assigned the base with higher quality, with a quality score equal to the difference of the base qualities from the original reads.

We trimmed adapter sequences by restricting the merged read start to the start of the forward read and the merged read end to the end of the reverse read. Only merged reads of 30 base pairs or longer were processed further; shorter reads were discarded. If a forward and reverse read had multiple alignments that met the minimum overlap and minimum resulting length, they were discarded.

FASTQ files were aligned using BWA's (118) "samse" command with parameters ($n=0.01$, $o=2$, $l=16500$) twice, against two references: the mitochondrial reconstructed sapiens reference sequence (MT RSRS (119)) and the full human reference genome (hg19). Sequence duplicates were removed using the Broad Picard MarkDuplicates tool (<http://broadinstitute.github.io/picard/>), requiring matching indices and barcodes.

To evaluate authenticity of ancient DNA, we used PMD Tools (120) to compute the frequency of deamination damage at the last two bases on both ends of the merged reads (**Table S2**; **Figure S2**). These two bases were then soft-clipped to remove damage for further analysis. We also estimated contamination using two methods: contamMix (121) for mitochondrial DNA (mtDNA) and ANGSD (122) applied to the X chromosome in males.

To analyze mtDNA haplogroups, we first generated a consensus sequence using SAMtools and BCFtools (123). We obtained a list of variants using SAMtools mpileup, requiring a minimum coverage of 2 and using a majority rule, and called haplogroups with HaploGrep2 (124), using PhyloTree build 17 (125). We created an additional consensus sequence for each individual which included damaged reads only, and also examined this sequence using HaploGrep2, looking for consistency between the damage-restricted and non-damage-restricted haplogroup calls. We obtained confident calls for individuals with at least $8\times$ mtDNA coverage (26 out of 32; **Table S7**).

For the autosomes, we determined pseudo-haploid genotypes by selecting a random allele at each SNP from overlapping reads having minimum mapping quality 10 and minimum base quality 20 at the SNP position. We assigned genetic sex based on the ratio of sequences mapping to the X and Y chromosomes (126). We called Y-chromosome haplogroups (**Table S6**) by determining the most derived mutation for each male individual, using the nomenclature of the International Society of Genetic Genealogy (<http://www.isogg.org>) version 13.295 (12 December 2018). We tested for family relatives on the basis of allelic identity rates as compared to unrelated individuals and did not detect any instances of closely related pairs.

Statistical analyses

To create our initial analysis dataset, we merged the new data with published genotypes of ancient individuals (22-27) and whole-genome sequence (WGS) data from

present-day groups (28, 31) at 1,150,531 autosomal SNP sites. We used this dataset for f -statistics (computed in admixtools (127)), MALDER (38, 39), and some *qpAdm* analyses (35, 36) (see below).

We performed PCA with smartpca, using the “autoshrink” and “lsqproject” options (128, 129). For this analysis, we merged the ancient and WGS data with published array-genotyped individuals from eastern and northeastern Africa (29, 30), yielding a total of 387,246 overlapping autosomal SNPs. In order to create axes that were informative about genetic relationships in eastern Africa but not overly influenced by features of specific groups (e.g., sample size, genetic drift, and ancient versus present-day sampling), we computed components using a minimal set of groups from ref. (28): southern African Khoesan (Ju|’hoan), Sudanese (Dinka), and non-Africans (French). We then projected all other individuals (present-day and ancient; see **Table S5**) onto the first two PCs, and we only consider the projected data for our results.

Our formal modeling of admixture was done using the *qpAdm* software (35). The program takes as input a test group, a set of reference (proxy source) groups (sometimes referred to as “left” groups), and a set of outgroups (sometimes referred to as “right” groups). By exploiting allele frequency correlations among these groups, the program estimates mixture proportions (together with standard errors derived from a block jackknife) for the test group under a model in which it has ancestry related to some or all of the reference groups, along with a p-value for the fit of the model. The only assumptions necessary are that the ancestry components present in the test group form clades with the respective reference groups, with respect to the given set of outgroups (i.e., the test group does not have outgroup-related admixture beyond what is present in the reference groups). In particular, the internal phylogeny relating the references and outgroups (which may involve admixture events) remains unspecified. If the phylogenetic assumptions for a given model are significantly violated (i.e., the test group has some ancestry more closely related to one of the outgroups than to the reference groups), the p-value will be low, and diagnostic statistics can be used to determine which outgroup(s) are implicated. The program is also very flexible in its ability to accommodate both ancient (including low-coverage) and present-day data, variable sample sizes, and large amounts of genetic drift.

For our *qpAdm* analyses (unless otherwise noted), we utilized a set of seven outgroups, including three non-African groups (French, Iranian, and the ancient Loschbour forager from Luxembourg (130)) and four African groups (Mende, Aka, Mbuti, and the ancient South African hunter-gatherers reported by refs. (22, 23)). For models involving present-day Hadendowa (29), we used a merged dataset comprising 563,968 autosomal SNPs. We restricted to a subset of five Hadendowa individuals (IDs 187, 188, 192, 209, and 218) forming a tight cluster in PCA (excluding four individuals shifted in the direction of northern African and Levantine individuals and two individuals with more Sudan-related ancestry) near the middle of the larger intermediate cluster of northeastern Africans (and near the right-hand end of the cline defined by eastern African Afro-Asiatic speakers). We used Mota (and sometimes Kenya LSA) to represent the forager-related ancestry in eastern Africans based on the relative orientations of the forager and Afro-Asiatic clines (and supported by *qpAdm*). For the robustness examples (**Table S9**), we used additional data from ancient Yamnaya steppe pastoralists from Samara, Russia (35, 117); the ~45,000 BP Ust'-Ishim individual from Russia (131); and present-day individuals from the 1000 Genomes Project (132).

For MALDER, we used the following set of reference groups: Dinka, Mende, Ju|’Hoan, Mbuti, Aka, Mursi, Agaw, Mozabite, Druze, Iranian, French, Mota, ancient Tanzanian forager from Zanzibar (~1300 BP) (22), ancient Malawi forager from Fingira (~2500 BP) (22), and ancient Chalcolithic-period individuals from Peqi’in in Israel (25). All tested pairs of individuals provided only one significant date except for I8814 and I8920, who yielded three separate dates, although we conservatively report only the two-wave results. In the other cases, the lack of multiple significant dates is likely due to insufficient statistical power; the single inferred dates would thus represent (weighted) averages over the full

historical admixture process. We computed standard errors for calendar dates of admixture by combining uncertainty from sample dates (average of the individuals in each pair) and admixture times (standard error from MALDER in generations, multiplied by 28 years per generation). For the PN mean admixture date, we averaged the (single-wave) results for all five Kenya PN pairs, weighting by the ratio of the Z -score and the standard error (133).

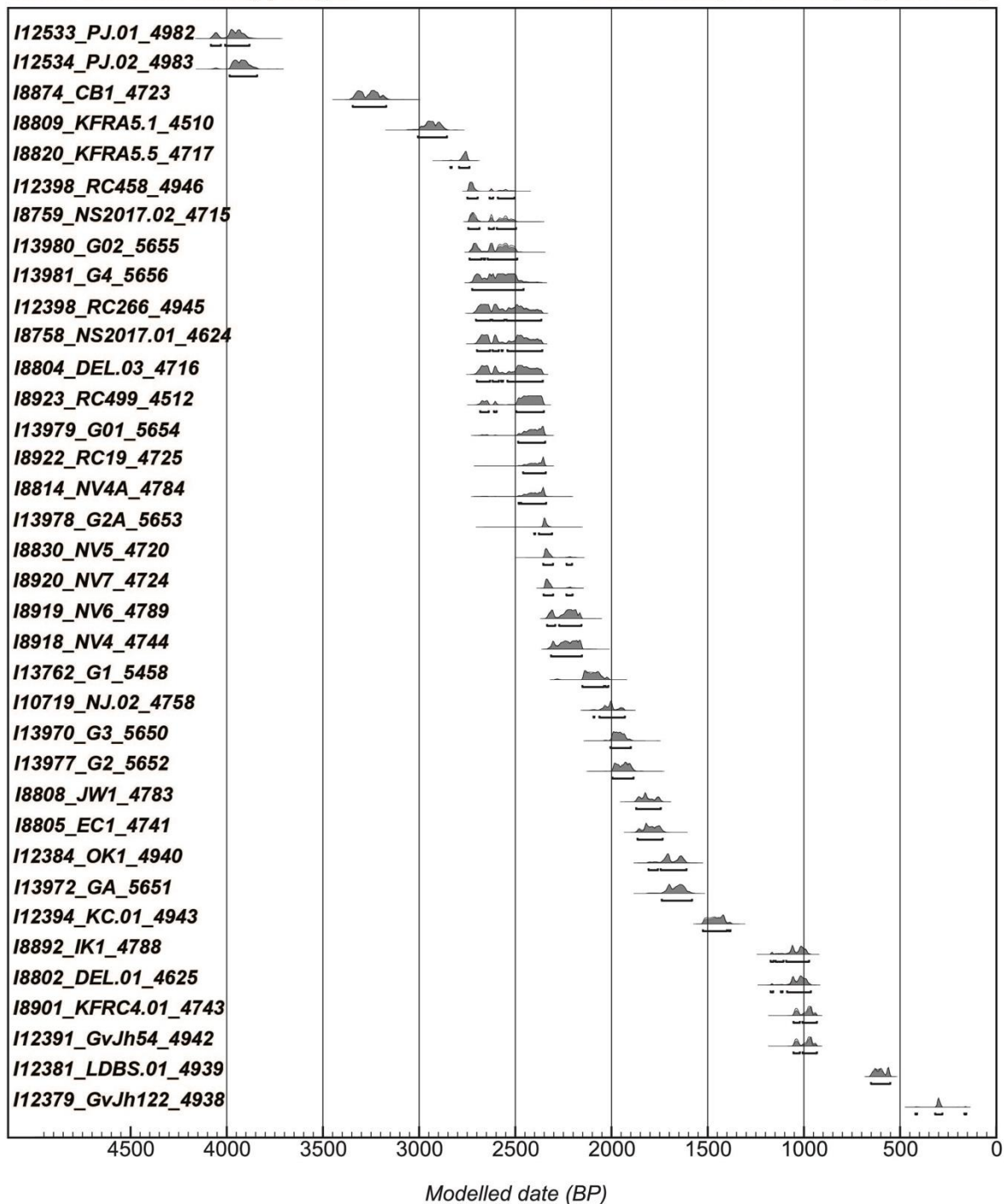


Fig. S1. Calibrated dates for the subset of 36 samples (from 35 individuals; I12398 was dated twice) for which usable aDNA was obtained and where radiocarbon dating was successful. Date labels list the individual number, skeletal sample code, and PSU AMS lab number. Dates were calibrated using OxCal version 4.3.2 (50) using a uniform prior (U(0,100)) to model a mixture of two calibration curves: IntCal13 (51) and SHCal13 (52).

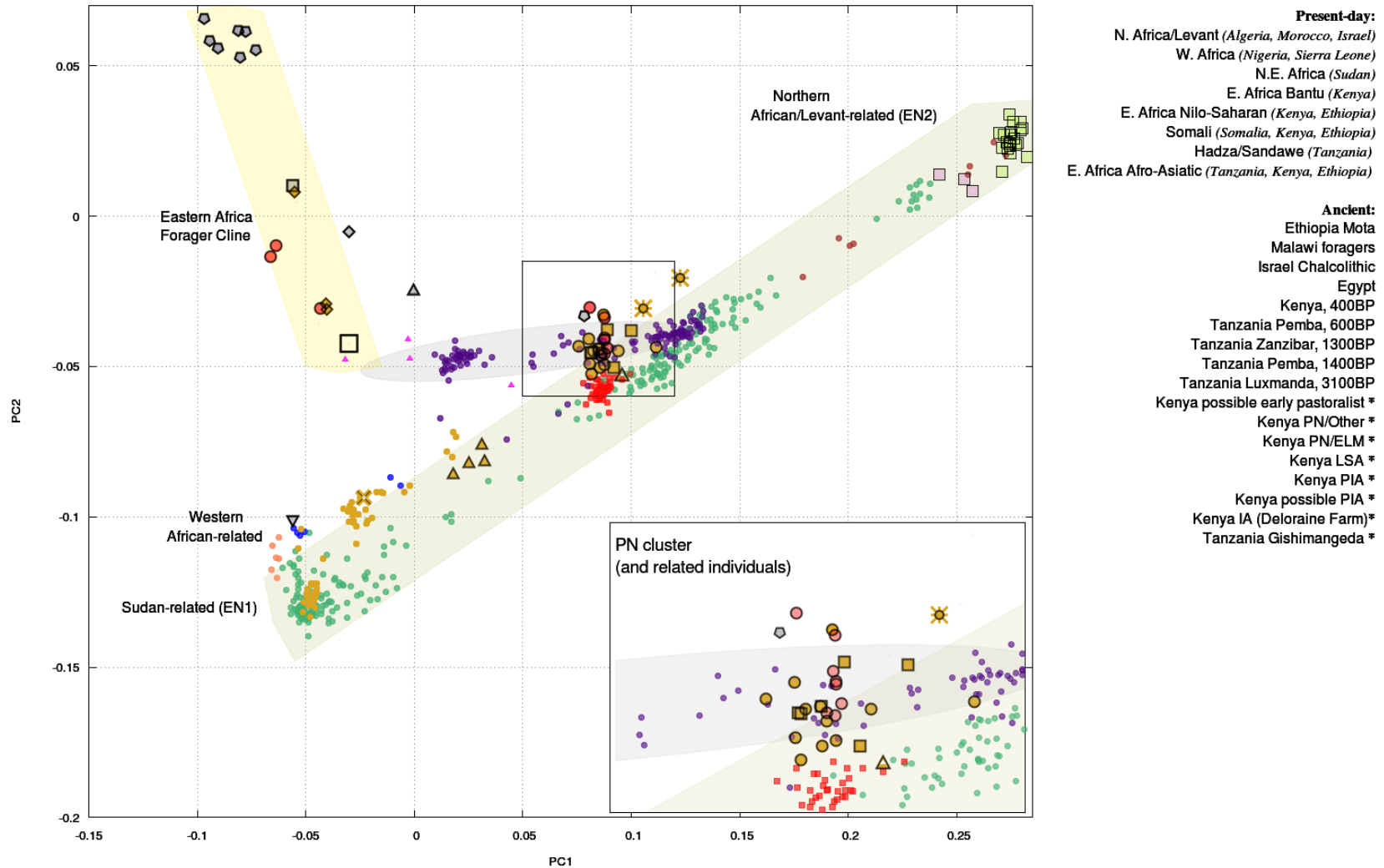


Fig. S2. Alternative PCA. Shaded regions are drawn to highlight notable clines of ancestry. Ancient individuals reported in this study are indicated in the legend with asterisks. Dates for published ancient individuals outside of Kenya and Tanzania are ~4500 BP for Mota, ~8100-2500 BP for Malawi foragers, ~6500-5800 BP for Israel Chalcolithic, and ~3600-2000 BP for Egypt. ELM, Elmenteitan; LSA, Later Stone Age; PN, Pastoral Neolithic; IA, Iron Age; PIA, Pastoral Iron Age. See **Fig 2** and **Table S5** for the primary PCA (from which this version differs by the addition of Yoruba in the set of groups used to compute the axes; the results are very similar).

Table S1. (separate file)

Skeletal samples analyzed or collected for analysis and not sampled in this study

Table S2. (separate file)

Detailed library-level information for samples passing through screening process

Table S3. (separate file)

Radiocarbon dates and carbon and nitrogen stable isotope values obtained on human skeletal collagen samples

Table S4. (separate file)

Comparison of radiocarbon calibration results

Table S5. (separate file)

List of individuals and groups used in PCA

Table S6. (separate file)

Y chromosome haplogroups and derived SNPs

Table S7. (separate file)

mtDNA haplogroups

Table S8. (separate file)

qpAdm admixture model results

Table S9. (separate file)

qpAdm robustness examples

Table S10. (separate file)

Allele-sharing statistics testing PN relationships

Table S11. (separate file)

MALDER results for pairs of ancient individuals

Table S12. (separate file)

Phenotype-associated SNP genotypes

Data S1. (separate file)

Genotype datasets used for analyses