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Stable dietary isotopes and mtDNA from Woodland period southern Ontario people: results from a tooth sampling protocol

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ABSTRACT

Bioarchaeological research must balance scholarly commitment to the generation of new knowledge, descendants' interests in their collective past, and the now common practice of rapid re-interment of excavated human remains. This paper documents the first results of a negotiated protocol built on the retention of one tooth per archaeologically derived skeleton, teeth that can then be used for destructive testing associated with ancient DNA and stable isotope investigations. Seven archaeological sites dating from the 13th to 16th centuries provided 53 teeth, 10 of which were subdivided between DNA and isotope labs. All tooth roots yielded haplogroup results, and five provided more detailed sequence results. Stable isotopes of carbon and nitrogen document heavy reliance on maize among all individuals, as well as reliance on a diverse range of fish. This work establishes baseline mtDNA information for Northern Iroquoians, and confirms the value of using dental tissues for dietary reconstruction. Particularly when human remains are fragmentary or co-mingled, this approach holds promise for ongoing incorporation of bioarchaeology into reconstructions of past peoples' lives.

1. Introduction

The lower Great Lakes of North America were the focus of dramatic cultural developments prior to European contact. The Transitional to the Late Woodland period, ca. 1500 to 350 BP, saw the development of distinctive Iroquoian and Algonquian speaking cultural groups. Southern Ontario was the home of the Wendat (known to the French as the Huron), the Tionontaté (Petun), and the Attiwandaronk (Neutrals), all of whom spoke Northern Iroquoian languages, as well as a number of Algonquian-speaking groups including the Odawa, Nipissing, and Algonquian (Fig. 1). There were interactions, as well, with the Iroquois Confederacy (Haudenosaunee) who lived in clustered tribal groupings across central New York State and included (from west to east) the Seneca, Cayuga, Onondaga, Oneida and Mohawk. All of these nations had unique cultural traits and histories owing to their geographic separation and development in distinct tribal territories, which they continued to occupy into the historic period.

Northern Iroquoian society often forms a distinct focus of Late Woodland archaeology. In Ontario, the Late Woodland period is subdivided into Early (A.D. 900–A.D. 1300), Middle (A.D. 1300–A.D. 1400) and Late Iroquoian Periods (A.D. 1400–A.D. 1650). Maize

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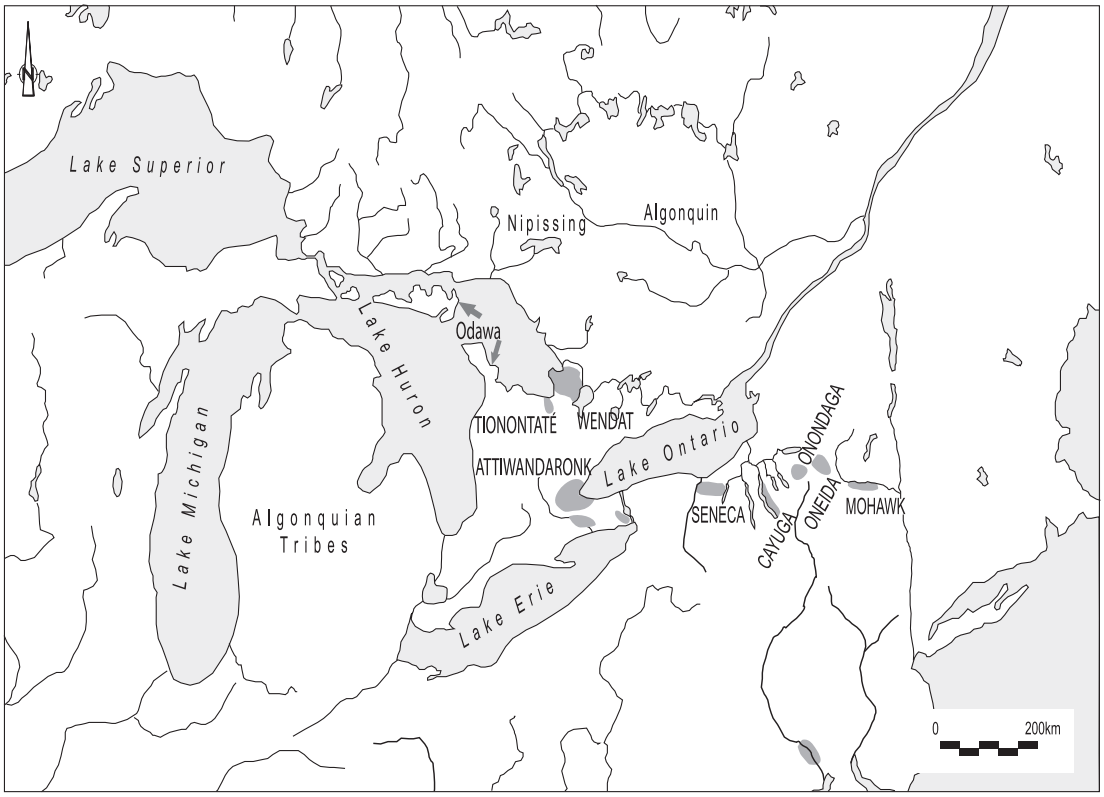

Fig. 1. Distribution of First Nations populations of the Great Lakes, prior to disruptions associated with European contact. Labels in CAPS indicate broader language groups of the region.

agriculture was an important organizing principle as large palisaded base settlements around which maize was grown developed from about A.D. 1000 onward (Pihl et al., 2008). Around the turn of the fourteenth century, a fully developed agricultural system including large, year-round occupied villages, new socially integrative institutions and distinctive material culture all point for the first time to a recognizably "Iroquoian" pattern as described in early European reports of their life. Population growth followed (Warrick, 2008). By the mid-fifteenth century, communities were coalescing in the face of violent conflict. By the early sixteenth century, these communities had consolidated with populations between 1500 and 2000 people with new fully integrated identities (Birch and Williamson, 2013). One of the distinguishing socially integrative features of ancestral and historic Huron-Wendat populations was the Feast of the Dead, associated with secondary burial in ossuaries of as many as 500 deceased community members (Williamson and Steiss, 2003).

Early to mid-seventeenth century accounts from explorers and missionaries provide information that can form a basis for hypothesis building (Thwaites, 1896-1901; Trigger, 1976). This approach must be supplemented, or even challenged, when sites are several hundred years older and when topics of interest are beyond the scope of historic observations.

Among First Nations descendant communities, interest in ancestral populations is growing. Permission to study the remains of archaeologically discovered ancestors is often granted, contingent on conditions that only small fragments of human tissue be retained and that questions deemed pertinent to the descendants be addressed. In part because of the analytical limitations of comingled ossuary remains, a protocol of retaining one tooth per person is a pragmatic approach. The approximate age and the sex of the person can be estimated from the morphology of the maxilla or mandible from which a permanent molar is taken, or the age in the case of a deciduous tooth. Insofar as modern teeth are removed as part of dental treatment and tooth loss is seen as a typical life experience, teeth are rarely seen as being imbued with spiritual power, so that this approach is acceptable to many descendants. The research described here arose as an exploratory project, designed to maximize the relevant information that could be obtained from a single human tooth. This project also marks the first opportunity to examine the mitochondrial DNA composition of ancient Northern Iroquoian speakers. Relatively little is known about the relationship of these groups to other aboriginal populations of Northeast North America. As the mtDNA of Algonquian groups of this region becomes better known (Dewar et al., 2010; Shook and Smith, 2008), information about northern Iroquoians is a missing piece of the puzzle.

The teeth come from excavations in which the human remains were reinterred after brief descriptive study and tooth sampling. In some cases, study was limited to in-ground examination. One tooth per person was retained with permission of the relevant First Nations groups, following a consultative process consistent with the Ontario Cemeteries Act. Each archaeological site in the study illustrates different research opportunities and challenges. Only one tooth from one site is of probable Attiwandaronk ancestry; all others are probable Wendat. One site, Staines Road, provided numerous teeth but they are from a disturbed context that compromised the generation of population information. Another site, Mantle, yielded two different contexts for human remains: a formal cemetery of primary interments and loose teeth found within the living site. Despite varying contexts, study of the teeth can generate information about the proportion of the diet that came from maize and the sources of animal protein. Despite differences in the excavation contexts, the study provides

methodological information about the isotopic offsets among human collagen, dentin and enamel tissues in a consistent and comparable format.

This study also provides information about the mitochondrial DNA of the ancestors of Northern Iroquoian speakers, contributing to our understanding of the peopling of Northeast North America. North and South America are home to some of the earliest studies of mtDNA and its distributions. The first four haplogroups identified (A, B, C, and D), based on differences from the Anderson or Cambridge Reference Sequence, are found in both modern and ancient native populations. Two more mtDNA lineages have been described in ancient remains from the Americas, haplogroups X (Malhi and Smith, 2002), and M (Malhi et al., 2007) which have so far been reported only in North America, but the latter haplogroup has not been reported in any modern population. Together, these six haplogroups are thought to comprise the vast majority, if not all, of New World mtDNA diversity.

Prior studies of dietary patterns using carbon and nitrogen isotopes from bone collagen and dental tissues have documented a rather rapid adoption and ultimately heavy reliance on maize (Katzenberg et al., 1995; Schwarcz et al., 1983, 1985; van der Merwe et al., 2003b). Investigating the nature of the transition to maize horticulture is important not only to answering questions regarding nutrition and health, but also to understanding the evolution of Iroquoian cultural complexity. High proportions of maize, perhaps high enough to be detrimental to health, may have been transient (Katzenberg et al., 1995; Schwarcz et al., 1985; van der Merwe et al., 2003b). Recent studies also report that neighbouring, non-Iroquoian groups ingested substantially more maize than would be expected in that their settlement systems lack year-round occupied villages (Dewar et al., 2010; Watts et al., 2011). These possibilities introduce questions about the temporal and spatial patterning of dietary regimens. Early work on isotopic dietary signals was based on bone collagen. This is a protein and as such, the carbon it contains comes primarily from protein foods, with some contribution from dietary carbohydrates and fats. The carbon isotope signal in collagen is therefore skewed in favour of protein foods. In this region, riverine, lacustrine and anadromous fish species were important sources of dietary protein. Their diverse isotopic values make the interpretation of human protein-based tissues challenging. Wishing to clarify the dietary carbohydrate contribution of maize to Iroquoian communities, researchers have explored apatite values from bone (Harrison and Katzenberg, 2003) and tooth crowns (van der Merwe et al., 2003b). The latter study of the early fourteenth century Moatfield ossuary proposed a design that focuses on dental tissue, including tooth enamel apatite and collagen from dentin. The potential for distinguishing isotopic differences between individuals who relied on different food sources or who grew up in different regions introduces the possibility of tracking local group identity, especially during periods of coalescence. The dynamic nature of the cultural landscape also generates questions about population origins and relationships.

2. Materials and methods

2.1. The archaeological sites

The teeth used in this study were derived from seven archaeological sites located in southern Ontario (Table 1, Fig. 2).

The Damiani site (Borden designation AlGv-231) is a 1.5 ha late fifteenth century village situated in the upper reaches of the Humber River in the City of Vaughn comprising 22 long houses, a palisade, and hundreds of features. Its location, structure and artifactual assemblage indicate Wendat cultural affinity. Five burial features were excavated by Archaeological Services Inc. (ASI) in 2009. Of the five, only one was of an adult (burial 2). The burial of infants and juveniles beneath house floors is a common feature of Iroquoian sites. Teeth available for study include a second maxillary molar from the adult woman and a deciduous maxillary molar from a child of about three years of age.

Table 1
Archaeological sites providing human teeth for this study.

Site name	Borden number	Approx. Date	Sources
Damiani	AlGv-231	AD 1450–1500	ASI 2012
Hidden Spring	AlGu-368	AD 1450–1500	ASI 2010
Hutchinson	AkGt-34	Mid-14th century	Robertson 2004
Mantle	AlGt-334	AD 1500–1530	Birch and Williamson 2013
Staines Road	AkGt-65	Late 13 century	Williamson and Steiss 2003
Teston Road	AlGv-2	AD 1450–1500	ASI 2005
Wainfleet	N/A	Late Woodland	ASI 2009

The late fifteenth century Hidden Spring site (Borden designation AlGu-368), excavated by ASI in 2008, is located about 15 km east of Damiani in the Don River watershed and is from a similar time period. Portions of the two long houses that were excavated included three burial features. Each feature included at least some poorly preserved adult remains, as well as juveniles in two of the three. Sex and specific ages at death remain unknown. The sampled teeth include three first permanent molars (two mandibular and one maxillary), one maxillary third molar and, from one of the juveniles, a deciduous second mandibular molar. In two of the adult teeth, some adhering alveolar bone was also available.

The fourteenth century Hutchinson site (Borden designation AkGt-34), excavated by ASI in 2001, is located along the Rouge River in the City of Toronto, Ontario. Two long houses were identified along with two small separate mortuary areas that contained intact burials as well as fragmentary skeletal remains of at least twelve individuals. It has been proposed that the site was used to prepare the dead for burial (Robertson, 2004). Given its immediate proximity to the Staines Road ossuary and their similarity in age, it is possible this was a preparation site for that ossuary. One tooth from each of five adults, plus alveolar bone from one of the five, were available for study. Sex and specific ages at death remain unknown.

The early sixteenth century Mantle site (Borden designation AlGt-334), located near Stouffville, Ontario, York Region, is the most structurally complex of the sites in this study. Excavations by ASI were undertaken from 2002 to 2005 (Birch and Williamson, 2013). The 2.94 ha occupation site included 98 overlapping long houses, 51 of which may have been occupied at one time (Birch and Williamson, 2013:78), providing housing for 1500–2000 people. From fragmentary human remains found within the village, nine permanent teeth were available for study. A sampling preference for right first molars combined with dispersal of teeth from across this very large site makes double sampling of any one person very unlikely. Mantle represents the coalescence of multiple small villages into one well-planned and well-integrated community (Birch and Williamson, 2013:53–64). Beyond the village palisade was a cemetery with at least thirty-four primary interments. Exceptionally cosmopolitan artifacts from the village, combined with single, primary interments – an interment style unusual for ancestral Wendat – contribute to a conclusion that people from quite far away may have lived and died at Mantle. Analysis of the skeletons was limited to only on-site observations, plus the removal of one tooth from a number of the adult burials. Samples include the maxillary first molar from one woman, and three first molars and one second premolar from each of four men.

The Staines Road ossuary (Borden designation AkGt-65) was discovered at a locale adjacent to the Hutchinson site in northwest Scarborough (eastern metropolitan Toronto). Investigated and

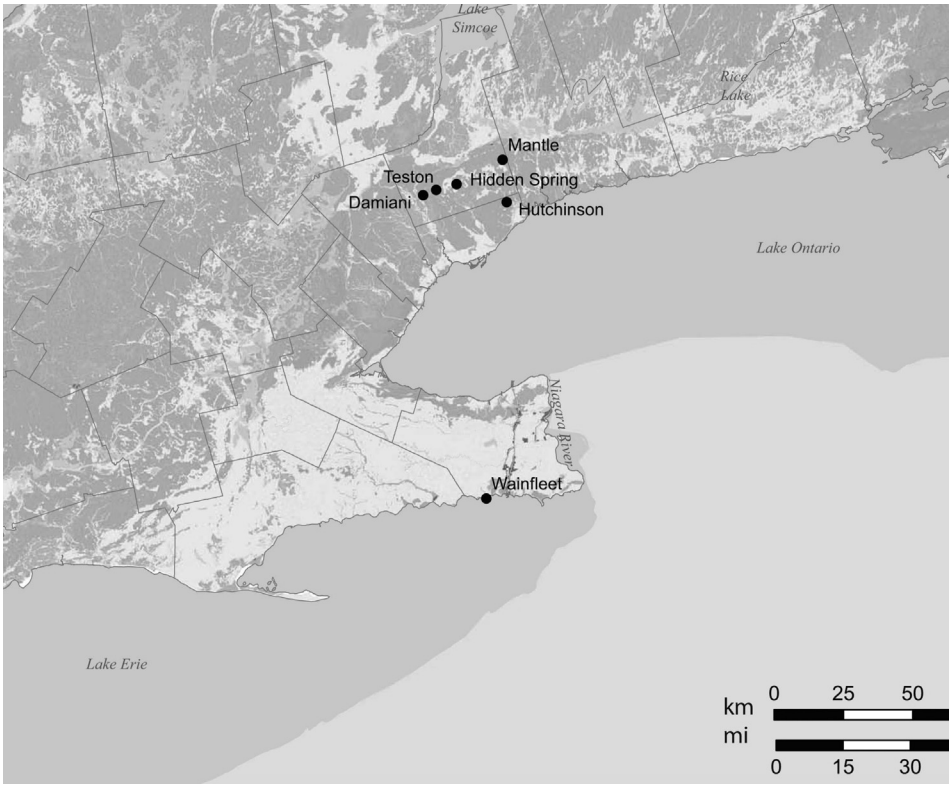

Fig. 2. Regional map illustrating site locations. Shading reflects surficial geology, lighter tones being clays and darker tones primarily till soils, highly relevant variables to communities dependent on maize crops.

excavated by ASI in 2001, this ossuary of at least 308 individuals had been removed from its original location, and fragmented, perhaps mechanically, then mixed into a pile of soil fill and modern garbage. Its aboriginal origin was confirmed through a radiocarbon date from bone collagen (Beta-156359) and the associated $\delta^{13}C$ value of −11.3. The date between cal B.P. 920 and 680, which must apply to all of the remains as most Wendat ossuaries are formed during one ceremonial event (Williamson and Steiss, 2003), makes the site a very early example of ossuary burial, so its large size is especially surprising. Soil adhering to the human remains as well as site taphonomy suggests the original ossuary pit was adjacent to the deposit. Of the numerous loose teeth that have been retained from this large collection, thirteen right first maxillary molars and two left first molars were chosen for this study. Sex and specific ages at death remain unknown.

The mid- to late fifteenth century Teston village site and ossuary (Borden designation AlGv-2) are located on the west bank of the West Don River in the City of Vaughan. While the village is situated on flat, high tableland, the ossuary is located on a small knoll on the tableland, one to two hundred metres to the southwest of the village (the precise limits of which are not known). The ossuary was discovered in 2005 during construction associated with the widening and relocation of Teston Road. It was found to have a diameter of 280 cm and only a portion of the feature was disturbed. From these disturbed materials a number of permanent maxillary molar teeth were retained for future study, consistent with the site disposition agreement. Displaced remains were returned to the ossuary and it has been permanently protected. Ossuaries of this size have been known to contain the remains of 300–400 individuals (Williamson and Steiss, 2003).

A maxillary first permanent molar from the Wainfleet site has also been included. It comes from an isolated burial of a man investigated by police and documented by ASI in 2009, in Wainfleet, Ontario, Niagara Region. The locale and associated artifacts suggest that this burial is ancestral to Attiwandaronk (Neutral) people.

2.2. Documentation

Prior to destructive testing, dental measurements were taken of maximum dimensions, as well as analogous dimensions at the cement-enamel junction. Each tooth was photographed in a standard format with scale from two perspectives, and a negative impression was made of each crown. A selection of ten teeth from four of the sites was sent to the Molecular Anthropology Laboratory at the University of California in Davis, California, where portions of the roots (approximately 0.5 g) were removed for analysis. The tooth crowns were returned to the University of Toronto, from which the entire sample of 53 teeth was transported to the University of Cape Town Archaeometry Laboratory for isotopic analysis.

2.3. Ancient DNA methods

The Molecular Anthropology Laboratory (MAL) at the University of California in Davis, California, maintains a positively pressurized clean room that is separated from the modern DNA extraction and PCR laboratories and to which access is strictly controlled. Precautions to minimize risk of contamination at every step of the extraction process include UV irradiating all supplies, performing duplicate independent extractions from, and amplification of, each sample, daily bleaching of all surfaces and tools within the room, use of protective clothing and running negative controls at each step. The mtDNA haplogroup and HVSI&II control region sequences

of anyone having any contact with the samples and aDNA facilities in which the analyses were conducted were noted, to ensure that any source of laboratory contamination of the aDNA samples could be identified.

The MAL conducted two independent DNA extractions on each sample, following the standard protocols described in Kemp et al. (2006). Each sample was cleaned with 6% sodium hypochlorite (full strength household bleach; Kemp and Smith, 2005), and soaked in 2 mL of molecular grade 0.5 M, pH 8.0, EDTA (Gibco) for at least 48 h (an average time of seven days) to remove calcium. Three milligrams of Proteinase K (Invitrogen, Fungal Proteinase K) was added to each sample and incubated at 65 °C overnight (an average time of 18 h). A three-step phenol-chloroform extraction was carried out including two extractions with equal volumes (2 mL) of phenol:chloroform:isoamyl alcohol (24:24:1; OmniPur), followed by one extraction with an equal volume of chloroform:isoamyl alcohol (24:1; Amresco). The DNA was precipitated in a solution of isoproponol and 5 M ammonium acetate overnight (for at least 12 h) to remove PCR inhibitors. The DNA was pelleted by centrifugation at 3100 rpm for at least 45 min, washed in 1 mL of 80% purified ethanol, centrifuged for at least another 45 min, and allowed to air-dry for 15–20 min. Each sample was purified using a Wizard PCR Preps Purification System (Promega) following the manufacturer's instructions, save for the final elution of the DNA using 1200 ul of ddH_2O.

Regions containing the mutations diagnostic of haplogroups A, B, C, and D were amplified at least twice from each extraction using the polymerase chain reaction (PCR) and primers, cycling conditions and methods cited in Kemp et al. (2006). After amplification of a PCR product of the expected size was confirmed by electrophoresis on 6% polyacrylamide gels, the DNA samples were digested with the Hae III (for haplogroup A) and AluI (for haplogroups C and D) restriction enzymes (see Kemp et al., 2006 for further detail). The amplicons were incubated for at least 4 h at 37 °C and then run on a gel to check for digestion. Visual inspection of digestions for each sample reveals the presence or absence of the restriction sites characteristic of haplogroup A, C, or D. Haplogroup A is identified by a Hae III site gain at np 663, haplogroup C by an Alu I site gain at np 13261, and haplogroup D by Alu I site loss at np 5176. Haplogroup B, identified by a 9-base pair deletion in region V of the mtDNA genome, is confirmed by its shorter fragment length after 6% polyacrylamide gel electrophoresis. Haplogroup X was not tested for using restriction fragment analysis, as it would have been indistinguishable from M. Snow who conducted the analysis and belongs to haplogroup X. As all of the samples were identified as belonging to other haplogroups, and none of the sequences demonstrate any haplogroup X diagnostic markers when sequenced, this was not problematic to this research.

Fisher's exact tests were conducted using Genepop (Raymond and Rousset, 1995) to compare haplogroup frequency distributions from relevant pairs of populations. Interpretation of the results were completed with the sample size in mind, particularly how it may influence the statistical significance of haplogroup similarities between populations. Samples that could be assigned a haplogroup were sequenced to independently confirm their haplogroup assignments and provide more detailed genetic characterization of similarities among these samples and those from other populations.

Sequencing was carried out by amplifying four overlapping 150-bp segments of the D-loop of the mtDNA control region from position 16045–16394, according to the Anderson reference sequence (Anderson et al., 1981). Samples were sequenced using an ABI 3130 DNA sequencer. The primers and parameters used throughout this process are those given in Kemp et al. (2007). The completed sequences were analyzed and concatenated using the Sequencher and Mesquite (Maddison and Maddison, 2007) computer programs and their mutations were compared with those expected based on their haplogroup assignments to confirm those assignments. Samples assigned to haplogroup A and B are expected to exhibit most of their haplogroup's characteristic HVSI mutations, described in Table 5.

The DNA sequences containing derived mutations were included in a haplotype median joining (MJ) network constructed using default parameters in Network 4.6.1.1 (Bandelt et al., 1999) together with other samples cited in Table 5. The MJ Network predicts the order in which specific mutations occurred that differentiate samples from the basal haplotype(s) in the network. Sequences that share derived mutations appear on the same branch of the network and reflect close ancestor/descendant relationships.

2.4. Dietary stable isotope methods

Three different types of tissue were prepared and analyzed for this research: bone, enamel and dentin. The laboratory work was done in large part by a group of six students during a University of Toronto "Science Abroad" course, hosted by the University of Cape Town. To analyze the $\delta^{13}C$ and $\delta^{15}N$ in the collagen of bone and dentin, the collagen was extracted using standard procedures employed for many years in the stable isotope laboratory at the University of Cape Town (Sealy, 1997, 2010). Small chunks (with diameters of a few millimetres) of dentin or bone are surface-cleaned with fine sandpaper, decalcified in ca. 2% HCl, rinsed in distilled water, then treated overnight with 0.1 M sodium hydroxide to remove humic acids. If rootlets are present, they are removed from the wet collagen by hand, using fine tweezers. The collagen samples are soaked in distilled water until the pH remains unchanged, then freeze-dried. The dry collagen is weighed and the % yield (% of the starting weight of whole bone or dentin) calculated. Approximately 0.6 mg of dry collagen is weighed into a tin cup that is tightly folded to exclude air. Each sample is analysed in duplicate. The tin capsules are loaded into an automated elemental analyzer and combusted at 1020 °C. The resultant gases are introduced into a Thermo-Finnigan Delta Plus XP or a Delta V Plus mass spectrometer via a continuous flow (Conflo) inlet, using helium as the carrier gas. Standard deviations of repeated measurements of a homogeneous laboratory standard (Merck gelatine, $n = 12$) are less than 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Results are reported relative to international standards Vienna PDB and AIR.

The $\delta^{13}C$ in tooth enamel carbonate was prepared and analysed as follows: For each tooth, a diamond-tipped drill was used to burr a line on either the buccal or lingual surface. This line extended from the occlusal surface to the cement–enamel junction, in order to sample tooth enamel formed over the duration of development of the crown. Care was taken to remove only enamel and not the underlying dentin. About 5 mg of the resultant enamel powder is collected and transferred to a microcentrifuge tube. In the stable isotope laboratory, the pre-treatment protocol used is that of Lee-Thorp et al. (1997), with slight modifications, as specified below. The powder is reacted with 1.75% sodium hypochlorite for 45 min, then washed three times with distilled water. It is treated with 0.1 M acetic acid for 15 min to remove diagenetic carbonates, then again washed three times with distilled water and freeze-dried. The pre-treated powders are analysed on a Finnigan-MAT 252 stable isotope ratio mass spectrometer coupled to a Finnigan Gas Bench II. Carbon dioxide is produced from enamel apatite by reaction with 100% phosphoric acid at 72 °C, then swept in a stream of helium through hygroscopic Nafion™ tubing to remove water, then through a glass capillary gas chromatograph, a second water trap, and finally into the mass spectrometer. The standard deviation of repeated measurements of homogeneous material (NBS 18, $n = 12$)

is lower than 0.2‰ for $\delta^{13}C$, and frequently lower than 0.1‰. Results are reported relative to the international standard Vienna PDB.

3. Results

3.1. Ancient DNA

All ten of the teeth that were sent to the MAL, were assigned to three of the six Native American haplogroups (haplogroups A, B, C), and for seven (70%) of the samples, this haplogroup assignment was replicated to ensure the extracted DNA was endogenous to the sample (Table 2). In no instance was there any contradiction between the first and second extraction. Due to the nature of this study and clear in-house confirmation of results, replication in a separate lab was not deemed necessary.

Five (50%) of the ten samples belonged to haplogroup A, four (40%) belonged to haplogroup B, and a single sample (10%) belonged to haplogroup C. The sample size is too small to allow for statistical comparison of these results to other populations in the region, as sampling bias would play a significant role.

It was possible to obtain replicated sequence data from the first hypervariable region for five (50%) of the 10 samples (GenBank accession numbers KF536720–KF536724). Four of these samples belonged to haplogroup A and were sequenced between np 16037–16403. A single haplogroup B sample was sequenced between np 16058–16399. The haplogroup B sample carried a shorter sequence range due to a failure to replicate sequence data at the primer pairs that covered the final portion of the HVR.

The sequences were compared to other modern and ancient samples from populations in the region (see Table 3) according to which haplogroup they were assigned. No exact matches were detected between the samples presented here and other populations in the region.

Of the four haplogroup A samples, all carried an additional mutation at np16325 (Table 4). This mutation is shared with thirty-seven other samples from the region, including samples from: Norris Farms, Wisconsin Chippewa, Sisseton/Wapheton Sioux, Quapaw, Micmac, Cheyenne, Wisconsin Chippewa, Turtle Mountain Chippewa, and Minnesota Chippewa. This mutation is found within many different populations from a broad region, and several different language families, and therefore provides limited information about any population relationships the Iroquoian haplogroup A samples may bear.

Among the haplogroup A samples, two samples carried mutations with np 16111: Wainfleet and Damiani B2 (Table 4). The mutation at np 16111 is typical of the Native American haplotype A2, which is one of the founding Native American lineages in the New World and found throughout many populations. Three samples exhibited derived mutations with the potential to reveal ancestor/descendant relationships when compared with other samples and all three sequences were confirmed by replication. The haplogroup A sample Mantle B8 carried additional derived mutations at np 16377, 16401 and 16403. Teston Road sample 1 also carried a derived mutation at 16051. The single haplogroup B sample, Mantle B24, was defined by the typical haplogroup B mutations at np 16189 and 16217, and also carried the additional mutations at np 16182 and 16223.

The MJ networks for haplogroups A and B are given in Figs. 3 and 4, respectively. These networks include the three samples cited above with derived mutations (Mantle B8 and Teston Road1 for haplogroup A and Mantle B24 for haplogroup B) together with reference samples cited in Table 3. The three derived mutations in Mantle B8 (16377, 16401 and 16403) define a branch of the

Table 2
Summary of results from the ten tooth roots sampled for mitochondrial DNA.

Sample ID	Haplogroup	Replication	Sequence	GenBank accession number
Mantle B2	C			
Mantle B7a	B	X		
Mantle B7b	B			
Mantle B8	A	X	X	KF536720
Mantle B24	B	X	X	KF536721
Teston Rd 1	A	X	X	KF536722
Teston Rd 2	B	X		
Damiani B4	A			
Damiani B2	A	X	X	KF536723
Wainfleet	A	X	X	KF536724

Table 3
North American populations used for comparison with haplogroup A and B sequences generated. Data was collected from Shook and Smith (Shook and Smith, 2008), and Bolnick and Smith (2007).

Population (abbrev.)	Sample name	N	Ancient	Language	Haplogroup
HIND (Glacial Kame)	Hind	3	Y		A
Morse	Morse	1	Y		A
Orend	Orendorf	4	Y		A
MNC	Minnesota Chippewa	4		Algonquian	A
TMC	Turtle Mountain Chippewa	55		Algonquian	A
WCH	Wisconsin Chippewa	24		Algonquian	A
Cheyen	Cheyenne	9		Algonquian	A
CheyenArap	Cheyenne/Arapaho	2		Algonquian	A
ChippSW	Chippewa SW	2		Algonquian	A
Delaware	Delaware	1		Algonquian	A
Iowa	Iowa	1			A
Kikapoo	Kikapoo	2		Algonquian	A
Micmac	Micmac	2		Algonquian	A
Orcche	Oklahoma Red Cross Cherokee	4		Iroquoian	A
Pawnee	Pawnee	1		Caddoan	A
Potowato	Potawatomi	1		Algonquian	A
Quapw	Quapaw	1		Siouan	A
Sac&Fox	Fox	1		Algonquian	A
Shawnee	Shawnee	2		Algonquian	A
StilcCh	Stillwell Cherokee	2		Iroquoian	A
SWSioux	Sisseton/Wapheton Sioux	9		Siouan	A
Wchippew	Wisconsin Chippewa	1		Algonquian	A
Ojibwa	Ojibwa	1		Algonquian	A
NorFarm	Norris Farms	11	Y		A
PyrLnsm		2			A
HW	Hopewell Mound Group	14	Y		A
Total		**160**			
HIND (Glacial Kame)	Hind	4	Y		B
Zimmer	Zimmer Site (Glacial Kame)	2	Y		B
TMC	Turtle Mountain Chippewa	8		Algonquian	B
MCH	Minnesota Chippewa	4		Algonquian	B
Cheyen	Cheyenne	3		Algonquian	B
Kickapoo	Kikapoo	1		Algonquian	B
ORCChe	Oklahoma Red Cross Cherokee	3		Iroquoian	B
Pawne	Pawnee	1		Caddoan	B
Stilwch	Stillwell Cherokee	7		Iroquoian	B
Swsioux	Sisseton/Wapheton Sioux	4		Siouan	B
Ojibwa	Ojibwa	1		Algonquian	B
NorFarm	Norris Farms	5	Y		B
PyrLnsm		4			B
HW	Hopewell Mound Group	3	Y		B
Total		**50**			

Table 4
Mutations observed in sequences listed in Table 3. Numbering is based on the Anderson reference sequence (Anderson et al., 1981). Dashes (–) indicate the sample was the same as the Anderson sequence at that location. Base pair locations marked with an asterisk (*) as mutations characteristic of haplogroup A, and those marked with a pound sign (#) are characteristic of haplogroup B. Derived mutations are shown in bold italic. The C > T mutation at 16223 is diagnostic of haplogroup A, but derived in members of haplogroup B.

Sample ID	Haplogroup	16051	16111	16182	16183	16189#	16217#	16223*	16290*	16319*	16325	16362*	16377	16401	16403
Anderson		A	C	A	T	T	T	C	C	G	T	T	C	C	C
Mantle B2	A	–	–	–	–	–	–	T	T	A	C	C	T	T	T
Teston Rd#1	A	G	–	–	–	–	–	T	T	A	C	C	–	–	–
Damiani B2	A	–	T	–	–	–	–	T	T	A	C	C	–	–	–
Wainfleet	A	–	T	–	–	–	–	T	T	A	C	C	–	–	–
Mantle B24	B	–	–	C	C	C	C	T	–	–	–	–	–	–	–

haplogroup A network that the sample shares with two sequences from prehistoric sites in the Illinois River Valley: one sample from the Morse site and two samples from the Orendorf site. The Morse site represents the Red Ocher tradition of the early Woodland period, 3000–2500 ybp (Morse and Morse, 1964), while the Orendorf site is a much younger Middle Mississippian site. The derived mutation 16182 in the Mantle B24 sample defines a branch of the haplogroup B MJ network shared with two aDNA samples from the Hind site in SW Ontario (Essex County) belonging to the Glacial Kame tradition (Donaldson and Wortner, 1995) that is approximately coeval with the Red Ocher tradition in the Illinois Valley. The co-occurrence of these derived haplogroup A and B mutations in the sites described here are consistent with the hypothesis of a relationship between the earlier Red Ocher and Glacial Kame traditions (Ritzenthaler and Quimby, 1962; Donaldson and Wortner, 1995). Studies of additional samples from these sites might clarify, especially if whole mtDNA genome sequences could be generated, might provide a test of this hypothesis.

3.2. Dietary stable isotopes

Nearly all samples yielded well preserved collagen. Indicators of collagen quality, i.e. percentages of C and N by weight, and atomic C:N ratios for all samples are given in Table 5. Three bone collagen samples yielded atomic C:N ratios outside the range 2.9–3.6. The isotopic results for those samples are considered unreliable (Ambrose 1990; van Klinken 1999) and have been discarded. The remaining C:N ratios ranged from 3.1 to 3.4, with an average of 3.13, not far from 3.2, which is the composition of fresh collagen. Three molar roots (noted on Table 5) were sampled at both the upper and

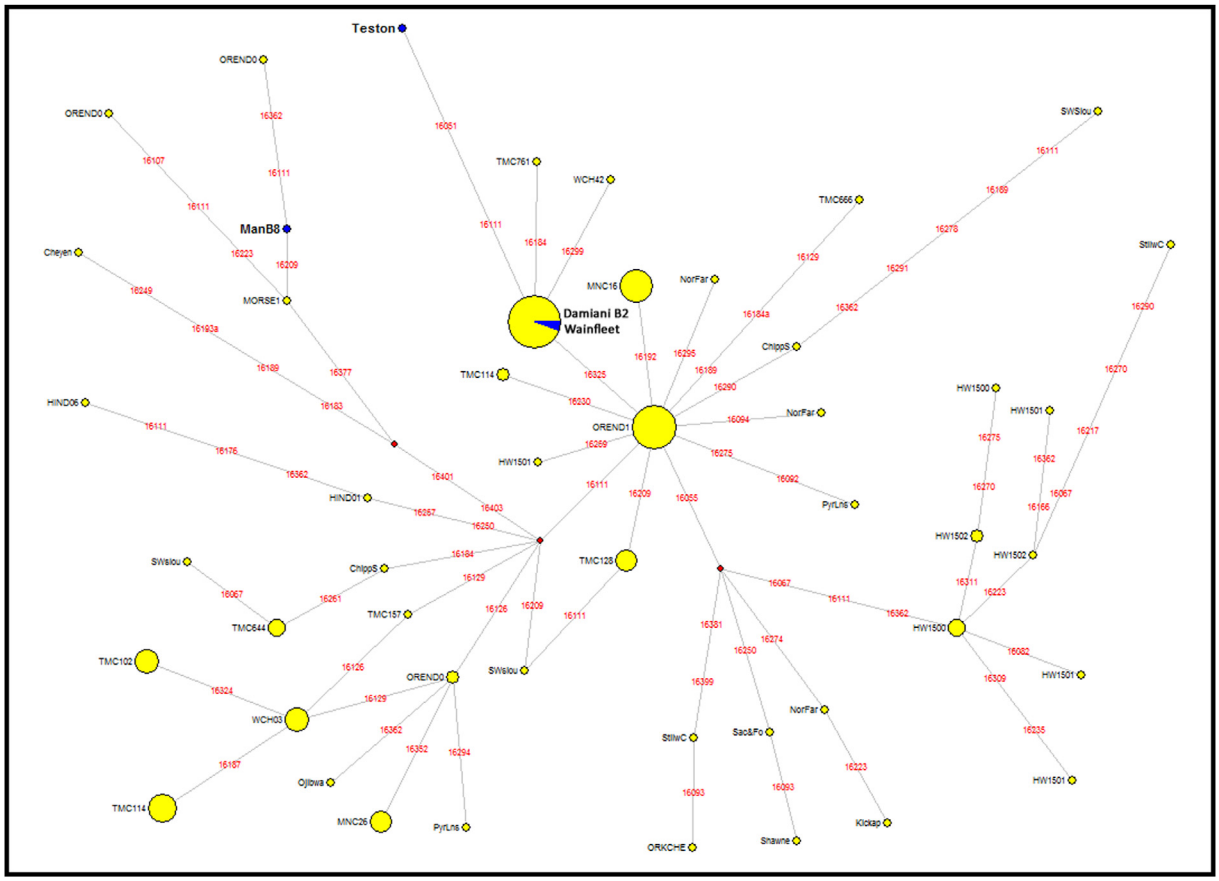

Fig. 3. Haplotype MJ Network for haplogroup A samples reported in this and other studies. Mutations that differentiate adjacent haplotypes are shown along branches. Haplotypes are represented by yellow circles whose size is proportional to the sample size and median vectors are identified by small red circles. Sample abbreviations are those provided in Table 4.

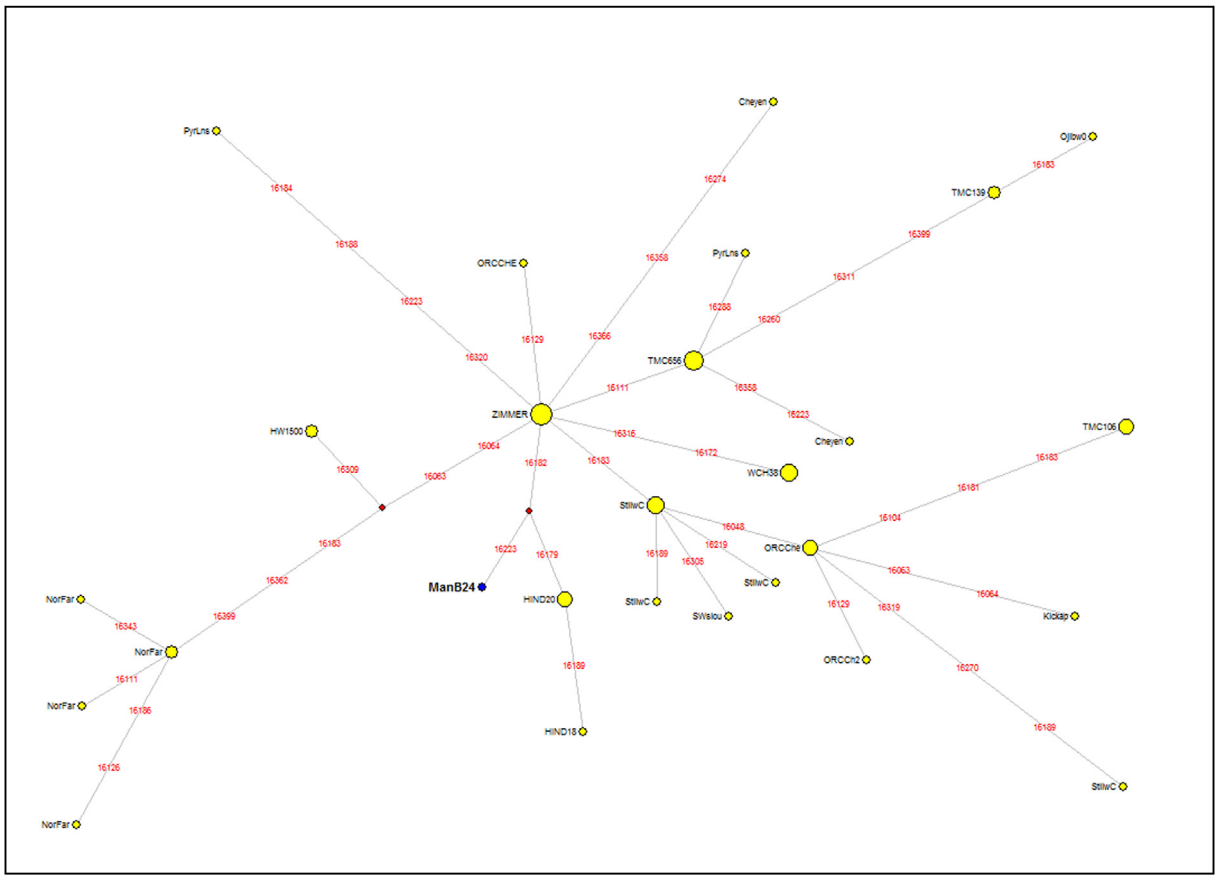

Fig. 4. Haplotype MJ Network for haplogroup B samples reported in this and other studies. Mutations that differentiate adjacent haplotypes are shown along branches. Haplotypes are represented by yellow circles whose size is proportional to the sample size and median vectors are identified by small red circles. Sample abbreviations are those provided in Table 4.

lower ends (cemento-enamel junction and apex), to explore possible dietary differences during the years while the root was forming. Averaged values for those pairs are reported here. The reported isotopic results can be regarded with confidence, without concern for diagenesis. Isotopic values from 53 teeth are summarized by each of the seven sites in Table 6, Figs. 5 and 6.

3.2.1. Nitrogen

Mean $\delta^{15}N$ for dentin from all 53 humans in this study is 11.5 ± 1.0‰, with a range from 9.8 to 13.4‰. For 13 individuals, adhering alveolar bone was also analysed. Mean $\delta^{15}N$ for dentin is 11.6 ± 0.5‰, and for bone 11.5 ± 0.7‰ (Table 6). Values for the two tissues are the same, within measurement error. The same pattern was noted at the Moatfield ossuary (van der Merwe et al., 2003a; van der Merwe et al., 2003b). Among the three pairs of within-tooth values, the differences were 0.5, 1.0 and 1.5‰, from a maxillary M^1, mandibular M_2 and maxillary M^2 respectively. The first two values, both from Staines Road, are within measurement error, given that dentin is not an entirely homogenous material. The M^2 from Teston Road has values of 11.6‰ at the crown and 10.1‰ at the apex, reflecting a dietary shift during mid-childhood.

$\delta^{15}N$ values for dentin for all 53 individuals fall in the same general range as those reported for southern Ontario peoples prior to European contact (van der Merwe et al., 2003a). Candidate dietary proteins include beans, maize (although lacking the essential amino acid lysine), hunted meat, meat of domesticated dogs and fish from nearby lakes and rivers. $\delta^{15}N$ values of plants are very variable, depending on processes of nitrogen cycling in soils. In relatively humid environments such as this, plants typically have $\delta^{15}N$ values close to zero, with a range of a few per mille either side. In Mesoamerica, cultivated beans have recently been reported to have a $\delta^{15}N$ value of 4.0 ± 2.6‰ ($n = 13$) (Warinner et al., 2013), with lowland crops marginally more enriched than highland. The most important source of meat was white tailed deer. Analysis of $\delta^{15}N$ in dentin from 45 archaeological white-tailed deer teeth from sites in the lower Great Lakes yielded a mean value of 5.9 ± 0.9‰ (Pfeiffer et al., 2013). This is in good agreement with the value of 5.5 ± 0.6‰ ($n = 8$) reported by Katzenberg (1989), who also reported archaeological dog bones to have mean $\delta^{15}N$ of 9.6 ± 0.2‰ ($n = 8$). Consumers typically have $\delta^{15}N$ 3–4‰ higher than their food (Hedges and Reynard, 2007) so that humans relying on beans, maize, deer and similar terrestrial foods might be expected to have $\delta^{15}N$ no higher than 10‰, unless they were consuming a great many dogs. In fact, most of the humans analysed in this study have more positive $\delta^{15}N$, almost certainly as a result of the consumption of fish. Bones of many types of fish have been recovered from archaeological sites; a few have $\delta^{15}N$ values comparable to those of deer, but the most desirable food species have values well over 8‰ (Katzenberg, 1989; van der Merwe et al., 2003b). Values for humans in this study are consistent with dietary reliance on diverse types of fish including salmon, trout, burbot (a form of cod) and walleye, all of which have $\delta^{15}N$ values above 10‰.

Dentin $\delta^{15}N$ values are different among the various sites (Fig. 5), with the most positive mean values at Hutchinson and the least positive at Mantle indicating fish was clearly an important item of

Table 5
Isotope values and collagen quality indicators for all tooth enamel, dentin and bone samples in this study. Values for dentin from UCT 13680, 13687 and 13694 are the means of two samples of tissue from the cement–enamel junction and the tip of the root.

Site Name	Identifier	Tooth type	UCT no.	$\delta^{13}C$			$\delta^{15}N$		wt %C		wt % N		C/N	C/N	mtDNA
			Tooth/Bone	Enamel	Dentin	Bone	Dentin	Bone	Dentin	Bone	Dentin	Bone	Dentin	Bone	
Mantle-cemetery	B8	L mand M1	13660	−4.3	−13.1		10.6		43.5		15.9		3.2		X
Mantle-cemetery	B2	L max M1	13661	−4.6	−10.8		10.6		41.8		15.3		3.2		X
Mantle-cemetery	B24	R max M1	13662	−3.4	−10.6		10.4		35.5		13.0		3.2		X
Mantle-cemetery	B7a	R max M1	13663	−2.7	−11.6		9.9		42.6		14.8		3.4		X
Mantle-cemetery	B7b	R mand PM2	13664	−5.4	−12.7		12.1		44.2		16.3		3.2		X
Mantle-village	435-180 surface	L max M1	13665	−5.7	−9.8		10.2		42.9		16.0		3.1		
Mantle-village	465-155 layer 1	R mand M1	13666	−3.3	−9.9		10.4		41.8		15.6		3.1		
Mantle-village	435-180 Q5, fill	L max M2	13667	−2.9	−9.1		11.1		41.7		15.6		3.1		
Mantle-village	485-240 trench	L max C	13668	−7.4	−10		10.5		42.4		15.6		3.2		
Mantle-village	495-129 fill	L mand PM2	13669	−3.2	−11.6		10.7		42.9		15.4		3.3		
Mantle-village	522-141 charcoal	L max M1	13670	−3.9	−9.5		9.8		42.6		15.5		3.2		
Mantle-village	335-160 fill	R max PM1	13671	−2.7	−8.8		10.5		41.9		15.5		3.2		
Mantle-village	465-155 Q4, layer 1	R mand M3	13672	−3	−10.7		10.6		28.7		10.7		3.1		
Mantle-village	500-165 fill	R max M3	13673	−2.4	−11.2		11.5		41.5		15.1		3.2		
Mantle-village	499-133 fill	R mand M1	13674	−3.2	−9.2		10		41.6		15.3		3.2		
Staines	Sample 1	R max M2	13675/13724	−5.7	−9.7	−11.9	12	12	42.6	43.1	15.5	14.8	3.2	3.4	
Staines	Sample 2	R max M1	13676/13725	−6.2	−11.3		12.6		43.8	9.4	15.7	2.7	3.3	4.1	
Staines	Sample 3	R mand M1	13677/13726	−5.9	−10.7	−11.9	11.8	12	41.6	41.6	15.0	14.6	3.2	3.3	
Staines	Sample 4	R max M1	13678/13727	−3	−9.2	−12.5	10.9	12.8	41.1	36.3	15.0	12.9	3.2	3.3	
Staines	Sample 5	R mand M1	13679	−1.9	−11.3		12		42.1		15.4		3.2		
Staines	Sample 6	L max M1	13680	−2.1	−11.4		12.3		43.2		15.7		3.2		
Staines	Sample 7	L mand M1	13681/13728	−3.2	−10.9	−14.1	11.3	10.6	42.2	42.0	15.6	14.8	3.2	3.3	
Staines	Sample 8	R mand M1	13682	−3.1	−11.9		12.7		41.8		15.3		3.2		
Staines	Sample 9	R mand M2	13683	−2.9	−13.4		11.6		43.1		15.4		3.3		
Staines	Sample 10	R max M1	13684	−4.5	−10.2		11.5		43.0		15.5		3.2		
Staines	Sample 11	L mand M1	13685	−3.8	−10.5		11.4		28.1		10.0		3.3		
Staines	Sample 12	R max M1	13686	−5.9	−11.4		12.9		43.3		15.8		3.2		
Staines	Sample 13	R mand M2	13687	−4	−9.6		11.3		36.8		13.4		3.2		
Staines	Sample 14	R max M1	13688	−3.4	−13.2		11.7		43.5		15.8		3.2		
Staines	Sample 15	R max M1	13689	−3.9	−12		12.5		43.0		15.7		3.2		
Teston	Sample 1	L max M2	13690	−3.9	−9.8		11.2		41.8		15.5		3.2		X
Teston	Sample 2	R max M2	13691	−3.7	−10.6		10.9		45.0		16.5		3.2		X
Teston	Sample 3	L max M1	13692/13716	−4.1	−11.7	−11.9	11.6	11	42.1	23.2	15.2	8.1	3.2	3.4	
Teston	Sample 4	L max M1	13693/13717	−5	−9.9		10.4		23.0	4.6	8.6	1.2	3.1	4.3	
Teston	Sample 5	R max M2	13694/13718	−2.1	−11.3	−12.5	10.9	11.8	42.6	24.4	15.8	8.6	3.2	3.3	
Teston	Sample 6	L max M1	13695/13719	−2.1	−11	−12	12.4	11.6	39.2	41.8	13.9	15.1	3.3	3.2	
Teston	Sample 7	L max M1	13696/13720	−4.2	−11.5	−12.5	11.6	10.7	43.2	25.0	16.0	8.8	3.2	3.3	
Teston	Sample 8	L max M1	13697/13721	−5.4	−11.6	11.9	11	11.3	46.3	32.0	16.5	11.5	3.3	3.2	
Teston	Sample 9	L max M1	13698/13722	−2.2	−11		9.8		42.5	5.2	15.1	1.3	3.3	4.6	
Teston	Sample 10	L max M2	13699/13723	−4.6	−11.4	−11.8	10.9	10.9	35.5	35.0	13.2	12.6	3.2	3.2	
Hidden Spring	B1	L mand M1	13700/13714	−3.9	−11.4	−13.3	12.5	11.9	42.1	31.0	15.6	10.5	3.2	3.4	
Hidden Spring	B2a	L max M3	13701	−3.3	−10.7		12.3		42.6		15.7		3.2		
Hidden Spring	B2b	R mand dm2	13702	−3	−12.8		10.8		42.1		14.4		3.4		
Hidden Spring	B3a	L mand M1	13703	−4.8	−11.9		12.2		43.2		16.2		3.1		
Hidden Spring	B3b	L max M2	13704/13713	−2.2	−10.7	−13	11.6	11.8	43.0	34.4	15.8	12.1	3.2	3.3	
Damiani	B2	L max M2	13705	−2.9	−9.7		12		42.8		15.6		3.2		X
Damiani	B4	R max dm1	13706	−3.8	−11.9		12.2		41.5		13.9		3.5		X
Hutchinson	B1	L max M3	13707	−2.8	−11.4		12.4		41.1		15.2		3.2		
Hutchinson	B2	R max M3	13708	−5.2	−10.5		13.8		35.4		13.2		3.1		
Hutchinson	B2a	R max M2	13709/13715	−3.3	−10.5	−12.2	12	10.4	42.8	19.2	15.7	6.5	3.2	3.4	
Hutchinson	F88	L mand M2	13710	−3.1	−11.6		13.4		42.1		15.7		3.1		
Hutchinson	F92	R max M3	13711	−2.5	−13.4		13.2		43.2		15.5		3.3		
Wainfleet	Sample 1	R max M1	13712	−3.5	−9.4		11.5		34.8		12.6		3.2		X

diet at Hutchinson but may not have been so at Mantle. This is a pattern consistent with the quantities of fish bone recovered from regional pre-coalescent period sites (AD 1300–1450) compared to later coalescent or post-coalescent sites (Birch and Williamson, 2013:104). Pre-coalescent sites in the same drainage as Mantle have significant percentages of fish bone in their faunal assemblages ranging from 33% to 50%. Similarly aged sites in the Don and Rouge River drainage systems to the west have 50–60% representation of fish. The fourteenth-century Robb site in the Rouge River system, near to the Hutchison and Staines Road sites, and possibly one of the contributing populations to the Staines Road ossuary, had an even higher representation of 70% fish. Fish represented 8% and 14% of the animal bone from the large, coalescent Draper and Parsons sites respectively and only 6% at Mantle. While there are many taphonomic factors that might affect the survival and recovery of fish bone, these percentages for the coalescent and post-coalescent Mantle sites are consistent with these isotopic data and suggest that fishing was not as important an activity as it had been in pre-coalescent times. This was perhaps because considerably more effort was needed to grow sufficient maize to feed expanding and aggregated populations as well as to hunt deer, not just for meat but also for the thousands of deerskins necessary to clothe coalescent and post-coalescent populations (Birch and Williamson, 2013:111–118). Moreover, Mantle and its successor villages were situated at greater distances from Lake Ontario than earlier sites. Substantially more effort would have been needed to acquire and transport large quantities of processed lake fish northward along non-navigable waterways.

Table 6
Values of $\delta^{13}C$ from enamel apatite and $\delta^{13}C$ and $\delta^{15}N$ from dentin collagen, summarized by individual sites and the total sample; mean, standard deviation in parentheses, and sample size provided.

Archaeological site	$\delta^{13}C$ enamel apatite (‰)	$\delta^{13}C$ dentin collagen (‰)	$\delta^{15}N$ dentin collagen (‰)
Damiani ($n = 2$)	−3.4 (0.6)	−10.8 (1.6)	12.1 (0.1)
Hidden Spring ($n = 5$)	−3.4 (1.0)	−11.5 (0.9)	11.9 (0.7)
Hutchinson ($n = 5$)	−3.4 (1.1)	−11.5 (1.2)	13.0 (0.7)
Mantle Cemetery ($n = 5$)	−4.1 (1.1)	−11.8 (1.1)	10.7 (0.8)
Mantle Village ($n = 10$)	−3.8 (1.0)	−10.0 (0.9)	10.5 (0.5)
Staines Road ($n = 15$)	−4.0 (1.4)	−11.1 (1.2)	11.9 (0.6)
Teston Road ($n = 10$)	−3.7 (1.2)	−11.0 (0.7)	11.1 (0.7)
Wainfleet ($n = 1$)	−3.5	−9.4	11.5
Total ($N = 53$)	**−3.8 (1.2)**	**−11.0 (1.1)**	**11.5 (1.0)**
Moatfield (vdM et al., 2003)	−4.2 (1.6)	−11.3 (1.4)	12.6 (0.9)

3.2.2. Carbon

Mean $\delta^{13}C$ for dentin collagen from all 53 humans in this study is -11.0 ± 1.1‰, with a range from −8.8 to −13.4‰. Among the three pairs of within-tooth values, the differences were 0.2, 0.1 and 0.3‰ for dentin from a maxillary M^1, mandibular M_2 and maxillary M^2 respectively. These are not significantly different. Comparison of $\delta^{13}C$ in dentin and bone is informative, since dentin forms at the time when the tooth develops, and therefore reflects diet in early life. Bone is slowly resorbed and reformed throughout life, so its isotopic values are likely to reflect diet somewhat later in life. Examination of the tissues from individuals in this study shows that dentin is more enriched: mean $\delta^{13}C$ for dentin is -10.9 ± 0.8‰, and for bone -12.4 ± 0.7‰ ($t(12) = 5.16$, $p < 0.05$) (Table 6; values are for 13 individuals for whom both tissues were available). If these data are combined with similar comparisons for 11 individuals from the nearby Moatfield ossuary (van der Merwe et al., 2003a; van der Merwe et al., 2003b), the picture remains the same: dentin is more enriched than bone ($t(23) = 4.28$, $p < 0.05$). The only study from further afield that reports $\delta^{13}C$ values in both dentin collagen and bone collagen from the same individuals does not show this pattern (France and Owsley, 2013). The difference may derive from shifts in dietary emphasis during peoples' lifetimes, with greater reliance on C_3 foods in later life. A larger sample size will be required to investigate this possibility in more detail. The dentin values of the 13 individuals in Table 6 do not diverge from those of the other forty, so the bone values can be used to compare with previously published bone-based studies.

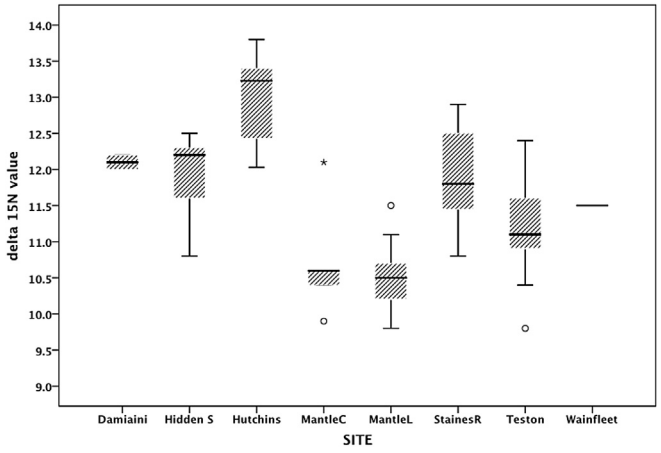

Fig. 5. Values for $\delta^{15}N$ (‰) from dentin collagen from each archaeological site. The median value is indicated by the line inside each box; box extends from the 25th (bottom) to 75th (top) percentiles, whiskers extend to 2.5th percentile or minimum value (bottom) and 97.5th percentile or maximum value (top), circles and star indicate outliers.

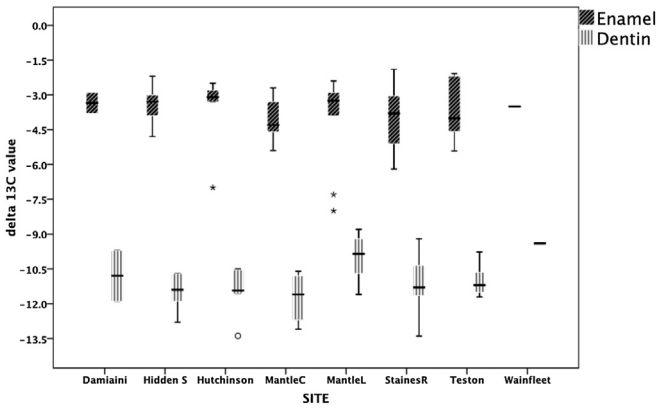

Fig. 6. Values for $\delta^{13}C$ (‰) from enamel apatite and dentin collagen from each archaeological site. The median value is indicated by the line inside each box; box extends from the 25th (bottom) to 75th (top) percentiles, whiskers extend to 2.5th percentile or minimum value (bottom) and 97.5th percentile or maximum value (top), circle and stars indicate outliers.

Mean $\delta^{13}C$ for enamel from all 53 humans is -3.8 ± 1.2‰, with a range from −1.9 to −7.4‰ (Fig. 6). The mean value for $\delta^{13}C$ in enamel from Moatfield is very similar, at -4.2 ± 1.6‰. The Staines Road site, which is thought to predate Moatfield, has mean enamel $\delta^{13}C$ of -4.0 ± 1.4‰ ($n = 15$). Hence, these values reflect no clear temporal trajectory in the magnitude of maize consumption. These values are generally about 1‰ less negative than bone apatite values from Iroquoian sites of the same era (Harrison and Katzenberg, 2003).

Values for $\delta^{13}C$ and $\delta^{15}N$ from dentin as well as $\delta^{13}C$ from enamel are very similar to those reported from the 44 teeth studied from the early fourteenth century Moatfield ossuary (van der Merwe et al., 2003b). Standard deviations are also quite similar, despite the broader temporal and spatial scope of the current study.

With regard to reconstructing the proportion of the diet contributed by maize, this study brings attention to the imprecision of using $\delta^{13}C$ of bone collagen as a proxy for maize intake if people also ate aquatic foods. Wild foods in this region are C_3, as are beans and squash. Forty-five archaeological deer teeth (this study) yielded mean $\delta^{13}C_{dentin}$ of -22.4 ± 1.0‰; since flesh is approximately 2‰ more negative than collagen, deer meat would be expected to have a value of about −24‰. Domesticated dogs had mean $\delta^{13}C$ of -11.1 ± 0.9‰ (for bone collagen) (Katzenberg, 1989). Elevated $\delta^{13}C$ values in human tissues likely derive primarily from maize consumption, since this was a staple food, with some contribution from dog meat and fish. $\delta^{13}C$ of archaeological fish bones from this area range from −11.5 to −24.9‰, although most lie between −16 and −21‰ (Katzenberg, 1989; van der Merwe et al., 2003a,b). To date, there is no evidence for the dietary dominance of any particular species. Fish are, therefore, enriched in ^{13}C compared with other foods except maize. As a result, if maize intake is held constant, more fish will lead to more negative $\delta^{13}C$ values.

Carbon in bone and dentin collagen derives primarily from the protein component of the diet, with some contribution from carbohydrates and fats. Carbonate in tooth enamel is a better index of average $\delta^{13}C$ of the whole diet (Ambrose and Norr, 1993; Howland et al., 2003; Tieszen and Fagre, 1993). We might, therefore, expect fish to reflect more strongly in collagen than in enamel carbonate. Plotting the difference between $\delta^{13}C_{dentin}$ and $\delta^{13}C_{enamel}$ against $\delta^{15}N_{dentin}$ is inconclusive ($y = 0.3288x + 8.8202$, $r^2 = 0.098$; one outlier omitted); there appears to be too much complexity in the system to track inputs in detail in this way. Nevertheless, as we

explore these questions more closely, it becomes apparent that some aspects of earlier estimates of proportional maize contribution to the diet need to be reconsidered.

4. Conclusions

The purpose of this research was to demonstrate the efficacy of one tooth per person as a protocol for addressing pertinent archaeological questions. Results generate insights into fundamental questions about the population background of Northern Iroquoians and their subsistence approaches. While the sample size for DNA analysis was small, the preservation was quite good, allowing for both haplogroup and haplotype assessment. While the samples did not provide a shared haplotype sequence with any modern population, additional sampling may allow for this type of finding. In general, the DNA results point to a great deal of diversity in the sampled sites, and the individuals tested are nested within the modern and ancient populations in this region that have previously been sampled. Derived mutations in several samples assigned to haplogroups A and B represent potentially tribal-specific mutations that may be identifiable in ancestors and/or descendants of the prehistoric populations sampled. Dietary isotopes show complex patterns of exploitation of the Great Lakes fishery and reliance on maize as a staple foodstuff throughout the region, with an apparent decrease in importance of fish in the diet of aggregated populations. Exploration of enamel apatite here and in future studies should help to clarify the timing and magnitude of shifts in this subsistence package.

Upon reflection, these results speak to the need for a population-based methodology in future research. Except in special circumstances (e.g., see Kemp et al., 2007) testing a small number of recent humans for solely mtDNA (and not the entire genome) provides an inadequate characterization of a population. The diversity seen in both the mitochondrial DNA results and the isotopic results is substantial. The Middle and Late Woodland periods were times of population movement, mixing and diversification in the Lower Great Lakes. The dynamism of the period raises the bar for researchers, making the precision and accuracy of our work ever more important.

From the one tooth per person, there is sufficient additional tissue to support the quantification of environmental isotopes such as strontium and oxygen, work that is underway with the samples studied here. Stronger relationships between researchers and descendant communities are crucial if we are to collectively learn about the past. While the discovery of human remains may be destabilizing in many regional contexts, it is important that field archaeologists pursue permission for some level of study, including the retention of tissue samples. We suggest that a single human tooth can be used to generate important information that would have been otherwise unavailable.

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