Evolutionary Consequences of Recently Founded Aleut Communities in the Commander and Pribilof Islands

By

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M.A., University of Kansas, 2001

Submitted to the Department of Anthropology and the
Faculty of the Graduate School of the University of Kansas
In partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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Chairperson

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Evolutionary Consequences of Recently Founded Aleut Communities in the Commander and Pribilof Islands

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Abstract

This study uses molecular genetic markers to investigate the genetic consequences of the founding and other historic events on the Aleut gene pool. Maternal markers (mtDNA RFLPs and sequencing), paternal markers (Y chromosome SNPs and STRs), and biparentally-inherited markers (autosomal STRs, and classic genetic markers from the literature) are characterized to address the questions: 1) is there reduced genetic diversity in recently founded Aleut communities compared to the parental Aleutian Aleut population? 2) How reproductively isolated are these communities? 3) Is there symmetry in maternal versus paternal gene flow? 4) What is the genetic effect of the interaction genetic drift and gene flow? 5) Which of the three aggregates differentiates most from the parental population? Maternal markers for all Aleut populations belong to Native American mtDNA haplogroups A and D, indicating there was no non-Native female gene flow into the population, for individuals claiming Aleut maternal ancestry. In contrast, the majority of paternal markers (73% to 90%) are of non-Aleut origin, due to gene flow from Russians and other non-Aleut males. The Bering community exhibits considerably reduced mtDNA diversity, demonstrated by the fixation of haplogroup D, and gene diversity=0.29, compared to other Aleuts (St. George=0.56, St. Paul=0.72, and Aleutian Aleuts=0.77). This is likely the result of Bering experiencing a founder effect, followed by its closure from other Aleut populations after the U.S. purchase of Alaska in 1867. Meanwhile, the Pribilof communities remained in contact with the Aleutian inhabitants. The low gene diversity, however, is not demonstrated by the paternal markers for the communities (Bering=1.0, St. Paul=0.9591, St. George=0.9167, and Aleutian Aleuts=0.9565), or the autosomal markers (Bering Aleuts= 0.776, and Bering mixed Aleuts=0.882). The results indicate genetic drift may be acting on the maternal lineages, while the opposing evolutionary force of gene flow is affecting the paternal markers. Autosomal markers are intermediate, falling within the rage of other Native American and Siberian populations. This study demonstrates that due to unique historic events, the Bering community has differentiated the most from the parental Aleut population, but that St. Paul and St. George have also experienced evolutionary genetic change due to their founding.

This dissertation is dedicated to Aaron and Sofia.

Acknowledgements

I thank the Aleuts, and Alice Petrivelli in particular, for their interest and participation in this project. Without them, this research would not have been possible.

I also thank my committee members: Dr. Michael Crawford, Dr. Steve Benedict, Dr. Jack Hoffman, Dr. James Mielke, and Dr. Alan Redd, as well as Dr. R.J. Mitchell; my collaborators on this project: Dr. Michael Crawford, Dr. Dennis O'Rourke, Dr. Victor Spitsyn, Dr. Ranjan Deka, Dr. Guangyun Sun, Dr. Eric Devor, Dr. Reena Roy, and Mark Zlojutro; my fellow students including: Kristine Beaty, Geetha Chittor, Virginia Hatfield, Anne Justice, Phil Melton, Chris Phillips, Jennifer Rack, and Kristin Young; the office staff: Judy Ross, Carol Archinal, and Kathleen Womack; and my family for their continuing support and encouragement.

This research was funded by NSF grants #OPP-990690 and # OPP-0327676 awarded to Dr. M.H. Crawford, and the following scholarships: Carroll D. Clark, Graduate Research Fund, Ida H. Hyde, and Sigma Xi.

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CHAPTER ONE: INTRODUCTION

While there are studies that have investigated the evolutionary and genetic effects of small, isolated populations using classic genetic or molecular markers, few of these have focused on recently aggregated populations with founders from known regions. According to Sewall Wright (1969), when a population is subdivided into smaller subpopulations, and these subdivisions remain isolated, they will differentiate genetically from both the founding population and each other, and become more homogeneous. Studies from the literature that have documented these effects in human populations include a publication by Thangaraj *et al*. (2003) on the Andaman Islanders, and research by Keyeux *et al*. (2002) on an isolated South American population from Columbia, both of which noted a marked decrease in genetic variability among these groups.

However, the best example is provided by studies of the small island community of Tristan da Cunha, for which the history and genealogy are well-documented (Roberts 1965). After its establishment in 1816, this small island population underwent several periods of expansion, interrupted by two major bottlenecks. The first was the result of emigration after the death of the community's founder in 1853, which reduced the population by half, and the second was due to an 1885 disaster at sea that killed 15 adult men, and was coupled with the subsequent emigration of additional community members, reducing the population size by one-third. According Roberts (1968), genetic drift, in the form of these drastic population reductions, had a profound impact on the composition of the Tristan da Cunha gene

pool. It eliminated the genetic contribution of some of the original founding ancestors to the population, and changed the relative genetic contributions of the remaining ancestors.

Based on historic records, the living population of Tristan can be traced to seven female and eight male original founders. The accuracy of these records was tested using mitochondrial DNA (mtDNA) markers to trace maternal lineages, and Y chromosome markers to trace paternal lineages (Soodyall *et al*. 2003, 1997). Discrepancies were reported in that one pair of documented "sisters" had mtDNAs from different lineages and therefore were not maternally related, and instead of the seven paternal lineages that were predicted, nine different Y chromosome lineages were in fact present. One of these appears to be the result of a new mutation to an existing lineage, the other, however, is of non-island origin, indicating undocumented paternal gene flow into the Tristan da Cunha community. These studies demonstrate the utility of molecular markers in testing the accuracy of historic records, and the effect of genetic drift, as well as gene flow, on small, isolated populations.

As demonstrated in this example, gene flow may also change the genetic composition of populations. Gene flow, or admixture, has the opposite effect on populational genetic variation when compared to genetic drift. It acts to increase heterozygosity within subdivisions, and decrease the variation among them, resulting in population subdivisions that more closely resemble one another genetically. Byard *et al*. (1983) documented increased heterozygosity measures for admixed individuals

in Eskimo communities in Alaska (Savoonga and Gambell on St. Lawrence Island; and Wales), which were the result of European gene flow into the populations.

The historically founded Aleut communities of Bering Island, in the Commander Islands, Russia, and St. Paul and St. George, in the Pribilof Islands, Alaska, provide another such opportunity to study the effects of genetic drift and gene flow in small island populations. These populations are recently aggregated, and there is good historical documentation of where their founders originated, and the demographic changes they have experienced since their establishment. All three of these communities were established by Russians at the height of the North Pacific fur trade. Aleuts were relocated from their homeland in the Aleutian Archipelago, an island chain that stretches from the Alaska Peninsula to Kamchatka, Siberia. In 1788, Aleuts were taken from Unalaska and Atka, in the eastern and central Aleutians, to the Pribilof Islands for the purpose of hunting Northern Fur Seals at their summer breeding grounds. Later, between 1825 and 1828, Aleuts were brought to the Commander Islands from Atka and Attu, in the central and western Aleutians, where the communities of Bering and Medni were founded in order to supply American-bound fur hunting expeditions, and in 1969 the two communities were consolidated at the Bering location. In addition to Aleuts, there were a number of Russian soldiers on Bering, and individuals were also relocated there from the Kurile Islands, Kodiak, Sitka, and Kamchatka. Similarly, the Pribilof Island Aleut communities have experienced an influx of Russian, European-American, Eskimo, and Athabascan individuals.

This study uses molecular genetic markers, and classic genetic data taken from the literature, in order to investigate the evolutionary consequences of the founding and other unique historic events on the genetic composition of the Bering, St. Paul, and St. George Aleut communities, and make comparisons to the parental Aleutian Islands Aleut population. Mitochondrial DNA analysis is used to characterize maternal lineages in these populations, paternal lineages are characterized using Y chromosome DNA analysis, and autosomal DNA and classic genetic blood group and protein markers are analyzed in order to characterize the bi-parentally-inherited nuclear gene pool. Specifically, this study tests: 1) is there a reduction in the genetic diversity of these three recently founded Aleut populations in comparison to the parental Aleut population? 2) How reproductively isolated are these communities, given their geographic locations? 3) Is there symmetry in gene flow? In other words, is there equal contribution of non-Aleut males and females to the communities? 4) What is the genetic effect of the interaction of two forces of evolution: genetic drift and gene flow? 5) Which of the three aggregates differentiates the most from the parental Aleut population, and why?

The chapters that follow include a review of the literature, the materials and methods used in this study, a summary of results, and discussion. Chapter two provides background on molecular markers, and on the Aleut populations including archaeological, linguistic, genetic, and historic information. The field research, laboratory, and analytical methods are presented in chapter three. Chapter four presents the results for the analysis of molecular and classic genetic markers, and

these are discussed in chapter five. Finally, the conclusions are summarized in chapter six.

CHAPTER TWO: LITERATURE REVIEW

This chapter provides an overview of molecular genetic markers (focusing on mitochondrial and Y chromosome DNA markers) that may be used in anthropological studies, and background information on the Aleuts and their historically established communities in the Commander and Pribilof Islands.

Molecular Genetics Review

The human genome consists of 46 chromosomes, 44 of which are called autosomes and are biparentally inherited. The remaining two are the sex chromosomes, XX in females and XY in males. There are approximately 30,000 genes in humans, most of these are located on the autosomes, with 1336 on the X chromosome, and 307 on the Y chromosome (OMIM 2007). The majority of the human genome, approximately 98.5% of nuclear DNA, is made up of non-coding regions that are not under the functional constraints of genes and therefore may exhibit great variation. This DNA largely consists of repetitive DNA sequences that are generally considered to be selectively neutral. DNA is also present outside the nucleus in the mitochondria (mitochondrial DNA), and it is strictly maternally inherited.

The effective population size (N_e), or estimated breeding size of a population, is largest for the autosomal markers, which are present in two copies in females and males. For the X chromosomal loci, N_e is ¾ of the total effective population size (two copies for females, and one copy for males), ¼ this number for Y chromosomal loci

(one copy per male), assuming an equal number of males and females, and ¼ the number for mitochondrial DNA loci.

There are a number of different types of polymorphisms in the human genome. Single nucleotide polymorphisms (SNPs) are the substitution of one nucleotide base for another. Single base insertions and deletions (indels) are sometimes lumped together with SNPs into the category of biallelic markers. Larger indels are also present. Repetitive DNA sequences include those repeated in tandem arrays, such as microsatellites, also called short tandem repeats (STRs), which are repeats of one to six base pairs that are usually not more than 350 base pairs in length, and minisatellites, or variable number of tandem repeats (VNTRs), which consist of repeat units of ten to 100 base pairs, strung together up to 1000 base pairs long. Retroelements are another form of repetitive DNA. These are sequences inserted by reverse transcriptase into the genome and include the *Alu* family, found only in

Table 1 Average mutation rate for different types of DNA markers (Rubicz et al. 2006, adapted from Jobling et al. 2004)

DNA Marker	**Mutation rate per locus per generation**
Some expanded polymorphic microsatellites	$<10^{0}$
Minisatellites	10^{-2} to 10^{-1}
Microsatellites	10^{-4} to 10^{-3}
Some structural polymorphisms	10^{-5} to 10^{-4}
Base substitutions (SNPs)	10^{-8} to 10^{-7}
Retroelement insertions	10^{-11} to 10^{-10}

humans and other primates. The mutation rates for some of these markers are given in Table 1. SNPs evolve slowly, while micro- and mini-satellites evolve more rapidly.

Mitochondrial DNA Markers

Several features of mitochondrial DNA (mtDNA) make it particularly useful for anthropological studies. These include its maternal inheritance and elevated mutation rate. Mitochondrial DNA (mtDNA) is a double-stranded, circular molecule (Figure 1) located in the energy-producing mitochondria of the cell, and is believed to be of bacterial origin (Margulis 1981). There are hundreds to thousands of mtDNA copies per cell, which is important for ancient DNA studies where the tissue recovered is often degraded. Its maternal inheritance means that a mother will pass it to all of her children, and her daughters will pass it on to future generations, but her sons will not. And, because it does not undergo recombination (intergenerational reshuffling of genetic material) it can be used to trace maternal lineages. The mtDNA molecule consists of roughly 16,569 base pairs, including a coding region with 37 genes and two noncoding hypervariable regions (HVS-1 and HVS-2) of around 400 bp each. It is estimated that the coding region mutates at a rate of 3.2% per million years (Francalacci *et al*. 1999), which is approximately five to ten times faster than nuclear DNA. The hypervariable region or D-loop has an even faster evolutionary rate of 8.4% per million years (Vigilant *et al*. 1989). These elevated mutation rates are in part due to its lack of repair mechanisms, and they are useful for comparing closely related populations such as humans.

Restriction fragment length polymorphism (RFLP) markers have traditionally been used in anthropological studies to define mtDNA lineages, also called haplogroups, and HVS-1 sequences provide information about diversity within the haplogroups, allowing for a higher resolution analysis of population relationships.

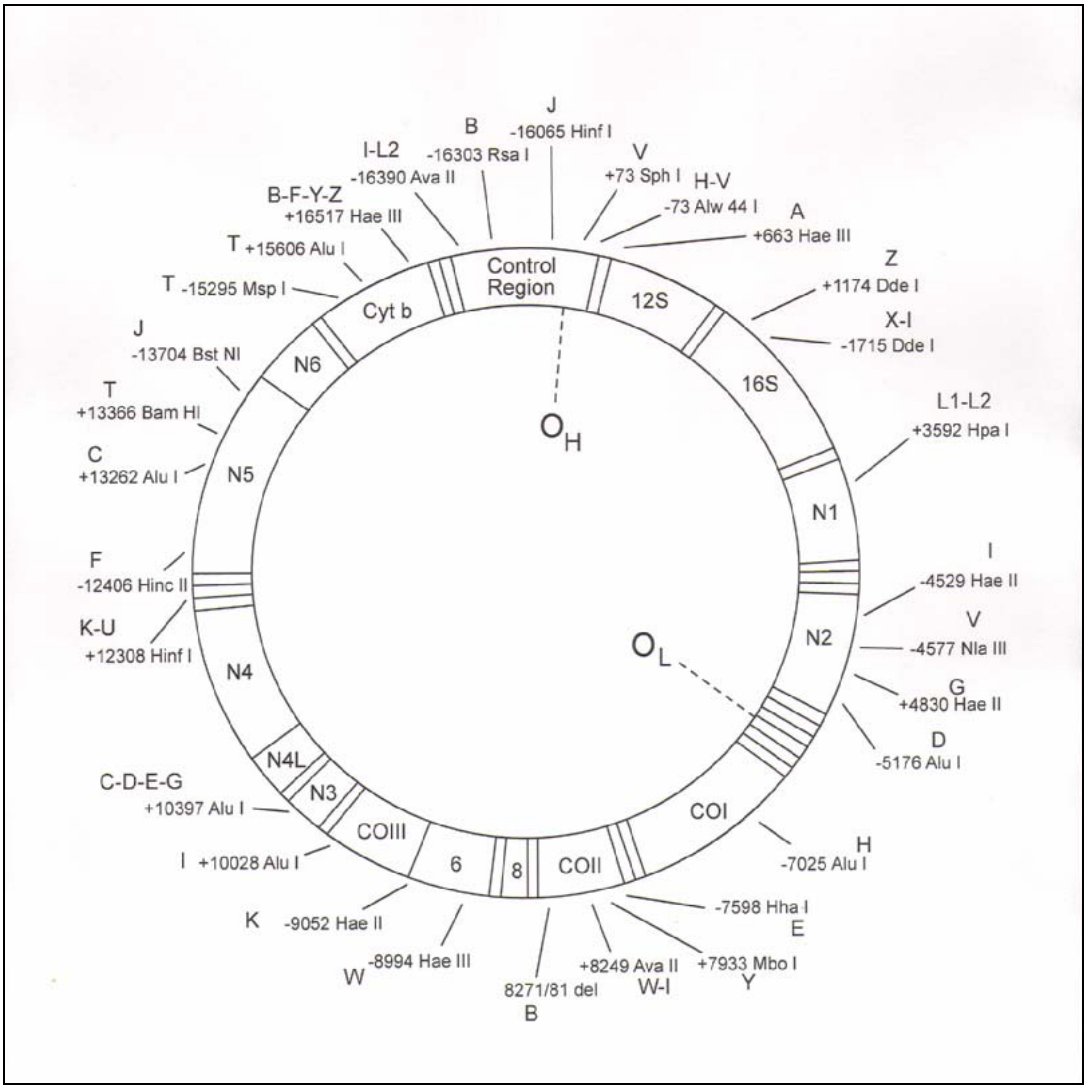

Figure 1 Mitochondrial DNA molecule with RFLP sites (Rubicz et al. 2006)

The origin and diversification of Native American populations has been extensively investigated using mtDNA markers to construct maternal lineages. These lineages are lumped into five major haplogroups: A, B, C, D, and X (Table 2). Although many populations indigenous to the Americas are polymorphic for all four haplogroups, some geographic trends are evident. Haplogroup A is present in highest frequencies in the North, among Arctic and Subarctic populations such as the Dogrib, Haida, and various Eskimo groups, it decreases in the Southwestern United States, and increases again in Central America, for example, among the Alta Mixtec and Teribe (Lorenz and Smith 1996, Merriwether *et al*. 1995, Torroni *et al*. 1994, Torroni *et al*. 1993, Ward *et al*. 1993). Haplogroup B is usually absent in northern populations except where due to admixture, it can be found in the Southwestern U.S. among the Navaho and Pima, it increases in Central America, and decreases in South America (Schurr *et al*. 1990, Torroni *et al*. 1993). Haplogroup C is present in low frequencies among some northern populations and is absent from others, is not found in Central America, and is highest in South America, for example among the Yanomama (Torroni *et al*. 1993, Horai *et al*. 1993). Haplogroup D is similarly distributed, with low frequencies among most northern populations with the exception of Aleuts where it is found at over 70%, and it is highest in South American groups, including the Wapishana (Rubicz *et al*. 2003, Torroni *et al*. 1993). The fifth haplogroup, X, has been characterized at low frequencies among several North American populations (Brown *et al*. 1998).

Table 2 HVS-I sequences defining Native American hapolgroups (after Melton 2004)

Haplogroup	16189	16223	16278	16290	16298	16319	16327	16362	**RFLP site**
CRS*	**T**	**C**	**C**	**C**	**T**	**G**	**C**	**T**	
A		T		T		A		C	+663 *HaeIII*
B	C								9 bp deletion (8271-8281)
C		T			C		T		-13259 *HincII*, +13262 *AluI*
D		T						C	-5176 *AluI*
X		T	T						-1715 *DdeI*

*Cambridge reference sequence (Anderson *et al.* 1981)

MtDNA has been used to investigate questions concerning the peopling of the New World. Although most researchers agree with an Asian origin for Native American populations, there is some disagreement over the timing of the initial migration(s) into the New World, the number of migrations involved, and the Asian source or sources of this (these) migration(s). Early studies using mtDNA RFLPs to date the first entry of humans into the New World produced dates ranging from 35,000 to 20,000 cal yr BP for haplogroups A, C, and D (Torroni *et al*. 1992, Torroni *et al*. 1994, Schurr 2004) and 17,000 to 13,000 cal yr BP for haplogroup B (Brown *et al*. 1998). Recent studies, based on mtDNA sequences, have produced more refined dates of 20,000 to 14,000 cal yr BP (Schurr 2004, Silva *et al*. 2002), which overlaps with dates proposed by the pre-Clovis archaeological model. According to this model, humans first entered the New World from Siberia around 14,000 BP or earlier (Dillehay 2000, Dixon 1999). This date is in contrast to the Clovis-first model that states the first Americans arrived shortly before 11,500 years BP, spreading their

biface and blade technology across North America between 11,200 and 10,900 BP (Bonnichsen and Turnmire 1992).

Discussions concerning the number of migrations into the Americas often cite a model proposed by Greenberg *et al*. (1986) that states there were three major migrations of humans into the New World from Siberia which correspond with three major language groupings. The earliest of these is represented by the Amerindian speakers who covered a vast geographic range including South America, and spoke a diverse set of languages. The second migration brought the Na-Dene speakers of interior Alaska, Canada, and the Northwest coast, and the final migration was that of the Eskimo-Aleut speakers, who occupy peripheral regions of the Americas and coastal Siberia. This model is criticized by linguists for lumping many diverse languages together into the Amerindian category, and the current mtDNA data do not appear to support it. Although some mtDNA studies have proposed three migrations, they do not correspond with Greenberg *et al*.'s classification. For example, Torroni *et al*. (1994) suggested there were three migrations: the first two contributed to the diverse Amerindian populations, and the third brought the Na-Dene and Eskimo-Aleuts. Other studies have suggested there were two migrations, the first carrying mtDNA haplogroups A, C, and D, which today are widespread in the Americas and are genetically diverse, and the second carrying haplogroup B, which is mainly absent from Na-Dene and Eskimo-Aleuts (Torroni *et al*. 1993, 1992, Schurr and Wallace 1999). This was based on the assumption that haplogroup B was less diverse, and therefore younger than the other haplogroups, which no longer appears to be the case

(Schurr 2004). It has also been proposed that the fifth haplogroup, X, may represent a separate migration from that carrying A-D (Torroni *et al*. 1993, Smith *et al*. 1999). It is also possible that there were several migrations into the Americas that all carried haplogroups A-D and which took place at different times (Schurr 2004). A single migration model is supported by a number of studies (Merriwether *et al*. 1995, Forster *et al*. 1996, Kolman *et al*. 1996) and appears to be gaining popularity among researchers (Silva *et al*. 2002, Jobling *et al*. 2004), although currently there is no consensus.

The molecular data have also been used to pinpoint Old World source populations for the New World colonizers. MtDNA haplogroups A, C, and D are fairly common in Siberia and throughout much of Asia. However, mtDNA haplogroup B is rare in Siberia, but is present in Southeast Asian populations. All four of these New World haplogroups are present in Mongolia, making it a possible source location (Merriwether *et al*. 1996, Kolman *et al*. 1996). Haplogroup X appears to be absent from most of Asia, although it has been described in the Altai populations of southern Siberia (Derenko *et al*. 2001).

Y Chromosome DNA Markers

Anthropologists use Y chromosome DNA markers as the paternal compliment to mtDNA. The Y chromosome (Figure 2) is passed exclusively from a father to his sons, and because the majority of it (~95%) does not recombine, it can be used to trace paternal lineages. In males, recombination between sex chromosomes takes place only at the very tips, at the pseudoautosomal regions (PAR1 and PAR2). The

non-recombinant region is referred to as the NRY, or MSY for the male-specific region. Approximately one half to two-thirds of the Y chromosome is composed of heterochromatin, a type of chromosomal material that is very condensed and genetically inactive, and that contains highly repetitive sequences. The genetically

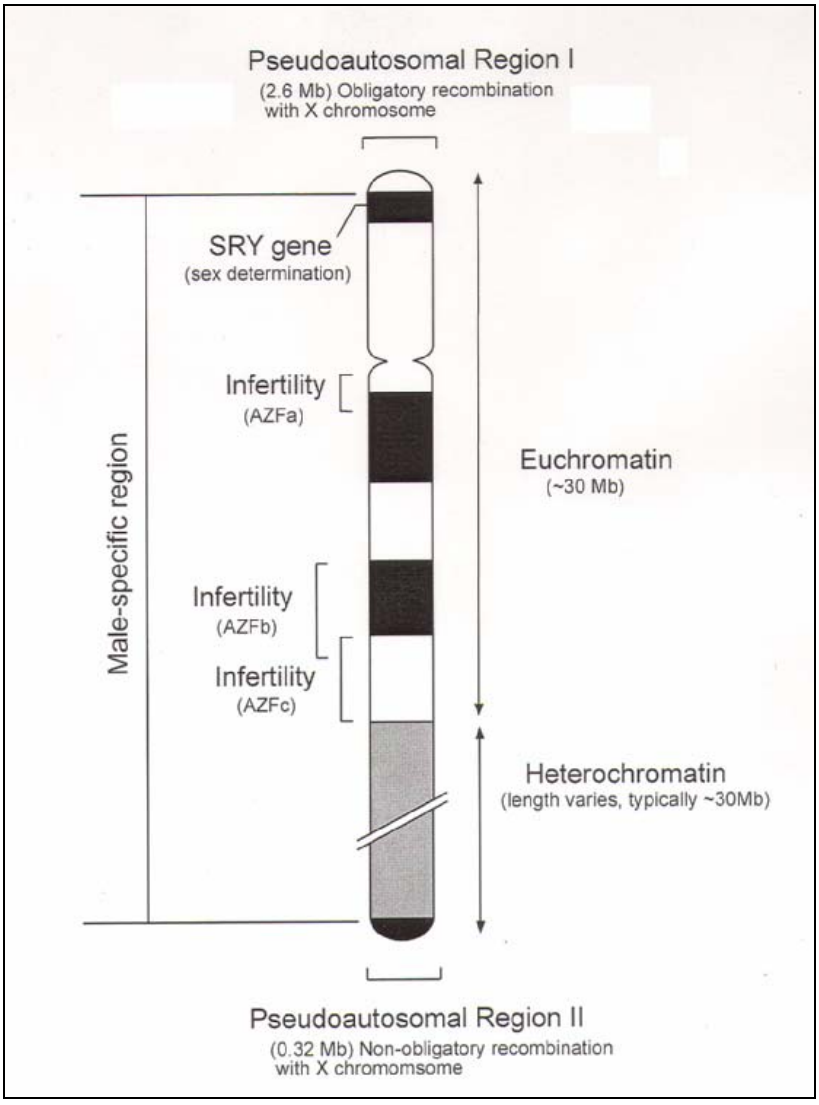

Figure 2 Y chromosome molecule (Rubicz et al. 2006)

active part of the Y, or euchromatin, contains the SRY (sex-determining region of the Y) gene which produces a transcription factor that turns on other genes involved in the development of the male testes from unspecified gonads (Graves 2002), and the AZFa, AZFb, and AZFc regions that have genes vital for sperm development. The MSY does not contain genes necessary for survival, since these are not shared with the X chromosome. In comparison to other nuclear DNA, it has been suggested the Y DNA mutates at a faster rate because it passes exclusively through the male germ-line, which undergoes a greater number of genome replications than the female germ-line, and therefore presumably accumulates mutations more quickly (Miyata *et al.* 1987, Hughes *et al*. 2005). Due to small effective population size (N_e) and male mating strategies, the Y is particularly vulnerable to genetic drift, which may contribute to geographic specificity of these markers (Seielstad *et al*. 1998). At the global level, male (Y DNA) and female (mtDNA) markers display the same level of diversity (Wilder *et al*. 2003).

The Y chromosomal polymorphisms most widely used in anthropological genetics studies include STRs and slowly-evolving biallelic markers. The biallelic markers include SNPs and insertion/deletion events (indels), which are believed to have occurred only once in humans, and are sometimes referred to as unique mutational events (UMEs). Biallelic markers can be used to construct Y-chromosome haplogroups (paternal lineages), while the STRs can be used to characterize the diversity within haplogroups and aid in the resolution of phylogenies, similar to the use of mtDNA markers (Figure 3).

The majority of Native American Y chromosomes belong to haplogroup Q (Hammer and Zegura 2001). Within this haplogroup, the American-specific Q-M3 lineages (also designated Q3) are present in all Native American populations, and are clinally distributed, with the highest frequency in South America (Schurr and Sherry 2004, Lell *et al*. 2002, Karafet *et al*. 1999). Q3 is hypothesized to have originated

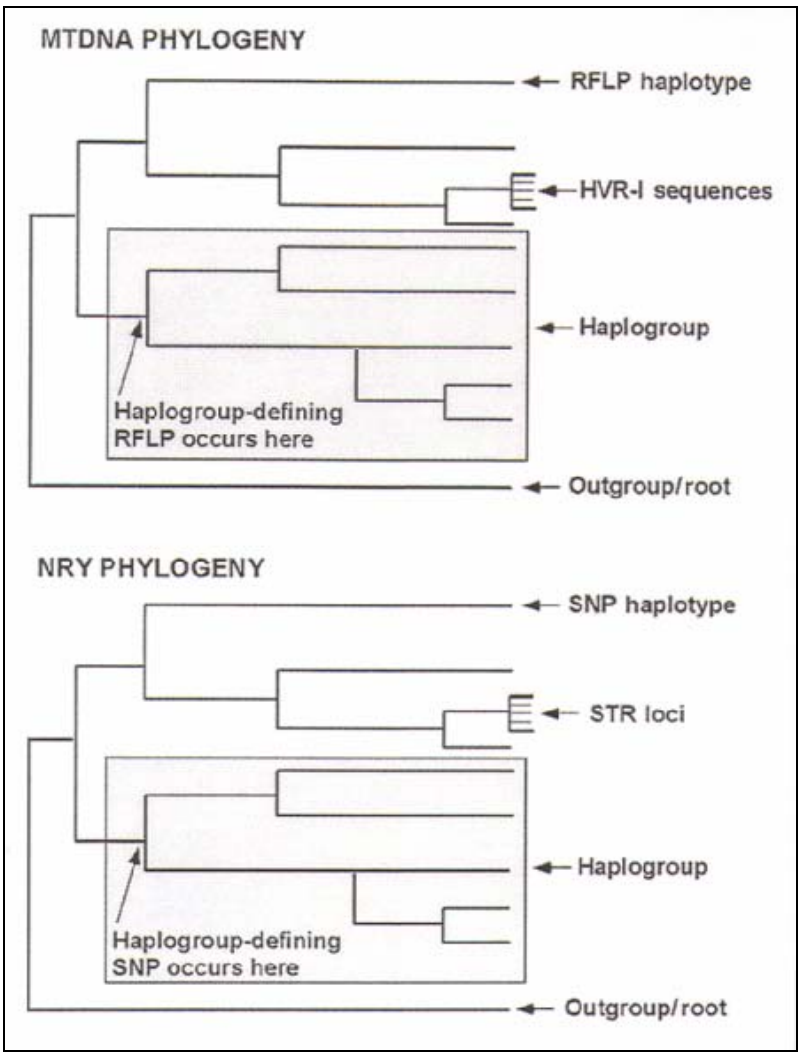

Figure 3 MtDNA and NRY (MSY) phylogenies (Schurr 2004)

either within the Americas or Beringia. P-M45 is another widely-distributed Y lineage present among Native Americans (comprising ~ 30% of the Y haplotypes), from which the Q-3 lineages appear to be derived (Schurr and Sherry 2004). Other Y lineages present in Native American populations include R1a1-M17, F-M89, and C-M130.

Similar to the mtDNA data, the Y chromosome data have been applied to questions concerning the peopling of the Americas including the timing of entry, number of migrations, and Asian source(s) for New World populations. The American-specific Q3 lineage has been dated at ~13,800 cal yr BP using SNP data, and 30,000 to 7,600 cal yr BP with STR data (Schurr 2004, Forster *et al*. 2000, Karafet *et al*. 1999, Underhill *et al*. 1996). Using a SNP called the Q-M242 marker that appears to be associated with human entry into the New World, Seielstad *et al*. (2003) and Bortolini *et al*. (2003) estimated its age at 18,000 to 15,000 BP, which they claim is a more precise measurement. The Y chromosome dates compliment those obtained by the mtDNA data, and lend additional support for a pre-Clovis archaeological peopling model.

The Y chromosome data are interpreted as representing either a single migration, or two separate migrations. Of the six major Y haplogroups shared between Native American and Siberian populations, DE-M1, Q-M3, R1a1-M17, P-M45, N3-M46, and C-M30, only two, P-M45 and Q-M3, appear to have been part of the first settlement of the Americas (Schurr 2004). Q-M3 is the most frequent American paternal lineage, it is widely distributed, and it is directly descended from

P-M45. P-M45 has an even broader distribution throughout the New World. It has been proposed that these two lineages were present in the first migration. The other lineages may have been part of a second expansion from Beringia (which includes Northeastern Siberia and Alaska), or may be the result of later admixture. Thus, the Y results appear similar to those based on mtDNA, neither of which support the three migrations model proposed by Greenberg *et al*. (1986) using linguistic data.

Y markers appear to derive from two different sources: P-M45a markers from central Siberia, and P-M45b markers from northeastern Siberia. Lineages derived from P-M45a, include: Q* and Q3. Those derived from P-M45b include: C and R1. While both the mtDNA and Y DNA trace a possible source population to central Siberia, the Y data differ in that they trace a second potential source population to Northeastern Siberia or Beringia, while the mtDNA may have a secondary source population in Mongolia.

Population Background

Archaeologists believe the Aleuts settled the Aleutian archipelago sometime after 12,000 BP, when this area became habiTable after the last ice age (see Figure 4). Questions concerning the manner in which the islands were settled, whether there was population continuity or replacement, if there was regional differentiation, and if there was contact between people of the Aleutians and outside groups is discussed in this section. Also presented is the linguistic, morphological, and genetic evidence available for this population, including their relationship to other Native Americans and North Asians, and their differentiation along this 1200 mile chain of islands, prior

to contact with Russians in the eighteenth century (Woodbury 1984, McCartney 1971, Rubicz 2001). The Commander and Pribilof Islands, located to the west and north of the Aleutians, respectively, were uninhabited during prehistoric times. Aleut communities were established in these locations by Russians, for the purpose of provisioning expeditions to the Aleutian region, and hunting fur bearing animals.

Archaeology and Prehistory

The Aleutian Islands lie to the south of Beringia and stretch from the Alaska Peninsula toward Kamchatka. The eastern-most Aleutians were at one time incorporated into the southeastern terminus of the Bering Land Bridge, which

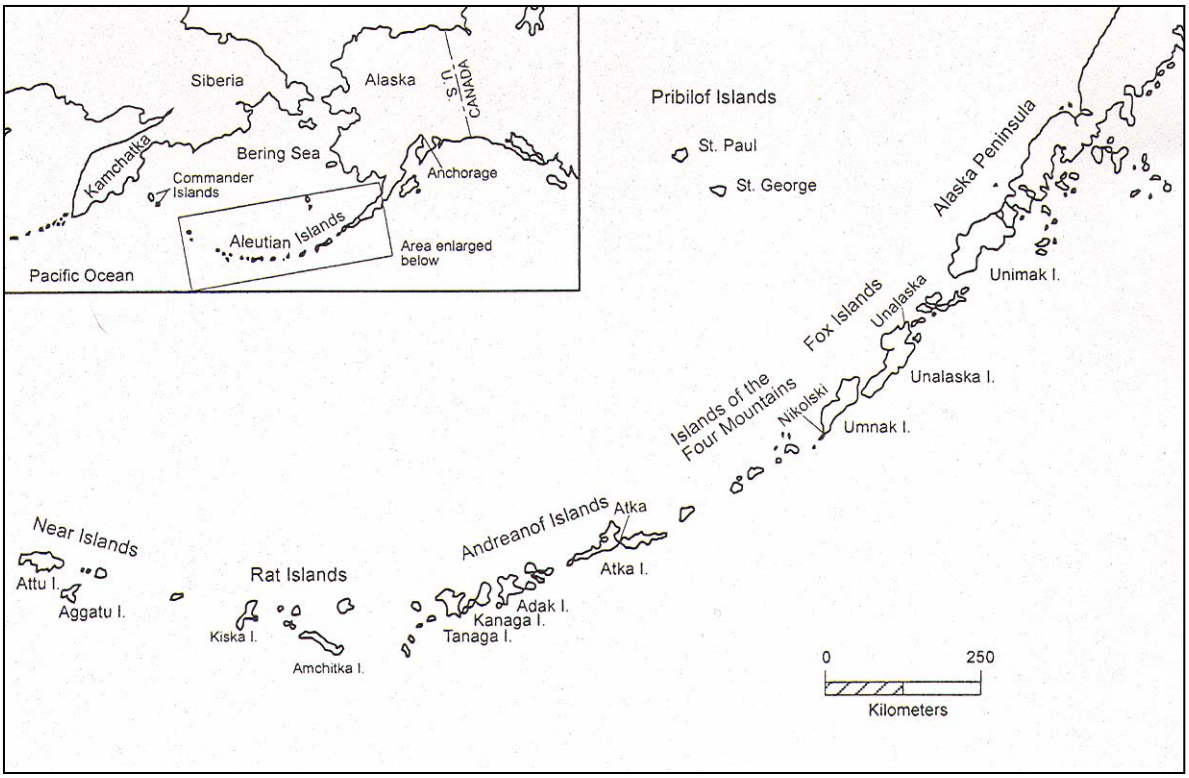

Figure 4 Map of the Aleutian Islands (Rubicz et al. 2003)

connected Alaska and Siberia most recently between 25,000 and 14,000 years ago (or perhaps later) and is thought to be a likely route for the earliest human migrations into the New World (Hopkins 1982, Elias *et al*. 1996, Hoffecker *et al*. 1993). Given their location between the continents of Asia and America, and the presence of archaeological sites in the island chain dating back nearly 9,000 years, the Aleutian Islands and their inhabitants play an important role in our understanding of human prehistory of the New World.

The Aleutians are a chain of volcanic islands that were formed by the subduction of the North Pacific plate beneath the North American plate (Black 1980). The area experiences volcanic activity, particularly in the east, where there is active underthrusting. As a result, the eastern islands tend to be larger and closer together than those in the west. Uplifting, caused by isostatic rebound from glacial unloading after the last ice age, as well as plate tectonics, has resulted in some coastlines rising between one and 180 meters above the modern sea level (Black 1966). Other coastal areas have been submerged by the rising sea, which did not stabilize at its current level until about 5,000 years ago. Because of the variable effect of these different processes throughout the region, each island must be considered to have its own unique geological history (Jordan 2001).

The location of the Aleutian Islands between the Bering Sea and Pacific Ocean allows them to maintain moderate year-round temperatures and stay free of pack ice during the winter. Adverse weather conditions, including heavy fog, rain,

sleet, snow, and hurricane-force winds are common (Lantis 1984). The islands are devoid of trees and many other terrestrial resources, and so their inhabitants have traditionally relied on the sea for subsistence. An upwelling of nutritious ocean water from the Aleutian trench supports a rich marine biomass, including invertebrates, fish, and sea mammals (Black 1976). Villages in the Aleutians were typically located on the north shores facing the Bering Sea where marine mammal hunting and fishing resources were more plentiful than the Pacific (Lantis 1984). Aleut men are said to have been excellent hunters, which they accomplished from their baidarkas (sea kayaks made from sea lion skin, driftwood and whalebone (Dyson 2000)). According to Coltrain *et al*. (2006) 95% of the Aleut diet was marine, based on values obtained from sTable carbon and nitrogen isotope analyses. Their dwellings were semi-subterranean 'barabaras' similar to those of North Asian populations.

Settlement of the Aleutian Islands

At the time of Russian maritime explorations of the North Pacific and Bering Sea in the early 1700s, it was believed the Aleutian Islands were settled by an expansion of peoples from the Kamchatka Peninsula (Laughlin 1951, Liapunova 1996). These early migrants are thought to have traveled by sea to Attu, the western-most Island in the Aleutian archipelago, and then spread east throughout the rest of the chain. Today, Russian and Japanese researchers continue to view the Aleutians as an extension of Asia, believing they were peopled from both the west and east, and that there were multiple migration events occurring at various times (Black 1983, Arutinov and Sergeev 1975, Dikov 1965, 1979). American scholars, on the other

hand, believe the Aleutians were settled by a migration from the east (Harper and Laughlin 1982, Hrdlička 1945, Jochelson 1925, Laughlin 1980). Laughlin (1980) proposed that an ancestral population crossed the Bering Land Bridge and after reaching Alaska, split into the Eskimos, who moved north, and the Aleuts, who moved southeast into the Aleutians.

The earliest archaeological sites in the Aleutian Islands are in the eastern part of the region, which supports the theory of a peopling event from Alaska rather than Kamchatka. These are the Anangula Blade, Russian Spruce, and Oiled Blade sites, dating between 8,500 and 7,000 rcybp.

The Anangula Blade Site, dated at 8,500 rcybp, is the oldest known Aleutian site. It is located on the small island of Anangula, across from Nikolski village on Umnak Island, in the Fox Islands group (McCartney and Turner 1966). Stone tools at this location were exposed in blowouts on top of a bluff 17 meters above the modern sea level (Laughlin and Marsh 1951). Anangula people probably had a maritime economy, based on the coastal location of the site and the resources available in the region today (Aigner 1976). They may have hunted sea mammals and birds, practiced deep sea fishing from boats, collected invertebrates and algae from coastal areas, and gathered roots and hunted terrestrial mammals. Extensive archaeological work at Anangula has recovered over 50,000 stone artifacts, making it one of the largest early Holocene assemblages in Alaska (McCartney and Veltre 1996, Hatfield 2002). Represented at the site are the remains of a unifacial blade and core industry, which primarily consists of knives, end scrapers, and transverse burins, but also includes

abraders, stone bowls, carved stone lamps, ochre grinders, fishing line weights, and small incised stones (Dumond 1987, McCartney and Veltre 1996). Although a large number of artifacts were recovered, there is only a thin cultural layer (less than 30 cm thick), which suggests humans occupied this location for a short time (McCartney and Turner 1966, Aigner and Fullem 1976). Abandonment of the site appears to have been in response to heavy ash fall from nearby volcanic activity, which choked the local source of drinking water (McCartney and Turner 1966, Black 1975).

The two other early Aleutian sites, Russian Spruce and Oiled Blade, are located on Hog Island near Unalaska Island, 200 km away from Anangula. These sites are contemporaneous with the Anangula Blade site, and are dated to 8000 BP. The Russian Spruce site, a short-term occupation of blade-making people, is located on ancient beach terraces 23 meters above the modern sea level (Dumond and Knecht 2001). The site was excavated from 1997-1999, during which time 624 artifacts were recovered. These artifacts are in many ways similar to those found at the Anangula Blade site, including cores prepared by the same procedures, and the absence of bifaces.

The Oiled Blade site is located 200 meters southeast of the Russian Spruce site, and 35 meters above the current sea level (Knecht and Davis 2001). During the excavation of this site in 2001 approximately 800 artifacts were recovered. Although the content of the Oiled Blade and Russian Spruce sites is similar, there are a few differences. The stratigraphy of Oiled Blade is deeper and more complex, and fewer microblades and more burins and burin spalls were recovered. Grooved net sinkers

and an oil lamp were also found at the site. Both sites are topped by a pyroclastic flow from nearby Mt. Makushin, which would have destroyed all life in the area. However, it is not clear if the sites were occupied up until the occurrence of this catastrophic event.

The earliest archaeological evidence in the central Aleutians is over 6,000 years old and comes from Adak in the Andreanof Island group. The ADK-171 site is located 20 meters above the modern sea level, on a terrace overlooking the lagoon (O'Leary 2001). Excavations identified a cultural layer dated at 6000-4000 BP, which contained the shell and bone remains of cockles, mussels, clams, sea birds, bird eggs, sea mammals (including sea otters), fish, and sea urchins. Three tools, thought to be associated with this layer were also recovered. These included two small stemmed points made on flakes, with unifacial retouch, and a small unifacial scraper. The other early Clam Lagoon site (ADK-012/013) was dated at 4,500 rcybp and consists of a house depression and a few flakes and tools (Hatfield 2002, O'Leary 2001).

The oldest sites in the western Aleutians are located on Amchitka in the Rat Island group, and are dated to 4000 BP. Excavations have recovered elements of a bifacial and unpatterned flake core technology, which includes burins, burin spalls, projectile points, scrapers, gravers, choppers, and abraders (Hatfield 2002). Bone and microblade technologies were not present. The limited archaeological work that has been done in the Near Islands, the western-most Aleutian Island group, indicates that those sites are even younger. Shemya Island contains the oldest site, around 3000 BP. This is the ATU-061 site, excavated in 1990 and 1994 (Lefevre *et al*. 2001). It is

located 100 meters away from the current shoreline, near a stream draining a lake. The site contains two cultural units, an earlier midden, into which a later house feature was apparently constructed. Faunal and artifact samples were collected for analysis, but have not yet been studied. The Cairn Creek site (ATU-193) provides the oldest dates for Attu, the Aleutian Island nearest to Asia. Dates for this site, which include house features and storage pits, are around 2000 BP (Corbett *et al*. 2001, Lefevre *et al*. 2001).

In addition to the relatively late dates associated with sites in the western Aleutians as compared with those in the eastern part of the region, which supports the theory of a peopling event from the east, there is no evidence for a prehistoric occupation of the Commander Islands. The Commander Islands, Bering and Medny, would have been a likely stopover for people migrating into the western Aleutians from Kamchatka. However, Hrdlička's brief survey of the islands did not produce any archaeological sites or artifacts (Hrdlička 1945). He also noted the presence of the Stellar Sea Cow, which he took as further evidence they were uninhabited. It is likely that had the Commanders been occupied, the local population would have hunted these animals to extinction, as they did in the Aleutians.

Replacement or Continuity in the Aleutians

The question of cultural continuity in the Aleutian Islands was first raised by Hrdlička (1945). During his archaeological investigations of the islands from 1936-1938, he noted morphological differences among the past inhabitants of the region, and suggested there had been a population replacement. He was, however, unable to

identify any cultural changes that would mark the arrival of later migrants. Laughlin (1975) strongly argued for both physical and cultural continuity in the Aleutians, and suggested the inhabitants of the 8,000 year old Anangula site had later occupied the nearby Chaluka site, dated at 4,000 BP. His argument was criticized by investigators who pointed out substantial technological differences between Anangula and all later sites, which they believed supported a model for cultural discontinuity (McCartney 1984).

Recent archaeological investigations in the eastern islands have stressed cultural continuity in the Aleutians. Excavations at the Margaret Bay site, near Unalaska Island, appear to bridge the gap between Anangula and later sites. The 'transitional culture' of Margaret Bay contains both blades, which are characteristic of the earliest Aleutian sites, and the bifaces that are present in later assemblages (Knecht and Davis 2001). Margaret Bay is a large site, with abundant archaeological features and artifacts representing an occupation that spans more than 3,000 years (Knecht *et al*. 2001). Carbon-14 dates for this site fall between 3110 ± 60 BP and 5470 ± 140 BP, which translate to approximately 3000-6700 calibrated years BP. Over 13,000 artifacts have been catalogued for this site, including both bone and stone tools. Several house remains and numerous shellfish, fish, bird and mammal bones have also been described.

Based on the addition of material from Margaret Bay and other recently excavated sites to that of existing collections, Knecht and Davis (2001) were able to

construct a prehistoric sequence for the eastern Aleutians that stresses cultural continuity (Table 3).

Table 3 Newly defined prehistoric phases in Eastern Aleutian prehistory (adapted from Knecht and Davis 2001)

Phase	Approximate Chronology	Unalaska Sites	Umnak Sites	Diagnostic Artifacts and Features
Early Anangula	9000-7000 BP	Russian Spruce site, Oiled Blade site	Anangula Blade site	Abundant blades, unifacial tools, transverse burins, large end scrapers, Grooved cobble sinkers, ochre grinders, stone bowls, oil lamps. Tent-like houses on shallow depressions?
Late Anangula	7000-4000 BP	Margaret Bay (levels 4,5) Agnes Beach (lower level) Airport site, Powerhouse site Cahn site	Sandy Beach Bay, Idaliuk Bay, Anangula Village	Abundant blades, stemmed points, bilateral-barbed harpoons with line guards, first bifacial tools. Shallow semisubterranean houses
Margaret Bay	4000-3000 BP	Margaret Bay (levels 2,3) Amaknak Bridge Tanaxtaxak (basal level) Agnes Beach (upper level)	Chaluka (basal level)	Blades, ASTt-like tools, stone bowls, plummets, angle and polished burins. First appearance of labarets, unilateral barbs on harpoons, bone socket pieces, net sinkers, exotic lithics. Ovoid-round stone-walled houses
Amaknak	3000-10000 BP	Summer Bay Cahn's site' D' Amaknak	Chaluka (middle levels)	Appearance of stemmed, notched lithics, elaborate barbing on bone, toggling hunting implements Harpoons, asymmetrical knives, spall scrapers, umqan. Rectangular houses?
Late Aleutian	1000-200 BP	Tanaxtaxak Eider Point Reese Bay Bishop's House	Chaluka (upper levels)	Abundant ground slate, ulus, limited chipped stone inventory, multiple-room and long houses, Fortified refuge rocks

Regional Differentiation

According to McCartney (1971), there appear to be regional cultural differences among the archaeological assemblages recovered from the Aleutian Islands, with greater similarity between the eastern and central island groups, and relative isolation of the Near Islands at the western end of the archipelago. Inhabitants of the Near Islands were separated from their closest neighbors, the Rat Islanders to the east, by the largest inter-island distance of the entire chain. This geographic isolation apparently resulted in the unique character of Attu, Agattu, and Shemya artifacts. Within the Near Island group, uniformity of technology and artifact styles suggests sustained contact among the region's inhabitants. Social contact with outsiders is thought to have been limited, coming primarily from the Rat Islands. Contact with people from other areas was probably rare and culturally insignificant.

The Near Island archaeological assemblages, according to McCartney (1971), differ from those of the central and eastern regions in terms of stylistic characteristics, rather than classes of bone and stone tool types. Intensive use of circle and dot decorations separates western bone artifacts from those of other regions. Additional western characteristics include unilaterally barbed simple dart points, asymmetrically pointed tangs, and indented blade lashing areas on harpoon tips. Obsidian artifacts are not present in the Near Island assemblages because none of the islands in the far west have volcanoes, and apparently trade with people from other areas with access to obsidian was restricted. There is an emphasis on projectile points in Near Islands stone tool types, as well as some scraper types, including long triangular section side

scrapers. Parallel flaking on projectile points and scrapers occurs in this region, but not elsewhere in the Aleutians, and is considered a stylistic embellishment, not a functional feature. These characteristics were tentatively described by McCartney (1971) as belonging to a Near Island Phase, although he stressed that further research was needed to clarify this.

Researchers have since noted a lack of blades and microblades in the central and western Aleutian Islands (Hatfield 2002). This suggests there may have been a social or environmental barrier west of the Fox Islands group, although their apparent absence may be due to insufficient sampling outside of the eastern Aleutians. Central and western sites are characterized by unpatterned flake, bifacial, and bone technologies present in the Late Anangula and Margaret Bay phases described by Knect and Davis (2001) for the eastern Aleutians. The earlier Early Anangula phase and later Amaknak and Late Aleutian phases do not appear to have spread westward.

Veltre and McCartney (2001) also describe regional variation in Aleutian settlement patterns. According to their study, long houses, which are defined as being at least 20 meters in length, were present only in the Islands of the Four Mountains and Fox Islands groups in the east. This appears to indicate the presence of social stratification in the more heavily populated east, where 75-80% of the Aleuts are said to have resided at the time of Russian contact (Dumond 2001, Venaiminov 1984).

Contact versus Isolation

There is disagreement over whether the Aleuts evolved in isolation after their initial peopling of the region, or if they had contact with other populations. The

former point of view has been predominantly supported by researchers from the Americas, while the latter is favored by Russian and Japanese scholars, who view the Aleutians as an extension of Asia, with significant contact from Northeast Asia (including the Kamchatka and Chukchi Peninsulas, and the Kurile Islands), China, and Japan (Black 1983). Although the Americanists reject the idea of any significant influence from groups to the west of the Aleutians, several agree that there was probably contact with populations in the east.

In the nineteenth century, a distinct linguistic boundary between Aleuts and Eskimos residing on the Alaska Peninsula was described as being located near 159° W. longitude (Dall 1870). Russian Church records indicated that Eskimo settlements extended south to Port Heiden on the Bering Sea side of the Peninsula, just east of that line (Dumond 2001). In 1871 French ethnographer Alphonse Pinart was informed the boundary on the Pacific side was traditionally located between Kupreanof Point and Kuiukta Bay. However, there has been some confusion over the placement of these boundaries because Russian administrators loosely used the term "Aleut" to describe both the Yupik Eskimo speakers of Kodiak and the Alaska Peninsula, and the Aleut speakers of the Aleutians and lower Alaska Peninsula (Dumond 1974). Archaeological investigations appear to support the placement of the Eskimo-Aleut boundary just to the east of Port Moller, based on the existence of three distinct cultural regions adjacent to one another on the Alaska Peninsula (Dumond 1992).

Although researchers such as Laughlin (1980) once believed the eastern boundary of the Aleut zone to be impenetrable, there is growing evidence for cultural

exchange at various times during the prehistory of the region. A tentative relationship between the Aleutian Late Anangula phase (~6000 BP) and the Pacific Eskimo Ocean Bay phase was suggested by Dumond (1987), and there may be a connection between the Aleutian Margaret Bay phase (~3000 BP) and the Eskimo Arctic Small Tool tradition (Dumond 2001, Knecht and Davis 2001).

From 1500 BP until the time of Russian contact in 1741, there is evidence for increased cultural interaction between Aleuts and peoples to the east, including Pacific Eskimos from the Alaska Peninsula and Kodiak Island, the Tlingit of the Pacific Northwest coast, and possibly the Tanaina of mainland Alaska (Holland 2001, Moss and Erlandson 1992, Dumond 1987). Cultural interactions may be identified archaeologically by unexpected differences in faunal remains, the presence of different tool styles, types and/or manufacturing techniques, and the use of exotic materials for stone or bone artifacts.

According to some investigators (Holland 2001, Moss and Erlandson 1992, McCartney 1984) interactions over the last 1500 years have resulted in a continuity of cultures located throughout the eastern Aleutians, Alaska Peninsula, Kodiak Island, and further east. These cultural interactions may have been the result of migration, hunting expeditions, trade, and warfare (Holland 2001).

In the western Aleutians, contact with Asian groups would have been more difficult due to a watery boundary extending 200 miles from Attu to the Commander Islands, the nearest landfall before reaching the Kamchatka Peninsula. There are, however, stories of Aleut travels to Asia, and Asian strandings in the Aleutians. The

first Russian explorers to reach the Near Islands, in 1747, reported that "foreigners" had visited the islands shortly before their arrival (Black 1983). According to descriptions, these "foreigners" may have been Chinese. There are numerous accounts of non-Russian shipwrecks in the region, including at least three that are known to have been Japanese. Korean amber was recovered from a burial in the eastern Aleutians, and the use of metal during pre-Russian contact times has been documented.

Commander Islands History and Demography

In 1741 the Commander Islands (Figure 5) were discovered by Vitus Bering and his crew on their return journey from the Americas. They had set out in June from the Kamchatka Peninsula with two ships, the *St. Peter*, commanded by Bering, and the *St. Paul*, under the command of Alexei Chirikof (Jochelson 1933). These two ships of Bering's second expedition became separated 16 days after their departure. Both ships made contact with the inhabitants of the Aleutian Islands before heading back to Siberia. Bering's crew suffered from scurvy, and 12 of his men died by the time they reached Bering Island in November. Their ship was tossed up on the shore of this island, and they were forced to stay for the winter. Bering Island was uninhabited at this time. The men dug underground pits in which to live and survived off the meat of sea mammals. The conditions were harsh, and in all, only 47 of 77 original crew members survived through January. Bering himself died in December and was buried on the island. George William Stellar, the naturalist and physician on the trip, wrote a detailed description of Bering Island, and noted there was another

Figure 5 Map of Bering and Medni Islands

island to the east (now called Medni or Copper Island). In August of 1742 the remaining crew members built a boat out of scraps from the *St. Peter* and sailed to Petropavlovsk (Torrey 1983). They brought with them sea otter pelts they had collected, which made them a small fortune and triggered a rush for furs in the Commander and Aleutian Islands.

Within two years of the return of Bering's crew, fur hunters from Siberia, called promyshlenniki, reached Bering Island (Vanstone 1984). The Commanders

became a provisioning station for expeditions leaving for the Aleutians in search of fur bearing animals, including sea otters, foxes, and fur seals (Laughlin 1980, Jochelson 1933). In 1781 merchants from eastern Siberia formed a company to exploit the American fur trade, which out-competed all other fur trading companies, and by 1799 became formally known as the Russian American Company (Vanstone 1984). All Aleuts fell under the administration of this company.

Between 1825 and 1828 Aleuts were forcibly relocated from the central and western Aleutian Islands to the Commanders to work for the Russian American Company. Aleut families from Atka were taken to Bering Island, and Medni was settled by Aleuts from Attu (Rychkov and Sheremetyeva 1972, Lantis 1984). The number of Aleuts on Bering in 1825 was only 45, but increased to 110 in 1826 as additional Aleuts were brought there by the Russians (Rychkov and Sheremetyeva 1972). This is in addition to the fifteen to thirty Russians who had settled in the Commanders prior to the Aleut relocations. The communities of Bering and Medni continued to grow, as individuals were also brought by Russians from the Pribilofs (originally settled by Aleuts from the eastern Aleutian community of Unalaska), Fox Islands, and Sitka (Rychkov and Sheremetyeva 1972).

The Aleut populations of Bering and Medni underwent admixture with Russians and probably with other non-Aleuts brought to the islands. The governor of Bering recommended that Russian men marry Aleut women, officially as a way of increasing fertility, but undoubtedly also as a means of controlling the population. Records indicate that a small number of Kodiak Eskimos and some “Creoles” (either

Russian-Tlingit or Russian-Aleuts) were also brought to the Commanders (Rychkov and Sheremetyeva 1972). Russia sold Alaska and the Aleutian Islands to the U.S. in 1867, effectively isolating the Commander Island Aleuts from their relatives to the east. In 1873 the Russian American Company turned over its control of the Kurile Islands to Japan, and brought 6 Ainus to Medni and 8 to Bering. At the same time 3 men and 6 women were brought from Kamchadal.

The Commander Island Aleut populations have fluctuated in size over the years, and have also undergone relocations. In 1879 there were 310 Bering Aleuts and 190 Medni Aleuts (Rychkov and Sheremetyeva 1972), see Table 4. The population reached an all time high in 1892, with a total size of 626 individuals (330 from Bering, and 296 from Medni). Afterwards there was a decrease in the population size, due to famine brought by the Civil War period, chronic alcoholism, and serious diseases such as tuberculosis. On two occasions Aleuts were moved to Kamchatka, and later to islands in the Sea of Okhotsk (Rychkov and Sheremetyeva 1972). In 1874 one hundred and seven Aleuts were brought to Kamchatka, but by 1888 only 26 had survived (mostly women and children), and they were returned to Medni Island. Then again in 1917 thirty-six people (young families with children) were taken to the Karagin Bay area, with similar consequences. Bering and Medni Aleuts became citizens of the Soviet Union in 1917, and with the socialist reorganizations watched their communities grow (due to an influx of individuals from other regions) (Liapunova 1975, Rychkov and Sheremetyeva 1972). At this time there was also an increase in mixed marriages among the Aleuts. The two Aleut communities were

Table 4 Commander Islands Aleut population sizes (after Rychkov and Sheremetyeva 1972)

Year	Total Aleuts	Bering Total	Bering Females	Bering Males	Medni Total	Medni Females	Medni Males
1890	619	345	175	170	274	141	133
1891	609	328	170	158	281	144	137
1892	626	330	166	164	296	149	147
1893	621	332	---	---	289	---	---
1894	612	326	163	163	286	139	147
1895	598	354	177	177	244	118	126
1896	605	356	180	176	249	118	131
1897	609	353	180	173	256	126	130
1898	612	342	---*	---	270	---	---
1899	546	292	---	---	254	---	---
1900	532	279	---	---	253	---	---
1901	530	278	---	---	252	---	---
1902	523	267	---	---	256	---	---
1903	509	255	---	---	254	---	---
1904	512	250	---	---	252	---	---
1905	514	263	---	---	251	---	---
1906	499	357	125	132	242	118	124
1907	520	275	140	135	245	119	126
1908	519	272	135	137	247	118	129
1909	501	267	135	132	234	115	19
1910	505	271	134	137	232	120	112
1917	449	262	132	133	187	---	---
1921	377	206	---	---	171	---	---
1922	381	210	---	---	171	---	---
1923	364	204	---	---	100	---	---
1957	---	---	---	---	87	45	42
1969	---	164	97	67	---	---	---

*--- indicates missing data

consolidated in 1969, when Medni Aleuts were relocated to the village of Nikolskye on Bering Island. Today the Aleuts on Bering Island consist of roughly 300 individuals, the majority of which appear to be admixed (personal observation).

Pribilof Islands History and Demography

Although the Pribilof Islands (see Figure 4, p.19)were unihabited at the time of Russian discovery, the Aleuts apparently knew of their existence. According to Aleut legend, Igadik, the son of an Unimak Island chief, was forced to run ahead of a storm in his kayak for several days (Torrey 1983, Veniaminov 1984). He finally came to an island whose beaches were covered with fur seals and their nursing pups. Igadik stayed on the island, which he named Amiq, for one year before returning to his home in the Aleutians. During his stay he collected fur pelts, and on clear days could see another island to the south.

The two islands, now called St. Paul and St. George, were discovered by Russians in 1786 and were named for their navigator Gerassium Pribylov (Lantis 1984, Torrey 1983). These islands are the summer residence and breeding grounds of the Northern fur seal, which was of great economic interest to the Russians, particularly after the decimation of sea otters in the Aleutian Islands. The Russians took Aleut hunters from Unalaska and Umnak Islands (in the eastern Aleutians) and established settlements in the Pribilofs (Black 1983) for the purpose of harvesting fur pelts. A fortune was made on the sale of these furs, by both the Russian treasury, and the fur trading companies that operated in the area. By 1796 the fur seal population was drastically reduced, although indiscriminate harvesting of their pelts continued until 1848, when protection was given to the female seals so that the herds could be replenished (Torrey 1983). In 1825 the village of St. Paul was established at its current location, and in 1830 the village of St. George was consolidated at its current

location. Both villages centered around Russian Orthodox churches, reflecting the importance of this religion in the region.

In 1867 Alaska was purchased from Russia by the US, and with it the Aleutian and Pribilof Islands. The Aleuts became US citizens, but they were still required to hunt fur seals, first under the Alaska Commercial Company, then for the Northern Commercial Company, and finally under the US Department of Fisheries. By 1874 the Pribilof Island Aleuts numbered 340 (222 on St. Paul, and 118 on St. George), see Table 6. Although the size of these communities has fluctuated over time, St. Paul has always remained the larger of the two.

With the invasion of the Aleutian Islands by the Japanese in 1942, and capture of the small community of Attu, the US government evacuated all remaining Aleuts to the Alaskan mainland. The Aleuts were kept separated by community, and placed in five different locations in Southeast Alaska. Four hundred and seventy-seven Pribilof Aleuts were taken to Funter Bay, west of Juneau (Lantis 1984). The Office of Indians Affairs was responsible for all of the relocated Aleut communities, except for those from the Pribilofs, which were under the charge of the Fish and Wildlife Department (Kohlhoff 1995). The conditions were poor for Aleuts at their new locations, with inadequate housing (Pribilof Island Aleuts were housed at an abandoned cannery and gold mining camp) and scarce food and fresh water. Even so, the Aleuts were forcibly detained in these internment camps for the duration of the war. In January of 1943 the US government allowed 151 Aleut men and school boys to return to the Pribilofs for the seal harvest, stating that seal oil would not gel as

easily in cold climates, and that fur seal coats would keep soldiers warm. In 1944 the Pribilovians were allowed to return home, and by June of 1945 all Aleuts were repatriated.

Once back in the Pribilofs, the Aleut people struggled for independence (Torrey 1983). In the 1940s and 1950s they put their efforts toward gaining economic and educational equality with the rest of Alaska. In the 1960s they established their own village-level governments, and finally in the 1970s they were given legal title to their land. As indicated in Table 5 (Lantis 1984) by 1970 the Pribilof Aleut population had grown to 640 individuals, a number of which (29) were non-Aleuts. Today there is an estimated population size of 500 for St. Paul, and 250 for St. George (personal observation). The influx of non-Aleuts appears to be a growing trend, as travel between the Pribilofs, Aleutian Islands, and Alaskan mainland has become more readily available. And with these increased population movements comes the higher likelihood of admixture in the Aleut population.

Table 5 Pribilof Islands Aleut population size in 1970 (Lantis 1984)

Year	Place	Native	Other	Total
1970	St. Paul	428	22	450
1970	St. George	156	7	163

Table 6 Pribilof Islands population 1872-1946 (unpublished document located in Museum of the Aleutians, Unalaska, AK)

Year	Total for Pribilofs	St. Paul	St. George
1872	---	217	---
1873	---	217	---
1874	340	222	118
1875	360	244	116

1876	353	260	93
1877	360	262	98
1878	373	278	95
1879	363	275	88
1880	357	265	92
1881	333	231	102
1882	322	219	103
1883	342	230	112
1884	348	237	111
1885	348	237	111
1886	330	219	111
1887	326	214	112
1888	337	218	119
1889	315	219	96
1890	282	193	89
1891	295	203	92
1892	279	190	89
1893	276	189	87
1894	278	188	90
1895	287	198	89
1896	288	198	90
1897	289	198	91
1898	283	190	93
1899	287	186	101
1900	290	186	104
1901	244	157	87
1902	243	157	86
1903	249	160	89
1904	253	161	92
1905	257	164	93
1906	262	168	94
1907	263	170	93
1908	267	177	90
1909	285	190	95
1910	295	198	97
1911	289	190	99
1912	301	195	106
1913	304	194	110
1914	309	192	117
1915	314	193	121
1916	311	192	119
1917	316	193	123

1918	322	199	123
1919	310	188	122
1920	316	188	128
1921	310	188	122
1922	321	193	128
1923	315	181	134
1924	320	179	141
1925	322	184	138
1926	344	202	142
1927	332	189	143
1928	352	205	147
1929	359	215	144
1930	364	222	142
1931	376	232	144
1932	385	232	153
1933	387	230	157
1934	392	234	158
1935	388	227	161
1936	398	239	159
1937	419	256	163
1938	422	253	169
1939	443	267	176
1940	446	261	185
1942	467	285	182
1942	480	295	185
1943	420	241	179
1944	430	254	176
1945	427	257	170
1946	490	314	176

Language

The Aleut language is one of 10 languages belonging to the Eskimo-Aleut language family, today spoken by an estimated 720 individuals residing in Alaska and the Commander Islands (Ruhlen 1991). The Aleut and Eskimo branches are thought to have at one time belonged to a single ancestral Proto-Eskimo-Aleut or Eskaleut language, from which they are estimated to have diverged between 5,000 and 11,000

years ago (Greenberg *et al*. 1986). The Eskimo branch is further subdivided into the Yupik branch, spoken in northeastern Siberia and along the Pacific coast of Alaska south of Norton Sound, and the Inuit-Inupiaq branch, spoken to the north of Norton Sound and across the arctic shores of Alaska and Canada all the way to Labrador and Greenland (Woodbury 1984). Aleut is subdivided into two mutually intelligible dialects, eastern and western (Woodbury 1984). The eastern dialect is spoken from Nikolski eastward, including the Pribilof Island communities of St. George and St. Paul. The western dialect is further subdivided into two subdialects, Atkan which is spoken in the central Aleutians, and Attuan, once spoken in the now depopulated western-most Aleutians (Bergsland 1959). Both western subdialects are also spoken in the Commander Islands (Bergsland 2001). According to Alice Petrivelli, there were at one time up to seven different subdialects of the Aleut language spoken throughout the archipelago (Petrivelli, personal communication).

Morphology

Morphologically Aleuts are similar to Eskimos and other peoples from the Bering Sea region. As a cold-climate adaptation, they share medium to sub-medium stature with tall relative sitting heights and small hands and feet. According to Laughlin (1980) Aleuts and Eskimos are distinct from other populations in having a very broad, low ascending mandibular ramus. Both have sinodont dentition, characterized by higher frequencies of incisor shoveling, single-rooted upper first premolars, and 3-rooted lower first molars (Powell 1993, Turner 1985). Worldwide,

Aleuts have the highest frequency of 3-rooted lower first premolars, a trait that is sexually dimorphic, being more prevalent among males, only in this population (Szathmary and Ossenberg 1978, Turner 1985). Although dental analyses group Aleuts with Eskimos and Northeast Asians (Turner 1985, Powell 1993), analyses of cranial traits suggest a closer relationship to American Indians rather than Eskimo or Siberian populations (Szathmary and Ossenberg 1978, Ossenberg 1992, Ousley 1995).

Based on observations of differences in cranial morphology, Hrdlička (1945) suggested there had been a population replacement in the Aleutians. He described the earlier "pre-Aleut" population as having dolicocephalic (long and narrow) skulls, and the later "Aleut" population as having brachycephalic (short and round) skulls. According to Hrdlička, these two populations represented separate migrations into the Aleutian Islands from the east. The "pre-Aleuts" who appeared to be related to the Sioux Indians, were replaced by the "Aleuts" who resembled the Siberian Tungus. Despite the apparent physical discontinuity, Hrdlička was unable to identify cultural change associated with the arrival of the later migrants. This was in agreement with earlier cultural investigations by Dall (1877) and Jochelson (1925).

Laughlin and Marsh (1951) re-examined the Aleutian material, determined the physical remains belonged to Eskimoid stock, and proposed the similarity between the "pre-Aleuts" and "Aleuts" represented the evolution of the former into the latter group. They replaced the term "pre-Aleut" with "Paleo-Aleut" and "Aleut" with "Neo-Aleut" to emphasize continuity between the inhabitants. Further investigation

by Laughlin indicated the two head morphologies were still present among the living Aleuts (Laughlin 1980). People in the western Aleutians retained the dolicocephalic skull shape, while those in the central and eastern islands possessed crania that had evolved into the brachycephalic form. Laughlin explained these differences as the evolution of the brachycephalic trait in the more densely populated eastern part of the region, followed by its slow dispersal into the smaller communities to the west. By the time of Russian contact in mid-1700, this trait had not yet reached the western-most islands. According to Laughlin, there was both physical and cultural continuity in the Aleutian Islands after their original settlement of the region nearly 9,000 years ago.

This question of physical continuity in the Aleutians was explored by Coltrain *et al*. in a recent paper (2006). They obtained radiocarbon dates on 80 samples from individuals who had previously been categorized as either Paleo-Aleut or Neo-Aleut, from three locations: Chaluka, Kagamil, and Ship Rock, all in the Eastern Aleutians on or near Umnak Island. All but one of the Chaluka samples were Paleo-Aleut, and all of the Kagamil and all except two of the Ship Rock samples were Neo-Aleut. The samples sorted out by date, with those dated between 3,635 and 1,000 BP all belonging to the Paleo-Aleut group. The Neo-Aleuts first appeared on Umnak after this date, where they were fully contemporary with the Paleo-Aleuts. This falsifies the population replacement hypothesis proposed by Hrdlička. The Neo-Aleuts are suggested by Coltrain *et al*. (2006) to be a migration of closely related peoples from the east, who had a higher level of social complexity. These individuals are associated

with complex mortuary practices (they mummified their dead), appear to have relied more heavily on sea mammal hunting than the Paleo-Aleuts whose diet consisted of lower-trophic-level marine foods, and may be responsible for longhouses and refuge rocks which appear after 1,000 BP in eastern Aleutians. They likely lived along side the Paleo-Aleuts who remained in the region and continued to bury their dead as inhumations for nearly another thousand years.

Genetics

Classic genetic markers have been described for Aleuts residing in the Commander and Pribilof Islands, including blood groups and serum protein systems (Rychkov and Sheremetyeva 1972, Majumder *et al*. 1988). For the ABO blood group system, Aleuts have high frequencies of ABO*O and ABO*A alleles, and low frequencies of ABO*B. This is similar to Eskimo groups, and differs from North and South American Indian populations (Laughlin 1980). The Aleuts have high frequencies of MNS*Ms, moderate frequencies of MNS*MS and MNS*Ns, and low frequencies of MNS*NS, also similar to the Eskimos (Rychkov and Sheremetyeva 1972, Majumder *et al*. 1988). Aleuts, like other Native Americans, are characterized by an absence of Rh- phenotypes, and they have high frequencies of the cDE and CDe alleles. For the Diego blood group, Aleuts have a high frequency of the Di*A gene, which is unusual for North Americans, although it is characteristic of South American populations. They have nearly equal frequencies of the haptoglobin genes HPA*1 and HPA*2, similar to American Indian groups including the Haida, Apache,

and Assiniboin, and, like other Native American and Siberian populations, nearly 100% of the AL*A serum albumin gene (Szathmary and Ossenberg 1978).

Rychkov and Sheremetyeva (1972) compared gene frequency data for the ABO and MN blood groups of Bering and Medni Aleuts with those of Aleutian Aleuts from: 1) Attu-Atka; 2) Unalaska; and 3) the central Aleutian Islands. They found that both Medni and Bering Aleuts were genetically closest to Aleuts from Unalaska. This was unexpected, given that Medni was originally founded by Aleuts from Attu, and Bering by Aleuts from Atka. However, later relocations of Aleuts from other parts of the Aleutian chain and Pribilofs to the Commanders may have obscured earlier affinities. It is unfortunate that additional markers were not available for the Aleutian Aleuts for their analysis because frequencies from just two blood group systems is not very informative. Analysis of 31 classic genetic markers by Majumder *et al*. (1988) indicated the two Pribilof Island Aleut communities, St. Paul and St. George, were genetically very close, and that they differed considerably from the Kodiak Island Eskimo groups to which they were compared.

More broadly, analyses of classic genetic markers have variously grouped Aleuts with Eskimos, American Indians, or Siberians. Harper (1980) found the Aleuts be to closely related to Eskimos, Ousley (1995) grouped them with American Indian populations, while Szthmary and Ossenberg (1978) demonstrated their affinity to Chukchi and Asiatic Eskimos. Rychkov and Sheremetyeva (1972) concluded that genetically the Aleuts were closely related to both Native American and North Asian populations.

Molecular data currently available for the Aleuts consists of mtDNA RFLPs and HVS-I sequences (Rubicz 2001, Rubicz *et al*. 2003, Zlojutro *et al*. 2006). RFLP analysis demonstrates Aleut mtDNAs belong to two of the five New World founding haplogroups: they consist of 71.5% D and 28.5% A (Rubicz 2001, Rubicz *et al*. 2003). This haplogroup pattern is unusual among Native North Americans, where D is normally absent, and A is present in high frequencies. Aleuts shared control region sequences with other circumarctic populations, but lacked the Eskimo-specific 16265G mutation. Analysis of sequence data indicated the Aleuts were most closely related to the Chukchi and Siberian Eskimos, rather than to Native American or Kamchatkan populations. These molecular data support Laughlin's (1980) hypothesis for a peopling event of the Aleutian Islands from the East. Zlojutro *et al*. (2006) identified three star-like clusters of Aleut sequences through network analysis, corresponding to A3, A7, and D2 subhaplogroups, and proposed they represent two population expansions, the first at 19,900 BP (A3) and the second at 5,400 BP (A7 and D2). AMOVA analysis (Rubicz 2001) revealed population substructuring along the Aleutian chain, with significant genetic differences between the individuals tracing their maternal ancestry to western, central, and eastern Island groups.

Summary

This chapter provided an overview of background information on the Aleuts, including their pre-history, and the history of their recently founded communities in the Commander and Pribilof Islands. Archaeological evidence indicates the Aleutian

Islands were first peopled by a migration from the east approximately 9,000 years ago, which reached the central Aleutians by 6000 BP, and the western-most island of Attu by 2000 BP. The original settlers of this region are likely the ancestors of modern Aleuts, as there is currently no convincing evidence for a population replacement. Genetically, Aleuts are closest to the Chukchi and Siberian Eskimo populations of Kamchatka. Contact with populations outside the Aleutians appears to have occurred mainly in the east, with peoples from Kodiak, the Alaska Peninsula, and mainland Alaska. There is archaeological, linguistic, and genetic evidence for differentiation of Aleuts between eastern, central, and western regions of the island chain. Given this population subdivision, it is possible Aleuts residing in the historically established communities of Bering, St. Paul, and St. George differ genetically from their Aleutian Island relatives. Bering Aleuts originally came from Atka, in the Central Aleutians, and were later joined by Western (Attu) Aleuts from Medni. St. Paul and St. George were originally settled by Aleuts from the Eastern Aleutians. Molecular genetic markers can be used to address the question of founder effect in the establishment of these communities, as well as the impact of intergenerational drift (given that the communities have always remained small), and gene flow (records indicate there was considerable Russian admixture) on their evolution. Of particular use to this study are the Y chromosome DNA and mtDNA markers, tracing paternal and maternal lineages, that may also be used to investigate whether there are differences between male and female histories of the three communities.

CHAPTER THREE: MATERIALS AND METHODS

This chapter describes the sampling and analytical methods used in this study. DNA was extracted from blood and cheek cell samples and characterized for mitochondrial, Y chromosome, and autosomal markers including: restriction fragment length polymorphisms (RFLPs); HVS-I region sequencing; single nucleotide polymorphisms (SNPs); and short tandem repeats (STRs). In addition, classic genetic markers were taken from the literature. Admixture estimates and heterozygosity values were calculated, R-matrix analysis was performed, MDS plots were created, heterozygosity versus distance from the centroid (r_{ii}) was plotted, and networks and phylogenetic trees were constructed.

Sampling Methods

During the summers of 1999, 2000, and 2004, cheek cell samples (for DNA extraction and analysis) and questionnaires were collected by Dr. Michael Crawford, Rohina Rubicz, and Aleut elder Alice Petrivelli from 215 self-designated Aleut participants residing in the Alaskan communities of St. Paul, St. George, Atka, Nikolski, Unalaska, and Anchorage, (see Table 7). In the summer of 2001, Dr. Michael Crawford, Rohina Rubicz, and a Russian research team led by Dr. Victor Spitsyn, collected genealogical information and blood samples from 256 participants on Bering Island and the Kamchatka Peninsula in Siberia (Table 8). In addition to the Aleut and mixed Aleut participants of Bering Island, other Siberian participants were of Russian, Koryak, Even, and other ethnicities. These non-Aleut individuals provided comparative data for the study. Permissions for this study were granted by

the University of Kansas Advisory Committee on Human Experimentation (ACHE), the Aleut Corporation, the Aleutian/Pribilof Islands Association, the Russian Academy of Sciences, and the tribal counsels of each community. Participants signed informed consent statements (see Appendix A) and were provided with contact information for the researchers in case questions arise in the future. Sample sizes for Aleut communities were considered adequate given that, with the exception of Anchorage, they represent approximately 20 or more percent of the populations.

Table 7 Number of Aleut participants by community in Alaska

Anchorage	*Atka*	*Nikolski*	*St. George*	*St. Paul*	*Unalaska*	*Total*
29	20	17	34	68	47	215

Table 8 Number of Siberian participants (from Bering Island and Kamchatka)

Bering Aleut	*Mixed Aleut*	*Russian*	*Koryak*	*Even*	*Kamchatka Mixed*	*Other*	*Total*
35	41	30	63	21	49	17	256

Laboratory Methods

DNA Extraction

DNA from the buccal samples was extracted by three different methods. The 1999 samples, which were collected with OraSure swabs, were extracted in the laboratory using OraSure kits (Analytical Genetic Testing Center, Denver, CO) according to the manufacturer's instructions. After finding the first method yielded small quantities of DNA, the 2000 and 2004 samples were collected using sterile wooden applicators that were then rinsed in sterile TE and extracted in the laboratory

using a phenol-chloroform method. This consisted of an overnight digestion at 55ºC in 100µl of extraction cocktail per sample (5X STE, 25µl 10% SDS, 25µl proteinase K (20mg/ml), and 325µl ddH_2O). Next, 250µl cold (4ºC) 5M potassium acetate was added to precipitate the protein, which was pelleted and discarded. The remaining proteins and fats were extracted from the aqueous DNA phase with two phenol:chloroform:isoamyl alcohol (24:24:1) extraction steps. The DNA was precipitated with two volumes of cold 95% ethanol, pelleted and washed with 75% ethanol, air-dried, and resuspended using 50µl sterile 1X TE, pH 8.0, and stored at 4ºC. 3) A second set of samples was collected from the 2004 participants in order to test a Chelex DNA extraction method in the field. These were mouthwash samples obtained by having individuals swish 10ml of water around their mouths. Samples were poured into 15ml collection tubes and let stand for 15-20 minutes to allow the cells to settle on the bottom. A bulb pipette was used to transfer cells into two 2.0ml microcentrifuge tubes. The samples were pelleted in a microcentrifuge at full speed for five minutes. The aqueous phase was poured off and discarded, and 100µl of 10% Chelex resin was added to each tube and vortexed. The suspended samples were incubated in a heat block at 100ºC for 10 minutes, in order to break up the cells and release the DNA and proteins. The tubes were next placed on ice for three minutes, allowing the Chelex to bind with everything except for the DNA. Samples were spun down in the microcentrifuge at full speed for 5 minutes in order to pellet the Chelex-bound material. The aqueous phase, containing the DNA, was transferred to a clean 0.5ml tube and stored at 4ºC.

Blood samples collected from participants in Siberia in 2001 were kept refrigerated, and stored in a cooler with ice packs during transport, until their extraction at the University of Kansas Biological Anthropology Laboratory. DNA was extracted from the blood samples using a third method: the Super Quik-Gene extraction kit (University of Kansas). Whole blood samples were centrifuged at 2500rpm for 20 minutes. The buffy coat and approximately 1ml of the underlying red blood cells were transferred to a 15ml tube, which was then filled to 10ml with cold 1xRBC lysis buffer, and gently mixed for 15 minutes. Samples were centrifuged at 2500rpm for 20 minutes and the supernatant was decanted into a blood waste container. The pelleted white blood cells were rinsed with an additional 2-3ml of RBC lysis buffer, which was also discarded. Next, 1.5ml WBC lysis buffer was added to the samples and they were vortexed to break up the pellets. Samples were incubated in a 55ºC water bath for 30 minutes. After incubation 0.2ml of 10% SDS and 0.5ml protein precipitating reagent were added to each sample, which was then vigorously shaken by hand for 30 seconds and returned to the water bath for an additional 15 minutes. Samples were centrifuged at 2500rpm for 20 minutes, and the precipitated protein appeared as white pellets at the bottom of the tubes. The clear aqueous phase containing the DNA was transferred to 15ml tubes and mixed with two volumes of room temperature ethanol. Gentle inversion of the tubes caused the DNA to precipitate as stringy white fibers, which were transferred, using plastic inoculation loops, to 1.5ml tubes containing 100µl TE buffer. Samples were stored at 4ºC.

Mitochondrial DNA Analysis (RFLPs and Sequencing)

Mitochondrial DNA laboratory analysis consisted of restriction fragment length polymorphism (RFLP) analysis and sequencing of the first hypervariable segment (HVS-1) of the control region. For the RFLP analysis, regions of the mtDNA genome containing diagnostic restriction sites were amplified by PCR (polymerase chain reaction). PCR can potentially create millions of copies of a particular segment of DNA by heating double-stranded DNA to denature it, cooling it to allow specific primers to anneal to the target sequence, extending the primers using DNA polymerase in order to create a new, complementary strand of DNA, and then repeating the process many times. Ingredients used for the PCR reactions (per sample) consisted of: 2.0µl 10X PCR buffer; 1.2µl $MgCl_2$ (25 mM); 1.6µl dNTP (deoxynucleotide) mix (10mM); 0.1µl *Taq* polymerase (5U/µl); 0.6µl forward primer (10pmol/ µl); 0.6µl reverse primer (10pmol/ µl); 2.0µl to 5.0µl DNA dilution (for a final concentration of 50 to 100ng; and ddH_2O to bring the final reaction volume to 25µl. All PCR ingredients were purchased from Promega (Madison, WI), except for the primers which were synthesized by IDT (Coaralville, IA). The primers and annealing temperatures used in this study are listed in Table 9. Reactions were run in either a PE Applied Biosystems Gene Amp 2400 or 9700 according to the following thermal profile: an initial denaturation at 94ºC for one minute; and then 35 cycles of denaturing at 94ºC for 40 seconds, annealing for 30 seconds, and extension at 75ºC for 45 seconds; a final extension of 5 minutes at 75ºC, and a hold at 4ºC.

After amplification, the DNA was digested with restriction enzymes (see Table 9). These are enzymes produced by bacteria that recognize and cleave specific DNA sequences (typically four to six bases long) in a consistent pattern. The presence or absence of particular cut sites are used to define the mtDNA haplogroups (Table 9). Restriction digest reactions (per sample) consisted of: 2.0µl 10X buffer (provided by the manufacturer); 1.0µl 100X BSA (bovine serum albumin); 0.5µl restriction enzyme (New England Biolabs, Beverly, MA); 7.5µl PCR DNA; and 9.0µl ddH_2O for a total sample volume of 20µl. Samples were digested for ten to eighteen hours at 37ºC, and reactions were stopped by adding 5µl of 3X loading dye (Promega, Madison, WI). They were then electrophoresed on 3% NuSieve gels (ISC BioExpress, Kaysville, UT) made with 1X TBE and stained with ethidium bromide, at 97 volts for 2 hours. Electrophoresis sorts DNA fragments by length, with the shorter fragments migrating more rapidly toward the positive node (DNA is negatively charged). The DNA fragments were measured against a size standard (25bp DNA step ladder from Promega, Madison, WI). Gels were illuminated under UV light and photo documented.

Approximately 400 base pairs (np 16000 to 16400) of the mtDNA control region were sequenced on an automated capillary electrophoresis system using the Sanger dideoxy sequencing method (Sanger 1977). This consists of synthesizing DNA strands in a single direction, using a PCR cocktail containing fluorescently-labelled dideoxynucleotides (ddNTPs), with a different color for each of the four bases (A, G, T and C). Incorporation of the ddNTPs causes termination of a growing

DNA strand (due to lack of the 3′ hydroxyl group needed for attachment of the following nucleotide), resulting in DNA fragments of varying lengths that are end-labeled with the fluorescent markers. The fragments are separated out electrophoretically on an automated sequencer, and their labeled ddNTPs excite as they pass a stationary laser. The output is chromatogram data that are recorded by a computer.

Table 9 Primers for mtDNA RFLP and HVS-I sequencing analysis

Haplogroup	Primer Pair	Sequence (5′→ 3′)	AT*
A (+*Hae*III 663)	535FOR 725REV	CCCATACCCCGAACCAACC GGTGAACYCACYGGAAGGGG	57ºC
B (+*Hae*III 8250)	8149FOR 8366REV	ACCGGGGGTATACTAACGGT TTTCACTGTAAAGAGGGTTGTTGG	53ºC
C (-*Hinc*II 13259 & +*Alu*I 13262)	13172FOR 13383REV	GCTTAGGCCCTATCACCA GTTGTGGATGATGGACCC	51ºC
D (-*Alu*I 5176)	5151FOR 5481REV	CTACTACTATCTTCGCACCTG GTAGGAGTAGCGTGGTAAG	53ºC
G (+*Hae*II 4830 & +*Hha*I 4831)	8239FOR 8363REV	CCTTGAAATAGGGCCCGT CACTGTAAAGAGGTGTTGG	50ºC
H (-*Alu*I 7025)	6958FOR 7049REV	CCTGACTGGCATTGTATT TGTAAAACGACGGCCAGTTGATAG GACATAGTGGAAGT	58ºC
K (-*Hae*II 9052)	8931FOR 9102REV	ACCCCTTATCCCCATACTAGTTA TTACTAGAAGTGTGAAAACGTAGG	51ºC
U (+*Hinf*I 12308)	12216FOR 12338REV	CACAAGAACTGCTAACTCATGC ATTACTTTTATTTGGAGTTGCACCA AGATT	55ºC
HVS-I	15976 FOR 16422 REV 16401REV	CCACCATTAGCACCCAAAGCTAAG AATGATTTCACGGGAGGATGG TGATTTCACGGAGGATGGTG	55ºC

*AT = annealing temperature

DNA templates were created for the sequencing reaction using the PCR protocol and thermal profile previously mentioned (for RFLP analysis). All samples

were sequenced in both (forward and reverse) directions, with earlier samples using the 16422 REV primer, which was later replaced by the 16401 REV primer because it gave cleaner results. Primers and annealing temperatures for the sequencing templates are listed in Table 9. Reactions were purified using the QIAquick kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. 100µl Buffer PB was mixed with 25µl PCR product, placed in a spin column, and centrifuged at 13,000 rpm for one minute in order to bind the DNA to the positively charged filter. Each sample was washed with 750µl Buffer PE and centrifuged at 13,000 rpm for one minute. Spin columns were placed in clean tubes, and the DNA was eluted using 50µl ddH_2O per sample and centrifuging for one minute at 13,000 rpm.

The DNA templates were sequenced at two different locations: 1) Integrated DNA Technologies (IDT), Coralville, IA, under the direction of Dr. Ric Devor; and 2) at the University of Kansas Sequencing Lab by Dr. Mike Grose. At IDT, Big Dye Sequencing kits and an ABI 310 Sequencer (Applied Biosystems, Foster City, CA) were used. The sequencing reaction (per sample) included: 4.0µL Big Dye Ready Reaction Mix; 2.0µL Big Dye 5X Sequencing Buffer; 1.0µL either forward or reverse primer; and 4.0µL DNA template. Samples were run using the thermal profile: an initial denaturation at 96ºC for 30 seconds; 25 cycles of denaturation at 96ºC for 10 seconds, annealing at 50ºC for five seconds, and extension at 55ºC for four minutes; and a hold at 4ºC. Unincorporated ingredients were removed from samples by running them through gel purification columns, and the samples were then dried by speed-vac. 20µl of ABI template suppression buffer was added to each sample, the

samples were heated to 95ºC for three minutes, and then snap-cooled on ice. Samples were transferred to ABI tubes and loaded onto the ABI 310 sequencer, and the resulting chromatograms were recorded by computer. Samples run by Dr. Grose at the KU sequencing lab were run using Big Dye Sequencing kits and an ABI 3130 Sequencer (Applied Biosystems, Foster City, CA), see protocol above.

The mtDNA sequences were edited using the program BioEdit (Ibis Therapeutics, Carlsbad, CA) and compared to the Cambridge reference sequence (the published human mtDNA sequence) published by Anderson et al. (1981). Nucleotides deviating from the reference sequence were recorded as mutations.

Y Chromosome Analysis (STRs and SNPs)

Male samples in this study were characterized for Y chromosome short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs). The STR analysis was done at two different locations: by Dr. Guangyun Sun in Dr. Ranjan Deka's laboratory in the Department of Environmental Health at the University of Cincinnati Medical Center.; and by Dr. Reena Roy at the St. Louis County Police Crime Laboratory. Dr. Sun characterized the Bering and Kamchatka samples for eleven Y STRs: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a&b, DYS438, and DYS439 (see Table 10), using the Y-Plex^{TM} 12 kit (ReliaGene Technologies, Inc., New Orleans, LA), according to the manufacturer's instructions. The following PCR ingredients (per sample) were used: 10.0µL of 2.5X Y-PLEX^{TM} 12 Primer Mix; 0.5µL AmpliTaq Gold^{TM} (5 units/µL); 0.5~3ng DNA; and ddH_2O to bring the final reaction volume to 25µL. Amplification

reactions were run a Perkin Elmer 9600 PCR thermal cycler according to the following parameters: initial incubation at 95ºC for 10 minutes; 30 cycles of denaturation at 94ºC for one minute, annealing at 59ºC for one minute, and extension at 70ºC for one minute; a final extension at 60ºC for 60 minutes, and then a hold at 4ºC. An ABI 377 DNA sequencer was used for electrophoresis and detection of amplified products. The amplified products were denatured using formamide loading solution (formamide/blue dextran/internal standard GeneScan-500 ROX from Applied Biosystems in the ratio 5:1:1), heated at 95ºC for 5 minutes and snap cooled on ice for five minutes. Samples were loaded on a 4% polyacrylamide denaturing sequencing gel and elecrophoresed for 2.5 hours at 3000V and 51ºC. GeneScan 3.1 and Genotyper 2.5 (Applied Biosystems) were used for sizing and genotyping. Dr. Roy characterized the Aleutian Aleut males for seventeen Y STRs, including the eleven previously mentioned, and in addition: DYS437; DYS448; DYS45; DYS458; and YGATAH4 (see Table 10). The Quantifiler™ Human Male DNA Quantitation kit (Applied Biosystems) was used to detect the amount of male DNA in each sample. Approximately 0.3 to 0.5 ng of DNA was amplified in a 12.5µL volume of the volume of the reaction mixture using the AmpFiStr® Y filer™ kit (Applied Biosystems). An ABI 310 Genetic Analyzer and GeneMapper® v3.2 (Applied Biosystems) were used to identify the Y alleles.

Y chromosome SNP analysis was done at two locations: by the author in Dr. Ranjan Deka's laboratory in the Department of Environmental Health at the University of Cincinnati Medical Center; and by the author at the University of

Kansas. In Dr. Deka's laboratory, Y SNPs were characterized hierarchically, because Y STR data were not yet available for the samples. Laboratory analyses included RFLPs and primer-specific PCR. Only blood samples (from the Bering and Kamchatkan populations) were successfully amplified for the Y SNPs at this location. Aleutian Aleut buccal samples were whole genome amplified (WGA) at the same time (due to concern there was little DNA available in the samples), but unfortunately they failed to produce consistent results for the Y SNP markers. Markers run in Dr. Deka's lab included: YAP, RPSY (M130), and M89, M9, M175, TAT (M46), M45,

Table 10 Y chromosome STRs used in this study

STR	*Repeat Sequence**	*Ref.*
DYS19	$(TAGA)_3tagg(TAGA)_n$	5
DYS389I	$(TCTG)_3\,(TCTA)_n$	2, 3
DYS389II	$(TCTG)_n(TCTA)_nN_{28}(TCTG)_3\,(TCTA)_n$	2, 3
DYS390	$(tcta)_2(TCTG)_n\,(TCTA)_n(TCTG)_n(TCTA)_ntca(tcta)_2$	2, 3
DYS391	$(tctg)_3(TCTA)_n$	2, 3
DYS392	$(TAT)_n$	2, 3
DYS393	$(AGAT)_n$	2, 3
DYS385a,b	$(aagg)_{6-7}(GAAA)_n$	2, 3
DYS437**	$(TCTA)_n(TCTG)_{1-3}(TCTA)_4$	1
DYS438	$(TTTTC)_1(TTTTC)_{0-1}(TTTTC)_n$	1
DYS439	$(GATA)_n$	1
DYS448**	$(AGAGAT)_nN_{42}(AGAGAT)_n$	4
DYS456**	$(AGAT)_n$	4
DYS458**	$(GAAA)_n$	4
DYS635**	$(TCTA)_4(TGTA)_2(TCTA)_2(TGTA)_2(TCTA)_2$ $(TGTA)_{0,2}(TCTA)_n$	6
YGATAH4**	$(AGAT)_4CTAT(AGAT)_2(AGGT)_3(AGAT)_n$ $N_{24}(ATAG)_4(ATAC)_1(ATAG)_2$	6

* GenBank top strand ** STRs characterized only for Alaskan Aleuts

References: 1. Ayub *et al*. 2000; 2. De Knijff *et al*. 1997; 3. Kayser *et al*. 1997; 4. Redd *et al*. 2002; 5. Roewer et al. 1992; 6. White *et al*. 1999

M173, and M3. PCR ingredients, thermal profiles, and digestion reaction ingredients are listed in appendix C (nearly every marker required a different protocol).

By the time the Y SNP analysis was completed for the male samples, at the University of Kansas, they had been characterized for Y STRs. To expedite the laboratory analysis, STR haplotype matches were looked up on the following website: http://www.ysearch.org, and the samples were tested for corresponding Y SNP haplogroup(s) only. Reaction mixtures (per sample) included: 5µL of 5X flexi buffer; 4.3µL $MgCl_2$; 0.5µL dNTP mix; 0.2µL GoTaq polymerase; 5.0µL ddH_2O; 2.5µL forward primer; 2.5µL reverse primer; and 5.0µL DNA dilution. PCR ingredients were purchased from Promega (Madison, WI), except for the primers which were synthesized by IDT (Coaralville, IA). The primers and annealing temperatures used in the Y analysis are listed in Table 11. Samples were run on a PE Applied Biosystems

Table 11 Primers for Y SNP analysis

Y SNP (Haplogroup)	Primer Pairs	Sequence (5′→ 3′)	AT*
P39 (Hap C)	P39FOR P39REV	AGAAGGACTGCCTCAGAATGC GTTCGAAAGGGGATCCCTGG	60ºC
P2 (Hap E3)	P2 FOR P2 REV	GATGCAAATGAGAAAGAACT CTAAAAACTGGAGGGAGAAA	62ºC
M170 (Hap I)	M170FOR M170REV	TGCTTCACACAAATGCGTTT CCAATTACTTTCACCATTTAAGACC	60ºC
M253 (Hap I1a)	M253 FOR M253 REV	GCAACAATGAGGGTTTTTTTG CAGCTCCACCTCTATGCAGTTT	62ºC
12f2 (Hap J)	12F2FOR 12F2REV	CTGACTGATCAAAATGCTTACAGATC GGATCCCTTCCTTACACCTTATAC	64ºC
M231 (Hap N)	M231 FOR M231 REV	CCTATTATCCTGGAAAATGTGG ATTCCGATTCCTAGTCACTTGG	64ºC
P36 (Hap Q)	P36 FOR P36 REV	TGAAGGACAGTAAGTACACA TAAGTCCATTGATCTACAGA	62ºC

M3 (Hap Q3)	M3 FOR M3 REV	TAATCAGTCTCCTCCCAGCA AAAATTGTGAATCTGAAATTTAAGG	60ºC
SRY10381b (Hap R1a)	R1A FOR R1A REV	CCACAACCTCTTTCATC AATAAAAATCCCGTAAAATA	55ºC
M269 (Hap R1b)	M269FOR M269REV	CTAAAGATCAGAGTATCTCCCTTTG AAATTGTTTTCAATTTACCAG	58ºC

*AT = annealing temperature for primer pair

Gene Amp 2400 according to the following thermal profile: an initial denaturation at 94ºC for one minute; and then 35 cycles of denaturing at 94ºC for 40 seconds, annealing for 30 seconds, and extension at 72ºC for 45 seconds; a final extension of 5 minutes at 72ºC, and a hold at 4ºC. The resulting DNA templates were cleaned using QIAquick kits (see previous description), and sequenced by Dr. Grose at the University of Kansas Sequencing Lab. The sequences were aligned in BioEdit, and the presence of SNP mutations were recorded and used for Y haplogroup placement of the samples.

Autosomal DNA Analysis

Autosomal STRs (for Bering and Kamchatka samples) were run by Dr. Guangyun Sun using the Profiler Plus kit (Applied Biosystems, Foster City, CA) in Dr. Ranjan Deka's laboratory in the Department of Environmental Health at the University of Cincinnati Medical Center. The nine STRs characterized for these samples are listed in Table 12 and include: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820. PCR ingredients (per sample) included: 0.5~2.5ng of genomic DNA; 9.5µL AmpF1STAR PCR reaction mix; 5µL primer set solution; and 0.5µL AmpliTaq Gold^{TM} DNA polymerase. Amplifications were run in a Perkin Elmer 9600 PCR thermal cycler according to the following thermal profile: initial incubation at 95ºC for 11 minutes; 26 cycles of denaturation at

94ºC for one minute, annealing at 59ºC for one minute, and extension at 72ºC for one minute; a final extension at 60ºC for 45 minutes, and then a hold at 4ºC. An ABI 377 DNA sequencer was used for electrophoresis and detection of amplified products. The amplified products and the AmpF1STR allelic ladder were separately mixed with the same volume of formamide loading solution (formamide/blue dextran/internal standard GeneScan-500 ROX from Applied Biosystems in the ratio 5:1:1), denatured at 95ºC for five minutes and snap cooled on ice for five minutes. Samples were loaded on a 4% polyacrylamide denaturing sequencing gel and elecrophoresed for 2.5 hours at 3000V and 51ºC. GeneScan 3.1 and Genotyper 2.5 (Applied Biosystems) were used for sizing and genotyping.

Table 12 Autosomal STR loci used in this study

STR Locus	*Chromosomal Location*	*Repeat Sequence*	*Reference*
D3S1358	3p	AGAT	Li et al. 1993
vWA	12p12-pter	TCTA	Kimpton et al. 1992
FGA	4q28	CTTT	Mills et al. 1992
D8S1179	8	TATC	Oldroyd et al. 1995
D21S11	21	TCTA	Sharma and Litt 1992
D18S51	18q21.3	GAAA	Urquhart et al. 1995
D5S818	5q21-31	AGAT	Hudson et al. 1995
D13S317	13q22-31	TATC	Hudson et al. 1995
D7S820	7q	GATA	Green et al. 1991

Analytical Methods

Genetic Diversity and Neutrality Tests:

Gene diversity measures and neutrality test scores for the haplotypic data were calculated in Arlequin ver. 3.1 (Schneider *et al*. 2000). Gene diversity (Nei 1987) was

calculated for the mtDNA sequences and Y chromosome STR data. It is equivalent to the expected heterozygosity for diploid systems, and is defined as:

$$H = \left(\frac{n}{n-1} \right) \left(1 - \sum_{i=1}^{k} p_i^2 \right) \quad (1)$$

where n represents the sample size, k is the number of haplotypes, and p_i is the frequency of the ith haplotype. This is a relatively sTable measurement that is thought to be less responsive to genetic drift and recent demographic events (Nicholson *et al*. 2002, Helgason *et al*. 2003). For the mtDNA sequence data nucleotide diversity (Nei and Li 1979) was calculated as:

$$\pi = \sum_{ij}^{q} x_i x_j d_{ij} \quad (2)$$

where q is the total number of alleles, x_i is the frequency of the i-th allele in the population, and d_{ij} is the number of nucleotide differences between alleles i and j.

Neutrality test statistics, Tajima's D and Fu's F_s, were used to determine whether the mtDNA sequence data demonstrated departures from the null model (i.e., constant population size and absence of natural selection). Tajima's D (Tajima 1989) is based on the infinite-site model without recombination and is appropriate for use with short DNA sequences. It is defined as:

$$D = \frac{\theta\pi - \theta s}{\sqrt{V(\theta\pi - \theta s)}} \quad (3)$$

where $\theta=2N_e\mu$ (for haploid data), where N_e is the effective population size and μ is the mutation rate; $\theta\pi$ represents the mean number of pairwise differences between

sequences (π); and θs is based on the number of observed segregating sites. If there are a large number of low frequency mutations, the result will be larger values of θs relative to $\theta\pi$ and negative *D* values. Negative *D* values are indicative of populations that have undergone expansion. Conversely, populations that have undergone genetic bottlenecks should have positive *D* values, since they will have a larger number of intermediate and high frequency mutations, thus inflating $\theta\pi$ relative to θs. Significant *D* scores can be produced by other factors, however, including mutation rate heterogeneity or selection (Tajima 1989a, Aris-Brosou and Excoffier 1996).

Fu's F_s (1997) is also based on the infinite-site model without combination but uses haplotype distribution information rather than mutation frequencies, and is estimated as:

$$F_s = \ln\left(\frac{S'}{1-S'}\right) \tag{4}$$

where $S' = PR(K \geq k_{obs}\ \theta=\theta\pi)$, in other words S' is the probability of observing a random neutral sample with *k* as the number of alleles equal to or smaller than the observed value given $\theta\pi$. Fu's F_s is less conservative than Tajima's *D*, and may produce large negative values in response to large population expansion events. Similar to Tajima's *D*, positive values may be indicative of genetic drift (Fu 1997).

For the autosomal STR loci, Arlequin ver. 3.1 (Schneider *et al*. 2000) was used to calculate the observed and expected heterozygosities, and to test for deviations from Hardy-Weinberg equilibrium. The later were evaluated by the method of Guo and Thompson (1992), which is analogous to Fisher's exact test, but uses a modified

(computationally efficient) version of the Markov-chain random walk algorithm. DISPAN (Ota 1993) was used to calculate the coefficient of gene differentiation for each locus:

$$G_{ST} = (H_T - H_S)/H_T \quad (5)$$

where H_T is the gene diversity among subpopulations (e.g., average of the allele frequencies for the total data set) , and H_S is the gene diversity within subpopulations (i.e., the average of the gene diversities for the individual populations) (Nei 1987).

Network Analysis

Networks were constructed for both the mtDNA sequence data and Y chromosome STR haplotypes using Network ver. 4.0 (www.fluxus-engineering.com) in order to determine the relationship of Aleut haplotypes to each other, and for the Y data, among the haplotypes of comparative populations as well. Networks for mtDNA haplogroup A and D sequences were generated separately, using the median-joining method (Bandelt *et a*l. 1999). This analysis produces gene trees (networks) with circles that are proportional to the number of haplotypes represented. It is able to resolve parallelisms and reversals between haplogroups, but also incorporates parallelisms that cannot be solved (i.e., by a single mutational pathway) as reticulations, thereby generating the most parsimonious trees. The A and D networks were then joined together based on a phylogeny of East Asian HVS-1 sequences (Zlojutro *et al*. 2006, Kivisild *et al*. 2002), and separate colors were given to lineages present among the Aleutian, Bering, St. George and St. Paul communities, in order to visualize their distribution among the four communities. Networks for the Y

chromosome STR lineages were constructed separately for the major haplogroups present among the Aleuts and comparative Russian and Kamchatkan populations: Q, R, I, and N. For this and the analyses that follow, the Y STR DYS389I repeats were subtracted from DYS389II repeats, in order to treat the two as separate loci (since DYS389I is included in the DYS389II PCR product). The output from the reduced median network analysis (Bandelt *et al*. 1995) was used for input in constructing the median-joining network, as a means of reducing large, phylogenetically unrealistic reticulations in the network (Zegura *et al*. 2004, Hurles *et al*. 2002).

Neighbor-Joining Trees:

Phylogenetic trees were constructed as a way of visualizing the relationship of Aleut communities to one another and to comparative populations. The neighbor-joining (NJ) method was used because it produces more accurate results when comparing closely related populations, such as humans, and it does not assume an evolutionary clock (Saitou and Nei 1987). For the mtDNA RFLP haplogroup frequency data, the Y chromosome SNP haplogroup frequency data, and the autosomal STR frequency data, NJ trees were constructed from DA distances (Nei *et al*.1983) using the program DISPAN (Pennsylvania State University, PA). Bootstrap tests were performed to test the robustness of the trees (Felsenstein 1985). For the mtDNA sequences, a Tamura and Nei (1994) distance matrix was calculated using Arlequin ver. 3.1 (Schneider *et al*. 2000). This output corrects the percentage of nucleotides by which two haplotypes differ, and takes into consideration different rates of transitions and transversions, making a distinction between purine transition

rates and pyrimidine transition rates. Slatkin's linearized F_{st} distances, based on the stepwise mutation model for microsatellites (Slatkin 1995), were calculated for the Y STR haplotypes using Arlequin. These distance matrices were used to construct NJ trees in the NTSYSpc2.1 program (Applied Biostatistics, Inc., Setanket, New York). The robustness of the trees was tested by generating cophenetic matrices and comparing them to the original distance matrices using Mantel tests (Mantel 1967).

R-matrix Analysis:

The autosomal STRs and classic genetic data (taken from the literature) were analyzed using the R-matrix method and ANTANA program (Harpending and Rogers 1984), as another way of visualizing the genetic relationships among Aleut communities and with comparative populations. The R-matrix is a variance-covariance matrix of genetic similarity and dissimilarity between populations that is constructed according to the formula:

$$R_{ij} = (p_i - \bar{p})(p_j - \bar{p})/\bar{p}(1 - \bar{p}) \qquad (6)$$

where r_{ij} is the kinship coefficient for every allele, p_i and p_j are allele frequencies for populations i and j, and $\bar{p}$ is the weighted mean frequency of allele p in the matrix (Harpending and Jenkins 1973). The final R-matrix is averaged over all alleles. Minitab ver. 12.0 (Minitab, Inc., State College, Pennsylvania) was used to transform the matrix by PCA so that the variation explained by the first two eigenvectors was maximized. The results are displayed as a two-dimensional plot of the eigenvalue scaled by the square root of the corresponding eigenvector, and populations that are genetically most similar will group together. For the classic genetic markers, an S-

matrix of alleles was computed from eigenvectors of the R-matrix and plotted in order to assess their relative contributions to the distribution of populations. R-matrix analysis is most informative when multiple loci are examined, and so single-locus markers (mtDNA sequences and Y chromosome STRs) were instead analyzed using multidimensional scaling.

Multidimensional Scaling Plots:

Multidimensional scaling plots were constructed for the mtDNA sequences and Y chromosome STRs using the NTSYSpc2.1 program (Applied Biostatistics, Inc., Setanket, New York), to visualize the population genetic relationships in two-dimensional space. For the mtDNA sequences, Tamura and Nei (1994) distances were used as input for the MDS analysis, and for the Y STR data, Slatkin's linearized F_{st} distances (Slatkin 1995) were used (see above section on NJ trees for details). MDS is an ordination method similar to principal coordinates solution (PCO), in which the dissimilarity of n objects is represented in k-dimensional space, so that the distances between points in the projected space correspond to the observed distances of the original matrix as closely as possible (Kruskal 1964a, b). This method tends to be more accurate for preserving small inter-point distances than PCA, because the latter maximizes variances thereby giving greater weight to larger distances. Therefore, MDS is more appropriate for comparison of the Aleut subpopulations with one another, and with neighboring comparative populations. Initially, the MDS algorithm begins with a set of points produced by PCA, it computes distances (d^*_{ij}) between all pairs of points (ij), which are then compared to the original distances (d_{ij}).

A monotome function (d^f_{ij}) is fitted to the variables, and deviations are computed as a normalized sum of squared deviations. A stress value is used to measure goodness of fit of the projected distances to the original distances fitted with the monotone function:

$$Stress = \sqrt{\frac{\sum \left(d^*_{ij} - d^f_{ij}\right)^2}{\sum d^{*2}_{ij}}} \quad (7)$$

The projected points are adjusted in order to lower the stress value. Minitab ver. 12.0 (Minitab, Inc., State College, Pennsylvania) was used to plot the final MDS coordinates.

Heterozygosity vs r_{ii}:

To assess the effects of systematic (e.g., admixture) versus stochastic (e.g. genetic drift) processes on the study populations, heterozygosity versus distance from the centroid (r_{ii}) plots were constructed for the autosomal STRs and classic genetic markers. The following formula was used:

$$r_{ii} = \left(p_i - \bar{p}\right)^2 / \bar{p}\left(1 - \bar{p}\right) \quad (8)$$

where r_{ii} is the distance from the centroid for a particular allele in the *i*th population, p_i is the frequency of the allele in the *i*th population, and $\bar{p}$ is mean frequency of the allele for all populations. Mean heterozygosity and r_{ii} values were calculated using the ANTANA program (Harpending and Rogers 1984), and were regressed in Minitab ver. 12.0 (Minitab, Inc., State College, Pennsylvania). Because heterozygosity measurements are inappropriate for haplotypic data, gene diversity

values were substituted for the mtDNA sequences and Y chromosome STRs. The relationship between heterozygosity and r_{ii} should be linear, according to Harpending and Ward (1982), with deviations indicating a particular population may have experienced either gene flow of genetic drift, depending on its location relative to the theoretical regression line.

Admixture Estimates:

Admixture estimates were calculated for the autosomal STRs using the mY coefficient in Admix ver. 2.0 (Bertorelle and Excoffier 1998, Dupanloup and Bertorelle 2001). The mY coefficient uses allele frequency information (assuming that the allele frequencies present in hybrid populations are lineal combinations of the allele frequencies present in the parental populations), and also incorporates molecular information. For this analysis, Aleuts and Russians were identified as potential parental populations for the hybrid population of mixed Aleuts on Bering Island. A lower triangular matrix specifying molecular distance as the squared difference in allele size (appropriate for microsatellite data), and the allele frequencies for the parental and hybrid populations were used to calculate mY.

For the Y chromosome data, Aleut paternal admixture estimates were calculated by hand, assuming that Y SNP haplogroup Q represents the Native American component, and all other haplogroups are the result of non-Aleut gene flow into the population. Admixture estimates for the maternal markers was unnecessary for the majority of Aleut communities, given that all individuals claiming Aleut ancestry on the maternal side had mtDNA lineages belonging to haplogroups A or D,

with the exception of the mixed Aleut sample from Bering. Admixture estimates for that group were hand-calculated.

Sewall Wright's Statistics:

In order to investigate the possible effect of genetic drift on the small-sized Aleut populations, several statistical methods developed by Sewall Wright (1931, 1969) were implemented. This includes the calculation of the harmonic mean of the Aleut communities:

$$\frac{1}{N_e} = \frac{1}{t}\sum_{i=1}^{t}\frac{1}{N_i} \tag{9}$$

which adjusts for fluctuations in population size over time. Population reductions are disproportionately important, having the largest impact on effective population size (N_e). For this study, N_e was estimated as 0.3 of the total population size. In addition, the variance due to stochastic processes was estimated, in order to test whether intergenerational drift could be responsible for the absence of mtDNA haplogroup A lineages in the Bering Aleut population, using the formula:

$$\sigma_x^2 = q(1-q)/2N_e \tag{10}$$

with q representing the frequency of haplogroup A in the parental Aleutian Islands Aleut population.

CHAPTER FOUR: RESULTS

This chapter presents the results of the analyses that were performed using mitochondrial DNA RFLPs and sequences, Y chromosome SNPs and STRs, autosomal STRs, and classic genetic marker data. Analytical methods that were used include: diversity and neutrality measures; construction of phylogenies, R-matrix analysis, and multidimensional scaling; heterozygosity versus distance from the centroid plots; admixture estimates; and several of Sewall Wright's statistical methods, as described in chapter 3.

Mitochondrial DNA

Restriction Fragment Length Polymorphisms

The mitochondrial DNA RFLP analysis of the Aleut samples supports earlier studies (Rubicz *et al*. 2003, Zlojutro *et al*. 2006) that characterized the Aleut population as having only two of the five Native American founding haplogroups: A and D. The combined Aleut sample in this study (n=226) has lineages that are 30.5% A and 69.5% D. When the historically founded Aleut communities are considered separately (Table 13), St. Paul has the highest frequency of haplogroup A, at 40.7%, and the lowest frequency of D, at 59.3%. This community most closely resembles the Aleutian Aleuts (38.9% A and 61.1% D), and both differ from St. George, which has only 17.2% A (and 82.8% D). Bering stands apart from the other Aleut communities because of its complete lack of haplogroup A and fixation of haplogroup D, supporting earlier findings by Derbeneva *et al*. (2002). In comparison to the other populations in Table 13, the Aleuts are distinct in having the highest frequency of

haplogroup D. Old Harbor Eskimos from Kodiak, and Gambell Eskimos have the next highest frequencies of D. Many of the comparative populations

Table 13 Frequencies of mtDNA haplogroups based on RFLPs

	n	Hap A	Hap B	Hap C	Hap D	Hap other	Ref
Aleutian Aleuts	108	38.9%	0%	0%	61.1%	0%	1,2
St. Paul Aleuts	54	40.7%	0%	0%	59.3%	0%	1,2
St. George Aleuts	29	17.2%	0%	0%	82.8%	0%	1,2
Bering Aleuts	35	0%	0%	0%	100%	0%	1
Asian Eskimo	50	80.0%	0%	0%	20.0%	0%	3
Coastal Chukchi	46	23.9%	0%	21.7%	8.7%	45.7%	4
Dogrib	154	90.9%	0%	2.0%	0%	7.1%	5
Old Harbor Eskimo	115	61.7%	3.5%	0%	34.8%	0%	5
Ouzinkie Eskimo	41	73.2%	0%	4.9%	14.6%	7.3%	5
Gambell Eskimo	50	58.0%	0%	14.0%	26.0%	2.0%	5
Savoonga Eskimo	49	93.9%	0%	0%	2.0%	4.1%	5
Even	63	0%	0%	33.3%	19.1%	47.6%	1
Haida	25	96.0%	0%	0%	4.0%	0%	6
Inuit	30	96.7%	0%	0%	3.3%	0%	7
Itel'men	47	6.4%	0%	14.9%	0%	78.7%	8
Koryak	155	5.2%	0%	36.1%	1.3%	57.4%	8
Ojibwa	28	64.3%	3.6%	7.1%	0%	25.0%	6

References: 1=this study; 2=Rubicz *et al*. 2003; 3=Torroni *et al*. 1993b; 4=Sukernik *et al*. 1996; 5=Merriwether *et al.* 1995; 6=Torroni *et al*. 1993a; 7=Lorenz and Smith 1996; 8=Schurr *et al*. 1999

have high frequencies of haplogroup A. Haplogroup C and "other" haplogroups are also present among many of the populations, while haplogroup B is nearly absent.

Control Region Sequencing

The mitochondrial DNA HVS-1 sequencing results for the Aleuts (n=226) are presented in Table 14. Table 15 provides a breakdown of sequences by community. There are twenty-six different mtDNA haplotypes, which are characterized by twenty-three variable sites. Only two mutations are characterized as transversions, one T→A and the other T→G, while the rest are transitions. AL20 is the most common haplotype among the Aleuts, present in 122 individuals, followed by AL01 in 31 individuals. Thirteen of the haplotypes are present only in single individuals. Sequences belonging to haplogroup A exhibit the most diversity, and are represented by eighteen different haplotypes, while there are only eight different haplotypes belonging haplogroup D. The Bering Aleuts have mtDNA sequences that belong to only two different haplotypes that are separated by a single mutation (16311C). St. George has the next lowest number of different haplotypes, with only two lineages belonging to haplogroup A. The St. Paul sample has the largest number of different haplotypes for the historically established populations, and overall the Aleutian Aleuts have both the largest sample size and number of different mtDNA lineages. Sequencing results for the mixed Aleut population (n=39) on Bering Island are presented in Table 16. Twenty five individuals have mtDNA lineages belonging to the Aleut AL20 and AL23 haplotypes, and the remainder represent non-Aleut female gene flow from neighboring native populations and Russians. Table 17 presents

Table 14 ***HVS-I sequence data for Aleuts***

SAMPLES	16051	16064	16092	16111	16129	16181	16189	16192	16212	16223	16234	16261	16271	16290	16304	16308	16311	16319	16324	16359	16362	16368	16399	
CAM	A	T	T	C	G	A	T	C	A	C	C	C	T	C	T	T	T	G	T	T	T	T	A	
AL01	.	.	.	T	.	.	.	T	G	T	.	.	.	T	.	.	.	A	.	.	C	.	.	
AL02	G	.	.	T	.	.	.	T	G	T	.	.	.	T	.	.	.	A	.	.	C	.	.	A
AL03	.	.	.	T	.	.	.	T	.	T	T	.	.	T	C	.	.	A	.	.	C	.	.	
AL04	.	.	.	T	.	.	.	T	.	T	.	.	.	T	.	.	.	A	.	.	C	.	.	
AL05	.	.	.	T	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C	.	.	
AL06	.	.	.	T	.	.	.	T	.	T	T	.	.	T	.	.	.	A	.	.	C	.	.	
AL07	.	.	.	T	.	.	.	T	G	T	.	.	.	T	.	.	C	A	.	.	C	.	.	
AL08	.	.	.	T	.	.	C	.	G	T	.	.	.	T	.	.	.	A	.	.	C	.	.	
AL09	.	.	.	T	.	.	.	T	G	T	.	.	.	T	.	.	.	A	.	C	C	.	.	
AL10	.	.	.	T	.	.	.	.	G	T	.	.	.	T	.	.	.	A	.	.	C	.	.	
AL11	.	.	.	T	.	.	C	.	.	T	T	.	.	T	C	.	.	A	.	.	C	.	.	
AL12	.	.	.	T	.	.	.	.	.	T	T	.	.	T	C	.	.	A	.	.	C	.	.	
AL13	.	.	.	T	.	.	.	.	.	T	.	.	.	T	.	.	.	A	.	.	C	.	.	
AL14	.	.	.	T	.	.	.	T	.	T	.	T	.	T	.	.	.	A	.	.	C	.	.	
AL15	.	.	.	T	.	.	.	T	.	.	.	T	.	T	.	.	.	A	.	.	C	.	.	
AL17	.	C	.	T	.	.	.	T	.	T	.	.	.	T	.	.	.	A	.	.	C	.	.	
AL18	.	.	.	T	.	.	.	T	.	T	.	.	.	T	.	.	C	A	.	.	C	.	.	
AL19	.	.	.	T	.	.	.	T	.	T	.	.	.	T	.	.	.	A	G	.	C	.	.	
AL20	.	.	.	.	A	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	C	.	.	
AL21	.	.	.	.	A	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	C	.	G	D
AL22	.	.	.	.	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	
AL23	.	.	.	.	A	.	.	.	.	T	.	.	C	.	.	.	C	.	.	.	C	.	.	
AL24	.	.	.	.	A	.	.	.	.	T	.	.	C	.	.	C	.	.	.	.	C	C	.	
AL25	.	.	.	.	A	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	C	.	.	
AL26	.	.	A	.	A	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	C	.	.	
AL27	.	.	.	.	A	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	

Table 15 Aleut HVS-1 sequences by community

Haplogroup	CR Sequence	Aleutian Aleut[1,2]	St. Paul Aleut[1,2]	St. George Aleut[1,2]	Bering Aleut[1]	Total
A	AL01	15	12	4	0	31
	AL02	5	0	0	0	5
	AL03	5	0	0	0	5
	AL04	4	4	0	0	8
	AL05	3	2	0	0	5
	AL06	1	1	0	0	2
	AL07	1	0	0	0	1
	AL08	0	1	0	0	1
	AL09	2	0	0	0	2
	AL10	1	0	0	0	1
	AL11	1	0	0	0	1
	AL12	1	0	0	0	1
	AL13	1	0	0	0	1
	AL14	0	0	1	0	1
	AL15	0	1	0	0	1
	AL17	0	1	0	0	1
	AL18	1	0	0	0	1
	AL19	1	0	0	0	1
D	AL20	48	26	19	29	122
	AL21	8	3	0	0	11
	AL22	3	2	2	0	7
	AL23	4	1	0	6	11
	AL24	1	0	1	0	2
	AL25	1	0	0	0	1
	AL26	0	0	2	0	2
	AL27	1	0	0	0	1
Total		108	54	29	35	226

1=this study; 2=Rubicz *et al.* 2003

Table 16 Bering Mixed Aleut mitochondrial DNA HVS-1 sequences

	16069	16093	16114	16124	16126	16129	16163	16170	16186	16189	16192	16194	16223	16244	16256	16270	16271	16292	16294	16298	16304	16311	16318	16327	16362	16390	Hap	n
Cam.ref	C	T	C	T	T	G	A	A	C	T	C	A	C	G	C	C	T	C	C	T	T	T	A	C	T	G		
MX01	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	T	T	.	.	C	3
AL20	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	C	.	D	19
AL23	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	C	.	.	.	.	C	.	.	C	.		6
RU02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	H	1
MX02	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.		1
MX03	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	T	.	.	.	.	.	.	.	.	.	.	K	3
MX04	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.		1
MX05	.	.	A	.	.	.	.	G	.	.	T	.	.	.	T	T	.	T	T	.	.	.	.	.	.	.		1
MX06	.	.	.	.	.	C	G	.	T	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	OT	3
MX07	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1

OT=other haplogroups

Table 17 Even mitochondrial DNA HVS-1 sequences

Cam ref	16017 T	16093 T	16124 T	16129 G	16172 T	16185 C	16189 T	16207 A	16223 C	16224 T	16232 C	16260 C	16261 C	16265 A	16288 T	16290 C	16291 C	16298 T	16326 A	16327 C	16362 T	16390 G	Haplogroup	n
EVEN01	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	C	.	T	.	.	C	5
EVEN02	.	C	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	T	.	.		4
EVEN03	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	C	.	T	.	.		2
EVEN04	.	.	.	.	.	.	C	.	T	.	.	.	T	.	.	.	.	C	.	T	.	.		1
EVEN05	.	C	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	C	.	T	.	A		1
EVEN06	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	C	T	T	.	.		1
EVEN07	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	T	.	.		1
EVEN08	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	T	.	.		1
EVEN09	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	C	.	T	.	.		1
EVEN10	.	C	.	.	.	.	.	.	T	.	T	.	T	.	.	T	.	.	.	.	C	.	D	10
EVEN11	.	.	.	.	.	.	.	.	T	.	T	.	T	.	.	T	.	.	.	.	C	.		1
EVEN12	.	C	.	.	.	.	.	.	T	.	.	.	T	.	.	T	.	.	.	.	C	.		1
EVEN13	.	C	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	5
EVEN14	C	C	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.		1
EVEN15	.	.	.	A	.	T	C	.	T	C	.	T	.	.	.	.	.	C	.	.	.	.	OTHER	5
EVEN16	.	.	.	A	.	T	.	.	T	C	.	T	.	.	.	.	.	C	.	.	.	.		5
EVEN17	.	.	.	A	.	T	C	.	T	C	.	.	.	.	.	.	.	C	.	.	.	.		2
EVEN18	.	.	.	A	C	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	.		2

sequences for the Even population (n=49) of Kamchatka, which are used in this study for comparative purposes. Mitochondrial DNA sequences were also characterized for the Koryaks and Russians, however, since they were not used in the analysis, they are presented in Appendices B and C.

MtDNA Network Analysis

The median-joining network analysis of the Aleut mtDNA sequences is presented in Figure 6. Aleut sequences all belong to the A2 (16111T, 16223T, 16290T, 16319A, and 16362C) and D2 (16129A, 16223T, 16271C, and 16362C) subhaplogroups defined by Forster *et al*. (1996). A2 is present among Native Americans and the populations of the Chukchi Peninsula (Shields *et al*. 1993, Starikovskaya *et al*. 1998, Saillard *et al*. 2000). A 16192T transition is characteristic of most of the A2 sequences, and defines the A3 subhaplogroup. Within A3 there is an Aleut-specific subclade with a 16212A mutation, which in Zlojutro *et al*. (2006) is designated A7. D2 lineages are mainly restricted to Eskimos-Aleut groups and the Chukchi. Lineages present at the highest frequencies for the total Aleut population are the D2 root and A7, both of which form the centers of star-like clusters. These, along with a third star-like cluster at the A3 root, are characteristic of populations undergoing expansion. When the Aleut communities are examined separately, Bering shows a striking lack of mtDNA diversity, with the presence of only two different D2 lineages. St. George is a little more diverse, with four different D haplotypes, but only two A haplotypes. The majority of the different A lineages are present among the St. Paul Aleuts and Aleutian Aleuts, which overall are the most diverse.

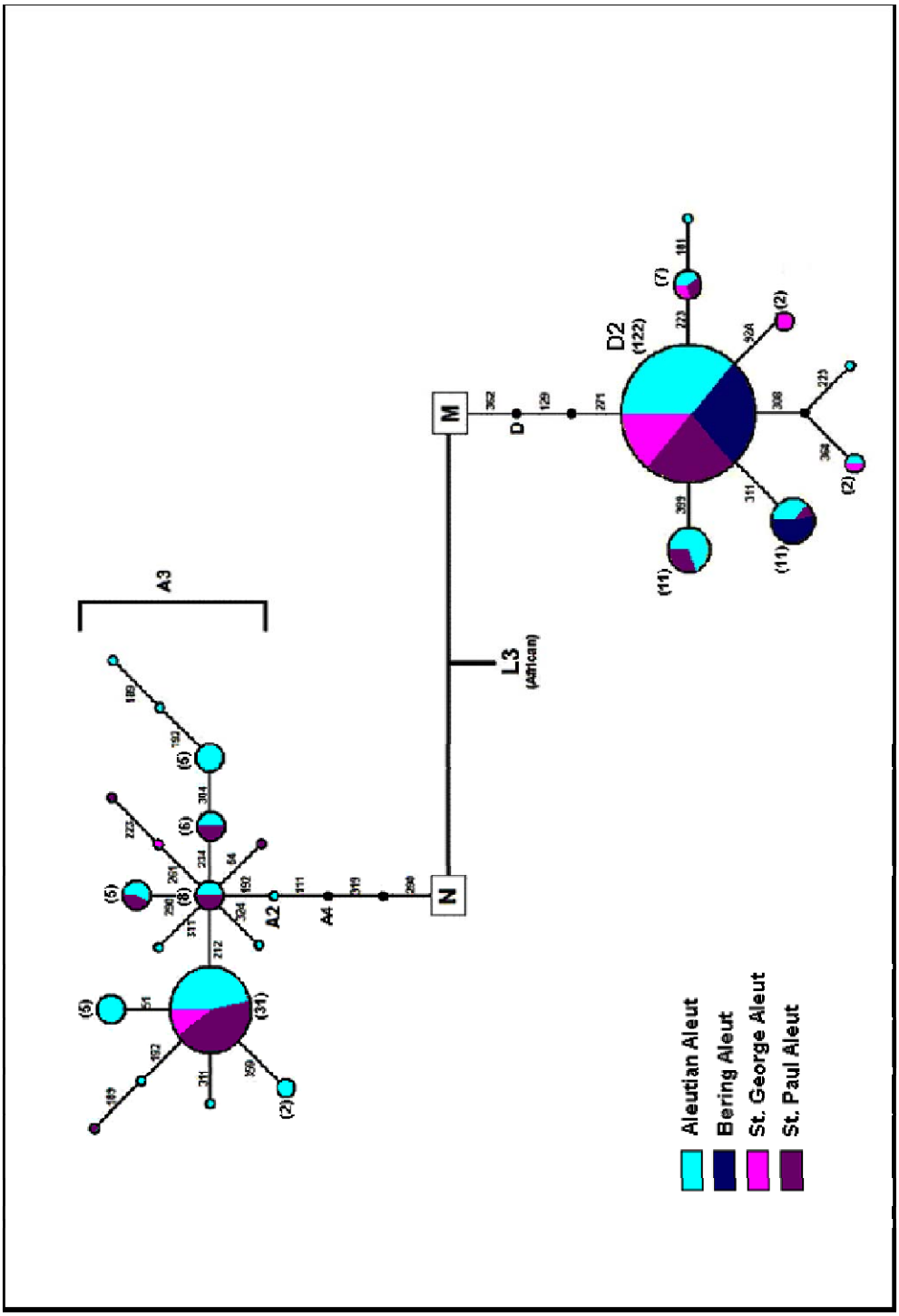

Figure 6 Network of Aleut mtDNA sequences

Diversity and Neutrality Measures

The results of the mitochondrial DNA diversity and neutrality measures for the Aleut communities and comparative populations are presented in Table 18. Nucleotide diversity is lowest for the Bering Aleuts (0.0007) followed by Siberian Eskimos (0.0010), West Greenland Eskimos (0.0051), and St. George Aleuts (0.0068). The Aleutian Aleuts and St. Paul Aleuts have similar nucleotide diversity measurements, of 0.0110 and 0.1010, respectively. The Bering Aleuts have by far the lowest gene diversity value (0.2924), and the St. George Aleuts have the next lowest value of 0.5591. The remaining populations all have gene diversities that fall between 0.7085 and 0.9443, with the Aleutian Aleuts and St. Paul Aleuts at the low end of this range. The two measures of selective neutrality, Tajima's D and Fu's F_s, are not significant for any of the Aleut populations. These results differ from those of

Table 18 Diversity and neutrality measures for mtDNA sequence data

Population	Nucleotide diversity	Gene diversity	Tajima's D statistic	Fu's F_s statistic
Aleutian Aleut[1]	0.0110 (0.0061)	0.7750 (0.0374)	0.4788	-3.9309
Bering Aleut[1]	0.0007 (0.0009)	0.2924 (0.0845)	0.3034	0.7599
St. Paul Aleut[1]	0.0101 (0.0057)	0.7191 (0.0531)	0.6110	0.0395
St. George Aleut[1]	0.0068 (0.0041)	0.5591 (0.1015)	-0.6056	1.0386
Chukchi[2]	0.0139 (0.0075)	0.8736 (0.0279)	-0.0676	-2.9492
Siberian Eskimo[2]	0.0010 (0.0056)	0.7286 (0.0456)	-0.1788	-0.4764
Koryak[3]	0.0162 (0.0085)	0.9443 (0.0082)	-0.6767	-17.4206*
Itel'men[3]	0.0128 (0.0070)	0.9295 (0.0220)	-0.6471	-4.3268
W.G. Eskimo[4]	0.0051 (0.0033)	0.7462 (0.0348)	-0.6601	-2.6550
Athabascan[6]	0.0073 (0.0046)	0.9048 (0.0482)	-1.1370	-6.0710*
Haida[5]	0.0080 (0.0048)	0.7085 (0.0606)	-1.2050	-1.4487
Bella Coola[5]	0.0151 (0.0082)	0.9038 (0.0203)	0.2018	0.3435
Even[1]	0.0164 (0.0088)	0.9201 (0.0195)	0.7049	-2.5325

* $P<0.05$ References: 1. This study; 2. Starikovskaya *et al.* 1998; 3. Schurr et al. 1999; 4. Saillard *et al*. 2000; 5. Ward *et al*. 1993; 6. Shields et al 1993

Zlojutro *et al*. (2006) that characterized the total Aleut population as having a significantly negative F_s value (-6.678, $p<0.05$), indicating the population may be undergoing an expansion. In the present study, although not significant, positive values for the historically-founded Aleut communities (except for St. George, with Tajima's D = -0.6056) suggest they may be experiencing genetic drift. For Fu's F_s, the Aleutian Aleuts are the only population with a negative value. The Koryaks and Athabascans both have statistically significant negative values for Fu's F_s, indicating they are expanding or perhaps being impacted by evolutionary forces.

Phylogenetic Trees and Multidimensional Scaling

A neighbor-joining tree based on mtDNA RFLP haplogroup frequencies for the populations in Table 13 is presented in Figure 6. The Aleut populations all cluster together, with Bering and St. George splitting off together from the Aleutian Aleuts, and St. Paul branching off directly from the Aleutian Aleuts. Based on this phylogeny, the Aleuts appear most closely related to the Old Harbor Eskimos of Kodiak Island, and the Asian Eskimos of Chukotka. The populations of Kamchatka (Even, Koryaks, and Itel'men) all cluster together, and branch off from the neighboring Chukchi population. Other Eskimo groups are dispersed among North American Na-Dene populations, indicating that this phylogeny, which is based on the frequencies of only four mtDNA haplogroups, may not be very informative.

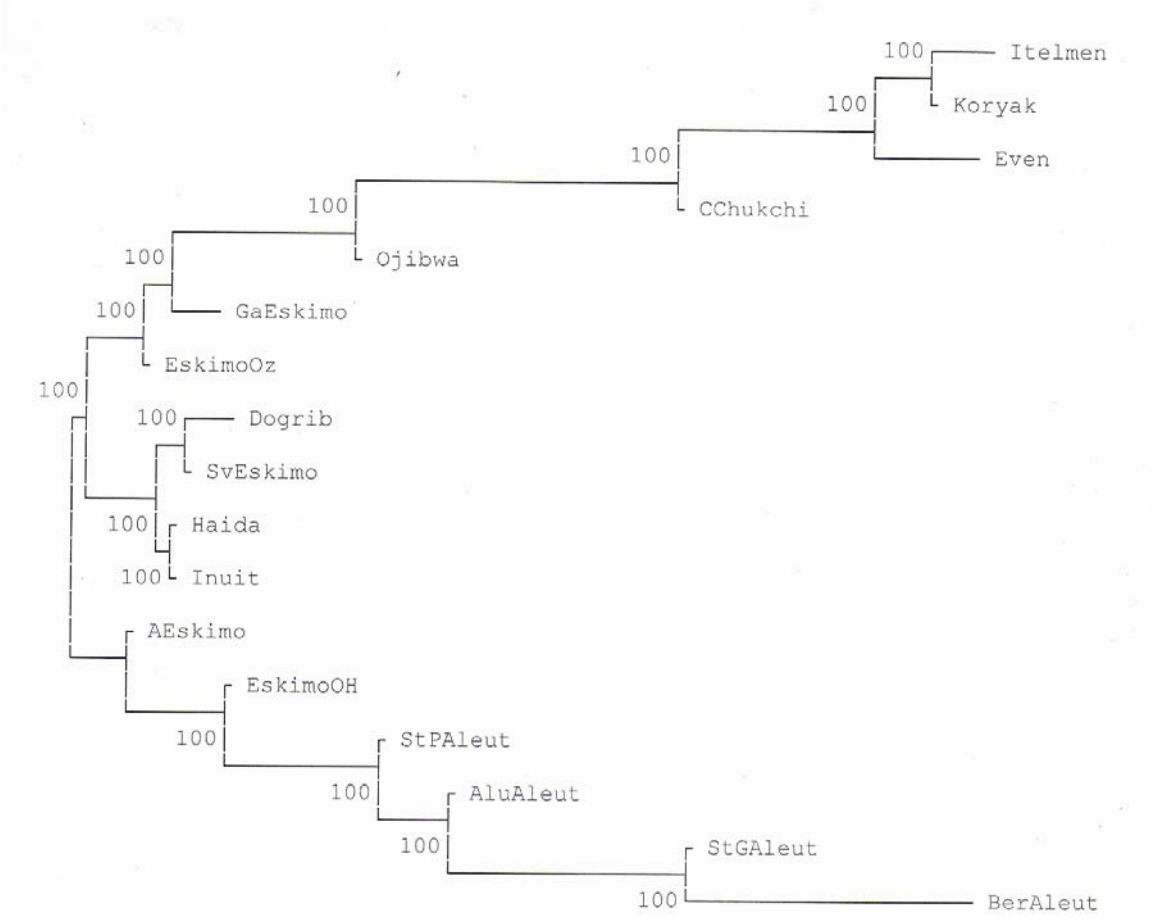

Figure 7 Neighbor-joining tree based on mtDNA RFLPs

A neighbor-joining tree based on the mtDNA sequence data for the populations listed in Table 18 is presented in Figure 7. Its correlation with the original distance matrix is high (r=0.9053, p=0.001), indicating it is a good fit. The relationships among the Aleut populations are similar to the tree based on mtDNA haplogroup frequencies, with the two smaller populations (Bering and St. George) sharing a branch off of the Aleutian Aleut population, and the St. Paul Aleuts branching off separately. The Aleuts share their own branch of the tree, and are genetically most similar to the cluster that includes the Chukchi, Eskimos and North

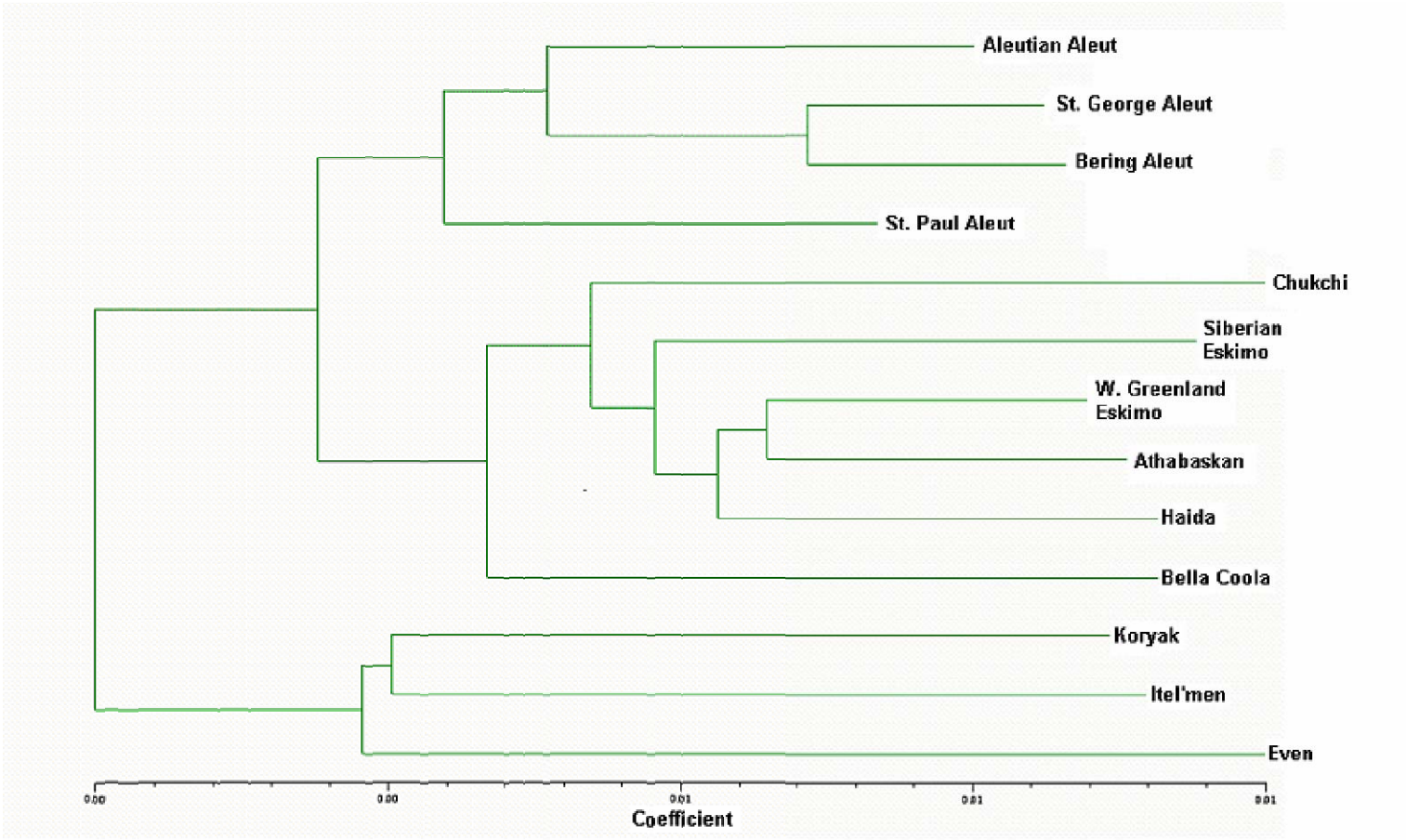

Figure 8 Neighbor-joining tree based on mtDNA sequences

American populations. The Kamchatkan groups are distinct from all other populations, and resemble each other genetically.

Figure 9 is an MDS plot based on mtDNA sequences using the populations from Figure 8, and the combined Aleut sample. The stress value of 0.03725 is well below the upper bound of 0.133, indicating the plot is a good fit with the original distance matrix (Sturrock *et al*. 2000). Along the first axis, the Aleuts are closer to the cluster of Chukotkan (Siberian Eskimo and Chukchi) and North American populations, rather than the Kamchatkan populations (Even, Koryak, and Itel'men). The second axis separates the Aleuts from all other populations, indicating they are genetically distinct. When the Aleuts are separated out by community (Figure 10), the fixation of haplogroup D in the Bering sample appears to skew the distribution of populations. Along the first axis, the Aleutian Aleuts and St. Paul Aleuts lie between the Chukotkan and North American populations, and the Kamchtkans, while the St. George Aleuts and Bering Aleuts are located on the other side of the Kamchtakan populations, away from the majority of populations in the graph. The stress value for this plot is 0.05388, indicating it is a good fit with the original matrix as it is well under the upper limit of 0.199 (Sturrock *et al*. 2000).

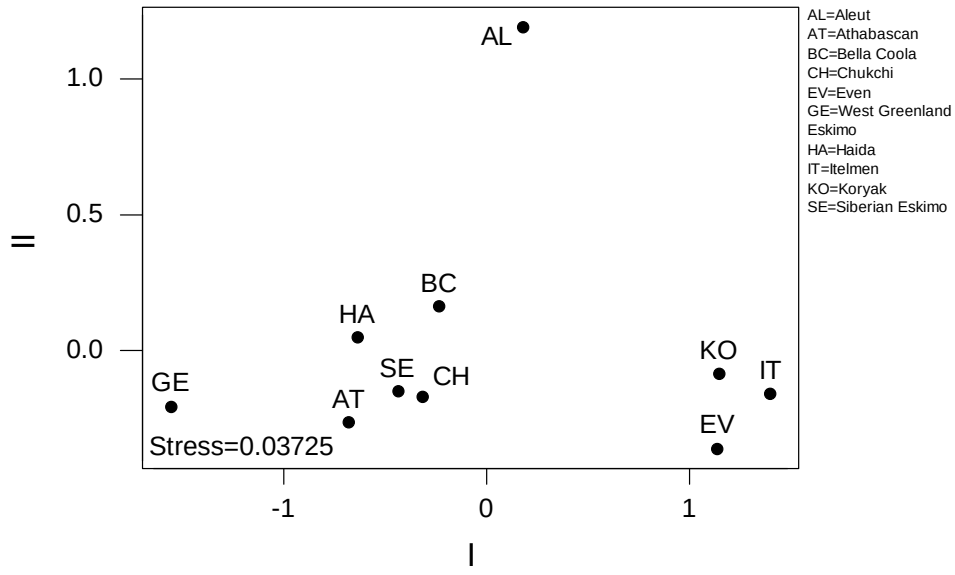

Figure 9 MDS plot based on mtDNA sequences with Aleuts combined

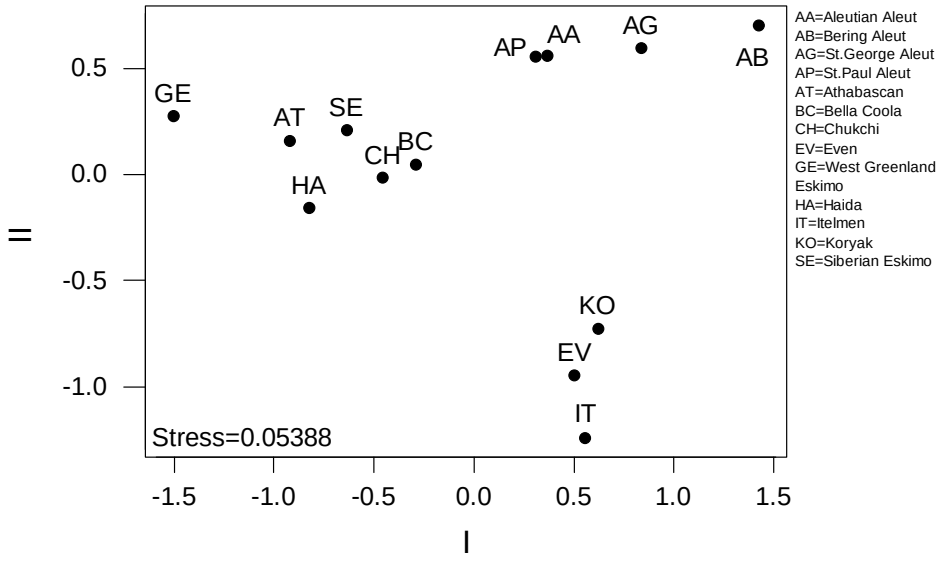

Figure 10 MDS plot based on mtDNA sequences with Aleuts separated

Diversity versus rii

The plot of diversity versus rii is presented in Figure 11. The historically established Aleut communities all lie below the theoretical regression line, indicating they lack mtDNA sequence diversity in comparison to other populations. This is especially true for the Bering Aleuts, who are located at the bottom of the graph. The Aleutian Aleuts, on the other hand, fall just above the line, indicating they have greater mtDNA diversity. The Chukchi are located in the upper left-hand corner, indicating they may have experienced more gene flow than the other populations. The Even and Bella Coola are furthest from the centroid of the alleleic array.

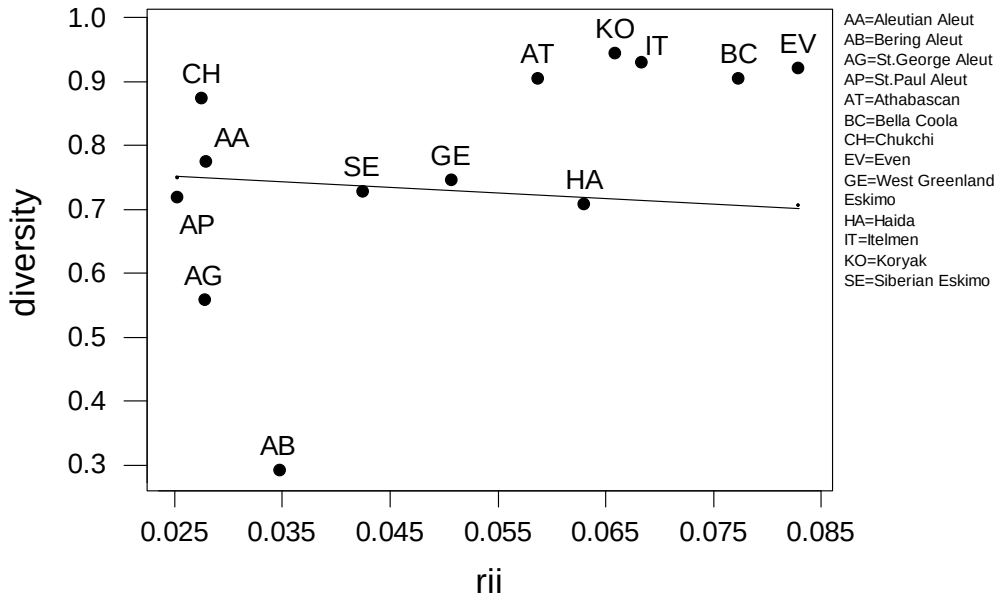

Figure 11 Diversity vs rii plot based on mtDNA sequences

Y Chromosome DNA

Y Chromosome SNPs

Results of the Y chromosome SNP analysis are presented in Table 19. The Aleut Y lineages belong to the following haplogroups: Native American Q; European haplogroups I, J, and R; Eurasian haplogroup N; and "other" as of yet undetermined haplogroups. The Bering Aleuts have the highest percentage of Native American Q, at 27.27%. Y lineages among the St. George Aleuts belong to only two haplogroups: Q (11.11%) and R (88.89%). The sample from St. Paul has the lowest frequency of Q, at 10.00%, with the remaining 90% representing non-Native admixture into the population. The Aleutian Aleuts have 13.04% Q lineages, and the rest belong to haplogroups I, R, and "other". The mixed Aleut sample from Bering has no lineages belonging to the Native American Q haplogroup, indicating a large amount of gene flow into this group through the paternal side. The Russian sample is mainly made up of N, R, and I haplogropups, with smaller percentages of C and "other". The Koryaks have halpogroups N and C, with smaller amounts of I, R, and "other". Nearly all of the Even Y lineages belong to haplogroup C (90%), with a smaller amount of Q (10%).

Table 19 Y SNP haplogroups

	sample size	C	E	I	J	N	Q	R	Other
Aleutian Aleuts	23	0.0000	0.0000	0.1304	0.0000	0.0000	0.1304	0.6957	0.0435
St. George Aleuts	9	0.0000	0.0000	0.0000	0.0000	0.0000	0.1111	0.8889	0.0000
St. Paul Aleuts	20	0.0000	0.0000	0.1500	0.0500	0.2000	0.1000	0.3000	0.2000
Bering Aleuts	11	0.0000	0.0000	0.0909	0.2727	0.1818	0.2727	0.0909	0.0909
Bering Mixed Aleuts	6	0.0000	0.3333	0.1667	0.0000	0.0000	0.0000	0.3333	0.1667
Russians	15	0.0667	0.0000	0.2000	0.0000	0.3333	0.0000	0.3333	0.0667
Koryaks	11	0.2727	0.0000	0.0909	0.0000	0.4546	0.0000	0.0909	0.0909
Even	10	0.9000	0.0000	0.0000	0.0000	0.0000	0.1000	0.0000	0.0000

Y Chromosome STRs

The Y chromosome haplotypes for Aleutian Aleuts, St. Paul Aleuts, and St. George Aleuts are presented in Table 20. These samples were characterized for the following seventeen loci: DYS19, DYS389I, DYS38911, DYS390, DYS391, DYS392, DYS393, DYS385a&b, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS635, and YGATAH4. The Aleutian Aleuts (n=24) have 17 different haplotypes, one of which (AA14) is shared with two individuals from St. Paul (AP13) and two from St. George (AG6). All other haplotypes are population specific. The St. Paul Aleuts (n=19) have 13 different haplotypes, and St. George (n=9) has only six haplotypes.

The results of the Y chromosome STR analysis for the Bering Aleuts, Russians, Even, and Koryak populations are presented in Table 21. These populations were characterized for only eleven loci: DYS19, DYS389I, DYS38911, DYS390, DYS391, DYS392, DYS393, DYS385a&b, DYS438, and DYS439. The Bering Aleuts (n=11) have eleven different haplogroups, one of which (AB7) is shared with the Russians (RU5). This same haplotype matches up with the truncated St. Paul Aleut haplotype AP6. The Russian sample (n=27) has twenty-two different Y haplotypes, including one (RU17) that matches up with truncated St. Paul Aleut haplotype AP12. The Even (n=10) and the Koryaks (n=11) both have six different haplotypes each, one of which they share (Even 4 & Koryak 6).

Table 20 Aleutian Aleut, St. Paul Aleut, St. George Aleut Y STR haplotypes

Y STR Haplotypes (DYS19-DYS389I-DYS38911-DYS390-DYS391-DYS392-DYS393-DYS385a,b-DYS438-DYS439-DYS437-DYS448-DYS456-DYS458-DYS635-YGATAH4)

Aleutian Aleuts (n=24)

1. 13-12-28-23-10-14-13-15,17-11-12-16-20-17-16-23-11 (2)
2. 13-13-29-22-10-15-13-15,17-11-12-14-19-17-17-22-12
3. 14-12-28-22-10-11-13-14,14-10-11-16-21-16-16-20-12
4. 14-12-28-23-11-13-13-12,14-12-12-13-19-15-17-23-12
5. 14-13-28-24-11-14-13-12,24-12-13-15-19-18-17-23-12
6. 14-13-30-24-11-15-13-11,15-12-12-15-19-18-17-23-12
7. 14-13-29-21-11-13-13-11,14-12-12-15-19-17-17-23-12 (2)
8. 14-14-30-24-11-13-13-11,14-12-12-15-19-16-17-23-12
9. 14-14-30-25-11-13-14-11,14-12-13-15-19-15-16-23-12 (2)
10. 15-13-29-24-10-12-15-15,16-10-11-15-20-15-16-19-11
11. 15-13-29-25-10-11-14-12,14-11-12-14-20-15-14-23-13
12. 16-13-30-25-11-11-13-11,15-11-11-14-20-15-15-24-13 (3)
13. 16-13-31-24-11-11-13-14,15-10-13-15-20-15-17-24-11
14. 16-14-30-25-10-11-13-11,14-11-11-14-20-16-16-23-12 (AP13, AG6)
15. 16-14-30-25-10-11-13-11,14-11-11-14-20-17-16-23-11 (3)
16. 17-13-33-24-11-11-12-14,15-10-13-15-20-15-18-24-11
17. 18-13-31-25-10-11-14-10,14-11-10-14-19-17-16-23-12

St. Paul Aleuts (n=19)

1. 13-14-30-24-10-14-13-15,15-11-11-14-20-15-18-22-11 (2)
2. 13-14-31-23-10-14-14-15,17-11-13-16-20-15-18-24-11 (2)
3. 14-12-28-23-10-11-13-14,14-10-11-16-20-15-15-22-11
4. 14-12-29-22-10-11-13-13,14-11-11-16-20-14-15-22-11
5. 14-13-29-24-10-13-13-12,14-12-12-15-19-15-18-24-12
6. 14-14-30-23-11-14-14-11,13-10-10-14-19-15-16-22-12
7. 15-12-28-23-11-14-13-12,13-10-11-14-18-17-15-19-12
8. 15-12-30-24-10-13-13-12,20-10-11-14-21-14-17-21-12 (3)
9. 15-13-29-23-11-14-14-12,13-10-10-14-19-14-17-22-11
10. 15-13-30-24-11-13-13-11,14-12-11-15-19-16-17-23-12 (2)
11. 16-12-28-24-10-11-12-13,17-9-11-16-19-13-18-22-11
12. 16-13-29-25-10-11-13-11,14-11-11-14-20-17-17-23-11
13. 16-14-30-25-10-11-13-11,14-11-11-14-20-16-16-23-12 (2) (AA14, AG6)

St. George Aleuts (n=9)

1. 13-14-31-24-10-14-14-15,17-10-13-15-11-16-17-22-12
2. 14-13-29-23-12-13-13-11,14-12-12-15-19-17-17-23-11
3. 14-14-30-23-10-13-13-11,14-12-12-15-19-16-18-23-11
4. 15-13-32-25-11-11-13-10,14-11-10-14-20-17-16-24-12 (2)
5. 16-13-30-25-10-11-13-11,14-11-10-15-20-16-14-23-12 (2)
6. 16-14-30-25-10-11-13-11,14-11-11-14-20-16-16-23-12 (2) (AA14, AP13)

Table 21 Bering Aleut, Russian, Even, and Koryak Y STR haplotypes

Y STR Haplotypes (DYS19-DYS389I-DYS38911-DYS390-DYS391-DYS392-DYS393-DYS385a,b-DYS438-DYS439)

Aleut from Bering (n=11)

1. 13-14-30-24-10-15-14-13,19-11-11
2. 13-14-31-24-10-15-14-13,19-11-11
3. 14-13-29-23-10-11-12-14,16-9-11
4. 14-13-29-23-10-14-13-15,17-11-11
5. 14-13-30-24-10-15-14-15,21-11-13
6. 14-13-30-24-11-13-12-11,14-12-12
7. 14-14-30-23-11-14-14-11,13-10-10 (same as Russian 5)
8. 14-14-30-23-11-14-13-11,11-10-10
9. 14-14-31-24-10-14-14-13,19-11-12
10. 15-13-31-23-11-11-14-14,15-10-13
11. 15-14-30-23-10-11-12-14,17-9-12

Russian from Bering and Kamchatka (n=27)

1. 13-13-31-24-10-11-13-18,20-10-13 (2)
2. 14-12-27-22-10-11-13-13,13-10-11
3. 14-12-28-23-10-11-13-14,14-10-10
4. 14-14-29-23-10-14-13-12,13-10-10
5. 14-14-30-23-11-14-14-11,13-10-10 (same as Bering Aleut 7)
6. 14-14-30-23-11-14-14-12,13-10-10
7. 14-14-30-23-11-15-14-11,11-10-10
8. 14-14-30-24-11-13-14-11,15-13-13
9. 14-14-30-24-11-14-14-11,13-10-10
10. 15-12-28-22-10-11-13-13,14-10-11
11. 15-13-28-23-10-11-14-11,18-10-12

12. 15-13-29-22-10-11-14-13,14-9-12
13. 15-13-29-25-10-11-13-11,15-11-11 (2)
14. 15-13-30-25-11-11-13-11,14-11-10 (2)
15. 15-14-31-23-11-12-14-11,13-10-10
16. 16-13-29-24-10-11-14-11,14-11-11
17. 16-13-29-25-10-11-13-11,14-11-11
18. 16-13-29-25-10-11-13-12,14-11-11
19. 16-13-30-25-10-11-13-11,14-11-10
20. 16-13-30-25-11-11-13-11,14-11-10 (3)
21. 16-13-31-24-10-11-13-14,15-10-12
22. 16-14-31-25-11-11-13-11,14-11-10

Even from Kamchatka (n=10)

1. 14-14-30-23-11-14-14-11,13-10-11
2. 16-13-29-23-9-11-13-12,12-10-11
3. 17-13-29-23-9-11-13-12,12-10-11 (2)
4. 17-13-29-24-9-11-13-12,12-10-11 (4) (same as Koryak 6)
5. 17-13-29-24-9-11-12-11,12-10-12
6. 17-13-29-25-9-11-13-9,12-10-12

Koryak from Kamchatka (n=11)

1. 14-14-30-23-10-14-13-11,14-10-11
2. 14-14-30-23-11-14-14-11,13-10-11 (5)
3. 15-13-30-26-11-11-13-11,14-11-10
4. 16-13-26-25-9-11-13-12,12-10-12 (2)
5. 17-12-28-25-11-11-14-13,16-10-11
6. 17-13-29-24-9-11-13-12,12-10-11 (same as Even 4)

Y Chromosome Network Analysis

Four networks based on Y STRs present among the combined Aleut sample, Russians, and combined Kamchatkan populations (Koryaks and Itel'men) are presented in this section. The networks are each composed of Y STRs representing a single haplogroup, for the four most common haplogroups present in the samples: Q, R, N, and I. Native-American haplogroup Q (Figure 12) is present only among the Aleuts, and is absent from the comparative populations characterized in this study. European haplogroup R (Figure 13) represents the largest number of Y STR haplotypes, and is present in each of the study groups. The majority of the haplotypes in the R network are Aleut, indicating there has been a significant amount of Russian/European male admixture into the population. Five of these lineages are present among the Russians, one of which is shared with the Aleuts. Figure 14 presents the network for haplogroup I, the other major European haplogroup represented in this study. Again, the majority of the haplotypes are present among the Aleuts, with Russian lineages scattered throughout, and a single Kamchatkan haplotype. Eurasian haplogroup N (Figure 15) is mainly composed of Russian (five) and Aleut (four) Y lineages, one of which is shared between the two groups. There is a single Kamchatkan haplotype, representing two individuals, in this network.

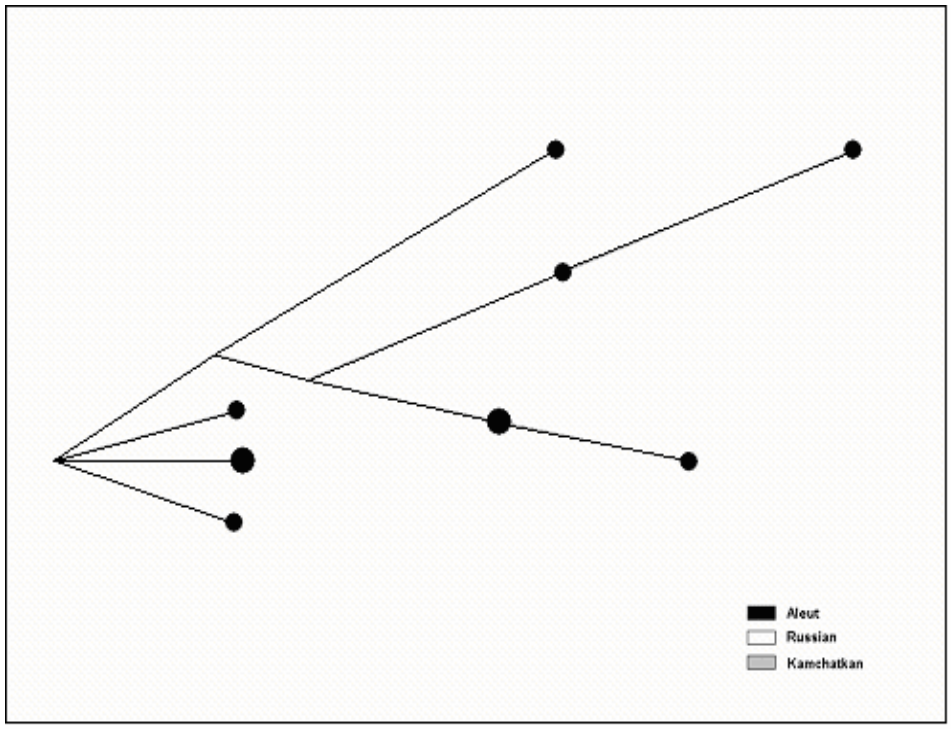

Figure 12 Network of Y chromosome haplogroup Q STRs

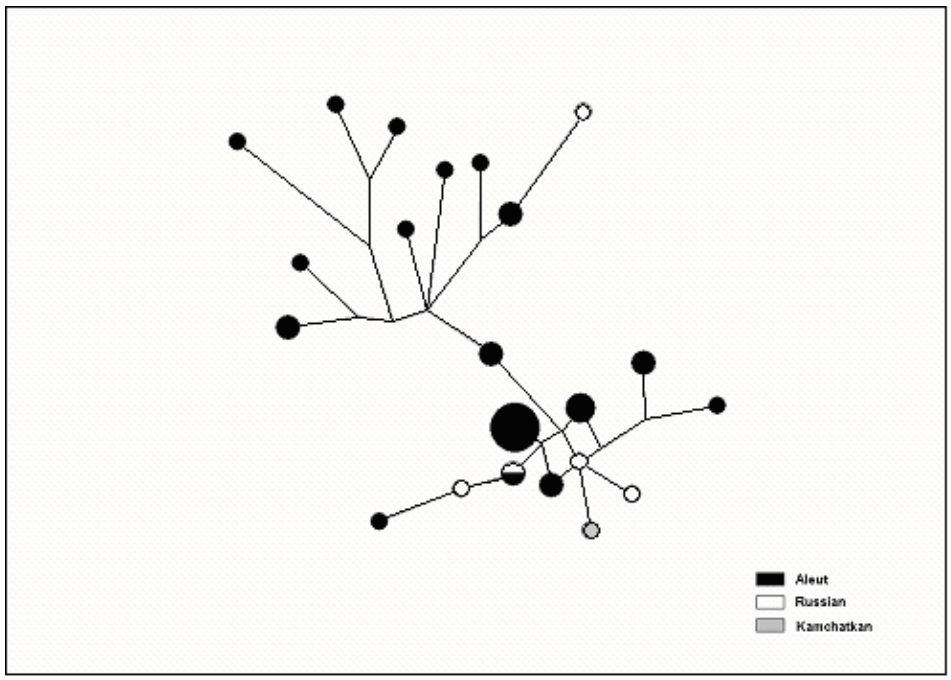

Figure 13 Network of Y chromosome haplogroup R STRs

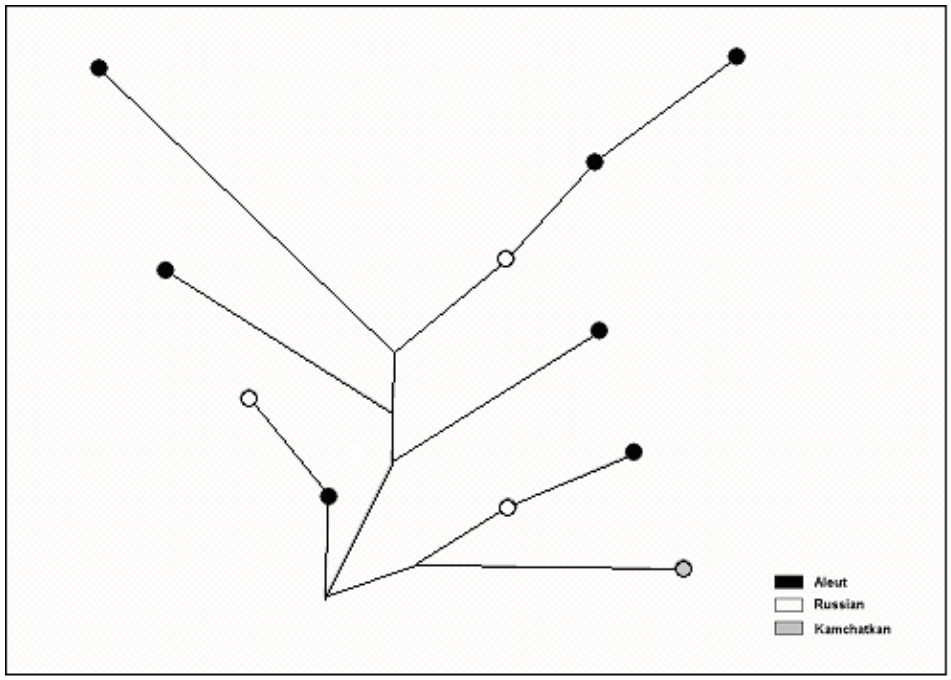

Figure 14 Network of Y chromosome haplogroup I STRs

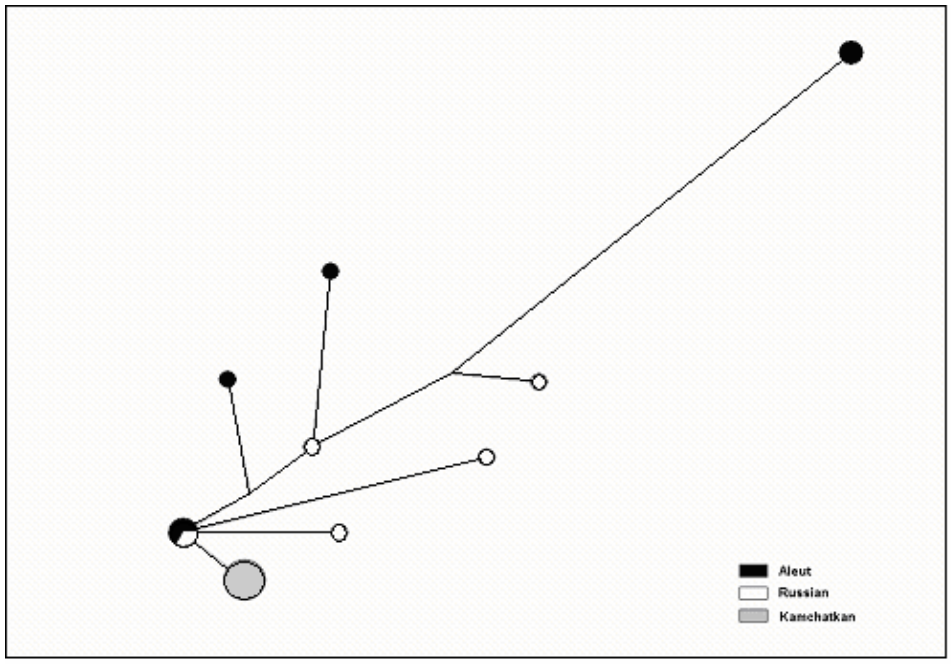

Figure 15 Network of Y chromosome haplogroup N STRs

Diversity Measures

Gene diversity measures, by population, for each Y STR locus and for the entire haplotype are presented in Table 22. The most informative locus, in other words the locus with the highest mean diversity measure is DYS285b (0.7480), while the locus with the lowest diversity measure is DYS393 (0.4719). The Bering Aleuts have the highest haplotype diversity measure (1.0000 +/-0.0388), indicating that each Y chromosome in the sample belongs to a different lineage. The Aleutian Aleuts and St. Paul Aleuts have similar haplotype diversities, of 0.9565 +/-0.0250 and 0.9591 +/-0.0388, respectively, and St. George has a slightly lower measure of 0.9167 +/-0.0725. The population with the lowest haplotype diversity is the Koryaks (0.8000 +/-0.1138).

Phylogenetic Trees and Multidimensional Scaling

A neighbor-joining tree constructed from Y STR haplogroup frequency data is presented in Figure 16. The populations used in this analysis include: the Aleuts, Russians, Koryaks, and Even from the present study; and the following populations taken from the literature: Siberian Eskimos, Alaskan Eskimos, Greenland Eskimos, Tanana, Cheyenne, Sioux, Southwest, Pima, Pueblo, Apache, Navajo, Mixtec, Zapotec, and Mixe (Karafet *et al*. 2006). The population relationships appear to be heavily influenced by non-Native (most likely European) male admixture. The Aleutian Aleuts and St. George Aleuts appear most closely related to the Sioux, while the Bering Aleuts and St. Paul Aleuts are genetically closer to the Siberian Eskimos, Russians, and Koryaks.

Table 22 Gene diversity measures for Y STRs

Locus	Aleutian Aleuts[1]	Bering Aleuts[1]	St.Paul Aleuts[1]	St.George Aleuts[1]	Russians[1]	Even[1]	Koryak[1]	Greenland Eskimos[2]	Mean
DYS19	0.7536	0.5818	0.7719	0.7778	0.7350	0.3778	0.6909	0.7153	0.6755
DYS389I	0.6196	0.5455	0.6959	0.5556	0.5698	0.2000	0.6182	0.6680	0.5735
DYS389II	0.7246	0.5818	0.6784	0.8056	0.7464	0.2000	0.6000	0.7890	0.6519
DYS390	0.7283	0.5091	0.6842	0.5556	0.7322	0.6444	0.6727	0.7011	0.6588
DYS391	0.5181	0.5091	0.4094	0.5556	0.5128	0.2000	0.5636	0.3197	0.4396
DYS392	0.6920	0.7818	0.7018	0.5556	0.4416	0.2000	0.5455	0.7366	0.6062
DYS393	0.4239	0.7273	0.4328	0.2222	0.4615	0.3778	0.5455	0.5303	0.4719
DYS385a	0.6739	0.8182	0.7836	0.5556	0.5897	0.5111	0.5636	0.7438	0.6668
DYS385b	0.7862	0.8727	0.8538	0.5556	0.8091	0.5333	0.7091	0.8585	0.7480
DYS438	0.6377	0.7636	0.6959	0.5556	0.5897	0.0000	0.1818	0.6799	0.5089
DYS439	0.7029	0.8000	0.4561	0.7778	0.6638	0.3556	0.4727	0.5171	0.5961
Haplotype diversity	0.9565 ±0.0250	1.0000 ±0.0388	0.9591 ±0.0277	0.9167 ±0.0725	0.9829 ±0.0154	0.8444 ±0.1029	0.8000 ±0.1138	0.9859 ±0.0058	

1 = this study; 2 = Willuweit et al (2007)

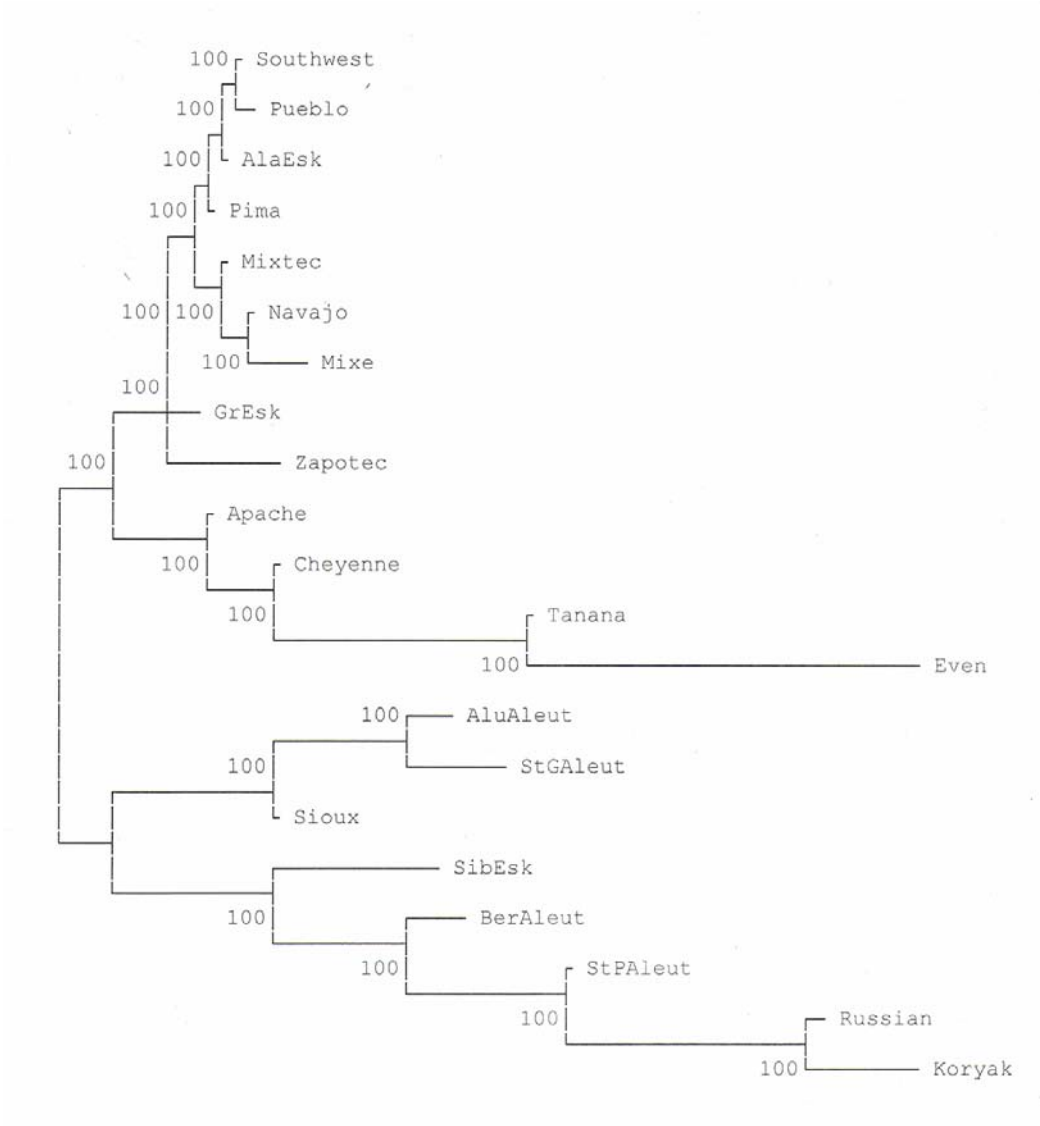

Figure 16 Neighbor-joining tree based on Y SNPs

The neighbor-joining tree based on Y STRs was a poor fit with the original distance matrix (r=0.1455, p=0.1109), indicating it is not a good representation of the phylogenetic relationships of the populations. Therefore it is not included here, but rather can be found in appendix F.

An MDS plot for the Y STRs is presented in Figure 17. The Aleut populations form a loose cluster with Greenland Eskimos, Russians, and Amerindians. The Aleutian Aleuts and St. George Aleuts are closer genetically, while the St. Paul Aleuts fall between the Aleutian Aleuts and Bering Aleuts. Outliers in the MDS plot include the Even, Japanese, and Sino-Tibetan populations. The proximity of the Aleut populations to the Russians is another indication they, like other Native Americans, have experienced a high level of non-Native paternal gene flow.

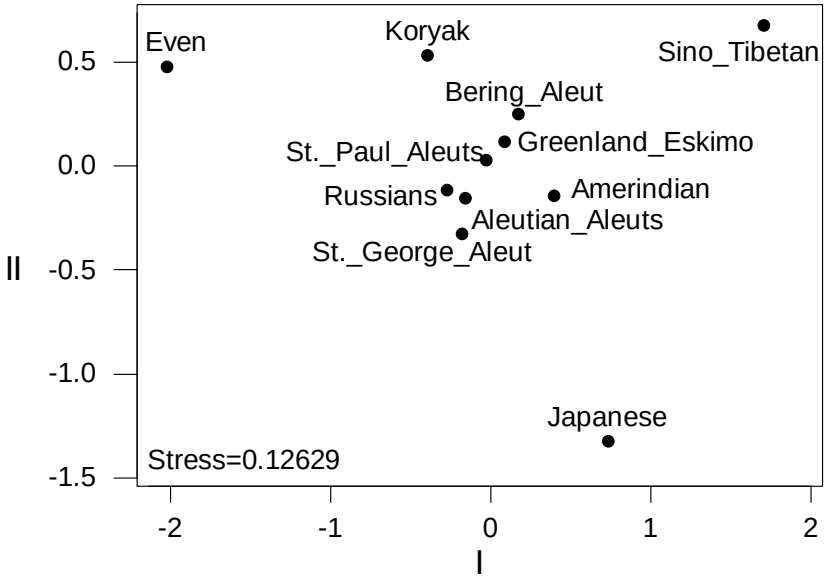

Figure 17 MDS plot based on Y STRs with Aleuts separated

Diversity versus r_{ii}

The plot of diversity versus distance from the centroid (r_{ii}) for the Y STR data is presented in Figure 18. The Aleutian Aleut and St. Paul Aleut populations are located above the theoretical regression line in the upper left-hand corner of the plot, indicating they have likely experienced a large amount of admixture. The Russians and Greenland Eskimos also appear to be highly admixed. While the St. George and Bering Aleuts have high diversity measures, they are a little further from the centroid. The Koryaks fall just below the theoretical regression line, while the Even are outliers, being furthest to the right side of the plot and having the highest rii values.

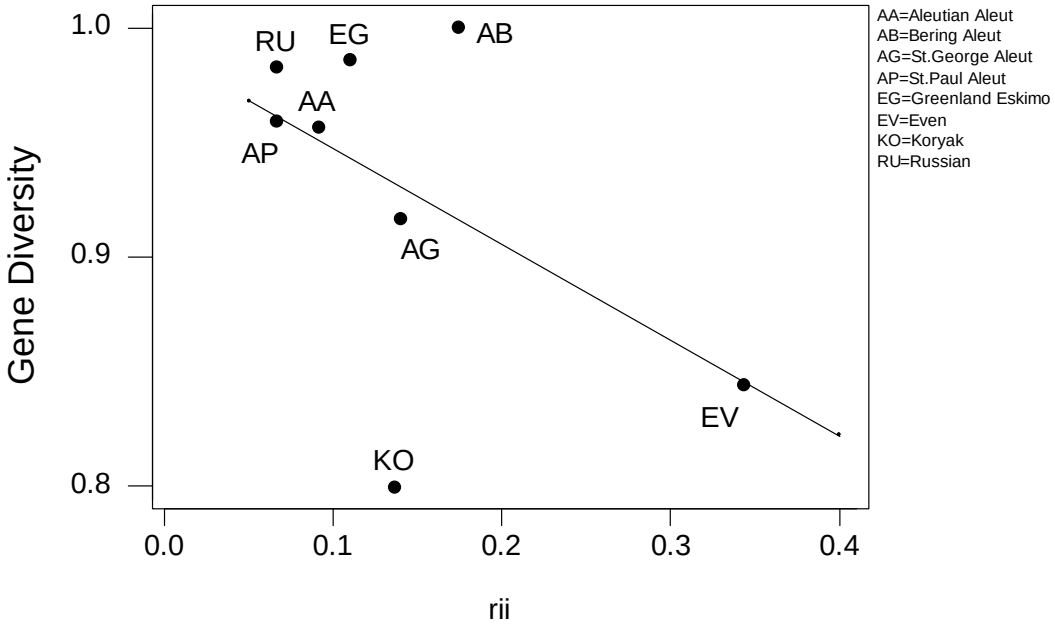

Figure 18 Diversity vs r_{ii} plot based on Y chromosome STRs

Autosomal STRs

The distribution of autosomal STR allele frequencies for the Bering Aleuts, the mixed Aleut population of Bering, the Russians, Even, and Koryaks is presented in Table 23. The observed heterozygosity scores range from a low of 0.471 for the D21S11 locus in the Aleuts, to a high of 0.939 for D3S1358 in the mixed Aleuts. Several of the STR loci show significant departures from Hardy-Weinberg equilibrium. These loci all have significantly lower observed compared to expected heterozygosity values, including: D21S11 and D13S317 ($p \leq 0.01$) for the Aleuts; FGA ($p < 0.05$) in the mixed Aleut sample; D8S1179 and D21S11 ($p < 0.05$) in the Even; and D21S11 and D13S317 ($p < 0.05$) in the Koryaks. These results indicate that there could be non-random mating and genetic sub-structuring within these populations, or that they may be impacted by evolutionary forces (i.e., genetic drift).

Table 23 Autosomal STRs: Observed allele frequency distributions

D3S1358	Aleut (N=34)	Mixed Aleut (N=33)	Russian (N=32)	Even (N=59)	Koryak (N=22)
14	0.0441	0.1212	0.1875	0.0085	0.0000
15	0.5735	0.4242	0.2656	0.2712	0.4091
16	0.0882	0.1061	0.2813	0.4576	0.3409
17	0.0882	0.1818	0.1563	0.2034	0.2500
18	0.1764	0.1667	0.1094	0.0593	0.0000
19	0.0294	0.0000	0.0000	0.0000	0.0000
H (Obs)	0.647	0.939	0.813	0.644	0.864
H (Exp)	0.631	0.745	0.797	0.690	0.703
P	0.886	0.152	0.868	0.196	0.106
vWA					
13	0.0000	0.0152	0.0156	0.0000	0.0000
14	0.2206	0.1667	0.1094	0.1356	0.0909
15	0.1176	0.1515	0.0625	0.0678	0.0455
16	0.1765	0.1667	0.1875	0.2373	0.2045
17	0.2941	0.2273	0.2969	0.3644	0.5227
18	0.0882	0.1970	0.2656	0.0932	0.0909
19	0.1029	0.0758	0.0313	0.0424	0.0455

20	0.0000	0.0000	0.0313	0.0593	0.0000
H (Obs)	0.765	0.970	0.781	0.746	0.591
H (Exp)	0.829	0.838	0.801	0.785	0.698
P	0.135	0.160	0.651	0.981	0.488
FGA					
18	0.0441	0.0000	0.0000	0.0085	0.0000
19	0.1765	0.1212	0.1250	0.0424	0.0909
20	0.0882	0.1061	0.1250	0.0085	0.0000
21	0.1029	0.1667	0.2344	0.0169	0.0455
22	0.1324	0.1364	0.2188	0.1949	0.1364
23	0.2941	0.2121	0.1094	0.2966	0.2955
23.2	0.0000	0.0152	0.0000	0.0000	0.0000
24	0.1179	0.0909	0.0781	0.3475	0.3182
24.2	0.0000	0.0152	0.0000	0.0000	0.0000
25	0.0147	0.0455	0.0938	0.0763	0.1136
26	0.0147	0.0303	0.0156	0.0000	0.0000
27	0.0147	0.0606	0.0000	0.0000	0.0000
H (Obs)	0.882	0.848	0.906	0.746	0.909
H (Exp)	0.842	0.881	0.852	0.758	0.788
P	0.155	0.043*	0.921	0.419	0.987
D8S1179					
8	0.0000	0.0303	0.0000	0.0000	0.0000
9	0.0441	0.0000	0.0000	0.0000	0.0000
10	0.1471	0.0909	0.0938	0.0254	0.1136
11	0.1029	0.0606	0.0781	0.0424	0.0000
12	0.1029	0.1212	0.2500	0.1271	0.0455
13	0.3088	0.4848	0.3438	0.5424	0.5000
14	0.1912	0.1364	0.1563	0.1525	0.3182
15	0.1029	0.0606	0.0313	0.1102	0.0227
16	0.0000	0.0152	0.0313	0.0000	0.0000
17	0.0000	0.0000	0.0156	0.0000	0.0000
H (Obs)	0.735	0.697	0.781	0.576	0.636
H (Exp)	0.829	0.726	0.790	0.658	0.648
P	0.325	0.870	0.817	0.037*	0.960
D21S11					
25.2	0.0000	0.0000	0.0000	0.0085	0.0000
27	0.0000	0.0000	0.0000	0.0085	0.0227
28	0.0882	0.1970	0.2344	0.0000	0.0455
29	0.4265	0.3788	0.2032	0.1949	0.2045
29.2	0.0294	0.0000	0.0000	0.0000	0.0000
30	0.2206	0.1667	0.1094	0.5169	0.5455
30.2	0.0735	0.0758	0.0469	0.0339	0.0227
31	0.0441	0.0303	0.1250	0.1441	0.0227
31.2	0.0588	0.0303	0.1563	0.0085	0.0455
32	0.0000	0.0152	0.0000	0.0000	0.0000
32.2	0.0294	0.0455	0.0625	0.0847	0.0909

33.2	0.0294	0.0606	0.0625	0.0000	0.0000
H (Obs)	0.471	0.727	0.906	0.559	0.636
H (Exp)	0.849	0.799	0.862	0.672	0.662
P	0.000**	0.782	0.658	0.010**	0.044*
D18S51					
11	0.0000	0.0000	0.0156	0.0000	0.0000
12	0.1029	0.1212	0.0625	0.0085	0.0227
13	0.1471	0.0758	0.1250	0.3220	0.2955
14	0.3235	0.2121	0.1719	0.1780	0.2500
15	0.1176	0.2273	0.1719	0.0932	0.2273
16	0.0882	0.1970	0.1250	0.0678	0.1136
17	0.0441	0.0606	0.1875	0.2627	0.0000
18	0.1618	0.0758	0.0625	0.0254	0.0682
19	0.0000	0.0303	0.0156	0.0000	0.0000
20	0.0000	0.0000	0.0000	0.0000	0.0227
21	0.0147	0.0000	0.0313	0.0424	0.0000
22	0.0000	0.0000	0.0156	0.0000	0.0000
23	0.0000	0.0000	0.0156	0.0000	0.0000
H (Obs)	0.882	0.879	0.719	0.763	0.864
H (Exp)	0.825	0.847	0.880	0.818	0.798
P	0.602	0.644	0.062	0.734	0.090
D5S818					
7	0.0147	0.0000	0.0156	0.0932	0.1136
9	0.0000	0.0758	0.0781	0.0339	0.0000
10	0.0588	0.0758	0.0781	0.1525	0.2273
11	0.5000	0.4848	0.2500	0.3220	0.2500
12	0.1912	0.2424	0.3750	0.2627	0.1136
13	0.0882	0.1212	0.1875	0.1356	0.2955
14	0.0294	0.0000	0.0156	0.0000	0.0000
15	0.1176	0.0000	0.0000	0.0000	0.0000
H (Obs)	0.676	0.758	0.625	0.763	0.864
H (Exp)	0.698	0.690	0.763	0.787	0.781
P	0.708	0.837	0.190	0.183	0.187
D13S317					
8	0.1765	0.2576	0.0781	0.4153	0.2955
9	0.2794	0.0758	0.0781	0.2881	0.2045
10	0.0294	0.0909	0.0625	0.1102	0.2273
11	0.4118	0.3030	0.4375	0.0932	0.2273
12	0.0882	0.1212	0.2813	0.0085	0.0227
13	0.0000	0.1212	0.0469	0.0593	0.0227
14	0.0147	0.0303	0.0156	0.0085	0.0000
15	0.0000	0.0000	0.0000	0.0169	0.0000
H (Obs)	0.500	0.727	0.750	0.746	0.636
H (Exp)	0.732	0.829	0.722	0.783	0.808
P	0.010*	0.299	0.070	0.177	0.035*

D7S820					
7	0.0147	0.0152	0.0313	0.0000	0.0000
8	0.2794	0.3333	0.1250	0.1695	0.0455
9	0.0294	0.0909	0.1406	0.0254	0.1364
10	0.1912	0.2576	0.3438	0.2542	0.4091
11	0.3235	0.1515	0.2188	0.2627	0.3864
12	0.1618	0.1364	0.0938	0.1864	0.0227
13	0.0000	0.0152	0.0469	0.1017	0.0000
H (Obs)	0.618	0.848	0.875	0.814	0.636
H (Exp)	0.795	0.786	0.803	0.799	0.678
P	0.082	0.432	0.854	0.080	0.183

Diversity Measures

Table 24 presents gene diversity measures of the autosomal STR loci for the study populations and comparative populations from the literature. The total genomic diversity (H_t) for the populations ranges from 0.709 for D3S258 to 0.859 for FGA. This is fairly high, and the majority of the diverisyt is attributable to within population variability (H_s). G_{st}, a measure of interpopulation variability, accounts for a smaller percentage of the total genomic diversity, ranging from 4% for the D18S51 locus to 9.3% for the D13S317 locus, with an average of 6.4% for the total data set. The average gene diversity for the Bering Aleuts is 0.776, which falls within the range of Native American and Siberian populations of 0.648 (for the Kogi of South America) to 0.784 for the Salishan. The mixed Aleut sample from Bering has an elevated average gene diversity value relative to the Aleuts of 0.802, which is likely a result of gene flow from Russians, who have a value of 0.818, and possibly other populations i.e., Siberians, European Americans, and Scandinavians). Second to the Russians, the Asian populations have the highest average gene diversity measures for the sample, ranging from 0.801 for the Japanese, to 0.813 for the Chinese.

Table 24 Gene diversity for autosomal STR loci

Population	FGA	vWA	D3S158	D5S818	D7S820	D8S1179	D13S317	D18S51	D21S11	Average
Aleut[1]	0.855	0.825	0.640	0.708	0.776	0.837	0.733	0.837	0.770	0.776
Aleut Mix[1]	0.894	0.850	0.756	0.701	0.796	0.737	0.822	0.859	0.800	0.802
Russian[1]	0.865	0.813	0.803	0.773	0.811	0.802	0.733	0.892	0.868	0.818
Even[1]	0.758	0.787	0.683	0.789	0.805	0.663	0.732	0.793	0.676	0.743
Koryak[1]	0.806	0.695	0.685	0.809	0.693	0.663	0.802	0.817	0.677	0.739
Athabaskan[2]	0.856	0.749	0.730	0.686	0.796	0.707	0.802	0.837	0.759	0.769
Inupiat[2]	0.866	0.809	0.601	0.757	0.771	0.762	0.751	0.808	0.814	0.771
Yupik[2]	0.845	0.783	0.595	0.696	0.759	0.673	0.722	0.792	0.806	0.741
N Ontario[3]	0.879	0.697	0.623	0.634	0.657	0.688	0.775	0.794	0.849	0.733
Salishan[3]	0.877	0.805	0.638	0.785	0.727	0.744	0.804	0.853	0.825	0.784
Chinese[3]	0.869	0.806	0.724	0.777	0.778	0.855	0.818	0.867	0.822	0.813
Japanese[3]	0.853	0.801	0.702	0.796	0.773	0.840	0.808	0.869	0.766	0.801
Korean[3]	0.838	0.810	0.710	0.758	0.786	0.849	0.821	0.860	0.808	0.804
Vietnamese[3]	0.882	0.792	0.729	0.783	0.758	0.863	0.776	0.844	0.831	0.806
Arsario[4]	0.679	0.648	0.672	0.728	0.370	0.809	0.611	0.819	0.878	0.691
Kogi[4]	0.617	0.520	0.763	0.627	0.471	0.719	0.508	0.813	0.796	0.648
Ijka[4]	0.750	0.719	0.582	0.788	0.475	0.733	0.766	0.829	0.739	0.709
Wayuu[4]	0.873	0.760	0.604	0.708	0.719	0.859	0.794	0.917	0.802	0.782
H_S[a]	0.805	0.740	0.662	0.720	0.690	0.747	0.735	0.817	0.773	0.743
H_T[b]	0.859	0.782	0.709	0.775	0.758	0.787	0.811	0.851	0.820	0.795
G_{ST}[c]	0.062	0.053	0.065	0.071	0.089	0.051	0.093	0.040	0.057	0.064

a. Gene diversity within subpopulations; b. Gene diversity among subpopulations; c. Coefficient of gene differentiation
References: 1. This study, 2. Budowle *et al.* 2002; 3. Budlowle *et al.* 2001; 4. Guarino *et al.* 1999

Phylogenetic Tree and R-Matrix Analysis

A neighbor-joining tree based on the nine autosomal STR loci is presented in Figure 19. The Aleuts and mixed Aleuts are grouped with the Russians, suggesting there has been gene flow between these populations. The Asian populations (Chinese, Vietnamese, Japanese, and Koreans) are all grouped together at the top of the tree. The Kamchatkan populations (Koryaks and Itel'men) share a branch, and the Eskimo populations (Inupiat and Yupik) are grouped together. The native South Americans, Ijka, Arsario, and Kogi, cluster together at the bottom of the tree. A fourth South American population, the Wayuu, is located between the other South Americans and several North American populations (Northern Ontario, Salishan, and Athabascan).

Figure 20 shows an R-matrix plot with the same populations that were used to construct the neighbor-joining tree. The first two eigenvectors in the R-matrix analysis account for 39.9% of the total variation. In this Figure, the mixed Aleut sample is located between the Aleuts and Russians. The Yupik and Inupiat cluster nearby, along with other Native American populations. The Asian groups are all clustered together near the Koryaks and Even of Kamchatka. The South American populations: Kogi, Arsario, and Ijka, are separated from all other populations along the first axis, with the Ijka appearing to be the most genetically distinct, as it separates out from the other South Americans along the second axis.

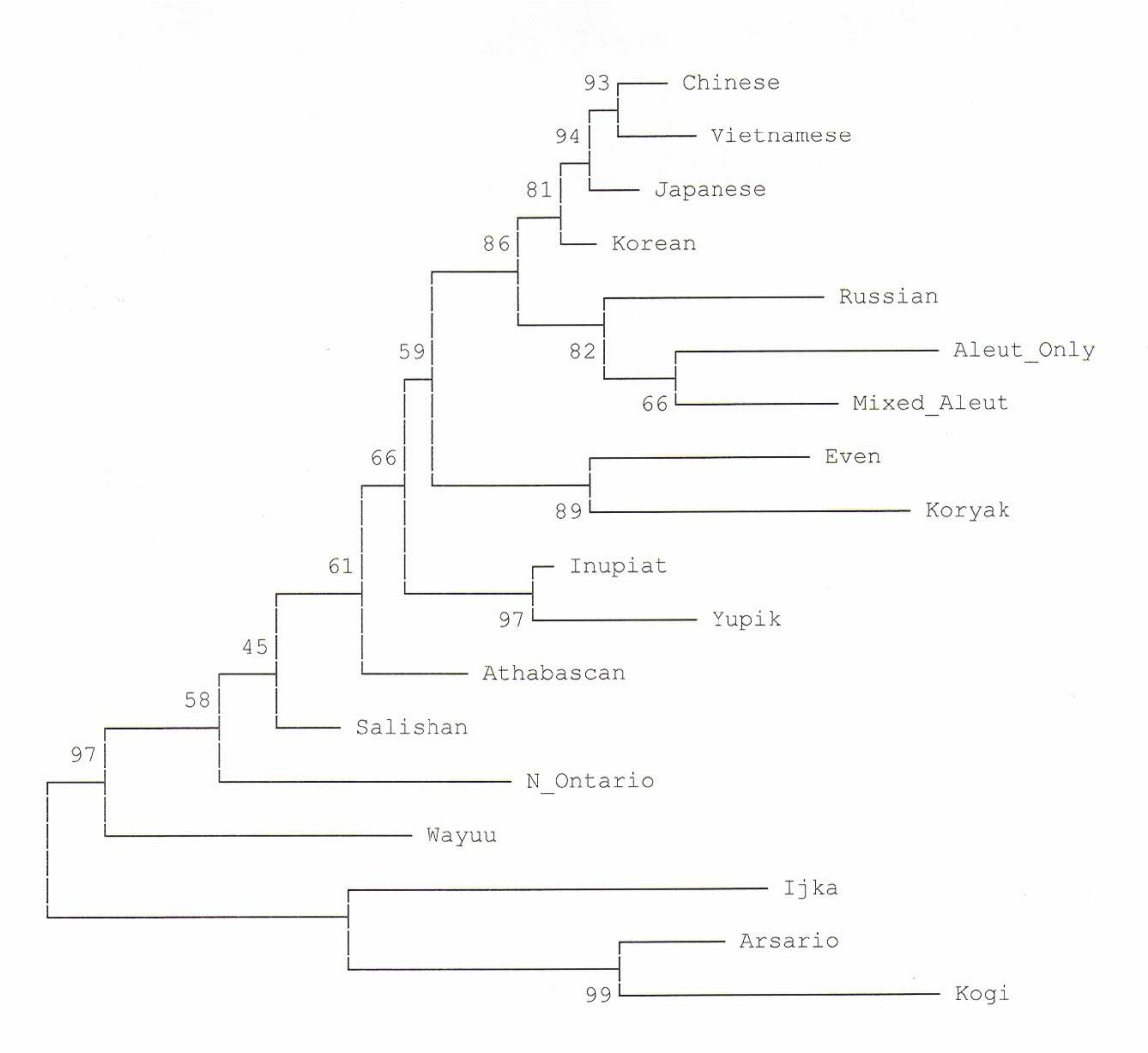

Figure 19 Neighbor-joining tree based on autosomal STRs

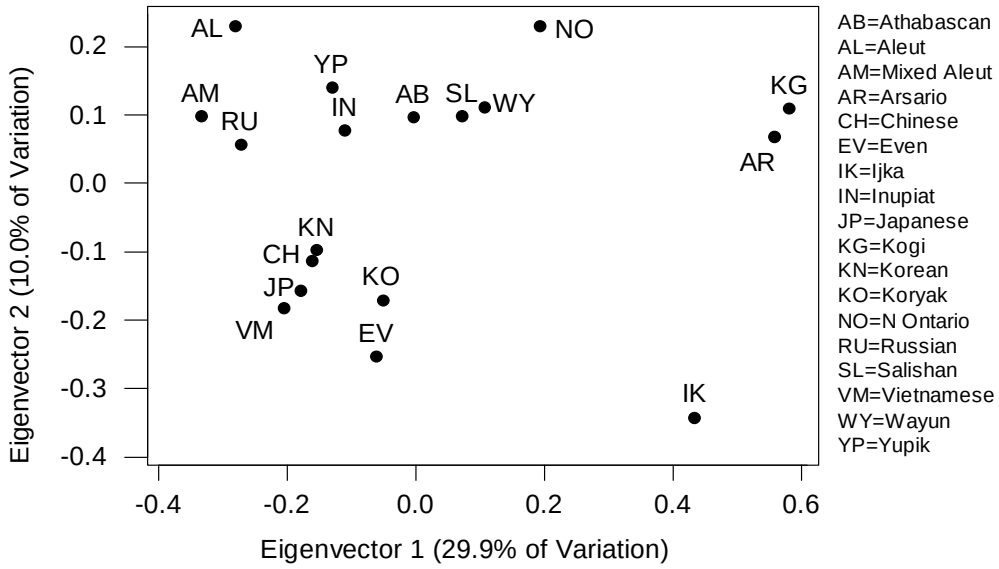

Figure 20 R-matrix plot based on autosomal STRs

Heterozygosity versus r_{ii}

A plot of heterozygosity versus distance from the centroid of the allelic array for the autosomal loci is presented in Figure 21. The Asian populations, Salishan, Athabascan, and Inupiat, are located above the theoretical regression line in the upper left-hand corner, suggesting they have all experienced a certain degree of admixture. The Russians and mixed Aleuts are clustered together further to the right in the graph, but near the top, indicating they both have high heterozygosity values. The Aleuts are located further to the right, and therefore further from the centroid. The Koryaks are located slightly below the theoretical regression line. The populations that appear to have been impacted by genetic drift are the South American Arsario, Ijka, and Kogi.

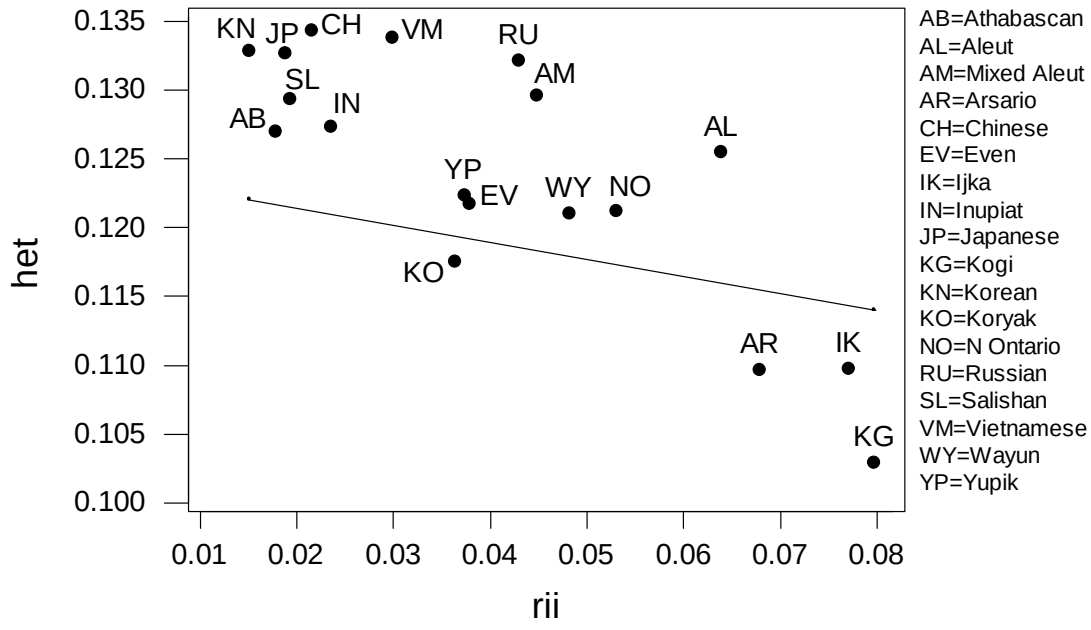

Figure 21 Heterozygosity vs r_{ii} plot based on autosomal STRs

Classic Genetic Markers

Classic genetic markers for the historically established Aleut communities of St. Paul, St. George, Bering, and Medni were taken from the literature (Majumder *et al*. 1988, Rychkov and Sheremetyeva 1972). Comparative populations used in the analysis include: Chukotan populations (Noonyamo and Siryeniki Chukchi and Eskimo); Kodiak Island Eskimo populations (Akhlok, Larson Bay, Old Harbor, and Ouzinikie); St. Lawrence Island Eskimos (Gambell and Savoonga); King Island Eskimos; and Eskimos from Wales, Alaska (Crawford *et al*. 1981, Crawford and Enisco 1992, Majumder *et al*. 1988).

R-matrix and Plot of Alleles

The R-matrix plot and plot of alleles for sixteen populations based on nine alleles from five different blood group systems are presented in Figures 22 and 23. The Aleut populations of St. George, Bering, and Medni are all grouped together. Along the first eigenvector, which accounts for 37.3% of the total variation, they are closest to the Wales, King Island, Savoonga, and Gambell Eskimo populations. The St. Paul Aleuts are genetically distinct from the other Aleut populations, and cluster instead with the Siryeniki Chukchi and Larson Bay Eskimos from Kodiak. The Asian Eskimo populations of Noonyamo and Siryeniki are the most divergent genetically, when both eigenvectors are considered (accounting for 70.7% of the total variation).

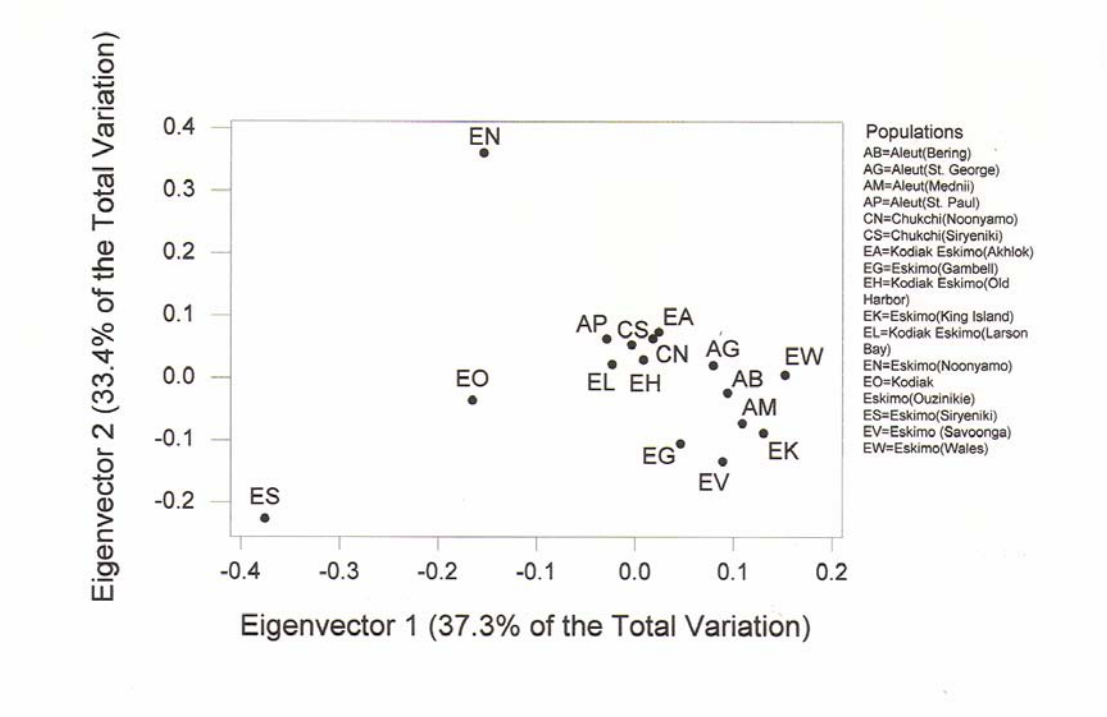

Figure 22 R-Matrix plot based on classic genetic markers

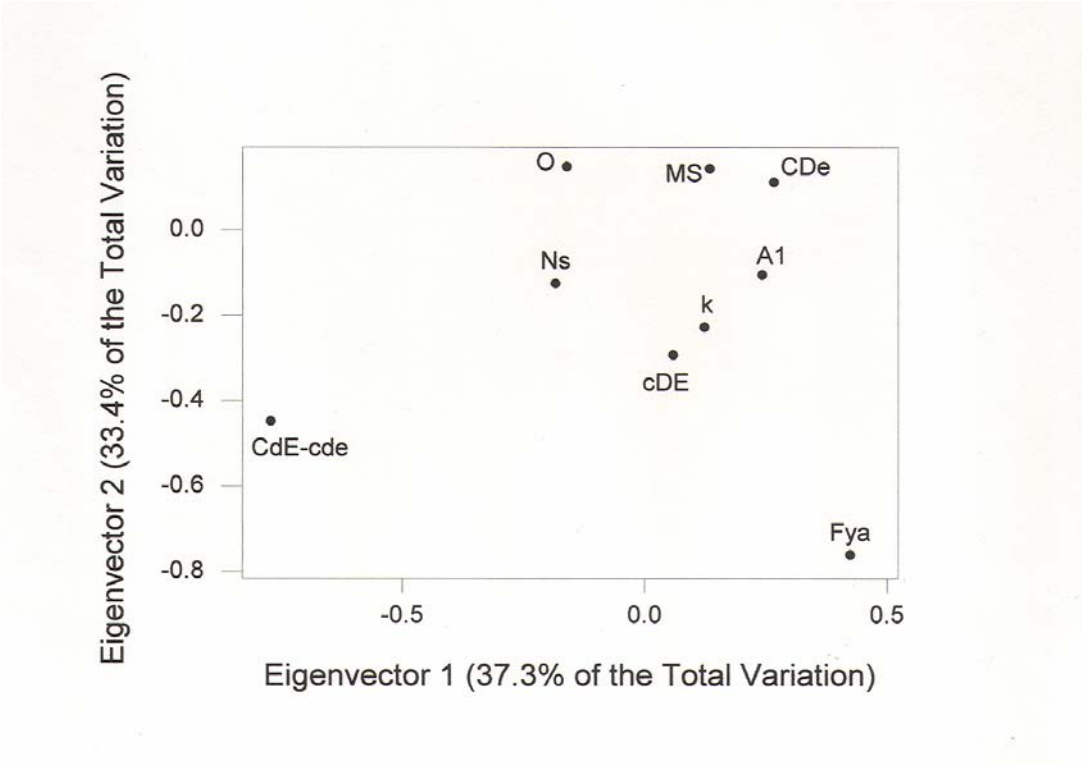

Figure 23 Plot of alleles for classic genetic markers

Heterozygosity vs r_{ii}

Figure 24 presents the plot of heterozygosity versus distance from the centroid (r_{ii}) for the populations based on the classic genetic data. According to these results, the St. Paul and Bering Aleuts appear to have experienced the most admixture of the Aleuts populations. The St. George Aleuts are located on the theoretical regression line, while the Medni Aleuts are located below it, indicating they have the lowest heterozygosity measure of the Aleut groups depicted here. Other populations likely experiencing admixture include the Noonyamo Chukchi, Larson Bay Eskimos, Old Harbor Eskimos, Gambell Eskimos, and King Island Eskimos. The Asian Eskimo

populations of Siryeniki and Noonyamo are located to the far right of the graph, indicating they are the furthest from the centroid.

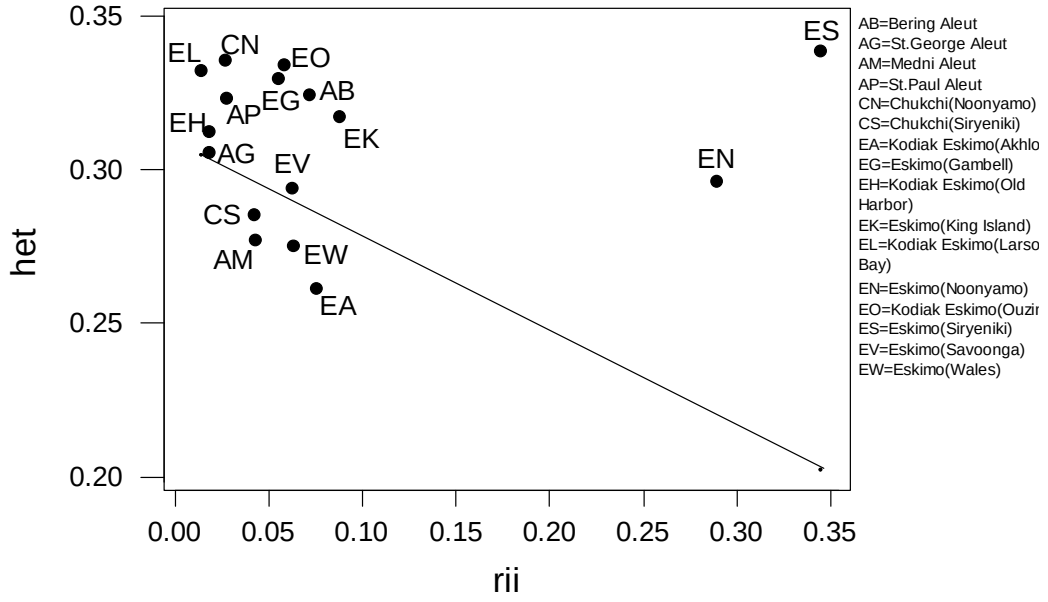

Figure 24 Heterozygosity vs r_{ii} plot based on classic genetic markers

Admixture Estimates

Admixture estimates based on mtDNA data are unnecessary for the majority of the Aleut populations presented in this study, given that the maternal lineages of individuals claiming Aleut maternal ancestry all belong to Native American haplogroups A and D. The one exception is the mixed Aleut sample from Bering Island (n=39), where only 64.1% of the lineages belongs to haplogroup D and can therefore be considered of Aleut ancestry. The remaining 35.9% of lineages belong to

haplogroups C, H, K, and "other", and represent Russian and other non-Aleut native maternal gene flow into the population.

Estimates of paternal gene flow into the Aleut populations are presented in Table 25. For the total Aleut sample, only 14.93% of the Y lineages can be considered native, with the remaining 85.07% of lineages representing non-Aleut admixture with Russians, Europeans, and/or Asians. When considered individually, the highest percentage of Aleut lineages are present in the Bering sample (27.27%). St. George Aleuts have a lower percentage of haplogroup Q (11.11%) than the Aleutian Aleuts (13.04%), and the St. Paul sample has the fewest Q lineages (10.00%), indicating there has been the most non-native male gene flow into this historically established population. The mixed Aleut sample from Bering Island does not have any Y lineages belonging to haplogroup Q, meaning the paternal contribution to this group is entirely non-Aleut (based on a small male sample size of six individuals).

Table 25 Admixture estimates based on Y haplogroup data

Population	n	Aleut (haplogroup Q)	Non-Aleut (other haplogroups)
Total Aleut	63	14.93%	85.07%
Aleutian Aleut	23	13.04%	86.96%
St. Paul Aleut	20	10.00%	90.00%
St. George Aleut	9	11.11%	88.89%
Bering Aleut	11	27.27%	72.73%
Mixed Aleut	6	0.00%	100.00%

Admixture estimates based on autosomal STR data were calculated only for the Bering Island community. Given that the mixed Aleut sample from Bering (n=33) is mainly of Aleut-Russian ancestry, the ADMIX 2.0 program was used to estimate

the contribution of these two parental populations to its nuclear gene pool. The results indicate that the Aleut contribution is 60.1%, and that of the Russians is 39.9%.

Sewall Wright's Statistics

Table 26 presents the harmonic means and effective breeding sizes for the historically established Aleut communities of Bering, St. Paul, and St. George. The harmonic mean, which adjusts for fluctuations in population size, was calculated for four generations, using sample sizes presented in Table 4 (chapter 2) for the Bering community. Both the harmonic mean of 272 and effective population size of 81 (using the standard 0.30 of the population size) were identical to the values calculated by Rychkov and Sheremetyeva (1972). This is not surprising given that the Table on which these estimates were based was taken from their publication. However, their estimates for the Pribilof Islands were much lower than the present study. Rychkov and Sheremetyeva (1972) estimated the harmonic mean of the Pribilof communities combined as 182, and N_e =31. This study estimates the harmonic means for St. Paul and St. George as 238 and 202, and effective population sizes as 71 and 61, respectively. The discrepancy between the two studies is partly due to the fact that their calculations were based on estimates of population size published by Russian priest Ivan Veniaminov in 1840, while those of the current study span a more recent time period (after 1872, see Table 3, chapter 2), during which there was apparently an increase in the number of individuals residing in these communities. In addition, their N_e was only 0.17 of the population size (compared to 0.30 used here). They based their N_e on the number of baidarkas (kayaks) recorded by Veniaminov, which was

thought to represent the number of men between the ages of 20 and 55, in other words, of reproductive age. According to Rychkov and Sheremetyeva (1972) there were more women than men in the communities at that time. The estimates of effective population size presented here are intermediate to the values calculated for other Aleut communities, ranging from 33 for Unga Aleuts to 127 for Unalaska Aleuts (Rychkov and Sheremetyeva 1972).

Table 26 Harmonic mean and effective population size

Population	Harmonic Mean	N_e
Bering	272	81
St. Paul	238	71
St. George	202	61

In order to test whether inter-generational genetic drift might have caused the loss of mitochondrial DNA haplogroup A from the Bering community, the variance due to stochastic processes was estimated using q=0.389 (the frequency of A for the Aleutian Aleuts presented in this study) and N_e=81. This gives a value of $\sigma^2_x = \pm$ 0.001 per generation, and 0.007 for seven generations (given a generation time of 25 years, and that Aleuts were first brought to Bering in 1825). Therefore, it is unlikely that the absence of haplogroup A among Bering Aleuts is due to intergenerational drift, but rather that this community was founded by closely related individuals (families) that were not representative of the larger Aleut population and lacked A to begin with. In other words, this is most likely due to founder effect.

CHAPTER FIVE: DISCUSSION

Genetic Composition and Gene Diversity

The mitochondrial DNA lineages present among the historically founded Aleut populations and the Aleutian Aleuts belong to Native American haplogroups A and D. The one exception to this is the mixed Aleut community of Bering, which will be discussed later. The mtDNA control region sequence data of this study are presented in part in Rubicz (2001), Rubicz *et al*. (2003), and Zlojutro *et al*. (2006). These studies indicate that as a group, the Aleuts are distinct from other populations in the North Pacific region due to their high frequency of haplogroup D. They lack the Eskimo-specific 265G mutation and share control region sequences with and appear to be most closely related to the Chukchi and Siberian Eskimo populations of Chukotka, rather than to Alaskan Eskimos or populations of the Kamchtaka Peninsula (the Koryaks, Itel'men, and Even). Genetic discontinuity between the Aleuts and Kamchatkan populations is supported by the results of SAMOVA analysis (Crawford 2007). Zlojutro *et al*. (2006) demonstrated that the network of Aleut mtDNA sequences is composed of three star-like clusters (A3, A7, and D2) that represent two expansion events. The first of these (consisting of A3 lineages) appears to have expanded at approximately 19,900 B.P. and represents a population ancestral to Eskimos, Aleuts, and Na-Dene, while the second event (composed of A7 and D2 lineages) is Aleut-specific and took place around 5,400 B.P. Recent research indicates that Aleuts residing in the Eastern-most communities have high frequencies of mtDNA haplogroup A and lower D frequencies, possibly due in part to admixture

with Eskimo populations during historic times (Crawford 2007). It now appears that the mtDNA haplogroups are linearly arranged, with haplogroup D highest among the western Aleut communities and haplogroup A highest in the East. There is a significant correlation between the mtDNA lineage distribution and geography based on Mantel tests ($r=0.72$, $p>0.000$), and spatial autocorrelation analysis indicates an isolation by distance model best describes the distribution of maternal genes along the Aleutian Island chain (Crawford 2007).

Information provided by the maternal markers in the present study indicates that all three of the historically founded Aleut populations, St. Paul, St. George, and Bering, have, to varying degrees, differentiated genetically from the other Aleuts who remained in the Aleutian archipelago, and from each other. Of these three communities, the mtDNA haplogroup frequencies and sequences of St. Paul are most similar to those of the Aleutian Aleuts. St. Paul has a slightly higher percentage of A, and only eleven different mtDNA lineages, compared to the twenty-one lineages present among Aleutian Aleuts. Even though there is slightly less genetic variability at the mtDNA locus for St. Paul, as measured by gene diversity, its overall similarity to the Aleutian Aleuts likely reflects the fact that it is the largest of the Pribilof Islands communities, and being on the U.S. side, it has remained in contact with the larger Aleut population.

The other Pribilof Islands community, St. George, has a much lower frequency of haplogroup A mtDNAs (17%), and only two different A haplotypes. Overall, St. George is characterized as having six different mtDNA lineages, and it

has a much lower level of gene diversity of 0.6, as compared to 0.8 for the Aleutian Aleuts. The differentiation of this community from the parental Aleut population, and also compared to the community of St. Paul, may be partly due to its smaller population size. It appears that genetic drift is responsible for the lack of diversity, particularly among A lineages, in this population.

Bering is the most divergent of the Aleut communities at the mtDNA locus, due to its fixation of haplogroup D. Haplogroup A lineages are absent from this population, and within haplogroup D, there are only two different lineages, 16129A-16223T-16271C-16362C and 16129A-16223T-16271C-16311C-16362C, which are separated by a single mutation. The low level of mtDNA variation is reflected in its unusually small gene diversity value of 0.2924. This value is particularly low when compared to other, non-Aleut, populations in the region, whose measures in this study range from 0.7 for the Haida, to 0.9 for the Koryaks. Genetic drift and the isolation of the Commander Islands Aleut communities from their U.S. counterparts are likely responsible in part for the decrease in mtDNA diversity among Bering Aleuts.

The fixation of haplogroup D in the Bering population and its high frequency (83%) in St. George have resulted in these communities resembling each other at the mtDNA locus, as demonstrated by the phylogenetic trees based on mtDNA RFLPs and sequence data. In the MDS plot, Bering is closest genetically to St. George, which in turn is intermediate between Bering and the Aleutian Aleuts. These analyses also demonstrate the genetic similarity between St. Paul and the Aleutian Aleuts. Overall, based on mtDNA data, the Aleut populations are genetically distinct from

other North American and Siberian populations, clustering with one another rather than non-Aleuts. Overall, this is likely due to the relative isolation of the Aleut population.

The Y chromosome lineages present among the Aleuts are largely of non-Native origin (I, J, N, R and "other"), representing gene flow predominantly from Russian and European males. Only the haplogroup Q lineages in these populations can be considered Native American, and therefore Aleut. This interpretation is based on Y chromosome STR and SNP research by Zegura *et al*. (2004) that indicates of the three Y chromosome haplogroups prevalent among Native Americans (accounting for 96% of Y chromosomes in a sample of 588 individuals), only Q and C represent early founding lineages, while the presence of R is likely the result of more recent European male gene flow. Haplogroup C is rare among members of the Eskimo-Aleut language family (its presence was noted in two individuals from the isolated Greenlandic Inuit Ittoqqortoormiit settlement), and as it is absent from the Aleuts in this study, the Native American paternal contribution to these samples is solely represented by haplogroup Q.

Based on Y haplogroup frequency data, the St. George community most closely resembles the parental Aleutian Aleut population. They have high frequencies of European haplogroup R (89% and 70%) and low frequencies of Q (11% and 13%, respectively). Both of these populations cluster with the Sioux in the NJ tree constructed from Y SNP data, which also has a relatively high R frequency (50%) and lower frequency of Q (25%). This relationship is due to shared non-Native gene

flow into the populations. In this analysis, the Aleut communities of St. Paul and Bering are more similar to each other than either is to the Aleutian Aleuts. They have similar frequencies of N (20% and 18%), and both have Q, R, I, and "other" haplogroups. Bering and St. Paul cluster with the Russians, Koryaks, and Siberian Eskimos, due to their sharing of haplogroups that are non-Native. Similarly, in the MDS plot St. George Aleuts are nearest to the Aleutian Aleuts, which cluster with the Russians and Amerindians. St. Paul is located between the Aleutian Aleuts and Bering Aleuts, and is also near the Greenland Eskimos.

The diversity measures based on Aleut Y STRs are high, which is likely a product of Russian/European admixture. The Bering Aleuts have the highest haplotype diversity (1.0), the result of each Y chromosome for which STR data are available belonging to a separate haplogroup. This is followed by similar estimates for St. Paul Aleuts and Aleutian Aleuts (0.96), and slightly lower diversity for St. George (0.92). These elevated Y diversity measures are contrary to the expectation for a reduction in gene diversity among the historically founded populations, and to the lower diversity levels exhibited by the maternal markers.

The autosomal STR markers, which are available only for the Bering community, are intermediate between the maternal and paternal markers. The mixed Aleut population of Bering is largely Aleut, with a smaller Russian component, likely introduced through the paternal side. On the phylogenetic tree, both the mixed Aleut and Aleut from Bering cluster with the Russians, a similar picture is provided by the R-matrix plot, in which the mixed-Aleut are intermediate between the Russians and

Aleuts, but slightly more proximal to the Russians. Heterozygosity measures for the mixed Aleut sample (0.88) are elevated in comparison to the Bering Aleuts (0.77), as would be expected for admixed individuals. Both of these values fall within the range of other Native American and Siberian populations. It should be noted that several of the autosomal markers in this study were characterized as having significantly lower observed than expected heterozygosity measures in some of the study populations, possibly as a result of genetic drift acting on these loci.

Research by Moscoso *et al*. (2007) also using autosomal markers (HLA), found no difference between Bering individuals who identified themselves as having one versus two Aleut parents. Analysis of HLA (human leukocyte antigen) markers resulted in the same genetic distance measures for ethnic Aleuts and admixed-Aleuts, and clustered both groups with the same populations (Saami, Finns, and Buryats) in the neighbor-joining trees. This suggests that there is a high level of Russian admixture into the Bering Aleut community, and also that self-identification of ethnicity in this population may not be accurate.

The analysis of classic genetic markers is not very informative, but does show the historically founded populations of Bering, Medni, and St. George clustering together in the R-matrix plot, with St. Paul appearing to have differentiated from these communities genetically. This is possibly a result of elevated non-Aleut male gene flow into this population. This is similar to the mtDNA results, where Bering and St. George appear to be closer genetically to each other than either is to St. Paul.

Reproductive Isolation of Aleut Communities

According to Crawford (2007), circumpolar, Arctic and Subarctic populations tend to be small in size and relatively isolated from one another. This is due in part to the ecology of the region which is unable to sustain large numbers of people. Travel in this part of the world is often difficult because of vast distances between settlements, seas that are impassible during certain times of year, mountains, icy conditions and extreme cold. Such factors often have an impact on the genetic structure of populations living at these locations. One example is Siberia, where the indigenous populations have been reproductively isolated from each other along an east-west axis, although an extensive river system has facilitated human movements in a north-south direction. As a result, there is an east-west gradient in the distribution of their genes.

Island communities tend to be closed to outside genetic influences, and for the small populations of the Aleutian archipelago this appears to have been the case during the time prior to Russian contact. The island chain spans a vast geographic range of 1200 miles, and travel between islands by the inhabitants is often difficult due to stormy seas, fog, rain, snow, sleet, and gale-force winds. This has likely contributed to the gradient of mtDNA haplogroups, which as previously mentioned is predominantly A among eastern inhabitants, and D among those in the west. But just how reproductively isolated are the historically-founded Aleut communities?

Even considering their remote location to the north of the Aleutian chain, the Pribilof Islands communities have remained in contact with their Aleutian neighbors

since their original founding. Aleuts were first brought to the Pribilofs in 1788 from Unalaska and Atka (in the eastern and central Aleutians) for the purpose of hunting fur seals for the Russians (Reedy-Maschner 2007, Elliot 1886). Recruitment of individuals from the Aleutians continued because there was a demand for workers for dangerous jobs related to the harvesting of fur seal pelts, which continued after sea otter hunting was banned in the Aleutian chain in 1911. This is reflected in the St. Paul and St. George Aleut communities exhibiting a variety of mtDNA haplogroup A and D sequence motifs, and C haplogroups observed by Merriwether *et a*l. (1995), possibly reflecting Athapascan admixture.

The situation was different, however, for the Commander Island Aleut communities on the Russian side. After Alaska (including the Aleutian and Pribilof Islands) was sold to the U.S. in 1867, the communities of Bering and Medni were effectively isolated from their relatives in the Aleutian Islands. The two communities were consolidated at the Bering location in 1969. Although the fixation of mtDNA haplogroup D in the modern Bering Aleut community is most likely due to founder effect, the lack of maternal gene flow from other Aleut communities may also be a contributing factor to the absence of haplogroup A lineages.

Evidence for paternal gene flow indicates the closure of the Bering community was not symmetric. A large percentage (73%) of non-Aleut Y lineages characterized in the Bering Aleut community is of Russian origin. This makes sense in light of historic documents indicating the governor of Bering encouraged Russian men (mainly soldiers) to marry Aleut women, as a way of increasing fertility and out

of concern for potential inbreeding among the Aleuts. Undoubtedly, this was also a means of controlling the population.

There has also been an influx of non-Native males into the Aleutian region, in pursuit of economic opportunities. First to arrive were Russian and Siberian fur traders. Federova (1973) describes the marriage of Aleut women to Russian men as way of reducing hostility and purposefully creating a "Creole" class, similar to the situation on Bering Island. The Creole class was christened as Russians, and Creole women preferentially married Russian or Creole men. These events resulted in the introduction of Russian male Y chromosomes into the Aleut population, while contributing to the preservation of Aleut maternal lineages. With a shift in economic focus in the Aleutian chain to fishing, a large number European-Americans and Scandinavians entered the region and married into Aleut villages. The largest Scandinavian impact was in the eastern Aleut communities, resulting in the development of commercial fishing industries (Reedy-Maschner 2007). Today, an estimated 87% of Aleutian Aleut male lineages are of non-Aleut origin.

Economic opportunities in the Pribilof Islands have also continued to draw non-Native males. St. Paul has always been the larger of the two communities, with more job opportunities, first related to seal hunting, and later the fishing and crabbing industries, and today this community has its own fish processing plant. There is an estimated 90% of non-Aleut male lineages in the St. Paul Aleut community, and 89% for St. George. While the community of Bering is the most closed of the historically-

founded Aleut communities, all three communities have experienced substantial non-Aleut gene flow through the paternal side.

Comparison of Markers (Maternal versus Paternal Histories)

The Aleut population histories vary considerably between the mitochondrial and Y Chromosome DNA markers. The maternal picture is one of a Native population with little or no female gene flow into the communities from outside sources, as demonstrated by the presence of only A and D mtDNA haplogroups. The lack of mtDNA diversity, particularly among the Bering Aleuts, appears to be the result of genetic drift and isolation from other Aleut communities. The paternal markers, in contrast, tell a story of substantial non-Aleut male admixture into the communities, accompanied by the presence of Russian and European haplogroups and increased diversity measures. This has largely erased the prehistoric phylogenetic relationships among Aleuts and other Native groups, producing phylogenies that do not make sense based on what is known of the ethnohistories and linguistic affiliations of the populations, and instead reflects shared non-Native admixture into the populations.

This disparity between female and male markers has also been shown in studies of other Native populations. For example, Bosch *et al*. (2003) found that in a sample of Greenlandic Inuit, while there was no maternal European contribution to the population (based on mtDNA analysis), approximately 58% of the Y lineages were of European origin. These male markers were traced to Scandinavia, and are thought to be either the result of gene flow from Icelandic Norse settlers 500 years

ago or eighteenth century Danish-Norwegian colonists into the Inuit population. Similarly, studies by Carvajal-Carmona *et al*. (2000), Mesa *et al*. (2000), and Santos *et al*. (1999) all found differences in admixture estimates for mtDNA and Y chromosome markers among Native American populations, indicating there was directional mating preferentially involving European men and indigenous women.

Shortly after Russians first made contact with the inhabitants of the Aleutian Islands in 1741, there was an influx of fur traders into the region in search of sea otter pelts. These individuals were exclusively male, and the majority were of Russian and Native Siberian ancestry (Black 1984). Conflict between Aleuts and these new arrivals was frequent, resulting in casualties on both sides. As the fur trade intensified between 1769 and 1775, violent encounters with Russian crews escalated, and Aleut men were specifically targeted (Reedy-Maschner 2007). It appears the killing of Aleut men was partly in order to gain access to Aleut women. As the Russians expanded eastward, they established permanent settlements, and forced Aleut men out of their villages to hunt otters and fur seals. This enabled the Russians to take over Native villages and marry Aleut women. According to Russian Orthodox priest Ivan Veniaminov, who lived in Unalaska and traveled throughout the region in the 1820s and 1830s, the number of Aleut women outnumbered Aleut men in most communities (Veniaminov 1984). These historic events help to explain the loss of many Aleut male lineages, and the introduction of Russian, and to a lesser degree Native Siberian, male Y chromosomes into the Aleut communities.

After the U.S. purchased Alaska in 1867, all Russians on the U.S. side were given three years to leave, or automatically assume citizenship (Reedy-Maschner 2007). Although many departed, they left behind their Russian Orthodox faith, which was adopted by the Aleuts, their surnames, and paternal lineages. The Alaska Commercial Company took over the fur hunting industry in the Aleutian region, although it was not as profitable as in previous years, and cattle and reindeer herds were introduced on many of the islands. This drew European-American and Scandinavian men into the region in pursuit of jobs. A policy of only allowing Native Alaskans and non-Native men married to Native Alaskan women the right to hunt was meant to conserve the dwindling sea otter population, but instead encouraged cross-cultural marriages. As the economic focus in the Aleutian chain shifted to cod fishing, an increasing number of Scandinavian men arrived, and married into Aleut villages.

It thus appears the loss of Aleut male lineages can be explained partially by violence suffered at the hands of Russian fur hunting crews, and the relocation of Aleut males. Preferential non-Native male and Aleut female marriage patterns introduced Russian, Scandinavian, and European-American male lineages into the populations, while maintaining the native Aleut maternal markers.

Genetic Drift and Admixture

As mentioned previously, the opposing forces of genetic drift and gene flow appear to be shaping the diversity present among the historically-founded Aleut communities, operating at the mtDNA and Y chromosome DNA loci, respectively.

For the mtDNA locus, positive scores for the neutrality test statistics (Tajima's D and Fu's Fs) for the historically founded Aleut populations (the one exception being St. George for Tajima's D), along with the low gene diversity values, indicate that the evolutionary force of genetic drift may be operating on the mtDNA locus in these groups. The most dramatic effect of genetic drift among the Aleut populations is seen in the fixation of mtDNA haplogroup D in the Bering Aleuts. According to a study of Commander Island Aleuts by Derbeneva *et al*. (2002), the absence of haplogroup A mtDNAs from the Bering Aleuts is due to the selective "genocide" of individuals carrying haplogroup A. But, given that A lineages are present in nearly 40% of the Aleutian Aleut individuals in this study (representing the parental population), it is unlikely that there was selective "genocide" against such a large number individuals in the Commander Islands, and that this completely erased their genetic contribution to the Bering gene pool. Intergenerational drift is also a poor explanation for the fixation of haplogroup D, given that Bering was founded only 175 years ago, representing approximately eight generations. Based on $N_e = 81$, the variance for mtDNA haplogroup A of 0.007 for this time frame does not adequately explain its loss from the Bering community. A better explanation is that the absence of haplogroup A lineages is the result of founder effect, where the original Aleut transplants to the Commander Islands consisted of families from Attu and Atka whose members had a disproportionately large number of mtDNA D2 haplotypes, and therefore were not representative of the parental Aleut population. Aleut individuals arriving from other locations in the Aleutian and Pribilof Islands to

the Commanders after their initial peopling appear not to have made a significant genetic contribution to modern population, or perhaps they also carried high frequencies of haplogroup D with them. An alternative explanation is that the historic records documenting the settlement of the Commander Islands are in error. Although less pronounced, genetic drift also appears to have had an impact on the Aleut community of St. George, resulting in fewer mtDNA lineages and decreased gene diversity in that population.

The evolutionary force of gene flow (admixture) has had a significant impact on the distribution of Y chromosomes among the historically founded Aleut communities and the parental Aleutian Aleut population, replacing Native Y lineages and increasing diversity measures. Admixture estimates range from 90% for the St. Paul Aleuts, to 73% for the Bering Aleuts. The Aleutian Aleuts are estimated as having nearly 87% non-Aleut paternal lineages. For the mixed Aleut population of Bering, 100% of the Y lineages are of non-Native ancestry. These paternal lineages are primarily of European and Russian ancestry, although it is possible there has been a small amount of Asian gene flow, as represented by the presence of Eurasian haplogroup N, and possibly "other" haplogroups.

For the mtDNA lineages, non-Aleut admixture estimates were only necessary for the mixed Aleut sample from Bering, given that all other Aleut mtDNA were Native A and D lineages. In this group, 64% of the haplogroups were of Aleut ancestry. The remaining 36% of the mtDNAs appear to represent admixture with non-Aleut native women, possibly brought to the Commander Islands by Russians from

surrounding regions such as Kamchatka, and more recent gene flow from Russian women (historic documents do not indicate that Russian women were part of the original founding of the Commander Island communities). Although the sample of St. Paul Aleuts presented in this study has only A and D lineages, this is not the case for a study by Merriwether *et al*. (1995). In their study, in addition to the A and D lineages, C and "other" lineages were also present. Rubicz *et al*. (2003) proposed the C lineages were the result of recent admixture with Athabascans from mainland Alaska (10.5%), while the "other" lineages (0.5%) were likely of European origin. These observations are based on the fact that only A and D lineages were present in the sample collected by Rubicz *et al*. (2003) for which genealogical information were collected, and in the analysis of ancient Aleut samples by Hayes and O'Rourke (2000).

Autosomal admixture estimates for the mixed Aleut sample of Bering were calculated as being 60% Aleut and 40% Russian. This largely reflects the asymmetric contribution of Russian males to the gene pool, although for the mixed Aleut sample, Russian women also appear to have made a contribution.

The plots of heterozygosity versus distance from the centroid (rii) were used in this study to examine the interactions of gene flow and genetic drift and their impact on the various markers used in this study (mitochondrial, Y chromosome, and autosomal). At the mtDNA locus, all of the historically-founded populations fall below the line, indicating there is a marked decrease in gene diversity, particularly among the Bering Aleuts. Only the Aleutian Aleuts have a slightly higher than

expected gene diversity measurement. For the Y chromosome locus, the Aleut populations are located in the upper left corner, indicating they have experienced a substantial amount of male admixture, especially the St. Paul and Aleutian Aleuts. The plot for the autosomal DNA markers places both mixed Aleuts and Aleuts of Bering above the theoretical regression line, indicating higher heterozygosity measures probably due to admixture, although they are further from the centroid. Similarly, the classic genetic markers plot places all of the historically founded Aleut populations on or above the line, with the exception of Medni Aleuts. These results are again suggestive of increased male gene flow into the Aleut populations resulting in higher than expected gene diversity measures at the Y locus, decreased diversity measures at the mtDNA locus, and intermediate values for the autosomal DNA markers.

Differentiation of Aggregated Populations

Of the historically founded Aleut communities, Bering has differentiated most from the parental Aleut population. The most dramatic difference is seen in the fixation of mtDNA subhaplogroup D2 in this community, which appears to be the result of a founder effect, followed by isolation from the parental Aleut population after 1867. Bering was founded by Aleut individuals from the western and central regions of the Aleutian archipelago, who apparently had a disproportionate number of D lineages. Although recent research has shown that mtDNA D haplogroups are present in higher frequencies in the western Aleutians (Crawford 2007), this does not explain the total absence of haplogroup A among Bering individuals. It is possible the

Aleuts who were relocated to the Commander Islands were closely related (members of the same family) rather than a random sample of individuals, and thus were not representative of the parental population.

A similar situation of population fission along familial lines was described by Crawford *et al*. (1989) among Mennonite communities. They found that the fission-fussion model best described the genetic diversity for these groups. The Mennonite groups were originally founded as a composite of individuals from the Netherlands, Germany, Switzerland, Moravia, Alsace, and Tirol. The Alexanderwohl community settled in the U.S. in 1874, and underwent fission upon arrival, splitting into the Nebraska Henderson and Kansas Alexanderwohl communities. In 1909, Tabor split off from the Kansas Alexanderwohl Mennonites, and then in 1920, Goessel split off from the same parental population. The characterization of classic genetic markers in these populations demonstrated that while the Kansas Alexanderwohl Mennonites were similar genetically to western European populations, the Tabor and Goessel offshoots were genetically distinct. Given the short generation time (2 to 3 generations) the most likely explanation for these differences was that the fissioning of these populations represented a nonrandom division of the Mennonite gene pool along family lines. Indeed, only 5% of the original surnames were present in the offshoot communities.

For the other historically founded Aleut populations, St. Paul is most similar to the Aleutian Aleut communities, based on the maternal data, while St. George

clusters more closely with the Bering community because of their higher percentage (83%) of haplogroup D.

On the paternal side, the Bering community is again distinct, having the largest number of male lineages belonging to Native American haplogroup Q (27%). For these data, St. George (not St. Paul) is closest genetically to the parental Aleutian Aleuts population. They have similar frequencies of European haplogroup R, and Native American haplogroup Q. The Bering and St. Paul communities resemble one another due to the presence of male haplogroups N, Q, R, I, and "other".

In conclusion, the historic founding of the Bering, St. Paul, and St. George Aleut communities has had genetic consequences. All three communities differ from the parental Aleutian Aleut population, as exhibited by decreased mtDNA diversity measurements, likely the result of genetic drift operating in these small island populations. This is most dramatic for the Bering population, which is genetically the most divergent of the aggregated communities, and whose members have mtDNAs belonging only to haplogroup D. Most likely the loss of haplogroup A lineages is due to a kin-structured founding of the Commander Islands, followed by their isolation from other Aleut communities after the sale of Alaska to the U. S. The Y chromosome markers for all of the Aleut communities exhibit high gene diversity measures and are mainly of non-Native origin, indicating that the evolutionary force of gene flow has impacted this locus. The loss of Aleut male lineages can be partially explained by violence suffered by Aleut males at the hands of Russian fur hunting crews, and their relocation from villages to prime fur mammal hunting grounds.

Preferential non-Native male and Aleut female marriage patterns introduced Russian, Scandinavian, and European-American male lineages into the Aleut communities, while maintaining the native Aleut maternal markers.

CHAPTER SIX: CONCLUSION

This study characterized the mitochondrial, Y chromosome, and autosomal DNA diversity for the historically established Aleut populations located in the Commander and Pribilof Islands, in order to determine how their founding and other historic events have impacted their genetic diversity. Questions concerning whether these populations have experienced reduced genetic diversity, how closed the island communities are to outside influence, whether there has been symmetry in gene flow, the genetic effects of genetic drift and gene flow and their interaction, and which of the recently founded Aleut communities differentiates most from the parental Aleuts, are addressed.

Theoretically, the genetic variation present in small island populations should be reduced. For the autosomal STRs, the average diversity of Bering Aleuts was 0.776, and 0.882 for the mixed Aleuts, both of which are similar to the diversity values for other Native American and Siberian populations. The Y chromosome gene diversity for Bering Aleuts (1.0) was slightly higher than that of other Aleuts (St. Paul=0.9591, St. George=0.9167, and Aleutian Aleuts=0.9565) and comparative populations. This reflects a disproportionate amount of Russian male gene flow into the Bering community. There was also non-Aleut gene flow into the other Aleut communities as well. In contrast, at the mitochondrial DNA locus, the Bering Aleuts had gene diversity=0.29, compared to 0.72 in St. Paul, and 0.56 in St. George. Given that the Aleutian Aleuts have gene diversity=0.77, there is a tremendous reduction in genetic variation on the maternal side for the Bering community. This is the result of

founder effect along with the closure of the Bering gene pool in 1867 when the Aleutian Islands were purchased from the Russians. After this time, St. Paul and St. George continued to experience gene flow from the other islands.

Island populations tend to be closed, and travel, particularly in the subarctic Aleutian Islands zone, is especially challenging. There are large inter-island distances, and adverse weather conditions are frequent. The closure of the Bering gene pool due to its loss of contact with the American Aleut communities was not symmetric. Gene flow from non-Aleut males was substantial compared to little female gene flow into the population. This is seen by the elimination of mtDNA haplogroup A and fixation of haplogroup D in the community. Despite their isolated location in the Bering Sea, St. Paul, and to a lesser degree, St. George continued to experience admixture with Aleuts from the archipelago, and also with non-Aleut individuals through the paternal side.

This asymmetry in female versus male gene flow into the Aleut populations is reflected in the mtDNA and Y chromosome DNA markers. The female side, with the exception of the Bering mixed Aleut populations, was exclusively Native American. Only mtDNA haplogroups A and D were present. On the other hand, the male lineages were largely of non-Native origin. These results are similar to studies of other Native American populations, and reflect a preferred marriage pattern of European males and indigenous women. In the case of the Aleuts, non-Native males marrying into the communities were mainly of Russian, European-American, and Scandinavian ancestry. The small percentage of Aleut male lineages present in the

modern Aleut populations can also be attributed to their decrease in numbers during the early Russian period at the hands of fur hunting expedition crews along with their removal from Aleut villages for the pursuit of fur bearing mammals.

It appears that the self-identification of ethnicity by participants is not completely accurate. Individuals from the Bering community claiming two Aleut parents had a substantial amount of non-Aleut admixture. This is demonstrated by a study of bi-parentally-inherited HLA markers that found no difference between ethnic Aleuts and admixed Aleuts, both of which clustered with European, Scandinavian, and Asian populations, rather than Eskimo, Na-Dene and Amerindians (Moscoso *et al*. 2007). In the present study, autosomal STR data for Bering demonstrated that both the Aleuts and mixed Aleuts were closest genetically to the Russian sample, again indicating there has been considerable gene flow from that population.

The evolutionary force of genetic drift appears to be operating at the mtDNA locus to decrease the amount of genetic variability in the study populations. For the Y chromosome locus, the introduction of foreign male lineages has not only increased the heterozygosity of paternal markers, it has also obscured the phylogenetic relationships among the Aleuts and other Native groups. The autosomal markers are intermediate between these two extreme pictures, with increased heterozygosity measures for the mixed Aleut population of Bering Aleuts in comparison to the Bering Aleuts, but also lower than expected heterozygosities for some of the autosomal loci.

Of the historically founded populations in this study, the community of Bering in the Commander Islands, Russia, was the most divergent for both the maternal and paternal loci. In addition to the fixation of the mtDNA D2 subhaplogroup, Bering individuals had the highest percentage of Y chromosomes belonging to Native American haplogroup Q. The other two study populations, St. Paul and St. George, were more similar genetically to the parental Aleutian Aleut population. St. Paul more closely resembled the Aleutian Aleuts at the mtDNA locus, and St. George was genetically the closest to the Aleutian Aleuts at the Y chromosome locus. These two historically founded populations remained in contact with each other and the Aleuts of the Aleutian chain.

This research demonstrates that unique historic events can have evolutionary consequences through the re-distribution of genes in human populations. The founding of Bering, St. George, and St. Paul has resulted in the genetic differentiation of these communities. While each community is characterized by its own gene frequency distribution for the mtDNA and Y chromosome loci, the general trend is for decreased mtDNA versus increased Y chromosome heterozygosity levels. The loss of Aleut Y lineages from the study populations, along with gene flow from non-Native males, and the fixation of mtDNA haplogroup D in the Bering community, have irreversibly changed the genetic landscape of this region.

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Appendix A: Informed Consent Statement

The Department of Anthropology at the University of Kansas supports the practice of protection for human subjects participating in research. The following information is provided for you to decide whether you wish to participate in the present study. You should be aware that even if you agree to participate, you are free to withdraw at any time without penalty.

We are interested in reconstructing the origins and migrations of the Aleut people, using molecular genetic information. You will be participating in one session that should require approximately one half hour of your time. During that time you will be interviewed about your family relations and history and either two buccal smears or a blood sample will be taken. The buccal smear technique consists of a sterile wooden applicator being gently stroked across the cheeks and gums, followed by rinsing the mouth with distilled water.

The DNA extracted from the buccal smears will be used solely to reconstruct the history of the Aleut people. Although participation will not directly benefit you, we believe that the information will be useful in revealing the origins of Aleut people and their connections to Siberian, Inuit, and Native American populations. All DNA will be used up in the analysis. Only personnel working directly on the Aleut project will have access to the DNA.

Your participation is solicited although strictly voluntary. We assure you that your name will not be associated in any way with the research findings. The information will be identified only by a code number.

If you would like additional information concerning this study before or after it is complete, please feel free to contact me by phone or mail.

Sincerely,

Michael H. Crawford, Ph.D.
Principal Investigator
Department of Anthropology
University of Kansas, Lawrence, KS 66045
785-864-4170

_____

Signature of participant agreeing to participate

With my signature I affirm that I am at least 18 years of age and have received a copy of the consent form.

Appendix B: Samples Sizes for Study Populations by Molecular Marker

Y SNPs:

Population	n	Reference
Siberian Eskimo	22	Karafet et al. 2006
Alaskan Eskimo	7	Karafet et al. 2006
Greenland Eskimo	60	Karafet et al. 2006
Tanana	12	Karafet et al. 2006
Cheyenne	44	Karafet et al. 2006
Sioux	44	Karafet et al. 2006
Southwest	10	Karafet et al. 2006
Pima	24	Karafet et al. 2006
Pueblo	18	Karafet et al. 2006
Apache	96	Karafet et al. 2006
Navajo	78	Karafet et al. 2006
Mixtec	28	Karafet et al. 2006
Zapotec	16	Karafet et al. 2006
Mixe	12	Karafet et al. 2006
Russian	15	this study
Even	10	this study
Koryak	11	this study
Bering Aleut	11	this study
Aleutian Aleut	23	this study
St. Paul Aleut	20	this study
St. George Aleut	9	this study

Y STRs:

population	n	reference
Russian	27	This study
Even	10	This study
Koryak	11	This study
Greenland_Eskimo	69	Willuweit et al. 2007
Aleutian_Aleuts	24	This study
Bering_Aleuts	11	This study
St.Paul_Aleuts	19	This study
St.George_Aleuts	9	This study

mtDNA RFPLs:

Population	n	Reference
Aleutian Aleuts	108	this study
St. Paul Aleuts	54	this study
St. George Aleuts	29	this study
Bering Aleuts	35	this study
Asian Eskimo	50	Torroni et al 1993b
Coastal Chukchi	46	Sukernik et al 1996
Dogrib	154	Merriwether et al 1995
Old Harbor Eskimo	115	Merriwether et al 1995
Ouzinkie Eskimo	41	Merriwether et al 1995
Gambell Eskimo	50	Merriwether et al 1995
Savoonga Eskimo	49	Merriwether et al 1995
Even	63	this study
Haida	25	Torroni et al 1993a
Inuit	30	Lorenz and Smith 1996
Itel'men	47	Schurr et al. 1999
Koryak	155	Schurr et al. 1999
Ojibwa	28	Torroni et al 1993a

mtDNA HVS-I Sequences:

Population	n	Reference
Aleutian Aleut	108	this study
Bering Aleut	35	this study
St. Paul Aleut	54	this study
St. George Aleut	29	this study
Chukchi	65	Starikovskaya et al. 1998
Siberian Eskimo	77	Starikovskaya et al. 1998
Koryak	147	Schurr et al. 1999
Itel'men	46	Schurr et al. 1999
West Greenland Eskimo	82	Saillard et a. 2000
Athabascan	21	Shields et al. 1993
Haida	41	Ward et al. 1993
Bella Coola	40	Ward et al. 1993
Even	49	this study

Autosomal STRs:

Population	n	reference
Aleut Only	34	this study
Mixed Aleut	33	this study
Russian	32	this study
Even	59	this study
Koryak	22	this study
Athabascan	101	Budowle et al. 2002
Inupiat	109	Budowle et al. 2002
Yupik	100	Budowle et al. 2002
N Ontario	125	Budowle et al. 2001
Salishan	93	Budowle et al. 2001
Chinese	111	Budowle et al. 2001
Japanese	153	Budowle et al. 2001
Korean	103	Budowle et al. 2001
Vietnamese	213	Budowle et al. 2001
Arsario	21	Guarino et al. 1999
Kogi	21	Guarino et al. 1999
Ijka	14	Guarino et al. 1999
Wayuu	14	Guarino et al. 1999

Appendix C: Y SNP laboratory analysis completed at Dr. Deka's Laboratory

1) YAP Insertion Protocol:

a) PCR mix (per sample): 0.5µL DNA; 5.5 µL ddH_2O; 1.0µL 10X buffer; 1.0µL dNTPs; 0.4µL Mg^{++}; 1.0µL YAP primers*; 0.5µL TRITON; 0.1µL Taq

b) Thermal Profile:94ºC for 2 minutes; 35 cycles of 94ºC for 1 minute, 51ºC for 1 minute, 72ºC for 1 minute; 72ºC for 5 minutes; final hold at 4ºC

c) Ran out on 2% agarose gel, stained with ethidium bromide, at 130 milliamps for 45 minutes (fragments w/Yap = 455bp, fragments w/o YAP = 150bp)

*YAP FOR: CAGGGGAAGATAAAGAAATA
YAP REV: ACTGCTAAAAGGGGATGGAT

2) RPSY (M130) Protocol:

a) PCR mix (per sample): 1.0µL DNA; 4.6 µL ddH_2O; 1.0µL 10X buffer; 1.0µL dNTPs; 0.8µL Mg^{++}; 1.0µL M130 primers*; 0.5µL TRITON; 0.1µL Taq

b) Thermal Profile:94ºC for 2 minutes; 40 cycles of 94ºC for 30 seconds, 55ºC for 30 seconds, 72ºC for 30 seconds; 72ºC for 5 minutes; final hold at 4ºC

c) Digestion mix (per sample): 2.2µL Mg^{++}; 0.4µL Bsl I; 0.5µL ddH_2O (ran digest overnight)

d) Ran out on 2% aragose gel for 45 minutes (no cut = samples w/ M130: C→T, 205 bp band. Ancestral = 162 + 43 bp bands)

*M130 FOR: TATCTCCTCTTCTATTGCAG
M130 REV: CCACAAGGGGAAAAAACAC

3) M89 Protocol:

a) PCR mix (per sample): 0.5µL DNA; 5.5 µL ddH_2O; 1.0µL 10X buffer; 1.0µL dNTPs; 0.4µL Mg^{++}; 1.0µL M89 primers*; 0.5µL TRITON; 0.1µL Taq

b) Thermal Profile:94ºC for 2 minutes; 35 cycles of 94ºC for 30 seconds, 55ºC for 30 seconds, 72ºC for 30 seconds; 72ºC for 5 minutes; final hold at 4ºC

c) Digestion mix (per sample): 2.2µL Mg^{++}; 0.4µL Nla III; 0.5µL ddH_2O (ran digest overnight)

d) Ran out on 3.2% aragose gel (cut = samples w/ M89: T→C, 67 bp + 20 bp bands. Ancestral = 87 bp band)

*M89 FOR: ACAGAAGGATGCTGCTCAGCTT
M89 REV: GCAACTCAGGCAAAGTGAGACAT

4) M9 Protocol:

a) PCR mix (per sample): 1.0µL DNA; 5.0 µL ddH_2O; 1.0µL 10X buffer; 1.0µL dNTPs; 0.4µL Mg^{++}; 1.0µL M9 primers*; 0.5µL TRITON; 0.1µL Taq

b) Thermal Profile:94ºC for 2 minutes; 35 cycles of 94ºC for 30 seconds, 56ºC for 30 seconds, 72ºC for 30 seconds; 72ºC for 5 minutes; final hold at 4ºC

c) Digestion mix (per sample): 2.2µL Mg^{++}; 0.4µL Bam HI; 0.5µL ddH_2O (ran digest overnight)

d) Ran out on 4.0% aragose gel for 75 minutes (no cut = samples w/ M9: C→G, 210 bp band. Ancestral = 190 + 20 bp bands)

*M9 FOR: GCAGCATATAAAACTTTCAGG
M9 REV: GCTTGAGCAAAGTTAGGTTTT

5) M175 Protocol:

a) PCR mix (per sample): 1.0µL DNA; 5.0 µL ddH_2O; 1.0µL 10X buffer; 1.0µL dNTPs; 0.4µL Mg^{++}; 1.0µL M175 primers*; 0.5µL TRITON; 0.1µL Taq

b) Thermal Profile:94ºC for 2 minutes; 35 cycles of 94ºC for 30 seconds, 56ºC for 30 seconds, 72ºC for 30 seconds; 72ºC for 5 minutes; final hold at 4ºC

d) Ran out on 3.0% aragose gel for 45 minutes (no cut = samples w/ M175: 5 bp deletion: 112 bp band. Ancestral = 117 bp band)

*M175 FOR: TTGAGCAAGAAAAATAGTACCCA
M175 REV: CTCCATTCTTAACTATCTCAGGGA

6) TAT (M46) Protocol:

a) PCR mix (per sample): 1.0µL DNA; 5.0 µL ddH_2O; 1.0µL 10X buffer; 1.0µL dNTPs; 0.4µL Mg^{++}; 1.0µL M46 primers*; 0.5µL TRITON; 0.1µL Taq

b) Thermal Profile:94ºC for 2 minutes; 35 cycles of 94ºC for 30 seconds, 56ºC for 30 seconds, 72ºC for 30 seconds; 72ºC for 5 minutes; final hold at 4ºC

c) Digestion mix (per sample): 2.2µL Mg^{++}; 0.4µL Nla III; 0.5µL ddH_2O (ran digest overnight)

d) Ran out on 3.5% aragose gel for 45 minutes (no cut = samples w/ M46: T→C, 112 bp band. Ancestral = cut)

*TAT FOR: GACTCTGAGTGTAGACTTGTGA
TAT REV: GAAGGTGCCGTAAAAGTGTGAA

7) M45 Protocol:

a) PCR mix (per sample): 1.0µL DNA; 5.0 µL ddH_2O; 1.0µL 10X buffer; 1.0µL dNTPs; 0.4µL Mg^{++}; 1.0µL M45 primers*; 0.5µL TRITON; 0.1µL Taq

b) Thermal Profile:94ºC for 2 minutes; 40 cycles of 94ºC for 30 seconds, 51ºC for 30 seconds, 72ºC for 30 seconds; 72ºC for 5 minutes; final hold at 4ºC

c) Digestion mix (per sample): 2.0µL NEBuffer 4; 0.4µL Bam HI; 7.6µL ddH_2O (ran digest overnight)

d) Ran out on 3.0% aragose gel for 60 minutes (no cut = samples w/ M45: G→A, 162 bp band. Ancestral = 140 + 22 bp bands)

*M45 FOR: ATTGGCAGTGAAAAATTATAGCTA
M45 REV: TGCCTTTGCTACAACTCTCCTA

8) M3 Protocol (primer-specific PCR):

a) PCR mix w/ T (per sample): 1.0µL DNA; 5.0 µL ddH_2O; 1.0µL 10X buffer; 1.0µL dNTPs; 0.4µL Mg^{++}; 1.0µL (T) primer; 0.5µL TRITON; 0.1µL Taq

b) PCR mix w/ C (per sample): 1.0µL DNA; 5.0 µL ddH_2O; 1.0µL 10X buffer; 1.0µL dNTPs; 0.4µL Mg^{++}; 1.0µL (C) primer; 0.5µL TRITON; 0.1µL Taq

c) Thermal Profile:94ºC for 2 minutes; 35 cycles of 94ºC for 30 seconds, 55ºC for 30 seconds, 72ºC for 30 seconds; 72ºC for 5 minutes; final hold at 4ºC

d) Ran out on 2.0% aragose gel (band = samples w/ M3: C→T. Ancestral = no band)

M3 FOR: TAATCAGTCTCCTCCCAGCA
M3 REV: AAAATTGTGAATCTGAAATTTAAGG

9) M173 Protocol (run at KU):

a) PCR mix w/ T (per sample): 2.0µL DNA; 10.8 µL ddH_2O; 2.5µL 10X buffer; 0.5µL dNTPs; 4.0µL Mg^{++}; 2.5µL FOR primer; 2.5µL REV primer; 0.2µL Taq

b) Thermal Profile:94ºC for 2 minutes; 35 cycles of 94ºC for 30 seconds, 44ºC for 30 seconds, 72ºC for 30 seconds; 72ºC for 5 minutes; final hold at 4ºC

c) Digestion mix (per sample): 2.0µL NEBuffer 4; 1.0 µL BSA; 0.5µL HpyCH4VI; 9.0µL ddH_2O (ran digest overnight)

d) Ran out on 3.0% aragose gel for 120 minutes (no cut = samples w/ 173 A→C. Ancestral = cut)

*M173 FOR: AAGAAATGTTGAACTGAAAGTTGAT
M173 REV: AGGTGTATCTGGCATCCGTTA

Appendix D: Koryak mitochondrial DNA HVS-I sequences

	16093	16111	16124	16129	16223	16232	16242	16261	16265	16290	16298	16319	16327	16362	Haplogroup	n
Cam ref	T	C	T	G	C	C	C	C	A	C	T	G	C	T		
KOR01	.	.	.	.	T	.	T	.	.	T	.	A	.	.	A	6
KOR02	.	.	.	.	T	.	T	.	.	.	.	A	.	.		1
KOR03	.	T	.	.	T	.	T	.	G	T	.	A	.	C		1
KOR04	.	T	.	.	T	.	.	.	G	T	.	A	.	C		1
KOR05	.	.	C	.	T	.	.	.	.	.	C	.	T	.	C	4
KOR06	C	.	.	.	T	T	.	T	.	T	.	.	.	C	D	1
KOR07	C	.	.	A	T	.	.	.	.	.	.	.	.	.		1
KOR08	.	.	.	A	T	.	.	.	.	.	.	.	.	.		1

Appendix E:

Russian mitochondrial DNA HVS-I sequences

	16069	16126	16129	16163	16172	16183	16189	16223	16230	16234	16239	16256	16261	16270	16278	16286	16293	16296	16298	16304	16311	16319	16325	16362	HP	n
Cam.	C	T	C	T	G	C	T	C	C	C	C	C	C	A	C	A	A	C	C	T	T	C	C	T		
RU01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H	2
RU02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		2
RU03	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1
RU04	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C		1
RU05	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1
RU06	.	C	.	.	.	C	.	.	.	.	.	.	.	G	.	.	G	.	.	.	C	.	.	.		1
RU07	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	T	T	.		1
RU08	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	K	3
RU09	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.		1
RU10	.	.	A	.	.	.	.	.	.	.	T	.	.	.	T	.	.	T	.	.	.	.	.	.		1
RU11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	OT	1
RU12	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1
RU13	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.		1
RU14	.	.	.	C	.	.	A	C	.	T	.	.	T	.	.	.	.	.	.	T	.	.	.	.		1
RU15	T	.	.	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1
RU16	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.		1

OT=other haplogroups

Appendix F: Neighbor-joining tree based on Y STRs