ANCIENT MTDNA SEQUENCES FROM PREHISTORIC NORTH AMERICAN ARCTIC POPULATIONS

by

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ABSTRACT

This study set out to sequence the hypervariable segment-I (HVS-I) of the mitochondrial genome from prehistoric skeletal remains associated with Aleut, Sadlermiut, Dorset and Thule groups in Northern North America in an effort to gain insight into their genetic prehistories. Sequences obtained from said ancient populations (Aleut *n*=6; Sadlermiut *n*=7; Thule (partial sequences) *n*=3) were compared to each other as well as those from contemporary and prehistoric populations in the surrounding area. The prehistoric populations under investigation harbored matrilineages typically found in circum-Arctic populations throughout time: A2, A2a, A2b1, D2/D2a'b/D2a/D2a1 and D4b1a2a1. Ancient Aleuts exhibited HVS-I polymorphisms associated with haplogroups A2a, D2 and D2a'b, while the Sadlermiut were characterized as A2b1 and D4b1a2a1. Partial Thule HVS-I sequences indicate A2 but preclude definitive assignment to A2, A2a or A2b1 until the remaining portion of HVS-I is sequenced.

The results indicate ancient Aleuts across time exhibit affinities with the Unangaŷ (modern Aleuts); however, population movement or genetic exchange with neighbors to the east cannot be ruled out at this time. Ancient Aleuts were also found to have a greater matrilineal genetic similarity to Chukotkan populations (Chukchi and Siberian Yuit), rather than those from Kamchatka (Koryak and Itel'men). This genetic similarity/dissimilarity provides additional corroboration for colonization of the Aleutian archipelago being initiated from the east rather than the west. The isolated eastern Arctic Sadlermiut population, on the other hand, was shown to have affinities with contemporary Eskimo (Inuit and Iñupiat). The implications of this points towards the Sadlermiut having Neo-Eskimo rather than Paleo-Eskimo ancestry and echoes previous findings of matrilineal discontinuity in the eastern Arctic. The mtDNA (mitochondrial deoxyribonucleic acid) profiles of the ancient populations in this study are also congruent with results from other mtDNA studies indicating the genetic prehistory of the Neo-Eskimo was distinct

from that of the Paleo-Eskimo and inhabitants of the Aleutians. Overall the findings in this study speak to matrilineal genetic relationships of prehistoric and contemporary populations in the most northern stretches of the New World while touching upon population movements in the region.

In memory of my father Andres Arismendi Jr.

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CHAPTER 1

INTRODUCTION

Human existence in the harsh, unforgiving and extreme conditions encountered in the remote northerly expanses of North America would be inconceivable for most. The exceptions to this are the indigenous peoples known as the Eskimos, Aleuts and Athabascans whose predecessors successfully adapted to and inhabited the Arctic region. The conventional designations of Aleut and Eskimo have been used for indigenous peoples of the Aleutian Island chain and the rest of the northern Arctic, respectively. Although contemporary Eskimos in Greenland and Canada identify themselves as Inuit, while those in North/northwest and southwestern Alaska, respectively, prefer Iñupiat and Yupik (Damas, 1984). Nonetheless Eskimo is still used in lieu of the foregoing autonyms when collectively referring to the Inuit, Iñupiat and Yupik peoples of the circum-Arctic region. Along the same lines, Unangax̂ as well as its eastern (Unangan) and central/Atkan (Unangas) dialect variations are the self-designations used by the indigenous peoples of the Aleutians (Lantis, 1984; Veltre and Smith, 2010).

Populations in the Arctic are said to have been both the first as well as the last of the indigenous New World populations in North America to be discovered by Europeans (Collins, 1984:8). Following contact with Europeans, Eskimos, Unangax̂, and other prehistoric populations in the region captivated the interest of scholars and nonscholars alike. Anthropologists have drawn on areas such as ethnography, linguistics, anthropometrics, archaeology, skeletal biology as well as genetics in an effort to gain insights into contemporary as well as prehistoric indigenous groups in the North American Arctic. Information garnered through these lines of inquiry is invaluable to commanding a better understanding of the cultural traditions and histories of these contemporary and prehistoric populations and potentially unraveling the intricate web of

relationships of the peoples in the Arctic through time.

Explorations and interdisciplinary research efforts in the Arctic frontier have broadened our understanding of northern North American prehistory. Circumpolar indigenous peoples of the Arctic, like other New World populations, were found to exhibit strong affinities with Asiatic peoples (Turner, 1983; Powell, 1993; Crawford, 1998). However, the populations in northern North America, in particular the Eskimos, are believed to represent later arrivals into the New World relative to other Native Americans (Greenberg et al., 1986; Schurr et al., 1990; Shields et al., 1993; Starikovskaya et al., 1998; Saillard et al., 2000; Rubicz et al., 2003; Schurr and Sherry, 2004; Perego et al., 2009). Surveys throughout the region have uncovered a complex cultural chronology in the archaeological record associated with prehistoric groups such as the ancient Aleut, Neo-Eskimo Thule, Paleo-Eskimo (Saqqaq and Dorset) and Sadlermiut (Dumond, 1984). These groups have garnered much attention from anthropologists who have studied archaeological sites as well as material goods and ancient human remains. To date there are indications that contemporary Eskimos and Unangaŷ are most likely the descendants of the Neo-Eskimo Thule and ancient Aleuts, respectively. Uncertainties still remain concerning how the prehistoric populations in this region relate to each other as well surrounding populations that could potentially be addressed through additional ancient DNA (deoxyribose nucleic acid) investigations.

While great strides have been made in the study of the populations of the North American Arctic the quest to resolve unanswered questions continues. The purpose of this dissertation is to add to the growing body of knowledge regarding the peoples of the northern stretches of the New World centered on several prehistoric populations in this region. More specifically this study will sequence the hypervariable segment-I (HVS-I) of the mitochondrial genome of individuals affiliated with the prehistoric Aleut, Sadlermiut, Dorset and Thule groups. Examining the genetic prehistories of these ancient circum-Arctic populations in relation to populations in the region (both ancient and contemporary) would afford insight into their relationships to one another as well as questions surrounding colonization and population dispersals in the region.

Genetic analyses of mtDNA (mitochondrial DNA) genomes from contemporary and

prehistoric indigenous populations have identified primarily haplogroups A and D to be present in the peoples of northern North America (Shields et al., 1993; Merriwether et al., 1995; Starikovskaya et al., 1998; Saillard et al., 2000; Derbeneva et al., 2002; Hayes, 2002; Hayes et al., 2003; Rubicz et al., 2003; Smith et al., 2005; Helgason et al., 2006; Zlojutro et al., 2006; Gilbert et al., 2008; Raff et al., 2010). Throughout this territory the populations generally exhibit appreciable frequencies of haplogroup A, while haplogroup D when present constitutes a very minor maternal genetic component, as seen in the Inuit of Canada and Greenland (Helgason et al., 2006). However, among the Unangaŷ prehistoric Aleut and Sadlermiut the haplogroup frequency profile is reversed, with generally higher frequencies of D relative to A (Merriwether et al., 1995; Hayes, 2002; Derbeneva et al., 2002; Hayes et al., 2003; Rubicz et al., 2003; Zlojutro et al., 2006; Smith et al., 2009; Zlojutro et al., 2009; Crawford et al., 2010). Additionally, the prehistoric Neo-Eskimo Thule and two prehistoric Paleo-Eskimo groups (Dorset and Saqqaq) are monomorphic for haplogroups A and D, respectively (Hayes, 2002; Hayes et al., 2003; Gilbert et al., 2008). Considering only a limited number of ancient Paleo-Eskimo (Dorset *n*=3, Saqqaq *n*=1) have been genetically analyzed to date and the low levels of haplogroup D (~5%) present amongst contemporary Eskimos, sampling could account for the maternal haplogroup monomorphism in the Thule and Paleo-Eskimos (Hayes, 2002; Hayes et al., 2003; Gilbert et al., 2008).

Examination of mtDNA genomes from indigenous populations throughout the New World and across time (contemporary and prehistoric) have revealed mtDNA haplogroup patterning was established early on and has remained stable for at least 4,000-3,500 y BP (years before present) (O'Rourke et al., 2000; Raff et al., 2011). Deviations from similar haplogroup profiles and/or frequencies are thought to be the result of sampling errors and/or evolutionary processes such as genetic drift and gene flow (Malhi et al., 2004). In the eastern circum-Arctic region the significantly different genetic profiles based on discrete marker analysis of the Thule and Dorset are thought to reflect the displacement of the Paleo-Eskimo Dorset by the Neo-Eskimo Thule with the Sadlermiut possibly being a remnant Dorset population with Thule gene flow, which would be consistent with the changes in material culture observed in the archaeological record (Hayes,

2002; Hayes et al., 2003). In the Aleutians there is a perceptible change in haplogroup frequency through time (Smith et al., 2009). The earliest ancient Aleuts (pre-1,000 BP) exhibit statistically significant different haplogroup frequencies compared to later Aleuts (both ancient post-1,000 BP Aleuts and Unangax̂). This difference in haplogroup frequencies between the earliest ancient Aleuts and later Aleuts coincides with changes observed in mortuary practices among ancient Aleuts, which could possibly be due to different migration scenarios (see Smith et al., 2009). Sequencing of the mtDNA HVS-I region would provide a greater degree of maternal haplogroup resolution that could either refine or revise interpretations drawn from discrete marker haplogroup frequency data.

Sequence analysis of the mitochondrial genome of the Unangax̂, Inuit of Greenland and Canada, ancient South Alaskans from Mink Island and Port Moller as well as the ancient Saqqaq have identified mtDNA polymorphisms associated with haplogroups D2 and D3, which are unique in comparison to those observed in Native Americans who predominantly are characterized by haplogroup D1 (Bandelt et al., 2003; Rubicz et al., 2003; Helgason et al., 2006; Zlojutro et al., 2006; Tamm et al., 2007; Gilbert et al., 2008; Raff et al., 2010). Other samples from a prehistoric site in south Alaska (Brooks River) have HVS-I sequence mutations associated with haplogroups B2 and D1, which could be the result of gene flow between peninsular populations and their neighbors to the east (Raff et al., 2010). The HVS-I mutation motifs used to characterize the haplogroups traditionally designated as D2 and D3 in the literature are now respectively referred to as D2a'b and D4b1a2a1: these latter designations will be used here for said haplogroups (van Oven and Kayser, 2008; mtDNA tree build #16). This revision of the nomenclature for some haplogroup names reflects the refinement in the global phylogeny of the human mtDNA tree using polymorphisms observed throughout the entire mtDNA genome—both the control and coding regions (van Oven and Kayser, 2008).

Haplogroup D4b1a2a1 has generally been detected amongst Inuit groups of Greenland and Canada, while subtypes of D2 (D2a'b, D2a and D2a1a) have been observed in the Unangax̂, ancient South Alaskans and the Saqqaq sample (Derbeneva et al., 2002; Rubicz et al., 2003; Helgason et al., 2006; Zlojutro et al., 2006; Gilbert et al., 2008; Zlojutro, 2008; Zlojutro et al.,

2009; Raff et al., 2010). In the eastern Arctic the observance of D4b1a2a1 in the Inuit and D2a1 in the Paleo-Eskimo Saqqaq is thought to reflect genetic discontinuity in the region perhaps associated with the arrival of the Neo-Eskimos (Gilbert et al., 2008). Haplogroups A2, A2a and A2b1 have also been identified in the populations in this northernmost region (Rubicz et al., 2003; Helgason et al., 2006; Zlojutro et al., 2006; Gilbert et al., 2007; Gilbert et al., 2008; Zlojutro, 2008; Zlojutro et al., 2009). In the New World A2 is present among several Pan-American haplogroups, with subbranches A2a and A2b1 generally found in the most northern populations (Gilbert et al., 2007; Achilli et al., 2008; Gilbert et al., 2008; Perego et al., 2010).

As described above, mtDNA discrete marker analysis to date has identified haplogroups A and D amongst the prehistoric Aleut and Sadlermiut while the Dorset and Thule are respectively monomorphic for haplogroup D and A (Hayes, 2002; Smith et al., 2009). However, without HVS-I sequences from said populations it remains unclear which of the following haplogroups and their respective subhaplogroups A1, A2 (A2a, A2b1), D1, D2 (D2a'b), D4b1 or D4b1a2a1 are harbored by these most northern ancient populations. This study will undertake the task of sequencing the HVS-I segment of the mitochondrial genome of the prehistoric Aleut, Sadlermiut, Dorset and Thule. The sequences obtained from these ancient populations will be compared to each other as well as sequences culled from the literature of both contemporary and prehistoric populations in the surrounding area.

Sequence data of these prehistoric populations would provide additional genetic information, expanding our understanding regarding the nature of the ancestor/descendant relationships in the region as well as colonization events. The specific research questions to be addressed in this study using HVS-I sequence information include: 1) Which haplogroups are present among the ancient populations under study? 2) What is the nature of the relationships of the haplogroup D's in the ancient populations to each other and contemporary populations? 3) Are the genetic matrilineal relationships indicative of common origin, population replacement or admixture? 4) How does the genetic information garnered in this study augment, change and/or influence our understanding of human colonization and dispersal in the Arctic?

The outline of the remaining four chapters in this dissertation includes a literature review,

methods and materials, and a summary of the results followed by a discussion and summary of findings. More specifically, Chapter 2 discusses anthropological genetics and its application to the investigation of the peopling of the New World as well as providing background information on the prehistoric populations central to this research—Aleut, Sadlermiut, Dorset and Thule. The provenience of the samples analyzed in this study followed by an overview of the laboratory and statistical methods employed during this investigation are covered in Chapter 3. In Chapter 4, the results of mtDNA sequencing and statistical analyses are presented. This dissertation concludes with a discussion and summary of findings in Chapter 5.

CHAPTER 2

BACKGROUND

Anthropological Genetics

Molecular Genetics

The molecular investigation into human population structures and their genetic histories has been made feasible with advances in both technology and molecular biology. Anthropologists use genetic analyses as an additional tool in their arsenal to investigate questions regarding the origin of modern humans (Cann et al., 1987; Ingman et al., 2000; Jorde et al., 2000), human evolutionary relationships among Neanderthals, Cro-Magnon and *H. sapiens* (Krings et al., 1997; Krings et al., 1999; Krings et al., 2000; Ovchinnikov et al., 2000; Caramelli et al., 2003; Serre et al., 2004), peopling of the Americas (Schurr et al., 1990; Torroni et al., 1993a; Merriwether et al., 1995; Forster et al., 1996; Tamm et al., 2007; Achilli et al., 2008; Perego et al., 2010), as well as exploring prehistoric population movements and replacements (Kaestle and Smith, 2001; Hayes, 2002; Hayes et al., 2003; Smith et al., 2009). Some of the genetic markers examined in molecular anthropology studies of Native American populations include classical markers, mtDNA, autosomal markers including STRs (short tandem repeats), SNPs (single nucleotide polymorphisms) and sex chromosomal marker such as those located in the nonrecombining region of the Y chromosome (NRY). Many contemporary population studies of indigenous populations of the New World have focused on mtDNA and Y chromosome analyses, while aDNA studies have mostly concentrated on mtDNA. Additionally, advances in DNA sequencing technology have facilitated the characterization and reporting of whole genomes (mitochondrial and nuclear) from modern individuals such as in the 1000 Genomes Project as well as samples from prehistoric contexts (e.g., Paleo-Eskimo Saqqaq, the Tyrolean ice mummy

Ötzi) (Ermini et al., 2008; Gilbert et al., 2008; Rasmussen et al., 2010; 1000 Genomes Project Consortium, 2010, 2012; Keller et al., 2012).

Mitochondrial DNA (mtDNA)

The human mitochondrial genome is a circular double-stranded molecule comprised of approximately 16,569 bp (base pairs) located within the mitochondria, the cells' energy-generating cytoplasmic organelles (see Figure 2.1). The entire sequence of the human mtDNA genome has been established and is comprised of two regions (Anderson et al., 1981; Andrews et al., 1999; Behar et al., 2012). The first region is known as the coding region and contains 37 genes; of these, 22 are tRNAs (transfer ribonucleic acids), 13 are proteins and two additional RNAs (ribonucleic acids). Meanwhile, the second region is referred to as the noncoding displacement loop (d-loop), or the control region, which is responsible for transcription and replication. There are three hypervariable regions or segments (HVR or HVS) within the control region, HVS-I spans from 16,024 np (nucleotide position) to 16,365 np, HVS-II from 73 np to 340 np and the rarely sequenced or reported HVS-III region from 438 np to 574 np (Jobling et al., 2013; Butler, 2005).

The 1 to 16,569 mtDNA nucleotide position numbering system came about when the first complete human mtDNA genome was sequenced (Anderson et al., 1981), commonly referred to as the Cambridge Reference Sequence (CRS). Andrews et al. (1999) retained this numbering system after they carried out a reanalysis effort. Resequencing confirmed all but 11 nucleotide polymorphism sites from the original sequence reported by Anderson et al. (1981), all of which were located outside of the control region. The amended CRS is now referred to as the revised Cambridge Reference Sequence (rCRS) and is routinely used as a standard to compare human mtDNA sequences, with differences noted as nucleotide position and base change. Recently Behar et al. (2012) have introduced the Reconstructed Sapiens Reference Sequence (RSRS), which represents the ancestral mitogenome of modern humans and have proposed the RSRS replace the rCRS. Over 18,000 complete human mtDNA sequences were compared to the available full mtDNA genomes of five *Homo neanderthalensis*. As such, the RSRS represents

the deepest root of the human mtDNA phylogeny known to date and is positioned at the base preceding the split of the human basal haplogroups L0 and L1'2'3'4'5'6. However, the inclusion of additional mitogenomes from additional Neanderthals and other *Homo* representatives could potentially refine the ancestral states of mtDNA nucleotide positions in the RSRS (Malyarchuk, 2013). Those in the scientific community suggest transitioning to the RSRS would be premature for some spheres of mtDNA research (e.g., population genetics, forensics) and perhaps unwarranted as the rCRS is simply an "an arbitrary extant sequence selected for notational purposes" and not meant to be a consensus or wild-type (WT) sequence (Salas et al., 2012; Bandelt et al., 2014:69).

The mutation rate of mtDNA is five to ten times higher than nuclear DNA (Brown et al., 1979). Across the mtDNA genome the mutation rate is variable, with the rate of mutation in the control region nearly ten times higher than that of the coding region (Howell et al., 2007; Pakendorf and Stoneking, 2005). The mutation rate of the mtDNA genome can be used as a molecular clock for estimating population history events. However, comparing these estimates is complicated by the use of different methods for estimating mtDNA mutation rates as well as methods of calibrating the molecular clock (Cann et al., 1987; Vigilant et al., 1991; Horai et al., 1995; Siguroardottir et al., 2000; Howell et al., 2003; Santos et al., 2005; Kivisild et al., 2006; Endicott and Ho, 2008; Henn et al., 2009). Additionally, broad confidence limits associated with these time estimates suggest a degree of caution should be exercised when drawing inferences from these results in the reconstruction of population histories.

Several attributes of the mitochondrial genome make it appealing and useful as a means of addressing genetic histories of both contemporary and prehistoric populations in anthropological studies. The mtDNA genome is inherited uniparentally through the female. This means the mother passes her mtDNA on to all of her progeny, sons and daughters alike, which is passed on to subsequent generations by only her daughters (Giles et al., 1980). Also, unlike nuclear DNA, mtDNA does not undergo the process of recombination. The uniparental mode of inheritance coupled with the nonrecombining nature of mtDNA enables researchers to follow and reconstruct the matrilineal histories of individuals and populations. Another characteristic of the mtDNA

genome is its high copy number per cell, with an average of 500 copies per cell (but as many as 1,000 copies) compared to the two copies of nuclear DNA per cell (Robin and Wong, 1988; Satoh and Kuroiwa, 1991). The mtDNA genome's feature of high copy number is advantageous when analyzing samples, either contemporaneous or ancient, where the integrity of the DNA is compromised.

Studies of human mtDNA genetic histories initially employed the use of restriction fragment length polymorphisms (RFLP) and one length polymorphism located in region V (between the cytochrome oxidase II and lysine tRNA genes) of the mitochondrial genome (Cann et al., 1987; Schurr et al., 1990; Ballinger et al., 1992; Torroni et al., 1992; Chen et al., 1995; Torroni et al., 1996). The observed mitochondrial diversity signaled the presence of lineages, now referred to as haplogroups, defined by the combined presence and absence of restriction sites and a length polymorphism. The collection of molecular data in subsequent human mtDNA studies assayed mtDNA for discrete markers and/or direct sequencing of the mtDNA of either portions of the control region (HVS-I, HVS-II) or the entire mtDNA genome (Torroni et al., 1993a; Chen et al., 1995; Torroni et al., 1996; Ingman et al., 2000; Herrnstadt et al., 2002). The efforts of these studies identified mutation motifs in the control region corresponding with the haplogroups identified via discrete marker analysis as well as the extent of diversity present within these haplogroups. Additionally, the global structuring of haplogroups/macrohaplogroups was brought into focus with the discovery of continental patterning of mtDNA haplogroups as well as the development of migration models of human populations (see Figure 2.2) (Schurr et al., 1990; Chen et al., 1995; Torroni et al., 1996; Ingman et al., 2000; Maca-Meyer et al., 2001; Kivisild et al., 2002; Reidla et al., 2003; Shriver and Kittles, 2004; Helgason et al., 2006).

It has been well established that the indigenous populations of the New World possess mitochondrial haplogroups A, B, C and D, accounting for ~97% of contemporary Native American mtDNAs (Brown et al., 1998). These four haplogroups, A-D, are considered to be Pan-American because they are found in indigenous peoples throughout the Americas including North, Central and South America. Individuals both contemporary and prehistoric who could not be assigned to one of these four haplogroups were initially grouped into the category 'other.' These 'other'

haplotypes were believed to represent: 1) haplogroups introduced via admixture with Europeans and/or African Americans, 2) mutations in one of the diagnostic areas where the primer rests, 3) contamination of the sample or 4) a novel mtDNA haplogroup present at very low frequencies (Eshleman et al., 2003).

Further analysis assigned many of these 'other' undetermined haplotypes to haplogroup X, which is the fifth founding matrilineage observed in ~3% of modern Native Americans (Smith et al., 1999). Typically haplogroup X is either low or absent in most indigenous groups but has been observed in populations in northern North America in particular those in the Great Plains and Great Lakes areas, with frequencies peaking in the latter region (Perego et al., 2009). Recognition of haplogroup X as the fifth Native American matriline was solidified when it was detected among the prehistoric Oneota, a Native American tribe in Illinois (Stone and Stoneking, 1998). The existence of additional founding matrilineages among Native Americans remains to be seen, but if present they exist at extremely low frequencies. The implementation of whole mtDNA genome sequencing has identified a number of sublineages (e.g., A2a, A2b1, B2, C1b, C1d, D1, D2, D3, D4h3a, X2a) associated with the New World haplogroups found in the Americas, and this number may potentially increase with additional whole mtDNA genome sequences (Perego et al., 2010).

Identifying possible source populations for the indigenous peoples of the New World has also interested anthropologists. Genetic analyses point to Asia as the most likely region for the source population. Classical markers indicated Native Americans have genetic affinities with North Asian populations (for review see Crawford, 1998). Several candidates for source populations based on mtDNA analyses pointed to Eastern Siberia (A, C and D are present), while Central East Asia, Tibet, Central China and Mongolia are also possibilities because of the observance of four (A, B, C and D) mtDNA haplogroups (Ballinger et al., 1992; Torroni et al., 1993b; Torroni et al., 1994; Kolman et al., 1996; Eshleman et al., 2003). The identification of haplogroup X as an additional Native American matriline introduced the possibility of the peoples in the Lake Baikal-Altai mountain region as another possible source population of Native Americans (Derenko et al., 2001). The X observed among the Altains (X2e), however, is intermediate to the X haplotypes

observed among Native Americans (X2a) and Europeans (Brown et al., 1998; Derenko et al., 2001).

The New World mtDNA haplogroups, A-D, are present among the Native Americans of North, Central and South America regardless of geographical or temporal distribution as well as language classification. However, there is considerable variation in the distribution of these haplogroups, and their frequencies amongst Native North Americans are geographically patterned (O'Rourke et al., 2000; Raff et al., 2011). In general, Native American populations in North America exhibit a decrease in haplogroup A from north to south, but increases west to east (Merriwether et al., 1995; Schurr and Sherry, 2004; Kemp and Schurr, 2010). In North America haplogroup A is the most common and reaches its highest frequency in northern North America (Arctic and sub-Arctic) and eastern U.S. populations (Lorenz and Smith, 1996). The Unangax̂ (modern Aleuts) are the exception in northern North America because across the archipelago and lower Alaska Peninsula they exhibit high frequencies of D (62.2%) relative to haplogroup A (37.8%) (Merriwether et al., 1995; Rubicz et al., 2003; Zlojutro et al., 2006; Zlojutro et al., 2009; Crawford et al., 2010). Haplogroup B reaches high frequencies in southwest U.S. and is nearly absent in northern North American populations as it has been detected in contemporary and prehistoric individuals in southwestern Alaska (Merriwether et al., 1995; Lorenz and Smith, 1996; O'Rourke et al., 2000; Raff et al., 2010). Haplogroups C and X are found at high frequencies in the east, with X found almost exclusively in North America (Brown et al., 1998; Malhi et al., 2004). Lastly, haplogroup D tends to reach high frequencies in the Aleutians, central California, the Great Basin and Columbia Plateau (Malhi et al., 2004).

Studies have examined whether or not there is any patterning of mtDNA haplogroups using Greenberg's (1987) linguistic divisions of Amerind, Na-Dene and Eskimo-Aleut (Lorenz and Smith, 1996; Hunley and Long, 2005). Lorenz and Smith (1996) found some instances where there was correspondence between haplogroup frequencies and shared ancestry based on language group affiliation for some North American tribes. However, there were also occurrences of genetic similarities between tribes that were the result of geographic proximity. Others have found some correspondence between genetic and linguistic classifications across shared

languages found in several language classification schemes at shallow branches of the language classification scheme but not deeper branches (Hunley et al., 2007). The equivocal findings from investigations into the relationship between genes and languages have been attributed to reservations surrounding language classifications as well as the tree-like evolution of languages (Bolnick et al., 2004; Hunley and Long, 2005; Hunley et al., 2007).

The number and timing of the indigenous peoples coming into the New World have been other topics of interest and continue to be matters of scholarly debate. Some of the first mtDNA studies hypothesized the four maternal lineages (A, B, C and D) were introduced to the Americas via independent migrations (Horai et al., 1993). This initial hypothesis was dismissed due to the presence of either most or all of the haplogroups in various frequencies in indigenous populations of the New World and comparable diversity levels observed among the haplogroups and between populations (Torroni et al., 1993a; Merriwether et al., 1995; Bonatto and Salzano, 1997a,b; Lorenz and Smith, 1997). In addition, when randomly sampling populations in present day Eastern Siberia there is a high probability of selecting individuals possessing one of the three haplogroups observed in the New World (A, C or D with B being absent) (Eshleman et al., 2003). Thus, various studies argued these findings indicated a single wave migration rather than four independent migrations of four separate populations each monomorphic for a single haplogroup (Merriwether et al., 1995; Bonatto and Salzano, 1997a; Silva et al., 2002).

Alternative hypotheses have also been offered regarding the peopling of the Americas. Some have argued Amerinds are the result of a single wave of migration, while the Na-Dene and Eskimo-Aleuts represent a later expansion of the same population that gave rise to Amerinds but had differentiated prior to this expansion (Forster et al., 1996). Torroni et al. (1992) argued the Amerinds and Na-Dene represented two independent migrations from Asia, the latter more recently than the former (Torroni et al., 1992). There is general acceptance the circum-Arctic populations represent later arrivals into North America (Schurr et al., 1990; Shields et al., 1993; Starikovskaya et al., 1998; Saillard et al., 2000; Rubicz et al., 2003; Schurr and Sherry, 2004; Perego et al., 2009).

The migration routes taken by those who peopled the Americas has been another point of

interest that continues to be a source of debate. An overland journey from Siberia across the now partially submerged Beringian landmass (connected Siberia and Alaska when sea levels were lower during the Pleistocene) and continuing southward via an ice-free inland corridor has long been considered to be the principal migration route into the New World. The discovery of sites in North and South America predating the Clovis culture coupled with the estimated timing of the opening of the ice-free corridor bolstered the plausibility of alternative routes such as a coastal entry into the New World (see Goebel et al., 2003). In forward simulation model analyses a coastal migration model was favored over a single rapid colonization through an ice-free corridor (*blitzkrieg* model) (Fix, 2002; Fix, 2005). These results were based on the simulated genetic values (F_{ST} and haplogroup frequencies), with the coastal model, rather than the *blitzkrieg* model, being more consistent with the patterning of observed genetic variation (Fix, 2002; Fix, 2005).

A compelling theory originally coined the “out of Beringia” model is now referred to as either the Beringian Incubation Model (BIM) or the Beringian Standstill. It provides an alternative account for the mtDNA patterning seen among New World populations (Bonatto and Salzano, 1997a:1870; Tamm et al., 2007). The premise of the BIM is that Beringia played a more centralized role in the peopling of the Americas than merely providing a corridor or gateway from the Old World into the New World. After their arrival in Beringia the ancestors of the indigenous peoples of the New World are believed to have remained there for some time (perhaps as long as 15,000 years) in isolation (Tamm et al., 2007). During this standstill the forebears of the New World genetically differentiated from their Asiatic relatives prior to entering and rapidly populating the Americas (Tamm et al., 2007). The analysis of whole mitogenomes from Asia and the Americas seems to be consistent with the theory of an isolation period in Beringia prior to the colonization of the New World based on the accumulation of mutations distinguishing Native American mtDNA founding haplotypes from their Asiatic sister clades along with the uniform distribution of founding haplotypes throughout the Americas (Tamm et al., 2007; Achilli et al., 2008; Fagundes et al., 2008a,b; Kitchen et al., 2008). There is also evidence for “more recent bi-directional gene flow between Siberia and the North American Arctic” based on the distribution of

D2 sister clades (D2a and D2b) as well as D4b1a2a1 and A2a in northern North America, Greenland and Siberia (Tamm et al., 2007:1).

Findings from paleoecological studies provide indirect evidence that seemingly suggests shrub tundra patches in Beringia (conceivably central Beringia) could have potentially served as a refugium for the ancestral population of the Americas during their stopover (see Hoffecker et al., 2014). Analyses of seafloor sediments (for pollen, plant and insect fossil material) and aDNA from fauna and flora indicate the climate in Beringia supported a mosaic of tundra vegetation habitats (e.g., shrub, steppe tundra) capable of sustaining a mélange of fauna, which secondarily implies humans could have survived there as well (Elias and Crocker, 2008; Hoffecker et al., 2014; Willerslev et al., 2014; Pringle, 2014). Archaeological sites indicating a long-term human presence in Beringia dating to the LGM (Last Glacial Maximum) would provide incontrovertible support for the BIM. Unfortunately the majority of such sites are likely underwater, although "some might be found in areas of the LGM shrub tundra refugium that remain above sea level, such as low-lying portions of southwestern Alaska and eastern Chukotka" (Hoffecker et al., 2014:980).

Other peopling models for Native American populations have been put forth involving dual migration routes via the ice-free corridor and along the coast (Schurr and Sherry, 2004; Perego et al., 2009). Schurr and Sherry (2004) posited the first pioneers into the New World traveled along the Pacific coast and introduced the Pan-American mtDNA haplogroups A-D, since a land route was likely inaccessible and/or impassable until the end of the last glacial maximum (LGM). A second migration followed an interior route along the ice-free corridor once glaciers receded, which is believed to have introduced haplogroup X into North America (Schurr and Sherry, 2004). In the same vein, Perego et al. (2009) made a case for two migration routes based on the frequency distribution of following two haplogroups D4h3 and X2a both of which are rare throughout the Americas. Given that haplogroup D4h3 is restricted to the western coast of both North and South America it could have been brought to the New World via a coastal migration. The distribution of haplogroup X2a in the Great Plains and Great Lakes regions suggests it expanded into northern North America through the ice-free corridor.

The analysis of mtDNA RFLP data has generated time depths of 35,000 to 20,000 yr for haplogroups A, C, D, and X (Torroni et al., 1992; Torroni et al., 1993a; Schurr, 2004). The younger estimated age (17,000 to 13,000 yr) of haplogroup B led to the belief that it may have been introduced to the Americas by a separate and later migration (Torroni et al., 1993a). The analysis of mtDNA sequence data has provided ages for haplogroups A-D between 20,000 to 15,000 yr, which is similar to estimates reported elsewhere (Forster et al., 1996; Bonatto and Salzano, 1997b; Lorenz and Smith, 1997; Silva et al., 2002; Achilli et al., 2008; Fagundes et al., 2008b; Perego et al., 2009).

The examination of contemporary New World populations has provided a wealth of data that serves as a framework for aDNA studies to put their results into context. As a result, prehistoric groups can be compared to contemporary indigenous populations who serve as a benchmark for evaluation. Populations within the same region have been shown to exhibit similar haplogroup patterning which appears to have been established and remained stable for at least 4,000 to 3,500 yr BP (O'Rourke et al., 2000; Raff et al., 2011). Due to this established stability over a long period of time period differences in haplogroup frequencies and/or haplogroup profiles are generally attributed to recent population movement into the area, gene flow, genetic drift, sampling error or any combination of these factors (reviewed in Malhi et al., 2004).

The analysis of aDNA has been used to address prehistoric population migrations and replacements based on archaeological, biological and linguistic evidence. One of the early population aDNA studies characterized the mtDNA of individuals recovered from a 700-year-old cemetery in central Illinois used by the Oneota people (Stone and Stoneking, 1993, 1998). Sequencing results of these individuals identified the presence of five haplogroups A, B, C, D and X, which as described earlier have been observed in contemporary Native American populations. Additionally, Stone and Stoneking (1993, 1998) determined the level of diversity and frequencies of the four principal haplogroups (A-D) were comparable to those observed in populations in the surrounding area. This genetic similarity between indigenous populations, both contemporary and prehistoric, was taken as an indication that patterns of mtDNA variation were established fairly early and were not profoundly impacted by population reduction at the time of European

contact (Ubelaker, 1992; Stone and Stoneking, 1998).

In the Northeast, Shook and Smith (2008) genetically assessed human remains from four prehistoric populations and compared them to other Native American groups (both prehistoric and contemporary) to gain insight into population histories and genetic relationships in the region. The prehistoric populations were recovered from two sites located in the Central Illinois River Valley, Morse (dated ~2,700 BP) and Orendorf (dated ~800 BP). Also included in the study were individuals from several sites located in southwestern Ontario including Glacial Kame (remains pooled from three sites: Hind, Satori and Zimmer) dated to ~2,900 BP and Great Western Park dated to ~800 BP. The findings of the study determined haplogroup frequency distributions among prehistoric samples were not significantly different from one another, including the Oneota (~650 BP) and Ohio Hopewell (~1,700 BP), but were significantly different from most contemporary Native American populations (Stone and Stoneking, 1993, 1998; Mills, 2003). Nearly all of the prehistoric populations clustered with other prehistoric and contemporary populations save for Glacial Kame and Morse, which represented the oldest sites included in the study. These two populations exhibited high frequencies of B and low frequencies of A relative to other North Eastern populations (Shook and Smith, 2008).

Haplogroup frequency analysis indicated a gradual change in frequency over time, with an increase in haplogroup A and decrease in haplogroup B frequencies. Sequencing results for HVS-I revealed fewer shared haplotypes as well as more unique haplotypes among prehistoric populations compared to contemporary populations. The decrease in diversity over time may have been the result of drift and/or population crash at the time of European contact. The presence of shared haplotypes across geographical space and time indicates regional continuity maintained by gene flow and the continued presence of some peoples/populations in some areas for nearly 2,000 years (Shook and Smith, 2008).

In the American Southwest Carlyle et al. (2000, 2003) used discrete markers to genetically characterize prehistoric Anasazi (*n*=38) from several archaeological sites in Utah, New Mexico and Arizona spanning over 1,500 years. The ancient Anasazi exhibited high frequencies of haplogroup B, with low frequencies of A and C regardless of geographic or temporal distribution,

which suggests matrilineal continuity among the sampling of ancient Anasazi. Additionally, the results indicated the ancient Anasazi had affinities with contemporary Puebloans as well as the ancient Fremont from the eastern margin of the Great Salt Lake Wetlands, rather than geographically proximal historic period Athabascan or Numic speaking peoples (Parr et al., 1996; Carlyle et al., 2000; Carlyle, 2003). The alignment of the Anasazi with the Fremont lends support to the possibility the latter was a northern splinter of the Anasazi culture and neither group was associated with the Numic expansion. Additionally, the data suggest the matrilineal continuity between the Anasazi and contemporary Puebloans, and the genetic ties between these groups had not been eclipsed by a recent influx of Athabascans (Navajo and Apache) into the Southwest.

Kaestle and Smith (2001) examined two prehistoric populations from western Nevada to evaluate the hypothesis of an expansion of Numic speakers into the Great Basin. Both western Nevada populations, Pyramid Lake and Stillwater Marsh, exhibited similar haplogroup frequencies to one another and were comparable to those observed among contemporary Californian Penutians. Phylogenetic analyses of the pooled ancient western Nevada populations grouped them closer linguistically to contemporary Californian Penutians and geographically with contemporary Californian groups rather than the modern day Northern Uto-Aztecan language group (which includes the Numic language) and the Great Basin geographic group. The observed maternal genetic discontinuity between the ancient western Nevadans and contemporary Numic speakers in the region was considered to be evidence supporting the expansion of Numic speakers into the Great Basin.

Cabana et al. (2008) used a computer simulation approach to test the Numic expansion using mtDNA data reported in previous studies (Lorenz and Smith, 1996; Kaestle and Smith, 2001). Various ranges for several demographic parameters (e.g., population structure, effective population size, population growth, gene flow, number of generations separating samples) were tested to determine if the level of haplogroup frequency variation observed in the ancient western Nevada (Stillwater Marsh) population and contemporary Northern Paiute population were attributable to in situ microevolutionary processes or population replacement. The results of the

simulations supported population replacement with models where the Pre-Numic population was large with high levels of gene flow. However, the study also found some models where the present level of genetic differences between populations could be accounted for with small Pre-Numic populations coupled with low levels of genetic exchange and drift. Additional samples from other geographic locations and time frames are necessary to unravel the prehistoric peopling events in the Great Basin.

The molecular analysis of individuals from two Puebloan sites in New Mexico, Tommy and Mine Canyon, has provided additional data suggesting continuity among populations within the Greater Southwest region (Snow et al., 2010). Haplogroup frequency data revealed a statistically significant difference between the two sampled sites. The Tommy site exhibited high frequency of haplogroup B, typical of Southwest populations, and clustered with the prehistoric Fremont and Anasazi as well as contemporary Jemez and Zumi. Meanwhile, the haplogroup frequency profile of the Mine Canyon group revealed high frequency of haplogroup A, uncharacteristic of populations in the Southwest and low frequency of haplogroup B. As a result, the Mine Canyon samples clustered with other populations exhibiting high frequencies of A such as Mesoamerican (Mixtec, Nahua and Maya) and Southern Athabascan (Apache and Navajo) populations. Although haplogroup frequency data of these two populations alone may signal genetic discontinuity, HVS-I sequencing data from the two prehistoric Puebloan populations indicate otherwise. Population continuity in the area was corroborated by the observance of shared HVS-I haplotypes between the prehistoric New Mexico Puebloans and contemporary populations throughout the Southwest but not Mesoamericans. The observed differences in haplogroup frequencies are attributed to intermittent episodes of gene flow coupled with genetic drift.

In California aDNA analysis was used to test the Penutian-Hokan linguistic hypothesis and determine if there was genetic evidence of the migration(s) of Penutian speakers (~4,500 yr BP) into the Central Valley and the displacement/replacement of older Hokan speaking populations residing in the area (Eshleman, 2002). The prehistoric individuals analyzed were believed to be representatives of early Penutian speakers and were recovered from three sites (Cecil [Bear Creek], Cook and Applegate) in California's Central Valley that belong to two traditions,

Windmiller (Cecil dates to ~3,200 to 2,800 yr BP) and Middle Horizon (Cook and Applegate date to ~2,000 to 1,700 yr BP). All three groups exhibited high frequencies of haplogroups C and D that were not statistically different from one another and shared HVS-I haplotypes, which suggests genetic continuity among the three sites. Genetic distances using haplogroup frequency distributions revealed the ancient Californians more closely resembled the Takic speakers (a branch of the Uto-Aztecan language family in southern California) than any of the other populations, including contemporary Penutian and Hokan speaking groups. The data presently available do not appear to have provided support for the Penutian-Hokan linguistic hypothesis. Instead, the data indicated the ancient Californians might not be representatives of early Penutian speakers based on the genetic discontinuity between the prehistoric Californians and contemporary Penutian speakers in California. Additionally, the timing of the posited migration of the Penutian speakers into California may have been a more recent event than previously thought, perhaps some time after the Middle Horizon.

In the eastern Arctic the archaeological record indicates the displacement of the Paleo-Eskimo Dorset tradition by the Neo-Eskimo Thule tradition. Hayes (2002) characterized the mtDNA of these two groups using discrete markers and determined a statistically significant difference in mtDNA frequencies between the two groups. The haplogroup profiles were notably different with the Dorset (an archaeologically defined Paleo-Eskimo material culture) and the Thule (Neo-Eskimo ancestors of modern Inuit) monomorphic for haplogroup D and A, respectively. Although it is quite possible all of the genetic variability in these two prehistoric populations (Dorset and Thule) may not have been captured completely given the limited skeletal material associated with the Dorset (see Table 1 in Lynnerup et al. 2003) culture and the low frequency of D ~5% (25/497) amongst present day descendants of the Thule (Saillard et al., 2000; Helgason et al., 2006; Gilbert et al., 2008). However, “the vagaries of sampling the prehistoric record for genetic analysis” make it difficult to pinpoint the necessary sample size to capture said haplogroups in these populations (Smith et al., 2009:422). Nonetheless, computer simulations using maternal haplogroup data from contemporary groups (Siberian Yuit and Eastern Inuit) treated as sister populations to provide insight into the Thule expansion identified a

narrow window of conditions (e.g., range of migration rates and female effective population sizes, level of bottleneck severity at time of expansion) that could give rise to matrilineal haplogroup monomorphism in a population (Marchani et al., 2007). These findings suggest the A monomorphism in prehistoric Thule could be possible under certain population histories (Marchani et al., 2007). Together the observed differences between the Dorset and Thule with respect to genetic matrilineage profiles and material culture provide strong indicators pointing towards population replacement.

The genetic analysis of a Paleo-Eskimo Saqqaq individual also suggests genetic discontinuity between the Paleo-Eskimo and contemporary Inuit of Greenland and Canada, the descendants of the Thule (Helgason, 2006; Gilbert et al., 2008). Full mtDNA sequencing characterized the ancient Saqqaq individual as D2a1, while the contemporary Inuit populations of Greenland and Canada exhibit A2 (A2, A2a, A2b1) and D4b1a2a1 (Gilbert et al., 2008). Sublineages of the D2a1 haplotype that characterized the Saqqaq has also been observed among other contemporary Beringian populations including the Unangax̂ (D2a1a) and Siberian Sireniki Yuit (D2a1b) suggesting a shared prehistoric matrilineal relationship (Derbeneva et al., 2002; Gilbert et al., 2008). The presence of haplogroup D amongst the Paleo-Eskimo Saqqaq and Dorset could signal a shared genetic matrilineal ancestry. However, the exact nature of the genetic relationship between the Dorset and Saqqaq remains uncertain until further analysis, such as mtDNA sequencing, is performed on prehistoric Dorset individuals.

In the western Arctic (Hayes, 2002; Smith et al., 2009), discrete marker analysis was employed to address Hrdlička's population replacement theory, according to which the brachycranic Neo-Aleuts displaced the dolichocranic Paleo-Aleuts in the Aleutians around 1,000 AD. The initial analysis (n=30) detected no difference between the two populations and both populations had comparable frequencies of haplogroups A and D to those observed among the Unangax̂ (Hayes, 2002). However, when the results from Hayes' (2002) initial study were combined with additional ancient Aleut (n=39) samples, there was an observed increase in frequency of haplogroup A among pre-1,000 AD Aleuts relative to post-1,000 AD Aleuts (Smith et al., 2009). Whether or not the shift in haplogroup frequencies reflects an influx of people into the

archipelago, kin-structured movements within the archipelago or "a redistribution of existing populations resulting in significant local founder effects" remains to be seen (Smith et al., 2009:422). Larger sample sizes and additional genetic analyses are necessary to adequately address Hrdlička's population replacement hypothesis.

Population Background

Aleutians/Aleuts

The Aleutian archipelago is a chain of nearly 100 islands extending from the terminus of the Alaska Peninsula approximately 1,900 km westward towards the Kamchatka Peninsula and is sandwiched between the Pacific Ocean and Bering Sea (McCartney, 1984). The arcuate chain of volcanic islands was formed as a result of tectonic activity, more specifically the subduction of the North Pacific Plate beneath the North American Plate. From east to west the Aleutian Islands are separated into five groups: Fox Islands, Islands of the Four Mountains, Andreanof Islands, Rat Islands and Near Islands. The eastern islands are generally clustered closer together and are larger in both size and elevation relative to the central and western islands (McCartney and Veltre, 1999). Across the treeless island chain, the region experiences an oceanic climate with annually moderate temperatures, fog, rainfall, sleet, snow, sudden violent storms and fierce gale-force winds (Lantis, 1984; McCartney and Veltre, 1999). The area is also subjected to volcanic and seismic activity occasionally triggering tsunamis (McCartney and Veltre, 1999). Upwellings in the surrounding waters of the Aleutians enrich the waters with nutrients supporting a diverse marine ecosystem the prehistoric and contemporary populations in the area came to rely on to sustain their way of life.

The Aleutian tradition has been found in the Aleutian archipelago and represents nearly 4,000 years of continuous cultural occupation (McCartney, 1984). However, several archaeological sites in the eastern Aleutians provide evidence of human presence dating as early as 8,000 BP (Laughlin and Aigner, 1966; Aigner, 1970; Dumond and Knecht, 2001; Knecht and Davis, 2001). The cultural zone of the Aleuts spans the region of the Aleutian archipelago, but historically they also occupied the Shumagin Islands as well as the lower western section of the

Alaska Peninsula (McCartney and Veltre, 1999).

Semipermanent Aleut settlements were located close to shore and were communities of either communal or single-family barabaras, semisubterranean houses made of driftwood and whale bones covered by layers of sod and grass and outfitted with hatches in the roof used for ventilation and an entranceway (Johnson and Wilmerding, 2001). The semisubterranean houses afforded their tenants a living environment with a semiconstant temperature as well as protection from the harsh oceanic climate, in particular high winds, cold and rain (McCartney and Veltre, 1999). Barabaras were heated and lit using lamps fueled by oil from sea mammals (Laughlin, 1980). Clothing made of bird skins or sea mammal furs and waterproof parkas (*kamleikas*) constructed from sea mammal gut provided the Aleuts protection against the elements (Laughlin, 1980). Throughout the Aleutians mortuary practices are varied. Several methods for the treatment of the deceased include cave burials with individuals either bundled or extended, *umqans* (burial mounds with V-shaped channels on hillsides), bundled burials located within houses, isolated burials, as well as evidence of dismemberment, curation of skulls and cremation (Hrdlička, 1945; Hatfield, 2010).

Tools found in tool kits associated with the Aleutian tradition include, but are not limited to, stone lamps, needles, awls, adzes, scrapers, abraders, stemmed points (bifacially trimmed), various knife forms including tanged and untanged knives, and ulu blades as well as an elaborate bone industry (Dumond, 1987; Fagan, 1995). Additionally, the Aleuts were capable of maritime travel and open water hunting using baidarkas, a seafaring kayak-style vessel made of driftwood and bone then covered by skins stitched together with sinew (McCartney and Veltre, 1999). The Aleut tool kit coupled with maritime capabilities facilitated their ability to exploit the abundantly rich ecosystem in the surrounding area. Food sources for the Aleut diet consisted of cetaceans (whales and porpoises), pinnipeds (seals and sea lions), sea otter, fishes (Irish lord, Pacific cod, rock greenling, halibut, salmon), marine invertebrates (sea urchin, mussels, clams, scallops, periwinkles, chiton and limpets) as well as birds and eggs (McCartney, 1984; Byers et al., 2011).

Stable isotope analysis of prehistoric Aleuts from Chaluka, Ship Rock and Kagamil revealed Paleo- and Neo-Aleuts coexisted at these sites for ~500 years post-1,000AD and confirmed their

reliance on a marine centered diet (Coltrain et al., 2004; Byers et al., 2011). Differences in diet were observed based upon cranial affiliation (Paleo- and Neo-Aleut) as well as time and geographic location (Byers et al., 2011). The results determined those from Chaluka, mostly Paleo-Aleuts, relied more on near shore prey such as greenling, Irish lord and Pacific halibut. Meanwhile individuals on Ship Rock and Kagamil, regardless of cranial affiliation, relied on higher trophic open water prey such as pinnipeds and cetaceans (Coltrain et al., 2004; Byers et al., 2011). Additionally, a slight shift in subsistence practice was observed on Chaluka during recent times (post-1,000 BP) with an increased reliance on open water fishes (Byers et al., 2011). However, it is currently unclear what these observed differences in diet between sites and through time represent, such as an influx of open water hunters into the area, differences in social class or changing resources (Byers et al., 2011).

Among researchers there had been the question regarding the peopling of the archipelago. Current archaeological and genetic evidence (discussed below) has led to the wide acceptance of the colonization of the Aleutian archipelago being initiated from the east and progressing westward (Laughlin, 1980; Dumond, 1987). In light of the current data, the east to west peopling model quashes an earlier alternative view held by a few who contended multiple migratory events into the Aleutians that were multidirectional, with entry into the west from Kamchatka through the Commander Islands and the eastern entrance from the edge of the Bering platform (see Black, 1983). To date the oldest archaeological sites in the Aleutians are located on the eastern islands of the Aleutian archipelago. The Anangula Blade site on Anangula Island is the earliest evidence for human presence in the Aleutian archipelago and has been dated to approximately 8,400 BP (Laughlin, 1963; Laughlin and Aigner, 1966; McCartney and Turner, 1966; Aigner, 1970). Two additional early eastern Aleutian sites are located on Hog Island approximately 200 km away from the Anangula Blade site. The two sites are the Russian Spruce site (UNL-115) dated to 8,050 BP and, 200 m uphill from it, the Oiled Blade site (UNL-318) dated to 8,000 BP (Dumond and Knecht, 2001; Knecht and Davis, 2001).

Moving westward into the central Aleutians, the Tutiakoff site (ADK-171) is the earliest archaeological evidence in the Andreanof Island group located on Adak Island (O'Leary, 2001;

Hatfield, 2010). The Tutiakoff site is located on a terrace 20 m above the eastern shore of Clam Lagoon and has been dated to approximately 6,000 to 4,000 BP (O'Leary, 2001). In the Near Islands, the most western group of islands in the Aleutians, the oldest archaeological evidence to date is the ATU-061 site found on Shemya dating from 3,500 to 3,000 BP (Lefevre et al., 2001; Hatfield, 2010). This particular site is located 100 m inland from the current shoreline on the southwest coast of the island (Lefevre et al., 2001). Meanwhile Cairn Creek (ATU-193) is the oldest site discovered to date on Attu Island, the most western island of the chain in the Near Islands group and has been dated to 2,200 BP (Corbett et al., 2001; Lefevre et al., 2001).

The distribution of the archaeological sites in the archipelago, with sites dating as old as 8,400 BP in the east and progressively younger ones in the west, is the evidentiary lynchpin used by scholars in support for a westward migration initiated from the Alaskan mainland. Additional support of a westward expansion along the island chain is the dearth of prehistoric human occupational sites on the Commander Islands. These particular islands are the most western islands of the chain and could have potentially served as a stopping point while traversing eastward. Although the east to west colonization of the Aleutians is now widely accepted, there is still uncertainty regarding interaction with peoples outside the Aleutians as well as the number of migrations into the island chain.

There has also been some disagreement as to whether or not those in the Aleutians developed in isolation or had contact with outside groups (Black, 1983). There is mounting archaeological evidence attesting to population movements and/or ideas that filtered from east to west in the Aleutians suggesting outside influences from the east. The case for outside influences rests upon the appearances of bifacial technology, Arctic Small Tool tradition (ASTt) tools and exotic materials such as jet and slate (Hatfield, 2010). The manifestation of bifacial technology in the Aleutians may have been the result of local innovation or contact with neighbors since previous technologies persisted in the region even after its introduction into the area (Hatfield, 2010). In the Aleutians, bifacial technology is found in the eastern islands ~7,000 BP and then filtered westward to the central and western Aleutians ~6,500 BP.

The presence of ASTt-like tools in the region suggests those in the easternmost Aleutians

were most likely influenced by individuals east of the Aleutians (Hatfield, 2010). However, the adoption of ASTt elements was confined to the eastern Aleutians ~4,000 to 3,500 BP and did not extend westward to the central or western Aleutians. The ASTt appeared approximately 4,500 to 4,000 BP and its geographic range spanned from the northern margin of Alaska and Canada up into the Canadian Arctic archipelago and Greenland (Dumond, 1987; Friesen, 2004). Additional regional traditions subsumed under the ASTt umbrella include: Denbigh, Saqqaq, Pre-Dorset, Dorset, Independence I, Ipiutak, Norton and Choris (Maxwell, 1984; Maxwell, 1985; Bielawski, 1998; Friesen, 2004; Hatfield, 2010). The roots of the ASTt are unclear but may have stemmed from peoples of either North America or Siberia and northeastern Asia (Anderson, 1984; Powers and Jordan, 1990; Hatfield, 2010). Its manifestation is believed to mark the arrival and/or appearance of ancestral Eskimo/Inuit cultures (Fagan, 1995).

The presence of exotic materials, such as jet and slate, is initially observed in the eastern Aleutians around ~1,000 BP and later on in the central and western Aleutians post-1,000 BP. Jet is typically associated with the Alutiiq culture from Kodiak Island (see Hatfield, 2010). Meanwhile ground slate artifacts are characteristic of the Neo-Eskimo group the Thule who are believed to have originated in northwest Alaska and expanded eastward to Greenland (Mathiassen, 1927; Ford, 1959; Taylor, 1963; Stanford, 1976; McCartney, 1977; McGhee, 1984a,b; Dumond, 1987). Both materials, jet and slate, are considered to be an indication of the influence of and/or interaction with peoples eastward of the Aleutians.

In the Aleutians Hrdlička (1945) identified two cranially distinctive groups, the Pre-Aleuts and Aleuts, while analyzing skeletal remains recovered from Chaluka Midden on Umnak Island and burial caves on Kagamil and Ship Rock Island. Hrdlička (1945) postulated the dolichocranic (high-vaulted and narrow) Pre-Aleuts, found mostly at Chaluka Midden, were either replaced or absorbed by the brachycranic (low-vaulted and broad) Aleuts, found primarily in burial caves on Kagamil and Ship Rock, when the Aleuts migrated from the Alaskan mainland sometime around 400 to 500 years prior to Russian contact. Hrdlička's replacement argument rests upon the recovery of Aleut skeletal remains from the stratigraphic layer overlying the Pre-Aleuts at Chaluka Midden (Hrdlička, 1945).

The idea of population replacement has been debated among those studying the peoples of the Aleutians. Hrdlička's classification scheme of Pre-Aleuts and Aleuts was later changed to Paleo-Aleut and Neo-Aleut, respectively, "to emphasize sequence rather than replacement" (Laughlin and Marsh, 1951; Turner et al., 1974:141). Additionally, it was also suggested the arrival of the Neo-Aleuts occurred circa 1,000 BP (Laughlin and Marsh, 1951). The change in cranial form has been suggested by some as having occurred in situ rather than being the product of population replacement (Laughlin, 1963; Laughlin, 1975; Laughlin, 1980). Laughlin and Marsh (1951) observed larger population sizes in the eastern islands, which they argued provided conditions that offset the effects of drift and allowed for the selection of the Neo-Aleut brachycephalic cranial form. Meanwhile the smaller populations in the west would have been more sensitive to the effects of drift thereby resulting in the retention of dolichocephalism in the Paleo-Aleuts. Additionally, Paleo- and Neo-Aleuts were contemporaneous on Umnak Island post-1,000 BP with both cranial types observed among the Unangaχ throughout the island chain (Laughlin and Marsh, 1951; Coltrain et al., 2006).

Biodistance analysis of nonmetric cranial traits in Aleut samples reveals an affinity with Athabascans and Northwest Coast Indians (Szathmary and Ossenberg, 1978; Ossenberg, 1992). Similar results were observed using discrete dental traits where Aleuts clustered with Athabascans (Turner, 1983; Scott, 1994). Anthropometric data grouped Aleuts with Labrador Indians, Northwest Coast Indians and several Yupik speaking Southwestern Alaska Eskimos (Bristol Bay, Kuskokwim and St. Lawrence Island) (Szathmary, 1979; Ousley, 1995). Craniometrically the Paleo-Aleuts were like the Plains Indians (Sioux more specifically), while the Neo-Aleuts were like the Siberian Tungus, Kodiak Eskimos, Northwest Coast Indians, Athabascans, St. Lawrence Island Eskimos and Lower Kuskokwim River Eskimos (Hrdlička, 1945; Heathcote, 1986; Ousley, 1995).

Ousley and Jones' (2010) analysis of craniometric data of ancient Aleuts discovered increased craniometric variability, changes in cranial morphology and a reduction of stature through time, which are most likely the result of skeletal plasticity. Although morphological variations with respect to geographical location were detected there was no discernible clinal

pattern to the variation and the relationship between morphology and time proved to be stronger than morphology and geography (Ousley and Jones, 2010). Analysis of Aleut dentition revealed minor dental variation between eastern and western Aleuts but such dental variation was not observed when comparing Neo- and Paleo-Aleuts. The absence of dental variation between the Neo- and Paleo-Aleuts was interpreted as evidence supporting the lack of a late migration into the islands. However, these findings were based on small sample sizes (Turner, 1967; Turner, 1974). Additionally, some have argued the criteria used by Turner to classify the ancient Aleut crania may have been misguided and the statistical methods used did not have sufficient power to detect differences between the two groups (Ousley and Jones, 2010).

Modern and ancient mtDNA analysis of discrete markers has established both the Unangax̂ and ancient Aleuts exhibited haplogroups A and D (Hayes, 2002; Rubicz et al., 2003; Zlojutro et al., 2006; Rubicz, 2007; Smith et al., 2009; Zlojutro et al., 2009). Earlier studies had shown contemporary and prehistoric Aleuts had comparable frequencies of haplogroup A and D (Hayes, 2002; Rubicz et al., 2003). Within the Aleutians exists a clinal distribution of haplogroups A and D across the island chain. The eastern communities exhibit higher frequencies of haplogroup A thought to be associated with gene flow with Eskimo populations while haplogroup D frequencies increase as one moves westward along the archipelago, which is the result of founder effect and genetic drift due to forced relocation of Unangax̂ to western communities during Russian occupation (Zlojutro et al., 2009; Crawford et al., 2010). Despite this regional variation amongst the Unangax̂, the ancient Aleuts and Unangax̂ are remarkable because they both demonstrate high frequencies of haplogroup D relative to surrounding populations who are characterized by elevated frequencies of haplogroup A.

Ancient Aleuts, regardless of cranial affiliation (Paleo- or Neo-Aleut), are indistinguishable from one another since individuals from both groups have the same haplogroups observed amongst the Unangax̂ in the region (Hayes, 2002; Smith et al., 2009). An increase in sample size revealed ancient Aleuts predating 1,000 AD exhibited higher frequencies of haplogroup A relative to later Aleuts (post-1,000 AD) who displayed elevated frequencies of haplogroup D than haplogroup A (Smith et al., 2009). Radiometric data attest to the coexistence of Paleo- and Neo-

Aleuts at these sites for ~500 years post-1,000 AD but the comparison of ancient DNA and craniometric data revealed no association between the transition in matrilineal molecular variation and morphological changes over time in either Aleut crania or body size (Coltrain et al., 2006; Ousley and Jones, 2010). However, these authors acknowledge the possibility that such gene flow could be obscured by the plastic nature of skeletal components in response to changes in environmental conditions (Ousley and Jones, 2010). Additionally, if gene flow was largely nonmaternal then an association between morphology and molecular variation may not be detectable using mtDNA.

Mitochondrially the Unangax̂ have genetic affinities with populations from the Chukotkan Peninsula, more specifically the Siberian Yupik and the Chukchi (Rubicz et al., 2003). Sequencing has revealed the Unangax̂ are A2 but are largely the A2 root with the additional mutation of 16192T, which in previous papers had been referred to as A2a1/A2a/A3 (as A3 in Rubicz et al., 2003; A2a in Helgason et al., 2006; A2a1 in Gilbert et al., 2008). The Unangax̂ also have A2 haplotypes with additional polymorphisms that appear to be Aleut specific. For instance, the haplotype characterized by the A2a root plus the 16212A transition, which in previous papers has been referred to as A2a1a and A7 (Rubicz et al., 2003; Zlojutro et al., 2006; Zlojutro et al., 2009). There is one other subhaplotype known as A2b1, characterized by the A2 root with the additional 16265G polymorphism, which is present in only two Unangax̂ individuals from Nelson Lagoon (Zlojutro, 2008; Zlojutro et al., 2009). The A2b1 subtype, also known as A2b and A6, is largely observed in the Inuit of Canada and Greenland but has also been observed in Siberian Yupik, Iñupiat and the Chukchi (Shields et al., 1993; Helgason et al., 2006; Zlojutro et al., 2006). Complete mtDNA sequencing has identified D subtypes D2a and D2a1a among the Unangax̂, using solely HVS-I mutations the D subtype observed is D2a'b with several haplotypes comprised of the D2a'b motif with one or two additional mutations (Derbeneva et al., 2002; Rubicz et al., 2003; Zlojutro et al., 2006; Zlojutro et al., 2009). The HVS-I mutation motif associated with the D2a'b subtype has been observed in the Chukotkan populations (Chukchi and Siberian Yupik) and ancient south Alaskans from Mink Island and Port Moller, as well as an ancient Saqqaq sample (Shields et al., 1993; Starikovskaya et al., 1998; Derbeneva et al., 2002; Gilbert et al.,

2008; Raff et al., 2010). Sequencing of the mtDNA genomes of ancient Aleut samples is necessary to assess which haplogroups they exhibit (A2, A2a, D2, D2a'b etc.) relative to their contemporary counterparts, the Unangax̂, as well as surrounding circum-Arctic populations to address the matrilineal genetic relationships of these populations.

Dorset and Thule

The Dorset complex represents a late Paleo-Eskimo culture that existed in the eastern Arctic around 2,500 yBP and persisted for nearly two millennia (Maxwell 1985). They developed in situ from the Pre-Dorset without outside cultural influences from the east or an influx of immigrants (Maxwell, 1984). The cultural range of the Dorset spans along the coastlines of eastern Canada, extending northwards into the Canadian High Arctic Archipelago and western Greenland (Maxwell, 1984). The material culture of the Dorset has its basis in the ASTt, which appeared in the eastern Arctic around 4,500 to 4,000 BP (Dumond, 1987; Friesen, 2004).

Lithics associated with the Dorset material culture are characterized as a microlithic complex with delicately flaked stone tools including triangular shaped projectile points with fluted distal ends, multiple notched slate knives and chert end blades as well as burin-like tools (Maxwell, 1985). Hunting technology used by the Dorset involved the use of harpoons with harpoon heads made of antler, ivory and sometimes hard driftwood (Maxwell, 1985). Line holes were gouged as opposed to drilled, which is a distinctive characteristic of Dorset harpoon heads (Jenness, 1925). Features used to distinguish the Dorset from their predecessors, the Pre-Dorset, include the introduction of bone sled shoes, snow knives, and ice creepers as well as an increased presence of stone lamps. The appearance of this winter hunting gear may signify an economic shift towards a greater emphasis on sea-ice hunting of seals and walrus during the winter and spring (Maxwell, 1984). Much of this is in part related to unstable and colder climatic conditions in the Eastern Arctic during the time of the Dorset (Barry et al., 1977). Additionally, the disappearance of the bow and arrow as well as dogs from Dorset cultural assemblages suggests a decline in winter stalking of caribou.

Dorset settlements were located closer to the coast and were primarily low density

occupations with seasonal dwellings of tents and snow houses as well as semisubterranean sod houses and elongated rectangular houses (Dumond, 1987; McGhee, 2001; Odess, 1998). The subsistence pattern of the Dorset is believed to have been dependent upon a dualistic economy. The more common faunal remains identified in Dorset middens included various species of seal, walrus, caribou, muskox and polar bear, Arctic char, Lake trout as well as rookery birds such as murres, guillemots and dovekies (Maxwell, 1985). Stable isotope analysis of three Dorset samples grouped two of the Dorset (T-1 and Tayara) with the Sadlermiut, who were heavily reliant on seal and sea birds, while the third Dorset (Angekok) sample plotted with the Thule who relied on terrestrial taxa as well as the intake of marine mammals with varying trophic levels (Coltrain et al., 2004).

Although there are a number of documented Dorset sites, a limited number of known Dorset remains have been recovered (see Lynnerup et al., 2003; Brown, 2011). The scarcity of remains has been attributed to the Dorset possibly leaving the remains of their deceased uncovered on the sea ice or tundra (Maxwell, 1985). Mortuary practices of the Dorset are varied, with remains recovered from a variety of contexts including no features (surface finds or midden deposits), stone rings, burial pits, caves and crevices (Brown, 2011). The recovered remains are generally incomplete and disarticulated, which is taken as indication of the remains being exposed prior to burial (Brown, 2011). Artifacts associated with Dorset remains are either functional or symbolic in nature. Functional items are related to either subsistence activities such as harpoon heads, end blades and fore-shafts or maintenance activities including needles, skin scrapers and steatite cooking pots (Brown, 2011). Meanwhile, symbolic items are either functional miniatures such as model harpoon heads or purely symbolic such as carved swimming bear figurines or amulets/pendants of seals, walruses and bears (Brown, 2011).

Outside of mortuary items the Dorset are known for their art-like carvings. Some examples recovered from Dorset sites include full size masks, little maskettes, dolls with removable appendages, rectangular plaques of polar bears with canines overlapping, and figurines of animals, birds and fish. Many of these carvings, though small in size, are accurately proportioned with attention to anatomical detail and engraved with straight lines that were parallel, crossed,

formed X's or chevrons (Maxwell, 1985). In the western Arctic small figures decorated by the ancient Aleut exhibit similar decoration motifs with crosshatched patterns and chevrons (Knecht et al., 2001). The exact purpose of these artistic items remains uncertain but is thought to be for personal enjoyment as well as magical or shamanistic rituals (Maxwell, 1985).

Given the limited completeness of Dorset remains, the use of three craniometric measurements from the only two complete Dorset crania (Pumbley Cove and Crow Head Cave, Newfoundland) grouped the Dorset with the Birnirk of Kugusugaruk (Alaska), Thule/Inuit of Labrador, and Siberians of Old Bering Sea (Utermohle, 1984). Although the Dorset individuals are distant from the Sadlermiut and Thule, Utermohle (1984) suggests similarities in cranial measurements indicate some type of distant ancestral Beringian relationship between the groups. Others are in agreement with the Dorset being Eskimoid rather than Native American (Oschinsky, 1964; Laughlin, 1979; Anderson and Tuck, 1974).

The remains of three Dorset were analyzed using discrete markers and identified haplogroup D in two Dorset (T-1 and Angegok) while the third Dorset (Tayara) is presumed to also belong to haplogroup D since it does not belong to haplogroups A, B and C (Hayes, 2002; Hayes et al., 2003). This haplogroup profile is strikingly different from the Thule, who were monomorphic for haplogroup A, while the Sadlermiut (see below) exhibited both haplogroups A and D (Hayes, 2002; Hayes et al., 2003). Additional genetic analysis such as mtDNA sequencing is necessary to determine which haplogroups may be present in the Dorset, which will elucidate their maternal genetic relationship to surrounding Arctic and sub-Arctic groups who belong to D4b1a2a1 (Canadian and Greenland Inuit) or subgroups of D2 (Saqqaq and Unangax̂) (Rubicz et al., 2003; Helgason et al., 2006; Zlojutro et al., 2006; Gilbert et al., 2008; Zlojutro et al., 2009). Sometime around 1,000 to 800 yBP the Thule (a Neo-Eskimo group originating from northwestern Alaska) began moving eastward, disrupting nearly three millennia of isolation of the eastern Arctic Paleo-Eskimos (Friesen and Arnold, 2008). The Thule successfully infiltrated the Canadian High Arctic, Labrador and Greenland and they have continued to persist to today in the area and are known as the Inuit (Maxwell, 1985). The eastward expansion of the Thule is believed to have coincided with the Medieval Warm Period (ca. 1,150 to 600 BP) but it is uncertain what exactly precipitated

the eastward dispersal of the Thule (Taylor, 1963; LeBlanc et al., 2004). Several possible reasons offered include “warmer climates, reduced seal population, population pressure from the west, and increased emphasis on hunting the bowhead whale, which were already moving eastward” as well as the availability of meteoric iron in northwestern Greenland (Taylor, 1963; Schledermann and McCullough, 1980; McGhee, 1984a; Maxwell, 1985:253).

Many of the areas the Thule came to inhabit had been previously occupied by the Dorset, which is marked by an abrupt change in material culture. The Thule used dog traction as well as the umiak and kayak as means for hunting and transporting people and their household goods. The Inuit also used the aforementioned gear, which was absent amongst the prehistoric Dorset (Maxwell, 1985). Thule technology is sophisticated and easily distinguished from that of the Dorset with the presence of the bow and arrow, ground-slate blades, bow drill, throwing boards, spears, toggling and nontoggling harpoon heads (some specific to certain hunting locations or species), floats used with harpoons which are indicated by the presence of carved plugs, swivels and plug mouthpieces (McGhee, 1984b). Another notable distinction between the Thule and Dorset is seen in the line holes of their harpoon heads; the former had drilled holes while those of the latter were gouged (Jenness, 1925). Elements of the Thule material culture including tool kit (particularly harpoon head styles), architectural layout of dwellings, and their maritime economy nod to the western roots of the Thule tradition that is believed to have emerged from the Birnirk tradition (Taylor, 1963; Schledermann and McCullough, 1980; Anderson, 1984; McGhee, 1984b; Dumond, 1987).

The Thule tool kit enabled them to hunt a wide array of game in the open waters, sea ice as well as on land throughout the ecosystems in the Arctic and sub-Arctic (Maxwell, 1985). Thule subsistence is thought to have included whale given their open-water hunting technology and the use of whalebone in housing structures (Mathiassen, 1927; Maxwell, 1985). Additional support for Thule whale hunting stems from biometric evidence of whale skeletal material at central Canadian Arctic sites that indicated the Thule focused their efforts on hunting smaller whales such as yearlings (Savelle and McCartney, 1994). In addition to whale, the Thule also sought out various species of seals along with caribou and walrus (Maxwell, 1985). The presence of hares,

fox, wolves, birds, muskox (in certain regions) as well as eggshells and clamshells in Thule assemblages indicates they procured a wider range of foodstuffs relative to the Dorset who had resided in the same localities (Maxwell, 1985). Stable isotope analysis of Thule remains from Kamarvik and Silumiut found the Thule's economic strategy included a reliance on marine mammals (ringed seal and bowhead whale) and caribou (Coltrain et al., 2009).

Thule settlements consisted of multiple sod and stone winter dwellings with whale-bone integrated into the frame with flagstone flooring and elevated sleeping platforms overlaid with gravel or stone slabs and the use of coursed stonework (McCartney, 1977; McGhee, 1984b). Snow houses and tents were also used by the Thule as evidenced by the presence of snow knives as well as tent rings at Thule sites (McCartney, 1977; Maxwell, 1985). Additional features identified at Thule culture sites include boat rests, *inuksuit* (stone landmarks), meat caches, stone traps for foxes or smaller animals and burial cairns (McCartney, 1977).

The mortuary practices of the Thule were markedly different from those used by the Dorset. As mentioned previously the Dorset are thought to have left their dead exposed on either land or ice prior to leaving the remnants of the remains on the surface, in stone rings, burial pits, caves or crevices (Maxwell, 1985; Brown, 2011). The Thule, on the other hand, "buried" their dead in stone cairns, which were carefully constructed and camouflaged to resemble "simple stone piles" (Merbs, 1997:251). Stone burial cairns were built around the flexed remains of the deceased who was either clothed or covered in animal skins and laid directly upon the ground (bedrock and nonbedrock) surface (McCartney, 1977; Maxwell, 1985; Merbs, 1997). Metric and nonmetric skeletal and dental analyses have shown prehistoric Thule remains aggregated with each other as well as with the Thule/Inuit (Greenland, Labrador and Central Arctic) and the Sadlermiut of Southampton (Mayhall, 1979; Utermohle and Merbs, 1979; Utermohle, 1984; Ossenberg, 2005). Additionally these analyses also demonstrated that the Thule have a strong affinity with the prehistoric Birnirk of Northern Alaska, which lends additional support to the Thule's connection to the west (Mayhall, 1979; Utermohle and Merbs, 1979; Utermohle, 1984; Ossenberg, 2005).

Thule art was also different from that observed amongst the Dorset with Thule figurine carvings being mostly oriented towards human and avian figures (Thomson, 1979; Maxwell,

1985). The most commonly carved figure is that of a female while the second is usually a depiction of a floating bird in the form of a loon (Maxwell, 1985). Human figurines were made of wood or ivory and either had stumps for arms or no arms with female representations having topknots along with distinctive pelvic and chest markings (Thomson, 1979; Maxwell, 1985). Swimming figurines, typically carved of ivory and sometimes bone, depicted either a loon or a loon body with a human female torso and head that was decorated with either rows or lines of dots (Thomson, 1979; Maxwell, 1985). Other Thule design elements, in addition to dots, include "Y" designs, lines, holes and pictographs of people in hunting or camping scenes (Thomson, 1979; Crandall, 2000).

The Thule also crafted toys and decorated certain classes of utilitarian objects for men and women. Toys were in the form of miniatures of just about everything used by adults but made for children using "bone, ivory, antler, wood and baleen" and included the likes of kayaks, umiaks, dogsleds, men's knives, fish spears, bows and arrows, lances as well as ulus and soapstone lamps (Maxwell, 1985:293). Women's decorated utilitarian objects included combs and needle cases, with combs being the most commonly decorated item used by women (Maxwell, 1985). Meanwhile the men's items that have been seen decorated include harpoon heads (with the exception of lance heads or sockets), men's knives (except for snow knives), fish lures, drag handles, toggles, quiver handle ends, pail handles and drill bows (Maxwell, 1985).

The fate of the Dorset as well as the question of cultural interaction between the Dorset and the Thule has been debated amongst anthropologists. The overlaps in carbon dates for Dorset and Thule coupled with attributes viewed as cultural borrowing are considered to provide support for cultural interaction between the two cultures. Examples often cited as evidence corroborating cultural borrowing include harpoon head morphology, the adoption of bone and ivory sled shoes, snow knives for snow houses and the use of soapstone for lamps and pots by the Thule from the Dorset (Maxwell, 1980; McGhee, 1984b; Maxwell, 1985; Parks, 1993). Aside from harpoon morphology, most of the technologies thought to have been adopted by the Thule from the Dorset have been observed at Alaskan sites (Alaskan Thule and Birnirk) predating the Thule migration eastward (Ford, 1959; Stanford, 1976; Parks, 1993).

Addressing the question of cultural interaction between the Dorset and the Thule has been complicated by the very nature of the sites themselves. In many instances Thule and Dorset sites overlie one another with little or no sterile soil separating one cultural occupation after another resulting in accidental comingling of cultural items (Park, 1993). A more conservative review of eastern Arctic dates almost entirely eliminated the 1,000-year overlap between the Dorset and Thule by removing dates considered problematic due to contamination, questionable source of dating material (e.g., raw bone, antler), geographic location and association (see Park, 1993).

A third tenuous line of evidence for Dorset-Thule interaction is the Inuit legend of the Tuniit. The legend of the Tuniit is used by the Neo-Eskimos to describe the Paleo-Eskimo people they encountered who inhabited the eastern Arctic prior to their arrival. The Tuniit are portrayed as a strong, gentle and peaceful people who spoke a language that sounded like baby talk. The cultural affiliation of the Tuniit is usually associated with the Dorset; however, it has also been used to describe the Sadlermiut (Mathiassen, 1927; McGhee, 2001).

Sadlermiut

The Sadlermiut are an aberrant eastern Arctic group whose place in Arctic prehistory has intrigued many. Sadlermiut occupations have been found on the islands of Southampton, Walrus and Coats in north Hudson Bay (Collins, 1955, 1956a, 1957). Having lived in relative isolation the Sadlermiut were considered “strange and primitive” by surrounding groups and had minimal contact with both surrounding groups and European travelers (Comer, 1910; Collins, 1956b:669; Clark, 1980). Neighboring Inuit groups looked down on the Sadlermiut because they had difficulty communicating with them since the Sadlermiut spoke an unfamiliar dialect (Collins, 1956b). The clothing of the Sadlermiut is characterized by fringeless parkas, as well as polar bear skin pants worn by men and nearly hip high women’s boots. Even though there are differences in stylistic attributes the Sadlermiut clothing falls well within the continuum of Inuit style clothing in the region (Rowley, 1994). During the winter of 1902-1903, the Sadlermiut sadly met their demise soon after they encountered a European whaling ship and contracted an

infectious disease, presumably typhus, typhoid or dysentery (Taylor, 1959; Ross, 1977; Rowley, 1994). Of the estimated 56 Sadlermiut residing on Southampton Island only five survived the epidemic that decimated their people. Among the survivors were four children and one woman (Rowley, 1994).

Various theories have been offered regarding the origins of the Sadlermiut. There has been speculation of the Sadlermiut having originated on Southern Baffin Island, somewhere from the east such as Fox Peninsula or east coast of Foxe Basin (Mathiassen, 1927; see Taylor, 1959). Others have proposed the Sadlermiut represent a remnant Thule group or developed in situ from a cultural group with Thule ancestry, while others have posited the Sadlermiut were a remnant Dorset population influenced by the Thule (Mathiassen, 1927; Collins, 1956a; Clark, 1980; Maxwell, 1984). The material culture of the Sadlermiut indicates affinities with both the Thule and Dorset. The chipped chert stone tools of the Sadlermiut bear some resemblance to those observed in the Dorset tool kit with the caveat they are not as finely flaked as the Dorset's (Clark, 1980; Maxwell, 1984). Bone, ivory and antler implements of the Sadlermiut exhibit similarities to those found among Thule assemblages (Clark, 1980). The Sadlermiut used sod, whale bone and limestone slabs to construct their winter houses (with 1-3 rooms) with sleeping platforms (either elevated or lower relative to the outdoor ground-level) and cold trap entrances; however, the coursed stone work seen in Thule dwellings was absent from Sadlermiut houses (Taylor, 1960). Lamps and cooking vessels used by the Sadlermiut were constructed of limestone slabs secured together with baleen and mortar made of hair, ground limestone and blood (Mathiassen, 1927; Taylor, 1960). Similarly constructed vessels have been observed at Neo-Eskimo sites where soapstone is unavailable (Rowley, 1994). Mortuary practices of the Sadlermiut are similar to those used by the Thule and involved the use of burial cairns or placing the deceased on the surface surrounded by a ring of stones (Collins, 1956b; Rowley, 1994).

Faunal remains recovered from Native Point had a limited number of birds, shell and fish but were dominated by mammals, in particular seal (49.8%) followed by caribou (23.8%) and walrus (10%) (Taylor, 1960). Stable isotope analysis of protohistoric Sadlermiut remains identified their reliance on high trophic level marine taxa such as ringed seals and sea birds (Coltrain et al.,

2004; Coltrain, 2009). The diet of the Sadlermiut mirrored some Dorset (T-1 and Tayara) but was unlike the Thule of Kamarvik and Silumiut and a Dorset individual (Angekok) who generally relied on terrestrial and marine taxa with various trophic levels such as the ringed seal and bowhead whale (Coltrain et al., 2004; Coltrain, 2009).

Analysis of craniometric data determined the Sadlermiut have affinities with the Thule populations in Northeastern Greenland, Baffin Island as well as eastern Canada, more specifically Naujan, Kamarvik and Silumiut in the northwest margin of Hudson Bay (Utermohle and Merbs, 1979; Utermohle, 1984). Nonmetric cranial data also grouped the Sadlermiut with other eastern Arctic Inuit groups including Thule from Greenland and Canada, especially Labrador (Ossenberg, 2005). Similar findings were echoed by Mayhall (1979) whose dental analysis of metric and discrete traits clustered the Sadlermiut with individuals from Kamarvik and Silumiut.

Genetic analysis of mtDNA using RFLP markers revealed the Sadlermiut possessed haplogroups A (55.6%) and D (44.4%), while surrounding ancient populations such as the Thule and Dorset were respectively monomorphic for haplogroups A and D (Hayes et al., 2003). The RFLP characterization of the prehistoric eastern arctic populations provided support for the possibility of the Sadlermiut being a remnant Dorset population with Thule gene flow. Full mtDNA genome sequencing has identified haplogroup D among the Saqqaq, more specifically D2a1 (Gilbert et al., 2008). Contemporary Inuit populations in Canada and Greenland have varying frequencies of A and D present, in particular A2 (A2, A2a, A2b1) and D4b1a2a1 (Helgason et al., 2006). Meanwhile ancient Aleuts and their contemporaries (Unangax̂) also have haplogroups A and D present with D2a'b, D2a, D2a1a and A2 (A2, A2a, A2b1) observed in the modern population (Derbeneva et al., 2002; Hayes et al., 2003; Rubicz et al., 2003; Zlojutro et al., 2006; Smith et al., 2009; Zlojutro et al., 2009). Additional genetic analysis such as mtDNA sequencing may clarify the Sadlermiuts' relationship to other populations in the genetic landscape of the Arctic and whether or not they are a remnant Dorset population with Thule admixture.

Summary

This chapter provided an overview of the applications of genetics, specifically the HVS-I region of the mtDNA genome, to anthropological studies addressing issues related to the peopling of the New World. The findings of these genetic studies undergird the consensus of the Asiatic affinities of the indigenous peoples from the Americas. There is also agreement on the presence of five haplogroups, A, B, C, D and X, among the native peoples of the New World with the identification of a growing number of sublineages using whole mtDNA genome sequencing. However, there is still much debate surrounding the number and timing of migrations as well as points of entry into the Americas. Nonetheless, the information garnered from the genetic analysis of contemporary New World populations has provided a frame of reference to contextualize the findings from aDNA studies of prehistoric populations. The molecular characterization of prehistoric native peoples has revealed genetic continuity in some instances based on similarities of genetic mtDNA haplogroup profiles as well as shared haplotypes between ancient and contemporary populations. Instances where differences have been observed amongst populations either contemporary and/or prehistoric are often associated with gene flow, population replacement and/or displacement, genetic drift as well as population reduction.

Background information was also presented for several prehistoric populations from northern North America including the Aleuts in the Aleutian Islands as well as the Dorset, Thule and Sadlermiut in the eastern Arctic. Archaeological evidence indicates the Aleuts have had nearly 4,000 years of continuous occupation in the archipelago but several sites in the eastern Aleutians indicate a human presence in the area as early as 8,000 BP. It appears the Aleuts were not as culturally isolated as previously thought and have experienced influences from outside of the Aleutians based on ASTt like tools and the presence of exotic materials such as jet and slate. Skeletal evidence indicates the Aleuts are similar to Athabascans and Northwest Coast Indians while discrete dental evidence indicates similarities to Athabascans. Also evidenced in the Aleut skeletal material is the presence of two craniometrically distinct groups, the dolichocephalic Paleo-Aleuts and the brachycranic Neo-Aleuts. The Paleo-Aleuts are craniometrically similar to the Plains Indians (Sioux in particular), while the Neo-Aleuts are most like the Siberian Tungus,

Northwest Coast Indians and Eskimos (Kodiak, St. Lawrence Island and Lower Kuskokwim River). Ancient Aleuts, like their successors the Unangaŷ, exhibit haplogroups A and D regardless of cranial affiliation and temporal distribution. HVS-I sequences of the Unangaŷ, have identified haplogroups A2 and D2a'b and are matrilineally similar to Chukotkan populations.

The Dorset was a Paleo-Eskimo group in the eastern Arctic who existed for several millennia until the Neo-Eskimo Thule began their expansion eastward around 1,000 to 800 yBP. As they traversed eastward into Canada and Greenland the Thule brought with them their material culture (e.g., tool kit, art, mode of transportation) and mortuary practices that were distinct from the Dorset population that resided in the area the Thule had expanded into. Archaeologically delicately chipped microlithic tools founded in the ASTt characterize the Dorset tool kit. Even though there are a number of known Dorset sites, very few Dorset remains have been recovered. The limited Dorset skeletal remains available appear to be more Eskimoid rather than Native American. Discrete marker analysis of three prehistoric Dorset samples identified haplogroup D for two samples while the third is neither A, B or C, which is in stark contrast to the haplogroup profile of the Thule who are monomorphic for haplogroup A. It is uncertain which D subtype(s) may be present amongst the Dorset until mtDNA sequencing is performed. Sequencing of a Paleo-Eskimo Saqqaq individual identified the D2a1 subtype, with similar lineages observed amongst the Unangaŷ and Siberian Sireniki Yuit. The D2a1 mtDNA lineage is distinct from the D4b1a2a1 observed in contemporary Inuit of Greenland and Canada, which suggests matrilineal discontinuity in the region. The Sadlermiut are a conundrum whose origin and relationship to surrounding populations have intrigued anthropologists. The Sadlermiut demonstrate archaeological similarities to both the Dorset and Thule. Meanwhile skeletal and dental evidence indicates similarities to the Inuit/Thule. To date, discrete marker analysis has identified nearly equal frequencies of haplogroups A and D in the Sadlermiut from Southampton but it remains to be seen which subtypes occur within this population. MtDNA sequencing of Sadlermiut individuals has the potential to shed some light on their origins as well as their relationship to surrounding groups, particularly the Dorset and Thule.

Some similarities noted between the Aleuts and the Dorset include the observance of ASTt

tools amongst the Dorset and ASTt influenced tools in the Aleutian tool kit. There are also similarities with respect to decoration motifs, including crosshatched patterns and chevrons, observed in small figurines decorated by ancient Aleuts as well as the Dorset. One noted similarity between the Dorset and Sadlermiut is the presence of chipped stone tools but those of the Sadlermiut are not as finely flaked as those of the Dorset. However, the Sadlermiut in general exhibit many attributes found amongst the Thule including mortuary practice, construction of stone lamps and cooking vessels, as well as bone, ivory and antler technology.

One definitive attribute the Aleut, Dorset and Sadlermiut do have in common is the presence of haplogroup D. The characterization of haplogroup D in the Aleuts, Dorset and Sadlermiut sets them apart from surrounding circum-Arctic populations who exhibit high frequencies of haplogroup A. Sequence analysis of the mtDNA genome of these prehistoric populations (Aleut, Dorset, Thule and Sadlermiut) will identify which subhaplogroups are present thereby providing additional insights into the matrilineal relationships of these circum-Arctic groups to one another as well as to contemporary populations in the region, which may also elucidate peopling events in the region.

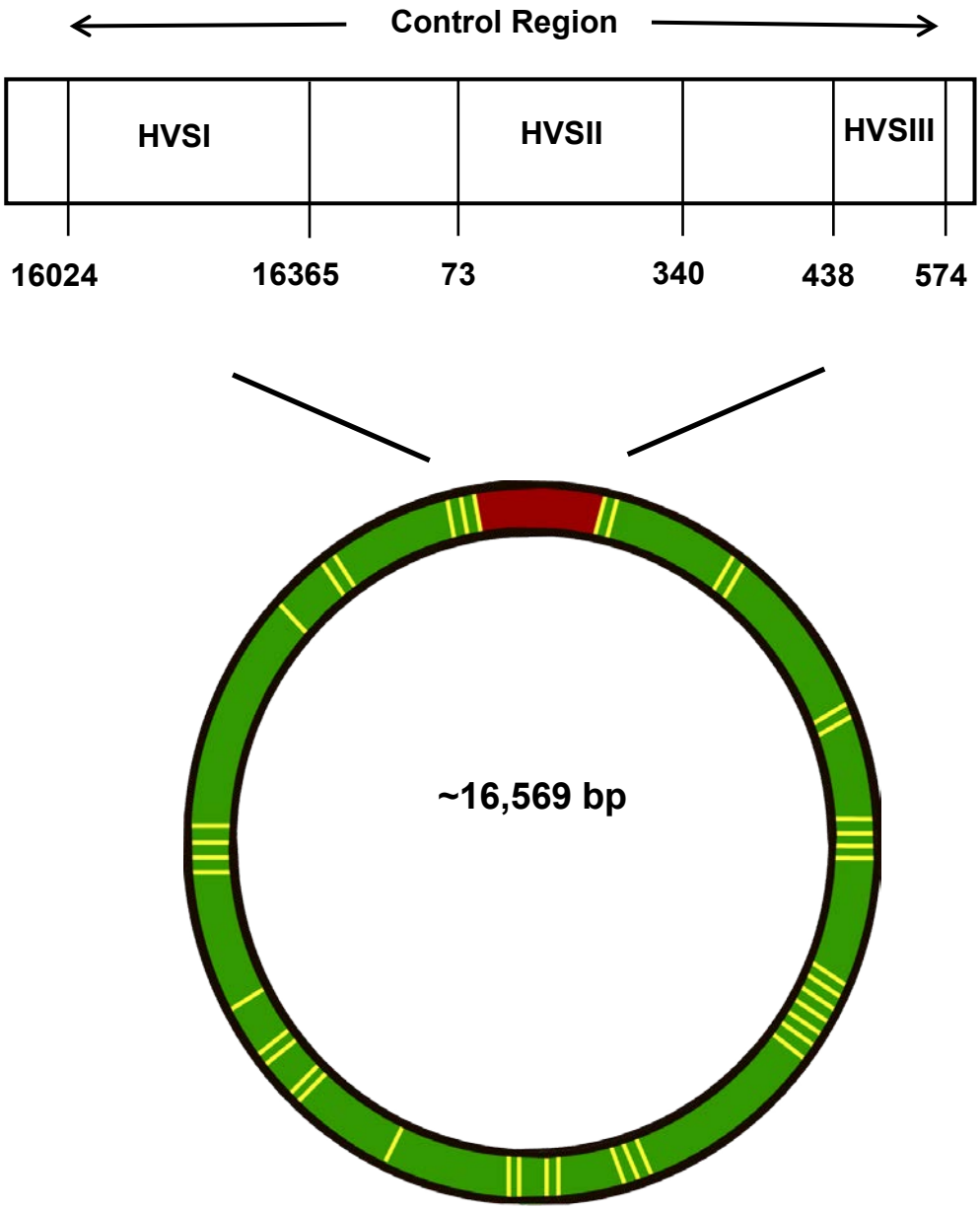

Figure 2.1. Human mtDNA map. Green and red regions indicate the coding and control region, respectively. The control region (red) is expanded above to show the three different hypervariable regions. Adapted from Butler, 2005; Jobling et al., 2013, created with PowerPoint.

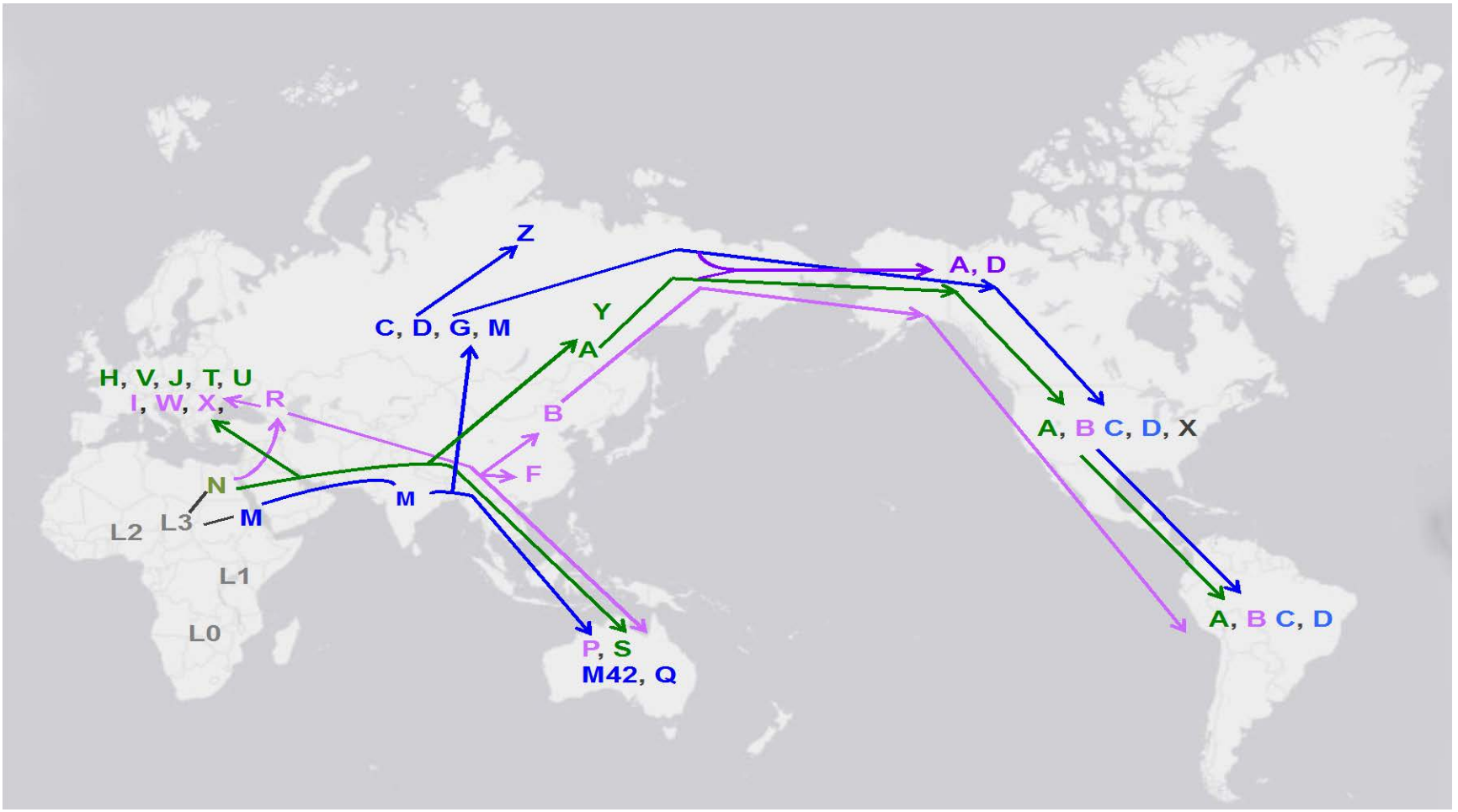

Figure 2.2. Human mtDNA haplogroups and their possible migration routes. Adapted after http://www.mitomap.org/pub/MITOMAP/MitomapFIgures/WorldMigrations.pdf, created using ArcGIS and ArcMap by ESRI, Adobe Photoshop and PowerPoint.

CHAPTER 3

METHODS AND MATERIALS

Samples

The ancient Aleut rib samples analyzed in this study are from the Aleut physical anthropology collection housed at the National Museum of Natural History (NMNH) in Washington, DC. The prehistoric Eastern Arctic fragmentary rib and tarsal samples of the Sadlermiut, Dorset and Thule were collected from the Canadian Museum of Civilization (CMC) in Hull, Quebec. Each sample was individually weighed, placed in separate sealable plastic bags and labeled. Throughout the collection process, the collector MG Hayes (MGH) took precautions to reduce the risk of self-contamination by wearing protective gear (e.g., masks, gloves, sleeves). In this study, mtDNA was extracted from sixteen ancient Aleut samples with the following proveniences and sample sizes: Chaluka Midden (*n*=10), Kagamil Island (*n*=2) and Ship Rock Island (*n*=4) (see Table 3.1 for sample information and Figure 3.1 for site locations). Aleš Hrdlička (1945) and his students recovered the ancient Aleut remains over the course of several field seasons (1936-1938) and are from three separate sites located on the more easterly islands of the Aleutian archipelago. Skeletal remains were recovered from interred burials at Chaluka Midden outside of Nikolski village on the western side of Umnak Island, whose terrain is characterized by treeless undulating grasslands dotted with bogs and freshwater lakes (Denniston, 1966). Chaluka Midden is a deeply stratified mound of considerable size, spanning over 200 m in length, 61 m in width and reaching a maximum depth of 6.8 m. The oldest radiocarbon dates from the Chaluka site based on charcoal samples 60 cm and 85 cm "above native sterile bottom," respectively, dated to 3,750±180 and 3,600±180 radiocarbon years BP (Laughlin and Reeder 1962:857). The nearly 4,000 years of continuous Aleut occupation of the site has yielded a rich and extensive collection

of material culture, faunal remains, structures and human skeletal remains (Hrdlička, 1945; Laughlin and Marsh, 1951; Denniston, 1966).

Mummified remains were recovered from burial caves located on Kagamil Island and Ship Rock Island but it is uncertain how long the burial caves were used at either location (Hrdlička, 1945; Keenleyside, 1994). Associated artifacts (e.g., glass beads, canvas) at both locales and pathological evidence (e.g., leprosy, syphilis, smallpox) on remains from Kagamil indicate the burial caves to have been in use as late as the early Russian contact period (Hrdlička, 1945; Laughlin, 1980; Frohlich and Laughlin, 2002). Radiocarbon dating of remains from Kagamil (*n*=32) and Ship Rock (*n*=16) found them to predate Russian contact (Coltrain et al., 2006). However, these dated remains only represent a subset of those recovered from the burial caves (Kagamil *n*=226; Ship Rock *n*=101) and the authors note they "may not have captured the full temporal range of these sites" (Hunt, 2002; Coltrain et al., 2006:544).

Remains recovered from the burial caves were clothed, placed in a flexed fetal position, wrapped in layers of skins (seal or sea lion) and sometimes woven mats or basketry (Laughlin, 1980; Frohlich and Laughlin, 2002; Hunt 2002). The bundle was subsequently secured with a cord of braided sinew (Laughlin, 1980). Mummy bundles were either placed on planks supported by a framework of posts driven into the walls of the cave, or placed on the ground and covered by rocks and planks (Laughlin, 1980). Laughlin (1980) described these burial caves as "museums" because of the cultural items (stone and organic materials) associated with the mummified remains as well as the preserved remains themselves (Laughlin, 1980:96). Of the mummified remains collected by Hrdlička from the burial caves on the islands of Kagamil and Ship Rock, only the most intact mummified bundles collected from Kagamil remain (Hunt, 2002). The mummy bundles from Kagamil are curated at the NMNH in their original state of preservation from the time of collection while the rest of the mummified remains were macerated down to the skeleton per Hrdlička's direction (Hunt, 2002).

The Sadlermiut (*n*=10) remains were archaeologically recovered from the Native Point (KkHh-1) site, which is located on the southeastern side of Southampton Island in the northern end of Hudson Bay (see Table 3.2 and Figure 3.2 for eastern Arctic skeletal sample locations)

(Collins, 1956a,b, 1957). It is uncertain as to the total length of time this settlement was used but the end of occupation was marked by the demise of the Sadlermiut in the early 20th century (1902-1903) (Mathiassen, 1927; Collins, 1957). The Sadlermiut were not the only ones to have used the site, as there was evidence it had also been used by the Thule and Aivilingmiut (a contemporary Inuit population) (see Taylor, 1960). The Native Point site was the subject of an archaeological field expedition during several field seasons from 1954 to 1956 (Collins, 1956a; Taylor, 1959; Merbs and Wilson, 1962). The focus of the 1956 field season was on the recovery of Sadlermiut remains, examining Sadlermiut winter houses as well as the collection of faunal elements and Sadlermiut material culture (Taylor, 1959). Over an area covering 0.12 km^2 one hundred stone house ruins were documented at the Native Point site and a comparable number of burials were said to dot the area (Collins, 1956b; Taylor, 1960). Human remains at the site were typically enclosed in burial cairns built of stone but some remains were simply placed on the surface and surrounded by a stone circle (Collins, 1956b; Rowley, 1994). Faunal assemblages indicate the Sadlermiut were heavily reliant on seal and caribou followed by walrus (Collins, 1956a; Taylor, 1960). Laughlin returned with Merbs and several others in 1959 and recovered more than 150 additional skeletal remains from Native Point (Merbs and Wilson, 1962).

The Dorset are represented by three individuals whose remains were archaeologically recovered from three separate sites: the Tayara (KbFk-7) site on Sugluk Island, the Angekok (JIGu-2) site on Mansel Island in Hudson Bay and a rock enclosure near the T-1 plateau site (KkHh-3) on Southampton Island (see Table 3.2 and Figure 3.2—Eastern Arctic skeletal sample locations). The Tayara site is situated on an elevated beach along the western shore of Sugluk Island in the Hudson Strait and appears to have been occupied approximately between 800 to 300 BC based on the similarity of recovered artifacts to those from dated sites (Taylor, 1968). The Tayara site produced an abundance of faunal remains, cultural artifacts as well as some human remains (more specifically a fragmentary mandible, several teeth and three rib fragments); however, the site lacked structural remains (e.g., houses, burials, food caches) (Taylor, 1968). Taylor recovered the Angekok (JIGu-2) Dorset remains from Mansel Island. Although no formal report was written for this site (per Coltrain et al., 2004:44), the remains as well as the site were

designated as later Dorset on the basis of associated artifacts (Oschinsky, 1960; Taylor, 1968). The third Dorset sample, T-1, was originally catalogued as Sadlermiut among the Native Point (KkHh-1) assemblage on Southampton Island but was later reclassified as a Dorset burial by Coltrain et al. (2004) based on the age of the remains (1,992±41 radiocarbon years BP). Collins collected the remains from a rock enclosure less than a mile away (northwest) from the Dorset Tunermiut site, also known as the T-1 plateau site (KkHh-3) on the southeastern coast of Southampton Island (Collins, 1956a; Coltrain et al., 2004). The T-1 site is located on a seventy-foot high plateau that has sparse, low growing vegetation and was radiocarbon dated to 2,000-2,792 BP using charred bone (Collins, 1956a; Collins, 1957; Coltrain et al., 2004). Save for the shallow middens that covered an area over 0.08 km^2 on the northern and western sections of the plateau as well as some hearths and irregularly arranged flat stones (possibly flooring), the T-1 site was void of any other discernible structures such as burials, cairns, house ruins or pits (Collins, 1956a; Collins, 1957). Seal followed by walrus and fox dominated the faunal assemblage; however, the abundance of bird bones indicates a seasonal (summer) occupation of this site (Collins, 1956a; Collins, 1957).

The Thule (*n*=9) skeletal remains were recovered from two archaeological sites, Silumiut (KkJg-1) and Kamarvik (LeHv-1), which are situated along the coast of the Canadian mainland in northwestern Hudson Bay (see Table 3.2 and Figure 3.2—Eastern Arctic skeletal sample locations). These sites were excavated in the late 1960s during the Northwest Hudson Bay Thule Project (McCartney, 1977). The Silumiut site is located on an island in northwestern Hudson Bay that during low tide is connected to the Canadian mainland, while Kamarvik is located on a peninsula approximately 181 km northeast of Silumiut (McCartney, 1977). The topography at both locales is similar, rugged exposed bedrock with the formation of lakes and ponds in low irregular basins (McCartney, 1977). Both sites were on grass covered gravel sections situated at the highest elevations (on either hill tops or slopes) providing expansive views of surrounding coastal areas (McCartney, 1977). The dwellings at both sites were typical of Thule winter villages as well as tent rings indicating seasonal (summer/autumn) usage (McCartney, 1977). Additional structures identified at both sites include boat rests, *inuksuit* (stone landmarks), meat caches and

burial cairns (McCartney, 1977). Burial cairns dotted the tundra surface at both sites with nearly 200 burial cairns documented at Silumiut and over 100 at Kamarvik (McCartney, 1977). Rocks were used to build the cairns that entombed an individual who was either clothed or covered in animal skins and laid in a flexed position directly on the bedrock or sometimes on nonbedrock surfaces such as "cobbles, pebbles, gravel, sand, clay, and a combination of these materials" (Merbs, 1997:259). Local conditions and taphonomic processes affected the preservation and condition of the skeletal remains, which varied from very good to very poor (see Merbs, 1997). The artifact assemblages at both locales are comparable with the collection from Kamarvik being smaller relative to that from Silumiut (McCartney, 1977). A number of recalibrated dates using wood samples from Silumiut provided dates of AD 1224 (2 sigma range AD 1039-1296) and AD 1293 (2 sigma range AD 1163-1427), while nine other intercept dates ranged from AD 1216-1657 (2 sigma AD 1000-modern) using other wood and caribou antler samples (see Coltrain et al., 2004). Meanwhile, a wooden sample from a midden provided a recalibrated date of AD 1221 (2 sigma range AD 1018-1387) for the Kamarvik site (McCartney, 1977; Coltrain et al., 2004).

Comparative Sequences

Ancient DNA Sequences

Sequences from other ancient populations in the geographic region were culled from the literature for comparison to the ancient populations examined in this study. The ancient south Alaskans samples are from the Hot Springs site near Port Moller, Alaska on the Bering Sea coast of the Lower Alaska Peninsula area, the Brooks River area in the northern region of the Alaska Peninsula and Mink Island off of the southern aspect of mainland Alaska (Raff et al., 2010). A single individual represents the ancient Saqqaq, which was recovered from an early Paleo-Eskimo site dating to 3,900 to 3,100 14C BP (based on conventional 14C dating on twigs framing the cultural area, while the hair was in a cultural level dating to 3,680±85 to 3,310±80 14CBP) on the small island of Qeqertasussuk in southern Disko Bay in western Greenland (Gilbert et al., 2008).

Contemporary DNA Sequences

The sequences from various contemporary populations included for comparison to the ancient populations of this study were garnered from the literature. The Unangax̂ (contemporary Aleut) sequences used in this study come from Rubicz et al. (2003) and Zlojutro et al. (2006). Additional circum-Arctic populations included in comparative population analyses include the Chukchi and Siberian Yuit (Shields et al., 1993; Starikovskaya et al., 1998; Derbeneva et al., 2002), Greenlandic Inuit (Saillard et al., 2000; Helgason et al., 2006; Gilbert et al., 2008), Canadian Inuit (Helgason et al., 2006), Koryak and Itel'men (Schurr et al., 1999), Han and Mongolian (Yao et al., 2002; Kong et al., 2003), northern North American Native American groups Haida, Bella Coola and Nuu-Chah-Nulth (Ward et al., 1991; Torroni et al., 1993; Ward et al., 1993) and Iñupiat (Alaskan Eskimos) (Shields et al., 1993).

Laboratory Methods

Contamination and Quality Control

By nature the polymerase chain reaction (PCR) is a robust process and is sensitive to low levels of DNA. As a result, it is imperative to implement precautions to avoid the inadvertent introduction of extraneous DNA. In ancient DNA (aDNA) studies, this is of particular concern since contamination from contemporary sources can potentially provide DNA of better quality compared to the degraded state of aDNA. The inadvertent introduction of exogenous contemporary DNA can ultimately result in the preferential amplification of the contaminant DNA over endogenous DNA. Stringent precautions have been recommended in aDNA studies in attempts to reduce the possibility of contamination as well as to provide means to detect any occurrences of contamination (Handt et al., 1994; Cooper and Poinar, 2000; Hofreiter et al., 2001).

In this study, the contamination precautions taken include the use of disposable gowns, face masks, hairnets, sleeves and gloves whenever handling a sample and throughout all extraction, PCR and post-PCR procedures. In addition, aDNA dedicated work area surfaces and equipment were cleaned with a 10% bleach solution followed by 100% ethanol, and when possible were

irradiated with ultraviolet (UV) light overnight. Ultrapure grade reagents were used and when possible were aliquoted for single use. Extraction and pre-PCR procedures were performed in laminar flow hoods equipped with UV lights located in physically separated clean rooms with positive pressure and overhead UV lights, which were dedicated to aDNA analysis. In addition, controls were included in all extractions as well as all amplifications to detect contamination. In the event contamination was detected, the experiment was discarded and precautions were taken to avoid additional contamination occurrences. Mitochondrial DNA profiles of personnel with lab access were determined and none belong to the haplogroups found in the ancient samples under investigation. Lastly, multiple sequences (either forward, reverse or both) were obtained for all samples and second independent extractions were performed.

Ancient DNA (aDNA) Extraction and Purification

Surface decontamination of the samples (0.30-0.36 gm) was performed prior to extraction by immersing the sample for 15 minutes in a 0.5% bleach solution. Next, the sample was rinsed thoroughly with ultrapure water, air-dried for 15 minutes and sealed in a UV-irradiated Seal-A-Meal® bag. The packaged sample was immersed in liquid nitrogen, crushed and transferred to a UV-irradiated 2 mL tube.

A silica matrix-based extraction method, after Baker et al. (2001), was used to extract the DNA from the manually reduced bone samples. Briefly, 1.5 mL of the extraction buffer [10 M GuSCN, 0.1 M Tris-HCl (pH 6.4), 0.2 M EDTA (pH 8.0), 1.3% Triton X-100] was added to the sample and incubated overnight at 60 °C with slight agitation. The following day 450 µL of the supernatant was transferred to a 2.0 mL UV-irradiated tube and purified with a silica-based slurry using the GENECLEAN® II Kit (Qbiogene, Inc., Carlsbad, CA). Samples were treated per manufacturer's instructions and eluted in 150 µL of ultrapure molecular grade water and stored at -20 °C until analysis.

For a handful of recalcitrant Dorset and Thule samples a modified extraction method was used in an attempt to obtain sequence data for these samples. The modified method implemented a decalcification step to chemically reduce the bone sample in lieu of manual

reduction of the bone sample. Samples were incubated overnight in EDTA with gentle agitation and the following day the samples were treated with a proteinase K digestion. The silica-based extraction method described above using the GENECLEAN® II Kit (Qbiogene, Inc., Carlsbad, CA) was performed per manufacturer's instructions with the exception of extending incubation times from 15 minutes to 30 minutes. Samples were subsequently eluted in 150 µL of ultrapure molecular grade water and stored at -20 °C until analysis.

Polymerase Chain Reaction (PCR)

The 50 uL reactions contained the following mixture of reagents from Applied Biosystems (Foster City, CA), unless otherwise specified, 2 U AmpliTaq Gold®, 1X of GeneAmp® 10X PCR Gold Buffer, 200 µM each dNTP, 2 mM $MgCl_2$, 10 pmol of each primer (Bio-Synthesis, Inc., Lewisville, TX) and 1-3 µL of extracted DNA. The hypervariable segment I (HVS-I) region (16,055-16,410 np) of the mitochondrial genome was amplified using three overlapping primer pairs when possible. Otherwise it was amplified in four overlapping sections, to obtain a 395 bp (base pair) fragment (see Table 3.3 for primer sequences after Handt et al., 1996). Thermalcycling parameters involved a four-minute enzyme activation step at 95 °C followed by 50 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds. A 12% polyacrylamide gel was used to resolve 20 µL of amplified product alongside a size standard. Amplicons of the appropriate size were bandstabbed (see Wilton et al., 1997) and amplified using the PCR and thermalcycling parameters described previously for 15 cycles. Prior to sequencing, the amplified product was purified using the QIAquick® PCR Purification Kit (QIAGEN®, Inc., Germantown, MD) per manufacturer's instructions.

Sequencing

QIAquick® purified samples were quantified visually on a 12% polyacrylamide gel by comparing the intensity of the sample's amplicon relative to known amounts of DNA in a quantitative DNA size standard ladder. The GenomeLab™ DTCS Quick Start Kit (Beckman and Coulter, Inc., Fullerton, CA) was used per manufacturer's instructions for the 10 µL sequencing

reactions using 3 to 11.5 ng of DNA in a 96-well plate (Beckman and Coulter Inc., Fullerton, CA). The following thermal cycling conditions were used for 30 cycles for the sequencing reactions: 96 °C for 20 seconds, 50 °C for 20 seconds and 60 °C for 4 minutes, followed by a holding step at 4 °C. Afterwards, 10 µL of molecular grade water was added to each well containing a sample and subsequently purified via ethanol precipitation per manufacturer's protocol for plate precipitation. The purified sequence amplification products were analyzed via capillary electrophoresis on a CEQ™ 8000 Genetic Analysis System plate (Beckman and Coulter Inc., Fullerton, CA) according to manufacturer's specifications using the LFR-1 method, with a decrease in separation time from 85 minutes to 45 minutes.

Statistical Analysis

Median-Joining Networks

The phylogenetic relationships of the HVS-I mtDNA sequences of ancient and contemporary populations were computed and graphically displayed via the median-joining (MJ) network analysis (Bandelt et al., 1999) using Network 4.6.1.2 (http://www.fluxus-engineering.com). The MJ network algorithm accommodates intraspecific multistate data (such as amino acid sequences), which qualifies it as an analytical method applicable to the human mtDNA sequences in this study. In addition, the mtDNA genome satisfies the median-joining networks stipulations of infrequent occurrence of ambiguous states and absence of recombination. First, the MJ algorithm begins with the construction of a minimum spanning network with all of the minimum spanning trees combined into one network. The algorithm proceeds by introducing a limited number of median vectors, which represent sequences that are either extinct ancestral sequences or extant sequences not yet sequenced. The MJ algorithm can handle ambiguous character states but it does not resolve ties when sequence pairs arranged in increasing order have the same distances. The resulting network produced by the MJ algorithm contains all of the most parsimonious trees, with reticulations denoting alternative mutational pathways due to mutational events such as parallel mutations. Sometimes these cycles, especially those on the periphery, can also signify issues with the data with regard to data entry or sequence alignment.

Multidimensional Scaling

The mtDNA relationships of ancient and contemporary populations in the geographic area were graphically represented in reduced-dimensional space using the ordination technique of multidimensional scaling (MDS) with the software program NTSYSpc2.21j (Rolf, 2010). Genetic distances between populations were calculated for mtDNA HVS-I sequences using Nei's (Nei and Li, 1979) average number of nucleotide differences between populations (D_A).

$$D_A = \hat{\pi}_{12} - (\frac{\hat{\pi}_1 + \hat{\pi}_2}{2}), \quad [3.1]$$

where $\hat{\pi}_{12}$ is the average number of pairwise differences between populations 1 and 2, and $\hat{\pi}_1$ and $\hat{\pi}_2$ are the average pairwise differences within populations 1 and 2. Genetic distances were calculated using Arlequin 3.5.1.3 and served as the distance matrices used for MDS (Excoffier and Lischer, 2010).

Multidimensional scaling analysis is a distance-based ordination method that provides a graphical representation of proximity patterns among *n* objects/variables with the corresponding number of points (*n*) in *k*-dimensional space using dissimilarities. The goal of MDS is to realize a configuration of interpoint distances in *k*-dimensions that approximates the distances observed between objects/variables in the original matrix as closely as possible (Kruskal, 1964a,b). Multidimensional scaling uses an iteration method and arrives at a solution once it either attains the minimum stress or reaches a prespecified number of iterations for the analysis. Both MDS and PCA (Principal Component Analysis) are ordination methods. However MDS preserves interpoint distances more faithfully than PCA. In practice, PCA assumes a linear relationship and maximizes the percentage of the variation detected thereby placing greater weight on larger distances. In MDS, the relationship between the distances in *k*-dimensional space and those observed is monotonic. What the rank ordering of dissimilarities translates to in the MDS plot is similar objects/variables with smaller distances would be located closer together while dissimilar objects/variables with larger distances would be positioned farther apart. Thus, the proviso of a monotonic relationship among distances preserves the "nearness" of small interpoint distances,

which is a valuable attribute when analyzing regional and/or geographically proximal populations.

The MDS process begins with a configuration of points in *k*-dimensions, with *k* being established a priori by the investigator. The preliminary configuration can be either random or from another ordination method (e.g., the first *k* principal coordinates). Once the initial configuration has been generated, the interpoint distances (d^*_{hi}) for all pairs of points (*hi*) are calculated and compared to the original distances (d_{hi}) using a monotonic regression. The monotonic regression is a procedure that begins by evaluating whether or not there is a one-to-one association between the rank ordering of the original distances (d_{hi}) to the ordering of the corresponding calculated interpoint distances (d^*_{hi}), such that whenever $d_{hi} < d_{jk}$ then $d^*_{hi} \leq d^*_{jk}$. At any time when the calculated interpoint distances (d^*_{hi}) violate the aforementioned monotonic inequality (e.g., you have $d_{hi} < d_{jk}$ and $d^*_{hi} \geq d^*_{jk}$) the monotonic regression process then calculates a set of pseudo-distances ($\hat{d}_{hi}$), also known as fitted distances/values, estimated distances or disparities (Rohlf, 1972; Kruskal and Wish, 1978; Cox, 1982). The disparities ($\hat{d}_{hi}$) are obtained by averaging the d^*_{hi} values of consecutive violators of the monotonic condition with the d^*_{hi} value of most recent nonviolator. This calculated ($\hat{d}_{hi}$) value is then assigned to all points within this particular block of violators and most recent nonviolator (Härdle and Simar, 2007). At the conclusion of the monotonic regression procedure, the disparities ($\hat{d}_{hi}$) satisfy the requirement of having a monotonic relationship with the original distances (d_{hi}), so whenever $d_{hi} < d_{jk}$ then $\hat{d}_{hi} \leq \hat{d}_{jk}$. The disparities ($\hat{d}_{hi}$) are then used to calculate a value known as Stress, which assesses the nonmonotonicity of a configuration. In other words, Stress is a 'goodness of fit' indicator of how representative the configuration is of the data, with a value between 0 and 1 and the smaller the value the better the fit. The calculation of Stress is as follows:

$$Stress = \sqrt{\frac{\Sigma(d^{*}_{hi} - \hat{d}_{hi})^2}{\Sigma d^{*2}_{hi}}}, \qquad [3.2]$$

where d^{*}_{hi} are the calculated interpoint distances for all pairs of points (*hi*) in the original configuration and ($\hat{d}_{hi}$) are the disparities calculated in the monotone regression procedure.

In order to improve upon the stress of the original configuration, the coordinates of each point are adjusted in ordination space by a small amount in the direction of steepest descent, where the stress value decreases most rapidly. The process of calculating the ordination matrix, regressing interpoint distances to the monotonic function of original distances and recalculating the stress value is repeated until a predetermined number of iterations has been reached or a lower stress value cannot be realized.

Figure 3.1. Ancient Aleut site locations in the Aleutian Islands. The smaller rectangle in the Aleutian Islands expanded to show detail of site locations for samples. Map adapted from Hayes 2002, using ArcGIS and ArcMap by ESRI, Adobe Illustrator and Adobe Photoshop.

Figure 3.2. Site locations for ancient Eastern Arctic populations. Dorset (Tayara, T-1 and Angekok), Sadlermiut (Native Point) and Thule (Kamarvik and Silumiut) samples. Map adapted from Hayes 2002, using ArcGIS and ArcMap by ESRI, Adobe Illustrator and Adobe Photoshop.

Table 3.1. Ancient Aleut sample information.

Curation Number	Population	Site	Sex	Age BP[a]	Cal Age AD Intercept[a]	Cal 2σ Range AD[a]	RFLP Haplogroup Designation[b]
378462	Neo-Aleut	Ship Rock	M	1071±39	1462	1346-1580	D
378474	Paleo-Aleut	Ship Rock	M	1361±45	1244	1082-1346	D
378464	Neo-Aleut	Ship Rock	M	1372±39	1236	1091-1327	A
378471	Neo-Aleut	Ship Rock	M	1446±44	1165	1043-1281	D
377901	Neo-Aleut	Kagamil	M	1206±51	1365	1275-1459	A
377902	Neo-Aleut	Kagamil	M	1214±58	1361	1258-1462	D
378606	Neo-Aleut	Chaluka	F	962±48	1557	1452-1666	A
378613	Paleo-Aleut	Chaluka	F	977±38	1543	1451-1652	D
378609	Paleo-Aleut	Chaluka	M	1392±39	1218	1078-1309	D
378616	Paleo-Aleut	Chaluka	F	2124±67	488	307-658	D
378633	Paleo-Aleut	Chaluka	M	2025±44	588	446-639	D
378639	Paleo-Aleut	Chaluka	F	2044±44	570	436-680	D
378624	Paleo-Aleut	Chaluka	M	3708±57	BC 1431	BC 1608-1257	A
378625	Paleo-Aleut	Chaluka	M	3722±88	BC 1447	BC 1686-1201	A
378626	Paleo-Aleut	Chaluka	M	3754±54	BC 1482	BC 1655-1318	A
378630	Paleo-Aleut	Chaluka	F	3758±42	BC 1485	BC 1645-1352	A

a. Dates are from Coltrain, 2009; Coltrain et al., 2006; Coltrain et al., 2004.
b. RFLP haplogroup assignments are from Hayes, 2002; Smith et al., 2009.

Table 3.2. Ancient Eastern Arctic sample information.

Curation Number	Population	Site	Sex	Age BP[a]	Cal Age AD Intercept[a]	Cal 2σ Range AD[a]	RFLP Haplogroup Designation[b]
XIV-C:749	Dorset	T-1	F	1992±41	423	325-539	D
XIV-C:340	Dorset	Angekok	UNK	1216±35	1248	1165-1297	D
XIV-B:003	Dorset	Tayara	UNK	2260±50	245	99-390	Not A, B or C
XIV-C:230	Sadlermiut	Native Point	M	684±40	1687	1642-1826	D
XIV-C:117	Sadlermiut	Native Point	M	690±55	1677	1548-1836	A
XIV-C:167	Sadlermiut	Native Point	M	714±42	1680	1627-1817	D
XIV-C:174	Sadlermiut	Native Point	M	725±50	1669	1536-1811	A
XIV-C:147	Sadlermiut	Native Point	F	728±41	1628	1488-1673	D
XIV-C:148	Sadlermiut	Native Point	F	806±37	1632	1503-1679	A
XIV-C:098	Sadlermiut	Native Point	F	822±32	1641	1521-1683	D
XIV-C:126	Sadlermiut	Native Point	M	836±59	1613	1466-1691	D
XIV-C:153	Sadlermiut	Native Point	UNK	856±41	1577	1484-1675	A
XIV-C:145	Sadlermiut	Native Point	F	875±38	1485	1436-1573	A
XIV:C:531	Thule	Kamarvik	F	594±41	1671	1630-1809	A
XIV-C:584	Thule	Kamarvik	F	768±49	1461	1411-1577	A
XIV-C:560	Thule	Kamarvik	UNK	846±43	1428	1334-1475	A
XIV-C:533	Thule	Kamarvik	M	852±38	1432	1393-1476	A
XIV-C:388	Thule	Silumiut	F	672±39	1475	1432-1619	A
XIV-C:356	Thule	Silumiut	M	716±44	1465	1422-1612	A
XIV-C:412	Thule	Silumiut	F	730±55	1487	1427-1643	A
XIV-C:380	Thule	Silumiut	UNK	762±44	1483	1423-1619	UNK
XIV-C:376	Thule	Silumiut	UNK	1130±50	1219	1063-1219	A

a. Dates are from Coltrain, 2009; Coltrain et al., 2006; Coltrain et al., 2004.
b. RFLP haplogroup assignments are from Hayes, 2002; Smith et al., 2009.

Table 3.3. Primers used for HVS-I sequencing.

Primer Position	Primer Sequence (5' to 3')[a]
L16055	GAAGCAGATTTGGGTACCAC
H16139	TACTACAGGTGGTCAAGTAT
L16131	CACCATGAATATTGTACGGT
H16218	TGTGTGATAGTTGAGGGTTG
L16209	CCCCATGCTTACAAGCAAGT
H16303	TGGCTTTATGTACTATGTAC
L16287	CACTAGGATACCAACAAACC
H16410	GCGGGATATTGATTTCACGG

a. Primers are after Handt et al., 1996.

CHAPTER 4

RESULTS

HVS-I Results

Ancient Aleuts

In this study six ancient Aleut samples (Table 4.1) successfully generated sequences for the 311 bp (base pair) amplicon spanning 16,060 np to 16,370 np (nucleotide position) of the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999). Among the six sequenced ancient Aleuts, a total of four haplotypes were observed and characterized by fourteen polymorphic sites that were all transitions. Sequences of the ancient Aleuts indicate haplogroups A and D, corroborating the restriction fragment length polymorphism (RFLP) results observed in previous studies (Hayes, 2002; Hayes et al., 2003; Smith et al., 2009). The ancient Aleut, like their contemporary counterparts the Unangax̂, exhibited haplogroups associated with D2 and A2 (Rubicz et al., 2003; Zlojutro et al., 2006). More specifically, the mtDNA haplogroups noted among the sequenced ancient Aleuts included D2 (*n*=1), D2a'b (*n*=4) and A2 (*n*=1). When the ancient Aleuts were separated according to cranial affiliation (Paleo-/Neo-Aleut), these haplogroups were distributed in the following manner between the two groups with D2a'b (*n*=3) in the Paleo-Aleut while the Neo-Aleut were D2 (*n*=1), D2a'b (*n*=1) and A2 (*n*=1). Haplogroup frequencies were the same as those determined for cranial affiliation when the Aleuts were separated according to time (pre-/post-1,000AD). The pre-1,000AD Aleut had D2a'b (*n*=3) while the post-1,000AD Aleut were comprised of D2 (*n*=1), D2a'b (*n*=1) and A2 (*n*=1). The suite of polymorphisms used to characterize haplogroup D2 include 16129A and 16362C, while haplogroup D2a'b is defined by 16129A, 16271C and 16362C (after van Oven and Kayser, 2008).

Meanwhile A2 is distinguished by the following motif of polymorphisms 16111T, 16290T, 16319A and 16362C (after van Oven and Kayser, 2008).

The most common sequence noted amongst the ancient Aleuts was the very one used to characterize the D2a'b haplogroup; this particular haplotype was observed in 67% of the samples sequenced to date. Among the populations analyzed (both contemporary and ancient), this HVS-I motif defining D2a'b has also been identified amongst the Chukchi, Siberian Yuit and Unangaŷ (modern Aleuts). The other two D haplotypes characterized in the ancient Aleuts sampled were not encountered in any of the other comparative populations, either contemporary or prehistoric. The haplotype for Aleut 378633 expressed the polymorphisms for the D2a'b haplogroup along with several additional polymorphisms. Aleut 378471 was classified as haplogroup D2 since the HVS-I sequence of this sample lacked the 16271C polymorphism used to establish membership to haplogroup D2a'b.

In this study, there was a single ancient Aleut sample exhibiting all of the mutations associated with the A2 haplogroup root definition, along with two additional mutations 16192T and 16234T. The contemporary Unangaŷ are also known to harbor these latter two polymorphisms (16192T and 16234T), with the 16192T transition occurring extensively (~91%) among Unangaŷ A2 sequences (Rubicz et al., 2003; Zlojutro et al., 2006; Zlojutro et al., 2009). The A2 root and the 16192T transition was used to define the A3 haplogroup present in the Unangaŷ population, which was renamed A2a1 (Rubicz et al., 2003; Zlojutro et al., 2006; Zlojutro et al., 2009). Helgason et al. (2006) have also reported this particular haplotype (A2 root along with the 16192T polymorphism) as A2a among the Inuit peoples from Greenland and Canada. The A2a haplotype has primarily been observed among the Unangaŷ and Eskimo/Inuit as well as some Beringian groups; however, it has also been detected in Na-Dene speaking Apache and Navajo and has been attributed to the migration of northern Athabascans to the Southwest (Basso, 1983; Shields et al., 1993; Torroni et al., 1993a; Starikovskaya et al., 1998; Schurr et al., 1999; Saillard et al., 2000; Malhi et al., 2003; Helgason et al., 2006; Tamm et al., 2007; Volodko et al., 2008; Achilli et al., 2013). A second independent extraction was performed for all of the ancient Aleut samples of which three (378462, 378616, 378639) were successfully verified via the

second independent extraction.

Sadlermiut

Sequences were successfully amplified for the 311 bp amplicon for seven Sadlermiut samples (Table 4.1) spanning 16,060 np to 16,370 np of the rCRS (Andrews et al., 1999). Four haplotypes were observed among the seven sequenced Sadlermiut samples in this study, with a total of six polymorphic sites all of which were transitions. The Sadlermiut sequences were assigned to haplogroups A2b1 and D4b1a2a1, which corroborates RFLP results of a previous study indicating the Sadlermiut harbored haplogroups A and D (Hayes, 2002; Hayes et al., 2003). Haplogroup A2b1 is characterized by the following polymorphisms: 16111T, 16265G, 16290T, 16319A and 16362C (after van Oven and Kayser, 2008). Meanwhile, the following motif of polymorphisms was used to define D4b1a2a1: 16173T, 16319A and 16362C (after van Oven and Kayser, 2008).

The frequencies of the haplogroups encountered amongst the Sadlermiut are as follows: A2b1 (*n*=4) and D4b1a2a1 (*n*=3). The haplotype defining haplogroup A2b1 was the most frequently observed haplotype (at 57%) amongst the Sadlermiut sampled in this study. The A2b1 haplogroup, also referred to as A2b by Helgason et al. (2006), has also been reported in the Chukchi, Siberian Yuit, Iñupiat, Inuit of Canada and Greenland, as well as two Unangaŷ from Nelson Lagoon (Shields et al., 1993; Zlojutro et al., 2006; Zlojutro, 2008; Zlojutro et al., 2009). Of the Sadlermiut sequenced to date belonging to haplogroup D4b1a2a1, two (Sadlermiut 147 and Sadlermiut 098) exhibited haplotypes not observed in any of the other comparative populations. Also, one of the Sadlermiut samples (Sadlermiut 167) typed as D4b1a2a1 possessed an additional polymorphism (16093C) while the other Sadlermiut samples (Sadlermiut 147 and Sadlermiut 098) were either heteroplasmic (T/C) at the 16093 site or lacked the polymorphism altogether. This particular D4b1a2a1 haplotype, consisting of the 16093C sequence variant associated with the D4b1a2a1 root, has been identified in contemporary Inuit groups from Canada and Greenland (Helgason et al., 2006). This particular D4b1a2a1 haplotype has also been referred to as D3 by Helgason et al. (2006) and was among a group of haplotypes referred

to as D4b1a2a1a by Derenko et al. (2010) observed in very low frequencies in several northern Asian populations such as the Chukchi and Koryaks (see Derenko et al., 2010 supplemental table S5). Over half of the Sadlermiut samples were confirmed by a second independent extraction with three of the samples (147, 167, 153) confirmed for the "full" 311bp HVSI sequence and one sample (174) confirmed by a partial sequence with two of the three fragments successfully sequenced. Meanwhile the sequencing results for three Sadlermiut samples were not successfully confirmed for the second independent extraction despite various amplification efforts and manipulations to a host of PCR parameters including the amount of DNA extract used, dilution of DNA extract, number of amplification cycles and concentrations of reagents (primer, $MgCl_2$, dNTPs and AmpliTaq Gold® DNA Polymerase).

Dorset and Thule

Of the three Dorset samples examined in this study, sequence data were obtained for the Angekok Dorset sample (Dorset 340) for the 311 bp amplicon spanning 16,060 np to 16,370 np of the rCRS (Andrews et al., 1999). A partial sequence of 238 bp was obtained for the T-1 Dorset sample (Dorset 749) spanning 16,060 np to 16,297 np. For the T-1 sample (Dorset 749), however, the fragment for primer pair 16287/16410 was not successfully amplified despite various amplification attempts. A range of manipulations to several PCR parameters were performed in an effort to obtain sequence data including varying concentrations of reagents (magnesium, primers, AmpliTaq Gold® DNA Polymerase), annealing temperatures, number of cycles as well as varying amounts of DNA extract and diluting the DNA extract. The 16287/16410 primer pair flanks the stretch of sequence containing polymorphisms used to assign several D haplogroups such as D1 (16,325 np and 16,362 np), D2 (16,362 np) or D4b1 (16,319 np and 16,362 np).

Of the sequence data available for the two Dorset samples in this study, a total of two haplotypes were determined (Table 4.1). There were five polymorphic sites observed all of which were transitions. The Dorset sequences were monomorphic for haplogroup D, corroborating RFLP results of a previous study (Hayes, 2002). The T-1 Dorset sample (Dorset 749) appeared

to potentially belong to D4b1a2a1; meanwhile, the Angekok sample (Dorset 340) exhibited the polymorphisms associated with haplogroup D2a'b. The polymorphisms observed for the partial T-1 Dorset sequence (Dorset 749) included 16093G, 16173T and 16223T which closely resembles the D4b1a2a1 subtype observed in the Inuit of Greenland and Canada. The third Dorset sample (Tayara) never provided any sequence data for any of the four overlapping fragments of HVS-I analyzed in this study. Discrete marker analysis determined this particular Dorset sample (Tayara) to belong to neither haplogroups A, B nor C (Hayes, 2002; Hayes et al., 2003). Despite repeated amplifications and extractions the mtDNA segment used to characterize haplogroup D in the Tayara sample proved to be unsuccessful (Hayes 2002; Hayes et al., 2003). This could be due to two possibilities: the Tayara individual "is a member of either haplogroup D or X, the latter the rare fifth Native American haplogroup" (Hayes, 2002:5). Additional testing is required to determine haplogroup membership of the Tayara Dorset individual.

As noted above, the Dorset samples proved to be remarkably recalcitrant and required various amplification attempts that involved manipulations to several different PCR parameters (see Table 4.2). A total of 470 amplifications (see Table 4.2) were carried out between the nine extractions done on the Dorset samples and sundry modifications to PCR factors. Nearly half (*n*=200) of the 470 amplifications were performed on the Tayara Dorset sample (Dorset 003) alone. Despite the number of amplifications the Tayara sample failed to provide any sequence data between the three separate extractions, with the third attempt employing a modified extraction protocol. As mentioned in the Methods and Materials chapter (see section Ancient DNA (aDNA) Extraction and Purification in Chapter 3), the alterations to the extraction protocol involved a chemical reduction of the sample in lieu of a mechanical reduction and extended incubation times throughout the silica-based extraction process. Briefly, the bone samples were incubated overnight in EDTA with slight agitation and the following day the samples were treated with a proteinase K digestion. The samples were subsequently extracted using the same silica-based extraction method as the nonrecalcitrant samples with the exception of extending incubation times throughout the process from 15 to 30 minutes. Independent extractions were carried out for each of the Dorset samples, which unfortunately did not provide sequencing

results to confirm sequence data obtained from the initial extractions of these samples.

Partial sequences were obtained for three out of the nine Thule samples for a short segment of HVS-I using primer pair 16055/16139 (Table 4.1). The 16111T transition was the only polymorphism noted in the sequence data for all of the Thule samples. Amongst the most northern populations, the 16111T mutation is associated with the A2 haplogroup and its associated subhaplogroups (A2a, A2b, A2b1 etc.). However, at this time it remains unclear which A haplogroups are present in the Thule. The Thule results presented are promising given a previous study that identified haplogroup A among the Thule using discrete markers (Hayes, 2002). Like the Dorset, the Thule samples also proved to be challenging. None of the Thule samples provided any data with the first two extractions. Partial sequences were obtained only when a third extraction was performed on a subset of the Thule samples (412, 584 and 388) using the modified extraction protocol discussed in the previous paragraph. The partial Thule sequences were not reproduced by an independent extraction.

Discrete Marker Success Versus Sequencing Success

Discrete marker analyses in previous studies of prehistoric circumpolar populations (Aleut, Sadlermiut, Dorset and Thule) unambiguously assigned anywhere from 67% (2/3 Dorset) to 94% (18/19 Sadlermiut) of the samples undertaken for study (see Table 4.3) (Hayes, 2002; Hayes et al., 2003; Smith, 2005; Smith et al., 2009). In this investigation a subset of samples from the aforementioned studies yielded sequencing results (see Table 4.3) ranging from 33% (3/9 Thule) to 70% (7/10 Sadlermiut) with a handful of sequences replicated via independent extraction. Differential successes between these molecular markers (discrete markers and HVS-I) have been reported by other aDNA studies examining these markers in parallel. Some studies have had better success with discrete markers relative to sequencing (e.g., Alzualde et al., 2005; Mooder et al., 2006; Shook and Smith, 2008), while others have had a converse experience in the successfulness of these markers (e.g., Stone and Stoneking, 1998). Occurrence of this phenomenon of differential successes between molecular markers as well as within markers (e.g., not all overlapping HVS-I fragments sequencing for a sample, not all discrete markers

working for a sample) is generally glossed over or altogether overlooked.

The usual suspect for amplification failures is the very nature of aDNA itself. Endogenous aDNA is typically fragmented and present in low quantities with the quality of the aDNA compromised via enzymatic (nucleases) and chemical (oxidation and hydrolysis) processes (Pääbo et al., 1989; Handt et al., 1994; Hofreiter et al., 2001). Endonucleases cleave DNA into short fragments, while nonezymatic processes such as oxidation and hydrolysis inflict damage to the DNA via several mechanisms resulting in an assortment of modifications and lesions to endogenous DNA (Lindahl, 1993; Hofreiter et al., 2001; Poinar, 2003; Alaeddini et al., 2010). Downstream the accrual of lesions caused by enzymatic, oxidative and hydrolytic attacks could impact the successfulness of DNA amplification (Butler, 2005; Alaeddini et al., 2010). For instance, the accumulation of abasic sites and nicks in DNA regions targeted for amplification could result in fragmentation of DNA, inability of primers to anneal to the DNA or the DNA polymerase unable to extend beyond abasic site(s) (Butler, 2005; Evans, 2007). Hydrolytic deamination can result in either amplification failure or misincorporation of bases depending upon whether the DNA polymerase used had proofreading capabilities (e.g., *Pfu*, Vent DNA polymerases) or was a nonproofreading (e.g., *Taq* DNA polymerase) polymerase, respectively (Evans, 2007).

Factors that impact the rate of DNA degradation include exposure to high temperatures, humidity, water, oxygen, pH of depositional medium and sunlight. Other culprits known to inflict additional damage to DNA include insect, fungal and bacterial agents (Eglinton and Logan, 1991; Poinar et al., 2003). Thus, ideal conditions for aDNA preservation are a rapid burial with dark, dry, anaerobic conditions, with low temperatures, microorganism-free environment and neutral or alkaline pH (Burger et al., 1999; Bollongino et al., 2008). As a result, the treatment of remains prior to burial, depositional environment, local environment and taphonomic conditions could potentially be factors affecting DNA preservation in the Arctic samples included in this study. For instance the Dorset are thought to have left the remains of their deceased exposed on the surface prior to burial (Brown, 2011). The preservation and condition of Thule remains from Kamarvik and Silumiut were found to vary from very good to very poor due to the effects of local

conditions and taphonomic processes (see Merbs, 1997).

Even if samples have been recovered from ideal conditions endogenous DNA could still undergo degradation following excavation. The phenomenon of postexcavation degradation has been noted in some studies where differential success in aDNA amplification was observed in skeletal elements recovered from excavations of the same site performed on two separate occasions (Götherström, 2001; Pruvost et al., 2007). Samples excavated more recently amplified successfully while those excavated at an earlier time (anywhere from either nearly sixty years to a century earlier) did not. Postexcavation treatment (e.g., washing bone samples with water and allowing them to air dry in the sun, maceration) and storage conditions (e.g., temperature in storerooms and laboratories, transportation between locales) of recovered skeletal element are factors thought to influence the survivability of aDNA in samples excavated at different times (Götherström, 2001; Pruvost et al., 2007). Optimally the window of time from sample recovery in situ to aDNA analysis should be small and tissue samples slated for aDNA analysis should be stored at dry and cold conditions, preferably in a freezer. Many of the circum-Arctic samples in this study were excavated during the early to mid-twentieth century and were collected for aDNA analysis by MG Hayes from skeletal collections housed at museums. Since their recovery from museum collections, the ancient circum-Arctic samples have been housed in secured metal specimen cabinets in a classroom setting. Ideally these samples should be housed in a cold temperature-controlled environment in the event they will be used in any additional aDNA analyses in the future. Precautions such as this could retard any further molecular decay from chemical or enzymatic processes.

Inhibitors (e.g., heme, calcium, collagen, humic acids) present in the DNA extract (endogenous to the sample or introduced by the environment or lab processing) are another assiduous factor that can either reduce amplification efficiency or impede it altogether (Butler, 2005). Potential mechanisms for how these inhibiting agents affect amplification include: 1) degrading DNA or reducing the recovery of DNA during extraction, 2) binding to the DNA, 3) interacting or binding to the polymerase and 4) interfering with the availability or binding to cofactors (e.g., magnesium) necessary for amplification (Wilson, 1997). Dilution of aDNA extract,

use of PCR additives such as BSA (bovine serum albumin), increasing concentrations of other PCR cocktail components (DNA polymerase or magnesium) and additional purification of DNA extract prior to amplification are some of the methods employed when combating PCR inhibitors (Tuross, 1994; Bickley et al., 1996; Scholz et al., 1998; Opel et al., 2010). However, as results from this study can attest these methods are not always foolproof. Amplification failures can still occur if additional DNA is lost via further purification of the DNA extract, over dilution of available endogenous DNA leading to issues with reproducibility of results and amplification failure, or if inhibitor concentration levels are too great to overcome with these approaches.

Additional reasons for differential success with various genetic markers between studies and laboratories could also be related to the efficiency of DNA recovery methods and possibly choice in polymerases. DNA extraction methods that implement a demineralization step [EDTA, EDTA plus proteinase K (PK)] have been shown to be very effective in terms of liberating DNA from bone facilitating in its recovery from osseous samples (Żołędziewska et al., 2002; Rohland and Hofreiter, 2007; Campos et al., 2012). Differences in sample success could possibly be due to this aspect of the extraction process as both Smith (2005) and Hayes (2002) chemically reduced bone samples with EDTA followed by the addition of PK. All the samples in this study, on the other hand, were physically reduced with only a handful of cantankerous samples reduced chemically using EDTA and PK when amplifications from initial extractions were unsuccessful. The modification made to sample reduction (from manual to chemical) methodology during the extraction process in this study provided promising though limited success with the samples extracted using this modified technique.

It has been established there are differential sensitivities/resistances of various DNA polymerases to the presence of inhibitors in DNA samples (Eilert and Foran, 2009; Monroe et al., 2013). Performances of some DNA polymerases have been found to be more robust (e.g., Omni Klentaq LA (Klentaq polymerase), hot start *Taq* variants like *Ex Taq* HS and *Tth*) in their ability to overcome inhibition (Abu Al-Soud and Rådström, 1998; Eilert and Foran, 2009; Monroe et al., 2013). Meanwhile other polymerases (e.g., *Tfl*) have been found to be more susceptible to the effects of inhibitors (Abu Al-Soud and Rådström, 1998; Eilert and Foran, 2009; Monroe et al.,

2013). In this study a different DNA polymerase (AmpliTaq Gold®) was used in lieu of the Deep Vent® polymerase used by Hayes (2002) and Smith (2005) in their studies, which could potentially be another factor in differential success with the ancient Arctic samples. The AmpliTaq Gold® polymerase was chosen for this study because of its properties related to amplification efficiency and specificity, characteristics valued when analyzing compromised samples as encountered in the forensic community and in aDNA studies (Moretti et al., 1998; Yang et al., 2003). Notwithstanding the foregoing properties AmpliTaq Gold® can be susceptible to the inhibitory effects of certain substances (e.g., hemoglobin, humic acid, collagen, calcium ions) found in extracts from blood and bone that cannot always be mitigated by the addition of BSA (Abu Al-Soud and Rådström, 1998; Eilert and Foran, 2009; Monroe et al., 2013).

Amplification failures involving DNA samples from forensic and aDNA contexts can be attributed to several different issues ranging from endogenous DNA damage to the presence of inhibitors. Taphonomic processes, local environments and conditions can also affect the preservation of aDNA in samples recovered from colder climates. Further degradation of aDNA is possible following excavation if ancient samples are not properly stored postexcavation. Additional factors such as extraction efficiency and DNA polymerase could also potentially play a role in differential successes with different molecular markers between studies. At this point in time the specific underlying cause(s) behind the differential success of HVS-I sequencing in this study and discrete markers in previous studies (Hayes, 2002; Smith, 2005) remain unclear. Additional analyses of the DNA extracts from these particular aDNA studies could possibly shed light on this matter. For instance, real time PCR (qPCR) could be used to assess the presence and levels of inhibitors in the aDNA extracts which could be related to the DNA extraction and purification techniques employed. A better understanding of the reasons behind amplification failure coupled with more efficient aDNA extraction methodologies and advances in molecular technology could be key in garnering additional sequences from these circum-Arctic samples in the future.

Median-Joining Network Analysis

Haplogroup A

A network was constructed using HVS-I sequences characterized as belonging to haplogroup A (Figure 4.1) from the Sadlermiut, ancient Aleuts, ancient South Alaskans (Port Moller, Mink Island and Brooks River) as well as the Inuit of Greenland and Canada, Iñupiat and Unangax̂. All four of the Sadlermiut samples (denoted by red) were designated as haplogroup A2b1, which is defined by the A2 root with the 16265G polymorphism. As revealed by the network this particular haplogroup, A2b1, was primarily observed among the Inuit of Greenland and Canada while infrequent among the Unangax̂. Meanwhile, the haplotype of the single ancient Aleut (Neo-Aleut/post-1,000AD) in this study (represented by blue) was characterized as the A2a (A2 root with the 16192T polymorphism), which is also present in the Unangax̂, Iñupiat and Inuit of Greenland and Canada. There is also one A2a haplotype in particular that appears to be Aleut specific with the 16212G polymorphism as it is not observed in any of the other comparative populations. The other ancient samples in the network were also characterized as A2, including ancient South Alaskans from Mink Island, Port Moller and Brooks River. The median-joining network illustrates there are a handful of A2 haplotypes and subhaplogroups (A2a and A2b1) present among contemporary and prehistoric populations throughout the Arctic/sub-Arctic.

Haplogroup D

A median-joining network analysis of haplogroup D sequences (Figure 4.2) was carried out using HVS-I sequences from the Sadlermiut, Dorset, prehistoric Aleuts, Saqqaq, ancient South Alaskans (Port Moller and Mink Island), Iñupiat, Unangax̂, as well as Greenland and Canadian Inuit populations. The ancient Aleut exhibited one haplotype associated with haplogroup D2 while the remainder were D2a'b. The latter of these two haplogroups was also present among the Unangax̂, ancient Alaskans (Port Moller and Mink Island), the Saqqaq as well as the Angekok Dorset sample from Mansel Island. The three Sadlermiut samples belong to haplogroup D4b1a2a1 and are represented by the color red. Among the Sadlermiut samples characterized as D4b1a2a1 in this study, one of the Sadlermiut samples shared the D4b1a2a1 haplotype that

also exhibits the 16093C polymorphism. As illustrated by the median-joining network (Figure 4.2), this particular D4b1a2a1 haplotype dominates the haplogroup D landscape observed in modern Greenlandic and Canadian Inuit but is also present in the Alaskan Iñupiat. The other two Sadlermiut samples were also characterized as D4b1a2a1, but as mentioned earlier, one does not possess the 16093C transition while the other is heteroplasmic at that site. Meanwhile, the partial sequence of the T-1 Dorset from Native Point harbored mutations that tentatively place it in the D4b1a2a1 haplogroup. A short segment of the T-1 Dorset sample that spans a section of HVS-I containing several nucleotide positions of interest, specifically 16,319 and 16,362, could not be sequenced. If transitions at those positions (16319A and 16362C) were to be observed, then the T-1 Dorset would be characterized with the same D4b1a2a1 haplotype observed in contemporary Greenlandic and Canadian Inuit as well as the Sadlermiut. The median-joining network (Figure 4.2) aptly highlights the limited distribution of haplogroup D among Arctic/sub-Arctic populations, which is restricted to a small number of haplogroups (D2, D2a'b and D4b1a2a1) and associated haplotypes.

Genetic Distances (D_A)

Pairwise genetic distances (D_A) were calculated for the prehistoric populations in this study, with the exception of the Dorset given their small sample size, as well as other prehistoric and contemporary populations in order to gain insight into the maternal genetic relationships of these populations. The computed pairwise genetic distances (D_A) for seventeen populations are presented in graphical form and are represented by the white-blue gradient below the diagonal, with smaller and larger genetic distances respectively represented by white and progressively darker blue color-filled squares (see Figure 4.3). As can be seen from Figure 4.3, the ancient Aleuts have the smallest pairwise genetic distances with the Mink Islanders (0.000) and the Unangax̂ (0.146) (see Appendix for values). The largest genetic pairwise distances for the ancient Aleuts were observed with the Inuit of Greenland (3.979) and Canada (3.749). As for the Sadlermiut, they exhibited the smallest pairwise genetic distances with the Canadian Inuit (0.168) and the Chukchi (0.574). The largest pairwise genetic distances for the Sadlermiut were with the

Itel'men (4.479) and Koryak (3.934). Comparable results were observed when the ancient Aleuts were separated according to cranial affiliation (Paleo-/Neo-Aleut), which coincidentally contained the same samples when they were grouped according to temporal distribution-- post-/pre-1,000 AD, respectively (see Figure 4.4). The Paleo-Aleuts had the smallest pairwise genetic distances with the Mink Islanders (0.680) and Neo-Aleuts (0.696), while the largest distances were with the Inuit of Greenland (6.719) and Canada (6.259) (see Appendix for values). As for the Neo-Aleuts they exhibited the smallest distances with the Mink Islanders (0.000) and the Unangax̂ (0.000), whilst the largest distances were with the Itel'men (2.177) and Koryak (1.696). The Sadlermiut, on the other hand, were revealed to share the smallest pairwise genetic distances with the Canadian Inuit (0.168) and the Chukchi (0.574), whereas the largest distances were with the Paleo-Aleut (4.977) and Itel'men (4.479).

Additional matrices of pairwise genetic distances were calculated by separating HVS-I sequences of both contemporary and prehistoric populations as either haplogroup A or D. The Sadlermiut were the only prehistoric population included in the genetic distance matrix calculated from sequences characterized as haplogroup A (Figure 4.5; see Appendix for values). The other prehistoric groups (ancient Aleuts and Mink Islanders) only had a single sample classified as haplogroup A and were not included in this analysis due to small sample sizes. The Sadlermiut shared the smallest genetic distances with the Inuit of Canada (0.128) and Greenland (0.487), while the largest distances for the Sadlermiut were observed with the Evenki (2.661) and Mongolians (2.353).

Genetic distance matrices calculated using HVS-I sequences classified as haplogroup D from prehistoric and contemporary populations (see Figure 4.6) revealed the ancient Aleuts exhibited the smallest genetic distances with the Unangax̂ (0.000) and Chukchi (0.088) (see Appendix for values). The largest genetic distances for the ancient Aleuts were with the Inuit of Canada (5.397) and Greenland (5.397) as well as the Sadlermiut (4.281). As for the Sadlermiut, the smallest genetic distances were with the Inuit of Canada (0.354) and Greenland (0.354). Meanwhile the largest genetic distances for the Sadlermiut were observed with the Unangax̂ (4.443) and ancient Aleuts (4.281). Similar results were encountered when the ancient Aleuts

were separated according to cranial affiliation (see Figure 4.7). The Aleuts (both Paleo- and Neo-Aleuts) again exhibited the largest genetic distances with the Inuit of Canada (Paleo = 6.008 and Neo = 4.538) and Greenland (Paleo = 6.008 and Neo = 4.538) (see Appendix for values). The Sadlermiut once more had the largest genetic distances with the ancient Aleuts (Paleo = 4.864 and Neo = 3.465) as well as the Unangax̂ (4.443). The smallest genetic distances for the Paleo-Aleuts were with the Unangax̂ (0.025) and Neo-Aleuts (0.077), while the Neo-Aleuts had the smallest distances with the Chukchi (0.000) and Unangax̂ (0.017). The Sadlermiut again exhibited the smallest genetic distances with the Inuit of Greenland (0.354) and Canada (0.354).

Multidimensional Scaling

Multidimensional scaling (MDS) plots were used to assess the relationship of the ancient populations under study to other ancient and contemporary circum-Arctic populations, with exception of the Dorset due to small sample size. The MDS plot of the ancient populations compared to other populations (MDS Figure 4.8) had a stress level of 0.059. For seventeen data points in two dimensions the upper bound is 0.254 indicating the plot produced was neither random nor without structure (Sturrock and Rocha, 2000). The plot revealed a distinct grouping where the ancient Aleuts clustered with Unangax̂ and ancient south Alaskans from Mink Island. This clustering is in agreement with the calculated pairwise population genetic distances, which as discussed previously revealed the ancient Aleuts have the smallest pairwise population genetic distances with the Mink Islanders and the Unangax̂ (Figure 4.3; see Appendix for values). Along the first and second component the Koryak, Itel'men, Han, Mongolian and Evenki populations were contained to the lower left quadrant of the plot and were distinct from the rest of the populations possessing primarily haplogroups A and D (Figure 4.8). The MDS plot captures the distinctness of these populations that was also evident with their pairwise population genetic distances (Figure 4.3; see Appendix for values). For all five populations (Koryak, Itel'men, Han, Mongolian, Evenki), their largest genetic distances were exhibited with the Inuit of Greenland and Canada. Additionally, the MDS plot shows the Koryak and Itel'men clustering together along the lower left quadrant away from the other Arctic and sub-Arctic populations. This separation is

consistent with the genetic pairwise distances for both the Unangax̂ and Sadlermiut whose largest values were observed with the Itel'men and Koryak. Along the first component, the populations high in haplogroup D formed a cluster comprised of the ancient Aleuts, Unangax̂ and ancient South Alaskan populations, which was separated from other populations at the extreme end of the spectrum such as the Inuit from Greenland and Canada who exhibit higher frequencies of A. Those populations in between, such as the Chukchi, Siberian Yuit, and ancient Sadlermiut, as well as the North American populations including Haida, Athabascan, Nuu-Chah-Nulth and Bella Coola, exhibited higher frequencies of A relative to the other haplogroups present in those populations. These populations generally had low frequencies of haplogroup D and a handful exhibited additional haplogroups such as B, C and other. As mentioned previously the clustering of the Unangax̂, Mink Islanders and ancient Aleuts echoes these populations' pairwise genetic distances where they display the smallest genetic distances between each other (Figure 4.3; see Appendix for values). Additionally, the separation observed amongst the populations mentioned above with high frequencies of A and the cluster consisting of the ancient Aleuts, Mink Islanders and Unangax̂ was also reflected in their pairwise population genetic distances (Figure 4.3; see Appendix for values). More specifically, the ancient Aleuts and Mink Islanders exhibit the largest genetic distances with Inuit of Canada and Greenland. Amongst the populations exhibiting high frequencies of A, the Unangax̂ had large genetic distances with the Inuit of Greenland and Canada. The Sadlermiut were subsumed within the grouping of populations possessing high frequencies of A. Among these populations with high frequencies of A, the smallest pairwise population genetic distances for the Sadlermiut were observed with the Inuit populations (Greenland and Canadian Inuit) and the Chukchi.

Similar results were observed in the MDS plot when the ancient Aleuts were grouped according to cranial affiliation, Paleo-/Neo-Aleut, which contained the same samples, respectively, for temporal distribution of the ancient Aleuts post-/pre-1,000 AD (Figure 4.9). The Neo-/post-1,000 AD Aleuts again clustered with Unangax̂ and ancient south Alaskans from Mink Island. This grouping in the MDS plot corresponds to the Neo-Aleuts having the smallest genetic distances with the Mink Islanders and the Unangax̂ (Figure 4.4; see Appendix for values).

Meanwhile, the Neo-Aleuts exhibited the largest distances with the Itel'men and Koryak. The Paleo-/pre-1,000 AD Aleuts still clustered with the aforementioned populations although not as tightly, which was most likely attributed to their being nearly monomorphic for D2a'b in this study. Even though they were not as closely grouped with the other Aleuts (Unangax̂ and Neo-Aleuts) or Mink Islanders in this MDS plot, the Paleo-Aleuts shared the smallest pairwise population genetic distances with these populations. The MDS plot reveals the Sadlermiut were again in the mix of populations exhibiting high frequencies of A relative to D. This grouping captures the pairwise genetic distances of the Sadlermiut, whose smallest genetic distances were with the Canadian Inuit and the Chukchi, whereas their largest distances were with the Paleo-Aleut and Itel'men (Figure 4.4; see Appendix for values).

To gain additional insight into the relationships among the populations, an MDS plot was constructed using haplogroup A sequences from ancient populations and surrounding populations (Figure 4.10). The stress of the plot was 0.071, with an upper bound of 0.217 for fourteen objects in two dimensions, which suggested the plot was neither random nor without structure (Sturrock and Rocha, 2000). Along the first dimension, the populations fell into clusters with respect to composition of A haplogroups. Located in the center of the plot were the Native American populations (Bella Coola, Nuu-Chah-Nulth and Haida), who possessed haplogroup A2. The Native American populations were intermediate to the grouping of populations who on the one hand possessed A and A4 haplogroups (Han, Mongolian and Evenki), while on the other hand, was the grouping of populations (Sadlermiut, Inuit of Canada and Greenland, Siberian Yuit, Chukchi, Athabascan, Koryak and Unangax̂) who were comprised of the A2a, A2b1 with a minor component of A2 and A. The grouping of the Sadlermiut with the other Inuit and Chukotkan (Chukchi and Siberian Yuit) populations reiterates the pairwise population genetic distances for the Sadlermiut who exhibited the smallest genetic distances with the Inuit of Canada and Greenland (Figure 4.5; see Appendix for values), whereas the largest distances for the Sadlermiut were observed with the Evenki and Mongolians. Along the second component, the Arctic/sub-Arctic populations fell along a gradient of A2 haplogroups. At the top of the MDS plot (Figure 4.10) were the Sadlermiut who were monomorphic A2b1, while at the bottom of the plot

were the Koryak who possessed A2a and A. In between these two extremes were the Canadian and Greenlandic Inuit, Siberian Yuit, Chukchi, Athabascans and Unangaŷ comprised of varying frequencies of A2b1, A2a and A2.

An additional MDS plot was constructed based solely on haplogroup D sequences for the populations as a means to further analyze the relationships of the ancient populations relative to other populations (Figure 4.11). The stress of this MDS plot was 0.027, which is below the maximum stress of 0.183 for twelve objects in two dimensions (Sturrock and Rocha, 2000). Along the first component, a cluster containing the Mongolian and Han was intermediate to the North American Nuu-Chah-Nulth and Bella Coola on one hand that possessed D and D1 while on the other side were the Arctic/sub-Arctic populations primarily composed of D2a'b and D4b1a2a1. Along the second component there was separation of two clusters. The first cluster towards the lower left of the MDS plot was composed of Siberian Yuit, Chukchi, ancient Aleuts, Unangaŷ and the ancient South Alaskan samples from Mink Island. The Siberian Yuit and Chukchi have D2a'b and some D4b1a2a1, while the ancient Aleuts and the Unangaŷ possessed D2a'b. The MDS grouping reflects smallest genetic distances observed between ancient Aleuts and the Unangaŷ and Chukchi (Figure 4.6; see Appendix for values). Meanwhile, the ancient South Alaskans from Mink Island were primarily D2 with one individual exhibiting polymorphisms associated with D4b1 (referred to as D3 in the text) and shared the smallest genetic distances with the Chukchi and ancient Aleut (Figure 4.6; see Appendix for values) (Raff et al., 2010). The largest genetic distances for the ancient Aleuts were with the Inuit of Canada and Greenland as well as the Sadlermiut (Figure 4.6; see Appendix for values). Closer to the top of the MDS plot was a second cluster comprised of the Sadlermiut as well as Inuit of Greenland and Canada, which exhibited the D4b1a2a1 haplotype with the D4b1a2a1 mutations and the additional 16093C polymorphism. This grouping is a reflection of the smallest genetic distances between the Sadlermiut and the eastern Inuit peoples of Canada and Greenland (Figure 4.6; see Appendix for values). The distinctness of the eastern circum-Arctic populations (Sadlermiut and Inuit) from those in the west (Unangaŷ, ancient Aleut, Chukotkans and Mink Islanders) in the MDS plot (Figure 4.11) corroborates the calculated genetic distances amongst these groups (Figure 4.6; see Appendix

for values). More specifically the largest distances for the ancient Aleuts, Unangax̂ and Mink islanders are with the Inuit (Canada and Greenland) and Sadlermiut, while those for the Sadlermiut are with the Unangax̂ and ancient Aleut.

A comparable scattering of populations was observed when ancient Aleuts were separated according to cranial affiliations (Paleo- and Neo-Aleut) and temporal distribution (pre- and post-1,000 AD Aleuts) (Figure 4.12). The Neo-Aleut group contained the same set of samples for post-1,000 AD Aleut, while the Paleo-Aleut group contained the same group of samples for pre-1,000 AD. The Paleo-/pre-1,000 AD Aleuts and Neo-/post-1,000 AD Aleuts again clustered with the Chukotkan populations and the Unangax̂. Meanwhile the eastern Arctic populations (Inuit and Sadlermiut) again formed a cluster separated from the rest of the populations. This MDS clustering again captures the essence of the genetic distances of these populations (Figure 4.7; see Appendix for values), with the smallest distances of the Sadlermiut occurring with the Inuit of Greenland and Canada and the largest with the Aleuts (both Paleo- and Neo) and the Unangax̂. In the same vein, the Aleuts (both Paleo- and Neo-) as well as the Mink Islanders exhibited the largest distances with the Inuit of Greenland and Canada. The clustering of the western Arctic populations in the MDS plot (Figure 4.12) was also congruent with the genetic distances of these populations as the smallest genetic distances for the Paleo-Aleuts were with the Unangax̂ and Neo-Aleuts, while the Neo-Aleuts exhibited the smallest distances with the Chukchi and Unangax̂. As for the Mink Islanders, they exhibited the smallest genetic distances with the Neo-Aleuts and Chukchi.

Table 4.1. HVS-I sequence data for ancient populations.

		HVS-I Nucleotide Positions																					
Sample		16092	16093	16111	16129	16151	16173	16179	16192	16218	16220	16223	16234	16265	16268	16271	16286	16290	16301	16319	16362	Haplogroup
rCRS		T	T	C	G	C	C	C	C	C	A	C	C	A	C	T	C	C	C	G	T	
Aleut 378462		.	.	.	A	.	.	.	.	.	.	T	.	.	.	C	.	.	.	.	C	D2a'b
Aleut 378471		.	.	.	A	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	C	D2
Aleut 378606		.	.	T	.	.	.	.	T	.	.	T	T	.	.	.	.	T	.	A	C	A2a
Aleut 378616		.	.	.	A	.	.	.	.	.	.	T	.	.	.	C	.	.	.	.	C	D2a'b
Aleut 378633		C	.	.	A	T	.	T	.	T	.	T	.	.	.	C	T	.	T	.	C	D2a'b
Aleut 378639		.	.	.	A	.	.	.	.	.	.	T	.	.	.	C	.	.	.	.	C	D2a'b
Sadlermiut 147		.	Y	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	A	C	D4b1a2a1
Sadlermiut 167		.	C	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	A	C	D4b1a2a1
Sadlermiut 148		.	.	T	.	.	.	.	.	.	.	T	.	G	.	.	.	T	.	A	C	A2b1
Sadlermiut 153		.	.	T	.	.	.	.	.	.	.	T	.	G	.	.	.	T	.	A	C	A2b1
Sadlermiut 174		.	.	T	.	.	.	.	.	.	.	T	.	G	.	.	.	T	.	A	C	A2b1
Sadlermiut 098		.	.	.	.	.	T	.	.	.	.	T	.	.	T	.	.	.	.	A	C	D4b1a2a1
Sadlermiut 145		.	.	T	.	.	.	.	.	.	.	T	.	G	.	.	.	T	.	A	C	A2b1
Dorset 340 (Angekok)		.	.	.	A	.	.	.	.	.	.	T	.	.	.	C	.	.	.	.	C	D2a'b
Dorset 749 (T-1)	PartialSeq	.	C	.	.	.	T	.	.	.	.	T	.	-	-	-	-	-	-	-	-	D4b1a2a1
Thule 412	PartialSeq	.	.	T	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A2 (?)
Thule 584	PartialSeq	.	.	T	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A2 (?)
Thule 388	PartialSeq	.	.	T	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A2 (?)

Table 4.2. Number of amplifications per extraction for a sample with variations of PCR parameters.

Sample with Number of Extractions	Variations of PCR Parameters with Number of Amplifications				Total Number of Amplifications per Extraction	Overall Total Number Amplifications
	Standard PCR	More DNA or DNA Dilution	Reagent Concentration[a]	Multiple Parameters[b]		
Dorset 749 (T-1)						
1st Extract	9	23	6	31	69	
2nd Extract	4	16	4	14	38	
3rd Extract	0	0	2	2	4	
						111
Dorset 340 (Angekok)						
1st Extract	4	22	6	24	56	
2nd Extract	8	24	10	57	99	
3rd Extract	0	0	2	2	4	
						159
Dorset 003 (Tayara)						
1st Extract	7	24	5	18	54	
2nd Extract	9	20	12	67	108	
3rd Extract	4	12	6	16	38	
						200
					Overall Total	470

a. Concentration of reagents such as $MgCl_2$ and primers.

b. Multiple parameters changed at one time including concentration of $MgCl_2$, primer concentration, more DNA, dilution of DNA, number of cycles and annealing temperatures.

Table 4.3. Success of different genetic markers. Discrete marker success versus HVS-I sequencing success.

Ancient Population	Discrete Markers[a]	HVS-I Sequence[b]	Independent Extraction Verification of HVS-I Sequences[c]
Aleut	77% (65/84)	38% (6/16)	50% (3/6)
Sadlermiut	94% (18/19)	70% (7/10)	57% (4/7)
Dorset	67% (2/3)	67% (2/3)	0% (0/2)
Thule	85% (17/20)	33% (3/9)	0% (0/3)

a. Discrete markers of ancient Aleuts are from Hayes 2002, Hayes et al. 2003, Smith 2005, Smith et al., 2009. Ancient Sadlermiut, Dorset and Thule are from Hayes 2002, Hayes et al., 2003.
b. HVS-I sequences of ancient samples (subset from Hayes 2002) performed in this study. Complete sequences for Aleut and Sadlermiut and one Dorset. Nearly complete sequence for one Dorset sample (3/4 HVS-I fragments obtained) and partial sequences for all Thule (1/4 HVS-I fragments sequenced).
c. Verification of HVS-I sequences from an independent extraction. All are complete sequences with exception of one Sadlermiut, which is a nearly complete sequence (3/4 HVS-I fragments sequenced).

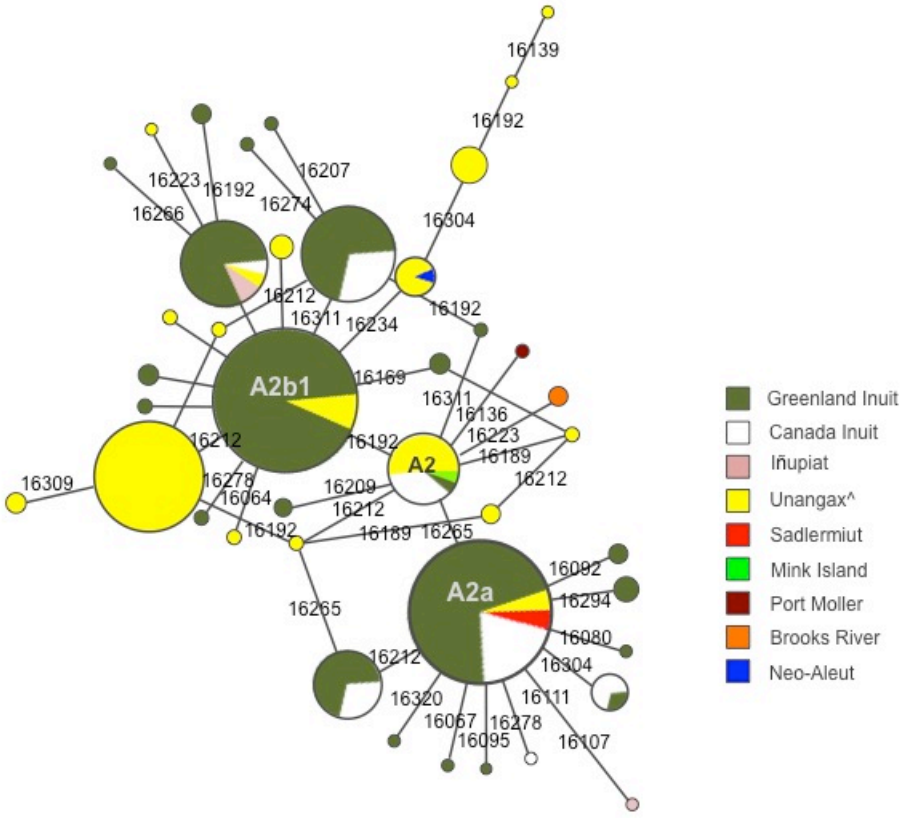

Figure 4.1. Haplogroup A median-joining network. Populations include Sadlermiut, ancient Aleuts, ancient South Alaskans (Brooks River, Mink Island and Port Moller), Unangax̂, Iñupiat, Canada Inuit and Greenland Inuit.

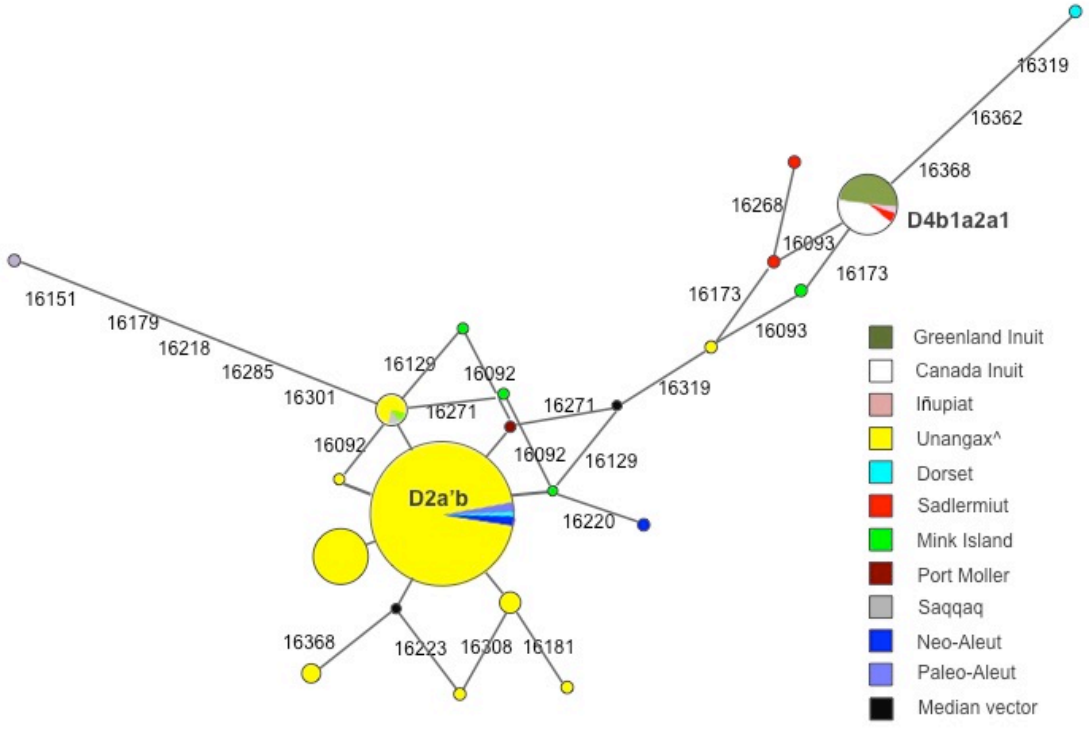

Figure 4.2. Haplogroup D median-joining network. Populations include Sadlermiut, ancient Aleuts, ancient South Alaskans (Brooks River, Mink Island and Port Moller), Saqqaq, Unangax̂, Iñupiat, Canada Inuit and Greenland Inuit.

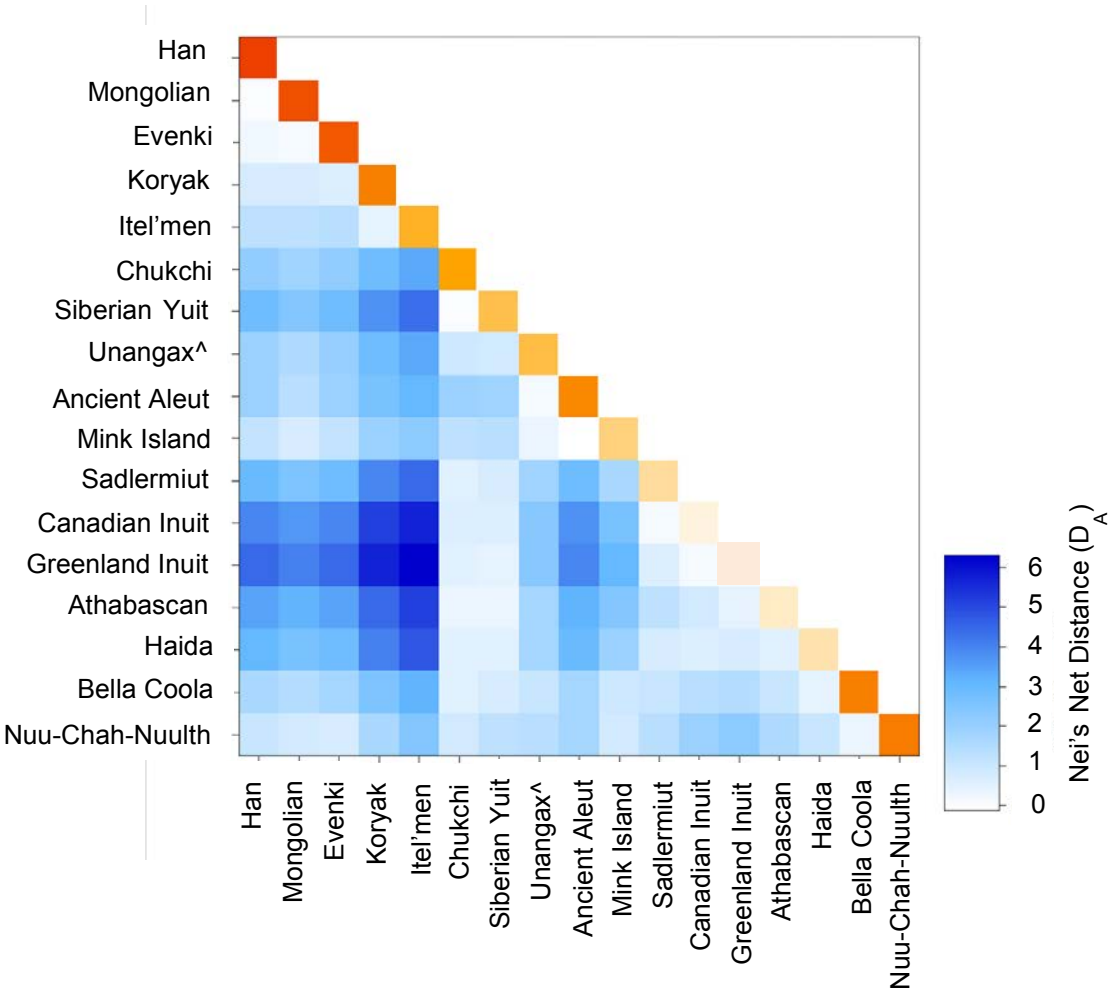

Figure 4.3. Nei's corrected average (D_A) number of pairwise distances between 17 populations.

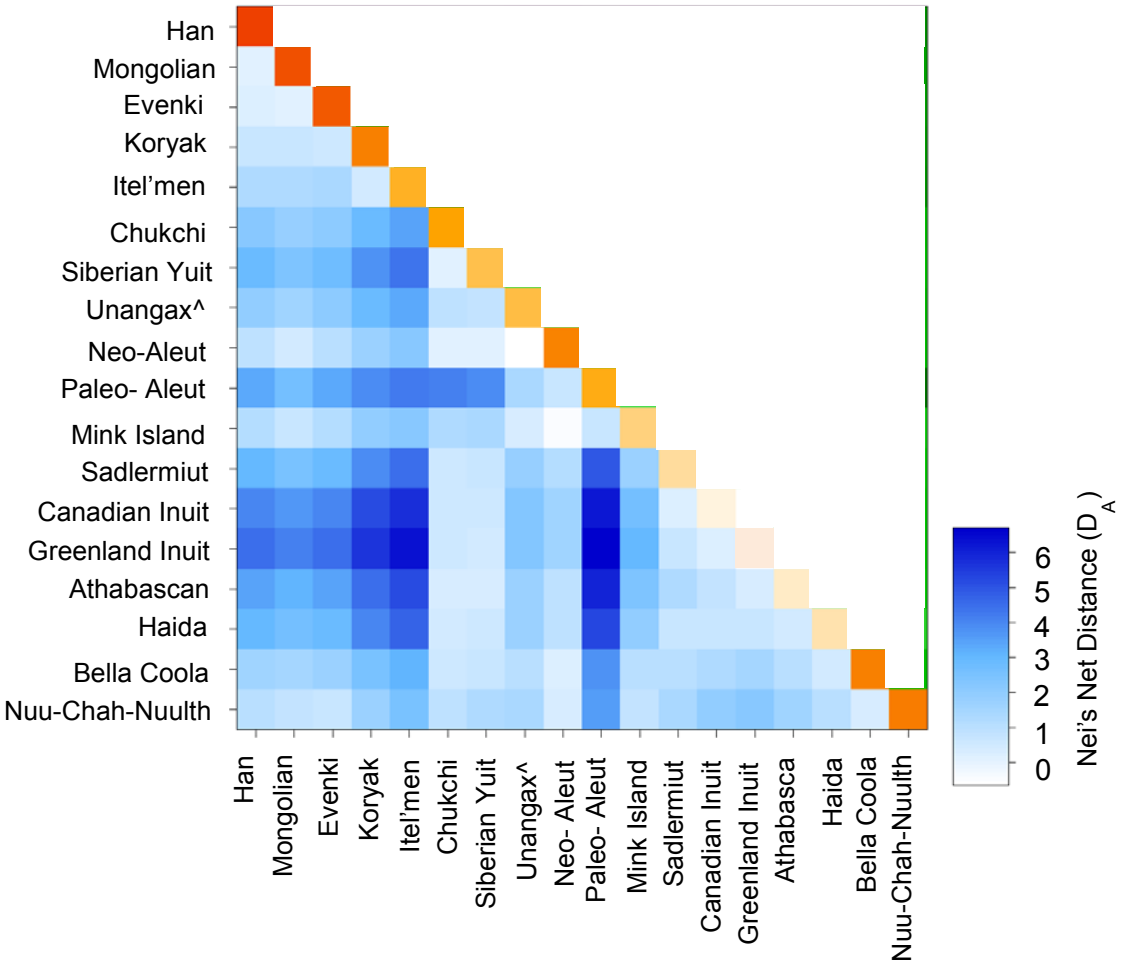

Figure 4.4. Nei's corrected average (D_A) number of pairwise distances between 18 populations. Ancient Aleuts separated according to cranial affiliation (Paleo-/Neo-Aleut).

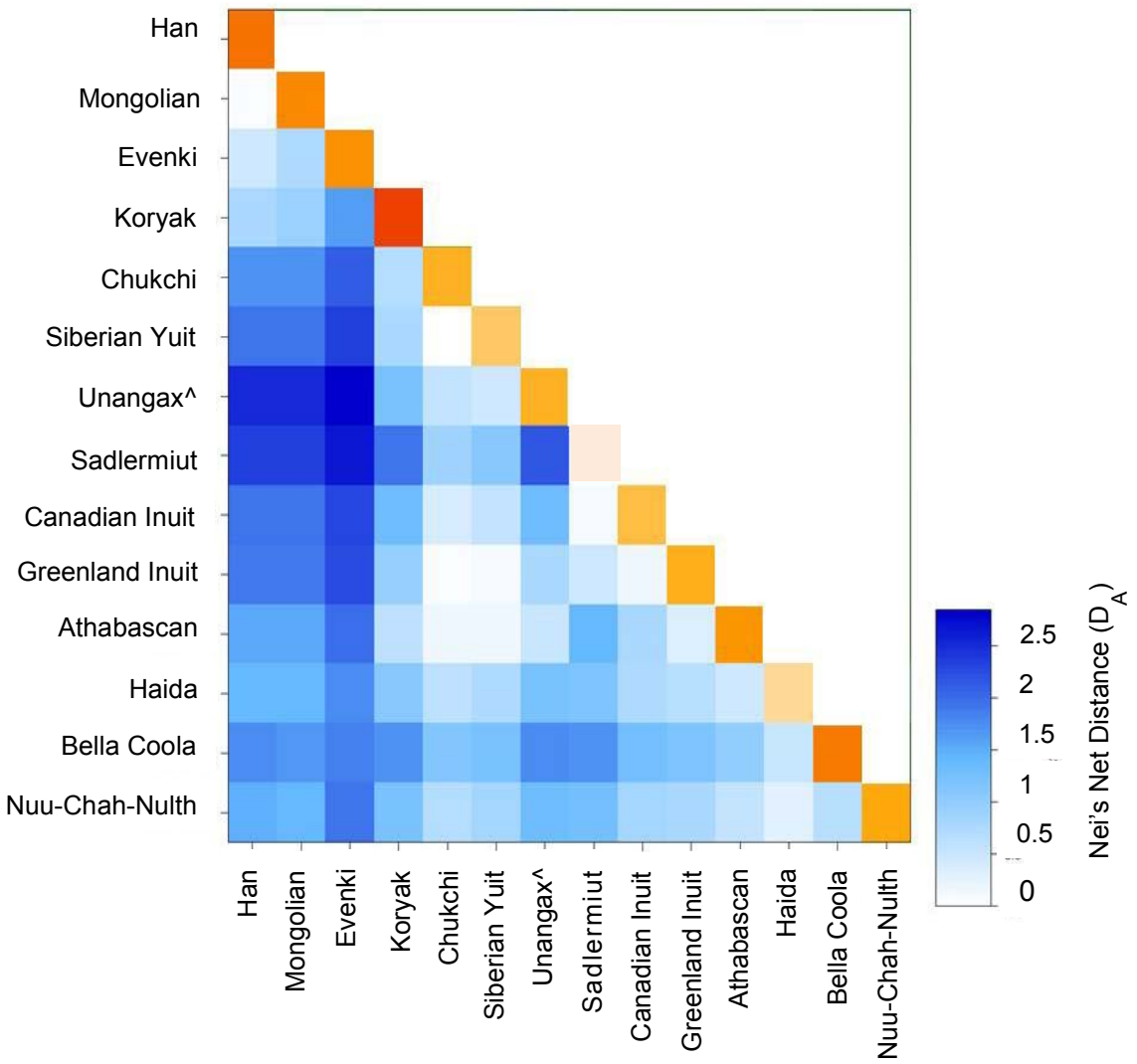

Figure 4.5. Nei's corrected average (D_A) number of pairwise distances between 14 populations for haplogroup A sequences.

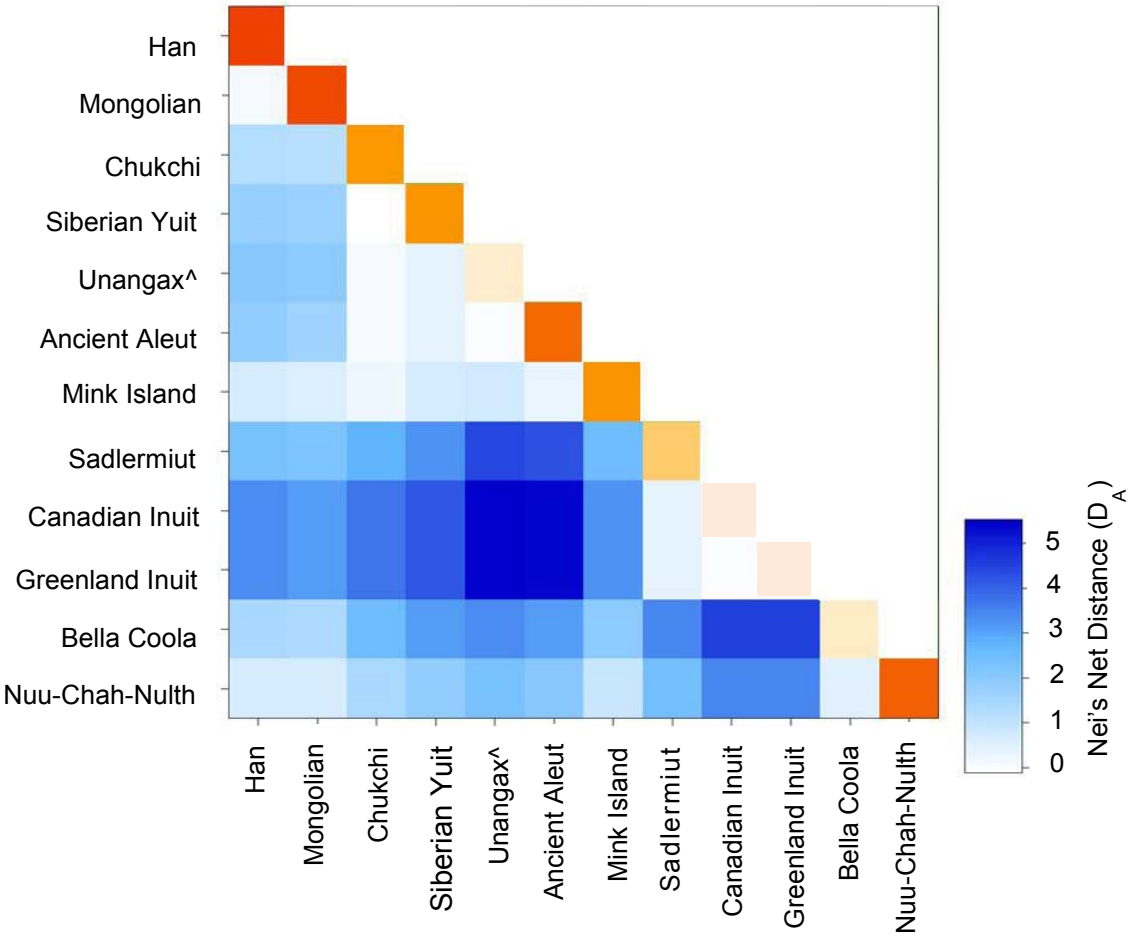

Figure 4.6. Nei's corrected average (D_A) number of pairwise distances between 12 populations for haplogroup D sequences.

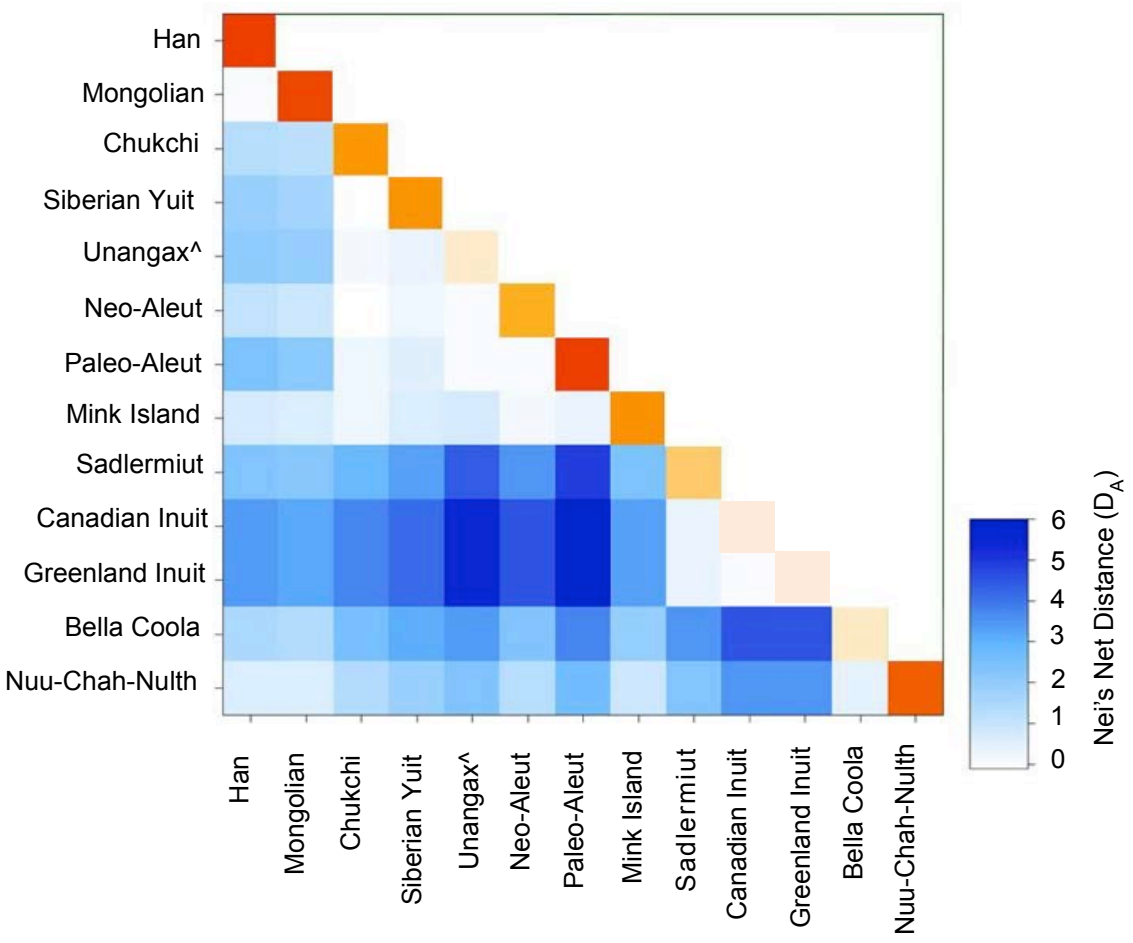

Figure 4.7. Nei's corrected average (D_A) number of pairwise distances between 13 populations for haplogroup D sequences. Ancient Aleuts separated according to cranial affiliation (Paleo-/Neo-Aleut).

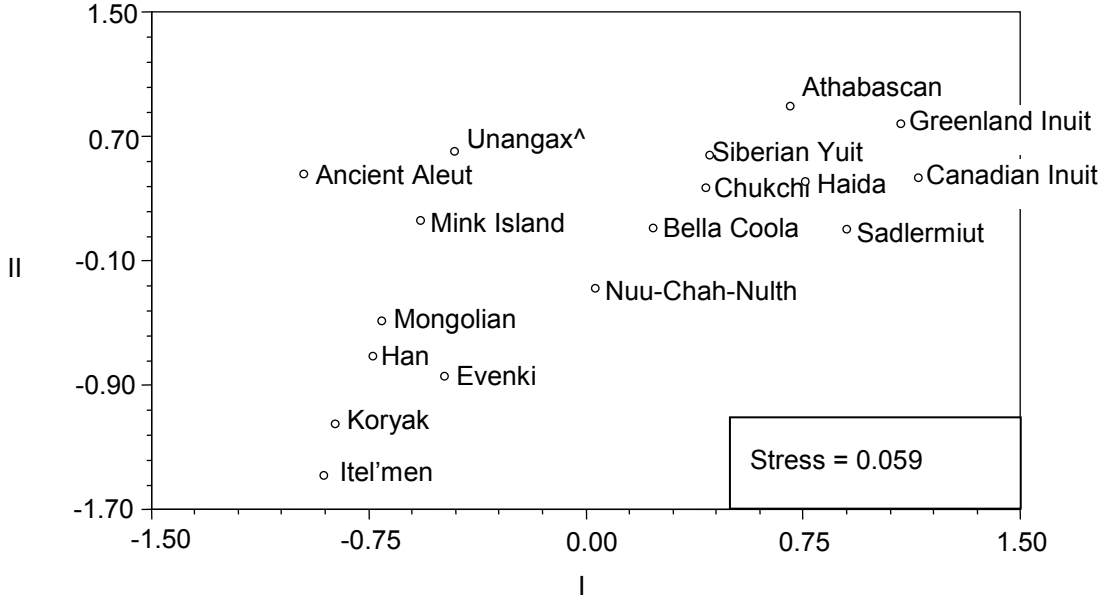

Figure 4.8. MDS plot of genetic distances between 17 populations. Sequences for all haplogroups.

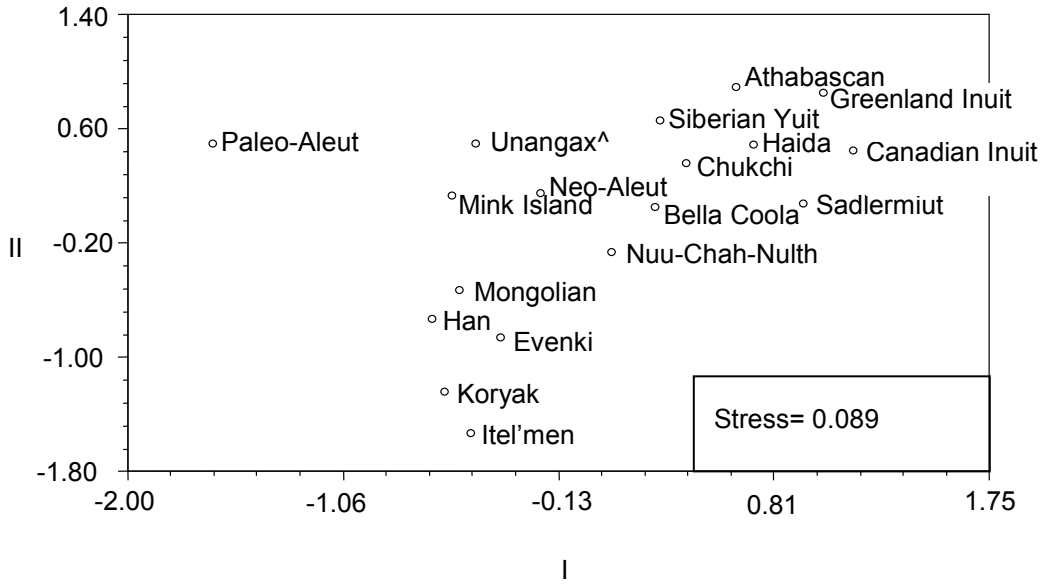

Figure 4.9. MDS plot of genetic distances between 18 populations. Sequences for all haplogroups, ancient Aleuts separated by cranial/temporal affiliation.

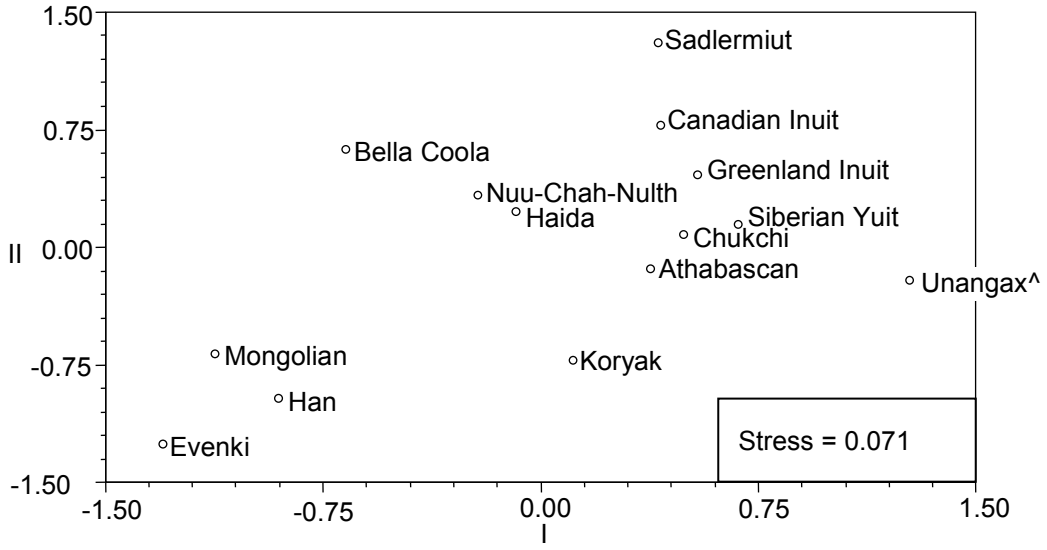

Figure 4.10. MDS plot of genetic distances between 14 populations for haplogroup A sequences.

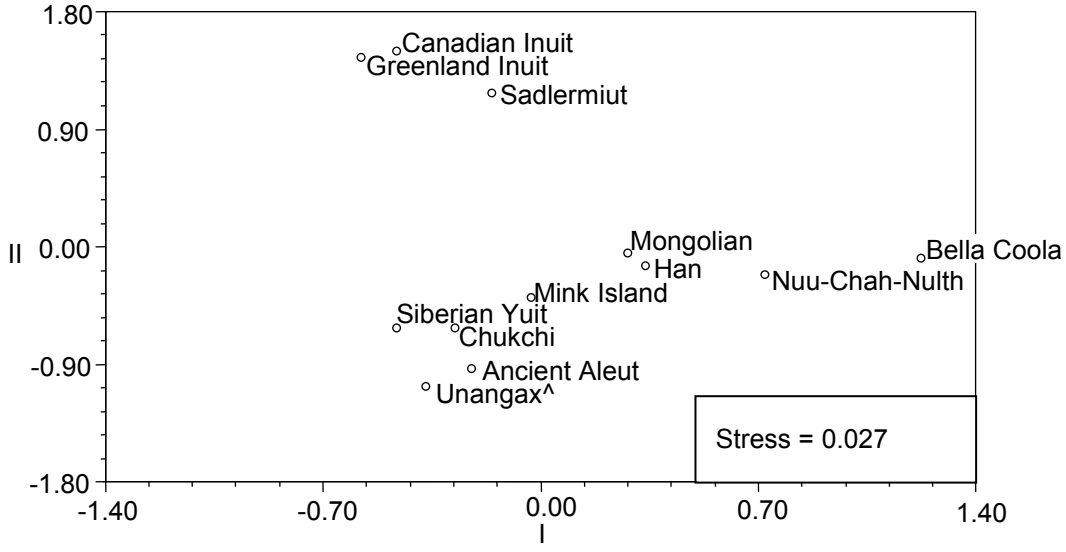

Figure 4.11. MDS plot of genetic distances between 12 populations for haplogroup D sequences.

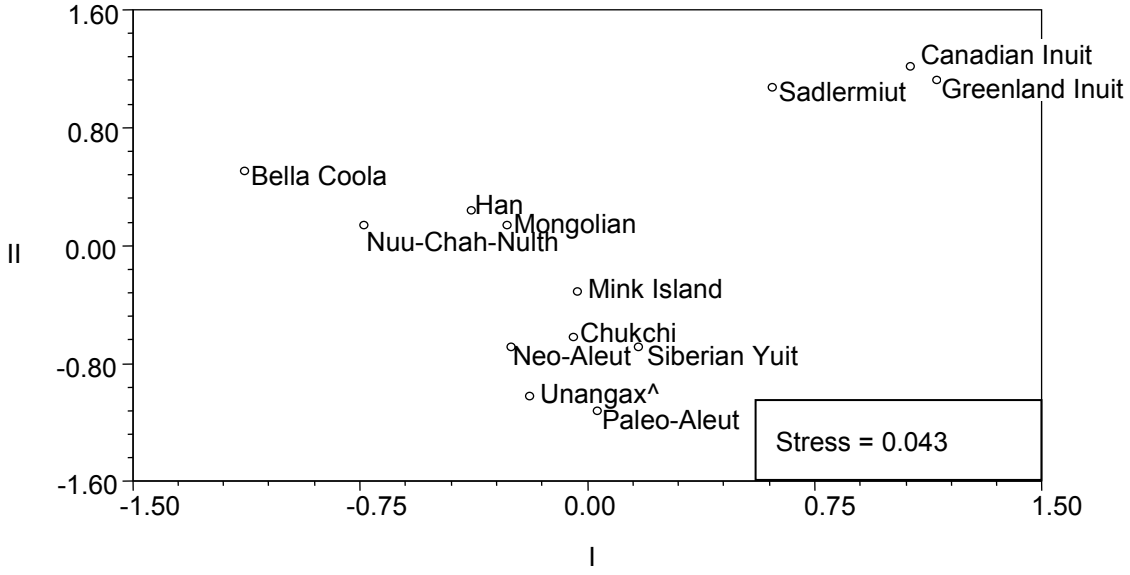

Figure 4.12. MDS plot of genetic distances between 13 populations for haplogroup D sequences. Ancient Aleuts separated according to cranial/ temporal affiliation.

CHAPTER 5

DISCUSSION AND SUMMARY

Discussion

This study characterized the mtDNA HVS-I region of four prehistoric Arctic/sub-Arctic populations, the Aleut, Sadlermiut, Dorset and Thule. The goal was to determine which haplogroups, with a special emphasis on haplogroup D, were present amongst these groups as well as to explore the genetic relationships between prehistoric and contemporary populations in the region and contextualize these relationships within the broader framework of human colonization and dispersals in the Arctic. Through the course of this research sequences spanning a 311bp region of HVS-I (16,060np to 16,370np) were successfully obtained for six ancient Aleuts, seven Sadlermiut and one Dorset from Angekok (Dorset 340), while partial sequences were determined for three Thule and the T-1 Dorset (Dorset 749).

Scholars have argued entry into the Aleutians was multidirectional from Kamchatka and the Bering platform, while others have advocated entry was initiated in the east from the Alaskan mainland and/or Bering platform (Laughlin, 1980; Black, 1983; Dumond, 1987). Archaeological evidence supports the east to west peopling scenario based on the distribution of archaeological sites with the oldest sites located in the east and becoming progressively younger moving westward along the island chain (reviewed in Hatfield, 2010). The absence of a prehistoric presence on the most westward grouping of islands in the Aleutians (Commander Islands), a potential stop-over point from Kamchatka to the Aleutians, strengthens the case for the east to west peopling scenario (Hrdlička, 1945). Studies of mtDNA in contemporary populations have also added additional corroboration for an east to west population movement by establishing the Unangax̂ have genetic affinities with the Chukchi and Siberian Yuit of the Chukotkan Peninsula

rather than Native American or Kamchatkan (Koryak and Itel'men) populations (Rubicz et al., 2003; Zlojutro et al., 2006; Zlojutro et al., 2009).

A genetic association with the Chukotkan populations also appears to extend and include ancient eastern Aleuts, regardless of temporal distribution or cranial classification. Support for this finding rests upon the ancient Aleuts clustering with the Chukotkan populations in MDS analyses, as well as shared haplogroups, namely A2a and D2a'b. Previous studies have identified the A2a haplogroup (referred to by some as A2a1) amongst Inuit and Beringian populations as well as Na-Dene speaking Apache and Navajo (Helgason et al., 2006; Zlojutro et al., 2006). Meanwhile, the HVS-I polymorphisms defining D2a'b have been identified in the Siberian Yuit, Chukchi, ancient Saqqaq and ancient South Alaskan populations (Shields et al., 1993; Starikovskaya et al., 1998; Derbeneva et al., 2002; Gilbert et al., 2008; Raff et al., 2010). Ancient Aleuts, even when subdivided according to cranial/temporal classifications, exhibited small genetic distances with Chukchi and Siberian Yuit populations when sequences belonging to haplogroup D lineages were analyzed. The matrilineal genetic affiliation of the ancient Aleuts with the Chukotkan (Siberian Yuit and Chukchi) rather than Kamchatkan populations builds upon the mounting archaeological as well as contemporary genetic evidence supporting an east to west colonization of the Aleutian Island chain after entering the New World.

Archaeologists have demonstrated remarkable cultural continuity for nearly 4,000 years in the Aleutian archipelago and perhaps even longer with a handful of sites dating as early as 8,400 to 8,000 BP (Laughlin, 1963; Laughlin and Aigner, 1966; McCartney and Turner, 1966; Aigner, 1970; Dumond and Knecht, 2001; Knecht and Davis, 2001). The matrilineal sequence information garnered from ancient Aleuts in this study as well as that from the contemporary people of the Aleutians adds another dimension to the evidence for continuity within the Aleutians. Sequencing results of this study corroborated earlier discrete marker findings by identifying HVS-I mtDNA polymorphism motifs associated with haplogroups A and D amongst the ancient Aleuts (Hayes et al., 2003; Smith et al., 2009). More precisely, the ancient Aleuts were characterized by haplotypes associated with haplogroups A2a, D2 and D2a'b. Median-joining network analyses demonstrated the ancient Aleuts shared these same matrilineal haplogroups, in

particular A2a and D2a'b as well as a haplotype associated with haplogroup A2a, with the Unangax̂—the descendants of the ancient Aleuts who continue to reside in the same region as their ancestors. The median-joining network for sequences belonging to haplogroup D also revealed two D haplotypes among the ancient Aleuts not observed in contemporary populations analyzed in this study.

The genetic affinity of the ancient Aleuts with the Unangax̂ was also underscored by the findings from genetic distances as well as MDS analyses. Genetic distance matrices revealed the ancient Aleuts had the smallest genetic distances with the Mink Islanders and the Unangax̂ (Figure 4.3 and Appendix). These results were echoed in the MDS analyses (Figure 4.8, Figure 4.9, Figure 4.11 and Figure 4.12) where the ancient Aleuts clustered with the Unangax̂ and Chukotkan populations as well as ancient south Alaskans from Mink Island. Comparable results were observed when the ancient Aleuts were separated according to temporal and cranial affiliation for the comparison of all haplogroups. Both Paleo- and Neo-Aleuts exhibited the smallest genetic distance (Figure 4.4 and Appendix) with the Mink Islanders, while the second smallest distance for each group was respectively with the Neo-Aleuts and Unangax̂. The grouping of populations for the MDS plot (Figure 4.9) involving the separation of ancient Aleuts according to cranial/temporal affiliation was similar to what was observed when they were not separated, although the Paleo-Aleuts were not as closely clustered with the Unangax̂ and Mink Islanders. This observation may be due to all of the Paleo-Aleuts sequenced to date belonging to haplogroup D as none of the four Paleo-Aleuts characterized as haplogroup A produced readable sequences. Thus this 'monomorphism' is not a true representation of the Paleo-Aleuts who are known to exhibit haplogroups A and D, 42% and 58%, respectively (Hayes, 2002; Hayes et al., 2003; Smith et al., 2009).

Genetic similarity between indigenous populations, contemporary and prehistoric, based upon comparable mtDNA haplogroup frequency distributions coupled with shared interior nodes in a phylogenetic network has been interpreted as an indication of common ancestry (Lawrence et al., 2010). In the Aleutians the initial findings suggest a shared genetic ancestry between the ancient Aleuts and the Unangax̂ based upon generally high frequencies of D relative to A, same

haplogroups, shared haplotype, small genetic distances as well as the ancient Aleuts clustering with the Unangax̂ MDS analyses. Additional D haplotypes were found among the ancient Aleuts (both Paleo- and Neo-) not shared with contemporary populations in the area, which may indicate the ancient Aleuts experienced some loss in diversity over time due to drift and/or population reduction at the time of nonnative contact. At the present time, though, postmortem damage to ancient template cannot be entirely ruled out. However, the observance of increased diversity in prehistoric indigenous groups relative to contemporary populations in the same area has been reported elsewhere (Shook and Smith, 2008; Raff et al., 2010). In the southwestern region of Alaska Raff et al. (2010) identified additional haplogroups including B and other D haplogroups along the Alaska Peninsula. There were also other A2 and D (D2a'b and D4b1) haplotypes not seen in contemporary or prehistoric populations in the surrounding region (Raff et al., 2010). In this study the findings from a handful of eastern ancient Aleut sequences provides encouraging evidence for the matrilineal genetic affinities between the ancient Aleuts and the Unangax̂, which potentially offers another component to the evidence for continuity within the Aleutian archipelago.

Although there is marked continuity in the Aleutians the presence of exotic materials such as jet and slate as well as some features of the Aleutian tool kit showing some semblance to the ASTt are perceived as archaeological indicators of outside influences (Davis and Knecht, 2005; Davis and Knecht, 2010). Their presence also signifies the inhabitants of the archipelago were not as isolated as previously thought (Aigner, 1970). Through time there are perceptible differences in haplogroup frequencies in the Aleutians. Pre-1,000 AD Aleuts were found to exhibit higher frequencies of A relative to D whereas the converse of this haplogroup frequency distribution pattern is observed among post-1,000 AD Aleuts, which was not attributable to genetic drift (Smith et al., 2009). However, no appreciable differences in haplogroup frequencies with respect to cranial affiliation have been detected (Hayes, 2002; Hayes et al., 2003; Smith et al., 2009). Among the Unangax̂ an east to west gradient for haplogroup A was identified with elevated frequencies of A in the eastern Aleutian Islands which decreases as one moves westward and is thought to reflect perhaps an episode of "Aleut and Eskimo admixture at some

historical time" (Crawford, 2007:212). Variation in stable isotope signatures have also been observed between geographical locations as well as in recent times (post-1,000 AD) at Chaluka (Byers et al., 2011). The archaeological evidence of outside cultural influences coupled with differences in haplogroup frequencies and stable isotope signatures may be associated with an influx of peoples into the Aleutians that may be unrelated to cranial affiliation.

The available HVS-I data for the ancient and contemporary Aleuts reveal they are remarkably similar with respect to matrilineages regardless of time as well as cranial affiliation amongst the ancient Aleuts. For instance, the median-joining network for sequences belonging to haplogroup D (Figure 4.2) illustrates the presence of haplogroup D2a'b amongst the Unangax̂ as well as ancient Aleut samples sequenced to date. Additionally, haplogroup D2a'b was observed in ancient Aleuts on both Ship Rock and Chaluka regardless of cranial classification (Paleo-/Neo-Aleut) or temporal distribution (pre-/post-1,000 AD). Genetic distances and the MDS plot using sequences belonging to haplogroup D (Figure 4.7 and Figure 4.12) illustrate the Paleo- and Neo-Aleuts do have small genetic distances with one another and were clustered within the same grouping of populations. The A2a ancient Aleut sample in this study was from Chaluka and was classified as a Neo-Aleut and post-1,000 AD Aleut when using cranial and temporal classifications, respectively. The median-joining network for sequences belonging to haplogroup A (Figure 4.1) revealed the ancient Aleut shared a particular haplotype of A2a with a handful (*n*=7) of Unangax̂. Unfortunately, none of the other samples characterized as A via discrete marker analysis yielded readable sequences in this study and remains to be seen which A2 subtypes are harbored by other prehistoric Aleuts (either Paleo-/Neo- or pre-/post-1,000 AD) in this region.

Perceptible differences in mtDNA haplogroup profiles are identified in the Aleutians through time but the available mtDNA HVS-I data indicates the ancient Aleuts and Unangax̂ are characterized by a genetic similarity across space and time. The observance of shared haplogroups and haplotypes between the ancient Aleuts and Unangax̂ suggests the cultural exchange of material goods with peoples outside the Aleutians may not have involved a considerable level of matrilineal genetic exchange. However, if the influx of peoples into the

Aleutians was either male driven or if they were matrilineally similar to the ancient Aleuts then such a movement would be imperceptible using HVS-I sequencing. Given that haplogroups A2 and D2a'b were recently characterized in prehistoric individuals along the Alaska Peninsula, it is possible for those who moved into the island chain to have similar HVS-I sequences to those among the Aleuts (Raff et al., 2010). However, additional haplogroups (B2 and D1) were identified among the most northeastern mainland ancient south Alaskans along the peninsula that have not been observed in contemporary or prehistoric Aleut populations. The implications of these findings suggest movement into the Aleutians from the Alaska Peninsula is plausible though it did not involve noncircumpolar populations.

The work done by Raff et al. (2010) highlights the interest in the relationship of the peoples of the Aleutians to those along the Alaska Peninsula. The cultural zone of the prehistoric Aleuts spans the region of the Aleutian archipelago. But at the time of Russian contact they historically occupied the Shumagin Islands as well as the western lower end of the Alaska Peninsula "west of approximately 159ºW longitude" (McCartney and Veltre, 1999:504). The nature of the prehistoric cultural interface between ancient Aleuts and their neighbors to the northeast along the lower Alaska Peninsula prompted research to identify the presence of this boundary and the degree of its permeability (McCartney, 1974; Dumond et al., 1975). Although a clear fixed cultural margin between ancient Aleuts and their neighbors to the northeast has yet to be identified, archaeological features and similarities across artifact classes indicate people along the lower Alaska Peninsula experienced contact with neighboring groups from either direction (Aleuts from the southwest and Eskimos from the northeast) (Dumond, 1974; Maschner, 1999; Maschner, 2004; Hatfield, 2010).

Prehistoric individuals from several sites along the southern Alaska Peninsula sequenced for HVS-I were found to harbor haplotypes primarily affiliated with haplogroups A2 and D2a'b (Raff et al., 2010). Additional haplogroups including D4b1 (in the paper referred to as D3), B2 and D1 were also identified amongst the ancient southern Alaskan populations, with the latter two haplogroups not yet observed in prehistoric or contemporary populations of the Aleutians (Raff et al., 2010). When the sequences of the ancient southern Alaskans were compared to the ancient

and modern inhabitants of the Aleutians, median-joining networks (Figure 4.1 and Figure 4.2) revealed individuals from the Mink Island and Port Moller sites shared haplogroup D2a'b with the ancient Aleuts and the Unangax̂. The Mink Islanders also exhibited a haplotype of D2a'b that was characterized in the ancient Paleo-Eskimo Saqqaq and the Unangax̂ as well as HVS-I variants associated with haplogroup D4b1. Ancient Alaskans from all three locales also shared the A2 haplogroup with the Unangax̂, but also exhibited A2 haplotypes not yet observed in prehistoric and contemporary populations in the Aleutians (Figure 4.1). Even though haplogroups A2a and A2b1 are frequently observed among Arctic/sub-Arctic populations they were not identified among the ancient south Alaskan samples characterized to date (Raff et al., 2010). When all sequences were considered, the ancient south Alaskans from Mink Island were found to have matrilineal affinities with the ancient Aleuts (Paleo-/Neo-) and Unangax̂ based on genetic distances (Figure 4.3 and Figure 4.4) and MDS analyses (Figure 4.8 and Figure 4.9). Results were comparable when sequences only belonging to haplogroup D were considered (Figure 4.6, Figure 4.7, Figure 4.11 and Figure 4.12), with the Mink Islanders exhibiting genetic affinities with the ancient Aleuts (Paleo-/Neo-) and Chukchi as well as the Unangax̂ and Siberian Yuit.

The peoples in this region (both the Aleutians and Alaska Peninsula) seem to have experienced a loss of diversity over time with the observance of additional haplotypes and/or haplogroups in prehistoric populations in the Aleutians as well as south Alaska not seen in present day peoples in the same area. The presence of additional haplogroups (B2 and D1) in the peninsular region suggests contact with indigenous populations from the east who introduced these haplogroups into this region via genetic exchange (Raff et al., 2010). While these haplogroups (B2 and D1) have not yet been found in prehistoric or contemporary inhabitants of the Aleutians, the ancient Aleuts do exhibit additional haplotypes not seen among their descendants. Additional matrilineages [C1 (Native American); H, K and U5a1 (European); M7b2 (Japanese and Korean)] have been noted in some eastern Unangax̂ (9.5%) but not in any of the prehistoric individuals in the Aleutians (Zlojutro et al., 2009). The presence of the haplogroups has been attributed to postcontact matrilineal gene flow from non-Unangax̂ sources (Zlojutro et al., 2009).

The sharing of A and D haplogroups (A2 and particularly D2a'b) between the peoples of the archipelago and those along the southern aspects of the Alaska Peninsula could indicate they at one point had a shared maternal genetic history, genetic exchange occurred between individuals in these proximally located locales (eastern Aleutians and the Alaska Peninsula), people from the peninsula migrated into the archipelago or some combination of these situations. The scenario of migration into the Aleutians is considered to be possible by Raff et al. (2010) given the antiquity of some of the individuals from the Hot Springs site near Port Moller (calibrated 2σ range of 3,547-1,388 BP) along with the elevated frequency of haplogroup D (Mink Island 83.3%, Hot Springs 66.6%) and shared D2a'b lineages with the Unangax̂ (Coltrain, 2010; Raff et al., 2010). Considering the combination of the aforementioned factors Raff et al. (2010) postulate the influx of peoples into the Aleutians nearly 1,000 years ago may have come from the Bering coast of the Alaska Peninsula, particularly since this movement is associated with elevated frequencies of D in the Aleutians (Smith et al., 2009; Raff et al., 2010). Smith et al. (2009) had also suggested the shift in haplogroup frequencies among prehistoric Aleuts could reflect an incursion of people into the archipelago but offered other possible scenarios of population history including kin-structured movements within the archipelago or "a redistribution of existing populations resulting in significant local founder effects" (Smith et al., 2009:422). Caution was stressed by Smith et al. (2009) as the perceived shift in prehistoric Aleut haplogroup frequencies for A and D among the pre- and post-1,000 AD Aleuts was based upon a limited number of (*n*=11) individuals predating 1,000 BP. Along the same lines, Raff et al. (2010) also noted the haplogroup frequency profiles of the south Alaskan groups in their study could potentially shift with the inclusion of more samples.

Although only a limited number of prehistoric samples from this region have been sequenced to date, there is evidence of shared haplogroups and similar or same haplotypes (based on HVS-I sequences) between the ancient eastern Aleuts (regardless of geography or time), Unangax̂ and ancient Alaskans. The genetic likeness observed among the peoples living in this region could be due to similar genetic ancestry of the initial settlers of the region, with those in the most northern area of the Alaska Peninsula experiencing gene flow from Native Americans. Aspects of

the material culture in the region suggest contact between the inhabitants of the Aleutian Islands and southern Alaskans, but it is unclear whether or not this contact was intermittent, recurrent or even one-sided with limited movement of those along the peninsula into the island chain at a later time. Thus, at this time the available genetic data are unable to fully address the nature of the relationship between the inhabitants of the Aleutian Islands (both contemporary and prehistoric) and the ancient South Alaskans. The cultural interface between the populations along the Alaska Peninsula and the timing as well as degree of interaction (including genetic exchange) between the people in this region also remains unclear. The similarity in haplogroup and haplotypes among the people in this region underscores the need for increased sample sizes across time and space as well as further genetic investigation to address the questions of population interaction in this region as well as movement into the Aleutians. Full mitogenome sequencing coupled with NRY marker analysis would offer finer grained detail with respect to the matrilineages and patrilineages present in contemporary and prehistoric individuals throughout the Aleutians and Alaska Peninsula which would afford further insight into the genetic prehistories of the people in this region.

The relationship of the Sadlermiut relative to surrounding populations, both prehistoric and extant, has been a source of interest and intrigue among researchers. The Sadlermiut lived in relative isolation with minimal contact with outsiders and were considered “strange and primitive” by neighboring groups who had difficulty communicating with them given their unfamiliar dialect (Comer, 1910; Collins, 1956b:669; Clark, 1980). The material culture of the Sadlermiut suggests affinities with the Paleo-Eskimo Dorset as well as the Neo-Eskimo Thule, while the Sadlermiut’s manner of dress is considered to fall within the spectrum of Inuit clothing style in the region (Clark, 1980; Maxwell, 1984; Rowley, 1994). Skeletal and dental analyses of the Sadlermiut, on the other hand, reflected an association with the Neo-Eskimo groups of Greenland and Canada (Mayhall 1979; Utermohle and Merbs, 1979; Utermohle, 1984; Ossenberg, 2005). Stable isotope analysis identified the Sadlermiut diet as most comparable to some of the Dorset (Tayara and T-1) but unlike another Dorset sample (Angekok) as well as the Thule of Canada (Kamarvik and Silumiut) (Coltrain et al., 2004). The isotopic signatures of the Sadlermiut are also generally

unlike those reported for the marine diet oriented Greenlandic Thule (Nelson et al., 2012).

Discrete mtDNA analysis suggested the Sadlermiut could have been a remnant Dorset population with Thule admixture (Hayes, 2002; Hayes et al., 2003). The grounds for this scenario are based upon the last two populations characterized as monomorphic for haplogroups A and D, while the Sadlermiut harbored both haplogroups A (55.6%) and D (44.4%) (Hayes, 2002; Hayes et al., 2003). Populations in the most northern stretches of the New World are characterized by high frequencies of A while the distribution of D has been limited to appreciable frequencies among those residing in the Aleutians (contemporary and prehistoric) and nominal frequencies in Inuit and Iñupiat peoples (Shields et al., 1993; Merriwether et al., 1995; Saillard et al., 2000; Hayes, 2002; Hayes et al., 2003; Helgason et al., 2006). The absence of haplogroup D amongst the Thule could be due to sampling given the low frequencies of haplogroup D (~5%) among their contemporary counterparts (Helgason et al., 2006; Gilbert et al., 2007). There have only been a limited number of Paleo-Eskimos that have been recovered with only a few analyzed genetically in some of the earlier studies, with the Saqqaq (*n*=1) and Dorset (*n*=3) having been characterized as being monomorphic for haplogroup D (Hayes et al., 2003; Gilbert et al., 2007). However, with such small sample sizes it is difficult to gauge what the estimated levels of lineage diversity would be among the Paleo-Eskimos. Also, with the populations in the region (prehistoric and contemporary) characterized by high frequencies of haplogroup A it would be remarkable if the Paleo-Eskimos were to be completely monomorphic for D. Given the distribution of these two haplogroups (A and D) in prehistoric and contemporary populations throughout the region along with similarities in material culture it remains plausible that the Sadlermiut are the product of admixture between the Thule and Dorset.

The HVS-I sequences of the Sadlermiut in this study were affiliated with haplogroups A and D, which is in agreement with the earlier findings from the discrete marker analysis (Hayes, 2002; Hayes et al., 2003). However, the HVS-I matrilineages present in these Arctic and sub-Arctic populations demonstrate the case made by discrete marker analysis for the Sadlermiut representing a remnant Dorset population with gene flow from the Thule may not be as straightforward as previously thought. The Sadlermiut samples sequenced to date were

determined to belong to D4b1a2a1 and A2 (more specifically A2b1), and both of these haplogroups have been observed in the Inuit of Greenland and Canada as well as the Iñupiat of Northern Alaska, Siberian Yuit and Chukchi (Shields et al., 1993; Starikovskaya et al., 1998; Helgason et al., 2006; Derenko et al., 2010). Since haplogroup D4b1a2a1 is generally associated with the Inuit (Greenland and Canada) and Iñupiat in the Arctic/sub-Arctic it is thought to be among a set of haplogroups (which possibly includes A2b1) associated with the more recent arrival of the Neo-Eskimos (Tamm et al., 2007; Gilbert et al., 2008; Achilli et al., 2013).

Median-joining network analysis identified shared haplotypes and haplogroups between the Sadlermiut and the Inuit of Canada and Greenland and Iñupiat of northern Alaska, rather than the Saqqaq or any of the other ancient and contemporary comparative populations. Genetic distance analyses revealed the Sadlermiut had smaller genetic distances with Inuit (Canada and Greenland) and Chukotkan (Chukchi and Siberian Yuit) populations for analyses involving sequences from all haplogroups. While analyses considering only sequences belonging to either haplogroup A or D found small genetic distances between the Sadlermiut and Inuit (Canada and Greenland) groups. Along the same lines MDS analyses consistently clustered the Sadlermiut with Inuit populations, especially in the MDS plots for haplogroup D (Figure 4.6 and Figure 4.7) and A (Figure 4.5).

The available genetic data and analyses revealed the Sadlermiut exhibit genetic affinities with Inuit and Iñupiat populations (descendants of Neo-Eskimo Thule) rather than the Paleo-Eskimo Saqqaq, which complements results recently reported in the study by Raghavan et al. (2014). The Sadlermiut having a closer genetic affinity to the Neo-Eskimo rather than Paleo-Eskimo also echoes previous finding of matrilineal discontinuity in the eastern Arctic (Gilbert et al., 2008). The shared mtDNA haplogroups and haplotype between the Sadlermiut and the contemporary Inuit suggest the Sadlermiut could have potentially developed in situ from a cultural group with Thule ancestry or were an isolated prehistoric splinter population of the Neo-Eskimo Thule. These findings are also congruent with archaeological similarities as well as the morphological (skeletal and dental) analyses grouping the Sadlermiut with the Neo-Eskimo Thule. Ethnographic and historic accounts describe the Sadlermiut as a people who lived in relative isolation from

surrounding groups, had limited interaction with both surrounding groups and European travelers and were a small population prior to their demise in 1902-1903 (Comer, 1910; Mathiassen, 1927; Collins, 1956b). This isolating behavior could have led to the distinctive genetic profile of the Sadlermiut relative to prehistoric and contemporary populations in the region, which would have been shaped by genetic evolutionary forces such as founder effect and genetic drift.

The genetic affinity of the Sadlermiut with the Neo-Eskimos would be bolstered with additional genetic evidence from additional Neo-Eskimos and Paleo-Eskimos. Unfortunately, the available partial Thule sequences generated to date did not span the HVS-I region that contains the diagnostic SNPs to differentiate between haplogroups A2, A2a and A2b1. Preliminary partial sequence data of the Thule from Kamarvik and Silumiut identified the 16111T polymorphism, which in Arctic/sub-Arctic populations exhibiting haplogroup A is associated with haplogroup A2. An earlier study (Gilbert et al., 2007) identified haplogroups A2a and A2b1 in 15th-century Greenland Eskimo mummies, which resembles findings reported more recently (Raghavan et al., 2014) in Neo-Eskimo Thule (A, A2a, A2b, A2b1 and D4b1a2a1) from northern North America and Greenland. At this time, the available genetic data indicates a matrilineal genetic semblance of the Sadlermiut with contemporary and prehistoric Neo-Eskimo rather than the Paleo-Eskimo.

Two of the three Dorset samples produced sequences. One partial sequence spanned 238 bp while the other 'full' sequence encompassed 311 bp. The Dorset samples proved to be quite recalcitrant involving multiple amplifications with a variety of manipulations to different PCR parameters (see Table 4.2) and exhausting the available extract of some of the samples in an effort to produce sequences. Neither Dorset sequence was successfully confirmed in this study with additional sequence results obtained from either the first extraction or from two subsequent independent extractions. Modifications were made to the extraction method for the third extraction of the Dorset samples in attempt to replicate the Dorset sequencing results (see Methods and Materials). There was no evidence of contamination detected in either the extraction or PCR controls included in every experiment. However, given the extensive handling of these two particular samples, the difficulty to obtain sequence data and the inability to reproduce the results despite considerable testing brings into question the authenticity of these

Dorset sequences. With that being said, the author is reluctant to provide much interpretation of the Dorset results until additional Dorset individuals are sequenced and the available Dorset sequences can be confirmed.

The Dorset samples in this study were characterized as D2a'b and D4b1a2a1. The HVS-I variants associated with D2a'b have been found in other Arctic/sub-Arctic populations including the Paleo-Eskimo (Saqqaq), ancient Aleuts and Unangaŝ (Rubicz et al., 2003; Zlojutro et al., 2006; Gilbert et al., 2008; Zlojutro et al., 2009). Given that the same HVS-I variants have been observed in other prehistoric and contemporary Arctic/sub-Arctic populations, the characterization of D2a'b amongst the Paleo-Eskimo Dorset group is within reason. Additionally, archaeologists have noted the possible connection between the prehistoric Dorset and Aleut based on observed similarities in their respective material cultures. More specifically, incised stone carvings in the (eastern) Aleutians have been described as "hauntingly Dorset in appearance" because they exhibit design elements such as the chevron and crosshatched motifs found on Dorset figurines (Knecht et al., 2001; Davis and Knecht, 2005:62). Decorative carved linear markings (incised cross (X or +), short and long parallel lines, human faces on the sides) found on some ancient (eastern) Aleut lance-heads and barbed harpoon heads bear resemblance to motifs found on embellished Dorset artifacts (Collins, 1940; Quimby, 1945). Further indication of a connection between the two traditions is found in the eastern Aleutians with the incorporation of ASTt elements in the Aleut material culture coupled with the observance of stone-lined house features in some Aleut homes that bear a striking resemblance to axial features/mid-passage hearths in Dorset dwellings (Knecht et al., 2001; Knecht and Davis, 2005; Knecht and Davis, 2008; Hatfield, 2010).

Haplogroup D4b1a2a1, on the other hand, has been associated with the Inuit of Greenland and Canada, the Iñupiat of Northern Alaska, Sadlermiut, Thule as well as some Siberian and northeastern Asian populations (Shields et al., 1993; Helgason et al., 2006; Derenko et al., 2010; Raghavan et al., 2014). Archaeological similarities between the Thule (the ancestors of the modern Inuit/Eskimo) and Dorset along with overlapping radiocarbon dates from Dorset and Thule archaeological sites in the eastern Arctic suggest potential contact between the two groups.

As a result, the occurrence of gene flow between the Dorset and Thule is plausible. However, the Dorset characterized as D4b1a2a1 in this study has been directly dated to Cal AD 423 and predates all of the Thule (Cal AD 1219 to 1637) samples analyzed in both this and previous studies (Hayes, 2002; Hayes et al., 2003). Again it should be stressed these findings are tentative since the sequences of these Dorset samples have not yet been confirmed with sequence data from either the initial extraction or two additional independent extractions in this study. Recently, though, the haplogroup assignment of the Angekok Dorset sample was further refined from D2a'b (this study) to D2a1 (Raghavan et al., 2014).

Given the limited availability of Paleo-Eskimo materials, the Saqqaq represented by a single individual and the scarcity of Dorset remains (*n*=3 in this study), it is uncertain whether additional haplogroups will be identified among these Paleo-Eskimo groups especially if present at low frequencies. Even with an expanded set of Dorset (*n*=19) analyzed in a recent study only sublineages associated with D (namely D2a1, followed by D2a and D) have been identified in this Paleo-Eskimo group (Raghavan et al., 2014). At this time it is unclear if the determination of haplogroup D4b1a2a1 in the Paleo-Eskimo Dorset T-1 sample will be proven to be legitimate, which if found to be true would mean its arrival in the eastern Arctic may not be as 'recent' as previously thought. This would also imply non-Neo-Eskimo groups may have had this haplogroup present at low frequencies and reached 'appreciable' frequencies in the east through either stochastic processes or waves of gene flow from the west. However, at the moment this scenario seems unlikely given the questionable nature of the Dorset T-1 sequence in this study and the expanded set of Paleo-Eskimo assigned to sublineages of D2 (Raghavan et al., 2014).

There is continued interest in the prehistory of the Eskimo-Aleuts as there are still questions surrounding their origins, number of migrations as well as the relationships of these populations to each other (both contemporary and prehistoric) and surrounding populations. A theory regarding Eskimo-Aleut prehistory articulated by Laughlin (1980) contended ancestors of the Eskimo-Aleuts migrated along the southern coast of the Bering Land Platform and diverged in southwestern Alaska following the inundation of Beringia and those who traveled west became the Aleuts while those to the east the Eskimos (Laughlin, 1980:77). While the Eskimo-Aleuts

have similarities in language and morphology (physically adapted to cold climate), results are discordant based on anthropometric, cranial and dental morphology/traits and classical genetic markers that group the people of the Aleutians with the Eskimo/Inuit or with other populations (Native American and/or Siberian) (Hrdlička, 1945; Marsh and Swadesh, 1951; Szathmary and Ossenberg, 1978; Szathmary, 1979; Harper, 1980; Laughlin, 1980; Turner, 1983; Heathcote, 1986; Ossenberg, 1992; Powell, 1993; Scott, 1994; Ousley, 1995; Ossenberg, 2005).

Overall there is general acceptance the populations of the circum-Arctic represent a separate but more recent arrival into North America (Schurr et al., 1990; Shields et al., 1993; Starikovskaya et al., 1998; Saillard et al., 2000; Rubicz et al., 2003; Schurr and Sherry, 2004; Perego et al., 2009; O'Rourke and Raff, 2010). Phylogeographic investigations have traced the maternal lineages (A2a, A2b1, D2a'b/D2a/D2a1/D2a1a and D4b1a2a1) of the circum-Arctic populations to Siberian and/or Beringian sources (Derbeneva et al., 2002, Derenko et al., 2007, Tamm et al., 2007, Gilbert et al., 2008, Volodko et al., 2008, Derenko et al., 2010, Kumar et al., 2011; Dryomov et al., 2015). In the Old World Arctic, the aforementioned haplogroups along with related sublineages have been observed in the Chukchi and Siberian Yuit. Contemporary populations in the New World Arctic such as the Unangax̂ are primarily A2, A2a and D2a'b (D2a and D2a1a), while the Inuit are largely associated with A2a, A2b1 and D4b1a2a1. As noted above prehistoric populations (ancient Aleuts and Sadlermiut) in the respective regions of their descendants generally exhibit comparable maternal haplogroup profiles, though fluctuations in frequency in some regions have been observed over time. Studies of ancient groups in the Arctic/sub-Arctic have also noted the presence of additional haplogroups amongst ancient southern Alaskans B2 and D1 that are thought to be due to admixture with Native Americans (Raff et al., 2010). There is also evidence of maternal genetic discontinuity between the D2 sublineages (D2, D2a, D2a1) found in ancient Paleo-Eskimo (Saqqaq and Dorset) and the haplogroups observed among both the prehistoric Sadlermiut (D4b1a2a1 and A2b1) as well as prehistoric and contemporary Inuit with D4b1a2a1 along with A2a and A2b1 (Gilbert et al., 2008; Raghavan et al., 2014).

Currently, the maternal genetic data suggest the Eskimo-Aleuts represent more recent migrations separate from that of the ancestral Native Americans responsible for distributing the

Pan-American lineages throughout the New World. Supporting evidence includes the limited number of founding haplogroups (A2a, A2b1, D2a'b/D2a/D2a1/D2a1a and D4b1a2a1) among the circum-Arctic populations, the geographic distribution of said haplogroups among Old and New World populations and the reported coalescence dates of these founding haplogroups being generally younger than those among other indigenous groups of the New World (Gilbert et al., 2008; Volodko et al., 2008; Achilli et al., 2013). Additionally, there is evidence indicating at least two population dispersal events in the Arctic/sub-Arctic region. The first involves the bidirectional movement of individuals carrying D2a1 (HVS-I polymorphism motif is D2a'b) (and possibly A2a) into the Aleutians and eastward across Canada and into Greenland as evidenced by the D2a1 in the Paleo-Eskimo Saqqaq. The second dispersal was even more recent and involves the movement of haplogroup D4b1a2a1 (and possibly A2b1), which presumably were carried eastward from Alaska to Greenland by the Neo-Eskimos as these haplogroups are associated with contemporary and prehistoric Iñupiat/Inuit groups. A larger sampling of mitogenomes from prehistoric and contemporary populations across the region could refine or perhaps modify our understanding of the genetic prehistories and movements of these Arctic/sub-Arctic populations throughout the northern stretches of North America.

Summary

The initial findings presented in this dissertation have provided some insight into the genetic prehistories of several populations in the circum-Arctic region. The HVS-I sequencing results of the ancient Aleuts identified haplogroups A2a, D2 and D2a'b. The Unangax̂, the descendants of the ancient Aleuts residing in the Aleutians, are also known to harbor haplogroups A2a and D2a'b along with A2—based on HVS-I sequences. Shared haplogroups along with haplotype provide evidence for the matrilineal genetic similarity between the ancient Aleuts and the Unangax̂. Like the Unangax̂, ancient Aleuts also exhibited a genetic affinity with Chukotkan populations (Chukchi and Siberian Yuit) rather than Kamchatkan populations (Itel'men and Koryaks). The genetic semblance of the ancient Aleuts with Chukotkan populations provides additional support for a westward colonization of the Aleutian archipelago from Alaska rather than eastward from

Kamchatka.

The Aleutians are characterized by cultural continuity as evidenced by archaeology but now also include the maternal likeness between prehistoric and contemporary inhabitants of the island chain. However the presence of exotic materials and ASTt-like tools suggests interactions/contact with other groups outside the archipelago. Meanwhile changes in haplogroup frequency profiles through time in the region are thought to indicate an influx of new people into the island chain. Available sequence data identified haplogroup D2a'b to be present among the ancient Aleuts regardless of cranial (Paleo-/Neo-Aleut) and temporal (pre-/post-1,000 AD) affiliation along with a shared A2a haplotype between the ancient Aleut (Neo-/post-1,000 AD) with the Unangax̂, which would indicate matrilineal continuity in the region, though recently, haplogroup D2a'b has also been observed among ancient south Alaskans, which could be due to similar maternal genetic histories and/or genetic exchange. At this point in time the data available cannot resolve the question of population movement into the Aleutian region or the relationship between the ancient Aleuts and their neighbors to the east along the Alaska Peninsula. The genetic similarity across time and morphology among the people of the Aleutian Islands as well as across geography (Aleutians and Alaska Peninsula) highlight the necessity for additional sampling complimented by an expanded set of genetic markers (including NRY markers, autosomal markers (SNPs) and whole mtDNA genome sequencing) to advance our understanding of population relationships and movements throughout the region.

The Sadlermiut were characterized with haplogroups A2b1 and D4b1a2a1. The D4b1a2a1 observed in the Sadlermiut exhibited the additional sequence polymorphism 16093C, which is a haplotype observed among contemporary Siberian and Neo-Eskimo Iñupiat/Inuit groups. Additionally, haplogroup A2b1 is also affiliated with Chukotkan and Iñupiat/Inuit groups. The genetic similarity between Sadlermiut and contemporary Neo-Eskimo groups suggests the Sadlermiut may represent a population with Thule ancestry known to have isolated themselves from surrounding populations. This isolation coupled with founder effect may explain why the Sadlermiut exhibit nearly equal frequencies of haplogroups A and D, while surrounding Inuit populations have appreciably higher frequencies of A and depressed frequencies of D.

The characterization of A2a and A2b1 among 15th-century Greenlandic mummies who were described as “characteristically Inuit” as well as other recently characterized Thule samples (for A, A2a, A2b, A2b1 and D4b1a2a1) provides additional support for the genetic semblance of the Sadlermiut with the Inuit (Gilbert et al., 2007:852; Raghavan et al., 2014). The partial genetic sequences available in this study for ancient Thule have been initially designated as A2. However, the haplogroup assignment of these Thule could potentially be refined even further to A2a and/or A2b1 if the DNA segment containing the diagnostic SNPs for these haplogroups can be successfully amplified and sequenced. The genetic data indicate different genetic histories as the HVS-I polymorphism motif associated with D2a’b was observed among the peoples of the Aleutians (prehistoric and contemporary) and the Paleo-Eskimo Saqqaq in Greenland while D4b1a2a1 has been characterized amongst the Sadlermiut and Eskimo groups (prehistoric and contemporary) from Chukotka to Greenland. It would be worthwhile to analyze additional genetic markers and samples from more archaeological sites and temporal time frames to enhance our understanding of the population dynamics in the circum-Arctic region.

The enigmatic Dorset continue to safeguard their secrets from the academic community. Despite this researcher’s best efforts the position of the Dorset within the genetic landscape of the Arctic could not be resolved. Sequence data were obtained for two of the Dorset samples and indicated haplogroups D2a’b and D4b1a2a1. Unfortunately any conclusions drawn from the Dorset samples are eclipsed by the problematic nature surrounding the Dorset sequencing results. This is chiefly due to the difficulties in obtaining these sequences as well as the inability to confirm either of these sequences with sequence data from either the same extract or independent extracts. Recently, though, advances in molecular genetic technology (e.g., next generation sequencing) have afforded researchers the opportunity to unravel some of the mysteries of the Dorset, such as sorting out their relationship to other Eskimos including Paleo- and Neo-Eskimos (Raghavan et al., 2014). Findings from the study indicate the Paleo-Eskimo groups (e.g., Saqqaq, Dorset) belong to one continuous population despite variations in material culture and that the matrilineal history of the Paleo-Eskimo was independent of that of the Neo-Eskimo Thule (Raghavan et al., 2014). However, questions pertaining to the fate of the Dorset

such as whether or not the Thule absorbed the Dorset they encountered at the time of the Paleo-Eskimo—Neo-Eskimo transition or if the genetic legacy of the Dorset perished along with the loss of the last of its members remain unsettled.

As discussed earlier, the maternal genetic data to date indicate the Eskimo-Aleut represent more recent migrations in the New World apart from that of the ancestors of the Amerindians. Distribution of the circum-Arctic matrilineages (A2a, A2b1, D2a'b/D2a/D2a1/D2a1a and D4b1a2a1), particularly those ascribed to D, among indigenous populations in the region signal that there have been at least two migratory events. The first involved the distribution of D2a1 (and possibly A2a) across the northern stretches of the New World as this particular haplogroup in the New World is found in populations from the Aleutians to Greenland, as this haplogroup is affiliated with the Unangaχ and the Paleo-Eskimo Saqqaq. The second appears to mark the dispersal of the Neo-Eskimos, as haplogroup D4b1a2a1 (and possibly A2b1) has been associated with Iñupiat/Inuit groups. The findings from this study appear to dovetail with this mtDNA oriented view on the peopling of the most northern stretches of the New World. Haplogroups D2a'b and A2a were identified amongst the ancient Aleuts while D4b1a2a1 and A2b1 were associated with the Sadlermiut who are genetically indistinguishable from contemporary and prehistoric Neo-Eskimos. The unique genetic maternal profiles of contemporary and prehistoric groups imply the genetic prehistory of the Neo-Eskimos was distinct from that of the Paleo-Eskimo and prehistoric/contemporary Aleut. Sequence data from the HVS-I region have proven to be informative but provide only a partial perspective on the peopling of the circum-Arctic region.

Despite the small sample sizes in this study the preliminary information garnered from the prehistoric Aleut, Sadlermiut and Thule sequences has helped shed additional light on their matrilineal relationship to contemporary and prehistoric populations adding to the growing body of knowledge of ancient populations in the circum-Arctic region (Hayes et al., 2003; Gilbert et al., 2007; Gilbert et al., 2008; Smith et al., 2009; Raff et al., 2010; Raghavan et al., 2014). It should be noted that the findings of this study, as well as a nearly all of those of other studies referenced above (with exception to Raghavan et al., 2014), are based on sequence data from a limited

number of prehistoric individuals (e.g., a single Paleo-Eskimo, a limited number of ancient south Alaskans, Sadlermiut, Aleut, Thule and Greenlandic mummies) who surely do not completely capture the genetic diversity present in their respective prehistoric populations. The findings in this study, though tempered by small sample sizes for the populations under investigation, have provided insight into these populations as well as the groundwork for future studies to build upon.

The Dorset and Thule samples proved to be rather fastidious when it came to obtaining sufficient DNA from the extracts for successful amplification as well as readable sequences. The DNA extraction method was modified from manual reduction of the sample to a chemical reduction for the third extraction attempt of these samples. This modification showed promise for the Thule samples as partial sequences were obtained for some of these samples. Unfortunately the same cannot be said for the Dorset samples as no sequence data were acquired from these particular samples even after using the modified DNA extraction method. The success for the Thule samples could be linked to using a chemical reduction of the sample as the efficacy of DNA extraction methods employing a demineralization step (EDTA, EDTA plus proteinase K) has been established elsewhere (Żołędziewska et al., 2002; Rohland and Hofreiter, 2007; Campos et al., 2012). Recently though it has been suggested aDNA recovery could be maximized if both fractions of the bone extract (EDTA soluble and nonsoluble) were utilized, which may help with getting sufficient quality DNA from Dorset samples that could potentially lead to readable sequences (Campos et al., 2012).

Advances in sequencing technology along with improvements in DNA capture methods may enable researchers to delve further into population relationships and peopling events in the northern stretches of the New World. In particular, the advent of high throughput sequencing methods such as next generation sequencing (NGS) is providing molecular anthropologists a means to target entire mitogenomes and nearly complete genomes from ancient samples that may have been previously inaccessible via classical methodologies. Several advantages of NGS technology include its ability to handle the shorter fragment lengths of aDNA as well as the low concentration of endogenous aDNA (Stoneking and Krause, 2011). This type of sequencing methodology has been successfully applied to an historic Aboriginal Australian man (Rasmussen

et al., 2011), ancient human individuals including a Paleo-Eskimo Saqqaq individual (Gilbert et al., 2008; Rasmussen et al., 2010), Ötzi the mummified Tyrolean Iceman (Ermini et al., 2008; Keller et al., 2012), an infant Clovis (Anzick-1) boy from western Montana (Rasmussen et al., 2014), ancient individuals from the Northwest Coast of North America and the New World Arctic (Cui et al., 2013; Raghavan et al., 2014), ancient European hunter-gatherers and farmers (Sánchez-Quinto et al., 2012; Skoglund et al., 2012; Olalde et al., 2014), early modern humans from Russia and China (Krause et al., 2010a; Fu et al., 2013), an anatomically modern human from Siberia (Raghavan et al., 2013), as well as extinct hominins (Neanderthals, Denisovans, Sima de los Huesos) (Green et al., 2006; Green et al., 2008; Briggs et al., 2009; Green et al., 2010; Krause et al., 2010b; Reich et al., 2010; Meyer et al., 2012; Meyer et al., 2013; Prüfer et al., 2014).

The genetic analyses of ancient genomes using NGS have provided insights into evolutionary histories, interaction patterns, affinities and phylogenetic relationships of contemporary and ancient groups such as prehistoric hunter-gatherers and farmers, prehistoric and contemporary populations as well as modern humans and extinct hominins (Green et al., 2006; Green et al., 2008; Briggs et al., 2009; Green et al., 2010; Krause et al., 2010a,b; Reich et al. 2010; Meyer et al., 2012; Sánchez-Quinto et al., 2012; Skoglund et al., 2012; Fu et al., 2013; Meyer et al., 2013; Raghavan et al., 2013; Raghavan et al., 2014; Olalde et al., 2014; Prüfer et al., 2014). The information garnered from NGS has also afforded researchers an idea of the physical characteristics of individuals from extinct groups, some represented by a few fragments of skeletal material, through the reconstruction of their phenotypic characteristics (Gilbert et al., 2008; Keller et al., 2012; Olalde et al., 2014). An individual's genetic predisposition to diseases (e.g., coronary heart disease) and other genetic traits (e.g., lactose intolerance) as well as the presence of infectious pathogens (e.g., Lyme disease) have also been ascertained using NGS technologies (Gilbert et al., 2008; Keller et al., 2012). With respect to future work in the circum-Arctic region, expanding samples sizes across a greater geographical and temporal scale along with improvements in extraction methods and DNA capture and sequencing methods will provide investigators the means to access mitogenomes and nuclear information (autosomal and Y

SNPs) from contemporary and ancient Arctic peoples to further refine their genetic prehistories and peopling events in the region.

For instance, full mtDNA genome analysis of prehistoric and contemporary individuals in this region would provide increased resolution of mtDNA phylogenies (as seen in contemporary populations and the ancient Saqqaq individual) by identifying mtDNA variation throughout the entire mitochondrial genome (Tamm et al., 2007; Achilli et al., 2008; Gilbert et al., 2008; Volodko et al., 2008; Perego et al., 2010; Raghavan et al., 2014; Dryomov et al., 2015). To date whole mitogenome sequencing has characterized haplogroups D2a and D2a1a among the Unangaŷ from the Commander Islands, while the eastern Arctic Paleo-Eskimos primarily belong to haplogroups D2a1 and D2a and contemporary Eskimo harbor A2a and A2b1 and D4b1a2a1 with similar haplogroups observed in 15th-century and prehistoric Neo-Eskimo (A2a, A2b, A2b1) (Derbeneva et al., 2002; Gilbert et al., 2008; Raghavan et al., 2014; Dryomov et al., 2015). Ancient Aleut HVS-I polymorphisms correspond to haplogroups A2a, D2 and D2a'b, while the Unangaŷ primarily exhibit haplogroups A2, A2a and D2a'b. Ancient south Alaskans associated with haplogroups A and D possessed HVS-I polymorphisms corresponding to A2, D2a'b and D4b1. Meanwhile HVS-I sequences from prehistoric Sadlermiut were characterized by haplogroups A2b1 and D4b1a2a1, and partial Thule sequences preliminarily indicate A2. However, the haplogroups of these ancient and contemporary groups could be refined even further (e.g., A2a, D2a, D2a1) via whole mitogenome sequencing.

More precise classification of matrilineages would enhance our understanding of the matrilineal relationships and affinities of both contemporary and prehistoric populations across time and space in the most northern stretches of the New World. In the Aleutians this could address whether contact with peoples outside the Aleutians based on the presence of ASTt-like tools, exotic materials (jet and slate) was also accompanied with any genetic exchange particularly in the most eastern Aleut communities. Sequences from securely dated samples may also enable anthropologists to ascertain whether such contact was responsible for shifts in haplogroup frequencies between pre- and post-1,000 AD Aleuts as well as an increase in haplogroup A frequencies in eastern Unangaŷ that may be associated with an influx of people

from the east. The relationship between the inhabitants of the Aleutians and the groups of individuals along the Alaska Peninsula could also be clarified.

Recently a study garnered low coverage whole genomes (~0.3X) from Paleo-Eskimo and Neo-Eskimo using NGS (Raghavan et al., 2014). Analysis of remains belonging to different Paleo-Eskimo groups (Pre-Dorset, Dorset and Saqqaq) in Canada and Greenland were characterized as being more genetically similar to one another rather than other groups based on mitochondrial and autosomal markers. Implications of this finding point to the Paleo-Eskimo groups "represent(ing) a continuum of the same single ancestral population" across time and space despite variations in material culture (Raghavan et al., 2014:1255832-5). Meanwhile, Neo-Eskimo Thule from Canada and Greenland were found to have greater genetic affinities with contemporary Inuit of Greenland than the Saqqaq. This provides additional support for the Neo-Eskimo Thule being the biological and cultural ancestors of contemporary Inuit (Mayhall, 1979; Collins, 1984; McGhee, 1984; Hayes, 2002). The Sadlermiut were also found to be more genetically similar to the Thule and Inuit than the Saqqaq, which mirrors the findings in this study. Overall, the implications of the results from this recent study indicate the Paleo-Eskimo "likely represent a single migration pulse into North America from Siberia, separate from the ones giving rise to the Inuit and other Native Americans" (Raghavan et al., 2014:1020). The authors also found evidence of limited genetic exchange between the Paleo-Eskimo and Neo-Eskimo dating ~4,000 years ago, which most likely occurred in Beringia prior to these groups moving eastward at separate times. However, later episode(s) of gene flow between the Dorset and the Thule after the Thule arrived in the eastern Arctic cannot be ruled out at this time. Additional samples could potentially address the fate of the Dorset and determine whether or not the Thule absorbed the Dorset they encountered at the time of Neo-Eskimo—Paleo-Eskimo transition or if the Dorset lineage disappeared in the east with the last Paleo-Eskimo. Also mitogenomes as well as autosomal information obtained from contemporary and prehistoric Aleuts could be compared to those of the Paleo-Eskimo (Dorset and Saqqaq, who have similar matrilineages to the D2a/D2a1a observed among the Unangax̂), which could potentially better our understanding of the genetic relationships and prehistories of these groups.

Additional insight into genetic relationships of contemporary and prehistoric populations across time and space in the Arctic/sub-Arctic region could also be garnered from the analysis of uniparentally inherited NRY (nonrecombining region of the Y chromosome) markers. Male inherited NRY markers such as SNPs (single nucleotide polymorphisms) and STRs (short tandem repeats) could be used to assess which Y chromosome haplogroups and haplotypes are present in these populations. The analysis of high-resolution NRY markers would afford insights into the paternal genetic prehistories of the ancient populations of the Arctic/sub-Arctic relative to their surrounding populations (both contemporary and prehistoric).

It has been established that there are two Y patrilines autochthonous to New World populations, haplogroups Q and C, which are commonly found in Old World populations in Northern Eurasia as well as Siberia (Karafet et al., 2008). An initial analysis of Y chromosomal SNP and STR markers identified haplogroup Q (Q-P36.2 and Q-M3) among the Unangax̂ (Zlojutro et al., 2009; Rubicz et al., 2010a,b). High frequencies of nonindigenous Y haplogroups (I, J, R, N, C and other) were also detected which were an indication of extensive postcontact nonnative male gene flow into the area (Zlojutro et al., 2009; Rubicz et al., 2010a,b). The Y profile of the ancient Paleo-Eskimo Saqqaq individual assigned him to paragroup Q1a*, which provided additional support for his Beringian affinities as this haplogroup has also been detected in the Koryaks (Rasmussen et al., 2010; Malyarchuk et al., 2011). The Q1a6 patriline, a subbranch of Q1a* redefined by marker NWT01, was recently identified in a western Arctic Canadian Inuit group (Inuvialuit) in the northern Northwest Territories (Dulik et al., 2012). This particular lineage (Q1a6) is thought to be “largely confined to the Inuvialuit, Inuit and Iñupiat populations” and could potentially be present among the Unangax̂, Canadian Inuit and maybe even the Saqqaq although confirmation of this awaits further analysis of additional markers (the NWT01 locus and an expanded set of Y chromosomal markers) (Dulik et al., 2012:8473). The implication for the distribution of the Q lineages (Q1a* and Q1a6) in these Arctic and sub-Arctic populations was initially thought to indicate a Siberian origin (Rasmussen et al., 2010). Although Dulik et al. (2012) argue “an origin in northwestern North America with a subsequent back migration across the Bering Strait is equally likely” given the elevated levels of diversity and frequencies of these

NRY haplogroups in Eskimo speaking populations and the absence of other NRY haplogroups (e.g., C3c, N1c) among said populations that are commonly observed in coastal Siberian populations (Dulik et al., 2012:8475).

High resolution Y marker analysis of contemporary and prehistoric circum-Arctic groups will be necessary to fully characterize the patrilines present in these populations in order to sort out paternal affinities and potentially afford insights into male mediated movements in the region. This information would provide a paternal perspective to complement the maternal histories garnered from mtDNA. Of particular interest would be the determination of the patrilineal haplogroups and haplotypes harbored by contemporary and prehistoric populations throughout the region as well as their distribution through space and time. Findings from Y chromosomal analysis of prehistoric populations (e.g., Aleuts, south Alaskans) would speak to their patrilineal affinities to other populations (contemporary and prehistoric) as well as expand our understanding of population stability and male driven peopling processes in the area. It would also be of interest to see if the patrilineal affinities mirrored those observed with mtDNA in these groups.

Another possible molecular avenue to delve further into the genetic prehistories of Arctic and sub-Arctic populations would be the examination of autosomal SNPs in prehistoric and present day individuals. This would provide a means to assess the affinities of the prehistoric populations across time with contemporary populations. Securely dated samples with high sequence depth and genome coverage may allow researchers to assess the magnitude and timing of admixture with other indigenous and/or nonindigenous groups, as well as perhaps gauge the impact of European contact on indigenous levels of genetic diversity among New World peoples prior to and following European contact. Autosomal analysis could also be useful in sex identification with subadult or fragmentary remains, genetic predispositions to diseases and other genetic traits as well as phenotypic features of individuals from extinct populations (e.g., Paleo-Eskimo Saqqaq). The SNP analysis of an ancient Saqqaq revealed the Beringian affinities of this individual's diploid genome that was most like several populations in northeast Siberia, more specifically the Nganasan, Koryak and Chukchi (Rasmussen et al., 2010). Also of interest was the determination of no detectable European or Native American admixture in the Saqqaq

genome unlike the contemporary Inuit (Greenland) and Unangax̂ who exhibit admixture from both European and Native American sources (Rasmussen et al., 2010). The autosomal analysis also provided information on a host of phenotypic characteristics (A+ blood type, dry earwax, shovel-shaped incisors, dark thick hair, brown eyes, a complexion unlike fair Europeans and adapted to colder climate as indicated by SNPs linked to metabolism and body mass index) which provided a glimpse of a man who was a member of a prehistoric population from long ago who left behind little to no skeletal evidence (Rasmussen et al., 2010).

Advances in technology (NGS) coupled with improvements in aDNA extraction methodologies holds promise for future avenues of research concerning population histories in the circum-Arctic region—particularly for unsuccessful ancient samples using traditional methodologies. A growing body of literature has demonstrated the successful application of NGS technology using ancient samples. As the costs of sequencing using NGS platforms continues to decline, its use with prehistoric and contemporary population studies is becoming more and more feasible. The characterization of full mitogenome sequences, coupled with SNP data (Y and autosomal) from contemporary and prehistoric populations spanning the region will be necessary to decipher the palimpsest of human migrations throughout the circum-Arctic region as well as reconstruct the genetic histories of these populations.

APPENDIX

GENETIC DISTANCE MATRICES

Table A.1. Nei's corrected average (D_A) number of pairwise distances between 17 populations.

	Han	Mongolian	Evenki	Koryak	Itel-men	Chukchi	Siberian Yuit	Unangax^	Ancient Aleut	Mink Island	Sadler-miut	Canadian Inuit	Green-land Inuit	Athabascan	Haida	Bella Coola	Nuu-Chah-Nulth
Han	***																
Mongolian	0.055	***															
Evenki	0.243	0.148	***														
Koryak	0.694	0.723	0.596	***													
Itel'men	1.253	1.282	1.333	0.470	***												
Chukchi	2.129	1.873	2.108	2.844	3.392	***											
Siberian Yuit	2.811	2.431	2.797	3.784	4.376	0.073	***										
Unangax^	1.984	1.556	2.064	2.873	3.370	0.928	0.799	***									
Ancient Aleut	1.918	1.309	1.945	2.616	3.022	1.920	1.789	0.146	***								
Mink Island	1.179	0.738	1.145	1.906	2.219	1.216	1.334	0.373	0.000	***							
Sadlermiut	2.930	2.562	2.812	3.934	4.479	0.574	0.689	1.865	2.831	1.671	***						
Canadian Inuit	3.995	3.664	3.959	5.138	5.697	0.591	0.623	2.299	3.749	2.622	0.168	***					
Greenland Inuit	4.435	4.099	4.428	5.652	6.311	0.523	0.461	2.341	3.979	3.000	0.646	0.171	***				
Athabascan	3.451	3.152	3.416	4.483	5.161	0.285	0.293	1.757	3.181	2.425	1.233	0.831	0.388	***			
Haida	3.006	2.687	2.877	4.047	4.743	0.485	0.559	1.733	2.913	1.899	0.717	0.647	0.704	0.503	***		
Bella Coola	1.655	1.441	1.704	2.506	3.128	0.517	0.716	1.078	1.745	0.971	1.053	1.313	1.465	1.034	0.408	***	
Nuu-Chah-Nulth	1.000	0.797	0.735	1.667	2.471	0.858	1.229	1.341	1.778	0.842	1.338	1.936	2.207	1.562	0.992	0.367	***

Table A.2. Nei's corrected average (D_A) number of pairwise distances between 18 populations. Ancient Aleut separated by cranial affiliation.

	Han	Mon-golian	Even-ki	Kor-yak	Intel-men	Chuk-chi	Siberian Yuit	Unan-gax^	Neo-Aleut	Paleo-Aleut	Mink Island	Sadler-miut	Cana-dian Inuit	Green-land Inuit	Athab-ascan	Haida	Bella Coola	Nuu-Chah-Nulth
Han	***																	
Mongolian	0.055	***																
Evenki	0.243	0.148	***															
Koryak	0.694	0.723	0.596	***														
Itel-men	1.253	1.282	1.333	0.470	***													
Chukchi	2.129	1.873	2.108	2.844	3.392	***												
Siberian Yuit	2.811	2.431	2.797	3.784	4.376	0.073	***											
Unangax^	1.984	1.556	2.064	2.873	3.370	0.928	0.799	***										
Neo-Aleut	0.883	0.425	0.972	1.696	2.177	0.112	0.056	0.000	***									
Paleo-Aleut	3.372	2.610	3.336	3.953	4.285	4.146	3.938	1.350	0.696	***								
Mink Island	1.179	0.738	1.145	1.906	2.219	1.216	1.334	0.373	0.000	0.680	***							
Sadlermiut	2.930	2.562	2.812	3.934	4.479	0.574	0.689	1.865	1.102	4.977	1.671	***						
Canadian Inuit	3.995	3.664	3.959	5.138	5.697	0.591	0.623	2.299	1.656	6.259	2.622	0.168	***					
Greenland Inuit	4.435	4.099	4.428	5.652	6.311	0.523	0.461	2.341	1.657	6.719	3.000	0.646	0.171	***				
Athab-ascan	3.451	3.152	3.416	4.483	5.161	0.285	0.293	1.757	0.862	5.916	2.425	1.233	0.831	0.388	***			
Haida	3.006	2.687	2.877	4.047	4.743	0.485	0.559	1.733	0.949	5.294	1.899	0.717	0.647	0.704	0.503	***		
Bella Coola	1.655	1.441	1.704	2.506	3.128	0.517	0.716	1.078	0.173	3.734	0.971	1.053	1.313	1.465	1.034	0.408	***	
Nuu-Chah-Nulth	1.000	0.797	0.735	1.667	2.471	0.858	1.229	1.341	0.386	3.587	0.842	1.338	1.936	2.207	1.562	0.992	0.367	***

Table A.3. Nei's corrected average (D_A) number of pairwise distances between 14 populations for haplogroup A sequences.

	Han	Mongolian	Evenki	Koryak	Chukchi	Siberian Yuit	Unangax^	Sadlermiut	Canadian Inuit	Greenland Inuit	Athab-ascan	Haida	Bella Coola	Nuu-Chah-Nulth
Han	***													
Mongolian	0.081	***												
Evenki	0.473	0.715	***											
Koryak	0.772	0.898	1.631	***										
Chukchi	1.716	1.704	2.121	0.682	***									
Siberian Yuit	1.945	1.922	2.337	0.788	0.000	***								
Unangax^	2.496	2.495	2.846	1.230	0.555	0.486	***							
Sadlermiut	2.341	2.353	2.661	1.945	0.870	1.069	2.167	***						
Canadian Inuit	1.937	1.941	2.281	1.297	0.372	0.536	1.318	0.128	***					
Greenland Inuit	1.884	1.882	2.247	0.949	0.057	0.125	0.769	0.487	0.151	***				
Athabascan	1.526	1.533	1.968	0.619	0.134	0.147	0.499	1.422	0.793	0.319	***			
Haida	1.392	1.380	1.756	1.074	0.596	0.726	1.225	1.164	0.729	0.660	0.471	***		
Bella Coola	1.764	1.668	1.853	1.692	1.113	1.222	1.766	1.700	1.275	1.198	1.020	0.494	***	
Nuu-Chah-Nulth	1.487	1.398	1.917	1.202	0.690	0.814	1.332	1.264	0.829	0.760	0.574	0.303	0.652	***

Table A.4. Nei's corrected average (D_A) number of pairwise distances between 12 populations for haplogroup D sequences.

	Han	Mongolian	Chukchi	Siberian Yuit	Unangax^	Ancient Aleut	Mink Island	Sadlermiut	Canadian Inuit	Grenland Inuit	Bella Coola	Nuu-Chah-Nulth
Han	***											
Mongolian	0.068	***										
Chukchi	1.288	1.168	***									
Siberian Yuit	1.822	1.702	0.000	***								
Unangax^	2.073	1.951	0.128	0.355	***							
Ancient Aleut	1.860	1.609	0.088	0.371	0.000	***						
Mink Island	0.654	0.567	0.239	0.625	0.712	0.247	***					
Sadlermiut	2.323	2.211	2.783	3.296	4.443	4.281	2.455	***				
Canadian Inuit	3.342	3.141	3.716	4.159	5.533	5.397	3.244	0.354	***			
Greenland Inuit	3.342	3.141	3.716	4.159	5.533	5.397	3.244	0.354	0.000	***		
Bella Coola	1.464	1.373	2.520	3.068	3.363	3.140	1.981	3.433	4.527	4.527	***	
Nuu-Chah-Nulth	0.638	0.626	1.397	1.870	2.282	2.050	0.889	2.368	3.471	3.471	0.435	***

Table A.5. Nei's corrected average (D_A) number of pairwise distances between 13 populations for haplogroup D sequences. Ancient Aleut separated by cranial affiliation.

	Han	Mongolian	Chukchi	Siberian Yuit	Unangax^	Neo-Aleut	Paleo-Aleut	Mink Island	Sadlermiut	Canadian Inuit	Greenland Inuit	Bella Coola	Nuu-Chah-Nulth
Han	***												
Mongolian	0.068	***											
Chukchi	1.288	1.168	***										
Siberian Yuit	1.822	1.702	0.000	***									
Unangax^	2.073	1.951	0.128	0.355	***								
Neo-Aleut	1.067	0.933	0.000	0.190	0.017	***							
Paleo-Aleut	2.426	2.098	0.253	0.530	0.025	0.077	***						
Mink Island	0.654	0.567	0.239	0.625	0.712	0.144	0.355	***					
Sadlermiut	2.323	2.211	2.783	3.296	4.443	3.465	4.864	2.455	***				
Canadian Inuit	3.342	3.141	3.716	4.159	5.533	4.538	6.008	3.244	0.354	***			
Greenland Inuit	3.342	3.141	3.716	4.159	5.533	4.538	6.008	3.244	0.354	0.000	***		
Bella Coola	1.464	1.373	2.520	3.068	3.363	2.321	3.724	1.981	3.433	4.527	4.527	***	
Nuu-Chah-Nulth	0.638	0.626	1.397	1.870	2.282	1.239	2.630	0.889	2.368	3.471	3.471	0.435	***

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