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Community-engaged ancient DNA project reveals diverse origins of 18th-century African descendants in Charleston, South Carolina

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In this study, we present the results of community-engaged ancient DNA research initiated after the remains of 36 African-descended individuals dating to the late 18th century were unearthed in the port city of Charleston, South Carolina. The Gullah Society of Charleston, along with other Charleston community members, initiated a collaborative genomic study of these ancestors of presumed enslaved status, in an effort to visibilize their histories. We generated 18 low-coverage genomes and 31 uniparental haplotypes to assess their genetic origins and interrelatedness. Our results indicate that they have predominantly West and West-Central African genomic ancestry, with one individual exhibiting some genomic affiliation with populations in the Americas. Most were assessed as genetic males, and no autosomal kin were identified among them. Overall, this study expands our understanding of the colonial histories of African descendant populations in the US South.

paleogenomics | trans-Atlantic slave trade | Colonial North America | haplotype | ancestry

Significance

Through the Anson Street African Burial Ground Project, we have investigated the lives of thirty-six 18th-century individuals from Charleston, South Carolina, using bioarchaeological and genomic methods. We report low coverage genomic data from 18 individuals that reveal a diversity of ancestral connections across western Africa, including one individual with genomic affiliations with Fulani peoples of West Africa. Overall, this study describes the largest number of ancient genomes from African-descended persons in North America, substantially increasing our understanding of African diversity and history in colonial America. This study further demonstrates how ancient DNA research can be conducted in close collaboration with stakeholder communities, which is a critical step in building an ethical framework for the field of paleogenomics.

From the 16th to the 18th centuries AD, over 15 million persons were abducted from the African continent during the trans-Atlantic slave trade, of which nearly 300,000 were taken to eastern North America (1). Information about the origins of these individuals has been largely examined through archival records, which suggest that most were purchased by European slave traders along the coasts of West and West-Central Africa before being transported across the Atlantic Ocean through the Middle Passage (2). This detailed demographic information has, in large part, been inferred from information available in the Trans-Atlantic Slave Trade Database, which has digitally archived over 36,000 British slave ship records (3). In cases in which limited or no documentary records exist, however, ancient DNA (aDNA) analysis has provided critical and complementary information about the African origins of enslaved individuals and the larger trans-Atlantic slave trade network (4). As examples of this work, ancient autosomal and mitochondrial DNA (mtDNA) of colonial African individuals from the Caribbean (5) and the Americas (6–9) have indicated that they had predominantly West or West-Central African ancestries.

While aDNA methods have advanced our ability to recover information about the genetic ancestral origins of archaeological individuals, Black, feminist, and Indigenous Science and Technology Studies frameworks emphasize the importance of community collaboration, accountability, and activism in African diasporic historical studies (10–13), as modeled by such groundbreaking projects as the New York African Burial Ground (NYABG) Project (14) and the Estate Little Princess Archaeology Project (15). The NYABG in particular made a strong case for the empowerment of community members to decide research direction and outcomes (16).

We employed these frameworks in the Anson Street African Burial Ground (ASABG) Project, a community-initiated and -engaged study designed to elucidate the lives of the 36 individuals whose remains were accidentally unearthed and subsequently removed during construction of the Charleston Gaillard Center in downtown Charleston, South Carolina. The Gullah Society, Inc., a community grassroots organization whose members later formed the ASABG Project team, hosted a "Community Conversations" series to assess what the Charleston African American community, who considered these individuals to be their communal ancestors, felt should be done with their remains. Based on community feedback, the Gullah Society advocated for the reburial of these individuals, henceforth referred to as the Anson Street Ancestors, in the grounds of their original resting place. The community also wanted to learn as much as possible about these ancestors and sanctioned genomic research with these remains (9). Questions from the community included the following: Is there any documentation of the ancestors? Were they interred with care? Were women and children buried there? Were they recently transported

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to North America? Were they related to each other? Importantly, community members were interested in understanding more about the ancestral origins of the ancestors.

In 2017, the Gullah Society and Charleston African American community discussed and agreed to conduct possible aDNA analysis of the Anson Street Ancestors. The Gullah Society contacted two of the authors (R.E.F. and T.G.S) with experience conducting aDNA projects in colonial North American contexts and modern DNA research with African descendant communities and who were committed to carrying out community-centered research. A research plan was then codeveloped and executed in collaboration with community stakeholders (9). We held regular community meetings and educational events to update stakeholders on research progress in an effort to develop trust and relationships through transparent communication. This work culminated in a reinterment ceremony of the 36 ancestors in 2019 (17). Community input also stimulated the creation of a naming ceremony, whereby Yoruba priests bestowed honorary names to the 36 ancestors prior to their reburial. These symbolic names were sourced from Lorenzo Dow Turner's Africanisms of the Gullah Dialect (18) and chosen based on previous mitogenome findings, osteological evidence, and spiritual guidance (9). Accordingly, we refer to individual ancestors by their honorary names (italicized) along with their burial numbers throughout the text.

Previous bioarchaeological and archival research provide a preliminary understanding of the histories of the Anson Street Ancestors (9). Despite poor preservation, osteological analyses suggested that these individuals were likely of African ancestry. Archival and archaeological artifact analyses suggested that the burial ground was active approximately between AD 1760 and 1790. Subsequent documentary research indicated that the burial ground at Anson Street was located far from known interment locations for Free People of Color in Charleston and notably on property of White landowning individuals. Although no direct documentation of the identity or enslavement status of the Anson Street Ancestors has yet been found, despite our extensive efforts, it is likely that they were enslaved given their interment location. Complete mitochondrial genome (mitogenome) analysis further confirmed that all but one ancestor had African matrilineal ancestry, with the remaining individual having North American matrilineal ancestry. These mitogenome results revealed important insights into the broader demography and background of the ancestors (9) but lacked the depth of an autosomal-based analysis.

For these reasons, we conducted a more extensive genomic analysis of the Anson Street Ancestors. This study reports data for 18 low-coverage genomes and 31 uniparental haplotypes from these individuals. We integrated this genomic information with previously published archaeological, osteological, and genetic findings and compared these data to demographic information collated from the Trans-Atlantic Slave Trade Database. We also assessed the genomic data in relation to previously reported strontium isotopic evidence to more deeply investigate residence patterning at the ASABG. These results demonstrate how community-engaged aDNA research can be effectively undertaken and provide the most extensive understanding of the genomic ancestry of presumably enslaved African-descended individuals in North America published to date.

Results

Trans-Atlantic Slave Trade Database Analysis. Based on our analysis of Trans-Atlantic Slave Trade Database records, we estimate that, between AD 1700 to 1810, a total of 174,793 recorded individuals were abducted from ports in the upper and lower regions of the West African coast (Senegal to Nigeria), the West-Central African coast (Cameroon to Angola), and, to a lesser degree, Mozambique in Southeast Africa, and disembarked in Charleston (Fig. 1*A* and Dataset S1). When analyzed by region, almost one-third of these individuals (27.4%) came from ports located in West-Central Africa and St. Helena, with approximately half (47.35%) embarking from ports in West Africa (Fig. 1*B* and Dataset S1).

Our analysis of database records by decade further indicates that the African origins of abductees disembarking in Charleston differed over time. For example, the greatest number of individuals were brought into Charleston prior to the 1750s originated from the coast of West-Central Africa, whereas afterward, slave traders shifted to acquiring more individuals from ports on the upper West African coast (Fig. 1 *C* and *D*). Additionally, a dramatic increase in the number of abducted individuals is also observed

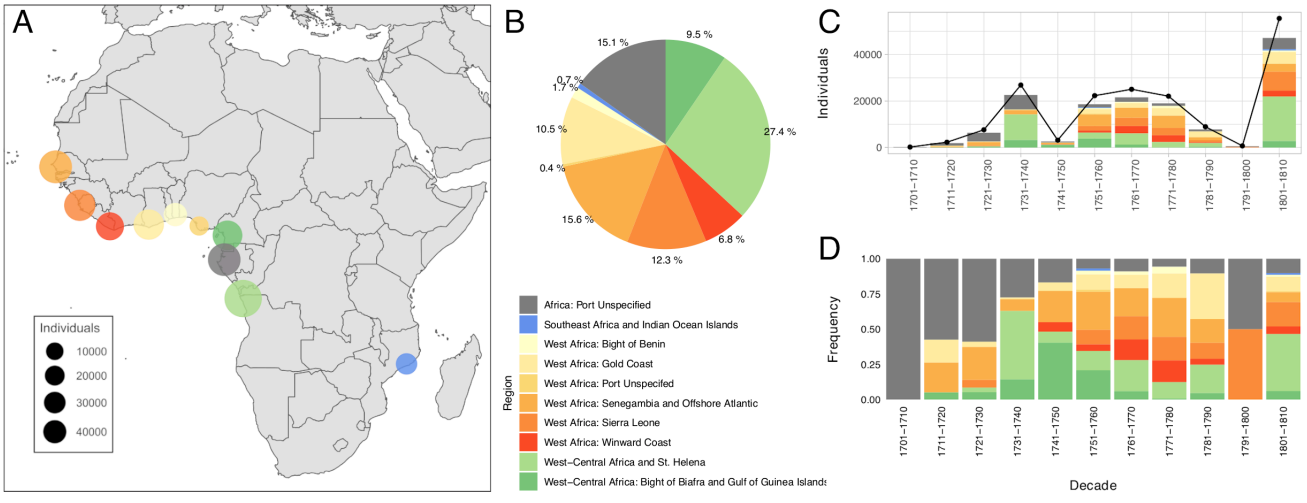

Fig. 1. Distribution of abducted individuals brought into the port of Charleston, as assessed from the Trans-Atlantic Slave Trade Database (accessed 22 March 2021) (Dataset S1). All colors correspond to regional ports of embarkation. (*A*) Map of Africa displaying the number of embarked individuals by regional port of embarkation, with node size proportional to the number of individuals. (*B*) Frequency of the total number of embarked individuals by regional port of embarkation. (*C*) Number of abductees, by decade, who embarked from Africa and disembarked in Charleston. The line graph represents the number of individuals who left Africa, while the bar plot represents the number of individuals who arrived in Charleston, colored proportionally by regional port of embarkation. The space between these points represents the loss of life during the Middle Passage. (*D*) Frequency, by decade, of individuals who disembarked in Charleston, colored by regional port of embarkation.

between AD 1801 and 1809, during which time enslavers transported nearly half (46.6%) of the total number of individuals brought into Charleston during the entire span of the trans-Atlantic slave trade (Fig. 1*C*). This surge was likely stimulated by an AD 1803 resolution passed by the South Carolina state legislature to resume the importation of enslaved Africans before the federal government enacted the Act Prohibiting Importation of Slaves in AD 1808 (19), reflecting the city's deep entrenchment in the socioeconomic institution of slavery.

Sequencing and aDNA Authentication. Mitochondrial and nuclear human DNA were obtained from 31 of 36 Anson Street Ancestors, resulting in 0.002 to 1.23× genomic coverage (Table 1 and Dataset S2). The petrous pyramid of the temporal bone, tooth, or postcranial skeletal elements (metatarsals/carpals, phalanges, or ribs) were selected for analysis based on their availability and preservation, with the petrous elements ultimately yielding the most useable data. aDNA extracts contained variable levels of mitochondrial contamination, as estimated using ContamMix (20) and Haplocheck (21), with the majority displaying a low level of mitochondrial contamination. Four individuals with high levels of mitochondrial contamination were removed from downstream analyses (Dataset S3). Nuclear contamination as assessed using ContamLD (22) was minimal (<9%) for ancestors whose nuclear DNA was sequenced with adequate coverage (n = 30) (Dataset S3).

Sequencing reads were analyzed using MapDamage (23) and PMDtools (24), which indicated signs of aDNA damage (Dataset S4). MapDamage (23) profiles indicated an asymmetrical nucleotide misincorporation pattern, with reduced levels of C to T substitution rates at 5' ends, but elevated G to A rates at 3' ends. We hypothesize that the unusual pattern is due to the use of Phusion High Fidelity DNA Polymerase (NEB) during library amplification. This polymerase is unable to read through uracil nucleobases but preserves damage to "CpG" methylated ends (23, 25). Elevated CpG methylation levels at both 5' and 3' ends were detected using PMDtools (24), verifying aDNA authenticity (*SI Appendix*,

Table 1. Summary table representing 18 of the Anson Street Ancestors, with age and enamel strontium values previously generated (9)

Name	ID	Age	Bone sample	Genetic sex*	Nuclear coverage	mtDNA haplogroup	MSY haplogroup	Ancestry	Strontium (enamel)
Banza	CHS01	Young adult	Petrous	XY	0.696X	L3e3b1	E1b1a-CTS668	W-C Africa	Non-local
Lima	CHS03	Middle adult	Petrous	XY	0.7563X	L3b3	E1b1a-M4671	W Africa	Local
Kuto	CHS04	Older adult	Petrous	XY*	0.5439X	L2a1a2	E1b1a-CTS2198	W-C Africa	Nonlocal
Anika	CHS10	Young adult	Petrous	XY*	1.0137X	L2b1	E1b1a-CTS6126	Sub-Saharan Africa	Local
Nana	CHS11	Young adult	Petrous	XX	1.1328X	L2b3a	–	W Africa	Local
Zimbu	CHS13	Middle adult	Petrous	XY	1.0641X	L3e1e	E1b1a-CTS5497	W-C Africa	Nonlocal
Wuta	CHS16	Adult	Petrous	XY*	1.2251X	L3e2b +152	E1b1a-CTS7305	Sub-Saharan Africa	Local
Daba	CHS17	Adult	3rd molar	XY	0.3908X	L2c	E1b1a-M4273	W Africa	Nonlocal
Fumu	CHS19	Middle adult	Petrous	XY	0.8206X	L3e2b +152	B2a1a- Y12201	Sub-Saharan Africa	Local
Lisa	CHS22	Young adult	Petrous	XY*	0.1581X	H100	E1b1a-Z6020	W Africa	Local
Ganda	CHS23	Adult	Petrous	XY*	0.1155X	L1c1c	E1b1a-CTS5612	W Africa	Nonlocal
Coosaw	CHS24	Adolescent	Petrous	XY*	0.1323X	A2	E2b1a-CTS2400	Admixed: W Africa and Native American	Local
Kidzera	CHS28	Infant	Petrous	XX*	0.1458X	L2a1a2c	–	W-C Africa	Local
Pita	CHS29	Adult	Petrous	XY*	0.1851X	L3e2b	E1b1a-M4287	Sub-Saharan Africa	Local
Tima	CHS31	Adult	Petrous	XX	0.0849X	L3e1e	–	W-C Africa	Local
Jode	CHS33	Child	Petrous	XY*	0.2625X	L2a1a2c	E1b1a-CTS4975	Sub-Saharan Africa	Local
Ajana	CHS34	Adult	Petrous	XX*	0.0796X	L2a1i	–	W-C Africa	Local
Isi	CHS36	Adult	Petrous	XX*	0.1653X	L3e2a	–	W-C Africa	Local

Age estimates are based on osteological analysis, with age group designations following Buikstra and Ubelaker (1994): infant (0 to 3 y), child (3 to 15 y), adolescent (12 to 20 y), young adult (20 to 35 y), middle adult (35 to 50 y), and older adult (50+ y). Enamel strontium residency status is based on Charleston local vs. nonlocal estimates, with non-local indicating that the individual was not born in Charleston. Ancestry characterization was based on cumulative results from the MSY (male-specific Y chromosome), mitogenome, PCA, ADMIXTURE, and F3 statistical analyses of autosomal data, with W-C Africa indicating West-Central Africa, and W Africa indicating West Africa. If regional ancestry was not consistent among all analyses, then the ancestor was characterized as having sub-Saharan African ancestry. Only ancestors with enough genomic coverage for genomic ancestry analyses are represented in the table.
*Individuals whose genetic sex estimation differed from osteological sex estimation, either due to a different assignment or because osteological sex was not able to be estimated, are noted with an asterisk (*).

Fig. S1 *A*–*D* and Dataset S4). Overall damage estimation rates were also calculated using PMDtools (24) and indicated between 8.57 and 16.61% damage, also typical of degraded DNA samples (Dataset S4).

PCA and Admixture Analyses. To assess the biparental ancestries of the Anson Street Ancestors, we called single-nucleotide polymorphisms (SNPs) against the Human Origins reference panel (Dataset S1). Only genomes with greater than 2,000 transversion-only SNPs were used in subsequent autosomal analyses for conservative estimations of ancestry. Eighteen of the ancestors' genomes met this criterion. SmartPCA (26) results indicated that most of the ancestors fell within the projected dimensions of reference African populations. Notably, *Lisa* (CHS22) was positioned lower than the rest of the ancestors along the second principal component but within the projected range of the reference populations (Fig. 2*A*). By contrast, *Coosaw* (CHS24) did not project onto any specific population cluster and instead was positioned between reference populations from Africa and the Americas.

To further investigate this ancestry distribution, ADMIXTURE (27) was run between K = 4 and K = 16 with 100 bootstrap replicates, with K = 12 having the lowest CV value. The ancestors showed predominately African ancestry, except for *Coosaw* (CHS24), who displayed approximately a third of their admixture profile similar to populations found in the Americas (Fig. 2*B*), consistent with previous findings (9).

Variants were also called against a custom panel of 78 African reference populations, encompassing 3,553 individuals, to obtain a detailed understanding of inferred African ancestry of the 18 ancestors (*SI Appendix*, Table S1 and Dataset S5). SmartPCA (26) results indicated that the majority of individuals cluster with West and West-Central reference populations (Fig. 3*A*). Again, *Lisa* (CHS22) and *Coosaw* (CHS24) were separated from the rest of the ancestors along the first principal component, although both fell within the range of reference populations from The Gambia.

Variants were then called against West and West-Central African reference populations to further delineate their African ancestry. SmartPCA (26) results indicate a parsing of ancestry between ancestors with West African and West-Central African ancestry along PC1 (Fig. 3*B*). Accordingly, nine ancestors contained negative PC1 values that were more closely associated with West-Central African reference populations, with *Kuto* (CHS04), *Banza* (CHS01), and *Zimbu* (CHS13) all aligning closely with reference populations from Gabon. Nine ancestors contained positive PC1 values associated with West African reference populations. More specifically, *Lima* (CHS03), *Ganda* (CHS23), *Nana* (CHS11), and *Daba* (CHS17) aligned closely with Ghanaian, Ivory Coast, and Sierra Leonian reference populations. In addition, *Lisa* (CHS22) projected near Fula populations in The Gambia. To further verify the designations between West and West-Central African reference populations in the ancestors, an additional SmartPCA (26) was run with the addition of Southern African reference populations (*SI Appendix*, Fig. S2). The splitting of ancestries between West and West-Central African reference populations fell along PC2, with the ancestors grouping similarly with West and West-Central African reference populations.

ADMIXTURE (27) was also run against the custom African reference population panel using K = 4 to K = 18 with 100 bootstrap replicates, with K = 12 having the lowest CV value. The results indicate a similar splitting of ancestries between West and West-Central African reference populations as observed in the PCA projections, with *Ajana* (CHS34), *Kuto* (CHS04), *Banza* (CHS01), and *Zimbu* (CHS13) displaying similar admixture profiles to West-Central African reference populations from Gabon (Fig. 3*C*). By contrast, *Ganda* (CHS23) and *Daba* (CHS17) show profiles similar to West African reference populations, corresponding to their PCA projections. *Lisa* (CHS22) displays a unique admixture profile in comparison to the other ancestors that is similar to populations in The Gambia, and, in particular, the Fula ethnic group (*SI Appendix*, Fig. S3).

F Statistics. To ascertain similarities in levels of genetic drift between the worldwide reference populations and the Anson Street Ancestors as a proxy for genetic affinities, we calculated outgroup F3 statistics using qp3Pop (28) in the form F3(Ust'Ishim; CHS#, Y), designating Ust'Ishim as the outgroup to West African populations (8). To conservatively estimate population affinity, only results from individuals with greater than 12K SNPs in common with reference populations were used (29). The results largely verified estimations of West and West-Central African ancestries associated with the Bantu expansion (Dataset S6 and *SI Appendix*, Fig. S4).

To further assess the affinity of populations with similar Fst levels, D Statistics were run using qpDstat in the form D (Chimp, CHS#; Target, Y), with Y representing the African populations on the Human Origins Panel and the target population consisting of the population with the highest F3 value from the previous analysis (Dataset S7). Of the seven ancestors tested, only *Nana* (CHS11) and *Wuta* (CHS16) who associated with the Yoruba and Esan of Nigeria, respectively, were not significantly rejected (*SI Appendix*, Fig. S5).

The lack of congruency with contemporary African reference populations could be due to the high amount of population turnover in Africa as a result of the trans-Atlantic slave trade or the lack of a representative sampling of populations from the African continent (8, 30). While current efforts are underway to ethically assay African genomic diversity (31, 32), more representative reference populations are needed to accurately estimate ancestral contributions for aDNA analyses in African diasporic contexts.

Genetic Sex and Kinship. Genetic sex was estimated in individuals with low contamination (33), which revealed that 21 of 27 individuals analyzed were chromosomal males (Table 1 and Dataset S8). These assignments largely agreed with osteological estimates (9), only differing in three individuals for which there was poor skeletal preservation.

Autosomal kinship was estimated using LcMLkin, which calculates genotype likelihoods to estimate the probability of identity-by-descent against the 1000 Genomes reference panel (34, 35) (Dataset S9). The results revealed no autosomal kinship among the individuals analyzed, as assessed to the third degree. Our previous mtDNA analysis indicated that the adult *Isi* (CHS36) and the child *Welela* (CHS37) shared the same mtDNA haplogroup (9), suggesting some degree of possible maternal kinship. Unfortunately, low genomic coverage for *Welela* (CHS37) prevented further autosomal assessment of this relationship.

Uniparental Ancestries. Mitogenome variants were called by independently mapping them to the NC_012920.1 reference genome and generating haplotypes using Haplogrep (36). The results verified previous mitogenome findings, including the L3e2a haplotype shared between *Isi* (CHS36) and *Welela* (CHS37), which suggested that they were maternally related (9) (Table 1 and *SI Appendix*, Table S2). Of the 27 mitogenomes analyzed, 24 individuals displayed variants characterizing haplogroups L0-L3 currently found in African populations. These basal haplogroups are also found in contemporary African American populations (37–39), suggesting possible genetic continuity with colonial African communities. *Lisa* (CHS22) contained an H1cb1a mtDNA that has also been observed in

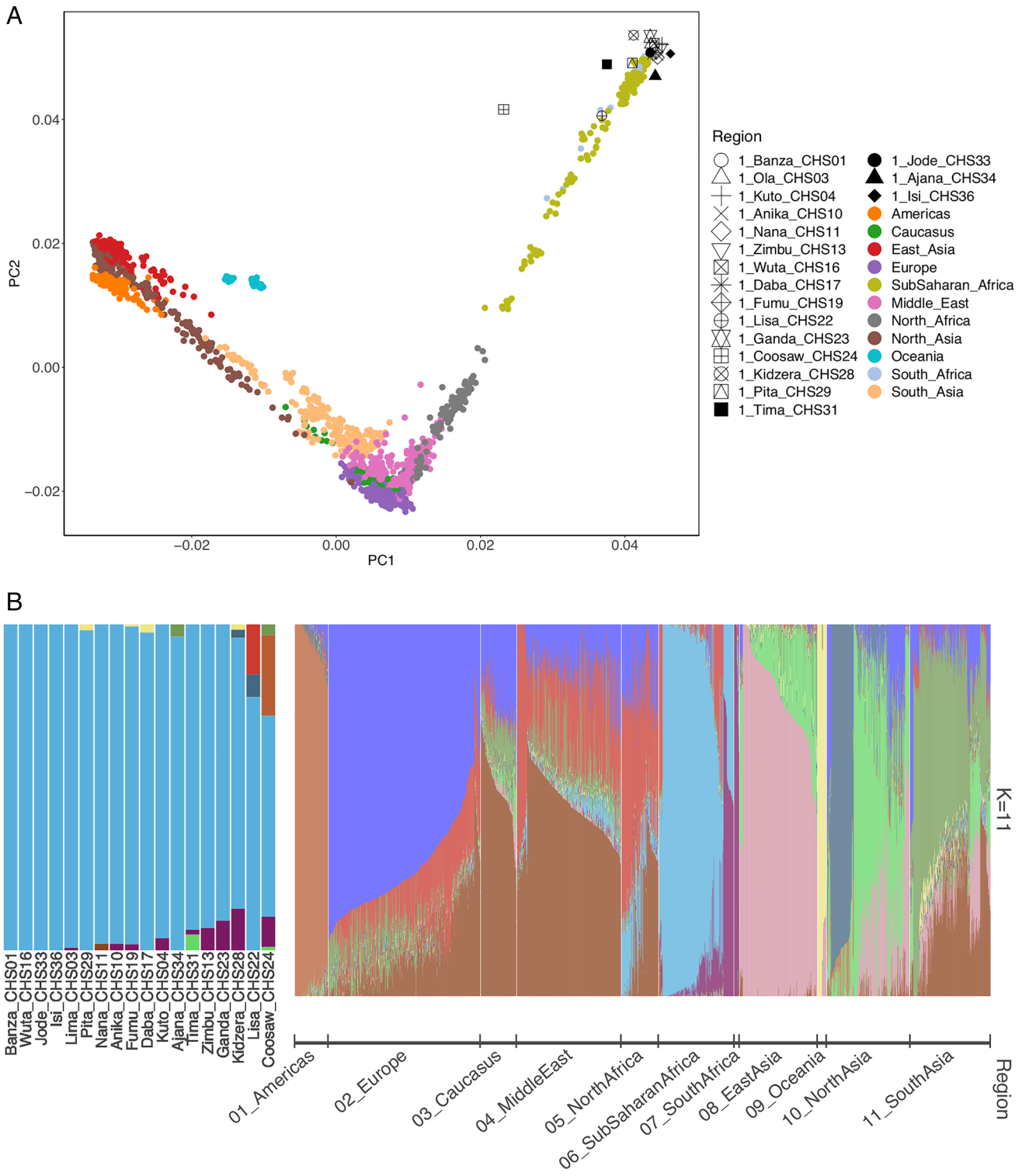

Fig. 2. Autosomal ancestries of the Anson Street Ancestors estimated against the Human Origins reference panel. (*A*) The "first" and second principal components comparing the genetic relationship of the ancestors to other African populations. (*B*) ADMIXTURE results at K = 11 for the ancestors relative to comparative populations.

Fulani populations in Niger, Guinea, Burkina Faso, and Mali (9, 40). *Coosaw*'s (CHS24) A2 haplogroup was additionally confirmed, indicating that this individual's ancestry with populations in the Americas minimally derives from their direct maternal line.

Haplogroups of the nonrecombining region of the male-specific Y chromosome (MSY) were characterized using Yleaf for chromosomal males (41) (Table 1 and Dataset S10). No individuals shared an MSY haplotype, with the majority of individuals having haplotypes belonging to haplogroup E1b1a. This haplogroup is commonly found among present-day sub-Saharan Africans and African Americans and is associated with the spread of Bantu-speaking populations in West Africa (42, 43). Additionally, *Coosaw* (CHS24) displayed an E2b1a haplotype and

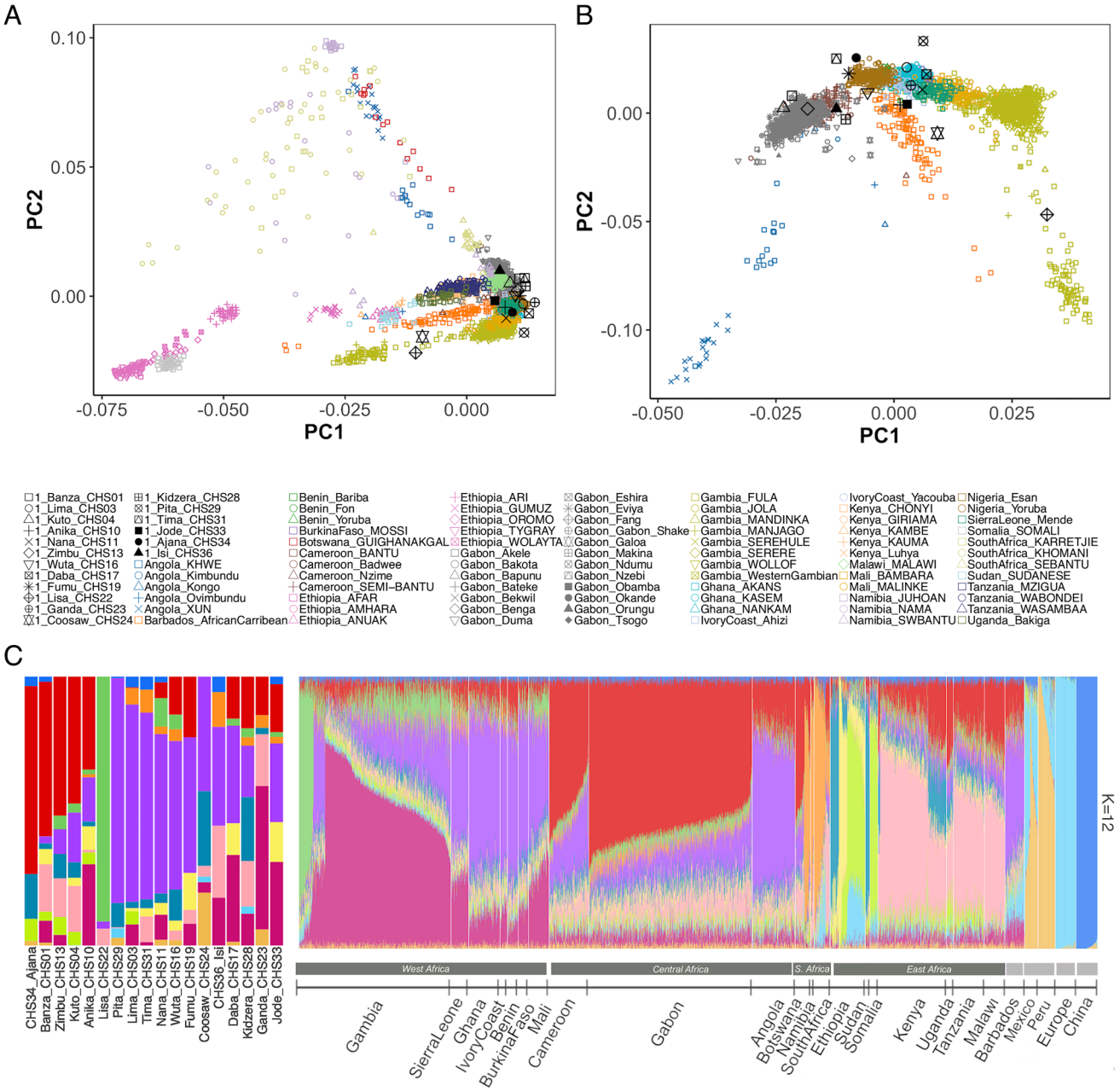

Fig. 3. Characterization of African ancestry for the Anson Street Ancestors against the custom African reference panel. (*A*) A PCA plot of African ancestry for the Anson Street Ancestors. (*B*) A PCA plot showing the extent of West and West-Central African ancestry in the Anson Street Ancestors. (*C*) An ADMIXTURE plot (K = 12) of the ancestors compared to populations from the African reference panel.

Fumu (CHS19) a B2a1a haplotype. Both these MSY lineages are also associated with Bantu expansions, although today they are found at low frequencies in sub-Saharan Africa (44, 45).

Discussion

This study infers a range of ancestral origins from West or West-Central Africa among the Anson Street Ancestors living in 18th-century Charleston. PCA and ADMIXTURE analyses suggest that five ancestors were likely affiliated with present-day West African reference populations and seven others with West-Central African populations (Table 1). Notably, autosomal and mtDNA evidence suggests that *Lisa* (CHS22) likely shared ancestry with Fulani populations in The Gambia. The predominance of sub-Saharan African ancestries among the ancestors is also reflected in their MSY and mitogenome lineages. These findings align with our analyses of the Trans-Atlantic Slave Trade Database reports, which indicate that most individuals brought to Charleston came from ports in West and West-Central Africa during the 18th century (Fig. 1).

To more deeply analyze the genomic origins of the Anson Street Ancestors in the context of individual histories of forced migration, we integrate previously published enamel strontium isotopes into this discussion to examine residency and ancestry (Table 1) (9). Since strontium is absorbed into enamel from local groundwater and food sources during development, its isotopic signature provides a means of estimating geographical residence during childhood (46).

Previous isotopic findings indicated that six ancestors had very high enamel strontium values that were inconsistent with the Charleston population mean and instead overlapped with values reported in populations from the West African coast (9). This result suggested that they were not born in the Charleston area and

instead were likely recently transported through the Middle Passage. Of these individuals, five adults contained high-enough DNA coverage for autosomal analysis. *Daba* (CHS17) and *Ganda* (CHS23) projected within the PCA range of West African populations, while the remaining three individuals, *Banza* (CHS01), *Kuto* (CHS04), and *Zimbu* (CHS13), were similar with populations in Gabon. Interestingly, the latter three were interred next to each other (*SI Appendix*, Fig. S6). Based on the estimated osteological age and burial internment range, these individuals could have been born in Africa by AD ~1680 at the earliest and AD ~1770 the latest (*SI Appendix*, Fig. S7 and Dataset S11). The distribution of African ancestries among the first-generation African individuals indicates that they were being transported from disparate areas of the African continent during the last half of the 18th century.

Strontium enamel analysis also indicated that 13 ancestors may have been born outside of Africa, representing second or greater generations of African-descended individuals in Charleston. These same ancestors also show genomic affiliations with either West, West-Central, or sub-Saharan African reference populations. As noted before, the genomic history of one of these ancestors, *Coosaw* (CHS24), reveals admixture with populations in the Americas. *Coosaw's* (CHS24) A2 mtDNA haplogroup and significant autosomal admixture with African and Indigenous North American populations attest to the histories of interactions among marginalized populations in the colonial South. Indigenous peoples in 18th-century South Carolina included groups such as the Catawba and Cherokee, among others (47). Historical sources document social interactions between Indigenous North American and African persons in communities of free and/or escaped Africans on the frontier or in enslavement settings during the 17th and early 18th centuries (48). In the early 17th century, Indigenous North American individuals were enslaved by European colonizers prior to the rise in the trans-Atlantic slave trade in the region (49). As European systems of forced labor became more deeply entrenched in the Carolina landscape during the 18th century, racialized boundaries simultaneously hardened and documented interactions between Indigenous North American and African individuals increasingly followed colonial European systems of power (48).

While the exact circumstances of *Coosaw*'s (CHS24) family history may never be fully reconstructed without direct archival documentation, the presence of mtDNA haplogroup A2 and autosomal admixture with populations in the Americas attests to a multigenerational presence of African-descended individuals in the region who interacted with Indigenous North American individuals in the early colonial South. These findings also support the low percentage of North American ancestry in contemporary African American populations in the United States (50, 51), which are attributed to admixture events during the 18th and 19th centuries (52, 53).

Our genomic findings further illuminate how conditions of enslavement may have impacted biological kinship patterns at the burial ground. With the exception of the maternal haplotype shared between *Isi* (CHS36, an adult female) and *Welela* (CHS37, a 6-to-8-y-old child), no other evidence of close biological kinship among the ancestors was evident (9). While only a subset of the ancestors with enough genomic coverage were included in the analysis (18 of 36 ancestors), these results suggest an opportunistic interment pattern over time rather than one organized by biological kin. The absence of close biological relatedness among the Anson Street Ancestors is also consistent with other autosomal aDNA findings from enslaved African individuals (5, 8, 54), reflecting the nature of structural violence experienced in enslavement.

However, other expressions of community or cultural kinship, as evidenced by the grave goods and artifacts of adornment found buried with the ancestors (9), demonstrate connections extending beyond the corporeal realm. For example, the child *Welela* was buried with coins over their eyes (CHS37), and the infant *Omo* (CHS18) was buried with a bead next to their cranium. These objects likely represent acts of care through material adornment by those who honored them from life and into death (9).

This study provides important insights into the history of African-descended individuals in Charleston during the 18th century and the trans-Atlantic slave trade more broadly. Whole genome analyses reveal the ancestor's connections to populations in West and West-Central Africa, confirming archival research based on slave ship registers (1), while also adding additional details about the intersecting histories with Indigenous North American populations (48). The integration of aDNA and strontium isotopic analyses further allows for greater precision in reconstructing the individual histories of the Anson Street Ancestors, informing their African regional ancestries and intercontinental migration histories.

Finally, we would like to emphasize that these results were generated from a community-centered study that investigated questions important to Charleston's African descendant community concerning the origins and histories of their ancestors. The engagement process centered the community as primary stakeholders in the research process, thereby prioritizing their needs and necessitating accountability from the researchers working with the remains of the ancestors. Overall, this study presents an example of how paleogenomic research can be conducted in collaboration with community stakeholders.

Materials and Methods

Ethics and Community Outreach. DNA analysis was undertaken in collaboration with the Charleston African descendant community and the Gullah Society, Inc., a nonprofit dedicated to the preservation and protection of African Burial Grounds (which later formed the ASABG Project team), to learn more about the Anson Street Ancestors. A project plan including the return of all skeletal and genetic material at the end of the study, expected timelines for study completion, educational outreach with local schools (elementary, middle and high school, and university), and measures for capacity building through training of a College of Charleston student at the University of Pennsylvania were agreed upon. In-person presentations, as part of Community Conversations, occurred periodically (every 3 to 4 mo) in Charleston over the course of the study to update the community about the results and make transparent the methods of DNA and analytical analyses. Additional details about the community engagement process are described in Fleskes et al. (9).

The resulting sequence reads are available via the European Genome-phenome Archive (EGA) repository (https://www.ebi.ac.uk/ega) under the accession number EGAS00001006693 with controlled access, in line with the community's decision for additional protection against commercial use of the data.

Trans-Atlantic Slave Trade Database. Demographic estimates from the Trans-Atlantic Slave Trade Database (accessed March 22, 2021) were obtained by selecting Charleston as the principal landing place for all dates from AD 1700 to 1810. The data were viewed in the "Tables" tab, selecting "10-y period" for the row designation, the "Principal place of purchase" for the column designation, and either "Sum of Embarked Slaves" to obtain embarkation estimates or "Sum of Disembarked Slaves" for disembarkation estimates in the cell designation (Dataset S1). Frequency information was calculated by dividing the target population number with the total number of individuals. The results were visualized using the ggplot2 package (55) in RStudio v4.0 (56).

aDNA Methods and Sequencing. DNA samples were collected from the 36 ancestors, with preference for petrous-temporal bones or molar teeth, and transported to the University of Tennessee-Knoxville (UTK) (Dataset S2) (9). Targeted regions of the bone were isolated using a Dremel handheld saw and the surfaces

decontaminated prior to grinding using a SPEX SamplePrep 6770 Freezer/Mill. Between 0.2 to 0.3 grams of bone powder were used for DNA extraction as per Dabney et al. 2013 (57). Double-stranded DNA libraries were prepared using a modified NEBNext Ultra II library preparation protocol with partial USER treatment and quantified using a Bioanalyzer High Sensitivity DNA assay and quantitative qPCR (Applied Biosystems). Ancestors with high enough DNA concentration (n = 35) underwent whole genome enrichment (myBaits) and were test sequenced on an Illumina MiSeq at UTK's Next-Gen Illumina Sequencing Core Facility. Of those, 31 were subjected to deeper sequencing using a NovaSeq (4 lanes) and HiSeq 4000 (2 lanes) at the University of Pennsylvania's Next Generation Sequencing Genomics Core.

Sequence Processing and Authentication. Sequencing adapters, low-quality bases, and ultrashort fragments (<30 base pairs) were removed using Adapter Removal v2 in the single-end mode (58). FastQC v0.11.9 was used to verify the removal of all adapters and assess the overall quality of sequence reads. Reads were mapped to the hg19 reference genome using BWA-aln v0.7.17-r1188, specifying "-l 1000 -n 0.01 -o 2" parameters (59). Mapped reads were merged between sequencing runs and filtered for quality (>30), duplicates, and reads with multiple mappings using SAMtools v1.9 (60). Coverage and mapping statistics were obtained using Qualimap v2.2.1 (61).

Damage estimates were calculated using MapDamage v2.2.0-2, and PMDtools v0.50 at 0 and 3 damage thresholds (23, 24). Damage at CpG methylation sites was calculated using the "platypus" option and overall damage rates calculated using the first and CpG options in PMDtools v0.50 (24). For *Banza* (CHS01), *Anika* (CHS10), *Nana* (CHS11), and *Zimbu* (CHS13), the first 100,000 reads were used. Results were used to estimate nuclear contamination with ContamLD (22) and ContamMix v1.0-10 (20) for mtDNA contamination, verified using Haplocheck v1.3.3 (21) (*SI Appendix*, section S1.1).

Reference Populations and Variant Calling. The Human Origins Panel (62), 1000 Genomes Panel (35), and a custom panel of African reference populations (63–65) were assembled and filtered for variant calling using PLINK (66) (Dataset S5). PileupCaller was used to call pseudo-haploid genotypes, keeping only transitions with >30 mapping and base quality scores (*SI Appendix*, section S1.2).

Principal Component and ADMIXTURE Analyses. Principal component analysis was conducted on merged datasets using SmartPCA v16, including all outliers and using lsqproject (26). The results were visualized in RStudio v4.0 using ggplot2 (55, 56). Merged datasets were pruned for linkage-disequilibrium in PLINK using indep-pairwise "50 10 0.1" for admixture analysis (66). ADMIXTURE v1.3 was run with 100 bootstrap replicates and visualized in R using pophelper (27, 67).

F and D Statistics. F and D statistics were run using Admixtools v.7.0.2 against population on the Human Origins panel (68, 69). F3 outgroup statistics were calculated using qp3Pop from the AdmixTools package (28) following F3(Ust'Ishim; CHS#, Y), where Y is the Human Origins comparative population, and the outgroup is Ust'Ishim. Ust'Ishim, a 45,000-y-old individual from Siberia, has both East Asian and West Eurasian ancestry components (70) and, hence, was an appropriate outgroup for West African populations (8). We specified the parameter "inbreed: YES" and considered results only with greater than 12K overlapping SNPs and significant Z scores (>3) for quality control measures. For ancestors with significant F3 outgroup results, we tested for further population affinity using D statistics in the qpDstat program from the AdmixTools package (28), following D (Chimp, CHS#; Target, Y). The population with the highest F3 outgroup value was selected as the target population, and Y corresponded to the African reference populations on the Human Origins Panel.

Genetic Sex and Kinship Estimation. Genetic sex was estimated from filtered mapped reads using ry_compute.py, which compares the ratio of Y chromosomal mapped reads to those mapped on both the X and the Y chromosome (33). Relatedness was estimated using lcMLkin, which calculates the coefficient of relatedness between pairs of individuals using genotype likelihoods (34). Genotype likelihoods were calculated using SNPbam2vcf.py script included in the package, against target SNPs in the 1000 Genomes reference panel, trimmed every 50,000 bases and filtered for SNPs with greater than 5% allele frequency (*SI Appendix*, section S1.4).

Uniparental Ancestries. Mitogenome sequences were mapped using BWA-aln against the NC_012920.1 reference sequence (59). Reads were merged and filtered according to the protocols described in Sequence Processing. Genotype likelihoods were calculated using SAMtools v1.9 mpileup, using only reads with mapping and base call qualities >30 (60). Variants were called using BCFtools v1.9 (71) specifying for haploid ploidy and filtered for quality (>30) and depth (>5) using RTG tools v3.10.1 vcffilter. Haplotypes were characterized using Haplogrep v2.1.25 against the Phylotree Build 17 and mitogenome coverage calculated using Qualimap v2.2.1 (36, 61, 72). Mitochondrial contamination was assessed using ContamMix v1.0-10 (20) and Haplocheck v1.3.3 (21).

Y chromosomal reads in the filtered bam files were isolated and indexed using SAMtools for Y haplogroup characterization using Yleaf (41). Markers were called against positions in the ISOGG database using reads with >30 quality scores. For a marker to be called, at least five reads and a minimum of 90% consistency between reads were required.

Data, Materials, and Software Availability. DNA sequences data have been deposited in EGA (EGAS00001006693). All study data are included in the article and/or *SI Appendix*. Some study data available (Following community stakeholder decision, the aDNA data will be uploaded to the EGA with controlled access. This means that persons wishing to download the data will have to sign a consent form that they will not use the data for commercial purposes.)

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