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Origins of an Unmarked Georgia Cemetery Using Ancient DNA Analysis

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Key words: Genetics, African American Cemetery, Mitochondrial Haplotypes.

Short Title: Origins of a Historical Cemetery

Abstract

Determining the origins of those buried within undocumented cemeteries is of incredible importance to historical archaeologists and in many cases, the nearby communities. In the case of Avondale Burial Place, a cemetery in Bibb County, Georgia, in use from 1820 to 1950, all written documentation of those interred within it has been lost. Osteological and archaeological evidence alone could not describe, with confidence, the ancestral origins of the 101 individuals buried there. In the present study, we utilize ancient DNA extraction methods to investigate the origins of Avondale Burial Place through the use of well-preserved skeletal fragments from 20 individuals buried in the cemetery. Through examination of hypervariable region I in the mitochondrial genome (HVR1, mtDNA), we determine haplotypes for all 20 of these individuals. A total of 18 of the 20 individuals belong to the L or U haplogroups, suggesting that Avondale Burial Place was most likely used primarily as a resting place for African Americans. After the surrounding Bibb County community expressed interest in investigating potential ancestral relationships to those within the cemetery, a total of eight potential descendants provided saliva in order to obtain mtDNA HVR1 information. This phase of the study revealed that two different individuals from Avondale Burial Place matched two individuals with oral history ties to the cemetery. Using the online tool EMPOP, we calculated the likelihood of these exact matches occurring by chance alone (<1%). The present findings exhibit the importance of genetic analysis of cemetery origins when archaeological and

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osteological data are inconclusive for estimating ancestry of anonymous historical individuals.

Avondale Burial Place was unearthed in 2008 during a road expansion in Bibb County, Georgia (Figure 1). New South Associates (NSA) under contract with the Georgia Department of Transportation (GDOT) uncovered 101 human burials but no grave markers of any kind (Matternes et al., 2012). An initial challenge was determining what community was responsible for its construction and then linking the cemetery to the appropriate community. Historical reviews of communities present in southern Bibb County during the nineteenth and twentieth centuries were conducted by both the GDOT and NSA (Matternes et al. 2012).

The cemetery was not tied to any church, burial society, or other known bodies, nor did it seem to be affiliated with any nineteenth or twentieth century landowners. A review of census records indicated that historically there had always been an African-American presence in the area; however the burial sites for many of these enclaves remained unknown. There were no maps or other records of the cemetery and the lack of headstones hampered identification of specific decedents within the facility. The cemetery's position in the landscape, in an unusable corner of an agricultural field, adjacent to a wetlands area, and away from habitation complexes, was similar to places commonly employed by African-American communities (Martin 2010; Matternes and Richey 2014; Matternes and Smith 2014). While many nineteenth and twentieth century communities viewed the cemetery as a means of social expression to the world at large, many African-American communities perceived burial grounds as private, family-oriented facilities and placed them in the discreet, out of public view locations (Honerkamp and Crook Jr. 2012; Parsons 1923; Sloane 1991). GDOT and NSA investigations suggested that the burial ground was used by African-American communities, but the near absence of historic records on the cemetery and highly ephemeral surface representation precluded making any definitive assertions about the cemetery's place in society.

Archaeological evidence suggests Avondale Burial Place was used by African Americans between the 1820 and 1950 with the greatest component of the assemblage likely deposited after the Civil War (Matternes et al. 2012). A concentration of graves was present in the central portion of the complex with later period graves radiating outward. These simple, undecorated coffins with few to any accouterments suggested the Avondale Burial Place had its beginnings as a slave cemetery (Matternes et al. 2012). The graves were all oriented east-west, a common practice of both European-Christian and African-influenced burials. In the case of European-Christians, burials are usually positioned in an east-west alignment with the head facing west in order to allow the dead to face Jesus on his arrival; it is also symbolically attached to rebirth (Milbauer 1989). Early African

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burials in the U.S. are oriented in the same east-west direction but were intended to synchronize the dead with the structure of the universe (Genovese 1972; Pollitzer 1999). Other features, including vaulted graves and the use of hexagonal coffins are shared among other African American cemeteries within the Southern United States (Matternes et al. 2012).

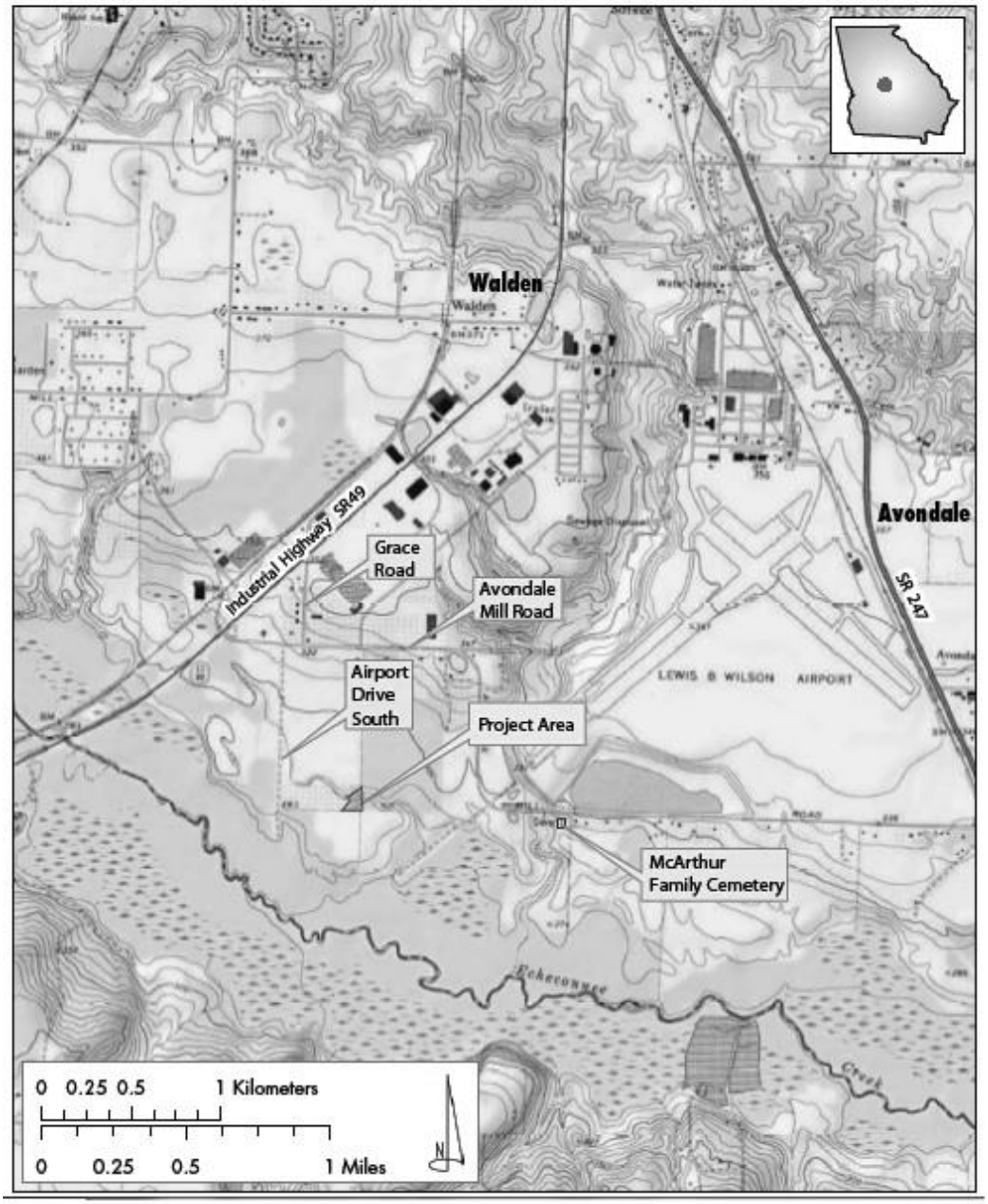

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Figure 1. The location of Avondale Burial Place (noted as Project Area) within Bibb County, Georgia (from Matternes et al. 2012).

While grave markers had not survived, a light scatter of glass and white-bodied ceramic fragments were found on the cemetery's surface. Objects of this nature were commonly placed on the top of graves as a means of controlling the spirit and encouraging its passage to the world of the dead and were important decorations for Africans in the new world (Evans et al. 1969; King 2010; Puckett 1926; Vlach 1990). Within the graves, pierced silver coins (Figure 2a) and seed bead necklaces were used as charms, designed to improve health and protect the wearer from magic (Capozzoli 1997; Crist et al. 2000; Davidson 2004; Evans et al. 1969; Puckett 1926; Stine et al. 2000). Beads are considered important personal artefacts within African American interments but many within Avondale Burial Place were shattered by post-depositional forces prior to recovery and thus the total amount could not be properly estimated. White, blue, black, and clear beads were identified from four interments and were between 1.5 to 2.0 millimeters in diameter (Figure 2b). Additionally, a sheet copper pendant depicting a crescent moon and a star may have reflected African-based Islamic ideas within the community. Stars are commonly viewed as human souls while the crescent moon symbolizes the beginning of a new life after crossing the Kalunga line into the world of the dead (Puckett 1926; Thompson 1984; Thompson and Cornett 1981) (Figure 2c). Gomez (2011) has demonstrated that pre-emancipation Muslim traditions were a part of African American heritage.

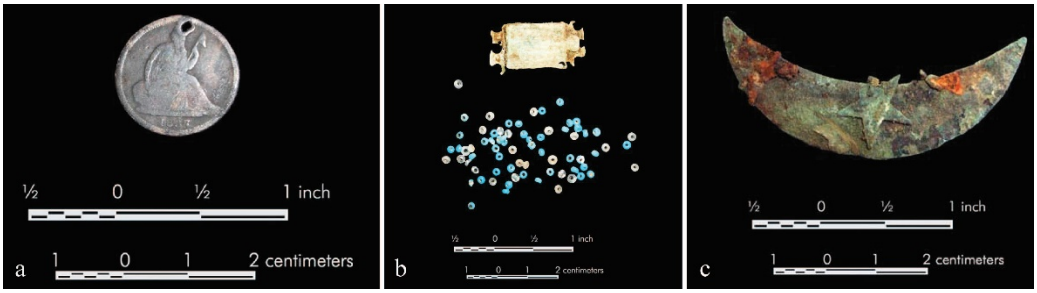

Figure 2. (**a**) Pierced silver coins (from F034, not used for genetic analysis) were among the charms used by African Americans to improve health and ward off deleterious magic (**b**) Mixed color beads from one of the burials (F055, not used for genetic analysis) and a necklace clasp. These small glass artefacts, usually referred to as seed beads, have strong ties to West Africa. (**c**) A copper pendant from F007 depicting a star and crescent moon, also thought to be of West African tradition (Matternes et al. 2012).

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The burial of boots on top of one coffin related to African-American traditions where footwear served as a means of protecting the dead from malevolent spirits (Davidson 2010). Personal items including combs, dolls, tobacco pipes, purses, jewelry, and wedding bands, were placed with the dead to prevent the spirit from returning to their former homes, searching for things they needed in the next world (Fenn 1985; Matternes et al. 2010; Roedinger 1981; Thompson and Cornett 1981; Vlach 1991). While not a uniquely African-American feature, leaving coins on the eyes of the dead as a means of keeping the eyelids closed was a common phenomenon in African-American graves (Crissman 1994; Matternes et al. 2010; Rose and Santeford 1985; Shogren et al. 1989). Overall, the material remains recovered during excavation suggest that the burial site was a mix of lowland/coastal African American and upland-like funeral practices (Matternes et al. 2012).

The poor preservation of the 101 individuals recovered from Avondale Burial Place allowed for few conclusions regarding the lives of those buried there. Skeletal preservation was marginal and definable skeletal features were found in 64% of the 101 graves (Matternes et al. 2012). Bones of the legs and skull were the most represented, but unfortunately many indicators of age, sex, health, and ancestry found on delicate bones like the ribs, pubis, face, feet, and hands were least represented (Matternes et al. 2012). As a result, only 26 individuals could be identified for sex (16 females, 10 males). Age was estimated for 63 individuals using a combination of dental, cranial, skeleton developmental, vertebral and os coxae markers (0-9.99yrs: 35, 10–19.99yrs: 5, 20–29.99yrs: 3, 30–39.99yrs: 7, 40–49.99yrs: 7, >50yrs: 6). Overall skeletal preservation was documented for each bone using the Haglund and Sorg (1997), which uses a numerical scale ranging from 1.00 (bone in perfect condition) to 8.00 (completely decomposed, only organic stain present) with a score of 0 indicating a missing skeletal element. Scores at Avondale Burial Place ranged from 2.70 to 8.00 with an average non-zero score of 6.004 for all 101 individuals (Matternes et al., 2012). A total of 10 crania from Avondale Burial Place were complete enough to be examined for ancestral affiliation using a variety of metric (Ousley and Jantz 1996) and nonmetric techniques (Bass 1987; Rhine 1990; White and Folkens 2000) (Table 1). Examinable crania features were consistent with morphologies that would have been classified as African American by the local community. Faces were dominated by widened nasal apertures with well-developed nasal gutters that merged with moderately projecting zygomatics. These were features generally associated with people with ancestral ties to Africa (Bass 1987; Rhine 1990; White and Folkens 2000). Metric classification using discriminant function analysis also identified African populations as major contributors to expressed cranial dimensions. Aside from an os acromiale on the left scapula of F026, no

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pathological markers were present. Os acromiale is a genetic condition when the lateral end of the acromion fails to unite with the rest of the scapula (Mann and Hunt 2005), a feature found to be more common among blacks than whites

Table 1. Information on the preservation and nonmetric/metric assessment of the individuals used for genetic analysis.

Burial	Preservation score	Nonmetric Assessment	Metric Assessment	Notes (from Matternes et al., 2012)
F001	4.60			Shovel shaped incisors
F003	3.60			
F005	2.70			
F016	4.80	AA	AA/C	
F017	4.80			
F018	5.20	AA	NA	Skull broken, so very few measurements possible - results are suspect
F023	4.90	AA	NA	Skull broken, so very few measurements possible - results are suspect
F024	5.10	C/AA	C/AA	Individual may reflect Caucasian admixture
F025	6.80			
F026	4.20	AA	NA	Nasal infection may have influenced nasal dimensions
F030	6.00			
F031	5.40	AA	AA	
F033	5.50			
F035	5.10	AA	I	Very few observations possible
F036	5.40			
F038	4.00	C/AA	AA	Individual may reflect Caucasian admixture
F039	5.80			
F051	6.50			
F096	4.40			
F100	7.80			

AA=African American
C=Caucasian
NA=Native American
I=Intermediate

(Sammarco 2000). Other health related pathologies on skeletal remains within Avondale Burial Place were consistent with the African American health conditions from Central Georgia at the time, including the presence of tendon ossification, hypercementosis, bowing of long bones due to rickets, and dental

pathologies like malocclusions, hypoplasias, and periodontal disease (Matternes et al. 2012). The lack of conclusive archaeological and osteological evidence as to the origins of the cemetery led to one final line of evidence using ancient DNA techniques at the University of Oklahoma.

Mitochondrial DNA from skeletal remains has been successfully used in a number of studies to better understand to population history and origin(s) of those buried within them (Adachi et al. 2009; Lee et al. 2009; Shook and Smith 2008; Stone and Stoneking 1998). Despite the poor preservation at Avondale Burial Place, it is still possible to recover portions of the mitochondrial genome using certain ancient DNA analysis techniques. In the present study, we estimated the maternal ancestry of those buried within Avondale Burial Place through an examination of the mitochondrial hypervariable region I (mtDNA HVR1). Using this information it is possible to discern an individual's haplotype, a set of DNA polymorphisms that provide valuable insights into ancestral origin. Remains from 20 individuals, ranging in age from juvenile to adult, were selected for DNA extraction and mtDNA HVR1 amplification based on overall preservation (more in Materials and Methods).

Contact was made with potential living descendants who had ancestral ties to the area's African-American communities. There are very few surviving records about southern Bibb County's African Americans and these descendants expressed considerable interest in what the cemetery could reveal about their heritage. Potential living descendants were also deeply concerned about whether the remains were truly those of their ancestors or whether they belonged to other unknown individuals and communities. Within the African American community there is a strong movement to restore the historical, cultural, and genealogical information in historic cemeteries like that of Avondale Burial Place (Carrier, 2004). While historical, archaeological, and osteological information leaned strongly towards Avondale Burial Place representing an African-American burial site, no definitive evidence of this conclusion was found. Likewise, no evidence was found in the cemetery that specifically named decedents or linked the burial ground with the potential descendant community. Before accepting the cemetery as part of their own heritage, potential descendants desired further confirmation that these were truly the graves of their former kinsmen.

A total of eight individuals with a history of slave ancestors in Bibb County provided saliva for mtDNA analysis. These individuals, living within and outside of Bibb County, are all hereby referred to as Bibb County participants. In order to establish potential maternal ancestry, we use the online tool EMPOP (version 2.3, release 11) (Parson and Dur 2007) to determine if the haplotypes between volunteers and interred individuals appeared at a higher probability than expected by chance alone. Genetic data provided an independent and potentially

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more specific means of verifying what the archaeological and osteological data had to say about the ancestry of these individuals.

Materials and Methods

The University of Oklahoma Laboratories of Molecular Anthropology and Microbiome Research (LMAMR) were initially contacted by GDOT to extract DNA from the skeletal remains of individuals from Avondale Burial Place. Skeletal material from burials was selected for genetic analysis based on the following criteria: 1) skeletal material which would yield the greatest amount of DNA (ideally well preserved teeth with an intact root), 2) skeletal material that could be linked to a single burial site with confidence (only a single individual within a site feature), and 3) skeletal material that could be recovered with the minimal amount of contamination from the excavation team (Matternes et al., 2012). A total of 19 teeth and a vertebral fragment from 20 well preserved skeletons underwent DNA extraction at LMAMR (Table 2). Teeth or bone were pulverized using sterile tools by researchers equipped with sterile gowns, hair nets and marks. DNA was extracted in an ISO 7 (class 10,000) HEPA-filtration, positive pressure clean room using previously established salting out extraction methods (Tito et al. 2012; Tito et al. 2008). In brief, the salting out protocol involved lysing the pulverized skeletal material overnight, followed by a Proteinase K (Invitrogen) incubation, and a subsequent washing and eluting of the DNA (Tito et al. 2012; Tito et al. 2008). Seven of these samples (F001, F017, F024, F035, F051, F096, F100) were replicated at Washington State University (WSU) using a modified protocol of Kemp et al.(2007) described by Moss et al. (2014).

The University of Oklahoma Institute Review Board approved the collection of eight living individuals, recruited by NSA, who could trace their family history back at least two generations and whose oral family history suggested ancestors who were slaves in Bibb County. Saliva was collected from these individuals using the Oragene®-DNA (DNA Genotek; OG-250) collection kit and DNA was extracted from each sample in accordance with the manufacturer protocol.

At LMAMR, PCR amplifications were performed for the HVR1 to sequence nucleotide positions (nps) 16011-16382, using the primers listed in Table 3. The mtDNA HVR1 PCR amplification was completed using previously established protocols (Grier et al. 2013; Tito et al. 2011). Agarose gels stained with ethidium bromide were used for visualization of HVR1 PCR products. Amplicons of the appropriate size were purified with exonuclease I-shrimp alkaline phosphatase and were sequenced in an ABI 3730XL capillary sequencer. For those individuals with ambiguous haplotypes (F003, F005, F023, F024, F031, F033, F039, F051, F096) PCR products were cloned using TOPO TA Cloning®

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Kits (Invitrogen) following the manufacturer instructions and sequenced again using an ABI 3730XL capillary sequencer. At WSU, DNA extractions were tested

Table 2. Avondale Burial Place skeletal material used for genetic analysis.

Burial	Sample for DNA testing	Skeletal Sex	Skeletal Age
F001	Molars (2)	*	9.5-14.5y
F003	Molars (2)	F	14-16y
F005	Molar (1) & Premolar (1)	M	35-40y
F016	Molar (1) & Premolar (1)	M	50-59y
F017	Molar (1) & Premolar (1)	*	10-12y
F018	Molar (1) & Premolar (1)	F	30-50y
F023	Molar (1) & Vertebrae frag (1)	F	25-35y
F024	Molars (2)	M	45-49y
F025	Molar (1)	*	12.5-17.5y
F026	Molar (1)	M	39-45y
F030	Incisors (3)	*	4-8y
F031	Molar (1) & Premolar (1)	M	20-30y
F033	Molar (1) & Premolar (1)	F	50-59y
F035	Molar (1) & Premolar (1)	M	45-49y
F036	Incisor (1) & Molar (1)	*	<7y
F038	Molar (1)	M	20-29y
F039	Molar (1)	F	38-52y
F051	Premolars (2)	F	<30y
F096	Molar (1) & Premolar (1)	F	35-45
F100	Incisors (2)	*	Adult?

( ) = number of bone/tooth fragments
* = unknown due to preservation or child burial

for the presence of PCR inhibitors following Grier et al. (2013). As none of the samples were demonstrated to contain sufficient levels of inhibition to preclude amplification, PCR amplification of four segments of the mitochondrial HVR1 and sequence preparation was conducted using previously established methods (Kemp et al. 2007).

These raw HVR1 data were cleaned and compiled using Sequencher 4.10.1. The resulting modern and ancient HVR1 sequences were compared to mthap (HVR1 region only) (dna.jameslick.com/mthap/) in order to determine the best haplogroup match (Lick 2014). Additionally, the online tool EMPOP (version 2.3, release 11) (Parson and Dur 2007) was used to assess whether haplotype matches between Bibb County participants and Avondale Burial Place

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historical individuals are unlikely to have occurred by chance from a random sample of African Americans. EMPOP settings included nps 16024-16365,

Table 3. HVR1 primer sequences.

Primer Pairs	DNA Sequence	Region	Length	Ancient/Modern
15986F	GCACCCAAAGCTAAGATTCTAATTT	HVR-1	168bp	Ancient
16153R	CAGGTGGTCAAGTATTTATGGT			
16106F	GCCAGCCACCATGAATATTGT	HVR-1	146bp	Ancient
16251R	GGAGTTGCAGTTGATGTGTGAT			
16190F	CCCCATGCTTACAAGCAAGT	HVR-1	166bp	Ancient
16355R	GGGATTTGACTGTAATGTGCTATGT			
16232F	CACACATCAACTGCAACT	HVR-1	173bp	Ancient
16404R	GGTGGTCAAGGGACCCCTATCT			
15971F	CTTTAACTCC ACCATTAGCA	HVR-1	1082bp	Modern
484R	TGAGATTAGTAGTATGGGAG			

disregarding length variants at np 16193. Bonferroni corrections (Dunn 1961) were used to conservatively estimate the probability of an exact HVR1 match from Avondale Burial Place to a potential twenty-first century descendant.

Results

The primary objective of this study was to determine the geographic origins of those interred within Avondale Burial Place. Eighteen of the 20 remains (90%) exhibited HVR1 haplotypes that belong to either haplogroup L or haplogroup U, both of which indicate African Ancestry (Table 4) (Figure 3). The haplotypes of seven of these individuals were confirmed by Washington State University. The most abundant haplogroups were L2 (n=7) and L3 (n=7) and L1 (n=2). Two Avondale Burial Place burials exhibited HVR1 haplotypes belonging to the H2 mitochondrial lineage. Overall, these results suggest that the Avondale Burial Place cemetery was used predominantly as an African American cemetery.

The secondary objective of this research was to identify potential familial relationships between Bibb County residents and those within in Avondale Burial Place using HVR1. The eight potential descendants possessed mtDNA HVR1

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sequences belonging to African ancestral haplogroups: L1 (n=3), L2 (n=2), L3 (n=1); a Eurasian haplogroup T (n=1) and an Asian haplogroup D (n=1). Those

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Table 4. Sample haplotype information.

ID	16017	16075	16086	16111	16126	16129	16145	16148	16163	16168	16172	16183	16184	16186	16187	16188	16189	16209	16218	16223	16230	16239	16260	16264	16270	16278	16286	16292	16293	16294	16309	16311	16320	16327	16355	16360	16362	Haplogroup	Matches
Ref	T	T	T	C	T	G	G	C	A	C	T	A	C	C	C	C	T	T	C	C	A	C	C	C	C	C	C	C	A	C	A	T	C	C	C	C	T		
ANCIENT HAPLOTYPE INFORMATION																																							
F001	-	-	-	-	-	A	-	-	-	-	-	-	-	-	T	-	C	-	-	T	-	-	-	-	-	T	-	-	G	T	-	C	-	-	-	T	-	L1c1	^
F003	-	-	-	A	-	-	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	T	-	-	-	T	-	T	-	-	-	C	-	-	T	-	-	L2e	
F005	-	-	-	T	C	-	-	-	-	-	-	-	-	-	T	-	C	-	-	T	-	T	-	-	T	T	-	-	G	-	-	C	-	-	-	-	-	L1b	
F016	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	T	-	-	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	L3f1b	
F017	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H2	
F018	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	T	G	-	-	-	-	-	-	L2a1c	
F023	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H2	
F024	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	T	-	-	-	L3e1	
F025	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	T	-	-	T	T	-	T	-	-	-	-	-	-	-	-	-	-	-	U6a7b1	
F026	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	T	G	-	-	-	-	-	-	L2a1	
F030	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	T	T	-	-	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	L3f1b	
F031	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	T	-	-	-	-	-	T	-	-	-	T	G	-	-	-	-	-	-	L2a1f	#
F033	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	T	-	-	-	-	-	T	-	-	-	T	G	-	-	-	-	-	-	L2a1f	#
F035	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	T	G	-	-	-	-	-	-	L2a1	
F036	-	-	-	A	-	-	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	T	-	-	-	T	-	T	-	-	-	C	-	-	T	-	-	L2e	
F038	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	T	T	-	-	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	L3f1b	
F039	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	L3e2	
F051	-	-	-	-	-	-	-	-	-	-	C	C	-	-	-	-	C	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	L3e2b	
F096	-	-	-	-	-	A	-	T	-	T	C	-	-	-	T	G	C	-	-	T	G	-	-	-	-	-	-	-	-	-	-	C	T	-	-	-	C	L0a1	
F100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	T	T	-	-	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	L3f1b	

Table 4. Sample haplotype information. (continued)

ID	16017	16075	16086	16111	16126	16129	16145	16148	16163	16168	16172	16183	16184	16186	16187	16188	16189	16209	16218	16223	16230	16239	16260	16264	16270	16278	16286	16292	16293	16294	16309	16311	16320	16327	16355	16360	16362	Haplogroup	Matches
Ref	T	T	T	C	T	G	G	C	A	C	T	A	C	C	C	C	T	T	C	C	A	C	C	C	C	C	C	C	A	C	A	T	C	C	C	C	T		
AV002	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	T	-	-	T	G	-	-	-	-	-	-	L2a1	
AV003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	T	-	-	-	-	-	T	-	-	-	T	G	-	-	-	-	-	-	L2a1	#
AV006	C	-	-	-	-	A	-	-	G	-	-	-	-	-	T	-	C	-	-	T	-	-	-	-	-	T	-	-	G	T	-	C	-	-	-	T	-	L1c1	
AV007	-	-	-	-	-	A	-	-	-	-	-	-	-	-	T	-	C	-	-	T	-	-	-	-	-	T	-	-	G	T	-	C	-	-	-	T	-	L1c1	^
AV009	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	D	
AV010	-	-	-	-	-	A	-	-	-	-	-	-	-	-	T	-	C	-	-	T	-	-	-	-	-	T	-	-	G	T	-	C	-	-	-	T	-	L1c1	^
AV013	-	-	-	-	C	-	-	-	G	-	-	-	-	T	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	T1	
AV014	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	L3e1	

Sample haplotypes were designated using the best hit for HVR1 in the mthap database
F = Historic samples
AV = Modern samples
Matches (# and ^) denote an identical haplotype between historic and modern samples

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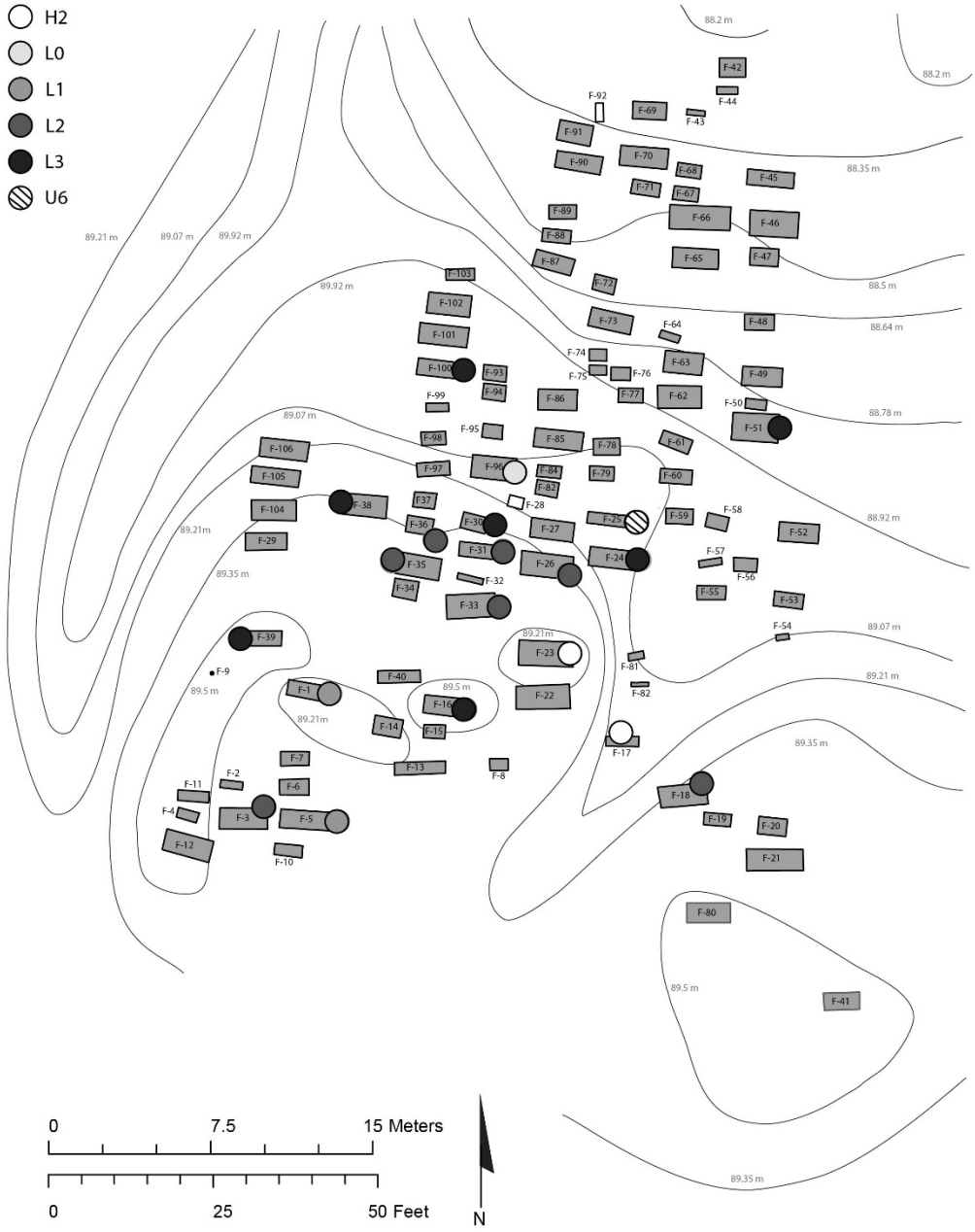

Figure 3. Location of burials within Avondale Burial Place and the haplotypes of those individuals selected for analyses (amended from Matternes et al. 2012).

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identical haplotype sequences between individuals from Avondale Burial Place and potential descendants (F001, F031, AV003, AV007 and AV010) are specifically denoted in Table 4. These shared sequences were compared to the online tool EMPOP (version 2.3, release 11) (Parson and Dur, 2007). Using EMPOP, we set the parameters to search those haplotypes with exact matches to the "USA" geographic affiliation and with an "African" metapopulation. The results provided in Table 5 represent the number of exact matches of each HVR1 mtDNA haplotype from Avondale Burial Place in relation the to the total haplotypes in EMPOP (n=34,617) prior to repeated number correction. Requiring exact haplotypes matches is a conservative criterion because recent mutation can be a confounding factor. Nevertheless, this conservative criterion finds putative relationships between historical and living participants. In this study, historical haplotypes were compared to eight unique modern haplotypes; therefore, a reasonable critical value after Bonferroni correction for each historical haplotype would be 0.05/8=0.00625. The haplotype observed in F001, AV007 and AV010 has a probability less than this critical value (Table 6).

Table 5. Haplotype matches in the EMPOP database.

Haplogroup	Burial	Total haplotypes with exact matches
H2	F017	1
H2	F023	56
L0	F096	7
L1	F001	6
L1	F005	5
L2	F003	1
L2	F018	8
L2	F026	95
L2	F031	24
L2	F033	24
L2	F035	95
L2	F036	1
L3	F016	1
L3	F024	11
L3	F030	3
L3	F038	3
L3	F039	3
L3	F051	64
L3	F100	3
U6	F025	0
Geographic Affiliation: USA, Metapopulation: African		
Total African Haplotypes in America: 2,823		
Total Haplotypes in EMPOP: 34,617		

Table 6. **Probability of randomly sampling haplotypes from 2,823 African American sequences available in the EMPOP database.**

Haplotype Comparisons	Observations in EMPOP	Probability
F001 AV007 AV010	6	0.0022
F031 AV003	24	0.0085

Discussion

Origins of Avondale Burial Place The presence of individuals belonging to haplogroup L (and U) within Avondale Burial Place suggest that the cemetery was used as an interment for African Americans. The results of this study confirmed that those interred within Avondale Burial Place shared genetic features associated with people of African origins. These people would have been identified locally as 'colored' or 'negro'. These results would be consistent with African Americans living in rural southern environments where physical and social factors limited access to the resources needed to maximize their quality of life. They lived during a period where the American death ritual emphasized a considerable outlay of capital for social display, but the use of home-made, locally made, and conservatively adorned coffins and attire implied that capital may not have been readily available for these rituals (Matternes et al. 2012; Sloane 1991). As noted earlier, inclusion of personal possessions, charms, and other objects reflected a continuation of traditions that likely had their origins in pre-Emancipation African and African-American communities. These genetic data in addition to the complementary archaeological, osteological, and oral history information helped validate, with some accuracy, the African American origins of the cemetery when little written information was available.

Close Familial Relationships with Avondale Burial Place. The genetic information from potential twenty-first century descendants was also compared to burials from Avondale Burial Place in order to assess familial relationships. Based on EMPOP sequences, the likelihood that these two sets of exact matches, F001, AV007 and AV010, and F031 and AV003 occur by chance within this cemetery is less than 1%. These historical and modern HVR1 haplotype comparisons are compelling, but should only be used for exploratory purposes. One limitation to this analysis is the use of only the mitochondrial hypervariable I region, but due to the overall preservation of the skeletal material, we did not make an attempt to obtain deeper autosomal data. Essentially, these data provide a proof of concept that with additional genome-level information, family relationships between these

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recently discovered unmarked cemeteries and living descendants can be estimated. Although only 20 burials were genetically examined in this study, this research gives a compelling reason for further evaluation of familial relationships in Avondale Burial Place using a deeper genome characterization. A future, genome-level characterization would 1) allow for unprecedented outreach opportunities, and 2) facilitate family mapping within this cemetery to further evaluate potential descendants.

The HVR1 mtDNA information helped confirm that the living conditions reflected in Avondale Burial Place's skeletons were those of the African-American community. Degenerative conditions including osteoarthritis, tendon ossification, and degenerative joint disease emphasized that many adults were engaged in physical labor that literally wore their bodies out (Matternes et al. 2012). They likely consumed a diet high in calories but short in nutrition that lowered disease resistance and resulted in rickets, hypercementosis, short stature, and contributed to dental hypoplasias and periodontal disease (Matternes et al. 2012). This latter malady also reflected limited access to hygienic living environments. The harshness of these conditions were reflected in an assemblage where less than ten percent of the cemetery population lived past age 50 and over 55% of the assemblage died before reaching their tenth birthday (Matternes et al. 2012). Avondale Burial Place was a community living in considerable physical stress. Life outside of Avondale Burial Place was likely perceived as a 'greener pasture' by its community members.

Oral history from the descendant community placed their ancestors in southern Bibb County and genetic results confirmed this part of the family's history. Likewise, abandonment of the cemetery occurred largely during the early twentieth century and corresponded with the general abandonment of the region by the descendant community (Matternes et al. 2012). Movement of Avondale Burial Place away from central Georgia in search of better opportunities was part of an African American exodus from the south generally referred to as the Great Migration (Wilkerson 2010).

The results of this study provided a sense of closure for many members of the descendant community. Prior to discovery of the Avondale Burial Place, descendants had spent at least 20 years searching for the graves of their ancestors. Discovery offered hope and genetic analysis confirmed that the bones in Avondale Burial Place were those of their ancestors. Likewise this study's results grounded the traditions, material culture, and quality of life reflected in the cemetery as features of the family's past. Important parts of their heritage, which had never been recorded or were lost by the passage of time, had now been recovered. The world of Avondale Burial Place was not a vignette from another unrelated community, but was unquestionably that of their own people. A missing chapter in family history had been restored and descendants were secure in

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knowing that the bones they honored were those of their kinsmen. With this study, we utilized genetic evidence to expose the origins of Avondale Burial Place and potential ancestral relationships when osteological and archaeological evidence was inconclusive. When the remains were finally reinterred, family member and reverend, Herman 'Skip' Mason observed: "These must be very special people. We only get one funeral and these folks get two" (Matternes et al. 2012:435) (Figure 4).

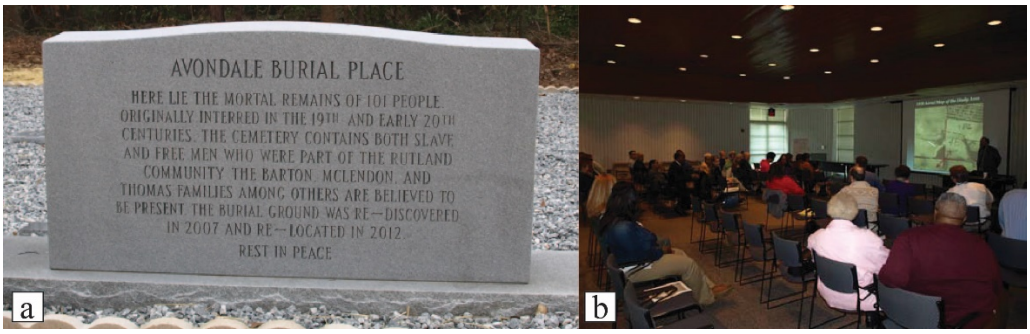

Figure 4. (a) Memorial stone placed beside the reinterment area, located next to Bethel AME church in Byron, Georgia. (b) Presentation of the findings at a public forum in Bibb County, Georgia (Matternes et al., 2012).

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