

# Brief Communication: Haplogroup X Confirmed in Prehistoric North America

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**ABSTRACT** Haplogroup X represents approximately 3% of all modern Native North American mitochondrial lineages. Using RFLP and hypervariable segment I (HVSI) sequence analyses, we identified a prehistoric individual radiocarbon dated to  $1,340 \pm 40$  years BP that is a member of haplogroup X, found near the Columbia River

Using RFLP and hypervariable segment I (HVSI) sequence analyses, a mitochondrial DNA haplogroup that represents approximately 3% of all modern Native North American samples (Forster et al., 1996; Brown et al., 1998; Smith et al., 1999) and whose known worldwide distribution also includes West Asia and Europe (Torroni et al., 1996) has been identified. Due to the absence of haplogroup X in East Asia, presumed to be the home]] group in North America (Brown et al., 1998), suggested the possibility of an ancient European migration to North America. Stanford (1997) suggested that early Holocene Europeans used a trans-Atlantic route to colonize the Americas and that Clovis technology was derived from the Solutrean tradition of the Iberian Peninsula. The presence of haplogroup X in North America might also be due to recent European admixture, since this haplogroup is found in high frequency in the Ojibwa (Brown et al., 1998; Malhi et al., 2001), among whom nuclear markers exhibit evidence of extensive European admixture (Szathmary and Auger, 1983).

Most of these hypotheses have been refuted by more recent research. Derenko et al. (2001) reported the presence of haplogroup X in Altaian populations from southern Siberia, where the other four Native American founding haplogroups are also present. Therefore, the Altai are the only known modern ethnic group whose membership represents all five Native American haplogroups and, assuming the New World was colonized by a single migration, constitute a possible origin of the founders of Native America. Despite unsuccessful attempts to extract ancient mtDNA from the Kennewick specimen

in Vantage, Washington. The presence of haplogroup X in prehistoric North America, along with recent findings of haplogroup X in southern Siberians, confirms the hypothesis that haplogroup X is a founding lineage. *Am J Phys Anthropol* 119:84–86, 2002. © 2002 Wiley-Liss, Inc.

(Kaestle, 2000; Merriwether and Cabana, 2000; Smith et al., 2000b), other early Holocene skeletons in North America with cranial features unlike modern Native Americans (e.g., from Wizard's Beach and Hourglass Cave) exhibit haplogroups that are found in Native Americans but not in Europeans (Stone and Stoneking, 1996; Kaestle and Smith, 2001). These lines of evidence, together with recent criticism of similarities between the Clovis and Solutrean cultures (Straus, 2000) that were cited by Stanford (1997), strongly suggest that haplogroup X did not reach the Americas via recent European migration.

The wide geographic and linguistic distribution of haplogroup X in modern Native North American groups (Forster et al., 1996; Brown et al., 1998; Smith et al., 1999) and the predominance among those of a characteristic mutation in HVSI of the control region not found in European or Asian members of haplogroup X (the G → A transition at np 16,213), imply that it is a founding Native American lineage. In addition, the presence of three (allegedly full-blood) Algonquian-speaking Native Americans (Smith et al., 2000a; Malhi et al., 2001) who exhibit both the haplogroup X with the transition

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at np 16,213 and the rare Albumin marker, Albumin\*Naskapi, also only found in North America, suggests that haplogroup X is not found in North America due to recent European admixture.

The most convincing evidence that haplogroup X is not the result of Viking or even more recent European admixture would be its presence in ancient Native Americans. Ancient samples from the Norris Farms site (Stone and Stoneking, 1998), the Win-

dover site (Hauswirth et al., 1994), and the Amazon Basin (Ribeiro-Dos-Santos et al., 1996) exhibit the characteristic HVSI control region markers found in individuals assigned to haplogroup X, but they could not be confidently assigned that haplogroup because they were not tested for the *AccI* restriction site at np 14,465. We confirmed the presence of haplogroup X in one prehistoric sample excavated at a site on the Columbia River near Vantage, Washington and radiocarbon dated to  $1,340 \pm 40$  years BP. Extensive precautions were taken to limit contamination of this sample and to detect any such contamination when it did occur. To eliminate surface contamination, the tooth was soaked in 10% bleach for 10 min, followed by ultraviolet light (254 nm) irradiation. Extraction and amplification setups were performed in a dedicated ancient DNA laboratory that is routinely bleach-sterilized. Negative controls were included at various stages of the extraction and amplification setup processes. Multiple extractions were performed on the tooth to confirm haplogroup assignment. For a complete review of the extraction method and precautions used to prevent contamina-

tion of the sample, see Smith et al. (2000b). Extraction and most amplification setup negative controls exhibited no evidence of contamination. When negative controls did exhibit contamination, the data