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mtDNA Variation among Greenland Eskimos: The Edge of the Beringian Expansion

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The Eskimo-Aleut language phylum is distributed from coastal Siberia across Alaska and Canada to Greenland and is well distinguished from the neighboring Na Dene languages. Genetically, however, the distinction between Na Dene and Eskimo-Aleut speakers is less clear. In order to improve the genetic characterization of Eskimos in general and Greenlanders in particular, we have sequenced hypervariable segment I (HVS-I) of the mitochondrial DNA (mtDNA) control region and typed relevant RFLP sites in the mtDNA of 82 Eskimos from Greenland. A comparison of our data with published sequences demonstrates major mtDNA types shared between Na Dene and Eskimo, indicating a common Beringian history within the Holocene. We further confirm the presence of an Eskimo-specific mtDNA subgroup characterized by nucleotide position 16265G within mtDNA group A2. This subgroup is found in all Eskimo groups analyzed so far and is estimated to have originated <3,000 years ago. A founder analysis of all Eskimo and Chukchi A2 types indicates that the Siberian and Greenland ancestral mtDNA pools separated around the time when the Neo-Eskimo culture emerged. The Greenland mtDNA types are a subset of the Alaskan mtDNA variation: they lack the groups D2 and D3 found in Siberia and Alaska and are exclusively A2 but at the same time lack the A2 root type. The data are in agreement with the view that the present Greenland Eskimos essentially descend from Alaskan Neo-Eskimos. European mtDNA types are absent in our Eskimo sample.

Introduction

Eskimos are the native people of the Arctic from northeastern Siberia to Greenland. The Siberian and western Alaskan Eskimos are also known as the Yupik, whereas the Eskimos of northern Alaska, Canada, and Greenland are the Inuit (in Alaska also called Inupiat) (see Kaplan [1990]). As to the etymology of the name "Eskimo," see Goddard (1984). Recent archeological research would place the beginnings of Eskimo culture traditions in western Alaska (Kuzitrin Lake) at ~5,500 years ago (Harritt 1998). The Eskimo-Aleut language phylum, deeply rooted within the Siberian language mesh, may have arrived on the shores of Alaska about that time (see Fortescue [1998]).

Modern Eskimo-Aleut and Na Dene mtDNA types belong predominantly to mtDNA groups A (+663*Hae*III) and D (−5176*Alu*I) and are thus less diverse than Amerind mtDNA types further south that range over haplogroups A, B, C, D, and X (Shields et al. 1993; Torroni et al. 1993; Merriwether et al. 1994; Forster et al. 1996; Brown et al. 1998). The phylogeny of circumarctic mtDNA group A2 (distinguished by nucleotide position [np] 16111T, numbering as done by Anderson et al. [1981]) has been taken to indicate a reexpansion from a Beringian glacial refuge for common ancestors of Na Dene and Eskimos (Forster et al. 1996). Classical genetic studies also showed similarities between Alaskan Na Dene and Eskimos (Szathmary 1993). Nevertheless, the linguistic division between Na Dene (*sensu lato,* i.e., including Haida) and Eskimo-Aleut is mirrored in some mtDNA markers. One control region marker (nps 16233G and 16331G) is typical for a subset of Na Dene mtDNA A2 types (Torroni et al. 1993), and Starikovskaya et al. (1998) noted that the np 16265G subclade of mtDNA group A2 distinguishes Eskimo from Na Dene mtDNA. High percentages of mtDNA group D2 (characterized by −3315*Hae*III, +8683*Alu*I, 16129A, and 16271C) in Alaskan and Siberian Eskimos seem to differentiate them from Na Dene, but it is unclear whether this extends to group D found in Aleuts (Merriwether et al. 1994), because D2 is indistinguishable from Amerind mtDNA group D1 (characterized by 16325C) by low-resolution RFLP analysis. This subclassification of A and D follows Forster et al. (1996).

Here, we present mtDNA data of 82 individuals from the previously poorly sampled east and west Greenland

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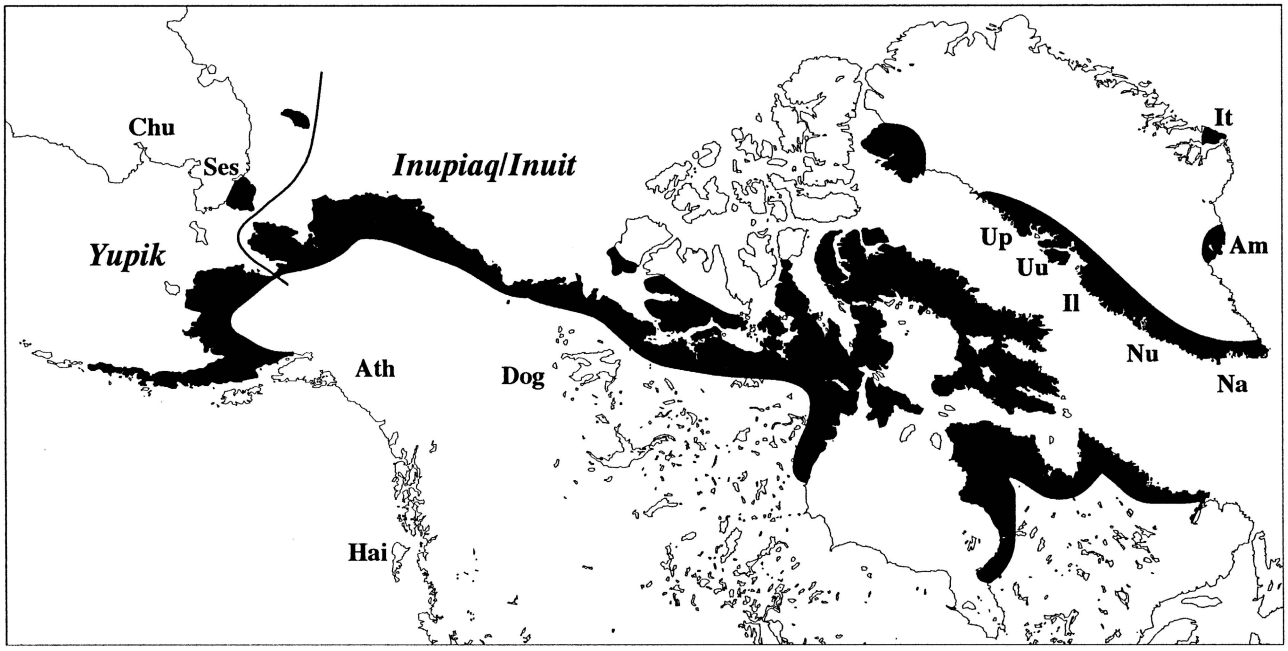

Figure 1 Map of circumpolar regions, including sample locations of Greenland Eskimos. The locations of the Eskimo language groups Yupik and Inupiaq/Inuit are blackened. The origins of the new Greenland Eskimo samples published here are indicated by the following abbreviations, with the Danish names in parentheses: Up, Upernavik; Uu, Uummannaq (Umanak); Il, Ilulissat (Jakobshavn); Nu, Nuuk (Godthåb); Na, Nanortalik; Am, Ammassalik (Angmagssalik); It, Ittoqqortoormiitt (Scoresbysund). The two medieval Norse colonies were situated near Nuuk (Western Settlement) and Nanortalik (Eastern Settlement). Previously published samples referred to in the text and in figure 2 are also indicated (language phylum affiliation in brackets): Ses, Siberian Eskimo (Eskimo-Aleut); Chu, Chukchi (Chukotko-Kamchatkan); Ath, Athapaskan (Na Dene); Dog, Dogrib (Na Dene); Hai, Haida (Na Dene). The map is based on Berthelsen et al. (1992).

Eskimos (Inuit) to determine whether the proposed A2 and D2 markers span the entire geographic range of Eskimos. After identifying Eskimo-specific mtDNA clades, we apply genetic dating to uncover their prehistory. We point out problems in our earlier mtDNA mutation-rate calibration and present a method for estimating the variance of the phylogeny-based coalescence time ρ. The ultimate aim of this study is to clarify the genetic prehistory of Greenland. The oldest radiocarbon dates in Greenland are from the 4,500-year-old Paleo-Eskimo cultures (throughout, we present radiocarbon dates in calibrated years before A.D. 2000; for calibration, see Stuiver et al. [1998]). However, there is reason to believe that there has been no genetic contribution from these early settlers to the founders of the present Eskimos, because these and subsequent archeological cultures end with periodical climatic cooling phases, which in turn suggests periodic extinctions (Fitzhugh 1984). We are furthermore interested in the genetic fate of the medieval Greenland Viking colonies, which were founded in A.D. 985, flourished with $\leqslant$2,000 inhabitants, and then vanished around A.D. 1500 (Kleivan 1984; Lynnerup 1998).

Material and Methods

Sampling

Eighty-two blood samples were collected from Greenland Eskimos, from the west coast (Nanortalik, Nuuk, Ilulissat, Uummannaq, and Upernavik) and from the east coast (Ammassalik and Ittoqqortoormiit). The sample areas are shown in figure 1, and the sample sizes are specified in table 1. The present project has been approved by the Committee for Scientific Investigations in Greenland (registration number 505-16).

DNA Extraction and Amplification

Mitochondrial DNA was amplified directly from whole blood, using a rapid alkaline DNA-extraction protocol (Rudbeck and Dissing 1998) with a 5-min incubation at 75°C with 0.2 M NaOH. A 1262-bp segment, encompassing the entire mtDNA control region, was amplified by PCR, using the following primers (Pharmacia): 5′-ATACACCAGTCTTGTAAACC-3′ (L15907) and 5′-TTGAGGAGGTAAGCTACATA-3′ (H599). The reaction mix contained 5 μL of DNA extract, 10 pmol of each primer,

Table 1

mtDNA Types in Greenland Eskimos

Up	Uu	Il	Nu	Na	Am	It	HVS-I[a]	RFLP[b]
							1111111111111111	1
							6666666666666666	13591
							0011222222223333	03003
							8919125666790116	03056
							0312236156804192	47322
							h	okcna
Cambridge Reference Sequence							ATCCACCCACCCTTGT	+++++
Sample Size[c]								
3	2	4	4	3	2	1	..TT.T.....T..AC	++++u
...	5	3	2	...	...	...	..TT.T.T...T..AC	++++u
...	...	...	1	...	...	...	..TT.T.T.T.T..AC	++++u
1	...	1	...	...	2	4	..TT.T.....T.CAC	++++u
...	...	...	...	1	...	...	..TT.TT....T..AC	++++u
...	...	...	...	...	1	...	..TT.T....TT..AC	++++u
5	3	5	6	1	5	10	..T..T..G..T..AC	uuuu-
1	...	1	1	...	...	...	..T.GT..G..T..AC	uuuu-
1	...	1	...	...	...	...	..T..T..G..TC.AC	uuuu-
...	1	...	...	...	...	...	.CT..T..G..T..AC	uuuu-
...	...	...	1	...	...	...	G.T..T..G..T..AC	uuuu-

[a] h = heteroplasmy (at position 16266).

[b] o = *Hin*cII; k = *Rsa*I; c = *Dde*I; n = = *Hae*II; a = *Alu*I; u = undetermined.

[c] Abbreviations of place names are as in the legend to figure 1.

100 μM of each dNTP (Boehringer Mannheim), 2.5 mM of $MgCl_2$, 10 mM TrisHCl pH 7.8, 50 mM KCl, 0.01% gelatine and 1 unit of *Taq* polymerase (PE Biosystems) in a final volume of 50 μL. The reaction program consisted of 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 2 min.

Sequencing

PCR products were purified with spin columns H-300 (Pharmacia) using the standard protocol. The two following fluorescein-labeled sequencing primers were used: 5′-CTCCACCATTAGCACCCAAAGC-3′ (L15975) and 5′-TGATTTCACGGAGGATGGTG-3′ (H16420). The thermosequenase fluorescent-labeled primer cycle sequencing kit (Amersham) with 7-deaza dGTP was used following the instructions of the manufacturer, with 25 cycles of denaturation at 95°C and annealing extension at 60°C. The sequencing reactions were run on an ALFexpress automated sequencer (Pharmacia) and the data processed with the ALFwin Sequence Analyzer 2.10 software (Pharmacia). The sequences obtained were compared with the Cambridge reference sequence (Anderson et al. 1981) using the alignment program included in the software. Sequences were determined from np 16030 to np 16370.

RFLP Typing

We analyzed five restriction sites found to be polymorphic in group A mtDNAs from Siberian Eskimos and Chukchi (Starikovskaya et al. 1998). The following four pairs of primers were used for the restriction analysis of the sites 1004*Hin*cII, 3337*Rsa*I, 5003*Dde*I, and 9052*Hae*II, respectively: L336 (336–355) and H1727 (1727–1708), L3014 (3014–3035) and H3758 (3758–3736), L4793 (4793–4810) and H5128 (5128–5109), and L8797 (8797–8816) and H9463 (9463–9441). A mismatch forward primer, L11331 (11331–11360): 5′-CCAACAACTTAATATGACTGGCTTACACAA-3′, was designed for the assay of the *Alu*I site at np 11362 in order to avoid the appearance of the constant *Alu*I site at np 11350 in the PCR product. The underlined nucleotide indicates the mismatch—G instead of A. The reverse primer was H11516 (11516–11497).

Time Estimates

A genealogy of n individuals is, mathematically speaking, represented by an ultrametric tree, in which the lengths of links are scaled to time and each interior node corresponds to a coalescence event. Assume that there are, altogether, $k \leqslant 2n - 2$ links of lengths $t_1,...,t_k$ time units and that the clade defined by the ith link carries n_i individuals ($i = 1,...,k$). Then the coalescence time t can be expressed as $t = (n_1t_1 + n_2t_2 + ... + n_kt_k)/n$.

Let u be the mutational rate expressed as the expected number of (scored) mutations in the sequenced segment per time unit. One may associate to the ith link a Poisson-distributed random variable X_i with parameter $\mu_i = t_iu$ (yielding the expected number of mutations along that link). The random variable $X = (n_1X_1 + n_2X_2 + ... + n_kX_k)/n$, then, has the expected value $E(X) = (n_1t_1u + n_2t_2u + ... + n_kt_ku)/n = tu$ and the variance $V(X) = (n_1^2t_1u + n_2^2t_2u + ... + n_k^2t_ku)/n^2$ (assuming independence of $X_1,...,X_k$).

Given a sample of n sequences with observed mutations, we simply take an inferred tree (such as a most-parsimonious tree) with a specified root and m links, say, as an estimate for the unknown genealogy and take the number l_i of observed mutations along the ith link as an estimate for t_iu. If more than one plausible tree exists, the following computation should be carried out for each candidate tree. Under the hypothesis that the tree under consideration is correct, $\rho = (n_1l_1 + n_2l_2 + ... + n_ml_m)/n$ is an unbiased estimator for the age of the specified root. Note that ρ is just the average distance to the root, as employed by, for example, Morral et al. (1994). Moreover, we see that $\sigma^2 = (n_1^2l_1 + n_2^2l_2 + ... + n_m^2l_m)/n^2$ serves as an estimator for the variance. Observe that $\sigma^2 \geqslant \rho/n$, where equality holds exactly when the star index, as defined by Torroni et al. (1998),

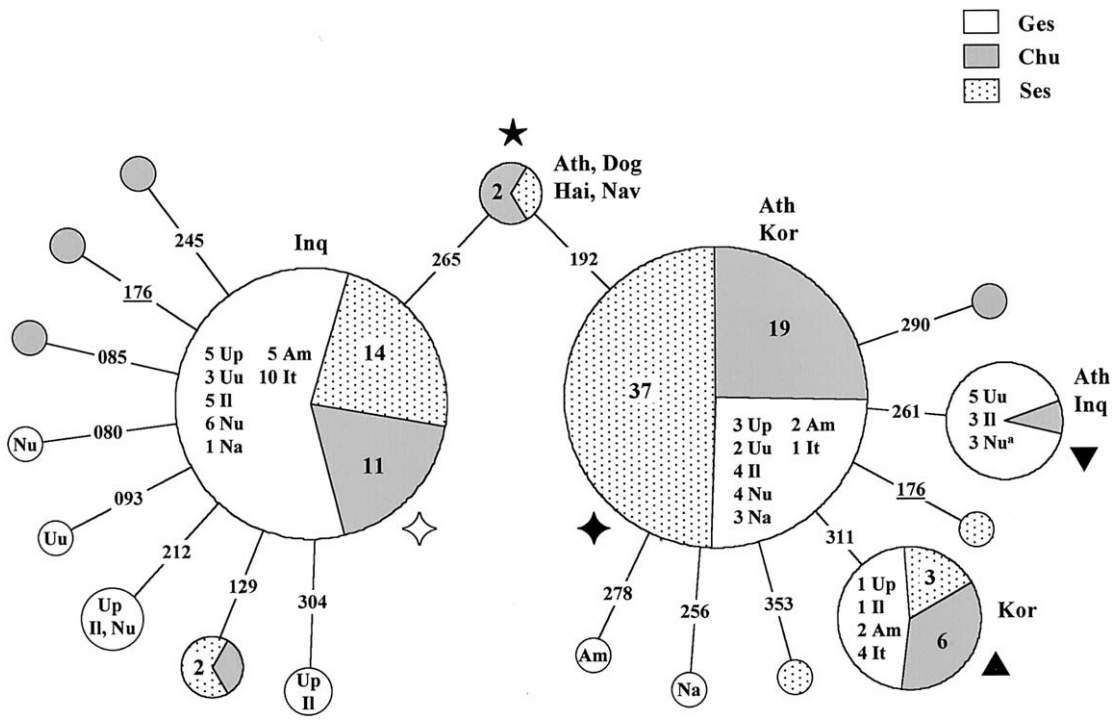

Figure 2 The unique most-parsimonious tree for mtDNA group A2 from Greenland and Siberian samples, comprising 82 Greenland Eskimos (Ges [our sample]), 59 Siberian Eskimos (Ses), and 44 Chukchi (Chu) (Starikovskaya et al. 1998). The displayed mtDNA sequences range from np 16030 to np 16370. The inscribed two-letter codes specify sample locations in Greenland, and the three-letter codes next to some nodes indicate sequence matches with other Siberian and American samples (Inq, Inupiaq; Kor, Koryaks; Nav, Navajo—for other abbreviations, see legend to fig. 1). The area of each circle is proportional to the number of individuals. Mutated positions (−16,000) are indicated along the links. The node labeled with ★ designates the root for A2 types. The two nodes labeled ★ and ♦ are the founders for the Na Dene/Eskimo reexpansions; all five labeled nodes—★, ♦, ◇, ▼, ▲—are the founders for the Eskimo dispersal to the east. [a]One Nu individual is heteroplasmic at np 16266.

equals 1 for this sample. This is attained exactly when the sample corresponds to a "perfect star" phylogeny, in which the tips represent single individuals and the central (root) node harbors the remainder of the sample. In general, ρ/σ^2, rounded to the nearest integer, would indicate the size of a perfect-star sample with approximately the same values of ρ and σ^2 as the given sample. The closer $\rho/n\sigma^2$ comes to the optimal value of 1, the more "starlike" the sample is. We therefore call the ratio ρ/σ^2 the "effective star size" and $\rho/n\sigma^2$ the efficiency (for coalescence-time estimation) of the sample. We will represent age estimates in the form $\rho \pm \sigma$ for mtDNA clades and expansion events originating from reconstructed founder sequences (after collapsing the mutations separating these founder sequences).

Reassessment of the mtDNA Mutation Rate

Forster et al. (1996) had chosen the Beringian reexpansion, radiocarbon dated in Alaska at 11,300 years ago (Kunz and Reanier 1994), as an mtDNA calibration benchmark. This date coincides with the end of the Younger Dryas glacial interlude, dated at 11,400 calendar years ago (Björck et al. 1996), and postdates the flooding of the Bering land bridge ~12,500 years ago (Elias et al. 1996). However, the calibration suffers from major weaknesses because of the suboptimal quality of the sequence data. Forster et al. (1996) had previously noticed that the Beringian mtDNA sequences published by Shields et al. (1993) show hypervariability at positions towards the ends of the sequences, particularly at np 16362. This kind of variation had not been observed in Amerinds by other workers, and it is also absent in the data of Starikovskaya et al. (1998) and in the present Greenland data (compare our fig. 2 with fig. 3 in Forster et al. [1996]). We conclude that a number of mutation events used for the calculation by Forster et al. (1996) are probable artifacts. When the Shields et al. (1993) sequences are removed from the A2 phylogeny and are replaced by the A2 sequences of Starikovskaya et al. (1998), another problem becomes apparent: the phylogeny is no longer starlike (after the founder types are collapsed), because of pronounced founder effects after the initial reexpansion. This would greatly increase the variance, which was already considerable. For example,

if we choose the Younger Dryas (ending 11,400 years ago) as a calibration benchmark for the hypervariable segment I (HVS-I) mutation rate and optimistically assume an efficiency of 0.5, then a sample size of >1,400 is required to obtain a calibration with a standard deviation of 5% of the ρ value. However, to facilitate comparison with previously published age estimates, analogous to uncalibrated radiocarbon years, we retained the scale $\rho = 1$ transition in 20,180 years within nps 16090–16365 in human mtDNA. This rate is in the range of calibrations based on primate mtDNA (Vigilant et al. 1990). Diversity detected by 14-enzyme RFLP analysis of the complete mtDNA was related to control region diversity (nps 16090–16365) by multiplication with the empirical factor 1.21 (Torroni et al. 1998). We prefer this factor to the lower factor of Macaulay et al. (1999), because RFLP diversity is underestimated in the data of Starikovskaya et al. (1998), who—presumably for technical reasons—do not register variation at the np 16310*Rsa*I site.

Results

mtDNA Variation in Greenland

The sequencing and RFLP results for the Greenland mtDNA samples are shown in table 1. Despite the wide geographic coverage of our sample areas, all sequences belong to mtDNA group A2, and, within this group, there is no evidence of association between geographic origin (east, south, west) and mtDNA haplotype. One major A2 subgroup characterized by a transition at np 16265 (and by a loss of the *Alu*I site at np 11362) has been proposed by Starikovskaya et al. (1998) as an mtDNA marker for Eskimos/Chukchi. This is confirmed by the Greenland data: the 16265G subclade of A2 is found at high frequency at both ends of the Eskimo range, from Siberia (37%) to Greenland (51%), but neither in Amerinds nor in Na Dene sampled to date (384 and 69 samples respectively, reviewed by Forster et al. [1996]). In agreement with the notion of an Alaskan origin for the Eskimo Thule culture (Mathiassen 1927), our Greenland sample is a subset of the Alaskan mtDNA pool, lacking the A2 root type (defined by nps 16111T, 16223T, 16290T, 16319A, 16362C, and +663*Hae*III), and any D types (D2 being defined by nps 16129A, 16223T, 16271C, 16362C, and +10394*Dde*I, +10397*Alu*I, −5176*Alu*I, −3315*Hae*III, +8683*Alu*I; D3 being defined here by nps 16223T, 16319A, 16362C, and +10394*Dde*I, +10397*Alu*I, −5176*Alu*I, −10180*Taq*I; inferred from Starikovskaya et al. [1998]). Note that the latter type was erroneously allocated to group A (because of 16319A, in the absence of RFLPs) by Forster et al. (1996). The mtDNA group D is found at 2%–70% in Alaska (Merriwether et al. 1994), and the A2 root type, rare in Eskimos, is frequent in Alaskan Na Dene (Shields et al. 1993).

Finally, we conclude that our Greenland sample has no admixture from European mtDNA, as no European sequence would harbor the combination of mutations characterizing the A2 mtDNA subgroup. Thus, we see no trace of female admixture of Viking or more recent Danish origin.

Phylogeography of mtDNA Group A2

Figure 2 shows the unique most-parsimonious tree for Eskimo and Chukchi A2 sequences, on the basis of our Greenland sequences and the published Siberian Eskimo and Chukchi sequences (Starikovskaya et al. 1998). Furthermore, nodes shared with the Na Dene–speaking Haida (Ward et al. 1993) and Athapaskans (Shields et al. 1993) are labeled. The extensive overlap of deep Eskimo and Na Dene mtDNA nodes within the A2 phylogeny indicates a common ancestral Na Dene and Eskimo mtDNA pool. Canadian Haida mtDNA—although lacking most of the Amerind mtDNA groups A1, A2, B, C, D1, and X (Forster et al. 1996)—is least similar to Eskimo mtDNA: only the A2 root node is shared. Alaskan Athapaskan mtDNA is more similar to Eskimo and Chukchi mtDNA in sharing the A2 root node as well as the two nodes characterized by transitions at np 16192 and at nps 16192 and 16261 (see fig. 3 of Forster et al. [1996]). The 16192T subclade, therefore, seems to be Beringian-specific (Schurr et al. 1999), if sporadic occurrences elsewhere (Stone and Stoneking 1998) are considered parallelisms. Each Eskimo and Chukchi mtDNA sample is characterized by a high frequency (34%–51%) of the 16265G subclade, which is thus the phylogenetically deepest Eskimo mtDNA marker found so far. Our partial RFLP typing of the Greenland Eskimo samples confirms that −11362*Alu*I is diagnostic for this clade (table 1) (Starikovskaya et al. 1998). We further tested, by RFLP analysis, whether any phylogenetically peripheral RFLP types are shared between our Greenland sample and the Siberian sample of Starikovskaya et al. (1998). The RFLP results in table 1 demonstrate that there is no sharing with Siberian mtDNA RFLP types, except for the two RFLP ancestral types (distinguished by 11362*Alu*I). This distinction between shared and private polymorphisms can then be exploited in a founder analysis for age estimates.

Age and Variance Estimation of Founding Nodes

The A2 phylogeny (fig. 2) is not starlike, which greatly reduces the precision of genetic age estimates. For example, the coalescence of Eskimo mtDNA group A2 dates to 24,800 ± 14,500 years ago (table 2) and either reflects the emergence, in Beringia, of the A2 ancestor

Table 2

Estimated Ages of Beringian/Alaskan Ancestral Nodes and Founder Nodes Shared with Greenland in the Phylogeny of mtDNA Group A2

Nodes/Populations	Marker	n	ρ/σ^2	$\rho/n\sigma^2$	$\rho \pm \sigma$	Age (years)
A2 root (★)	HVS-I	185	2.4	.01	1.23 ± .72	24,800 ± 14,500
16192 node (◆):						
Ges, Ses, Chu	HVS-I	109	8.5	.08	.31 ± .19	6,300 ± 3,900
Ses, Chu	HVS-I	69	10.6	.15	.19 ± .13	3,800 ± 2,700
Ses, Chu	RFLP	69	8.0	.12	.32 ± .20	7,800 ± 4,900
Ges	HVS-I	40	4.5	.11	.53 ± .34	10,600 ± 6,900
16265 node (◇):						
Ges, Ses, Chu	HVS-I	73	32.1	.44	.15 ± .07	3,000 ± 1,400
Ses, Chu	HVS-I	31	14.1	.45	.16 ± .11	3,300 ± 2,200
Ses, Chu	RFLP	31	…	…	0	0
Ges	HVS-I	42	18.0	.43	.14 ± .09	2,900 ± 1,800
Nodes (★, ◆, ◇, ▼, ▲):						
Ges, Ses, Chu	HVS-I	185	98.7	.53	.09 ± .03	1,700 ± 600
Ses, Chu	HVS-I	103	58.9	.57	.08 ± .04	1,600 ± 700
Ses, Chu	RFLP	103	11.9	.12	.21 ± .13	5,200 ± 3,300
Chu	HVS-I	44	44.0	1.00	.09 ± .05	1,800 ± 900
Chu	RFLP	44	17.6	.40	.09 ± .07	2,200 ± 1,800
Ses	HVS-I	59	39.3	.67	.07 ± .04	1,400 ± 800
Ses	RFLP	59	6.6	.11	.31 ± .22	7,500 ± 5,300
Ges	HVS-I	82	41.0	.50	.10 ± .05	2,000 ± 1,000

NOTE.—Data from Starikovskaya et al. (1998) (Ses, Chu) and our data (Ges); see figure 2 for abbreviations and symbols. $\rho = 1$ is translated to 20,180 years for HVS-I (Forster et al. 1996) and to 24,420 years for RFLP (Torroni et al. 1998), the heteroplasmic variant np 16266T and variation at the hypervariable site 16517*Hae*III being disregarded.

common to Amerinds, Na Dene, and Eskimos or a later founding event. The large variance of this estimate does not permit a discrimination of these two possibilities and underlines that it is inadvisable to base a date on a molecular phylogeny which has evolved with severe bottlenecks; the age of the A2 root is determined with the equivalent of only 2.4 independent lineages (the effective sample size here), in spite of an actual sample size of 185 (table 2). Dating younger nodes in figure 2 is similarly difficult. The age of the 16192T subclade (characteristic for Eskimos, Chukchi, and Alaskan Athapaskans, but not the Haida further south) is estimated as 6,300 ± 3,900 years (table 2). This age presumably reflects a time when the ancestors of Athapaskans and Eskimos were not yet differentiated at the mitochondrial level. The age of the 16265G subclade is estimated as 3,000 ± 1,400 years (table 2) and probably postdates the global separation between the Athapaskan and Eskimo mtDNA pools, notwithstanding that later local contacts may have occurred (Hosley 1981). To obtain a date for the maximum age for the Eskimo mtDNA expansion from the putative Beringian source, we performed a founder analysis of the two geographically most widely separated samples, namely Siberia and Greenland. The separation of the Siberian Eskimo and Greenland A2 types from the shared ancestral mtDNA pool is dated at 1,600 ± 700 and 2,000 ± 1,000 years ago, respectively, with an average of 1,700 ± 600 years (table 2). However, all these dates are subject to large variances, which can be illustrated by comparing the RFLP diversities of the same nodes (table 2).

Discussion

Circumarctic mtDNA Phylogeography and Linguistics

The recently published data on Siberian Eskimo and Chukchi mtDNA (Starikovskaya et al. 1998) agree with the scenario proposed by Forster et al. (1996) that American Beringian (Eskimo and Na Dene) mtDNA differs markedly from Amerind mtDNA further south because of a postglacial Beringian demographic reexpansion from a reduced surviving mtDNA pool. Moreover, the mtDNA data of Starikovskaya et al. (1998) finally demonstrate that Beringian mtDNA group D2 is not derived from Amerind mtDNA group D1, but originates from Beringian contact with Siberia. Such contact would then be an obvious explanation why the languages and Asian morphological features of Eskimo and/or Na Dene are seen as the result of more recent waves of migration (Greenberg et al. 1986). This recent contact which brought mtDNA D2 types however did not lead to genetic replacement but only to substantial admixture, 2%–70% in the overview of Merriwether et al. (1994),

of the resident Beringian mtDNA. The "Beringian Gateway" acted both as a bottleneck and a spread zone, promoting cultural contacts during propitious periods but leaving the autochthonous mtDNAs predominant, so that "the spread of genes and the spread of languages have been out of step" (Fortescue 1998). Thus, neither a monolithic one-wave nor a clear-cut three-wave scenario is appropriate for modeling the North American settlement process in all its facets. One of the major problems remaining to be solved on the genetic side is the arrival time of mtDNA group D2. Certainly D2 must already have been present in North America 1,000 years ago, because one New Mexican Apache (Na Dene) in the sample of Torroni et al. (1993) has a D2 type, which a maternal ancestor evidently acquired before the southward migration of Na Dene tribes (Haskell 1987). Further control-region sequencing, particularly of Aleut mtDNA group D, will help clarify this issue.

Within the Beringian mtDNA A2 phylogeny, the mtDNA types of the Canadian Haida (Na Dene) seem to have split away at an early stage, as they share no types with Alaskan Na Dene and Eskimos/Chukchi except for the A2 root. In particular, they seem to have missed the latest expansion(s) of the 16192T subclade, dated at an average of 6,300 ± 3,900 years ago. An early split agrees with the fact that they are geographically and linguistically distant from most Na Dene tribes (see Fortescue [1998], p. 186). Not so the Alaskan Athapaskans, who have a high percentage of the 16192T subclade, which is widespread on both the American and the Siberian side of the Bering Strait (Schurr et al. 1999). The occurrence of the 16265G subclade of A2 is more restricted. It is found only in the Eskimos and the Chukchi and is dated at 3,000 ± 1,400 years ago. Although the variance estimates of the ages of the two types overlap considerably, the geographic and ethnic distributions of their derivatives strongly suggest that the 16265G type is, in fact, much younger than the 16192T type. Indeed, the phylogeny (not shown) of the more diverse Na Dene and Kamchatkan variants of the 16192T subclade (Shields et al. 1993; Torroni et al. 1993; Schurr et al. 1999) yields an age of ⩾22,000 years; however, this, in turn, may well be an overestimate, because the Na Dene sequences of Torroni et al. (1993) were preselected for diversity. It is plausible that the 16192 mutation originated in the Beringian population at least before the end of the Younger Dryas, but the major spread of this A2 type occurred later.

Settlement Scenarios for Greenland

Although global climate has been exceptionally stable since the end of the Ice Age 11,400 years ago, analysis of Greenland ice cores has shown that minor cooling events still occur approximately every 2,600 years (O'Brien et al. 1995). Cold phases starting 8,400, 5,600, 2,900, and 500 years ago lasted for several centuries, the most recent event (the "Little Ice Age") being suspected of extinguishing the Viking colonies in Greenland (Pringle 1997). Fitzhugh (1984) noted that each intervening warm phase corresponded to radiocarbon dates of new emerging cultures in Greenland: 4,500 years ago, the Paleo-Eskimo cultures Independence I and Saqqaq came to Greenland. Only small stylistic variations in stone implements differentiate these two cultures. During the first migration, north Greenland's inhospitable Peary Land was settled. Following in the wake of Independence I, the Saqqaq culture arrived on the west coast. A thousand years later, the Independence II culture also appeared in north Greenland. Shortly after Independence II, the Dorset culture spread south, along the west coast. There seems to be no consensus on the relationship of these Greenland Paleo-Eskimo cultures to other subarctic cultures (e.g., see Harritt [1998]). In contrast, the Neo-Eskimo Thule culture evidently spanned the entire geographical range from Siberia to Greenland (Ackerman 1984; Jordan 1984). The advance guard of the Thule culture arrived around A.D. 1200 and, during the next 300 years, spread across both west and east Greenland (Gulløv 1997). The Thule culture's superiority was based upon hunting sea mammals from umiaqs (large skin-covered boats). Icelandic Norsemen, farmers of the Viking culture, founded the Eastern Settlement in south Greenland in A.D. 985 and subsequently established the Western Settlement in the Nuuk region (fig. 1). The Western Settlement was probably deserted in the 1300s, whereas the Eastern Settlement lasted into the 1400s (Kleivan 1984).

The mtDNA founder types identified and dated here indicate periods when severe bottlenecks must have occurred, considering the shallow time depths of <10,000 years. The dating of founding events hinges upon the adequate reconstruction of the founder mtDNA pool. Although the impoverishment of mtDNA diversity in the Circumarctic assists in identifying most founder types in question, there is still a good chance to have missed a founder or two by the approach we have chosen. As can be learned from the Icelandic case (Helgason et al. 2000), sufficient sampling is important for reliable age estimates. For example, if we had only compared the mtDNA pools of east Greenland and Siberian Eskimos, we would have missed the variant with 16261T in A2 as a founder for Greenland Eskimos. Even with perfect sampling, one should bear in mind that the mtDNA pool of the past Alaskan source does not need to match completely the pioneer mtDNA pool having entered Greenland: an mtDNA type may have been lost along the long way to Greenland, or a new mutant may have arisen. It

is thus quite plausible that the variant with 16212G found in three locations in west Greenland was actually a Thule founder. If so, the founder age of Greenland would drop from 2,000 to 1,200 years. The true founder age(s) therefore may be slightly younger than the ages presented in the last eight rows of table 2. In order to model whether those who brought the most recent major culture, the Thule culture, to Greenland could exclusively have given rise to the mtDNA variation in our present Greenland sample out of an mtDNA pool of early Neo-Eskimos, we ran forward simulations with the following settings: two major founder types, at 40% sample size each (corresponding to the 16192T and 16265G variants), and two minor types, at 10% sample size each (corresponding to the two peripheral types in the 16192T clade shared by different populations—see fig. 2); mutation rate: 1 transition per 20,180 years; generation time 25 years; and running time 50 generations. An almost constant effective population size, with random fluctuation up to 5% per generation, was specified, except for a 10% decrease during the Little Ice Age and a subsequent rebound. We found that effective female population sizes of 200–400 were sufficient to retain the four founder types. Much smaller or larger population sizes typically have lost or gained too many types, respectively, by the end of the simulation. Thus, a plausibly small number of people could have migrated from Alaska to Greenland in the time frame of the Thule culture without losing the mtDNA founder types seen in the Greenland mtDNA pool today, even though our simulation scenario (no population substructure, nearly constant population size) were chosen to make this outcome difficult to attain. No simulation can rule out that the mtDNA variation in Greenland today is to some extent a palimpsest of different migrations. But a recent, mainly Thule-culture origin of Greenland and Siberian Eskimo mtDNA variation would be in keeping not only with our genetic dates but also with the fact that the two Eskimo language subgroups, Inuit-Inupiaq and Yupik, represent language continua (Woodbury 1984).

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