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South from Alaska: A Pilot aDNA Study of Genetic History on the Alaska Peninsula and the Eastern Aleutians

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Abstract The Aleutian Islands were colonized, perhaps several times, from the Alaskan mainland. Earlier work documented transitions in the relative frequencies of mtDNA haplogroups over time, but little is known about potential source populations for prehistoric Aleut migrants. As part of a pilot investigation, we sequenced the mtDNA first hypervariable region (HVRI) in samples from two archaeological sites on the Alaska Peninsula (the Hot Springs site near Port Moller, Alaska; and samples from a cluster of sites in the Brooks River area near Katmai National Park and Preserve) and one site from Prince William Sound (Mink Island). The sequences revealed not only the mtDNA haplogroups typically found in both ancient and modern Aleut populations (A2 and D2) but also haplogroups B2 and D1 in the Brooks River samples and haplogroup D3 in one Mink Islander. These preliminary results suggest greater mtDNA diversity in prehistoric populations than previously observed and facilitate reconstruction of migration scenarios from the peninsula into the Aleutian archipelago in the past.

Hrdlička's (1945) inference that the modern population of the Aleutian Islands derived from a migration of people from mainland Alaska through the Alaska Peninsula who replaced the original inhabitants of the island chain approximately 1,000 years ago was predicated on his assessment of change in cranial form over time. Hrdlička considered the earliest population in the island chain, represented at the time by the Chaluka midden burials [Aigner 1978; Aigner et al. 1976; Laughlin 1963; McCartney and Turner 1966; Veltre and Smith 2010 (this issue)], to be Pre-Aleut, whereas the later population was indistinguishable from the modern Aleut population and hence was termed simply Aleut. Subsequently, Laughlin and Marsh (1951) used the terms Paleo-Aleut and Neo-Aleut to refer to Hrdlička's two prehistoric morphological groups. We adopted Laughlin's terminology (Smith et al. 2009) to unambiguously distinguish the prehistoric population(s) of the Aleutian archipelago (i.e., Paleo- and Neo-Aleuts) from the modern population of the region (Aleuts).

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Hrdlička's interpretation of Aleutian prehistory has been challenged on both archaeological (Laughlin 1980; Laughlin and Marsh 1951) and linguistic grounds [Woodbury 1984; see also Berge 2010 (this issue)] but has been tested only rarely with the type of data that led to its formulation, that is, biological data (e.g., Moorrees 1957; Ousley 1995; Turner 1967, 1974, 1991). Although Laughlin collected some data on biological variation among Aleut communities in the 1950s (Laughlin and Marsh 1951), no real effort to document the pattern of biological variation among Aleut populations, or their possible progenitors, was attempted until the initiation of recent genetic studies [Coltrain et al. 2006, 2010 (this issue); Crawford et al. 2010 (this issue); Hayes 2002; Hayes et al. 2003; O'Rourke et al. 2000; Rubicz et al. 2003; Smith et al. 2009; Zlojutro et al. 2006).

With the advent of new molecular genetic methods in the 1980s, it became possible to access DNA in prehistoric samples and therefore to characterize patterns of genetic variation in prehistory. This innovation made possible the more direct assessment of ancestor-descendant relationships and the direct testing of archaeologically derived hypotheses regarding prehistoric population movements. In earlier work we documented the distribution of mtDNA haplogroups in prehistoric inhabitants of the eastern Aleutians (Hayes 2002; Hayes et al. 2003; Smith et al. 2009), compared them to the distribution of mtDNA variation in contemporary Aleut communities (Rubicz et al. 2003; Zlojutro et al. 2006), and made inferences regarding the origin of Aleut populations and the nature of prehistoric colonizations to the area. In the current pilot study we report sequence variation in the first hypervariable region (HVRI) of the mitochondrial genome of prehistoric inhabitants from three different regions (two on the Alaska Peninsula and one further north in Prince William Sound) in an effort to refine our understanding of population movement from the mainland to the Aleutian Islands over time. Many of the samples examined molecularly here have also been directly dated and characterized for isotopic profile [Coltrain 2010 (this issue)].

Materials and Methods

Samples. The samples studied here come from three regions and several archaeological sites on or near the Alaska Peninsula (Figure 1). The oldest material comes from the Hot Springs site located on the Bering coast of the Alaska Peninsula near Port Moller, Alaska (Dumond et al. 1975; Okada and Okada 1974; Weyer 1930; Workman 1966). This large site is well known archaeologically and has been intensively studied in recent years to elucidate the prehistoric cultural boundary between Aleut and Yupik Eskimo peoples on the Alaska Peninsula (Dumond 1987a; Maschner 1999). Samples from Mink Island derive from the excavation of a single archaeological site by the National Park Service in the 1980s (Jeanne Schapf, personal communication, 2004). Mink Island is a small island located in the North Pacific Ocean just off the Alaska mainland in Prince William Sound. It is included here so that we have a representative of a more northerly prehistoric population not on the peninsula proper. Finally, a group of samples from the interior of the northern region

Figure 1. Geographic location of Alaska Peninsula archaeological sites.

of the peninsula come from excavations directed by Don Dumond of the University of Oregon in the Naknek/Brooks River area (Dumond 1987b; Harritt 1988; Hilton 1998). A number of samples derive from excavations at the Paugvik site on the Naknek River, a part of the Brooks River drainage (Dumond 1981), and the remainder of this group comes from excavations of a cluster of sites along the Brooks River itself (Dumond 1987b; Harritt 1988).

The genome of choice for ancient DNA analyses has traditionally been the mitochondrial genome, and it is also targeted in this pilot project. Unlike the biparentally inherited genome of the cell nucleus, the small (16,569 base pairs) circular genome of the mitochondria is exclusively maternally inherited. Lacking the molecular editing machinery of the nuclear genome, mtDNA accumulates substitutions at a rapid rate, permitting examination of a population's genetic history in an archaeological time frame. Importantly, each mitochondrion contains potentially hundreds of copies of the mtDNA molecule, and with numerous mitochondria per cell, thousands of mtDNA molecules may be present for every copy of the nuclear genome. This high copy number feature of mtDNA makes it especially useful for aDNA studies because it maximizes the likelihood of amplifying a target sequence from even seriously degraded molecular material.

The large number of substitutions in mtDNA result in many single nucleotide polymorphisms (SNPs), which can be correlated into a few larger groupings called haplogroups. Although many haplogroups exist in populations throughout the world, only five major pan-American haplogroups are known to have been present in the founding population of the Americas, with potentially an additional six minor haplogroups (A2a, A2b, D2a, D3, C4c, D4h3a) playing a role during the initial colonization or subsequent post–Last Glacial Maximum expansions out of Beringia. Four of these five major pan-American mtDNA haplogroups (A2, B2, C1, and D1) are widely distributed in indigenous American populations, whereas haplogroup X2a has a limited geographic distribution, confined to North America, particularly the Great Lakes region (Brown et al. 1998; Perego et al. 2009; Smith et al. 1999). We use HVRI SNPs to define lineages of mtDNA haplogroups following O'Rourke and Raff (2010), van Oven and Kayser (2009), and Gilbert et al. (2008).

DNA Extraction and Purification. Approximately 0.25–0.50 g of dry-weight bone was decontaminated with ultraviolet light and a 10-min bleach treatment and rinsed with 0.2 μm filtered water. The bone was decalcified and digested overnight with a 55°C incubation in 0.5 M EDTA (pH 8.5), and 1 mg/ml Proteinase K. The digested sample was extracted using the Geneclean for Ancient DNA Kit (MP Biochemicals, Solon, Ohio) following the manufacturer's protocol. For each extraction an extraction blank was run concurrently with the ancient samples. Reported sequencing results are only for those samples that had a corresponding nonamplifying extraction blank.

Amplification and Sequencing. Samples were amplified using the polymerase chain reaction (PCR) in 50 μL reactions containing 5 μL of 10× PCR buffer (ABI, Carlsbad, California), 3 μL of 25 mM $MgCl_2$ (ABI), 4 μL of 10 mM dNTPs (ABI), 0.1 μL BSA, 0.5 μL each of the forward and reverse primers (20 μM; see Table 1 for primer sequences), 32.3 μL H_2O, 0.6 μL AmpliTaq Gold DNA Polymerase (ABI), and 4 μL template. Reactions were hot-started for 10 min at 95°C before the first amplification cycle, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 51°C for 30 s, and extension at 72°C for 30 s. A final extension of 5 min at 72°C followed the 40-cycle amplification reaction. Ten microliters of the PCR product was visualized on 2.5% agarose gel adjacent to a size standard. For each PCR reaction a PCR blank was run concurrently with the ancient samples. Samples were processed for sequencing only if the corresponding PCR blank did not amplify. Chosen amplified PCR products were cleaned using either the QIAquick PCR Purification Kit (Qiagen, Germantown, Maryland) or the UltraClean PCR Clean-Up kit (MoBio Laboratories, Carlsbad, California). Purified PCR products were sequenced on both strands at the University of Utah HSC Core Facilities. Sequences were manually corrected; cases of heteroplasmy are reported as Y (pyrimidines) or R (purines) pending cloning to identify contributing sequences.

Table 1. Primer Sequences Used for HVRI Amplification and Sequencing

Primer Designation	*Sequence 5′–3′*
HVR1_P1F	GTTCTTTCATGGGGAAGCAG
HVR1_P1Rc	TTGATGTGGATTGGGTTTTT
HVR1_P2Fb	AAAACCCAATCCACATCAAA
HVR1_P2R	GGGTGGGTAGGTTTGTTGG
HVR1_P3F	CCCACTAGGATACCAACAAACC
HVR1_P3R	ATTGATTTCACGGAGGATGG

Contamination and Quality Control. Contamination from modern nucleic acids is a constant risk when working with aDNA (Cooper and Poinar 2000; Gilbert et al. 2003, 2005; Handt et al. 1994). We took several measures to both reduce the possibility of contamination and detect it when it did occur. These measures included the following: (1) Only bone samples without pathogenic lesions were chosen for extraction. This selection reduces the risk for the interior of the bone samples to become contaminated in situ and during routine handling of the samples by prior investigators. (2) All samples were pretreated with bleach to remove surface contamination before aDNA extraction. (3) Protocols used freshly bleached and/or ultraviolet-light cross-linked dedicated instruments, labware, and aerosol-resistant filtered pipettor tips. Reagents were certified DNA-free by the manufacturer and aliquoted into single-use volumes. (4) Pre- and post-PCR activities were performed in separate laboratories located at opposite ends of the building, each fully dedicated to aDNA analysis. Amplifications of modern DNA positive controls are never performed in this building. (5) Laboratory personnel were masked, coated, sleeved, and gloved. All garments were bleached before entry into the pre-PCR laboratory, and gloves and sleeves were periodically bleached throughout the laboratory work. (6) Preamplification procedures were performed in a HEPA-filtered, positively pressured Airclean 600 sterile enclosure equipped with an internal ultraviolet light, housed in a HEPA-filtered, positively pressured, fully ultraviolet-light-equipped pre-PCR room. (7) The thermal cycler used for all amplifications is located in a separate room of the building, isolated from both the pre- and post-PCR laboratories. (8) As previously described, negative extraction and PCR controls were used to identify false-positives. (9) Sequences were confirmed with a replicate amplification for each amplicon from a second extraction. If the replicate was not identical in sequence to the original, a third extraction, amplification, and sequence reaction was performed to resolve the ambiguity.

A fuller explanation of laboratory procedures and methods can be found in Hayes (2002) and Smith et al. (2009).

Results

We obtained full or partial HVSI sequences from eighteen samples: four from Port Moller, eight from Brooks River, and six from Mink Island (Table 2).

Table 2. Sequence Variants Observed in HVRI Sequences of Three South Alaskan Archaeological Samples

Site	*Sample*	*Coverage*	*16092*	*16093*	*16111*	*16129*	*16136*	*16189*
	REF	16012–16422	T	T	C	G	T	T
Port Moller	HS 82-3	16012–16422	.	.	.	.	.	.
Port Moller	HS 84-2	16012–16422	.	.	T	.	C	.
Port Moller	HS 82-2*	16012–16296	.	.	Y	A	.	.
Port Moller	HS 82-5	16012–16422	.	.	.	.	.	.
Brooks River	NAK2B4*	16162–16415	–	–	.	.	.	.
Brooks River	NAK2B3*	16055–16254, 16266–16422	.	.	.	.	.	.
Brooks River	NAK2B1	16012–16422	.	.	T	.	.	
Brooks River	NAK2B2*	16162–16425	.	.	T	.	.	.
Brooks River	BR1 Bur2*	16023–16253	.	.	.	.	.	C
Brooks River	BR1 B11961*	16023–16256, 16266–16422	.	.	T	A	.	.
Brooks River	BR5 Bur2*	16012–16254, 16260–16425	.	.	.	.	.	C
Brooks River	BR9 Bur1	16012–16422	.	.	T	.	.	.
Mink Island	XMK-030 Southwest slope	16012–16422	C	.	.	.	.	.
Mink Island	XMK-030 Unit 15A/Bur2	16012–16422	.	.	T	.	.	.
Mink Island	XMK030 misc. fragments	16012–16422	.	C	.	.	.	.
Mink Island	XMK030 Unit 10A/Bur2	16012–16422	C	.	.	A	.	.
Mink Island	XMK030 Unit 15b/Bur4	16012–16422	.	.	.	A	.	.
Mink Island	XMK030 Bur3/IndA	16012–16422	C	.	.	A	.	.

*, Shortened or incomplete coverage.
R, purine heteroplasmy.
Y, pyrimidine heteroplasmy.

16217	*16223*	*16224*	*16271*	*16290*	*16298*	*16311*	*16319*	*16325*	*16327*	*16362*	*Haplogroup*	*^{14}C Date (BP)*
T	C	T	T	C	T	T	G	T	C	T	rCRS	
.	T	.	C	.	.	.	.	.	.	C	D2	N/A
.	T	.	.	T	.	.	A	Y	.	C	A2	2070
.	T	.	C	.	–	–	–	–	–	–	D2	
.	.	C	.	.	.	C	.	.	.	.	Unknown	N/A
.	.	C	.	.	C	.	A	C	T	C	D1?	N/A
.	.	.	.	T	.	.	A	.	.	C	A2	N/A
	.	.	.	T	.	.	R	.	.	C	A2	N/A
.	.	.	.	T	.	.	A	.	.	C	A2	N/A
C	.	.	.	.	.	.	.	.	.	.	B	1295
.	.	.	.	Y	.	.	R	.	.	.	A2	N/A
C	.	.	.	.			.	.	.	B		938
.	.	.	.	T	.	.	A	.	.	C	A2	1318
.	T	.	C	.	.	.	.	.	.	C	D2	1029
.	T	.	.	T	.	.	A	.	.	C	A2	1135
.	T	.	.	.	.	.	A	.	.	C	D3	1110
.	T	.	.	.	.	.	.	.	.	C	D2	1078
.	T	.	.	.	.	.	.	.	.	C	D2	1215
.	T	.	C	.	.	.	.	.	.	C	D2	1130

Of these sequences, we could preliminarily assign haplogroups to seventeen individuals: Eight belonged to haplogroup D (including six D2 individuals, one D3 individual, and one D1 individual), seven belonged to haplogroup A (all A2), and two individuals belonged to haplogroup B2. Haplogroups C and X were not observed.

Sample sizes in this pilot study are too small to allow for meaningful statistical comparisons, but some useful preliminary observations can be made. As expected, the most frequent haplogroups present are haplogroups A and D, as was also true in the earlier eastern Aleutian study (Smith et al. 2009). But two individuals belonging to haplogroup B2 were also present in the Brooks River collection. Although extreme caution must be exercised because of the small sample sizes (e.g., Smith et al. 2009), the relative haplogroup frequencies appear to differ considerably between the three sites (Table 3).

The haplotypes present showed considerable sequence diversity. Mitochondrial DNA haplotypes A2a and A2b are frequent in Arctic populations, but we did not observe the diagnostic transitions at np 16192 and np 16265 for these two haplogroup A sublineages in any of our samples. Either these samples represent greater haplotype diversity than expected or key SNPs are missing because of a damaged template. We are aware that a damaged template is present in our samples (the D1 sample from the Brooks River area, for example, has ample evidence of damage) but detailing the extent of such damage to sequences requires analyses beyond the scope of this pilot project (Axelsson et al. 2008; Briggs et al. 2007; Ho et al. 2007).

It is worth noting that the full HVRI sequence of one Mink Island sample (XMK030-B3) was previously sequenced by R. Malhi under a contract with the National Park Service, and our sequence of this sample is identical to the one reported by Malhi (n.d.). In addition, L. Marchani provided partial sequence data on three different Mink Island samples (XMK030 Unit 10a/Bur2, XMK030 Unit 15b/Bur4, and XMK030 Unit 15a/Bur2) as part of a feasibility study on these specimens three years ago in another laboratory, and these sequences were also replicated in the current study. Thus several sample sequences reported here have been independently replicated by other investigators in other laboratories, providing evidence of authenticity of and confidence in the reported ancient sequences.

Discussion

The Hot Springs Site. The smallest set of remains examined derives from the Hot Springs site located near Port Moller, Alaska, on the Bering Sea coast near the midpoint of the peninsula (see Figure 1). As indicated by Coltrain [2010 (this issue)], these samples come from the most ancient of the three sites examined. The mean age of samples examined from this site is more than 2,000 years, but at least two samples are dated to approximately 3300 BP. Perhaps the age of the remains from this site helps to explain why it proved more difficult to obtain

Table 3. Haplogroup Frequencies Present in Samples from Peninsula Sites

Site	*Haplogroup A (%)*	*Haplogroup D (%)*	*Haplogroup B (%)*
Mink Island	16.7	83.3	0
Port Moller	33.3	66.6	0
Brooks River	62.5	12.5	25

nucleic acids for analysis from here than from samples from either Mink Island or the Brooks River area. Only three of four Hot Springs site samples examined resulted in sufficient sequence data to provisionally assign haplogroup lineages. As indicated in Table 3, two of these samples represent lineages from mtDNA haplogroup D, one was lineage A2, and the fourth sample contained no SNPs unambiguously associated with any of the mtDNA haplogroups.

Both of the haplogroup D lineages belong to lineage D2, although one does not exhibit the transition at np 16129 typically associated with this lineage and the other is characterized by an incomplete HVRI sequence (terminating at np 16296) but is also clearly a representative of lineage D2.

The single individual from the Hot Springs site representative of haplogroup A has SNPs indicative of lineage A2, a pan-American lineage widely distributed throughout both North and South America, including both ancient and modern Aleuts. Additional sequence data are required to determine whether this individual is lineage A2a or A2b, because the SNP differentiating these sublineages is not present in this sample. As with diagnostic SNPs absent in other sequenced samples, this may be the result of template damage (Briggs et al. 2007; Ho et al. 2007). Finally, one Hot Springs site sample exhibited transitions at np 16224C and np 16311C but was otherwise characterized by the Cambridge Reference Sequence. These two SNPs are not diagnostic for any of the founding mtDNA haplogroups in the Americas.

Brooks River Area. The eight samples from the Brooks River area of the interior peninsula provided unexpected results. Five of the samples had HVRI sequences representative of mtDNA lineage A2. A single individual represented haplogroup D, and with a transition at np 16325C appears to represent lineage D1 rather than the more commonly observed D2 in the Aleutian region (although evidence of postmortem sequence damage in this sample makes the inference of lineage D1 less secure than we would like). The high frequency of haplogroup A and the possible presence of haplogroup D1 here suggest less of an Aleut influence and perhaps greater similarity to Yupik Eskimo populations of the region. A haplotype network for the haplogroup D lineages observed across these three sites is given in Figure 2. It is clear that the distribution of lineages is geographically structured in these few samples, with the putative D1 lineage in Brooks River being most divergent from the lineages at the other two archaeological sites. Nevertheless, the other sites are also distinct in the network, with the reticulations

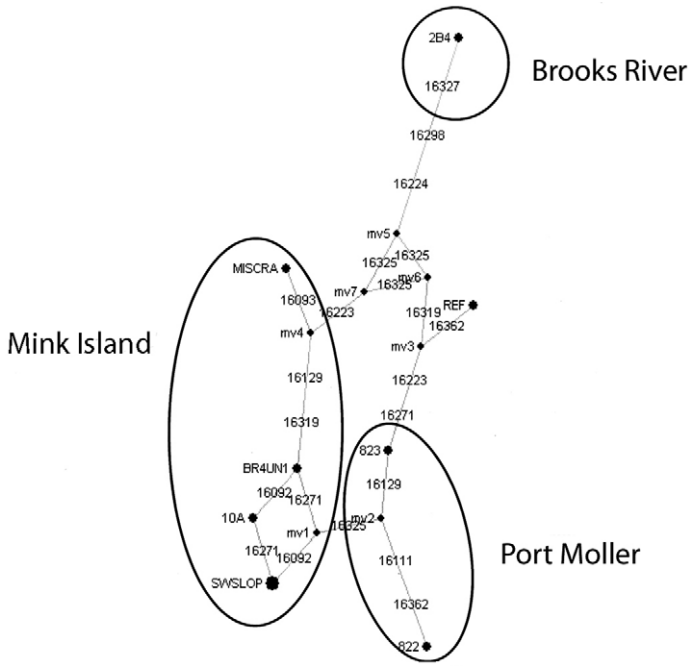

Figure 2. Median network of D haplogroups from three sites in southern and southwestern Alaska.

indicative of small sample sizes that likely have not captured the full extent of the sequence diversity present in these lineages in prehistory. It is worth noting that the network for haplogroup A does not exhibit the same geographic structure (data not shown).

The most unexpected result from these analyses, however, is the presence of two HVRI sequences consistent with mtDNA haplogroup B2. These two samples share transitions at np 16189C and np 16217C that are diagnostic for haplogroup B2 (lineages B2 and B4 cannot be distinguished with HVRI, but B2 is the expected American founding haplogroup and we infer it here). Given that neither sample exhibits any other SNPs observed in other peninsula samples or any SNPs that suggest any other haplogroup, the inference of haplogroup B2 for these two samples is most likely, if unexpected. Haplogroup B2 is common among populations farther south in the Americas and is present in coastal populations of southwest British Columbia (e.g., Bella Coola and Nuu-Chal-Nuth; Ward et al. 1991) but has never been reported in precontact northern North American populations and has been found only rarely in contemporary populations of the region (Helgason et al. 2006; Saillard et al. 2000). Haplogroup B2 is also apparently absent from northern populations in Siberia (Shields et al.

1993). However, Merriwether et al. (1995) did report three haplogroup B2 individuals from Old Harbor on Kodiak Island, which lies adjacent to the northern peninsula region that contains the Brooks River area, just across Shelikof Strait. Both of the haplogroup B samples reported from the Brooks River area of the peninsula were directly dated by Coltrain [2010 (this issue)], with one dating to 799 BP and the other to 493 BP, thus precluding modern intrusive burials as a source of the unexpected haplogroup.

Both sequences have been replicated, and none of the laboratory personnel possess this mtDNA lineage, suggesting that recent contamination is an unlikely explanation. Moreover, no ancient samples possessing this haplogroup have been analyzed in our laboratory, so some form of crossover contamination is even less likely. We acknowledge that this result is unexpected and has not been reported for other groups in northern North America. However, if it is a contaminant, it seems likely to have been introduced before collection for molecular analyses. The pilot analyses conducted to date on these samples are not sufficient to determine whether this may be the case.

Because we have no reason to believe that these results are spurious, they imply that the prehistoric population at the northern end of the Alaska Peninsula 800–1,000 years ago was characterized by a greater degree of genetic diversity than has typically been observed, even in many modern samples from the area. It also implies a greater degree of movement of populations and/or individuals, perhaps across substantial distances, and rates of admixture not generally associated with prehistoric populations. Although the presence of haplogroup B in modern Kodiak Island communities (Merriwether et al. 1995; Merriwether and Ferrell 1996) could have been the result of recent migration and admixture, the presence of the same rare haplogroup in the region in precontact times implies a modern link to a deeper history.

Mink Island Site. The Mink Island samples are from the youngest site examined, are not from the peninsula but rather from Prince William Sound just off the northeast coast of the Kenai Peninsula, and have an average age of only about 500 years. Like the Brooks River samples, all the Mink Island material yielded sufficient DNA for HVRI sequencing and mtDNA lineage assessment. Of the six samples examined, five represented haplogroup D and one was from lineage A2. Four of the haplogroup D individuals were lineage D2. However, all are missing at least one diagnostic SNP for this haplogroup (np 16129A or np 16271C) while exhibiting the other. Moreover, three of the four share a transition at np 16092C that is widely distributed in modern Arctic populations. The other haplogroup D individual in this collection possessed a transition at np 16319A that may indicate membership in lineage D3, although one informative SNP for this haplogroup (np 16173) was not observed because it lay too close to an amplification primer for a clear sequencing read. Regrettably, this SNP was not known when the primers were designed. Nevertheless, the overall sequence motif of this sample is more consistent with haplogroup D1 than D2.

Collectively, these late prehistoric sequences may provide additional insight into the previously observed transition in mtDNA frequencies in the eastern Aleutians after about 1000 BP. Using data from the Chaluka Midden site on Umnak Island, Smith et al. (2009) demonstrated that before 1000 BP haplogroup A was the predominant haplogroup in the eastern Aleutian region. Based on a small number of samples (n = 11) dating to between 1000 BP and 3500 BP, these investigators identified eight samples as haplogroup A (73%) and only three (27%) as haplogroup D. This was a clear reversal of frequencies observed in post–AD 1000 samples and in modern Aleuts [Crawford et al. 2010 (this issue); Zlojutro et al. 2006]. The Hot Springs site at Port Moller yielded only three samples that could be identified with respect to lineage and two of these were lineage D2. Unfortunately, these two samples were not directly dated, but if they are of comparable age to the dated Hot Springs site samples [Coltrain 2010 (this issue)], they would be expected to be contemporaneous with the earlier material at Chaluka. This inference, if correct, suggests that individuals bearing haplogroup D lineages were present not far from the eastern Aleutian region but had yet to move further out in the archipelago in significant numbers, if the early Chaluka material is reflective of the mtDNA variation in the region at the time. At the least it is logical to conclude that haplogroup A occurred at significantly higher frequency than haplogroup D in the early eastern Aleutians, but the same appears not to be true only a few hundred kilometers eastward at Port Moller, although this tentative inference is tempered by the small number of samples examined from this site to date.

The Mink Island site represents the most recent occupation examined here, and the distribution of mtDNA haplogroups mirrors that in the eastern Aleutians, with a predominance of haplogroup D, despite the great distance separating them. Thus by 500 BP or so, the Aleut pattern of a high frequency of haplogroup D and a low frequency of haplogroup A may have become established throughout the southern Alaska coastal region. Whether the expanded mtDNA variability exhibited by the few Brooks River samples is common to a broader geographic region in southern Alaska in prehistory awaits enhanced sampling from additional early sites in the region.

A Temporal Perspective. The mtDNA haplogroup frequency shift documented by Smith et al. (2009) in the Chaluka midden site series at about 1000 BP could be the result of a population bottleneck, that is, the result of genetic drift following a population crash. Indeed this is essentially the conclusion reached by Laughlin in his reexamination of Hrdlička's replacement hypothesis. Laughlin and Marsh (1951) noted that the dolicho-/brachycranic dichotomy observed by Hrdlička (1945) also characterized the geographic diversity of contemporary Aleut populations. They invoked selection in the larger eastern Aleutian region as the mechanism that gave rise to a change in cranial shape but small population size and genetic drift for the persistence of dolichocephaly in modern Aleuts of the region. Thus they attributed a considerable portion of the diversity observed in the Aleutians, both geographically and temporally, as the result of stochastic

forces in populations of small size and low density, at least in the central and western Aleutians. The data reported by Crawford et al. [2010 (this issue)] seems to mirror Laughlin's view of contrasting variation between the western/central Aleutian islands and the eastern islands of the chain. In the absence of samples from the central and western Aleutians for aDNA study, it is not possible to directly test this model of Aleutian population history. However, it was possible to test for the effects of small size and drift in the mtDNA haplogroup frequency data available from the eastern Aleutians.

Using Cabana et al.'s (2008) drift simulation program Nsitu, Smith et al. (2009) tested for the effects of drift on changes in haplogroup frequencies over time and statistically rejected drift as the sole mechanism structuring haplogroup frequencies in the eastern Aleutian data (based on published frequencies). Thus the most parsimonious explanation of the ancient genetic data from the eastern Aleutians appears to be a population expansion of (perhaps) culturally different but genetically related (i.e., possessing only mtDNA haplogroups A and D in any appreciable frequency) people into the eastern Aleutians about 1,000 years ago, followed by amalgamation of the two previously distinct gene pools. The most recent genetic data on contemporary Aleut communities suggests that this expansion and amalgamation has been overlain recently by admixture with non-Aleut populations [Crawford et al. 2010 (this issue); Zlojutro et al. 2006].

The pilot data presented here suggest that populations inhabiting areas of the Alaska Peninsula before or immediately after the documented transition in haplogroup frequencies in the eastern Aleutians were characterized by a greater degree of genetic variation than previously assumed. They could easily have served as a platform for migration and change in the Aleutian region to the west. Moreover, the early dates associated with the Hot Springs site coupled with the (presumed) relatively high frequency of haplogroup D lineages shared with modern Aleuts suggest that the movement of peoples into the archipelago approximately 1,000 years ago, which appears to be associated with an increase in haplogroup D frequency, may have been a migration of coastal peoples derived from Bering coast populations on the peninsula.

The presence of haplogroups B2 and D1 in the Brooks River samples suggests a resident population in the interior of the peninsula that likely had greater contact with populations farther east or elsewhere on the mainland—populations that were characterized by the full suite of haplogroups found in many Native American populations and that would have also served as a source population for new genetic diversity as peninsula populations moved westward toward the islands in the Aleutian chain late in prehistory.

Finally, we want to emphasize the pilot nature of the data on prehistoric populations presented here. Collectively, they represent only 17 individuals who lived in three different geographic areas of south Alaska over a span of perhaps 3,000 years. We cannot assume that they are precisely representative of the populations of which they were members. As a group, they exhibit nearly equal frequencies of haplogroups A and D—not too dissimilar from pooled ancient

samples from the eastern Aleutians or from modern Aleut populations. However, as noted by Smith et al. (2009), increasing sample sizes per site revealed both site differences in haplogroup frequencies as well as temporal changes. Given the temporal and isotopic distinctions between these sites, described by Coltrain [2010 (this issue)], we think it best for the moment to consider them as distinct populations. The Brooks River population, in particular, appears distinctive with respect to the other peninsula and Aleut samples, both ancient and modern, examined by us and others [Crawford et al. 2010 (this issue); Rubicz et al. 2003; Smith et al. 2009; Zlojutro et al. 2006]. Thus the interior of the peninsula may represent a greater linkage with the larger Native American gene pool of the continent than the smaller, marine-adapted populations living on both the Bering and Pacific coasts. As such, it may represent a conduit for genetic exchange from outside the region and a source of diversity carried by late prehistoric migrants to the islands. In this scenario the eastern reaches of the peninsula are important to our understanding of the population dynamics of genetic change in the Aleutian system and to critical tests of Hrdlička's craniometric-based hypothesis of population replacement and the distribution of Paleo- and Neo-Aleuts in the archaeological record. Further work to increase the samples sizes of the peninsula samples as well as the sequence coverage beyond HVRI will be critical to robust tests of population history models of the region.

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Literature Cited

Aigner, J. S. 1978. *The Lithic Remains from Anangula, an 8500-Year-Old Aleut Coastal Village*. Tübingen, Germany: Archaeologica Venatoria Institut für Urgeschichte der Universität Tübingen.

Aigner, J. S., B. Fullem, D. Veltre et al. 1976. Preliminary reports on remains from Sandy Beach Bay, a 4300–5600 BP Aleut village. *Arctic Anthropol.* 13:83–90.

Axelsson, E., E. Willerslev, M. T. P. Gilbert et al. 2008. The effect of ancient DNA damage on inferences of demographic histories. *Mol. Biol. Evol.* 25:2181–2187.

Berge, A. 2010. Origins of linguistic diversity in the Aleutian Islands. *Hum. Biol.* 82(5–6):557–581.

Briggs, A. W., U. Stenzel, P. L. F. Johnson et al. 2007. Patterns of damage in genomic DNA sequences from a Neanderthal. *Proc. Natl. Acad. Sci. USA* 104:14,616–14,621.

Brown, M. D., S. H. Hosseini, A. Torroni et al. 1998. mtDNA haplogroup X: An ancient link between Europe/Western Asia and North America? *Am. J. Hum. Genet.* 63:1852–1861.

Cabana, G. S., K. Hunley, and F. A. Kaestle. 2008. Population continuity or replacement? A novel computer simulation approach and its application to the Numic expansion (western Great Basin, USA). *Am. J. Phys. Anthropol.* 135(4):438–447.

Coltrain, J. B. 2010. Alaska Peninsula stable isotope and radioisotope chemistry: A study in temporal and adaptive diversity. *Hum. Biol.* 82(5–6):613–627.

Coltrain, J. B., M. G. Hayes, and D. H. O'Rourke. 2006. Hrdlička's Aleutian population-replacement hypothesis: A radiometric evaluation. *Curr. Anthropol.* 47(3):537–548.

Cooper, A., and H. N. Poinar. 2000. Ancient DNA: Do it right or not at all. *Science* 289:1139.

Crawford, M. H., R. C. Rubicz, and M. Zlojutro. 2010. Origins of Aleuts and the genetic structure of populations of the archipelago: Molecular and archaeological perspectives. *Hum. Biol.* 82(5–6):695–717.

Dumond, D. 1981. *Archaeology on the Alaska Peninsula: The Naknek Region, 1960–1975*. University of Oregon Anthropological Papers 21. Eugene: Museum of Natural and Cultural History, University of Oregon.

Dumond, D. E. 1987a. A reexamination of Eskimo-Aleut prehistory. *Am. Anthropol.* 89:32–56.

Dumond, D. E. 1987b. *Prehistoric Human Occupation in Southwestern Alaska*. University of Oregon Anthropological Papers 36. Eugene: Museum of Natural and Cultural History, University of Oregon.

Dumond, D., L. Conton, and H. Shields. 1975. Eskimo and Aleuts on the Alaska Peninsula: A reappraisal of Port Moller affinities. *Arctic Anthropol.* 12:49–67.

Gilbert, M. T. P., H.-J. Bandelt, M. Hofreiter et al. 2005. Assessing ancient DNA studies. *Tr. Ecol. Evol.* 20:541–544.

Gilbert, M. T. P., T. Kivisild, B. Grønnow et al. 2008. Paleo-Eskimo mtDNA genome reveals matrilineal discontinuity in Greenland. *Science* 320:1787–1789.

Gilbert, M. T. P., E. Willerslev, A. J. Hansen et al. 2003. Distribution patterns of postmortem damage in human mitochondrial DNA. *Am. J. Hum. Genet.* 72:32–47.

Handt, O., M. Höss, M. Krings et al. 1994. Ancient DNA: Methodological challenges. *Experientia* 50:524–529.

Harritt, R. 1988. *The Late Prehistoric of Brooks River, Alaska*. University of Oregon Anthropological Papers 38. Eugene: Museum of Natural and Cultural History, University of Oregon.

Hayes, M. G. 2002. *Paleogenetic Assessments of Human Migration and Population Replacement in North American Arctic Prehistory*. Ph.D. dissertation, University of Utah.

Hayes, M. G., J. B. Coltrain, and D. H. O'Rourke. 2003. Mitochondrial analyses of Dorset, Thule, Sadlermiut, and Aleut skeletal samples from the prehistoric North American Arctic. In *Mummies in a New Millennium: Proceedings of the 4th World Congress on Mummy Studies*, N. Lynnerup, C. Andreasen, and J. Berglund, eds. Copenhagen: Danish Polar Center, 125–128.

Helgason, A., G. Palsson, H. S. Pedersen et al. 2006. mtDNA variation in Inuit populations of Greenland and Canada: Migration history and population structure. *Am. J. Phys. Anthropol.* 130:123–134.

Hilton, M. 1998. Integrating natural and cultural resource management in Katmai National Park and Preserve. *CRM* [Cultural Resource Management] 9:17–19.

Ho, S. Y. W., T. H. Heupink, A. Rambaut et al. 2007. Bayesian estimation of sequence damage in ancient DNA. *Mol. Biol. Evol.* 24:1416–1422.

Hrdlička, A. 1945. *The Aleutian and Commander Islands and Their Inhabitants*. Philadelphia: Wistar Institute of Anatomy and Biology.

Laughlin, W. S. 1963. Eskimos and Aleuts: Their origins and evolution (physiological and cultural adaptation to facilitate the evolutionary success of Eskimo-Aleut stock). *Science* 142:633–645.

Laughlin, W. S. 1980. *Aleuts: Survivors of the Bering Land Bridge*. New York: Holt, Rinehart & Winston.

Laughlin, W. S., and G. H. Marsh. 1951. A new view of the history of the Aleutians. *Arctic* 4:75–88.

Malhi, R. n.d. *Report on the Extraction of DNA from the XMK-030-B1, B2, B3a, and B3b Teeth*. Final report to the U.S. National Park Service.

Maschner, H. 1999. Prologue to the prehistory of the lower Alaska Peninsula. *Arctic Anthropol.* 36:84–102.

McCartney, A. P., and C. G. Turner II. 1966. Stratigraphy of the Anangula unifacial core and blade site. *Arctic Anthropol.* 3:28–40.

Merriwether, D. A., and R. E. Ferrell. 1996. The four founding lineage hypothesis: A critical re-evaluation. *Mol. Phylogenet. Evol.* 5:241–246.

Merriwether, D. A., F. Rothhammer, and R. E. Ferrell. 1995. Distribution of the four founding lineage haplotypes in Native Americans suggests a single wave of migration for the New World. *Am. J. Phys. Anthropol.* 98:411–430.

Moorrees, C. F. A. 1957. *The Aleut Dentition: A Correlative Study of Dental Characteristics in an Eskimoid People*. Cambridge, MA: Harvard University Press.

Okada, H., and A. Okada. 1974. Preliminary report of the 1972 excavations at Port Moller, Alaska. *Arctic Anthropol.* 11(suppl.):112–124.

O'Rourke, D. H., M. G. Hayes, and S. W. Carlyle. 2000. Spatial and temporal stability of mtDNA haplogroup frequencies in Native North America. *Hum. Biol.* 72(1):15–34.

O'Rourke, D. H., and J. A. Raff. 2010. The human genetic history of the Americas: The final frontier. *Curr. Biol.* 20:R202–R207.

Ousley, S. D. 1995. Relationships between Eskimos, Amerindians, and Aleuts: Old data, new perspectives. *Hum. Biol.* 67:427–458.

Perego, U. A., A. Achilli, N. Angerhofer et al. 2009. Distinctive but concomitant Paleo-Indian migration routes from Beringia marked by two rare mtDNA haplogroups. *Curr. Biol.* 19:1–8.

Rubicz, R. C., T. G. Schurr, P. L. Babb et al. 2003. Mitochondrial DNA variation and the origins of the Aleuts. *Hum. Biol.* 75(6):809–835.

Saillard, J., P. Forster, N. Lynnerup et al. 2000. mtDNA variation among Greenland Eskimos: The edge of the Beringian expansion. *Am. J. Hum. Genet.* 67:718–726.

Shields, G. F., A. M. Schmiechen, B. L. Frazier et al. 1993. mtDNA sequences suggest a recent evolutionary divergence for Beringian and northern North American populations. *Am. J. Hum. Genet.* 53:549–562.

Smith, D. G., R. S. Malhi, J. Eshleman et al. 1999. Distribution of mtDNA haplogroup X among native North Americans. *Am. J. Phys. Anthropol.* 110:271–284.

Smith, S., M. G. Hayes, G. Cabana et al. 2009. Inferring population continuity versus replacement with aDNA: A cautionary tale from the Aleutian Islands. *Hum. Biol.* 81(4):19–38.

Turner, C. G., II. 1967. *The Dentition of Arctic Peoples*. Ph.D. dissertation, University of Wisconsin, Madison.

Turner, C. G., II. 1974. The use of prehistory for direct comparative baselines in the study of Aleut microevolution and adaptation. In *International Conference on the Prehistory and Paleoecology of the Western North American Arctic and Subarctic*, S. Raymond and P. Schledermann, eds. Calgary, Canada: University of Calgary Archaeological Association, 205–215.

Turner, C. G., II. 1991. *The Dentition of Arctic Peoples*. New York: Garland.

Van Oven, M., and M. Kayser. 2009. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum. Mutat.* 30:E386–E394. http://www.phylotree.org; doi: 10.1002/humu.20921.

Veltre, D. W., and M. A. Smith. 2010. Historical overview of archaeological research in the Aleut region of Alaska. *Hum. Biol.* 82(5–6):487–506.

Ward, R. H., B. L. Frazier, K. Dew-Jager et al. 1991. Extensive mitochondrial diversity within a single Amerindian tribe. *Proc. Natl. Acad. Sci. USA* 88:8720–8724.

Weyer, E., Jr. 1930. *Archaeological Materials from the Village Site at Hot Springs, Port Moller, Alaska*. Anthropological Papers of the American Museum of Natural History, v. 21, pt. 4. New York: American Museum of Natural History, 237–279.

Woodbury, A. C. 1984. Eskimo and Aleut languages. In *Handbook of North American Indians*, v. 5, *Arctic*, D. Damas, ed. Washington, DC: Smithsonian Institution, 49–63.

Workman, W. 1966. Prehistory of Port Moller, Alaska Peninsula, in light of fieldwork in 1960. *Arctic Anthropol.* 3:132–153.

Zlojutro, M., R. Rubicz, E. J. Devor et al. 2006. Genetic structure of the Aleuts and circumpolar populations based on mitochondrial DNA sequences: A synthesis. *Am. J. Phys. Anthropol.* 129:446–464.