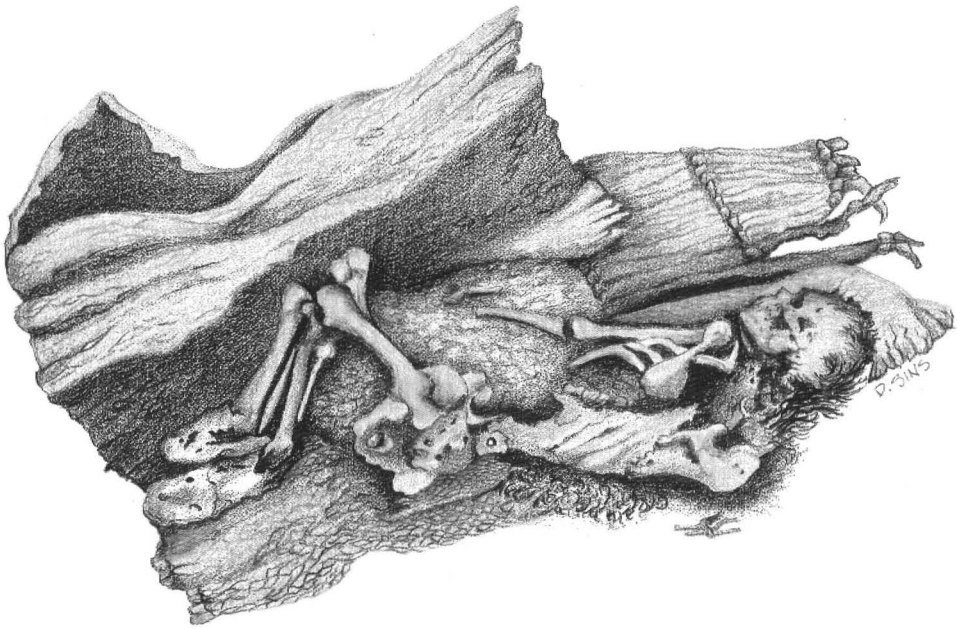
Nevada

Historical Society Quarterly



Spring 1997

NEVADA HISTORICAL SOCIETY QUARTERLY

The *Nevada Historical Society Quarterly* solicits contributions of scholarly or popular interest dealing with the following subjects: the general (e.g., the political, social, economic, constitutional) or the natural history of Nevada and the Great Basin; the literature, languages, anthropology, and archaeology of these areas; reprints of historic documents; reviews and essays concerning the historical literature of Nevada, the Great Basin, and the West.

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MOLECULAR ANALYSIS OF ANCIENT NATIVE AMERICAN DNA FROM WESTERN NEVADA

Frederika Kaestle

Anthropological interest in the peopling of the Great Basin has a long history. By now we are all aware of the debate regarding the Numic expansion hypothesis (Madsen and Rhode 1994). There are many arguments regarding how, when, and where the Numic spread occurred. A central problem remains: Was the Numic spread a spread of people, or simply a spread of language and technology? This article will address this question with some new data — mitochondrial DNA (mtDNA) from both modern and ancient Native American groups.

First I will give some general background about mtDNA and the techniques involved, and then the actual data. MtDNA is found in cells, but not in the nucleus, where most DNA is located. The mtDNA is found in the mitochondria, which are organelles found in the cytoplasm of the cell which help the cell produce energy. MtDNA is maternally inherited. Males and females both inherit mother's mtDNA, but only females pass it on to their children. MtDNA also mutates at a much faster rate than nuclear DNA, and so is informative on questions of recent prehistory, such as the peopling of the Americas and prehistoric movement in the Americas.

MtDNA is a circular molecule. We can detect the mutations in mtDNA in several ways. The first is by using restriction enzymes. A restriction enzyme is an enzyme that recognizes a very specific series of nucleotides (usually four to eight), and then cuts at that site. The presence of a restriction site that is normally absent, or the absence of a restriction site that is normally present, is indicative of a mutation in that region of DNA. Another type of mutation is the actual loss or gain of some number of nucleotides, such as the 9 base pair deletion.

New technology now allows us to access even small amounts of DNA, for example, the damaged DNA that survives in ancient individuals. This process, called the polymerase chain reaction, or PCR, uses the same mechanisms that your body uses to copy DNA when cells divide. With PCR, we can make millions of copies of a short segment of target DNA in a few hours.

The author is at the University of California, Davis. She wishes to thank Amy Dansie and Donald Tuohy of the Nevada State Museum for the opportunity to be involved in this research.

Using these techniques we can identify many mitochondrial lineages around the world. If we examine modern Asians, the putative ancestors to Native Americans, we can see that there are many different "versions" of mtDNA in Asia, but we are specifically interested in the four lineages that also appear in modern Native Americans. The more generally accepted labels for these four lineages are A, B, C, and D. Each lineage is united by a single mutation found in all individuals possessing that mitochondrial lineage.

We detect these mutations, in three cases, by cutting with a restriction enzyme. Figure 2 shows PCR products, which are DNA fragments, that have been separated in an electric current through a gel matrix. The fragments move through the gel at a rate that is dependent on their size: the larger the fragment, the slower it moves. Thus, fragments that have been cut can be seen as two small bands, while those that have not been cut are seen as one larger band. As can be seen in Figure 2, lineage A is identified by a HaeIII site gain at nucleotide position (np) 663, lineage C by a HincII site loss at np 13259, and lineage D by an AluI site loss at np 5176. Lineage B is identified by the loss of nine nucleotides in a row (a 9 bp deletion).

As can be seen in the world-wide distribution, shown in Figure 3, these four lineages are not present in the same frequencies in all groups. In Asia, although lineages A, B, C, and D are present in many groups, other mitochondrial lineages predominate. Not all contemporary Native American groups have these four lineages in the same frequencies (Figure 4). In fact, some tribes lack one or more of the lineages completely. This variation in frequency of the four lineages, or haplogroups, allows us to explore questions of relatedness between tribes, and also ancestor-descendant relationships between ancient and modern populations. Groups that are closely genetically related should have similar frequencies of the four lineages, while groups with very different frequencies are probably not closely related to each other.

The PCR technology allows us to examine the DNA of ancient individuals. For this study, we focused on twenty-one prehistoric individuals from the Pyramid Lake region in Western Nevada. These individuals have been ^{14}C dated to between 860±75 and 9,2225±60 B.P. Thus far, I have successfully extracted and amplified mtDNA from twenty of these individuals, identifying the mtDNA lineage in fifteen of them. Figure 5 shows the distribution of the four lineages in the Pyramid Lake individuals. All four lineages are present in our sample. Note that the oldest individual, from Wizards Beach and dated to 9225±60 B.P., belongs to the C lineage. This lineage identification can be seen on this electrophoretic gel photo (Figure 6). No other individual in this sample belongs to that lineage (see Figure 6). The frequency of that lineage in the modern inhabitants of the area, the Northern Paiute, is about 11 percent. Because we, to date, have only one sample from this time period in this area, a statistical analysis of lineage affinity is not possible. However, I also plan to sequence another portion of the mitochondrial DNA, called the D-loop, which is hypervariable. This means that the mutation rate for this region is

much higher (10X) than elsewhere in the mtDNA. I can then compare the sequence of this ancient individual with those of the other ancient individuals from the area, and also with modern Native Americans at a level that may allow finer resolution.

We can also look at the Pyramid Lake individuals as one group for analysis. Obviously, since the sampled skeletal material spans thousands of years, this is an artificial population. However, if we look at the temporal distribution of the mitochondrial lineages (Figure 7), no clear pattern emerges, except that the only individual possessing the lineage C marker is the oldest individual in the sample (9,225 B.P.). I have tried dividing the samples into two groups by age at 2,000 B.P., 3,000 B.P., and 4,000 B.P. and see no obvious discontinuities. To my knowledge, these represent the oldest examples of all lineages except B. In addition, if we accept the hypothesis of the recent Numic expansion, none of the samples should post-date this expansion. The frequencies of the four mitochondrial lineages in the ancient Pyramid Lake samples can be compared with those in modern Native American groups. This might clarify the identity of the pre-Numic inhabitants of the Great Basin if the frequencies of the lineages in modern tribal groups are similar to those of their ancestors. It seems unlikely that the pre-Numic population from Pyramid Lake is ancestral to any modern population from which its frequencies of the four mitochondrial lineages differ significantly.

The distribution of modern tribes in western North America that have been tested for these four mtDNA lineages can be seen in Figure 8. For analysis these had to be grouped in some way. The modern samples were sorted into both language groups and geographic regions for estimating and comparing frequencies of the four mitochondrial lineages (Figure 9). This was done because the relationship between genes and language or geography is often complicated. Populations may share genes with other groups in a geographic region, with other groups that speak related languages, or some combination. The patterns seen here may give us clues to the direction of gene flow in prehistory. Language groups consist of eight subsets of three larger groups: Penutian, Hokan and Uto-Aztecan. The samples were also sorted into the following four geographic regions: California, Baja, the Great Basin, and the Southwest.

Figure 10 shows the frequencies of the four lineages in the modern language groups and those in the ancient Pyramid Lake individuals. I have also included another ancient population, the Fremont. This data was generated in Dennis O'Rourke's laboratory at the University of Utah at Salt Lake. Those samples are from archaeological sites on the margin of the Great Salt Lake in Utah and have been dated to between 730 and 1,500 B.P. As can be seen, some language groups completely lack one lineage. Some geographic relationships may also be seen here (e.g., most language groups lacking lineage D are found in the Southwest). A comparison can be made more easily with Figure 11. The most obvious similarity is seen between the Pyramid Lake individuals and the modern California Penutian language group. However, the next two groups, the Northern Uto-Aztecan and

Northern Hokan language groups, although lacking lineage A do possess similar frequencies of the remaining three lineages. It is interesting that the prehistoric Fremont individuals appear quite distinct from any of the modern samples.

Figure 12 compares the frequencies of the four lineages in the modern geographic groups with those in the ancient samples. Again, differences between some modern groups and the ancient Pyramid Lake sample can be seen, and become more apparent when depicted in chart form (Figure 13).

I plan to add more samples to both the ancient and modern groups. I will then apply statistical tests to hypotheses of the relationships between these ancient and modern groups. For example, I will also be sequencing the hypervariable D-loop region for many of the ancient samples and doing a phylogenetic analysis of the ancient and modern sequences. This more detailed analysis may provide more information regarding patterns of prehistoric population movement in the Great Basin.

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FIGURES

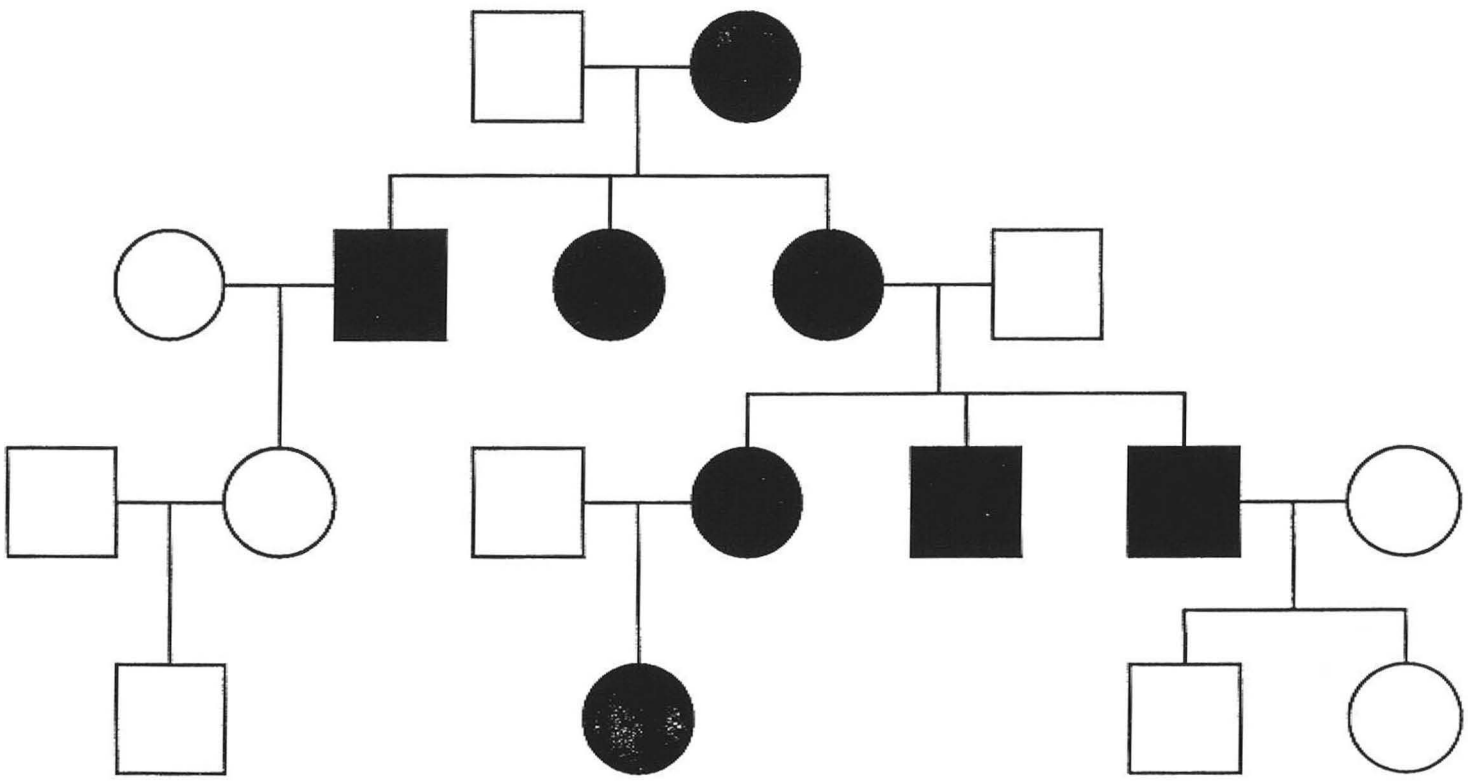

FIGURE 1. Mitochondrial DNA inheritance.

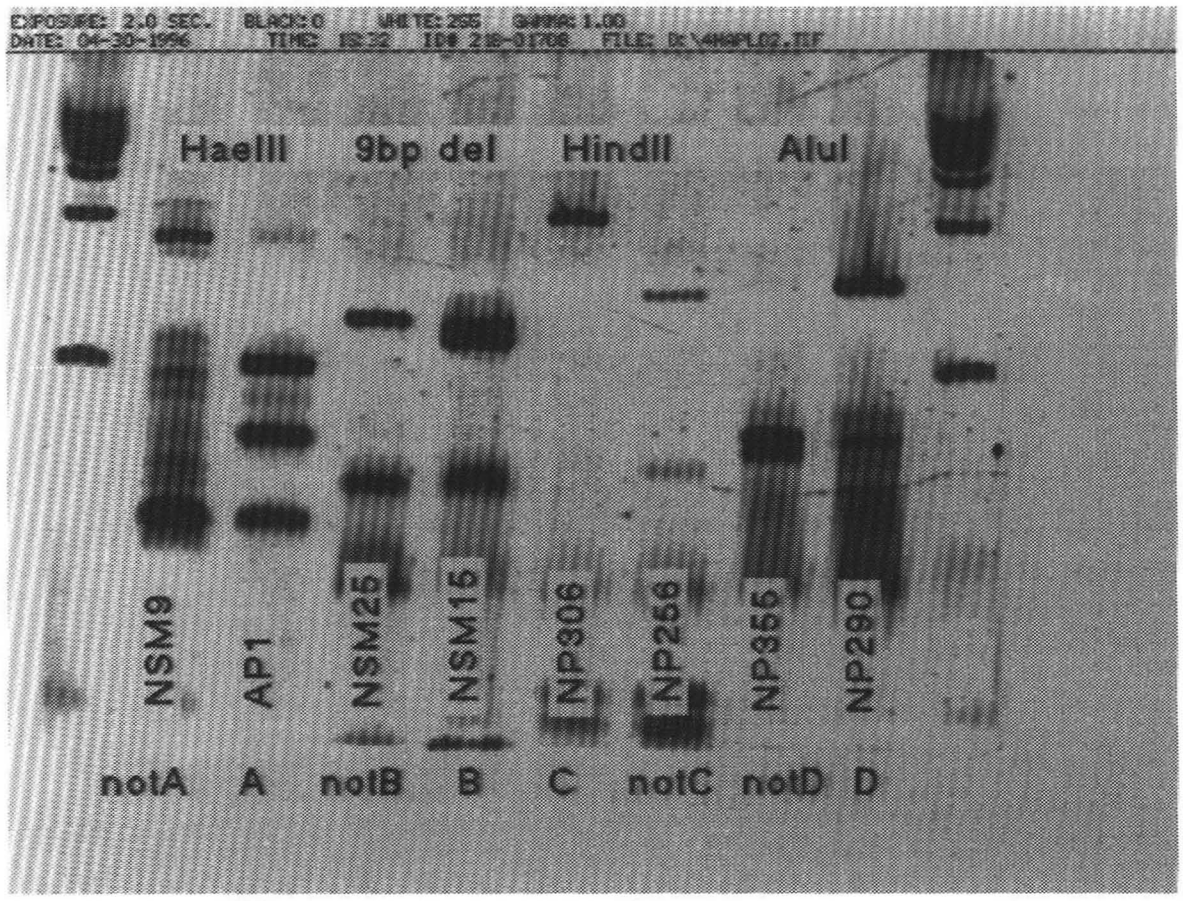

FIGURE 2. Identification of the four Native American mitochondrial lineages. Lanes one and ten contain size markers. In lanes two and three are the PCR products for the fragment containing the marker for lineage A, exposed to the HaeIII restriction enzyme. Note the Apache individual in lane three possesses the HaeIII restriction site (was cut) and thus belongs to lineage A. In lanes four and five are the PCR products for the fragment containing the marker for lineage B. Note the ancient individual from Pyramid Lake in lane five possesses the 9 bp deletion (the fragment is 9 nucleotides shorter than a non-lineage B fragment) and thus belongs to lineage B. In lanes six and seven are PCR products for the fragment containing the marker for lineage C, exposed to the HincII restriction enzyme. Note the modern Northern Paiute individual in lane six was not cut by the HincII enzyme, and thus belongs to lineage C. Lanes eight and nine contain the PCR products for the fragment containing the marker for lineage D, exposed to the AluI restriction enzyme. Note the modern Northern Paiute individual in lane nine does not possess the AluI site (did not cut) and therefore belongs to lineage D.

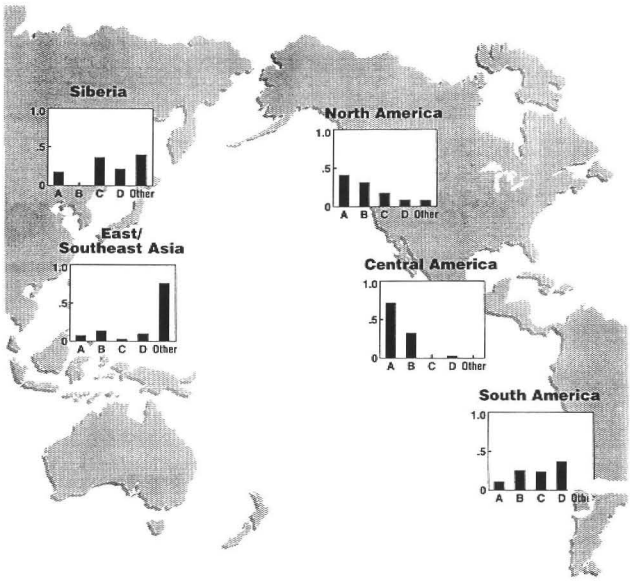

FIGURE 3. Frequency distributions of mtDNA haplogroups in Asia and the New World (after Lorenz and Smith 1996:318).

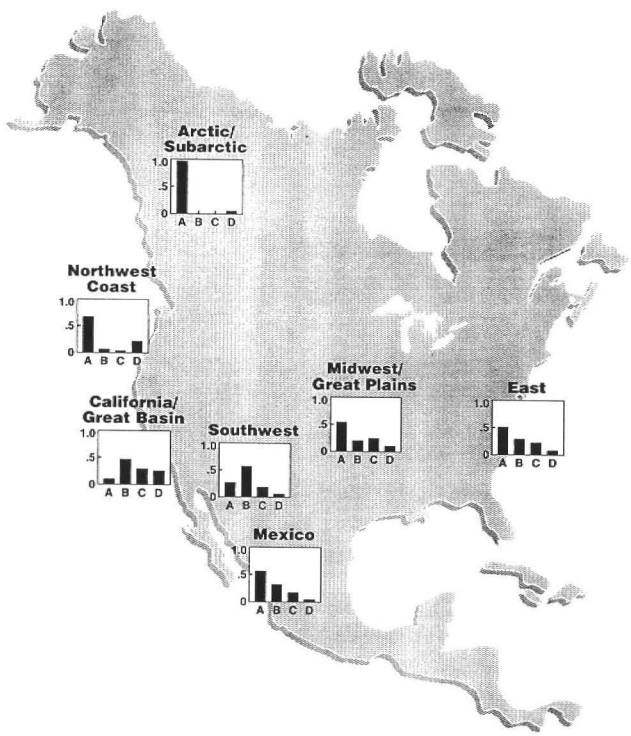

FIGURE 4. Modern frequency distributions of the four mtDNA haplogroups by geographic region in North America (after Lorenz and Smith 1996:314).

Lineage assignment of Prehistoric western Nevada samples

ID	date in yBP	# extractions	HaeIII (A)	9 bp del (B)	HindII (C)	AluI (D)	Lineage
NSM-1	3725 +/- 105	3	+	-	+	+, -	?
NSM-2	860 +/- 75	2	-	+	+	+	B
NSM-6	2935 +/- 140	2	-	-	+	-	D
NSM-7	4745 +/- 115	2	-	-	+	-	D
NSM-8	2735 +/- 100	2	-	-	+	-	D
NSM-9	2435 +/- 85	2	-	-	+	-	D
NSM-10	1620 +/- 50	2	-	-	+	-	D
NSM-11	1490 +/- 50	2	-	-	+	-	D
NSM-12	1820 +/- 180	3	-	+	+	+	B
NSM-13	1360 +/- 80	2	-	+	+	+	B
NSM-14	1520 +/- 90	2	+	-	+	+	A
NSM-15	5905 +/- 125	2	-	+	+	+	B
NSM-16	9200 +/- 60	2	-	-	-	+	C
NSM-17	3630 +/- 60	3	-	-	+	-	D
NSM-18	2430 +/- 100	2	-	-	+	-	D
NSM-19	3165 +/- 370	1	-	-	+	-	D
NSM-20	1500	2	-	-	+	-	D
NSM-21	1950 +/- 100	2	-	+	+	+	B
NSM-22	3540 +/- 95	2	-	+	+	+	B
NSM-23	4505 +/- 105	2	+	-	+	+	A
NSM-25	4435 +/- 110	2	-	-	+	+	other

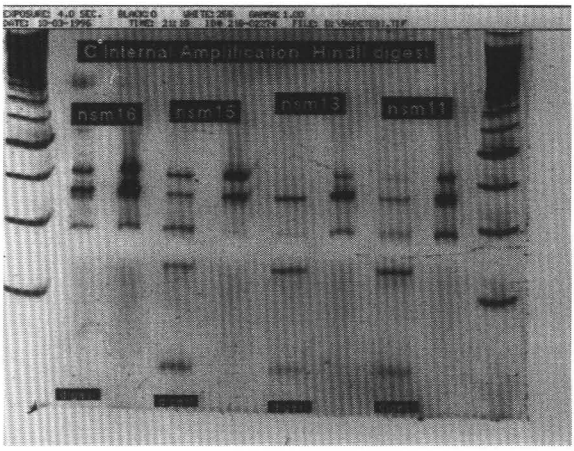

FIGURE 6. Identification of lineage C in Pyramid Lake individuals. Lanes one and ten contain size markers. Lanes three, five, seven, and nine contain the PCR product for the fragment containing the HincII marker for lineage C for individuals nsm-16, nsm-15, nsm-13, and nsm-11 respectively. Lanes two, four, six, and eight contain the same PCR product after exposure to the HincII restriction enzyme. Note that the fragments from individuals nsm-15, nsm-13, and nsm-11 all were cut by the HincII, while the fragment from individual nsm-16 was not. The lack of the HincII site is the marker for lineage C. Thus only nsm-16 belongs to lineage C.

FIGURE 7

Chronological Patterning of Lineage Distribution in Pyramid Lake Samples?

	A	B	C	D
Oldest	4505	5905	9515	4745
Youngest	1520	1360		2435

Ages of samples by lineage:

A: 1520, 1820, 3165, 4505 yBP

B: 1360, 3540, 5905 yBP

C: 9515 yBP

D: 2435, 2735, 4745, (and two undated, probably between 2000 and 5000 yBP)

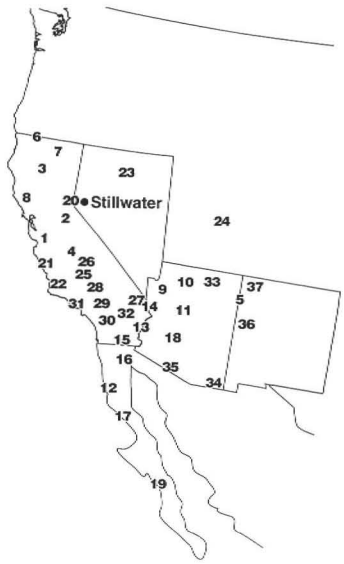

FIGURE 8. Modern tribes in western North America that have been tested for mtDNA lineage.

group	subgroup	sub-subgroup	number tribal members sampled	geographic group
Penutian	California Penutian		5 Costanoan (Ohlone)	California
			1 Miwok	California
			1 Maidu	California
			1 Wintun	California
			9 Yokuts	California
	Zuni		20 Zuni	Southwest
Hokan	Northern Hokan		1 Karok	California
			1 Achumawi	California
			3 Pomo	California
	Yuman		5 Walapai	Southwest
			5 Havasupai	Southwest
			5 Yavapai	Southwest
			8 Paipai	Baja
			19 Quechan	Southwest
			3 Mojave	Southwest
			16 Kamia (Tipai)	Baja
			3 Cocopa	Baja
			3 Kiliwa	Baja
			13 Cochimi	Baja
	Washo		28 Washo	Great Basin
	Central Coast Hokan (Salinan-Seri)		2 Salinan	California
			9 Chumash	California
Uto-Aztecan	Northern Uto-Aztecan	Numic	98 N. Paiute/Shoshone	Great Basin
			1 Kawaiisu	Great Basin
			4 Tubatulabal	California
		Takic	4 Kitanemuk	not included
			1 Gabrielino	not included
			1 Luiseno	not included
			1 Juaneno	not included
			1 Fernandeno	not included
			1 Cahuilla	not included
		Hopi	4 Hopi	Southwest
	Southern Uto-Aztecan (Sonoran)		37 Pima	Southwest

FIGURE 9. Modern samples sorted by language groups and geographic regions for estimating and comparing the four mitochondrial lineages.

Group	Subgroup	N	%A	%B	%C	%D
Ancient	**Ancient Western Nevada**	19	10	36	5	53
	Fremont	30	0	83	10	7
Penutian	**California Penutian**	17	12	41	6	41
	Zuni	20	20	70	10	0
Hokan	**Northern Hokan**	5	0	40	20	40
	Yuman	80	4	63	34	0
	Washo	28	0	54	36	11
	Central Coast Hokan	11	46	18	9	27
Uto-Aztecan	**Northern Uto-Aztecan**	116	0	42	15	43
	Southern Uto-Aztecan	37	5	57	38	0

FIGURE 10. Frequencies of the four lineages in the northern language groups and in the ancient western Nevada indivisual.

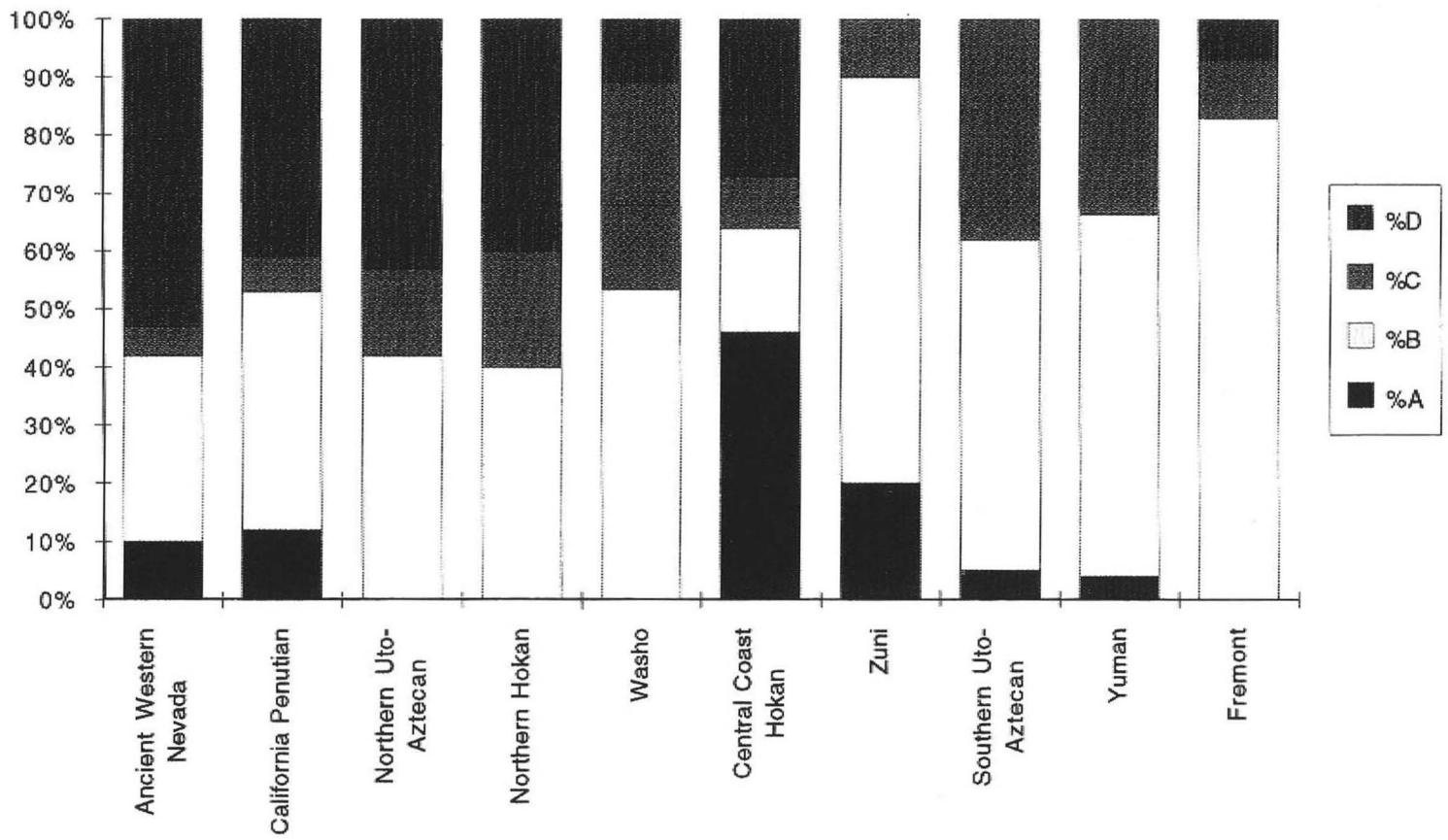

FIGURE 11. Lineage frequencies in ancient populations and modern language groups.

Geographic Group	N	%A	%B	%C	%D
Ancient Western Nevada	19	10	32	5	53
Fremont	30	0	83	10	7
California	37	18	35	11	35
Baja	43	2	67	30	0
Southwest	98	8	61	31	0
Great Basin	127	0	44	17	39

FIGURE 12. Comparison of the frequencies of the four lineages in the modern geographic groups to those in the ancient samples.

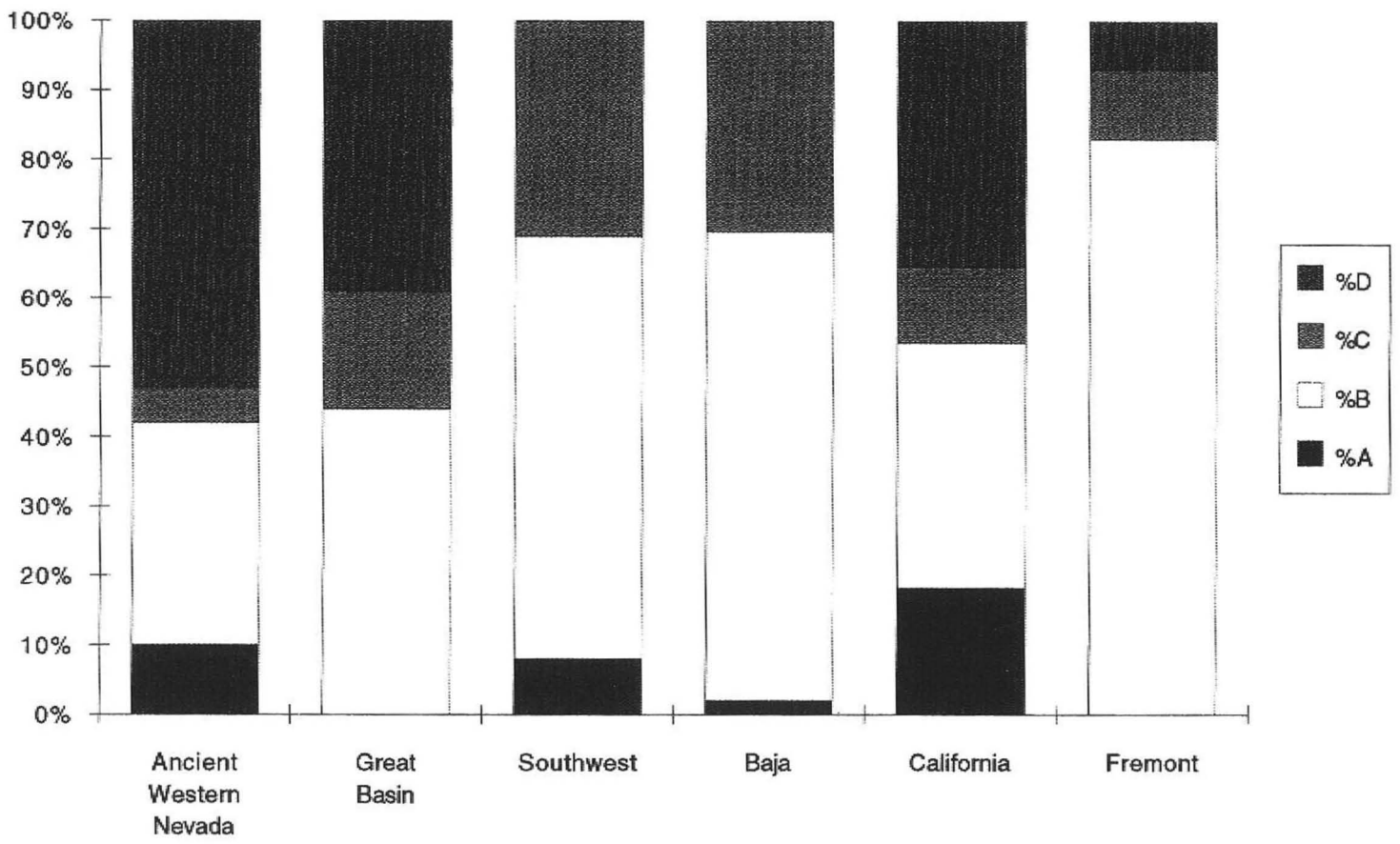

FIGURE 13. Lineage frequencies in ancient populations and modern geographic groups.