Report

Current Biology

Historical genomes elucidate European settlement and the African diaspora in Delaware

Highlights

- Genomic data indicate eight individuals of European and three of African ancestry
- Individuals were interred in separate burial groupings based on genomic ancestry
- Paternally related parent and child of African descent were identified
- Maternally related grandparent, parent, and child of European descent were found

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In brief

Fleskes et al. conduct ancient genomic analysis of a colonial period archaeological site in Delaware (USA). They investigate genetic ancestry of eleven burials and identify genetic kinship among individuals of both European and African descent. This study enlarges our understanding of frontier settlement and the trans-Atlantic slave trade.

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Report

Historical genomes elucidate European settlement and the African diaspora in Delaware

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SUMMARY

The 17th-century colonization of North America brought thousands of Europeans to Indigenous lands in the Delaware region, which comprises the eastern boundary of the Chesapeake Bay in what is now the Mid-Atlantic region of the United States.[1] The demographic features of these initial colonial migrations are not uniformly characterized, with Europeans and European-Americans migrating to the Delaware area from other countries and neighboring colonies as single persons or in family units of free persons, indentured servants, or tenant farmers.[2] European colonizers also instituted a system of racialized slavery through which they forcibly transported thousands of Africans to the Chesapeake region. Historical information about African-descended individuals in the Delaware region is limited, with a population estimate of less than 500 persons by 1700 CE.[3,4] To shed light on the population histories of this period, we analyzed low-coverage genomes of 11 individuals from the Avery's Rest archaeological site (circa 1675–1725 CE), located in Delaware. Previous osteological and mitochondrial DNA (mtDNA) sequence analyses showed a southern group of eight individuals of European maternal descent, buried 15–20 feet from a northern group of three individuals of African maternal descent.[5] Autosomal results further illuminate genomic similarities to Northwestern European reference populations or West and West-Central African reference populations, respectively. We also identify three generations of maternal kin of European ancestry and a paternal parent-offspring relationship between an adult and child of African ancestry. These findings expand our understanding of the origins and familial relationships in late 17th and early 18th century North America.

RESULTS

Genomic sequencing results

Human genomic and mitochondrial DNA (mtDNA) were extracted from the petrous pyramid of the temporal bone for each of the 11 Avery's Rest individuals. The DNA extracts underwent whole-genome enrichment, generating between 0.17 and 0.64× genomic coverage (Table 1; Data S1B). Estimates of present-day DNA sequence contamination were calculated, with X chromosome contamination[6] for chromosomal males below 4.5% and present-day mtDNA contamination[10] ranging between 0.73% and 15.38% (Table 1; Data S1G). Four individuals (AR03, AR07, AR09, and AR10) exhibited >5% mtDNA contamination. Consequently, we restricted sequence reads to include only those with damage using PMDtools (PMD > 3) for all subsequent population analyses (PCA, ADMIXTURE, F3 statistics, and kinship analyses) to determine if there were any discrepancies in our findings (Figure S1; Data S2Q–S2V). Although the results were affected by the decrease in the number of useable sequence reads, they were not substantially different from initial estimates.

The overall percentage of damage at the first base position ranged between 5.16% and 21.33%, consistent with partial USER treatment during library preparation. Damage patterns were initially characterized using MapDamage,[11] which identified increased G-to-A substitution rates at the 3′ end, but decreased C-to-T rates at the 5′ end. This asymmetrical pattern of damage is likely due to the use of Phusion High-Fidelity DNA Polymerase (NEB) during library amplification, which cannot read through uracil bases but preserves signatures of damage to CpG methyl groups.[11–13] PMDtools[7] was run using the "platypus" option and detected elevated levels of damage to CpG methyl groups at both 5′ and 3′ ends, suggesting damage attributable to DNA degradation (Figure S1; Data S1G).

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Table 1. Archaeological, osteological, and sequencing results summary for the Avery's Rest individuals

ID	Burial location	Age[a] (years)	Coverage	Mapped reads (Human Origins panel)	Contamination[b] (%)	Damage[c] (%)	Sex[d]	mtDNA coverage	mtDNA haplogroup	NRY haplogroup
AR01	south	30–40	0.56×	11,797	4.80%	7.22%	M	177.20×	T2a1a8	R1b1a-Y16765
AR02	south	4–6 months	0.58×	15,882	0.73%	12.33%	M	176.83×	H1af1a	R1b1a-S378
AR03	south	35–45	0.55×	12,533	10.91%	7.09%	M	189.75×	W	R1b1a-Z30233
AR04	south	40–50	0.50×	11,997	3.64%	7.36%	M	174.25×	U5b2a1a1a	R1b1a-S1746
AR05	south	35–45	0.45×	10,482	1.59%	6.85%	M	168.14×	H11a	I2a1b-FGC3617
AR06	south	50–60	0.43×	11,225	1.65%	7.69%	F	157.10×	H1af1a	–
AR07	south	35–45	0.17×	4,919	15.38%	8.14%	F	142.61×	H1af1a	–
AR08	south	30–40	0.32×	8,351	4.58%	8.11%	M	167.85×	J1c2p	I1a2a-BY211
AR09	north	32–42	0.25×	7,275	7.18%	10.30%	M	157.72×	L3e3b	E1b1a-CTS2447
AR10	north	27–37	0.37×	10,198	7.18%	21.33%	M	170.05×	L0a1a2	E1b1a-Z5974
AR11	north	4.5–5.5	0.64×	14,898	4.58%	5.16%	M	186.98×	L3d2	E1b1a-Z5974

See also Figures S1 and S2, Table S1, and Data S1B–S1G.
[a]Estimation based on osteological assessments in Fleskes et al.[5]
[b]mtDNA contamination estimates using contamMix[6]
[c]Damage estimation using PMDtools, showing percentage of damaged reads >3[7]
[d]Here, we estimate sex using Skoglund et al.[8] based on generated data related to chromosomal sex. Note that this program only estimates XX or XY states and does not account for other chromosomal sex karyotypes. While the authors contextualize sex using binary assignments of X and Y chromosomes, we recognize that sex is a far more complicated multidimensional variable.[9]

Principal component analysis

To ascertain the genomic ancestries of the Avery's Rest individuals, we called single nucleotide polymorphisms (SNPs) against transversion sites on the Affymetrix Human Origins panel (Table S1). SmartPCA[14] results reinforced previous findings that showed a clear separation of the 11 individuals into two groups based on continental ancestry, with eight individuals in the southern burial group clustering in PCA space with European and Middle Eastern reference populations and the three individuals in the northern group projecting with African reference populations (Figure 1A). To verify this outcome, SmartPCA[14] was also run only with damaged reads (PMD > 3),[7] with the results showing a similar data projection (Figure S2).

For the eight individuals projecting near European populations (AR01–AR08), we investigated their genomic similarity with only European reference populations in the Human Origins panel (Table S1). The SmartPCA[14] results showed that they projected close to reference populations in northwestern Europe (Figure 1B). To obtain a more detailed assessment of European ancestry, an additional SmartPCA[14] was run using only European populations on the Human Origins panel. Individuals with European ancestry clustered closest to British and French populations (Figure S3A).

The genomic similarities of the three individuals with African ancestry were further evaluated using a custom panel of 78 populations and 3,558 individuals (Table S1). SmartPCA[14] results indicated that these individuals cluster with different regional African reference populations (Figure 2B). Individual AR09 projected toward the middle of the PCA, with admixed African descendant populations from Barbados. Individual AR10 fell within the projected range of West-Central African Bantu-speaking populations, while AR11 clustered between West and East African populations.

To obtain better resolution of their African ancestries, we ran SmartPCA[14] using data from West, West-Central, and Southern African reference populations. Overall, the three individuals showed similarity to west and west-central biogeographic groups along the second principal component. AR09 projected close to West African populations, while AR10 clustered with West-Central African populations. In contrast, AR11 did not closely align with any specific population (Figure S3B). However, this approach assumes that present-day African reference populations are a comprehensive representation of the diversity that existed during the colonial period.[15] This is problematic as populations are not static entities, population distributions in continental Africa were disrupted by the trans-Atlantic slave trade, and African reference populations are underrepresented in public databases.[16]

Inter- and intra-continental admixture

To investigate inter-continental population admixture for the individuals at Avery's Rest, their genomic data were analyzed using ADMIXTURE[17] for K = 3 to K = 8 against transversion sites in the Human Origins panel. The results showed no major continental admixture for any of these individuals (Figure 1C). We investigated admixture patterns for the African-descended individuals by running ADMIXTURE[17] with the custom African panel and the 1000 Genomes Project European-American (CEU), Han Chinese (CHS), Mexican (MXL), and Peruvian (PEL) populations, from K = 4 to K = 12 (Figure 2C). The resulting admixture profile for AR10 was similar to those for reference populations from West-Central Africa, namely Cameroon and Gabon. By contrast, AR09 and AR11 did not display admixture profiles similar to any contemporary African populations in the panel, although the predominant cluster in AR09 appeared to be highly represented in West African reference populations. The admixture profile of AR11 also contained a combination of all African clusters and did not align closely with any regional group. This pattern suggests that either this individual had

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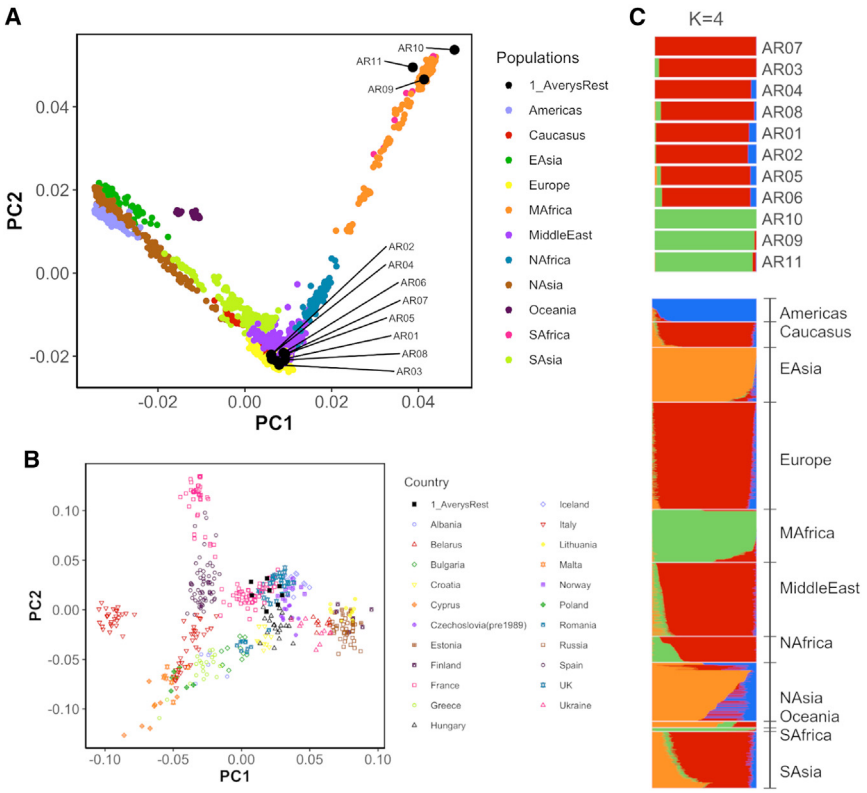

Figure 1. Genomic ancestries of Avery's Rest individuals against the global Human Origins panel

(A) PCA results showing separation of individuals with European and African ancestry components.

(B) PCA results for the eight European-affiliated Avery's Rest individuals, plotted against European populations in the Human Origins panel.

(C) ADMIXTURE results at K = 8 for the Avery's Rest series.

See also Figures S2 and S3 and Data S1C.

genetic contributions from several different African populations or the primary source population was not represented in the reference panel.

We then ran outgroup F3 statistics using popstats[18] for all Avery's Rest individuals against populations in the Human Origins reference panel to ascertain patterns of shared genetic drift. We specified F3(AR, Target Population; Mbuti) against all populations in the Human Origins reference panel, whereby Mbuti represents an equal outgroup to West African and European populations.[15,19] The results mirrored findings from the SmartPCA[14] and ADMIXTURE[17] analyses. There were higher F3 values with Northern or Western European populations for individuals AR01 through AR08 and higher F3 values for Bantu-speaking populations in Africa for individuals AR09, AR10, and AR11 (Data S2A–S2K; Figure S4). All values contained significant *Z* scores (>3).

To obtain a finer delineation of the admixture profiles of the three African-affiliated individuals, we ran outgroup F3 statistics using the arrangement F3(AR, Target Population; Mbuti).[18] The results showed higher F3 values with significant *Z* scores (>3) across western, southern, and central Africa and did not reveal strong associations with any one population (Data S2L–S2N; Figure S4). In addition, we ran admixture F3 statistics with AR09 and AR11 to test whether they were admixed with any of the African reference populations, where F3(AR, Test Population; Target Population), but found no evidence for it (Data S2O and S2P).

Chromosomal sex estimation

Chromosomal sex was estimated for all individuals from filtered reads.[8] The results showed that two females and nine males were buried at Avery's Rest, verifying osteological assessments of sex for the adults[5] and clarifying that the infant of European ancestry (AR02) and child of African ancestry (AR11) were chromosomal males. The two females of European ancestry were interred in the southern group (Table 1; Data S1F).

mtDNA and Y chromosome diversity

To investigate the maternal and paternal lineages of these individuals, we characterized their mitogenome and male-specific Y chromosome (MSY) sequences. The eight individuals in the southern burial group displayed mtDNA haplogroups found broadly across western Europe (H, J, U, T, and W), including the United Kingdom (Table 1; Data S1E).[20,21] Three of these individuals (two adult females [AR06 and AR07] and the infant male [AR02]) share haplogroup H1af1a, indicating a strong likelihood of maternal relatedness. The three individuals in the northern group had mtDNAs from distinctly African haplogroups, namely, L0a1a2, L3d2, and L3e3b. Of these, persons carrying L3e3 and L0a1a2 mtDNAs are present in Central Africa,[22,23] and those with L3d mtDNAs are found at high frequencies among West African populations, although the sublineage L3d2 has also been observed in East African populations.[24,25] L0a1 and L3e3 have also been found in other historical African-descended individuals from South Carolina (United States) and St. Helena (South Africa),[26,27,28] and all three mtDNA haplogroups have been reported in contemporary African-descended populations in the Americas.[29–32]

MSY haplogroups for chromosomal males (AR01, AR02, AR03, AR04, AR05, AR08, AR09, AR10, and AR11) were predicted from genomic sequence data using Yleaf[33] and showed similar patterns of ancestral distributions (Table 1; Data S1D). The southern burial group possessed a diverse set of European paternal lineages, with MSY sublineages of haplogroups I1, I2, and R1b1a being identified. I1a is commonly found in populations in northern Europe,[34] I2 in western and eastern Europe,[35] and R1b1a throughout western Europe.[36] By contrast, the three individuals in the northern burial group had Y chromosomes belonging to the African haplogroup E1b1a. This MSY haplogroup is observed in west, central, and southern African populations,[37] as well as in African-descended populations in the Americas.[36,38,39] In addition, the adult male (AR10) and child

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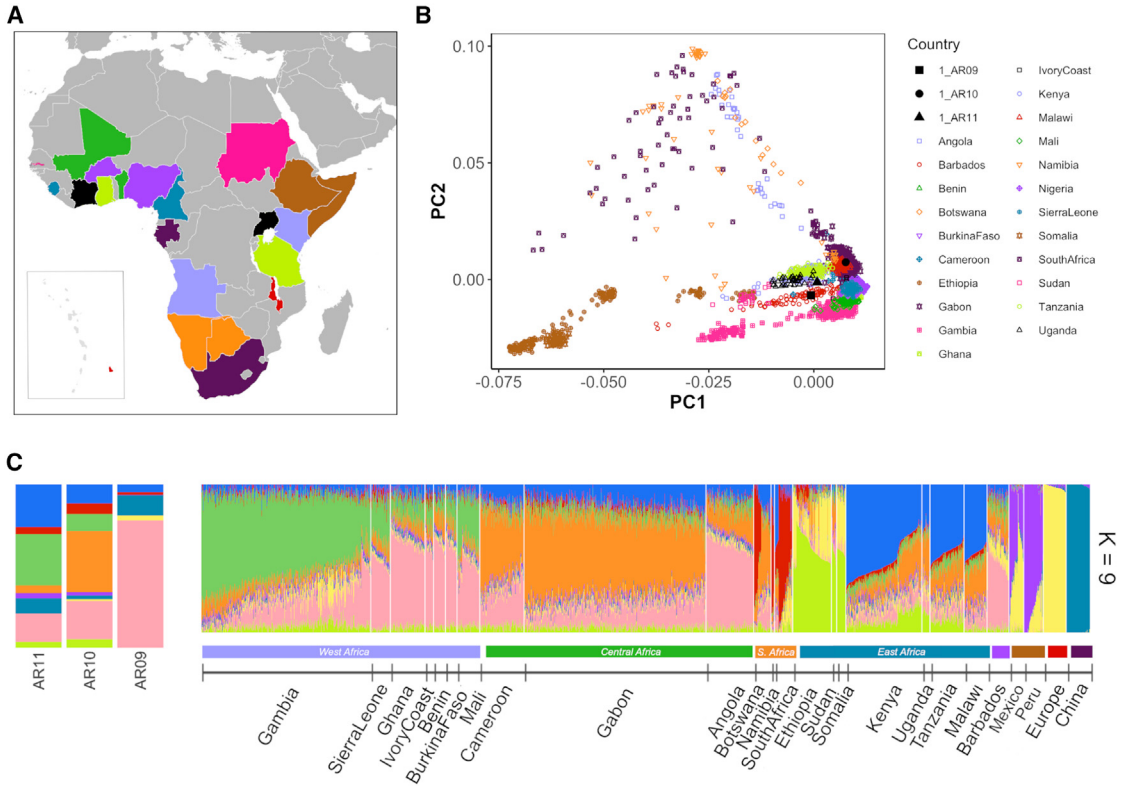

Figure 2. African ancestry profiles for AR09, AR10, and AR11 against a custom African reference panel

(A) Map of Africa with the countries used in the reference panel colored according to the accompanying PCA plot. The subpanel displays the Lesser Antilles Islands, for which the island of Barbados is highlighted.

(B) PCA plot of the three Avery's Rest individuals with African ancestry.

(C) ADMIXTURE profiles of the three individuals with African ancestry at K = 9.

See also Figures S2 and S3, Tables S1 and S2, and Data S1.

(AR11) shared the same terminal marker in the E1b1a haplotype. While not conclusive evidence of a paternal genetic relationship, this finding does not exclude the possibility.

Genetic kinship

To explore the degree of genetic relatedness among Avery's Rest individuals, we assessed autosomal kinship using two primary approaches. We estimated first-degree relationships using average pair-wise distance with READ[40] and observed three first-degree relationship pairs (AR06 and AR07; AR07 and AR02; AR10 and AR11) (Figure 3; Data S3A and S3B). These pairs shared either the same mtDNA (H1a1af1 for AR02, AR06, and AR07) or MSY (E1b1a1-Z5974 for AR10 and AR11) haplogroup. For the individuals with African ancestry, the fact that they did not share the same mtDNA haplogroup ruled out a full-sibling relationship within the context of first-degree relatedness with identical MSY haplogroups. Thus, this evidence supports a paternal parent-offspring relationship between the African-descended adult male (AR10) and child (AR11).

We further characterized the first-degree relationship for only the individuals with European ancestry (AR02, AR06, and AR07) using NgsRelate.[41] This program requires input allele frequencies generated from contemporary populations, which could not be accurately approximated for the African individuals. For the European individuals, we used the British (GBR) and Northern European (CEU) populations from the 1000 Genomes Project to estimate allele frequencies for the intersecting SNPs.[42] The R0, R1, and KING-coefficient statistics for the AR06-AR07 and AR02-AR07 pairs fell within the range of parent-offspring relationships, as reported by Waples and colleagues (Figure 3; Data S3C).[43] In addition, NgsRelate identified a second-degree relationship between AR06 and AR02. Since these individuals share the same mtDNA haplogroup, it is likely that AR06, AR07, and AR02 had an inter-generational maternal relationship, specifically that of grandmother, mother, and son.

DISCUSSION

Our findings at the Avery's Rest site illustrate how the entangled web of colonialism spans the larger Atlantic world and provide important insights into early colonial settlement, kinship, and the trans-Atlantic slave trade in Delaware at the end of the 17th century. This case study provides the first genomic evidence of the demographic makeup of European colonial migrants and their occupation in Delaware during the 17th century, which had previously only been estimated from archival records and archaeological evidence. Our results show the European-descended individuals were most genetically similar to populations

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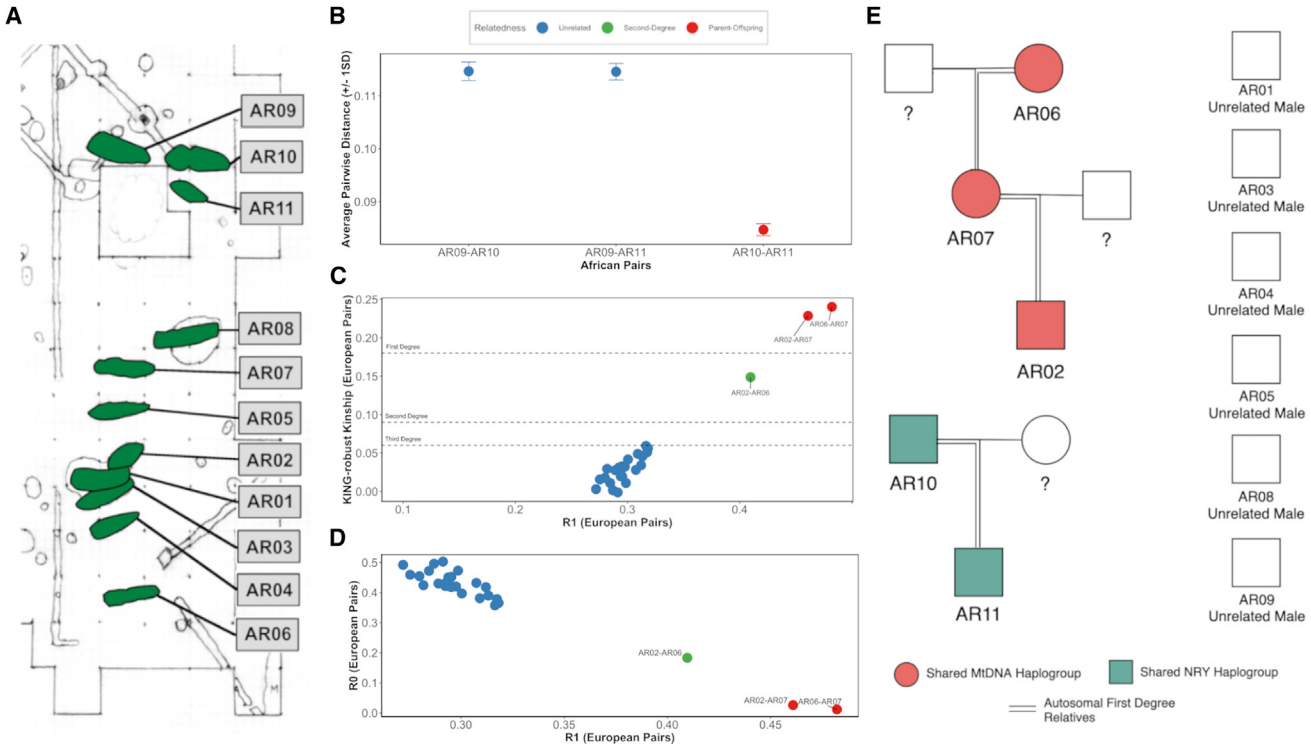

Figure 3. Kinship estimation for the Avery's Rest individuals

(A) Burial locations at the Avery's Rest site. Individuals AR01–AR08 are located in the southern burial cluster; individuals AR09–AR11 are located in the northern cluster. The space between the dots represents a 5-foot scale. Note that due to the scale of the map, burials AR01 and AR03 appear to overlap but are buried parallel to each other.

(B) READ results for the African individuals, with error bars showing one standard error for average pairwise distance.

(C and D) (C) NgsRelate results for the 2DSFS method, displaying R1 and the KING-Coefficient, and (D) R1 and the R0 values for the European-affiliated individuals.

(E) Constructed pedigree chart showing relationships among Avery's Rest individuals. Squares indicate males and circles represent females, as estimated in the Chromosomal sex estimation section of the STAR Methods.

See also Figure S2 and Data S1D–S1F and S3A–S3D.

from Great Britain and France, which correlates with historical records showing British colonial settlement in Delaware following Dutch occupation of Indigenous lands.[1] Five non-genetically related males were buried along with three consecutive generations of matrilineal kin, representing a grandmother, mother, and son. This demographic profile supports historiographical findings of a blended or mosaic organizational structure for European colonists at early colonial Chesapeake and Mid-Atlantic settlements, in which unrelated males and small groups of biological kin formed social and labor units.[2]

Besides providing insight into the demography of colonial Delaware, the identification of individuals of European and African ancestry at Avery's Rest reflects mortuary practices in North American frontier settings. These individuals were interred in closely spaced, but separate, burial clusters divided by continental-level ancestry. Their interment location further suggests that interactions between European and African persons may have been more integrated than suggested by mortuary practices in later antebellum cemeteries, where burials were often interred in more separated locations based on race.[44–46] The distinct clustering of the burial ground by continental ancestry nevertheless suggests that frontier lifeways from the beginning were governed by settler-colonial practices of racialized enslavement.[3,47]

The presence of African individuals at Avery's Rest is itself significant, as few enslaved Africans are known to have entered the Delaware region between 1665 and 1713 CE.[1,4] However, two adult African individuals at Avery's Rest were recorded as part of an estate re-evaluation case petitioned on 7 October 1690 CE concerning debts to the estate of John Avery after his death in September 1682 CE.[48] The stated reason for re-evaluation was that the two African males had died shortly before the initial 1683 CE evaluation was conducted.[48] It is possible, although uncertain, that the two adult African individuals found at Avery's Rest are those referenced in the record. Previous osteological analysis provided information on the cause of death for AR10.[5] Their cranium displays evidence of a facial fracture to the right zygomatic arch and maxilla caused by blunt force trauma at or near the time of death. The location of the fracture on the cranium is consistent with interpersonal violence.

The identification of a patrilineal parent-offspring relationship between adult AR10 and child AR11 represents the earliest demonstration of biological kinship among African-descended individuals using ancient DNA at a North American colonial site. The

side-by-side interment of AR10 and AR11 suggests that biological kinship may have dictated burial placement of the son (AR11), whose death likely followed the father's (AR10). Burial placement suggests that AR09, who was buried adjacent to the fence line similar to most of the other burials (Figure 3), was interred prior to AR10 and AR11. The father-son relationship is notable as families could be, and often were, separated at the will of their enslavers, reflecting the structural violence embedded in the institution of slavery.[49] This discovery is especially critical for understanding histories of African-descended individuals during the early colonial period, for which evidence of biological relatedness in the archival record is limited.

In addition, this study offers important insights into the demographic histories of African individuals forcibly enslaved and brought to peripheral locations in the colonial Atlantic world. For the father-son pair (AR10-AR11), our results indicate that the father (AR10) had ancestry tracing to west-central Africa, whereas his son (AR11) did not show any clear association with a specific African reference population. This finding may suggest that the mother of AR11, who was not found at the site, originated from a different population in Africa. The other unrelated adult African male (AR09) likely had ancestral origins in west Africa. These results align with other ancient genomic analyses of African-descended individuals in North America and the Caribbean[50,51] and contemporary African Americans[16,52,53] and broadly reflect the larger patterns of west and west-central African origins in the trans-Atlantic slave trade.[54] Furthermore, the genomic data suggest that the networks embedded in the trans-Atlantic slave trade were consistent between early core and peripheral locales in English colonial North America.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.04.069.

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AUTHOR CONTRIBUTIONS

R.E.F., T.G.S., and D.W.O. designed research; R.E.F., D.W.O., K.S.B., K.G.B., and D.R.G. performed research; R.E.F. analyzed data; R.E.F., T.G.S., D.W.O., G.S.C., K.S.B., K.G.B., and D.R.G. wrote the paper; and T.G.S., D.W.O., and G.S.C. supervised research.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR01 / 7S-G-57-AVERYSREST-01
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR02 / 7S-G-57-AVERYSREST-02
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR03 / 7S-G-57-AVERYSREST-03
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR04 / 7S-G-57-AVERYSREST-04
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR05 / 7S-G-57-AVERYSREST-05
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR06 / 7S-G-57-AVERYSREST-06
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR07 / 7S-G-57-AVERYSREST-07
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR08 / 7S-G-57-AVERYSREST-08
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR09 / 7S-G-57-AVERYSREST-09
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR10 / 7S-G-57-AVERYSREST-10
Archaeological Individual	This study / The Avery's Rest Archaeological Site / The Smithsonian Institution's National Museum of Natural History	AR11 / 7S-G-57-AVERYSREST-11
Chemicals, peptides, and recombinant proteins		
DNA AWAY	Molecular BioProducts	Cat# 7010
Bleach	Clorox	N/A
Agarose	Fisher Scientific	Cat# BP1356-500
Gel Red	Phenix	Cat# RGB-4103
Tris base	Fisher Scientific	Cat# BP152-1

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Boric Acid	Sigma Aldrich	Cat# B7660-500G
0.5 M EDTA	Teknova	Cat# E0306
Tween-20	Sigma Aldrich	Cat# P9416-50ML
Proteinase K	Sigma Aldrich	Cat# P2308-25MG
Guanidine Hydrochloride	Teknova	Cat# G0316
Isopropanol	Walgreens	Cat# 715455
Sodium Acetate	Sigma Aldrich	Cat# 71196-100ML
1M Tris-HCl	Invitrogen	Cat# 15568025
NEBNext Ultra II Library Preparation Kit	New England Biosciences	Cat# E7645S
USER enzyme	New England Biosciences	Cat# M5508
Phusion High-Fidelity DNA Polymerase	New England Biosciences	Cat# M0530S
Kapa HiFi Hot Start ReadyMix	Roche Diagnostics	Cat# 07958927001
10 mM Tris-Cl, 0.05% TWEEN-20 solution	Teknova	Cat# T1485
NEBNext Multiplex Oligos for Illumina	New England Biosciences	Cat# E7600S
dNTP	New England Biosciences	Cat# N0447S
DMSO	New England Biosciences	Cat# M0530S
BSA	New England Biosciences	Cat# B9000S
Critical commercial assays		
PowerUp SYBR Green Master Mix	Thermo Scientific	Cat#: A25742
MinElute PCR Purification Kit	QiaGEN	Cat# 28006
myBaits Expert Whole Genome Enrichment Kit v4	Arbor Biosciences	Cat# 302508
Agilent High Sensitivity DNA Kit	Agilent Technologies	Cat# 5067-4626
MiSeq Reagent Kit v2 (300-cycles)	Illumina	Cat# MS-102-2002
NextSeq 500/550 High Output Kit v2.5 (150 Cycles)	Illumina	Cat# 20024907
NovaSeq 6000 S2 Reagent Kit v1.5 (100 cycles)	Illumina	Cat# 20028316
Deposited data		
Raw data	This study	SRA: PRJNA812783
Software and algorithms		
AdapterRemoval v2	Schubert et al.[55]	https://github.com/MikkelSchubert/adapterremoval
BWA	Li et al.[56]	https://bio-bwa.sourceforge.net/
samtools	Li et al.[57]	https://samtools.sourceforge.net/
Qualimap 2	Okonechnikov et al.[58]	http://qualimap.conesalab.org/
MapDamage 2.0	Jónsson et al.[11]	https://ginolhac.github.io/mapDamage/
contamMix	Fu et al.[59]	N/A
ANGSD	Moreno-Mayar et al.[6]	https://github.com/ANGSD/angsd
PMDtools	Skoglund et al.[7]	https://github.com/pontussk/PMDtools
PLINK	Purcell et al.[60]	https://zzz.bwh.harvard.edu/plink/
READ	Kuhn et al.[40]	https://bitbucket.org/tguenther/read/src
NGSRelate	Korneliussen et al.[41]	https://github.com/ANGSD/NgsRelate
pileupCaller	N/A	https://github.com/stschiff/sequenceTools
Haplogrep 2	Weissensteiner et al.[61]	https://haplogrep.i-med.ac.at/
yleaf	Ralf et al.[33]	https://github.com/genid/Yleaf
SmartPCA	Patterson et al.[14]	https://www.hsph.harvard.edu/alkes-price/software/
ADMIXTURE	Alexander et al.[17]	https://dalexander.github.io/admixture/
popHelper	Francis et al.[62]	http://www.royfrancis.com/pophelper/articles/

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
popstats	Patterson et al.[18]	https://github.com/pontussk/popstats
lcMLkin	Lipatov et al.[63]	https://github.com/COMBINE-lab/maximum-likelihood-relatedness-estimation
Other		
Zymo Spin V	Zymo Research	Cat# C1012-50

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Raquel Fleskes (raquel.fleskes@uconn.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Genomic sequence data have been deposited at the Sequence Read Archives and are publicly available as of the date of publication. Accession numbers are listed in the key resources table. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Archaeological site

The Avery's Rest archaeological site encapsulates an early nexus of European settler colonial migration and the African diaspora. The site is located near present-day Lewes, Delaware (United States). Archival research indicates that the site was owned by the Avery family between circa A.D. 1675 to 1725, whose members migrated from the neighboring British colony of Maryland.[64] Archaeological excavations began at the site in 2006, and eventually revealed 11 burials comprised of a southern grouping of eight individuals situated 15–20 feet from a northern cluster of three individuals.[5] All individuals buried at Avery's Rest are oriented east/west, with the head facing east, and aligned with a fence line running north/south. Previously published osteological estimations of age and biological sex indicate the presence of an infant, child, women and men at the site between the ages of 6 months to over 40 years of age.[5] All burials are thought to date to the Avery family occupation period or slightly later.[65]

Ethics statement

Consultation with representatives of Avery family descendants, the Harmon Family, who are the current property owners of the Avery's Rest site, and the Delaware Division of Historical and Cultural Affairs were conducted. Permission was obtained from the Smithsonian Institution National Museum of Natural History Department of Anthropology Collections Advisory Committee for DNA sampling in 2019. Outreach programming and public presentations were conducted through the Delaware Division of Historical and Cultural Affairs, as well as The Delaware Archaeological Society, Inc.

METHOD DETAILS

Contamination control

All laboratory analyses took place in the ancient DNA (aDNA) clean room of the Molecular Anthropology Laboratories at the University of Tennessee-Knoxville (UTK). The aDNA facility is constructed to minimize exogenous contamination through a four-chambered design, overhead UV lights, and positive-pressured HEPA-filtered airflow. Full PPE was used during all procedures conducted in the clean room laboratory. All surfaces, equipment, and gloves were wiped down using a 10% household bleach solution and/or DNA *AWAY* (MBP) followed by 70% ethanol before and after each use. All disposable lab supplies and small equipment were additionally subjected to UV radiation in a UV crosslinker for 30 min before use. During sample processing, gloves were changed, and all surfaces were fully cleaned using bleach and ethanol solutions between each sample to prevent cross-contamination. Negative controls were used during extraction and library preparation. The negative extraction control consisted of a blank sample tube filled with lysis buffer and no bone powder, which was carried through the extraction and library preparation process with the 11 Avery's Rest bone samples. An additional negative control (ultra-pure water) was used during the library preparation protocol. The negative controls were subject to identical protocols and quantification measures detailed in the next two sections. All were negative.

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DNA sample collection

Sampling for individuals AR01 and AR03-AR10 took place at the Smithsonian Institution's National Museum of Natural History, using the minimally invasive petrosal drilling method, given that the craniums were intact.[66] A chemical fume hood was retrofitted for DNA sampling by fully decontaminating all surfaces before and after each sample using a 10% solution of household bleach followed by 70% ethanol. All sampling materials were similarly decontaminated and placed in a UV crosslinker for 10 min on each side before use.[67] Full personal protective equipment (PPE) was worn during sampling and gloves were changed between each sample collection. Bone powder was generated using a Dremel 8200 drill with a diamond-tipped bit.

Individuals AR02 and AR011 were not represented by fully intact crania. For this reason, the temporal bones from these individuals were transported to the UTK ancient DNA laboratory to allow for greater control over the sampling environment to reduce the chance of outside contamination. Petrosal samples were drilled inside a dead air box (Bone Clone Zone) that was fully cleaned with DNA *AWAY* (MBP) followed by 70% ethanol between each sample. The petrous pyramid was drilled from the interior aspect of the cranium to allow for easier access.

DNA extraction and library preparation

Between 0.18-0.24 g of petrous bone powder was collected in sterile 2 mL O-ring tubes for DNA extraction as per Dabney et al. (Data S1A).[68] Sample incubation occurred in an EDTA-based buffer for 24 h at 56°C with gentle shaking. DNA was then isolated using MinElute spin columns (Qiagen). Extracts and negative controls were verified through mtDNA PCR amplification and agarose gel electrophoresis.[5]

Dual-indexed sequencing libraries were prepared using a double-stranded library preparation protocol (NEBNext Ultra II) modified for degraded, low-template DNA samples. Libraries were prepared from 25 μL of DNA extracts, and subject to 3 μL of USER enzyme to partially reduce damage while still maintaining damage signatures for authentication purposes. Following ligation, libraries were dual-indexed using NEBNext Multiplex Oligos for Illumina (NEB). A StepOne Real-Time quantitative PCR (qPCR) system (Applied Biosystems) using a SYBR-Green master mix (BioRad) with library-specific primers and standards were used to verify adaptor ligation success and determine the relative target cycle number. Following qPCR results, libraries were amplified using Phusion High-Fidelity DNA Polymerase (NEB) for 15 or 16 cycles, depending on qPCR results (Data S1A). Amplified libraries were cleaned using a MinElute spin column protocol (Qiagen), following spin speeds and duration as outlined in Winters.[69]

Quantification of the amplified indexed libraries, including positive and negative controls, was achieved by via a three-step process. First, the dsDNA concentrations were estimated using a Nanodrop Fluorospectrometer 3300 (Thermo Scientific). Second, the presence of adapter dimers was checked through the electrophoresis of 3 μL of extract on a 3% agarose gel. Third, a High Sensitivity Bioanalyzer assay (Agilent Technologies) was run at the UTK Division of Biology Sequencing Facility.

Enrichment and sequencing

Libraries were enriched using the myBaits Whole Genome Enrichment Kit v4 (Arbor Biosciences). Samples were independently incubated at 60°C for 48 h, with all relevant steps executed at 60°C. On-bead libraries were amplified using the HiFi Hot Start ReadyMix (Kapa Biosystems) for 14 cycles. Enriched libraries were cleaned using a modified spin-column clean up and analyzed using a High Sensitivity Bioanalyzer assay. DNA samples were pooled and sequenced on Illumina MiSeq v2 (150PE), followed by deeper sequencing on an Illumina NextSeq 500 (75SR) and NovaSeq 6000 S2 (100SR).

QUANTIFICATION AND STATISTICAL ANALYSIS

Sequence Data Processing and Authentication

AdapterRemoval v2 was used to trim adapters and filter reads based on quality scores (>30) and sequence length (>30).[55] FASTQ files were merged by sample ID before mapping to the human reference genome (hg19) with Burrows-Wheeler Aligner (BWA) aln using "l 1000 -n 0.01 -o 2" parameters, followed by BWA samse.[56] Reads with <30 mapping quality scores, duplicates, and reads with multiple mappings were removed using samtools.[57] Coverage and average read length were calculated using Qualimap 2 (Data S1B).[58]

The authentication of aDNA sequences was first evaluated by assessing their damage patterns using MapDamage 2.0.[11] Noting the atypical damage profile likely resulting from library preparation methods, we additionally assessed damage at CpG methylation sites using the "platypus" option in PMDtools.[7] Overall damage rates were calculated for the first 100,000 sequencing reads for each sample using the "first" and "CpG" options in PMDtools. Contamination estimates from present-day DNA sources were calculated using contamMix[59] for mitogenome data and ANGSD[6] for X-chromosome data (Data S1G).

Reference panels and Variant calling

Variants were called against the complete Human Origins panel, a European Human Origins panel, and a custom panel of African populations (Data S1C). The Human Origins reference panel was used to determine the extent of European and African continental ancestry and admixture.[70] Variants were filtered following the protocol from Chang (2020) using PLINK.[71,60] Specifically, variants were filtered for SNPs (–snps-only), missingness data (geno 0.1, mind 0.1), close relatives (king-cutoff 0.177), minor allele frequency (maf 0.05), and markers that deviate from Hardy–Weinberg equilibrium (–hwe 1e-25, keep-fewhet). All rsIDs were changed to chromosome and position number for consistency between datasets. This resulted in 425,308 variants for 2,056 individuals in the Human

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Origins panel (Table 1; Data S1C). To assess European continental ancestry, we retained only the European populations in the Human Origins panel and filtered as described above. This resulted in the same number of variants and 450 individuals.

To obtain a more detailed understanding of African ancestry, a representative panel of 3,553 African individuals from 22 countries were assembled from the literature (Table S1).[42,72–77] Before merging them, individual datasets were filtered as described above and a SNP list of updated rsIDs was generated in PLINK. A list of SNPs common between all datasets was generated in R using the respective lists. Each panel was then filtered for only these common SNPs and merged in PLINK. For quality purposes, the merged dataset was filtered again, resulting in 239,562 variants (Data S1C).

For admixture analyses of the three individuals with estimated African ancestry, we added the European-American (CEU), Han Chinese (CHB), Mexican (MXL), and Peruvian (PEL) populations from Phase 3 of the 1000 Genomes Project to the custom African panel to account for inter-continental admixture.[42] The data from the selected 1000 Genomes populations were filtered as described above, and only the SNPs found on the list of common variants in the custom African panel were kept. This filtered dataset was then merged with the custom African panel and filtered again before variant calling and admixture analysis.

1000 Genomes Project data were also used to call genotypes for kinship analyses, given the significantly larger number of variants.[41] After filtering, 2,493 individuals and 6,835,358 variants in the 1000 Genomes Panel were used (Data S1C).

Pseudo-haploid genotypes for the Avery's Rest individuals were independently called for each panel using samtools mpileup and pileupCaller (https://github.com/stschiff/sequenceTools), removing transitions and keeping SNPs with >30 mapping and base quality.[57] Subsequently, each panel was filtered for overlapping SNPs with the Avery's Rest genotypes and merged to the study individuals for downstream analyses.

Uniparental markers

The mitogenome sequences were independently mapped against the NC_012920.1 reference sequence using the mapping parameters outlined above in Sequence Data Processing and Authentication. Coverage was calculated using Qualimap 2.[58] Haploid variants were compiled using bcftools mpileup, filtering for map and base quality scores (>30), and called using bcftools call.[57] Variants were filtered for >5 depth. Haplogroups were called using the resulting VCF files with Haplogrep 2 (Data S1E).[61]

The mitogenome results generated from next-generation sequencing (NGS) mostly confirmed the previous mtDNA haplogroup calls generated from control region sequences in our previous study, while also increasing their phylogenetic resolution.[5] The mtDNA haplogroups calls from two individuals (AR08 and AR11) differed from the previous Sanger sequencing results (AR08: H1 [Sanger] to J1 [NGS]; AR11: L3i [Sanger] to L3e [NGS]) generated from metacarpal or rib bone DNA extracts, respectively. This discrepancy could have resulted for different reasons, including higher error rates in Sanger sequencing for degraded DNA samples, or increased sequencing resolution with NGS technology.[78]

For individuals with Y-chromosomes, paternal haplogroups were determined using yleaf, filtering for >30 quality scores, read depth >5, and calling only known ISOGG variants (Data S1D).[33]

PCA and admixture analyses

Using autosomal data, principal components were calculated using SmartPCA, specifying no outliers and using lsqproject: YES.[14] For admixture analyses, we first transformed the filtered comparative reference panels to make all sites homozygous to match the ancient data using a custom script (https://github.com/cegamorim/). We then additionally filtered for LD using indep-pairwise parameters "50 10 0.1" in PLINK.[60] We ran the program ADMIXTURE with 100 bootstrap replications for the designated values of K.[17] ADMIXTURE results were visualized using popHelper in R.[62]

F3 statistics

F3 statistics were run with popstats using the heterozygosity correction (Figure S4; Data S2A–S2V).[18,19] Outgroup F3 statistics were run for all individuals against the Human Origins Reference panel using (AR, Target Population; Mbuti) to ascertain fine scale regional ancestry (Figure S4A). We also formally tested for shared genetic drift for the three African-descended individuals (AR09-AR11) with the Target Population representing the populations on the African panel (Figure S4B). The Mbuti samples were sourced from the publicly available 1240K dataset on the Reich lab website.[79,80] To formally test for inter-African admixture for individuals AR09-AR11, F3 statistics were run specifying (AR, Test Population; Target Population) for all combinations of populations represented on the African reference panel/

Chromosomal sex estimation

The chromosomal sex of each individual was estimated by calculating the ratio of Y chromosome mapped reads compared to those mapped to both the X- and Y-chromosome reads (Data S1D).[8]

Kinship estimation

We used two main approaches to assess autosomal relatedness amongst the Avery's Rest individuals. First, READ was used to obtain a basic assessment of relatedness (Data S3A and S3B). READ was developed to estimate relatedness for low coverage sequences by calculating pairwise differences from pseudo-haploid genotype calls, which is ideal for aDNA studies.[40] However, READ assumes that all individuals derive from the same population and can be biased by high numbers of related individuals. To account for this issue, we independently ran READ for the European and African individuals genotyped against the 1000 Genomes reference

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panel. We ran READ using the 'max' option for trios for the African-affiliated individuals, given the sample size of three. Due to the lower coverage of the samples, we used READ to characterize first-degree relationships with confidence. Individuals with second-degree relationships could not be verified using additional methods described below, and thus were not counted.

Second, we analyzed the data with NgsRelate, which produces R0, R1, and kinship coefficient values that can distinguish between parent-offspring and sibling first-degree relationships (Data S3D).[41,43] NgsRelate uses allele frequencies from similar contemporary populations to estimate relatedness coefficients. This is also a drawback of this method, however, since an appropriate comparative population may not be available for archaeological individuals.[81] Given the diverse ancestral composition of the three African individuals at Avery's Rest, a representative population could not be approximated.

For the individuals with European ancestry at Avery's Rest, we selected the European-American (CEU) and British (GBR) 1000 Genomes populations to generate representative allele frequencies.[42] The data from these populations had been previously filtered as previously described, and were used to create a sites file to calculate genotype likelihoods in ANGSD, specifying '-gl 2 -domajorminor 3 -minMapQ 30 -domaf 1 -minmaf 0.05 -doGlf 3'.[82] The resulting genotype likelihood positions file was used to select the positions with which to estimate allele frequencies in the CEU and GRB populations for NgsRelate.

To add additional support for the READ and NgsRelate analyses, we ran lcMLkin, which does not require estimated allele frequencies from contemporary populations.[63] Instead, lcMLkin estimates genotype likelihoods in a maximum likelihood framework to estimate kinship coefficients (K0, K1, K1, Pairwise) in a given population. Genotype likelihoods were estimated using the SNPbam2vcf python script against the 1000 Genomes panel. To estimate how the number of sites analyzed impacted the results of lcMLkin, we thinned every (A) 25K, (B) 50K, and (C) 100K sites and filtered (–maf >0.5) using vcftools, where K equals 1,000 sites.[83] This resulted in (A) 104,724, (B) 53,064, and (C) 26,732, out of a possible 6,835,358 sites, respectively (Data S3E and S3F).

Given the small number of individuals at Avery's Rest, all individuals were analyzed together in lcMLkin two separate times. The first run identified related individual pairs (PI_HAT > 0.05), which were then used to identify the founders for the second run using the parameter -u. At all different SNP levels, relatedness between AR09 and AR10, between AR07 and AR02, and between AR07 and AR06 was estimated. The kinship coefficient was consistently higher for AR09 and AR10 than between the European pairs (Data S3G). These estimates are likely affected by the increased genomic diversity in the African-affiliated individuals relative to the European individuals in the analysis. However, separating the individuals by ancestry resulted in too small of population numbers for valid analysis. Therefore, the interpretation of the lcMLkin results were taken with caution and used only to verify the existence of biological relationships identified in READ and NgsRelate.

Our consideration of the choice of kinship estimation relied on the nature of the data analyzed. Avery's Rest represents a small 'population' comprised of two genetically diverse groups. The small sample size and proportionally higher levels of relatedness with the groups can introduce biases into analyses such as READ and lcMLkin if they are not corrected for. The importance of representative allele frequencies for NgsRelate has been noted by others,[81] and is reiterated for the African-affiliated individuals analyzed in this paper. The African-affiliated individuals at Avery's Rest likely represent a diverse group of persons with West and West-Central African ancestry. African populations are known to contain the highest global levels of genetic diversity, even within regional areas.[84] This fact, combined with low sample numbers, makes determining a representative African population to estimate allele frequencies very difficult. Therefore, we support the use of multiple approaches to estimate kinship for aDNA samples to avoid the potential pitfalls noted above for a more robust estimation of biological relatedness.

Effects of contamination

For individuals with greater than 5% mtDNA contamination (AR03, AR07, AR09, and AR10), we restricted for reads with damage (>3) using PMDtools.[7] This reduced the number of reads for all individuals (1,505,427 for AR03; 686,121 for AR07; 1,187,443 for AR09; and 1,411,230 for AR10). The damage restricted data was then analyzed to compare against downstream analyses (PCA, ADMXITURE, outgroup F3-statistics, and kinship analyses) to deduce if the impact of contamination impacted results. We first ran SmartPCA[14] following previously outlined protocols against the Human Origins Panel and Custom African Panel, respectively, which increased noise in the PCA projection but did not produce significant discrepancies in regional ancestry assignment (Figures S2A and S2B). We then conducted ADMIXTURE, as described previously, against the Human Origins Panel (K = 3 to K = 8) and the custom African Panel (K = 3 to K = 14). We also observed slight noise in the admixture profiles but did not produce significant discrepancies (Figures S2C–S2F). We then conducted outgroup F3-statistics[18] using Mbuti as the designated outgroup. The resulting F-statistic and z-scores against each reference population were slightly different (Data S2Q–S2V), which is attributed to the reduced number of SNPs used in the comparison, but did not change interpretations of the findings. Taken together, the regional characterization of either African or European genomic similarity for the Avery's Rest individuals did not change.

In relation to the possible effect of contamination on kinship designations, only one individual within each of the identified kin groups displayed higher rates of mtDNA contamination (AR07 for the European-descended kin group; AR10 for the African-descended kin group). These individuals show the same previous basal mtDNA haplogroup calls as our previous paper, which used different skeletal elements (metatarsals, carpals, etc.) for control region sequencing of the mtDNA.[5] The results are commensurate with identical sequences using different sequencing resolutions.

Damage restricted data slightly changed kinship designations by altering the degree of relatedness between individuals (Figures S2G–S2H). We conducted READ[40] and NgsRelate[41] using the damage restricted sites, following previously outlined protocols. First, NgsRelate[41] was conducted for the European individuals, which still identified all three relationship pairs, but reduced the R1 and KING-relatedness coefficients (Data S3D). This had an effect of reducing the degree of relatedness between AR02-AR07 from

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a first degree to a second degree relationship. Alternatively, READ[40] analysis increased pairwise relatedness estimates, but maintained the same relationship pairing. For the African-descended individuals, READ[40] analysis resulted in identifying a first degree relationship between AR09 and AR10. We attribute this to the fact that pairwise estimates can be biased by the number of comparative sites, of which both AR09 and AR10 had a significantly reduced number. Therefore, we argue that using damage restricted sites for the Avery's Rest samples result in too little coverage for reliable relatedness estimations, but still demonstrated previously identified genetic kin relationships.

To verify the Y chromosome haplogroups designations, which were important in the characterization of the parent-offspring relationship between AR10 and AR11, we also reanalyzed the Y chromosome haplogroups using Yleaf[33] (Data S1D). The effect of the damage restriction significantly reduced the number of available sites, thereby reducing the resolution in Y chromosome haplogroup calls. This has the effect of eliminating some haplogroup calls from being made entirely, as well as removing the overlapping terminal marker that was shared between AR10 and AR11. However, the same broad haplogroups designations remained. Because of this, we believe that the higher resolution data is needed for this analysis.