Am. J. Hum. Genet. 53:549–562, 1993

mtDNA Sequences Suggest a Recent Evolutionary Divergence for Beringian and Northern North American Populations

Gerald F. Shields,* Andrea M. Schmiechen,* Barbara L. Frazier,† Alan Redd,†,[1] Mikhail I. Voevoda,‡ Judy K. Reed,* and R. H. Ward†

*Institute of Arctic Biology, University of Alaska, Fairbanks; †Department of Human Genetics, University of Utah, Salt Lake City; and ‡Institute of Internal Medicine, Russian Academy of Medical Sciences, Siberian Branch, Novosibirsk

Summary

Conventional descriptions of the pattern and process of human entry into the New World from Asia are incomplete and controversial. In order to gain an evolutionary insight into this process, we have sequenced the control region of mtDNA in samples of contemporary tribal populations of eastern Siberia, Alaska, and Greenland and have compared them with those of Amerind speakers of the Pacific Northwest and with those of the Altai of central Siberia. Specifically, we have analyzed sequence diversity in 33 mitochondrial lineages identified in 90 individuals belonging to five Circumpolar populations of Beringia, North America, and Greenland: Chukchi from Siberia, Inupiaq Eskimos and Athapaskans from Alaska, Eskimos from West Greenland, and Haida from Canada. Hereafter, we refer to these five populations as "Circumarctic peoples." These data were then compared with the sequence diversity in 47 mitochondrial lineages identified in a sample of 145 individuals from three Amerind-speaking tribes (Bella Coola, Nuu-Chah-Nulth, and Yakima) of the Pacific Northwest, plus 16 mitochondrial lineages identified in a sample of 17 Altai from central Siberia. Sequence diversity within and among Circumarctic populations is considerably less than the sequence diversity observed within and among the three Amerind tribes. The similarity of sequences found among the geographically dispersed Circumarctic groups, plus the small values of mean pairwise sequence differences within Circumarctic populations, suggest a recent and rapid evolutionary radiation of these populations. In addition, Circumarctic populations lack the 9-bp deletion which has been used to trace various migrations out of Asia, while populations of southeastern Siberia possess this deletion. On the basis of these observations, while the evolutionary affinities of Native Americans extend west to the Circumarctic populations of eastern Siberia, they do not include the Altai of central Siberia.

Introduction

Most serious students of human history support an Asian origin for New World natives (Szathmary 1985). However, there are unresolved questions concerning the number and timing of migrations into the New World, where in Asia they may have arisen, their composition, and the ultimate fate of the descendants of these early Americans (Owen 1984; Greenberg et al. 1986; Greenberg and Ruhlen 1992; Szathmary 1993). While archaeological and linguistic data can provide considerable insights into these early migrations, a molecular approach gives two additional dimensions: (i) it has the advantage of defining the genetic affinities of the contemporary populations inhabiting the region through which the migrations occurred, and (ii) it also provides an evolutionary perspective from which presumed times of divergence, and relative ordering of population origins, can be inferred. In this respect, sequence analysis of mtDNA has been exceptionally informative in defining the evolutionary relationships

Received December 22, 1992; final revision received April 28, 1993.

Address for correspondence and reprints: R. H. Ward, Ph.D., Department of Human Genetics, University of Utah, Salt Lake City, UT 84112.

1. Present address: Department of Anthropology, Pennsylvania State University, University Park, PA.

0002-9297/93/5303-0001$02.00

of populations that have diverged during the past 100,000 years (Wilson et al. 1985). There are several advantages of using mtDNA to address questions of the evolutionary origins of New World populations. The rapid rate of evolutionary change of the mitochondrial molecule (Brown et al. 1979), especially the mitochondrial control region, means it will be informative for the relatively short time period during which New World populations are thought to have arisen. Its maternal, essentially haploid, mode of inheritance (Giles et al. 1980) means that molecular phylogenies can be estimated without the ambiguity caused by recombination. In addition, because molecular variability of mtDNA has been used to define evolutionary relationships in many contexts (Wilson et al. 1985), including an analysis of the origin and subsequent radiation of aboriginal humans within the Old World (Stoneking et al. 1986; Cann et al. 1987; Di Rienzo and Wilson 1991; Vigilant et al. 1991), the distribution of sequence variants within New World populations can be placed within an established evolutionary framework. Finally, because mtDNA is a haploid, nonrecombining molecule, the average number of nucleotide differences in pairwise sequence comparisons can be used to estimate the time depth during which the molecular divergence occurred (Nei 1987). Since molecular divergence will generally precede population divergence (Nei 1987), these estimates of molecular time depths can be used to establish an approximate upper bound for population divergence times.

Recent studies of mtDNA have attempted to determine the number of molecular genetic lineages that entered the New World, as well as to evaluate the molecular diversity of Amerindian populations (Wallace et al. 1985; Schurr et al. 1990; Ward et al. 1991; Torroni et al. 1992; Horai et al. 1993; Ward et al., in press). However, apart from one exception (Shields et al. 1992), the mtDNA of contemporary populations indigenous to Beringia and adjacent regions has not been studied. Here we follow Hoffecker et al. (1993) in defining Beringia as extending from just east of the Verkhoyansk Range in Siberia (130° E) to the Richardson Mountains just west of the Mackenzie River (135° W). Since all populations worldwide north of 55° N are termed "Circumpolar," we use the term "Circumarctic" to refer to that subset of Circumpolar populations that inhabits the area from the western extremity of Beringia (130° E) to Greenland. While these Circumpolar populations have definite affinities with Amerinds, the Circumpolar peoples west of 130° E clearly do not. According to the most recent linguistic classification (Ruhlen 1991), there are three major linguistic phyla contained in the region that we define as "Circumarctic"—Chukotko-Kamchatkan (Krauss 1988), Eskimo-Aleut, and Na-Dene—while the somewhat controversial classification of Greenberg (1987) places all remaining languages of the Americas within a single phylum, Amerind. Therefore, we have obtained mtDNA sequence data from five populations belonging to all three major linguistic groups. Three of these populations were sampled from Beringia: Chukchi from northeastern Siberia and Inupiaq Eskimos and Athapaskans from Alaska. We also sampled Eskimo from West Greenland and the Na-Dene–speaking Haida from British Columbia, Canada. For comparison, we obtained mtDNA sequence data from three Amerind-speaking tribes (Bella Coola, Nuu-Chah-Nulth, and Yakima) of the Pacific Northwest and, as an outlier group, a sample of Altai from central Siberia. Two of the Pacific Northwest tribes, the Nuu-Chah-Nulth and Bella Coola, speak Mosan languages thought to be closely related (Wakashan and Salishan, respectively), while the Yakima, living east of the Cascades Range, speak a more distantly related Penutian language. The linguistic origins of the Altai sample are harder to define, since the villages from which the samples were drawn have historically been influenced by both Turkic-speaking groups and Samoyedic-speaking groups. Finally, we also sequenced six Siberian Eskimos and extended our ongoing survey of the 9-bp deletion in region V (Ward et al. 1991; Shields et al. 1992) to two additional Siberian populations, the Evenk and the Buriats.

Results of this survey of mitochondrial sequence variation allow us to (1) obtain a better understanding of the evolutionary relationships between these populations, (2) determine the relative levels of sequence divergence within and among Circumarctic populations and thus estimate the relative time depth of their origin and subsequent radiation, and (3) determine, within Siberia, the geographic extent of shared ancestry with Amerind populations. In addition to providing a phylogenetic glimpse of the process of human entry into the New World, this study also provides an initial assessment of the distribution of molecular genetic data in the rapidly vanishing gene pools of the aboriginal groups that inhabit the Beringian region.

Subjects, Material, and Methods

Populations

mtDNA was purified from whole blood of 17 Altai from the villages of Ust-Kan (4 individuals), Ulagan (6 individuals), and Chibit (7 individuals) of the former Gorno-Altai Autonomous Territory, Siberia; 7 Chukchi

from the villages of Ust-Belaia (4 individuals), Kanchalan (2 individuals), and Uelen (1 individual) of the former Chukchi Autonomous Territory, Chukotka, and 6 Siberian Eskimos from the villages of New Chaplino (5 individuals) and Lorino (1 individual) of the Chukotka Peninsula. DNA was isolated by cesium-chloride density-gradient centrifugation (Carr and Griffith 1987; Shields and Wilson 1987) from placenta of 5 Inupiaq mothers and 21 Athapaskan mothers who gave birth to their children at Fairbanks Memorial Hospital. The 5 Inupiaq Eskimos came from the villages of Shaktoolik (1 individual), Barrow (2 individuals), Anaktuvuk Pass (1 individual), and Tanana (1 individual), while the 21 Athapaskans came from nine communities in interior Alaska: Tanana (6 individuals), Fairbanks (5 individuals), Hughes (2 individuals), Fort Yukon (3 individuals), with one individual each coming from St. Mary's, Nulato, Ruby, Koyukuk, and Stevens Village. DNA was also extracted from serum samples of 17 Eskimos from West Greenland and from whole blood samples of 42 Yakima from the Yakima Indian Reservation in Washington State. We thus obtained new mtDNA sequence data for a total of 115 individuals. In addition to the new sequences generated from these samples, we extended the analysis to the existing sequence data for 63 Nuu-Chah-Nulth (Ward et al. 1991), 41 Haida, and 40 Bella Coola (Ward et al., in press). The total data set thus included mtDNA control region sequences for 259 individuals, of which 97 belonged to five Circumarctic populations, 145 belonged to three Amerind-speaking populations, and 17 belonged to the linguistically and geographically remote Altai.

We interviewed donors regarding their knowledge of their maternal ancestry; all stated that their grandmothers and great-grandmothers belonged to their respective native groups. Although estimates of genetic admixture are not available for all of these populations, estimates of Caucasian admixture for Beringian populations fall into the range of 2%–5% (Alekseyev 1979; Ferrell et al. 1981). Since these estimates are based on nuclear markers, and since mitochondrial admixture would be affected only by gene flow from non-native females, we feel that foreign admixture of mtDNA in our samples is undoubtedly less than 5%.

Amplification and Sequencing of DNA

We used PCR (Saiki et al. 1985; Kocher et al. 1989) to amplify a segment of mtDNA which included the first 360 nucleotides of the mitochondrial control region, corresponding to region I in Vigilant et al. (1989). DNA was amplified for sequencing by using one of the following two sets of primers: L15926: 5′-TCAAAGCTTACACCAGTCTTGTAAACC-3′ and H16498: 5′-CCTGAAGTAGGAACCAGATG-3′ (Kocher et al. 1989) or L15997: 5′-CACCATTAGCACCCAAAGCT-3′ and H16401: 5′-TGATTTCACGGAGGATGGTG-3′ (Ward et al. 1991). The numbers of the primer designations identify the 3′ ends according to the reference sequence (Anderson et al. 1981), while "L" and "H" designate the light and heavy strands of the mtDNA molecule, respectively. Single-stranded DNA was obtained for sequencing by means of asymmetric PCR, with the limiting primer diluted 1:50. DNA sequences were obtained for both strands of the control region, via the dideoxy chain-termination procedure of Sanger et al. (1977), by using Sequenase kits (U.S. Biochemicals), with the limiting primer of the preceding asymmetric amplification being used as the sequencing primer. We surveyed for the presence of the 9-bp deletion in region V by using the primer pair A L8196: 5′-ACAGTTTCATGCCCATCGTC-3′ and B H8316: 5′-ATGCTAAGTTAGCTTTACAG-3′ (Wrischnik et al. 1987) to amplify a 121-bp segment of region V in 8 Evenk, 10 Buriats, 17 West Greenland Eskimos, and 42 Yakima. Absence of the deletion (i.e., presence of two identical copies of the CCCCCTCTA 9-bp sequence) was verified through direct sequence analysis (Shields et al. 1992), and the results were compared to all published studies.

Phylogenetic Analysis

For the majority of the analyses, direct pairwise comparison of sequences was used to generate the distribution of pairwise sequence differences, since the application of the multiple-hit correction gave essentially identical results for all instances where only transitions occurred. For sequence comparisons involving transversions, we also calculated pairwise sequence differences by using Kimura's multiple-hit correction (Kimura 1981) and an estimated transition:transversion ratio of 30:1 (Ward et al. 1991). The distribution of pairwise sequence differences was then evaluated within populations, between populations, and for all Circumarctic populations combined. Were this procedure to be carried out for allele frequency data, it would be appropriate to correct the between-population dispersion for the amount of within-population dispersion. However, since the distribution of sequence differences is a direct function of evolutionary time, such a procedure would fail to be meaningful. Hence, we simply present the direct comparisons of the distribution of pairwise sequence differences between populations. We also computed the distribution of pairwise sequence differences within an "average Amerind-speaking tribe" by separately calculating the intratribal

distribution for each of the three Amerind-speaking tribes and then by combining the resulting proportions to obtain a simple average, with each tribe being weighted equally.

Pairwise differences can be translated into divergence times by one of two methods. The most commonly used method relies on the fossil record to estimate divergence times between humans and chimpanzees. When applied to sequence data for the mitochondrial control region, this method gives an estimate of approximately 1% sequence divergence/30,300 years, when a transition:transversion ratio of 30:1 is assumed (Ward et al. 1991). Alternatively, the mutation rate can be estimated directly, by using a model based on the coalescent (Lundstrom et al. 1992). By extension of the coalescent model of Kingman (1982), the ancestry of a random sample of n DNA sequences can be modeled by two components: (*a*) the genealogy of the n sequences, where the distribution of evolutionary time between the nodes in the ancestral tree is defined by an exponential random variable with parameter $j(j-1)/2$, where j is the number of distinct ancestors in the sample and (*b*) the number of nucleotide substitutions along the branches of the tree, where the substitutions occur at a rate of $N_f\mu$ along each branch, where N_f is the effective population size (females for mitochondria) and μ is the substitution rate per nucleotide per generation. When this latter method is applied to data from an Amerind population, the divergence time for the human mitochondrial control region is estimated as approximately 1% sequence divergence/8,950 years. Although this latter estimate is substantially faster than the former, we use this estimated rate of control region sequence divergence because it is based on firmer statistical principles and does not rely on a potentially imprecise estimate for the temporal separation of humans and chimpanzees.

Molecular phylogenies were estimated for the 33 unique mitochondrial lineages found in the Circumarctic populations by using both maximum-likelihood methods (Felsenstein 1981) and maximum-parsimony algorithms, using the PHYLIP package (Felsenstein 1991). A "mitochondrial lineage" is defined by the unique combination of nucleotides in a particular sequence, whereas a "clade" refers to a cluster of lineages for which there is statistical support of a monophyletic origin (Ward et al. 1991). The existence of mitochondrial clades was evaluated statistically by bootstrap analysis of the parsimony trees (Felsenstein 1985), by using 1,800 bootstrap iterations. Finally, the set of mean pairwise sequence differences between populations was used to estimate an inferred population phylogeny, by using the least-squares criteria of the FITCH algorithm in PHYLIP (Felsenstein 1991).

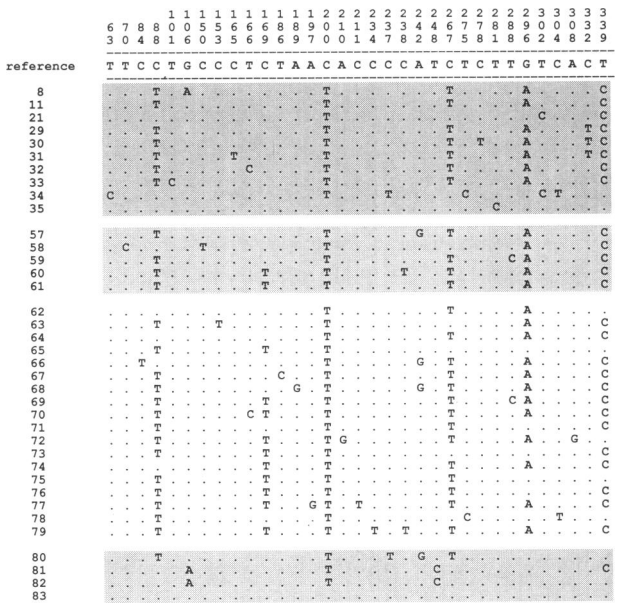

Figure 1 Thirty-seven Circumarctic lineages for the control region of mtDNA. The shading is used to highlight lineages that have been observed in previous studies (8–35; Ward et al. 1991, and in press), newly described lineages that occur in more than one population (57–61), and lineages that were not used in the statistical analysis (see text). The remaining lineages (62–79) are newly described and were observed in only one population. The positions define variable nucleotides in the control region, where position 1 corresponds to position 16086 in the human reference sequence published elsewhere (Anderson et al. 1981). The nucleotides in the reference sequence are indicated by the top line, while dots in the subsequent lines indicate identity with the reference sequence. Nos. on the left define the identity of sequences consecutively discovered in this laboratory. The 27 newly described sequences have been deposited in the GENBANK data base (accession nos. L20157–L20183).

Results

Control Region Sequences

Circumarctic populations.—When the six Siberian Eskimos are included, the first 360 nucleotides of the control region defined 37 lineages in the total sample of 97 Circumarctic individuals (fig. 1). The top 10 sequences (shaded) have been reported elsewhere: lineages 8, 11, and 21 initially were described in the Nuu-Chah-Nulth (Ward et al. 1991), and lineages 29–35 were described in the Haida (Ward et al., in press). Of the 27 previously undescribed lineages, 5 (57–61) were observed in more than one Circumarctic population, while 22 were observed in only a single Circumarctic population. Three of the last four lineages were

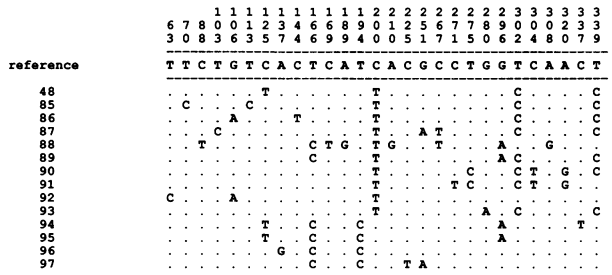

Figure 2 Fourteen previously undescribed mtDNA control region sequences observed in the Yakima. The format is identical to that of fig. 1. These sequences have been deposited in the GENBANK data base (accession nos. L20184–L20197).

excluded from subsequent statistical analyses, because we could not exclude the possibility of recent admixture and because they either are identical to the Cambridge consensus sequence (lineage 83 found in a single West Greenland Eskimo) or are broadly divergent from other Circumarctic lineages (81–82 found in two Siberian Eskimos). Finally, because the exclusion of lineages 81 and 82 resulted in an inadequate sample size for the Siberian Eskimo population, we also excluded the remaining lineage (80) found in this population. The remaining 23 previously undescribed Circumarctic lineages (57–79) are characterized by 23 variable positions, all involving transitions. Fourteen of these transitions represent unique derived sites (autapomorphies), since they occur in only a single lineage.

Nine of the lineages depicted in figure 1 occur in more than one Circumarctic individual. The lineages with multiple occurrences, and the number of times they occur, are as follows: lineage 11 (27 individuals), lineage 21 (2 individuals), lineage 29 (10 individuals), lineage 34 (3 individuals), lineage 57 (4 individuals), lineage 58 (2 individuals), lineage 60 (6 individuals), lineage 61 (13 individuals), and lineage 71 (2 individuals). All other lineages were observed in only a single individual. Four of the lineages with multiple occurrences are found in only a single population: lineage 21 (2 Haida), lineage 29 (10 Haida), lineage 34 (3 Haida), and lineage 71 (2 Athapaskans). The other five lineages with multiple occurrences that are found in more than one population are discussed at greater length below.

Amerind populations.—The data for the 28 lineages found in a sample of 63 Nuu-Chah-Nulth have been reported elsewhere (Ward et al. 1991), as have the data for the 10 lineages found in the sample of 40 Bella Coola (Ward et al., in press). The sample of 42 Yakima identified a total of 20 lineages, of which 5 had been described elsewhere (Ward et al. 1991), in the Nuu-Chah-Nulth: lineages 1 (2 individuals), 11 (1 individual), 21 (1 individual), 24 (1 individual), and 27 (11 individuals). In addition, lineage 59, found in the Inupiaq (fig. 1), was also found in a single Yakima. The remaining, previously undescribed 14 lineages, found in 25 individuals of the Yakima sample, are presented in figure 2. All of the 28 substitutions observed in these 14 new lineages are transitions, 15 of them involving single unique derived sites, while 4 of them (C at 166, T at 200, C at 302, and C at 339) occur in at least 6 of these lineages. Apart from lineage 95 (which occurs in 7 individuals) and lineage 96 (which occurs in 6 individuals), the lineages described in figure 2 were observed in only a single individual.

Altai.—Figure 3 presents the 16 lineages observed among the 17 Altai studied. Lineage Alt 01 was observed in two individuals, while all other lineages occurred only once in this sample. Twenty-eight substitutions occur among the Altai, of which 16 are single unique derived sites. However, 3 of these 16 autapomorphic substitutions are transversions (G→C at position 106, A→T at position 254, and A→T at position 320). Also, a fourth transversion (A→T) was noted at position 242 in lineage Alt 10, in addition to the transition (A→G) also observed at this position in lineage Alt 14. The high number of unique lineages observed in this small sample (16 lineages in 17 individuals), plus the presence of four transversions, suggests that, relative to Amerind and Circumarctic populations, the Altai are a more genetically diverse population.

Within-population sequence differences: Circumarctic populations.—Table 1 presents within-population mean pairwise sequence differences for the Circumarctic populations. No transversions were observed, and, with the exception of the Inupiaqs, mean values for all

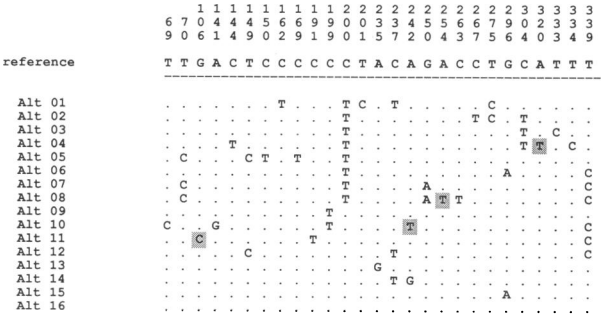

Figure 3 Sixteen mtDNA control region lineages observed in the Altai. Format is identical to that of figs. 1 and 2. Shaded positions indicate transversions. These sequences have been deposited in the GENBANK data base (accession nos. L20198–L20213).

Table 1

Within-Group Mean Pairwise Sequence Differences for the First 360 Nucleotides of the mtDNA Control Region Observed in Five Circumarctic Populations

Group	No. of Individuals	No. of Lineages	Mean	SD
Chukchi	7	6	2.57	1.29
Athapaskans	21	12	2.47	1.63
West Greenland Eskimos	17	9[a]	2.06	1.61
Inupiaqs	5	4	4.00	1.94
Haida	41	10	2.49	3.18

[a] While 10 lineages were observed, lineage 83 was excluded from this analysis, because it was presumed to be present because of admixture.

groups are low, suggesting a recent origin for these populations. Since we believe that the Inupiaq values may have been adversely influenced by sampling error, we have not estimated molecular divergence times for this group. With the estimated mutation rate corresponding to approximately 1% sequence divergence/8,950 years (Lundstrom et al. 1992; see Subjects, Material, and Methods), an average of 5,100–7,100 years would be required to generate the sequence diversity observed within these Circumarctic populations. While this time period refers to the ancestry of the molecular lineages rather than to the absolute ages of the populations, it suggests an upper limit for the origin of these populations. Also, under the assumption of comparable population sizes and similar levels of population subdivision and migration, the similarity in average molecular divergence found within these populations suggests that their evolutionary ages are also similar.

Within-population sequence differences: Altai and Amerind populations.—Table 2 lists the within-population mean pairwise sequence differences for the three Amerind-speaking populations and for the Altai populations. In each instance, the mean within-population pairwise sequence differences are considerably higher than those of Circumarctic populations. The sequence differences for the Amerind-speaking groups involve only transitions and are, therefore, directly comparable to the sequence differences calculated for the Circumarctic populations. The mean pairwise sequence difference within Amerind tribes ranges from 4.86 (Yakima) to 5.32 (Nuu-Chah-Nulth), or nearly twice the values observed within Circumarctic populations. While the Amerind tribes also exhibit similar within-population values, suggesting a similar evolutionary history for all three groups, their average pairwise sequence differences correspond to a larger time depth of 12,100–13,200 years. The comparison with the Altai sequences is less straightforward, since, as noted above, the 16 lineages observed in this population contain four transversions. Hence, a simple pairwise sequence difference calculation is an inadequate measure of the amount of mitochondrial divergence that has occurred within this group. However, when a transition:transversion ratio of 30:1 is used to estimate sequence divergence, the resulting estimate (5.33% ± 5.0% sequence divergence) has an unacceptably large standard error. In all likelihood, a sample size of 17 is too small to provide a reliable estimate of the distribution of molecular diver-

Table 2

Within-Group Mean Pairwise Sequence Differences for Control Regions of the Altai Population and of Three Amerindian Populations

Group	No. of Individuals	No. of Lineages	Mean	SD
Altai	17	16	5.52[a]	2.01[b]
Nuu-Chah-Nulth	63	28	5.32	2.70
Bella Coola	40	10	5.02	3.12
Yakima	42	20	4.86	3.15

[a] When a ratio of 1:30 is used for transversions:transitions, this value becomes 19.2.
[b] When a ratio of 1:30 is used for transversions:transitions, this value becomes 18.0.

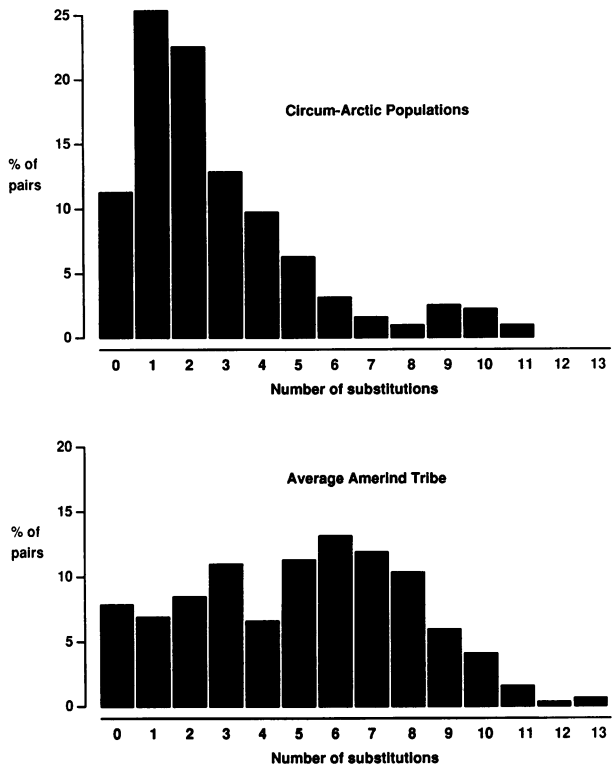

Figure 4 *Top,* Distribution of mean pairwise nucleotide substitutions observed in the control regions of mtDNA of Circumarctic populations. *Bottom,* Distribution of the average mean pairwise nucleotide substitutions in control regions of mtDNA of three populations of Amerind (Bella Coola, Nuu-Chah-Nulth, and Yakima). This fig. was obtained by calculating the distribution of pairwise nucleotide differences in each tribe separately and then by taking the average for all three tribes.

gence in this group. Accordingly, we did not attempt to estimate a mitochondrial time depth for this group, although it is bound to be substantially greater than the time depth of the other populations in this survey.

Pairwise sequence comparisons.—Figure 4 (*top*) describes the distribution of pairwise sequence differences within Circumarctic populations and demonstrates sequence similarity among these far-flung groups. The mean pairwise sequence difference is 2.80 (SD = 2.45) or 0.8% sequence difference for this area of the control region. Sequence similarity among Circumarctic populations contrasts markedly with the average pairwise sequence diversity for Amerind-speaking tribes (fig. 4, *bottom*). The mean of the average distribution of pairwise sequence differences within Amerind tribes is 6.1, corresponding to 1.7% difference for this area of the control region. Moreover, most pairs of lineages within Amerind tribes differ by 5–13 substitutions, whereas most of the pairwise comparisons within the total Circumarctic sample are characterized by zero to three mutations. This distribution of mitochondrial sequence differences implies a relatively short period of evolutionary divergence, as well as relative homogeneity, among the Circumarctic populations as compared with the Amerind populations.

Lineage sharing between tribes.—When lineages 80–83 are excluded (fig. 1), there are a total of 92 distinct lineages in this study. As indicated in table 3, 12 of these lineages are shared between two or more of the populations. It is notable that these shared lineages tend to fall into two groups: those lineages found predominantly among Amerind tribes and four lineages (57, 58, 60, and 61) found only among Circumarctic populations. However, six lineages are shared between the Circumarctic groups and the Amerind tribes, of which three (lineages 8, 11, and 21) occupy nodal positions in the molecular phylogeny (fig. 5) and are therefore presumed to be ancestral lineages. Of the other two lineages found in both groups, lineage 59 falls into cluster II of the Amerind phylogeny (Ward et al. 1991), while lineage 34 is presumed to have entered the Haida by admixture from the Bella Coola (Ward et al., in press).

Lineage 11, the most frequently shared lineage, is not only widespread within Amerind speakers (found in all three tribes) but is also present in a large number of Haida (20) and more than one-fourth of the Athapaskans sampled. This suggests that it may be ancestral to many of the lineages found in North America. Similarly, lineage 61 may represent an ancestral Circumarctic lineage, since it is both frequent and found in three widely scattered populations (Athapaskans, West Greenland Eskimos, and Chukchi).

Population sequence differences.—Table 4 gives the average between-group pairwise sequence difference values, which can be used as an approximate estimate of the upper limit of population divergence (Nei 1987). It is clear that the four Circumarctic populations (Chukchi, Eskimos, Athapaskans, and Haida) have the smallest values, suggesting that they are the most closely related populations. It is notable that the between-group sequence difference values for these four populations are all very similar and that they are equivalent to their within-group difference. By contrast, the three Amerind groups have between-group mtDNA sequence difference values that are twice as large, suggesting that Amerind speakers diverged substantially before the separation of the Circumarctic groups. While the pairwise sequence differences between the

Table 3

Lineages Shared by Two or More Tribes of This Study

	Tribe								
Lineage	Nuu-Chah-Nulth	Bella Coola	Yakima	Haida	Athapaskans	Inupiaqs	West Greenland Eskimos	Siberian Eskimos	Chukchi
1	3	2	...	...	...	...	...	...	...
22	3	2	...	...	...	...	...	...	...
27	1	...	9	...	...	...	...	...	...
8	2	8	...	1	...	...	...	...	...
11	5	3	1	20	6	...	...	...	1
21	3	5	2	2	...	...	...	...	...
34	...	3	...	3	...	...	...	...	...
59	...	...	1	...	...	...	1	...	...
57	...	...	...	...	...	1	2	1	...
58	...	...	...	...	...	1	1	...	...
60	...	...	...	...	3	2	1	...	...
61	...	...	...	...	2	...	7	2	2

Altai and the other groups appear to be similar to the values for the Amerind populations, when the presence of the four transversions is taken into account, these values double. While we have no valid statistical technique for estimating evolutionary time depth from sequences with transversions, it appears that the Altai are very distinct from the other populations in this study.

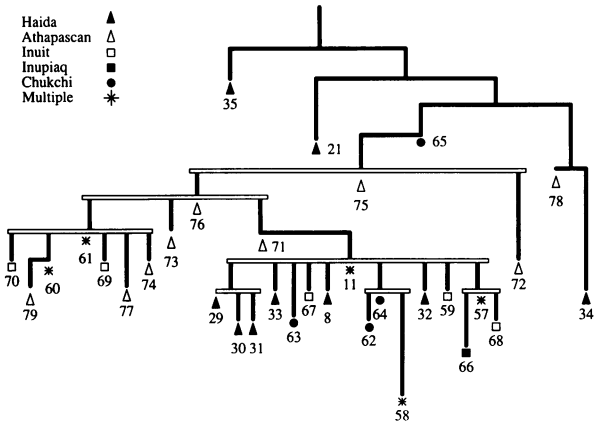

Figure 5 Maximum-likelihood tree of Circumarctic populations. Unshaded bars represent multifurcations in which branching orders cannot be resolved. Lineages identified by an asterisk (*) refer to Circumarctic lineages which are also found in other New World groups (see table 3). Lineage 57 is shared with another Inupiaq, two West Greenland Eskimos, and a Siberian Eskimo. Lineage 58 is shared with another Inupiaq and a West Greenland Eskimo. Lineage 60 is shared with three other Athapaskans, two Inupiaqs, and a West Greenland Eskimo. Lineage 61 is shared with two other Athapaskans, seven West Greenland Eskimos, two Siberian Eskimos, and two Chukchi. Lineage 11 (originally described in a Nuu-Chah-Nulth) is shared with 4 other Nuu-Chah-Nulth, 3 Bella Coola, 1 Yakima, 20 Haida, 6 Athapaskans, and 1 Chukchi.

Phylogenetic analyses.—In order to assess whether the Circumarctic populations contained major clades or clusters of mtDNA lineages comparable to those found within Amerind tribes (Schurr et al. 1990; Ward et al. 1991; Torroni et al. 1992; Horai 1993), we carried out a phylogenetic analysis of the 33 mtDNA lineages that are unambiguously Circumarctic in origin. This analysis also allows an assessment of whether the Circumarctic mtDNA lineages assort on the phylogenetic tree in accordance with their population affiliation, as might be expected if each population has been evolving in isolation for some time. Figure 5 presents a phylogenetic tree based on maximum likelihood analysis for these lineages. The most obvious feature of this tree is that most lineages originate from multifurcations in which the branching order cannot be defined (unshaded bars). Moreover, there is no clear-cut clustering of lineages in terms of the populations from which they were sampled. For example, while two Haida lineages are separated out at the top of the tree, most other Haida lineages reside in an unresolved cluster at the bottom of the tree which also includes Chukchi, West Greenland Eskimo, and Inupiaq lineages. Overall, the results of the bootstrap analyses (1,800 iterations) indicate that there are no clades with greater than 45% support and, further, that these weakly defined lineage clusters include no more than 2–3 lineages. The lack of well-defined lineage clusters, plus the intermingling of lineages among different populations, argues against a long-term separation among these Circumarctic popula-

Table 4

Between-Group Sequence Difference Values for Control Regions of All Populations

	Mean ± SD for						
	Altai	Chukchi	West Greenland Eskimo	Athapaskan	Haida	Nuu-Chah-Nulth	Bella Coola
Chukchi	5.97[a] ± 1.84						
West Greenland Eskimo	7.24[a] ± 1.68	2.53 ± 1.52					
Athapaskan	6.78[a] ± 1.93	2.50 ± 1.52	2.35 ± 1.65				
Haida	6.53[a] ± 1.81	2.72 ± 2.20	2.81 ± 2.51	2.91 ± 2.53			
Nuu-Chah-Nulth	6.19[a] ± 1.84	4.62 ± 2.31	5.36 ± 2.83	5.12 ± 2.75	4.75 ± 2.95		
Bella Coola	7.05[a] ± 2.25	4.24 ± 2.09	4.58 ± 2.44	4.56 ± 2.46	4.10 ± 2.95	5.53 ± 2.89	
Yakima	6.29[a] ± 2.00	6.44 ± 2.31	7.42 ± 2.02	7.31 ± 2.00	6.94 ± 1.96	6.49 ± 2.11	6.96 ± 2.30

[a] When adjusted for a transversion:transition ratio of 1:30, these values become increased by 6.9 substitution units because of the presence of four transversions in the Altai lineages.

tions. We also note that lineages 11 and 61 fall on nodes of the tree, in support of the hypothesis that they are ancestral lineages. This is also true for two of the other shared lineages (57 and 60). Lineage 58 is the only lineage shared among Circumarctic populations that does not fall on a node of the Circumarctic molecular phylogeny and is thus the only lineage which suggests recent migration between Circumarctic populations—in this case between two Eskimo groups.

Figure 6 presents an inferred population tree derived from average sequence divergence values for all sequences of Circumarctic populations in this study, as well as for the Nuu-Chah-Nulth, Bella Coola, and Yakima, plus the Altai. This inferred population tree emphasizes the broad divergence of the Altai from both the Circumarctic and Amerind populations. However, the five Circumarctic populations essentially form a polychotomy, with relatively little genetic divergence separating the nodes that link them and without statistical support for any one population's having diverged before any other. The relatively short branch lengths leading to each of these populations are also consistent with the hypothesis of a relatively recent origin for Circumarctic populations. By contrast, the branch lengths leading to the Amerind populations are considerably longer, and the internodes between populations are significantly greater than zero. This is consistent with a much greater age for the Amerind populations and also suggests they have evolved in relative isolation.

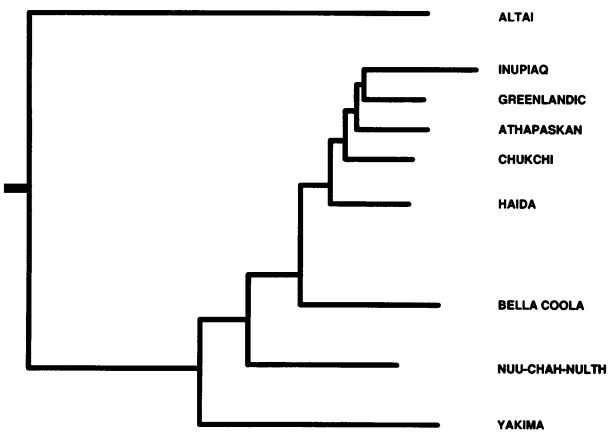

Figure 6 Inferred family tree based on least-squares analysis of the mean pairwise sequence differences between mtDNAs of populations (Felsenstein 1991). "Greenlandic" refers to the West Greenland Eskimo. Siberian Eskimos are not included because of their small sample size (see text).

Region V Deletion

Figure 7 shows the distribution of the region V deletion among indigenous eastern Asians and northern North Americans. Five of the lineages found in the Yakima (lineage 27 and lineages 94–97) corresponding to 26 individuals (62% of the sample) possess the deletion. The Altai (Shields et al. 1992) possess the deletion at frequencies similar to those of southeastern Asians, as do the Buriats (present study) and Mongols (Sambuughin et al. 1991) of south-central Asia. Although some sample sizes for some of these groups are low (Lamut [1 individual], Nanai [1 individual], Koryak [1 individual], Chuvan [2 individuals], and Yukagir [4 individuals]), there is the consistent finding that all Circumarctic populations lack the deletion. Conversely, as in-

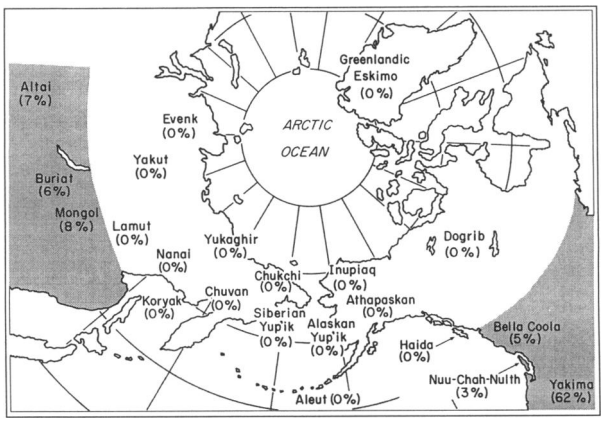

Figure 7 Percentage distribution of the region V 9-bp deletion in Circumarctic populations. Sources for data are as follows: Dogrib (Torroni et al. 1992); Nuu-Chah-Nulth (Ward et al. 1991); Haida and Bella Coola (Ward et al., in press); Mongols (Sambuughin et al. 1991); and Altai, Nanai, Lamut, Koryak, Yukaghir, Chukchi, Chuvan, Siberian Yup'ik, Inupiaqs, Alaskan Yup'ik, Aleut, and Athapaskans (Shields et al. 1992). Shading indicates geographic regions where the region V 9-bp deletion has been observed to occur.

dicated by the shaded area in the figure, all populations that have been examined south of 55° N possess the deletion, irrespective of whether they are in Asia or America.

Discussion

In this study, we have used mtDNA sequence variation to infer population affiliations, as well as evolutionary divergence, for those populations whose evolutionary history has some bearing on the origins of New World populations. The two major groups compared here, Amerind tribes and Circumarctic populations, present contrasting patterns in the diversity, and geographic distribution, of their mtDNA control region sequences. Low values for pairwise sequence differences within and between the far-flung Circumarctic populations contrast with higher difference values for the three Amerind-speaking tribes localized to the Pacific Northwest. In the absence of extensive admixture, it is difficult to think of plausible biological processes that could give rise to population divergences that are deeper than the molecular divergences contained within the population. Extensive admixture seems unlikely in view of the relatively low levels of lineage sharing, especially for "nonancestral" lineages that occur on the tips of the molecular phylogeny. Hence, the most plausible explanation for these observations is that the evolutionary radiation of these Circumarctic groups, in concert with the evolution of their mitochondrial lineages, occurred within a shallow time depth. Low sequence diversity, coupled with the broad geographic distances over which some Circumarctic populations (e.g., Alaskan Inupiaqs and West Greenland Eskimos) have become established, suggests that the establishment of these far-flung populations occurred during a relatively short period of time. By contrast, the mtDNAs of Bella Coola, Nuu-Chah-Nulth, and Yakima are broadly divergent, even though the present geographic distribution of these people is confined to a relatively small region. This suggests that these Amerind tribes are much older than the Circumarctic tribes and have undergone considerable localized genetic differentiation. In this context we note that, while all three tribes are grouped in Greenberg's (1987) Amerind linguistic phylum, two tribes, Bella Coola and Nuu-Chah-Nulth, speak the closely related languages of Salishan and Wakashan, respectively, while the Yakima belong to the more distantly related Penutian phylum. Recent radiation of Circumarctic populations is also supported by the analysis of mean pairwise sequence differences among the various groups. mtDNAs of Circumarctic populations have a low (2.80) mean pairwise number of sequence differences separating them, whereas mtDNAs of Amerinds have a relatively high (6.1) mean pairwise number of sequence differences, with a substantial proportion of the pairwise differences involving from 5 to 13 nucleotides. By contrast, most mtDNAs of Circumarctic individuals either are identical or differ from one another by only a few (0–3) substitutions.

Relative recency of Circumarctic populations is further substantiated by the maximum likelihood phylogeny for the 34 lineages found in these five populations (fig. 5). The most obvious feature of the maximum likelihood tree is that, for the majority of the lineages, the branching order cannot be resolved, suggesting recent origin by a series of independent mutations, with little time for branching order to become established. Moreover, lineages observed in distinct populations are intermingled among the branches of the tree, with no obvious clustering of lineages by the geographic location, or linguistic affiliation, of the tribe from which they were ascertained. For example, individual lineages of Chukchi, West Greenland Eskimos, Athapaskans, and Haida are scattered throughout the tree. This pattern is exactly what would be expected for populations which have recently undergone a demographic expansion and which are in the early stages of evolutionary divergence.

The inferred population tree, based on average be-

tween-population sequence differences, also suggests a close evolutionary relationship between the five Circumarctic populations (fig. 6). In fact, there appears to be no clear separation among Circumarctic populations in this tree; nodes at branch points in the Circumarctic data set are so shallow that their phylogeny could be considered a polychotomy. One notable aspect of this inferred population tree is that it links both Na-Dene–speaking groups in the New World (Alaskan Athapaskans and northwest coast Haida) with Eskimos and Chukchi, rather than with the three Amerind populations of the Pacific Northwest. While this contrasts with some analyses based on classical genetics (Schell et al. 1978; Harper 1980; Harper and Laughlin 1982), osteology (Levin 1963), and linguistics (Swadesh 1964; Krauss 1979; Greenberg 1987; Cavalli-Sforza et al. 1988; Greenberg and Ruhlen 1992), it reaffirms the earlier conclusions of Szathmary and Ossenberg (1978), who demonstrated that a careful analysis of classical genetic markers indicated a close relationship between Athapaskans (Na-Dene–speaking) and Eskimos. Szathmary (in press) has recently included Gm allele frequencies in her analyses of the phylogenetic relationships of Athapaskans to Eskimos and Amerindians and shows that Athapaskans are intermingled with Eskimos and Siberian Chukchi in similar fashion to the relationships depicted in figure 6. Hence, despite the fact that sequence data from mtDNA represent only a single "gene tree," they appear to confirm the results obtained by analyzing a set of the more informative classical genetic markers. When both data sets are taken together, it appears that, despite their geographic separation, Circumarctic populations form a cohesive biological entity that includes the Na-Dene–speaking Haida and Athapaskans, as well as West Greenland Eskimos, Inupiaqs, and Chukchi. By contrast, the Amerind-speaking groups (Bella Coola, Nuu-Chah-Nulth, and Yakima) are genetically distinct.

Our sample sizes for Haida ($n = 41$), Alaskan Athapaskans ($n = 21$), and West Greenland Eskimos ($n = 17$) are reasonably large and appear adequate for these types of comparisons (Hey 1991; Ward et al., in press). Therefore, it is unlikely that the similarity in the distribution of sequences from these three groups is an artifact of sampling error. Further, we do not believe that the close association between Na-Dene speakers and West Greenland Eskimos is a consequence of extensive non-native admixture: there is simply no evidence for this occurrence among the sequences themselves, and, because of the different histories of these three populations, non-native admixture would be more likely to increase genetic differences between the groups than to obscure them. Moreover, it is difficult to argue for recent admixture between these groups, in view of both their extensive geographic separation and the small number of lineages they share. All three instances of lineage sharing (lineage 11 found in Haida and Alaskan Athapaskans; lineages 60 and 61 found in Alaskan Athapaskans and West Greenland Eskimos) involve lineages which are situated on nodes of the molecular phylogeny and thus appear to be ancestral. Hence, we interpret the occurrence of these lineages in multiple populations as indicative of common ancestry rather than of recent admixture. While the sample sizes for the other two Circumarctic groups are much smaller, their similar pattern of lineage distribution suggests that the same reasoning applies to these groups as well.

However, while the influence of recent admixture can be discounted, the consequences of very early interactions between the Circumarctic groups are more difficult to assess. Burch (1979) suggested an early association between Alaskan Inupiaqs and Athapaskans on the basis of archaeological finds, and Dumond (1987) suggests that the archaeological distribution of blades and microblades is a reflection of extensive cultural ties between Eskimos and groups to the south. Hence, two possible scenarios exist. One possibility is that, as the progenitors of the contemporary Eskimos moved across what is now Arctic Alaska and Canada, ancient and extensive gene flow may have occurred between populations that originally differed genetically. This gene flow would have had to have been sufficiently extensive to have obliterated the original mitochondrial lineages that presumably existed in the ancestral Na-Dene populations. Alternatively, as suggested by the mitochondrial data, all Circumarctic populations had their genetic origins about the same time, and the cultural differences that subsequently occurred were accompanied by relatively little biological differentiation. While we favor the second hypothesis, resolution of the true relationships of "northern" native populations of North America awaits the more detailed study of additional native groups in this region.

Here it should be noted that the majority of the Circumarctic lineages exhibit close affinities with those Amerind lineages that fall into cluster II, as defined by Ward et al. (1991). The only major exceptions are lineages 34 and 35, both of which are presumed to have entered the Haida by admixture (Ward et al., in press). Of the remaining 31 lineages, 27 have so far been observed only in Circumarctic populations, while 4 are found in one or more Amerind-speaking tribes. Hence, the overall affiliation of the unique Circumarctic lineages with the cluster II Amerind lineages suggests that

these Circumarctic populations originated from an Asiatic population whose own ancestors had previously contributed to a substantial fraction of the lineage ancestry of contemporary Pacific Northwest Amerind populations.

The inferred phylogenetic relationships of the nine populations included in this study (fig. 6) suggest that Altai are only distantly related to Circumarctic and Amerind populations. Additionally, all four of the transversions observed in the present study occur among Altai. This suggests a greater evolutionary time depth for the Altai compared with that for both Circumarctic and Amerind populations. The fact that Altai appear broadly divergent from all other groups of this study, as well as the fact that 7% of Altai possess the deletion in region V (Shields et al. 1992), suggests that they are not directly related to the eastern Siberian groups in this study, which contrasts with the interpretation based on immunological allotypes (Schanfield et al. 1980). Our observations corroborate an earlier finding, based on blood group frequencies, that there is a substantial degree of genetic divergence between the Altai and other groups of eastern Siberia (Crawford and Enciso 1982).

Distribution of the region V deletion among indigenous populations allows additional predictions about relationships among these groups. Indigenous populations of northeastern Siberia (Lamut, Evenk, Yukagir, Chuvan, Koryak, Yakut, Nanai, Chukchi, and Siberian Eskimos) lack the 9-bp deletion, as do Inupiaq Eskimos, Yup'ik Eskimos, Aleuts, Athapaskans, West Greenland Eskimos, Haida, and Dogrib of the New World. By contrast, Buriats and Mongols of southeastern Siberia possess the 9-bp deletion at frequencies which suggest an evolutionary affinity with many Asian, Southeast Asian, and Pacific populations (Hertzberg et al. 1989; Stoneking and Wilson 1989; Harihara et al. 1992). In general, we find it noteworthy that there seem to be two distributions for the 9-bp deletion: Circumarctic populations uniformly lack the deletion, while populations to the south of 55° N (shaded area in fig. 7) possess the deletion in variable frequencies. In addition to the Asian populations mentioned above, the deletion is found in a wide variety of Amerind groups in North America—Nuu-Chah-Nulth (Ward et al. 1991), Bella Coola and Yakima (Ward et al., in press), and Pima and Maya (Torroni et al. 1992); Central America—all Chibchan groups (R. H. Ward, unpublished data); and South America (Horai et al. 1993; D. A. Merriwether and R. H. Ward, unpublished data). One explanation for this distribution is that the 9-bp deletion was originally present in Circumarctic populations but was subsequently lost due to drift. However, this explanation is somewhat improbable, because it requires that all lineages with the deletion were independently lost from the entire set of the isolated Circumarctic populations. Hence it is more likely that the lineages found in Amerind speakers are descended from the set of lineages that occurred in a genetically diverse set of early migrants. These early migrants, who introduced mitochondrial lineages with and without the deletion, are presumed to have reached the New World well before the subsequent radiation of an ancestral population lacking the deletion gave rise to the present-day Circumarctic populations. If sustained, our hypothesis would imply that mitochondrial sequence divergence could be used to place an upper limit on the time depth when the radiation of these two groups occurred. Intriguingly, our estimate of a 12,100–13,200-year time depth for the evolutionary divergence of mtDNA in Amerind populations happens to correspond to the most recent estimate for Clovis occupation, based on C-14 accelerator mass spectrometry analysis of artifacts (Hoffecker et al. 1993).

Acknowledgments

We dedicate this paper to the memory of the late Allan C. Wilson who, more than anyone else, encouraged molecular studies from a temporal perspective. We thank our tissue donors, and we hope that our observations may have particular meaning to them. Dr. Ralph Wells of Fairbanks Memorial Hospital helped coordinate the collection of placental tissues. T. I. Astakchova and V. L. Feigin helped coordinate collection of tissues from the Beringian regions of Siberia. We also wish to thank Mark Lathrop, Chris Salski, and Catherine Enel for collecting the samples from West Greenland Eskimo, and we thank Gerry Nepom for collecting the Yakima samples. We also wish to acknowledge the helpful comments we have received from Svante Pääbo, Emőke Szathmary, and an anonymous reviewer. This research has been supported by National Institutes of Health grant RO1 GM 41746 (to R.H.W.), an Alfred P. Sloan Sabbatical Supplemental Fellowship in Studies of Molecular Evolution (to G.F.S.), the Alaska/Siberia Medical Research Exchange of the University of Alaska (to G.F.S. and M.I.V.), and a UAF Chancellor's Resource Fellowship (to A.M.S.).

References

Alekseyev VP (1979) The genetic structure of Asiatic Eskimos and Coastal Chukchis compared to that of American Arctic populations. Arctic Anthropol 16:147–164

Anderson S, Bankier AT, Barrell BC, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, et al (1981) Sequence and

organization of the human mitochondrial genome. Nature 290:457–465

Brown WM, George M, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76:1967–1971

Burch ES Jr (1979) The ethnography of northern North America: a guide to recent research. Arctic Anthropol 16:62–146

Cann RL, Stoneking M, Wilson AC (1987) Mitochondrial DNA and human evolution. Nature 325:31–36

Carr SM, Griffith OM (1987) Rapid isolation of animal mitochondrial DNA in a small fixed-angle rotor at ultrahigh speed. Biochem Genet 25:385–390

Cavalli-Sforza LL, Piazza A, Menozzi P, Mountain J (1988) Reconstruction of human evolution: bringing together genetic, archaeological, and linguistic data. Proc Natl Acad Sci USA 85:6002–6006

Crawford MH, Enciso VB (1982) Population structure of circumpolar groups of Siberia, Alaska, Canada and Greenland. In: Crawford MH, Mielke JH (eds) Current developments in anthropological genetics. Vol 2: Ecology and population structure. Plenum, New York, pp 51–91

Di Rienzo A, Wilson AC (1991) Branching pattern in the evolutionary tree for human mitochondrial DNA. Proc Natl Acad Sci (USA) 88:1597–1601

Dumond D (1987) A reexamination of Eskimo-Aleut prehistory. Am Anthropol 89:32–56

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

——— (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

——— (1991) PHYLIP, version 3.4. University of Washington, Seattle

Ferrell RE, Chakraborty R, Gershowitz H, Laughlin WS, Schull WJ (1981) The St. Lawrence Island Eskimos: genetic variation and genetic distance. Am J Phys Anthropol 55:351–358

Giles RE, Blanc H, Cann HM, Wallace DC (1980) Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci USA 77:6715–6719

Greenberg JH (1987) Language in the Americas. Stanford University Press, Stanford

Greenberg JH, Ruhlen M (1992) Linguistic origins of native Americans. Sci Am 267(5):94–99

Greenberg JH, Turner CG, Zegura SL (1986) The settlement of the Americas: a comparison of the linguistic, dental and genetic evidence. Curr Anthropol 27:477–497

Harihara S, Hirai M, Suutou Y, Shimizu K, Omoto K (1992) Frequency of a 9-bp deletion in the mitochondrial DNA among Asian populations. Hum Biol 64:161–166

Harper AB (1980) Origins and divergence of Aleuts, Eskimos and American Indians. Ann Hum Biol 7:547–554

Harper AB, Laughlin WS (1982) Inquiries into the peopling of the New World: development of ideas and recent advances. In: Spencer F (ed) A history of American physical anthropology: 1930–1980. Academic Press, New York, pp 281–304

Hertzberg M, Mickleson KNP, Serjeantson SW, Prior JF, Trent RJ (1989) An Asian-specific 9-bp deletion of mitochondrial DNA is frequently found in Polynesians. Am J Hum Genet 44:504–510

Hey J (1991) The structure of genealogies and the distribution of fixed differences between DNA sequence samples from natural populations. Genetics 128:831–840

Hoffecker JF, Powers WR, Goebel T (1993) The colonization of Beringia and the peopling of the New World. Science 259:46–53

Horai S, Kondo R, Nakagawa-Hattori Y, Hayashi S, Sonoda S, Tajima K (1993) Peopling of the Americas, founded by four major lineages of mitochondrial DNA. Mol Biol Evol 10:23–47

Kimura M (1981) Estimation of evolutionary distances between homologous nucleotide sequences. Proc Natl Acad Sci USA 78:454–458

Kingman J (1982) On the genealogy of large populations. J Appl Probl 19A:27–43

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86:6196–6200

Krauss ME (1979) Alaska native languages: past, present and future. Paper presented at the joint US-USSR Symposium on the Peopling of the New World, Moscow and Leningrad, May

——— (1988) Many tongues—ancient tales. In: Fitzhugh WW, Crowell A (eds) Crossroads of continents. Smithsonian Institution, Washington, DC, pp 145–151

Levin MG (1963) Ethnic origins of the peoples of northeastern Siberia. In: Michael HN (ed) Arctic Institute of North America, anthropology of the north, no 3. University of Toronto Press, Toronto

Lundstrom R, Tavaré S, Ward RH (1992) Estimating substitution rates from molecular data using the coalescent. Proc Natl Acad Sci USA 89:5961–5965

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Owen RC (1984) The Americans: the case against an ice-age human population. In: Smith FH, Spencer F (eds) The origins of modern humans: a world survey of the fossil evidence. Alan R Liss, New York, pp 517–563

Ruhlen M (1991) A guide to the world's languages. Vol 1: Classification. Stanford University Press, Stanford

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354

Sambuughin N, Petrishchev VN, Rychkov YG (1991) DNA polymorphism in a Mongolian population: analysis of restriction endonuclease polymorphism of mitochondrial DNA. Genetika 27:2143–2151

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Schanfield MS, Alexyeeva T, Crawford MH (1980) Immunological allotypes of Asian populations. Hum Hered 30: 343–349

Schell LM, Agarwal SS, Blumberg BS, Levy H, Bennett PH, Laughlin WS, Martin JP (1978) Distribution of albumin variants Naskapi and Mexico among Aleuts, Frobisher Bay Eskimos, and Micmac, Naskapi, Mohawk, Omaha and Apache Indians. Am J Phys Anthropol 49:111–118

Schurr TG, Ballinger SW, Gan Y-Y, Hodge JA, Merriwether DA, Lawrence DN, Knowler WC, et al (1990) Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. Am J Hum Genet 46:613–623

Shields GF, Hecker K, Voevoda MI, Reed JK (1992) Absence of the Asian-specific region V mitochondrial marker in native Beringians. Am J Hum Genet 50:758–765

Shields GF, Wilson AC (1987) Calibration of mitochondrial DNA evolution in geese. J Mol Evol 24:212–217

Stoneking M, Bhatia K, Wilson AC (1986) Rate of sequence divergence estimated from restriction maps of mitochondrial DNAs from Papua New Guinea. Cold Spring Harb Symp Quant Biol 51:433–439

Stoneking M, Wilson AC (1989) Mitochondrial DNA. In: Hill A, Serjeantson S (eds) The colonization of the Pacific: a genetic trail. Oxford University Press, Oxford, pp 215–245

Swadesh M (1964) Linguistic overview. In: Jennings JD, Norbeck E (eds) Prehistoric man in the New World. University of Chicago Press, Chicago, pp 527–556

Szathmary EJE (1985) Peopling of North America: clues from genetic studies. In: Kirk R, Szathmary EJE (eds) Out of Asia: peopling the Americas and the Pacific. J Pacific Hist 19:79–104

——— Application of our understanding of genetic variation in native North America. In: Sing CF, Hanis C (eds) Genetics of cellular, individual, family and population variability. Oxford University Press, New York (in press)

——— (1993) Genetics of aboriginal North Americans. Evol Anthropol 6:202–220

Szathmary EJE, Ossenberg NS (1978) Are the biological differences between North American Indians and Eskimos truly profound? Curr Anthropol 19:673–701

Torroni A, Schurr TG, Yang C, Szathmary EJE, Williams RC, Schanfield MS, Troup GA, et al (1992) Native American mitochondrial DNA analysis indicates that the Amerind and the Na Dene populations were founded by two independent migrations. Genetics 130:153–162

Vigilant L, Pennington R, Harpending H, Kocher T, Wilson AC (1989) Mitochondrial DNA sequences in single hairs from a southern African population. Proc Natl Acad Sci USA 86:9350–9354

Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC (1991) African populations and the evolution of human mitochondrial DNA. Science 253:9350–9354

Wallace DC, Garrison K, Knowler WC (1985) Dramatic founder effects in Amerindian mitochondrial DNAs. Am J Phys Anthropol 68:149–155

Ward RH, Frazier BL, Dew K, Pääbo S (1991) Extensive mitochondrial diversity within a single Amerindian tribe. Proc Natl Acad Sci USA 88:8720–8724

Ward RH, Redd A, Valencia D, Frazier BL, Pääbo S. Genetic and linguistic differentiation in the Americas. Proc Natl Acad Sci USA (in press)

Wilson AC, Cann RL, Carr SM, George M, Gyllensten UB, Helm-Bychowski KM, Higuchi RG, et al (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. Biol J Linnaean Soc 26:375–400

Wrischnik LA, Higuchi RG, Stoneking M, Erlich HA, Arnheim N, Wilson AC (1987) Length mutations in human mitochondrial DNA: direct sequencing of enzymatically amplified DNA. Nucleic Acids Res 15:529–542