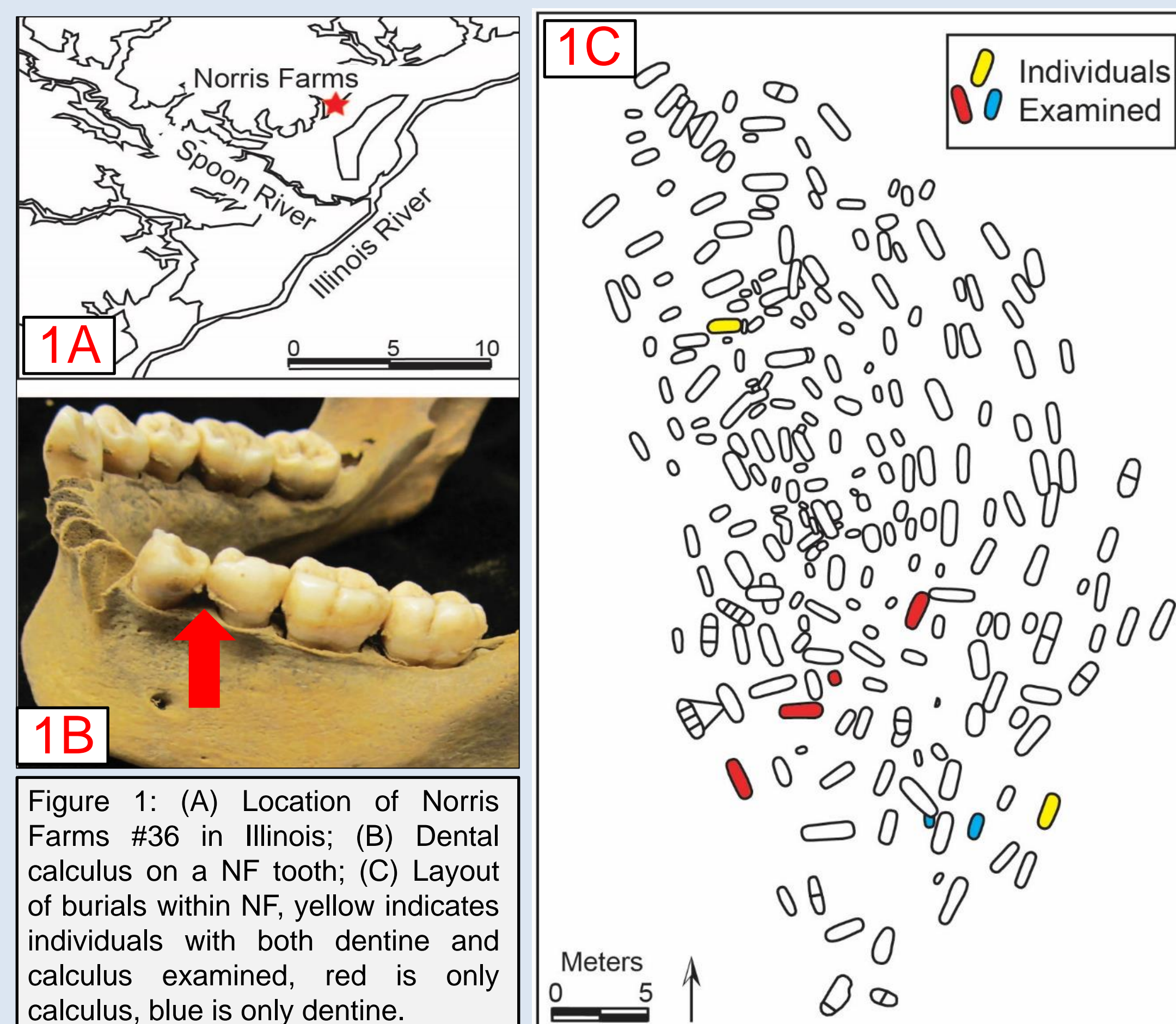


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Introduction

Until recently, ancient DNA research necessitated the destruction of skeletal material in order to extract human genetic information. The destruction of human remains has raised a number of ethical and cultural concerns for researchers and native communities alike. Research to date has shown calcified dental plaque (dental calculus) as being an excellent reservoir for microbial and dietary biomolecules. However, no research has shown it to be a viable resource for host DNA. Here we compare human mitochondrial DNA (mtDNA) preservation from both dental calculus and dentine by way of high-throughput mitogenomic enrichment sequencing. Material from eight individuals (Figure 1) from Norris Farms #36, a Mississippian period Oneota cemetery in Illinois dating back to 700BP, underwent analysis.



Results

Full mitogenomes were recovered from both calculus (7-33X) and dentine (168-342X) with proportions of endogenous DNA ranging from 0.3% to 0.6% and 1.0% to 7.0% respectively. Mitochondrial haplogroups were consistent with previously published data and capture enrichment yielded haplogroup assignments for three samples previously unsuccessful using traditional PCR methods. DNA yields from Norris farms, sequencing statistics and haplogroup assignments are shown in Tables 1-3. Damage plots indicate that DNA from ancient dental calculus is less degraded than DNA from dentine from the same individual (Figure 3).

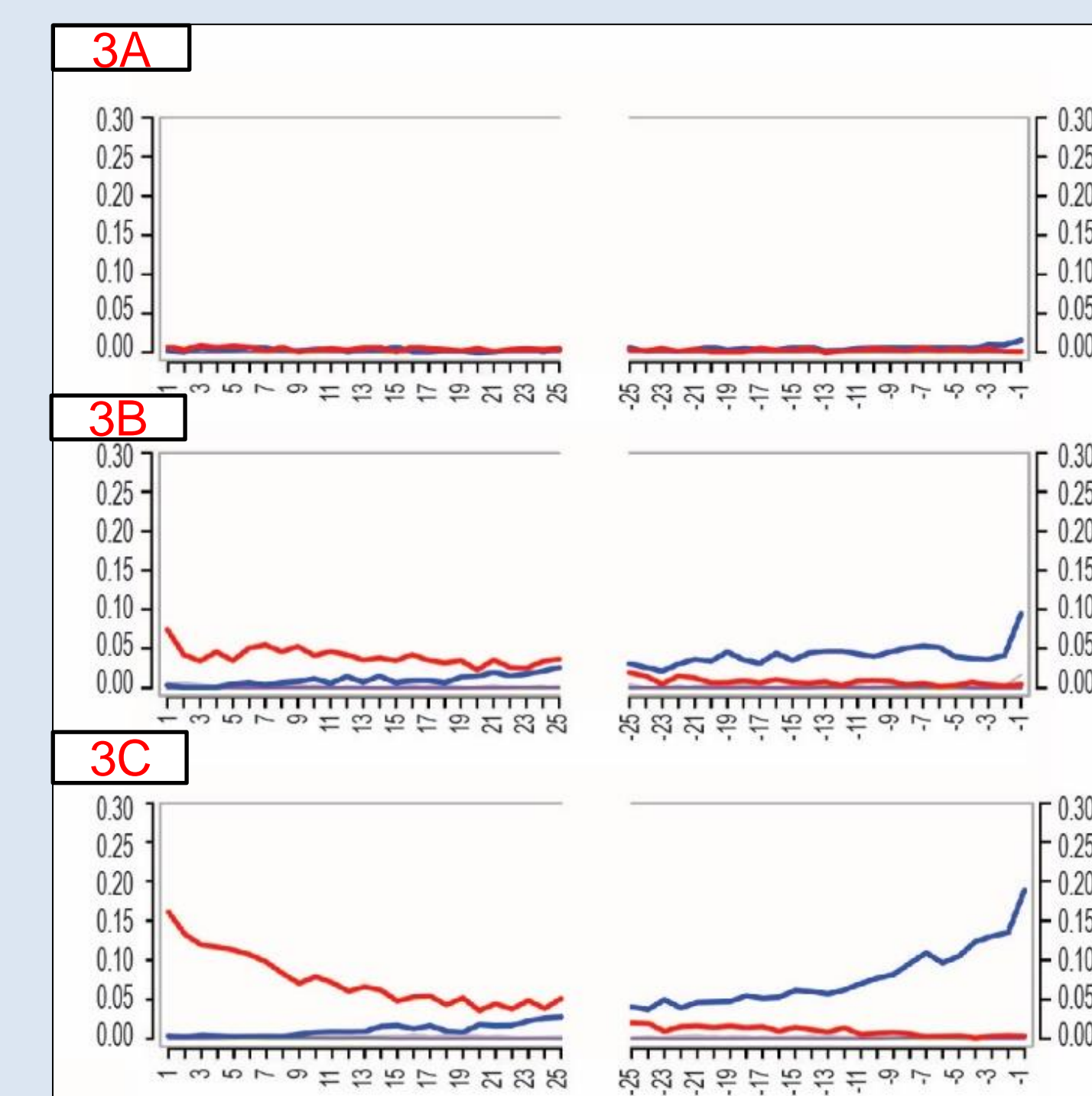


Figure 3: Damage plots for Norris Farms indicating deaminated sites (Red = C to T, Blue = G to A transition): A) Modern calculus; B) NF-217 calculus; C) NF-217 dentine.

Discussion

Dental calculus is an emerging archaeological source of host-associated biomolecules and contains over 200,000,000 microbial cells per milligram. It is abundant within archaeological collections, preserves across long-time periods and is nearly ubiquitous across human populations. Calculus is less porous than dentine and therefore less susceptible to environmental factors and degradation. Despite the low proportion of mitochondrial DNA within dental calculus, its substantially higher total DNA content means that per milligram, dental calculus contains more DNA than dentine. We demonstrate that although dentine contains a larger proportion of endogenous host DNA, dental calculus, even in small quantities (<20mg), is a viable alternative for host genomic material when dentine or bone are either unavailable or too degraded. With NAGPRA allowing federally recognized native American tribes the right to manage their ancestral remains, destructive analysis has become a highly sensitive topic. Here we show that high quality human genomic material can be recovered without any damage to skeletal remains. We hope these findings will reveal new and exciting opportunities for better understanding diverse aspects of the human past.

Methods

Ancient Norris Farms materials were UVed and pulverized in molecular cleanrooms adhering to standard operating procedures. Calculus (n=6) and dentine (n=4) samples were extracted using phenol-chloroform or Dabney et al. 2013 (Figure 2A). Resulting DNA was built into dual-indexed shotgun libraries. Libraries were amplified and quantified before undergoing an in-solution enrichment for human mtDNA (Figure 2B-C). Enriched libraries were sequenced using Illumina MiSeq V2 2x150bp chemistry (Figure 2D).

Sample ID	Sample size (mg)	Normalized DNA yield (ng/mg)	Total DNA yield (ng)
Dental Calculus			
NF-15c	23.0	12.5	286.8
NF-47c	16.0	37.5	571.2
NF-95c	22.9	19.6	449.4
NF-108c	21.3	69.0	1470.0
NF-217c	25.0	30.0	750.0
NF-262c	23.1	42.1	972.0
Dentine			
NF-47d	97.4	0.1	6.8
NF-217d	87.2	0.6	49.0
NF-53d	100.0	1.1	105.6
NF-293d	100.0	0.3	35.3
Non-Template Control			
N	-	-	-

Table 2: Sequencing statistics and sample quality metrics for mtDNA captured libraries

Sample ID	Total analysis ready reads	Total mtDNA reads	Unique mtDNA reads	Proportion endogenous mtDNA	Mean mtDNA coverage
Dental calculus					
NF-15c	507,781	4,187	1,598	0.35%	6.8
NF-47c	327,874	6,761	1,524	0.55%	7.1
NF-95c	1,283,872	29,838	3,145	0.36%	13.9
NF-108c	1,999,869	44,572	3,594	0.29%	21.4
NF-217c	2,036,850	72,331	4,239	0.34%	21.4
NF-262c	1,752,516	57,484	6,220	0.56%	33.8
Dentine					
NF-47d	4,066,147	1,521,276	33,572	1.15%	325.2
NF-217d	3,575,314	666,742	29,615	1.59%	169.7
NF-53d	405,553	172,723	29,013	6.96%	218.2
NF-293d	3,085,147	1,466,777	32,935	1.04%	341.5

Table 3: Mitochondrial haplogroup assignment of Norris Farms samples

Sample ID	Haplogroup assignment	Haplogroup rank	Stone and Stoneking (1998)
Dental Calculus			
NF-15c	A2f1	0.979	-
NF-47c	D1	0.921	D
NF-95c	B2	0.936	B
NF-108c	A2f1	0.998	A
NF-217c	A2+(64)+@153	0.904	-
NF-262c	B2	0.921	-
Dentine			
NF-47d	D1	0.961	D
NF-217d	A2+(64)+@153	0.937	-
NF-53d	X2a1a	0.907	X
NF-293d	X2a1a	0.939	X

Ozga AT, Nieves-Colon MA, Honap TP, Sankaranarayanan K, Hofman C, Milner G, Lewis Jr. CM, Stone AC, Warinner CW. Successful enrichment and recovery of whole mitochondrial genomes from ancient human dental calculus. *American Journal of Physical Anthropology* DOI:10.1002/ajpa.22960

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