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Patterns of Antemortem Tooth Loss in Late Prehistoric West-central Tennessee

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ABSTRACT The later prehistoric subsistence-settlement pattern in the Kentucky Lake Reservoir (KLR) of northern west-central Tennessee is of interest as human occupation inexplicably terminates by AD 1450 as part of a larger regional depopulation. Antemortem tooth loss (AMTL) collectively and by tooth type was identified in four site samples from the KLR. These are a Late Woodland (AD 600-900) sample (Hobbs) and three Middle Mississippian period (AD 1100- 1400) hierarchically organized and presumptively maize agriculturalist samples (Link/Slayden, Gray Farm, Thompson Village). AMTL prevalence in the Hobbs sample is consistent with a native crop and seasonal foraging economy. The AMTL in the Link/Slayden sample is more congruent with the pre-maize Late Woodland sample than the essentially contemporaneous Gray Farm site sample. Thompson Village, a later-dated satellite community of the Gray Farm polity, exhibits significantly fewer AMTL than Gray Farm. This may flag climate-influenced agricultural shortfall of dietary carbohydrates later in the occupation sequence. Additionally, males in the Gray Farm site sample have significantly more AMTL than males in the other two Mississippian samples. The patterns suggest regional, possibly shortfall mitigated, differences in maize intensification with a polity-specific male-focused maize consumption in the Gray Site.

Oral pathology has frequently been an effective barometer of community health and an attestable marker of subsistence strategy in archaeological contexts where material culture provides the interpretive context (e.g., Betsinger & Smith, 2018; Larsen, 1983; Larsen et al., 1991; Lukacs, 1992; Russell et al., 2013; Turner, 1979; Watson, 2008). Maize is a cariogenic carbohydrate (e.g., Bibby et al., 1951; Horton et al., 1985; Newbrun, 1979). Caries has been a particularly interpretively useful oral pathological condition in North America, enabling the flagging of maize-intensive agriculture (e.g., Emerson et al., 2005; Larsen, 1981; Powell, 1985; Watson, 2008). In the absence of adequate oral hygiene, crown or cervical carious lesions progress to penetrate the pulp cavity resulting in dental necrosis and ultimately to exfoliation of the tooth from the alveolar anchor. In more extensive assessments of caries prevalence (i.e., caries correction factor), antemortem tooth loss (AMTL) has been included as a proxy for carious teeth (Duyar & Erdal, 2003; Erdal & Duyar, 1999; Lukacs, 1995; Marquez-Grant, 2009).

Although AMTL is primarily attributed to the destructive pathogenesis of caries and periodontal

disease (Baelum et al., 1986; Kida et al., 2006; Müller & Hussein, 2017; Niessen & Weyant, 1989; Ong, 1998; Shaffer et al., 2013; van der Velden et al., 2015), there are other causes. These include rapid attrition, ablation, acidogenic response, and traumatic injury (Costa, 1980; Duyar & Erdal, 2003; Han & Nakahashi, 1996; Humphrey & Bocaege, 2008; Lukacs, 2007; Nelson et al., 1999; Niessen & Weyant, 1989; Pollard et al., 1997). Although traumatic injury may be a contributing factor in a few cases of AMTL in the late prehistoric samples from west-central Tennessee, the present study observed that attrition and ablation, as elsewhere in the Tennessee Valley (Smith, 1982), are absolutely not evident. As such, oral pathology is the most apparent contributor to AMTL. Previous assessment of caries prevalence in Late Prehistoric human osteologi-

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cal samples from northern west-central Tennessee did not identify a clear temporal trend of caries increase with agriculturalization suggesting regional variability in the reliance of maize relative to domesticated native seed crops (Smith & Betsinger, 2019), a not uncommon phenomenon elsewhere in the Mississippian world (e.g., Hart & Lovis 2013; Hutchinson et al., 1998; Scarry, 1993). A renewed look at oral health using AMTL data and a larger sample of sites may help clarify the lack of comparative oral health congruence with maize-intensive samples. Given the inexplicable abandonment of the region by AD 1450 as part of the large scale depopulation of the lower Ohio River Valley (“The Vacant Quarter”) (Cobb & Butler, 2002; Williams, 1990), the temporal and regional differences in AMTL may also provide some eco-political insight.

The maize-intensive subsistence economy in the eastern United States temporally associates with the Mississippian period (~AD 1000 – 1500) and life way, the apex of which (AD 1200-1400) is socio-politically characterized as having centralized (possibly chiefdom level) authority, a complex iconographic-rich cosmology, a well-organized aggregated village settlement pattern, shell-tempered pottery, and wall trench architecture (Bense, 2016; Cobb, 2003; King, 2002; King et al., 2007; Lewis et al., 1998; Peregrine 2013; Wilson, 2017). Since the Mississippian period material culture correlates are present in the late prehistory of upper west-central Tennessee, a maize-intensive agricultural subsistence economy has been proposed (Bass, 1985; Dye, 2004, 2007; Krus & Cobb, 2018; Lunn, 2013; Mainfort, 1996). The purpose of this assessment is to clarify why the Mississippian period Kentucky Lake collective site sample from Link [40HS6] and Slayden [40HS1] did not exhibit a caries frequency or pattern consistent with unequivocal maize-intensive comparative samples from Tennessee (Smith & Betsinger, 2019).

Materials and Methods

Archaeological context

In eastern North America, the time period spanning the Late Woodland (~AD 600-900) to the apex of the subsequent Mississippian period (~AD1200-1400) is one of marked regionally and temporally variable economic, settlement, and sociopolitical change (Bense, 2016; VanDerwarker et al., 2017; Fritz, 1990; Nassaney, 2001; Scarry, 1993; Wilson & Sullivan, 2017). The subsistence-settlement pattern of the Late Woodland is generally characterized by dispersed small villages within circumscribed terri-

tories that economically engaged in cultivating native seed crops as well as foraging strategies (i.e., the Eastern Agricultural Complex) (Bense, 2016; Fritz, 1990; Nassaney, 2001; Scarry, 1993). This pattern is archaeologically evident in central Tennessee (Shea, 1977). The geographic distribution and the ensuing time period of the Mississippian is defined by the transition to maize-reliant agriculture (Bense 2016; Fritz, 1992; Krus & Cobb, 2018; Lewis et al., 1998; VanDerwarker et al., 2017), palisaded aggregated villages with one or more variably functioning (e.g., domiciliary, mortuary, temple) platform mounds flanking a central plaza, and more complex, likely hierarchical, social organization (Bense, 2016; King & Freer, 1995; Schroedl, 1998). In the greater Southeast, the Mississippian period is also iconographically rich (i.e., “Southeastern Ceremonial Complex” or “Southeastern Ceremonial Exchange Network”) (Bense, 2016; King et al., 2007).

The typical Mississippian large aggregated settlement was organized around a central plaza that was flanked by flat-topped mounds which variably served as platforms for domiciles, temples, mortuary structures (e.g., charnel houses, mound burials), or community buildings (Bense, 2016; Lewis et al., 1998; Nash, 1968). Mound settlements were civic-ceremonial polity centers geographically surrounded by satellite communities and separated from other mound-centered polities by buffer zones (Bense, 2016; Dye, 2004; Hally, 1993).

The sites of Gray Farm (40SW1), Thompson Village (40HY5), Link Farm (40HS6)/Slayden (40HS1), and Hobbs (40HS44) are located in the Lower Tennessee River Valley of west-central Tennessee in the projected catchment area of what is now the Kentucky Lake Reservoir (KLR) (Figure 1). The sites were excavated between 1938 and 1944 as part of extensive salvage archaeological recovery operations conducted by TVA (Tennessee Valley Authority) and the Federal WPA (Works Progress Administration) program prior to the completion of the Kentucky Dam (Gilbertsville, Kentucky) (Dye, 2013). This section of the Tennessee River valley lies between the Western Tennessee Uplands on the west, and the steep hills of the Western Highland Rim on the east. The archaeological recovery of the sites’ material culture and skeletal material was hampered by time constraints, many years of agricultural activity, highway construction, and extensive looting (Bass, 1985). There are no monographic site reports for any of the five sites, but one or other of them has been the source of descriptive or synthetic archaeological assess-

ment (Bass, 1985; Dye, 2002; Lunn, 2013). Given the absence of carbon samples, the sites are relatively dated (ceramic sequences, mortuary patterning) (Bass, 1985).

The five site samples examined here consist of the Late Woodland period (~AD 500-900) site of Hobbs (Kuemin Drews, 2001) and four primarily Middle Mississippian period (~AD 1100-1350) sites that segregate into two geographically discrete polities (Bass, 1985; Dye, 2004). Hobbs is a mound mortuary site on the main channel of the Tennessee River downstream from the Link and Slayden sites (Figure 1). The Early-to-Middle Mississippian period sites of Slayden (~AD 1050-1250) and Link Farm (~AD 1250-1400) are located on opposite shores of the Duck River floodplain, geographically close to the confluence with the Tennessee River (see Figure 1) (Dye, 2002, 2007; Kuemin Drews, 2000; Lunn, 2013). The multiple mound site of Link Farm is argued to have expanded from the Slayden village site with the former becoming the administrative center for the Middle Mississippian period Link Farm polity (Dye, 2002, 2004; Lunn, 2013; Nash, 1968). Given the paucity of human remains recovered from Slayden, the site samples are combined for this study as Link/Slayden.

The Gray Farm polity is located near the confluence of the Big Sandy River and the Tennessee River (see Figure 1). The sites included here are the

multiple mound Gray Farm site (~ AD 1150-1400) (Dye, 2004) and the Thompson Village site which fissioned from the former during the late Middle Mississippian period (~post AD 1250) and remained occupied through the early Late Mississippian period (Bass, 1985) (~AD1350-1450). All aboriginal occupation of west-central Tennessee apparently ended before circa AD 1450 (Bass, 1985; Smith, 2010) as part of the phenomenon of Middle Mississippian period regional abandonment in the greater lower Ohio River Valley (the "Vacant Quarter") (Cobb & Butler, 2002; Krus & Cobb, 2018; Williams, 1983). The specific reasons for the abandonment are unclear, but interpretations include corollaries of climate change (i.e., the "Little Ice Age" [~ AD1400-1700]) (Grove, 2012; Mann et al., 2009, Meeks & Anderson, 2013) such as resource depletion, and/or endemic warfare (Bass 1985; Krus, 2013; Worne, 2011).

Although the Kentucky Lake Mississippian period site samples archaeologically exhibit the hallmarks of Mississippian settlement organization and iconography, they are not further sub-divisible into temporally and regionally defined cultural phases. This is partly an artifact of pre-excavation site disturbance which impeded stratigraphic control. Geographically they are west of the arguably aboriginal interaction-impeding Western Highland Rim geophysical feature as well as outside of the

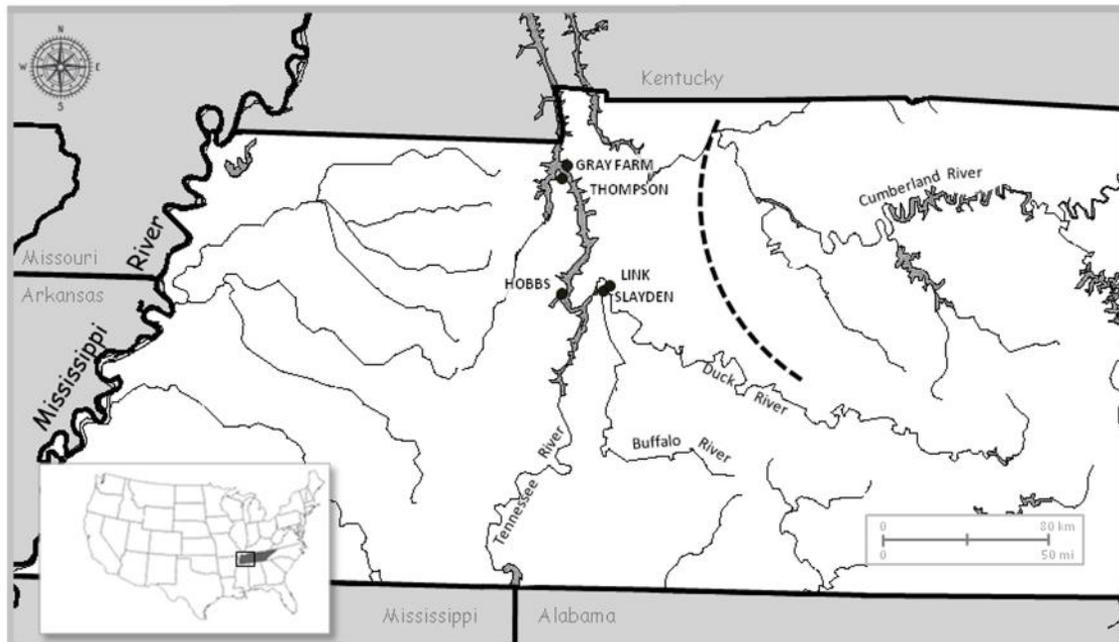


Figure 1. Map of the Kentucky Lake Reservoir sites utilized in the study. The western boundary of the Mississippian Period (AD 1000-1450) Middle Cumberland Culture is indicated by the dashed line.

Mississippian period (~AD 1050-1450) Middle Cumberland Region (MCR) (see Figure 1) of the central Cumberland River valley and its tributaries (Beahm, 2013; Dowd, 2008; Moore & Smith, 2001; Smith, 1992). There is also no archaeological evidence that the KLR samples had contact with Mississippian period sites in the Mississippi River drainage of west Tennessee (Mainfort, 1996; Mainfort & Moore, 1998). However, some shared cultural elements with the MCR (Dowd, 2008; Ferguson, 1972; Smith, 1992) are evident (e.g., infants interred within house structures and at least some use of limestone slabs to line graves (i.e., stone box burials) (Bass, 1985; Wamsley, 2018).

Sample and Diagnostic criteria

The skeletal age at death and differentiation by sex were previously determined using standard non-metric osteological protocols (Buikstra and Ubelaker, 1994) and the information is archived in a computer data base. The individuals regarded as adults in the AMTL assessment had least two of the third molars in occlusion *in vivo*. Mandibles and maxillae were the primary source of AMTL, but loose teeth were included if antimeres were present or if they were accounted for by alveolar bone (either as sockets or remodeled alveolar processes). Individ-

uals assessed consisted of the numbered burials; unaffiliated or extraneous teeth were excluded. Adults were segregated into three age-at-death categories: young (~18 years to ~35 years), middle age (~35 years to ~50 years of age), and old age (50+) years of age (Table 1).

Teeth were considered present when observed *in situ*, as loose teeth, and as un-remodeled alveolar sockets. Teeth were considered lost antemortem if the socket was porotic and failed to conform to the sharp alveolar contours of an *in situ* root, displayed abscessing with no accompanying (loose) tooth, and ultimately, by the remodeling/resorption of the alveolar corpus. Teeth were consolidated by arch and antimeres and evaluated for AMTL by categories of tooth type. Incisors and canines were collapsed into a single category (I/C), as were all premolars (PM). Molars were assessed by number (M1, M2, M3) and collectively.

Preservation ranged from fair to poor for all sites limiting the primary assessment to post-mortem presence by tooth type rather than pattern(s) of AMTL by individual (see Table 1). The Gray Farm sample is particularly poorly preserved; seventy percent of the interments with dental data are not ascribable to an age-at-death category and 23 individuals (~38 %) are assignable to a sex.

Table 1. Demographic overview of sample preservation and segregation by age-at-death and biological sex.

LINK FARM/SLAYDEN (Total n = 25 ²)								
young			m aged			old age		
males	females	sex-indet. ¹	males	females	sex-indet. ¹	males	females	sex-indet. ¹
1	3	2	4	3	1	0	3	0
GRAY FARM (Total n = 61 ^{2,3})								
young			m aged			old age		
males	females	sex-indet. ¹	males	females	sex-indet. ¹	males	females	sex-indet. ¹
0	1	0	9	2	2	2	2	0
THOMPSON (Total n = 103 ^{2,4})								
young			m aged			old age		
males	females	sex-indet. ¹	males	females	sex-indet. ¹	males	females	sex-indet. ¹
8	14	1	16	19	3	5	8	0
HOBBS (Total n = 8 ²)								
young			m aged			old age		
males	females	sex-indet. ¹	males	females	sex-indet. ¹	males	females	sex-indet. ¹
1	0	0	1	3	0	0	1	0

¹ sex-indeterminate

² sample Total includes individuals not assignable by either age or sex

³ 43/61 not ageable, 7/43 sex assignable but not ageable

⁴ 29/103 not ageable, 20/29 sex assignable but not ageable

Therefore, the primary intersite assessments are based on the presence of teeth (loose and *in situ*), alveolar evidence (pre- and postmortem loss), and AMTL.

Analytical methods

Fisher's exact test (<https://www.graphpad.com>) was used to test the prevalence differences for all teeth and by tooth category, and in the same categories when segregated by sex and/or age-at-death. No tests were undertaken for samples of less than ten. Small sample sizes prevented several comparisons. For those tests meeting the minimum sample size, the results are considered tentative.

Results

The Late Woodland Hobbs mortuary sample has the lowest total tooth sample prevalence of AMTL (14/153, 9.1%) (Table 2), but it is not significantly less ($p=0.4479$) than the Middle Mississippian Link/Slayden sample (46/391, 11.8%) (Table 3). However, the Hobbs sample has significantly fewer teeth lost antemortem than the Middle Mississippian site samples of Gray Farm ($p = 0.0001$) and Thompson Village ($p=0.0160$). The total tooth sample AMTL between the Gray Farm (32.7% and Thompson Village (16.7%) site samples is also significantly different ($p=0.0001$). When AMTL is further evaluated by the five tooth type categories, the Hobbs and Link/Slayden samples remain congruent (see Table 3). Hobbs differs in almost all tooth categories from Gray Farm and from the Thompson site sample in the collective loss of molars. Link/Slayden lost significantly fewer teeth than Gray Farm in all tooth categories except the first molar, and in many tooth categories compared to Thompson Village. Thompson, with overall fewer AMTL than the Gray Farm, differs from it in fewer incisiform and premolar tooth loss (see Table 3).

Age-at-death comparisons

Despite the case deficit in the young age at death (see Table 2), it is apparent that overall, the young adult cohorts for all four west-central Tennessee sites experience the lowest AMTL prevalence in each of the respective site samples. Where statistical tests are possible, there is a pattern of no intersite differences except for the greater AMTL of all molars in Gray Farm relative to Thompson (see Table 2). In the Middle Age cohort, the vulnerability of complex-crowned teeth to factors contributing to AMTL is evident. Hobbs and Link/Slayden exhibit a significant difference in the loss of M1, the molar longest in occlusion, as well as molars overall and the dentition overall. Hobbs,

however, has significantly fewer teeth lost in more categories than both Gray Farm and Thompson. The Link/Slayden site sample, in turn, has lost significantly fewer teeth than Gray Farm and molars compared to Thompson.

Thompson Village, with an overall tooth loss in the Middle Age category of 25% ($n=179/715$), is significantly lower than Gray Farm (89/165, 54%) in the loss of incisiform teeth (see Table 1) (7.8% versus 36.5%, 13.8% versus 49%), but congruent in the loss of individual molars. Meaning, the samples are evidently significantly different in the absolute number of molars lost, but not different in the proportion by molar type (see Tables 2 and 3). Few meaningful statistical comparisons were possible for many of the tooth categories in the Old Age cohort (i.e., samples < 10). All samples yielded few individuals in that age category (see Table 1) resulting in little available dental data.

Intrasite and intersite differences by sex

Site sample dissimilarities (see Tables 2 and 3) may be affected by sex-based differences in vulnerability to oral pathology (e.g., Lukacs & Largaespada, 2006). The samples were first compared for overall AMTL differences by sex (Table 4). In the collective adult sample, there are no sex differences in AMTL in the Hobbs and Link/Slayden samples. There are multiple categories of sex difference in the Gray Farm and Thompson samples. In the Gray Farm sample, males exhibit significantly more AMTL ($p=0.0001$) than females (43.5% versus 20.8%) and more AMTL in the anterior teeth; in the Thompson Village sample, females have more AMTL than males (~18% versus 12.6%) ($p=0.0022$). However, more tooth categories in the Thompson Village sample display the higher female AMTL prevalence.

Very few tooth class categories have a large enough Young Adult case sample to argue a pattern. However, the Gray Farm sample has more male AMTL for all molars and all dentition overall. Since most Young Adult site samples exhibit very few cases of AMTL (see Table 2) sex differences may be statistically undetectable. Sex differences do emerge in the Middle Age category (see Tables 4 and 5). At the Link/Slayden site, females lose significantly more molars overall ($p=0.0088$). Specifically, the Link/Slayden females lost 46% ($n=13/28$) while males lost 13% ($n=4/30$). Antithetically, there is very little sex difference in AMTL in the Gray Farm sample. It is restricted to the incisiform teeth (I/C) with males losing significantly more (males 50% [$n=13/26$], females 13.6% [$n=3/22$]). In the Thompson Village sample, first

Table 2. Raw frequencies of antemortem tooth loss by tooth category segregated by sex and by three skeletal age-at-death categories.

ANTEMORTEM TOOTH LOSS ¹												
LINK FARM/SLAYDEN												
<u>Tooth</u>	<u>TOTAL</u>		<u>males</u>		<u>females</u>		<u>young</u>		<u>m aged</u>		<u>old</u>	
I/C	3/124	2.4	0/17	0.0	3/77	3.9	1/28	3.6	0/37	0.0	2/10	20.0
PM	8/106	7.5	0/17	0.0	8/63	12.7	4/16	25.0	3/36	8.3	0/13	0.0
M1	19/64	29.7	4/19	22.2	5/29	17.2	2/10	20.0	11/22	0.0	3/6	50.0
M2	8/56	14.3	2/18	11.0	4/19	21.0	0/11	0.0	6/20	0.0	0/4	0.0
M3	8/41	19.5	5/17	29.4	2/13	11.1	0/16	0.0	0/26	0.0	0/5	0.0
ALL M	35/161	21.7	11/54	20.4	11/61	18.0	2/37	5.4	17/58	29.3	3/15	33.0
ALL DENT	46/391	11.8	11/88	12.5	22/201	10.9	6/81	7.4	20/120	6.7	5/38	13.2
GRAY FARM												
<u>Tooth</u>	<u>TOTAL</u>		<u>males</u>		<u>females</u>		<u>young</u>		<u>m aged</u>		<u>old</u>	
I/C	46/140	33.0	29/64	45.3	8/59	13.5	0/23	0.0	19/52	36.5	18/33	54.5
PM	38/124	30.6	15/40	37.5	6/42	14.3	0/15	0.0	22/45	49.0	4/14	28.6
M1	26/61	42.6	11/21	52.4	7/20	35.0	0/8	0.0	16/23	9.5	3/6	50.0
M2	22/59	37.3	9/26	34.6	7/20	35.0	1/7	14.3	16/23	69.5	1/5	20.0
M3	23/50	46.0	10/19	52.6	5/18	27.8	0/6	0.0	16/22	72.7	2/7	28.6
ALL M	71/170	41.8	30/66	45.5	19/58	32.7	1/21	4.8	48/68	70.6	6/18	33.0
ALL DENT	155/434	35.7	74/170	43.5	33/159	20.8	1/59	1.7	89/165	4.0	28/65	43.0
THOMPSON												
<u>Tooth</u>	<u>TOTAL</u>		<u>males</u>		<u>females</u>		<u>young</u>		<u>m aged</u>		<u>old</u>	
I/C	44/743	6.0	6/300	2.0	24/348	6.9	0/70	0.0	21/266	7.8	5/38	79.0
PM	49/554	8.8	8/228	3.5	26/257	10.1	4/56	7.1	26/189	13.8	10/29	34.5
M1	81/253	32.0	26/106	24.5	42/114	36.8	6/28	21.4	49/97	24.9	0/8	0.0
M2	79/247	32.0	31/102	30.4	37/113	32.7	4/23	17.4	40/85	47.0	3/8	37.5
M3	82/215	38.1	32/83	38.5	37/97	38.1	5/24	20.8	43/78	55.1	1/7	14.3
ALL M	142/715	33.8	89/291	30.6	116/324	35.8	15/75	20.0	132/260	0.8	4/23	17.4
ALL DENT	235/2012	11.7	103/819	12.6	166/929	17.9	25/201	12.4	179/715	25.0	19/90	21.1
HOBBS												
<u>Tooth</u>	<u>TOTAL</u>		<u>males</u>		<u>females</u>		<u>young</u>		<u>m aged</u>		<u>old</u>	
I/C	1/55	1.8	0/23	0.0	1/32	3.1	0/8	0.0	0/21	0.0	1/10	10.0
PM	4/35	11.4	0/6	0.0	4/13	30.1	0/6	0.0	0/15	0.0	4/7	57.1
M1	4/20	20.0	0/2	0.0	4/6	66.0	0/2	0.0	1/13	7.7	3/3	100.0
M2	3/24	12.5	1/5	20.0	2/17	11.8	0/3	0.0	0/10	0.0	3/3	100.0
M3	2/19	10.5	0/1	---	2/5	40.0	0/2	0.0	0/8	0.0	2/3	67.0
ALL M	9/63	14.3	1/8	12.5	8/28	28.6	0/7	0.0	0/31	0.0	9/9	100.0
ALL DENT	14/153	9.1	1/37	2.7	13/73	17.8	0/21	0.0	1/67	1.5	13/26	50.0

¹ Dentition was segregated by sex or age; sex-indeterminate dentition was included in the age-at-death samples.

Table 3. Pair-wise statistical comparisons of antemortem tooth loss based on the raw data from Table 1. No tests were undertaken if a sample was less than ten cases. Statistical significance ($p \leq 0.05$) is indicated by bold font.

	Link/Slayden x Gray Farm	Link/Slayden x Thompson	Gray Farm x Thompson	Gray Farm x Hobbs	Thompson x Hobbs	Link/Slayden x Hobbs
All						
I/C	0.0001	0.0093	0.0001	0.0001	0.3572	1.0000
PM	0.0001	0.5190	0.0001	0.0288	0.5440	0.3119
M1	0.1413	0.0592	0.1329	0.1083	0.3236	0.5672
M2	0.0196	0.0234	0.4444	0.0342	0.0611	0.7586
M3	0.0428	0.0908	0.3373	0.0103	0.0222	0.2130
All M	0.0001	0.0005	0.0607	0.0001	0.0001	0.2632
All Dent	0.0001	0.0001	0.0001	0.0001	0.0160	0.4479
Young						
I/C	1.0000	0.3519	0.4017	----	----	----
PM	0.3094	0.6704	0.6490	----	----	----
M1	----	1.0000	----	----	----	----
M2	----	0.2741	----	----	----	----
M3	----	0.0712	----	----	----	----
All M	0.6495	0.1040	0.0156	----	----	----
All Dent	0.2239	0.1681	0.0044	1.0000	0.1408	0.3410
Middle Age						
I/C	0.0001	0.0887	0.0001	0.0008	0.3810	1.0000
PM	0.0001	0.5865	0.0001	0.0004	0.2255	0.5462
M1	0.2307	1.0000	0.1100	0.0004	0.0056	0.0132
M2	0.0148	0.2136	0.0636	0.0003	0.0045	0.0741
M3	0.0001	0.0001	0.1515	----	----	----
All M	0.0001	0.0037	0.0039	0.0001	0.0001	0.0004
All Dent	0.0001	0.4091	0.0001	0.0001	0.0001	0.0012
Old Age						
I/C	0.0764	0.6251	0.0003	0.0261	1.0000	1.0000
PM	0.0978	0.0182	1.0000	----	----	----
M1	----	----	----	----	----	----
M2	----	----	----	----	----	----
M3	----	----	----	----	----	----
All M	0.0135	0.0002	0.2889	----	----	----
All Dent	0.6781	0.0780	0.0045	0.3643	0.5953	0.2073

¹ Fisher's exact test, $p \leq 0.05$; sample $n \geq 10$

molars and molars collectively are more commonly lost by females. Females lost ~57% of M1 ($n=29/51$) compared to 34% ($n=18/53$) in the males. The disparity drives the significance for all molars ($p=0.0272$) and collectively for all dentition (males $n=67/397$ [16.9%], females $n=97/405$ [24%]).

Since the three sites with testable samples may have samples that may have a temporal bias (e.g., Link/Slayden biased by some temporally later Link cases), it is possible that the AMTL varies by

sex between the sites (Table 6). Comparing the larger Middle Age category by sex and by site, females apparently vary very little between samples. Thompson females significantly differ from Gray Farm females for only the premolars (PM) ($p = 0.0382$, Thompson 10.6% [$n=12/113$], Gray Farm 31.2% [$n=5/16$]). There are also no statistical differences when all molars are pooled ($p=0.0727$) (Thompson 53% [$n=77/145$], Gray Farm 41% [$n=25/61$]). The scenario is different for the males. The Link/Slayden sample, with overall the fewest

teeth lost antemortem (7.7%), has fewer molar tooth loss than Thompson ($p=0.0426$) and exhibits significantly fewer tooth loss in all tooth categories from the Gray Farm sample (with a total tooth loss of 77.8%). Although both Gray Farm and Thompson village were occupied until regional abandonment, the Thompson site sample has a temporally later sample bias than Gray Farm. Thompson Village males have significantly fewer AMTL in all but third molar loss (see Table 5). Overall tooth loss for Thompson males is 16.9%.

Discussion

Although etiologically multifactorial, the primary clinical causes of AMTL are the progressive pathol-

ogies of caries and periodontal disease (e.g., Baelum et al., 1986; Kida et al., 2006; Müller & Hussein, 2017; Niessen & Weyant, 1989; Ong, 1998; van der Velden et al., 2015). Both processes are associated with the consumption of carbohydrates in combination with poor oral hygiene (e.g., Baumgartner et al., 2009; Chapple et al., 2017; Hix & O'Leary, 1976). As a proxy for pre-Columbian carbohydrate consumption, AMTL has the potential to augment archaeological information about diet and subsistence. In the context of the pre-Columbian skeletal samples from the Kentucky Lake Reservoir of northern west-central Tennessee, the insights are more than supplemental as archaeological assessment is limited and will likely con-

Table 4. Comparisons of antemortem tooth loss by sex for each site. No test was undertaken if either the male or female cohort consisted of less than ten cases. Statistical significance ($p = \leq 0.05$) is indicated by bold font.

	Link/Slayden	Thompson	Gray Farm	Hobbs
All Adults				
I/C	1.0000	0.0042	0.0002	1.0000
PM	1.0000	0.0043	0.0225	0.2554
M1	1.0000	0.0579	0.3499	----
M2	0.7057	0.7697	1.0000	1.0000
M3	0.3892	1.0000	0.1837	----
All M	0.3981	0.0827	0.1976	0.6478
All Dent	0.6908	0.0022	0.0001	0.0851
Young				
I/C	----	0.5762	1.0000	----
PM	----	1.0000	0.2308	----
M1	----	1.0000	----	----
M2	----	1.0000	----	----
M3	----	0.4423	----	----
All M	0.2881	1.0000	0.0420	----
All Dent	0.5918	0.5810	0.0223	----
Middle Age				
I/C	1.0000	0.7854	0.0131	----
PM	0.3416	0.8239	0.5166	----
M1	----	0.0297	1.0000	----
M2	----	0.0656	1.0000	----
M3	1.0000	0.5169	1.0000	----
All M	1.0000	0.0272	1.0000	----
All Dent	1.0000	0.0001	0.0646	----
Old Age				
I/C	----	0.1469	----	----
PM	----	0.0061	----	----
M1	----	0.1939	----	----
M2	----	0.2364	----	----
M3	----	0.1939	----	----
All M	0.1923	0.0021	----	----
All Dent	0.6882	0.0008	----	----

[†] sample $n \geq 10$

Table 5. The Middle Age category generated the largest samples of antemortem tooth loss segregable by sex. However, several tooth classes generated samples of less than ten teeth. These were not eligible for statistical comparisons.

	Link/Slayden		Gray Farm		Thompson	
MA Males						
I/C	0/12	0.0%	13/26	50.0%	6/146	4.6%
PM	0/10	0.0%	10/23	43.5%	10/108	9.3%
M1	3/10	30.0%	9/12	75.0%	18/53	34.0%
M2	1/10	10.0%	8/12	67.0%	15/50	30.0%
M3	0/10	0.0%	9/12	75.0%	18/40	45.0%
All M	4/30	13.3%	26/36	72.2%	51/143	35.7%
All Dent	4/52	7.7%	49/63	77.8%	67/397	16.9%
MA Females						
I/C	0/25	0.0%	3/22	13.6%	8/147	54.4%
PM	3/16	18.8%	5/16	31.3%	12/113	10.6%
M1	8/12	67.0%	6/8	75.0%	29/51	56.9%
M2	5/10	50.0%	6/8	75.0%	24/49	49.9%
M3	0/6	0.0%	5/7	71.4%	24/45	53.3%
All M	13/28	46.4%	17/23	73.9%	77/145	53.1%
All Dent	16/68	23.5%	25/61	41.0%	97/405	24.0%

¹statistical tests not undertaken if sample size ≤ 10

tinue to be so for the foreseeable future. Absent from the KLR archaeological record are radiocarbon dates, therefore, using attributes of the material culture, the sites have been temporally sequenced relative to each other (Bass, 1985; Dye 2002, 2003, 2004; Lunn, 2013). Pairing the occupation sequence with the AMTL data in the Kentucky Lake Reservoir yielded several potentially interpretively valuable patterns. Unfortunately, poor sample sizes restrict assessments by skeletal age at death. Therefore, the interpretations of the rate and pattern of AMTL should be considered tentative.

Temporal pattern

Hobbs, the earliest site sample evaluated here, dates to the Late Woodland period (~AD 500-900). Overall, there are fewer teeth lost antemortem by the Hobbs mortuary site relative to the Middle-to-Late Middle Mississippian Period Gray Farm and Thompson Village samples. The frequencies are consistent with the reconstruction of Late Woodland subsistence in the Tennessee River Valley as pre-maize horticulturalists with (at least) seasonal foraging (e.g., mast, fleshy fruits) (Bense, 2016; Crites, 1978; Emerson et al., 2000; Kline et al., 1982; McMahan, 1983). Cultigens utilized were the grasses native to the local environment. These were the oily (e.g., *Iva* [sumpweed], *Helianthus* [sunflower]) and starchy seeds (e.g., *Chenopodium* [goosefoot],

Table 6. For samples of at least ten individuals, statistical comparisons were undertaken between the site samples by sex. Statistical significance ($p = \leq 0.05$) is indicated by bold font.

	Link/Slayden v Thompson	Link Slayden v Gray Farm	Thompson v Gray Farm
Males			
I/C	1.0000	0.0026	0.0001
PM	1.0000	0.0303	0.0003
M1	1.0000	0.0237	0.0287
M2	0.2629	0.0115	0.0422
M3	0.0085	0.0016	0.2021
All M	0.0426	0.0001	0.0001
All Dent	0.1473	0.0001	0.0001
Females			
I/C	0.6048	0.0950	0.1577
PM	0.3986	0.6851	0.0382
M1	0.7462	----	----
M2	1.0000	----	----
M3	----	----	----
All M	0.5421	0.0858	0.0727
All Dent	0.3063	0.3272	0.0601

¹Tests undertaken on samples ≥ 10

Phalaris [maygrass], *Polygonum* [knotweed], *Hordeum* [little barley]), the latter of which are cariogenic and capable of initiation and progression of tooth decay (e.g., Lingström et al., 2000; Pollard, 1995). The AMTL results for Hobbs parallels the prevalence of caries (Smith & Betsinger, 2019). That is, there were significantly fewer carious teeth compared to maize-intensive samples.

The small Hobbs sample size did not permit age-at-death AMTL comparisons. Indeed, the sample was biased in favor of older individuals which, given the progressive nature of AMTL, might bias in favor of congruence with samples archaeologically identified as agriculturalist. However, when segregated by age-at-death in the larger Middle Age category, Hobbs sustains the pattern of significantly fewer AMTL for all tooth categories compared to Gray Farm and for the posterior teeth in Thompson.

The AMTL of Link/Slayden relative to Hobbs

Archaeologically, the Link/Slayden polity exhibits the material culture attributes of the Mississippian period (Bass, 1985; Dye, 2002, 2003, 2013; Lunn, 2013). The Link site (AD 1250-1400) is a civic-ceremonial mound center complex with a central plaza (Bass, 1985; Dye, 2007, 2012; Nash, 1968). Although Bass (1985) characterized Slayden as an expansion site from Link, recent ceramic analysis

suggests it is the earlier (circa AD 1050-1250) of the two (Dye, 2004; Lunn, 2013). The Link site was arguably abruptly abandoned circa AD 1400, based on the archaeological context of the Duck River Cache (Dye, 2007, 2012; Nash, 1968), a large assemblage of ritual performance objects (e.g., crown-form clubs, monolithic axes, raptor talon claw effigies) recovered from the site. The cache was arguably deliberately buried as a last act prior to (possibly warfare-related) site abandonment (Dye, 2007; Dye & King, 2007). Although there is a general congruence of AMTL between the Link/Slayden sample and the Late Woodland Hobbs sample (see Table 2), when the samples are segregated by age at death, the Mississippian Link/Slayden sample does exhibit more molar loss, particularly M1, the molar longest in occlusion. The M1 sample size drives the difference in all molars and all the dentition.

Previous assessment of the caries pattern and prevalence in the Hobbs sample indicated an overall higher prevalence of carious teeth compared to the Link/Slayden sample (although few tooth classes are significantly higher) (Smith & Betsinger, 2019). Considering the temporal and archaeological context of Link/Slayden, maize was unequivocally available as a productive cultigen. It is possible that as a shortfall-hedging strategy, food production within the Link polity included seasonal foraging and/or cultivation of native cultigens (e.g., Gremillion et al., 2008). It is also possible that the WPA era (Works Progress Administration, 1939-1943) (Dye, 2017) salvage recovery protocols at Link/Slayden biased in the direction of a temporally earlier (i.e., incipient/early maize adoption) mortuary sample. More speculative, but an aspect to consider given the archaeologically-based conclusion by Bass (1985) that the Link polity is ethnically different from Gray Farm, is regional variability in the symbolic or ritual role of maize or, in the routine maize processing and/or preparation (e.g., roasted, hominy, hoecake, and/or corn mush) which may have reduced the oral bioavailability of starch (e.g., Blitz, 1993; Briggs, 2016; Fritz & Lopincot, 2007; Katz et al., 1974; Peres, 2017; Raviele, 2011).

The AMTL differences between the Mississippian samples

In the large Middle Age cohort, the Link/Slayden site sample has significantly fewer AMTL than Gray Farm in all tooth categories except the first molars. This is unexpected given the apparently extensive temporal overlap of the sites, but may be

explained by the scenarios presented above. The Gray Farm (AD 1150-1400) site sample might be considered exemplary of maize-intensive Mississippian agriculturalization. Based on the prevalence of carious teeth by tooth type and age-at-death, the Gray Farm dental sample certainly allies with definitively maize-intensive Late Mississippian period sites from (at least) East Tennessee whereas Link/Slayden does not (Smith & Betsinger, 2019). However, in the Middle Age cohort, compared to the maize-intensive samples, the Gray Farm dental sample exhibits small lesion size and virtually no cases of pulp exposure. If the progression of tooth decay was comparatively rapid in the Gray Farm sample, the carious lesions achieving pulp penetration may have precipitated the earlier exfoliation of affected teeth, generating the high AMTL results seen here. The co-occurrence of larger lesions with fewer teeth lost antemortem can potentially be tested in the Late Mississippian period sites from East Tennessee. This faster rate could also explain the significant increase in the frequency of AMTL in the anterior teeth (I/C, PM) of Gray Farm relative to both Link/Slayden and Thompson Village (see Table 2). Caries and AMTL initially progress in the complex-crowned teeth with greater interstitial surface area (e.g., Broadbent et al., 2006; Carlos & Gittelsohn, 1965). The greater involvement of the anterior teeth in Gray Farm may signal an oral environment of advanced tooth decay (e.g., O'Sullivan & Tinanoff, 1993). Possible mitigating factors, as yet unknown, are dental issues such as LEH which might predispose the incisiform teeth to pulp-penetrating demineralization.

It can be assumed that maize was the primary cariogenic carbohydrate consumed by the community residing in the Gray Farm site. But evidently, the consumption was differential by, at least, sex (see Table 5), but not by the same sex. In the Middle Age category, Gray Farm males exhibit more AMTL than females (77.8 percent versus 41.0 percent) while the females of Link/Slayden have the higher frequency (23.5 percent versus 7.7 percent). Pregnant and nursing females are arguably more vulnerable to tooth decay and in archaeological contexts may signal reproductive stress (Ferraro and Vieira, 2010; Lukacs, 2011; Lukacs and Largaespada, 2006; Walter et al., 2016), but the statistically significant higher AMTL among females in the Link/Slayden sample ($p=0.0260$) may also suggest differential consumption of decay-causing carbohydrates (e.g., Larsen, 1983; Somerville et al., 2015). Greater maize consumption by females has been documented elsewhere in the Mississippian

world (Ambrose et al., 2003; Larsen, 1983). The higher rate of AMTL in the males of Gray Farm strongly suggests greater consumption. This is underscored by the between sample comparison by sex in the larger Middle Age category (see Tables 4 and 5). There are few differences in the prevalence of AMTL among the females for all tooth types and all teeth between Link/Slayden, Gray Farm, and Thompson Village. However, male AMTL is significantly higher in Gray Farm than either Link/Slayden or Thompson Village in the vast majority of statistical comparisons. The reason for this pattern might include the use of maize in male-predominated ritual feasting. Given the similarity of female AMTL across the site samples, it does not suggest a simple community pattern of differential maize dietary consumption. As stated earlier, Bass (1985) has argued that the Link and Gray Farm polities are ethnically different. This is based on the differences in mortuary protocols in addition to the considerable geographic distance between the two polities. This is certainly plausible given the large buffer zone between the polities in addition to an intervening polity with its own large mound center (Dye, 2004). However, the high frequency and male bias of AMTL is not repeated in the Thompson Village sample, a satellite community within the Gray Farm polity.

Based on the ceramic sequence and temporal pattern of domestic structure construction, the Gray Farm site was occupied earlier than the Thompson site with the founding of the Williams site (40HY1) (no AMTL data), part of the Gray Farm polity, straddling the two (Bass, 1985). Gray Farm, therefore, would have had a mortuary component earlier in time than Thompson Village. Both the Gray Farm and Thompson Village sites generated Late Mississippian period (post AD 1300) ceramic types which suggests both sites, as well as Link, were occupied until regional abandonment (“Vacant Quarter”) of the 14th and 15th centuries (Cobb & Butler, 2002). If maize adoption intensified with Mississippianization, as conventional wisdom suggests (Bense, 2016; Emerson et al., 2020), the Thompson AMTL frequencies for all tooth types should be greater than Gray Farm, even if not significantly so (see Tables 1 and 2). They are not. The results may be a by-product of sampling error (biased site recovery, differential preservation, and/or small sample sizes) or reflect local socioeconomic issues. Thompson Village was a satellite community within the Gray Farm polity and may not have engaged in, or had access to, the same civic-ceremonial activities of a mound center.

That is, if indeed the male AMTL bias at Gray Farm is reflective of non-dietary maize consumption. But the circumstance preceding regional abandonment may have also been a factor in the apparent better oral health of the temporally later Thompson Village sample.

Climate volatility

Maize cultivation may have been affected by a series of droughts that occurred in the Southeast beginning circa AD 1100 (Aharon et al., 2012; Benson et al., 2009) and in Tennessee in the Middle Cumberland River culture area (Figure 1) circa AD 1288-1388 (Meeks & Anderson, 2013). Agricultural shortfall may have episodically reduced overall carbohydrate consumption potentially causing nutritional stress or dietary shifts to foraged resources (e.g., Scopa Kelso, 2018). This may have been reflective of, or exacerbated by, the onset after AD 1300 of the climate phenomenon of the Little Ice Age (Bird et al., 2017; Naftz et al., 1996; Stahle & Cleaveland, 1994; Wilson, 2017). Nutritional stress may not be documentable,¹ but dietary shifts are certainly a plausible scenario based on (for example) the botanical evidence from the Illinois River Valley of the apparent juxtaposition of maize adoption and reduction in arboreal seed crop harvest (VanDerwarker et al., 2017). Mast and fleshy fruits may have been fall-back options in times of carbohydrate harvest insufficiency. This may be what was occurring in the Link polity and in the later occupation within the Gray Farm polity.

Political instability

Perhaps synergistically related to climate change, there is a wide-spread decline of polities in the late Mississippian period (after ~ AD 1300) (Anderson, 1994, 1996; Benson, et al., 2009; Bird et al., 2017; Wilson, 2017). Although particular mound centers as loci of civic and ceremonial functions did fluctuate over time and space (e.g., Anderson, 1996; Beck, 2003; Benson et al., 2009; Blitz, 1999; Wilson, 2017), in the Middle Cumberland River Valley to the East of the KLR samples, political destabilization occurs circa AD 1325 (Dye, 2004; Krus & Cobb, 2015; Vidoli, 2012) just prior to the regional abandonment. This is archaeologically manifested by the reduction in centralized authority and increase in settlement fortification (palisades) (Krus & Cobb, 2015). Warfare is argued to escalate during this time period and, in the KLR, the apparent abrupt abandonment of the Link site (i.e., burial of the Duck River Cache) has been attributed to imminent intergroup violence (Dye, 2004; Dye &

King, 2007). Bass argued that it is only in the later occupation sequence of the Mississippian sites that palisades are firmly documented (1985:216). Corroboratively, based on a shift in mortuary patterning, Bass argues the changes “indicate that the sociopolitical structure of these polities in the late Middle Mississippian Period... had altered to ones in which elite status or high rank was truncated and invested in and restricted to only one rank, an individual kin group or family which produced the chiefly line (1985:195).” Perhaps the absence of male-focused decay-inducing carbohydrate consumption in the Thompson Village sample compared to Gray Farm is reflective of this political decline with an overall reduction in AMTL in Thompson is suggestive of agricultural shortfall.

Conclusions

The trend towards maize intensification co-occurring with sociopolitical complexity is a general pattern within the Mississippian period (AD 1000-1550) in what is now the lower Midwest and much of the southeastern United States. However, the transition varies temporally and unevenly between sociopolitical and/or ecological contexts. Mississippianization abruptly ended in northern west-central Tennessee by AD 1450. Occupation of the area was abandoned as part of a much broader inexplicable depopulation of the lower Ohio River Valley inclusive of the lower Tennessee River Valley. The dearth of archaeological context for the Kentucky Lake Reservoir region of northern west-central Tennessee contributes to the conundrum of the apparent abandonment. Much basic subsistence information is archaeologically unclear but can be remediated by certain lines of bioarchaeological inquiry.

Although AMTL has a complex etiology, it has been an effective proxy for oral decay which has been bioarchaeologically observed to vary by subsistence strategy and the dietary dependence on starchy cultigens. The AMTL observed in the Kentucky Lake Reservoir yielded three patterns which segregate the site samples of Hobbs, Link/Slayden, Gray Farm and Thompson Village by temporal period and geographic distribution. The first pattern is the retention of a general Late Woodland (AD 600-900) AMTL loss (Hobbs site sample) in the Mississippian period Link polity (Link/Slayden site sample). The pattern does not indicate a maize-intensive subsistence strategy. It may reflect a strategically mitigated economic adjustment to include foraged resources consequential to demonstrable climate volatility (~AD 1400-1700). This subsistence strategy is similar to what has been archaeo-

logically reconstructed for the pre-maize Late Woodland period. However, this pattern may also reflect possible salvage archaeology recovery bias inadvertently favoring temporally earlier Link/Slayden burials, which may indeed have practiced a Late Woodland subsistence economy. Less likely, but culturally plausible given that Bass argued ethnic differences between the Link and Gray Farm polities, there may have been polity-variable food preparation techniques which reduce the opportunities for dental decay in the oral environment.

The second pattern is a regional difference in Mississippian period maize adoption. The caries data as well as AMTL of the Gray Farm site sample is consistent with maize-intensive agriculturalization and in contrast with the lower prevalence results of the Link polity. Indeed, AMTL is higher than the Thompson Village site sample, a temporally later-founded satellite community of the Gray Farm polity. It is possible that Gray Farm reflects a temporal window of successful maize overcrop which then declined just prior to regional abandonment. Alternatively, maize may have been siphoned from satellite communities to the Gray Farm site as it was the administrative capitol for the polity.

The third pattern is the higher overall prevalence of AMTL in the males of the Gray Farm sample. This contrasts with the Link/Slayden and Thompson Village samples which indicated more AMTL among the females. Interpretively important, the prevalence of AMTL is not significantly different between the females in the three Mississippian period site samples. Therefore, the Gray Farm male pattern may reflect specific male-focused civic-ceremonial roles which involved maize consumption that may have declined in the later Thompson Village context paralleling documented sociopolitical decline. Alternatively, the civic-ceremonial activity may not have been undertaken in satellite communities.

The patterns apparent in this study may be a consequence of sampling error from possible excavation bias as well as small sample sizes available for assessment. However, the patterns may indeed reflect the political landscape as well as the climate, ecological, and sociopolitical challenges of the thirteenth century prior to regional abandonment. Hopefully, more health status data can be marshaled to clarify the regional and temporal patterns.

¹Access to the osteological collections has now been terminated pending reburial

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The Prevalence and Possible Causes of Third Molar Agenesis in Post-Medieval Chichester

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ABSTRACT Third molar agenesis is a dental anomaly that occurs in approximately 25% of people worldwide and results in the complete absence of one or more of the third molars. A rise in the prevalence of congenitally absent third molars has been noted in modern clinical data, and it has been proposed as an evolutionary step in the dental reduction of the human dentition. Whilst research has been conducted in extant cohorts, relatively little has been published on third molar agenesis in archaeological assemblages. A post-medieval assemblage (AD 1550-1850), from Chichester, United Kingdom, was visually and radiographically analyzed to determine the prevalence of this anomaly. Mesiodistal and buccolingual measurements were taken on retained third molars to determine if there was an association between agenesis and reduced tooth size. Prevalence of agenesis was found to be comparatively high (42.7%) relative to contemporary and modern European samples, and tooth size reduction was documented. Consequently, it can be said that high rates of third molar agenesis are not simply a modern clinical phenomenon, as many prevalence rates in recent populations are lower. Temporal and regional patterns are, therefore, unclear. In order to better understand the trajectory and evolution of this anomaly, more archaeological assemblages ought to be examined.

Third molars are the last permanent tooth to develop, the most variable in size and morphology, and are also the most commonly congenitally absent tooth. According to Sujon et al. (2016), approximately 50% of modern (20th century onwards) human third molars are anomalous, either unerupted, partially erupted or absent. Congenital absence is known as dental agenesis, which results from a developmental anomaly in the dental epithelium or the underlying mesenchyme (Bhutta et al., 2014). Grewal's (1962) analysis of agenesis in the third molars of mice revealed that congenitally absent teeth begin as tooth germs but growth formation ceases at or before the cap stage of development, at which point the tooth germ resorbs. It may occur unilaterally, bilaterally, in combinations of three teeth, or completely, with all four absent. In their meta-analysis of modern data, Carter & Worthington (2015) found that 22.63% of people worldwide have some degree of third molar agenesis. The samples included in their analysis were gathered from various ethnicities and socioeconomic groups, with prevalence ranging from 5.32% - 56.0%.

The exact etiology of third molar agenesis is unknown, but a genetic component is well established (Carter & Worthington, 2015; Frazier-

Bowers et al., 2002), and it is thought that delayed growth or a lack of space in the jaw may result in epigenetic absence (Anderson & Popovich, 1981; Kajii et al., 2004; Suri et al., 2004). Disease and nutrition have also been shown to affect the eruption and formation of third molars (Anderson & Popovich, 1981; Garn et al., 1961; Suri et al., 2004), adding to the already complex etiology of this trait. Grüneberg's (1951) experiments with mice indicate that agenesis is the phenotypic result of the extreme end of a size continuum. Mice with absent third molars more often displayed small and variable remaining third molars, and as the dental lamina became smaller, the more likely growth and tooth formation were to cease development and resorb.

It has frequently been reported that third molar agenesis occurs more often in modern populations than in the past (Alam et al., 2014; Kajii et al., 2004),

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with some claiming the third molar is likely to disappear from the human dentition altogether (Raloti et al., 2013). A general reduction in tooth size has taken place throughout hominid evolution, with a rapid reduction in size occurring in the Upper Palaeolithic (50,000 – 10,000 YA) and again in the early Holocene (10,000 – 8,000 YA) (Hillson, 2005). While the impetus behind these changes is unclear, many associate the diminution of teeth with the atrophy of the masticatory complex due to increasingly soft diets, advancement of food processing techniques, and the diminished use of the mouth as a tool (Brace et al., 1987; Carlson & Van Gerven, 1977). The agriculturalization that took hold in the early Holocene is thought to have furthered this trend in dental reduction, leading to what may be a further evolutionary step in dental reduction, the congenital loss of the third molar (Sengupta et al., 1999).

In this study, the past prevalence of third molar agenesis is examined in a post-medieval assemblage from Chichester, providing new insights into patterns in agenesis and the role of dental size reduction and its occurrence. This investigation will also test whether this anomaly represents a recent secular trend and will add to our limited understanding of third molar agenesis in archaeological assemblages.

Materials

The skeletal assemblage under analysis comes from The Litten cemetery at Eastgate Square in Chichester, West Sussex. Chichester has a long history of occupation, with evidence of Roman defensive ditches found at The Litten cemetery (Hart, 2012), and continuous settlements recorded from the Anglo-Saxon period onwards (Dhaliwal et al., 2019). In the later medieval period (14th century), Chichester flourished as one of the more important ports in the country, with dominance over the wool trade and a strong agricultural economy (Hart, 2012). A grain-based economy continued in the post-medieval period (1550-1850), although the town's import declined as the wool trade waned. Chichester also appears to have experienced a population surge between 1670-1801, with the number of inhabitants doubling from 2,400 to 4,752 due to increasing trade with London and other domestic markets (Dhaliwal et al., 2019). This assemblage was excavated from a cemetery that seems to have been established in the 12th century with the construction of the chapel and altar of St. Michael, which are no longer standing. Interment officially ceased in 1859, although family plots remained

active until the end of the 19th century (Hart, 2012). The vast majority (66%) of human remains recovered date to the post-medieval period and represent a range of social strata, with the bulk of individuals (1,365), both from the medieval and post-medieval periods, buried in the simple shroud style (Rando, 2016). In the present study, only post-medieval skeletons were analyzed for third molar agenesis.

Excavation of the site began in advance of its redevelopment, with 93 burials excavated by Pre-Construct Archaeology Ltd. (PCA) in 2005 and 2006, and the remaining 1637 skeletons excavated by Archaeology South-East (ASE) between August of 2011 and January of 2012 (Hart, 2012). Four hundred and thirty skeletons from these excavations that have been retained for analysis at the University College London Institute of Archaeology due to high preservation levels or presence of pathological conditions. Of these skeletons, 311 matched the preservation levels required (alveolar bone and dentition present) to warrant examination and only 116 had a minimal level of antemortem tooth loss that allowed for inclusion in this study. Of these 116 skeletons, 89 had complete dentitions without any data missing. The remaining skeletons had missing data in either one (n=18) or two (n=9) of the dental quadrants. These skeletons were incorporated into the analysis when the lack of data did not affect the results (see below). In total, 46 males, 36 females and 34 skeletons of indeterminate sex were analyzed, comprising 83 adults and 33 subadults.

Methods

Selection, Visual Assessment, Aging and Sex Estimation

Skeletons were carefully selected according to a set of criteria designed to minimize the effects of antemortem tooth loss. Skeletons with fewer than four teeth lost antemortem were included in the analysis. In addition, only skeletons of a maximum age of a pubic symphysis phase 4 (Brooks & Suchey, 1990) and a auricular surface phase 4 (Lovejoy et al., 1985) were incorporated in order to mitigate a greater risk for antemortem tooth loss with increasing age. The age at which third molars initiate crown formation varies more than any other tooth (AlQahtani et al., 2010). AlQahtani et al. (2010) reported a median dental age of 8.5 years for the initiation of crown development, and Ubelaker (1989) provides a dental age of 10 years +/- 30 months for the initiation of crown mineralisation in both the maxillary and mandibular third molars. In this

study, only subadults with a minimum dental age of 12.5 years were included, following the dental age categories established by AlQahtani et al. (2010). According to Garn et al. (1963), 99% of third molars begin their cusp mineralisation by the age of 14 years. However, due to the relatively small number of individuals that fit the criteria for analysis in this assemblage, the dental development stage of 12.5 years, defined by AlQahtani et al. (2010), was selected as a minimum in order to maximize the available data.

Mandibles and maxillae were visually observed for the presence or absence of third molars using the following criteria to determine a lack of agenesis:

- The tooth is in the alveolus.
- The tooth was lost post-mortem, with a well-defined alveolus present.
- The tooth was lost antemortem but the alveolus is still in the process of resorbing, and no other pathological or taphonomic process could be responsible for the feature.
- The second molar in the particular quadrant has an identifiable distal approximal wear facet (indicating it had once been in contact with a third molar).
- An unerupted or impacted third molar is visible through radiographic analysis.

Third molar agenesis was diagnosed based on the absence of these criteria. If the maxillae or mandible met these requirements it was x-rayed to ensure that the third molar was not impacted, developing within the crypt, or had failed to erupt. If radiographic analysis did not reveal a third molar it was therefore determined to be congenitally absent. Impaction was assessed based on abnormal angulation of the tooth in the alveolus or crypt (after Raloti et al., 2013).

Sex determination was used to examine differences in size or agenesis prevalence. This was based on a combined assessment of pelvic morphological traits (after Phenice, 1969), including the greater sciatic notch and composite arch (after Bruzek, 2002), as well as measurements of the proximal humeral and femoral heads (maximum diameters after Bass, 1995) and an assessment of the sexually dimorphic features of the skull (after Ubelaker, 1989). The latter two methods were only employed if the features of the pelvis were slightly ambiguous, or if the pelvic bones were missing or too poorly preserved. The dimorphic traits of the pelvis are generally regarded to be more reliable indicators of sex than features of the skull (Bruzek,

2002). The skeletons were assigned sex of male, possible male, indeterminate, possible female, and female. However, due to the small size of the sample possible males and possible females were collated with the respective sex.

Measurements

Measurements of third molars were taken in accordance with the cervical method developed by Hillson et al. (2005) using specialized Paleo-Tech calipers (also developed by Hillson and colleagues, 2005). Cervical measurements are usually not affected by the level of crown wear, and as individuals with an advanced age were not included, tooth wear on third molars was generally not an issue. Individuals with carious lesions affecting the crown could also be included.

Mesiodistal measurements were taken by placing the tips of the calipers on the mesial and distal enamel, just occlusal to the cervico-enamel junction (CEJ) and at the midpoint between the buccal and lingual sides of the tooth (see Hillson et al., 2005). Buccolingual measurements were also taken on the buccal and lingual surface at the midpoint of the enamel, slightly occlusal of the CEJ, between the mesial and distal surfaces of the tooth. It is important to note that these measurements were taken at the midpoints and are not maximum measurements, however, if an enamel extension was present at the midpoint, the tip of the caliper was placed at whichever side of the extension provided the maximum measurement for the midpoint, following Hillson et al. (2005). The tips of the calipers that meet end-to-end were used with loose teeth and for the buccolingual measurements of teeth in the alveoli whenever possible. The caliper tips that meet at an angle were most useful for the mesiodistal measurements of teeth fixed in alveoli, and for the upper third molars, this measurement was approached lingually as these teeth tend to taper lingually, thereby ensuring a precise measurement.

Analysis

Inter- and intraobserver error tests were performed to ensure reproducibility and accuracy of results. Third molars, especially those in the upper dentition, have a variable morphology and can be difficult to measure due to their irregular and oblong crown morphology (Hillson et al., 2005). However, by ensuring the measurements are taken at the midpoint on the CEJ through careful and methodical application of technique, it is possible to achieve consistent results. Two observers unfamiliar with measurement technique of Hillson et al. (2005) took mesiodistal and buccolingual measure-

ments on the same set of ten third molars (five upper and five lower) following the system described above. The values were then compared using SPSS 21 software to determine mean difference and 95% confidence interval (CI). The buccolingual measurements with Observer 2 differed by as much as 0.5 mm, with one measurement revealing a 0.88 mm difference. However, the measurements of Observer 1 closely resembled those of the researcher and therefore these differences were not explored further. In addition, Observer 1 frequently reported slightly lower measurements than those of the researcher, most probably due to measurements taken on the CEJ or on the root surface, rather than on the enamel slightly occlusal to the CEJ. Intra-observer tests for mesiodistal measurements (MD=-0.098, SD=0.13481) remained close to ± 0.2 mm, a range ideal for tooth measurements, but the range for buccolingual measurements was slightly higher (MD=0.027, SD=0.21103). To correct for this, a larger sample size should be used in future studies in order to determine if the degree of error is acceptable.

SPSS 21 Statistics software was used to assess the prevalence of third molar agenesis in the Chichester assemblage and analyze patterns within the sample. The data were divided into three groups: no data missing, one quadrant missing,

and two quadrants missing. It is ideal to collect data on complete remains, but information was recorded on all three groups in order to gain as much data as possible.

T-tests were performed to determine whether size differences exist in the mesiodistal and buccolingual measurements of third molars between those with and without third molar agenesis. Difference in sizes between males and females were also compared statistically to determine the impact of sexual dimorphism on the results. Following this test, males and females were analyzed separately for size differences in third molars. T-tests were also used to determine if significant differences in size existed between the various distributions and patterns of third molar agenesis.

Results

The total prevalence of third molar agenesis in adult and subadult skeletons in the Chichester cohort with data present for all dental quadrants is 42.7% (n=38/89). When incorporating those with data missing from one quadrant the prevalence falls to 40.2% (n=43/107) and is slightly higher when including those with missing data in two quadrants at 41.4% (n=48/116) (Table 1). Subadults with complete data yielded a prevalence of 45.8% (n=11/24), and this remained consistent at 45.5%

Table 1. Agenesis prevalence recorded for all skeletons, separated into groups defined on the inclusion of missing data.

	Agenesis	N	Percent	95% CI
Skeletons with no missing data	Absent	51	57.3	± 10.28
	Present	38	42.7	± 10.28
	Total	89	100	
Including those with data missing from one dental quadrant*	Absent	64	59.8	± 9.29
	Present	43	40.2	± 9.29
	Total	107	100	
Including those with data missing from one and two dental quadrants*	Absent	68	58.6	± 8.96
	Present	48	41.4	± 8.96
	Total	116	100	

*Due to the small number of individuals in the assemblage, the inclusion of individuals with data missing was explored. No significant differences were found between prevalence in any of the groups, and it is therefore acceptable to use individuals with data missing as representative of the assemblage.

Table 2. Agenesis prevalence recorded for subadult skeletons, separated into groups defined on the inclusion of missing data.

	Agenesis	N	Percent	95% CI
Skeletons with no missing data	Absent	13	54.2	± 19.93
	Present	11	45.8	± 19.93
	Total	24	100	
Including those with data missing from one dental quadrant	Absent	17	56.7	± 17.73
	Present	13	43.3	± 17.73
	Total	30	100	
Including those with data missing from one and two dental quadrants	Absent	18	58.6	± 17.60
	Present	15	45.5	± 17.60
	Total	33	100	

(n=15/33) when subadult individuals with data missing were included (Table 2). Subadult prevalence is higher, but not significantly greater, $\chi^2 (1, n=89) = 0.13, p = 0.72$, than the 41.5% prevalence among adults with complete data in this assemblage (n=27/65) (Table 3). When adults with one (n=30/77) and two (n=33/83) dental quadrants of data missing were included, this lowered the prevalence of agenesis to 39.0% and 39.8%, respectively, although the difference between adult and subadult prevalence remained statistically non-significant, $\chi^2 (1, n=107) = 0.17, p = 0.68$, and

$\chi^2 (1, n=116) = 0.32, p = 0.57$.

Males in this assemblage show a 38.9% prevalence of agenesis (n=14/36), whereas females express a prevalence of 39.3% of third molar agenesis (n=11/28). Third molar agenesis in the maxilla was less common than third molar agenesis in the mandible, and the right side was more frequently affected by agenesis than the left (Table 4). The number of teeth missing followed a pattern in frequency of two, one, three, four, with agenesis of two molars occurring almost twice as frequent as one, and the absence of three and four was less

Table 3. Agenesis prevalence recorded for adult skeletons, separated into groups defined on the inclusion of missing data.

	Agenesis	N	Percent	95% CI
Skeletons with no missing data	Absent	38	58.5	± 11.98
	Present	27	41.5	± 11.98
	Total	65	100	
Including those with data missing from one dental quadrant	Absent	47	61.0	± 10.89
	Present	30	39.0	± 10.89
	Total	77	100	
Including those with data missing from one and two dental quadrants	Absent	50	60.2	± 10.53
	Present	33	39.8	± 10.53
	Total	83	100	

Table 4. The distribution of third molar agenesis between males, females, and the total assemblage, on the right and left sides and in the maxilla and mandible.

	Males			Females			Total (Including Indeterminate Sex)		
	Right	Left	Total	Right	Left	Total	Right	Left	Total
Maxilla	9	6	15	6	4	10	21	17	38 (46%)
Mandible	7	8	15	7	7	14	23	22	45 (54%)
Total	16	14	30	13	11	24	44	39	83

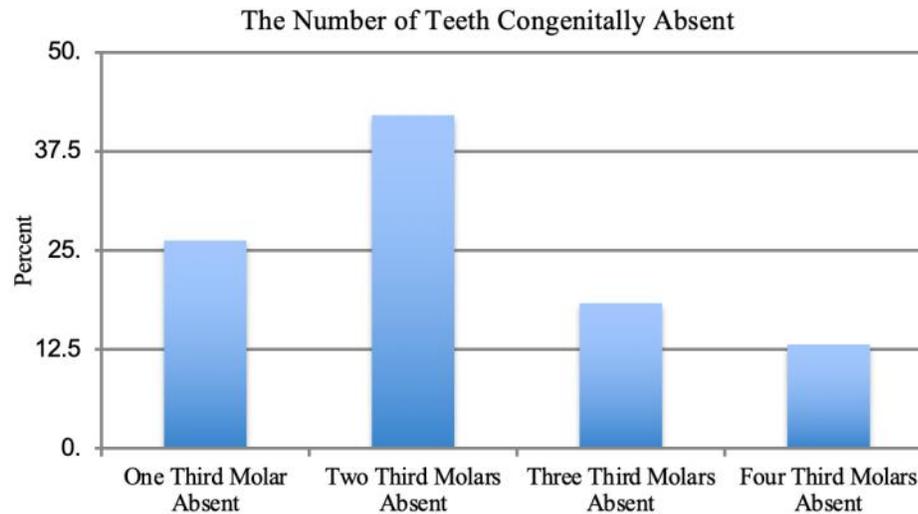


Figure 1. The frequencies in the number of third molars congenitally absent in individuals with agenesis and all data present in this assemblage. Two third molars absent occur much more often in this assemblage than one third molar absent, and three and four are least common.



Figure 2. A mandible demonstrating bilateral agenesis of the third molars from the Chichester assemblage (Author's own 2017).

common (Figure 1). Bilateral agenesis (Figure 2) occurred more frequently than unilateral, or both unilateral and bilateral agenesis in one dentition, for example if unilateral agenesis occurred in the upper arcade and bilateral agenesis in the lower

Table 5. Laterality of third molar agenesis in the Chichester assemblage. Bilateral agenesis occurs in over half of those with third molar agenesis.

	n	Percent
Unilateral	11	28.9
Bilateral	20	52.6
Both	7	18.4

arcade (Table 5).

Significant differences in tooth size were found between male and female third molars in this assemblage. The buccolingual dimensions of the ULM3, URM3, LRM3 and the mesiodistal dimensions of URM3, LLM3 and LRM3 (Table 6) produced significant differences between sexes, with mean male measurements being larger.

Two significant differences ($p < 0.05$) were found in the buccolingual dimensions of the ULM3 ($p = 0.048$, 95% CI [-1.04, -.005]) and URM3 ($p = 0.009$, 95% CI [-1.15, -.17]), in which those individuals with agenesis showed reduced dimensions compared to individuals without third molar agenesis (Table 7). When separated by sex, only males with agenesis retained a significant reduction in

size ($p < 0.05$) in the ULM3 buccolingual dimension (Table 8). Third molars visibly reduced in size and complexity, known as "vestigial third molars" as described by Nanda (1954), were noted in seven skeletons that also displayed third molar agenesis.

T-tests did not reveal significant differences in the mesiodistal or buccolingual dimensions of third molars between individuals with one or three third molars congenitally absent, bilateral maxillary or mandibular agenesis, and those without agenesis. There was a significant difference ($p > 0.05$) in the mesiodistal dimensions of LLM3 in those with three third molars missing and bilateral maxillary agenesis; however, the 95% CI for those with agenesis of three molars was not significant due to the small number of individuals with the measurements (Table 9).

Discussion

To date, the literature on third molar agenesis in past assemblages is extremely sparse, and while it is at times included in the skeletal analysis (Iseri & Uzel, 1993; Munson, 2001; Öhrström et al., 2015; Lieverse et al., 2014), it is not extensively discussed. Only a few studies (Castro, 1989; Henriksson et al., 2019; Nelsen et al., 2001; Sengupta et al., 1999; Voldanović, 2012) record assemblage-wide data on third molar agenesis as part of broader analyses of dental anomalies.

In this study, 116 post-medieval skeletons from The Litten Cemetery in Chichester were analyzed to determine the past prevalence of third molar agenesis and to test any association with reduction in molar size. 42.7% of adult and subadult skeletons with complete data ($n=51/89$) demonstrated M3 agenesis. When those with one or two dental quadrants missing are included (to test a larger dataset), this frequency lowered to 40.2% and 41.4%, respectively (see Table 1). While this difference may be attributed to the inclusion of more data, it is also possible that the difference is the result of missing data resulting in undiagnosed agenesis. However, the difference is not statistically significant, and it is therefore acceptable to include the individuals with incomplete data as members of the assemblage. The inclusion of data groups with missing dental quadrants was also explored for subadults and adults separately, and no significant differences in the prevalence of agenesis was found between these groups. In this assemblage, 45.5% ($n=18/33$) of subadults have third molar agenesis. This indicates that ante-mortem tooth loss is less likely to have an effect on the prevalence of agenesis as subadults are exposed for less time to the pathological processes

Table 6. Differences between the size of male and female third molars. Male third molars are significantly larger than female third molars (in bold).

	Sex	n	Mean	Sig. (2-tailed)*	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
ULM3 Buccolingual	Male	24	10.3717	0.005	0.82461	0.27705	0.26423	1.38499
	Female	17	9.5471					
URM3 Buccolingual	Male	17	10.2271	0.054	0.51984	0.26034	-0.00984	1.04951
	Female	18	9.7072					
LLM3 Buccolingual	Male	23	8.647	0.064	0.55629	0.29138	-0.03465	1.14723
	Female	15	8.0907					
LRM3 Buccolingual	Male	24	8.5521	0.015	0.60708	0.23828	0.1255	1.08866
	Female	18	7.945					
ULM3 Mesiodistal	Male	24	6.7313	0.164	0.27596	0.19442	-0.1173	0.66921
	Female	17	6.4553					
URM3 Mesiodistal	Male	17	6.7335	0.028	0.49353	0.2147	0.05672	0.93034
	Female	18	6.24					
LLM3 Mesiodistal	Male	24	8.8667	0.024	0.57042	0.24262	0.07926	1.06157
	Female	16	8.2963					
LRM3 Mesiodistal	Male	24	9.0913	0.017	0.64958	0.26195	0.12016	1.17901
	Female	18	8.4417					

Table 7. T-test results of size comparison between those with M3 agenesis and those without M3 agenesis.

	Agenesis	n	Mean	Sig. (2-tailed)*	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
ULM3 Buccolingual	Present	14	9.6021	0.048	-0.52331	0.25882	-1.04179	-0.00484
	Absent	44	10.1255					
URM3 Buccolingual	Present	14	9.5486	0.009	-0.66248	0.24341	-1.15138	-0.17358
	Absent	38	10.2111					
LLM3 Buccolingual	Present	9	8.0111	0.129	-0.46178	0.29957	-1.06291	0.13935
	Absent	45	8.4729					
LRM3 Buccolingual	Present	10	8.1040	0.461	-0.20722	0.279	-0.76591	0.35146
	Absent	49	8.3112					
ULM3 Mesiodistal	Present	14	6.4464	0.2	-0.25221	0.19454	-0.64191	0.1375
	Absent	44	6.6986					
URM3 Mesiodistal	Present	14	6.6021	0.817	-0.05207	0.22443	-0.50285	0.39872
	Absent	38	6.6542					
LLM3 Mesiodistal	Present	9	8.3522	0.232	-0.31674	0.26231	-0.84242	0.20895
	Absent	48	8.6690					
LRM3 Mesiodistal	Present	10	8.8960	0.68	0.12682	0.30555	-0.48505	0.73868
	Absent	49	8.7692					

*Significant differences found in the ULM3 buccolingual and the URM3 buccolingual measurements (in bold). All skeletons were used in order to increase the number of individuals analyzed. Separate analyses revealed similar results and therefore data groups were collated. Equal variances are assumed.

Table 8. T-test results of size comparison between males with M3 agenesis and males without M3 agenesis. Only the ULM3 shows significant differences in size (in bold). Equal variances are assumed.

	Agenesis	n	Mean	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
ULM3 Buccolingual	Present	5	8.7980	0.022	-1.15123	0.45548	-2.11680	-0.18566
	Absent	13	9.9492					
URM3 Buccolingual	Present	7	9.2929	0.118	-.62631	0.38043	-1.42894	0.17632
	Absent	12	9.9192					
LLM3 Buccolingual	Present	2	7.4500	0.209	-.81200	0.61855	-2.13040	0.50640
	Absent	15	8.2620					
LRM3 Buccolingual	Present	3	8.0800	0.697	0.16118	0.40688	-0.69365	1.01600
	Absent	17	7.9188					
ULM3 Mesiodistal	Present	5	6.3560	0.779	-0.11785	0.41199	-0.99123	0.75554
	Absent	13	6.4738					
URM3 Mesiodistal	Present	7	6.1900	0.882	-0.05417	0.35974	-.081315	0.70481
	Absent	12	6.2442					
LLM3 Mesiodistal	Present	2	8.3800	0.854	0.08313	0.44391	-0.85792	1.02417
	Absent	16	8.2969					
LRM3 Mesiodistal	Present	3	8.8800	0.438	0.41118	0.51857	-0.67831	1.50066
	Absent	17	8.4688					

Table 9. Example of analysis in size patterns between the distributions of agenesis in the dentition. The small number of individuals with measurements available for each tooth dimension in each group made it impossible to determine significant relationships between the variables.

Measurement	Type	n	Mean	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
LLM3 Mesiodistal	Bilateral Maxillary Agenesia	4	8.905	0.02	1.15	0.29535	2.00465
	Agnesia of Three Teeth	2	7.755	0.02	1.15	-3.3154	0.7904
LLM3 Buccolingual	Bilateral Maxillary Agenesia	4	8.57	0.149	0.795	-0.44409	2.03409
	Agnesia of Three Teeth	2	7.775	0.149	0.795	-1.77698	2.12698
LRM3 Mesiodistal	Bilateral Maxillary Agenesia	4	8.907 5	0.145	-1.2625	-3.3154	0.7904
	Agnesia of Three Teeth	1	10.17	0.145	-1.2625	-0.44409	2.03409
LRM3 Buccolingual	Bilateral Maxillary Agenesia	4	8.575	0.794	0.175	-1.77698	2.12698
	Agnesia of Three Teeth	1	8.4	0.794	0.175	-0.44409	2.03409

that stimulate antemortem tooth loss.

The prevalence of 42.7% in this assemblage is significantly higher ($p < .05$) than those reported for British clinical samples in which data was gathered from dental radiographs. Shinn (1976) found that 12.72% ($n=318/2500$) of patients referred to an orthodontic hospital in Southampton had third molar agenesis, whereas Gravely (1965) found that 25.9% ($n=21/81$) of patients exhibited third molar agenesis. From the Bristol Dental Hospital, Sengupta et al. (1999) found that 22% ($n=22/100$) of people were found to have third molar agenesis. In other groups of European ancestry prevalences of 28.2% (Krekeler et al., 1974), 28.5% (Trondle, 1973), 29.3% (Weise & Bruntzsch, 1965) and 33% (Elomaa & Elomaa, 1973) have been reported. In European-derived North American samples, frequencies of 25.7% (Keene, 1965), 22.3% (Thompson et al., 1974) and 31.5% (Harris & Clark, 2008) have been observed. The frequency of M3 agenesis found in this study is comparable to the 44% prevalence reported in extant Asian and Native North American populations (Carter & Worthington, 2015). Clinical accounts of third molar agenesis in Asia appear to be higher than most European groups: 30% in a Malaysian Malay population (Alam et al., 2014), 33% in a Chinese Malaysian population (Alam et al., 2014), 50% in Nepal (Upadhyaya et al., 2012), 32.3% in Japan (Endo et al., 2015) and 38.4% in Bangladesh (Sujon et al., 1984). Ren & Kumar (2014) also report a prevalence of agenesis of 48% of males and 64% of females from southern India, but only 25 individuals of each sex were analyzed, and therefore the small sample size may not be representative.

Prevalence rates in archaeological assemblages are also extremely variable, in addition to the way in which data are collected and reported. In the present study, third molar agenesis is reported per individual, but due to preservation requirements or research questions, other studies separate data by the upper and lower dental arcade, the dental quadrant, or as an overall tooth count, making statistical comparisons with such research difficult. The Late Antique ($n=117$) and early medieval ($n=245$) assemblages from eastern Croatia examined by Vodanović (2012) produced third molar agenesis prevalences of 30.21% and 15.64% respectively, with the change in frequency attributed to population replacement in the early medieval period. Radiographic assessment was not performed, and the frequency of third molar agenesis is presented separately for the upper and lower arcade, rather than for each individual. Without radio-

graphic assessment unerupted third molars may be mistaken for agenesis, creating the potential for a slightly higher prevalence than may otherwise be reported.

Castro (1989) found comparatively low prevalences of 7.6% in Gran Canaria, 10.8% in Tenerife, and 9.4% in the Canary Islands in archaeological assemblages dating from the 1st century B.C. – 14th century A.D. In this study, a total of 1,790 maxillae and 1,920 mandibles were visually analyzed for third molar agenesis. Due to the majority of mandibles having been separated from their skulls, Castro (1989) calculated the frequency of agenesis separately between the upper and lower dental arches. The author divided the total number of congenitally absent third molars by the total number of third molars that would be expected if third molar agenesis was absent in each individual to determine prevalence.

Nelsen et al. (2001) found third molar agenesis to be prevalent in 23.5% of individuals ($n=12/51$) from the Iron Age cemetery of Noen U-Loke, in Thailand. The authors did not use radiographic analysis. This prevalence is significantly lower, $\chi^2(1, n=140) = 5.2, p = 0.023$, than the prevalence of 42.7% recorded in the present study. The Noen U-Loke assemblage also has a high prevalence of lateral incisor agenesis, with 79% of individuals missing at least one lateral incisor. The authors hypothesize that endogamy and isolation likely factored in to the high prevalence of lateral incisor hypodontia. However, this does not appear to have a marked effect on the prevalence of third molar agenesis, as the modern worldwide average described by Carter & Worthington (2015) is 22%. In order to understand if endogamy and isolation affected the prevalence of third molar agenesis in this assemblage, analysis of other archaeological assemblages from the time period and the area with more genetic diversity would be necessary in order to confidently assess typical third molar agenesis prevalence.

The methods of archaeological analysis employed by Henriksson et al. (2019) in their analysis of medieval and modern Norwegian assemblages align closely with the present study. The authors used both radiographic and visual analysis to determine 36 of 130 medieval skeletons had third molar agenesis. A decrease in third molar agenesis from medieval (27.7%) to modern times (17.2%) was detected. The frequency of third molar agenesis found in the present study (42.7%) is significantly higher, $\chi^2(1, n=219) = 5.3, p = 0.021$, than the frequency recorded in the medieval Norwegian

assemblage. Henriksson et al. (2019) proposes that the higher rate of third molar agenesis in the medieval assemblage compared with the modern Norwegian sample may be due to the biological relationships present in the cemetery of St. Olav, as opposed to the unrelated sample of modern Norwegian 15 year olds. A strong genetic influence could also be present in the Chichester assemblage, and may be a primary factor in the relatively high frequency of 42.7%, although further analysis is required to explore this.

Sengupta et al.'s (1999) analysis of Victorian skeletons from the Spitalfields cemetery in London represents the closest archaeological comparison to the present study, both temporally and geographically. The frequency of third molar agenesis was determined by assessing each dental quadrant as a separate specimen, and both visual and radiographic analysis were used. The prevalence of third molar agenesis presented here (42.7%) is significantly higher, $\chi^2(1, n=140) = 5.2, p = 0.023$, than the prevalence of 23.5% recorded at Spitalfields ($n=12/51$), and is much greater than the frequency of 14% observed in the medieval burials at St. Peter's Church, Barton-on-Humber, also examined by Sengupta et al. (1999). Due to the proximity and temporal overlap with the Chichester assemblage, it is likely familial genetic predispositions towards third molar agenesis were present in the Chichester assemblage.

In addition to a genetic component, diet could have factored in to the rates of third molar agenesis in Chichester. In post-medieval Britain, the diet was heavily impacted by the industrial revolution of the 17th century, with food becoming sweeter and increasingly processed (Rando et al., 2014). Refined flour and white bread became popular, and in 18th - early 19th century London, potatoes, bread, and tea were a dietary staple (Mant, 2015). Increasingly processed diets reduce dental wear on the occlusal and interproximal surfaces of teeth. As teeth wear down more space becomes available in the jaw due to the mesial drift of teeth, and without this wear, dental crowding and impaction are more likely to occur (Sengupta et al., 1999). Rando et al. (2014) compared the mandibular morphology of medieval and post-medieval Londoners and found a decrease in the robusticity of bone associated with masticatory muscles in post-medieval skeletons. The strong association between the hardness of diet, cranio-facial development, and the resulting formation of dental anomalies has been demonstrated in the literature, and likely contributed to third molar agenesis in the Chichester assemblage (Corruccini et al., 1983;

Corruccini & Lee, 1984; John et al., 2012; Yamada and Kimmel, 1991). However, the Spitalfields assemblage (Sengupta et al., 1999) was also exposed to these influences and has a much lower prevalence (23%) of third molar agenesis. Therefore, dietary influences alone cannot account for the high prevalence rates found in the Chichester assemblage.

It is also relevant to consider the how the biocultural environment, the relationship between biological and cultural elements, may have impacted growth in post-medieval Chichester. Despite the resistance of tooth formation to growth disruptions (Hillson, 2005), delayed dental eruption is often reported in individuals with systemic disease, in the absence of essential nutrients, or in individuals living in a low socioeconomic setting (Cardoso, 2007; Suri et al., 2004). Delayed formation and eruption has also been correlated with increased frequency of third molar agenesis, and reduced morphological complexity in first and second molars (Anderson and Popovich, 1981). Research has shown that the pre-natal environment and the quality of breastfeeding during tooth development also affect the size of the third molars (Garn et al., 1980; Grüneberg, 1951; Grüneberg, 1963; Lumey and Stein, 1985). In Chichester in the early 17th century and again in 1665, the plague was present, and smallpox peaked in 1722, 1740, 1759 and 1775 (Morgan, 1992). In the 19th century, "the health of Chichester often lagged behind the rest of the country" (Morgan, 1991:23), with epidemics linked to water and sewage, such as cholera and typhoid fever, occurring at regular interval. Statistics from 1871-1880 put Chichester amongst the highest number of cases of consumption and typhoid fever in the country, and historical records detail poor sanitation and a lack of the necessary infrastructure for clean water supply and sewage drainage (Morgan, 1992). Such adverse conditions would certainly have disrupted growth, and may have also had an impact on the development of third molars.

Size Reduction and Agenesis

Third molars highly reduced in size, both in mesiodistal and buccolingual dimensions, and/or simplified in morphology, are often referred to as vestigial molars (Nanda, 1954), a term that implies an evolutionary trend towards dental reduction. These third molars are easily recognized upon visual assessment. In Nanda's (1954) analysis of vestigial third molars, all individuals with diminution also had third molar agenesis in other dental quadrants. Size reduction has also been demonstrated in

dentitions with agenesis of other tooth types (Baum & Cohen, 1971). Grüneburg (1951) proposed that agenesis is the most severe expression of a size continuum, in which the tooth germ falls below a critical threshold and formation ceases. From this evidence it might be expected that individuals in the Chichester assemblage who demonstrate third molar agenesis would have other third molars reduced in size and would be smaller upon comparison with those that do not have third molar agenesis.

In this assemblage, all individuals demonstrating vestigial third molars ($n=7$) (Figure 3) had third molar agenesis, except for one skeleton that was missing data on the URM3. It is likely the number of vestigial third molars in this assemblage would have been higher had post-mortem loss not been a factor, and if third molars in alveolar tooth crypts had been measured radiographically.



Figure 3. Left portion of a mandible demonstrating a third molar reduced in size and morphology (Author's own 2017). The remaining third molars are congenitally absent.

Buccolingual measurements of the maxillary third molars in individuals with agenesis were significantly smaller ($p < 0.05$) than those without agenesis in this study (Table 7). Maxillary third molars are more frequently reported congenitally absent than mandibular third molars in the literature (Carter & Worthington, 2015). Given that the buccolingual dimensions of maxillary third molars in this assemblage were smaller in those with agenesis, it is possible to infer maxillary molars are more vulnerable not only to agenesis, but to diminution as well; however, mandibular agenesis was found to be slightly more common in this assem-

blage (54% vs 46%, Table 4), though this difference was not statistically significant, $\chi^2(1, n=166) = 1.18, p = 0.278$.

Baum & Cohen (1971) collected buccolingual and mesiodistal measurements of all teeth, except third molars, from a clinical sample of European-derived ancestry in the Northeastern United States. They analyzed size reduction in the presence of dental agenesis in tooth types other than the third molar. In contrast to the present study, the authors found that mesiodistal dimensions demonstrated a statistically significant association with size reduction and agenesis in 70% of tooth types, excluding third molars. Buccolingual dimensions were, however, only reliable indicators of the association between size reduction and agenesis in measurements of the canines. Garn et al. (1968) investigated the relationship between buccolingual and mesiodistal dimensions. While the two are correlated, the results reveal more autonomy than commonality governing morphological expression, although the further distal in the dental arcade the tooth, the higher the correlation between the two dimensions. Therefore, it might be expected that both the mesiodistal and buccolingual dimensions of third molars would demonstrate an association with size reduction and agenesis. The fact mesiodistal measurements did not show a statistically significant association between size reduction and agenesis in this study may be due the small number of individuals in this cohort, the highly variable morphology of third molars, or it could be an indication that the relationship between buccolingual and mesiodistal dimensions is both population dependent and complex.

Another factor complicating results is the significant differences ($p < 0.05$) in size between the third molars of males and females (see Table 6). To explore this further, an analysis of the relationship between size and agenesis was conducted separately. Removing indeterminate sex from the pool of measurements eliminated 29% of the assemblage. Males ($n=46$) continued to present significantly smaller third molars in the presence of third molar agenesis compared to those without agenesis in the buccolingual dimension of the upper left third molar. The smaller female sample size ($n=36$) made testing the correlation between agenesis with smaller tooth size more difficult.

The final question of analysis in this study focused on detecting patterns in size reduction amongst those with third molar agenesis. Khalaf (2016) analyzed the relationship between size reduction and agenesis in all tooth types in individu-

als with mild (≤ 2 teeth congenitally missing), moderate (3-5 teeth congenitally missing) and severe (≥ 6 teeth congenitally missing) hypodontia. They found that size reduction in the remaining teeth increased with the severity of hypodontia. With this research in mind and Grewal's (1951) evidence of third molar diminution in mice, it was hypothesized that individuals in the Chichester assemblage with three third molars congenitally absent might have a smaller remaining third molar than those with less third molars congenitally absent. In addition to relationships in size within third molar agenesis, any differences that existed between certain groups of third molar agenesis and those without agenesis, for example those with three congenitally absent third molars and those without third molar agenesis, were tested to determine if size differences in third molars could be found between these groups. Unfortunately, this reduced the number of individuals in each measurement category and it was not possible to reach statistical significance (see Table 9). Size patterns within third molar agenesis have yet to be explored in modern or archaeological data, and therefore further testing is required.

Conclusions

Rates of third molar agenesis recorded in modern clinical data are often interpreted as a secular trend in which the third molar, now deemed redundant due to decreased dental wear, low masticatory stress and soft diets, will eventually cease development and potentially disappear from the human dentition. Although there is an established genetic component, the etiology is far from clear. Research on archaeological assemblages is vital in order to better understand the trajectory and origin of this phenomenon, and this study provides a valuable contribution to the relatively little that is known about third molar agenesis prevalence in the past. In post-medieval Chichester, third molar agenesis occurred in 42.7% of individuals. This result is higher than any reported for a clinical British sample, and it is also significantly higher than the prevalence reported from the Victorian Spitalfields assemblage (Sengupta et al., 1999), indicating that an inheritance pattern may be present amongst the skeletons from the post-medieval assemblage of the Litten cemetery in Chichester. While reduced dental wear and masticatory stimulation may contribute to the frequency of agenesis in this assemblage, a strong genetic influence combined with the adverse community health conditions may prove to be important etiological components of

third molar agenesis and avenues for future research.

Significant differences in the size of third molars between those with third molar agenesis and those without were found, although only two of the eight measurements analyzed were found to be significant. If third molars are indeed vestigial, more studies with larger sample sizes will be needed to further test any temporal trend. This includes the examination of archaeological as well as clinical samples.

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Odontometric Patterns in Human Mandibular Molars for Sex Estimation in a Forensic Context

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ABSTRACT Sex estimation is an important step in the identification of human skeletal remains, since it reduces the number of potential matches to approximately one-half. The os coxae and skull are considered the most dimorphic skeletal elements; however, when unavailable, teeth may be used alternatively. This study aims to evaluate the usefulness of specific odontometric parameters from the mandibular first molar – mesiolingual-distobuccal distance (MLDB), mesiobuccal-distolingual distance (MBDL) and mesiodistal distance (MD) - on sex estimation in a Portuguese sample composed of 135 mandibles (78 males; 57 females; ages at death ranging from 18 to 59 years old) selected amongst the 505 individuals of the Coimbra Identified Skeletal Collection (University of Coimbra). Since canines are considered the most accurate teeth for sexual assessment, comparison between first molar parameters and canines mesiodistal dimensions (MD) was performed. Statistical analysis showed sexual dimorphism in human first molars and cut-off points between male or female groups were determined. Using the first molar MLDB and MBDL, 60.7% and 65.2% of individuals were correctly classified, respectively. Highest sex estimation accuracy was achieved with canine MD, reaching 74.6%. Our results indicate that although mandibular molar dimensions are useful for sex estimation, the canine should be prioritized when available to perform this task.

In a forensic context, successful positive identification of human skeletal remains starts by determining the biological profile of the individual: sex, age at death, ancestry, and stature. Sex is usually one of the first parameters to be estimated, not only because other parameters and methods are sex dependent, but also because, when a reliable sex estimation can be obtained from the skeletal remains, only two possible outcomes are provided, male or female, thus lowering the number of possible individuals to whom the remains belong by approximately one-half (Scheuer, 2002; Scheuer and Black, 2007). In skeletal elements, sex estimation is usually obtained by studying the pelvis followed by the skull, as it is considered to yield the second highest percentage of sexual dimorphism; nonetheless, it was found, that postcranial metric measurements

can perform better than those from the skull, so these should be applied when the pelvis is unavailable (Spradley & Jantz, 2011). However, when both cranial and postcranial bones are absent or fragmented, other elements need to be considered (Bruzek & Murail, 2006; Ubelaker, 2008; İşcan &

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Steyn, 2013). In these circumstances, dentition can be a good alternative due to its hard, stable, and durable composition. Teeth are often well preserved and can endure fire, decomposition, and severe trauma; furthermore, their analysis may contribute to the biological profile and its sexual dimorphism is represented by the differing teeth dimensions between males and females (Tabor & Schrader, 2010; Cardoza, 2011; Vishwakarma & Guha, 2011).

The most common tooth dimensions for sexual estimation are the mesiodistal (MD) and buccolingual (BL) crown diameters, which are defined as the distance between the most mesial and most distal points of the crown, and the distance between the most buccal and most lingual points of the tooth, respectively (White & Folkens, 2005; İşcan & Steyn, 2013; Kondo & Manabe, 2016). Although most studies apply these linear dimensions, other measures, such as the Mandibular Canine Index (MCI), have also been evaluated (Scott & Turner, 1988; Rao et al., 1989).

Of the many studies performed worldwide, most identify the canine as the most sexually dimorphic tooth of the human dentition (Acharya & Mainali, 2007; Acharya & Mainali, 2008; Cardoso, 2008; Zorba et al., 2011; Angadi et al., 2013; Viciano et al., 2013; Khamis et al., 2014). This is one of the reasons why the MCI is so often applied. For example, Rao and collaborators (1989) obtained an overall accuracy of 85.9% in sex estimation when using MCI. Nevertheless, since the degree of sexual dimorphism is population specific, some studies presented low success when employing this technique, which led to the focus being shifted to different teeth and dental dimensions (White & Folkens, 2005; Zorba et al. 2012; İşcan & Steyn, 2013; Narang et al., 2015).

The posterior teeth, normally having more than one root, are considered by some researchers a better choice over anterior teeth since the former are more strongly attached to the dental arch and less prone to suffer postmortem loss, thus being more frequently found in fragmentary remains (İşcan & Steyn, 2013; Zorba et al., 2013; Narang et al., 2015). On the other hand, the linear diameters are not as easily measured in molars as they are in anterior teeth. This fact, combined with the compromise of wear and attrition on the linear diameters, guided Hillson and colleagues (2005) to come up with alternative dimensions for the posterior dentition. These included the crown diagonals: the distance between the most mesiolingual and most distobuccal points of the crown (MLDB) and distance between the most mesiobuccal and most distolingual

points (MBDL).

The disarticulation of the mandible from the skull is a consequence of the decomposition of the human body and since the temporomandibular joint is one of the first to disarticulate during decay, it is not uncommon to find an isolated mandible in forensic contexts (Pineiro, 2006; Cardoza, 2011; Cunha, 2014). In Portugal, there are very few studies on sexual dimorphism using teeth and none of them focus on the mesiodistal nor diagonal dimensions of the molars (Cardoso, 2008; Pereira et al., 2010; Gonçalves et al., 2014; Silva et al., 2016; Gouveia et al. 2017; Azevedo et al., 2019). Therefore, the aim of this study was to evaluate the sexual dimorphism in mandibular first molars using the mesiodistal and alternative diagonal dimensions and to compare it to the mandibular canine with the traditional mesiodistal distance, in a sample from the Portuguese population.

Materials and Methods

The sample was selected amongst the 505 individuals which compose the Coimbra Identified Skeletal Collection (20th century), housed at the Department of Life Sciences, University of Coimbra, according to the following selection criteria: Portuguese nationality; age at death ranging between 18 and 59 years old; mandibles presenting at least one well preserved first molar showing minimal signs of attrition (occlusal wear). The resulting study base comprised 135 human mandibles, 78 (57.8%) belonging to males and 57 (42.2%) to females.

The dimensions were taken in millimetres with a *Mitutoyo Digimatic* caliper, to the closest 0.01 mm. The following dimensions were measured on the left mandibular first molars: mesiodistal distance (MD), mesiolingual to distobuccal distance (MLDB), and mesiobuccal to distolingual distance (MBDL); when left teeth were absent, the antimere was used. Additionally, MD of right canines was taken for analysis and comparison with previous measures. All dimensions were measured according to the recommendations of Hillson and collaborators (2005).

The first molar was measured in 78 male and 57 female mandibles. Only 59 out of the 135 mandibles had preserved canines (33 from males and 26 from females). All measurements were collected by an experienced observer, each taken in triplicate in non-consecutive order and separated in time, to avoid bias. The first value was eliminated, and the definitive value was obtained from the arithmetic average of the two last measurements. The intraobserver error was calculated with Bland-Altman analyses (Myles & Cui, 2007). The comparison be-

tween sexes by the selected tooth sizes were performed by the independent sample t-test, after checking the Normality assumptions by Kolmogorov-Smirnov or Shapiro-Wilk tests. Receiver Operating Characteristics (ROC) curves were applied to estimate the more accurate cut-off value for each dimension, which discriminate males from females. Tooth measurements with a value under the cut-off point were considered to belong to female individuals, and values equal to or higher than the cut-off point to belong to male individuals. Each dimension has an associated correct sexual classification accuracy, given by the sensitivity value for correctly classifying males and specificity for females.

Since MLDB and MBDL were too correlated (multicollinearity), the joint performance of sex classification was assessed using a multivariate binary logistic regression with first molar MD and canine MD, by estimating the probability of an individual being a female. The regression model was constructed by enter-method approach. The Omnibus Test was used to check that the model holds an improvement with respect to the constant model. Additionally, goodness of fit was assessed by Hosmer-Lemeshow statistical test, and the percentage of data variance explained by the model was quantified by R-Square based statistics (Cox & Snell and Nagelkerk). The predicted probabilities of being a female were then used as the classification variable.

For the statistical analysis, version 25.0 of SPSS software (*Statistical Package for Social Sciences*) was used. The level of significance was established at 5%. A *p*-value with Bonferroni correction for multiple comparisons (four dimensions) of <0.05 was considered to indicate statistical significance.

Results

By the Bland-Altman analysis there were no relevant differences for the four dimensions (MLDB, MBDL, Molar MD and Canine MD) between the second and the third measurements, which were

not statistically significant ($p > 0.05$). The maximum absolute mean difference between the second and the third measurements was 0.013mm registered in the Molar MD dimension. With 95% of confidence, the mean differences for all dimensions are lower than 0.03mm. Moreover, the Bland-Altman analysis did not reveal a relationship between the differences obtained with the second and third measurements, and the magnitude of the measurements. It also revealed a non-dependence between the variation of those differences and the magnitude of the measurements.

Table 1 depicts the MLDB, MBDL, first Molar MD and Canine MD dimensions. All measurements were higher for males, and the mean differences were statistically significant ($p < 0.05$). In absolute terms, the larger differences (>0.41mm) were found in MD dimensions.

Figure 1 shows the ROC curves obtained for MLDB and MBDL dimensions. The MBDL dimension exhibits a larger area under the curve (AUC) than that of MLDB, regardless of the cut-off considered. Figure 2 shows the ROC curves obtained for first molar MD and canine MD dimensions. This figure clearly illustrates that the AUC were similar for almost cut-offs.

Table 2 presents the results of the AUC analysis obtained for each of the four dimensions. Interestingly, the AUC values are well above 0.5 for all variables, showing statistical significance in any case ($p < 0.05$). Accordingly, as shown in Figures 1 and 2, Table 2 points out that the most discriminative variables are first molar MD and canine MD with an AUC of 0.735 and 0.801, respectively. On the other hand, both MLDB and MBDL dimensions have much lower performance in sex discrimination (AUC values below 0.71).

The accuracy assessment in sex prediction was based on the optimal cut-offs (Table 3). Using the MBDL dimension which presented higher AUC than MLDB for all cut-offs, with the optimal cut-off of 11.44mm, 67.9% of the males were correctly classified, whereas females were accurately identified

Table 1. Descriptive statistics for MLDB, MBDL, first Molar MD, and Canine MD dimensions by sex.

Dimensions (mm)	Female (n=57)	Male (n=78)	<i>p</i>	95% CI (Difference) Lower-Upper bond
MLDB	11.18±0.55	11.51±0.56	0.004	0.14-0.52
MBDL	11.29±0.52	11.70±0.52	<0.002	0.23-0.59
First Molar MD	10.77±0.57089	11.19±0.56530	0.04	0.10-0.69
Canine MD	6.39±0.35408	6.83±0.47072	<0.002	0.23-0.66

Mean ±standard deviation; *p* represents the *p*-value with Bonferroni correction for four dimensions.

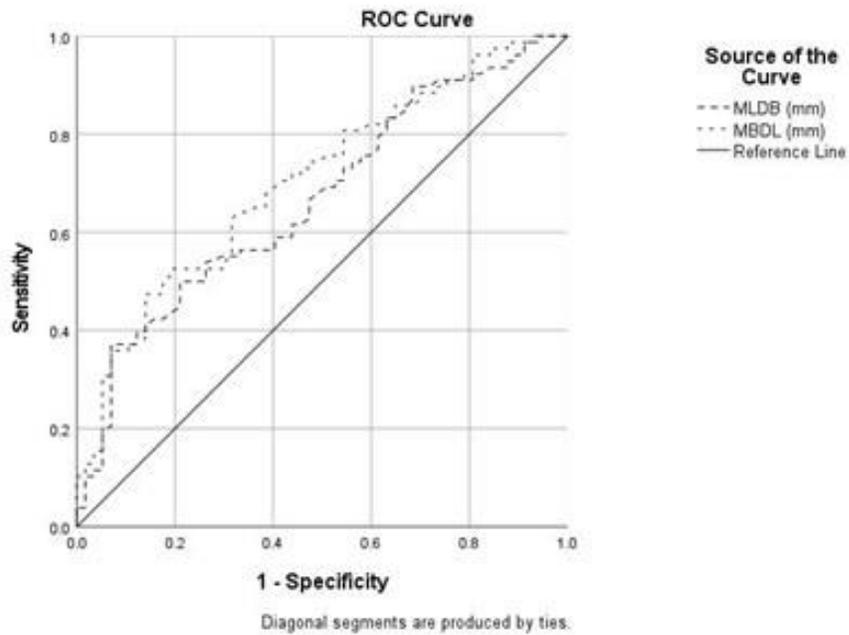


Figure 1. ROC curve analyses for the MLDB and MBDL dimensions. The identity line represents the curve with AUC equal to 0.5.

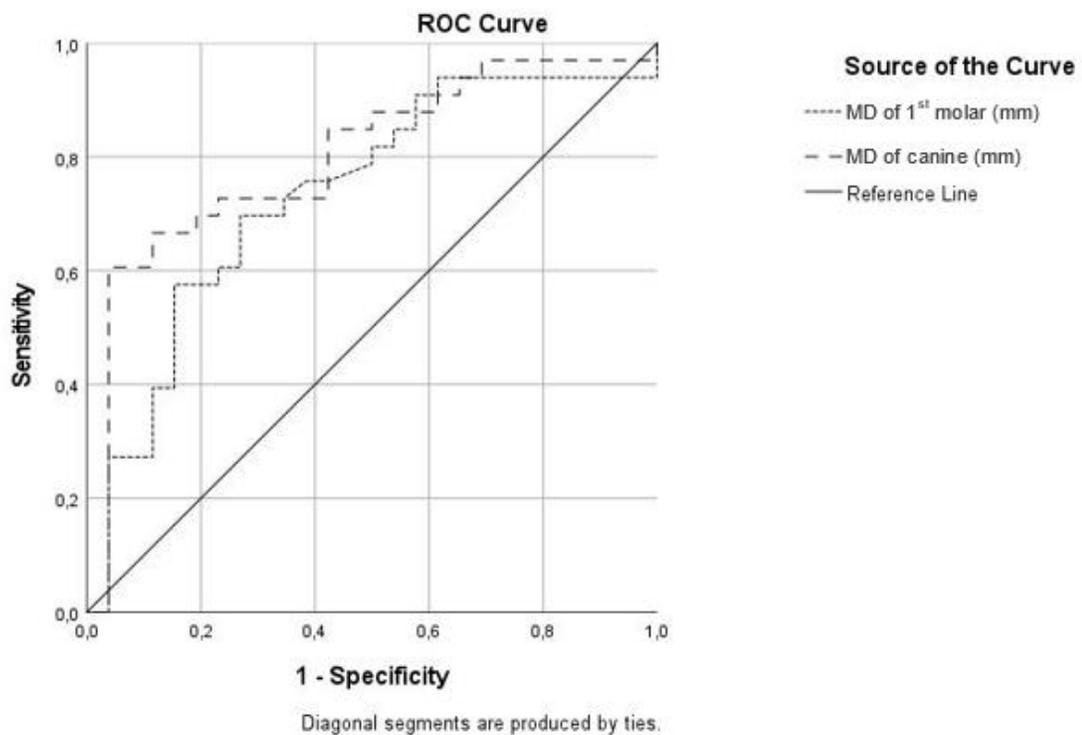


Figure 2. ROC curve analyses for Molar MD and Canine MD dimensions. The identity line represents the curve with AUC equal to 0.5.

Table 2. Area under the curve (AUC) and corresponding 95% confidence intervals (CI) for the MLDB, MBDL, first Molar MD and Canine MD dimensions.

Dimensions	AUC	CI _{95%} AUC	<i>p</i>
MLDB (mm)	0.668	0.577-0.759	0.004
MBDL (mm)	0.705	0.617-0.792	<0.002
First Molar MD (mm)	0.735	0.603-0.866	0.008
Canine MD (mm)	0.801	0.684-0.918	<0.002

p represents the *p*-value for the null hypothesis that the true AUC is 0.5 with Bonferroni correction for four dimensions.

Table 3. Overall accuracy in sex classification for the MLDB, MBDL, first Molar MD and Canine MD dimensions.

Dimensions (Optimal cut-off)	Accuracy		
	Female	Male	Total
MLDB (11.27mm)	30/57 (52.6%)	52/78 (66.7%)	82/135 (60.7%)
MBDL (11.44mm)	35/57 (61.4%)	53/78 (67.9%)	88/135 (65.2%)
First Molar MD (10.89mm)	17/26 (65.4%)	24/33 (72.7%)	41/59 (69.5%)
Canine MD (6.54mm)	20/26 (76.9%)	24/33 (72.7%)	44/59 (74.6%)
First Molar MD+Canine MD*	18/26 (69.2%)	23/33 (69.7%)	41/59 (69.5%)

*Multivariate binary logistic regression; the Omnibus Test of model coefficient ($\chi^2=15.502$; $df=2$; $p<0.001$)

in 61.4% of the cases. The corresponding overall accuracy was around 65%. As expected, a greater accuracy in sex estimation was obtained with the optimal cut-offs for the first molar MD and canine MD dimensions, respectively, 10.89mm and 6.54mm. For both dimensions, 72.7% of the males were correctly classified. Yet, the Canine MD presented better accuracy to identify the females with 76.9% accuracy, as opposed to 65.4% with the first molar MD. The Canine MD showed an overall performance of approximately 75%, followed by the first Molar MD (overall accuracy around 70%).

Multivariate binary logistic regression was then performed to assess the joint impact of the two predictors (first molar MD and canine MD) in sex classification. The Omnibus Test of model coefficient ($\chi^2=15.502$; $df=2$) was statistically significant, with $p<0.001$. Regarding the model goodness of fit, the result of the Hosmer-Lemeshow test indicates that the null hypothesis could not be rejected ($\chi^2=11.812$; $df=8$; $p<0.16$). The R Square based statistics was found to be 31% (Cox & Snell statistics).

Finally, the first molar coefficient was not shown to be statistically significant ($p=0.644$), and the coefficient estimates for canine MD ($B=-2.606$; Standard Error =0.988; Wald = 6.957; $df = 1$; $p=0.008$) was found to be negative and statistically significant. So, when the first molar MD and canine MD were considered together, 69.2% of females and 69.7% of males were correctly classified, with the overall accuracy being 69.5%.

Discussion

Sexual assessment is one of the first steps in the process of the identity reconstruction. When skeletal preservation is seriously compromised by taphonomic and/or anthropic reasons, teeth are of great value for sex estimation due to their strength and durability (Scheuer, 2002; Bruzek & Murail, 2006; Scheuer and Black, 2007; Ubelaker, 2008; İşcan & Steyn, 2013; Scott et al., 2018).

Most studies estimating the sex from teeth size focus on the canines as these are known as the most sexually dimorphic teeth. In addition, it is

considered the sturdiest and most durable tooth when faced with disease and trauma (Kaushal et al., 2003; White & Folkens, 2005), which explains why linear dimensions and other measures like crown indexes – such as the MCI – are so commonly applied (Rao et al., 1989; Kaushal et al., 2004; Zorba et al., 2011; Khamis et al., 2014; Azevedo et al., 2019). On the other hand, its increased probability of postmortem loss, compared to posterior teeth, due to its single root led researchers to start evaluating the sexual estimation accuracy in molars (İşcan & Steyn, 2013; Narang et al., 2015).

This study corroborates the results of previous research showing that the dentition exhibits sexual dimorphism. Statistically significant differences between males and females were found in mandibular first molars size. Furthermore, the use of molar alternative dimensions to the mesiodistal and buccolingual parameters, such as the diagonal dimensions, can be particularly useful when the teeth crowns are partially absent. In this regard, the MBDL dimension provided the highest overall estimation accuracy: 65.2% of correct classifications and an AUC of 70.5%. However, when considering all dimensions, Canine MD was the variable with the highest correct classification accuracy, namely 74.6% and an AUC of 80.1%. Both results are in accordance with the conclusions of studies from other populations, with diagonal dimensions having an accuracy range between 58.3% and 76.6% and mesiodistal dimensions a range between 63.9% and 85.8% (Karaman, 2006; Acharya & Mainali, 2009; Acharya et al., 2011; Zorba et al., 2013; Manchanda et al., 2015; Narang et al., 2015; Tabasum et al., 2017; Azevedo et al., 2019).

When both MD variables were considered in a multivariate binary logistic regression, the accuracy of sexual estimation was 69.5%, slightly lower than the value achieved with canine MD alone, and the first molar MD variable ceased to be statistically significant in this situation, which reinforces the significance of the canine. Nonetheless, these two dimensions were found to explain 31% of the existing variability between male and female individuals, which further corroborates that sexual dimorphism is present in the dentition.

Results from the first mandibular molar diagonal dimensions did not achieve a higher accuracy compared to the mesiodistal dimensions, leading to the conclusion that the canine should always be analysed when present. However, it is important to bear in mind that sample size was smaller when investigating the MD dimensions, which could

have led to a slight bias of results. In this sense, the molars prove to be a good option when combined with other elements and as a corroborating method. It is also important to mention that these results only apply to a Portuguese population of the early 20th Century since sexual dimorphism is population specific, as previously mentioned. Because sexual dimorphism varies across space and time, further studies should test this method in a contemporaneous Portuguese population (21st Century) to enable the investigation of secular trends in these odontometric parameters in this specific population.

Conclusions

The present results corroborate that the posterior dentition, namely odontometric parameters of the first mandibular molars, demonstrates sexual dimorphism in humans. The mesiodistal dimension of the canine was the variable that showed the highest sexual estimation accuracy, reaching levels of 74.6%. The diagonal dimensions of the molar, although less accurate, proved to be acceptable variables to be used in conjunction with other dimensions when the canine is unavailable, or when partial destruction of the molar crowns makes it impossible to use other parameters. Additional research of these variables should be carried out in other populations, both to further validate the usefulness of posterior teeth in forensic scenarios and to contribute to secular trend investigations in the dentition's sexual dimorphism.

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The Impact of Dental Wear on the Analysis of Morphological Affinities based on Dental Non-metric Traits

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ABSTRACT Dental wear is described as a limitation to dental morphological studies, as it obscures important crown trait features, resulting in significant differences on trait frequencies, an essential component for estimating biodistances. However, the actual impact of dental wear on biological distances still requires further characterization. We explore the impact of dental wear on morphological affinities for Brazilian pre-colonial series in the context of worldwide reference series. Twenty crown traits were scored using the Arizona State University Dental Anthropology System, and dental wear was quantified as an ordinal scale between 1 (no wear) and 8 (crown eroded). Eight crown trait frequencies are significantly associated with dental wear ($p < 0.05$), demonstrating its impact on their analysis. To explore this impact on biodistances, data were divided by wear categories (all teeth, low-wear, moderate/severe wear) and morphological affinities among series were compared through Euclidean distances, Mean Measure of Divergence, and Principal Component Analysis. Results show that the impact of wear is only meaningful when a sample contains many wear-biased traits with only moderate/severe wear. We conclude that, despite the impact of wear on individual trait frequencies, its impact on morphological affinities can be mitigated by including other variables or when comparisons focus only on large-scale biological differences.

Dental morphology has a strong genetic component which allows it to be used as a proxy for neutral genetic markers (Hubbard et al., 2015; Irish et al., 2020; Kimura et al., 2009; Rathmann et al., 2017; Rathmann & Reyes-Centeno, 2020). Dental non-metric traits are assumed to lack significant sexual dimorphism, have minimal influence from divergent selection, and have high heritability (Irish et al., 2020; Scott & Irish, 2013; Scott & Turner II, 1997; Turner II et al., 1991). The required methods to analyze and quantify dental morphology are also cost efficient, and since teeth are often found in the archaeological record and highly resilient to taphonomic processes (Hillson, 2005), they are a good alternative to reconstruct population biological affinities and human mobility on individual (Scott, Pilloud, et al., 2018), local (Scott et al., 2013; Turner II & Scott, 1977), regional (Irish et al., 2017; Rathmann et al., 2019; Sutter, 2009; Turner II, 1976), and global scales (Hanihara, 2008; Scott & Irish,

2017; Scott & Turner II, 1997; Sutter, 2005). A prolific example of the latter can be seen in studies discussing the peopling of the Americas (Greenberg et al., 1986; Huffman, 2014; Powell, 1995, 1997; Powell & Neves, 1998; Scott, Schmitz, et al., 2018; Stojanowski et al., 2013; Stojanowski & Johnson, 2015; Sutter, 2005; Turner II & Scott, 2013).

Dental morphology played an important role in discussing the settlement of the Americas since the first half of the 20th century (Dahlberg, 1945; Hrdlička, 1920, 1921). After the development of

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standardized methods for data collection, such as the Arizona State University Dental Anthropology System (ASUDAS), studies of dental non-metric traits increased significantly over the years (Powell, 1995; Scott & Irish, 2017; Scott & Turner II, 1997; Sutter, 2005; Turner II et al., 1991). However, as researchers studied different archaeological series, different conclusions were drawn about how the Americas were first settled by modern humans. Some studies argue that all Native Americans are more strongly related to each other than to any other group outside the Americas, and share a rather homogenous dental morphological pattern, related to Northeast Asians populations that first crossed the Bering Strait (Greenberg et al., 1986; Scott, Schmitz, et al., 2018; Scott & Turner II, 1997; Turner II, 1990; Turner II & Scott, 2013). Others suggest phenotypic variation within the Americas is larger, with some Native American groups biologically related to Southeast Asians, meaning that at least two distinct biological populations crossed the Bering Strait during pre-colonial times (Haydenblit, 1996; Huffman, 2014; Lahr & Haydenblit, 1995; Ortiz, 2013; Powell, 1995, 1997; Powell & Neves, 1998; Powell & Rose, 1999; Sutter, 2005, 2009).

This discrepancy in narratives has often been attributed to issues regarding the replicability of ASUDAS, as observer error is often an anticipated concern (Marado, 2017; Nichol & Turner, 1986; Wu & Turner, 1993). Also, the combination of which morphological traits are used to assess biological affinities may have an important influence on the results (Rathmann & Reyes-Centeno, 2020). Furthermore, dental wear has also been suggested as a noteworthy concern on its own, causing bias in scoring non-metric traits (Burnett et al., 2013; Burnett, 2016; Stojanowski & Johnson, 2015). Dental wear is a physiological phenomenon on which tooth enamel and dentine are gradually worn over time by attrition, abrasion and/or erosion mechanisms (Kaidonis, 2008). Many dental non-metric traits are features located in the tooth crown, so dental wear may gradually erase morphological details and impact scoring decisions (Scott et al., 2016). The effects vary for each particular trait and can result in the under-estimation of trait frequencies (i.e., attributing lower grades or absence to traits that should be scored as higher grades or present), or over-estimation of frequencies (i.e., higher trait expressions are scored regardless of wear, but lower/absent expressions are scored as missing data under the same circumstances) (Burnett et al., 2013; Burnett, 2016). If the error in the estimations of trait frequency is significantly

biased between teeth with low and moderate/severe wear, it violates the assumptions that samples have data missing completely at random (MCAR) (Burnett et al., 2013; Stojanowski & Johnson, 2015). Data MCAR is a central tenet in the reconstruction of population parameters based on samples, because it means missing values follow the same distribution as the observed values (Bhaskaran & Smeeth, 2014), and therefore information about the population has not been skewed by the data that was not observable.

Many non-metric dental traits have been shown to be susceptible to wear-related bias: shoveling UI1, cusp number LM2 (Burnett et al., 2013; Stojanowski & Johnson, 2015), distal accessory ridge UC, mesial canine ridge UC, accessory ridges UP, lingual cusp number LP2, hypocone UM2 (Burnett et al., 2013; Burnett, 2016), double shoveling UI1, enamel extensions UM1, deflecting wrinkle LM1 (Stojanowski & Johnson, 2015). However, the clear impact of wear-biased traits on multivariate analysis has not been formally evaluated. It is possible that a certain amount of error is acceptable as long as it does not change the interpretations of the results. In other words, the measured attributes are still valid as long as they are meaningfully reflecting real biological relationships (Houle et al., 2011).

Going back to the example about the peopling of the Americas, the debate around dental wear is particularly relevant. Although there is consensus that Native Americans share a recent common ancestor with Asians, there is no clear agreement about which Asian dental complex they are more related: 1) a specialized pattern which emerged approximately between 20 and 11 thousand years ago (kya) in Northeast Asia, with high frequencies of shoveling UI1, double shoveling UI1, one-rooted UP1, enamel extensions UM1, pegged/reduced/missing UM3, deflecting wrinkle LM1, three-rooted LM1; commonly referred to as the Sinodont pattern (Turner II, 1989, 1990); or 2) a generalized and more simplified pattern which appears between 25 and 40kya in Southeast Asia (Turner II, 2006), with lower trait frequencies of the same above-mentioned traits, and a higher frequency of four-cusped LM2; commonly described as the Sundadont pattern (Scott, Schmitz, et al., 2018; Turner II, 1990).

Some authors suggest that Native Americans have a different derived dental morphological pattern from both Sinodonts and Sundadonts (Scott, Schmitz, et al., 2018; Stojanowski & Johnson, 2015). While keeping ties to Sinodont groups such as Northeast Asians, Native Americans have even higher trait frequencies of some traits (e.g., shovel-

ing UI1, double shoveling UI1), which can be viewed as “super-Sinodont” (Scott, Schmitz, et al., 2018). In other words, it seems that there are considerable differences on the dental morphological patterns between Native American and Asian populations, which is even larger in some traits than the differences observed between Asian Sinodonts and Sundadonts (Scott, Schmitz, et al., 2018).

Since Sundadonty has lower frequencies of several morphological traits, particularly when compared to Native Americans, some scholars argue that the under-estimation of trait frequencies due to dental-wear bias is responsible for the close biological link between Native Americans and Southeast Asians (Scott, Schmitz, et al., 2018; Turner II & Scott, 2013), which has been noted in several independent studies and different archaeological series (Haydenblit, 1996; Huffman, 2014; Lahr & Haydenblit, 1995; Ortiz, 2013; Powell, 1995, 1997; Powell & Neves, 1998; Powell & Rose, 1999; Sutter, 2005, 2009).

To contribute to this discussion, and at the same time to illustrate the impact of dental wear in dental non-metric analyses, we present a case study of a Brazilian coastal series dated to between 10.0 and 1.0 kya. Our study subsets this dataset into different series based on dental wear degrees and compare their morphological affinities within a global reference framework, using a combination of only wear-biased traits, only unbiased traits, and all traits pooled together. These analyses aim to improve our understanding of the impact dental wear has in multivariate statistical analyses, and to explore if at any point dental non-metric traits stop being meaningful markers of biological relationships.

Materials and Methods

To quantify the impact of wear on the morphological affinities of Brazilian coastal populations, we analyzed 431 individuals from the South and Southeast Brazilian coast, dated between ~10.0 and 1.0 kya. Most of our sample comes from a broad archaeological context of shellmound builders, commonly known as *Sambaquis*, which have previously been shown to share a Native American dental morphological pattern (Turner II & Scott, 2013). Our sample includes individuals from the following archaeological sites: Capelinha 1 (n=7), Capelinha 2 (n=1), Itaoca (n=2), Estreito (n=5), Laranjal (n=9), Moraes (n=32), Piaçaguera (n=34), Tenório (n=24), Mar Virado (n=21), Cosipa 4 (n=2), Buracão (n=17), Galheta IV (n=6), Ilha de Espinheiros 2 (n=7), Enseada (n=26), Morro do Ouro (n=70), Itacoara (n=28), Rio Comprido

(n=48), Cabeçuda (n=12), Guaraguaçu A & B (n=69), and Matinhos (n=11).

A total of 20 crown traits from ASUDAS were scored (Scott & Irish, 2017; Turner II et al., 1991), and dental occlusal wear was noted according to Smith (1984). To improve sample sizes, we used the total tooth count method to calculate trait frequency: when available, both antimeres were scored for each trait, and sample frequencies were calculated by dividing the total number of positive expressions by the total number of teeth analyzed (Scott, 1980). While this approach may add redundant information to the data, as individuals are often scored twice (Scott, 1980; Scott & Irish, 2017; Turner II & Scott, 1977), previous studies have shown that results based on individual and total counting methods produce very similar results, and thus can be used for comparative purposes (Marado, 2014; Scott, 1980). As the main goal of this study is to explore the impact of wear bias on the estimations of morphological affinities, we opted for the method that would maximize the number of teeth and dental wear information included.

Intra-observer error of dichotomized traits was calculated with a subsample of 128 individuals, analyzed by the first author twice with approximately one month interval between analyses. Only teeth that were scored for dental wear were considered in this analysis, and Cohen’s Kappa coefficient of agreement was used to assess the level of agreement between analyses. Kappa’s values were classified as follows: 0.00-0.20 (slight agreement); 0.21-0.40 (fair agreement); 0.41-0.60 (moderate agreement); 0.61-0.80 (substantial agreement); 0.81-0.99 (almost perfect agreement) (Landis & Koch, 1977).

To test the impact of wear on morphological affinities among series, we only included teeth scored for both dental wear and morphological traits, and followed a similar approach to Burnett (2013): three categories of dental wear were established based on the scale of Smith (1984): low wear (Grades 1-3); moderate wear (Grades 4-5); and severe wear (Grades 6-8). As there were very low sample sizes of traits scored on teeth with severe wear, we combined teeth with moderate or severe wear. Afterwards, we compared trait presence and absence between low and moderate/severe wear groups using Fisher’s Exact tests.

Finally, we evaluated the morphological affinities among series through multivariate exploratory analyses, comparing our samples with other skeletal series from Southeast Asia, Asia, Circumpolar, North America, Mesoamerica, and South America (Scott & Irish, 2017). All data tables used for com-

parative purposes are available in Scott and Irish (2017). Furthermore, we split our sample into three series based on dental wear categories: 1) Brazilian coast, which includes all teeth regardless of dental wear; 2) Brazilian coast (low wear), which excludes teeth with moderate/severe dental wear; 3) Brazilian coast (Mod/Sev wear), which uses only teeth with moderate/severe occlusal dental wear. We recognize it is unlikely for a researcher to select only moderate/severe wear traits in any study on dental morphology. However, some archaeological series are very limited, and sometimes only composed by individuals with substantial amounts of dental wear. Thus, we use this series as a way to infer the maximum amount of error that can result from the use of only teeth moderately to severely worn out.

To assess possible trait correlations between groups, and check if correlations varied significantly between combinations of wear-biased and/or unbiased traits, Spearman correlations were calculated over trait frequencies of three different data sets: A) Only wear-biased traits; B) Only unbiased traits; and C) all traits combined. To mitigate the impact of multicollinearity, for each highly correlated pair of variables ($r \geq 0.7$), we removed one of those traits from the multivariate analyses.

Next, Euclidean distances and Mean Measure of Divergence without sample size correction were calculated for each of the three datasets, and represented through Kruskal Multidimensional Scaling. Mantel matrix correlation tests were applied to compare distance matrices generated by both methods for each dataset to test the level of similarities between them. The morphological affinities

were also explored through Principal Component Analysis, and the first two principal components were extracted from the average trait frequencies for the series and represented in a scatterplot.

Together, these different multivariate analyses allow us to evaluate the impact of wear biases in estimating morphological affinities (and biological relationships) among samples, by illustrating to what degree the inclusion of biased frequencies affect the overall pattern of affinities among series when inserted in a broader comparative framework. Furthermore, as we expect our samples to share a Native American dental complex, as suggested by Turner and Scott (2013), any deviation from this cluster may lead us to assume that dental wear can shift the results significantly, enough to bias our ancestry estimations at a worldwide scale, as suggested by some authors (Scott, Schmitz, et al., 2018; Turner II, 2006; Turner II & Scott, 2013).

All statistical analyses were done in R (R Core Team, 2020), with functions written by two of us (MH and DF), and complemented by the packages ggplot2 (Wickham, 2016), ggfortify (Tang et al., 2016), MASS (Venables & Ripley, 2002), vegan (Oksanen et al., 2013), and irr (Gamer et al., 2012).

Results

Figure 1 shows the intra-observer error for all the analyzed traits in this study. While most traits show substantial agreement or higher, three traits only reached moderate agreement (metaconule UM1, anterior fovea LM1, and groove pattern LM2), and so should be considered with caution. These traits are also traits that show significant bias from dental wear (Table 1), suggesting that dental

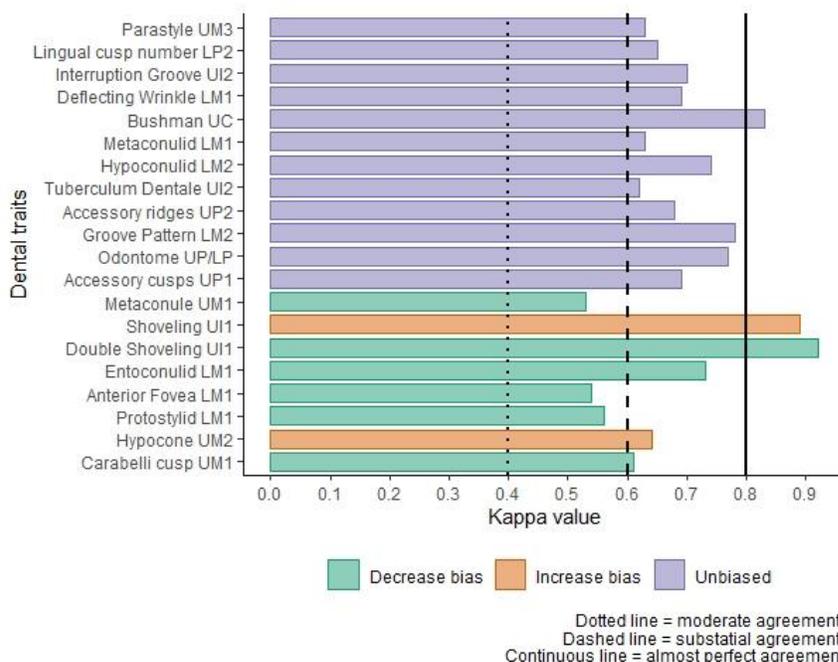


Figure 1. Bar plot with Cohen's Kappa coefficient of agreement for each morphological trait.

wear may play a role in the consistent scoring of these traits. However, it is worth noting that some traits with almost perfect agreement are also wear biased, one by underestimation (double shoveling UI1) and the other by overestimation of trait frequencies (shoveling UI1). Therefore, the role of wear on the replicability of trait analysis depends on the type of trait and should be assessed accordingly.

Figure 2 shows the distribution of dental wear grades for each scored morphological trait. As can be seen, the distribution of trait scores is largely similar between teeth with low and moderate/severe dental wear. Table 1 shows the sample sizes and trait frequencies for the series in this study, as well as the results for the Fisher Exact tests comparing trait frequency by wear degrees. There were 4,191 dental trait scores in total, 2,069 on teeth with low wear and 2,122 on teeth with moderate/severe wear. Half of the dental traits have larger sample sizes on teeth with low wear, and the other half have larger sample sizes on teeth with moderate/severe wear. However, in some traits there is a clear larger sample size for teeth with low wear

(e.g., deflecting wrinkle LM1 and accessory ridges UP2). Eight of the 20 traits (40%) show significant dental wear bias ($p < 0.05$). The effect of dental wear varies between traits: shoveling UI1 and hypocone UM1 are biased towards increased trait frequencies (25%, 2/8) whereas the remaining trait biases (75%, 6/8) resulted in the underestimation of their frequencies. Therefore, in the Brazilian context, as in other studies, dental wear is more prone to bias traits by underestimating their frequencies (Burnett et al., 2013).

In the analyses comparing the Brazilian series with the reference series, the following traits were excluded because they are not available from Scott and Irish (2017): anterior fovea LM1, accessory ridges UP2, and accessory cusps UP1. This resulted in a dataset of 17 traits, seven of which show significant wear bias. We calculated the absolute mean difference of each trait between all pairs of reference series and compared it with the frequency differences observed between low and moderate/severe wear groups (Table 2), to quantify the magnitude of the wear bias in the context of observed differences among series representing large

Table 1. Sample sizes, frequencies, Fisher's Exact test, and dental wear-bias effect for each dental morphological trait.

Variable	Grade Threshold	Brazilian Coast		Brazilian Coast (low wear)		Brazilian Coast (moderate/severe wear)		Fisher <i>p</i> . value	Bias effect
		n	f	n	f	n	f		
Shoveling UI1	3	168	0.857	100	<u>0.81</u>	68	<u>0.926</u>	0.043	Increase
Double Shoveling UI1	2	166	0.681	98	<u>0.745</u>	68	<u>0.588</u>	0.042	Decrease
Interruption Groove UI2	1	165	0.158	93	0.161	72	0.153	1	-
Tuberculum Dentale UI2	2	163	0.215	90	0.233	73	0.192	0.569	-
Bushman UC	1	175	0.051	84	0.048	91	0.055	1	-
Accessory cusps UP1	1	176	0.091	101	0.129	75	0.04	0.062	-
Accessory ridges UP2	2	84	0.476	77	0.494	7	0.286	0.437	-
Metaconule UM1	1	158	0.089	81	<u>0.136</u>	77	<u>0.039</u>	0.048	Decrease
Carabelli cusp UM1	3	229	0.127	78	<u>0.308</u>	151	<u>0.033</u>	<0.01	Decrease
Hypocone UM2	3	291	0.832	104	<u>0.644</u>	187	<u>0.936</u>	<0.01	Increase
Parastyle UM3	2	228	0.004	148	0.007	80	0	1	-
Lingual cusp number LP2	1	192	0.255	93	0.258	99	0.253	1	-
Deflecting Wrinkle LM1	2	71	0.479	65	0.477	6	0.5	1	-
Anterior Fovea LM1	2	103	0.563	76	<u>0.645</u>	27	<u>0.333</u>	<0.01	Decrease
Protostylid LM1	2	227	0.031	73	<u>0.082</u>	154	<u>0.006</u>	<0.01	Decrease
Entoconulid LM1	1	167	0.24	79	<u>0.316</u>	88	<u>0.17</u>	0.031	Decrease
Metaconulid LM1	1	244	0.111	81	0.099	163	0.117	0.829	-
Groove Pattern LM2	Y	260	0.123	95	0.095	165	0.139	0.332	-
Hypoconulid LM2	1	193	0.777	95	0.789	98	0.765	0.731	-
Odontome UP/LP	1	731	0.01	358	0.014	373	0.005	0.277	-

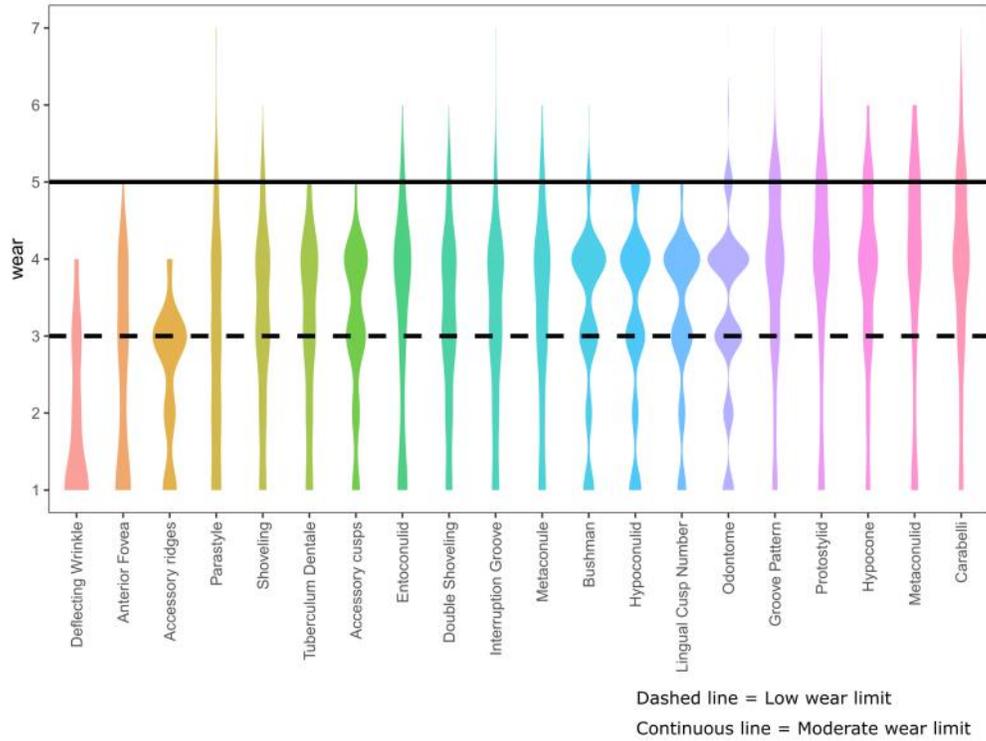


Figure 2. Violin plot showing the frequency of wear degrees for each morphological trait.

Table 2. Maximum trait frequency differences between low and moderate/severe wear subset in the Brazilian Coast groups, comparative information about absolute mean difference in trait frequency among worldwide series, and proportion of differences among reference series that exceed the wear bias observed for Brazilian series.

Traits	Frequency bias between low and moderate/severe wear Brazilian series	Absolute mean frequency differences among reference series	Proportion of differences among comparative series exceeding bias in Brazilian series
Shoveling UI1	0.116	0.263	0.638
Double shoveling UI1	-0.157	0.349	0.65
Interruption grooves UI2	-0.008	0.147	0.96
Tuberculum dentale UI2	-0.041	0.107	0.766
Bushman Canine UC	0.007	0.029	0.672
Metaconule UM1	-0.097	0.123	0.533
Carabelli cusp UM1	-0.275	0.138	0.134
Hypocone UM2	0.292	0.155	0.174
Parastyle UM3	-0.007	0.037	0.826
Lingual cusp number LP2	-0.005	0.25	0.991
Deflecting wrinkle LM1	0.023	0.174	0.929
Protostylid LM1	-0.076	0.047	0.217
Entoconulid LM1	-0.146	0.157	0.484
Metaconulid LM1	0.018	0.045	0.766
Groove pattern LM2	0.044	0.091	0.718
Hypoconulid LM2	-0.024	0.152	0.875
Odontome UP1/LP1	-0.009	0.037	0.835

Bold: Wear-biased traits that show absolute frequency bias larger than the absolute mean frequency differences among reference series.

continental biological profiles. Although seven wear-biased traits show significant wear bias (see Table 1), only three of them (Carabelli cusp, hypocone, and protostylid) show wear bias that exceeds the average difference in the reference series. Therefore, most traits in the Brazilian series show wear biases that are smaller than the majority of differences among the reference series. These three traits should be considered as the most problematic, and may lead to a more significant bias in the patterns of morphological affinities observed in our data.

Before running the multivariate analyses, Spearman correlation tests among the 17 traits were done to check for collinearity of variables. The correlation tests revealed a strong correlation ($r \geq 0.7$) between double shoveling UI1 and groove pattern LM2 (SI2). Therefore, groove pattern LM2 was removed from the analyses using all 17 traits (dataset C). When testing correlations among wear-biased (dataset A) and unbiased (dataset B) traits, no strong correlations were found (SI2), and no traits were removed from the analyses with these datasets.

The results of the multidimensional scaling based on Euclidean distances (Figure 3) and Mean Measures of Divergence (Figure 4) show very similar results, as the two distance measurements show extremely high correlations (Mantel correlation tests: $r=0.950$, $p \leq 0.001$ for biased traits; $r=0.960$, $p \leq 0.001$ for unbiased traits; $r=0.961$, $p \leq 0.001$ for combined traits). Each of the distances matrices produced in this study can be accessed in Supplementary Information 3 (SI3).

The analyses using datasets with biased (Figure 3A and Figure 4A) and with combined traits (Figure 3C and Figure 4C) show a cluster composed by Asian and Southeast Asian groups, a second cluster formed by North American and Circumpolar series, and a third cluster mostly formed by Mesoamerican and South American series. Greater Northwest coast is a constant outlier for North America, since it is within the expected variation for Mesoamerica/South America. Japan is also an outlier of the Asian cluster, standing between them and Mesoamerica/South Americans. Finally, in both Euclidean distances and Mean Measure of Divergence, the Brazilian coast series are within the Mesoamerica/South America cluster, with the wear bias pushing the series slightly away from this cluster.

However, the results using only unbiased traits (Figure 3B and Figure 4B) show important differences from the other analyses. In this case, there are only two clear clusters, one made of Asian and Southeast Asian groups, and another composed by

Circumpolar, North American, Mesoamerican and South American series. This reduced number of traits reduces the ability of the analysis to discriminate among most of the geographical regions represented in the reference dataset, which suggests that the inclusion biased traits may be important to infer population structure within the Americas. In other words, this exercise illustrates the fact that removing wear-biased traits may sometimes be more harmful to the study of morphological affinities than their inclusion. Regarding our particular samples, the Brazilian Coast series, although closer to the Native American cluster, is still considerably distant from it, which to some extent may highlight some degree of inter-observer error between the first author of this study and Christy Turner II, who analyzed the worldwide comparative samples (Scott & Irish, 2017). Nevertheless, the Brazilian series appear close to each other, irrespective of the degree of wear considered, which shows that wear bias by itself is not enough to cause the association of series with another geographic region, as suggested before (Turner II, 2006; Turner II & Scott, 2013).

The Principal Component Analyses (Figure 5) show very similar results to Euclidean Distance and Mean Measure of Divergence and helps to identify traits responsible for the population structure within the Americas discussed previously. Shoveling UI1 and double shoveling UI1 are particularly relevant traits to distinguish between Circumpolar/North America and Mesoamerican/South American series, with frequencies being higher on Central and South Native American groups (Figure 5A and Figure 5C). As these traits are missing on the unbiased dataset (Figure 5B), the distinction between Circumpolar, North Americans, Mesoamericans, and South Americans is not evident. Finally, overall, these results reinforce that despite significant differences in frequencies due to dental wear, these differences are not strong enough to change the relative pattern of morphological affinities of the Brazilian series when inserted in a large comparative framework.

Nevertheless, among Brazilian Coast series with different amount of wear, there is a pattern where the subset using only teeth with moderate/severe wear is more separated from other groups (the only method where this pattern is not observed is on the Principal Component Analysis). This suggests that although using only teeth with moderate/severe wear may not change the overall interpretations of the morphological affinities of the series, it is still adding error to the interpretations, especially if the analysis is concerned with patterns of associations within smaller geographic scales.

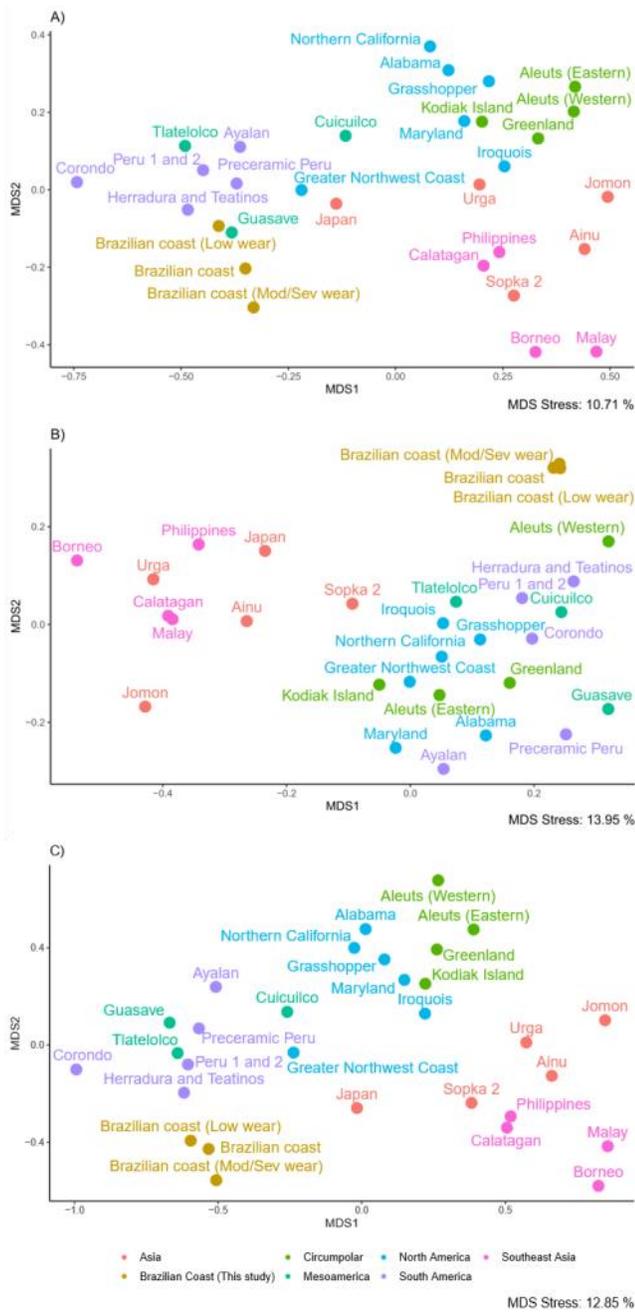


Figure 3. Kruskal non-metric multidimensional scaling of the Euclidean distance using: A) 7 wear-biased ASUDAS traits; B) 10 Unbiased ASUDAS traits; C) 16 ASUDAS traits (9 wear-unbiased and 7 wear-biased traits).

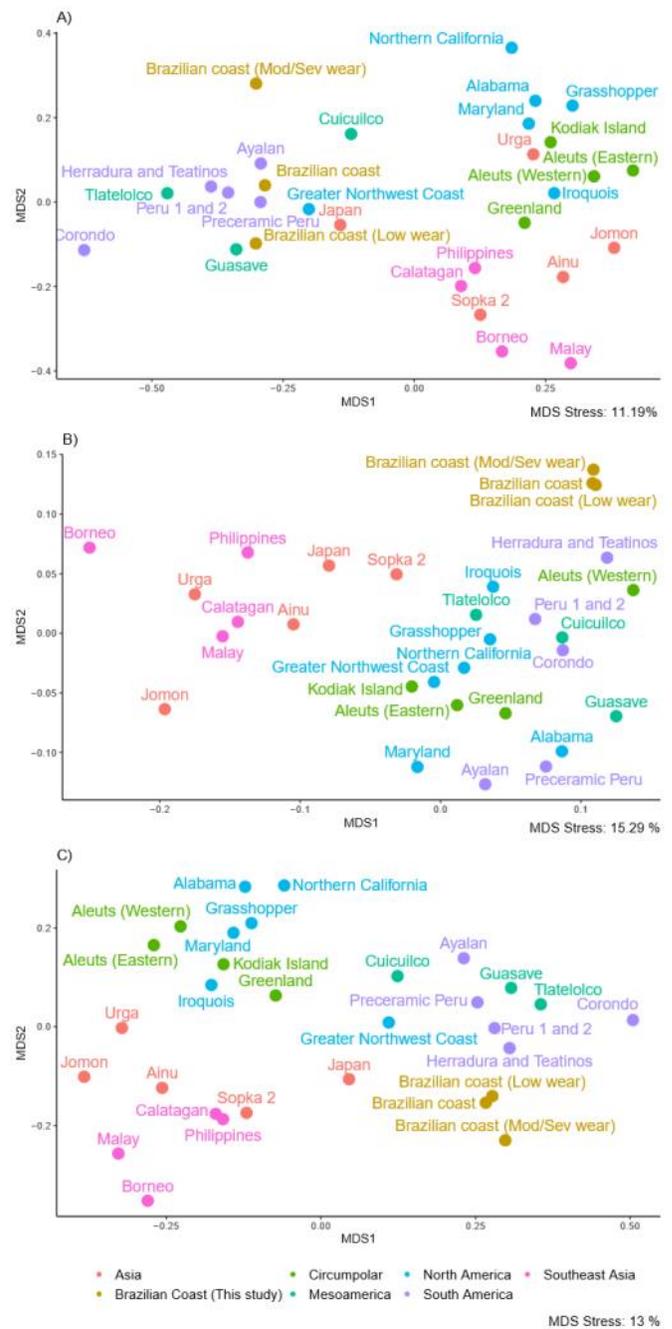


Figure 4. Kruskal non-metric multidimensional scaling of the Mean Measure of Divergence using: A) 7 wear-biased ASUDAS traits; B) 10 Unbiased ASUDAS traits; C) 16 ASUDAS traits (9 wear-unbiased and 7 wear-biased traits).

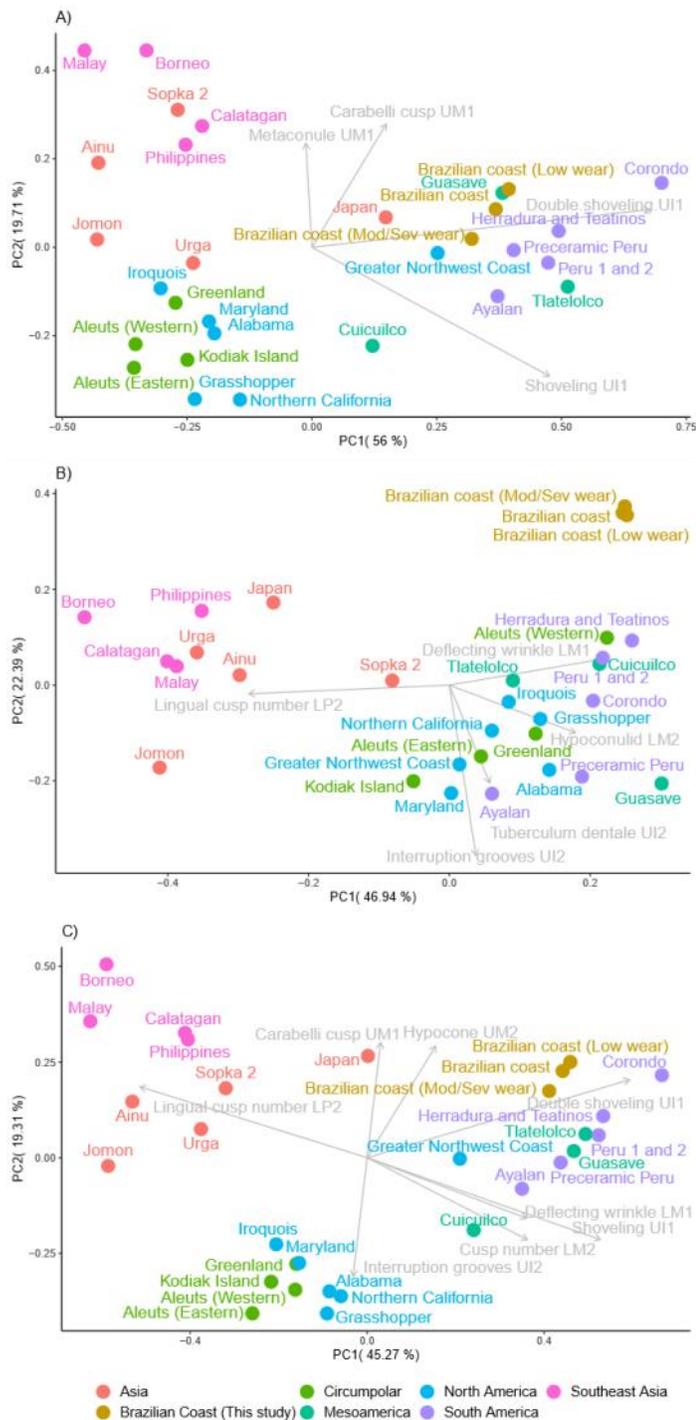


Figure 5. Principal component analysis. Gray arrows show variables most correlated ($r > |0.5|$) with each axis. A) 7 wear-biased ASUDAS traits; B) 10 Unbiased ASUDAS traits; C) 16 ASUDAS traits (9 wear-unbiased and 7 wear-biased traits).

Discussion

The results show that several traits in the Brazilian series present significantly different frequencies between low and moderate/severe wear groups (shoveling UI1, double shoveling UI1, metaconule UM1, Carabelli cusp UM1, hypocone UM2, anterior fovea LM1, protostylid LM1, entoconulid LM1). These differences can result in variation of up to 31.2% (anterior fovea LM1) of the frequency of the traits. However, when some of these traits are included in multivariate analyses along with other traits, this discrepancy is mitigated, as our series appear closely associated to each other in most analyses, despite the significant trait frequency differences among them. Discrepancies in the multivariate analyses are only relatively important when a series is composed exclusively of teeth with moderate/severe dental wear and when all traits show significant dental-wear bias. But even in these cases, our results do not indicate drastically different patterns of morphological affiliation of the Brazilian series. In reality, we see more important deviations from this pattern when biased traits are removed (see Figures 3B and 4B), suggesting that the removal of biased traits may not be always the ideal solution for studies of dental morphological affinities.

The main reason for these discrepant results, where individual traits show significant differences, but they do not impact the overall pattern of morphological affinities in multivariate space is due to the fact that individual trait frequencies have a small contribution to the overall position of the series in the multivariate space. Even though the frequency of traits can vary as much as 31.2% in some traits, this variation is only a small portion of the final distances between group or have a small contribution to principal component score of that group. Given that the wear bias for most variables is smaller than the average difference seen among the reference series (see Table 2), this small contribution of each trait to the final multivariate results does not significantly impact the pattern of morphological affinities among them. In other words, the wear bias in this case represents a small fraction of the total variance seen among series in the data.

These results support that, even though trait frequency differences should not be overlooked, wear-biased traits should still be considered in studies that are trying to contextualize the morphological affinities of series within larger comparative frameworks (i.e., in situations where it is ex-

pected that the wear bias is consistently smaller than the average differences among comparative series; see Table 2). Therefore, our results suggest that it is possible to contextualize better the validity of wear-biased traits in studies of morphological affinities, especially when these traits represent important components of the biological profile of populations. Shoveling UI1 and double shoveling UI1 are two examples of traits that have been noted to be wear biased in different independent studies (Burnett et al., 2013; Stojanowski & Johnson, 2015), including ours. However, they are also very important when characterizing dental variation patterns between Asian and Native American groups (Scott, Schmitz, et al., 2018; Turner II, 1990). When combined with other ASUDAS traits, although biased by dental wear (shoveling UI1, $p=0.043$, double shoveling UI1, $p=0.042$) they did not have a significant impact on the pattern of morphological affinities of the Brazilian series in relation to the Mesoamerica/South America cluster. Therefore, in response to the claims that dental wear may be responsible for the dental variation researchers have found within Native American groups (Turner II, 2006; Turner II & Scott, 2013), we argue that it seems rather unlikely, for it would require several traits to have wear-biased frequencies causing differences of the same order of magnitude of what is observed between continents, which is not the case in our analyses.

Our analyses do not show any strong morphological affinities among Native Americans and Southeast Asian groups (Scott & Turner II, 1997; Turner II & Scott, 2013). In this study, as in previous studies, Brazilian coast series are within the dental phenotypic variation of Native Americans (Turner II & Scott, 2013). This occurs in all multivariate analyses, independent of wear-biased traits, or sub-sampled series based on dental wear grades. This is another argument to take into account when excluding teeth or variables based in dental wear alone. In a large scale of analysis, if wear-bias is not very significant, and series are not composed exclusively by teeth with moderate/severe dental wear, removing worn teeth may cause the removal of important diagnostic traits, potentially resulting in more meaningful changes in morphological affinity patterns than if wear-biased traits are kept in the analyses. This is illustrated well by our analyses using only unbiased traits. Furthermore, this also offers some confidence to the interpretation of multivariate morphological affinities of series for which there is no precise information about their dental wear. Alt-

hough it is often a standard data-collection procedure, not many studies report dental wear grades in dental morphological studies. Our study shows that, although this would be optimal to interpret possible discrepancies between series, it does not imply that such comparisons should not be made when the scale of the variance in the comparison framework is larger than the variance that results from wear bias. Caution must be taken, however, when contextualizing populations within smaller regional contexts, or within populations that share strong morphological affinities, as in these cases the wear-bias can be higher than the differences that define the biological affinities among series. Therefore, the scale of analysis is essential in making the decision of whether to included wear-biased traits, and we recommend that future studies consider the relationship between the variance in the data that can be the result of wear-bias and the variance that is the result of difference between series. As long as the latter is larger than the former, wear-biased traits can be informative of morphological affinities and could be considered in the analyses.

Finally, we agree with previous claims that dental wear is more susceptible to downgrading morphological traits (Turner II, 2006; Turner II & Scott, 2013). Out of the eight identified wear-biased traits, only 25% were biased towards increasing their frequency (2/8), and the remaining 75% (6/8) resulted in the underestimation of the frequencies. As occlusal wear increases, the features of each crown trait become less pronounced, leading the observer to score lower grades, when they should have been scored as not observable. This may occur partially due to the unconscious necessity of an observer to reach substantial sample sizes.

Conclusions

Our study corroborates previous studies showing dental wear bias is a valid concern when analyzing dental non-metric traits, and its assessment should become standard procedure in future studies whenever possible (Burnett et al., 2013). However, while wear-biased traits have an impact on trait frequencies, when combined with other variables, and in a large scale of analysis, its impact may be not meaningful in interpreting the patterns of morphological affinities among series. This impact is directly dependent on the scale of analysis, and regional studies must be more cautious in the inclusion of wear-biased traits, as in contexts with relatively small differences among groups, wear-bias can become meaningful. In other words, the

scale of analysis is a key factor when deciding whether to use wear-biased traits.

We hope this study offers a more optimistic perspective about the impact of dental wear in dental morphological studies and gives a better perspective on how meaningful wear-related bias affects the interpretations of morphological affinities among past populations. Our study suggests that eliminating worn teeth by default may not always be the best solution, since it may exclude important discriminatory variables, or invalidate future studies due to a significant reduction on sample sizes.

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BOOK REVIEW

Bioarchaeology of Marginalized People. Edited by Madeleine L. Mant and Alyson Jaagumägi Holland. Academic Press, an imprint of Elsevier. 2019. 300 pp., \$84.95 (paperback). ISBN: 9780128152249.

This edited volume is among the latest to demonstrate how current bioarchaeological research supports the integration of robust, data-driven case studies with broader anthropological scholarship focused on marginalization. The volume includes chapters that interact with race, class, gender normativity, and sexuality.

Contributions model a range of current best practices in bioarchaeology. This includes advancing North American research not centering the United States, along with multi-authored and international collaborations. Furthermore, studies present innovative models of public and community engagement that are as varied as the definitions of marginalization employed by contributors throughout the book. Mant and Holland organize the types of marginalization covered in the book into the following categories: peripheralization, a loss of individuality, positivity through difference and the absence of context and forgetting. These are somewhat limiting descriptions, given that contributions present a broad range of conditions of marginalization and how they are constructed, experienced, and change across space and time. The first case in the book sets the tone for the complex ways that marginalization is tied to socio-historical, political and economic processes - including the interplay of local and extra-local dynamics.

"Mummies, memories, and marginalization: The changing social roles of a mummy from ancient to modern times," looks at shifts in meaning attributed to the "Lady Hudson" Roman-era mummy as she moves from the hands of collectors, to museums and ultimately the University of Western Ontario. Encounters with different publics go hand in hand with this circulation that bears on how her identity is shaped, and how people's identities are shaped by their engagement with her. These dynamics are different with collectors and museum goers than they are with university students learning from studying her. Nelson discusses Lady Hudson's marginalization according to her personal, mortuary, curated

and bioarchaeological identities. The extent to which these identities can be fully realized is based on the availability of information about her as an individual, changes in ethical perspectives on the acquisition of human remains and the technologies available for scientific translation. Therefore, her marginalization in various spheres differs according to how she is being engaged and consumed by researchers and various publics across space and time.

The fourth chapter, "Looking into the eyes of the ancient chiefs of shíshálh: The osteology and facial reconstructions of a 4000-year-old high-status family" addresses similar dynamics of marginalization according to how personal and curated identities are shaped. Clark et al. detail a case at the intersection of art, science, and community partnership that demonstrates how interpretations of past populations bear on the identities of descendants. Approaches to conducting research on past populations can contribute to the marginalization of their descendants. With that in mind, Clark et al. adopted a research model that involved bringing visibility to 4000 year-old high-status individuals unearthed at the Kwenten Makw'ali site that involved shíshálh descendants in the excavation, facial reconstruction, and museum exhibition. The presence of archaeologists from the shíshálh Nation emphasizes that the community-research binary implicit in community engagement discourses belies the reality that descendants are also researchers.

Several chapters present cases illustrating how bioarchaeological studies can contribute to anthropological studies of people's relationship to the state and state-society relations. The last chapter by Hackett and colleagues, "Innovation in population health intervention research: A historical perspective," addresses how the past itself can be marginalized in ways that obscure its role in current conditions of the state and disparities that people experience within it. The case centers on treating the past as a "laboratory" from which to collect health and health policy data to understand how continuities and changes in the distribution of resources at local and state levels over time impact health. Heather Battles speaks to marginalization arising from "forgotten historical moments" in her chapter, "In the shadow of war: The forgotten 1916 polio epidemic in New Zealand." One of the many unique contributions that this chapter makes to the volume is a discussion of the relationship that one's body has to the state. Battles demonstrates the context that bioarchaeology provides for understanding the long-term impact of historical events, including

how state formation and bodily formations are constitutive of one another.

Redfern and Hefner's "'Officially absent but actually present": Bioarchaeological evidence for population diversity in London during the Black Death, AD 1348-50" also takes up the issue of how the ability for people to be seen (or not) is impacted by how the state is conceptualized. For instance, Redfern and Hefner note how sexual taboos and normalized historical interpretations of the medieval period foster the "official" absence of African-descendant people." Their study demonstrates how bioarchaeological research can play a unique role in bringing visibility to "forgotten" groups of people through DNA and isotope analysis. Similarly, Shields Wilford and Gowland illustrate how changes in welfare ideologies and policies impacted the health of post-medieval workhouse inmates. Their analysis also highlights how the changes in Poor Laws lead to particularly gendered health disparities, given overseers' willingness to aid single mothers and widows. In "Health in equity and spatial divides: Infant mortality during Hamilton, Ontario's industrial transition, 1880-1912," Ludlow and Hackett also present a case on the gendered aspects of marginalization. Discourses around infant mortality in late 19th-early 20th century Ontario targeted mothers as the cause. However, the authors provide a counter-illustration of how changes in the social and physical environment correlated with diarrheal and respiratory-related infant mortality.

Their findings emphasize the fallacy of ties between health disparities and inherent biological or behavioral differences. Lovell and Palichuk round out gender and health discussions in the volume with "Task activity and tooth wear in a woman of ancient Egypt." Their case focuses on a woman excavated from ancient Mendes (Egypt) with a unique dental wear pattern. Difference between her wear pattern and those found among women in more domesticated contexts suggest that the woman was using her teeth as a tool for a specialized craft. The interpretive possibilities demonstrate how bioarchaeological research fits within current anthropological research that lends to disrupting notions of gender normativity.

Carlina de la Cova's case study of the Terry collection focuses on the significance of the Great Migration to the presence of African Americans in the sample. "Marginalized bodies and the construction of the Robert J. Terry anatomical skeletal collection: A promised land lost," argues that migration reflects the oppression taking place in the South, but

also the embodiment of human agency that led Blacks to seek better social and economic conditions elsewhere. This is an important contribution to scholarship on a well-studied populations largely used to illustrate disparities. de la Cova's chapter also addresses the way that the identities of skeletal collections shape and are shaped by their architects and researchers. Doubeck and Grauer address this latter point in their chapter, "Exploring the effects of structural inequality in an individual from 19th-century Chicago." The authors offer a case study of an individual from the Field Museum's anatomical collection with a unique emphasis on researcher "appreciation." Specifically, the authors use appreciation as a frame for deconstructing the notion that a researcher's engagement with human remains is purely scientific. This complements the detailed social context they provide for the skeleton as part of an agenda to expand our analyses of marginalized populations to include immigrant communities. In "Marginalized by choice – Kayenta Pueblo communities in the Southwest (AD 800-1500)," Debra Martin offers a unique perspective on the agency of marginalized groups in a case focused on the Kayenta of Northeastern Arizona. Researchers interpret these groups to be marginal based on evidence of minimal interaction with surrounding political and ceremonial centers. However, Martin offers a health profile indicating the material benefits of their "inward focus" in terms of social stability, fertility, and flexible subsistence. The Kayenta strategy for navigating the challenges of their physical environment reminds us of the importance of complicating our understanding of marginalization as it relates to human agency.

The volume is at its strongest where studies are presented in ways that lend to theoretical engagement without engaging the theory itself. The use of intersectionality is particularly sloppy, and perhaps needless for framing the presence of Black ancestry in medieval Europe. The sloppiness is not a matter of lacking adherence to a particular definition of intersectionality, which is a matter of debate itself. Arguably, the concept is not necessary for Redfern and Hefner to critique assumptions about: 1) the absence of Black ancestry in medieval European populations and 2) a singular experience among people identified as having Black ancestry. More generally, while definitions of marginalization vary between chapters, its presentation as an exacting force is rather consistent throughout the book. Apart from the Martin and de la Cova chapters, discussions tend not to address how agency factors

into dynamics of marginalization. Readers seeking more direct theoretical engagement can look to other recent volumes such as *Theoretical Approaches in Bioarchaeology*, which includes chapters authored by several contributors to *The Bioarchaeology of Marginalized People*.

None of the book's shortcomings detract from the robust presentation of case studies, models of collaboration and appropriate marginalization of the US in a volume focused on North American bioarchaeology. This is an excellent book for undergraduates and graduates because of the accessible writing on the part of the contributors, and the excellent guidance that the editors provide in the introductory and concluding chapters.

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Dental Anthropology

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