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RESEARCH ARTICLE

Ancient DNA and bioarchaeological perspectives on European and African diversity and relationships on the colonial Delaware frontier

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Abstract

Objectives: Ancient DNA (aDNA) and standard osteological analyses applied to 11 skeletons at a late 17th to early 18th century farmstead site in Delaware to investigate the biological and social factors of settlement and slavery in colonial America.

Materials and methods: Osteological analysis and mitochondrial DNA (mtDNA) sequencing were conducted for all individuals and the resulting data contextualized with archaeological and documentary evidence.

Results: Individuals of European and African descent were spatially separated in this colonial cemetery. The skeletal remains exhibited differences in osteological features and maternal genetic ancestry. A specific mtDNA haplotype appeared in a subset of the European-descended individuals suggesting they were maternally related. Individuals of African descent were not maternally related, and instead showed a diversity of haplotypes affiliated with present-day Western, Central, and Eastern regions of Africa.

Discussion: Along with the bioarchaeological and documentary evidence, the aDNA findings contribute to our understanding of life on the colonial Delaware frontier. Evidence of maternal relatedness among European-descended individuals at the site demonstrates kin-based settlements in 17th century Delaware and provides preliminary identifications of individuals. The maternal genetic diversity of the individuals with African descent aligns with the routes of the trans-Atlantic slave trade but broadens our understanding of the ancestries of persons involved in it. Burial positioning, osteological pathology, and lack of maternal kinship among individuals of African descent provide tangible evidence for the emergence of racialized labor and society in Delaware during the late 17th century.

KEYWORDS

bioarchaeology, colonization, mitochondrial DNA, trans-Atlantic slavery

1 | INTRODUCTION

One of the largest human migration events of the Early Modern Period (AD 1500–1800) involved the European colonization of North America, including the forced transport of enslaved Africans across the Atlantic Ocean (Eltis & Richardson, 2013). The biological and societal factors influencing colonial settlement (e.g., population diversity, demography, health, mortality, kinship) are only partially understood using documentary evidence. This is particularly true for the 17th century, the period following the founding of the first permanent English settlement on Jamestown Island in 1607 (Horn, 2018). The rise of tobacco as a cash crop in the Chesapeake region of the Mid-Atlantic and the concomitant necessity for a large labor force resulted in an influx of indentured servants, who were later supplemented and replaced by Africans as race-based slavery emerged in the colonies (Morgan, 2003). In addition, Chesapeake colonists experienced high rates of disease and mortality, which resulted in significant population replacement and slow natural increase (Tomlins, 2010). By the end of the 17th century, approximately 100,000 individuals of European descent and 20,000 persons of African descent were living among the native Chesapeake peoples on lands that eventually included the colonies of Delaware, Maryland, and Virginia (Richter, 2001; Tomlins, 2010).

Demographic factors relating to colonization, particularly kinship, varied by time period, region, and between and within groups, although there are limited resources to measure them. Bioarchaeological methods have traditionally provided the most direct means of determining ancestry and kinship for human remains, while archaeological analyses have inferred kinship through material evidence of household organization (Ensor, 2013; Gibb & King, 1991; Yentsch, 1994). Genetic evidence, however, can provide biological insight into ancestry and relationships of individuals recovered from unidentified burials (O'Sullivan et al., 2018; Schroeder et al., 2015).

In particular, ancient DNA (aDNA) methods have greatly expanded our ability to reconstruct the ancestry and migration history of past populations. Such methods have been used to evaluate the biohistories of individuals from North American colonial burial sites, providing crucial information about multiethnic cemetery organization (Byrnes et al., 2012; Lee, Anderson, Dale, & Merriwether, 2009) and the population affinity of African or African-descended individuals transported to the Canary Islands (Maca-Meyer et al., 2005; Santana et al., 2016) and the Caribbean (Schroeder et al., 2015) during the trans-Atlantic slave trade. aDNA analysis has also confirmed the identities and relationships of colonial Chesapeake remains in conjunction with detailed historical archaeological evidence, including investigations of high status individuals found at Historic St. Mary's City, Maryland (Reich et al., 2016), an 18th century Maryland family tomb (Owsley, Bruwelheide, Barca, Reidy, & Fleskes, 2018), and an 18th century European domestic site in Delaware (McKeown et al., 2014). These studies, in addition to the recovery of DNA from a clay tobacco pipe in 19th century Maryland (Schablitsky et al., 2019) and the developing analysis of 18th–19th century tooth samples from Montpelier, Virginia (Wright, Monroe, Reeves, & Hoffman, 2018), represent the only reported aDNA data from colonial Chesapeake sites at the time of submission.

A primary reason for the paucity of data from this period is that few 17th century Chesapeake sites have yielded well-preserved human skeletons. In Virginia, the majority of remains come from Jamestown Island (Kelso, 2006, 2017). Maryland human remains dating to the 1600s have been recovered from Historic St. Mary's City (Riordan, 2000), Patuxent Point (King & Ubelaker, 1996), and Leavy Neck (Leavy Neck, 2019). There is a relative scarcity of colonial period sites in Delaware. Three nonindigenous human skeletons dating to about AD 1675 have been documented at the site of Bay Vista near the site of Avery's Rest (Zebooker & Reinbold, 1999). Examination of the remains from some of these Chesapeake colonial sites is ongoing, but nearly all of these individuals are of European ancestry.

By contrast, individuals of African descent are relatively uncommon in the bioarchaeological record of the colonial period. With data from approximately 250 Africans and their descendants, the New York African Burial Ground study is unparalleled in providing information about African life in America (Blakey & Rankin-Hill, 2004). However, these remains originate from an 18th century urban context. Much less is known about African lives in 17th century frontier settlements.

This study presents genetic and bioarchaeological data from a well-preserved multiethnic cemetery of eleven graves found at the site of Avery's Rest (7S-G-57; AD 1674–1715). Located in present-day Sussex County, near the 17th century frontier Dutch settlement of Whorekill (present-day Lewes, DE) (Figure 1), this farmstead represents one of the earliest colonial settlements professionally excavated in Delaware.

Non-native occupation at this site began at least by the mid-17th century, when a 300-acre land grant for “Avery's Rest” was issued in AD 1666 to Captain John Avery, a mariner for the colony of Maryland who transported tobacco and food stores to Barbados and the West Indies (Sellers, 1901). The full 800-acre tract was granted in AD 1674 when John Avery, his wife Sarah, and their three daughters moved onto the property (Sellers, 1901, p. 11). The Averys' arrival followed the burning of the Whorekill settlement and surrounding farmsteads in December 1673, an act related to the final reclaiming of the territory by the English (de Valinger, 1950; Weslager, 1988; Weslager, 2016). After John Avery's death in AD 1682, his wife remarried and his daughters engaged in a court dispute over ownership of the Avery's Rest tract (Horle, 1991). The land was eventually partitioned between the daughters, one of whom lived at the site until AD 1715 (Horle, 1991). The 11 graves found at the site could represent Avery family members, servants, enslaved individuals, or others living and laboring on the farmstead.

Excellent bone preservation, a detailed archaeological excavation, and primary source documentation on land ownership facilitate a multi-faceted biohistorical investigation that includes mitochondrial DNA (mtDNA) sequencing. This study is the first to present genetic and bioarchaeological information from the individuals buried at this 17th century Delaware frontier farmstead, where residents coexisted

FIGURE 1 Map of Delaware within North America; Avery's Rest archaeological site is marked with a star. Map provided by americanhistory.si.edu.

in "a culturally diverse world in which complex relationships were formed for purposes of profit, status, and survival…" (Lukezic, 2013, p. 9).

2 | MATERIALS AND METHODS

2.1 | Bioarchaeological analysis

In 2006, prior to residential development, the Archaeological Society of Delaware collaborated with the Delaware Division of Historical and Cultural Affairs to excavate the Avery's Rest site. Fieldwork spanning multiple seasons revealed structures, fence ditches, a well, household refuse pits, including tobacco pipe fragments, ceramic sherds, and food remains, as well as 11 graves (Lukezic, 2013; McKnight & Jones, 2017). A southern group of eight interments was separated by approximately 20 ft from a northern cluster of three burials (Figure 2). Based on historical documents, material culture, and arrangement of the east–west aligned burials relative to structural features, the cemetery dates to the AD 1674–1714 Avery occupation.

Burial excavation was conducted in 2014 in accordance with the requirements of the Delaware Unmarked Human Remains Law (Title 7, Ch. 54). Each skeleton was inventoried and evaluated for osteological markers of age, sex, ancestry, and bone and dental pathology following established methods and guidelines (Buikstra & Ubelaker, 1994; Owsley & Jantz, 1989). Dental development and long bone growth measurements for juveniles were collected according to Moorrees, Fanning, and Hunt (1963a, 1963b) and Ubelaker (1989). Cranial and postcranial measurements were used for estimations of body size and ancestry (Howells, 1973; Ousley & McKeown, 2001; Zobeck, 1983). The resulting data are stored in the Human Skeleton Database at the National Museum of Natural History in Washington, DC (Barca, 2014).

2.2 | Mitochondrial DNA analysis

All sample processing, extraction, and PCR amplification procedures were conducted in the Ancient DNA Laboratory at the University of Tennessee-Knoxville (UTK) to prevent contamination with exogenous DNA. This lab is a BSL2-certified clean-room facility specifically designed to minimize exogenous contamination through the use of UV-lights, positive HEPA-filtered airflow, and separate gowning, extraction, and PCR rooms. Full personal protective equipment was worn during all procedures. Gloved hands were routinely washed with 10% bleach followed by 70% ethanol to prevent cross-contamination and changed after the preparation of each sample. All equipment was cleaned with bleach and ethanol, and all materials UV-irradiated before use.

All procedures were conducted in designated UV-irradiated biosafety cabinets. DNA extractions included a negative control, and PCR amplifications conducted with positive and negative controls. All post-PCR processes took place in the Modern DNA laboratory at UTK, with mandatory unidirectional movement of materials and personnel from the aDNA laboratory to the modern laboratory.

A well-preserved bone from each skeleton was selected for mtDNA analysis. Extraction and sequencing of mtDNA was performed for all 11 skeletons. For each sample, the first ~1 mm of bone surface was removed and 1.8 g of bone were collected using a Dremel 8200 drill. Samples were soaked in a 6% wt/vol sodium hypochlorite

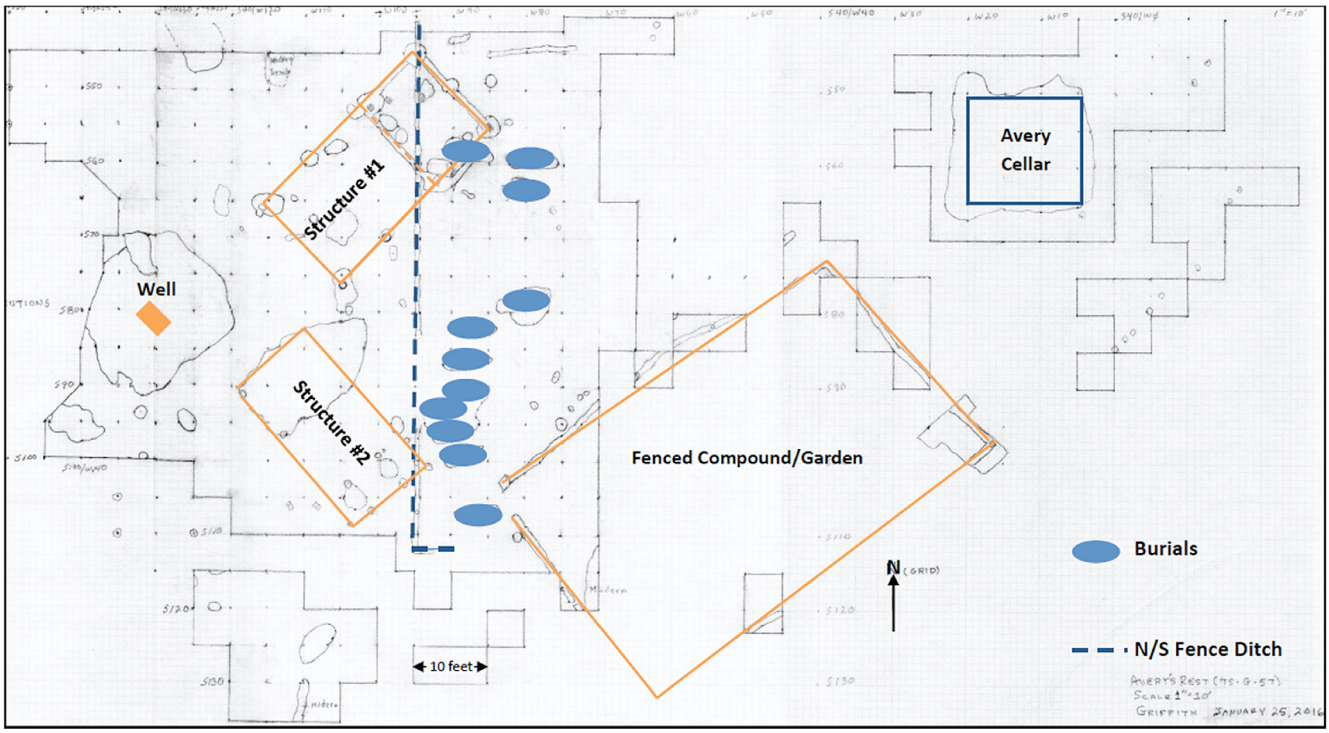

FIGURE 2 Site map of Avery's Rest (AR) displaying post holes, structures, a well, fence ditches, refuse features, and burials. Burials AR01–AR08 are located in the southern burial cluster and AR09–AR11 in the northern burial cluster

solution for 5 min to eliminate surface contamination and rinsed for 1 min with ddH_2O to remove bleach residue. The samples were air-dried and powdered using a Spek Freezer Mill. Two rounds of DNA extractions using a silica-spin column method (Dabney et al., 2013) were conducted over a 3-month period. For each sample, 0.2 g of powdered bone was incubated in an EDTA-based buffer with gentle shaking at 56°C for 22–22.5 hr, and extracted using Minelute spin columns (Qiagen).

For each sample, an 865 base pair (bp) section encompassing the entire hypervariable region I (HVRI) and most of hypervariable region II (HVRII) of the mtDNA control region (CR) was targeted for PCR amplification. For each sample, the region between nucleotide pair (np) 16,024 and np 300 was PCR amplified using 12 overlapping primer pairs (Kemp, 2006) for 60 cycles (Table S1), with 1 μL of extracted DNA per PCR reaction. PCR products were visualized using gel electrophoresis on 3% agarose gels using GelRed (Biotium). Post-PCR products were purified using Exonuclease I and Shrimp Alkaline Phosphatase (New England Biosystems), and quantified using the AccuBlue High Sensitivity dsDNA Quantitation Kit (Biotium) on the NanoDrop 3300 Fluorospectrometer (Thermo Scientific). DNA sequencing was performed using the Big Dye Termination Pre-Mix v. 3.1 (Applied Biosystems), and the sequences read on an ABI 3730 Gene Analyzer in the UTK Genomics Core facility.

The resulting mtDNA CR sequences were aligned against the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999) using Sequencer v. 5.4.1 (GeneCodes) at the University of Pennsylvania's Laboratory of Molecular Anthropology (LMAP). The ends of DNA sequence reads were trimmed and corrected due to aDNA end degradation, producing fragment sizes from 82 to 170 bp. All sequence variants were confirmed in two independent sequencing reads. Haplotypes, or the unique mutational composition of the mtDNA sequences, were called and used to define the haplogroups, or the larger maternal lineage, to which they belong, using Haplogrep 2.0 (Weissensteiner et al., 2016) against Phylotree Build 17 (van Oven, 2015).

2.3 | Haplogroup frequency analysis

Comparative mtDNA sequences for European and African populations were compiled from published papers and public databases. European data were obtained from the United Kingdom (n = 3,594), Netherlands (n = 785), Sweden (n = 2,679), and Finland (n = 843). These mtDNA datasets were evaluated for the frequency of each European haplogroup identified at Avery's Rest Table S2, as Swedish (including present-day Finland), Dutch, and English colonial powers each established settlements in Delaware to varying degrees during the 17th century (Tomlins, 2010). Haplogroup frequencies were calculated by dividing the total number of individuals with the study-reported European haplogroup by the total number of individuals in each comparative database (Table S2). Counts were generated from haplotype or sub-haplotype matches (e.g., H1afa1 being a sub-haplotype of H1af).

The African dataset encompassed the entire African continent to more broadly assay potential source populations. The 17,995 individuals in this dataset represent 366 populations from 42 countries, including only groups with more than five persons (Tables S3–S5).

TABLE 1 Avery's Rest bioarchaeological data

Burial	Burial cluster	Associated artifacts	Sex[a]	Ancestry	Age (years)	Notable pathology
AR01	Southern	54 coffin nails; 1 pin	Male	European	30–40	Spinal degeneration; Schmorl's depressions; tooth wear from pipe use
AR02	Southern	40 coffin nails; 16 pins	Indeterminate	European	4–6 months	N/A
AR03	Southern	40 coffin nails; 1 pin	Male	European	35–45	Healed depression fracture in frontal; lytic lesions in cranium and scapula; ulna abnormal bone formation and loss; degenerative changes in spine; Schmorl's depressions; tooth wear from pipe use
AR04	Southern	51 coffin nails	Male	European	40–50	Spinal degeneration; Schmorl's depressions; degenerative joint disease; tooth wear from pipe use
AR05	Southern	39 coffin nails	Male	European	35–45	Spinal degeneration; Schmorl's depressions; tooth wear from pipe use
AR06	Southern	60 coffin nails; 2 pins	Female	European	50–60	Spinal degeneration; Schmorl's depressions; enthesophyte formation
AR07	Southern	39 coffin nails; 2 pins	Female	European	35–45	Schmorl's depressions
AR08	Southern	72 coffin nails; 2 pins	Male	European	30–40	Spinal degeneration; Schmorl's depressions
AR09	Northern	47 coffin nails; 2 cuprous metal buttons	Male	African	32–42	Spinal degeneration; Schmorl's depressions; fibula periostitis; tooth wear from pipe use
AR10	Northern	40 coffin nails	Male	African	27–37	Spinal degeneration; Schmorl's depressions; perimortem facial fractures; tibiae and right fibula periostitis; tibiae bowing; tooth wear from pipe use
AR11	Northern	17 coffin nails; 1 pin[b]	Indeterminate	African	4.5–5.5	N/A

[a]Sex determination based on osteological assessments.
[b]The copper alloy pin from Burial 11 was found in the animal burrow running through the burial. The pin may have come from overlying soils.

Haplogroup frequencies were calculated by dividing the number of individuals with the study-reported African haplogroups by the total number of individuals in a particular population sample.

GPS location data for each African population were either provided in the original publication or closely approximated from population descriptions in the corresponding studies (Table S4). African mtDNA haplogroup frequency data were plotted with the corresponding GPS coordinates in ArcMap v. 10.4.1 (ESRI) using the Geospatial Analyst Inverse-Distance Weighting (IDW) mapping function with a power of two and visualized using geometric intervals. IDW interpolates the haplogroup frequencies by location data under the assumption that frequencies decrease with distance to spatially visualize haplotype distribution.

3 | RESULTS

3.1 | Bioarchaeological findings

All of the graves conformed to Christian burial practices (Figure 2). Each interment was oriented east-to-west with the body supine and hands positioned on the pelvis or along the sides of the torso (Table 1; Figure 3). Slight variations in burial locations along the east–west axis may be due to the seasonality of internment (D. Griffith, personal communication). Hexagonal-shaped coffins were identifiable by nails, soil stains, and, in one case, coffin wood residue identified to the genus *Pinus* spp. (pine) (McKnight, 2014). The coffin construction design differed for only one individual (AR08), where the floorboard was attached by driving the nails upward from the bottom into the sideboards, instead of nailing into the floorboard from the sides. Construction by a different carpenter, probably one of lesser experience, is suggested by the greater number of nails used to assemble this burial container. The alignment of this grave in relation to others in the southern cluster may also indicate that this burial postdates others in the cemetery (D. Griffith, personal communication).

Burial depths, measured from the base of the plow zone to the grave floor, ranged from 18″ and 23″ for an infant and child, to 44″ for adults. Adult burial depths varied, with one grave (AR10) being 6″ to 17″ shallower compared to others.

Burial artifacts were few in number. Cuprous alloy pins, likely related to burial clothes or shrouds, were found with seven

FIGURE 3 In situ images of burials demonstrate skeletal preservation. Photographs by Donald E. Hurlbert, Smithsonian Institution

individuals, and two cuprous buttons recovered in the pelvic region of an adult male (AR09) suggest breeches. Green, copper-oxide staining on the skulls of three adults without associated pins indicated that they, too, were shrouded. Only one individual, the shallowest of the adult burials, lacked evidence of a shroud.

The eight individuals buried in the southern cluster had biological features indicative of European descent, whereas the three individuals in the northern cluster had features consistent with African ancestry. The infant in the southern cluster was inferred to be of European descent based on its burial location. This tentative assessment was strengthened through mtDNA analysis (see below). This infant and the single child of African descent from the northern cluster were the only sub-adults in the cemetery. The nine adults ranged in age from 25 to 60 years old, and included seven males and two females. Of the seven adults with European ancestry, all were estimated to be 30 years of age or older. The two males of African descent were adults with estimated ages between 27 and 42 years (Table 1).

Skeletal markers indicated that the adults were routinely engaged in moderate to strenuous physical labor. Both European and African descended individuals displayed some degree of joint degeneration, although none was classified as severe. Herniated disc depressions (Schmorl's nodes) in vertebral endplates from axial loading and spinal compression (Burke, 2012; Resnick & Niwayama, 1978) were present in at least one vertebra of each adult. Four males displayed five or more vertebrae with endplate depressions. Cranial lesions characteristic of cribra orbitalia and possible anemia were expressed as slight porosity in the left orbital roof and posterior parietals of one adult. Additional lytic bone loss and a possible healed depression fracture were evident in the cranium and scapula of this same European-descended male (AR03).

A male with African ancestry (AR10) had perimortem trauma in the form of unhealed facial fractures (Figure 4a). The right cheekbone (zygomatic) had separated from the face along sharp breaks in the zygomatic arch, frontal bone above the zygomaticofrontal suture, and the maxilla, medial to the zygomaticomaxillary suture. This "tripod fracture" usually occurs from a facial impact, the result of a fall or assault (Wedel & Galloway, 2013, p. 148). The tibiae of this male also showed trace bowing of the shafts, possibly indicative of poor childhood nutrition (infantile, early stage rickets).

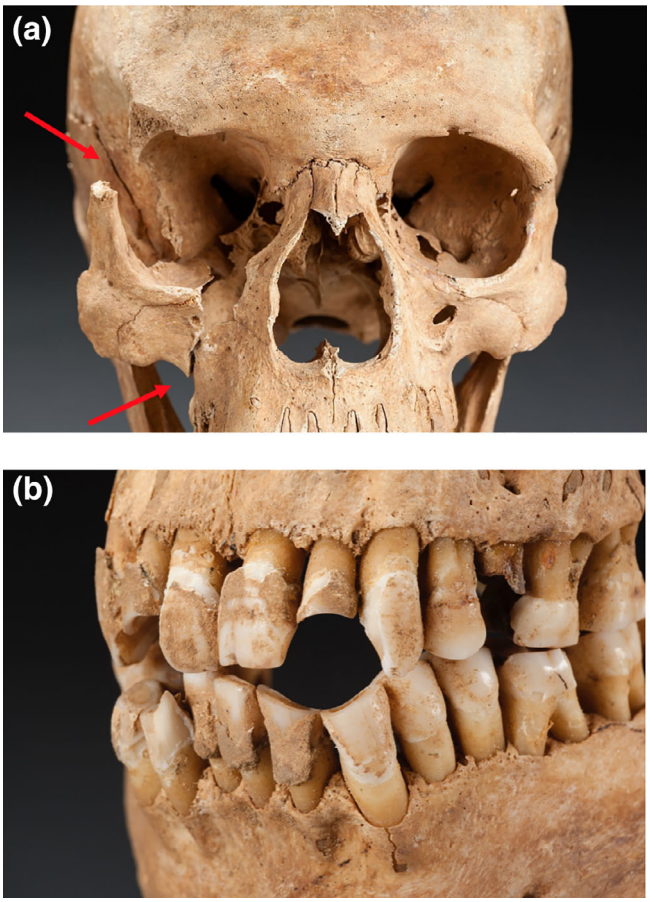

FIGURE 4 (a) Frontal view showing a "tripod" fracture in AR10. Arrows point to perimortem fractures in the frontal and right maxilla; (b) circular groove in the dentition of AR05 from using a clay tobacco pipe. Facets formed in the left lateral incisors and canines reflect approximate dimensions of the pipe stem. Photographs by Kate D. Sherwood, Smithsonian Institution

Notable in the dentition of Avery's Rest males, and a significant factor affecting their dental health, were distinctive tobacco pipe stem wear facets (Figure 4b). These grooves result from repetitive clenching of tobacco pipes between the maxillary and mandibular teeth (Meyer, Nicklisch, Held, Fritsch, & Alt, 2011; Ubelaker, 1996). All men had at least two facets, and both the left and right side dentitions of most of them were affected. Males of European descent had the most severe expression of pipe stem tooth wear. Similarly defined pipe facets have been documented at other rural colonial farmsteads dating to the second half of the 17th century (King & Ubelaker, 1996), coinciding with the rise of Chesapeake tobacco cultivation.

3.2 | Mitochondrial DNA findings

Genomic DNA was successfully extracted from all 11 individuals and the entire mtDNA CR was sequenced for each. The CR sequences obtained from the study samples did not match those of the sample handlers involved with the project (Table S6). The authenticated sequences are deposited in GenBank under accession numbers MN107021-MN107031.

The mtDNA data confirmed the osteological assessments of geographical ancestry and identified European and African haplotypes in the Avery's Rest individuals (Table 2). The mtDNAs from the eight individuals in the southern burial cluster belonged to five different European haplotypes (W4a1, H24, H1af, T, and U5b2a1a), with four individuals (two females, one male, and one infant) sharing the same H1af haplotype. The three individuals from the northern burial cluster had three different African haplotypes (L3e3, L3i2, and L0a1a).

The comparative European mtDNA dataset was checked for the frequency of each haplogroup identified at Avery's Rest (Table 3). The basal haplogroups from which the European Avery's Rest haplotypes derive represent maternal lineages (H, T, U, W) that are found throughout Europe (e.g., Richards, 2003; Simoni, Calafell, Pettener, Bertranpetit, & Barbujani, 2000) in a largely homogenous distribution (Loogväli et al., 2004; Tillmar, Coble, Wallerström, & Holmlund, 2010). Although fine-scale genomic population structure has been detected in northern European countries (e.g., Genome of the Netherlands Consortium, 2014), the same is not true for mtDNA diversity (Chaitanya et al., 2016). This pattern is consistent with the relative similarity of the macro-haplogroup frequencies in the comparative European mtDNA dataset (Table 3), with a slight difference being the higher percentage of haplogroup HV in the Netherlands.

The frequencies of the Avery's Rest mtDNA haplotypes in the present-day United Kingdom, Sweden, Finland, and Netherlands comparative database were negligible (Table 3). Excepting AR01 with a haplogroup T haplotype, all other Avery Rest's European mtDNA haplotypes did not exceed 0.59% in the comparative population database. Frequencies of the Avery's Rest haplotypes in the United Kingdom and Netherlands databases, both of which had longer and more permanent settlements in Delaware during the 17th century (Munroe, 2006), were slightly less, but within the margin of sampling error, than in the Swedish and Finnish databases. Yet, the frequency of the Avery's Rest mtDNA haplotypes combined with the overall similarity of the comparative European mtDNA dataset did not allow the assignment of the Avery's Rest haplotypes to a specific European country of origin.

While their specific geographic origins remained ambiguous, the European-descended individuals identified at Avery's Rest exhibited evidence of familial relationships within the cemetery. Four of the eight individuals shared the H1af haplotype, as identified in independent rounds of DNA extraction (see Mitochondrial DNA Extraction and Analysis). This haplotype is uncommon in European populations, having been reported only four times in the comparative samples from the United Kingdom and Sweden, and once in a European-

TABLE 2 mtDNA control region sequences in the Avery's Rest individuals[a]

Burial	Bone sample	Haplogroup	MtDNA sequence (16,024–300)
AR01	Third right metacarpal	*T*	T16126C, C16294T, T16519C, A73G, A263G
AR02	Right rib, 6–10 range	*H1af*	T16357C, T16519C, A263G
AR03	First right metacarpal	*W4a1*	T16093C, C16223T, C16286T, T16519C, A73G, G143A, A189G, T192C, C194T, T195C, T196C, T204C, G207A
AR04	Second left metacarpal	*U5b2a1a*	C16192T, T16311C, A73G, C150T, A263G
AR05	Second right metacarpal	*H24*	A16293G, T16311C, T195C, A263G
AR06	First right metacarpal	*H1af*	T16357C, T16519C, A263G
AR07	Third right metacarpal	*H1af*	T16357C, T16519C, A263G
AR08	Fourth right metatarsal	*H1af*	T16357C, T16519C, A263G
AR09	Fourth right metatarsal	*L3e3*	T16093C, C16223T, A16265T, T16519C, A73G, C150T, T195C, A263G
AR10	Fourth left metatarsal	*L0a1a*	G16129A, C16148T, C16168T, T16172C, C16187T, C16188G, T16189C, C16223T, A16230G, T16311C, C16320T, C64T, A93G, C167T, G185A, A189G, A200G, T236C, G247A, A263G
AR11	Right rib, 6–10 range	*L3i2*	C16223T, C16260T, G16361A, G16412A, G16456A, G16471A, A73G, T152C, A189G, T195C, A263G, C273T

[a]These sequences contain the entire HVRI (16,024–16,365) and a portion of the HVS2 (73–340) regions. Variants were identified by comparison with the rCRS (Andrews et al., 1999).

TABLE 3 European MtDNA haplogroup diversity in Avery's Rest individuals compared to populations known to have settled in 17th century colonial Delaware (English, Dutch, Swedish, Finnish)[a]

		United Kingdom		The Netherlands		Sweden		Finland	
Haplogroup	Avery's Rest haplogroup	#	%	#	%	#	%	#	%
A		3	0.08%	0	–	0	–	0	–
B		2	0.06%	1	0.13%	1	0.04%	0	–
C		0	–	0	–	7	0.26%	0	–
D		1	0.03%	0	–	1	0.04%	3	0.36%
G		0	–	0	–	1	0.04%	1	0.12%
HV		40	1.11%	183	23.31%	47	1.75%	4	0.48%
H		1,616	44.96%	185	23.57%	1,151	42.96%	312	36.94%
	H1af	*2*	*0.06%*	*0*	–	*2*	*0.07%*	*0*	–
	H24	*3*	*0.08%*	*1*	*0.13%*	*3*	*0.11%*	*5*	0.59%
I		132	3.67%	15	1.91%	63	2.35%	24	2.85%
J		419	11.66%	81	10.32%	189	7.05%	54	6.41%
K		313	8.71%	90	11.46%	191	7.13%	51	6.06%
L		3	0.08%	2	0.25%	8	0.30%	0	–
M		1	0.03%	2	0.25%	1	0.04%	0	–
N		6	0.17%	1	0.13%	7	0.26%	1	0.12%
R		2	0.06%	1	0.13%	2	0.07%	6	0.71%
T	*T*	*358*	*9.96%*	*89*	*11.34%*	*223*	*8.32%*	*37*	4.39%
U		446	12.41%	112	14.27%	498	18.59%	189	22.45%
	U5b2a1a	*6*	*0.17%*	*0*	–	*4*	*0.15%*	*5*	0.59%
V		143	3.98%	6	0.76%	195	7.28%	73	8.67%
W		53	1.47%	14	1.78%	37	1.38%	65	7.72%
	W4a1	*1*	*0.03%*	*0*	–	*4*	*0.15%*	*3*	0.36%
X		56	1.56%	3	0.38%	36	1.34%	12	1.43%
Z		0	–	0	–	21	0.78%	11	1.31%
Total		3,594	100%	785	100%	2,679	100%	843	100%

[a]European haplogroups at Avery's Rest (n = 8) are displayed as a subset to express their specific count and frequency. Comparative data are provided in Table S3.

descendant Bolivian population (Taboada-Echalar et al., 2013). Given the rarity of H1af, it is highly improbable that four unrelated individuals with the same mtDNA haplotype would be present at this small plantation site on the Delaware frontier. Accordingly, these H1af individuals likely shared recent, if not immediate, maternal kinship.

The African haplotypes were compared to the African mtDNA dataset with respect to their frequency and geographic distribution (Tables S3–S5). Haplogroup frequency data for L0a1, L3i, and L3e were generated, as well as corresponding sub-haplotypes, as they are more numerous than the L0a1a, L3i2, and L3e3 haplotypes alone. The absence of haplogroup sharing among the three individuals of African descent indicate that they are not related maternally and possibly originated in geographically dispersed populations in Africa. It is also possible that these persons originated from the same geographic area within a genetically heterogeneous population.

The larger haplogroups (L0a1 and L3e) that included the mtDNAs of the two adult males have wide distributions and occur in moderate frequencies in areas associated with 17th century slave trading ports in west and central Africa (Walsh, 2001, 2010) (Figure 5). L0a, the basal haplogroup from which L0a1 derives, was involved in the eastern Bantu expansion into the African continent, which may explain its high frequency around Mozambique (Beleza, Gusmão, Amorim, Carracedo, & Salas, 2005; Rito et al., 2013). It is absent from the central-western coast of Nigeria, Cameroon, and Gabon. Several of the founder types of L0a leading to L0a1 are also found in Angola, suggesting some connection between the eastern and western parts of southern Africa (Beleza et al., 2005; Salas et al., 2004).

Haplogroup L3e is distributed across central and southern Africa, and is also present along the western coast (Figure 5). Its derivative, L3e3, is found primarily in West Africa, although it is also present in southern and central regions (Salas et al., 2002; Soares et al., 2012). The distribution of L3e3 in the African continent likely results from persons involved in the southern Bantu agricultural expansions from West Africa carrying these mtDNAs into southwest and southern

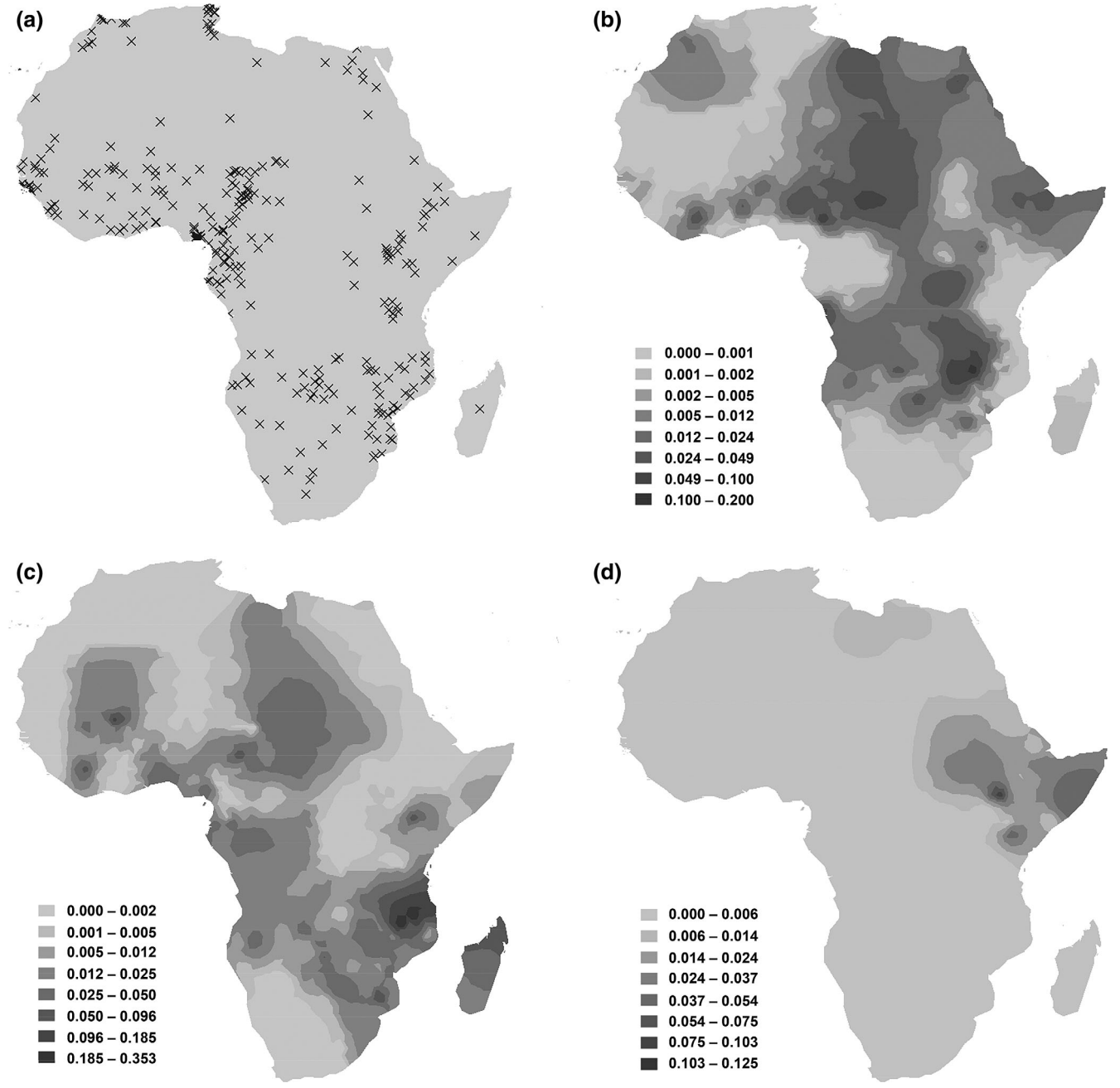

FIGURE 5 Map of the frequency distribution of the African mitochondrial haplotypes identified at Avery's Rest. Panel (a) displays the distribution of populations analyzed in the African continent. Interpolation maps using the frequency of (b) L0a1a and all subtypes, (c) L3e3 and all subtypes, and (d) L3i and all subtypes

Africa around 2,000 years ago (Bandelt et al., 2001; Plaza et al., 2004; Soares et al., 2012).

Haplogroups L0a and L3e have been identified in African-descendant populations in North, Central, and South America (Bandelt et al., 2001; Salas et al., 2004; Silva et al., 2006), indicating that persons with these haplogroups were involved in the trans-Atlantic slave trade. Specifically, L0a1a is identified in Cubans (Mendizabal et al., 2008) and Bermudians (Gaieski et al., 2011), while L3e3 has been reported in the Caribbean (Mendizabal et al., 2008; Salas, Carracedo, Richards, & Macaulay, 2005) and at high frequency in African-descent populations in Brazil (Carvalho, Bortolini, dos Santos, & Ribeiro-dos-Santos, 2008; González et al., 2006; Salas et al., 2002; Silva et al., 2006). In North America, both haplogroups Le3e and L0a1 have been identified in multiple studies assaying mtDNA diversity in contemporary African American populations (Diegoli et al., 2009; Ely, Wilson, Jackson, & Jackson, 2006; Johnson et al., 2015; Salas et al., 2004).

The L3i haplotype of the 5-year-old child (AR11) had not been reported in African-descended populations in the Americas prior to this study. This haplogroup is found almost exclusively in eastern Africa (Cerezo et al., 2016; Soares et al., 2012) (Figure 5), and the

distribution of L3i suggests that the parent haplogroup originated in the same region (Soares et al., 2012). While mostly restricted to east African populations (Coudray et al., 2009; Harich et al., 2010; Kivisild et al., 2004), L3i has been reported in Berbers (Coudray et al., 2009), a highly mobile group traversing the trans-Sahel, although it appears to be absent in Bantu-speaking populations (Batai, Babrowski, Arroyo, & Williams, 2013). East African haplogroups occur at low frequencies in populations of African ancestry in the Americas based on published data (Alves-Silva, Santos, Guimara, & Ferreira, 2000; Salas et al., 2004; Silva et al., 2006; Stefflova et al., 2011), but have not been reported in genetic diversity studies of contemporary African American populations (Diegoli et al., 2009; Ely et al., 2006; Johnson et al., 2015; Salas et al., 2002).

4 | DISCUSSION

Use of aDNA methods to study colonial period sites provides new information about the genetic diversity of, and relationships between, founder or colonizing populations and those of contemporary peoples. The European haplotypes identified at Avery's Rest (W4a1, H1af, H24, T) offer an example of mtDNA diversity associated with European colonization in one locality. Because European populations are not mitochondrially distinct from one another, it is difficult to define the specific geographic source of maternal ancestry for the European descended individuals. Moreover, three of the four haplotypes, W4a1, H1af, and H24, are infrequently reported in contemporary European data sets, further complicating efforts to determine how they came together at Avery's Rest.

Maternal ancestry of the three individuals of African descent provides a glimpse into the geographic reach of the slave trade from Africa to colonial America. The maternal genetic origin of these individuals appears to extend across a wide geographic area of present-day Africa, including the West, Central, and Eastern regions of the continent. Genomic analysis of these individuals will provide greater specificity in ancestry assessment and illuminate the biological history of enslaved persons living in the 17th century Chesapeake region. The diverse sources of these three haplogroups in Delaware during the early phase of the trans-Atlantic slave trade underscores the degree to which enslavement was an expansive, far-reaching process during this early period of colonial history.

The presence of individuals of African descent at Avery's Rest is significant in itself since, in 1700, Africans persons represented only about 5% of the population in Delaware (Williams, 1996). During the 17th and early-18th centuries, the Chesapeake region was not a primary destination for the trans-Atlantic slave trade (Morgan, 2003; Walsh, 2001), making the rural location of Avery's Rest site even more peripheral. The absence of major shipping ports in Delaware meant that most enslaved Africans individuals were purchased and brought to the region through small-scale trading with the English colony of Barbados (of which John Avery likely participated), or illegally with Dutch and Portuguese interlopers (Morgan, 2003). Alternatively, enslaved persons arrived at larger ports in other colonies, such as New Amsterdam (New York), Maryland, or Virginia, and were brought to Delaware via land transport (Morgan, 2005).

The adult males have mtDNA haplotypes (L01a1, L3e3) that are common in present-day West and Central Africa, known source regions for enslaved Africans brought to the Chesapeake centuries ago (Walsh, 2001, 2010). In contrast, the child's L3i2 haplotype is almost exclusively found in East African populations today. It is thus possible that this child, or more likely the mother, was brought to North America as a result of forced internal population movements in Africa, since persons were often captured far distances inland and transported to coastal shipping ports (Harich et al., 2010; Thornton, 1998). Alternatively, persons carrying L3i2 mtDNAs could once have been more widely geographically dispersed than reported in contemporary African populations (e.g., Behar et al., 2008). Further autosomal analysis will provide more information on the extent of east African ancestry in this individual.

As evident by our results, the use of aDNA methods in bioarchaeological research can expand knowledge of individual biohistories at archaeological sites (Matisoo-Smith & Horsburgh, 2012). This is particularly relevant for the individuals at Avery's Rest who share the somewhat uncommon H1af haplotype, suggesting they are maternally related. Historical records indicate that biological kin were increasingly involved in colonial European settlement during the 17th century (Menard, 1988; Rutman & Rutman, 1984; Williams, 1996). During the initial period of British settlement in Delaware (AD 1675–1715), it is estimated that almost 50% of migrants arrived in family groups, followed closely by young males (Tomlins, 2010). Many of these migrants were of the middling planter class seeking economic advancement through tobacco cultivation (Horn, 1994). The family unit, supplemented by indentured servants and tenant workers when available, served as the primary labor source during this time period, as few new landowners could afford enslaved labor (Horn, 1994; Williams, 1996).

While the mtDNA results do not provide conclusive proof that the eight individuals with European ancestry are members of the Avery family, identification along this line can be explored using primary source documents and results from the osteological analysis. Court records (Horle, 1991) indicate that John Avery and his family owned the property during the time period of the cemetery, as indicated by its placement relative to the site's structures and material culture (ca. AD 1675–1715). Documents suggest at least three members of the family died during this period and are likely buried on Avery land, including John Avery, his son-in-law, Hercules Shepheard, and his infant grandson, John Shepheard, the son of Hercules and Mary Avery (Horle, 1991). Thus, if the H1af haplotype represents the maternal genetic signature of the extended family, then the infant (AR02) could be the grandchild. These data provide a step toward identifying the two females with the H1af motif (AR06, AR07), as they could represent John's wife, Sarah Avery, or alternatively one of her daughters or her grandchildren. The demographic profiles of specific skeletal remains are consistent with known details of John Avery and his kin. This does not preclude the presence of other individuals, such as indentured servants or tenant

farmers, from having the same haplotype by chance, although the possibility is remote. Precise identifications will likely be clarified through additional autosomal DNA analysis, in conjunction with further genealogical research and stable isotope testing.

A limited documentary record precludes identification of the African individuals at Avery's Rest. Sussex County court documents refer to two enslaved males at Avery's Rest, not by name, but instead for their valuation as property in John Avery's estate (Horle, 1991). Since early Delaware farmsteads were typically limited to one or two enslaved laborers per household (Williams, 1996), it is possible that one or both of these individuals are the men in the cemetery. Even so, this court document tells nothing of their individual or collective identities. Like other Africans in the Americas, their stories are difficult to reconstruct due to the subaltern status of those in bondage (Sweet, 2009).

Bioarchaeological and osteological information from the study can be utilized to clarify additional aspects of these individuals' lives and relationships to others in the cemetery. The lack of burial artifacts, excepting a pair of cuprous buttons, combined with the uniformity of Christian-style burial, makes it difficult to discern individual or cultural agency in death. Nevertheless, physical separation of European and African burials suggests a degree of social segregation in life (Aufderheide, Neiman, Wittmers, & Rapp, 1981; Bell, 2005). In comparison to other late 17th and 18th century cemeteries, when discrete burials grounds for Africans emerged (Aufderheide et al., 1985; Handler, 1996; Jamieson, 1995), the relative closeness of the burial groupings suggests a lesser degree of social separation. The degree of integration at Avery's Rest can only be inferred from the archaeological record and within the context of the institution of slavery during the 17th century. Still, the presence of clothing for one man of African descent, and the shallow grave of the other, with no evidence of burial wrappings or a shroud, differs from their European-descent contemporaries, suggesting less formal burial treatment .

Osteological analyses further illuminate issues of social interaction in frontier landscapes. All adults at Avery's Rest exhibit developmental and arthritic changes indicative of moderate to heavy labor. All were engaged in physically demanding activities. Difficult to discern in this case is enslavement's possible impact on the allocation, duration, and types of labor undertaken. The growth of tobacco was undoubtedly involved, as evidenced by the extensive pipe-stem wear in their dentitions and artifacts found at the site, particularly for males of European descent. Differences in cultural attitudes toward tobacco use, or simply greater access to tobacco throughout their longer lifespans, may explain the differences between males of European and African descent. The perimortem injury noted for one African descended male (AR10) suggests a violent death. Combined with evidence of childhood nutritional stress, and his informal burial treatment, lesser regard in life is implied for this individual. However, trauma and disease are not confined to this individual. A male of European descent (AR03) exhibits healed cranial trauma in addition to disease.

It is unlikely that the events leading to the presence of these African descended individuals at Avery's Rest will ever be completely reconstructed. The mtDNA data expand these interpretations to identify different maternal genetic origins for these individuals. Yet, the lack of documentary records leaves interpersonal relationships to be inferred only by archaeological and osteological evidence. On the other hand, such results do not preclude cultural forms of kinship and community that are not observable in the bioarchaeological record. The lives and relationships within and between African and European descended persons at Avery's Rest thus demonstrate the intertwined complexity of early colonial settlements, governed by pre-existing social institutions and actualized on the 17th century Delaware frontier.

5 | CONCLUSION

This study has employed aDNA methods in the investigation of colonial period skeletal remains in the Mid-Atlantic region of North America, a region that has received little attention by geneticists. The results have implications for the future study of the colonial era by elucidating questions about the settlement, migrant origins, and bio-social relationships of individuals from specific sites, which may not be recognizable using historical, documentary, or archaeological evidence alone.

This study identifies maternal genetic kinship and illuminates genetic diversity in European and African descended persons at a 17th century Chesapeake site. For the individuals of European descent, maternal relatedness likely contributed to the settlement of and lifeways at Avery's Rest. Future autosomal analyses and genealogical research will further assist with the identification of members of the John Avery family who are potentially buried at the site, as well as possibly clarifying the parentage of the infant (AR02) and African child (AR11). Moreover, this study broadens narratives concerning the origins of African individuals in colonial North America, and demonstrates that multiple lines of evidence are required to more completely describe the relationships between European and African descended persons in the colonial period. The findings point to the need for expanded studies of historic period African and African American genetic diversity to account for the peripheral areas and periods involved in the trans-Atlantic slave trade.

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CONFLICT OF INTEREST

The authors do not declare any conflict of interest regarding this research.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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