Supporting Online Material

A 425bp fragment of the mitochondrial DNA control region was amplified in three overlapping fragments from bones, teeth or mummified soft tissue of ancient American dogs (primers Thr-L 5'-GAA TTC CCC GGT CTT GTA AAC C and dogDL-5 5'-CAT TAA TGC ACG ACG TAC ATA GG; dogDL-1g 5'GTG CTA TGT CAG TAT CTC CAG G and dogDL-2 5'GCA AGG GTT GAT GGT TTC TCG or dogDL-3 5'-CCC TTA TTG GAC TAA GTG ATA TGC AT; dogDL-4 5'-GCA TAT CAC TTA GTC CAA TAA GGG or dogDL-7 5'-TAT TAT ATC CTT ACA TAG GAC and DL-Hcan 5'-CCT GAG GTA AGA ACC AGA TG). Polymerase chain reaction and extraction conditions as well as precautions applied to the study of ancient DNA are as described in ref. *1*. Thirty-seven specimens from archaeological sites in Mexico (N= 6), Peru (N= 26) and Bolivia (N= 5) and dated by their archaeological context as pre-Columbian were analyzed. Complete sequences were obtained from 13 of these specimens. To confirm reliability of the sequences obtained, at least one fragment was replicated for each sample, three samples were extracted and sequenced two or three times (at UCLA by JAL) and one sequence fragment was independently extracted, amplified and sequenced with separate reagents at the University of Uppsala by CV. Sequences have been deposited into GenBank.

Phylogenetic trees were built using a neighbor-joining algorithm and a HKY85 model of evolution assuming variation in the rate of nucleotide substitutions across loci following a gamma distribution with parameter a= 0.5. Neighbor-joining trees were constructed assuming other models of sequence evolution, as well as trees using different optimality criteria (maximum parsimony and maximum likelihood). Trees were build using PAUP*4.10b (*2*). In all cases, the same framework topology was observed, with four well-differentiated groups of dog sequences as

in ref. *3*. See ref. *3* for analysis of statistical support for the four dog clades. A clade of American-specific sequences (clade *a*) was retained in all trees.

When ancestral haplotypes are likely to be present in a population, such as in dogs, networks may be a more efficient way to represent phylogenetic relationships since they allow for sequences at the internal nodes. For dog sequences in Clade I (Fig. 1) we have constructed a statistical parsimony network with the program TCS v1.13 (*4*), where all haplotypes are connected by branches representing single mutational events, and insertions/deletions were used as the fifth character state.

References

1. J. A. Leonard, R. K. Wayne, A. Cooper, *Proc. Natl. Acad. Sci.* **97**,1651 (2000).
2. D. L. Swofford, *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.* (Sinauer Associates, Sunderland, Massachusetts, 1998).
3. C. Vilà, *et al. Science* **276**,1687 (1997).
4. M. Clement, D. Posada, K. A. Crandall, *Mol. Ecol.* **9**, 1657 (2000).