ARCHAEOGENETICS OF THE GREAT LAKES REGION OF NORTH AMERICA

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aDNA	Ancient Deoxyribonucleic Acid
AD	Anno Domini
ASO	Ancient South Ontario
BAM	Binary Alignment Map
BC	Beforer Christ
bp	Base pair
BP	Before Present
BWA	Burrows-Wheeler Aligner
C	Carbon
Cal.	Calibrated
CRS	Cambridge Reference Sequence
CV	cross-validation
D-loop	Displacement loop
ddH_2O	double distilled water
DNA	Deoxyribonucleic Acid
EAGER	Efficient Ancient Genome Reconstruction
EBT	elution buffer + tween
EDTA	ethylenediaminetetraacetic acid
FA	Founder Analysis
GATK	Genome Analysis Tool Kit
HCl	Hydrochloric acid
Hg19	Human Reference Genom build 19
HVR	Hypervariable Region
IGV	Integrative Genomic Viewer
ISOGG	International Society of Genetic Genealogy
Kya	thousand years ago
LGM	Last Glacial Maximum
ML	Maximum Likelihood
mtDNA	Mitochondrial DNA
N	Nitrogen
NGS	Next Generation Sequencing
O	Oxygen
PAML	Phylogenetic Analysis by Maximum Likelihood
PB	Phosphate buffered
PBS	Phosphate buffered Saline
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
PE	Wash Buffer

rCRS	revised Cambridge Reference Sequence
READ	Relationship Estimation from Ancient DNA
ROH	Run of homozygosity
Rpm	Revolutions per minute
SE	Standard Error
SNP	Single Nucleotide Position
STR	Short Tandem Repeat
UDG	Uracil D Glycosylase
USER	Uracil Specific Excision Reasgent
UV	Ultraviolet
VCF	Variant Call File
WGS	Whole Genome Sequencing

ABSTRACT

The initial movement of people into the Americas began approximately 16,000 years ago via a land bridge connecting Siberia and Alaska. This land bridge, known as Beringia, provided some resources for the nomadic lifestyle of hunter-gatherers and was suggested to be the culture for early Native Americans. Proposal of an alternative route, the transatlantic route, was also hypothesized due to the similarity of archaeological findings, raising questions for the initial studies regarding the entry and arrival time of Native Americans in the Americas continent. However, despite broad agreement that the first settlers entered via Beringia, this dispersal is still poorly understood. Previous publications have hinted at the possibility of a rapid migration into the Southern coast of the Americas via a coastal route, whilst other studies indicate the availability of the Ice-Free corridor after the last glacial maximum (LGM) as an important factor for populating Americas. Although much progress has been made using archaeological evidence to understand the movement of Native Americans, the implementation of the genetic methodology using archaeological human remains was used to complement the archaeological evidence. This allows the differentiation of possible admixture events between Native Americans of different regions, in which the physical evidence sometimes fails to provide a coherent picture.

The great lake region in the north is one of the center points for the congregation of indigenous population, with many different cultures and linguistic populations occupying this area, and utilizing the resources for survival purposes. This area was also suggested to be a crucial location for trading, bringing goods and people from a far. Speculation of competitions between tribes to obtain resources was hypothesed based on the understanding of post-European colonization with little understanding of Indigenous culturalism and contribution

Here, I present low-coverage genome of two Maritime Archaic culture and nine Huron-Wendat individual predating the arrival of European in North America. Demonstrating genetic continuity between ancient populations and discovering mitochondrial haplogroups which appears to be specific to populations from the Great Lake region. The phylogeographic analysis of modern and ancient mitochondrial haplogroup of A and C, expresses different story suggesting perhaps a different introduction of these haplogroup into the Americas, demonstrating the uses of uniparental genomes shows greater detail of relationships between indigenous populations.

1. Archaeological period of Americas - The five stages

Pre-Columbian era – also known as the pre-contact era, a term that is used to encompass the five different stages, covering the period from the initial arrival of the indigenous people of the Americas during the Upper Paleolithic, to the arrival of Christopher Columbus in 1492 (Willey et al., 2001). The five stages are the Lithic, Archaic, Formative, Classic, and Post-Classic stage, in this chronological order. Each stage represents a period of cultural changes, these are generalized terminology to reference a particular period of time. The stages vary across different locations in the Americas, as each region has variations of cultures that experience changes at a different rate (figure 1). Isotopic analysis of climatic fluctuations and archaeological evidence of clearly define culture is used, to organize the archeological period of North America (D. G. Anderson et al., 2015a). Each stage is divided into further sub-stages to elaborate the minor cultural changes (Willey et al., 2001).

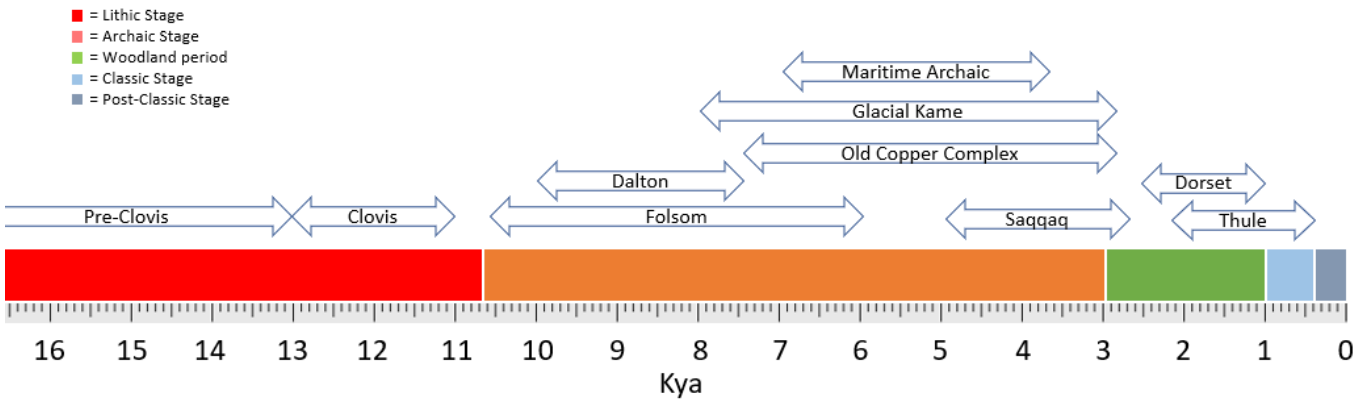

Figure 1. A timeline to demonstrate the five Archaeological period of North Americas and the approximate range each stage exhibit. Some traditions and cultures was illustrated in this figure to show various culture within each period, as well as approximate dates for each culture.

1.1. Lithic Stage

The lithic stage or Paleo-Indian stage is defined as the earliest period for people to enter and colonize the Americas between the Last Glacial Maximum (LGM) and 8500 BC (Willey et al., 2001). As the name implies, this stage represents the first appearance of flaked stone tools in the Americas, the main tools used for this period. The finding of flaked stone tools indicates that the initial group of indigenous people heavily relies on hunting as a way of survival, and this way of life was suggested to follow patterns of animal migration, living a nomadic lifestyle limited by the available resources (Buchanan et al., 2012). The environmental factor complements the available resources, as seasonal changes affect the search for the best resources, developing a mobile lifestyle (Goring-Morris & Belfer-Cohen, 2003), similarly can be said to the depletion of a particular food source (Tremayne & Winterhalder, 2017). As evidently shown that during the lithic stage, the extinction of the megafauna in North America coincides with the settlement of indigenous people (Koch & Barnosky, 2006; P. Martin, 1973). Whether

the causation of megafauna extinction was induced by the indigenous people remains unclear, as the assumption of hunting would have affected the megafauna population but unsure regards to the full impact (Haynes Jr. et al., 1998; Haynes Jr, 1964). The gathering of plants was also recorded in sites with small animal remains, highlighting mixed foraging strategies and the uses of different resources (Haynes, 2015). At this stage, no evidence of plant cultivation can be seen in all archaeological sites within the Lithic stage, demonstrating a hunter-gatherer style of living.

The Clovis culture appears at the end of the LGM and is considered as the ancestor of most indigenous cultures of the Americas (Waters & Stafford, 2007). It was first identified at the Clovis site in New Mexico with the oldest site found in Sonora, Mexico dated at 13,390 cal years BP (Sanchez et al., 2014). The distinctive features of a Clovis site are the bifacial fluted lanceolate point containing a concave base known as a flute, which can be found on either one or both surfaces of each blade. Creating the Clovis point requires a stone kit and striking technique known as flint knapping, in which a stone core is struck and creating a flake. Formation of the desire shapes and size would require further striking, and depending on the type of stone, different pressure must be applied for the successful creation of a Clovis point (Howard, 1990). The archaeological evidence so far has indicated a common use of chert as the main material, perhaps due to the ubiquity of this resource. Clovis points made from obsidian, quartzites, and jasper are considered a rarer find, demonstrating the manufacture of points from different materials, perhaps driven by the availability of local resources.

Across modern day US, the distribution of Clovis tools is considerably higher in the south-east than on the west coast (D. G. Anderson & Faught, 1998), suggesting the movement of hunter-gatherers from east to west, as well as the possible development of Clovis tool to have been originated in the south-east. Analysis of geographic and lithic distribution, indicates the movement from east to west was impeded by the Appalachian Mountains and would most likely require an easier pathway to travel westward through the Coastal Plain and Piedmont. The movement westward could have taken a long period according to the model proposed by Gardner (1983), as early indigenous people appears to be selectively mobile within a prescribed territory, mostly towards a known available resource, such as stone quarries and hunting location. These territories containing known resources were suggested to be an important social aggregation, which could lead to the formation of new groups and sharing of information prior to movement in a new location. Buchanan et al., 2016, postulate that these territories could have formed an early regionalization of Clovis culture across the USA. The lithic network generated by analyzing distinct raw materials and the structure of 84 Clovis assemblages, highlights the eastern Clovis population did not interact and exchange resources with groups from the west but had the opportunity to do so. In addition to this, the Clovis point form and shapes differ between eastern and western USA, supporting previous results of regional adaptations or isolation between Clovis populations (Hamilton et al., 2013). Whilst the lithic network from Buchanan et al., 2016, highlights a connection amongst all of the assemblages from the Northeast, Great Lakes, and midcontinent, the differences within North-eastern Clovis

assemblage suggests further sub-regionalization in the east. This could explain the high concentration of lithic artifacts found in the eastern USA, however, the low density of artifacts in the west can also be explained by the intensity of artifact used.

1.1.1. Pre-Clovis culture/unidentified Clovis point

At the beginning of the 1970s, evidence of archaeological sites predating Clovis began to emerge, causing the debate on the timing and places of initial human arrival and the number of migrations. Different viewpoints have been proposed to justify the finding of the earliest known stone tool technologies. First is the Fishtail projectile points reaching South America approximately 13,000 cal BP, suggested to be associated with Clovis tools, hinting at the diffusion of technology development from North to South America (Morrow & Morrow, 1999). The second is the dissociation of lithic tools assemblages between North and South America, as the environmental differences could lead to different adaptations and cultural changes, if this is true, then the development of technology would have occurred separately prior to their entry into the Americas (Thomas D Dillehay, 2000). Presently, several discoveries such as the Monte Verde archaeological site located in Chile dates to 14,500 cal BP (Tom D. Dillehay et al., 2015), Cactus Hill in Virginia dates to 18,000 BP (McAvoy & McAvoy, 1997), and many other sites (figure 2) provide strong support for the second argument (Aimola et al., 2014; Braje et al., 2017; Goodyear, 2005; Neves & Hubbe, 2005). The existence of similar age sites which predates Clovis technology, located in both northern and southern Americas indicates occupancy in both hemispheres, rectifying the idea of Clovis first as the ancestor of all indigenous American. These data also highlight a minimum of two separate migration into the New world and suggest a highly mobile movement into South America before 15,000 years ago (Tom D. Dillehay et al., 2015).

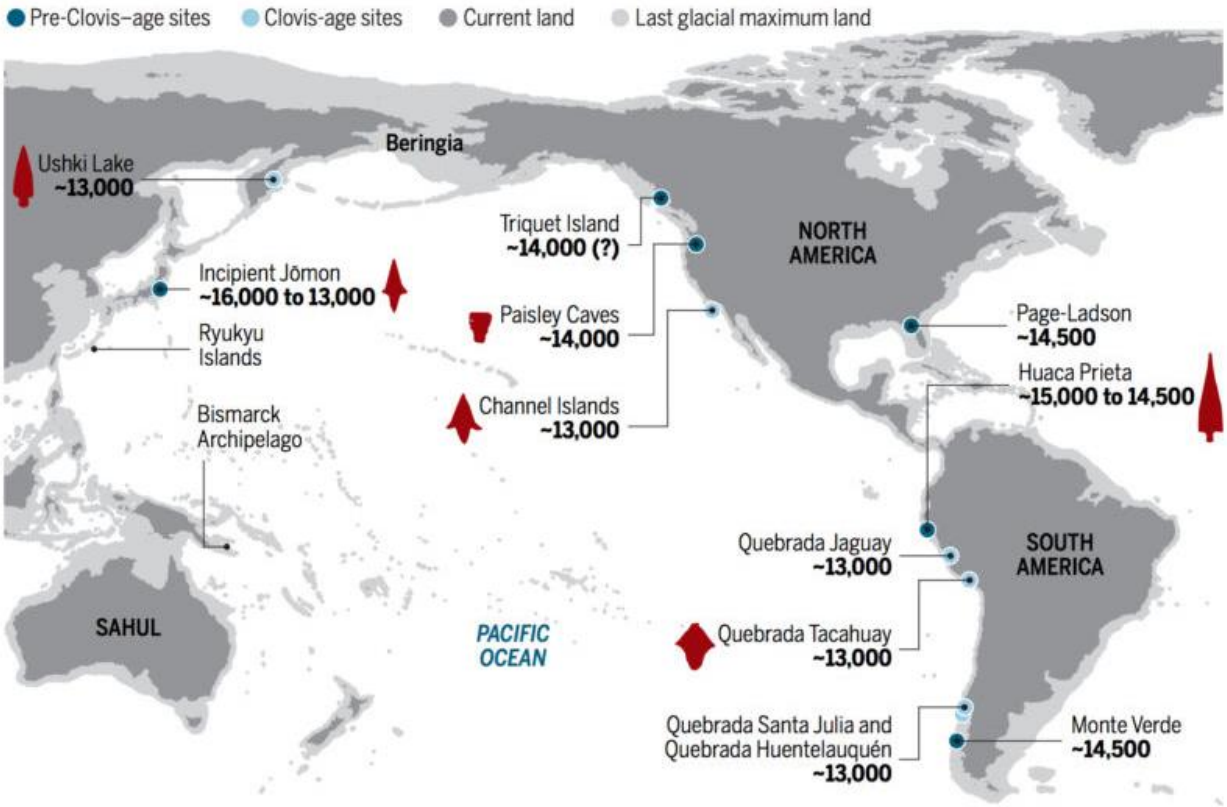

Figure 2. Map of archaeological sites containing stone tools pre dating Clovis (Braje et al., 2017). Premission to freely reproduced the image shown under the terms and conditions of Science's Creative common licenses (CC-BY 4.0).

1.1.2. Ways into America

1.1.2.1. Land bridge theory

In the early 19th century, Ales Hrdlicka, from his findings on the similarity of craniometry measurement of skeletal remains from Mongolia, Tibet, Siberia, Alaska, and the Aleutian Islands, proposes an East Asian origin for early Native American during the late Pleistocene/early Holocene (Hrdlička, 1908). Further evidence also supports this hypothesis of an East Asian origin by analyzing late Pleistocene megafaunas remains on the Aleutian Islands, concluding that the similarity between Asian and the Aleutians islands megafaunas can only be explained by distribution patterns of post-glacial migration (Guthrie, 1968). This led to further questions if early Native Americans were from Asia, how did they travel across the Bering Sea?

A comparison of $\delta^{18}O$ from deep-sea cores and ice cores located in Greenland, Artic, and Antarctica suggests an increased volume of ice throughout the LGM. This data identified the expansion of glaciers across the Atlantic Ocean pre-LGM, causing the lowering of sea levels as much as 150 meters (Clark & Mix, 2002; Lambeck & Chappell, 2001). The decrease in sea levels created a landmass connecting Alaska and North Eastern Siberia (Porter, 1964), this area is known as Beringia, provide accessibility for early Native Americans to travel by foot into the Americas. Evidently shown in present time, accessibility by foot is currently not possible, this

was also the scenario during the Pleistocene. The fluctuation of $\delta^{18}O$ values shown in ice core data highlights the sporadic changes in sea levels due to irregular warming and cooling period, affecting the movement of peopling by foot (Hu et al., 2010). Therefore, the window of opportunity for movement by foot is limited to a particular period of time. Other data also support this by analyzing, a study on ~120,000 years old long-horned bison from Snowmass, Colorado, identifying two waves of bison dispersal from Asia into North America. The first wave occurred approximately ~195,000-135,000 years ago, and the second wave approximately ~45,000-21,000 years ago. Suggesting the different temporal periods for the accessibility of Beringia (Froese et al., 2017).

The temperature fluctuation in Beringia affected the Paleo-environment greatly. Utilizing the fossil evidence and isotopic dating, the Paleo-environment of Beringia before its submergence can be identified. This can be seen in the tundra plants located in the Aleutians Islands and Chukotka region of Russia, suggesting some species of tundra plants once flourished (Brubaker et al., 2005). However, this does not necessarily suggest an ideal environment for humanity. The movement of people into Beringia most likely occurred during a warming period, when it is more favorable to support human activity (P. M. Anderson & V. Lozhkin, 2001; Koch & Barnosky, 2006). Alternatively, analysis of the Chukotka Pleistocene environment between 57,000-15,000 years ago, highlights the migration of megafaunas into East Beringia could be due to the unfavorable condition in west Beringia when compared to the East side (Kuzmina et al., 2011). Although it is more favorable for peopling to occur during a warming period, the possibility of travelling during a cold period cannot be disregarded.

More recent archaeological findings suggest an early human occupation for East Beringia, around 27,000 years ago. The archaeological sites, Yana RHS, located in North-East Siberia, containing evidence of lithic tools and foreshaft made from megafaunas remains, indicate the arrival of hunter-gatherers in this region during the LGM (Pitulko et al., 2004). Further evidence located in Canada at the Bluefish cave, also demonstrates the existence of human occupation around 24,000 years ago, finding both lithic assemblages and faunal remains (Bourgeon et al., 2017). This archaeological site supports the presence of people in unfavorable conditions, but it does not represent a direct link with Clovis or later Native Americans, whether these sites validate as potential failed migration is still undetermined (Potter et al., 2018).

1.1.2.2. Beringia Standstill

The evidence of people in eastern Beringia between 20,000 to 30,000 years ago demonstrates the arrival of people in this area, leading to the hypothesis of Beringia Standstill (Tamm et al., 2007). This hypothesis suggests the early peopling in eastern Beringia led to an extended period of isolation from western Beringia due to local climatic changes before the entry of eastern Beringian into the Americas. The separation suggests the occurrence of founder effect for the eastern population, as (Szathmary et al., 1978) proposes the genetic and physical similarities of two modern Native American groups was the causation of genetic exchange by founding

population in Beringia. Further studies also postulate the importance of Beringia as the location in which the founding population settled and genetically diversified prior to they enter into the Americas (Bonatto & Salzano, 1997). Although the idea of the Beringia Standstill was present decades ago, the popularity of the hypothesis gain weight via the study by (Tamm et al., 2007) utilizing mitochondrial DNA, speculating the common Native American haplogroups derived from Asia must have arisen after their ancestors left the old world, but before their dispersal into the new world.

1.1.2.3. Ice Free Corridor

During the Last Glacial Maximum (LGM) between 25,000 to 10,000 years ago, the late Wisconsin glaciation covered the North American Cordillera, Canada, Canadian Arctic Archipelago, and Greenland. This ice sheet reaches the southern point at the Rocky Mountain in Oklahoma during the LGM, creating a geographical barrier preventing the movement of people eastward and southward from east Beringia (A. S. Dyke et al., 2002). Although the presence of people in unfavorable conditions does not eradicate the idea of peopling crossing the ice sheet into Americas. The earlier hypothesis suggests the movement of people into the Americas would have occurred post glaciation when the improvement of environmental factors due to the warming period, causes the Cordilleran and Laurentide ice sheets to retreat (Fiedel, 2000; Hoffecker et al., 1993). The deglaciation of the two ice sheets was suggested to have occurred around 15,000 to 14,000 years ago, providing an opening known as the ice-free corridor, allowing human colonization of the Americas by foot (Arthur S Dyke, 2004; Pedersen et al., 2016). The evidence from geological samples indicate the opening of the ice-free corridor first occurred in the southern ice sheet due to the warmer condition, and eventually became accessible in the north at a later period, allowing migration to occur from north to south direction (Duk-Rodkin & Hughes, 1994; A. S. Dyke et al., 2002).

The availability of the ice-free corridor was previously considered as the sole entry route for the arrival of the first Americans, this is due to the emergence of Clovis culture shortly after the opening of the ice-free corridor (Sanchez et al., 2014). However, a question arises concerning the timing of entry and whether it is possible for this migration to occur. Firstly, the assumption of big game hunting from the hunter-gatherer lifestyle requires certain conditions for both hunters and faunas. Secondly, after the opening of the corridor, whether it is biologically viable and the constraint of time require for the establishment of biota is unknown, therefore the opening of the corridor does not necessarily indicate an immediate movement of people (Mandryk et al., 2001; White et al., 1979). Evidence from palynological and paleontological data have often been used to determine the colonization of plants and animal, to understand the timing in which the ice-free corridor became biologically viable for human migration. Radiocarbon dating and lake sediment cores are also used to understand the history of this corridor. These findings have demonstrated the opening of the corridor began around 15,000 to 14,000 years BP, but the deglaciation of the ice-sheet causes regional flooding preventing

habitable areas for up most 2000 years (Arthur S Dyke, 2004; Hickin et al., 2015). The vegetation of the corridor began approximately 12.6 cal. Kyr BP when both ice sheets were hundreds of kilometers apart, allowing faunas to occupy the area, reaching the requirement for the hunter-gatherer lifestyle.

1.1.2.4. Costal Route

In spite of the fact that the availability of the ice-free corridor contributed to the hunter-gatherer lifestyle, the evidence of human occupancy prior to the opening of the corridor showcase the migration of people during harsh condition or the possibility of a different pathway, allowing peopling to occur before the ice-free corridor became accessible (Beaudoin et al., 1996).

The Coastal route model (figure 3) implies the movement of people via the Beringia coast into the Americas due to a more favorable environmental condition compare to the interior inland migration corridors during the late and post-glacial period (Fladmark, 1979). Evidence from the sediment cores suggests an ice-free area in the North Pacific coastline during the late Pleistocene, this area potentially acted as a refugium for settlement and the earlier arrival of people (Josenhans et al., 1995). The lowering of sea level also contributed to a wider area by the coast for the possible settlement to occur, along with an optimal climatic environment, habitat for plants and animals likely supported the early migrant, leading to strong support for the Coastal Route (Josenhans et al., 1995; Luternauer et al., 1989). However, this does not necessarily dictate a large ice-free area, rather it suggests a pocket of locations that could have the potential to support refugia. Reaching these refugia locations requires further investigation and our current understanding is still under review.

One speculation of the coastal route suggests the possibility of marine transportation as a method of movement into the Americas, as the coastal movement by foot would be improbable as rough terrains and sea ice would hinder travelling (Erlandson & Braje, 2011). Evidence of maritime capabilities was hinted at in a few archaeological sites, such as the Alta and Baja island sites in California, showcasing the consumption of mollusk demonstrating inhabitants between 11,500 and 13,000 cal BP utilizes marine technology (Des Lauriers, 2006; Erlandson et al., 2005). The marine ecology along the coast was outlined to have facilitated the migration of maritime people, as modern analysis of present kelp forest contributes heavily to the biodiversity of a coastal community (Erlandson et al., 2007). However, it is unclear the existence of kelp forest during the LGM and how kelp forest was distributed. Additionally, a lack of coastal archaeological evidence to support this hypothesis due to the fluctuation of sea levels, causing the drowning of possible evidence which increases the difficulty in testing the hypothesis (Heusser, 1960). Although the physical evidence is not present, the coastal route still has high support as this hypothesis represents a favorable route for the existence of people predating the availability of the ice-free corridor.

1.1.2.5. Transatlantic Route

Alternative hypothesis regarding the origin of the first Native American was conjectured, suggesting the ancestors of the first Native American travelled from western Europe via the North Atlantic Ocean into the eastern Americas (Bradley & Stanford, 2012). The proposed journey took place around 18 and 24 cal ka BP, during the peak of the LGM with the expansion of ice sheets which extended from North America and the Atlantic coast of France, would require marine transportation, allowing early migrants to travel along the edge of the sea ice (Vettoretti & Peltier, 2013; Westley & Dix, 2008). The idea of this hypothesis was proposed by (Bradley & Stanford, 2012), due to the similarity of lithic technology between the Clovis culture and the Solutrean culture from France and Spain, additionally, current evidence of Siberian archaeological sites does not contain similar lithic assemblages as Clovis or their precursor (Westley & Dix, 2008). The distribution of Clovis tools found predominately on the east coast of the USA compared to the west is also in favor of this scenario (Smallwood & Small, 2015).

This hypothesis has been under heavy criticism due to insufficient archaeological and genetic evidence for this migration. (Straus, 2000) reviewed the chronology of Solutrean culture and the Clovis culture, addressing the discontinuity between the two cultures. The radiocarbon dating for the Solutrean culture dates between 17,000 to 22,000 years ago and for the earliest Clovis culture around 13,000 years ago, demonstrating an approximately 5000-year time gap.

The spread of the Ice sheet in western Europe during the LGM, constricts the geographic range of human occupation, resulting in the possible abandonment of the previously occupied region. This confines the region of Solutrean culture to be occupied in a restricted area, such as southern France and eastern and southern Iberian Peninsula based on the archaeological sites (Jochim, 1987; O'Brien et al., 2014). The most northern Solutrean site located at the Loire River Valley, dates to 18,000 to 19,000 BP, demonstrate the presence of settlement and possibly the most extreme condition in which people can occupy. Coincidentally, speculation of peopling in the new world also occurred around a similar time, therefore one argument to dismiss the Solutrean hypothesis is the distribution of Solutrean culture demonstrating a southward movement towards Iberia (Aura Tortosa et al., 2019). The occurrence of this migration would have occurred from northern Portugal and must travel 5,000km to reach the Americas, which does not appear feasible (O'Brien et al., 2014).

In order for the movement of Solutrean Culture across the Atlantic Ocean, a well develops maritime adaptation is required. Evidence from Iberia during the Solutrean period suggests the utility of marine resources can be seen in archaeological sites indicating the consumption of marine mollusk and fish. The presence of marine mammals was available during the Solutrean period, as cave art of penguin drawings and seal engraving was found in Cosquer Cave, southeast France (Clottes & Courtin, 1996), but no archaeological remains of marine mammals were found in Solutrean sites. The consumption of marine resources was not the main focus regarding foraging techniques due to findings of medium to large animal remains, suggesting favoritism of resources due to environmental constriction. Additionally, evidence of boat

making or the ability for seafaring is lacking from the archaeological sites, suggesting the inability of marine travel. However, this evidence could most likely perish as organic material use for boat making would have degraded (Bednarik, 1997).

If the usage of boats were available, how can people travel across the Atlantic Ocean and into America? Reconstruction of peak glacial sea surface temperature in the Atlantic was performed using 119 well-dated sediment cores and 947 modern analog samples, identifying the spread of sea ice in the North Atlantic Ocean to be further south across Icelandic Faeroe Ridge, with additional sea ice to be found in central-east Atlantic and around Azores high (Pflaumann et al., 2003; Sarnthein, 2003). Temperature as indicated by the oxygen isotopes ($\delta^{18}O$) values from deep-sea sediments comparing to ice core studies, ranges from 0°C to 11°C, proposed to be within the temperature range of Arctic species survival, implying that the availability of food source was present for hunter-gatherers during the travel across the sea ice (Sarnthein, 2003; Tynan & DeMaster, 1997). Further investigation utilizing genetic methodology could provide insight regarding the Solutrean hypothesis (Oppenheimer et al., 2014).

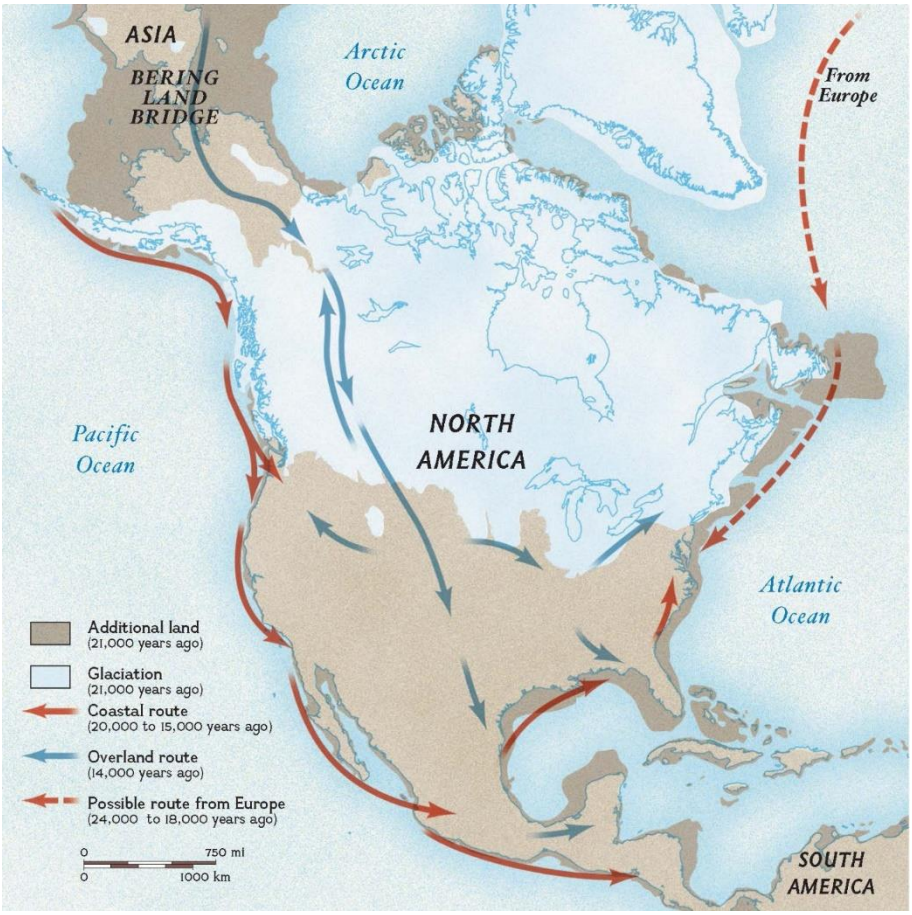

Figure 3. Map indicating the possible routes taken for the first people to enter the Americans. The coastal route, Ice Free corridor route and the Solutrean route. https://www.nationalgeographic.org/photo/bering-land-bridge/ Permission to freely reproduce the image shown under the terms and conditions of National Geographic terms and conditions.

1.2. Archaic Stage

The Archaic stage is divided into three separate periods, each consist of a further development indicating their transition from the Lithic stage, spanning between 10,000 to 3,000 BP, is the longest stage amongst the five stages, with different regions developing at a different rate (Kelley et al., 1958). The changeover from the Lithic stage led to the development of agricultural goods and a significant shift from the nomadic lifestyle. This becomes sedentary, living in large groups and consuming a range of plants and hunted game. Regional and local adaptations began, resulting in the increases of societies and the divergence of many cultural developments (figure 4). These points infer cultural development in various regions which differ from one region to the next, however, it does not suggest cultural isolation within a specific region. Instead, the sharing of regions between different cultures highlights complex interactions between neighboring groups.

1.2.1. Early Archaic Stage

The early Archaic stage last from 10,000 to 8,000 BP, coincides with the end of the last glacial period. Around this time, the raising of sea levels due to warming climate and deglaciation led to the closing of the land bridge in Beringia, preventing further access from North-Eastern Siberia by foot (White et al., 1979). The Laurentide and Cordilleran ice sheets continue to retreat, enabling a wider Ice-free corridor than the late Lithic stage. By this period, the area surrounding the ice-free corridor is speculated to be populated by faunas and other biotas, which are accessible by any hunter-gatherers (Froese et al., 2017; Jass et al., 2011). The deglaciation of the two ice sheets also caused an increase of floodplains located near the edge of ice-sheets and generating powerful river streams, which could hinder movement across water sources (G. W. Crawford et al., 1998; Thornbush & Desloges, 2011). Along the Great Lakes and shoreline, raise of water level led to the submergence and destruction of possible archaeological sites in North Eastern Canada, causing difficulty in understanding the history of this area during this period (A. S. Dyke et al., 2002; Mason, 1981; Siddall et al., 2003).

Potential in discovering submerged Lithic and Archaic sites have long been recognized by archaeologist (O'Shea & Meadows, 2009), however, in order to understand the occupancy of people surrounding the Great Lake region during this stage, information from neighboring regions within North America are used accompanied with the limited evidence to provide an understanding. Derivative of Clovis tools or the successor of Clovis culture can be identified across North America, with changes of lithic assemblages indicating further adaptations to the surrounding environment. The variation of lithic tools found, suggest regional diversification of cultures, perhaps indicating a regional development of tools and isolations due to separation caused by the environmental factors (J. A. Tuck, 1974). The lithic tools found within the early archaic stage shows a significant reduction in size compare to the lithic tools discovered in the Lithic stage, additionally side notches located at the base of each points highlights an improve adaptations, for the attachment of lithic points to create spears utilizing rope for further stable

attachment (D. G. Anderson et al., 2015b). Stone knappers during this period appears to rely on local stone resources and greater details in curation of specific stones for tool making (figure 4), unlike their predecessor which travel greater distances for lithic source (D. G. Anderson & Faught, 1998). Within the tool kit, a scraper can be found, suggesting the leather work and wood craving development, these evidences are particularly prominent within the Dalton culture tools assemblages located in south eastern North America (Goodyear, 1982). The method requires to create these tools based on archaeological findings indicates the usage of similar techniques as Clovis, therefore regarding Dalton culture and points similar to Dalton as the descendants of Paleoindians (Goodyear, 1982). The variation of Dalton-like points across North America suggests an early sign of territorial formation of cultures, evidence from Hi-Lo points found near the Great Lakes region are similar to the Dalton points found in the Great Plain and discover in adjacent areas, but are mutually exclusive with little overlap, showing evidence of contemporaneity (Ellis & Deller, 1982; Lothrop et al., 2016). Furthermore, the archaeological sites found during this period indicate an increased population size, perhaps due to more organized communities for food gathering strategies such as seasonal hunting (Jass et al., 2011; Surovell et al., 2016; Veltre et al., 2008).

Another hunter-gatherer community, the Plano cultures, occupied the majority of the Great Plain region of North America, overlaps with northern Dalton culture territories. The Plano culture a possible successor of Folsom tradition due to the similarity of point-making differs from the Dalton points as it is unfluted, demonstrating the establishment of segregation between the culture of the eastern and western Great Plain, although still containing the side notches (Dawson, 1983; Quimby, 1959). Different tool usage also highlights the variation of hunting strategy with the Plano culture retaining the hunting practices exhibited by their ancestors, whereas the Dalton culture utilized an increased number of plant resources such as nuts and berries and the broad range of game animals (D. G. Anderson et al., 2015a; C. S. Reid, 1980).

The ability of aquatic transportation was questioned for early archaic cultures, as the evidence from lithic tools demonstrates the capability to carve wood but lacking physical evidence of any boat making due to the deterioration of natural material (Erlandson et al., 2005; Erlandson & Braje, 2011). However, the spread of South-Eastern technology, such as the adzes, to the western hemisphere within North America greatly support the assumption of aquatic transportation, suggest either a mobile movement or the exchange of artifacts between different region (D. G. Anderson et al., 2010; Gaertner, 1994). This excludes the idea of regional isolation of cultures, rather the occurrence of regional interaction of resource exchange between separated groups. It is unknown the extent of social interactions between different regions.

An interesting development during this stage is the cultural practice of burial remains in a cemeterial manner. Although most burial during this period is usually found in small excavated pits containing some grave goods, some structure of cemeterial behavior can be identified

(Griffin, 1967). Excavation at the Sloan site located in North Eastern Arkansas recognized as a Dalton culture site is considered as the oldest cemetery in North America, containing human bone fragments and stone artifacts interred with the deceased (Condon & Rose, 1997; Morse, 2017). The alignment of artifacts facing a southwest direction suggests possible ritual as each burial located at the Sloan site exhibits the same behavior (Smallwood et al., 2018). It is important to point out at this period, there is a cultural difference between the east and the west, as well as subtle differences within each region as evidently shown in lithic assemblages and burial methods. Such as the mummification of bodies in Nevada showing evidence of afterlife concern and the use of atlatl in the Great Basin which does not appear in the Great Plain or the Great Lakes region (Barker et al., 2000; Edgar, 1997).

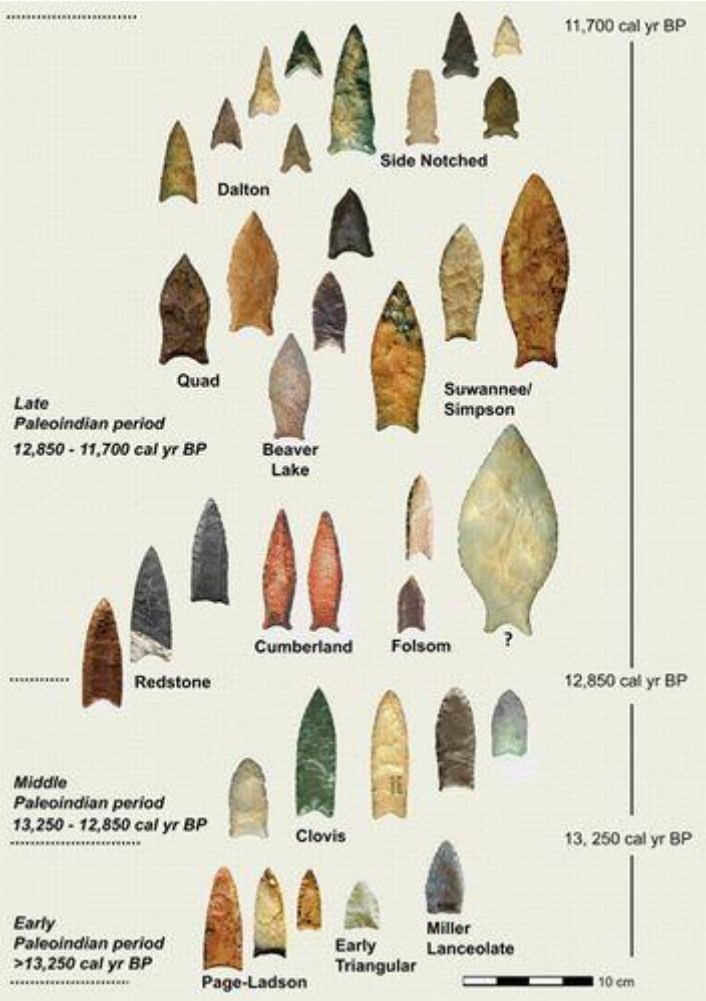

Figure 4. Lithic tools dated before and post Clovis, indicating the changes of lithic assemblages and the variation of tools through time. (Anderson and Smallwood, 2015). Permission to freely reproduce the image shown under the terms and conditions of Taylor & Francis reuses of its content for a thesis or dissertation.

1.2.2. Middle Archaic stage

Temperature fluctuation continues during the Middle Archaic stage and is considerably warmer with a drier climate than the Late Archaic stage (Dean et al., 1996). The warming of the climate in this period, known as Altithermal or Holocene climatic optimum, signals the start of the Middle Archaic stage around 6500 BC (Albanese & Frison, 1995; Antevs, 1948). Cultures from the Early Archaic stage continue to thrive under the warmer climate as indicated by the increased size of Middle Archaic archaeological sites compare to Early Archaic sites, demonstrating the increase of population density (Dejarnette, 1962; Meltzer, 1999). However, the increased population does not appear to correlate to an increase of archaeological sites found during the Middle Archaic, as the number of recorded sites in Middle Archaic appears lower than the Early Archaic stage (Dincauze & Mulholland, 1977). The inability to distinguish pertinent materials from later tools and the lack of recovered datable archaeological evidence was suggested as the cause for the decreasing number of Middle Archaic sites found (M. B. Collins, 1995; Ellis & Ferris, 1990; Mason, 1981). This also led to limited evidence regarding this period for the interior of North Eastern America, example, the John's Bridge site in northwestern Vermont was previously classified based on materials found as postdating 4000 BC but radio carbon dating place this site to be around 6000 BC (Thomas & Robinson, 1980). Difficulty in identifying material does not represent the lack of people occupying the Great Lake region, rather this causes the difficulty in understanding the possibility of continuous occupation and the changes of adaptation in this region from this period to the next.

1.2.2.1. Old Copper Complex

Given the limited information for the Great Lake region for this period, the available evidence became key in conjunction with evidence from the Late Archaic period to understand the complexity in this region. The emergence of metallurgy utilization grows to be quite important in Native North American societies, procuring copper to make weaponry and tools (Pleger, 2000). These societies, a series of many cultures collectively known as the Old Copper Complex, begun at the end of the Middle Archaic stage and continue into the Late Archaic, with many scholars believe the procurement of copper found in burial mound within the Mississippi Valley and the development of metallurgy to have begun at the Great Lakes because of the abundance of copper deposit found around Lake Superior (Loring, 1985; Pleger, 2000; Pompeani et al., 2015; Wittry & Ritzenthaler, 1956). Although the discovery of native copper goods is rare, the finding so far shed light on techniques used to craft tools, cold working copper nuggets, hammering, and annealing into the desired shape (Chastain et al., 2011; Ehrhardt, 2009). This is significantly different from Southern Indigenous American metal work, which uses a lost wax technique of molding to create the desired object (Bray, 1978). Copper deposits located at Lake Superior identified as an important source but most likely not the only primary source for native copper, as previous research highlights a different region of North America, such as Nova

Scotia and New Jersey, as the area of potential copper deposit for regional societies (Abbott, 1885; Anselmi et al., 1997; Duns, 1880). Therefore, the previous assumption of copper trading solely from the Great Lake may not be the most accurate, but it is important to illustrate the importance of Great Lake copper in this region to the surrounding area, as scholars believe the finalized product was made within the Great Lake and traded to the neighboring region rather than the raw resources (Bebber & Eren, 2018; Levine, 2007; Willoughby, 1935).

The usage and the creation of copper objects trading between societies show the rise of sociopolitical complexity and continue into the modern time (Levine, 2007; Pleger, 2000). The ability to exchange objects is fundamentally important to maintaining good relations and reaffirming alliances for ancient societies (Lattanzi, 2007). This also creates networks of exchange between communities which are more difficult to determine during the Middle Archaic, but are prevalent in the Late Archaic to the present, perhaps even establishing direct trading over long distances from the Great Lake to the Eastern and South Eastern coast of America basing on the later evidence of copper goods and burial practices from the Late Archaic stage (Bassett et al., 2019).

1.2.2.2. Glacial Kame Complex and The Red Ocher Culture

The Glacial Kame Complex was a complex found within the Great Lakes region, inhabiting Michigan, Ohio, Indiana, and Southern Ontario (Cunningham & Griffin, 1948). It is unknown the exact temporal placement of Glacial Kame due to a lack of dating, but evidence suggests the earliest formation of Glacial Kame could have begun during the Middle Archaic or the start of Late Archaic (Sciulli, Piotrowski, et al., 1984). This complex was given the name due to the mortuary practice of burying the dead in a kame, an irregularly shaped hill composed of sand and gravel, initially accumulated in a depression on a retreating glacier, when the glacier melt, the pile of sand and gravel falls on land forming an isolated hill. Within a Glacial Kame burial, many items can be found which are almost always non-utilitarian, such as sandal-shoe shell gorget which are characteristics of a Glacial Kame burial, variety of shells and shell beads, copper beads, bird stones, and stone pipes (Donaldson & Wortner, 1995; Sciulli, Piotrowski, et al., 1984). Some sites also exhibit worked and unworked animal bones from various different species, such as antler projectile points (Donaldson & Wortner, 1995). It is to be noted that some Glacial Kame burials indicate the practice of cremation and evidence of red ochre usage, demonstrating further burial practice attributes, although the usage of red ochre does not appear in all burials whether this demonstrates further symbolism for particular burial is unknown (Donaldson & Wortner, 1995; Sciulli, Lozanoff, et al., 1984; Sciulli, Piotrowski, et al., 1984). Pieces of pottery can also be found within some Glacial Kame burial, although the lack of dating prevents further understanding of when pottery usage was first established in the Great Lakes region, the earliest pottery usage within North America can be seen in Southeastern United States approximately 4500 BP, perhaps a connection between the Great Lakes and Southeastern America (Sassaman, 1998). The finding of projectile points amongst Glacial Kame

burial exhibits diverse styles with some burials lacking projectile points, leading to suggestion of different manufacturing process and alternative behavior develop within Glacial Kame complex.

Due to the variation of findings within the discovered Glacial Kame burials, speculation arises suggesting the discovery of Glacial Kame could be the amalgamation of more than one culture, or obtaining influence from neighboring traditions (Ritzenthaler & Quimby, 1962). The similarity exhibit between the burial practice of the Glacial Kame complex and the Red Ocher culture led to the possibility that the two cultures may not be separate. These two cultures overlap spatially and temporally in Michigan, Indiana, and Ohio, with the finding in both sites suggesting some form of relations with the early Old Copper complex, perhaps followers of basic cultural traditions (Ritzenthaler et al., 1956). The Red Ochre culture can also be found in Wisconsin, Illinois, and Iowa, spreading further southwest of the Great Lakes region than the Glacial Kame. As previously mentioned, the characteristic of Glacial Kame burial is the sandal shaped gorget and the usage of kame, which differs from a Red Ochre culture burial, as the latter burial contains turkey tail blades which do not appear in Glacial Kame burials and all Red Ochre burials, as the name implies, heavily featuring the usage of Red Ochre. (Cunningham & Griffin, 1948). Current evidence only utilizes these differences to distinguish between two cultures with no findings in both cultures containing the unique items of the other cultures, therefore these two cultures are still considered as two distinguishable cultures but are closely related (Ritzenthaler & Quimby, 1962). Although the overlapping region between the two cultures suggests potential interaction amongst each other, little is currently known regarding if such interaction or relationship existed.

1.2.2.3. Aquatic transportation

The establishment of trading amongst cultures in a different area would have required high mobility and one such method is the utilization of aquatic transportation along and across the river, suspected to have inaugurated during the middle archaic (Jefferies, 1995, 1997; R. M. Stewart, 1994). Aside from the evidence of copper trading, some late Middle Archaic sites located in the Ohio Valley containing grave goods that are not local to the region (Donaldson & Wortner, 1995; T. M. N. Lewis & Lewis, 1961; Rothschild, 1979). These items in the form of shell beads identified the presences of shells species associated with the Eastern coast, found in burials with the deceased, perhaps the usage of shell beads was used as a symbolic material to show case hierarchy of the buried individual due to the difficulty of obtaining the items (Rothschild, 1979). The utilization of shell beads is commonly found in Eastern Woodlands tribes of North America during the late Archaic and Woodland period, known as wampum which is a string of white and purple shell beads, and are used as storytelling and ceremonial gifts. E.g. The Hiawartha wampum belts, the symbolism of peace between the Iroquois Confederacy (Parker, 1909; Sullivan, 1992). Although the Hiawartha wampum belts are related to recent time, the importance of shell beads perhaps have a higher purpose during the Middle Archaic (Claassen, 2008; Pietak, 1998).

The finding of whole shells in numerous different coastal and lakeshore sites also supports the claim of aquatic transportation development during the Middle Archaic. Evidence of shell middens highlights a heavier reliance on fresh water marine resources, especially shellfish, during this period compared to the Early Archaic (Betts & Hrynick, 2017; Erlandson et al., 2005; Erlandson & Braje, 2011). Shell middens are known as dump sites containing mostly mollusk shells, demonstrate the adaptation of villager lifestyle and reliance on fresh water marine resources as a staple diet as well as the usage of areas for disposing of domestic waste. Animal bones and other lithic resources can also be found amongst shell middens showing a continuation of mixed hunting strategy from the Early Archaic Stage (Cook-Patton et al., 2014; Rick et al., 2011). The high volume of shells found within a shell midden emphasizes an increased foraging strategy for this particular resource which would require greater ability to gather sufficient quantity, the concentration of gathering fresh water resources could cause an unsustainable lifestyle if it is solely relied upon, therefore the utilization of aquatic transportation would enhance the collection of shells from numerous coastal sources to more inland location and would explain the large size of shell middens found in some archaeological sites (Álvarez et al., 2011).

Early archaeological work located in Northwest America also exhibits the usage of aquatic transportation (Chapman, 1943). The area of Columbia Plateau demonstrates a continuous occupational history for the past 7000 years with the local population establishing a fishing economy, capturing salmon, and trading with different tribes along the Columbia River (Chapman, 1943; Hutchinson & Hall, 2019). Evidence from Kettle Fall and Celio Falls, two important salmon fishing sites located on the Columbia River, became the center of trading as indigenous people from the west coast and people from the Great Plain located in the east, travel to the two locations to exchange for salmon or the right to procure this resource during the annual salmon run (Galm, 1994; Hutchinson & Hall, 2019; Knudson, 1980). The mass gathering of people or the trading amongst one another which can be identified during the initial European arrival, demonstrate the extensive networking between groups of different regions, most likely spanning from the Archaic stage to the presence (M. Stewart, 2004). One reason why these two sites became important area is due to the spiritual nature and the respect indigenous people have regarding their resources, as the method of procuring salmon as seen and described by oral history, inform us that only the salmon which did not make the fall was captured in order to maintain the salmon populations (Kay, 1985). This prevents over procurement of salmon, allowing the two sites to maintain the center for salmon trading between the west and east.

1.2.2.4. Maritime Archaic Tradition

Flourished across the Atlantic Canada coastline between 7000 and 3800 BP, the Maritime Archaic Tradition was named after the discovery of the Port au Choix site in northwestern Newfoundland dated to 4000 year old (W. Fitzhugh, 1978; J. Tuck, 1971). This traditional way of

life can be seen in Northeastern America and demonstrate the utilization of marine resources which appears to be pivotal for the indigenous people of the North-Eastern coast. The L'Anse Amour site being one example, featuring one of the oldest burial mounts in North America, located in southern Labrador possibly dated to 7000 years ago, consist of walrus tush, fish bones, and toggling harpoon (M A Priscilla Renouf, 1984). The sophistication of the weaponry during this period validates the importance and the reliance of marine resources for the first people of this region.

Current archaeological evidence suggests the idea of two variations within the Maritime Archaic tradition, as the difference between Maritime Archaic assemblages have become apparent, classifying into the Northern and Southern branch, with the Northern branch possibly the earlier of the two that inhibited southern Labrador (Bell & Renouf, 2004; W. Fitzhugh, 1978). The variations are suggested as the causation of regional differences with the southern variants intruding the Northern branch with new stone tool complexes that appeared in southern Labrador around 6000 BP.

The northern variant dates from 7500 BP to approximately 3500 BP can be found as far north in Saglek Bay and as far south by the shore of Labrador with no interior site found in Newfoundland (McGhee & Tuck, 1975). Within northern variant sites, evidence of residency such as longhouses were uncovered at Labrador archaeological sites, suitable for small families and potentially used as a place for a long-term commitment (W W Fitzhugh, 1985). Although some projectile points can be identified as the northern variants found in the shoreline of Newfoundland, suggesting evidence of marine travel into the island, a lack of evidence related to residency from possible northern variant archaeological sites located in Newfoundland suspect the northern variant did not settle in this location (W. Fitzhugh, 1978).

The southern variants can be found between the northern Black island and southern Bonavista Bay of interior Newfoundland (Carignan, 1975). Although evidence of northern branches can be found in Newfoundland, the southern branch was proposed to have first colonized the island of Newfoundland by 6000 BP (M A P Renouf & Bell, 2011). Whether the southern variants descended from the northern branch is unknown presently, however the lack of conflicts between the two that coexisted for 3000 years in the same region, sharing the same resources perhaps indicates social involvement which appears to keep the peace between the two variants (H. S. Reid, 2007). Overall, the Marine Archaic people were extremely well adapted to life by the coastline in Newfoundland and Labrador based on their technology and other items found, therefore the complete absence of the Marine Archaic tradition after 3000 BP is a mystery, considering how well the Marine Archaic people adapted to the surrounding of Atlantic Canada shore (J. A. Tuck, 1975).

1.2.2.5. The arrival of Paleo -Inuit

The origin of Inuit cultures has baffled many scientists for generations. Where did they come from? Are they descendants of the first people or recently entered American soil? Theories began to appear in the late 19th and early 20th century, studying the legends, cultural traditions, and linguistics suggesting Siberia be the home of the Inuit (Mathiassen, 1930; Thalbitzer, 1904). Furthermore, a publication by Steensby 1916, distinguished arctic and sub-arctic cultures, recognizing the arctic group as Paleo-Inuit suggested to have developed within central inland arctic Canada, spreading east into Northeastern Canada and Greenland and west wards into Alaska and the Bering Strait. This movement westwards supposably gave rise to a later subarctic culture possibly arose from the Bering strait known as the Neo-Inuit, gradually spread east into the Arctic, leading to the discrepancy of tools found allowing the distinguishment of Paleo and Neo-Inuit (Birket-Smith, 1947; Steensby, 1916). As the archaeological studies involving Inuit continue, the concept Steensby applied has been altered, dismissing the development of Paleo-Inuit in central inland arctic Canada due to similarity with the Arctic Small tool Tradition, which was developed along the Alaska Peninsula, and Old World traditions, such as lamp usage and methods of hunting (Larsen & Rainey, 1948). The finding which led Steensby to believe the development of Paleo-Inuit to be in central inland arctic was the suggested contacts with the indigenous people due to overlapping region between the different cultures (Birket-Smith, 1947), however, it is unknown the level of interactions between the early Paleo-Inuit and the Indigenous people in this area.

Evidently, contacts appear in north eastern woodlands but at a later period associated with the Dorset culture, a late Paleo-Inuit culture, as Red Paint culture containing distinctive features likely to derived from the Inuit (De Laguna, 1946). Further contacts were also speculated in Northern Labrador when the early Paleo-Inuit first appears around 4000 years ago, possibly replaced the Maritime Archaic Tradition as they exploited the same resources along the coastline of Labrador (Cox, 1978). Paleo-Inuit archaeology sites located in Northern and Central Labrador are often situated in the same location as Maritime Archaic tradition, with evidence of campsites previously occupied by the Maritime Archaic tradition replaced by the Paleo-Inuit, further hinting the arrival of the Paleo-Inuit displacing the Maritime Archaic Tradition (W. Fitzhugh, 1976; J. A. Tuck, 1976). However, the disappearance of Maritime Archaic Tradition may not be due to the arrival of Paleo-Inuit, as our current understanding is still limited, the coincidence of Paleo-Inuit occupying the same region favors this hypothesis (Schwarz, 1994).

Although it is to be noted that the Dorset culture differs from its predecessor, the pre-Dorset which utilizes bows and arrows as a hunting strategy whilst the Dorset culture does not make use of this strategy (Wells, 2012). The abandonment of bows and arrows technology by Dorset culture signifies the possibility of new adaptation to aquatic hunting, moving away from hunting land-based animals, as archaeological evidence identifies an increased assemblage of sea mammal items such as walrus and narwhals bone (William W Fitzhugh, 1980; Wintemberg, 1939). The Dorset culture appears to settle along the coastline of North Eastern Arctic, Labrador, and North Western Greenland correlates to the idea of mobile aquatic adaptation,

they were then replaced by the arrival of Neo-Inuit around 1,000 AD (Figure 5). It is unknown the reason for their disappearance, whether it is the competition with Neo-Inuit or climatic changes (Cox & Spiess, 1980).

The movement of Paleo-Inuit from the Old World via the Bering sea demonstrates a late migration outside of the Americas approximately 2500 BC (Krupnik et al., 2012). The Paleo-Inuit travelled across the Bering strait was suggested to require aquatic transportation in order to sail across the Bering Sea and into the Northern Arctic due to the rise of sea level which covers Beringia by the Late Archaic. The archaeological evidence of human presence at Qeqertasussuk in Greenland associated with a Paleo-Inuit culture known as Independence I or Saqqaq dated to approximately 2000 BC, validate the idea of boat usage to travel into Greenland (Grønnow & Sørensen, 2004; Knuth, 1954). However in regards to the usage of boats, evidence of the physical objects are still lacking but the movement across the Arctic coastline and the behavior

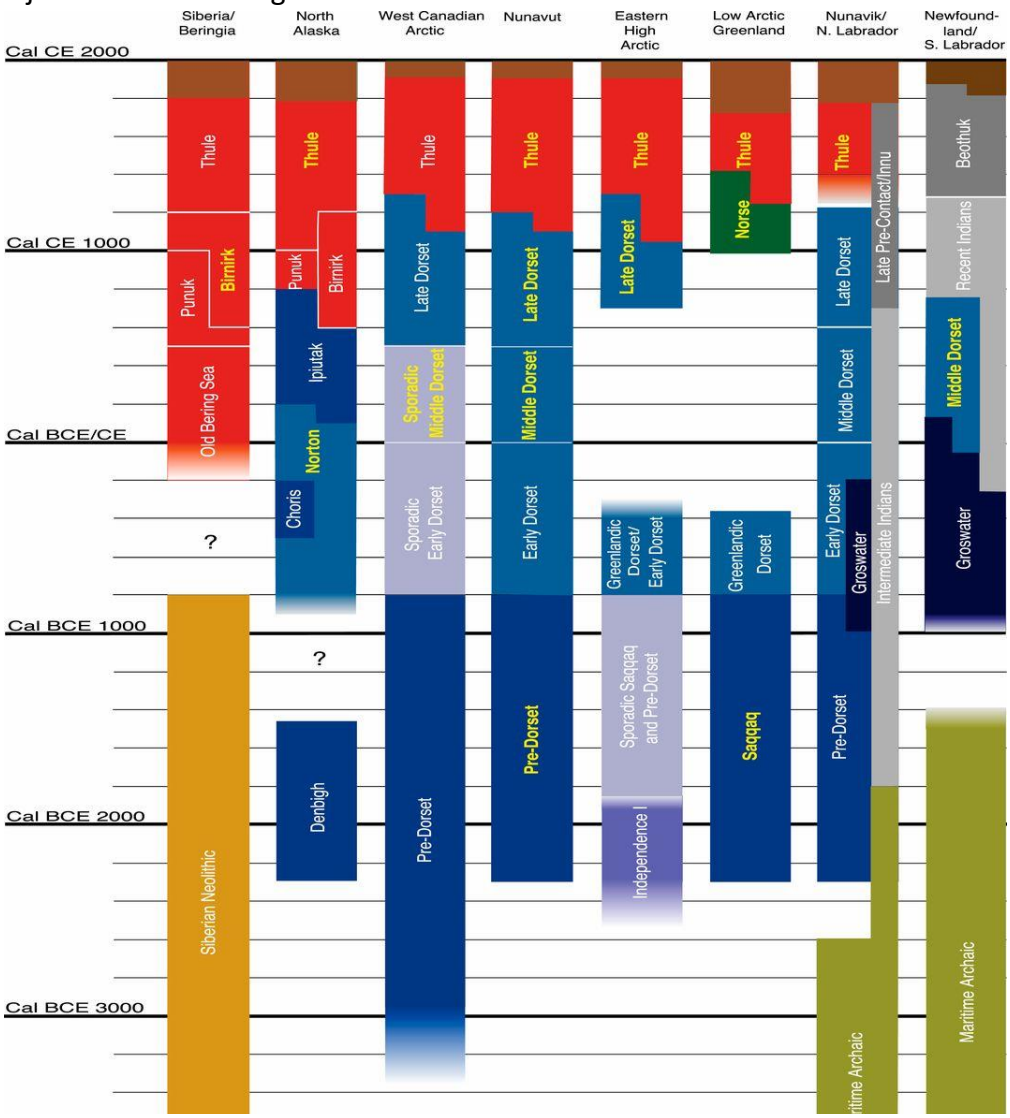

Figure 5. The New World Arctic and North Eastern Siberia prehistoric cultures in Chronological order. (Raghavan, et al, 2014). Premission to freely reproduced the image shown under the terms and conditions of Science's Creative common licenses (CC-BY 4.0).

of hunting sea mammals led to the assumption of aquatic vessels to facilitate this movement (Binford, 1978).

1.2.2.6. Spread of Neo-Inuit

Neo-Inuit tradition begun possibly around 2,200 years before the present, as expressed by the Old Bering Sea culture from Siberia. This gave rise to both the Birnik and Punuk culture which develops into the western Thule culture with contribution from Paleo-Eskimo traditions that can be found on both sides of the Bering Strait (M. Raghavan et al., 2014). The finding of Thule culture in Eastern North American Arctic around 1,000 AD, demonstrate an efficient method of transportation in the northern arctic using large skin boats and dog sleds, whilst introducing new hunting gear such as harpoons and bows for hunting whales (Maxwell, 1985; McGhee, 2001). This technology and the Thule culture replaces the Dorset in most regions, along with the declination of whaling post-European arrival, transforming into the modern Inuit cultures that we know today (McGhee, 2001).

The Thule culture in the east can be found around the coastline from the Hudson's Bay region towards Northern Newfoundland and into Greenland by the time of European arrival (figure 6). These locations in which the Thule occupied, overlap with indigenous American tribes (Kalkreuth et al., 2012). Whether a mutual relationship was formed between the two cultures is unknown today. It is to be noted that the Thule people were exceptionally skilled with crafting technology and were using iron before European contact, creating projectile points for arrows (McCartney & Mack, 1973). Speculation regarding the source of iron is relatively unknown, as the tool kits found in Thule culture do not exhibit any technology for gathering ores or evidence of heating for refinement (Morrison, 1987). Suggestions of meteoric resources were implied in order for Neo-Inuit to utilize iron (McCartney & Mack, 1973). Other suggestions were the potential iron trading between Neo-Inuit and Vikings during the Norse expansion into

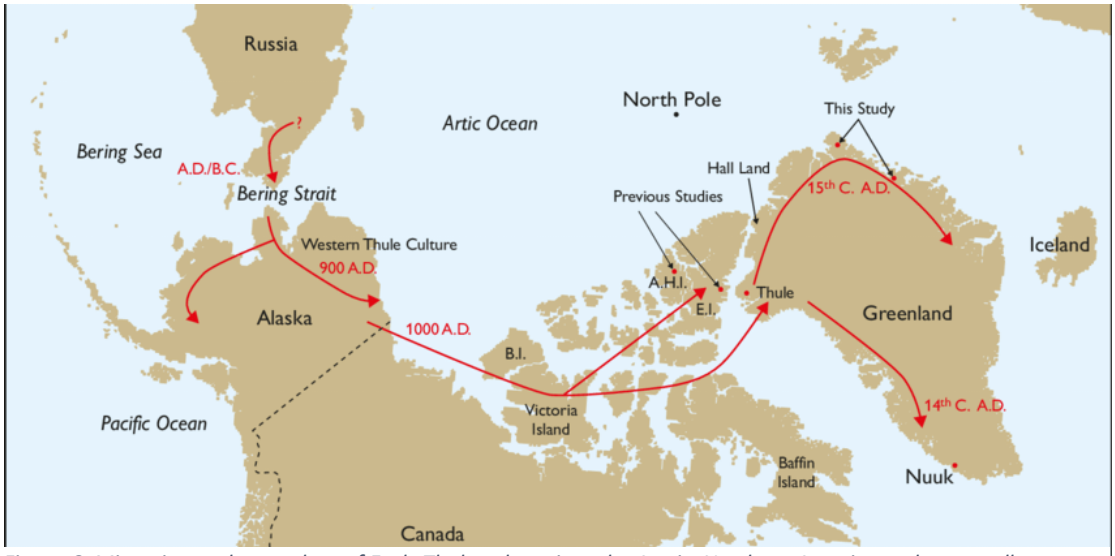

Figure 6. Migration and entry date of Early Thule culture into the Arctic, Northern America and eventually Greenland. (Kalkreuth et al., 2012). Permission to freely reproduce the image shown under the terms and condition of Geological Society of Denmark creative commons attribution (CC-BY 4.0)

Greenland when the two cultures met around 1000 AD as written in the Saga of the Greenlanders (William W Fitzhugh, 2000).

The movement of both Paleo and Neo-Inuit led to the spread of the Eskimo-Aleut language in the Northern Arctic. It is believed that the ancestral language of the Inuit and Aleut potentially separated when the Paleo-Inuit travels into America. It is to be noted that although the Eskimo-Aleut language is classified as one of the native languages of the Americas, it is placed in a different language family compared to the other language families in America. This is due to the similarity with Yupik languages spoken in far-east Russia, complimenting the hypothesis of a recent move into America.

1.2.3. Late Archaic stage

The progression of time sees the stabilization of climatic temperature during the Late Archaic, suspected to be similar to present-day environment (Fitting, 1968; Sears, 1952). The deglaciation of the ice sheets provides remnants of glaciers in the area such as the high arctic and alpine regions with sea levels liken to the current time (Vincent et al., 2001). The calming environment correlates to a more permanent settlement formation, as the Late Archaic stage spanning from approximately 3000 BC to 1000 BC, observes an increased usage of pottery, cooking slabs, and permanent housing (R. M. Stewart, 1994). Late Archaic cultures continue to hunt game and gather comparable to the Middle Archaic, however, the defining characteristic for the Late Archaic stage is the cultural and technological developments of farming and cooking ability (Dincauze, 1975; Versaggi et al., 2001). Although the ability to farm is not as intensively compare to the later stages of the woodland period, the ability to grow crops suggests the establishment of permeant residency. Evidence of shell middens also supports this idea of a settled lifestyle (Álvarez et al., 2011). Finding from funeral objects in the Great Lakes region continue to showcase items that are most likely traded from nearby cultures, such as the continuation of the Old Copper complex and the importation of shells from the Atlantic Coast (Erlandson et al., 2005). Cremation and burial also appear commonly around the Great Lakes region, with evidence of mound construction beginning in the region south of the Great Lakes (Kelley et al., 1958; Rothschild, 1979).

1.2.3.1. Agriculture development

The indigenous people in the Americas domesticated multiple different plant species, each species appears to have arisen independently in a different regions of the Americas, South America, Meso America, and North America (Olsen & Schaal, 1999; Price, 2009; B. D. Smith, 2006). The main agricultural crops for the indigenous people of North America are collectively known as the Three Sisters, which consist of maize, beans, and squash (Hart, 2008). These plants are usually found in close proximity to each other as indigenous people of North America develop the technique known as companion planting to aid the growth of each species

(Lewandowski, 1987). Although the development of agriculture enhanced survivability and provide a reliable food source, the start of agriculture in North America did not begin during the Middle Archaic. It most likely started in the Late Archaic as the cultivation of the Three Sisters in North-Eastern America suggest the presence of these plants was utilized for the improvement of the regional agroecosystem, not initially for agriculture purposes (Hart, 2003; Zhang et al., 2014).

It is to be noted that the domestication process of the Three Sisters did not originate in North-Eastern America. The presence and the cultivation of the Three Sisters in this region was the causation of trading between numerous cultures, using the Mississippi River and other river sources as methods of transporting crops as evidently shown in Late Archaic and Woodland period cultures (John P Hart, 2008; Pluckhahn et al., 2016). Additionally, the utilization of the Three Sisters can be found in different areas within the Americas. Cultivation of maize can be seen across the Americas and was speculated that multiple maize domestication events occurred due to the diversity found in a different region. In order to understand the high level of diversity in maize, a phylogenetic approach was implemented by (Matsuoka et al., 2002). Their findings concluded that maize developed from a single domestication event in southern Mexico as early as 9,000 years ago with two paths of dispersal, first path travel into northern Mexico and the other southward towards Guatemala and the lowlands of South America (Matsuoka et al., 2002). Similar dispersal pattern can be said for both squash and the common beans, as they both originated from domestication events in Mexico (B. D. Smith & Yarnell, 2009). However, the domestication of squash occurred perhaps 1,000 years before maize, and beans were the last plants of the Three Sisters that underwent the process of domestication with two possible points of origin in Mexico and Peru (B. D. Smith & Yarnell, 2009). The variation of the maze found is most likely the causation of further selection events after the initial domestication. Other agricultural plants were also farmed but only seems to appear during the late Archaic, but overall, the importance of agriculture in the Northeast can be seen in both Algonquian and Iroquoian linguistic cultures by the late Archaic, resulting in a more settled lifestyle.

1.2.3.2. Iroquoian vs Algonquian vs Athabaskan (Dene)

Language spoken by the indigenous peoples of the Americas contains many different family groups and dialects, as well as many unclassified languages and lost languages post-European contact. Previous studies attempted to group them into one distinctive group, but the variation between populations differs significantly to put them into one large linguistic family (L. Campbell, 2000). Within North America, the largest spoken linguistic family groups are the Uto-Aztecan, Na-Dené, and the Algic, with the Na-Dené and Algic containing the widest geographic distributions. Na-Dené speakers can be found ranging from Alaska to Washington and southwest America, commonly spoken in Navajo population, whilst the Algic speaker spread

from the majority of Northeastern Canada and Northeastern California comprise of the speaker from Algonquin, Cree, and Ojibwa population.

Na-Dené linguistic group contains the Athabaskan language which is the largest spoken group within the Na-Dené classification, can be split into three geographic groups: Northern, Pacific, and Southern Athabaskan. The Northern Athabaskan is the largest group in the Athabaskan family, spoken by indigenous peoples in Alaska, Yukon, and Northwest Territories. The Pacific Athabaskan was difficult to place as a separate family due to the similarity with Northern Athabaskan, further consideration is required to determine whether the Pacific branch is an internal branch of Northern Athabaskan. The Southern Athabaskan is also known as Apachean is spoken in Southwestern America the current home of the Navajo. Present evidence suggests Na-Dené languages potentially related to the Yeniseian language of Siberia as key evidence identify particular consonant articulations that exist in both language, this led to further discussion suggesting the origin of Na-Dené speakers are of recent migration approximately 8,000 years ago from Asia into the Americas continent (Vajda, 2012). This is still highly controversial and requires further study.

The wide distribution of the Algic or Algonquian-speaking population can be further divided into categories or subgroups, East, Central, and Plains Algonquian. It is believed that the Eastern Algonquian are the true group of speakers, as the Central and Plains Algonquian groups consist of linguistic elements which do not appear to have derived from Proto-Algonquian speakers, rather it could be local dialect with influence from nearby language contact (Goddard,

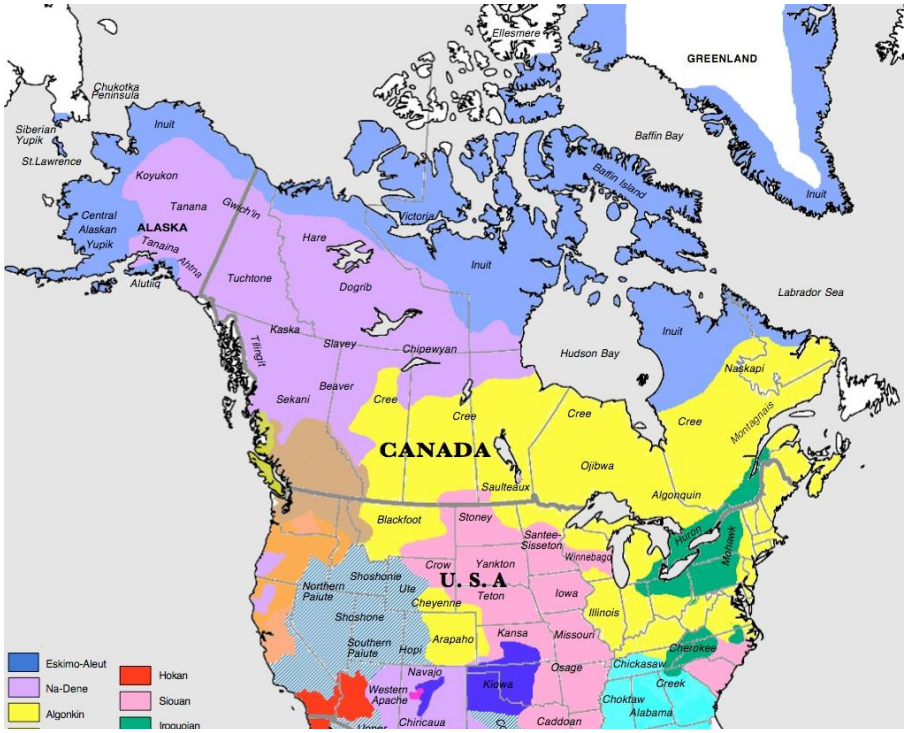

Figure 7. A map showing the distribution of the different major linguistic group found in North America prior to European arrival http://www.languagesgulper.com/eng/Northamer.html Premission was granted by the author for the usage of this image in the thesis under creative commons license 3.0 (CC BY-NC-ND 3.0)

1994). The common acceptance for the spread of the Algonquian language is that the ancestor of Algonquian, Proto-Algonquian, originated in the west and move eastward. Over time, migration causes diversity. and the Plains Algonquian in the west first deviated, followed by the Central Algonquian (Goddard, 1994).

Coincidentally, within Northeastern Canada, the Iroquoian language is spoken along the St. Lawrence River with some spoken in North Carolina, is surrounded by Algic speakers as shown in figure 7. The Iroquoian family can be divided into two divisions based on vocabulary and modern phonology, the Northern and Southern Iroquoian, with both in critical danger due to the lack of speakers remaining. Studies so far are poorly documented due to the lack of speakers and the migration of Iroquois post-European arrival.

1.3. Woodland period (1,000 BC to 1,000 AD)

Approximately 1,000 BC to the time of European contact in Eastern America is the Woodland period. This period is known for a developmental stage, especially around the lower Great Lakes of North America demonstrating the development of distinctive Iroquoian and Algonquian speaking cultural groups (Pfeiffer et al., 2014). Within the region of Southern Ontario, diverse cultures can be identified, with Northern Iroquoian speakers such as the Wendat, Tionontaté, and Attiwandaronk living in close proximity with Algonquian-speaking groups (figure 8). Additionally, further interaction with the Haudenosaunee (Iroquois Confederacy or the Five Nations) which comprise of Cayuga, Mohawk, Oneida, Onondaga, and Seneca tribes, cluster across the New York State, highlights the diversity of tribes within a small

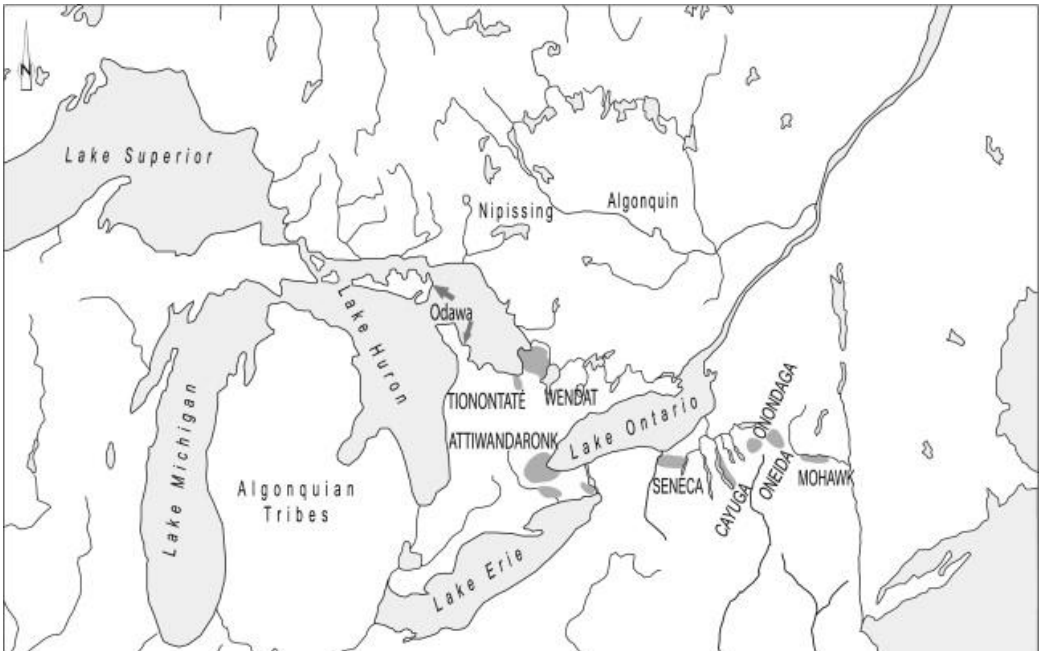

Figure 8. Map of the First Nations populations of the Great Lakes region prior to the arrival of European contact (Pfeiffer et al 2014). Permission to freely reproduce the image shown under the conditions of the journal of Archaeological Science creative commons license 3.0 (CC BY-NC-ND 3.0)

region all containing unique cultural histories in their own geographic tribal territories (Mann, 2000).

One key advancement during this period is the widespread usage of pottery, although it has been noted that pottery was used in other places during the Archaic period, the diversity of decoration and ways of making each style of pottery once again showcase the variety of cultural practices within this period (Kooiman, 2016). The increase of agriculture provided an important method for large settlements with maize as an important source of food to sustain all year round (Pihl et al., 2008). This coincides with an increase in population to support a more permanent village lifestyle, as demonstrated by the increased sizes of village and pottery usage (Heidenreich, 1971).

The Woodland period can be divided into three categories, early, middle, and late. The Early Woodland period shows cases many trends that were developed at the end of the Archaic Stage, which includes trading between different cultures over large distances and regional burial complexes exhibited in different areas. Hunting and gathering appear to be the main source of resource with some agriculture development, suggesting more permanent settlements than the Archaic stage (Fiedel, 2001; Pluckhahn et al., 2016). It is the usage of pottery that separates the Early Woodland period from late Archaic sites. The increased appearance of Mound burials in southern Lake Ontario highlight a change of burial practice exhibited in Glacial Kame/Red Ochre culture, it is unknown the causes for the variation, perhaps the influences from a different culture from trading introduced this method into some society (Silverberg, 1986).

The Middle Woodland period follows the tradition of the Early Woodland period but with a further increase of pottery, creating thinner and increase quality compared to earlier periods. The trading network once established during the Archaic appears to intensify from coastal area to local and interregional trading, as the burials mounds containing rare resources and numerous mortuary gift suggests the importance of certain burial compared to other mounds (Silverberg, 1986). Culture along the river flourished in the northeastern and mid-western Eastern Woodlands during this period, utilizing the river as a trade route, connected by a series of trading networks known as the Hopewell exchange system (Carr & Case, 2005). The overview of the Hopewell tradition is not a singular culture or a society, it is numerous different tribes dispersed over a large region, covering from modern-day Florida to the Northern shores of Lake Ontario, with each culture contributes to the high activity of trading (M. F. Seeman et al., 2019). Exchanged materials were then covert into products for further regional trading inland (Hill et al., 2018). One important note to consider is the harmonious trading between different territories, as this requires formal agreements with the possible linguistic barrier (Abrams, 2009).

Late Woodland period continues the traditions set by the earlier stages, intensification of trading over a greater distance and the gradual adaptation to bow and arrow can be seen throughout this stage (Emerson et al., 2000). An increase in agricultural production of Three

Sisters can be seen during the Mississippian period (Emerson & Lewis, 1999), this tradition similar to the Hopewell, a combination of different cultures and trades amongst each other along the Mississippian river towards the mid-western. The number of Late Woodland settlements increases, however, the size decreases suggesting population increase with diversification away from initial communities (I. D. Campbell & Campbell, 1994). This way of life continues until the Europeans arrived, with many regions of Eastern Woodlands adopted the Mississippian culture and retained this as shown in Iroquois culture (Richter, 1983).

1.4. Post-European contact

The arrival of Christopher Columbus in the Bahamas on 12th October 1492 ended the pre-Columbian period, accredited as the first contact with the indigenous people, although Vikings made contact with the North-Eastern indigenous population some 500 years prior to Christopher Columbus arrival (William W Fitzhugh, 2000). However, the first contact with Northern American was accredited to John Cabot when his voyage landed at North-Eastern America in 1497, with many different proposed landing locations ranging from Newfoundland, Nova Scotia, Labrador, and Maine as possible landing sites (P. Martin, 1973).

The declination of the Indigenous population corresponds to the settlement of European colonization, but the decrease could have occurred prior to the arrival of Europeans (Neumann, 1940; M. O. Smith, 1995). Speculation of conflicts between many neighboring tribes and cultures due to the lack of resources and territories led to disagreement amongst many tribes within North America. The formation of the Haudenosaunee confederacy potentially began 800 years ago with the suggestion of later establishment in northeast North America by The Great Peacemaker, Jigonhsasee and Hiawatha, connecting the five nations with later acceptance of Tuscarora forming the six nations we know today (Venables, 2010). Under the Haudenosaunee confederacy, rules otherwise known as the Great Law were agreed upon between the nations to mutually respect and prevent greater loss of lives, a symbolism of tree planting when the five Chief of the Nations gathered by the Onondaga Lake to signify the binding of the Haudenosaunee confederacy. The combination of the five nations also significantly strengthens the confederacy, increases military and political power against nearby nations.

European arrival initially established great relationships with indigenous people, developing trades of furs for metals and other resources. The conflicts between different European countries were introduced into indigenous tribes, as different tribes were in alliance with different European countries competing for the same resources. The depletion of resources and the high demand for furs lead to conflicts amongst tribes and with European allies, as the competition was too great. This generated wars over territories leading to a loss of indigenous population after the European settle within America (Bennett, 2018). The travel across the Atlantic Ocean into the Americas also brought in foreign disease which also affected the indigenous population causing a further decrease.

The Huron Nation was one of the Iroquoians speaking populations around the Lake Ontario region which was heavily affected due to European contacts. The Huron-Wendat ouccpied from the source of the St. Lawrence River to the East of Lake Huron, lived in longhouses up 90 meters in length (Creese, 2012). A society similar to other Iroquian population predominately consist of an economy based on the Three Sister and fish, with close trading amongst other population within the Huron Nation and Algonquin nations of Georgian Bay and Ottaway Valley (Hancock et al., 1995; Lovisek, 1991). The Huron people were not a tribe but a confederacy, containing the people of the Bear (Attignawantans), people of the Cord (Attigneenongnahacs), people of the Rock (Arendarhonons), and the people of the Deer (Tahontaenrats), all had mutual intelligible languages, enabling a full understanding of each other under the Iroquoian language family (Tooker, 1991). Prior to European arrival, the Huron developed conflicts with Haudenosaunee Confederacy, forcing the Huron Nation to abandon territories. When the Europeans arrived, the Huron nation was once again forced to travel due to further conflicts with Haudenosaunee and European diseases. These conflicts led to the death of many from the Hurons and other nearby nations, equally, many were adopted into the culture of the Haudenosaunee Nation, causing the loss of a smaller society (J. E. Lewis, 2004).

2. A brief history of Genetics

2.1. Start of Genetics

As defined in the Oxford dictionary, genetics is the science of heredity, dealing with resemblances and differences of related organisms resulting from the interaction of their genes and the environment. A branch of biology that analyses the genome of an organism. The origin of the first study of genetics is difficult to determine due to the rapid evolvement of the topic and the collaboration of great minds to reach the current understanding. One of the earliest studies related to genetics is by Gregor Mendel in the 19th century observing pea plants, identifying traits from parents' plants which was passed down to the offspring, a terminology which we understand as trait inheritance (Mendel, 1965). Although simplistic at the time, the thought process was groundbreaking with modern genetics still implies this understanding in modern research. Other revolutionary events which shaped the future of genetics is the discovery of deoxyribonucleic acid (DNA) located within the nucleus of a cell and the structure of the DNA double helix molecule during the mid-20th century (Avery et al., 1944; Watson & Crick, 1953), pave the way to all present genetic research. The backbone of DNA connected by a phosphodiester bond with each sugar attached to their complementary bases via hydrogen bonds, adenine (A) with thymine (T) and guanine (G) with cytosine (C), to create the double helix structure (Watson & Crick, 1953). Further research at a later time discovers the duplication of DNA was the causation of the enzyme known as DNA polymerase, which can introduce possible errors during the replication process unless it is corrected by the cells own repair system (Chatterjee & Walker, 2017; Kornberg et al., 1956). These errors are known as mutations, and it is critical in modern research to understand not just the causation of the mutations but the effects the mutations cause.

2.2. Technological advancement

2.2.1. Sanger Sequencing

In order to understand mutations or variations within a segment of a DNA, old studies utilize restriction enzyme which cut at specific locations to determine whether the fragments of DNA consist of a particular mutation. This method is limited as it can only identify the selective region and the enzyme can easily cut multiple positions which can cause false positives. Around the late 1970s, DNA sequencing was introduced by incorporating chain-terminating dideoxynucleosides during DNA replications a method known as Sanger sequencing (Sanger et al., 1977). This method requires a single-stranded DNA which is used as a template, DNA polymerase for replication, a DNA primer approximately 20bp complementary to the initial position of the template DNA to initiate synthetization, deoxynucleotide triphosphates (dNTPs) to elongate the replicating strand, and dideoxynucleotide triphosphates (ddNTPs) to prevent further elongation of the strand. The initial research by Sanger et al., 1977, showcases four separate reactions using one of the four dideoxynucleotides for each reaction and visualized using x-ray film, thus allowing the position of each base pair to be determined.

Over the past 20 years, the Sanger sequencing method has improved significantly, the four reactions which were first shown in the original research are now altered with dye-terminator sequencing and can be performed under one reaction. The usage of fluorescent-labeled dideoxynucleotides chain terminators emits different wavelengths allowing easier detections for more precise base pair detection. On top of this, automation of DNA sequencing instruments such as a DNA sequencer can perform multiple Sanger sequencing reactions and dispatch data automatically, speeding up the entire process. The development of this technology allows the first genome, a bacteriophage, as well as the first human genome to be sequenced (Fiers et al., 1976; Venter et al., 2001). However, regardless of the advancement of this technology, modern Sanger sequencing techniques exhibit limitation which affects the ability to distinguish the first 40bp in great accuracy, this also affects sequencing long-stranded DNA. Additionally, the cost of sequencing each base pair is significantly higher compared to other sequencing techniques, such as the Next-generation sequencing (NGS) methods (Shendure et al., 2017).

2.2.2. Polymerase Chain Reaction

Another advancement in genetic research is the development of Polymerase Chain Reaction (PCR) in the mid-1980s (Mullis et al., 1986). This technique is used commonly in different disciplines of genetic research in order to obtain millions of specific DNA copies from a very small quantity of DNA. The ability to acquire many copies of the desire DNA region is the reason this method is used in a range of genetic research from medical to forensics. The procedure requires a thermal cycler to undergo a heating and cooling process to commence the duplication process, a denaturing step to separate the double-stranded DNA into single strands, an annealing step allowing primers to align to their complementary region, and elongation steps for the extension of the replicating strand. This requires specific primers which are complementary to the target DNA region, a heat-stable DNA polymerase, and dNTPs for the elongation of replicating the strands. Many cycles can be performed thus allowing an exponential copy of the target region to obtain as shown in figure 9.

This method is extremely advantageous as previously mentioned to obtain high copies of target DNA from a small amount of DNA. However, it does have limitations, firstly information regarding the target sequence is required for the synthesizes of primers for successful amplification, otherwise, multiple regions would be amplified. Secondly, the sensitivity of PCR can amplify contaminate DNA causing misleading results for further investigation. Lastly, although it is rare, the introduction of mutation during PCR can occur as DNA polymerase can cause errors during the elongation stage (Zhou et al., 1991).

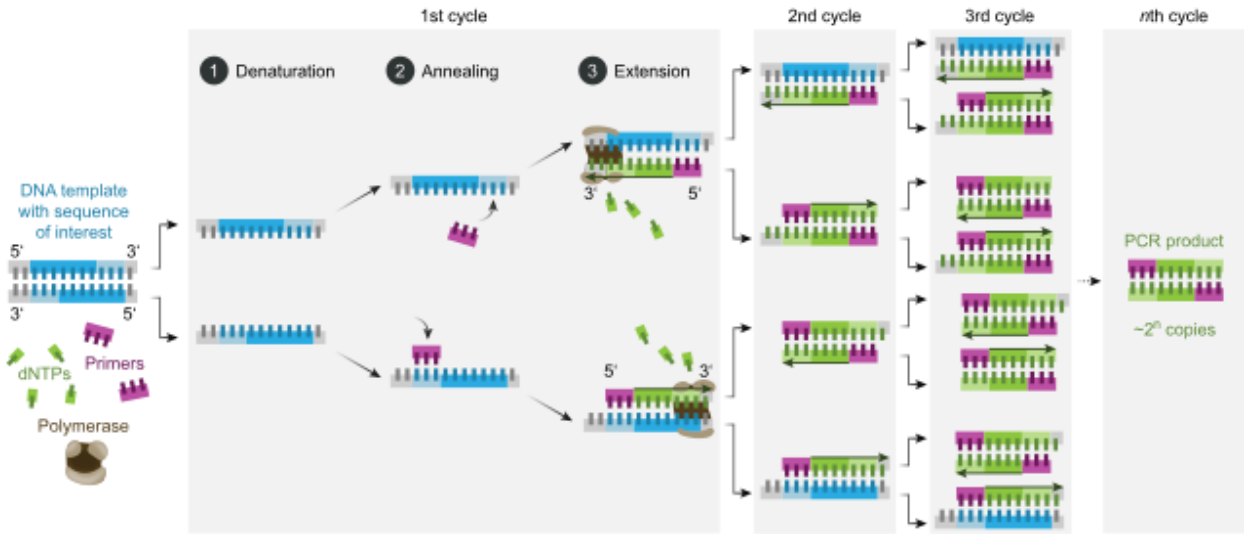

Figure 9. Schematic of PCR reaction to illustrate the duplication process of target sequences. Permission to freely reproduce the image shown under the conditions of creative commons license 3.0 (CC BY-NC-ND 3.0)

2.2.3. Next-generation sequencing

The advancement of sequencing technology has provided a method to reduce the cost of sequencing whilst generating genomes of species and organisms to high coverage. This new wave of sequencing technology is known as Next-generation sequencing (NGS), which requires three essential steps: amplification of the template DNA, perform the sequencing reactions, and analysis of the sequenced DNA. The Illumina dye sequencing, one method of NGS technology, commonly used for genomic studies, requires short fragments of DNA undergoes multiple amplification steps prior to sequencing to acquire great depth of each position to generate high coverages. Additionally, the ability to multiplex increases the number of samples running for each sequencing attempts speeding up the overall process of obtaining multiple genomes.

Presently, obtaining human whole genome data can be performed with different methods using NGS. There are two common ways vis the Illumina sequencing method, one is using probes to target region belong to a human, a method known as target enrichment capture, the second is shotgun sequencing, all DNA from the extracted sample are sequenced (Hofreiter et al., 2015). Both methods have their flaws, target enrichment focus on specific known mutation regions which means not the entire genome is sequenced, whilst the shotgun sequencing can amplify environmental DNA and other contaminants which would lower the overall coverage (Carpenter et al., 2013; Schubert et al., 2012).

Although the ability to sequence genomes is advantageous for many genetic studies, there is a limitation that can easily affect the results. Firstly, the number of programs available for the alignment process of sequenced data and the parameter for aligning against a reference

sequence can lead to bias with the results, especially when comparing with different studies. Secondly, commonly used databases such as Human Origin contain the genotyping of thousands of mutations, by focusing on these positions alone can lead to further biases (Patterson et al., 2006, 2012).

2.3. Population Genetics

The ability to pass on the genetics to offspring allows the mutations accumulated over time by both parents to be passed on. Over time, these mutations remain and are passed on to further generations whilst amassing further mutations within a population. The student of the different mutations within and with other populations is known as population genetics. By identifying these mutations, it is possible to decipher the origin and the distribution pattern of a population. These mutations are known as single nucleotide polymorphism (SNP), a substitution of a single base at a particular position within the genome. Different point mutations known as transition and transversion is used to categories specific mutation. When a purine is replaced by another purine or a pyrimidine replace by another pyrimidine, this is known as a transition mutation. Transversion mutations are the substitution of a purine into a pyrimidine or a pyrimidine into a purine. Further mutations such as the insertion and deletion of nucleotides can also be found within a genome.

In order to understand variations within a population, factors that can affect the gene pool such as interactions within and with other species, inbreeding within a population, and the number of founders from the original population can all affect the genetics of a population over time. Other factors, known as the positive selection, which is a process an individual or a population contains a particular allele benefited their survival to the environment, leading to the survival of these alleles to be passed on in future generations which can be detected in high frequency over time. Additionally, this can also have a negative effect, as the alleles which did not benefit an individual or a population would disappear over many generations. Genetic drift can also affect the allele frequencies detected, as the occurrence of stochastic events can offset or enhance the process of selection (Star & Spencer, 2013).

2.4. Beginning of Archaeogenetics

Population genetics of the past is difficult to determine as historical events and modern migration can alter the gene pool for modern-day populations causing complexity in understanding the origin of a particular population. The utilization of DNA isolated from ancient specimens, otherwise known as ancient DNA (aDNA) provides a snapshot of the genetics from the past (Pääbo et al., 2004). These ancient specimens such as bones, hair, teeth, and biological tissues are used to obtain aDNA, and dependent on the age, the quality, and the type of material used for DNA extraction, it transpires to the quality of aDNA extracted (Lassen et al., 1994; Rohland & Hofreiter, 2007b; Wayne et al., 1999). The ability to understand genetic

patterns at a particular point in time allows the formulation of new hypothesis which can then be tested or verifying existing hypothesis which one discipline may not provide enough evidence to support. The genetic snapshot in time also provides assistance in comprehending the variations between modern and ancient populations to simulate possible events that transpire from the past which sometimes the physical evidence misses.

The first publication using ancient DNA extracted DNA from the dried muscle of a museum specimen and targeted a fragment of the Quagga mitochondrial DNA, sequenced it to determine its genus as *Equus* (Higuchi et al., 1984). Further studies shortly follow, with more publications using the same technique as (Higuchi et al., 1984) in the 1980s for different species, including the first aDNA work on modern humans (Pääbo, 1984, 1985). This led to more studies and on much older samples with the first mitochondrial DNA sequences from a Neanderthal published in 1997 (Krings et al., 1997). Furthermore, since the 1990s, an explosion of aDNA studies, bring forth several high-profile publications declaring to have successfully retrieved and sequenced DNA from much older specimens. These studies claimed to have obtained DNA from the Cretaceous period, plants dated to millions of years old and insects preserved in amber have now been disproved due to replication failure, causing skepticism for this field of study (Cano & Borucki, 1995; Golenberg, 1991; M. B. Richards et al., 1995; Weyand & Bunnell, 1994). Nonetheless, the advancement in technology came to the rescue and ensure the credibility of the field with strict guidelines to verify the authenticity of ancient DNA (Cooper & Poinar, 2000).

2.4.1. DNA degradation

One characteristic of ancient DNA is DNA degradation caused by active nucleases when an organism passes away due to unregulated cell repair mechanisms, this causes DNA fragmentation and deamination which is a common post mortem modification (Hofreiter, Serre, et al., 2001; Pääbo et al., 2004). The effect of the degradation can increase depending on the environment in which the sample is stored, with lower temperatures decreases the rate of fragmentation (Eglinton & Logan, 1991). The fragmentation process can hinder the ability to sequence the sample as it can induce errors during the sequencing process and the alignment phase. There are different ways DNA can be damaged to prevent sequencing or induces errors, these are known as depurination, deamination, and polymerase blocking lesions. Depurination is the breakage of the chain caused by the hydrolysis of the β-N-glycosyl bond between a purine and a sugar, creating single-stranded nicks (Lindahl, 1993). Whilst deamination, also caused by hydrolysis altering cytosine to uracil, can be found at the end of a fragment with single-stranded nicks caused by depurination appears to be more prone to deamination (Briggs et al., 2007; Shapiro, 1981). On the other hand, polymerase blocking lesions are caused by the oxidations of pyrimidines or the crosslinking of DNA, physically interfere with polymerase transcribing the template DNA thus affecting the amplification process during PCR reactions (Pääbo et al., 2004).

Simply by assessing the sequence data for these ancient DNA damages, the authenticity of ancient DNA can be verified this way. The length of DNA is usually one method to determine if there is modern contamination as ancient DNA is expected to be shorter due to fragmentation (Sawyer et al., 2012). The identification of deamination at the 5' and 3' end of sequences can also verify whether the sequenced data is ancient, as the process of deamination causes a change from C to T at the 5' end and G to A at the 3' end (Hofreiter, Jaenicke, et al., 2001). Although DNA degradation affects the quality of sequence data, clarifying the authenticity of ancient DNA is far more important to maintain the credibility of this field.

A number of preventative measures needed to be taken for any ancient DNA studies to ensure authenticity. As previously mentioned, methods to detect authenticity are available, but to obtain ancient DNA from the ancient specimen, special precautions are a must. Such as specialized clean-room facilities dedicated to ancient DNA extraction to prevent modern DNA contamination, control amplifications and pre-PCR procedures in specific dedicated areas, protective clothing to avoid direct contact with ancient samples, and usage of negative controls to identify possible contaminations (Cooper & Poinar, 2000; Orlando & Cooper, 2014). These precautions do not guarantee the retrieval of aDNA due to the natural occurrences of degradation, although the estimation of DNA half-life was previously estimated as 521 years (Allentoft et al., 2012), recent studies indicate ancient DNA preservation appears to correlates with environmental factors and shown no direct correlation with time (Marciniak et al., 2015). Temperature appears as the most important factors regarding ancient DNA preservation, with cold, dry, and low-radiation conditions seemingly increase DNA survival rate, with some of the oldest ancient genomes published found in cold or permafrost climatic environment (Druzhkova et al., 2013; Froese et al., 2017; Orlando et al., 2013). The type of material also affects the degradation of DNA as certain samples are prone to environmental exposure which would affect the amount of endogenous DNA retrieval. For example, the petrous bone is a part of the temporal bone, position within the skull thus protecting from external degrading factors (Hansen et al., 2017; Pinhasi et al., 2015).

2.4.2. Advancement in Archaeogenetics

Cloning techniques applied by the earliest ancient DNA studies were a revelation, with the invention of PCR and Sanger sequencing spring forth the start of Archaeogenetics studies (Higuchi et al., 1984; Mullis et al., 1986; Sanger et al., 1977). Since then, this field has improved significantly in order to maximize the genetic information obtained from archaeological samples due to destructive sampling. By applying stricter measures in handling and sample storage it can help slow down degradation and prevent modern contaminant. Whilst different methods of extracting DNA from the archaeological samples have been constantly improved to obtain the maximum yield of DNA and maintaining the authenticity of ancient DNA. Such as the usage of spin column for extractions and purification process in order to get rid of potential inhibitors which could hinder PCR reactions, has been implemented in modern extraction

methods to optimize each step of the extraction process (Rohland & Hofreiter, 2007b; D. Y. Yang et al., 1998). Optimization of sequencing methods has also been implemented to speed up and lower the cost of sequencing with the technological development of NGS, as previous methods of cloning techniques and Sanger sequencing are extremely labor-intensive, time-consuming, and costly to generate a complete genome as shown in the human genome project (F. S. Collins et al., 2003).

The advancement of Archaeogenetics allows the publication of some phenomenal research, such as the first complete ancient human mtDNA genome and the first ancient human genome from the Holocene, a Palaeo Inuit individual from Greenland dated to 4000 years old (Gilbert et al., 2008; Rasmussen et al., 2010). Since then, increased sampling of archaeological biological material from different parts of the world and in different time periods have enabled a further expansion into understanding population genetics and migration pattern, although most focus on the genetics of European prehistory (Slatkin & Racimo, 2016). Presently, by targeting the petrous bone, retrieving endogenous DNA from samples found in unsuitable climates is now possible. Accompany by NGS, it's now possible to recover higher coverage genomes for these poor samples.

2.5. Uniparental genome

The mitochondrial DNA and Y chromosome are known as uniparental genetic markers, which are inherited maternally for mitochondrial DNA and paternally for the Y chromosome. Due to their uniparental inheritance and the lack of recombination, studying the uniparental genomes provide a sex-biased view on demographic events which is useful to determine potential founder or bottlenecks event (Lippold et al., 2014). By analyzing the mutation exhibit in the uniparental genome, reconstruction of different lineage clusters otherwise known as haplogroups allows the construction of phylogenies of a population. The implementation of the molecular clock or an estimation for the most recent common ancestor, complimenting the phylogeny build, would provide a method to infer demographic events (Jobling & Tyler-Smith, 2003).

2.5.1. Mitochondrial DNA

Mitochondrial DNA is a circular double-stranded molecule that exists within the mitochondria; a double-membrane intracellular organelle involved with several cellular functions. In particular, the mitochondrial is coined as the powerhouse of the cell, as it is involved in ATP synthesis, a crucial component for cellular metabolism (Ernster & Schatz, 1981; Siekevitz, 1957). The first human mitochondrial DNA complete sequence was published in the early 1980s which became the Cambridge reference sequence (CRS) (S. Anderson et al., 1981). This was reviewed due to positional errors leading to a new mitochondrial DNA reference sequenced published in the late 1990s, this new reference sequence is known as the revised Cambridge reference

sequence (rCRS) (Andrews et al., 1999). In 2012, a proposal of an alternative mitochondrial DNA reference sequence, the Reconstructed Sapiens Reference Sequence (RSRS), to refer to the ancestral genome of the mitochondrial Eve (Behar et al., 2012). This is due to the classification of rCRS belonging to the mitochondrial lineage of H2a2a1, and it is a leaf within a tree instead of the root. However, the rCRS is still the preferred reference sequenced used today, as the replacement of rCRS with RSRS would alter the haplotype called by rCRS when it was first used as a reference sequence. Additionally, this would affect other field of studies aside from phylogenetics studies (Bandelt et al., 2014).

The mitochondrial genome for humans is 16,569 bp long, compacted, lacks intron, and mostly for coding. The hypervariable region, also known as the control region or the D-loop, is located within the mitochondrial genome and for humans is 1,121 bp long (position from 16,024 to 576) (S. Anderson et al., 1981). The mutation rate for mitochondrial DNA is higher than the nuclear DNA due to free radicals of oxygen from respiration and the lack of protection from histones (W. M. Brown et al., 1979; Fernández-Silva et al., 2003). The variation of mutation rate also exhibit within the mitochondrial genome as the control region appears to evolve at a higher rate than the rest of the genome (S. Meyer et al., 1999). Utilizing the control region, early studies were able to distinguish haplogroups within a population, but recent studies have included the full mtDNA genome sequences in order to detect all the variations within the mtDNA genome within a given populations.

The accumulation of variations within mitochondrial DNA provides a suitable candidate for the phylogeographic approach due to the high mutation rate of mtDNA. As there are numerous copies of mitochondria within a eukaryotic cell and each mitochondrion contains several copies of mtDNA, mtDNA can be found in high copy number compared to nuclear DNA (Lee & Wei, 2005; Shuster et al., 1988). This favor aDNA studies due to an easier recovery compared to nuclear DNA, especially when archaeological samples are usually in poor condition. Although it is worth noting that certain samples would provide a higher yield of ancient mtDNA, such as the tooth cementum compared to the petrous bone (Hansen et al., 2017).

2.5.2. Mitochondrial phylogeography

Due to the unique methods in which mitochondrial DNA is inherited, a certain locus is inherited via the maternal lineage. These loci can be detected in clusters which are known as a haplotype. During the early studies of mitochondrial DNA, a monophyletic cluster of haplotype otherwise known as haplogroup was coined by (Antonio Torroni et al., 1993), in reference to a common ancestor of a particular lineage. The nomenclature of the mtDNA haplogroup is labeled in an alphabetical system, which are followed by numbers and letters to signified deeper branches (M. B. Richards et al., 1998). The first haplogroup was used to describe Native American populations, this macro-haplogroup were labeled as A, B, C and D (Antonio Torroni et al., 1993). Later haplogroups were labeled with other letters and do not appear to follow any particular logic in their labeling.

In order to construct the mtDNA tree, early studies utilize the restriction fragment length polymorphism (RFLP), to determine major haplogroups using specific restriction enzyme sites (Antonio Torroni et al., 1996). Since then, the improvement of sequencing techniques increases the resolution of the tree significantly, able to discern basal clades and younger branches which allows a better judgment into the placement of haplogroups and discovering haplogroups that appear to be specific to a particular region (M. Richards et al., 2002). Presently, there are approximately 24,000 complete published human mtDNA sequences that are used to construct a global phylogenetic tree using PhyloTree build 17 (Van Oven, 2015). Although the topology of the tree is built to a great extent, this tree was constructed with a modern mtDNA genome, lacking possible ancient DNA variates which could affect the topology of the tree (Fu et al., 2015, 2016).

The basal node of the human mtDNA tree is classified as L0 and L1'2'3'4'5'6, two African lineages, with the latter become widespread within and outside of Africa (Antonio Torroni et al., 2006). Majority of the human mtDNA variation outside of Africa falls under two haplogroup which branched off the macro-haplogroup L3, these two haplogroups M and N both dated between 50,000 to 70,000 years ago (Behar et al., 2012; Soares et al., 2009), with haplogroup M can be found in the South and East Asia and Oceania, whilst haplogroup N can be found in Europe, Asia and the Americas (figure 10).

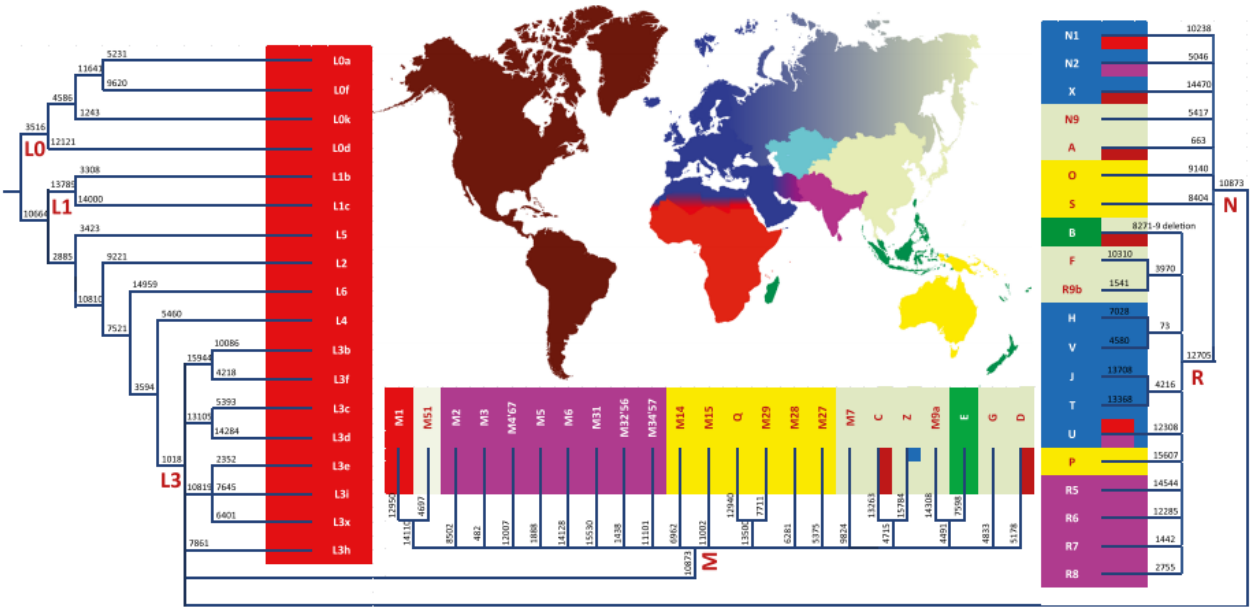

Figure 10. A map showing the distribution of mtDNA haplogroup with colour associating with the geographic location. (Kivisild, 2015). Permission to freely reproduce the image shown under the conditions of PMC commons license 3.0 (CC BY-NC-ND 3.0)

2.5.3. Indigenous American mtDNA variation

As previously mentioned, four indigenous American macro-haplogroup was discovered in the early 1990s (A Torroni et al., 1993), with further sequencing, identified the distinctive variation amongst the four major-haplogroup which are the subbranches of the macro-haplogroup A, B, C and D. The major haplogroup A2, B2, C1 (specifically C1b, C1c, and C1d) and D4 (specifically D4h3a) can be found across the Americas continent with rarer minor haplogroup C4c and X2a found in a certain geographic location within North America (Achilli et al., 2008; M. D. Brown et al., 1998; Merriwether et al., 1995; T G Schurr et al., 1990; Tamm et al., 2007). By understanding the phylogeography of these haplogroups within America and their divergence from their lineages, it is possible to trace back and determine the origin of the indigenous people when accompanied by archaeological evidence.

The archaeology hypothesized the origin of the indigenous people initially derived from Asia, either via the Beringia land bridge, or early development of aquatic transportation to enter North America prior to the availability of the ice-free corridor (Fladmark, 1979; Hoffecker et al., 1993). The initial studies on the mitochondrial haplogroup of the indigenous population have clarified that the major haplogroup exhibit maternal lineages derived from Asia, but the method of arrival is still unclear (figure 11). Tamm et al 2007, using the mitochondrial genome to identify the four founding haplogroups, suggesting ancestral indigenous population paused in Beringia, and obtained new variations leading to the separation of New World founder lineages from their Asian sister-clades, prior to their migration southward into the Americas continent. This finding favors the Beringia standstill hypothesis. Highlighting, the initial movement North to South via this method was suggested to be a rapid migration from a single source ancestral population and not a gradual diffusion, which follows by long term isolation of local populations leading to further variations causing regional haplotypes to be found (Tamm et al., 2007).

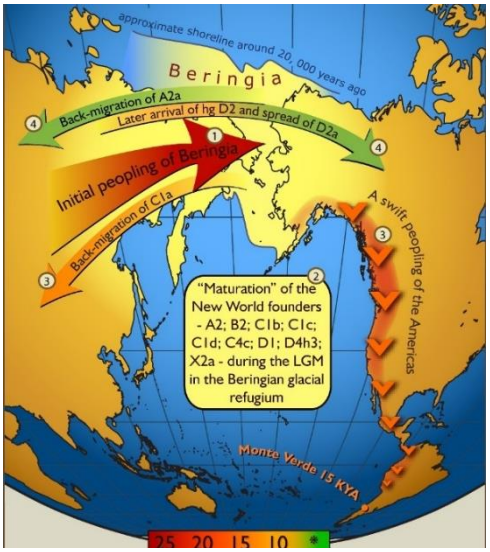

Figure 11. Possible maternal geneflow demonstrating potential back migration into Beringia after the arrival of people into Beringia. (Tamm et al., 2007). Permission to freely reproduce the image shown under the conditions of PLOS ONE commons license 3.0 (CC BY-NC-ND 3.0)

Alternatively, Perego et al, 2009, identified the minor haplogroup D4h3a, as a support for the coastal route due to its high frequency in the Southern indigenous population compared to the North, with this haplogroup mostly distributed along the west coast. This contrasts with the findings of Tamm et al, suggesting a possible two-wave expansion into North America which favors the Coastal route hypothesis. Speculating D4h3a exists within the first expansion, rapidly move into Southern America, and the second expansion either replaces or decreases D4h3a population in North America. There are numerous factors that can affect the distribution of the haplogroup, such as the admixture between regional population and population bottleneck which can reduce the genetic variations and the distribution of the haplogroups. Therefore, to understand past population history, a comparison of ancient and modern DNA with inference from archaeological evidence can aid in understanding past migration events (Llamas et al., 2016).

One such event that has been clarified is the presence of Paleo-Inuit population. Further mtDNA studies have identified additional haplogroup, specifically D2a, which exist in Inuit populations but do not appear in indigenous North American populations (Derenko et al., 2007). This haplogroup can be seen in both ancient and modern Inuit individuals, suggesting a lack of genetic exchange between Paleo-Inuit populations and Indigenous North-East American via the maternal lineages, although archaeological evidence indicates both populations have come into contact with each other when Paleo-Inuit travel into Greenland via the Arctic Sea (Derenko et al., 2007; Dryomov et al., 2015; Skoglund & Reich, 2016). This suggests the physical contact between populations does not necessarily equate to genetic exchange. Although speculation of recent gene flow between Northern indigenous Americans with Neo-Inuit of the Siberian coastline can be seen in the distribution of haplogroup A2a and A2b, additional investigation may provide insight into the cause of the phylogenetic connections (Flegontov et al., 2019; Tamm et al., 2007).

The usage of the uniparental genome is hugely powerful but by only interpreting the uniparental genome alone, not the whole picture can be printed. One such hypothesis arises with the idea that haplogroup X2a is derived from an ancient trans-Atlantic migration to the Americas (Oppenheimer et al., 2014; J. A. Raff & Bolnick, 2015). The haplogroup X2a is uniquely found in North America around the great lake region but the Macro-haplogroup, X, is most likely of West Eurasian origin with an ancestral source near the Mediterranean region as seen in figure 12 (M. D. Brown et al., 1998; Reidla et al., 2003). It is to be noted that currently sister clades of X2a is not found in Asia, and formal arguments have been in favor of X2a arriving in America via Beringia with the other major haplogroups (Fagundes et al., 2008; J. A. Raff et al., 2011), as current archaeological and genetic evidence unrelated to X2a favor the Asia origin scenario. Further study is needed to understand whether the X2a connection with the initial migration from Beringia is true, but the Solutrean hypothesis has largely been disregarded due to the overwhelming evidence from other research (Bradley & Stanford, 2004; O'Brien et al., 2014; Oppenheimer et al., 2014; J. A. Raff et al., 2011).

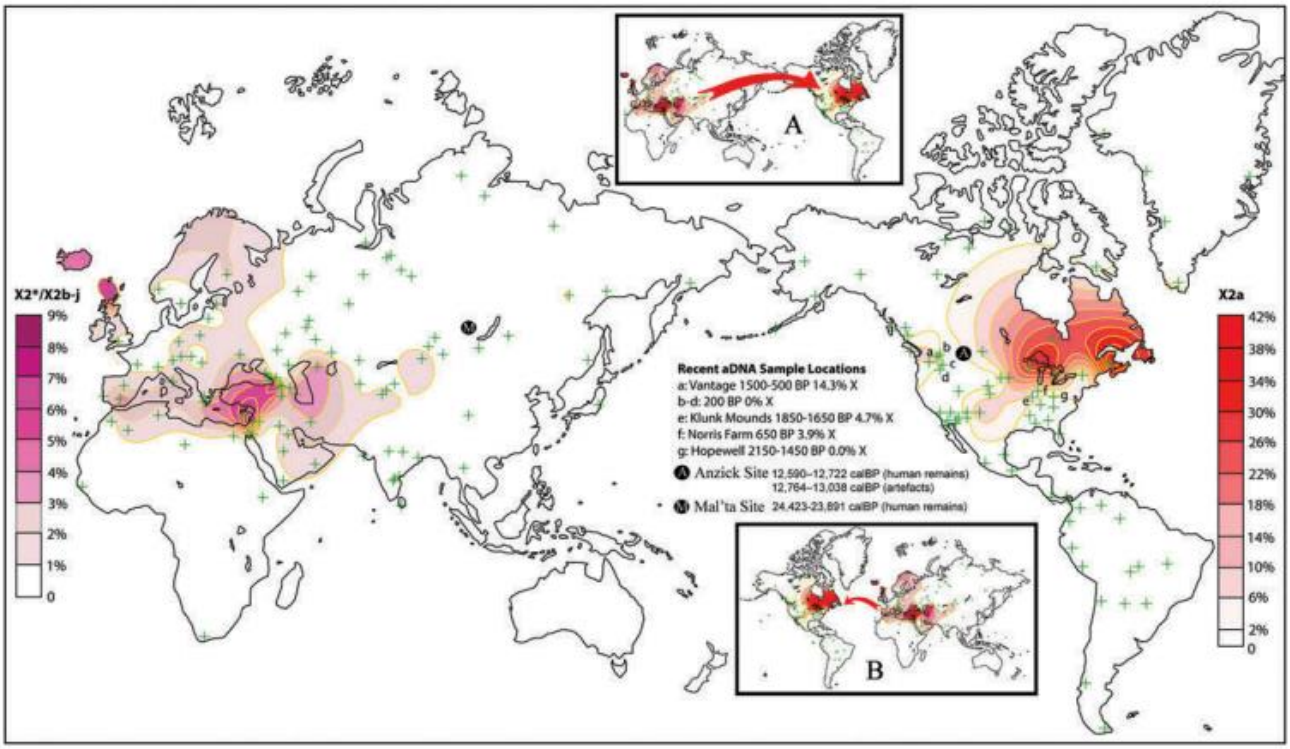

Figure 12. a map showing the distribution of haplogroup X2. A) showing the route require for X2 to enter America via Beringia. B) The Solutrean hypothesis using the Atlantic ocean route. (Oppenheimer, et al., 2014) Permission to freely reproduce the image shown under the conditions of Taylor & Francis reuses of its content for a thesis or dissertation.

2.5.4. Y Chromosome

The male-specific uniparental marker is the Y chromosome, consisting of two regions, the male-specific region (MSY) and pseudo-autosomal regions which are found at the ends of the chromosome (Skaletsky et al., 2003). The MSY encompasses 95% of the human Y chromosome, which equates to approximately 57 million bp (Jobling & Tyler-Smith, 2003). The early studies using the uniparental marker, Y chromosome, identify two types of polymorphisms that are used to define haplogroups (Y. C. Consortium, 2002). These are biallelic markers, such as SNPs, insertions and deletions, and short tandem repeats otherwise known as multiallelic markers (STR).

Discerning haplogroups in the early studies was challenging. The utilization of SNPs from previously studied markers can introduce ascertainment bias which led to inaccurate interpretation. Moreover, STR evolve at a high rate which can cause homoplasy and the mutation rates can vary amongst different STR in different position, introducing potential erroneous age estimation if a universal mutation rate is applied (Carvalho-Silva et al., 1999; Hallast et al., 2015). By combining both biallelic and multiallelic markers, a more reliable age estimation and Y chromosome topology can be performed (Y. C. Consortium, 2002; Jobling & Tyler-Smith, 2003). Similar to mtDNA, the advancement in NGS technology provide a method to sequence the entire Y-Chromosome which increases the resolution of the Y-Chromosome

phylogeny. This allows refinement of the tree and potential error which was previously undetected in the earliest studies (Batini et al., 2015; Hallast et al., 2015).

2.5.5. Indigenous American Y-chromosome variation

Recent advancements in Y-chromosome studies have provided an improved phylogenetic resolution to study the male demographic of indigenous Americans. This enables the detection of major haplotypes found within the non-admix modern individual suggesting the same major founder Y chromosome haplotype for both the North and South indigenous populations (Fabricio R Santos et al., 1996). Approximately over 90% of central and southern indigenous populations exhibit the same major haplotype, supporting the idea of founder effect in the development of these populations. Later studies viewing northern indigenous population, Eskimo and Na-Dene speaking populations have also exhibited the same major haplotype while it is in lower frequency compare to the southern population, high level of admixture may have contributed to this factor (M. H. Crawford, 2001; Fabrício R Santos et al., 1999; Underhill et al., 1996).

Majority of indigenous American Y-chromosomes belong to three different macro-haplogroups, haplogroup C, Q, and R. Haplogroup C and Q represent early Indigenous founding Y chromosome lineages with haplogroup R suggested to have derived from recent European admixture (Pinotti et al., 2019). However, this still requires further resolution as R haplotypes from some indigenous population, specifically around the great lake region does not appear to share with Europeans haplotype (Oppenheimer et al., 2014).

Within the Y-Chromosome phylogeny, haplogroup C is widely distributed around the world, and can be found in modern Australian, Asian and Indigenous American, additionally ancient Europeans as well (Bergström et al., 2016; Fu et al., 2016; Poznik et al., 2016; Roewer et al., 2013; Xue et al., 2006). Amongst haplogroup C, C3 defined by the marker M217 is found frequently in indigenous Siberians and Americans, clustering the two populations into one branch, suggesting a central Siberian lineage for indigenous American haplogroup C3, migrating to the Americas during the upper Pleistocene (Pinotti et al., 2019; Fabrício R Santos et al., 1999). Diving deeper into C3, a separate cluster for indigenous population between the north and the south with different ages estimation suggest the occurrence of both a rapid expansion and a short Beringian standstill within indigenous American founders (Pinotti et al., 2019). Haplogroup C is virtually limited to North America whilst haplogroup Q can be found throughout the Americas continent (Dulik et al., 2012).

Similar scenario has also been suggested for haplogroup Q (Malyarchuk et al., 2011). Most indigenous American Y chromosomes belong to this haplogroup which can also be found among indigenous Siberian populations (Grugni et al., 2019). In particular, haplogroup Q-M3 can be found predominantly over 80% of the South American population and ~50% in the North American population (Battaglia et al., 2013). There appears to be population isolation when viewing deeper into the Q-M3 branch, as some southern indigenous tribes consist of a higher proportion of particular Y-chromosome haplotype compares to neighboring tribes (Battaglia et

al., 2013). Addition to this, within haplogroup Q-M3, Q-M848 exhibits many branches enclose containing the ancient Kennewick sample, correlating to previous studies in confirming that haplogroup Q is a founding haplogroup (Pinotti et al., 2019; Rasmussen et al., 2015). It is to be noted that haplogroup Q is also found in the ancient Saqqaq individual but in a different branch within haplogroup Q, representing a later origin correlates to other data (Rasmussen et al., 2010).

2.6. Genome wide variation

The complete sequencing of the human genome by the International Human Genome Sequencing Consortium in 2004 provided a view to comprehend the structure and organization of our genome (I. H. G. S. Consortium, 2004). Consisting of 23 pairs of chromosomes, 22 pairs of autosomes and two sex chromosomes, containing over 6 billion bp. Each chromosome varies in length, includes large fraction of non-coding DNA and protein coding DNA (Ludwig, 2002). By analyzing the chromosomes, it is possible to genotype polymorphic sites in the human genome, which can be used for demographic and medical studies of the human populations (figure 13). The genome wide approach offers a method to evaluate and estimate admixture between different populations with distinct ancestries. Using programs such as ADMIXTURE analysis and Principal Component Analysis (PCA) with genotyped datasets (Patterson et al., 2006, 2012), ancestral components of individuals can be estimated using a maximum likelihood approach, which can be used to discern relatedness among individuals and to understand population structure (Novembre et al., 2008).

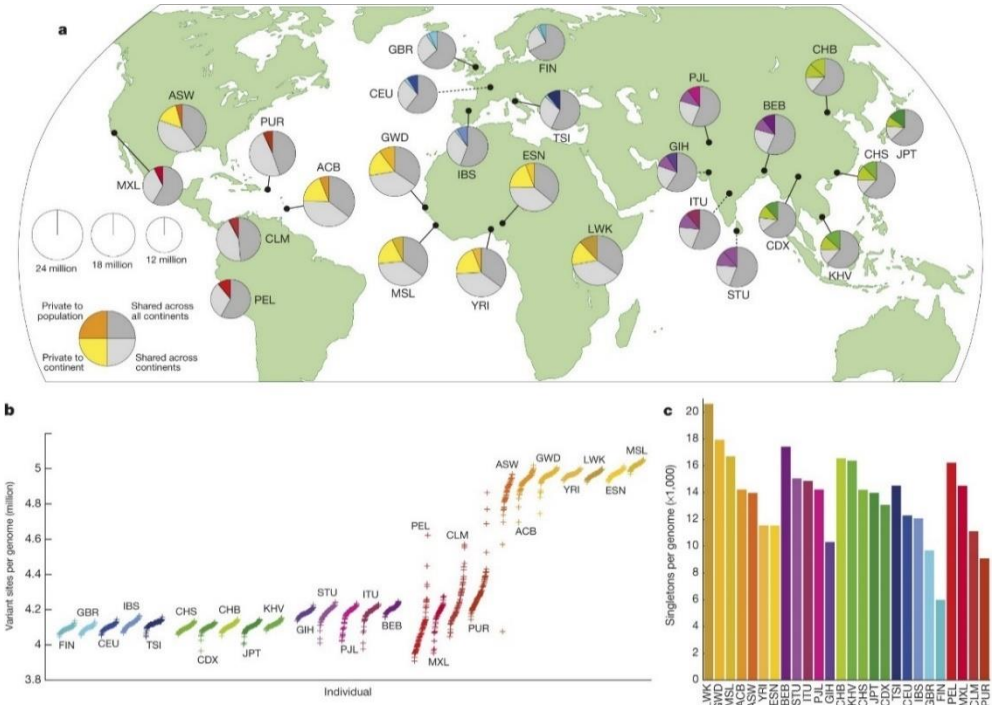

Figure 13. a) Genetics variants of different populations, each piechart represents private variants of a population in darker colour and lighter representing variant private to a continenetal area. Variant shared across continental areas in light grey and across all continent in dark grey. b) Shows the number of variant sites per genome and c) the average number of singletons per genome. (The 1000 Genomes Project Consortium 2015). Permission to freely reproduce the image shown under the conditions of Nature commons license 3.0 (CC BY-NC-ND 3.0)

2.6.1. Indigenous American genome wide variation

The settlement of the Americas as we understand so far based on the archaeological studies and uniparental genomes indicates the ancestor of the indigenous Americans travel via Beringia into the Americas continent at least 15,000 years ago. Interpretation from the two disciplines so far could not distinguish the number of migration events from Asia, and via which pathway the ancestral population first enter the continent (Fagundes et al., 2008; Kitchen et al., 2008). By studying the genome wide variation of indigenous populations, it provides a possible method to decipher the dispersal patterns of indigenous American. One complication in studying the indigenous American genetic history is the recent arrival of European and African since the discovery of the continent in 1492, causing admixture between these populations (Alexander et al., 2009). This is particularly challenging in order to learn about the historical relationship amongst the populations (Reich et al., 2012).

With the intention of understanding indigenous American population history, archaeogenetic is relied upon to trace potential waves of migration (Gravel et al., 2013; Posth et al., 2018). However, current aDNA data for America is limited due to the lack of material available for analysis. Excavated remains found associated with indigenous populations are returned to affiliated tribes and repatriated (figure 14), which can prevent archaeogenetic research to understand population structure and migration (Callaway, 2016). To facilitate and improve our current knowledge, recent communication and improvement in conversing with indigenous people allowed some research to be conducted, thus providing a glimpse into understanding the genetic history of the past.

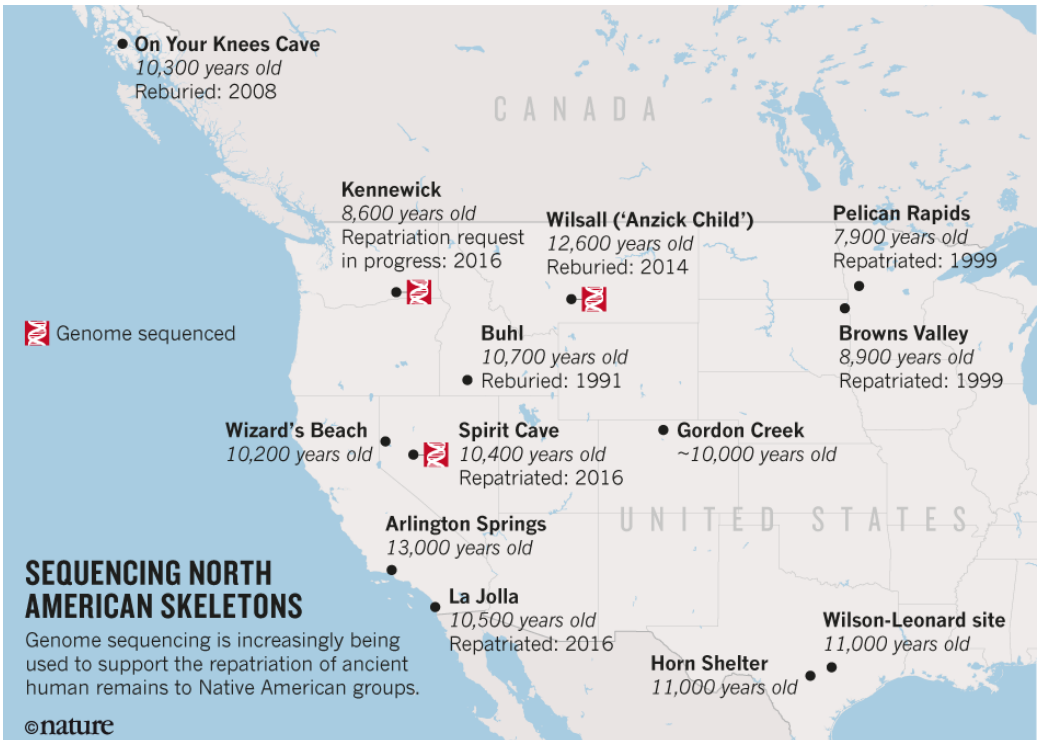

Figure 14. Map showing some of the oldest archaeological site with human remains found in North America. (Callaway, 2016). Permission to freely reproduce the image shown under the conditions of Nature commons license 3.0 (CC BY-NC-ND 3.0)

The single founder population hypothesis postulated by archaeologists and previous studies was the first to be questioned in recent times. Analysis of genome-wide data of 52 modern Native Americans with 17 Siberian groups demonstrates the majority of Native Americans derive their ancestry from a homogeneous ancestral population, with additional gene flow from Asia into America, thus rejecting the single migration model (Reich et al., 2012). To distinguish the complexity of multiple wave migration, the genome of the Anzick 1 child associated with Clovis artifacts in western Montana dated to 12,600 BP was analyzed (Rasmussen et al., 2014). The results demonstrate the Anzick ancestry derived from the same populations as central and south Native Americans and are closely related to them compared to Native Northern Americans. This proposes a similar scenario derived from some mtDNA studies, suggesting Native Americans primarily originated from a single source population with a secondary movement into North America (Perego et al., 2009; Rasmussen et al., 2014). Although the Anzick study provided a hint into the origin of Native American, more question arises as several scenarios of early divergence were left unanswered. The Kennewick individual, on the other hand, dated to ~8,500 years ago, showcases a similar story to Anzick, sharing a high degree of ancestry with Indigenous population from Central and South America compared to contemporary Native Americans, with Anzick more closely related to Central and Southern Native Americans than Kennewick man (Rasmussen et al., 2015). The finding of Kennewick being closely related to Southern Native Americans than Northern Native Americans hints at the idea that an additional Northern lineage potentially diverged from the original source population of Anzick and Southern Native American postdating Kennewick, or perhaps an early population structure within Americas (Rasmussen et al., 2015).

Recent findings in Central and South America, sequencing some very old genomes dated to 9,300 BP from Belize and 10,900 BP from Chile were compared with the Anzick individual, identifying distinct ancestry between these populations (Posth et al., 2018). However, the lack of specific affinity to Anzick when compared with later ancestry from South Americans demonstrate that South American is derived from one of two major ancient lineages in North America (Posth et al., 2018). Instigating the assumption that the Clovis culture and the Fishtail complex originate from the same events, would require further investigation by obtaining ancient genome associated with the fishtail complex (Pearson, 2017; Posth et al., 2018). These findings also support complex scenarios with possible sub-structure lineages with and without Anzick affinity, proposing that those with Anzick ancestry had a short-term contribution if the Clovis culture played a part to the peopling of South America.

This contrast with the finding from another publication which focuses on North America, results from the ancient genomes of California and Southwestern Ontario demonstrate the existence of two distinct ancestries, which contributed to the ancestral population that's been found in modern Central and South Americans (Scheib et al., 2018). The two ancestries, A and B, are distributed differently in Native populations, with ancestry A (ANC-A) found in Anzick associated with Southern American and ancestry B (ANC-B) found in ancient south Ontario individuals, representing the Northern lineages. The proportion of the ANC-A and ANC-B differs

in different populations, and if the two-ancestry contributed to Southern Americans, possible ancient substructure along with a separation time of a few thousand years prior to merging could lead to these particular results (Scheib et al., 2018). Although both studies contradict each other, both findings provide a strong statement of population continuity since the initial peopling of the different regions within the Americas (O'Connor, 2018).

Current evidence has been unable to distinguish precisely where and when the split between the North and South American, despite recent data suggesting the separation occur south of the ice-sheet as it fits well with the scenario these publications propose (Moreno-Mayar et al., 2018; Scheib et al., 2018). Realistically, the findings at the moment allow multiple models for the peopling of the Americas and have not been able to distinguish whether the separation of the Native American ancestor from ancient north Eurasian began in western or eastern Beringia (Potter et al., 2018). Geneflow between ancestors of Native Americans and Ancient North Eurasian were detected in Mal'ta individual dated to ~24,000 years BP hint at the origin of Native American deriving from an ancestral population in east Siberia (Maanasa Raghavan et al., 2013). Alternative scenarios suggest the split from Ancient Beringian occurred in eastern Beringia, potentially in Alaska, however, the current evidence favors the western Beringia scenario as there are currently a lack of ~20,000 years old American site whilst abundant evidence of human occupation in northeast Asia (Bourgeon et al., 2017; Buvit et al., 2016).

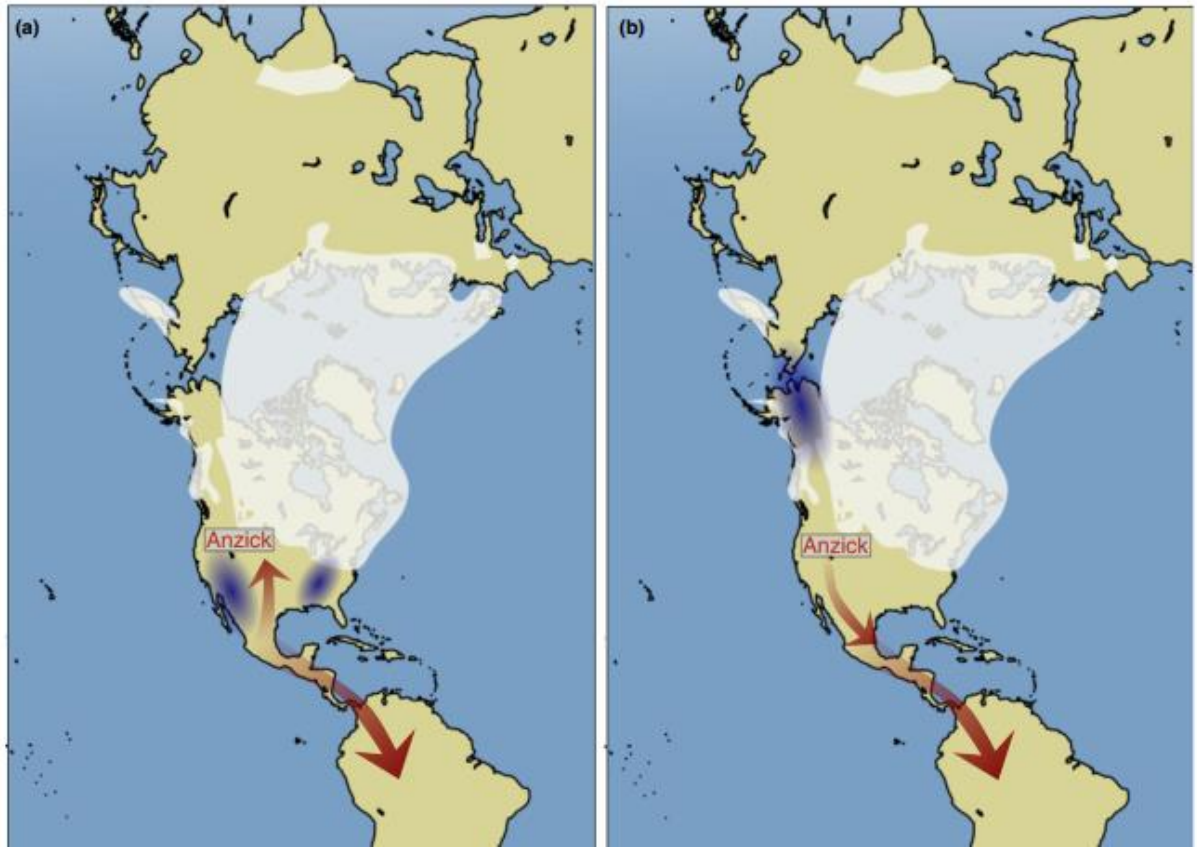

Figure 15. Two possible scenarios regarding to the spread of the initial people using Anzick genome. (Skoglund & Reich 2016). Permission to freely reproduce the image shown under the conditions of PMC commons license 3.0 (CC BY-NC-ND 3.0)

Analysis of the Upward Sun River genome located in Alaska, supports the claim that Native American descended from a single population with two deep branches (Tackney et al., 2015), an Ancient Beringian population possibly split off from East Asians between 22,000 to 18,000 years and the second branch between 17,500 to 14,600 years leading to the northern and southern American lineages (Moreno-Mayar et al., 2018). The findings that the southern lineages in Anzick suggest the split between the Northern and Southern lineage could date back to more than 12,600 years ago (Rasmussen et al., 2014). The southern lineage, including Anzick, present-day Central and Southern American populations, and the Northern lineage including populations from Algonquin, Cree, and Ojibwa (Skoglund & Reich, 2016). Currently, our understanding of the Northern lineage is limited, with many questions unanswered, one such question is where and how this lineage became incorporated into the Native American population. One possibility suggests the northern lineage arrived via the ice-free corridor, implying the split could occur north of the ice sheet and displace the southern lineage in America. Another possibility is the expansion of Clovis from a southern region and could later displace by the increased population of the Northern lineage as illustrated in figure 15 (Skoglund & Reich, 2016).

The ability to sequence the genome of ancient humans has clearly provided an insight into the past. Along with archaeological evidence, the two-discipline complement one another and help decipher or dismiss hypotheses. One such hypothesis is the arrival of the Paleo-Inuit population, which the Archaeological evidence suggests as a third entry into America approximately 5,000 years bp prior to European colonization. Investigation of the Saqqaq individual from Greenland provided evidence for a migration from Siberia into the Americas with a lack of Native American admixture suggesting genetic isolation and independently gave rise to modern Inuit populations (M. Raghavan et al., 2014; Rasmussen et al., 2010). Confirming the arrival of Paleo-Inuit as the third migrations into the Northern Arctic Americas. This led to further questions with the migration of Paleo-Inuit, contacts with first people must have occurred as previously raised by Archaeological and modern genome analysis due to overlapping region with Na-Dene speaking population, additionally, admixture between Paleo and Neo-Inuit is still currently unsolved (Reich et al., 2012; Skoglund & Reich, 2016). Recent publication has a focus on the North-west to resolve these particular questions, discovering that Paleo-Inuit related ancestry is ubiquitously within both Eskimo-Aleut and Na-Dene speaking populations, showing key connections to the spread of the Na-Dene people and the peopling of the Aleutian Islands and the migration of Inuit across the Arctic region (Flegontov et al., 2019). However, it is unknown if there is a genetic contribution of Paleo or Neo-Inuit into the North Eastern Indigenous population, with previous publication focusing on mtDNA suggests this genetic isolation too (Duggan et al., 2017).

3. Aims and Objectives.

Currently, there are limited genetic information for the population located in Northeastern America. This hindrance our understanding of the initial movement of the first people as well as understanding the spread of Native Americans into or out of this region. Furthermore, the arrival of European affected the genetic and cultural history of this region, and increased the difficulty in investigating the potential genetic relationships between the different linguistic groups at the Northeast coast.

Considering the Great Lakes region is a location where many different indigenous tribes gathered for resources, investigating the genetic composition of ancient and modern individuals in this region would provide vital information into the understanding of the relationship between cultures of the past and the present. In order to do so, a collection of ancient and modern samples was investigated by DNA sequencing and the data produced was compared with published data to shed light on possible admixture events among the different population groups in the area, as well as the distribution and frequency of mitogenomes in this region. Stable carbon and nitrogen isotopic analysis were also performed to create a snapshot of the Native American diet and to determine whether there is a particular diet and to understand the reasons for conjugating at the Great Lakes region.

4. Materials & Methodology

4.1. Archaeological Sites

Figure 16 Geographic location of archaeological sites for ancient DNA study. Purple represents the Glacial Kame Sites and Yellow represents the Huron Wendat sites. 1. Teston Road Ossuary 2. Staines Road Ossuary 3. Moatfield Ossuary 4. Turnbull Drive Ossuary 5. Hind Site 6. Sartori Site

4.1.1. Moatfield Ossuary

Located by the bank near a small stream, approximately 10km north of Lake Ontario in North York, Ontario, Canada. This sandy loam soil secondary burial site was found in 1997 during the revamping of a football field, discovering a singular artifact of a small clay turtle effigy and 87 individuals of which 58 were identified as adults, 44 maxillary teeth were obtained corresponds to 44 individuals (van der Merwe et al., 2003). It is unknown the reason for the practice of secondary burial among some Iroquoian groups, perhaps is for ceremonial purposes to remember the deceased or moving the ancestor to a new location due to degradation of the burial sites (Johnston, 1979). This site was radiocarbon dated using a bone fragment and two teeth, with the bone dated between ca. 1020-1220 A.D. and teeth between ca. 1160-1310 A.D., the discrepancy suggests that the bone was from either someone who passes away considerably earlier or an error with measurement (van der Merwe et al., 2003). This also suggests the possibility that this site was revisited, or additional remains were added if the age of the bone was true. Both ages identify this site to be a Late Woodland Iroquoian village, approximately 3 acres large.

4.1.2. Staines Road Ossuary

This site was found in close proximity to another Huron-Wendat site in northwest Scarborough in Eastern Toronto. A disturbed secondary deposit of an ossuary, 308 individuals was excavated in 2001 by the Archaeological Service Inc. (ASI). Investigation by the ASI suggests the individuals had been moved from their original location and were found mixed with sandy loam soil with modern garbage (Pfeiffer et al., 2014). Due to the disturbed nature of this site, radiocarbon dating using bone collagen of some individuals was used to confirm the origin of the individuals dating to ca. A.D. 1250 ± 50 (Pfeiffer et al., 2014). It is unclear whether all individuals are of the same period but was suggested to be similar as Huron-Wendat ceremonial events occurred as a singular event, a custom of the Wyandot people known as the Huron Feast of the Dead which occurs when the whole villages moved to a new location (Williamson & Steiss, 2003). This is to protect their dead when they move to a location, to mourn and celebrate the dead (E. R. Seeman, 2011).

4.1.3. Teston Road Ossuary

Teston road ossuary is approximately a 3-hectare village located in Vaughan, North York, Ontario, 30km north of Toronto. The area is within the Humber River watershed, located on the corner of Teston Road. This site was first discovered in 1925 by A.J. Clark, further investigation in the late 1980s led to the recovery of small artifacts similar to other Huron-Wendat sites suggesting this to be a Huron Wendat ossuary, occupied around 1450-1500 CE (ASI, 2010), this was further confirmed using radiocarbon dating (Pfeiffer et al., 2014). The ossuary itself was unearthed in 2005 during the construction of Teston road, several hundred commingled individuals were found in a secondary burial, characteristic of a Huron Wendat burial (Williamson et al., 2014).

4.1.4. Turnbull Drive Ossuary

The discovery of the Turnbull Drive Ossuary occurred in 2011 during the development of a Beach Club. This site, located at Turnbull Drive, Orillia city, Simcoe, on the west of Lake Couchiching, contains over 300+ commingled remains. Within the ossuary, lack of grave goods prevents a definite answer to determine whether this site is true of Huron-Wendat origin, but it is assumed as Huron-Wendat due to the location of sites, as it is found within the ancestral homeland of the Huron Nation (Scheib et al., 2018; Williamson et al., 2014). Similar to other Huron ossuary appears to be a secondary burial in a sandy loam soil and exhibit minimal disturbance by construction activities.

4.1.5. Hind Site

The Hind site was discovered at Mosa, Middlesex, approximately 4.8km of Wardsville, Ontario (figure 16), contains many burial features (figure 17). Excavation of the site began in 1968 and ended in 1977, leading to 86 five-foot unit dugs, searching for archaeological evidence from the original site first found by Mr. Herman Hind. A total of 24 burials were found, each containing different assortments of grave goods, copper beads, stone bifaces and tools, marine shells, a human-animal skeleton with some burial containing red ochre and a cigar shape stone pipe, a characteristic of a glacial kame burial (Cunningham & Griffin, 1948). Radiocarbon dating of bone from Burial 15 was performed, producing a date of 2875±75 BP, suggesting the Hind site as a late Archaic period or an early woodland period site (Donaldson & Wortner, 1995).

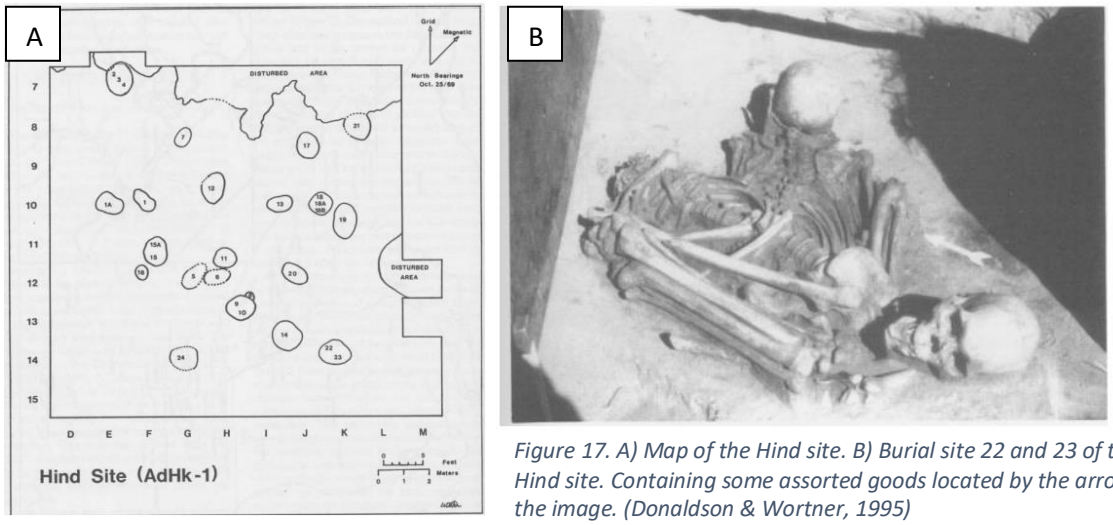

Figure 17. A) Map of the Hind site. B) Burial site 22 and 23 of the Hind site. Containing some assorted goods located by the arrow in the image. (Donaldson & Wortner, 1995)

4.1.6. Sartori Site

Situated south of Leamington Ridge next to Lake Erie, Mersea, Essex, Ontario, 45 burial was found in 1974. The individuals found to range from juvenile to adults consist of red ochre staining as well as various different items found in each grave. These artifacts include copper tools and beads, marine shell beads. Chery bifacial blade, animal bones, a limestone pipe, and an item appear to be a gorget (figure 18), which are characteristic of a Glacial Kame burial (Cunningham & Griffin, 1948). This site does not have a radiocarbon date, but it's associated with the same culture as the Hint Site, suggesting this site to be a late archaic or an early woodland period site (Donaldson & Wortner, 1995).

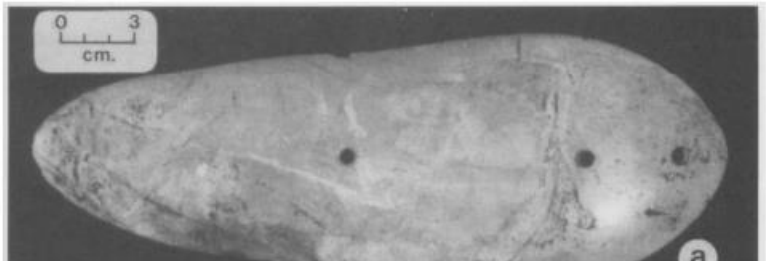

Figure 18. A sandal-sole Gorget found at the Sartori site. (Donaldson & Wortner, 1995)

4.2. Ancient Sample Preparation

A total of 81 teeth were sent to the University of Huddersfield by Dr. Ronald Williamson of the ASI, these were stored at the Ancient DNA facility in the University of Huddersfield (figure 19). The Ancient DNA facility situated within the University of Huddersfield is a dedicated clean area, specifically designed for preparing and processing ancient samples for ancient DNA analysis. It contains specific rooms for sample processing, preparation, DNA extraction, and library preparation for sequencing. The facility also has a high-tech air filtration system generating positive air pressure into the laboratory to prevent contaminated air from entering or circulating across rooms. Strict rules were applied to minimize the chances of contamination, these include the user of the facility to wear face mask, full-body suits, gloves, hairnets, and clean clothing at all times. Decontamination protocol was followed, all surfaces and tools were clean with LookOut DNA Erase (SIGMA Life Science) and via UV light irradiation prior and after usage. When a new sample was brought into the lab, each sample was cataloged and photographed, the surface of the sample was exposed to UV for an hour, 30 minutes on each side. This was then stored in labeled bag for further processing (Willerslev & Cooper, 2005).

4.3. Ancient Sample Processing

In the sample processing room, working exclusively on teeth, the surface of each tooth was cleaned using 29 µm aluminium oxide powder in a compressed air abrasive system (SWAM-Blaster®). Using a diamond tip saw, the root of each cleaned tooth was cut from the crown, the crown was then kept for dietary stable isotopic analysis, whilst the root was used for DNA analysis (Rohland & Hofreiter, 2007a). The crown was sent to the University of Oxford for carbon and nitrogen isotopic analysis, performed by Dr. Peter Ditchfield. The root of each tooth was placed inside a zirconium oxide grinding jar, which was then placed inside the Retsch® MM400 Mixer Mill and was grinded into a very fine powder at 30 Hz/s for 30 seconds. 0.2g of the bone powder was then transferred to a labeled O-ring tube, ready for extraction. The remaining power was put into storage within the Sample Processing room under 4˚C. The

Figure 19. A The schematic of the ancient DNA laboratory, indicating the separation of specific room for processing and preparation.

weighted tubed tubes containing the powder were then transferred to the extraction room for DNA extraction.

From the Huron-Wendat site, ten teeth were randomly selected from each site for ancient sample processing, for the Turnbull Ossuary and Teston Road Ossuary, all samples were processed as it contains nine teeth for Turnbull Ossuary and eleven teeth for Teston Road Ossuary. All samples were processed for Hind site and Sartori site as seen in (table 1).

ID Map	Site Name	Town, COUNTY	Date	Samples	Processed
1	Teston Road Ossuary	Vaughan, YORK	AD 1450-1500	11	11
2	Staines Road	Scarborough, YORK	AD 1300	35	10
3	Moatfield	MET. TORONTO	AD 1280-1330	18	10
4	Turnbull Ossuary	Simcoe, NORFOLK	AD 1400-1500	9	9
5	Hind Site	Wardsville, MIDDLESEX	BC 800-1000	6	6
6	Sartori	Mersea, ESSEX	BC 800-1000	1	1

Table 1. Indicating the number of samples obtained and the number of samples proceeded for each site.

4.4. DNA Extraction Process

Using an amalgamation of protocol from (D. Y. Yang et al., 1998) and (MacHugh et al., 2000), an extraction buffer was prepared (table 2) to demineralized the bone powder for the DNA extraction step. Transferring 1 ml of the extraction buffer to each tube containing bone powder and incubate at 37˚C for 24 hours in a rotating wheel. The rotating wheel prevents the clumping of bone powder as it rotates and mixes the bone powder and extraction solution. After the initial 24 hours, the mixed solution was centrifuged at 13,000 rpm for 10 minutes and the supernatant was transferred into a clean tube. This supernatant is kept and stored at 4˚C for future uses should the extraction process failed, this supernatant also contains PCR inhibitors due to the demineralized bone powder, by not using this supernatant for DNA extraction, it decreases the concentration of inhibitors and other unwanted byproducts from demineralization. Another 1 ml of extraction buffer was added to resuspend the pellet from the previous centrifugation step and replaces the supernatant that was taken, and the process of incubation was repeated for another 24 hours at 37˚C. This step was followed by a final centrifugation at 13,000 rpm for 10 minutes to obtain the supernatant again. The second supernatant was transferred to a 5 ml 30kDA columns containing 3 ml of 10 mM Tris-HCl. This was centrifuged at 2,500 rpm for 30 minutes; the filtered liquid was discarded. 3 ml of 10 mM Tris-HCl was added and centrifuged once more at the same setting as before. The solution remaining in the column was transferred to a clean 2 ml tube, this tube now contains impurified DNA.

The purification process uses the Qiagen QIAQuick MiniElute Purification kit to purify the extracted DNA. The protocol from this kit was used with a modification which includes two

500µl buffer PE wash steps, discarding the first flow-through. Prior to the final elution step, the sample was warmed at 37˚C for 15 minutes in order. The final elution step was performed with 100 µl of buffer EBT, a combination of buffer EB from the supplied kit, and 0.05% tween. The now purified DNA was transferred to a 2ml O-ring tubes and stored in the fridge at 4˚C. Controls were included during the sample processing and extraction stages in order to detect potential DNA contamination during the processing and extraction stage.

Reagents	Volume (µl)
EDTA (0.5M)	950
SDS (20%)	17
Tris-HCl (1M)	20
UV exposure for 15 Minutes	
Proteinase K (~20 mg/ml)	13
Total	**1000**

Table 2. Reagents require to make the extraction buffer for the demineralization of bone powder for DNA extraction.

4.5. Control Region Amplification

After the DNA extraction process, the control region of the mitochondrial DNA was targeted to determine the haplogroup of the Huron-Wendat samples. The region between the position 16190 to 16322 was targeted as it covers important positions to distinguish different haplogroup associated with the indigenous people of America, producing a fragment of 133bp for analysis (table 3). The control region amplification was set up in the Ancient DNA facility and the PCR amplification step was performed at the modern DNA laboratory (table 4 and 5). Negative control was applied to all PCR processes to determine possible contamination caused by the researcher, reagent used, or environmental factor. The PCR product was then purified using the protocol of Wizard SV Gel and PCR Clean-up System (Promega), to remove excess dNTPs and other PCR reagents which could affect the quality of sequencing. The samples were sent to Eurofins for Sanger sequencing and the results were analyzed using Sequencher and aligning with the rCRS to determine the haplogroup of the individuals. The table below is the protocol, primer, and reagent used for the amplification of the control region.

Oligo	Sequence (5'->3')	Tm	GC Content
F16190	ccccatgcttacaagcaagt	57.3	52.6
R16322	tggctttatgtactatgtac	51.2	35

Table 3. Primer used for the Sanger sequencing reaction to target the proportion of the rCRS.

Reagents	Initial Concentration	Volume per Reaction (ul)
Nuclease Free Water		16
$MgCl_2$	50mM	2.5
dNTP	10mM	1
Forward Primer	10µM	10
Reverse Primer	10µM	10
10x buffer	10x	5
Platinum Taq	5U/µl	0.5
DNA		5
Total		50

Table 4. Reagents used for each PCR reaction.

Steps	Temperature (°C)	Time	Cycles
1. Initial Denaturation	98	2 min	1
2. Denaturation	94	30 sec	35
3. Annealing	51	30 sec	
4. Extension	72	30 sec	
5. Final Extension	72	4 min	1
6. Hold	4	Forever	1

Table 5. PCR reaction protocol for short-range PCR

4.6. Library Preparation

Using the method outlined in (M. Meyer & Kircher, 2010) with modification adapted from (Cassidy et al., 2016) and (Scheib et al., 2018), the next-generation sequencing libraries were constructed. As ancient DNA is fragmented due to natural DNA degradation (Dabney et al., 2013), the DNA shearing steps were not needed for ancient DNA library construction. Some libraries which were used for screening did not undergo the UDG treatment, this is to provide a method for DNA damage patterns assessment to determine the authenticity of ancient DNA.

4.6.1. UDG treatment

Deamination is the post-mortem damage exhibited by ancient DNA and it affects the 3’ and 5’ ends of the DNA strand (Dabney et al., 2013). The USER (Uracil-Specific Excision Reagent) or UDG (Uracil DNA Glycosylase) treatment steps was recommended prior to library preparation to reduce the number of sequencing errors caused by cytosine deamination (Marciniak et al.,

2015; Rohland et al., 2015). The USER is a mixture of endonuclease VIII and UDG, the combination of both repairs the deamination site and breaks the phosphodiester backbone at abasic sites. Although this process further fragments the already shorten ancient DNA, it helps minimize the detection of post-mortem damage from sequencing reactions (Briggs et al., 2010). 5 µl of USER enzyme was added to 16.5 µl of extracted DNA and incubated for 3 hours at 37˚C.

4.6.2. Blunt-End Repair

Post reaction of the UDG treatment causes overhangs from the DNA fragments, therefore blunt-end repairs are a must prior to the adapter ligation step. A total of 21.5 µl of UDG treated DNA were added to 48.5 µl of blunt-end repair mix (NEBNext) which contain, 3.5 µl of End Prep Enzyme Mix, 7 µl of End Repair reaction buffer, and 38 µl of ddH_2O. This mixture total to 70 µl was incubated at 25˚C for 15 minutes, then 12˚C for 5 minutes. The purification steps come next, using the QIAquick MinElute purification kit (Qiagen). The DNA samples were added to each column along with 350 µl of buffer PB (binding buffer), followed by a centrifugation step at 13,000 rpm for 1 minute. Discarding the flow-through, 700 µl of PE buffer (wash buffer) were added to each column and was centrifuged again. The flow-through was discarded again and was centrifuged at 13,000 rpm for 1 minute to empty the column. The remaining flow-through was discarded once more, and the column was centrifuged at the same setting for 1 minute to dry the column. Placing the column in a new 1.5ml Eppendorf, the elution step was performed by adding 22 µl of EBT and centrifuged at 13,000 rpm for 1 minute.

4.6.3. Adapter Ligation

In order to ligate the adapter sequence to the DNA, a reaction mix total 40 µl was made for each sample containing, 4 µl of 10x T4 DNA ligase buffer (Thermo Scientific), 4 µl of 50% PEG 4000 (Thermo Scientific), 1 µl of 5 U/µl T4 DNA ligase (Thermo Scientific), 1 µl of 100 µM custom made adapter mix see table 6 (Sigma-Adrich), 10 µl of ddH_2O and 20 µl of DNA from the blunt-end repair steps. This was incubated at 22˚C for 30 minutes, followed by the purification once more with the QIAQuick MinElute purification kit.

Reagents	Volume (µl)
IS1_adapter_P5.F (500 µM) or IS2_adapter_P7.F (500 µM)	0.2
IS3_adapter_P5+P7.R (500 µM)	0.2
Oligo hybridization buffer (10x)	0.05
ddH_2O	0.05
Total	**0.5**

Table 6. Hybridization mix required for the adapter ligation step.

4.6.4. Adapter Fill-in

From the adapter ligation reaction, 20 µl of the product were mixed with 20 µl of polymerization mix (1 µl of 10mM dNTPs, 1.5µl Bst polymerase large fragment (8U/µl), 4 µl of 10x Thermopol reaction buffer, and 13.5 µl ddH_2O) This was incubated at 37˚C for 30 minutes, then 80˚C for 20 minutes as a termination step.

4.6.5. Amplification

The final step requires the addition of barcode indexes for multiplexing. The total volume of this reaction mixture is 50 µl, consisting of 1 µl of 10 µM IS4 primer, 2 µl of indexing oligoes (Sigma-Aldrich), 41 µl of Accuprime Pfx supermix (Thermoscientific), and 6 µl of product from adapter fill-in step. The mix was prepared on the ancient DNA facility, then transported to the modern laboratory for the amplification steps. The PCR step was performed as shown in table 7. The amplicon was purified using the QIAQuick MinElute purification kit and elute with 25 µl WEBT buffer.

Steps	Temperature (°C)	Time	Cycles
1. Initial Denaturation	95	5 min	1
2. Denaturation	95	15 sec	12
3. Annealing	60	30 sec	
4. Extension	68	30 sec	
5. Final Extension	68	5 min	1
6. Hold	4	Forever	1

Table 7. PCR protocol for the amplification of ancient genome.

4.6.6. Library Pooling

After making the library, the concentration of each library was checked using Qubit dsDNA HS Assay kit. Furthermore, the fragment size distribution of each library was determined following the protocol of the Agilent High Sensitivity DNA kit with a Bioanalyzer (Agilent). These steps were necessary to verify whether the library was successfully made. Libraries made without UDG treatment were sent to Trinity College, Dublin, for initial screening with Illumina MiSeq to determine the endogenous content of the samples. Further samples were then selected for the high endogenous content for library preparation and to undergo UDG treatment. The selected UDG treated samples were sent to Macrogen, Seoul in South Korea for Illumina HiSeq4000 sequencing. A total of 11 samples (table) were sent for next-generation sequencing.

4.7. Bioinfomatic Method

4.7.1. Data processing, trimming, and mapping

Samples sent for screening return as single end. Cutadapt v2.3 was used to trim the adapters discarding reads under 30bp using parameter (-m 30) after trimming, and allowing minimum overlapping of 1bp between the adapters and read using parameter (-O 1) (M. Martin, 2011). This was followed by the alignment process aligning to the human reference genome (hg19), replacing the mitochondrial genome within the reference sequence with rCRS (Andrews et al., 1999; 1000 Genomes Project Consortium, 2015). The program BWA 0.7.17 was used for alignment purposes, as recommended for ancient DNA, the seed disabled with (-l 16569) (Li & Durbin, 2009; Schubert et al., 2012). Further parameter was applied, with the edit distance set to 0.01 for more substitutions (-n 0.01) and gaps allowed (-o 2).

Samples sent for Marcogen were treated differently as they return as pair-end, containing two files for each library in the format of fq.gz. The forward and reverse reads were trimmed and merged using leeHom (Renaud et al., 2014), using the option -ancientdna. This was then aligned as the same as single-end reads to hg19 with the same parameter.

4.7.2. Quality filtering

MergeSamFiles from Picard tools v2.9.2 were used to merge BAM files by sample. Mapping quality of less than 20 along with PCR duplicates and reads below 30bp were removed using Samtools. The quality of each alignment was checked using Qualimap version 2.2.1 prior to quality filtering to determine the number of sequences mapped and unmapped, as well as the number of duplicated reads (Okonechnikov et al., 2016). This was repeated after the filtering process to decern successful quality filtering was applied. Using trimBam from bamUtil v1.0.14 Soft clipping of 3bp were applied to both ends of the sequences to reduce the effect of DNA damage during downstream analysis. Estimation of coverage for each library was determined using Qualimap.

4.7.3. Damage and contamination assessment

One method to determine the authenticity of ancient DNA is to assess deamination at both ends of the sequenced reads. Using mapDamage version 2.0.8.2 (Ginolhac et al., 2011) on both UDG and non-UDG treated libraries, the authenticity of ancient DNA was verified. All samples exhibited ancient DNA damage and do not exhibit modern DNA contamination, additionally, the non-UDG treated library contains damaged that was higher than the UDG treated libraries. This was expected as the UDG treatment would have removed or lessened the post-mortem damage exhibited by ancient DNA.

Further steps to clarify potential modern contamination or cross-contamination can be detected by checking the sequenced sample by libraries. Using the mitochondrial genome, each library was assessed to determine that it consists of one single mitochondrial haplotype and that this is consistent across all libraries of the same sample. The position called during the SNP

calling stage (chapter) was checked visually by IGV (Intergrative Genomics Viewer) (Robinson et al., 2011). The Sanger sequencing data previously performed were also used, adjacent with the NGS data, to check for possible contamination.

4.7.4. Uniparental classification

4.7.4.1. Determining Mitochondrial Haplogroup

For accurate determination of the mitochondrial DNA haplogroup, each library was aligned and use the same quality filtering process with the same parameter as (chapter 4.7.1 and 4.7.2) but using the rCRS as the reference sequence instead of the human referencing genome (hg19). This allows more reads to align onto rCRS which improves the depth of coverage. GATK version 3.7.0 HaplotypeCaller (McKenna et al., 2010) was used to determine the variant positions of the mitochondrial genome for each sample. This was shortly followed by utilising HaploGrep version 2.0 with guide tree build 17 from PhyloTree to determine the haplotype of each sample (Van Oven, 2015; Weissensteiner et al., 2016). IGV was used to visualise the variants and was used to check heteroplasmies, the presence of gaps within the sequenced data, and whether positions were accurately determined by checking the depth of the variant position.

4.7.4.2. Determining Y-Chromosome Haplogroup

The sex of each sample was determined using the scrip generated by (Skoglund et al., 2013), which was used to determine the ratio of sequences aligned to the X and Y chromosome. Once a sample is designated as male, Yleafv1.0 (Ralf et al., 2018) was used to assign to a Y-chromosomal haplogroup. Using the aligned and merged library which underwent the quality filter process as performed in chapter 4.7.2. The Y-chromosome assignment was checked using ISOGG 2019 and IGV.

4.7.5. Genotype calling

After the filtering process for quality using samtools, GATK version 3.7.0 was used to call all the bases at each site. Only biallelic SNPs which contain minimum base quality of 30 were considered. The ancient samples were called as pseudo-haploid and were compared with previously published datasets. The Affymetrix Human Origins dataset (Lazaridis et al., 2014; Patterson et al., 2012), containing 597,573 SNP positions for 166 populations with 2068 modern individuals. This was used for unsupervised admixture analysis and PCA. The 1240K SNP array panel was also used from David Reich lab (https://reich.hms.harvard.edu) for D-statistics and outgroup-f3 analysis.

4.7.6. Kinship analysis

The Huron-Wendat samples were compared to each other to determine any possible relationship within and between different archaeological sites. The program READ (Relationship Estimation from Ancient DNA) was used as it is designed and optimised with low coverage samples (Kuhn et al., 2018). It can determine whether the individual is related by first or second degree, as well as identifying unrelatedness. First-degree relation would indicate either the individuals tested is either a sibling or a parent and offspring relationship. Second-degree relation would indicate either an aunt/uncle and niece/nephew relationship or a grandparent and a grandchild relationship or the individuals are half-siblings.

4.7.7. Principal Components Analysis

The dataset from the Human Origin Project was used to generate the PCA plots along with Smartpca v.16000 within EIGENSOFT (Lazaridis et al., 2014; Patterson et al., 2006, 2012). The ancient samples were projected on top of the modern individuals from this dataset using the option "Isqproject: YES". The output of this was plotted using R to generate figure 29.

4.7.8. ADMIXTURE

In order to understand the different ancestry components of our ancient samples, ADMIXTURE v1.3.0 was used to differentiate the ancestry components of modern and ancient populations. Unsupervised ADMIXTURE analysis was performed with various numbers of ancestral populations represented by the variable K. The value of K was increased from 2 to 15 to view potential ancestries sharing between the modern and ancient populations. CV value, otherwise known as the cross-validation value, was used within the ADMIXTURE program for error estimation within the prediction model (Alexander et al., 2009). The value K with the lowest CV error was used. Random seeds were introduced for each run using the command -seed and were replicated ten times. The results were imported into R for plotting.

4.7.9. D-Statistics and f-Statistics

Within ADMIXTOOLS, qp3Pop v412 was used for Outgroup-f3 analysis using the 1240K SNP array panel (Patterson et al., 2012). Using the modern Mbuti population as an outgroup, the shared drift of the ancient samples and other modern populations were tested. Admixf3, another component of ADMIXTOOLS was also performed to test whether admixture can be detected in modern and ancient indigenous populations with our ancient individuals. $|Z| > 3$ were taken to be significant.

Further function of ADMIXTOOLS is the D-Statistic function which uses allele frequency to infer admixture between testing populations. Four populations is used with Mbuti as an outgroup population, this is illustrated in the arrangement of D(Mbuti, Pop X, Pop A, Pop B). Population X

is the population in which you would like to test against populations A and B. In order to determine whether admixture has occurred, a positive value would indicate admixture has occurred with population X and B, whilst a negative value highlights admixture between population X with population A.

4.8. Modern Mitochondrial samples

Extracted DNA of modern Native American individuals was sent over by our collaborator Prof Damian Labuda (University of Montreal). This consists of samples from three different populations from two major linguistic groups, 36 Cree and 37 Ojibwa samples, representing the Algonquian speaking population, and 37 Chipewyan samples, representing the Athabaskan speaking population, total to 110 modern samples.

4.8.1. Long-range PCR Amplification

The entire mitochondrial DNA was amplified using two reactions, using two separate primer sets, two overlapping fragments were generated. GoTaq Long-Range PCR Master Mix Kit (Promega) was used for the amplification steps, this master mix contains the hot-start DNA polymerase, dNTPs, $MgCl_2$, and buffer (table 8). THE PCR condition was first optimised according to the instruction protocol provided by the Master Mix kit, testing with different annealing temperatures for the primer. Please see tables 9 and 10 below for PCR protocol and primer information. To determine successful amplification, 1% agarose gel electrophoresis was used at 80 volts for 60minutes in 0.5x TBE buffer. The amplified product was visualized with the INGENIUS 3 (Syngene) system. Size of the amplicon was determined by comparing it to 1 kb DNA ladders (biolabs).

Reagents	Initial Concentration	Volume per Reaction (ul)
Nuclease Free Water		22
GoTaq Long range PCR MasterMix	2x	25
Forward Primer	10 pmol/ul	1
Reverse Primer	10 pmol/ul	1
DNA		1
Total		50

Table 8. PCR Reagents for long range PCR

Steps	Temperature (°C)	Time	Cycles
1. **Initial Denaturation**	94	2 min	1
2. **Denaturation**	94	30 sec	30
3. **Annealing**	55	30 sec	
4. **Extension**	65	9 min	
5. **Final Extension**	72	10 min	1
6. **Hold**	4	Forever	1

Table 9. PCR protocol for long range PCR

Fragment	Oligo	Sequence (5'->3')	Fragement size (bp)	Tm	GC Content
1	5871 for	gcttcactcagccattttacct	7959	58.4	45.5
	13829 rev	agtcctaggaagtgacagcga		60.3	50
2	13477 for	gcaggaatacctttcctcacag	9438	60.1	50
	6345 rev	agatggttaggtctacggaggc		60.9	50

Table 10. Primers used for long ranger PCR, to cover the entire mitochondrial DNA.

4.8.2. Purification, Quantification, and Pooling

The PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega), following the manufacturer's guidelines. This was performed to remove excess dNTPs and primers. Quantification of purified DNA was performed using 1 µl of product from each fragment with Qubit dsDNA HS Assay kit and Qubit 3.0 Fluorometer (ThermoFisher Scientific). After identifying the concentration of each fragment, this was diluted to 1 ng/µl and the two fragments of each sample were pooled together reaching a total volume of 40 µl. Each sample was then placed into a well on a 96-well plate and sent for next-generation sequencing on a MiSeq250 located at the Earlham Institute, Norwich.

4.8.3. Modern DNA analysis

The raw data obtained as paired-end fastq files, using the pipeline generated by (Peltzer et al., 2016) know as EAGER (Efficient Ancient Genome Reconstruction), the raw data was aligned to the reference sequence rCRS. BWA mem and HaplotypeCaller were selected in the pipeline to generate BAM files and VCFs. Setting the minimum coverage to 2 and the ploidy to 100 when using HaplotypeCaller allows easier detection of heteroplasmies. The pipeline EAGER also produces a quality report using Qualimap version 2.2.1 to check the quality and coverage of the sample. Each sample was checked further using IGV v2.4.16 to assessed and identify haplotype.

In order to determine the variants, IGV v2.4.16 was used to check the position based on the VCF file generated. The VCF was filtered by allele frequency to determine mutations. If the

allele frequencies are above 0.7, this was considered as true mutations, whereas if the allele frequencies fall between 0.3 and 0.69, these were considered as heteroplasmies. Frequency falling below 0.3 and appear different to the reference sequence were consider as potential sequencing error and not a true mutation.

Once the positions of true variants and heteroplasmies were verified, the list of mutations was converted into FASTA. This was then used along with the mitochondrial data-based compiled in (chapter) for the construction of maximum parsimony tree.

4.8.4. Mitochondrial DNA Database Compilation

Whole mitochondrial genome of modern and ancient published Native American Macro haplogroups was collected in order to construct the maximum parsimony tree, maximum calculation, and founder analysis. The database currently consists of 1079 sequences of haplogroup A, 3100 sequences of haplogroup B, 1376 sequences of haplogroup M8 (a macro-haplogroup containing haplogroup C, M8, and Z), 1816 sequences of haplogroup D, and 375 sequences of haplogroup X, totaling 7746 sequences, of which 1898 of these sequences belong to Native American haplogroup. This database contains geographical and population information of each sequence. Sequences with unknown origins are used for tree construction and maximum likelihood calculations and excluded for founder analysis.

4.8.5. Phylogenetic tree design

A phylogenetic tree is formed by using the mutations of mitochondrial sequences, originating from the reference sequence, creating branches that are connected by nodes and terminate at a tip. The tip at the end of the branches is the individual samples and the nodes generated are the branching points representing potential ancestral genome which was not represented by available samples. By understanding the origin of each sample, it is possible to decern potential demographic patterns.

The tree was build using the reference sequence rCRS to determine the mutations of our samples that define each branch. Using the software myPhyl version 5.003 with guide tree build 17 from Phylotree (Van Oven, 2015), a maximum parsimony tree was constructed. Within the tree, each branch displays mutations according to the reference genome. A mutation is listed on the branch to show the position in which it differs from the reference by a numerical value, and a DNA base before and after the numerical value to distinguish the occurrence of transitions or a transversion mutation. For the insertion and deletion, letter ins and del along with the numerical position of the reference sequences was used to indicate the occurrence of insertion and deletion. Recurring mutations are underlined, and back mutations are displayed with a @ sign. Hotspot mutations, mutations which occurs at a specific location in high frequency, are excluded when building the maximum parsimony tree as recommended by PhyloTree. A colour code was applied to the dataset and trees to distinguish between different geographic regions; this was coded as shown in table 11.

Colour for region	Countries for each region
Asia	Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan
East Asia	China, Japan, Mongolia, North Korea, South Korean, Taiwan
South Asia	Afghanistan, Bangladesh, Bhutan, Maldives, Nepal, India, Pakistan and Sri Lanka
Southeast Asia	Myanmar, Thailand, Laos, Cambodia, Vietnam, Singapore, Philippines and Malaysia
East Siberia	Eastern region of Russia: Chukotka, Kamchatka, Magadan, Sakhalin Oblast, Khabarovsk Krai, Primorsky Krai
Central Siberia	Central Region of Russia, south of the Kara and Laptev Sea: Sakha, Krasnoyarsk, Irkutsk Oblast, Tomsk Oblast, Yamalo-Nenets Autonomous Okrug, Khanty-Mansi-Autonomous Okrug
South Siberia	South Region of Russia, sharing the border with Mongolia, China and Kazakhstan: Amur Oblast, Zabaykalsky Krai Buryatia, Tuva, Altai, Altai Krai, Khakassia, Novosibirsk Oblast
Canada	This includes all 10 provinces and 3 territories of Canada
Greenland	Greenland, located in between the Arctic and Atlantic oceans
Mexico	This includes all 32 states in Mexico
America	The United States, consist of all 50 states, including Alaska
Central America	Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama and the Caribbean islands
South America	The continent of South America: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Uruguay and Venezuela
Near East	Iraq, Iran, Israel, Jordan, Kuwait, Lebanon, Palestinian territories, Oman, Saudi Arabia, Syria, Turkey, UAE and Yemen
North Caucasus	Adygea, Chechnya, Dagestan, Ingushetia, Kabardino-Balkaria, Karachay-Cherkessia and North Ossetia
South Caucasus	Armenia, Azerbaijan and Georgia
Europe Northwest	Austria, Belgium, Czechia, Denmark, France, Germany, Hungary, Ireland, Luxembourg, Netherlands, Norway, Poland, Slovakia, Sweden, Switzerland and United Kingdom
Europe Northeast	Belarus, Estonia, Finland, Lithuania, Moldova, Romania, Ukraine and Western Russia
Europe Southeast	Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Cyprus, Italy, Macedonia, Serbia and Slovenia
Iberia	Portugal and Spain
Unknown	Samples with unknown origin

Table 11. In order to distinguish the origin of the sample visually on the tree, a color scheme was introduced.

4.8.6. Age Calculation of Maximum parsimony tree

By producing the most parsimonious phylogenetic tree, the node ages were calculated using ρ-statistic and maximum likelihood. The calculation using *ρ*-statistic was performed using a mutation rate of one substitution for every 3624 years corrected for purifying selection (Soares et al., 2009) and the standard errors estimated in (Saillard et al., 2000). This method estimates the age by using the number of mutations within the phylogeny of the tree and the mutation rate provided.

Using the software PAML version 4.7 with the HKY85 mutation model, the generated maximum parsimony tree was used to calculate the maximum likelihood of each node (Hasegawa et al., 1985; Z. Yang, 2007). With the fast-evolving hypervariable region, two partitions were incorporated to differentiate the hypervariable region and the remaining mitochondrial sequences when calculating the maximum likelihood. The model HKY85 considers the different rate of transitions and transversions and does not equal base frequencies.

4.8.7. Founder Analysis

The founder analysis uses a phylogenetic approach to estimate migration events. By determining the source population and the sink population, this tool can quantify the genetic contribution of migration from the source to the sink using *ρ*-statistic (M. Richards et al., 2000). By using the estimated age obtained from each clade in the sink population, hypothetical migration times are projected.

An ideal founder should be an identical sequence type detect in both source and sinks population. Assuming all founders reflect source and sink relationships, two possible scenarios can affect the signal of the founder analysis. One is the gene flow from the sink population back into the source population (back migration), the other is the possible recurrent mutations within the mitochondrial genome. Criteria are introduced to the corresponding scenarios. These are known as f1 and f2 criteria, which specify that sequence matches in the source population should not be at the tips of the source phylogeny but should still have one (f1) or two (f2) derived branches. As it requires more branches to be considered as the founder, f2 is, therefore, a stringent criterion than f1.

Two files were required for the Founder analysis program, one is the maximum parsimony tree in the format of an xml file, the other is a text file consisting of the name of each sequence and a label to distinguish whether the sequence is considered as a source or a sink. Furthermore, a mutation rate of 1 mutation per 2600 years (Fernandes et al., 2015; Gandini et al., 2016) and a time interval from 0 to 30 ka with 200 years intervals was inputted, as the founder analysis does not allow the use of a time depend clock as used in Soares et al., 2009.

5. Results

5.1. Ancient Samples

5.1.1. Initial ancient DNA results

Sample ID	Site Information	Tooth	mtDNA
CE006	Staines Road	URM1	B
CE007		URM1	B
CE008		URM1	X2a
CE009		URM1	B
CE010		URM1	C
CE011		URM1	B
CE012		URM1	A
CE013		URM1	X2a
CE014		URM1	X2a
CE015		URM1	A
CE016	Moatfield	RM3	C
CE017		LM2	A
CE018		LM3	C
CE019		RM3	C
CE020		RM2	C
CE021		LM3	C
CE022		RM2	C
CE023		RM2	C
CE024		RM2	C
CE025		LM1	C
CE026	Teston Road	URM1	A
CE027		URM1	C
CE028		URM1	A
CE029		URM1	A
CE030		URM1	A
CE031		URM1	A
CE032		URM1	B
CE033		URM1	A
CE034		Forming molar	C
CE035		Forming molar	A
CE036		Forming molar	-
CE037	Turnbull	URM2	A
CE038		Molar	C
CE039		Molar	A
CE040		Molar	A
CE041		Molar	A
CE042		Molar	A
CE043		Pre-molar	A
CE044		Deciduous M1	A
CE045		Deciduous M1	A

Table 12. Ancient Sanger sequencing results for the Huron-Wendat individuals.

The DNA of all Huron Wendat samples was first extracted for initial mitochondrial haplogroup testing. Sanger sequencing was used as an initial test to determine the macro mitochondrial haplogroup. This method provided a low-resolution screening for successful extraction and the results were used in the later stage, to compare with the mitochondrial DNA results generated by shotgun sequencing, an approach to detect the possibility of contamination. This method also aids with the identification that all samples are most likely of indigenous American descent, as all sample exhibits the typical Native American haplogroup, aside from one individual, CE036, which provided insufficient data for further analysis as seen in table 12. This also provides a glimpse of expectation from the shotgun sequencing data, as the results from the Sanger sequencing identify the lack of haplogroup D across all sites, therefore, the shotgun sequencing results should comply with the Sanger sequencing results.

5.1.2. Screening results

The table 13 is the initial screening results of one library generated for each sample, a total of 36 individuals was sent for screening. The sequencing process was performed at Trinity College, Dublin as illustrated in section 4.6.6, to determine the endogenous content of each sample. With the obtained screening data, a strategy was proposed in search of the best samples for shotgun sequencing, as endogenous content for each ancient sample varies due to the different rate of DNA degradation caused by environmental factors. Other factors such as the material used for DNA extraction and non-endogenous DNA from the bacterial and fungal present in the environment, would also affect sequencing endogenous DNA of the sample (Kistler et al., 2017; Pääbo et al., 2004; Pinhasi et al., 2015; Sawyer et al., 2012). One factor which limited the experiments overall would be the material obtained, which happens to be teeth, as the petrous bone is widely agreed upon as the best material for ancient DNA study (Hansen et al., 2017). Nevertheless, the material obtained was used to extract DNA and the screening procedure was implemented to search for the sample containing the highest endogenous content for further sequencing. DNA extracted from the molar was selected as the preferred choice.

Prior to the establishment of the screening procedure, four samples were sent directly for shotgun sequencing, as the initial assumption of endogenous content must be high for the successful retrieval of Sanger sequencing results. These samples are CE013, CE019, CE032, and CE040, unfortunately, yield a lower percentage of endogenous content compared to the sample which was sent after the establishment of the screening procedure as seen in table 15.

In order to determine the endogenous content of each sample, the reads were trimmed and aligned to HG19 of the human reference genome. The number of aligned reads was then divided by the number of trimmed reads, obtaining a percentage that represents the endogenous content of the sample. This was also performed after the quality check using the parameter q30, to predict the final endogenous content and estimate the percentage of duplicate reads. The screening process also provides a method to determine whether the sample is indeed considered ancient by analysing the presence of deamination, the presence of

modern DNA contamination can also be identified by using this method. The screening results (table 13) indicates that all samples obtained were identified as ancient DNA and was not contaminated with modern DNA, as the screening results exhibit deamination at both ends of the sequenced reads.

Sample ID	Site Information	Number of Raw Reads	Number of Trimmed Read	Aligned Reads	Raw Endogenous (%)	Q30 Aligned Reads	Rate of Duplication (%)	Endogenous content Q30
CE007	Staines Road	78763	70579	2673	3.79	2326	0	3.3
CE008		93734	89609	853	0.95	722	0	0.81
CE009		79141	71481	411	0.57	353	0	0.49
CE010		75446	65976	25883	39.23	21259	0.02	32.22
CE011		125122	115097	5081	4.41	4383	0.02	3.81
CE012		71997	65590	807	1.23	657	0	1
CE014		70368	61333	216	0.35	173	0	0.28
CE015		66075	58979	5816	9.86	5077	0.02	8.61
CE016	Moatfield	83443	70811	1009	1.42	874	0.23	1.23
CE017		81997	73807	4059	5.5	3512	0.03	4.76
CE018		80447	68976	419	0.61	354	0	0.51
CE020		63255	57043	816	1.43	655	0	1.15
CE021		93699	80432	195	0.24	153	0	0.19
CE022		86024	76021	450	0.59	366	0.27	0.48
CE023		87104	75741	5925	7.82	4986	0.04	6.58
CE024		102984	92061	1837	2	1483	0	1.61
CE025		109567	94213	698	0.74	566	0	0.6
CE026	Teston Road	76960	64325	177	0.28	129	0	0.2
CE027		95878	74576	386	0.52	307	0	0.41
CE028		118619	101696	721	0.71	586	0	0.58
CE029		72866	61594	1126	1.83	923	0.11	1.5
CE030		73937	63016	28535	45.28	24555	0.07	38.94
CE031		78689	67883	29011	42.74	24572	0.07	36.17
CE033		72997	61072	3036	4.97	2608	0.04	4.27
CE034		74721	67420	107	0.16	72	0	0.11
CE037	Turnbull	84309	75811	352	0.46	233	0	0.31
CE038		69050	61141	1283	2.1	1099	0	1.8
CE039		98668	93018	93	0.1	68	1.45	0.07
CE041		113593	101250	570	0.56	457	0	0.45
CE042		89993	81479	508	0.62	396	0	0.49
CE043		114555	105721	123	0.12	69	0	0.07
CE044		83106	77466	35	0.05	16	0	0.02
BY004	Hind 19	64775	55934	326	0.58	260	0	0.46
BY005	Hind 22	66210	57826	4360	7.54	3479	0.06	6.01
BY006	Hind 18a	97058	65259	131	0.2	95	0	0.15
BY007	Sartori	55961	49576	12012	24.23	9956	0.04	20.07

Table 13. Screening results obtained to determine the endogenous content for the selection of further sequencing. Green highlighted individuals are the sample selected for further sequencing due to the high endogenous content.

5.1.3. Coverage Estimation

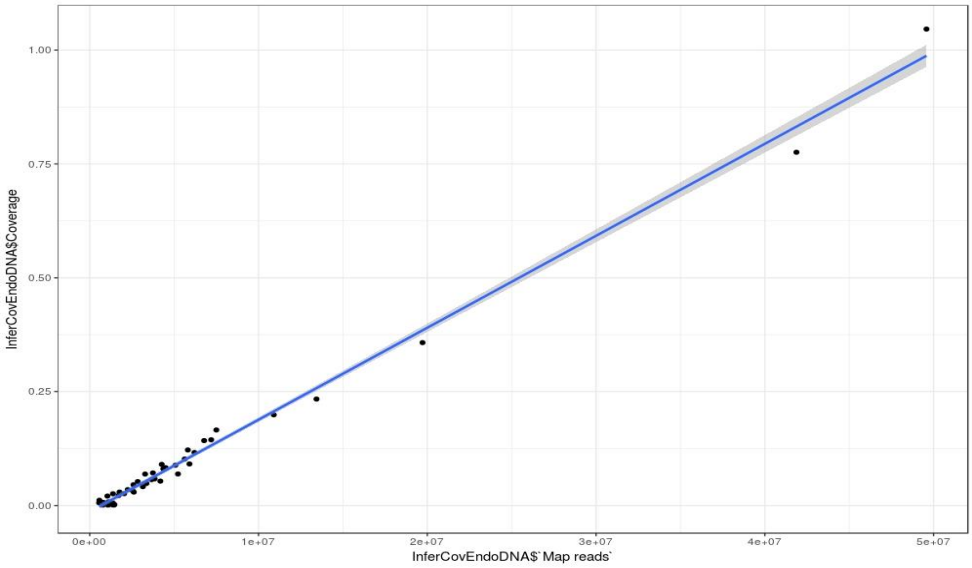

Figure 20. Using the data obtained from the entire lab group, the number of endogenous map reads of each sample was plotted against the coverage of the sample to generate a linear regression. This was used to estimate and calculate the endogenous content of screening results for the purposed of selecting the best samples for further sequencing. (Figure generated by Gonzalo Oteo Garcia)

Making use of the shotgun sequencing results generated by the entire lab group, figure 20 was created using the number of mapped reads, plotting against the average coverage depth of the sample, producing a linear regression. By finding out the endogenous content of the samples and understanding the maximum number of reads that can be generated per lane from Illumina Hiseq4000 (330 million reads per lane) (M. Meyer & Kircher, 2010), calculations was made to estimate the possible number of reads that can be generated based on the percentage of the endogenous content of each sample. This estimation utilizing the linear regression line provides an approximation of the coverage that can be generated based on the percentage of endogenous content from the screening results.

Samples that contain above 6% endogenous content were sent for shot-gun sequencing, as estimation of samples containing at least 6% endogenous content should yield the minimum of 0.2x overall coverage (table 13 & 14). This should provide minimum data for cluster0based analysis and determining uniparental markers (Patterson et al., 2012).

Majority of the samples have provided poor endogenous contents, containing less than the ideal endogenous content of 6%, therefore would yield less than 0.25x mean genome coverage. Three out of six samples from the Hind sites were sent for screening, the remaining three were not sent due to the low concentration of the library made for screening. Additionally, from the screening results, it was not possible to conduct further genomic analysis of each sample, this was due to insufficient reads covering the mitochondrial and whole genome, however, it was possible to determine that all screening individuals were indeed ancient DNA with no modern DNA contamination using mapDamage. Samples containing above 6% endogenous content

were selected for shotgun sequencing and are highlighted in green in table 14. An interesting point shown from the screening results demonstrates that although samples are found from the same site, the endogenous content of each sample varies. As seen in the Teston site, CE030 and CE031 consist of the highest endogenous content, whereas other samples from the same site illustrated poor endogenous content. This further supports that DNA degradation occurs differently amongst material buried in the same site (Gilbert et al., 2005). Furthermore, the screening results for deciduous teeth did not provide a higher endogenous content than molar, as CE034, CE043 and CE044 provided the lowest endogenous content across all sites, additionally only deciduous teeth samples failed the Sanger sequencing method and provided poor screening results. The quality of the sample from the Turnbull site was considered poor, this was reflected in the screening and shotgun sequencing results.

Sample ID	Site Information	Endogenous content Q30 (%)	Estimated Reads from Hiseq4000	Estimated Coverage
CE007	Staines Road	3.3	10,890,000	0.11x
CE008		0.81	2,673,000	0.026x
CE009		0.49	1,617,000	0.016x
CE010		32.22	106,326,000	1.07x
CE011		3.81	12,573,000	0.127x
CE012		1	3,300,000	0.033x
CE014		0.28	924,000	0.0093x
CE015		8.61	28,413,000	0.287x
CE016	Moatfield	1.23	4,059,000	0.041x
CE017		4.76	15,708,000	0.16x
CE018		0.51	1,683,000	0.017x
CE020		1.15	3,795,000	0.038x
CE021		0.19	627,000	0.0063x
CE022		0.48	1,584,000	0.016x
CE023		6.58	21,714,000	0.22x
CE024		1.61	5,313,000	0.054x
CE025		0.6	1,980,000	0.02x
CE026	Teston Road	0.2	660,000	0.0067x
CE027		0.41	1,353,000	0.014x
CE028		0.58	1,914,000	0.019x
CE029		1.5	4,950,000	0.05x
CE030		38.94	128,502,000	1.298x
CE031		36.17	119,361,000	1.205x
CE033		4.27	14,091,000	0.14x
CE034		0.11	363,000	0.0036x
CE037	Turnbull	0.31	1,023,000	0.01x
CE038		1.8	5,940,000	0.06x
CE039		0.07	231,000	0.002x
CE041		0.45	1,485,000	0.015x
CE042		0.49	1,617,000	0.016x
CE043		0.07	231,000	0.002x
CE044		0.02	66,000	0.0006x
BY004	Hind 19	0.46	1,518,000	0.015x
BY005	Hind 22	6.01	19,833,000	0.2x
BY006	Hind 18a	0.15	495,000	0.005x
BY007	Sartori	20.07	66,231,000	0.66x

Table 14. The estimated coverage of each sample based on the screening results obtained. Highlight in green are the individuals sent for further sequencing.

5.1.4. Shotgun Sequencing Results

Sample ID	Site Information	Number of Library	Q30 reads above 30bp	Whole Genome Coverage	SNPs called (600k)	SNPs called (1240k)	mtDNA Coverage	mtDNA	M/F	Y Chromosome
CE010	Staines Road	1	9,249,057	0.161	-	-	-	-	-	-
		2	9,254,342	0.161	-	-	-	-	-	-
		3	9,707,205	0.169	-	-	-	-	-	-
		4	8,061,624	0.139	-	-	-	-	-	-
		5	9,829,775	0.168	-	-	-	-	-	-
		6	9,937,234	0.170	-	-	-	-	-	-
		Total	54,711,746	0.947x	295,678	582,123	134.6x	C4c1	XX	N/A
CE013		1	51,560	0.001	-	-	-	-	-	-
		2	93,005	0.002	-	-	-	-	-	-
		3	58,831	0.001	-	-	-	-	-	-
		Total	201,824	0.004x	2,305	4,264	0.890x	X2a1	XX	N/A
CE015		1	2,842,774	0.069	-	-	-	-	-	-
		2	2,903,204	0.070	-	-	-	-	-	-
		3	3,023,512	0.073	-	-	-	-	-	-
		Total	8,697,623	0.212x	94,375	188,366	93.7x	A2i	XX	N/A
CE019	Moatfield	1	283,271	0.007	-	-	-	-	-	-
		2	372,739	0.008	-	-	-	-	-	-
		3	273,485	0.005	-	-	-	-	-	-
		Total	927,757	0.0205x	11,412	20,947	6.638x	C1c	XX	N/A
CE023		1	2,635,288	0.039	-	-	-	-	-	-
		2	2,119,953	0.032	-	-	-	-	-	-
		3	2,501,193	0.037	-	-	-	-	-	-
		Total	7,232,903	0.108x	54,404	108,157	41.3x	C1c	XX	N/A
CE030	Teston Road	1	12,444,809	0.269	-	-	-	-	-	-
		2	12,694,691	0.275	-	-	-	-	-	-
		3	11,369,186	0.247	-	-	-	-	-	-
		Total	36,303,011	0.786x	274,180	539,601	66.7x	A2i	XX	N/A
CE031		1	12,485,277	0.200	-	-	-	-	-	-
		2	12,442,023	0.200	-	-	-	-	-	-
		3	12,292,491	0.198	-	-	-	-	-	-
		Total	36,996,890	0.595x	229,845	452,351	44.7x	A2i	XX	N/A
CE032		1	247,621	0.006	-	-	-	-	-	-
		2	289,747	0.007	-	-	-	-	-	-
		3	213,472	0.004	-	-	-	-	-	-
		Total	749,536	0.017x	10,029	18,264	3.83x	B2	XX	N/A
CE040	Turnbull	1	56,579	0.001	-	-	-	-	-	-
		2	84,013	0.002	-	-	-	-	-	-
		3	79,655	0.001	-	-	-	-	-	-
		Total	219,379	0.004x	2,192	4,057	1.803x	A2i	XY	C2b1a1a
BY005	Hind22	1	4,557,874	0.097	-	-	-	-	-	-
		2	3,799,809	0.081	-	-	-	-	-	-
		3	4,984,451	0.107	-	-	-	-	-	-
		Total	13,263,592	0.283x	129,125	252,125	116.8x	B2	XY	Q1a2a1a1
BY007	Sartori	1	13,266,263	0.315	-	-	-	-	-	-
		2	14,550,167	0.345	-	-	-	-	-	-
		3	14,423,920	0.341	-	-	-	-	-	-
		4	10,920,471	0.253	-	-	-	-	-	-
		5	12,209,013	0.282	-	-	-	-	-	-
		6	13,378,801	0.310	-	-	-	-	-	-
		Total	77,368,885	1.816x	397,967	796,417	96.03x	B2	XY	Q1a2a1b

Table 15. The results of each library generated for sequencing are displayed. The merge libraries and duplication of reads were removed, and the final coverage along with the haplogroup of mitochondrial and Y-Chromosome can be seen.

The screening step was not performed for the following samples, CE013, CE019, CE032, and CE040, instead, these samples representing each Huron Wendat site were sent directly for shotgun sequencing. This was performed initially prior to the establishment of the screening procedure, as a random selection method to determine the quality of DNA from each site, therefore the estimation of endogenous content was not calculated prior to sequencing. The libraries generated for the four samples, CE013, CE019, CE032, and CE040 were treated differently compared to the screened samples, as the first library out of the three libraries for each sample did not undergo UDG treatment for the purpose of detecting deamination, a process to determine the authenticity of ancient DNA and modern DNA contamination. The results obtained from these four samples identify poor quality DNA preservation across the four Huron Wendat sites, demonstrating the need for the screening procedure with the remaining ancient samples to obtain quality data. The UDG treated libraries appears to provide more mapped reads than untreated libraries in compliance with previous publication (Briggs et al., 2010; Rohland & Hofreiter, 2007b).

Shotgun sequencing results can be seen in table 15, documenting the results for each library generated. Three libraries of each sample were generated per half lane for the Illumina Highseq4000. BY007 and CE010 were the exceptions, as the two samples came from the oldest sites and contain a high endogenous content, six libraries were sent in a single lane to obtain a higher coverage for BY007 and CE010. The estimated coverage previously calculated (table 13) was assuming each sample was sent for their own single lane, the estimation was adjusted by halving the calculated value to accommodate two samples sharing a single lane.

The prediction of overall coverage using endogenous content was helpful to understand an approximate coverage estimation. Across the samples, the overall coverage results underperformed when compared to the estimated coverage. This was most noticeable with CE010, as the estimation indicates this sample should obtain the maximum coverage of ~1.9x when the samples were sent for sequencing in a single lane, however the actual result obtained for the overall genome coverage for CE010 was 0.947x, a significant decrease. Similarly, CE030 and CE031 were sent sharing one lane, with the predicting results for both samples using the endogenous contents should be closer to 1x, but the results obtained indicate a lower overall genome coverage at 0.786x for CE030 and 0.595x for CE031. The only sample which performed above expectation was BY007, with the predicted overall coverage at approximately 1.5x and the results obtained indicate a higher coverage at 1.8x.

The underperformance of overall coverage obtained when compared to the estimated value is disappointing. However, the reason for the overestimation of coverage is due to the inconsideration of duplicate reads during the prediction stage. As the number of reads does correlate to the overall coverage, but during the filtering stage after the alignment to the reference sequence, duplicate reads were removed as it was considered a by-product of PCR during the amplification process. Therefore, the estimation method used is an inaccurate

method, as it considers a direct linear regression plotting the number of reads against overall coverage without assessing the true duplication rate of sequences.

5.1.5. Uniparental markers

5.1.5.1. Y-Chromosome

Using the sex identification script from Skoglund et al., 2013, the ratio of sequences aligning to the X and Y chromosomes were determined to identify whether the ancient individual sequenced is male. Yleaf was then used to determine the Y-Chromosome haplogroup of the individual that was identified as male. Out of all the individuals, only three samples were identified as male, these are CE040, BY005, and BY007 (table 15). Unfortunately, it was not possible to determine the sex of all individuals, with CE013 obtaining low overall coverage providing insignificant information to clarify whether the sample is male (Hallast et al., 2015; Skoglund et al., 2013). Additionally, for sample CE040, also obtaining low overall genome coverage of the sample, only a handful of mutations were applicable for Y chromosome haplogroup assignment. Nonetheless, based on the available position called using Yleaf, a Y chromosome haplogroup was assigned for CE040, perhaps with greater depth, a more accurate assignment can be determined for this individual.

The Y chromosome haplogroup determined for CE040, BY005, and BY007, each exhibit different haplotype but all are found in the indigenous population of the Americas. Haplogroup C2a1a1a can be found in several indigenous peoples of North America, including Na Dene, Algonquian, and Siouan speaking populations (Zegura et al., 2004). Q1a2a1 can be found in both Siberian populations as well as Indigenous American populations predominantly meso and southern Americans (Grugni et al., 2019; Rangel-Villalobos et al., 2008). However, more information for Northern indigenous Y Chromosome is required to understand the relationship between our samples with other populations.

5.1.5.2. Mitochondrial DNA

Although some samples consist of a low percentage of mapped reads, with the lowest overall coverage samples (CE030 and CE040) at 0.004x, it was still possible to obtain enough information for mitochondrial analysis. Using Haplogrep and manual check of mtDNA alignment with IGV provided a method to classify the low coverage of sample with higher precision, pointing to the mitochondrial haplogroup illustrated in table 15. Once more, sample CE013 proved to be more difficult compared to the other samples as the whole mitochondrial genome was not covered, however with the low mean mtDNA coverage of 0.890x, it was still possible to classify CE013 to be X2a1 as key diagnostic position was covered. The mtDNA of all samples identified all individuals to be indigenous American as it displays mtDNA typically associated with the indigenous American population (appendix 1). Four individuals were found to be A2i haplogroup, two belonging to C1c haplogroup, three basal B2 haplogroup, one C4c1 haplogroup, and one belongs to X2a1. Cross- validation between the shotgun sequencing and

Sanger sequencing identifies the same variants and haplogroup as each other, demonstrating a lack of contamination during the amplification and library-making steps.

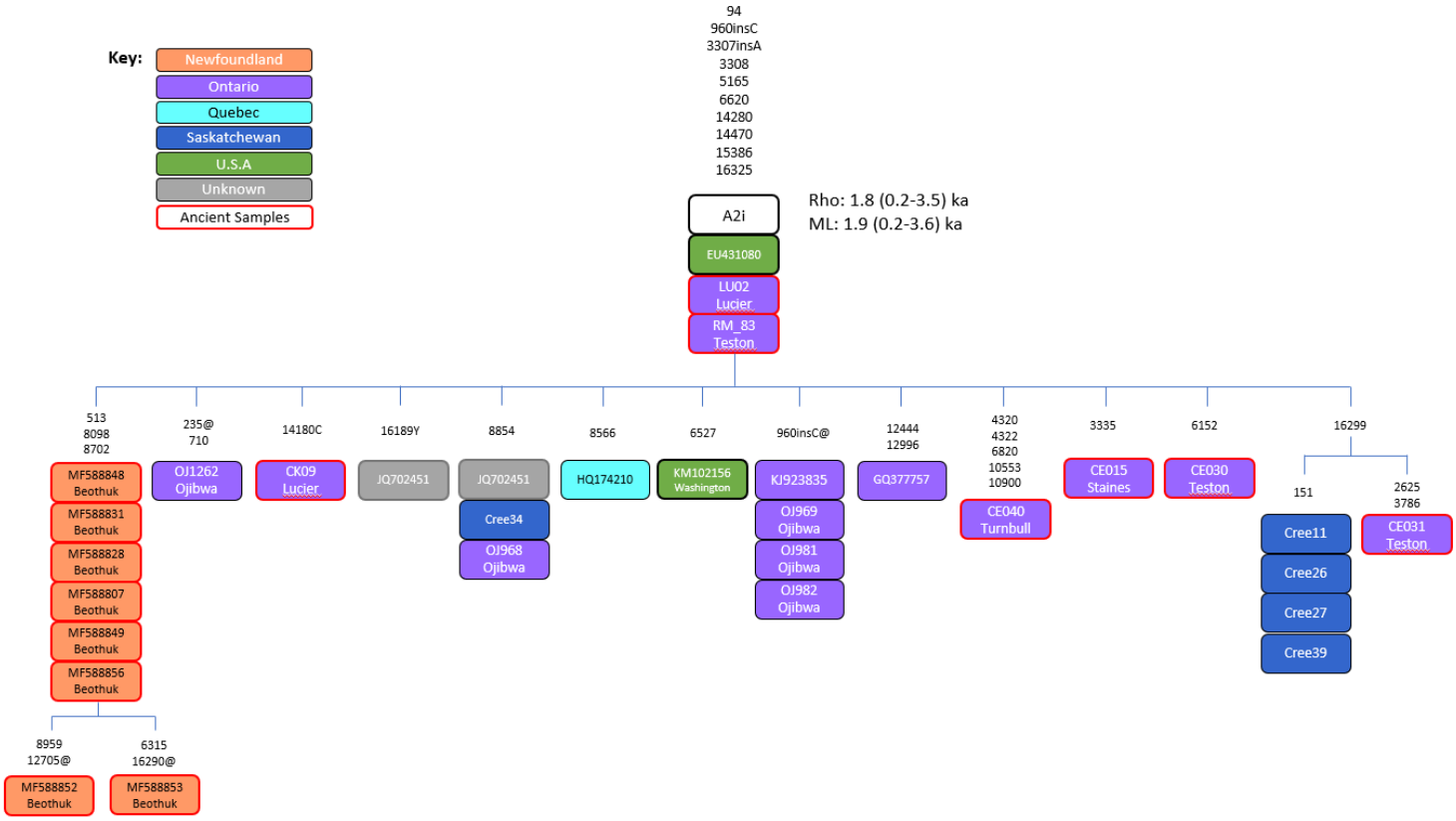

Figure 21. Maximum parsimony tree of the haplogroup A2i with Rho and maximum likelihood (ML) age estimation shown. Ancient DNA sequences are highlighted in red and each sample are coloured based on the geographic location.

The four individuals belonging to haplogroup A2i are CE015, CE030, CE031, and CE040. A2i in general are found in high frequencies in Canada and can be found in Cree and Ojibwe populations. This haplogroup appears to be exclusive to Northern indigenous Americans, with the majority of the ancient and modern individuals found in this haplogroup originated from Northeast Americas and only one modern individual found on the west coast in Washington state (figure 21).

Three published samples can be found located at the node of A2i, one sample is a modern individual from the U.S.A without further linguistic or more precise geographic information and the remaining two samples are ancient individuals found at the Lucier site and Teston site in Ontario. Unfortunately for the published ancient individual, LU02, the age of this individual is unknown, however, the Lucier site was suggested to be between 1200 to 1450 AD (Scheib et al., 2018), demonstrating the presence of A2i in Ontario in 1200 AD. Fitting into our maximum likelihood calculated age of haplogroup A2i at 1.8ka.

Deeper into the branch of A2i, ancient Beothuk individuals from Newfoundland cluster amongst each other, although the published Beothuk individuals are primarily from the terminal population within the last 300 years (Duggan et al., 2017), the ancient data demonstrate a wide distribution of haplogroup A2i, covering between Ontario and Newfoundland. The Beothuk population is now extinct, unfortunately, information regarding their dispersal only suggests this culture inhabited the area of Labrador and Newfoundland (Marshall, 1988). Further linguistic information related to Beothuk also did not provide enough evidence to class them into any linguistic family (L. Campbell, 2000; L. Campbell & Mithun, 2014). Therefore, Beothuk relationship with other indigenous people on the east coast is unknown at the current time.

The four ancient A2i Huron-Wendat samples spread across three different sites; all contain different private variants of this haplogroup and cluster with samples predominantly found in Ontario. The only exception is CE031, which appears to share a mutation at 16299 with a modern Cree individual from Saskatchewan. This is particularly interesting as Huron-Wendat is of the Iroquoian language family, whilst the Cree and Ojibwa population are known to be Algonquian-speaking family. The sharing of this haplogroup between two different language family suggests either the ancestor of both languages shared a common ancestor, or at some point in time, the maternal genetic of one language family were shared with another.

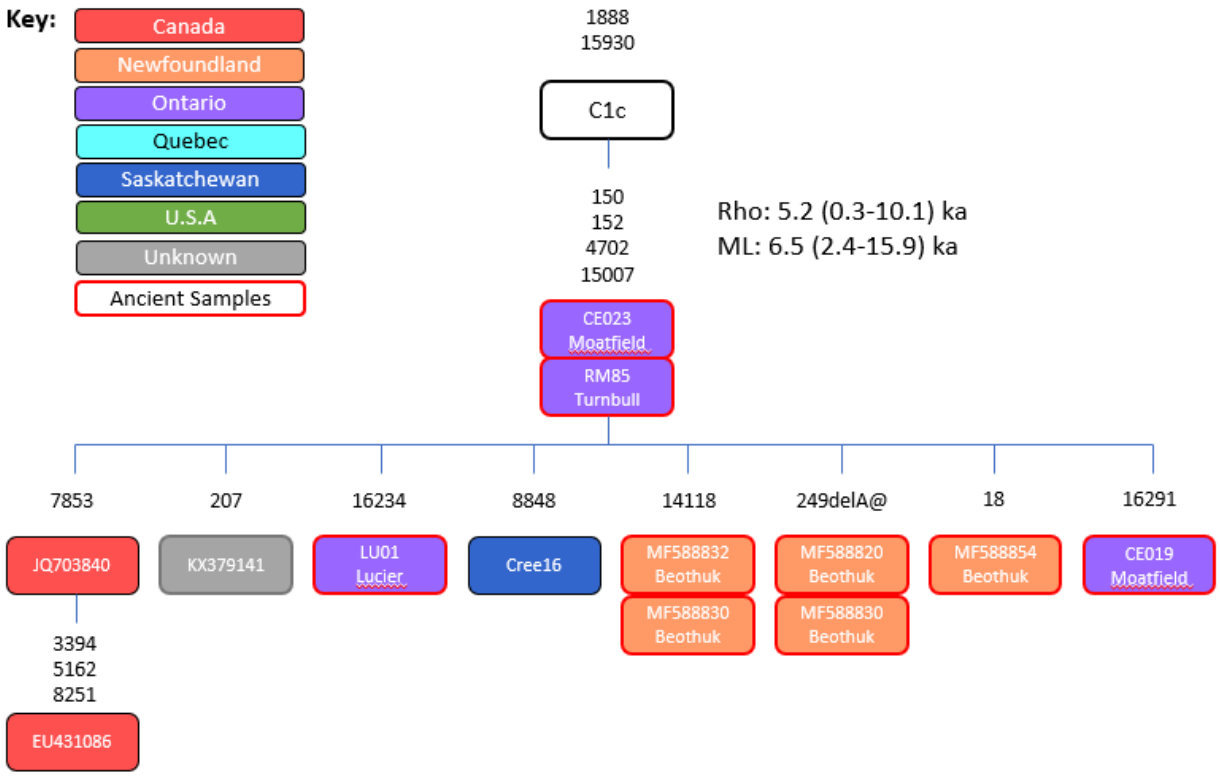

Figure 22. Maximum parsimony tree of haplogroup C1c+150, the topology of this tree was formed by the addition of ancient sequences. Rho and maximum likelihood (ML) estimated ages are shown. The colour of each samples represent the geographic location with ancient individuals highlights in red.

The two Huron-Wendat individuals, CE019 and CE023, both from the Moatfield site belong to the mtDNA haplogroup of C1c, more specifically C1c+150 (figure 22). This haplogroup dates to 6.5 ka consist of individuals exclusively from Canada, appears to have an eastern Canada distribution based on the generated and publicly available data. A higher number of ancient samples than modern samples can be found in this haplogroup, which was used to define the topology of this branch. Potentially indicating a higher frequency in the past than the present. Due to the lack of modern individuals for the haplogroup C1c+150, it is difficult to accurately discern modern geographic distribution of this haplogroup other than its presence in Canada and can be found in Cree population. This haplogroup has been found in other ancient samples in Ontario, as well as Beothuk individuals in Newfoundland.

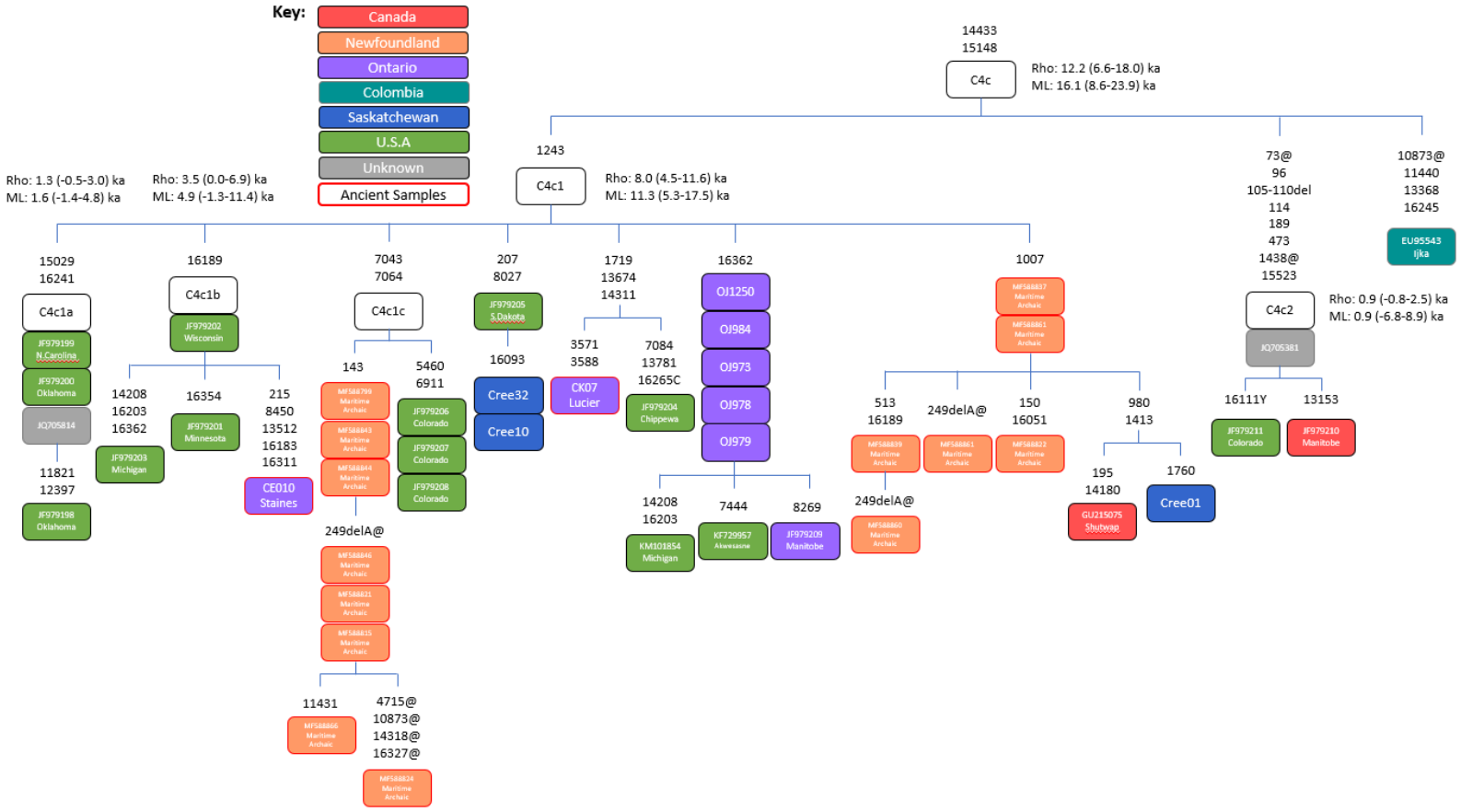

Figure 23. Maximum parsimony tree of C4c, containing both C4c1 and C4c2. C4c1 are the major haplogroup within C4c, containing sample predominantly found around the great lake and northeast coast. Rho and maximum likelihood ages are shown. Coloration of the samples represents the geographic location as shown in the key provided. Ancient DNA sequences are highlighted in red.

The haplogroup C4c dates to 16.1 ka and is considered as a rare haplogroup found predominately in North America (Achilli et al., 2013; Kashani et al., 2012; Tamm et al., 2007). Based on the geographic distribution of the modern individuals, most modern C4c are found surrounding south and east of the great lake region with C4c1, the major branch within the

haplogroup. Within this branch, our ancient Huron-Wendat individual, CE010, and the published Lucier sample CK07 demonstrate the presence of C4c1 haplogroup in the Ontario region approximately 800 years ago. Deeper within the branch of C4c1, haplogroup C4c1c, and the subbranch C4c1+1007 contains ancient individuals from Maritime Archaic culture from Newfoundland (Duggan et al., 2017). The ancient Maritime archaic samples confirm the presence of this haplogroup in Eastern Canada approximately 4000 years ago and are not the ancestor to the later inhabitant of Newfoundland as suggested in the finding by (Duggan et al., 2017). Therefore, it is interesting to identify the presence of modern individuals from the west and southern great lake region sharing mutations with the Maritime Archaic site. An interesting point to be identified is the lack of Beothuk individuals from the C4c haplogroup and that both individuals from Ontario are placed in different subbranches of C4c1, not sharing mutations with the Maritime Archaic.

The individual CE010 is classified as C4c1b, sharing this haplogroup with modern individuals from south and west of the great lake region. The modern individuals within C4c1b does not have further information regarding their history, preventing further understanding in determining their relationship with the sample CE010.

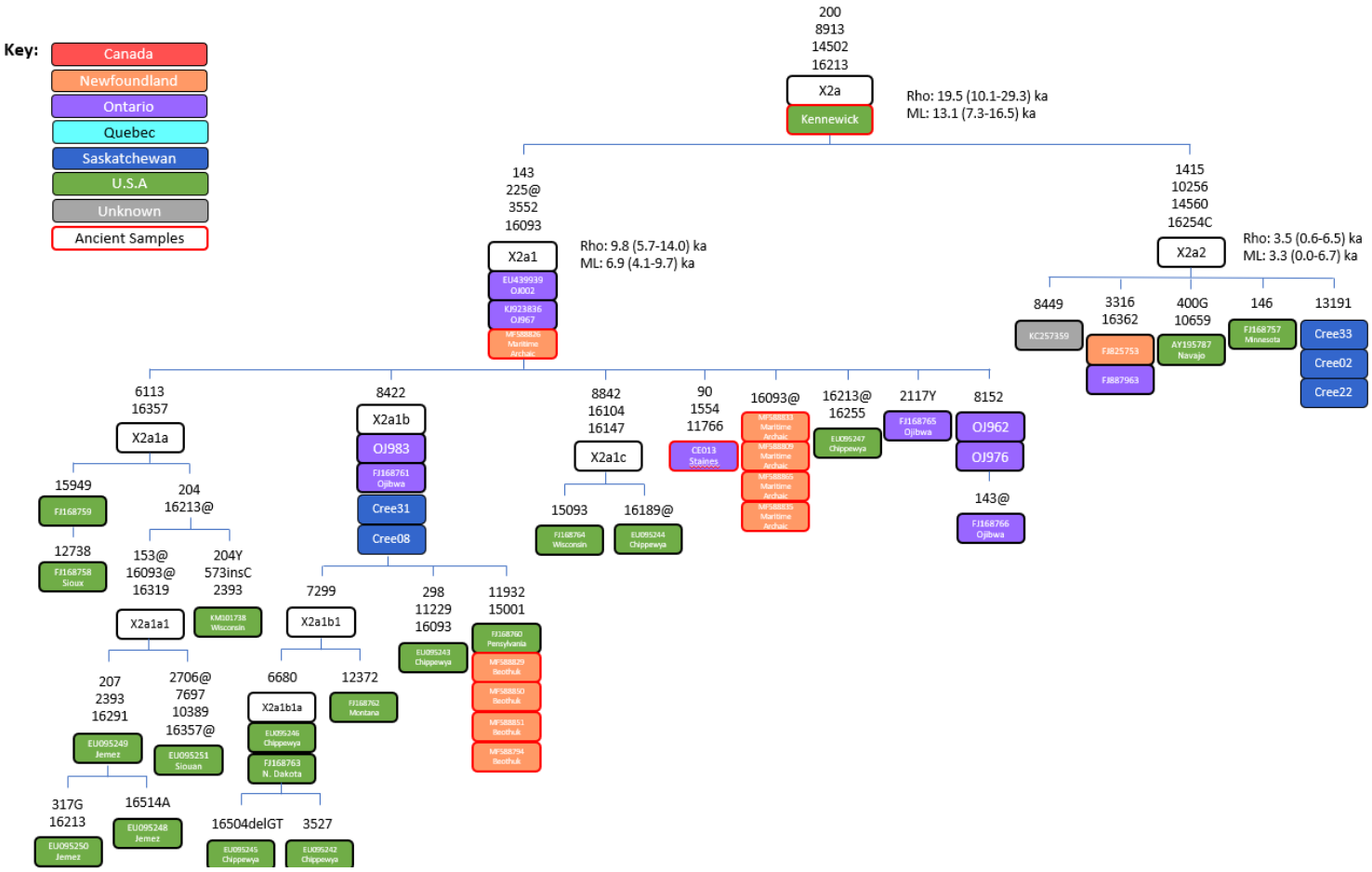

Figure 24. Maximum parismony tree of X2a.Rho and maximum likelihood ages estimation were shown. Coloured samples are represents the geographical location of the individual as illustrated with the key, and ancient individuals are highlighted in red

X2a is exclusively found in North indigenous Americans, consider rare due to the low frequency found amongst all North Indigenous American haplogroup. Suggested to be found in high frequency within the Ojibwa population (Scozzari et al., 1997; A Torroni et al., 1993). Although X2a in general indicate high frequencies at the great lake region (Perego et al., 2009), the oldest individual to be found as X2a is the Kennewick Man from the Washington state dated to ~8500 BP, highlight the presence of this haplogroup in North-Western Americas prior to European arrival (Rasmussen et al., 2015).

Sample CE013 was identified as X2a from both Sanger sequencing and NGS results. Although the sequencing result for CE013 was poor and not the whole mitochondrial genome was covered with the mtDNA coverage at 0.890x. It was possible to manually identify key position to place this as X2a1. The finding of at least one X2a individual from the given samples was anticipated, due to previous publications suggesting a high frequency of this haplogroup around the great lake region (M. D. Brown et al., 1998; Tamm et al., 2007). It is interesting that both Maritime Archaic and Beothuk samples from published data are found within X2a1. This demonstrates a wider presence of X2a than previously suggested, and perhaps relationships with individuals from the great lake and east coast of Canada.

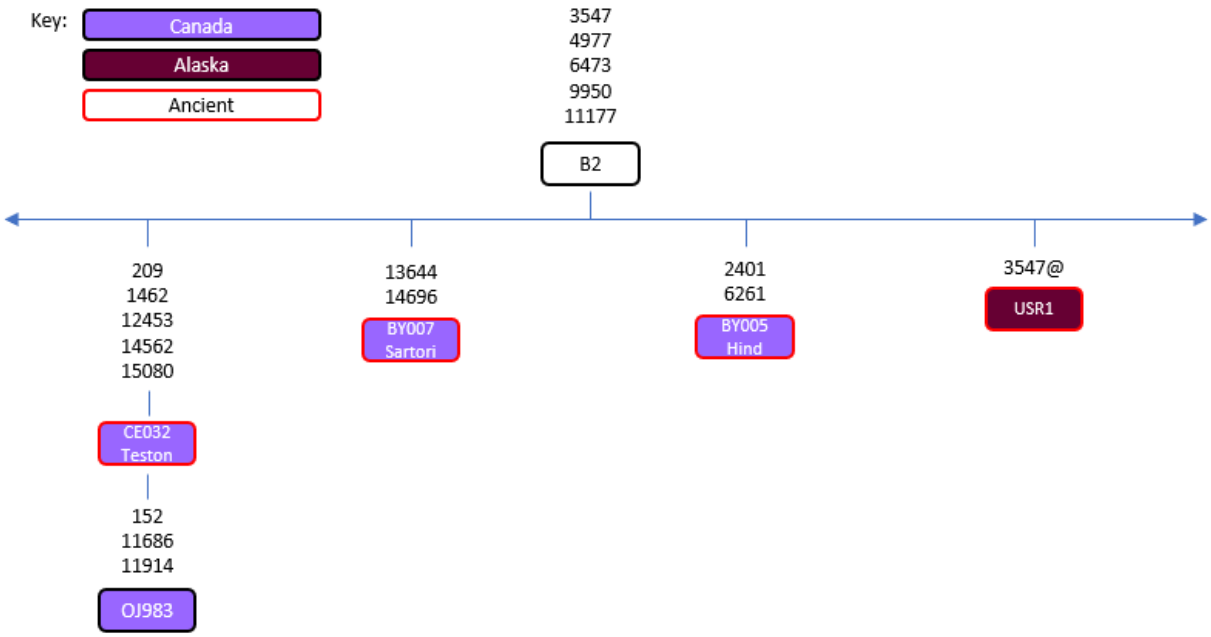

Figure 25. A schematic tree of B2 showing the position of our sequenced individuals and ancient individuals from North America.*

Our ancient samples, CE032, BY005, and BY007 have been classified as B2*, placing them at the top of the tree (figure 26). Although haplogroup B2 and its subbranches can be found be across the Americas continent (Brandini et al., 2018), it does not appear to be common in Canada. Within the entire B2 tree, 14 individuals are from Canada, with 12 out of the 14 being ancient

Canadian individuals. Demonstrating the rarity of this haplogroup in modern Canada or the commonness of haplogroup B2 in ancient Canada. It is interesting to see our three ancient individual places at the node of B2, with CE032 sharing mutations with modern Ojibwe individuals, showing continuity between ancient and modern Ojibwe individuals. BY007 and BY005 both contain private mutation which suggests that perhaps the diversity of B2 was much greater and was lost over time.

5.1.6. Dietary Isotope Analysis

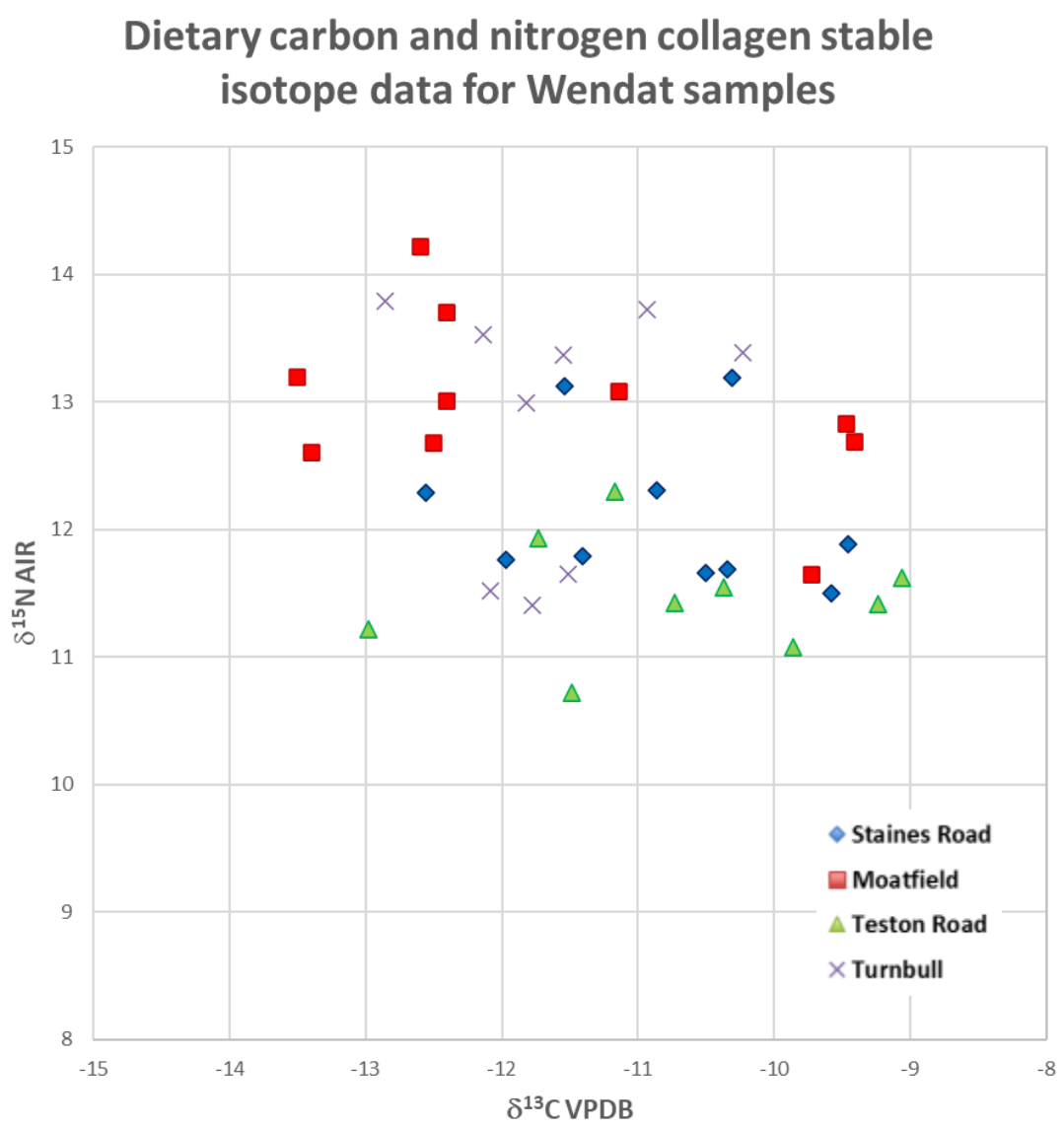

Figure 26. A graph showing the stable isotope results for the Huron-Wendat samples using the crown of adult molar, plotting $\delta^{13}C$ against $\delta^{15}N$

DNA code	Site	Era	$d^{13}C$ VPDB	$d^{15}N$ AIR	C:N
CE006	Staines Road	AD 1250	-11.97	11.76	3.3
CE007	Staines Road	AD 1250	-11.54	13.13	3.3
CE008	Staines Road	AD 1250	-10.86	12.31	3.2
CE009	Staines Road	AD 1250	-10.34	11.69	3.2
CE010	Staines Road	AD 1250	-10.50	11.66	3.3
CE011	Staines Road	AD 1250	-12.56	12.29	3.3
CE012	Staines Road	AD 1250	-9.46	11.89	3.3
CE013	Staines Road	AD 1250	-10.31	13.19	3.3
CE014	Staines Road	AD 1250	-9.58	11.50	3.3
CE015	Staines Road	AD 1250	-11.41	11.79	3.2
CE016	Moatfield	AD 1280-1330	-9.47	12.83	3.2
CE017	Moatfield	AD 1280-1330	-13.40	12.61	3.2
CE018	Moatfield	AD 1280-1330	-13.51	13.20	3.3
CE019	Moatfield	AD 1280-1330	-11.14	13.09	3.2
CE020	Moatfield	AD 1280-1330	-12.41	13.71	3.2
CE021	Moatfield	AD 1280-1330	-12.51	12.68	3.2
CE022	Moatfield	AD 1280-1330	-9.41	12.69	3.2
CE023	Moatfield	AD 1280-1330	-9.73	11.65	3.2
CE024	Moatfield	AD 1280-1330	-12.60	14.22	3.2
CE025	Moatfield	AD 1280-1330	-12.41	13.01	3.2
CE026	Teston Road	AD 1450	-12.98	11.22	3.2
CE027	Teston Road	AD 1450	-11.17	12.30	3.2
CE028	Teston Road	AD 1450	-11.49	10.72	3.2
CE029	Teston Road	AD 1450	-9.86	11.08	3.2
CE030	Teston Road	AD 1450	-10.73	11.43	3.2
CE031	Teston Road	AD 1450		no collagen	
CE032	Teston Road	AD 1450	-10.37	11.55	3.2
CE033	Teston Road	AD 1450	-11.73	11.93	3.2
CE034	Teston Road	AD 1450	-9.24	11.42	3.2
CE035	Teston Road	AD 1450	-9.06	11.62	3.3
CE036	Teston Road	AD 1450		no collagen	
CE037	Turnbull	AD 1400-1500	-12.14	13.53	3.3
CE038	Turnbull	AD 1400-1500	-12.08	11.52	3.2
CE039	Turnbull	AD 1400-1500	-11.78	11.41	3.3
CE040	Turnbull	AD 1400-1500	-11.55	13.37	3.3
CE041	Turnbull	AD 1400-1500	-10.93	13.73	3.3
CE042	Turnbull	AD 1400-1500	-11.51	11.65	3.3
CE043	Turnbull	AD 1400-1500	-11.82	12.99	3.3
CE044	Turnbull	AD 1400-1500	-12.86	13.79	3.7
CE045	Turnbull	AD 1400-1500	-10.23	13.39	3.3

Table 16. Dietary isotope results from the crown of adult molar of Huron-Wendat. Could not obtain results for CE031 and CE036 due to the lack of collagen for the two samples.

The analysis of carbon and nitrogen was performed by Dr. Peter Ditchfield at the research Laboratory for Archaeology located at the University of Oxford. 40 teeth samples from the four Huron-Wendat sites were sent for dietary isotope analysis, allowing comparison of carbon and nitrogen values to determine the type of food consumed by the indigenous population to detect whether there are variation amongst the Huron-Wendat population. Similar to other Northern Iroquoian, the Huron-Wendat were known to be farmers and seasonal hunters, hunting and fishing vary animal depending on the time of the year (Pfeiffer et al., 2014; Van Klinken, 1999). The role of the Huron-Wendat is also gender-specific, in which the women manage and maintain the growth of the crops, which varies from beans, maize, and squash, and the men predominately focus on hunting and fishing. Therefore, it is anticipated that the dietary isotope data would indicate this scenario.

From table 16, two individuals CE031 and CE036 did not provide enough collagen for further isotopic testing. Additionally, CE044 contains a C:N ratio of 3.7, the previous publication suggests that the ratio out of 2.9-3.6 are considered unreliable as natural levels of contamination could skew the ratio (Pfeiffer et al., 2014; Van Klinken, 1999). However, this was included in figure 26 but was not considered for further interpretation, totaling 37 individuals analyzed. All remaining individuals obtain a C:N ratio of 3.2 to 3.3, which is the composition of fresh collagen (Pfeiffer et al., 2014).

5.1.6.1. Nitrogen

The mean $\delta^{15}N$ value overall is 12.3178 and the mean value for each site: Staines Road (12.121), Moatfield (12.969), Teston Road (11.4744), and Turnbull (12.6988). Comparing to previous studies of $\delta^{15}N$ value of south Ontario people from the woodland period, the value obtained for all individuals fall appears similar to the value obtained in the previous publication (Pfeiffer et al., 2014; van der Merwe et al., 2003), indicating a similar type of food was consumed in this region prior to European contact. It was speculated that consumer has an increased $\delta^{15}N$ value of 3-4% than the food they consumed (Hedges & Reynard, 2007), therefore, the $\delta^{15}N$ value for all individuals are consistent with the consumption of fish when comparing the $\delta^{15}N$ value of land mammal and fishes of this region (van der Merwe et al., 2003; Warinner et al., 2013).

Despite all individuals are of the Huron-Wendat nation, the variation of $\delta^{15}N$ value indicates in table 16, demonstrates different trophic levels between each site and amongst different individuals from the same site. The variation between individuals of the same site could be due to preference of food for the individuals, as seen in some Turnbull individuals shows relatively low $\delta^{15}N$ value compared to the other individual of the same site, suggesting the presence of fish were available but not everyone consumed this. Additionally, it can also be interpreted with individual consuming fish of a lower $\delta^{15}N$ value as this varies between different species of fish, further research in this region could perhaps identify this possibility for favoritism of a particular species. The variation of $\delta^{15}N$ between different sites could be due to the available resources surrounding the area. Referring to figure 26, Moatfield and Turnbull site contains a

higher $\delta^{15}N$ value than Staines and Teston, implying a higher consumption of fish protein for the two sites. From the map as seen in the method figure 16, the three sites, Moatfield, Staines, and Teston sites are within close proximity with one another, therefore, it was assumed the three sites would share the same resources obtaining a similar $\delta^{15}N$ value. Overall, from the $\delta^{15}N$ value, indicate the availability of freshwater fish led to increase consumption and different site consumed different proportion leading to a non-uniform pattern, however, the consumption of other land-based mammals would have contributed to the $\delta^{15}N$ value, if the freshwater resources were the predominate diet, an even higher $\delta^{15}N$ value would have been expected.

5.1.6.2. Carbon

Cultivation and consumption of C_3 (beans and squash) and C_4 (maize) plants were documented during the early stages of European arrival, it was assumed that maize consumption was heavily relied upon during the offseason for hunting (Katzenberg et al., 1995; Watts et al., 2011). In order to understand this assumption, the $\delta^{13}C$ value from the four archaeological sites should fit within the $\delta^{13}C$ value range of C_4 plants (-16 to -10) (O'Leary, 1988). As this data produced uses dentin, it can detect the dietary of the individual during their youth as the formation of dentin occurs during the developmental stage of each tooth.

The mean $\delta^{13}C$ value for all 37 individuals is -11.1835 and the mean value for each site: Staines Road (-10.852), Moatfield (-11.659), Teston Road (-10.7367), and Turnbull (-11.505). This value fits in the range of C_4 plant consumption as well as showing similarity to the published data of other southern Ontario populations (Pfeiffer et al., 2014; van der Merwe et al., 2003). This is particularly interesting, as the $\delta^{13}C$ of freshwater fish also ranges from -16 to -21, and for a land mammal is around -24 due to the animal heavy diet of C_3 plants. Therefore, heavy consumption of fish or animal protein would provide a more negative $\delta^{13}C$ value. As our data produced is based on dentin, which measures the dietary of youth. Perhaps this shows a higher proportion of maize consumption at the early age of Huron-Wendat individuals. Although Moatfield and Turnbull site consist of a higher $\delta^{13}C$ value compared to Staines and Teston Road, this could be due to a heavier reliance on freshwater fish, as indicated by the $\delta^{15}N$ value too. Overall, all sites fit into the range of C_4 plant consumption and appear heavily reliant on maize products.

5.1.7. Kinship Analysis

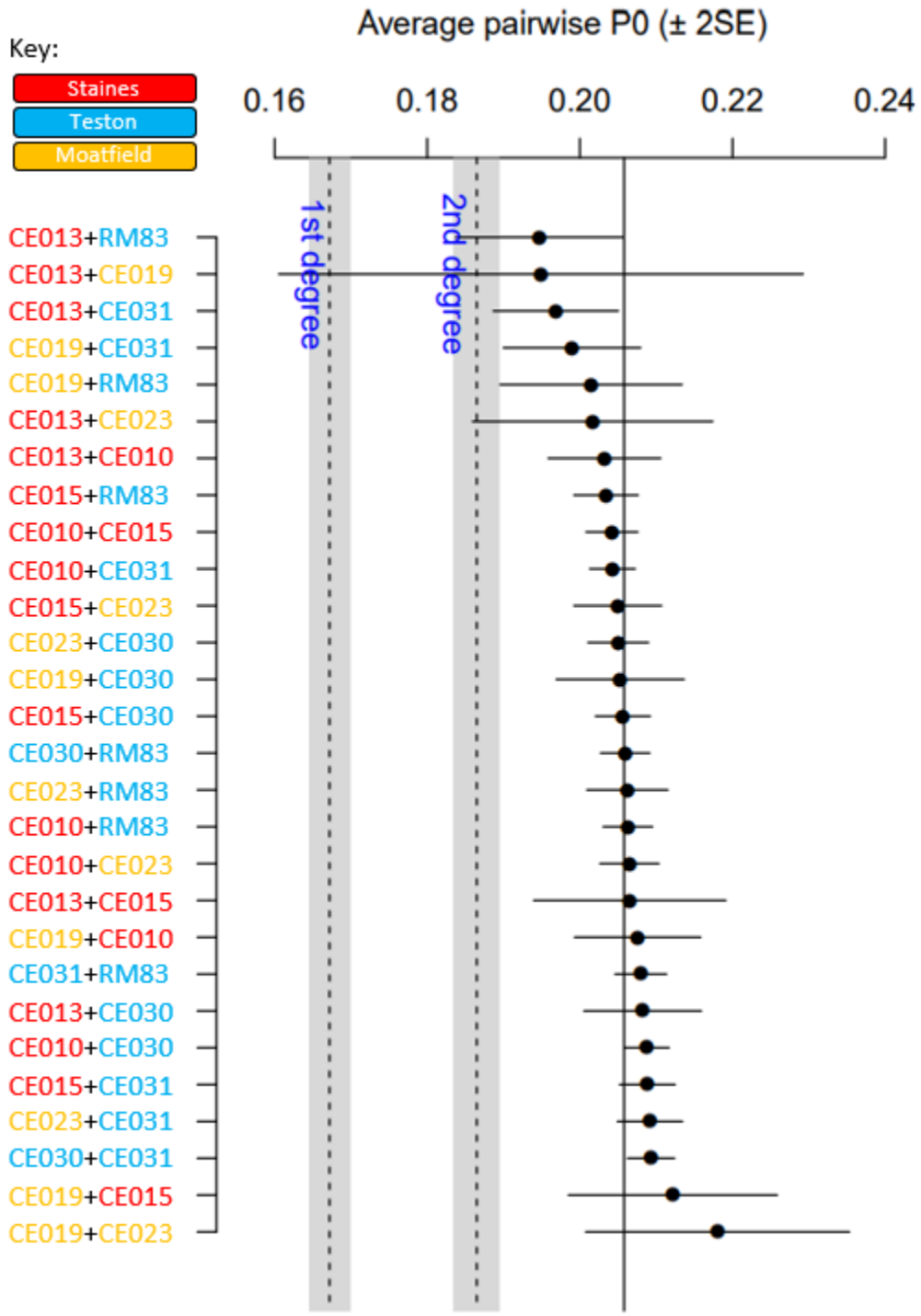

Figure 27. Results obtained from READ using shotgun data from our ancient Huron Wendat data and publish Huron Wendat data (RM83) from Scheib et al., 2018 to determine kinship between individuals of the same and other sites.

In order to determine familial relationships between samples (figure 28), READ was used for the purpose of detecting up to a second-degree relationship with low coverage samples (Kuhn et al., 2018). Although the uses of uniparental genome can be used to infer family relationships of maternal or paternal relationships, the actual degree of relationship is unknown. From the mitochondrial data, the different mitochondrial DNA exhibited in all samples indicate there is no first-degree maternal relationship, and as most of our individual are female, it is not possible to determine a first-degree paternal relationship. For the two individual BY005 and BY007, kinship analysis was not performed as both exhibit different mtDNA and Y-Chromosome. Additionally, kindship analysis was not performed comparing Maritime Archaic and Huron Wendat with READ due to the limitation of READ only providing information up to second degree relation. Kindship analysis for CE040 and RM85 from Scheib et al., 2018 was not performed due to poor overall coverage of the two sample. Comparison of samples between different sites was performed, although it was predicted that READ would not be able to distinguish the possible relationships between samples from different site as the date of each site differ and READ cannot provide further information above a second-degree relationship.

Sample CE013 and CE019 have the highest error bar, this is due to the low coverage of both samples at 0.004x and 0.0205x respectively. Therefore, unreliable to ascertain the relationship between these two samples. Additionally, samples between different and the same site do not appear to be related to each other, suggesting the burial of each site is not a family cemetery.

5.1.8. Principle Component Analysis

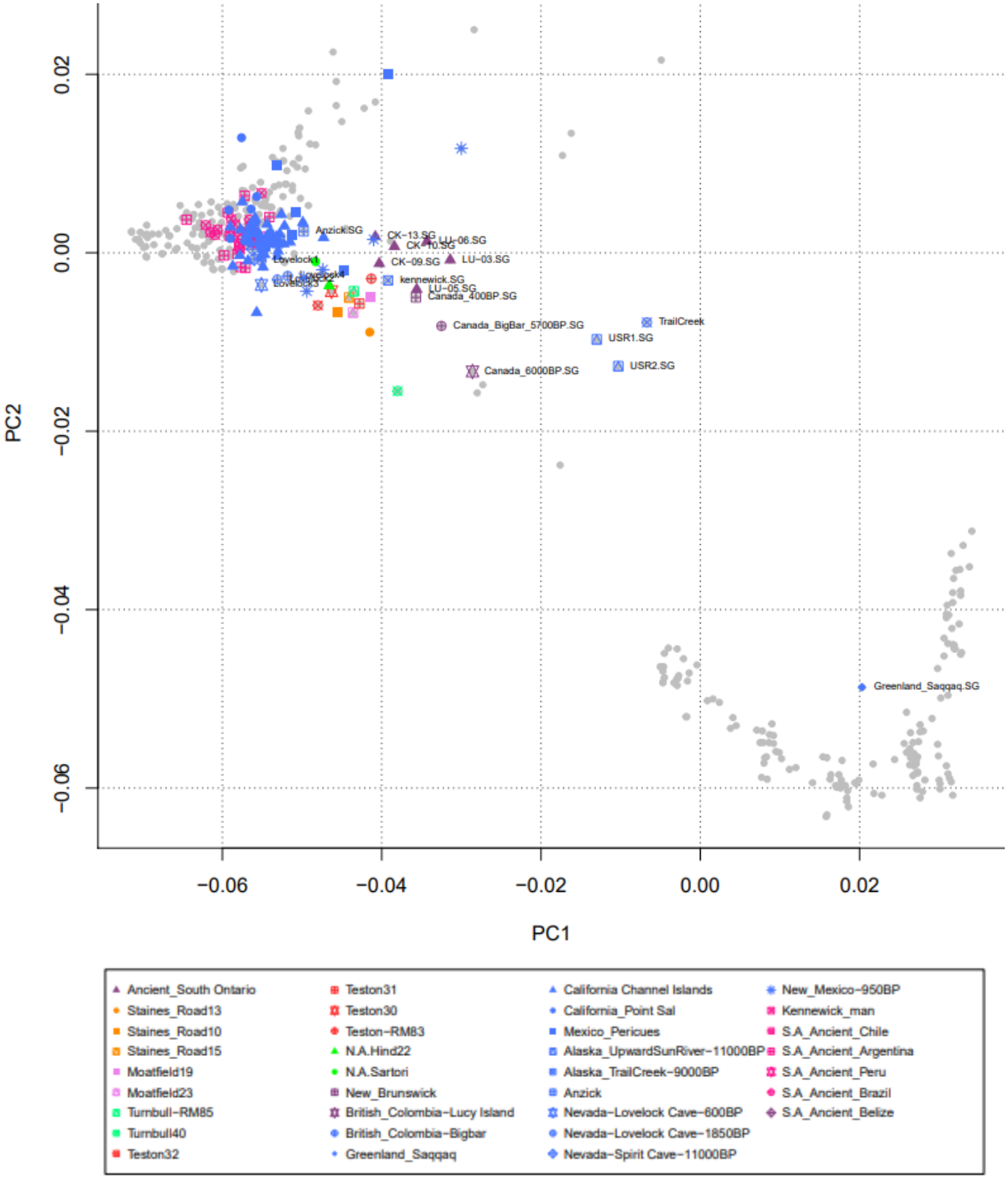

Figure 28. The PCA of the Americas using the subset of the Human Origins dataset (Lazaridis et al., 2014), the light grey indicates modern individuals with ancient individual in color projected on top. This was generated using R-Studio's package plot..

Merging of the sequenced individuals with a subset of the Human Origins dataset (appendix 39) containing modern Siberian and Native American as well as Ancient Native populations was first performed (Lazaridis et al., 2014) in order to perform PCA and Admxiture analysis to identify whether a pattern can be seen.

Viewing the PCA, all the sequenced individuals had enough known SNPs sharing with modern and ancient published populations to be plotted on the PCA (figure 29). Even with the low coverage of some samples such as CE013, CE032, and CE040, it was possible to place them into the PCA, clustering with modern and ancient Native populations. In the bottom right of the PCA are the modern Siberian and Eskimo populations with the top left population indicates the Native American population.

Majority of the ancient South American and North American cluster closely with each other and place on top of modern South American. Interestingly, some North American individuals including the ancient samples from the great lake region plotted slightly away from the main cluster. The individuals from Staines, Moatfield, Turnbull, Teston, Hind, and Sartori appear to cluster close to each other, only the sample CE040 (Turnbull 40) were plotted slightly away from this cluster, perhaps due to its low overall coverage at 0.004x. Curiously, the cluster containing our samples was plotted in between Kennewick and Anzick, two of the oldest ancient genomes from America, additionally, the Lucier individuals also from the South Ontario plotted further away from the great lake region cluster and closer to Kennewick.

5.1.9. Admixture

The ancestry composition of the ancient samples was analyzed using ADMIXTURE with published modern and ancient populations (figure 30 and 31). In order to accurately determine the components of our samples, the lower coverage individuals that are below 10,000 SNPs were not used during this analysis, this excludes the three individuals, CE010, CE032, and CE040.

All individuals sequenced comprise of two main components representing the Native Americas component in dark blue and an orange component which is found in Paleo and Neo Eskimo population. Additional components can be seen within higher K value of some individuals (Moatfield and Sartori), which appears to be associated with northeastern Siberian populations in grey and population from western Siberia in yellow. The admixture analysis was unable to separate North and South American components at higher K value. Comparing our sequenced individuals with each other, the Moatfield sample (CE019) appears different with the grey component appear to be missing, this is perhaps due to the low SNPs obtained. The samples appear relatively similar amongst each other, exhibiting the same component across all samples aside from Teston 31 (CE031), Moatfield 23 (CE023), and Santori (BY007), which exhibit additional components at higher K value.

Comparing our sequenced individuals to published samples from ancient south Ontario and Teston Road (Scheib et al., 2018), this exhibits the same component across all K-value in similar proportion with one exception. Looking at K=15, the published samples from Ancient South Ontario (Lucier Sites) contain a small fifth component in purple, which can be seen in other ancient individuals from San Nicolas located in southwestern America and southern American from Brazil and not in our samples from the same region. Additionally, a mint green component can be seen in our individuals from Moatfield and Teston (CE031), can be identified in Alaskan trail creek individuals, Ancient Beringian (USR1 and 2) and Saqqaq but not in the Ancient South Ontario.

Viewing the Anzick and Kennewick individual, a brown and white component can be seen in Kennewick but not in Anzick. This brown component can also be seen in Ancient South Ontario individuals but not in Glacial Kame (Hind 22 and Santori) and other samples aside from Teston31, which exhibit a very small proportion. This brown component can also be seen in western Canada individuals (Canada BigBar and Canada 6000 BP) and one of the individuals from Ancient Beringia (USR1).

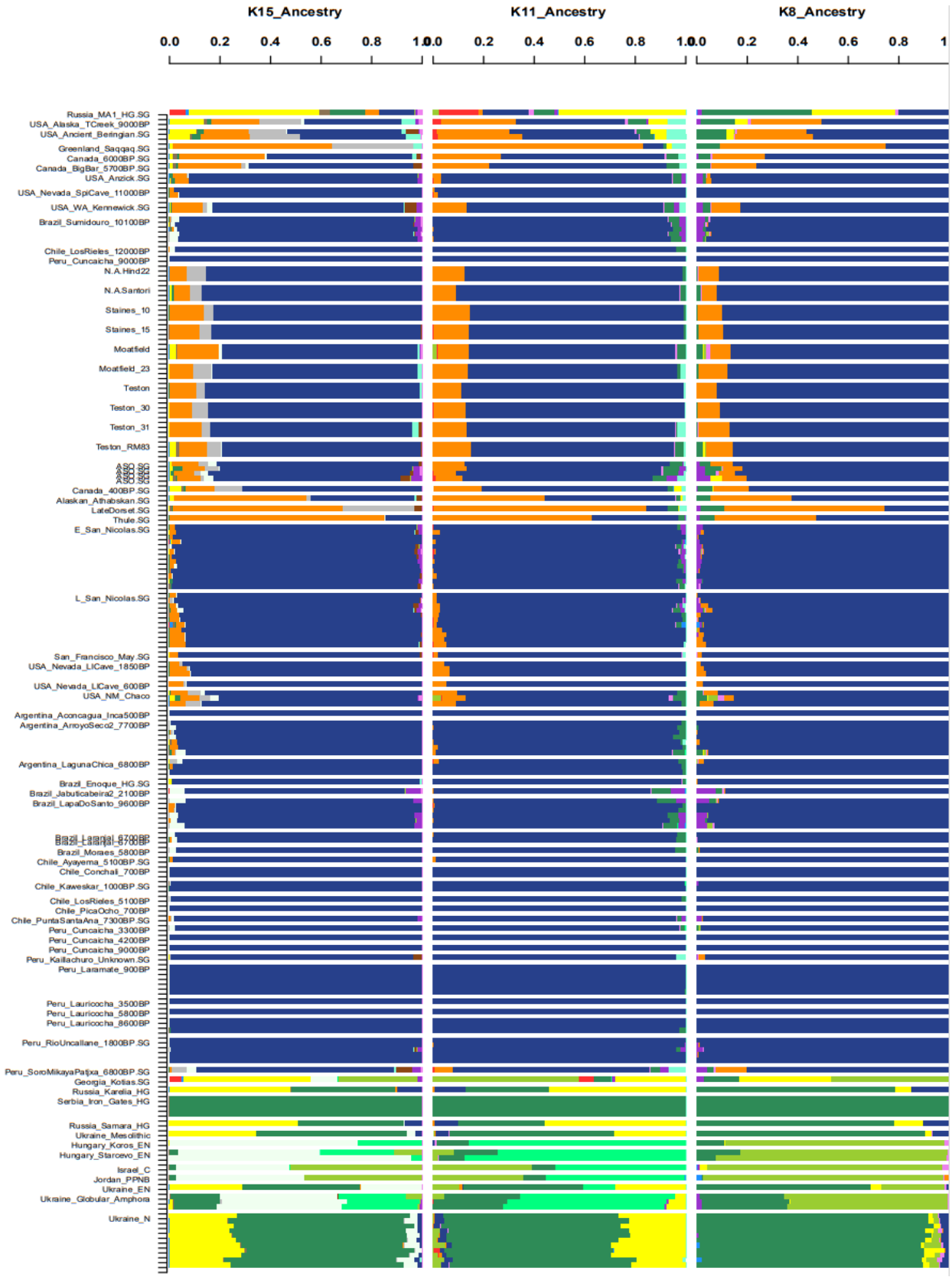

Figure 29. Admixture results (K=8, K=11, K=15) of our samples and published ancient and modern individuals, showing only the ancient individuals from the Americas and Siberia.

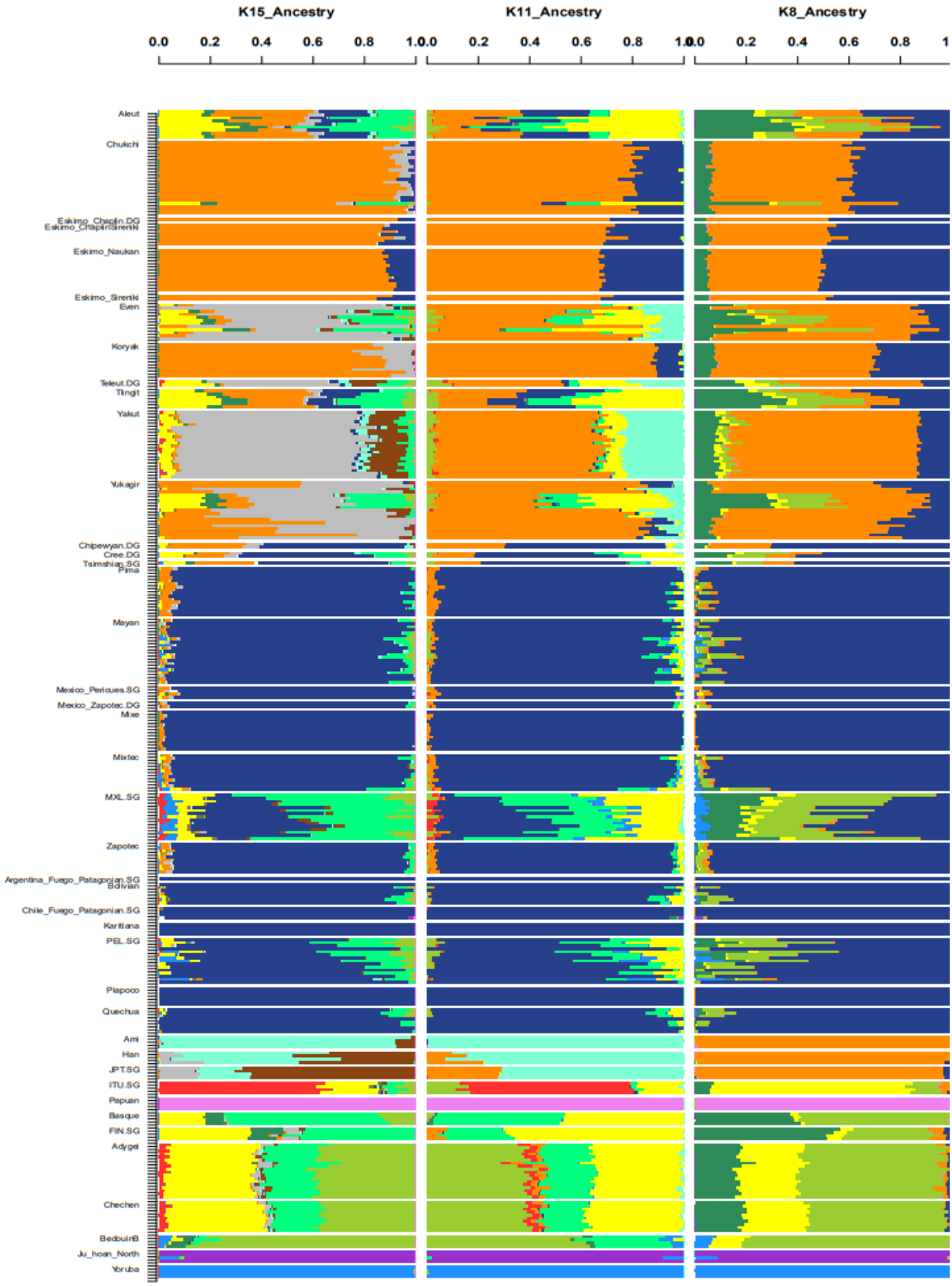

Figure 30. Admixture run of our samples (K=8, K=11, K=15) with published ancient and modern individuals, showing only the modern individuals from the Americas, Siberia, and European populations.

5.1.10. f-statistics

5.1.10.1. Outgroup-f3

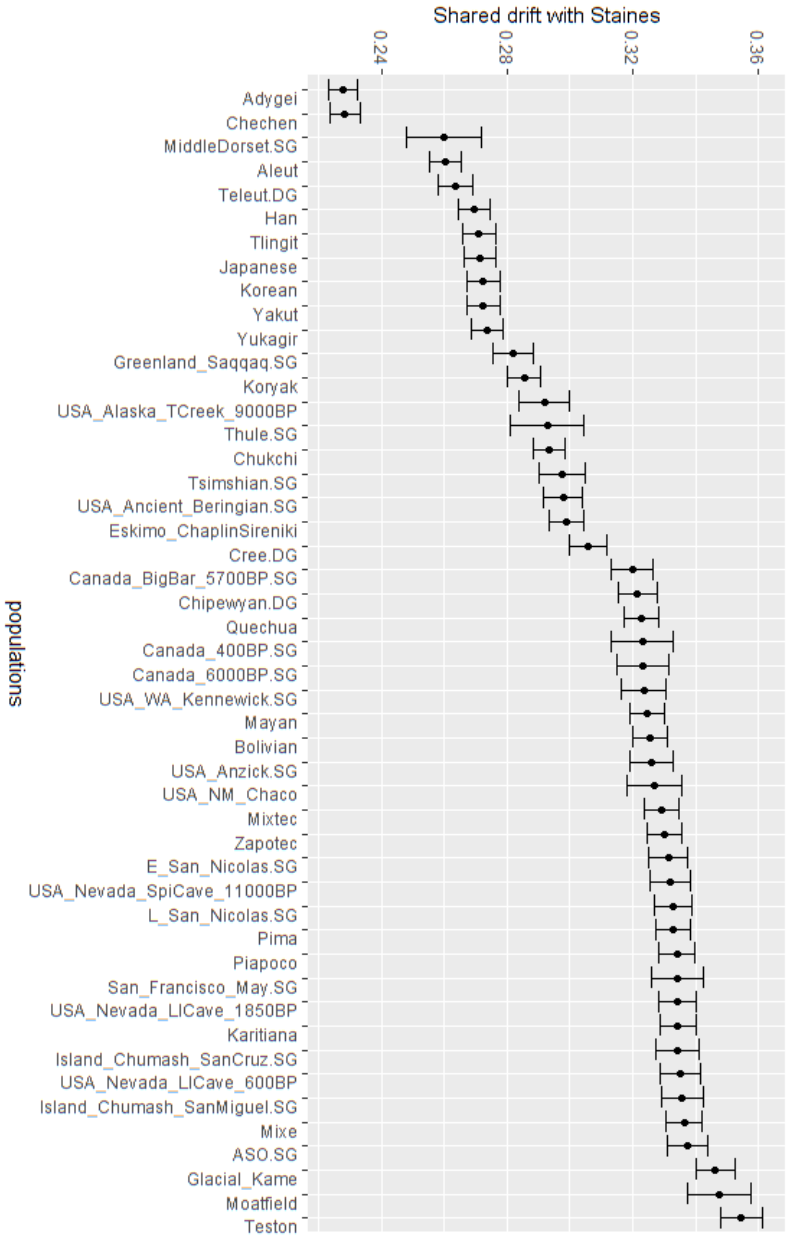

Figure 31. Outgroup f3 statistic for Staines Road in the format of f3(Mbuti, population, Staines)

Outgroup f3 was applied to the sequenced samples using Mbuti as an outgroup for the assessment to determine which indigenous population shares the greatest drift with the Huron-Wendat and Glacial Kam (figure 32-35) in the format of f3(outgroup, target population, test population). Staines road was tested and Teston, Moatfield, and Glacial Kame individual shares the highest drift (figure 32). Out of the published ancient populations of North America, Ancient

South Ontario (ASO) shares the highest drift with Staines and other Ancient Great Lake sites. Anzick and Kennewick share a similar proportion with Staines, this can also be said with ancient populations from the West Coast. It is interesting to see the Ancient Beringian (USR1+2), Alaska Trail Creek, and other Eskimo populations sharing significantly less with Staines, compared to the ancient populations that are closer to the Great Lake region. The sharing of drift with modern Chipewyan being higher than modern Cree is intriguing, perhaps this is due to admixture with Cree individuals and other populations. Surprisingly, Staines appear to show a high drift with modern Southern American population, such as the Karitiana then with Mayan and Zapotec.

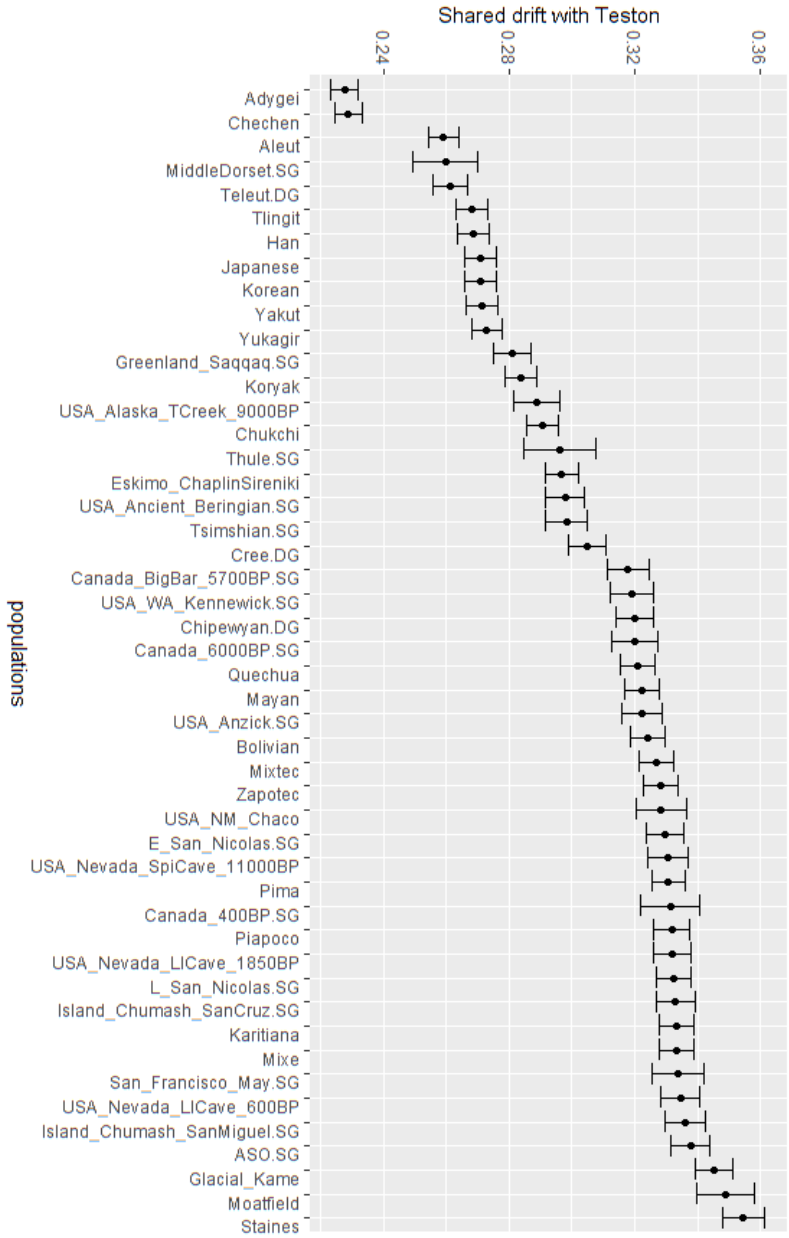

Figure 32. Outgroup f3 statistic for Teston Road in the format of f3(Mbuti, population, Teston)

The outgroup f3 was performed with Teston instead of Staines whilst keeping Mbuti as the outgroup (figure 33). Results obtained strikingly similar to the outgroup f3 results for Staines, with Staines, Moatfield, and Glacial Kame sharing the greatest drift with Teston. Populations around the Great lake regions appear to share the greatest drift, with Ancient South Ontario (ASO), sharing the highest drift with Teston amongst published ancient populations. Similar to the results from Staines, Eskimo population appears to share less of with Teston then the population that is closer to the Great Lake, with modern southern American sharing more than Chipewyan and Cree.

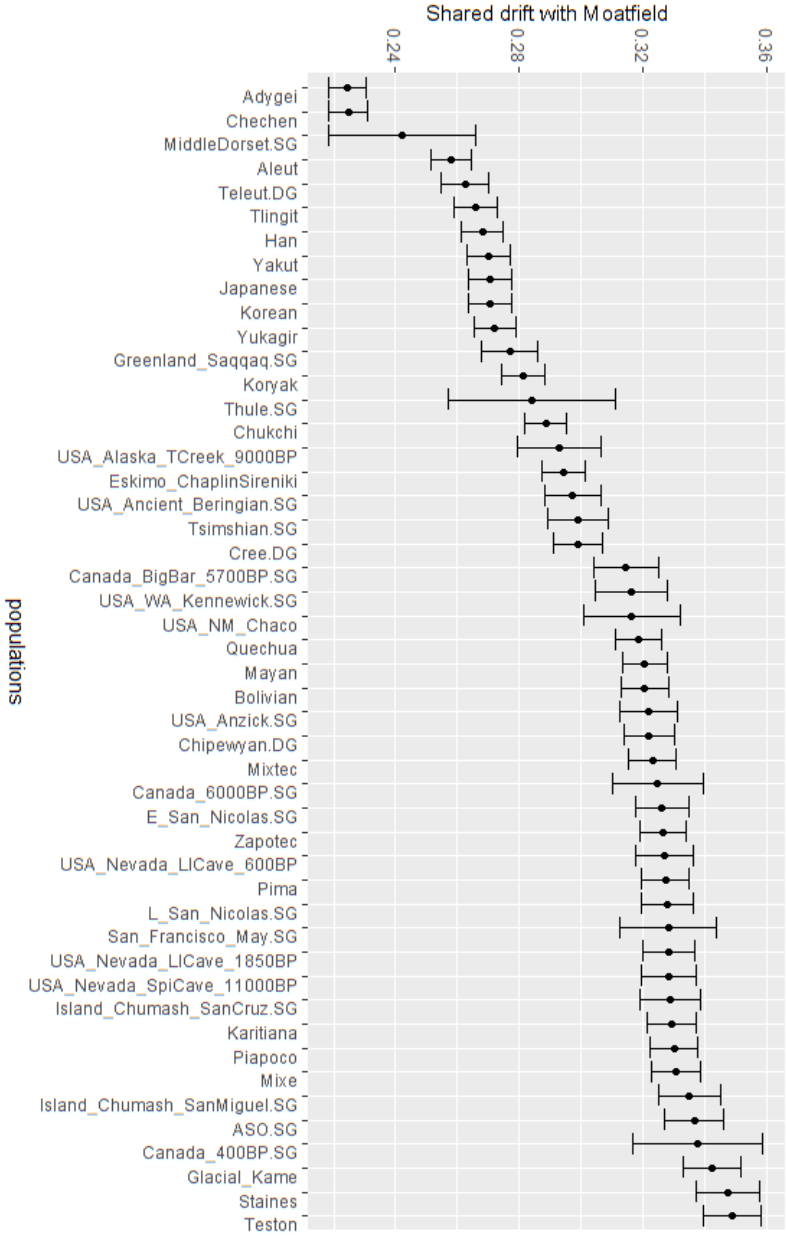

Figure 33. Outgroup f3 statistic for Moatfield site in the format of f3(Mbuti, population, Moatfield)

Despite the lower coverage of the Moatfield site, outgroup f3 can still be performed showing the greatest shared drift with Glacial Kame, Staines, and Teston site (figure 34). Close drift with Ancient South Ontario (ASO) can still be identified. The low coverage of Moatfield led to a larger error bar across the outgroup f3 compared to the other sites, but the pattern of share drift remains comparatively similar to the outgroup f3 results of Staines and Teston sites.

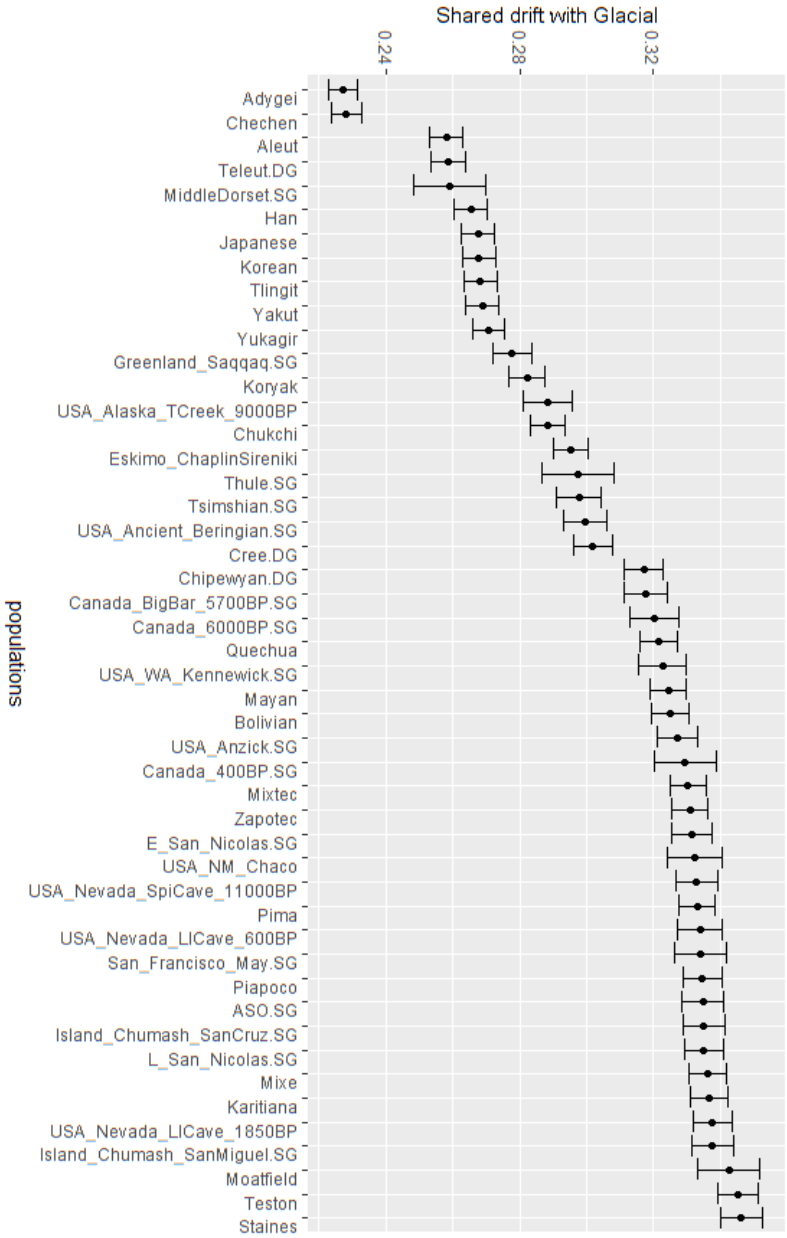

Figure 34. Outgroup f3 statistic for Glacial Kame in the format of f3(Mbuti, population, Glacial Kame).

When testing the shared drift with Glacial Kame, once again the Huron-Wendat individuals share the highest drift with Glacial Kame (figure 35). However, the Ancient South Ontario (ASO) still share a high degree of drift with Glacial Kame, but another ancient population such as the Nevada Lovelock cave and ancient Californian appears to share higher drift with Glacial Kame compared to Ancient South Ontario.

5.1.10.2. Admix-f3

Admix f3 was applied to determine whether the Huron-Wendat and Glacial Kame population was the causation of admixture between other ancient populations (figure 36). This test was also extended to test whether the Huron-Wendat and Glacial Kame population was admixed with other ancient populations to form the modern population around the great lake region (appendix 3-25). It is to be noted that if the statistic is negative, it provides proof that population C is admixed between population A and B. However, if the admixed f3 is positive, it does not prove that there is no admixture. Another point to consider when applying this test, is the occurrence of the admixture event, as it does not suggest when the admixture could have happened.

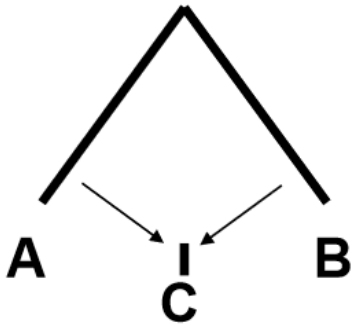

Figure 35. Schematic for admix f3, testing whether admixture occurred for population C between population A and B

When the Huron-Wendat and Glacial Kame population was used as population C and other ancient populations as populations A and B (appendix table 3-7), all results show positive f3. The test was repeated to the population around the Great Lake region, testing Ancient South Ontario as population A and Glacial Kame and Huron-Wendat as population B to determine admixture in the modern population at the Great Lake (appendix table 8-11). The admix f3 results also appear positive. Positive f3 results were also obtained when Huron-Wendat as population C with Ancient South Ontario and Glacial Kame as A and B.

The only results which appear negative were the combination of Huron-Wendat and Glacial Kame as A and Aleut, Late Dorset and Middle Dorset individual as B, to test modern Cree population as C (appendix 16-20). This suggests a potential relationship between Huron-Wendat, Glacial Kame, Aleut, Dorset and modern Cree population. However, the modern Cree population could be admix with other modern individuals with Eskimo ancestry. Therefore, this may not truly demonstrate the sharing of genetics between the ancient population into modern Cree population, as admix f3 does not show when the occurrence of admixture could have happened.

5.1.10.3. D-Statistic

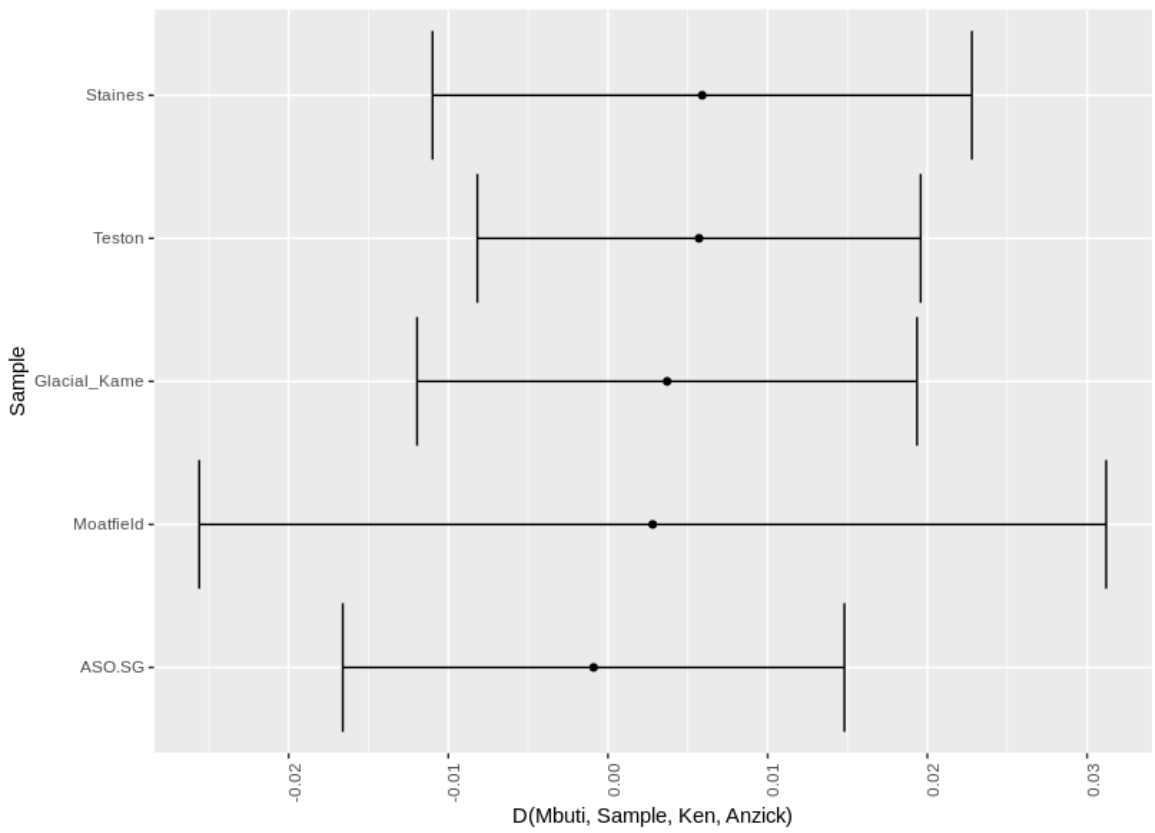

Figure 36. D-statistic to test the affinity of ancient Great Lake region population with Kennewick and Anzick. Value above 0 suggest closer affinity with Anzick than Kennewick, although the D-statistic calculation does not indicate this to be significant due to the |Z| value being less than 3.

D-Statistic was applied to determine the affinity of Huron-Wendat and Glacial Kame population to Anzick and Kennewick in the format of D(outgroup, testing population, Kennewick, Anzick) (figure 37). Although the results indicate that the Huron-Wendat and the Glacial Kame obtain geneflow from Anzick when compared to Kennewick, the |Z| being less than 3 does not indicate this to be significant (Appendix 26).

Further D-statistic was applied to test Huron-Wendat and Glacial Kame populations to Ancient South Ontario and other populations in North Americas, showing great affinity to Ancient South Ontario. However, when compared with the population generated with this study, it shows greater affinity with each other compared to Ancient South Ontario (Appendix 27-36). The Moatfield site had the lowest number of SNPs due to the low coverage of the individual, this causes a large error bar for all the D-Statistic calculated.

5.2. Modern mitochondrial data

Sample	Haplogroup
Cree01	C4c1
Cree02	X2a2+13191
Cree03	C1c+150
Cree04	A2+64
Cree05	A2f1a
Cree06	A2b1
Cree07	A2a5
Cree08	X2a1b
Cree09	A2a5
Cree10	C4c1
Cree11	A2i
Cree12	C1b+10031
Cree13	C1b+10031
Cree15	C1d1a1
Cree16	C1c+150
Cree18	D1+7747
Cree21	C1d1a1
Cree22	X2a2
Cree24	A2
Cree26	A2i
Cree27	A2i
Cree28	B2a4
Cree29	C1b+10031
Cree30	D1+7747
Cree31	X2a1b
Cree32	C4c1+207
Cree33	X2a2+13191
Cree34	A2i
Cree35	A2f1a
Cree36	A2n
Cree37	C1b+10031
Cree38	A2q
Cree39	A2i
Cree40	A2+64
Cree41	A2+64
NAD02	A2+64+6527
NAD04	A2+64+6527
NAD06	A2+64+6527
NAD07	A2+64+6527
NAD08	A2+64+6527

NAD09	A2+64+6527
NAD11	A2+64+6527
NAD14	A2+64+6527
NAD15	A2+64+6527
NAD16	A2+64+6527
NAD17	A2+64+6527
NAD19	A2+64+6527
NAD20	A2+64+6527
NAD21	A2+64+6527
NAD22	A2+64+6527
NAD24	A2+64+6527
NAD25	A2+64+6527
NAD26	A2+64+6527
NAD27	A2+64+6527
NAD28	A2a5
NAD29	A2+64
NAD31	A2+64+6527
NAD32	A2+64+6527
NAD33	A2+64+6527
NAD37	D1+15930
NAD43	A2+64+6527
NAD46	A2+64+6527
NAD54	A2+64+6527
NAD55	A2+64+6527
NAD56	A2a5
NAD59	A2+64+6527
NAD64	A2+64+6527
NAD87	A2+64+6527
NAD93	A2+64+6527
NAD95	A2+64+6527
NAD96	A2+64+6527
NAD101	A2+64+6527
Oj962	X2a1+8152
Oj967	X2a1
Oj968	A2i
Oj969	A2i
Oj970	C1b+10031
Oj971	A2i
OJ972	A2f1
Oj973	C4c1
Oj974	C1b+10031
Oj976	X2a1

Oj978	C4c1
Oj979	C4c1
Oj980	C1b+10031
Oj981	A2i
Oj982	A2i
Oj983	X2a1b
Oj984	C4c1
Oj985	B2+152
Oj1116	C1b+10031
Oj1117	C4c1
Oj1136	A2f1a
Oj1245	C1c+8700
Oj1250	C4c1+16362
Oj1262	A2i
Oj1288	A2f1

Table 17. The mitochondrial haplogroup of the modern individual send by our collaborator, Prof Damian Labuda from the University of Montreal. Sample labelled Cree is of the Cree population, NAD is from Na Dene population and Oj is from Ojibwe population.

From the 110 modern individual samples send over by our collaborator Prof Damian Labuda (University of Montreal), successful retrieval of mtDNA occurred to 97 of the individuals, all exhibiting mitochondrial haplogroup belonging to Native American populations (table 17 and appendix 2). Unfortunately, it was not possible to obtain DNA from 13 individuals due to the low quality of the samples. Both ancient and modern mitochondrial data obtained were added to the database, increasing the database from 1898 sequences to 2007. This database was used for the Maximum likelihood calculation and founder analysis in order to estimate the age of each haplogroup and identify and date migration into a new territory using mitochondrial data.

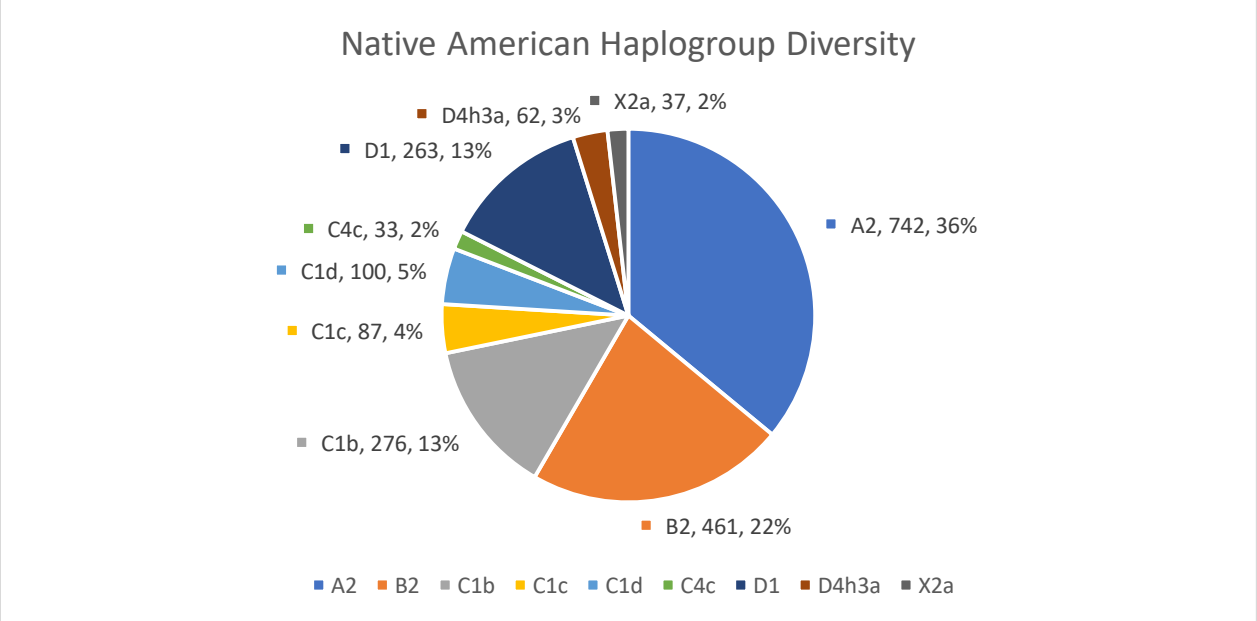

Figure 37. Pie chart showing the diversity of native American haplogroup collected within the database.

The database was created to provide a way to identify haplogroup diversity in the Americas. As seen from figure 38, 36% of the sequences belong to Haplogroup A2, 22% belong to haplogroup B2, haplogroup C1b, C1c, and C1d combine for 22%, Haplogroup D1 with 13% and the three rarer haplogroup D4h3a at 3%, C4c at 2% and X2a at 2%. Figure 38 shows the frequency of different mitochondrial haplogroup within Native American populations. When looking only at samples from Northern Americas (figure 39), haplogroup A2 still the majority haplogroup at 50%, haplogroup B2 at 13%, C1b, C1c and C1d total as 16%, D1 at 8%, and the rarer haplogroup C4c at 5%, D4h3a at 2% and X2a at 6%. Comparing figure 38 and 39, it would appear haplogroup diversity between North and South America differs, however, the database for Southern Native American mitochondrial is significantly larger (~1300 sequences) compared to Northern Native American (~700 sequences).

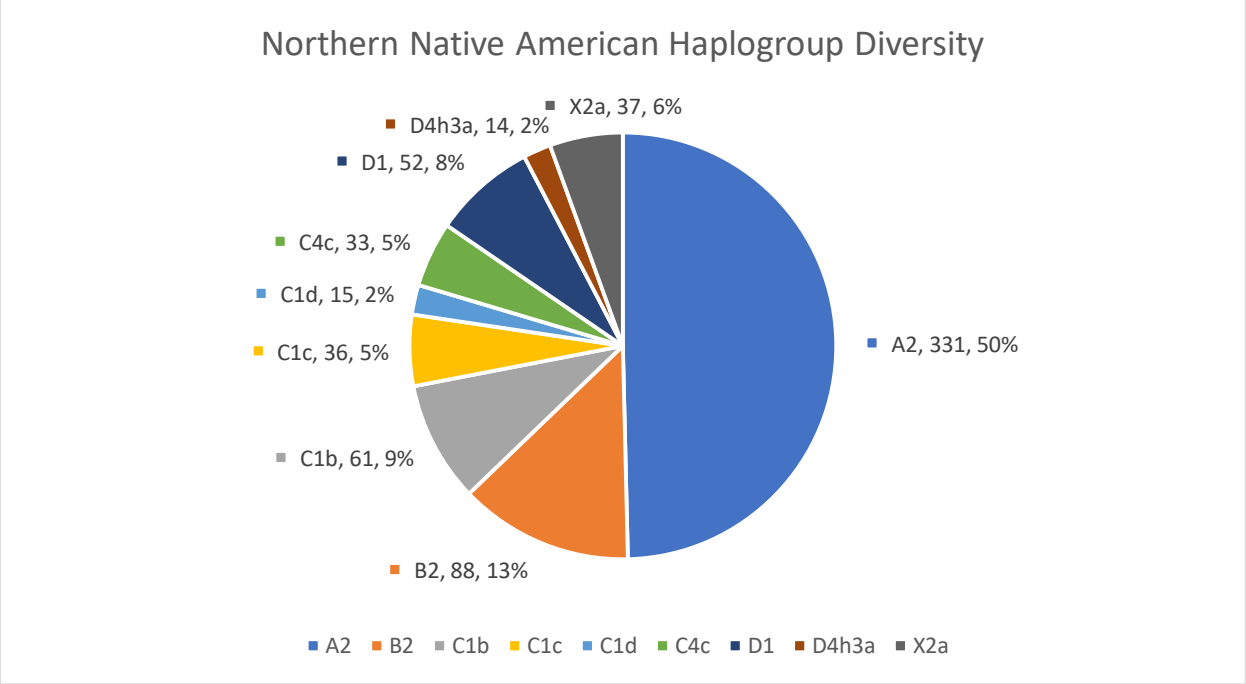

Figure 38. Pie chart of Native American mitochondrial haplogroup diversity from North America.

Viewing specifically at the mitochondrial DNA sequences recovered from our 97 modern individuals (figure 40), a large proportion of our individual exhibits haplogroup A2 at 63%, with Chipewyan population (NAD) exhibiting almost entirely haplogroup A2. Haplogroup C is the second largest haplogroup combining for a total of 23% which includes haplogroup C1b, C1c, C1d, and C4c. Haplogroup B2 and D1 appear to be less common within the Ojibwe, Cree, and Chipewyan populations, accumulating only 2% and 3 % respectively. X2a were found only in Cree and Ojibwe populations, totaling to 9% of the samples found in our 97 sequenced samples. Haplogroup D4h3a was not found amongst our samples. Identifying the mitochondrial haplogroup for these three populations shows Chipewyan samples from this study appears to be from the same family or individuals from the same maternal lineage, as majority of the sample exhibit the same haplogroup A2+64+6527. Ojibwe and Cree population shares some

maternal lineages with each other, these haplogroups are A2i, A2f1, C1b+10031, C4c1, and X2a1.

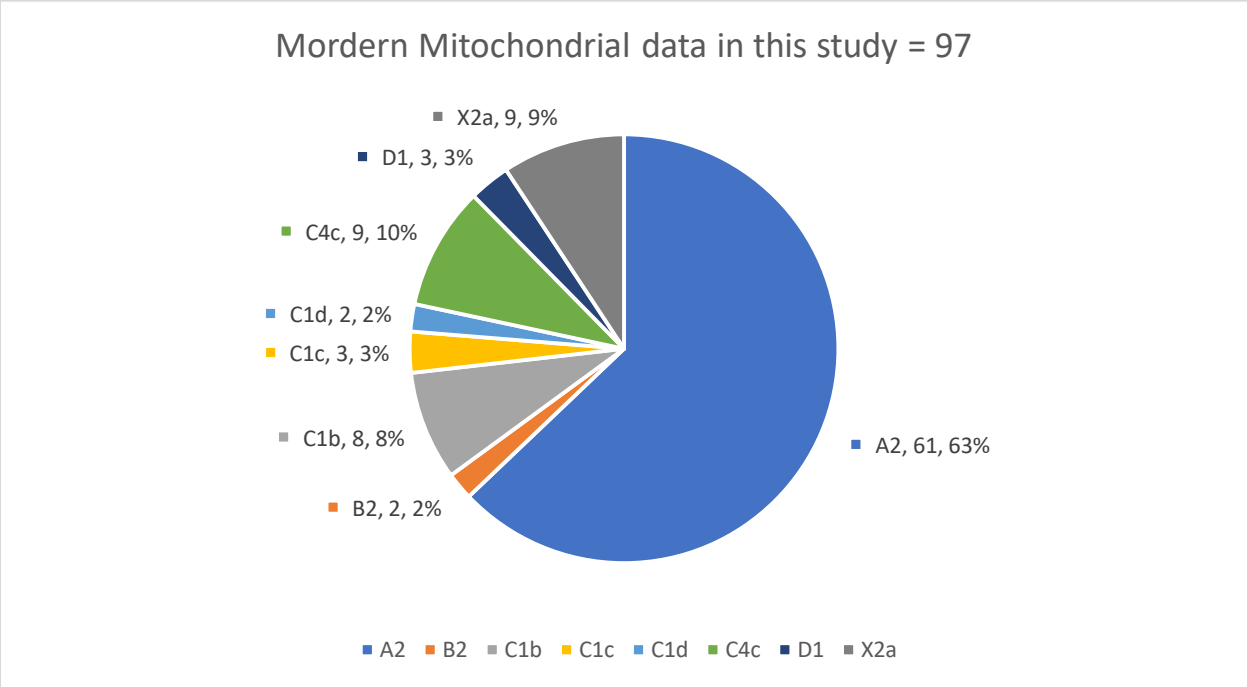

Figure 39. Pie chart of the haplogroup diversity sequenced samples generated in this study.

5.2.1. Haplogroup A2

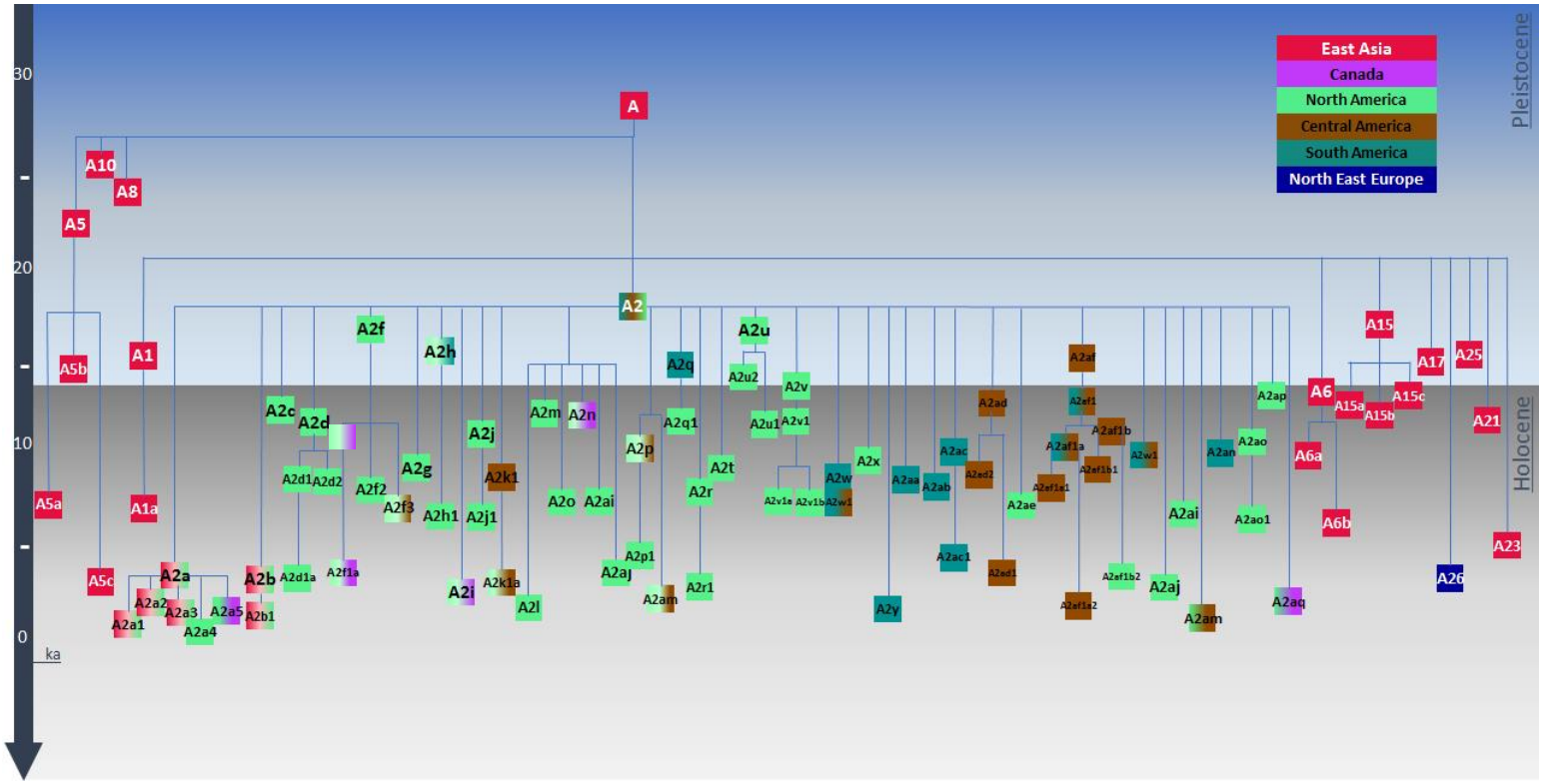

Figure 40. Schematic tree of the haplogroup A with estimated maximum likelihood age calculated using 1079 modern sequences of which 742 sequences belong to haplogroup A2. The colour represent the geographic location of which the haplogroup can be found.

Calculation for the age estimation using maximum likelihood and *ρ*-statistic for haplogroup A2 was performed by calculating the entire haplogroup A using a total of 1079 modern sequences, of which 742 were haplogroup A2 (appendix 37).

Haplogroup A arose within haplogroup N ~30 kya, most likely in Asia (figure 41). Within haplogroup A, subclades such as A5, A10, and A8, possibly appear during the LGM in East Asia. Interestingly, the diversity within Haplogroup A appears to increase in East Asia post ~20 kya, at the end of Pleistocene and seems restricted in East Asia.

The age calculated for haplogroup A2 is ~18-20 kya, similar to the calculated value estimated in previous studies (Achilli et al., 2008). This haplogroup consists of mostly Native American samples with many publications identify this haplogroup as one of the major Native American founding haplogroup (Merriwether et al., 1995; A Torroni et al., 1993). This remains to be true for the majority of subclades within haplogroup A2.

Within A2, separations of North America and South America subclades can be identified. A2f and A2u, the oldest haplogroups calculated, date to ~17 kya and contains samples largely from North America. Subclade A2f1, dated to ~11 kya within this, A2f1a, dated to ~3 kya, contains 50% of its samples from Canada, A2f2 dates to ~8 kya with individual originate from the U.S.A

and A2f3 dates ~ 7 kya, majority of this individual from this haplogroup originated within the USA except for one individual from Central America. A2f appears to be excluded from North Americas and shows a separation of A2f into different regions within North America. This is different from A2u, with subclade A2u1 and A2u2 dates to ~11 kya and ~15 kya, only containing samples from the South-west coast of America, indicating a restriction within this region.

A2af, appears to be the oldest Central/South American haplogroup, estimated to be ~15-16 kya. This haplogroup contains 26 individuals of which 19 are from Central America, representing 24% of the central American samples in A2. The subclade A2af1a date to ~11 kya, consists of a basal South American individual, and nested deeper within A2af1a. lies A2af1a1 and A2af1a2 date to ~9 kya and ~1.5 kya, consist of individual strictly from Central America. A2af1b, another subclade within A2af1 dates to ~12 kya, in which A2af1b2 contains two individuals from North America. This most likely represents modern migrants from Central or South America into North America, more information is needed to clarify this.

Haplogroup A2a and A2b are the two biggest haplogroups represented within A2 and date to ~4.5 kya and ~3.5 kya respectively. Both haplogroups contain samples from North Eastern Siberian populations, demonstrating admixture between North American and East Siberian populations most likely associating with Paleo and Neo Eskimo movement (Flegontov et al., 2019). Only the subclade A2a4 and A2a5 contains exclusively Native American individuals, in which our Cree and Chipewyan individual lies, both dates to ~350 and ~1900 year ago.

Two haplogroups appear restricted to the great lake and Northeastern Americas. These are A2i and A2f1a, in which the majority of our samples from Cree and Ojibwe fall. A2i dated to ~1.9 kya and A2f1 around ~2.8 kya.

5.2.2. Haplogroup C

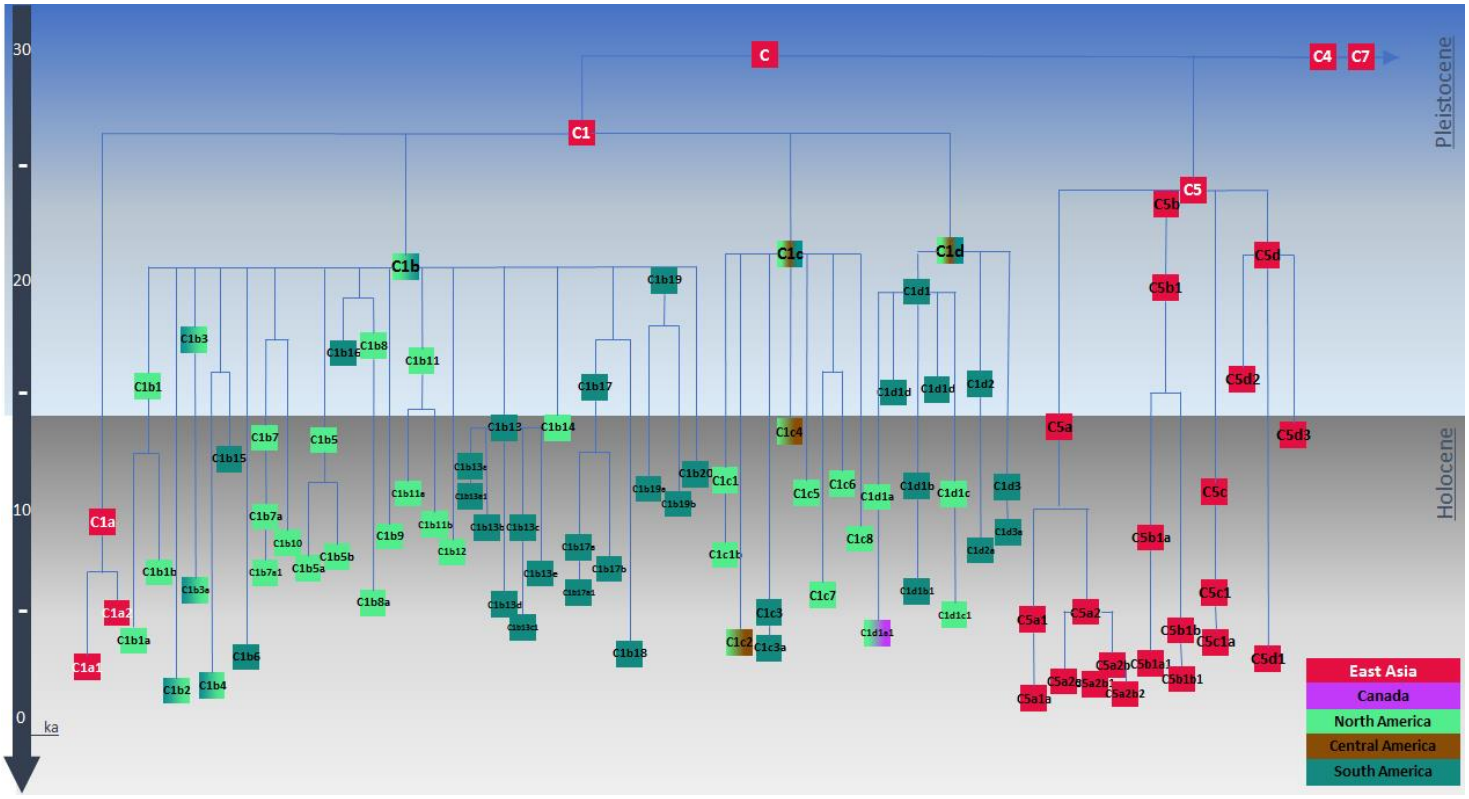

Figure 41. Schematic tree of haplogroup C, featuring C1 and C5, with maximum likelihood age calculated using 1104 modern sequences for the entirety of haplogroup C.Colouration of each haplogroup illustrate the geographic location in which it can be found.

Calculation for the maximum likelihood and *ρ*-statistic for haplogroup C1 and C4 was performed. In order to accurately calculate the age estimation for C1 and C4, the entire Haplogroup M8, the macro haplogroup of C was calculated, a total of 1311 sequences was used for this calculation in which 1102 of this belong to haplogroup C (appendix 38).

Haplogroup C1 and C4 date to ~26 kya and ~24 kya respectively, are the largest haplogroup within this tree combining for a total of 879 individuals, consist of samples from the Americas continent and East Asia. C5 and C7, dates to ~24 kya and ~23 kya respectively, containing a total of 225 samples in which all the samples within C5 and C7 are of Asia and East Asia origin.

C1b, C1c, and C1d are known Native American haplogroup, all dates to ~21 kya. C1b is the biggest haplogroup within C1 (figure 42), contain sequences from both North and South America with only one individual from Central America locating in C1b4. Within C1b, there are limited samples from Canada or North-East America. Containing one ancient Beothuk individual sharing mutations with a published modern sample from Los Angeles and C1b+10031, dates to

~3.5 kya, consists of individuals from Canada, which includes the modern samples generate in this study and ancient Maritime Archaic individual.

C1d consist of mostly South American samples with C1d1a and C1d1c contain samples exclusively from North America, both estimated to ~11 kya. Within C1d1a, it contains individuals from around the great lake region as well as individuals from Mexico and one of the Cree samples from this study. C1d1c consist of individuals from Mexico, Texas, and Michigan. The small proportion of North American individuals found in C1d perhaps suggest post-European migration from South America to the north.

C1c contains the majority of the samples from America and Mexico with C1c3 the exception consisting of samples from South America. Some C1c individuals can be found in Canada, clustering to the sample produced in this study with ancient Beothuk individuals at C1c+150. Ancient Maritime individuals can also be found in C1c. This suggests the presence of C1c in Canada prior to European arrival and has a greater dispersal.

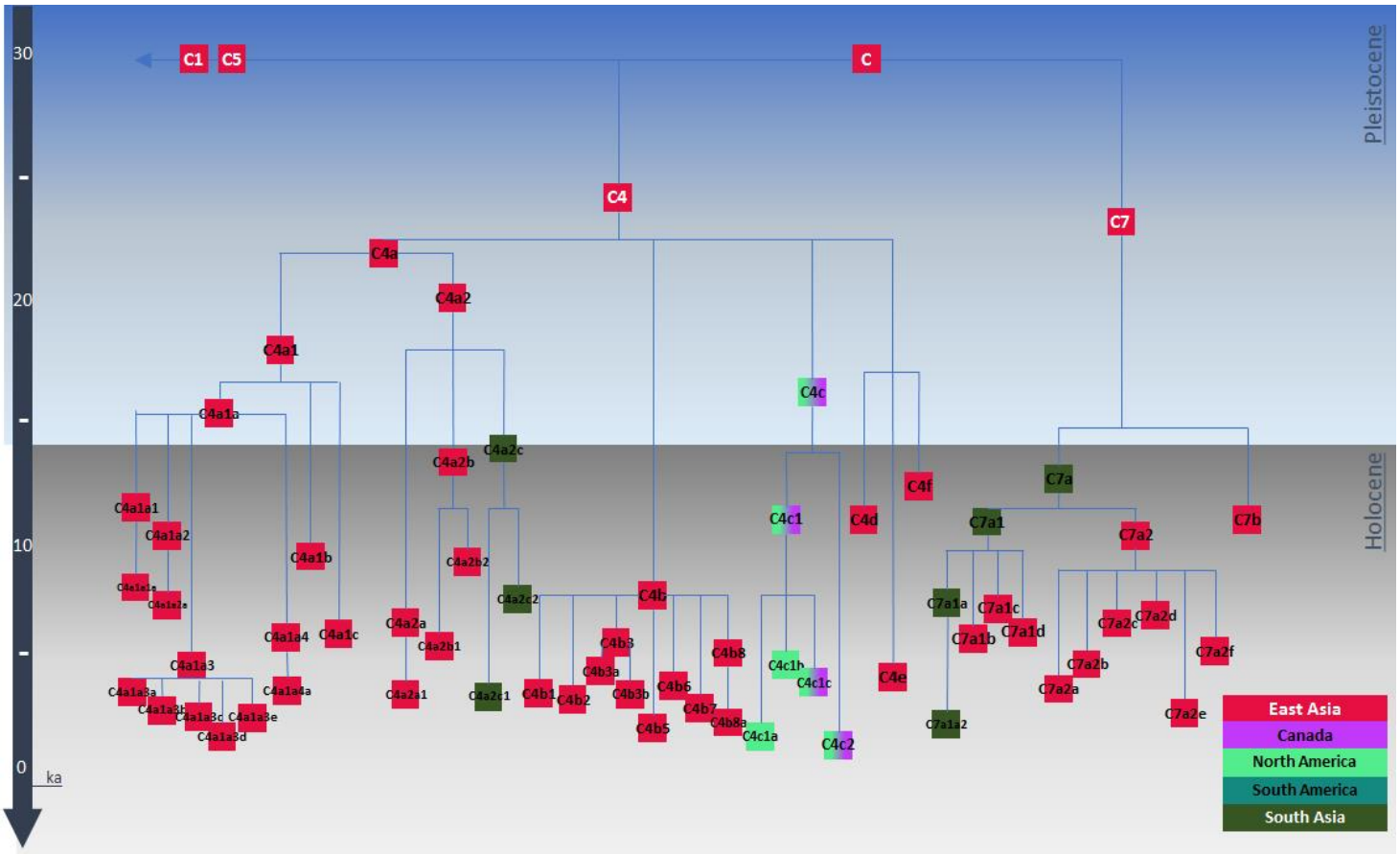

Figure 42. Schematic tree of haplogroup C, featuring C4 and C7, with maximum likelihood age calculated using 1104 modern sequences for the entire haplogroup C. The colour represent the geographic location in which the haplogroup can be found.

Haplogroup C4, dates to ~25 kya predominantly contain individuals from East Asia. The only haplogroup which contains Native American individuals is C4c calculated to ~16 kya are located exclusively from North America (figure 43). Within C4c, more precisely C4c1, contain Cree and Ojibwe samples from our study as well as ancient samples from Maritime Archaic culture, demonstrating its existence on the Northeast coast around 4,500 years ago.

5.3. Founder Analysis

The aim of using founder analysis is to determine whether an observable pattern can be identified for the major demographic events using mitochondrial data. Important initial decision for the founder analysis is to determine the source and the sink populations. Following the hypothesis of all Native Americans originate from Asia (Forster et al., 1996; Theodore G Schurr, 2004), source and sink populations were determined to stimulate possible scenarios. Considering three potential main sources for Native Americans. The first source is entire Asia, the second source is Siberia and the last in North America. The sink population selected were the Americas continent, North America, and South America (figure 44).

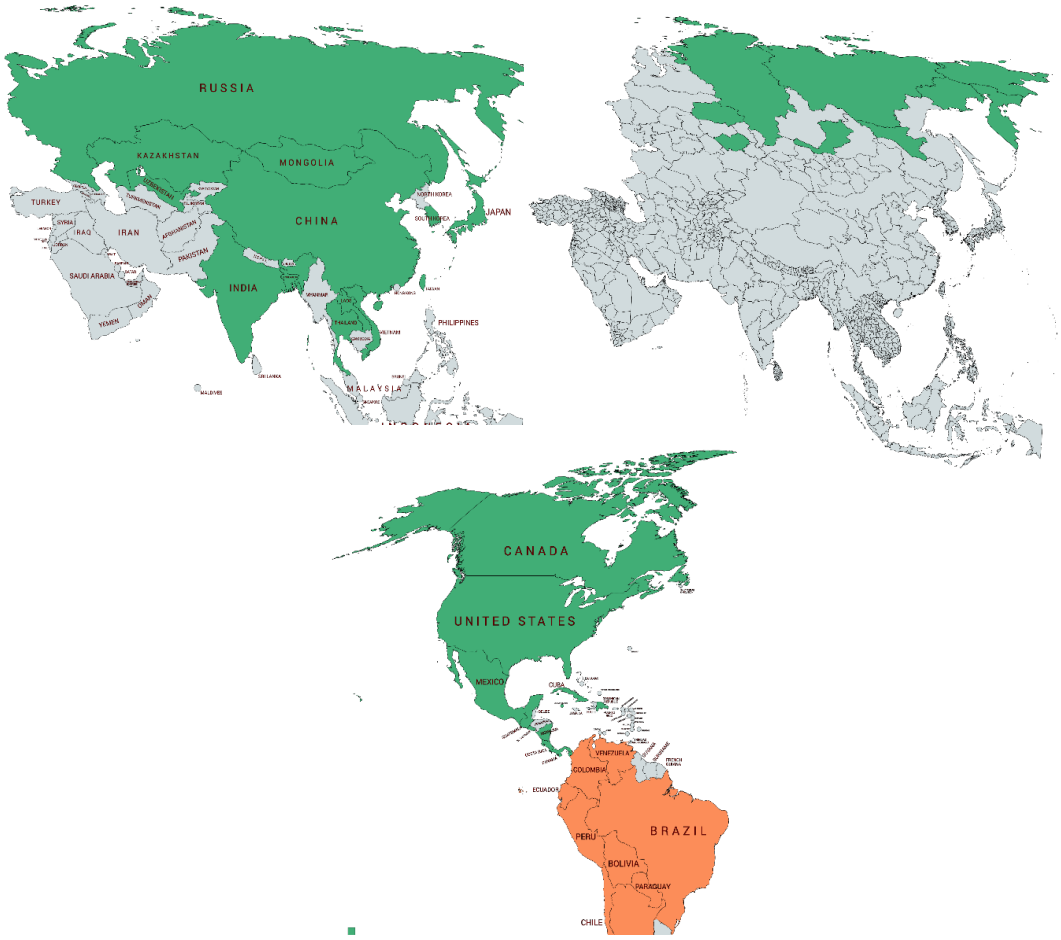

Figure 43. A map to show the area that was considered as Source population (Green) and Sink population (Orange) for the founder analysis. The whole Americas was use as a Sink population when testing with Siberian and Asia population as the source. When testing movement from North America to South America, North America was used as the source and South America as the sink.

5.3.1. Haplogroup A

Combining all the modern individuals belonging to haplogroup A, the founder analysis approach was applied to test out migration events using the mitochondrial genome. As seen in figure 46, firstly when East Asia was used as the source for migration and the entirety of the American continent as sink, two peaks can be seen with f1 and f2 criteria matching at ~19 kya and ~2 kya and a minor peak at ~9 kya. Investigating further the source for the initial migration of the first people, only the Siberian population was selected and using the same sink as before, which generate an identical figure. Demonstrating that the source for mitochondrial genome most likely derived from Siberian populations.

Further testing was performed using North and South America as the sink and keeping the source as Siberia. Interestingly, the peak at ~1.4 kya was seen in the figure when North America was the sink but disappear in Central and South America when the more stringent f2 was applied. Possibility caused by populations have subsequently experienced gene flow back towards Asia or that it has never reached to South America. A very small peak was seen around 9 kya, perhaps showing additional gene flow into the continent from Siberia, but this was not shown in the f2 peak for South America, perhaps suggesting a similar situation as the peak at 1.4 kya.

When using North America as the source and South America as the sink (figure 45), although the signal for both f1 and f2 matches with each other, the projected migration dates differ from the date produced when Asia and Siberia were considered as source populations. As only haplogroup A2 was considered for this analysis and the estimated age of A2 is ~18 kya, this could explain the reason why the first peak is at ~15 kya, however, the peak at ~4.5 kya is particularly interesting as that suggest further movement within the continent from North to South after the initial migration into the new world. Overall, for haplogroup A, possibly three migrations from Asia and Siberia into North America, and South America, two migrations.

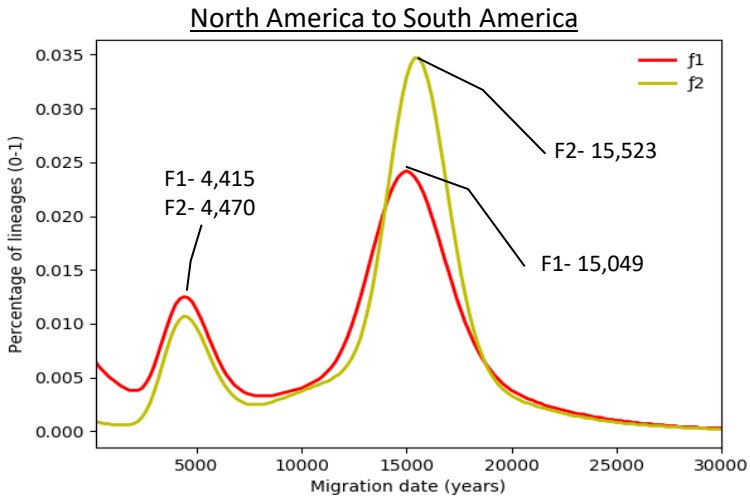

Figure 44. Founder analysis results of complete modern mitochondrial DNA sequences of haplogroup A using North America as a Source and South America as sink.

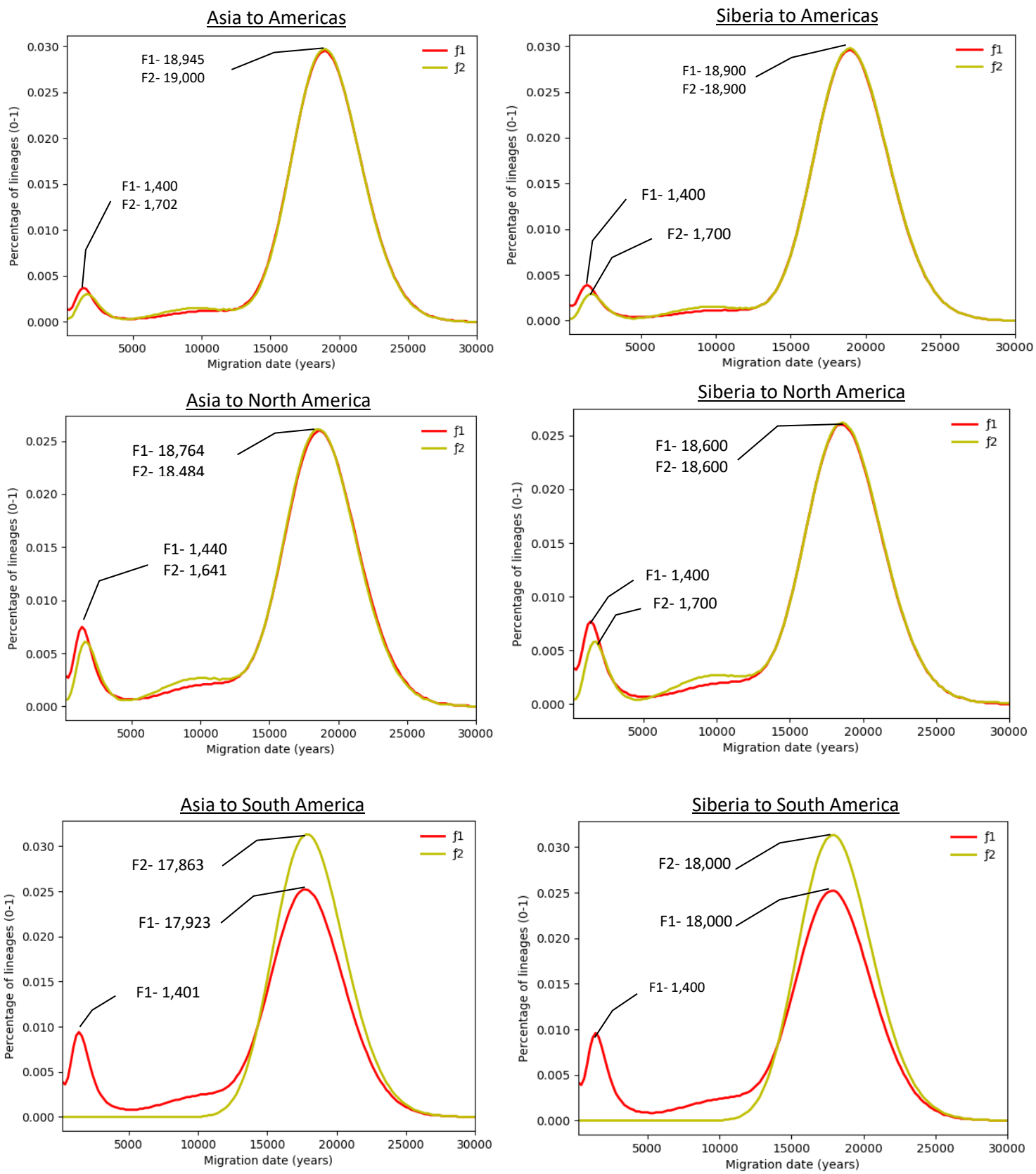

Figure 46. The founder analysis results of complete modern mitochondrial DNA sequences for haplogroup A, using Asia or Siberia as the Source and the Americas as sink.

5.3.2. Haplogroup C

The founder analysis approach was also applied to haplogroup C in order to understand their dispersal and whether it differs from haplogroup A. The same source and sink were applied as haplogroup A using both Siberia and entire Asia as the source and the North and South America as the sink.

When using Asian and Siberia as the source and the Americas continent as the sink (figure 48), f1, and f2 shows only one signal but at different migration date. When this is repeated with North America and South America as the sink, similar results occur, the only difference it's the migration date for South America appearing at a later date comparing to North America at ~24 kya for f2 and ~21 kya for f1, whereas North America is at ~20 kya for f2 and ~17.5 kya for f1. The singular peak shown across these scenarios shows a single migration from Siberia into America. This scenario can also be identified when North America is used as the source population for South America (figure 47), although the estimated age is ~16.5 kya, a significant decrease compared when Siberia and Asia are used as the source population. Additional peak located at 620 ya when North America is used as a source, this could be in relation to recent migration post-European contact.

Overall, haplogroup C tells a different story to haplogroup A. It would appear that only one migration occurs for haplogroup C and people remain in their particular region once they enter, whereas haplogroup A shows a more complex scenario, indicating further migration from Siberia into North America.

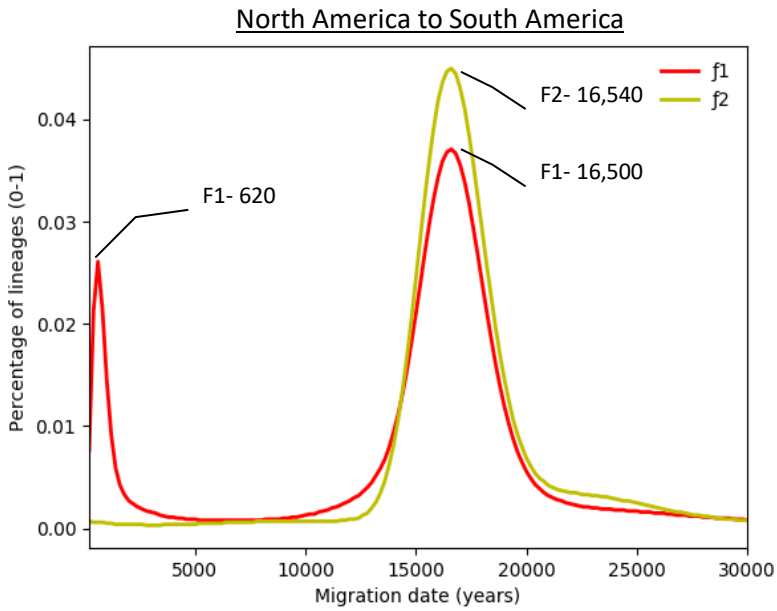

Figure 48. Founder analysis results of complete modern mitochondrial DNA sequences of haplogroup C using North America as a Source and South America as sink.

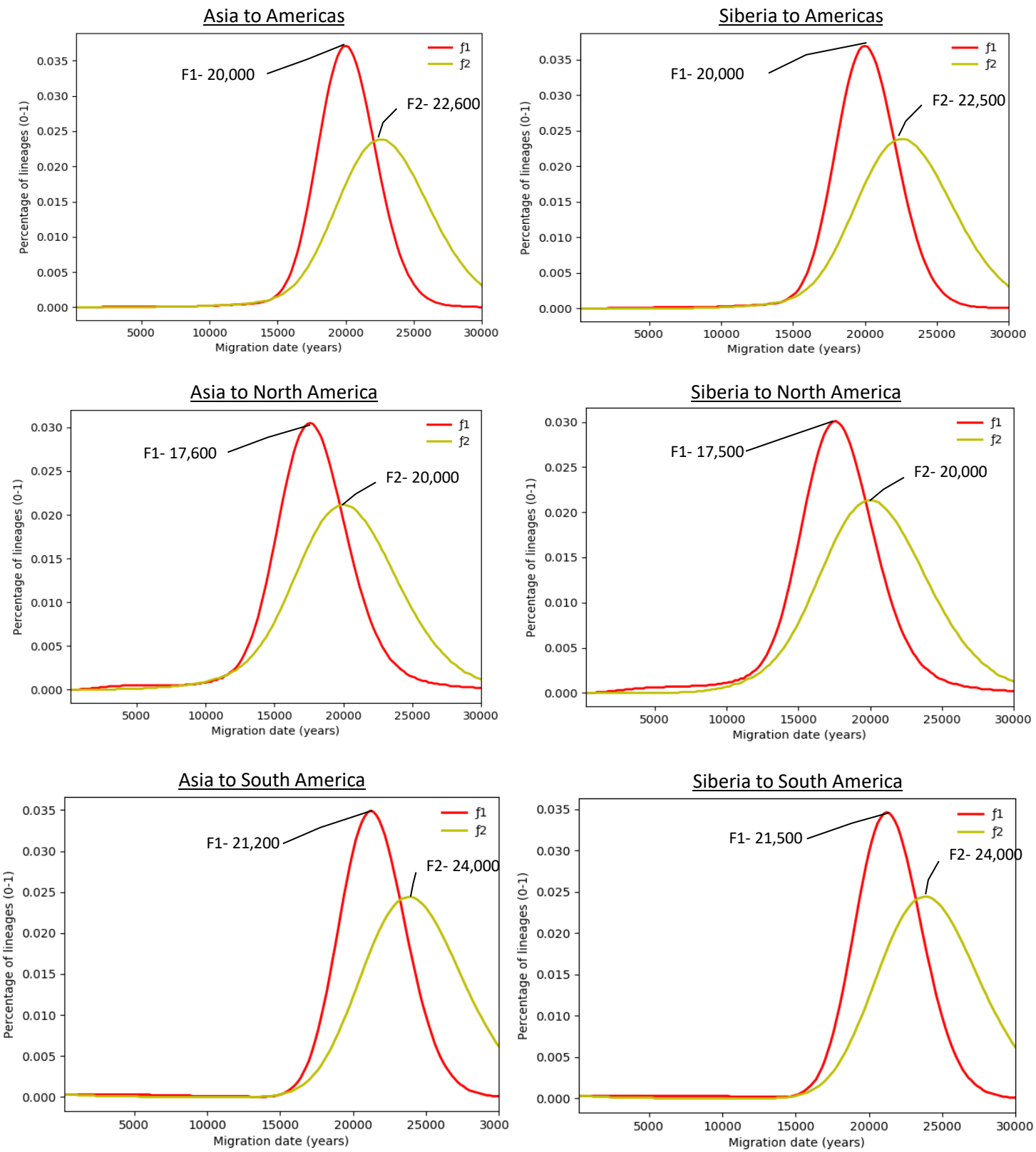

Figure 50. The founder analysis results complete modern mitochondrial DNA sequences for haplogroup C, using Asia or Siberia as the Source and the Americas as sink.

6. Discussion

6.1. Male to female ratio

Analyzing the sequenced individuals from this study, the majority of the Huron-Wendat individuals appear to be female (8 out of 9). The individuals selected were completely random across the 4 different sites, with majority of our individual found to be female, perhaps indicate a high ratio of female to male burial in each site. Additionally, the lack of personal items within the mass grave of Huron Wendat prevents the determination of role for each individual to associate whether the individual in this study is male or female. However, the chances of randomly selecting individuals that are female from each site, especially finding only female individuals from Staines, Moatfield, and Teston sites, suggest a possible high proportion of females within these mass graves for the three sites.

For the two sequenced Glacial Kame individuals, the Hind individual (BY005) was anatomically identified as male with grave goods associated with hunting, and Sartori individual was suggested to be a probable male with a flaking tool kit (Donaldson & Wortner, 1995). The genetic finding that both individuals are male corresponds to the archaeological and skeletal analysis, indicates that the traditional method of identifying the sex using the skull and other skeletal structure, along with archaeological finding provides insight for sex identification. Additionally, hunting could be a male dominant procedure for the Glacial Kame culture. The sex identification of the skeletal structure at the hind site identified burial consisting of singular female does not contain any grave goods or grave goods unassociated with hunting, such as burial 3, 5, 6 and 14, whilst grave which are associated with male contain bifaces and animal bones, such as burial 9, 19, 20, 22 and 23 (Donaldson & Wortner, 1995).

6.2. Uniparental markers

The Uniparental markers found are typical of Native American descent. Specifically, the mitochondrial data (figure 15 and 37) obtained for the sequenced individuals exhibit haplotypes that appear exclusively across the Great Lake and the Northeastern region of the Americas. Firstly, the diversity of haplogroups found from our sequenced ancient individuals appears in haplogroups A2i, B2*, C1c+150, C4c1, and X2a. Private mutation of individual from the same haplogroup of the same archaeological sites was found. Individual from the same site also exhibit different haplogroups from one another, suggesting the ossuary from each archaeological site contain a mix of people and not a family grave. This is particularly interesting, as the ossuary moves with the village in Huron-Wendat culture when the resource are low causing migration into other areas of the Great Lakes region (E. R. Seeman, 2011), this suggesting number of different haplogroups within each settlement, perhaps caused by this movement into a new territory. Additionally, the tribal or the clan status is passed on only via the maternity side, each clan would assume to have an increase of a particular haplogroup due to the matriarchal society of Huron Wendat. Therefore, a village appears to have members

from different clan, the ossuary appears to be for members of the village regardless of clan association. Whether a village consist of a large number of a particular clan member will need further investigation. Additionally, information regarding clans should be included in future research when collecting samples, to understand haplogroup diversity within clans of a particular nation.

The lack of haplogroup A2a, A2b, and D2a, which are associated with Eskimo populations, suggests that from the maternal lineage perspective, it was not integrated into the Huron-Wendat population (Ackerman, 1998; M. Raghavan et al., 2014; Saillard et al., 2000). The whole genome results from viewing PCA, ADMIXTURE, Outgroup-f3 and D-Stats also compliment the idea that Eskimo population did not integrate into Huron Wendat or Glacial Kame population. Trading between the Eskimo population from the Great lake was previously suggested (Lattanzi, 2007), the assumption of association with Eskimo does not correlate to genetic exchange for the ancient Huron-Wendat population or was not detected when viewing the maternal lineages.

Interestingly, viewing the ancient Huron-Wendat haplogroups, apart from haplogroup B2* and C4c1, the haplogroups containing ancient Huron-Wendat appears to be shared with Beothuk individuals. Given the fact that the ancient Lucier individuals, dates to between 1.2 to 4.2 ka (Scheib et al., 2018), also shares the same haplogroup with ancient Huron-Wendat and Beothuk, and the maternal genetic discontinuity between the Maritime Archaic and Beothuk in Newfoundland (Duggan et al., 2017). The Beothuk population could have migrated into Newfoundland from the Great Lake via the St. Lawrence River, perhaps before the discovery of Newfound by the Vikings 1000 years ago (William W Fitzhugh, 2000). Further archaeological dated sites could clarify this, as the oldest Beothuk site discovered in Newfoundland is approximately 500 years old (Duggan et al., 2017; Pastore, 1989). The clade C1c+150, consists of mostly ancient sequences which were used to form the topology of this branch, this reitierates the importance of ancient DNA data in forming the topology of trees.

The finding of haplogroup B2 in this region is curious, although from the Sanger sequencing results demonstrating the presence of haplogroup B in Staines and Teston sites. B2 is also a Pan American lineage, so finding this lineage is to be expected. Perhaps B2 was common, as this was also found in both our Glacial Kame individuals and other published control region of Glacial Kame individual (Schultz Shook & Smith, 2008). If maternal genetic continuity were to occur for this haplogroup for the great lake region, the assumption of the same clade would be shared between Glacial Kame and the Huron-Wendat samples, similar to Lucier and Huron-Wendat for C1c+150. This does appear to be true, but finding 5 B2 out of 40 Huron-Wendat individuals and 2 out of 110 modern individuals from the great lake suggest a decline of haplogroup B2 over time.

Overall, from viewing the mitochondrial DNA, continuity in sharing maternal lineages between the Lucier and the Huron-Wendat, as well as Beothuk individuals. This sharing of haplogroup demonstrates an ancient connection between the extinct culture of Beothuk and the Great

Lake region, perhaps due to trading of resources on a larger scale between the great lake and the East coast, eventually leading to the permanent settlement of Beothuk in Newfoundland.

6.3. Dietary Isotopes

The Huron-Wendat population had a mixed consumption of both freshwater marine resources and C_4 plants. As Huron-Wendat are known farmers recorded by the early European (Heidenreich, 1971), the heavy reliance on C_4 plants suggests a settled lifestyle around the great lake region with freshwater marine resources consumption when crops are not in season (Pfeiffer et al., 2014). Although the four sites are in close proximity with each other, the dietary of each site differs slightly, with Moatfield and Turnbull sites obtain a slight increase in freshwater marine diet then Moatfield and Turnbull (figure 27). The inability to distinguish the sex of each individual or the stature and clan status of individual in the study prevents clarity into understanding whether the occurrence of a sex-biased in terms of food consumption.

6.4. Kinship

The usage of READ can only provide the identification of first- and second-degree relationships. With the coverage of Huron-Wendat, the first- or second-degree relationships were not detected when compared across and within sites. Considering the ages of each site differ by 50 to 100 year, it was improbable to detect the first and second relationship across different sites. However, first- and second-degree relationship between samples within the same site was not detected. Although some individuals of the same site share the same haplotype, suggesting these individuals are related but the degree of relationship might be higher, and READ was unable to discern this.

6.5. Genetic Ancestry

Homogeneity appears across the Great Lake region when viewing the ADMIXTURE, PCA, f-statistic and D-statistic, with no noticeable difference between the ancient Huron-Wendat, Glacial Kame, and other ancient individuals from the Great Lake. The PCA demonstrates Glacial Kame and Huron-Wendat cluster closely with each other within the North America Cluster. This correlates to the components in ADMIXTURE that appears in higher K value (figure 30), the Great Lake region population contains components which appears in Eskimo, Ancient Beringian, and small proportion in Kennewick that does not appear in Anzick and other Ancient Southern American population. This Eskimo component is not visible from the mitochondrial data. This perhaps suggest further integration from the Northwest into the Great Lake region after Kennewick.

Admixture between different linguistic populations around the Great Lake and within the Huron-Wendat population was not detected when testing with Admix f3. This is peculiar as the

close proximity of the Huon-Wendat sites suggests sharing of territory, although it is to be noted that positive Admix f3, does not reject the absence of admixture, rather the negative Admix f3 confirm unambiguously the occurrence of admixture (Flegontov et al., 2019). Cree population shows admixture between Huron-Wendat and Mid Dorset population, suggesting some integration of Paleo Eskimo population from the Northern Artic into Northern Great Lake region. However, the occurrence of this admixture is unclear and perhaps inaccurately representing the timing of this admixture as modern Aleut also demonstrates admixture with Cree population when testing with Admix f3.

6.6. Modern Mitochondrial DNA

There is a clear indication that the three populations, Ojibwe, Chipewyan, and Cree, all exhibit Native American mitochondrial haplotype. Viewing deeper into the subclade of each haplogroup (table 17), only Ojibwe and Cree share a number of different haplotype (A2i, A2f1a, C1b+10031, and X2a). Perhaps this is due to both populations belong to the Algonquian language family whilst the Chipewyan population is within the Athabaskan family. The finding of X2a from the Ojibwe and Cree population was anticipated, and support previous publications that X2a is more commonly found at the Great Lake region despite its rarity within the Americas continent (Kashani et al., 2012; Reidla et al., 2003).

Ojibwe and Cree individuals share the same subclade as Ancient Huron-Wendat. This is particularly interesting as Algonquins were in conflict with Iroquois after European arrival (Bailey, 2016; Birch, 2010). The extensive trading network along the St. Lawrence River perhaps indicates a harmonious relationship between the Algonquins and Iroquois (Bohaker, 2006), leading to sharing of genetics between the two different linguistic groups. Additionally, the acceptance of Iroquois into Algonquins society could have occurred after the Iroquois War (Richter, 1983), which could lead to the integration or the sharing of this haplogroup between the two linguistic groups.

Viewing the haplogroup diversity of the Ojibwe population, A2a and A2b was not detected but were found in both Chipewyan and Cree. Considering A2a and A2b are associated with the Siberian population and the Northwest coast, the detection of this indicates an association with the Northwest. Whether this represents an integration of modern or ancient population mixing still requires further analysis.

The founder analysis for haplogroup A and C shows two different stories. Both demonstrate the initial movement into the Americas between 15 kya and 20 kya complimentary to previous proposed date from other publication (Posth et al., 2018; J. a Raff & Bolnick, 2014; Reich et al., 2012), but haplogroup A demonstrate further movement from Siberia into America around 1.8 kya. This corresponds to Paleo-Eskimo and Neo-Eskimo movement into Northern America (M. Raghavan et al., 2014; Rasmussen et al., 2010). A further minor peak can also be seen in the founder analysis of haplogroup A approximately 9 kya (figure 46), suggesting further movement

from Siberia into North America, which was previously unknown. This movement does not appear to affect South America.

7. Conclusion

In an attempt to complete the aim of this thesis, to understand the indigenous population of Northeastern America, two approaches were applied. The first was the usage of ancient individuals from the Ontario region by the Great Lakes and the second was to utilize the current information on the modern and ancient phylogeographic analysis of Native American mitochondrial haplogroup.

The homogeneity between the ancient individuals and the modern can be identify which reflects the mitochondrial data obtained, as it shows continuity between Huron-Wendat, Glacial Kame and the modern population from the Great Lakes. The finding from the Huron-Wendat individuals demonstrate a lack of kinship from the individuals that were tested but sharing of mitochondrial haplogroup can be seen across different sites, therefore these individuals must have a common ancestor via the maternal lineages. The sharing of haplogroup with Beothuk is unexpected, as it was unknown that the Great Lake population interacted with the extinct Beothuk population, this is probably originated from the same source population. This indicates a wider spread of Iroquois than previously thought.

The finding of additional components within the Great Lake population in higher ADMIXTURE demonstrate the complexity in understanding the population in this area. Especially finding an increase of a particular component after Kennewick demonstrates either the presence of further founder events or integration of population with this component into Great Lake society post-Kennewick. This perhaps is an over-interpretation as additional data, preferably from Mid-west Canada post-Kennewick would provide a clearer picture of the movement of indigenous people into the Great Lake region from the Northwest.

The usage of the uniparental genome shows greater detail of the relationship between indigenous populations, especially due to the lack of indigenous whole-genome data for North America. The linguistic barrier appears to prevent further sharing of maternal lineage between Chipewyan and Algonquian speakers but did not affect Iroquoian speakers. From viewing haplogroup A and C, both demonstrate as founding haplogroup, but each expresses a different story. Additional two possible expansions from Siberia into Northern Americas can be identified after the initial arrival of the first people, one at approximately 9 kya and 1.8 kya, which was detected only in haplogroup A but not for haplogroup C. More sampling and further sequencing are required to extend our understanding of further expansion into Northern Americas.

8. Future work

From the samples collected, only 11 whole genomes were sequenced in-depth. Further sequencing of more samples and in greater depth would provide enhance details into understanding the relationship within the population. Whole-genome database of Native Americans is required for greater analytical power, especially in understanding the admixed population in finer details. Cluster-based algorithms such as ADMIXTURE and ChromoPainter would provide greater detail on genetic ancestry, however, understanding the culture of other indigenous populations is key to discover and understand how indigenous populations mixed. Further work must imply this, in accordance with indigenous people, to understand the past. qpADM would be useful to apply in order to understand whether the Huron-Wendat and the Glacial Kame are related to Anc A or Anc B.

Maximum likelihood calculation and Founder analysis for Haplogroup B and D would be particularly interesting, as both haplogroups appear to be uncommon in North America. This would provide a greater understanding of the migration of people into Southern America.

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10. Appendix

10.1. Mitochondrial DNA mutation

10.1.1. Ancient individuals

Sample ID	Site Information	mtDNA haplogroup	Mutations
CE010	Staines Road	C4c1	A73G A215G 249d A263G 309.1C 315.1CC T489C A750G T1243C A1438G 2232.1A A2706G T3552A A4715G A4769G G6026A C7028T C7196A T8450C G8584A A8701G A8860G T9540C A9545G A10398G C10400T T10873C G11719A G11914A G11969A C12705T A13263G A13512G T14318C C14433T C14766T T14783C G15043A G15148A T15204C G15301A A15326G A15487T A16183C T16189C C16223T T16298C T16311C C16327T T16519C
CE013	Staines Road	X2a1	A73G G90A G143A A153G A263G 290-291delAA 523insAC A750G A1438G G1554T A2706G T3552C A4769G T6221C C7028T A8913G G11719A G11766A A12397G C12705T A13966G T14470C T14502C A15326G G16213A C16223T C16278T T16519C
CE015	Staines Road	A2i	C64T A73G G94A T146C A153G A235G A263G 309.1C 315.1C 522d 523d A663G A750G 960.1CCC A1438G A1736G A2706G 3307.1A T3335C T4248C A4769G A4824G C5165T T6620C C7028T G8027A C8794T A8860G G11719A G12007A C12705T A14280G T14470C C14766T A15326G C15386T C16111T C16223T C16290T G16319A T16325C T16362C T16519C

CE019	Moatfield	C1c	C19T A73G C150T T152C 249delA A263G 290-291delAA T489C A750G A1438G G1888A A2706G A3105CA T3552A A4702G A4715G A4769G C7028T C7196A G8584A A8701G A8860G T9540C A9545G A10398G C10400T T10873C G11719A G11914A C12705T A13263G T14318C C14766T T14783C C15007T G15043A G15301A A15326G A15487T G15930A C16223T C16291T T16298C T16325C C16327T
CE023	Moatfield	C1c	A73G C150T T152C 249d A263G 290d 291d 309.1C T489C A750G A1438G G1888A A2706G AC3105A T3552A A4702G A4715G A4769G C7028T C7196A G8584A A8701G A8860G T9540C A9545G A10398G C10400T T10873C G11719A G11914A C12705T A13263G T14318C C14766T T14783C C15007T G15043A G15301A A15326G A15487T G15930A C16223T T16298C T16325C C16327T
CE030	Teston Road	A2i	C64T A73G G94A T146C A153G A235G A263G 309.1C 522d 523d A663G A750G 960.1CCC A1438G A1736G A2706G 3307.1 T4248C A4769G A4824G C5165T T6152C T6620C C7028T G8027A C8794T A8860G G11719A G12007A C12705T A14280G T14470C C14766T A15326G C15386T C16111T C16223T C16290T G16319A T16325C T16362C T16519C
CE031	Teston Road	A2i	C64T A73G G94A T146C A153G A235G A263G 315.1C 522d 523d A663G A750G 960.1C A1438G A1736G C2625T A2706G 3307.1A T3786C T4248C A4769G A4824G C5165T T6620C C7028T G8027A C8794T A8860G G11719A G12007A C12705T A14280G T14470C C14766T A15326G C15386T C16111T C16223T C16290T A16299G G16319A T16325C T16362C T16519C
CE032	Teston Road	B2	A73G T209C A263G 309.1C G499A A750G A827G A1438G G1462A A2706G AC3105A A3547G A4769G G4820A C4880T C4881T T4977C C6473T C7028T C7401T C8655T A8860G T9950C C11177T G11719A T12453C G13590A C14562T C14766T A15080G A15326G C15535T A15953G T16217C T16519C
CE040	Turnbull	A2i	C64T A73G G94A T146C A153G A235G A263G 523delAC A663G A750G 955.2C A1438G A1736G A2706G 3105delC 3306.1A T4248C C4320T C4322T A4824G C5165T T6620C C6820T C7028T G8027A C8794T A8860G C10553T C10900T G11719A G12007A C12705T A14280G T14470C A15326G C15386T C16111T C16223T G16319A T16325C T16362C T16519C
BY005	Hind 22	B2	A73G T146C T152C A263G 309.1C 315.1C G499A A750G A827G A1438G A2401G A2706G AC3105A A3547G A4769G G4820A T4977C G6261A C6473T C7028T 8281d 8282d 8283d 8284d 8285d 8286d 8287d 8289d A8860G T9950C C11177T G11719A G13590A C14766T A15326G C15535T

			A15924G C16179T A16182C A16183C T16189C T16217C T16519C
BY007	Sartori	B2	A73G A263G 309.1C G499A A750G A827G A1438G A2706G A3547G A4769G G4820A T4977C C6473T C7028T 8281d 8282d 8283d 8284d 8285d 8286d 8287d 8288d 8289d A8860G T9950C C11177T G11719A G13590A C13644T A14696G C14766T A15326G C15535T 16183.1C T16189C T16217C T16519C

Appendix 1. The mitochondrial DNA mutation of the ancient individuals sequenced.

10.1.2. Modern individuals

Sample	Haplogroup	Mutation
Cree01	C4c1	73G 249d 263G 310d 311d 312d 313d 489C 750G 980C 1007A 1243C 1413C 1438G 1760A 2232.1A 2706G 3552A 4715G 4769G 6026A 7028T 7196A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12705T 13263G 14318C 14433T 14766T 14783C 15043A 15148A 15204C 15301A 15326G 15487T 16223T 16298C 16327T 16519C
Cree02	X2a2+13191	73G 153G 195C 200G 225A 263G 310C 750G 1415A 1438G 1719A 2706G 4769G 6221C 6371T 7028T 8860G 8913G 10256C 11719A 12397G 12705T 13191C 13966G 14470C 14502C 14560A 14766T 15326G 16189C 16213A 16223T 16254C 16278T 16519C
Cree03	C1c+150	73G 150T 152C 263G 489C 750G 1438G 1888A 2706G 3552A 4702G 4715G 4769G 7028T 7196A 8584A 8701G 8848C 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 12705T 13263G 14318C 14766T 14783C 15007T 15043A 15301A 15326G 15487T 15930A 16223T 16298C 16325C 16327T
Cree04	A2+64	64T 73G 146C 152C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 7028T 8027A 8334A 8794T 8860G 11719A 12007A 12705T 14783C 15326G 16111T 16211T 16223T 16290T 16319A 16362C 16519C
Cree05	A2f1a	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 1809C 2638C 2706G 3316A 4248C 4769G 4824G 7028T 7897A 8027A 8794T 8860G 11719A 12007A 12092A 12705T 12940A 14766T 14911T 15326G 15670C 16111T 16192T 16223T 16290T 16319A 16362C

Cree06	A2b1	73G 146C 153G 235G 263G 310C 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 7028T 8027A 8794T 8860G 11365C 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16265G 16278T 16290T 16319A 16362C
Cree07	A2a5	73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3330T 3552C 4248C 4769G 4824G 7028T 7797C 8027A 8794T 8860G 11719A 12007A 12166C 12705T 14766T 15326G 15664T 16111T 16189C 16223T 16233G 16290T 16319A 16331G
Cree08	X2a1b	73G 143A 153G 195C 200G 263G 750G 1438G 1719A 2706G 3552C 4769G 6221C 6371T 7028T 8422G 8860G 8913G 11719A 12397G 12705T 13966G 14470C 14502C 14766T 15326G 16093C 16189C 16213A 16223T 16278T 16519C
Cree09	A2a5	73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3330T 3552C 4248C 4769G 4824G 7028T 7797C 8027A 8794T 8860G 11719A 12007A 12166C 12705T 14766T 15326G 15664T 16111T 16189C 16223T 16233G 16290T 16319A 16331G
Cree10	C4c1	73G 207A 263G 489C 750G 1243C 1438G 2706G 3552A 4715G 4769G 6026A 7028T 7196A 8027A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12705T 13263G 14318C 14433T 14766T 14783C 15043A 15148A 15204C 15301A 15326G 15487T 16093C 16223T 16298C 16327T 16519C
Cree11	A2i	64T 73G 94A 146C 151T 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16299G 16319A 16325C 16362C 16519C
Cree12	C1b+10031	73G 263G 489C 493G 750G 1438G 2706G 3552A 4715G 4769G 7028T 7196A 8584A 8697A 8701G 8860G 9540C 9545G 10031C 10398G 10400T 10873C 11719A 11914A 12361G 12397G 12705T 13263G 14179G 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16223T 16298C 16325C 16327T 16519C
Cree13	C1b+10031	73G 249d 263G 290d 291d 489C 493G 523d 524d 750G 1438G 1452C 2706G 3552A 4715G 4769G 7028T 7196A 8584A 8701G 8860G 9540C 9545G 9686C 10031C 10398G 10400T 10873C 11719A 11914A 13263G 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16223T 16294T16298C 16325C 16327T
Cree15	C1d1a1	73G 263G 489C 750G 1415A 1438G 2706G 3552A 3757T 4715G 4769G 5147A 6297C 6340T 7028T 7196A 7343G 7697A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 12705T 13263G 14305A 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16051G 16223T 16298C 16325C 16327T 16519C
Cree16	C1c+150	73G 150T 152C 263G 489C 750G 1438G 1888A 2706G 3552A 4702G 4715G 4769G 7028T 7196A 8584A 8701G 8848C 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 12705T 13263G 14318C 14766T 14783C 15007T 15043A 15301A 15326G 15487T 15930A 16223T 16298C 16325C 16327T
Cree18	D1+7747	73G 200G 263G 489C 750G 1438G 2092T 2706G 3010A 4769G 4883T 4916G 5178A 7028T 7747T 8414T 8701G 8860G 8986G 9540C 9682C 10398G 10400T 10873C 11719A 12352G 12705T 13734C 13934T 14668T 14766T

		14783C 15043A 15301A 15326G 15616T 15760T 16184T 16223T 16325C 16362C
Cree21	C1d1a1	73G 249d 263G 290d 291d 315.1C 489C 523d 524d 750G 1415A 1438G 2706G 3552A 4715G 4769G 6297C 7028T 7196A 7343G 7697A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 12705T 13263G 14305A 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16051G 16223T 16298C 16311C 16325C 16327T 16519C
Cree22	X2a2	73G 153G 195C 200G 225A 263G 310C 750G 1415A 1438G 1719A 2706G 4769G 6221C 6371T 7028T 8860G 8913G 10256C 11719A 12397G 12705T 13191C 13966G 14470C 14502C 14560A 14766T 15326G 16189C 16213A 16223T 16254C 16278T 16519C
Cree24	A2	64T 73G 146C 152C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 7028T 8027A 8334A 8794T 8860G 11719A 12007A 12705T 14783C 15326G 16111T 16211T 16223T 16290T 16319A 16362C 16519C
Cree26	A2i	64T 73G 94A 146C 151T 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16299G 16319A 16325C 16362C 16519C
Cree27	A2i	64T 73G 94A 146C 151T 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16299G 16319A 16325C 16362C 16519C
Cree28	B2a4	73G 151T 263G 499A 750G 827G 1438G 2706G 3547G 4769G 4820A 4977C 6473T 7028T 8860G 9097G 9950C 11177T 11719A 12373G 13590A 14766T 15326G 15535T 16092C 16111T 16183C 16189C 16217C 16483A 16519C
Cree29	C1b+10031	73G 263G 489C 493G 750G 1438G 2706G 3552A 4715G 4769G 7028T 7196A 7754A 8584A 8701G 8860G 9540C 9545G 9686C 10031C 10398G 10400T 10873C 11719A 11914A 12705T 13263G 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16223T 16298C 16325C 16327T 16519C
Cree30	D1+7747	73G 200G 263G 489C 750G 1438G 2092T 2706G 3010A 4769G 4883T 4916G 5178A 7028T 7747T 8414T 8701G 8860G 8986G 9540C 9682C 10398G 10400T 10873C 11719A 12352G 12705T 13734C 13934T 14668T 14766T 14783C 15043A 15301A 15326G 15616T 15760T 16184T 16223T 16325C 16362C
Cree31	X2a1b	73G 143A 153G 195C 200G 263G 750G 1438G 1719A 2706G 3552C 4769G 6221C 6371T 7028T 8422G 8860G 8913G 11719A 12397G 12705T 13966G 14470C 14502C 14766T 15326G 16093C 16189C 16213A 16223T 16278T 16519C
Cree32	C4c1+207	73G 207A 263G 489C 750G 1243C 1438G 2706G 3552A 4715G 4769G 6026A 7028T 7196A 8027A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12705T 13263G 14318C 14433T 14766T 14783C 15043A 15148A 15204C 15301A 15326G 15487T 16093C 16223T 16298C 16327T 16519C
Cree33	X2a2+13191	73G 153G 195C 200G 225A 263G 310C 750G 1415A 1438G 1719A 2706G 4769G 6221C 6371T 7028T 8860G 8913G 10256C 11719A 12397G 12705T

		13191C 13966G 14470C 14502C 14560A 14766T 15326G 16189C 16213A 16223T 16254C 16278T 16519C
Cree34	A2i	64T 73G 94A 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8854A 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16319A 16325C 16362C 16519C
Cree35	A2f1a	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 1809C 2638C 2706G 3316A 4248C 4769G 4824G 7028T 7897A 8027A 8794T 8860G 11719A 12007A 12092A 12705T 12940A 14766T 14911T 15326G 15670C 16111T 16192T 16223T 16290T 16319A 16362C
Cree36	A2n	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3849A 4248C 4769G 4824G 7028T 8027A 8794T 8860G 9344T 10700G 11719A 12007A 12280G 12705T 14766T 15326G 15777A 15924G 16126C 16129A 16187T 16223T 16290T 16319A 16362C 16519C
Cree37	C1b+10031	73G 263G 489C 493G 750G 1438G 2706G 3552A 4715G 4769G 7028T 7196A 8584A 8697A 8701G 8860G 9540C 9545G 10031C 10398G 10400T 10873C 11719A 11914A 12361G 12397G 12705T 13263G 14179G 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16223T 16298C 16325C 16327T 16519C
Cree38	A2q	64T 73G 146C 153G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 7028T 8027A 8794T 8860G 10993A 11172G 11719A 12007A 12705T 14766T 15326G 16111T 16209C 16223T 16290T 16319A 16362C
Cree39	A2i	64T 73G 94A 146C 151T 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16299G 16319A 16325C 16362C 16519C
Cree40	A2+64	64T 73G 146C 189G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6410T 7028T 8027A 8794T 8860G 11719A 12007A 12705T 14182C 14766T 15326G 15644G 16093C 16111T 16223T 16290T 16319A 16362C 16519C
Cree41	A2+64	64T 73G 146C 152C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 7028T 8027A 8334A 8794T 8860G 11719A 12007A 12705T 14783C 15326G 16111T 16211T 16223T 16290T 16319A 16362C 16519C
NAD02	A2+64+6527	64T 73G 146C 153G 234G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD04	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16256T 16290T 16319A 16362C
NAD06	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD07	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C

NAD08	A2+64+6527	64T 73G 146C 153G 234G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD09	A2+64+6527	64T 73G 146C 153G 234G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD11	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD14	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD15	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 9533T 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD16	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD17	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD19	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16256T 16290T 16319A 16362C
NAD20	A2+64+6527	64T 73G 146C 153G 234G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD21	A2+64+6527	64T 73G 146C 153G 195C 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 12795A 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD22	A2+64+6527	64T 73G 146C 153G 195C 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD24	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD25	A2+64+6527	64T 73G 146C 153G 235G 263G 310C 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD26	A2+64+6527	64T 73G 146C 153G 195C 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 12795A 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD27	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C

NAD28	A2a5	73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3330T 3552C 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 15664T 16111T 16189C 16223T 16233G 16290T 16319A 16331G
NAD29	A2+64	64T 73G 146C 153G 235G 263G 297G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 7028T 8027A 8794T 8860G 11719A 12007A 12166C 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD31	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD32	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD33	A2+64+6527	64T 73G 146C 153G 195C 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD37	D1+15930	73G 263G 489C 750G 1438G 2092T 2706G 3010A 4769G 4883T 5178A 7028T 8414T 8701G 8860G 9540C 10398G 10400T 10873C 11719A 12705T 14668T 14766T 14783C 15043A 15301A 15326G 15930A 16223T 16325C 16362C
NAD43	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD46	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD54	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD55	A2+64+6527	64T 73G 146C 153G 234G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD56	A2a5	73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3330T 3552C 4248C 4769G 4824G 7028T 8027A 8794T 8860G 11719A 12007A 12166C 12705T 14766T 15326G 15664T 16111T 16189C 16223T 16233G 16290T 16319A 16331G
NAD59	A2+64+6527	64T 73G 146C 153G 235G 263G 310C 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD64	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD87	A2+64+6527	64T 73G 146C 153G 195C 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C

NAD93	A2+64+6527	64T 73G 146C 153G 235G 263G 310C 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD95	A2+64+6527	64T 73G 146C 153G 195C 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 12795A 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD96	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
NAD101	A2+64+6527	64T 73G 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 6527G 7028T 8027A 8794T 8860G 11176A 11719A 12007A 12705T 14766T 15326G 16111T 16223T 16290T 16319A 16362C
Oj962	X2a1+8152	73G 143A 153G 195C 200G 263G 310C 750G 1438G 1719A 2706G 3552C 4769G 6221C 6371T 7028T 8152A 8860G 8913G 11719A 12397G 12705T 13966G 14470C 14502C 14766T 15326G 16093C 16183C 16189C 16213A 16223T 16278T 16519C
Oj967	X2a1	73G 143A 153G 195C 200G 263G 309.1C 310C 750G 1438G 1719A 2706G 3552C 4769G 6221C 6371T 7028T 8860G 8913G 11719A 12397G 12705T 13966G 14470C 14502C 14766T 15326G 16093C 16183C 16189C 16213A 16223T 16278T 16519C
Oj968	A2i	64T 73G 94A 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8854A 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16319A 16325C 16362C 16519C
Oj969	A2i	64T 73G 94A 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16319A 16325C 16362C 16519C
Oj970	C1b+10031	73G 263G 489C 493G 750G 1438G 2706G 3552A 4715G 4769G 7028T 7196A 7754A 8584A 8701G 8860G 9540C 9545G 9686C 10031C 10398G 10400T 10873C 11719A 11914A 12705T 13263G 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16223T 16298C 16325C 16327T 16519C
Oj971	A2i	64T 73G 94A 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8854A 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16319A 16325C 16362C 16519C
OJ972	A2f1	64T 73G 146C 153G 235G 263G 310C 663G 750G 1438G 1736G 2638C 2706G 3316A 3816G 4248C 4769G 4824G 7028T 7897A 8027A 8794T 8860G 11719A 12007A 12705T 12940A 14766T 15326G 15670C 16111T 16192T 16223T 16290T 16319A 16362C
Oj973	C4c1	73G 263G 310C 489C 750G 1243C 1438G 2706G 3552A 4715G 4769G 6026A 7028T 7196A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12705T 13263G 14318C 14433T 14766T 14783C 15043A 15148A 15204C 15301A 15326G 15487T 16223T 16298C 16327T 16362C 16519C

Oj974	C1b+10031	73G 263G 489C 493G 750G 1438G 2706G 3552A 4715G 4769G 7028T 7196A 7754A 8584A 8701G 8860G 9540C 9545G 9686C 10031C 10398G 10400T 10873C 11719A 11914A 12705T 13263G 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16223T 16298C 16325C 16327T 16519C
Oj976	X2a1	73G 143A 153G 195C 200G 263G 310C 750G 1438G 1719A 2706G 3552C 4769G 6221C 6371T 7028T 8152A 8860G 8913G 11719A 12397G 12705T 13966G 14470C 14502C 14766T 15326G 16093C 16183C 16189C 16213A 16223T 16278T 16519C
Oj978	C4c1	73G 263G 310C 489C 750G 1243C 1438G 2706G 3552A 4715G 4769G 6026A 7028T 7196A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12705T 13263G 14318C 14433T 14766T 14783C 15043A 15148A 15204C 15301A 15326G 15487T 16223T 16298C 16327T 16362C 16519C
Oj979	C4c1	73G 263G 310C 489C 750G 1243C 1438G 2706G 3552A 4715G 4769G 6026A 7028T 7196A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12705T 13263G 14318C 14433T 14766T 14783C 15043A 15148A 15204C 15301A 15326G 15487T 16223T 16298C 16327T 16362C 16519C
Oj980	C1b+10031	73G 263G 489C 493G 750G 1438G 2706G 3552A 4715G 4769G 7028T 7196A 8584A 8701G 8860G 9540C 9545G 9686C 10031C 10398G 10400T 10873C 11719A 11914A 12705T 13263G 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16223T 16298C 16325C 16327T 16519C
Oj981	A2i	64T 73G 94A 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16319A 16325C 16362C 16519C
Oj982	A2i	64T 73G 94A 146C 153G 235G 263G 663G 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16319A 16325C 16362C 16519C
Oj983	X2a1b	73G 143A 153G 195C 200G 263G 750G 1438G 1719A 2706G 3552C 4769G 6221C 6371T 7028T 8422G 8860G 8913G 11719A 12397G 12705T 13966G 14470C 14502C 14766T 15326G 16093C 16189C 16213A 16223T 16278T 16519C
Oj984	C4c1	73G 263G 310C 489C 750G 1243C 1438G 2706G 3552A 4715G 4769G 6026A 7028T 7196A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12705T 13263G 14318C 14433T 14766T 14783C 15043A 15148A 15204C 15301A 15326G 15487T 16223T 16298C 16327T 16362C 16519C
Oj985	B2+152	73G 152C 209C 263G 310C 499A 750G 827G 1438G 1462A 2706G 3547G 4769G 4820A 4977C 6473T 7028T 8860G 9950C 11177T 11686T 11719A 11914A 12453C 13590A 14562T 14766T 15080G 15326G 15535T 16189C 16217C 16519C
Oj1116	C1b+10031	73G 263G 489C 493G 750G 1438G 2706G 3552A 4715G 4769G 7028T 7196A 7754A 8584A 8701G 8860G 9540C 9545G 9686C 10031C 10398G 10400T 10873C 11719A 11914A 12705T 13263G 14318C 14766T 14783C 15043A 15301A 15326G 15487T 16223T 16298C 16325C 16327T 16519C

Oj1117	C4c1	73G 249d 263G 315.1C 489C 750G 1243C 1438G 1719A 2232.1A 2706G 3552A 4715G 4769G 6026A 7028T 7196A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12705T 13263G 13674C 14311C 14318C 14433T 14766T 14783C 15043A 15148A 15204C 15301A 15326G 15487T 16223T 16265C 16298C 16327T 16519C
Oj1136	A2f1a	64T 73G 146C 153G 235G 257G 263G 750G 1438G 1809C 2638C 2706G 3316A 4769G 7028T 7861C 7897A 8027A 8794T 8860G 11719A 12007A 12092A 12705T 12940A 14766T 15326G 15670C 16111T 16192T 16223T 16290T 16319A 16362C
Oj1245	C1c+8700	73G 263G 489C 750G 1438G 1888A 2706G 3552A 4715G 4769G 7028T 7196A 8584A 8700T 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 12696C 12705T 13263G 14318C 14766T 14783C 15043A 15301A 15326G 15487T 15930A 16223T 16298C 16325C 16327T
Oj1250	C4c1+16362	73G 263G 310C 489C 750G 1243C 1438G 2706G 3552A 4715G 4769G 6026A 7028T 7196A 8584A 8701G 8860G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12705T 13263G 14318C 14433T 14766T 14783C 15043A 15148A 15204C 15301A 15326G 15487T 16223T 16298C 16327T 16362C 16519C
Oj1262	A2i	64T 73G 94A 146C 153G 263G 663G 710C 750G 1438G 1736G 2706G 3308C 4248C 4769G 4824G 5165T 6620C 7028T 8027A 8794T 8860G 11719A 12007A 12705T 14280G 14470C 14766T 15326G 15386T 16111T 16223T 16290T 16319A 16325C 16362C 16519C
Oj1288	A2f1	64T 73G 146C 153G 235G 263G 310C 663G 750G 1438G 1736G 2638C 2706G 3316A 3816G 4248C 4769G 4824G 7028T 7897A 8027A 8794T 8860G 11719A 12007A 12705T 12940A 14766T 15326G 15670C 16111T 16192T 16223T 16290T 16319A 16362C

Appendix 2. The mitochondrial DNA of modern individuals sequenced.

10.2. Tables for admix f3

A	B	C	f3	stderr	Zscore	nsnps
Anzick	Kennewick	Staines	3.62	0.0561	64.6	73683
Anzick	Kennewick	Moatfield	22.5	1.3	17.3	15644
Anzick	Kennewick	Teston	0.972	0.0136	71.5	93738
Anzick	Kennewick	Glacial_Kame	2.32	0.0321	72.3	93945

Appendix 3. Admix f3 results, testing whether Huron-Wendat and Glacial Kame is admixed between Anzick and Kennewick.

A	B	C	f3	stderr	Zscore	nsnps
USR	Kennewick	Staines	3.64	0.0566	64.3	78385
USR	Kennewick	Moatfield	22.8	1.33	17.1	16550
USR	Kennewick	Teston	0.963	0.0129	74.9	98455
USR	Kennewick	Glacial_Kame	2.3	0.0321	71.9	99354

Appendix 4. Admix f3 results, testing whether Huron-Wendat and Glacial Kame is admixed between USR and Kennewick.

A	B	C	f3	stderr	Zscore	nsnps
Saqqaq	Kennewick	Staines	3.65	0.0567	64.2	74365
Saqqaq	Kennewick	Moatfield	22	1.3	17	15748
Saqqaq	Kennewick	Teston	0.948	0.0127	74.3	93550
Saqqaq	Kennewick	Glacial_Kame	2.31	0.0307	75.1	94488

Appendix 5. Admix f3 results, testing whether Huron-Wendat and Glacial Kame is admixed between Saqqaq and Kennewick.

A	B	C	f3	stderr	Zscore	nsnps
USR	Anzick	Staines	3.74	0.0513	73	144675
USR	Anzick	Moatfield	23.2	1.01	22.9	30548
USR	Anzick	Teston	0.994	0.0119	83.3	183026
USR	Anzick	Glacial_Kame	2.35	0.0295	79.7	185230

Appendix 6. Admix f3 results, testing whether Huron-Wendat and Glacial Kame is admixed between USR and Anzick.

A	B	C	f3	stderr	Zscore	nsnps
Saqqaq	Anzick	Staines	3.72	0.0499	74.6	138178
Saqqaq	Anzick	Moatfield	22.6	1	22.6	29304
Saqqaq	Anzick	Teston	0.975	0.0111	87.9	175304
Saqqaq	Anzick	Glacial_Kame	2.35	0.028	83.9	177781

Appendix 7. Admix f3 results, testing whether Huron-Wendat and Glacial Kame is admixed between Saqqaq and Anzick.

A	B	C	f3	stderr	Zscore	nsnps
ASO	Kennewick	Staines	3.45	0.0584	59	58754
ASO	Anzick	Staines	3.49	0.046	75.9	108433
ASO	USR	Staines	3.55	0.0474	75	114513
ASO	Alaska_TCreek_9000BP	Staines	3.63	0.0708	51.2	39303
ASO	Nevada_LlCave_1850BP	Staines	3.54	0.0453	78.3	125027
ASO	Glacial Kame	Staines	3.49	0.0477	73.1	100281
ASO	Cree	Staines	3.54	0.0451	78.5	155881
ASO	Chipewyan	Staines	3.55	0.0453	78.2	142757
ASO	Canada_400BP	Staines	3.6	0.121	29.7	14083
ASO	Canada_6000BP	Staines	3.5	0.0728	48	36616
ASO	Thule	Staines	3.72	0.152	24.5	9412
ASO	LateDorset	Staines	3.57	0.0747	47.7	34551
ASO	MiddleDorset	Staines	3.64	0.137	26.5	9456
ASO	Teston	Staines	3.42	0.0473	72.4	98448
ASO	Moatfield	Staines	3.35	0.0997	33.6	16695

Appendix 8. Admix f3 results, testing if Staines is the results of admixture with Ancient South Ontario and other North America population.

A	B	C	f3	stderr	Zscore	nsnps
ASO	Kennewick	Teston	0.894	0.0123	72.4	74024
ASO	Anzick	Teston	0.907	0.0103	88	137017
ASO	USR	Teston	0.921	0.0098	94	143341
ASO	Alaska_TCreek_9000BP	Teston	0.931	0.0145	64.2	49284
ASO	Nevada_LlCave_1850BP	Teston	0.913	0.00911	100	153965
ASO	Glacial Kame	Teston	0.897	0.00967	92.7	125892
ASO	Cree	Teston	0.911	0.00893	102	187346
ASO	Chipewyan	Teston	0.913	0.00902	101	172816
ASO	Canada_400BP	Teston	0.916	0.0225	40.7	17737
ASO	Canada_6000BP	Teston	0.895	0.0146	61.4	46187
ASO	Thule	Teston	0.917	0.0281	32.7	11884
ASO	LateDorset	Teston	0.902	0.0153	58.8	43141
ASO	MiddleDorset	Teston	0.878	0.0277	31.7	11874
ASO	Staines	Teston	0.863	0.0103	83.4	98448
ASO	Moatfield	Teston	0.846	0.0185	45.8	20969

Appendix 9. Admix f3 results, testing if Teston is the results of admixture with Ancient South Ontario and other North America population.

A	B	C	f3	stderr	Zscore	nsnps
ASO	Kennewick	Moatfield	21.2	1.42	15	12346
ASO	Anzick	Moatfield	21.8	1.05	20.8	22931
ASO	USR	Moatfield	22	1.08	20.4	24168
ASO	Alaska_TCreek_9000BP	Moatfield	22.1	1.91	11.6	8344
ASO	Nevada_LlCave_1850BP	Moatfield	22	1.06	20.7	26641
ASO	Glacial Kame	Moatfield	22.2	1.15	19.3	21136
ASO	Cree	Moatfield	21.8	1.06	20.7	33374
ASO	Chipewyan	Moatfield	21.6	1.04	20.8	30525
ASO	Canada_400BP	Moatfield	17.3	2.11	8.21	2924
ASO	Canada_6000BP	Moatfield	20.5	1.72	11.9	7811
ASO	Thule	Moatfield	22.3	3.96	5.64	2051
ASO	LateDorset	Moatfield	20	1.71	11.7	7247
ASO	MiddleDorset	Moatfield	19.5	4.19	4.66	2064
ASO	Staines	Moatfield	21.9	1.3	16.9	16695
ASO	Teston	Moatfield	21.5	1.11	19.4	20969

Appendix 10. Admix f3 results, testing if Moatfield is the results of admixture between Ancient South Ontario and other North America population.

A	B	C	f3	stderr	Zscore	nsnps
ASO	Kennewick	Glacial Kame	2.22	0.0315	70.3	74644
ASO	Anzick	Glacial Kame	2.24	0.0282	79.4	138900
ASO	USR	Glacial Kame	2.27	0.0277	81.9	146212
ASO	Alaska_TCreek_9000BP	Glacial Kame	2.26	0.038	59.5	50100
ASO	Nevada_LlCave_1850BP	Glacial Kame	2.24	0.0253	88.3	158755
ASO	Cree	Glacial Kame	2.29	0.0252	90.7	197894
ASO	Chipewyan	Glacial Kame	2.29	0.0253	90.7	181605
ASO	Canada_400BP	Glacial Kame	2.34	0.0585	39.9	17806
ASO	Canada_6000BP	Glacial Kame	2.23	0.0399	55.9	46649
ASO	Thule	Glacial Kame	2.23	0.0711	31.4	12158
ASO	LateDorset	Glacial Kame	2.37	0.042	56.3	44085
ASO	MiddleDorset	Glacial Kame	2.32	0.0797	29.1	12214
ASO	Staines	Glacial Kame	2.24	0.0292	76.7	100281
ASO	Teston	Glacial Kame	2.24	0.0258	86.8	125892
ASO	Moatfield	Glacial Kame	2.25	0.0541	41.5	21136

Appendix 11. Admix f3 results, testing if Glacial Kame is the results of admixture between Ancient South Ontario and other North America population.

A	B	C	f3	stderr	Zscore	nsnps
ASO	Staines	Cree	0.0198	0.00233	8.49	155881
ASO	Moatfield	Cree	0.0207	0.00377	5.48	33374
ASO	Teston	Cree	0.019	0.00213	8.96	187346
ASO	Glacial Kame	Cree	0.0199	0.00245	8.1	197894

Appendix 12. Admix f3 results, testing whether modern Cree population is the admixture results of Ancient South Ontario with Huron-Wendat and Glacial Kame.

A	B	C	f3	stderr	Zscore	nsnps
ASO	Staines	Chipewyan	0.0813	0.0041	19.8	142757
ASO	Moatfield	Chipewyan	0.0773	0.00501	15.4	30525
ASO	Teston	Chipewyan	0.0804	0.00374	21.5	172816
ASO	Glacial Kame	Chipewyan	0.0809	0.00377	21.5	181605

Appendix 13. Admix f3 results, testing whether modern Chipewyan population is the admixture results of Ancient South Ontario with Huron-Wendat and Glacial Kame.

A	B	C	f3	stderr	Zscore	nsnps
Glacial Kame	Staines	Cree	0.0234	0.00259	9.04	183264
Glacial Kame	Moatfield	Cree	0.0253	0.00355	7.13	39248
Glacial Kame	Teston	Cree	0.0231	0.00218	10.6	220536

Appendix 14. Admix f3 results, determining if modern Cree population is caused by the admixture between Glacial Kame and Huron-Wendat.

A	B	C	f3	stderr	Zscore	nsnps
Glacial Kame	Staines	Chipewyan	0.0854	0.00404	21.1	167771
Glacial Kame	Moatfield	Chipewyan	0.0809	0.00492	16.4	35869
Glacial Kame	Teston	Chipewyan	0.0859	0.00369	23.3	203526

Appendix 15. Admix f3 results, determining if modern Chipewyan population is caused by the admixture between Glacial Kame and Huron-Wendat.

A	B	C	f3	stderr	Zscore	nsnps
Staines	Saqqaq	Cree	-0.00228	0.00268	-0.852	194940
Moatfield	Saqqaq	Cree	-0.00191	0.00381	-0.502	37798
Teston	Saqqaq	Cree	-0.00095	0.00241	-0.392	234944
Glacial Kame	Saqqaq	Cree	-0.00184	0.00255	-0.72	248404

Appendix 16. Admix f3 results, determining if modern Cree population is caused by the admixture between Glacial Kame or Huron-Wendat with Saqqaq.

A	B	C	f3	stderr	Zscore	nsnps
Staines	LateDorset	Cree	-0.00727	0.00324	-2.25	61269
Moatfield	LateDorset	Cree	-0.00433	0.00529	-0.819	12969
Teston	LateDorset	Cree	-0.00269	0.00295	-0.912	73384
Glacial Kame	LateDorset	Cree	-0.00491	0.00299	-1.64	77454

Appendix 17. Admix f3 results, determining if modern Cree population is caused by the admixture between Glacial Kame or Huron-Wendat with Late Dorset.

A	B	C	f3	stderr	Zscore	nsnps
Staines	MiddleDorset	Cree	-0.0132	0.0046	-2.87	16590
Moatfield	MiddleDorset	Cree	-0.0325	0.00995	-3.27	3604
Teston	MiddleDorset	Cree	-0.0132	0.00417	-3.16	19896
Glacial Kame	MiddleDorset	Cree	-0.0131	0.00427	-3.06	21056

Appendix 18. Admix f3 results, determining if modern Cree population is caused by the admixture between Glacial Kame or Huron-Wendat with Middle Dorset.

A	B	C	f3	stderr	Zscore	nsnps
Staines	Thule	Cree	0.00346	0.00475	0.728	17007
Moatfield	Thule	Cree	-0.00533	0.00989	-0.539	3718
Teston	Thule	Cree	0.000113	0.00398	0.028	20473
Glacial Kame	Thule	Cree	0.00527	0.00427	1.23	21642

Appendix 19. Admix f3 results, determining if modern Cree population is caused by the admixture between Glacial Kame or Huron-Wendat with Thule.

A	B	C	f3	stderr	Zscore	nsnps
Staines	Aleut	Cree	-0.0158	0.00202	-7.8	271889
Moatfield	Aleut	Cree	-0.0128	0.00259	-4.95	53057
Teston	Aleut	Cree	-0.0166	0.00178	-9.29	322681
Glacial Kame	Aleut	Cree	-0.0148	0.00184	-8.06	344383

Appendix 20. Admix f3 results, determining if modern Cree population is caused by the admixture between Glacial Kame or Huron-Wendat with modern Aleut.

A	B	C	f3	stderr	Zscore	nsnps
Staines	Saqqaq	Chipewyan	0.05	0.00443	11.3	177395
Moatfield	Saqqaq	Chipewyan	0.0395	0.0054	7.32	34366
Teston	Saqqaq	Chipewyan	0.0515	0.00414	12.5	215493
Glacial Kame	Saqqaq	Chipewyan	0.051	0.00434	11.7	226464

Appendix 21. Admix f3 results, determining if modern Chipewyan population is caused by the admixture between Glacial Kame or Huron-Wendat with Saqqaq.

A	B	C	f3	stderr	Zscore	nsnps
Staines	LateDorset	Chipewyan	0.0457	0.00456	10	56270
Moatfield	LateDorset	Chipewyan	0.038	0.00692	5.49	11900
Teston	LateDorset	Chipewyan	0.0521	0.00432	12.1	67915
Glacial Kame	LateDorset	Chipewyan	0.0489	0.00454	10.8	71222

Appendix 22. Admix f3 results, determining if modern Chipewyan population is caused by the admixture between Glacial Kame or Huron-Wendat with Late Dorset.

A	B	C	f3	stderr	Zscore	nsnps
Staines	MiddleDorset	Chipewyan	0.0509	0.00722	7.05	15332
Moatfield	MiddleDorset	Chipewyan	0.0243	0.0124	1.95	3335
Teston	MiddleDorset	Chipewyan	0.054	0.00588	9.19	18500
Glacial Kame	MiddleDorset	Chipewyan	0.0527	0.00606	8.69	19472

Appendix 23. Admix f3 results, determining if modern Chipewyan population is caused by the admixture between Glacial Kame or Huron-Wendat with Middle Dorset.

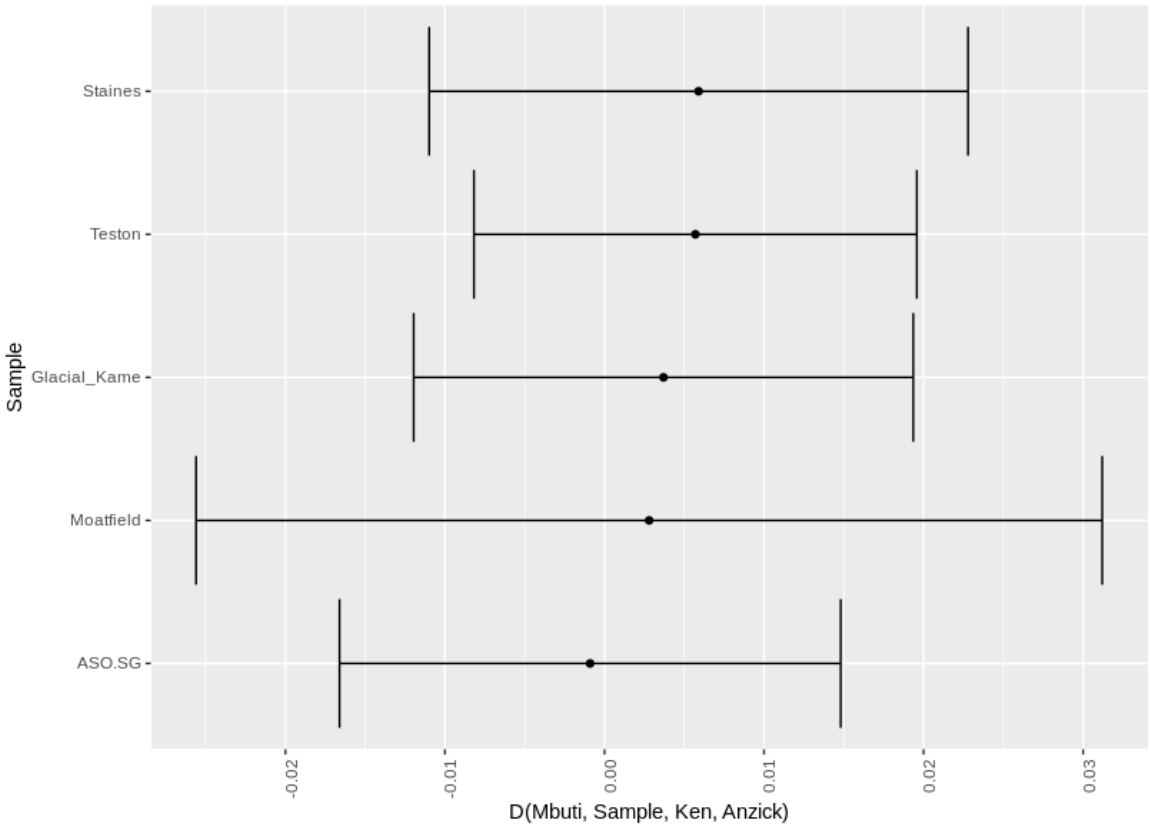
A	B	C	f3	stderr	Zscore	nsnps
Staines	Thule	Chipewyan	0.0608	0.00712	8.54	15358
Moatfield	Thule	Chipewyan	0.0535	0.0143	3.73	2982
Teston	Thule	Chipewyan	0.0602	0.00614	9.8	18595
Glacial Kame	Thule	Chipewyan	0.0609	0.00626	9.72	19466

Appendix 24. Admix f3 results, determining if modern Chipewyan population is caused by the admixture between Glacial Kame or Huron-Wendat with Thule.

A	B	C	f3	stderr	Zscore	nsnps
Staines	Aleut	Chipewyan	0.0497	0.00369	13.4	267128
Moatfield	Aleut	Chipewyan	0.0438	0.00407	10.8	52165
Teston	Aleut	Chipewyan	0.049	0.00337	14.6	317134
Glacial Kame	Aleut	Chipewyan	0.0513	0.00351	14.6	338397

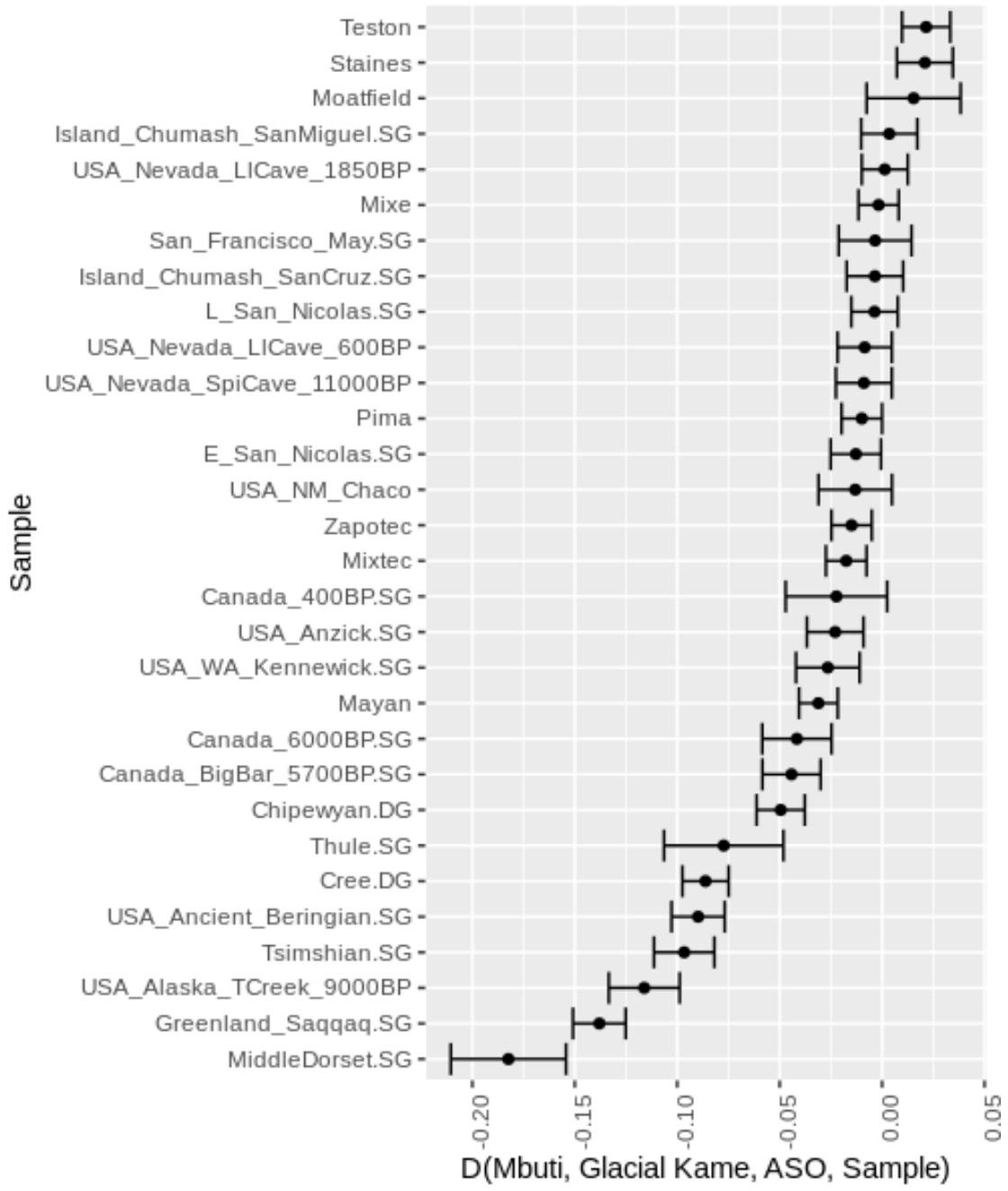
Appendix 25. Admix f3 results, determining if modern Chipewyan population is caused by the admixture between Glacial Kame or Huron-Wendat with modern Aleut.

10.3. D-Statistic



Outgroup	X	A	B	D-Stat	stderr	Zscore	BABA	ABBA	nsnps
Mbuti	Staines	Kennewick	Anzick	0.0059	0.00844	0.694	10410	10289	232596
Mbuti	Teston	Kennewick	Anzick	0.0057	0.00694	0.823	12155	12017	273352
Mbuti	Moatfield	Kennewick	Anzick	0.0028	0.0142	0.195	2056	2044	45673
Mbuti	Glacial_Kame	Kennewick	Anzick	0.0037	0.00783	0.467	12984	12890	291087
Mbuti	ASO.SG	Kennewick	Anzick	-0.0009	0.00785	-0.117	10796	10815	248432

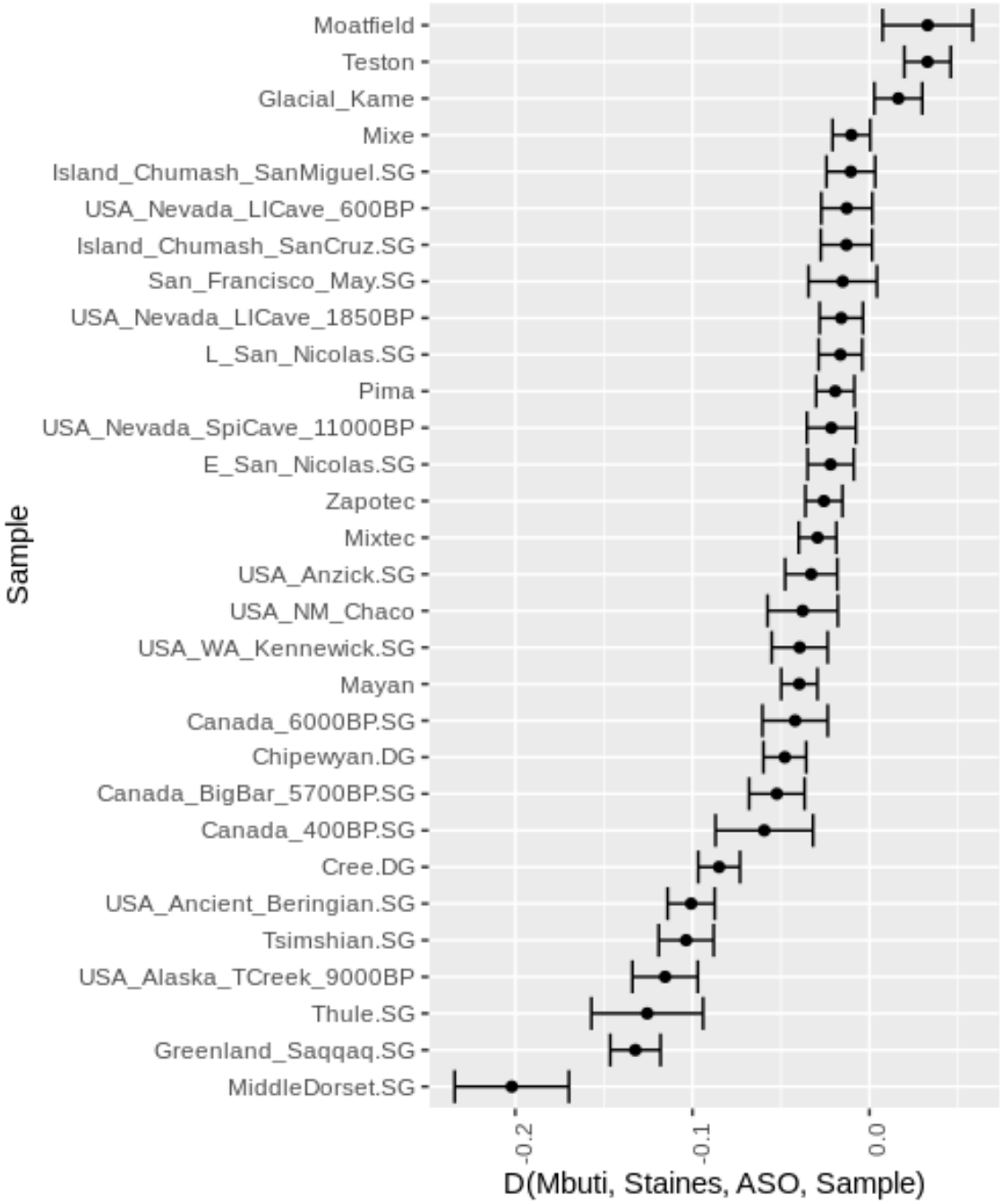
Appendix 26. D-statistic results. D(Mbuti, ancient Great Lake population, Kennewick, Anzick).



Appendix 27. D-statistic restuls of Glacial Kame and Ancient South Ontario with other Northern American population. Value below 0 suggests admixture between Glacial Kame and Ancient South Ontario and value above 0 suggests admixture between Glacial Kame and the sample population.

Outgroup	X	A	B	D-Stat	stderr	Zscore	BABA	ABBA	nsnps
Mbuti	Glacial Kame	ASO	Greenland Saqqaq	-0.138	0.006433	-21.457	16705	22055	402517
Mbuti	Glacial Kame	ASO	Cree	-0.0862	0.00564	-15.29	17540	20851	414124
Mbuti	Glacial Kame	ASO	Ancient Beringian	-0.0898	0.006487	-13.848	17988	21538	419506
Mbuti	Glacial Kame	ASO	Alaska TCreek 9000BP	-0.1161	0.00864	-13.44	6040	7627	147073
Mbuti	Glacial Kame	ASO	Tsimshian	-0.0966	0.007364	-13.119	17803	21610	419398
Mbuti	Glacial Kame	ASO	MiddleDorset	-0.1824	0.014058	-12.973	1414	2045	33989
Mbuti	Glacial Kame	ASO	Chipewyan	-0.0495	0.005879	-8.417	17732	19578	414124
Mbuti	Glacial Kame	ASO	Mayan	-0.0311	0.004732	-6.578	18248	19421	419514
Mbuti	Glacial Kame	ASO	Canada BigBar 5700BP	-0.0442	0.007111	-6.218	12412	13560	290648
Mbuti	Glacial Kame	ASO	Thule	-0.0773	0.014564	-5.309	1510	1764	35676
Mbuti	Glacial Kame	ASO	Canada 6000BP	-0.0416	0.008447	-4.922	6064	6590	140274
Mbuti	Glacial Kame	ASO	Mixtec	-0.0175	0.004957	-3.531	18369	19024	419514
Mbuti	Glacial Kame	ASO	Kennewick	-0.0265	0.007764	-3.413	9784	10317	227778
Mbuti	Glacial Kame	ASO	Anzick	-0.0229	0.0069	-3.315	18469	19334	418596
Mbuti	Glacial Kame	ASO	Zapotec	-0.0149	0.004919	-3.037	18371	18928	419514
Mbuti	Glacial Kame	ASO	E San Nicolas	-0.0128	0.006148	-2.087	18017	18485	413925
Mbuti	Glacial Kame	ASO	Pima	-0.0099	0.004942	-2.001	18372	18740	419514
Mbuti	Glacial Kame	ASO	Canada 400BP	-0.0223	0.012383	-1.801	2346	2453	55731
Mbuti	Glacial Kame	ASO	NM Chaco	-0.0131	0.008956	-1.466	4080	4188	93621
Mbuti	Glacial Kame	ASO	Nevada SpiCave 11000BP	-0.0089	0.006814	-1.303	18288	18616	419493
Mbuti	Glacial Kame	ASO	Nevada LlCave 600BP	-0.0085	0.006617	-1.28	18375	18689	419489
Mbuti	Glacial Kame	ASO	L San Nicolas	-0.0037	0.005664	-0.661	17946	18081	410470
Mbuti	Glacial Kame	ASO	Island Chumash SanCruz	-0.0035	0.006887	-0.508	17561	17684	399192
Mbuti	Glacial Kame	ASO	San Francisco May	-0.0034	0.008879	-0.386	4896	4930	112706
Mbuti	Glacial Kame	ASO	Mixe	-0.0017	0.004911	-0.352	18450	18514	419514
Mbuti	Glacial Kame	ASO	Nevada LlCave 1850BP	0.0013	0.005608	0.224	18519	18473	419504
Mbuti	Glacial Kame	ASO	Island Chumash SanMiguel	0.0035	0.006851	0.511	16097	15984	368737
Mbuti	Glacial Kame	ASO	Moatfield	0.0154	0.011462	1.344	2571	2493	59317
Mbuti	Glacial Kame	ASO	Staines	0.0209	0.006825	3.065	13329	12783	303685
Mbuti	Glacial Kame	ASO	Teston	0.0215	0.005883	3.655	15703	15042	358483

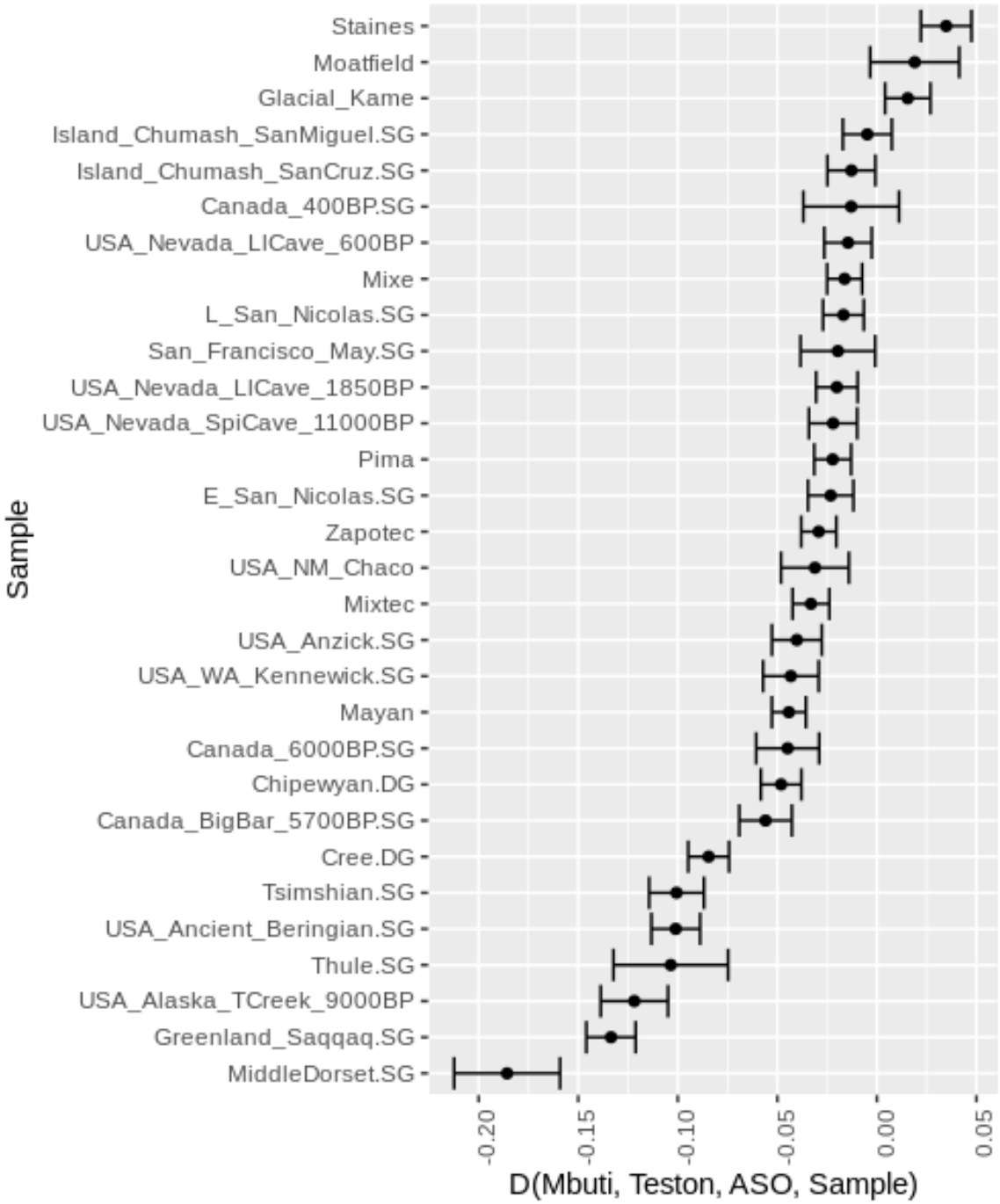
Appendix 28. D-statistic results of Glacial Kame and Ancient South Ontario with other Northern American population. |Z| > 3 and |Z| < -3 is consider significant. Value in red is |Z| < -3 and value in blue represent |Z| > 3.



Appendix 29. D-statistic restuls of Staines and Ancient South Ontario with other Northern American population. Value below 0 suggests admixture between Staines and Ancient South Ontario and value above 0 suggests admixture between Staines and the sample population.

Outgroup	X	A	B	D-Stat	stderr	Zscore	BABA	ABBA	nsnps
Mbuti	Staines	ASO	Greenland Saqqaq	-0.1322	0.007102	-18.617	13331	17394	319298
Mbuti	Staines	ASO	Ancient Beringian	-0.1007	0.006643	-15.16	14143	17311	332777
Mbuti	Staines	ASO	Cree.DG	-0.0849	0.005899	-14.393	13927	16512	328406
Mbuti	Staines	ASO	Tsimshian	-0.1035	0.007759	-13.343	14037	17279	332691
Mbuti	Staines	ASO	MiddleDorset	-0.202	0.016124	-12.526	1058	1593	26907
Mbuti	Staines	ASO	Alaska TCreek 9000BP	-0.1154	0.009231	-12.5	4836	6097	117500
Mbuti	Staines	ASO	Thule	-0.1255	0.01577	-7.96	1152	1483	28304
Mbuti	Staines	ASO	Chipewyan.DG	-0.0478	0.006044	-7.916	14075	15489	328409
Mbuti	Staines	ASO	Mayan	-0.0396	0.005112	-7.742	14327	15508	332784
Mbuti	Staines	ASO	Canada BigBar 5700BP	-0.0523	0.007833	-6.683	9823	10908	231779
Mbuti	Staines	ASO	Mixtec	-0.0293	0.005327	-5.508	14409	15280	332784
Mbuti	Staines	ASO	WA Kennewick	-0.0394	0.0079	-4.982	7694	8324	182544
Mbuti	Staines	ASO	Zapotec	-0.0257	0.005224	-4.921	14393	15153	332784
Mbuti	Staines	ASO	Canada 6000BP	-0.042	0.009216	-4.556	4811	5233	111780
Mbuti	Staines	ASO	Anzick	-0.0329	0.007355	-4.471	14457	15440	332064
Mbuti	Staines	ASO	Canada 400BP	-0.0594	0.013758	-4.318	1801	2029	44370
Mbuti	Staines	ASO	NM Chaco	-0.0377	0.009922	-3.795	3152	3399	74926
Mbuti	Staines	ASO	Pima	-0.0193	0.00539	-3.589	14415	14984	332784
Mbuti	Staines	ASO	E San Nicolas	-0.0219	0.006514	-3.36	14151	14784	328488
Mbuti	Staines	ASO	Nevada SpiCave 11000BP	-0.0215	0.006906	-3.115	14292	14921	332768
Mbuti	Staines	ASO	L San Nicolas	-0.0164	0.006145	-2.671	14040	14509	325791
Mbuti	Staines	ASO	Nevada LlCave 1850BP	-0.0159	0.006115	-2.6	14399	14864	332776
Mbuti	Staines	ASO	Mixe	-0.0102	0.005304	-1.922	14487	14785	332784
Mbuti	Staines	ASO	Island Chumash SanCruz	-0.013	0.007239	-1.797	13748	14111	317182
Mbuti	Staines	ASO	Nevada LlCave 600BP	-0.0128	0.007197	-1.775	14574	14951	332761
Mbuti	Staines	ASO	San Francisco May	-0.0151	0.009667	-1.562	3910	4030	90041
Mbuti	Staines	ASO	Island Chumash SanMiguel	-0.0105	0.006876	-1.533	12600	12869	292947
Mbuti	Staines	ASO	Glacial Kame	0.0163	0.006766	2.404	13329	12902	303685
Mbuti	Staines	ASO	Moatfield	0.0329	0.012733	2.584	2130	1994	47925
Mbuti	Staines	ASO	Teston	0.0328	0.006546	5.017	12672	11867	285543

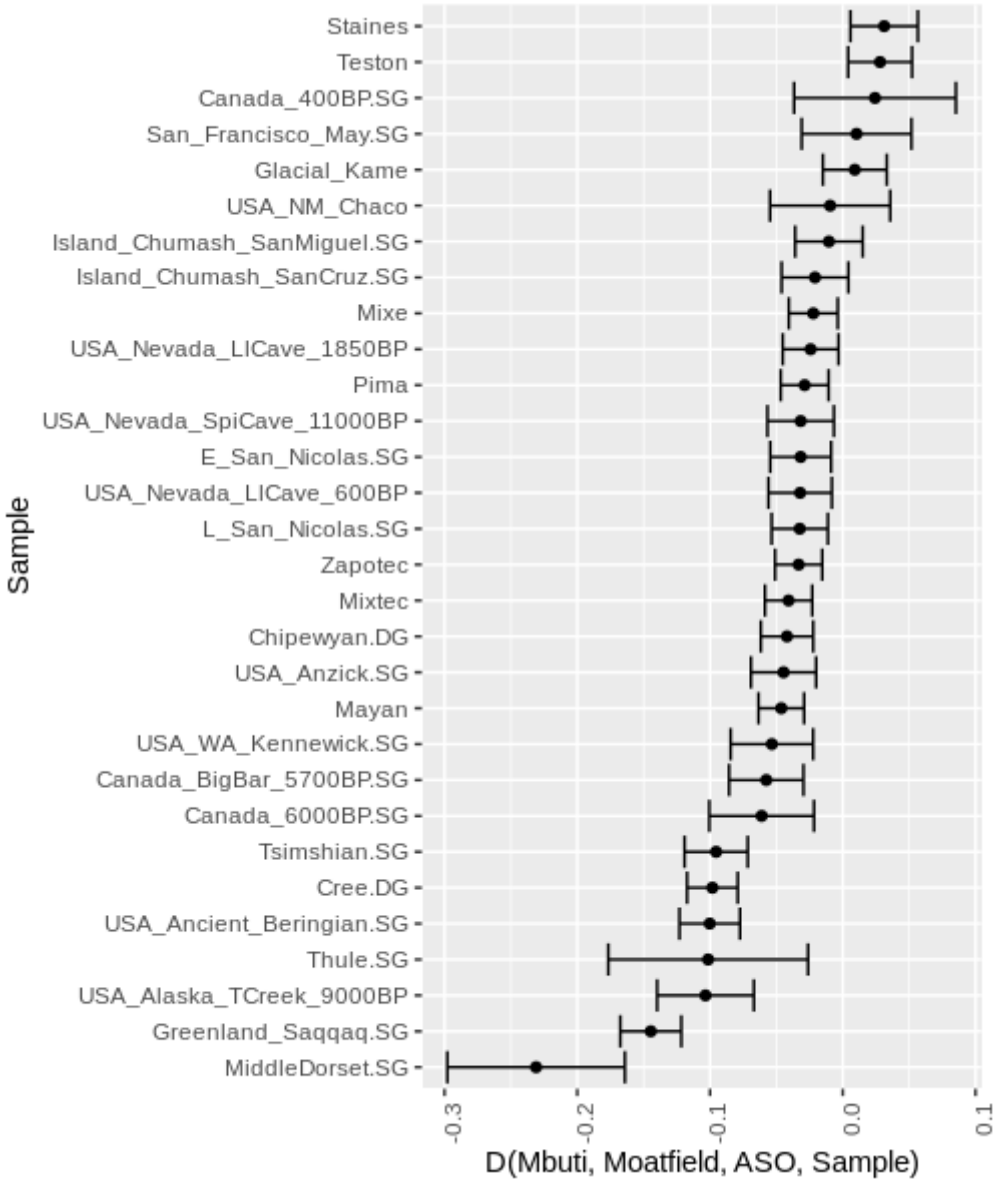
Appendix 30. D-statistic results of Staines and Ancient South Ontario with other Northern American population. |Z| > 3 and |Z| < -3 is consider significant. Value in red is |Z| < -3 and value in blue represent |Z| > 3.



Appendix 31. D-statistic restuls of Teston and Ancient South Ontario with other Northern American population. Value below 0 suggests admixture between Teston and Ancient South Ontario and value above 0 suggests admixture between Teston and the sample population.

Outgroup	X	A	B	D-Stat	stderr	Zscore	BABA	ABBA	nsnps
Mbuti	Teston	ASO	Greenland Saqqaq	-0.1335	0.006198	-21.539	15744	20596	377248
Mbuti	Teston	ASO	Ancient Beringian	-0.101	0.006112	-16.525	16609	20341	393077
Mbuti	Teston	ASO	Cree	-0.0845	0.005121	-16.507	16490	19536	387984
Mbuti	Teston	ASO	Tsimshian	-0.1006	0.00688	-14.622	16606	20320	392969
Mbuti	Teston	ASO	Alaska TCreek 9000BP	-0.1218	0.00845	-14.412	5647	7213	138104
Mbuti	Teston	ASO	MiddleDorset	-0.1857	0.013293	-13.969	1297	1889	31835
Mbuti	Teston	ASO	Mayan	-0.0442	0.004274	-10.35	16851	18411	393084
Mbuti	Teston	ASO	Chipewyan	-0.0481	0.005089	-9.445	16641	18322	387982
Mbuti	Teston	ASO	Canada BigBar 5700BP	-0.0559	0.006606	-8.457	11519	12882	272821
Mbuti	Teston	ASO	Mixtec	-0.0331	0.004604	-7.196	16932	18092	393084
Mbuti	Teston	ASO	Thule	-0.1035	0.014386	-7.193	1409	1735	33525
Mbuti	Teston	ASO	Zapotec	-0.0292	0.004399	-6.627	16938	17956	393084
Mbuti	Teston	ASO	Anzick	-0.0402	0.006259	-6.426	16915	18333	392214
Mbuti	Teston	ASO	WA Kennewick	-0.0432	0.006989	-6.188	9013	9828	214058
Mbuti	Teston	ASO	Canada 6000BP	-0.0448	0.007908	-5.661	5680	6213	131769
Mbuti	Teston	ASO	Pima	-0.0222	0.004632	-4.794	16992	17764	393084
Mbuti	Teston	ASO	E San Nicolas	-0.0232	0.005699	-4.066	16694	17486	387906
Mbuti	Teston	ASO	Nevada LlCave 1850BP	-0.0201	0.005221	-3.859	16962	17660	393072
Mbuti	Teston	ASO	Mixe	-0.0162	0.004398	-3.679	17006	17566	393084
Mbuti	Teston	ASO	NM Chaco	-0.0311	0.008519	-3.649	3758	3999	87875
Mbuti	Teston	ASO	Nevada SpiCave 11000BP	-0.022	0.006056	-3.626	16889	17647	393064
Mbuti	Teston	ASO	L San Nicolas	-0.0168	0.005122	-3.274	16588	17154	384660
Mbuti	Teston	ASO	Nevada LlCave 600BP	-0.0145	0.005954	-2.439	17139	17644	393064
Mbuti	Teston	ASO	Island Chumash SanCruz	-0.0128	0.006023	-2.127	16270	16692	374290
Mbuti	Teston	ASO	San Francisco May	-0.0196	0.009372	-2.094	4534	4716	105876
Mbuti	Teston	ASO	Canada 400BP	-0.0129	0.012008	-1.071	2254	2312	52497
Mbuti	Teston	ASO	Island Chumash SanMiguel	-0.0048	0.006147	-0.777	14958	15102	345761
Mbuti	Teston	ASO	Moatfield	0.019	0.011182	1.698	2454	2363	55909
Mbuti	Teston	ASO	Glacial Kame	0.0155	0.005693	2.72	15703	15224	358483
Mbuti	Teston	ASO	Staines	0.0348	0.006358	5.481	12672	11819	285543

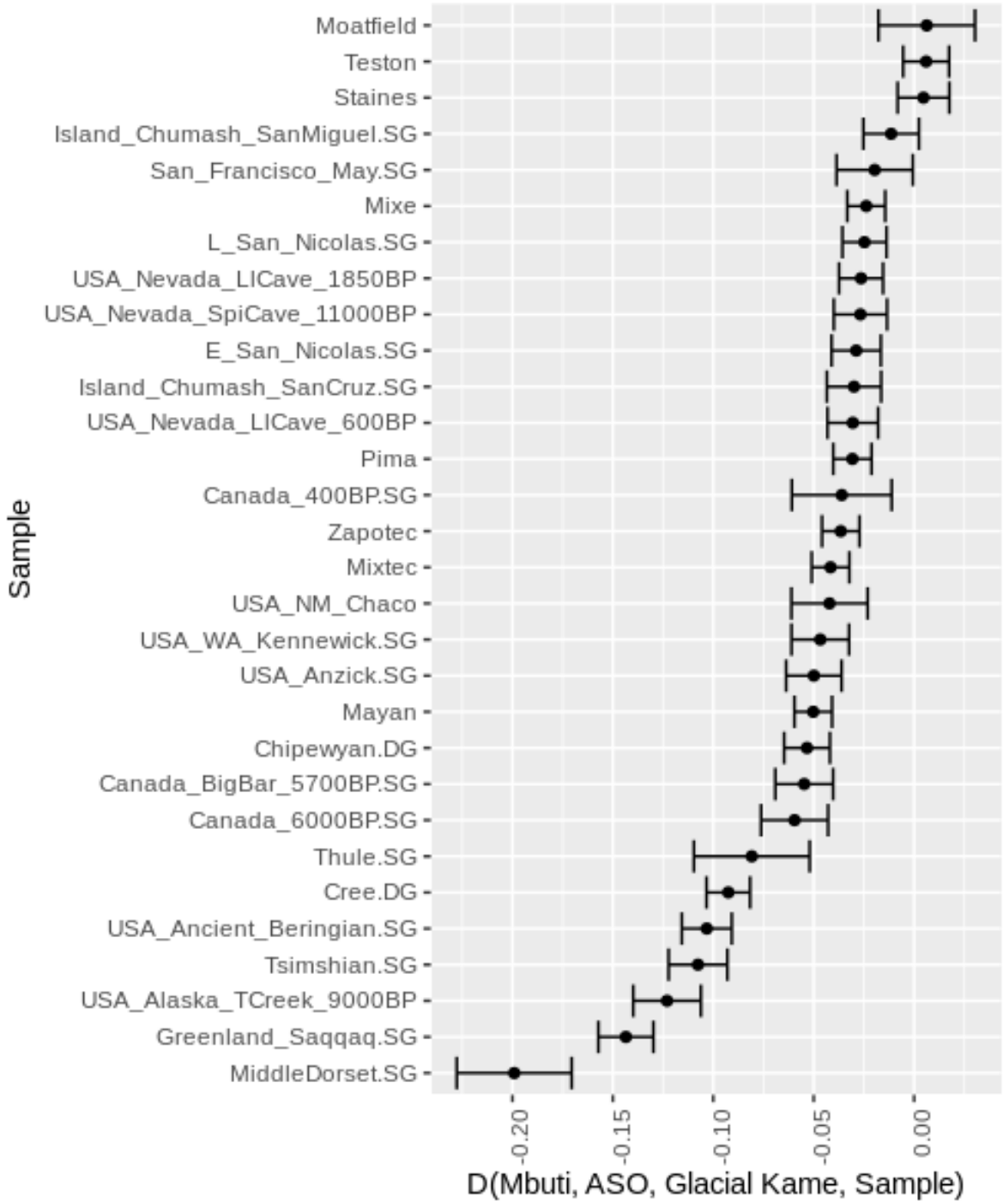
Appendix 32. D-statistic results of Teston and Ancient South Ontario with other Northern American population. |Z| > 3 and |Z| < -3 is consider significant. Value in red is |Z| < -3 and value in blue represent |Z| > 3.



Appendix 33. D-statistic restuls of Moatfield and Ancient South Ontario with other Northern American population. Value below 0 suggests admixture between Moatfield and Ancient South Ontario and value above 0 suggests admixture between Moatfield and the sample population.

Outgroup	X	A	B	D-Stat	stderr	Zscore	BABA	ABBA	nsnps
Mbuti	Moatfield	ASO	Greenland Saqqaq	-0.1446	0.011493	-12.578	2585	3459	62383
Mbuti	Moatfield	ASO	Cree	-0.0982	0.009533	-10.297	2672	3254	64196
Mbuti	Moatfield	ASO	Ancient Beringian	-0.1002	0.011399	-8.788	2740	3350	65065
Mbuti	Moatfield	ASO	Tsimshian	-0.0954	0.011898	-8.014	2776	3362	65047
Mbuti	Moatfield	ASO	MiddleDorset	-0.231	0.033441	-6.909	201	322	5340
Mbuti	Moatfield	ASO	Alaska TCreek 9000BP	-0.1034	0.018209	-5.677	992	1221	22894
Mbuti	Moatfield	ASO	Mayan	-0.0463	0.008588	-5.387	2787	3057	65065
Mbuti	Moatfield	ASO	Mixtec	-0.0408	0.008909	-4.58	2780	3017	65065
Mbuti	Moatfield	ASO	Chipewyan	-0.042	0.009863	-4.254	2761	3003	64197
Mbuti	Moatfield	ASO	Canada BigBar 5700BP	-0.0576	0.014004	-4.115	1896	2128	45388
Mbuti	Moatfield	ASO	Zapotec	-0.0332	0.008876	-3.736	2788	2979	65065
Mbuti	Moatfield	ASO	Anzick	-0.0446	0.012273	-3.632	2811	3073	64909
Mbuti	Moatfield	ASO	WA Kennewick	-0.0534	0.015535	-3.44	1491	1660	35738
Mbuti	Moatfield	ASO	Pima	-0.0287	0.009015	-3.186	2790	2955	65065
Mbuti	Moatfield	ASO	Canada 6000BP	-0.0611	0.01971	-3.102	942	1064	22034
Mbuti	Moatfield	ASO	L San Nicolas	-0.0324	0.010593	-3.061	2710	2892	63692
Mbuti	Moatfield	ASO	E San Nicolas	-0.0317	0.01145	-2.772	2722	2900	64184
Mbuti	Moatfield	ASO	Thule	-0.1014	0.037617	-2.695	229	281	5589
Mbuti	Moatfield	ASO	Nevada LlCave 600BP	-0.0321	0.011937	-2.692	2763	2947	65064
Mbuti	Moatfield	ASO	Nevada SpiCave 11000BP	-0.0317	0.012514	-2.532	2758	2938	65064
Mbuti	Moatfield	ASO	Mixe	-0.0222	0.009214	-2.409	2796	2923	65065
Mbuti	Moatfield	ASO	Nevada LlCave 1850BP	-0.0242	0.010503	-2.304	2777	2915	65064
Mbuti	Moatfield	ASO	Island Chumash SanCruz	-0.0209	0.012574	-1.659	2681	2795	62127
Mbuti	Moatfield	ASO	Island Chumash SanMiguel	-0.0104	0.012717	-0.814	2466	2517	57395
Mbuti	Moatfield	ASO	NM Chaco	-0.0095	0.022637	-0.42	657	670	14688
Mbuti	Moatfield	ASO	San Francisco May	0.0104	0.02067	0.502	786	770	17785
Mbuti	Moatfield	ASO	Glacial Kame	0.0091	0.012035	0.753	2571	2525	59317
Mbuti	Moatfield	ASO	Canada 400BP	0.0243	0.030473	0.796	378	360	8646
Mbuti	Moatfield	ASO	Teston	0.0281	0.012037	2.335	2454	2320	55909
Mbuti	Moatfield	ASO	Staines	0.0312	0.012691	2.462	2130	2001	47925

Appendix 34. D-statistic results of Moatfield and Ancient South Ontario with other Northern American population. |Z| > 3 and |Z| < -3 is consider significant. Value in red is |Z| < -3 and value in blue represent |Z| > 3.



Appendix 35. D-statistic restuls of Ancient South Ontario and Glacial Kame with other Northern American population. Value below 0 suggests admixture between Ancient South Ontario and Glacial Kame and value above 0 suggests admixture between Ancient South Ontario and the sample population.

Outgroup	X	A	B	D-Stat	stderr	Zscore	BABA	ABBA	nsnps
Mbuti	ASO	Glacial Kame	Greenland Saqqaq	-0.1435	0.006851	-20.943	2585	3459	62383
Mbuti	ASO	Glacial Kame	Cree	-0.0925	0.005423	-17.051	2672	3254	64196
Mbuti	ASO	Glacial Kame	Ancient Beringian	-0.1032	0.006219	-16.592	2740	3350	65065
Mbuti	ASO	Glacial Kame	Tsimshian	-0.1076	0.007309	-14.72	2776	3362	65047
Mbuti	ASO	Glacial Kame	Alaska TCreek 9000BP	-0.123	0.008422	-14.61	201	322	5340
Mbuti	ASO	Glacial Kame	MiddleDorset	-0.1991	0.014301	-13.919	992	1221	22894
Mbuti	ASO	Glacial Kame	Mayan	-0.0502	0.004685	-10.719	2787	3057	65065
Mbuti	ASO	Glacial Kame	Chipewyan	-0.0533	0.0057	-9.353	2780	3017	65065
Mbuti	ASO	Glacial Kame	Mixtec	-0.0416	0.004685	-8.888	2761	3003	64197
Mbuti	ASO	Glacial Kame	Zapotec	-0.0365	0.004669	-7.808	1896	2128	45388
Mbuti	ASO	Glacial Kame	Canada BigBar 5700BP	-0.0547	0.007197	-7.604	2788	2979	65065
Mbuti	ASO	Glacial Kame	Anzick	-0.0499	0.006899	-7.237	2811	3073	64909
Mbuti	ASO	Glacial Kame	Canada 6000BP	-0.0595	0.008337	-7.134	1491	1660	35738
Mbuti	ASO	Glacial Kame	WA Kennewick	-0.0467	0.007169	-6.509	2790	2955	65065
Mbuti	ASO	Glacial Kame	Pima	-0.0307	0.004772	-6.433	942	1064	22034
Mbuti	ASO	Glacial Kame	Thule	-0.0808	0.014411	-5.607	2710	2892	63692
Mbuti	ASO	Glacial Kame	Mixe	-0.0238	0.004724	-5.04	2722	2900	64184
Mbuti	ASO	Glacial Kame	Nevada LlCave 600BP	-0.0305	0.006301	-4.835	229	281	5589
Mbuti	ASO	Glacial Kame	Nevada LlCave 1850BP	-0.0264	0.005472	-4.829	2763	2947	65064
Mbuti	ASO	Glacial Kame	E San Nicolas	-0.0288	0.006133	-4.697	2758	2938	65064
Mbuti	ASO	Glacial Kame	L San Nicolas	-0.0247	0.005482	-4.508	2796	2923	65065
Mbuti	ASO	Glacial Kame	NM Chaco	-0.0421	0.00949	-4.436	2777	2915	65064
Mbuti	ASO	Glacial Kame	Island Chumash SanCruz	-0.0299	0.006757	-4.427	2681	2795	62127
Mbuti	ASO	Glacial Kame	Nevada SpiCave 11000BP	-0.0267	0.006626	-4.03	2466	2517	57395
Mbuti	ASO	Glacial Kame	Canada 400BP	-0.036	0.012428	-2.899	657	670	14688
Mbuti	ASO	Glacial Kame	San Francisco May	-0.0196	0.00949	-2.069	786	770	17785
Mbuti	ASO	Glacial Kame	Island Chumash SanMiguel	-0.0114	0.006911	-1.647	2571	2525	59317
Mbuti	ASO	Glacial Kame	Moatfield	0.0063	0.012026	0.528	378	360	8646
Mbuti	ASO	Glacial Kame	Staines	0.0047	0.006439	0.723	2454	2320	55909
Mbuti	ASO	Glacial Kame	Teston	0.006	0.005728	1.051	2130	2001	47925

Appendix 36. D-statistic results of Ancient South Ontario and Glacial Kame with other Northern American population. |Z| > 3 and |Z| < -3 is consider significant. Value in red is |Z| < -3 and value in blue represent |Z| > 3.

10.4. Maximum likelihood calculation

10.4.1. Haplogroup A2

	Rho			Maximum Likelihood		
Nodes	**Genetic distance**	**SEs**	**Age**	**Genetic distance**	**SEs**	**Age**
A2	6.594595	0.779483	17856.11	7.132677	0.533738	19392.33
A2ar	0.25	0.144338	642.0236	0.29113	0.169826	747.9269
A2+152	6.5	2.328699	17587.2	6.429114	2.159212	17385.92
A2+153!	3.333333	1.247219	8793.068	5.72555	2.377559	15398.88
A2+153!+3339	0.5	0.5	1286.949	0.752085	0.776346	1940.171
A2+16111!	5	1.274755	13370.48	6.065202	1.625474	16355.7
A2+16111!+7389	0.5	0.5	1286.949	0.679303	0.679303	1751.272
A2au	1.8	1	4686.605	1.528431	4.658075	3970.108
A2as	4.4	1.67332	11709.27	4.80364	1.601213	12825.19
A2as1	2	1.118034	5216.38	2.037908	1.285823	5316.993
A2at	2.538462	1.330124	6651.484	3.61486	1.21304	9558.079
A2at1	0.416667	0.220479	1071.652	0.412434	0.29113	1060.725
A2+64	6.486373	0.324413	17548.49	7.011373	0.363912	19045.03
A2av	0.5	0.353553	1286.949	0.630781	0.460955	1625.474
A2aw	2.5	1.118034	6548.554	4.051555	1.601213	10751.38
A2ax	4	1.178511	10610.09	4.80364	1.673996	12825.19
A2ax1	3.25	1.25	8567.269	3.032601	1.552692	7979.611
A2ax1+153!	1.666667	0.745356	4334.412	1.576952	0.776346	4097.883
A2ax2	2.5	1.118034	6548.554	3.226687	1.698256	8504.155
A2+64+150	1	0.745356	2585.428	1.140258	2.668689	2951.723
A2+64+150+14443	0.5	0.5	1286.949	0.436695	2.595906	1123.366
A2+64+152	4.75	0.790569	12676.51	5.652768	1.115997	15194.45
A2ay	3.333333	1.247219	8793.068	4.391206	1.528431	11685.03
A2+64+153!	5.823529	0.688512	15674.42	6.404853	0.921911	17317.07
A2az	2	1	5216.38	1.989386	1.018954	5188.221
A2bc	5	1.581139	13370.48	4.342684	1.528431	11551.36
A2ba	0.5	0.5	1286.949	0.509477	0.557999	1311.453
A2+64+16129	4.344828	0.951874	11557.26	5.677029	1.552692	15262.57
A2+64+16129+14971	0.5	0.5	1286.949	0.679303	4.779379	1751.272
A2bd	2.333333	1.20185	6103.274	1.455648	1.018954	3778.644
A2be	3.5	1	9245.555	5.167552	1.649735	13837.01
A2bf	2	1	5216.38	2.013647	0.946171	5252.594
A2bg	5.5	1.802776	14766.04	4.51251	1.261562	12019.64
A2+64+195	5	1.20185	13370.48	5.919637	1.771039	15945.07
A2bh	4.333333	1.452966	11525.61	4.949205	1.649735	13229.27
A2bi	0	0	0	0	4.463988	0

A2bj	1.666667	0.745356	4334.412	2.71721	1.431388	7130.693
A2bk	2.75	1.145644	7218.75	3.081122	1.722517	8110.595
A2bk1	2	1.054093	5216.38	2.11069	1.358605	5510.347
A2bl	6.75	1.887459	18298.64	6.429114	0.703563	17385.92
A2bl1	1.333333	0.666667	3457.413	1.334344	0.655042	3460.066
A2bm	1	0.745356	2585.428	1.043215	1.431388	2698.191
A2bn	4.5	1.5	11985.11	5.143291	1.407127	13769.38
A21	3.125	0.960143	8229.129	4.900683	1.892343	13094.48
A21a	1.833333	0.645497	4774.778	2.523124	1.50417	6610.43
A22	0.333333	0.333333	856.6771	0.412434	2.474602	1060.725
A23	1	0.745356	2585.428	1.50417	1.018954	3906.26
A23+73!	0	0	0	0	2.498863	0
A24	0.166667	0.166667	427.6924	0.218347	5.094769	560.5755
A25	4.142857	1.078548	11001.89	6.429114	2.207733	17385.92
A25a	0.5	0.5	1286.949	0.485216	0.800607	1248.73
A26	1.166667	0.763763	3020.791	0.873389	0.946171	2255.543
A26a	0.5	0.5	1286.949	0.29113	1.115997	747.9269
A2a	2.391304	0.549006	6258.016	1.843821	0.460955	4802.53
A2a7	0	0	0	0.412434	1.576952	1060.725
A2a8	0.444444	0.351364	1143.382	0.339651	0.31539	872.9644
A2a6	0.25	0.25	642.0236	0.29113	1.601213	747.9269
A2a9	1	0.707107	2585.428	0.970432	0.703563	2508.324
A2a1	0.25	0.25	642.0236	0.29113	0.29113	747.9269
A2a2	1.285714	0.820652	3332.536	1.21304	0.776346	3142.152
A2a2a	0	0	0	0	1.164519	0
A2a3	0.714286	0.377964	1842.037	0.752085	0.436695	1940.171
A2a4	0.142857	0.142857	366.5142	0.145565	0.145565	373.4703
A2a5	1.142857	0.597614	2958.519	0.776346	0.703563	2003.192
A2aa	2.875	0.875	7554.868	3.808947	1.285823	10087.44
A2aa1	2	1	5216.38	2.741471	1.261562	7195.842
A2aa2	1.5	1.06066	3895.289	1.50417	2.620167	3906.26
A2ab	3.25	1.030776	8567.269	3.517817	1.140258	9293.996
A2ab1	1.5	0.866025	3895.289	1.576952	0.970432	4097.883
A2ac	4.777778	1.222222	12753.49	4.585292	1.043215	12220.7
A2ac1	1.8	0.774597	4686.605	1.7953	0.946171	4674.176
A2ac1a	1	0.707107	2585.428	0.970432	0.727824	2508.324
A2ad	5.625	1.305038	15116.51	5.11903	0.921911	13701.79
A2ad1	1.333333	0.666667	3457.413	1.479909	1.115997	3842.439
A2ad2	3.5	1.322876	9245.555	3.129644	1.115997	8241.68
A2ae	3.25	1.299038	8567.269	2.474602	1.431388	6480.621
A2ae1	2	0.942809	5216.38	2.207733	2.862775	5768.517

A2af	8.807692	1.938019	24244.8	6.113723	0.727824	16492.76
A2af1	5.88	1.02528	15833.41	5.070509	0.727824	13566.66
A2af1a3	0	0	0	0	2.280516	0
A2af1a	4.066667	0.886942	10792.82	4.148598	0.752085	11017.65
A2af1a1	3.428571	0.808122	9051.487	3.542078	0.727824	9359.98
A2af1a2	0.5	0.5	1286.949	0.582259	0.557999	1499.784
A2af1b	6.1	1.24499	16453.98	4.706597	0.727824	12556.29
A2af1b3	0	0	0	0	3.420774	0
A2af1b1	3.6	1.131371	9517.614	2.935558	0.921911	7717.947
A2af1b2	1	0.707107	2585.428	0.824867	0.582259	2129.313
A2afb1a	0.5	0.5	1286.949	0.363912	0.339651	935.524
A2afb1b	0.5	0.5	1286.949	0.582259	0.873389	1499.784
A2ah	3	1	7891.662	3.760425	1.431388	9954.953
A2ai	2.333333	0.881917	6103.274	2.959818	1.067476	7783.325
A2aj	0.666667	0.471405	1718.501	1.091736	0.873389	2824.904
A2ak	5.333333	1.699673	14299.73	4.075816	1.140258	10817.91
A2al	4	1.414214	10610.09	4.148598	1.334344	11017.65
A2am	0.181818	0.128565	466.6377	0.218347	0.194086	560.5755
A2an	3.333333	1.414214	8793.068	3.881729	2.426081	10286.36
A2an+152	0	0	0	0	3.542078	0
A2ao	4.666667	1.414214	12445.76	4.585292	1.576952	12220.7
A2ao1	2	1	5216.38	2.280516	1.407127	5962.417
A2ap	3.6	1.296148	9517.614	5.482942	1.285823	14718.27
A2ap1	2.5	1.172604	6548.554	4.391206	1.431388	11685.03
A2aq	0.5	0.5	1286.949	0.485216	0.557999	1248.73
A2b	1.837209	0.952353	4785.033	1.334344	1.382866	3460.066
A2b1	0.95	0.217945	2455.066	0.849128	0.194086	2192.415
A2b1b	1.333333	0.816497	3457.413	0.582259	0.266869	1499.784
A2b1b+228	0.5	0.5	1286.949	0.266869	0.218347	685.4492
A2b1c	0.25	0.25	642.0236	0.242608	0.218347	622.9987
A2b1a	0.333333	0.333333	856.6771	0.194086	0.194086	498.1798
A2c	4.5	1.5	11985.11	5.11903	1.310084	13701.79
A2d	4.52381	1.00678	12050.84	4.269902	0.921911	11351.03
A2d1	3.363636	1.124483	8875.25	3.275209	1.067476	8635.543
A2d1b	2	1.054093	5216.38	2.086429	1.261562	5445.87
A2d1a	1.571429	0.820652	4083.332	1.334344	1.091736	3460.066
A2d2	2.666667	1.247219	6995.049	2.984079	1.358605	7848.728
A2f	8.055556	1.635826	22052.91	6.501896	0.557999	17592.59
A2f+676	4.2	1.428286	11158.84	4.51251	1.455648	12019.64
A2f1a	1.111111	0.544331	2875.53	1.115997	0.582259	2888.3
A2f1a2	1	0.707107	2585.428	0.655042	0.460955	1688.359

A2f1a1	0	0	0	0.266869	0.436695	685.4492
A2f2	3.25	1.089725	8567.269	3.129644	1.237301	8241.68
A2f2a	2	0.816497	5216.38	2.134951	1.018954	5574.851
A2f3	3.25	1.198958	8567.269	2.765732	1.043215	7261.015
A2f3a	2.666667	1.154701	6995.049	2.231994	1.018954	5833.124
A2g	3.090909	0.715819	8137.026	3.590599	0.994693	9492.021
A2g2	1	0.707107	2585.428	1.164519	0.776346	3015.172
A2g1	0	0	0	0	2.741471	0
A2h	6.307692	1.380335	17041.6	6.332071	0.873389	17110.68
A2h2	0.5	0.5	1286.949	0.557999	0.582259	1436.98
A2ay1	0	0	0	0	3.711903	0
A2h1	2.428571	0.936777	6357.571	2.523124	1.018954	6610.43
A2h1a	1.5	0.866025	3895.289	1.552692	0.824867	4033.983
A2h1b	0.5	0.353553	1286.949	0.533738	0.533738	1374.203
A2i	0.714286	0.319438	1842.037	0.752085	0.339651	1940.171
A2j	3.4	0.761577	8973.92	4.19712	1.115997	11150.93
A2j2	2	1	5216.38	2.620167	1.18878	6870.356
A2j3	2	1	5216.38	1.722517	0.873389	4481.842
A2j1	2	0.707107	5216.38	2.69295	1.18878	7065.571
A2k1	1.916667	0.989529	4995.425	3.081122	1.431388	8110.595
A2k1a	0.818182	0.374828	2111.929	0.752085	0.460955	1940.171
A2l	0.571429	0.202031	1471.743	0.679303	0.29113	1751.272
A2m	7.076923	1.287169	19232.64	5.313117	0.776346	14243.24
A2m2	3.6	1.624808	9517.614	3.105383	1.382866	8176.125
A2m1	5.2	1.574802	13927.49	4.852161	0.946171	12959.79
A2m1a	2.25	1.145644	5881.091	2.377559	1.50417	6221.312
A2m3	6.333333	1.666667	17114.26	4.22138	0.849128	11217.61
A2m3a	0.5	0.5	1286.949	0.582259	0.630781	1499.784
A2n	8.25	1.920286	22617.56	5.021987	0.89765	13431.63
A2n1	0.5	0.5	1286.949	0.31539	0.60652	810.432
A2n2	3	1.224745	7891.662	2.765732	1.285823	7261.015
A2o	2.5	0.957427	6548.554	2.814254	1.043215	7391.44
A2o1	0.666667	0.471405	1718.501	0.89765	0.655042	2318.698
A2p	3.307692	0.90691	8723.559	4.027294	1.261562	10684.88
A2p2	1.5	0.986013	3895.289	1.431388	1.916604	3714.875
A2p2a	0.8	0.632456	2064.662	0.655042	0.655042	1688.359
A2p1	1.75	0.829156	4554.44	1.698256	0.994693	4417.783
A2q	4.9	1.236932	13092.58	5.749811	1.479909	15467.08
A2q2	0	0	0	0	3.517817	0
A2q1	5.5	1.658312	14766.04	4.488249	1.358605	11952.67
A2r	2.5	0.866025	6548.554	3.105383	1.067476	8176.125

A2r1	1	0.57735	2585.428	1.091736	0.655042	2824.904
A2t	3.5	1.067187	9245.555	3.736164	1.115997	9888.745
A2t2	1.5	0.866025	3895.289	1.382866	0.752085	3587.417
A2t1	1	0.745356	2585.428	0.655042	3.833207	1688.359
A2u	6.818182	1.307954	18493.09	6.477635	0.679303	17523.67
A2u3	0	0	0	0	5.652768	0
A2u1	4.333333	1	11525.61	4.294163	1.091736	11417.79
A2u1a	3.5	1.322876	9245.555	2.984079	1.164519	7848.728
A2u1b	3	1.224745	7891.662	2.426081	1.043215	6350.915
A2u2	7	1.870829	19012.5	5.846854	0.946171	15740.08
A2v	4.642857	1.40153	12379.88	5.264595	2.013647	14107.73
A2v1	3.923077	1.127914	10399.47	4.294163	2.159212	11417.79
A2v1c	0.666667	0.471405	1718.501	0.727824	0.679303	1877.178
A2v1a	2.25	0.968246	5881.091	2.984079	1.018954	7848.728
A2v1b	3	1.224745	7891.662	2.911297	1.431388	7652.594
A2w	3.707317	0.606332	9810.053	2.838514	0.509477	7456.69
A2w4	0.5	0.5	1286.949	0.31539	0.436695	810.432
A2w5	0.5	0.5	1286.949	0.679303	0.655042	1751.272
A2w2	0	0	0	0	2.256255	0
A2w1	6.285714	1.26168	16979.34	2.71721	0.509477	7130.693
A2w1a1	4.857143	1.142857	12973.61	2.377559	0.557999	6221.312
A2w1b	2.75	1.030776	7218.75	1.50417	0.533738	3906.26
A2w1b1	1	0.707107	2585.428	0.509477	0.460955	1311.453
A2w1a	5.1	1.144552	13648.78	2.571646	0.533738	6740.342
A2w3	1	0.707107	2585.428	1.018954	0.655042	2634.875
A2x	3.333333	1.333333	8793.068	3.663382	1.455648	9690.271
A2y	0.5	0.204124	1286.949	0.533738	0.29113	1374.203
A2z	0.647059	0.256406	1667.664	0.557999	0.31539	1436.98

Appendix 37. Maximum likelihood and rho calculation of haplogroup A2 to estimate the age of each subclade.

10.4.2. Haplogroup C

	Rho			ML		
Nodes	**Genetic distance**	**SEs**	**Age**	**Genetic distance**	**SEs**	**Age**
C	9.53902	1.023137	26395.83	10.61822	1.564521	29604.79
C1	7.694639	0.788151	21008.58	9.515365	1.384986	26325.96
C1a	3.142857	1.195229	8277.394	3.411169	2.257014	9004.237
C1a+93	2.666667	0.971825	6995.049	2.693028	2.18007	7065.781
C1a2	2.5	0.935414	6548.554	2.103127	0.872028	5490.243
C1a2+9099A	2	1	5216.38	1.256746	0.718141	3256.62
C1a1	0	0	0	0.743789	0.769437	1918.627
C1b	6.152	0.480733	16600.95	7.566126	0.666845	20637.91
C1b+10586	5.333333	1.632993	14299.73	6.488915	1.128507	17555.71
C1b+10586+114	0.5	0.5	1286.949	0.641197	1.231098	1652.47
C1b+143	6.2	1.752459	16736.7	7.566126	3.411169	20637.91
C1b+143+16086	5.545455	2.059046	14893.41	6.155492	2.487845	16610.82
C1b15	3.25	1.25	8567.269	4.590971	2.872563	12236.4
C1b15+152	1	0.57735	2585.428	1.179803	1.718408	3055.158
C1b+146	5.769231	1.356553	15521.68	6.950577	2.154422	18871.18
C1b17	4.545455	1.164204	12110.63	5.668183	2.103127	15237.73
C1b17a	3.111111	0.87489	8191.6	2.923859	1.102859	7686.43
C1b17a1	3.75	1.25	9926.5	2.231366	0.923324	5831.451
C1b17a1+153	1.5	0.866025	3895.289	0.820732	0.461662	2118.561
C1b17a1+15728	1	0.707107	2585.428	1.128507	0.948972	2921
C1b17b	2.5	1.118034	6548.554	2.410901	1.615817	6310.359
C1b18	1	0.707107	2585.428	1.051563	1.025915	2719.986
C1b+152	3.5	1.322876	9245.555	5.69383	2.462197	15309.76
C1b+15758	5.5	1.767767	14766.04	6.30938	1.384986	17046.38
C1b+15758+1040	1.666667	1	4334.412	1.590169	2.282662	4132.705
C1b+15758+1040+9316	0.5	0.5	1286.949	0.384718	2.333958	989.1971
C1b19	8.6	1.886796	23637.45	7.566126	1.897943	20637.91
C1b19a	4.666667	1.490712	12445.76	4.154957	1.410634	11035.11
C1b19a+12717	0.5	0.5	1286.949	0.512958	0.538606	1320.454
C1b19b	3.5	1.322876	9245.555	4.129309	1.564521	10964.69
C1b+16189	5.333333	1.763834	14299.73	6.745394	3.411169	18285.51
C1b+16189+789	0	0	0	0	4.48838	0
C1b+16209	7.5	1.936492	20447.43	6.899281	1.641465	18724.61
C1b+16298!	7	1.870829	19012.5	6.822337	2.231366	18504.94
C1b+16311	6.409091	1.180944	17329.1	6.206788	1.667113	16755.9
C1b+16311+146	7.555556	2.119865	20607.45	5.899014	2.077479	15886.96
C1b+16362	7.888889	1.356284	21570.05	7.001873	1.487577	19017.86

C1b16	6	1.490712	16171.67	6.001605	1.538873	16176.19
C1b16+636	5.5	1.658312	14766.04	4.97569	1.538873	13302.88
C1b+16390	2	0.748331	5216.38	3.231633	1.256746	8517.544
C1b+16390+11150	0	0	0	0	10.90035	0
C1b+16390+6629	2	1	5216.38	2.205718	1.025915	5763.151
C1b+185	1.5	0.866025	3895.289	2.462197	1.308042	6447.45
C1b+195	3.5	1.322876	9245.555	5.69383	2.359605	15309.76
C1b+199	7.666667	1.732051	20927.85	7.566126	1.923591	20637.91
C1b+199+9	5.5	1.658312	14766.04	5.899014	1.718408	15886.96
C1b+199+9	2	1	5216.38	2.744324	1.795352	7203.503
C1b+214	3.5	1.322876	9245.555	3.744591	1.461929	9911.739
C1b+235	8	2	21891.84	5.591239	1.000268	15021.79
C1b+4242	2.25	1.339396	5881.091	3.411169	1.205451	9004.237
C1b+485	0	0	0	0	3.257281	0
C1b+6278	1	0.707107	2585.428	0.97462	0.666845	2519.241
C1b+7211	0.666667	0.471405	1718.501	1.000268	1.410634	2586.126
C1b+9686	0	0	0	1.33369	1.231098	3458.349
C1b20	3.5	1.322876	9245.555	4.616619	2.051831	12307.31
C1b1	4.2	1.341641	11158.84	5.745126	1.487577	15453.91
C1b1a	1.333333	0.816497	3457.413	1.615817	1.102859	4200.304
C1b1a+8392	0.5	0.5	1286.949	0.564253	0.564253	1453.17
C1b1b	2	1	5216.38	2.66738	1.410634	6996.963
C1b10	4.125	1.280869	10952.86	3.488112	1.33369	9213.241
C1b10+146!	0.5	0.5	1286.949	0.384718	0.35907	989.1971
C1b10+152	1	0.447214	2585.428	1.256746	0.743789	3256.62
C1b11	4.481481	1.68793	11934	6.283732	1.590169	16973.72
C1b11+194	3.461538	1.437042	9141.029	5.129577	2.128774	13731.17
C1b11b	2	1.320531	5216.38	3.436817	1.564521	9073.877
C1b11b+15924	1.047619	0.954759	2709.689	2.564788	1.128507	6721.976
C1b11b+15924+7757	0.05	0.05	128.1717	0.051296	0.051296	131.4949
C1b11a	5	1.802776	13370.48	4.206253	2.282662	11176.03
C1b11a+6086	1	0.745356	2585.428	0.769437	3.077746	1985.241
C1b11a+6086+16249	0.5	0.5	1286.949	0.333423	0.333423	856.907
C1b12	1.5	0.687184	3895.289	3.077746	1.487577	8101.477
C1b12+11419	0.5	0.5	1286.949	1.384986	1.436282	3592.983
C1b12+16509	0	0	0	0	3.411169	0
C1b13	4.409091	0.666494	11734.33	5.206521	0.84638	13945.67
C1b13a	5.833333	1.364225	15702.02	4.770507	0.872028	12733.34
C1b13a+15482	0	0	0	0	4.693563	0
C1b13a1	5.25	1.198958	14066.99	3.949774	0.872028	10472.54
C1b13a1+14587	3	1.224745	7891.662	3.052098	1.282394	8032.232

C1b13b	3.666667	1.105542	9699.224	3.693295	1.025915	9771.817
C1b13c	2.2	0.87178	5747.928	3.077746	1.33369	8101.477
C1b13c+5775	1	0.707107	2585.428	1.384986	1.205451	3592.983
C1b13c1	1.333333	0.666667	3457.413	1.667113	1.077211	4335.588
C1b13d	1.5	0.866025	3895.289	2.026183	1.384986	5285.867
C1b13e	2	0.707107	5216.38	2.744324	1.205451	7203.503
C1b14	4.5	1.274755	11985.11	5.155225	1.436282	13802.64
C1b14+709	0	0	0	0	3.924126	0
C1b2	0.205882	0.077816	528.5139	0.282127	0.128239	724.7389
C1b3	6.6	1.732051	17871.48	6.360675	1.154155	17191.77
C1b3a	3	1.290994	7891.662	2.513493	1.461929	6584.656
C1b3a+3468	0	0	0	0	2.051831	0
C1b4	0.3	0.223607	770.7771	0.256479	0.256479	658.7005
C1b4+152	0	0	0	0	104883.5	0
C1b5	4.25	1.118034	11296.29	4.950042	1.359338	13231.59
C1b5a	2.25	0.75	5881.091	2.975155	1.077211	7824.666
C1b5b	3.25	1.198958	8567.269	3.129042	1.051563	8240.053
C1b5b+255	0	0	0	0.384718	0.410366	989.1971
C1b6	0.666667	0.471405	1718.501	0.872028	0.666845	2252.001
C1b7	4.4	1.095445	11709.27	5.155225	1.410634	13802.64
C1b7+7606	2	1	5216.38	2.513493	1.590169	6584.656
C1b7a	3.285714	1.040016	8664.003	3.693295	1.410634	9771.817
C1b7a1	2.6	0.959166	6816.306	2.693028	1.69276	7065.781
C1b7a1+60	0.5	0.5	1286.949	0.461662	2.18007	1187.86
C1b7a1+8251	0.5	0.5	1286.949	0.564253	3.359873	1453.17
C1b8	8.2	1.907878	22472.23	6.463267	1.461929	17482.87
C1b8+195	1	0.707107	2585.428	0.872028	1.128507	2252.001
C1b8a	2.666667	1.154701	6995.049	2.462197	1.359338	6447.45
C1b8a+6473	1	0.707107	2585.428	0.948972	0.897676	2452.386
C1b9	2.222222	0.968644	5807.098	3.436817	1.69276	9073.877
C1b9+198	1.125	0.375	2911.832	1.256746	0.564253	3256.62
C1c	6.376623	0.581232	17237	7.873901	1.000268	21526.67
C1c+13153	8	2.160247	21891.84	7.104464	3.488112	19311.51
C1c+13153+185	0	0	0	0	4.667915	0
C1c+14581	7.2	1.83303	19585.32	7.02752	1.205451	19091.23
C1c+14581+153	0	0	0	0	3.539408	0
C1c+146	9	1.732051	24808.55	7.412239	1.795352	20194.87
C1c+150	6.5	1.695582	17587.2	7.258351	1.590169	19752.74
C1c+150+152	2	0.942809	5216.38	2.487845	1.744056	6516.038
C1c+150+152+7853	1.5	0.866025	3895.289	1.564521	1.282394	4065.137
C1c+152	6	1.732051	16171.67	7.386591	2.487845	20121.12

C1c+16274	5	1.311488	13370.48	6.258084	2.30831	16901.09
C1c+16274+152	1.5	0.866025	3895.289	1.744056	1.179803	4538.736
C1c+195	7.111111	1.247219	19330.55	7.386591	1.590169	20121.12
C1c+293	0.5	0.5	1286.949	0.872028	0.872028	2252.001
C1c+4784	1.5	0.866025	3895.289	2.18007	1.641465	5694.881
C1c+6575	0	0	0	0.564253	1.436282	1453.17
C1c1	3.285714	0.979379	8664.003	4.48838	1.538873	11953.03
C1c1b	2.4	0.8	6281.24	2.898211	1.025915	7617.355
C1c1b+11866	1	0.707107	2585.428	1.051563	0.718141	2719.986
C1c2	1.071429	0.276642	2771.858	1.384986	0.410366	3592.983
C1c3	2.142857	0.769309	5595.877	2.205718	0.769437	5763.151
C1c3a	2.333333	1	6103.274	1.461929	0.615549	3795.157
C1c3a+4435	1	0.707107	2585.428	0.820732	0.615549	2118.561
C1c4	4.25	1.198958	11296.29	5.052633	1.897943	13516.9
C1c5	3.666667	1.290994	9699.224	3.795887	1.667113	10051.77
C1c5+16354	1	0.707107	2585.428	1.564521	1.051563	4065.137
C1c5+195	0	0	0	0.307775	0.333423	790.8077
C1c6	3.5	1	9245.555	4.437084	1.513225	11811.51
C1c6+12092	2.5	1.118034	6548.554	3.103394	1.641465	8170.751
C1c7	2.333333	1.20185	6103.274	2.051831	2.359605	5353.963
C1c7+14979	0.5	0.5	1286.949	0.384718	2.359605	989.1971
C1c8	2	1	5216.38	3.15469	1.923591	8309.383
C1d	7.670213	1.279031	20938.08	7.950844	1.436282	21749.42
C1d+12717	0	0	0	0	8.694633	0
C1d+1282	2	1	5216.38	2.539141	1.590169	6653.302
C1d+151	7	1.870829	19012.5	7.232704	2.18007	19679.14
C1d+194	6.988372	0.973763	18979.24	7.51483	0.923324	20490.13
C1d+194+64	0	0	0	0	3.949774	0
C1d1	6.328571	0.615945	17100.77	7.207056	0.897676	19605.56
C1d1+115	1	0.707107	2585.428	1.179803	0.820732	3055.158
C1d1+12501	7	1.620185	19012.5	6.66845	1.077211	18066.3
C1d1+12501+16223!	6	1.699673	16171.67	6.232436	2.66738	16828.48
C1d1+12501+16223!+1719	0	0	0	0	4.437084	0
C1d1+16311	5.8	1.341641	15608.22	6.027253	4.744859	16248.57
C1d1+16311+16287	7.666667	1.914854	20927.85	5.514295	1.564521	14806.09
C1d1+16311+16287+3338	4	1.414214	10610.09	2.898211	1.154155	7617.355
C1d1+16362	5.153846	1.775907	13798.8	6.232436	2.564788	16828.48
C1d1+194!	5.8	0.935711	15608.22	6.386323	1.538873	17264.51
C1d1+194!+146	6.666667	1.943651	18061.22	6.052901	2.077479	16320.96
C1d1+194!+146+3831A	0.5	0.5	1286.949	0.461662	1.051563	1187.86
C1d1+194!+185	0	0	0	0	3.51376	0

C1d1+194!+6536	1	0.707107	2585.428	0.948972	0.948972	2452.386
C1d1+200	0.5	0.5	1286.949	1.205451	1.128507	3122.282
C1d1e	4.666667	1.333333	12445.76	5.924661	2.641732	15959.23
C1d1e+16327!	3.5	1.322876	9245.555	4.514028	2.513493	12023.83
C1d1+7269	8.333333	1.666667	22859.98	6.745394	0.97462	18285.51
C1d1a	3	1.249	7891.662	3.975422	1.667113	10542.76
C1d1a1	0.75	0.433013	1934.756	0.948972	0.897676	2452.386
C1d1b	3.166667	0.889757	8341.768	4.129309	1.564521	10964.69
C1d1b+3438	0	0	0	0	3.282929	0
C1d1b+9449	0.5	0.5	1286.949	0.589901	0.589901	1519.573
C1d1b1	1.875	0.572822	4885.062	2.103127	0.769437	5490.243
C1d1b1+204	0.5	0.5	1286.949	0.384718	0.384718	989.1971
C1d1c	3.916667	1.626602	10381.93	4.052366	2.693028	10753.6
C1d1c1	2.090909	1.064064	5457.774	1.69276	0.923324	4403.275
C1d1c1+16204	0.625	0.279508	1610.493	0.641197	0.35907	1652.47
C1d1c1+16242	0	0	0	0	2.333958	0
C1d1d	4.833333	1.013794	12907.55	5.616887	1.487577	15093.75
C1d1d+1452	4.5	1.5	11985.11	3.385521	1.487577	8934.625
C1d2	4.166667	1.34371	11067.27	5.873366	1.461929	15814.72
C1d2a	2	0.632456	5216.38	2.539141	1.025915	6653.302
C1d3	3.6	1.296148	9517.614	3.872831	2.051831	10262.03
C1d3a	3	1.224745	7891.662	3.02645	2.231366	7963.015
C1d3a+14992	0	0	0	0	3.077746	0
C1d3a+507	0	0	0	0	2.923859	0
C4	8.068889	1.210993	22091.58	8.48945	2.026183	23314.82
C4+152	6.083333	1.304373	16406.9	7.335295	2.026183	19973.69
C4f	3.25	1.145644	8567.269	4.950042	1.974887	13231.59
C4f+8152	1.666667	0.881917	4334.412	2.026183	2.282662	5285.867
C4f+8152+7106	0	0	0	0	3.052098	0
C4a	9.136364	1.516794	25209.12	8.079084	1.282394	22121.16
C4a1	6.45	1.673457	17445.2	6.899281	1.308042	18724.61
C4a1c	1.75	0.75	4554.44	2.333958	2.051831	6104.939
C4a1c+16093!	1.5	0.866025	3895.289	1.974887	1.410634	5149.762
C4a1a	4.592308	1.111718	12240.09	5.616887	1.33369	15093.75
C4a1a+152	0	0	0	0	2.616084	0
C4a1a+16129!	3	1.224745	7891.662	4.154957	2.051831	11035.11
C4a1a+195	3.377193	0.764165	8912.028	4.616619	1.154155	12307.31
C4a1a+195+16093!	3.304348	0.983801	8714.495	3.334225	0.897676	8795.485
C4a1a7	1.5	0.866025	3895.289	1.538873	1.179803	3997.598
C4a1a+195+16260	4.333333	1.452966	11525.61	3.462464	1.769704	9143.545
C4a1a+195+16260+146	0.5	0.5	1286.949	0.538606	3.385521	1386.797

C4a1a+195+263!	3	1.290994	7891.662	1.179803	2.410901	3055.158
C4a1a+195+263!+6221	0	0	0	0	2.795619	0
C4a1a+195+3212	0	0	0	0	3.462464	0
C4a1a1	6	1.761261	16171.67	4.84745	1.359338	12946.71
C4a1a1a	3.666667	1.080123	9699.224	3.205986	1.33369	8448.129
C4a1a1a+10775	0	0	0	0	2.641732	0
C4a1a1a+3552A!	0.5	0.5	1286.949	0.436014	0.641197	1121.609
C4a1a1a+5843	0.5	0.5	1286.949	0.436014	0.436014	1121.609
C4a1a2	3.714286	1.069045	9829.06	3.821535	1.179803	10121.83
C4a1a2+8137	0	0	0	0.48731	2.257014	1254.142
C4a1a2a	2.75	1.145644	7218.75	2.744324	1.256746	7203.503
C4a1a2a+16093!	1.666667	1	4334.412	1.33369	300.7727	3458.349
C4a1a2a+16093!+11176	0.5	0.5	1286.949	0	300.9779	0
C4a1a3	1.849315	0.440704	4817.07	1.718408	0.384718	4470.991
C4a1a3+152	0.5	0.5	1286.949	0.410366	6.052901	1055.388
C4a1a3+16093!	0	0	0	0.205183	296.2331	526.7155
C4a1a3e	1	0.824621	2585.428	0.589901	0.410366	1519.573
C4a1a3e+13857	0.25	0.25	642.0236	0.205183	0.282127	526.7155
C4a1a3a	1.294118	0.447987	3354.566	1.384986	0.384718	3592.983
C4a1a3a1	1.333333	0.62361	3457.413	1.051563	0.436014	2719.986
C4a1a3a1+3644	0.666667	0.471405	1718.501	0.692493	0.461662	1785.488
C4a1a3b	3.333333	1.247219	8793.068	1.487577	0.436014	3862.608
C4a1a3b+430	0	0	0	0	1.154155	0
C4a1a3c	1.375	0.795495	3566.765	0.923324	0.512958	2385.561
C4a1a3c+11447	1	0.895806	2585.428	0.461662	0.333423	1187.86
C4a1a3c+11447+11875	0.125	0.125	320.6479	0.102592	0.102592	263.1125
C4a1a3c+9bpdel9	0	0	0	0.256479	0.97462	658.7005
C4a1a3d	0.363636	0.28748	934.8128	0.538606	10.41304	1386.797
C4a1a3d+10885	0	0	0	0	10.41304	0
C4a1a4	2.5625	0.954021	6715.848	2.385253	0.97462	6241.857
C4a1a4a	1.785714	0.422577	4648.832	1.846648	0.512958	4810.01
C4a1a4a+12372	0.333333	0.333333	856.6771	0.410366	0.436014	1055.388
C4a1a4a+4688	0.5	0.5	1286.949	0.436014	1.025915	1121.609
C4a1a5	1.333333	0.816497	3457.413	1.436282	1.000268	3727.736
C4a1a5+8623	0.5	0.5	1286.949	0.512958	0.512958	1320.454
C4a1a6	0.5	0.5	1286.949	0.692493	0.692493	1785.488
C4a1b	2.166667	1.213352	5659.214	3.411169	1.718408	9004.237
C4a1b+9148	0	0	0	0	3.924126	0
C4a2	7.835616	1.579506	21415.93	7.489182	1.179803	20416.28
C4a2a	2.72	1.090688	7138.183	2.487845	0.97462	6516.038
C4a2a1	1.755102	0.488092	4567.921	1.538873	0.512958	3997.598

C4a2a1+16261	1.5	0.866025	3895.289	1.051563	1.025915	2719.986
C4a2a1+195	1.6	0.748331	4158.613	1.128507	1.128507	2921
C4a2a1+195+152	0	0	0	0	453424.9	0
C4a2a1+195+503	0.5	0.5	1286.949	0.307775	1.384986	790.8077
C4a2a1+47	1.5	0.866025	3895.289	1.051563	0.564253	2719.986
C4a2a1a	0.214286	0.159719	550.1278	0.282127	0.282127	724.7389
C4a2a1a+15301!	0	0	0	0	0.436014	0
C4a2a1b	1.4	1.029563	3632.413	0.512958	0.872028	1320.454
C4a2a1b+13818	0.555556	0.555556	1430.657	0.153887	0.948972	394.8528
C4a2a1b+13818+16000	0	0	0	0	0.948972	0
C4a2a2a+11398	0	0	0	0	0.564253	0
C4a2a2a+15658	0	0	0	0	0.564253	0
C4a2b	6.135135	2.092457	16553.27	5.155225	1.128507	13802.64
C4a2b+10304	4.338235	1.782084	11539.11	4.154957	1.025915	11035.11
C4a2b+16189	2.5	1.118034	6548.554	3.616352	2.128774	9562.141
C4a2b1	2	0.816497	5216.38	2.231366	0.97462	5831.451
C4a2b2	3.373134	1.506945	8901.016	3.718943	1.000268	9841.764
C4a2b2+13173	1.5	0.866025	3895.289	2.077479	1.821	5422.088
C4a2b2+188	0.5	0.5	1286.949	0.436014	2.487845	1121.609
C4a2b2c	0	0	0	0	3.949774	0
C4a2b2b	0.5	0.5	1286.949	0.436014	0.743789	1121.609
C4a2b2a	0.293103	0.107672	753.0111	0.461662	0.333423	1187.86
C4a2b2a+16037	0	0	0	0	6.694098	0
C4a2b2a+16311!	0	0	0	0	1215.556	0
C4a2c	5.272727	1.325216	14130.44	5.437352	1.615817	14590.63
C4a2c+207	5.307692	1.785875	14228.09	4.514028	2.949507	12023.83
C4a2c+207+16239	4.083333	1.63936	10838.53	3.51376	3.667648	9282.965
C4a2c+8443	1	0.707107	2585.428	2.077479	1.769704	5422.088
C4a2c1	1.272727	0.426401	3298.496	1.154155	0.641197	2988.064
C4a2c1+10724!	1	0.707107	2585.428	0.512958	1.128507	1320.454
C4a2c1+3394!	1	0.707107	2585.428	0.512958	0.48731	1320.454
C4a2c2	2	0.832993	5216.38	2.744324	1.846648	7203.503
C4a2c2a	1	0.790569	2585.428	1.025915	1.744056	2653.041
C4a2c2a+709	0	0	0	0	220852.7	0
C4a2c2a+8269	0.333333	0.333333	856.6771	0.333423	0.461662	856.907
C4a'b'c	8.107551	1.246195	22203.76	8.48945	1.359338	23314.82
C4b	2.931818	0.679837	7707.872	2.846915	0.692493	7479.29
C4b+16311	0.9375	0.757772	2422.493	1.33369	0.923324	3458.349
C4b+16311+189	0	0	0	0.282127	2.026183	724.7389
C4b+16497	0	0	0	0.769437	2.205718	1985.241
C4b+199	0	0	0	0.410366	1.564521	1055.388

C4b1	1.2	0.325246	3108.015	1.231098	0.333423	3189.436
C4b1+12311	0.9	0.9	2324.817	0.820732	0.538606	2118.561
C4b1+12311+16189	0	0	0	0.333423	674.642	856.907
C4b1+12870	0	0	0	0	1.282394	0
C4b1+14153	0.666667	0.471405	1718.501	0.948972	0.410366	2452.386
C4b1c	2	1	5216.38	0.948972	0.384718	2452.386
C4b1+6596	0	0	0	0	1.282394	0
C4b1+709	0	0	0	0	100128.2	0
C4b1a	1.285714	0.473804	3332.536	1.077211	0.35907	2786.961
C4b1a+15930	0	0	0	0	2.513493	0
C4b1b	0.333333	0.235702	856.6771	0.384718	0.333423	989.1971
C4b2	1.333333	0.785674	3457.413	1.846648	1.025915	4810.01
C4b2+195	0	0	0	0	437.04	0
C4b2a	0.5	0.372678	1286.949	0.48731	0.461662	1254.142
C4b2a+16311	0	0	0	0	1077.698	0
C4b3	3.1	0.948683	8161.582	2.487845	0.769437	6516.038
C4b3a	2	0.721393	5216.38	2.051831	0.897676	5353.963
C4b3a2	0.25	0.25	642.0236	0.461662	1.436282	1187.86
C4b3a+152	1.5	1.06066	3895.289	1.256746	1791.864	3256.62
C4b3a+152+1438!	0	0	0	0.256479	1792.736	658.7005
C4b3a1	0.8	0.632456	2064.662	1.231098	1.205451	3189.436
C4b3a1+16311	0	0	0	0.35907	936.8403	923.0368
C4b3b	2.25	1.299038	5881.091	1.359338	1.359338	3525.651
C4b3b+5894	0	0	0	0.230831	1.718408	592.6927
C4b5	0	0	0	0.769437	2.385253	1985.241
C4b6	1.5	0.612372	3895.289	1.744056	0.692493	4538.736
C4b7	0.5	0.372678	1286.949	1.051563	0.97462	2719.986
C4b7+13260	0	0	0	0.410366	1.154155	1055.388
C4b8	2.333333	1.105542	6103.274	2.051831	0.795084	5353.963
C4b8a	1	0.707107	2585.428	0.820732	0.564253	2118.561
C4c	4.578947	1.046032	12203.17	6.001605	1.384986	16176.19
C4c1	3.066667	0.6733	8071.561	4.257549	1.128507	11317.06
C4c1+16362	1	0.707107	2585.428	1.641465	3.616352	4267.931
C4c1c	0	0	0	0	2.616084	0
C4c1a	0.5	0.353553	1286.949	0.641197	0.615549	1652.47
C4c1b	1.333333	0.666667	3457.413	1.897943	1.231098	4945.823
C4c2	0.333333	0.333333	856.6771	0.333423	1.564521	856.907
C4d	3.333333	0.971825	8793.068	4.257549	1.949239	11317.06
C4d+152!	0	0	0	0	5.103929	0
C4d+16223!	2	0.942809	5216.38	2.846915	1.410634	7479.29
C4d+16223!+195	0	0	0	0	584.4384	0

C4e	1	0.707107	2585.428	1.461929	1.231098	3795.157
C5	7.078431	1.090309	19236.95	8.720281	2.359605	23988.99
C5+16093	6.341463	1.328765	17137.31	8.720281	2.128774	23988.99
C5a	4.485714	1.3465	11945.68	4.924394	2.231366	13160.33
C5a1	1.307692	0.480384	3390.159	1.641465	0.84638	4267.931
C5a1+1211	0.5	0.5	1286.949	0.564253	0.615549	1453.17
C5a1+16093	1.5	0.866025	3895.289	1.128507	0.897676	2921
C5a1+4216	0	0	0	0	2.564788	0
C5a1a	0.25	0.25	642.0236	0.307775	0.307775	790.8077
C5a2	2.181818	0.899954	5699.533	1.974887	3.308577	5149.762
C5a2a	0.4	0.4	1028.632	0.615549	3.795887	1586.006
C5a2a+16214	0	0	0	0	3.847183	0
C5a2b	1.411765	0.506019	3663.316	1.102859	0.48731	2853.965
C5a2b1	0.8	0.4	2064.662	0.692493	0.410366	1785.488
C5a2b2	0.75	0.75	1934.756	0.307775	0.769437	790.8077
C5a2b2+4892	0	0	0	0	0.820732	0
C5b	6.115385	1.642177	16497.45	8.720281	2.410901	23988.99
C5b1	5.12	1.381014	13704.49	7.15576	1.641465	19458.48
C5b1a	1.9	0.866025	4951.271	3.205986	1.231098	8448.129
C5b1a1	0.5	0.25	1286.949	0.692493	0.461662	1785.488
C5b1b	1.769231	1.158963	4605.259	1.282394	0.769437	3323.833
C5b1b1	0.916667	0.759203	2368.221	0.615549	0.410366	1586.006
C5b1b1+7498	0.111111	0.111111	284.9844	0.205183	0.205183	526.7155
C5c	4.055556	1.377733	10762.35	4.00107	2.257014	10613.02
C5c+16234	3.466667	1.302988	9154.962	3.359873	1.051563	8865.041
C5c+16291	1	0.707107	2585.428	1.590169	1.128507	4132.705
C5c1	2.384615	1.101371	6240.153	2.257014	0.872028	5899.78
C5c1a	1.5	0.645497	3895.289	1.461929	0.692493	3795.157
C5c1a+152	0.857143	0.349927	2213.266	1.000268	0.512958	2586.126
C5d	4.954545	1.359843	13244.11	7.899549	2.051831	21600.9
C5d3	4.333333	1.290994	11525.61	4.873098	1.974887	13017.89
C5d3+11092	1.5	0.866025	3895.289	2.385253	1.974887	6241.857
C5d1	0.625	0.306186	1610.493	0.538606	0.282127	1386.797
C5d1+14133	1	0.707107	2585.428	0.333423	0.256479	856.907
C5d1+1415!	0.25	0.25	642.0236	0.179535	0.538606	460.7689
C5d2	6.666667	1.943651	18061.22	6.283732	2.18007	16973.72
C5d2+195	0.5	0.5	1286.949	0	3.642	0
C7	6.07438	1.057238	16381.62	8.309915	1.949239	22791.83
C7+152	5.833333	1.936492	15702.02	7.950844	3.000803	21749.42
C7+152+449	0.4	0.282843	1028.632	0.48731	0.48731	1254.142
C7+16051	7.333333	1.885618	19968.06	7.617422	2.000535	20785.79

C7+16093	3.3	1.063015	8702.713	7.258351	3.385521	19752.74
C7+16093+16172	1.666667	0.53287	4334.412	1.949239	1.000268	5081.753
C7+16093+16172+16239	0	0	0	0	15.61956	0
C7+16093+16172+195	1.5	0.866025	3895.289	0.84638	0.512958	2185.266
C7a	5.070707	0.783463	13567.21	4.514028	0.641197	12023.83
C7a+11935	0.5	0.5	1286.949	0.897676	2.103127	2318.766
C7a+150	1.5	0.866025	3895.289	2.333958	1.923591	6104.939
C7a+16189	3.342105	0.626625	8816.853	3.795887	3.077746	10051.77
C7a+16189+12541	1.5	0.866025	3895.289	1.69276	1.179803	4403.275
C7a+16189+16092	0.333333	0.333333	856.6771	0.436014	0.641197	1121.609
C7a+16457	0	0	0	0	4.565324	0
C7a+214	0.5	0.5	1286.949	0.897676	0.923324	2318.766
C7a+234	1	0.707107	2585.428	1.000268	0.743789	2586.126
C7a+41	0.5	0.5	1286.949	0.538606	0.538606	1386.797
C7a+4233	0.375	0.216506	964.1246	0.48731	0.333423	1254.142
C7a1	6.096774	1.013437	16444.87	4.129309	0.564253	10964.69
C7a1b	2.555556	1.036375	6697.251	2.590436	0.692493	6790.68
C7a1b+222	1.5	0.866025	3895.289	1.615817	0.795084	4200.304
C7a1b+319	1.571429	0.795395	4083.332	2.026183	0.718141	5285.867
C7a1b+319+143	0	0	0	0	6.591506	0
C7a1a	4.818182	1.445998	12865.52	3.308577	0.743789	8725.958
C7a1a+151	2	1.20185	5216.38	1.821	1.231098	4742.148
C7a1a1	0	0	0	0	2.487845	0
C7a1a2	0.8	0.4	2064.662	0.743789	0.48731	1918.627
C7a1c	2.857143	0.728431	7506.809	3.180338	0.666845	8378.742
C7a1c+15670	0.333333	0.333333	856.6771	0.48731	0.512958	1254.142
C7a1d	4	1.414214	10610.09	2.718676	1.077211	7134.628
C7a2	3.424242	0.70222	9039.732	3.795887	0.897676	10051.77
C7a2b	1.571429	0.589015	4083.332	1.846648	0.872028	4810.01
C7a2b+16319	0	0	0	0	3.539408	0
C7a2b+8149	0	0	0	0	2.30831	0
C7a2e	1	0.790569	2585.428	1.154155	4.898746	2988.064
C7a2e+16239	0.333333	0.333333	856.6771	0.333423	0.512958	856.907
C7a2f	1.5	0.866025	3895.289	2.103127	1.154155	5490.243
C7a2c	2	0.902671	5216.38	2.795619	1.025915	7341.34
C7a2c+12672	0.714286	0.473804	1842.037	0.769437	0.564253	1985.241
C7a2c+12672+10086	0	0	0	0	0.97462	0
C7a2d	2.25	1.030776	5881.091	2.846915	1.051563	7479.29
C7a2d+16311	0.666667	0.666667	1718.501	0.769437	2120.413	1985.241
C7a2d+16311+8994	0	0	0	0	2121.08	0
C7a2a	1	0.515079	2585.428	1.384986	0.666845	3592.983

C7a2a+14226	0	0	0	0	1.846648	0
C7b	3.8	1.311488	10063.01	4.206253	1.949239	11176.03
C7b+15043!	3	1.224745	7891.662	3.231633	2.462197	8517.544
C7b+15043!+146	0	0	0	0	3.180338	0
C7b+15043!+16189	0	0	0	0	7.412239	0
CZ	15.36255	2.354282	44170.46	15.72215	2.821267	45302.98
JT	10.5	2.291288	29251.27	12.1571	3.257281	34249.71
L0	59.93146	6.975751	202168	63.65805	6.719746	16180.88
L0a	11.5	2.397916	32256.59	15.56827	3.949774	44817.87
L1-6	51.96532	6.387627	172391.4	56.83571	6.027253	90562.88
L2	5.5	1.658312	14766.04	6.617154	2.693028	17920.29
L2'3'4'6	34.97829	4.890059	110402.8	37.52286	4.180605	19498.26
L2-6	46.96819	5.988387	153891.4	47.06387	5.155225	54243.95
L3	23.98404	3.609885	72274.29	24.98104	2.385253	75639.01
L3'4'6	27.98768	4.12092	85908.58	30.29015	3.436817	93887.68
L3d	10	2	27761.53	13.84986	3.052098	39448.84
L3d2	5	1.581139	13370.48	7.976492	3.000803	21823.72
L3e	11.375	1.841365	31879.07	16.41465	3.359873	47494.51
L3e1	4.333333	1.20185	11525.61	6.463267	1.974887	17482.87
L3e2	7.8	1.509967	21312.94	10.59258	3.129042	29528.06
L3e2a	4	1.414214	10610.09	5.488647	2.359605	14734.24
L3e2b	6.333333	1.452966	17114.26	8.027788	2.257014	21972.39
L3f	16.5	2.872281	47765.58	18.97944	3.129042	55727.94
L4	16.5	2.872281	47765.58	18.90249	3.462464	55478.36
M	20.05809	3.062357	59242.81	20.08229	1.846648	59322.03
M2	15.45455	2.230517	44459.82	16.74807	2.051831	48554.58
M23	2.5	1.118034	6548.554	2.66738	1.384986	6996.963
M2a	10.25	1.903943	28505.31	12.747	2.462197	36051.1
M2a1	5.75	1.47902	15467.61	6.591506	2.693028	17847.32
M2a1a	3.333333	1.054093	8793.068	4.129309	1.69276	10964.69
M2a3	4.75	1.520691	12676.51	6.514563	3.077746	17628.57
M2a3+3337	0	0	0	0	4.924394	0
M2a3+9055	0.5	0.5	1286.949	0.538606	0.538606	1386.797
M2a'b	13.11111	2.165954	37168.61	15.31179	2.128774	44010.89
M2c+1888	4	1.414214	10610.09	4.257549	1.744056	11317.06
M3	8.769231	1.455453	24132.21	12.90089	3.180338	36522.88
M31	13	1.882938	36827.14	14.85013	2.18007	42563.26
M31a	10.5	2.20794	29251.27	12.51617	2.257014	35344.86
M31a1	1	0.707107	2585.428	1.282394	0.948972	3323.833
M31a2	2	1	5216.38	2.231366	1.128507	5831.451
M31b1	8	1.876166	21891.84	8.84852	2.333958	24364.37

M31b1+146	5	1.666667	13370.48	4.411436	1.590169	11740.8
M31b1+146+239	2	1	5216.38	1.69276	1.025915	4403.275
M31b1+3337	1	0.707107	2585.428	1.436282	1.538873	3727.736
M31b'c	11.28571	2.123724	31609.73	13.49079	2.30831	38338.38
M31c	2.5	1.118034	6548.554	3.257281	1.795352	8586.987
M36	10	1.695582	27761.53	13.13172	2.487845	37231.98
M36d	6.5	1.802776	17587.2	9.541013	3.02645	26401.72
M3a	4.4	1.131371	11709.27	7.64307	2.257014	20859.77
M3a1	2.666667	0.942809	6995.049	4.103662	1.564521	10894.3
M3a2	3.5	1.322876	9245.555	5.026985	1.949239	13445.54
M3c	11	2.254248	30749.67	12.23404	3.180338	34484.03
M3c1	6.8	1.6	18441.22	9.643605	3.334225	26705.01
M3c1a	3.5	1.322876	9245.555	5.206521	2.282662	13945.67
M3c1b	4.333333	1.374369	11525.61	9.643605	4.924394	26705.01
M3c1b1	1.5	0.866025	3895.289	9.643605	2.359605	26705.01
M3c2	3	1.224745	7891.662	5.924661	2.513493	15959.23
M44	2	1.054093	5216.38	3.385521	1.744056	8934.625
M44a	0.5	0.5	1286.949	0.641197	0.641197	1652.47
M50	16.4	2.497999	47448.01	17.41491	2.795619	50684.11
M50+1383	7.666667	2.134375	20927.85	7.668718	2.359605	20933.77
M50+1383+150	0.5	0.5	1286.949	0.48731	1.077211	1254.142
M50+16093	15	2.738613	43032.52	17.41491	3.462464	50684.11
M8	15.23036	2.256161	43755.1	15.72215	3.000803	45302.98
M8a	8.267857	1.42779	22669.48	9.33583	2.231366	25796.26
M8a1	2.571429	1.069045	6739.761	3.334225	2.154422	8795.485
M8a1+234	0	0	0	0	3.590704	0
M8a1a	1.333333	0.816497	3457.413	1.359338	1.513225	3525.651
M8a1a+16086!	0.5	0.5	1286.949	0.692493	1.231098	1785.488
M8a2	6.432432	1.185498	17395.34	6.104197	1.436282	16465.84
M8a2+152	7.04	1.406272	19126.94	5.719478	1.923591	15381.82
M8a2+499	0	0	0	0	6.514563	0
M8a2'3	7.22449	1.231612	19655.57	7.591774	1.487577	20711.84
M8a2a	7.583333	1.846543	20687.51	5.001338	1.179803	13374.2
M8a2a+11923	0.5	0.5	1286.949	0.641197	0.872028	1652.47
M8a2a1	6.4	1.667333	17303.31	4.154957	1.102859	11035.11
M8a2a1a	4.75	1.457738	12676.51	3.462464	1.051563	9143.545
M8a2a1a2	2.333333	1.105542	6103.274	2.462197	1.025915	6447.45
M8a2a1a2+10688	0.5	0.5	1286.949	0.538606	0.564253	1386.797
M8a2a1a1	1.6	0.8	4158.613	1.487577	0.718141	3862.608
M8a2a1a1+6216	1	0.57735	2585.428	1.025915	0.589901	2653.041
M8a2a1a1+146	0	0	0	0	42.83197	0

M8a2a1b	3	1.224745	7891.662	2.693028	1.102859	7065.781
M8a2b	1.833333	0.390868	4774.778	2.18007	0.564253	5694.881
M8a2c	1.333333	0.816497	3457.413	2.641732	2.359605	6928.173
M8a2c+3290	0	0	0	0	4.257549	0
M8a2d	2	1	5216.38	1.949239	1.231098	5081.753
M8a2e	3	1.224745	7891.662	4.308845	1.949239	11458.19
M8a3	5.583333	1.193152	14999.62	6.155492	1.282394	16610.82
M8a3b	5.333333	1.490712	14299.73	5.257816	1.487577	14088.81
M8a3b+152	3.5	1.322876	9245.555	3.334225	1.564521	8795.485
M8a3a	3.75	0.847791	9926.5	5.078281	1.154155	13588.3
M8a3a1	1.5	0.790569	3895.289	2.000535	1.846648	5217.8
M8a3a1+6164	0	0	0	0	2.590436	0
N	16.6	1.949359	48083.43	18.44083	2.821267	53984.15
R	14.5	3.041381	41469.64	16.61983	4584.527	48146.49
R2'JT	13.5	2.872281	38366.82	12.18275	20.72349	34327.79
Z	9.091503	1.534	25077.27	10.8747	2.641732	30373.38
Z+152	8.072848	1.18953	22103.07	9.233239	1.282394	25494.1
Z+152+151	5.833333	1.324187	15702.02	7.207056	2.359605	19605.56
Z1	7.19697	2.163061	19576.63	7.822605	1.641465	21378.3
Z1a	3.230769	0.90704	8515.204	3.462464	1.384986	9143.545
Z1a1	2.69697	1.164599	7076.36	2.641732	1.077211	6928.173
Z1a1a	0.769231	0.188422	1984.707	0.872028	0.256479	2252.001
Z1a1a+5206	0.5	0.5	1286.949	0.35907	0.333423	923.0368
Z1a1a+9693	0	0	0	0	0.897676	0
Z1a1b	0.428571	0.247436	1102.389	0.512958	0.436014	1320.454
Z1a2	3.307692	1.461538	8723.559	2.821267	1.487577	7410.301
Z1a2a	2.25	1.21621	5881.091	1.769704	1.769704	4606.51
Z1a2a+7419	0.777778	0.777778	2006.912	0.461662	2.205718	1187.86
Z1a2a+7419+14122	0	0	0	0.230831	2.231366	592.6927
Z1a3	0.722222	0.412011	1862.636	0.666845	3.103394	1718.964
Z1a3+9145	0.333333	0.333333	856.6771	0.384718	3.129042	989.1971
Z1a3+9151	0.5	0.5	1286.949	0.48731	3.103394	1254.142
Z1a3+9151+8997	0	0	0	0.205183	3.129042	526.7155
Z2	0.888889	0.351364	2295.888	1.102859	0.795084	2853.965
Z2+8521	0	0	0	0	1.359338	0
Z3	8	1.233588	21891.84	8.515098	1.205451	23389.63
Z3+13620	8.826087	2.008018	24298.66	8.00214	1.205451	21898.04
Z3+146	5.363636	1.523914	14384.43	8.36121	2.769972	22941.13
Z3+152!	6.5	1.802776	17587.2	6.899281	1.974887	18724.61
Z3+709	4.714286	1.203736	12577.58	6.232436	1.949239	16828.48
Z3a	5.909091	1.180069	15915.36	6.514563	1.205451	17628.57

Z3a+14476	4.454545	1.351461	11859.68	4.026718	1.795352	10683.3
Z3a3	2.833333	1.258306	7442.753	2.975155	5.001338	7824.666
Z3a3+10410	0.5	0.5	1286.949	0.589901	4.308845	1519.573
Z3a3+204	0	0	0	0	5.437352	0
Z3a1	3.555556	1.175889	9396.646	4.616619	1.461929	12307.31
Z3a1+11075	3	1.059457	7891.662	3.565056	1.205451	9422.498
Z3a1+12753	0	0	0	0	4.565324	0
Z3a1a	2	0.666667	5216.38	2.487845	1.000268	6516.038
Z3a1a+13174	0	0	0	0	2.66738	0
Z3a1a+16150	2	1	5216.38	1.769704	0.97462	4606.51
Z3a2	1	0.824621	2585.428	0.692493	0.97462	1785.488
Z3a2+15097	0.25	0.25	642.0236	0.333423	0.384718	856.907
Z3b	2.222222	1.01835	5807.098	3.000803	2.18007	7893.826
Z3b+146	1.285714	0.795395	3332.536	1.897943	14.38846	4945.823
Z3b+146+16189	0	0	0	0	2653.915	0
Z3b+146+16287	0.4	0.282843	1028.632	0.48731	1.359338	1254.142
Z3b+3394	3.5	1.322876	9245.555	3.565056	1.744056	9422.498
Z3c	2	1	5216.38	3.924126	2.282662	10402.34
Z3d	4.4	1.232883	11709.27	5.026985	1.69276	13445.54
Z3d+3396	2	1	5216.38	1.718408	0.948972	4470.991
Z3d+8020T	0	0	0	0	6.488915	0
Z4	4.826087	0.947587	12887.44	5.078281	2.154422	13588.3
Z4+151!	4	1.414214	10610.09	4.231901	1.615817	11246.53
Z4+214	0	0	0	0	14.72189	0
Z4+8292	0	0	0	0	5.82207	0
Z4a	5.214286	1.08091	13967.34	4.437084	1.256746	11811.51
Z4a+151!	6.375	1.515544	17232.39	4.129309	1.025915	10964.69
Z4a+151!+207	0	0	0	0	18.36389	0
Z4a+15773	0.5	0.5	1286.949	0.718141	3.15469	1852.042
Z4a1	6.166667	1.404358	16642.42	3.821535	1.000268	10121.83
Z4a1a	5.4	1.280625	14486.12	3.51376	0.923324	9282.965
Z4a1a2	2.5	1.118034	6548.554	1.897943	1.025915	4945.823
Z4a1a1	2	1	5216.38	1.179803	0.692493	3055.158
Z7	2.333333	0.707107	6103.274	2.026183	0.718141	5285.867
Z7+8348!	3.5	1.322876	9245.555	1.436282	0.615549	3727.736

Appendix 38. Maximum likelihood and rho calculation of haplogroup C to estimate the age of each subclade.

10.5. Published Genome used

Group Label	Number of Individuals	Average of 95.4% date range in calBP (defined as 1950 CE)	Country	Reference
AA	12	0	USA	Lazaridis2014
Abkhasian	9	0	Abkhazia	Lazaridis2014
ACB.SG	96	0	Barbados	1KGPhase3
Adygei	18	0	Russia	Patterson2012
Afar.WGA	5	0	Ethiopia	Lazaridis2014
Ain_Touta.WGA	3	0	Algeria	Lazaridis2014
523a.SG	1	125	USA	Scheib2018
Albanian	6	0	Albania	Lazaridis2014
Aleut	7	0	Russia	Lazaridis2014
Algerian	7	0	Algeria	Lazaridis2014
Altaian	7	0	Russia	Lazaridis2014
Ami	12	0	Taiwan	Lazaridis2014
Anatolia_C	1	5776	Turkey	Lazaridis2016
Anatolia_EBA	3	4604	Turkey	Lazaridis2017
Anatolia_EBA.SG	3	4700	Turkey	Damgaard2018
Anatolia_IA.SG	2	2900	Turkey	Damgaard2018
Anatolia_MLBA.SG	5	3800	Turkey	Damgaard2018
Anatolia_N	23	8100	Turkey	Mathieson2015
Anatolia_N_Boncuklu.SG	4	10078	Turkey	Kilinc2016
Anatolia_N_Kumtepe.SG	1	6682	Turkey	Omrak2016
Anatolia_N_Tepecik_Ciftlik.SG	4	8505	Turkey	Kilinc2016
Argentina_Aconcagua_Inca500BP	1	500	Argentina	MorenoMayar2018
Argentina_ArroyoSeco2_7700BP	7	8000	Argentina	PosthNakatsuka2018
Argentina_Fuego_Patagonian	4	100	Argentina	Raghavan2015
Argentina_LagunaChica_6800BP	3	7200	Argentina	PosthNakatsuka2018
Armenian	10	0	Armenia	Lazaridis2014
ASO.SG	4	1800	Canada	Scheib2018
Assyrian	11	0	Iran	Lazaridis2016
ASW.SG	66	0	USA	1KGPhase3
Atayal	9	0	Taiwan	Lazaridis2014
Australian	3	0	Australia	Lazaridis2014
Azeri.WGA	3	0	Azerbaijan	Lazaridis2014
Bahamas_Taino.SG	1	995	Bahamas	SchroederS2018

Baja_Mexico.SG	2	2700	Mexico	Scheib2018
Balkar	10	0	Russia	Lazaridis2014
Balochi	20	0	Pakistan	Patterson2012
BantuKenya	6	0	Kenya	Patterson2012
BantuSA	5	0	Botswana	Patterson2012
Bashkir.DG	3	0	Russia	Damgaard2018
Basque	20	0	France	Patterson2012
BEB.SG	86	0	Bangladesh	1KGPhase3
BedouinA	25	0	Israel	Patterson2012
BedouinB	19	0	Israel	Patterson2012
Belarusian	10	0	Belarus	Lazaridis2014
Belize_MayahakCabPek_9300BP	1	9285	Belize	PosthNakatsuka2018
Belize_SakiTzul_7400BP	2	7375	Belize	PosthNakatsuka2018
BIR.SG	9	0	India	Mondal2016
Bolivian	7	0	Bolivia	Lazaridis2014
Brahui	21	0	Pakistan	Patterson2012
Brazil_Botocudo.SG	2	300	Brazil	Malaspinas2014
Brazil_Enoque_HG.SG	1	3559	Brazil	Raghavan2015
Brazil_Jabuticabeira2_2100BP	1	2260	Brazil	PosthNakatsuka2018
Brazil_LapaDoSanto_9600BP	6	9600	Brazil	PosthNakatsuka2018
Brazil_Laranjal_6700BP	2	6700	Brazil	PosthNakatsuka2018
Brazil_Moraes_5800BP	1	5785	Brazil	PosthNakatsuka2018
Brazil_Sumidouro_10100BP	5	10100	Brazil	MorenoMayar2018
Bulgarian	10	0	Bulgaria	Lazaridis2014
Burbur.WGA	5	0	Morocco	Lazaridis2014
Burmese	10	0	Myanmar	Skoglund2016
Burusho	23	0	Pakistan	Patterson2012
Buryat.DG	2	0	Russia	Damgaard2018
Cambodian	8	0	Cambodia	Patterson2012
Canada_400BP.SG	1	387	Canada	Raghavan2015
Canada_6000BP.SG	1	6075	Canada	Raghavan2015
Canada_BigBar_5700BP.SG	1	5669	Canada	MorenoMayar2018
CDX.SG	99	0	China	1KGPhase3
CEU.SG	99	0	USA	1KGPhase3
Chane.DG	1	0	Argentina	Mallick2016
CHB.SG	103	0	China	1KGPhase3
Chechen	10	0	Russia	Lazaridis2014
Chile_Ayayema_5100BP.SG	1	5121	Chile	MorenoMayar2018
Chile_Conchali_700BP	2	700	Chile	PosthNakatsuka2018
Chile_Fuego_Patagonian.SG	4	100	Chile	Raghavan2015
Chile_Kaweskar_1000BP.SG	2	1000	Chile	delaFuenteS2018

Chile_LosRieles_12000BP	1	12000	Chile	PosthNakatsuka2018
Chile_LosRieles_5100BP	1	5100	Chile	PosthNakatsuka2018
Chile_PicaOcho_700BP	1	700	Chile	PosthNakatsuka2018
Chile_PuntaSantaAna_7300BP.SG	1	7293	Chile	MorenoMayar2018
Chile_Yamana_1000BP.SG	2	1000	Chile	delaFuenteS2018
China_Lahu	8	0	China	Patterson2012
China_Tianyuan	1	39475	China	Yang2017
Chipewyan.DG	2	0	Canada	Mallick2016
CHS.SG	108	0	China	1KGPhase3
Chukchi	24	0	Russia	Lazaridis2014
Chuvash	10	0	Russia	Lazaridis2014
CLM.SG	94	0	Colombia	1KGPhase3
Colonist.SG	1	291	USA	Scheib2018
Cree.DG	2	0	Canada	Mallick2016
Croatian	10	0	Croatia	Lazaridis2014
Cypriot	8	0	Cyprus	Lazaridis2014
Czech	10	0	Czechoslovakia	Lazaridis2014
Dai	10	0	China	Patterson2012
Daur	9	0	China	Patterson2012
Dolgan	4	0	Russia	Lazaridis2014
Druze	39	0	Israel	Patterson2012
E_San_Nicolas.SG	13	4000	USA	Scheib2018
Eskimo_ChaplinSireniki	7	0	Russia	Lazaridis2014
ESN.SG	99	0	Nigeria	1KGPhase3
Estonian	10	0	Estonia	Lazaridis2014
Even	9	0	Russia	Lazaridis2014
FIN.SG	99	0	Finland	1KGPhase3
French	54	0	France	Lazaridis2016
French_Polynesia_150BP	3	130	French Polynesia	Posth2018
GBR.SG	92	0	Great Britain	1KGPhase3
Georgia_Kotias.SG	1	9720	Georgia	Joness2015
Georgian	10	0	Georgia	Lazaridis2014
GIH.SG	106	0	USA	1KGPhase3
Gimi	9	0	Papua New Guinea	Skoglund2016
Greenland_Saqqaq.SG	1	3885	Greenland	Rasmussen2010
GWD.SG	113	0	Gambia	1KGPhase3
Han	43	0	China	Patterson2012
Hawaiian.DG	1	0	USA	Mallick2016
Hazara	14	0	Pakistan	Patterson2012
IBS.SG	107	0	Spain	1KGPhase3

Island_Chumash_SanCruz.SG	1	1052	USA	Scheib2018
Island_Chumash_SanMiguel.SG	2	650	USA	Scheib2018
Italian_North	20	0	Italy	Patterson2012
Itelmen	6	0	Russia	Lazaridis2014
ITU.SG	103	0	India	1KGPhase3
Japanese	29	0	Japan	Patterson2012
JPT.SG	104	0	Japan	1KGPhase3
Ju_hoan_North	5	0	Namibia	Patterson2012
Kalmyk	10	0	Russia	Lazaridis2014
Kankanaey	10	0	Philippines	Skoglund2016
Karitiana	13	0	Brazil	Patterson2012
Korean	6	0	South Korea	Lazaridis2014
Koryak	11	0	Russia	Lazaridis2014
Kumyk	8	0	Russia	Lazaridis2014
Kusunda	10	0	Nepal	Lazaridis2014
L_San_Nicolas.SG	10	2000	USA	Scheib2018
Lao	10	0	Laos	Skoglund2016
LateDorset.SG	1	800	..	Raghavan2014
Lezgin	9	0	Russia	Lazaridis2014
LSCI.SG	8	800	USA	Scheib2018
Mainland_Chumash_NewCuyama.SG	1	1344	USA	Scheib2018
Mansi	8	0	Russia	Lazaridis2014
Mayan	18	0	Mexico	Patterson2012
Mbuti	10	0	Congo	Patterson2012
Mexico_Pericues.SG	4	2000	Mexico	Scheib2018
Mexico_Zapotec.DG	2	0	Mexico	Mallick2016
Micronesian	10	0	Micronesia	Skoglund2016
MiddleDorset.SG	2	1550	..	Raghavan2014
Mixe	13	0	Mexico	Lazaridis2014
Mixtec	12	0	Mexico	Lazaridis2014
Mongola	6	0	China	Patterson2012
MXL.SG	67	0	USA	1KGPhase3
Nasioi	10	0	Papua New Guinea	Patterson2012
Naxi	9	0	China	Patterson2012
Nganasan	11	0	Russia	Lazaridis2014
Nogai	9	0	Russia	Lazaridis2014
Norwegian	11	0	Norway	Lazaridis2014
Oroqen	9	0	China	Patterson2012
Ossetian	10	0	Russia	Lazaridis2014
Papuan	14	0	Papua New Guinea	Patterson2012

PEL.SG	86	0	Peru	1KGPhase3
Peru_Cuncaicha_3300BP	1	3300	Peru	PosthNakatsuka2018
Peru_Cuncaicha_4200BP	1	4185	Peru	PosthNakatsuka2018
Peru_Cuncaicha_9000BP	1	9000	Peru	PosthNakatsuka2018
Peru_Kaillachuro_Unknown.SG	1	3950	Peru	Lindos2018
Peru_LaGalgada_4100BP	1	4105	Peru	PosthNakatsuka2018
Peru_Laramate_900BP	6	900	Peru	PosthNakatsuka2018
Peru_Lauricocha_3500BP	1	3500	Peru	PosthNakatsuka2018
Peru_Lauricocha_5800BP	1	5800	Peru	PosthNakatsuka2018
Peru_Lauricocha_8600BP	3	8600	Peru	PosthNakatsuka2018
Peru_RioUncallane_1800BP.SG	5	1800	Peru	Lindos2018
Peru_SoroMikayaPatjxa_6800BP.SG	1	6821	Peru	Lindos2018
Piapoco	4	0	Colombia	Patterson2012
Pima	14	0	Mexico	Patterson2012
PUR.SG	105	0	Puerto Rico	1KGPhase3
Quechua	5	0	Peru	Lazaridis2014
RapaNui	5	0	Chile	Skoglund2016
Russia_Abkhasian.DG	2	0	Russia	Mallick2016
Russia_Afanasievo.SG	2	4800	Russia	Allentoft2015
Russia_Alan.SG	6	1300	Russia	Damgaard2018
Russia_Aldy_Bel_IA	2	2650	Russia	Unterlanders2017
Russia_Andronovo.SG	4	3550	Russia	Allentoft2015
Russia_Crimea_Pantikapaion.SG	1	1622	Russia	VeeramahS2018
Russia_DevilsGate.SG	1	7624	Russia	Siskas2017
Russia_Early_Sarmatian_IA	4	2250	Russia	Unterlanders2017
Russia_EBA.SG	1	4627	Russia	Allentoft2015
Russia_EHG	1	5250	Russia	Mittniks2018
Russia_HG	1	6950	Russia	Mittniks2018
Russia_IA.SG	5	2253	Russia	Allentoft2015
Russia_Karasuk.SG	5	3429	Russia	Allentoft2015
Russia_Karelia_HG	2	8280	Russia	Mathieson2015
Russia_Khvalynsk_EN	3	6800	Russia	Mathieson2015
Russia_Kostenki14	1	37470	Russia	Fu2016
Russia_Kurma_EBA.SG	2	4710	Russia	Damgaard2018
Russia_Late_Sarmatian.SG	5	2176	Russia	Krzewinskas2018
Russia_LBA.SG	2	2821	Russia	Allentoft2015
Russia_Lokomotiv_EN.SG	4	7559	Russia	Damgaard2018
Russia_MA1_HG.SG	1	24305	Russia	Raghavan2013
Russia_Mezhovskaya.SG	3	3448	Russia	Allentoft2015
Russia_Nomad_Med.SG	1	1000	Russia	Damgaard2018
Russia_North_Ossetian	2	0	Russia	Mallick2016

Russia_Okunevo_BA.SG	14	4300	Russia	Damgaard2018
Russia_Poltavka	4	4627	Russia	Mathieson2015
Russia_Popovo_HG	1	6250	Russia	Mittniks2018
Russia_Potapovka	2	3897	Russia	Mathieson2015
Russia_SaltovoMayaki.SG	3	1115	Russia	Damgaard2018
Russia_Sarmatian.SG	7	2300	Russia	Damgaard2018
Russia_Shamanka_EBA.SG	5	4121	Russia	Damgaard2018
Russia_Shamanka_EN.SG	10	7941	Russia	Damgaard2018
Russia_Srubnaya	9	3675	Russia	Mathieson2015
Russia_Srubnaya_Alakul.SG	12	3743	Russia	Krzewinskas2018
Russia_Sunghir1.SG	5	32823	Russia	Sikora2017
Russia_Tagar.SG	8	2850	Russia	Damgaard2018
Russia_UstIda_EBA.SG	4	4827	Russia	Damgaard2018
Russia_UstIda_LN.SG	4	5610	Russia	Damgaard2018
Russia_Yamnaya_Kalmykia.SG	6	4706	Russia	Allentoft2015
Russian	22	0	Russia	Patterson2012
Samoan	8	0	Samoa	Skoglund2016
Selkup	10	0	Russia	Lazaridis2014
Serbia_EN	3	7440	Serbia	Mathieson2018
Serbia_Iron_Gates_HG	35	11465	Serbia	Mathieson2018
Serbia_Medieval_Gepidian.SG	1	1400	Serbia	VeeramahS2018
Serbia_N	1	7684	Serbia	Mathieson2018
Serbia_Starcevo_EN	1	7600	Serbia	Mathieson2018
She	10	0	China	Patterson2012
Surui	8	0	Brazil	Patterson2012
Teleut.DG	2	0	Russia	Damgaard2018
Tlingit	4	0	Russia	Lazaridis2014
Tofalar	13	0	Russia	Lazaridis2014
Tonga_2500BP	3	2495	Tonga	Posth2018
Tsimshian.SG	1	0	Canada	Raghavan2015
Tubalar	22	0	Russia	Lazaridis2014
Tuvinian	10	0	Russia	Lazaridis2014
Ukraine_EN	1	5047	Ukraine	Mathieson2018
Ukraine_Globular_Amphora	3	4753	Ukraine	Mathieson2018
Ukraine_Mesolithic	1	10371	Ukraine	Mathieson2018
Ukraine_N	26	7239	Ukraine	Mathieson2018
Ukraine_Scythian.SG	3	2550	Ukraine	Krzewinskas2018
Ukraine_Trypillia_Eneolithic	4	5736	Ukraine	Mathieson2018
Ukraine_Yamnaya	3	4950	Ukraine	Mathieson2018
Ukrainian	9	0	Russia	Lazaridis2014
Ulchi	25	0	Russia	Lazaridis2014

USA_Alaska_TCreek_9000BP	1	8956	USA	MorenoMayar2018
USA_Ancient_Beringian.SG	2	11435	USA	MorenoMayar2017
USA_Anzick.SG	1	12649	USA	Rasmussen2014
USA_Nevada_LlCave_1850BP	2	1880	USA	MorenoMayar2018
USA_Nevada_LlCave_600BP	1	600	USA	MorenoMayar2018
USA_Nevada_SpiCave_11000BP	2	10970	USA	MorenoMayar2018
USA_NM_Chaco	2	865	USA	Kennetts2017
USA_WA_Kennewick.SG	1	8770	USA	Rasmussen2015
Ust_Ishim.DG	1	45020	Russia	Pruefer2017
Vanuatu_1100BP	3	1105	Vanuatu	Posth2018
Vanuatu_1300BP_all	2	1260	Vanuatu	Lipson2018
Vanuatu_150BP_all	3	150	Vanuatu	Lipson2018
Vanuatu_2000BP	1	2030	Vanuatu	Posth2018
Vanuatu_2100BP	1	2115	Vanuatu	Posth2018
Vanuatu_2200BP	1	2215	Vanuatu	Posth2018
Vanuatu_2500BP	2	2505	Vanuatu	Posth2018
Vanuatu_2900BP_all	3	2870	Vanuatu	Lipson2018
Yakut	20	0	Russia	Patterson2012
Yi	10	0	China	Patterson2012
Yukagir	19	0	Russia	Lazaridis2014
Yukpa.SG	1	0	Venezuela	Raghavan2015
Zapotec	10	0	Mexico	Lazaridis2014

Appendix 39. List of published modern and ancient populations used for the PCA, Admixture and F-Statistic. Subset of the Human Origins dataset.