

- dependent lysis, because the calcium chelator EGTA, which blocks the perforin pathway, also inhibits up-regulation of FasL, as required in our studies with primary human T cells.
11. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) induces lysis of a murine fibrosarcoma cell line independently of perforin or Fas-FasL (19). This cytokine did not contribute to lysis of the CTLs used in this study because addition of blocking antibody to TNF- $\alpha$  did not inhibit the cytotoxicity.
  12. G. Berke, *Annu. Rev. Immunol.* **12**, 735 (1994).
  13. The MHC class I-restricted CD8<sup>+</sup> T cell lines that specifically recognize a defined influenza virus matrix protein presented by human leukocyte antigen (HLA) A2 (CD8.FP1, CD8.FP2) were generated from the blood of healthy HLA A.2<sup>+</sup> donors by stimulation of PBMCs with the peptide (10  $\mu$ g/ml) (gift of Cytel, San Diego, CA) (20). Lines were maintained by weekly stimulation with the peptide, with irradiated autologous PBMCs as feeder cells. Before the experiments, CD8<sup>+</sup> cells were enriched by immunomagnetic depletion of CD4<sup>+</sup> T cells and natural killer cells.
  14. S. H. E. Kaufmann, *Immunol. Today* **9**, 168 (1988).
  15. K. Benihoud, D. Bonardelle, P. Bobe, N. Kiger, *Eur. J. Immunol.* **27**, 415 (1997).
  16. T. S. Griffith, T. Brunner, S. M. Fletcher, D. R. Green, T. A. Ferguson, *Science* **270**, 1189 (1995); H. Yagita *et al.*, *Immunol. Rev.* **146**, 223 (1995).
  17. P. Laochumroonvorapong *et al.*, *Infect. Immun.* **65**, 127 (1997); A. Cooper, C. D'Souza, A. A. Frank, I. Orme, *ibid.*, p. 1317; R. J. Mazzaccaro, J. Flynn, B. R. Bloom, unpublished results.
  18. S. Pena *et al.*, *J. Immunol.* **158**, 2680 (1997); M. Anderson *et al.*, *EMBO J.* **14**, 1615 (1995); Y. Miyakawa *et al.*, *Infect. Immun.* **64**, 926 (1996).
  19. R. K. Lee, J. Spielman, D. Y. Zhao, K. J. Olsen, E. R. Podack, *J. Immunol.* **157**, 1919 (1996); M. Y. Braun, B. Lowin, L. French, H. Acha-Orbea, J. Tschopp, *J. Exp. Med.* **183**, 657 (1996).
  20. A. J. McMichael, F. M. Gotch, J. Santos-Aguado, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9194 (1988).
  21. N. Kayagaki *et al.*, *J. Exp. Med.* **182**, 1777 (1995).
  22. W. C. Liles, P. A. Kiener, J. A. Ledbetter, A. Aruffo, S. J. Klebanoff, *ibid.* **184**, 429 (1996).
  23. P. L. Coleman and G. D. Green, *Methods Enzymol.* **80**, 408 (1981).
  24. CD1<sup>+</sup> macrophages, which were infected with live *M. tuberculosis*, were labeled with 100  $\mu$ Ci of <sup>51</sup>Cr (ICN, Costa Mesa, CA) for 1 hour and plated in a 96-well V-bottom plate at a final concentration of 4000 targets per 100  $\mu$ l. Appropriate samples were incubated with blocking antibodies to CD1a, CD1b, or CD1c for 30 min before addition of the T cells. After a 9-hour incubation, target cell lysis was calculated by quantifying <sup>51</sup>Cr release in a gamma-counter. The data are given as percent specific lysis, calculated as [(cpm release from experimental - cpm spontaneous release)/(maximal release - cpm spontaneous release)  $\times$  100]. The spontaneous <sup>51</sup>Cr release by macrophages in the absence of T cells was <15%.
  25. CD1<sup>+</sup> macrophages were pulsed with soluble *M. tuberculosis* extract (5  $\mu$ g/ml) overnight, detached with 1 mM EDTA, and labeled with 100  $\mu$ Ci of <sup>51</sup>Cr for 1 hour. For inhibition of the interaction between FasL and Fas, the assay was done in the presence of blocking antibodies to FasL (21) (Pharmingen, San Diego, CA) or Fas (22) (Immunotech, Westbrook). Degranulation of the cytotoxic granules was induced by initial treatment of T cells with 25 mM Sr<sup>2+</sup> (Aldrich, Milwaukee, WI) for 18 hours. <sup>51</sup>Cr release was determined after a 4-hour incubation. Expression of Fas on the target cells was not affected by infection with *M. tuberculosis*, as determined by flow cytometry.
  26. CTLs (5  $\times$  10<sup>5</sup>) were incubated in the presence of 25 mM Sr<sup>2+</sup> for 10 hours in a final volume of 1.5 ml. The amount of N $\alpha$ -CBZ-L-lysine thiobenzyl (BLT)-esterase in the supernatant was determined by the BLT-esterase assay (23). The supernatants (20  $\mu$ l) were coincubated with 35  $\mu$ l of 1 mM BLT (Sigma), 35  $\mu$ l of 1 mM 5,5'-dithio-bis-(2-nitrobenzoic acid) (Sigma), and 10  $\mu$ l of a 0.1% Triton X-100 (Sigma) solution. After a 30-min incubation at 37°C, the absorbance at 405 nm was determined.
  27. P. Chomczynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987).
  28. FasL primer and probe sequences were designed as follows: FasL 5': CAGCTCTTCCACCTGCAGAAGG; FasL 3': AGATTCTCTAAAATTGATCAGAGAGAG; FasL probe: GAGCCGAGGAGTGTGGCCCATTTA-ACAGGG. PCR was performed with AmpliTaq polymerase (0.5 U/ml; Perkin-Elmer-Cetus) plus 2.5 mM MgCl<sub>2</sub> for 25 s of denaturation at 94°C followed by 45 s of annealing and extension at 65°C for 35 consecutive cycles. PCR products were resolved by agarose gel electrophoresis and transferred to nylon membranes (Amersham, Arlington Heights, IL). The membranes were then probed with an internal oligonucleotide probe labeled at the 5' end with  $\gamma$ -<sup>32</sup>P and polynucleotide kinase (Boehringer Mannheim, Ger-
- many) and visualized by autoradiography.
29. E. P. Miskovsky *et al.*, *J. Immunol.* **153**, 2787 (1994).
  30. We are indebted to M. Horwitz for use of his P3 laboratory and P. Sieling for continuous support and helpful discussions. Supported by the AIDS Stipendium; Deutsches Krebsforschungszentrum, Heidelberg (S.S.); NIH (R.L.M., S.A.P., B.R.B., M.B.B.); the Arthritis Foundation (S.A.P.); the Howard Hughes Medical Institute (B.R.B.); the Swiss Foundation for Grants in Medicine and Biology (J.-P.R.); and the United Nations Development Programme-World Bank-World Health Organization Special Program for Research and Training in Tropical Diseases (IMMLEP) and the Dermatologic Research Foundation of California.

30 January 1997; accepted 14 April 1997

## Multiple and Ancient Origins of the Domestic Dog

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Mitochondrial DNA control region sequences were analyzed from 162 wolves at 27 localities worldwide and from 140 domestic dogs representing 67 breeds. Sequences from both dogs and wolves showed considerable diversity and supported the hypothesis that wolves were the ancestors of dogs. Most dog sequences belonged to a divergent monophyletic clade sharing no sequences with wolves. The sequence divergence within this clade suggested that dogs originated more than 100,000 years before the present. Associations of dog haplotypes with other wolf lineages indicated episodes of admixture between wolves and dogs. Repeated genetic exchange between dog and wolf populations may have been an important source of variation for artificial selection.

The archaeological record cannot resolve whether domestic dogs originated from a single wolf population or arose from multiple populations at different times (1, 2). However, circumstantial evidence suggests that dogs may have diverse origins (3). During most of the late Pleistocene, humans and wolves coexisted over a wide geographic area (1), providing ample opportunity for independent domestication events and continued genetic exchange between wolves and dogs. The extreme phenotypic diversity of dogs, even during the early stages of domestication (1, 3, 4), also suggests a varied genetic heritage. Consequently, the genetic diversity of dogs may have been enriched by multiple founding events, possibly followed by occasional in-

terbreeding with wild wolf populations.

We sequenced portions of the mitochondrial DNA of wolves and domestic dogs. Initially, 261 base pairs (bp) of the left domain of the mitochondrial control region (5) were sequenced from 140 dogs representing 67 breeds and five cross-breeds and 162 wolves representing 27 populations from throughout Europe, Asia, and North America (Fig. 1) (6). Because all wild species of the genus *Canis* can interbreed (7) and thus are potential ancestors of the domestic dog, five coyotes (*Canis latrans*) and two golden, two black-backed, and eight Simien jackals (*C. aureus*, *C. mesomelas*, and *C. simensis*, respectively) were also sequenced.

The control region of wolves and dogs was highly polymorphic (Fig. 1). We found 27 wolf haplotypes that differed on average by  $5.31 \pm 0.11$  ( $\pm$ SE) substitutions ( $2.10 \pm 0.04\%$ ), with a maximum of 10 substitutions (3.95%). The distribution of wolf haplotypes demonstrated geographic specificity, with most localities containing haplotypes unique to a particular region (Fig. 1). Four haplotypes (W2, W7, W14, and W22) had a widespread distribution. In dogs, 26 haplotypes were found. Only haplotype D6 also occurred in

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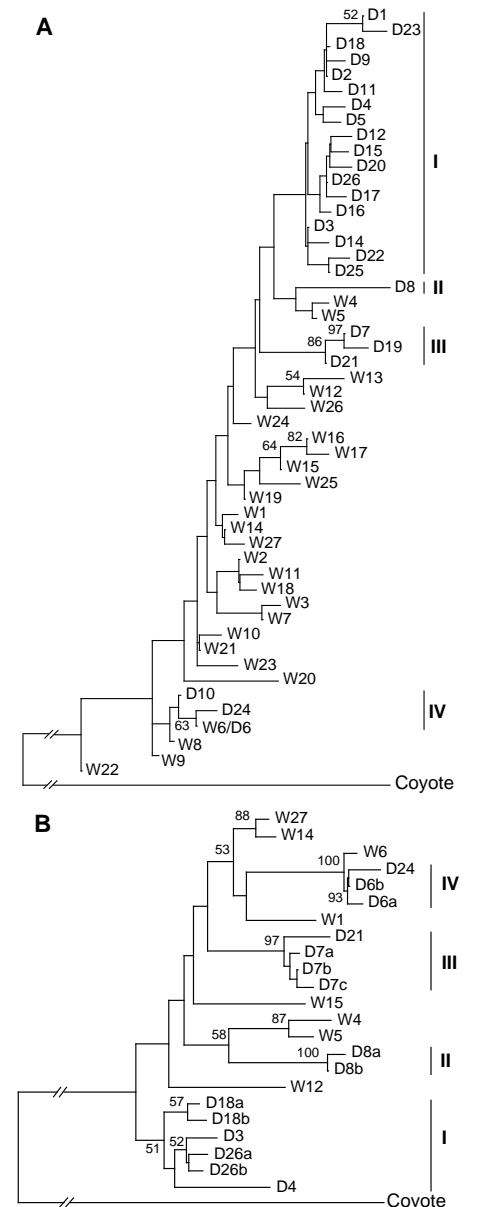
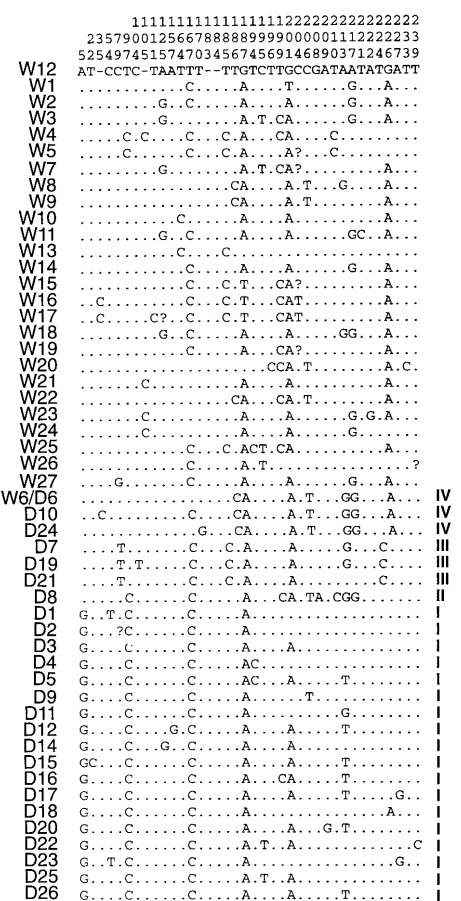
some gray wolves from western Russia and Romania (W6). Sequence divergence among dogs was similar to that found among wolves. Dog haplotypes differed by an average of  $5.30 \pm 0.17$  substitutions ( $2.06 \pm 0.07\%$ ), with a maximum divergence of 12 substitutions (4.67%). Mitochondrial haplotype diversity in dogs could not be partitioned according to breeds. For example, in eight German shepherds examined, five distinct sequences were found, and in six golden retrievers, four sequences were detected. Moreover, many breeds shared sequences with other breeds. For instance, dog haplotypes D4, D3, D5, and D1 were found in 14, 14, 9, and 7 breeds, respectively. No

dog sequence differed from any wolf sequence by more than 12 substitutions, whereas dogs differed from coyotes and jackals by at least 20 substitutions and two insertions. These results clearly support wolf ancestry for dogs. However, because mitochondrial DNA is maternally inherited, interbreeding between female dogs and male coyotes or jackals would not be detected. More limited studies of nuclear markers support the conclusion that the wolf was the ancestor of the domestic dog (8).

Several methods of phylogenetic analysis, including maximum likelihood (9), maximum parsimony (10), minimum spanning networks (11), and statistical

parsimony (12), were used to investigate relationships among sequences. All analyses supported a grouping of dog haplotypes into four distinct clades, although the topology within and among clades differed among trees (13). As exemplified by the neighbor-joining analysis (Fig. 2A), three of the four monophyletic clades defined a larger clade containing all but three dog

**Fig. 1.** Substitutions and deletions (–) observed in 261 bp of control region sequence from wolves (W) and dogs (D). The dog sequence D13 had the same sequence as D4 except for an insertion of a 67-bp tandem repeat. The numerals I, II, III, and IV indicate assignments to the four clades of dog sequences. Wolf localities: Bulgaria ( $n = 1$ , W7); Croatia ( $n = 5$ , W2); Estonia ( $n = 1$ , W10); France ( $n = 2$ , W4); Finland ( $n = 2$ , W10); Greece ( $n = 7$ , W2, W5, W8, and W9); Italy ( $n = 12$ , W4); Poland ( $n = 1$ , W3); Portugal ( $n = 19$ ; W1 and W2); Romania ( $n = 4$ ; W5 and W6); Russia ( $n = 3$ ; W6, W10, and W26); Spain ( $n = 46$ ; W1 and W3); Sweden ( $n = 2$ ; W2 and W10); Afghanistan ( $n = 3$ , W18); China ( $n = 3$ ; W14, W19, and W27); India ( $n = 1$ , W12); Iran ( $n = 6$ ; W16 and W17); Israel ( $n = 16$ , W11); Saudi Arabia ( $n = 7$ ; W7, W12, W13, W14, and W15); Turkey ( $n = 2$ , W2); Alaska ( $n = 3$ , W20); Alberta ( $n = 1$ , W22); Labrador ( $n = 3$ , W22); Mexico ( $n = 5$ , W25); Montana ( $n = 1$ , W22); Northwest Territories ( $n = 3$ , W22); and Yukon ( $n = 3$ ; W21, W23, and W24). Dog breeds: basenji ( $n = 1$ , D2); basset ( $n = 1$ , D6); boxer ( $n = 1$ , D4); Norwegian buhund ( $n = 1$ , D1); bulldog ( $n = 1$ , D6); Chinese crested ( $n = 2$ ; D2 and D25); chow chow ( $n = 3$ ; D1, D2, and D3); collie ( $n = 1$ , D1); border collie ( $n = 3$ ; D1 and D5); wirehaired dachshund ( $n = 3$ ; D5 and D10); Australian dingo ( $n = 4$ , D18); grey Norwegian elkhound ( $n = 9$ ; D3 and D8); Eskimo dog ( $n = 1$ , D23); German shepherd ( $n = 8$ ; D4, D5, D6, D7, and D19); greyhound ( $n = 1$ , D9); groenendaal ( $n = 1$ , D6); Mexican hairless ( $n = 6$ ; D3, D6, D21, and D26); hamiltonstövare ( $n = 1$ , D5); Afghanistan hound ( $n = 3$ , D6); Alaskan husky ( $n = 2$ ; D4 and D7); Siberian husky ( $n = 3$ ; D3, D7, and D18); jāmthund ( $n = 3$ ; D7 and D8); keeshond ( $n = 1$ , D5); kuvasz ( $n = 1$ , D4); Leonberger ( $n = 2$ ; D1 and D4); Norwegian lundehund ( $n = 1$ , D16); Mareema ( $n = 1$ , D6); Pyrenean mastiff ( $n = 1$ , D11); Newfoundland ( $n = 1$ , D4); otter hound ( $n = 1$ , D6); papillon ( $n = 2$ ; D3 and D4); poodle ( $n = 1$ , D3); toy poodle ( $n = 1$ , D6); pug ( $n = 1$ , D26); Chesapeake Bay retriever ( $n = 1$ , D13); flat-coated retriever ( $n = 3$ ; D4 and D10); golden retriever ( $n = 6$ ; D4, D6, D15, and D24); Labrador retriever ( $n = 6$ ; D4 and D12); Rhodesian ridgeback ( $n = 1$ , D26); rottweiler ( $n = 2$ , D3); Samoyede ( $n = 3$ ; D1, D4, and D5); St. Bernard ( $n = 1$ , D9); schipperke ( $n = 1$ , D4); giant schnauzer ( $n = 3$ ; D4 and D7); miniature schnauzer ( $n = 1$ , D9); English setter ( $n = 4$ ; D3 and D5); Irish setter ( $n = 3$ ; D1 and D9); New Guinea singing dog ( $n = 2$ , D18); shar ( $n = 1$ , D26); Icelandic sheepdog ( $n = 1$ , D3); Old English sheepdog ( $n = 1$ , D5); shiba inu ( $n = 1$ , D20); Cavalier King Charles spaniel ( $n = 1$ , D17); Irish water spaniel ( $n = 1$ , D6); springer spaniel ( $n = 1$ , D3); Tibetan spaniel ( $n = 1$ , D6); spitz ( $n = 1$ , D22); Japanese spitz ( $n = 1$ , D3);airedale terrier ( $n = 1$ , D7); border terrier ( $n = 2$ , D3); fox terrier ( $n = 2$ ; D3 and D14); Norfolk terrier ( $n = 2$ , D4); West Highland terrier ( $n = 2$ , D7); Tibetan terrier ( $n = 2$ ; D2 and D9); wachtelhund ( $n = 1$ , D5); whippet ( $n = 1$ , D3); Irish wolfhound ( $n = 2$ , D11); and crossbreeds ( $n = 5$ ; D1, D3, D4, D5, and D18).



**Fig. 2. (A)** Neighbor-joining tree of wolf and dog haplotypes (D13 excluded; see Fig. 1) based on 261 bp of control region sequence (17). **(B)** Neighbor-joining tree of 8 wolf and 15 dog genotypes based on 1030 bp of control region sequence. The suffixes a, b, and c after the haplotype labels were used to distinguish identical 261-bp sequences that have different 1030-bp sequences. Bootstrap support is indicated at nodes if found in more than 50% of 10,000 bootstrap trees.

haplotypes and a subset of wolf haplotypes (W4 and W5). Clade I included 19 of the 26 dog haplotypes. This group contained representatives of many common breeds as well as ancient breeds such as the dingo, New Guinea singing dog, African basenji, and greyhound (14). Clade II included dog haplotype D8, from two Scandinavian breeds (elkhound and jämthund), and was closely related to two wolf haplotypes found in Italy, France, Romania, and Greece (W4 and W5). Clade III contained three dog haplotypes (D7, D19, and D21) found in a variety of breeds such as the German shepherd, Siberian husky, and Mexican hairless. Finally, clade IV contained three haplotypes (D6, D10, and D24) that were identical or very similar to a wolf haplotype (W6) found in Romania and western Russia, which suggests recent hybridization between dogs and wolves. Many breeds contained representatives of more than one dog haplotype grouping (Fig. 1).

Because the overall bootstrap support for many of the internodes in Fig. 2A was low, 1030 bp of the control region were sequenced for 24 canids, including representatives of the four dog clades (15). Although the association of clades was different, the analyses of the longer sequences provided stronger support for the four monophyletic groupings of dog haplotypes (Fig. 2B) (13). A Wilcoxon signed-rank test was used to assess the monophyly of dog clades (16). Monophyly of all dog haplotypes can be rejected, and monophyly of clades I, II, and III is marginally rejected ( $P = 0.0004$  and  $P = 0.053$ , respectively). In both trees, dog haplotype clades II and IV are most closely related to wolf sequences from eastern Europe (Greece, Italy, Romania, and western Russia).

The coyote and wolf have a sequence divergence of  $0.075 \pm 0.002$  (17) and diverged about one million years ago, as estimated from the fossil record (18). Consequently, because the sequence divergence between the most different genotypes in clade I (the most diverse group of dog sequences) is no more than 0.010, this implies that dogs could have originated as much as 135,000 years ago (19). Although such estimates may be inflated by unobserved multiple substitutions at hypervariable sites (20), the sequence divergence within clade I clearly implies an origin more ancient than the 14,000 years before the present suggested by the archaeological record (21). Nevertheless, bones of wolves have been found in association with those of hominids from as early as the middle Pleistocene, up to 400,000 years ago (1, 22). The ancient dates for domes-

tication based on the control region sequences cannot be explained by the retention of ancestral wolf lineages, because clade I is exclusively monophyletic with respect to dog sequences and thus the separation between dogs and wolves has been long enough for coalescence to have occurred. To explain the discrepancy in dates, we hypothesize that early domestic dogs may not have been morphologically distinct from their wild relatives. Conceivably, the change around 10,000 to 15,000 years ago from nomadic hunter-gatherer societies to more sedentary agricultural population centers may have imposed new selective regimes on dogs that resulted in marked phenotypic divergence from wild wolves (23).

Although individual breeds show uniformity with respect to behavior and morphology, most breeds show evidence of a genetically diverse heritage because they contain different haplotypes. Moreover, dog sequences cluster with different groups of wolf haplotypes. Therefore, after the origin of dogs from a wolf ancestor, dogs and wolves may have continued to exchange genes. Backcrossing events could have provided part of the raw material for artificial selection and for the extraordinary degree of phenotypic diversity in the domestic dog. Domestic species of plants and animals whose wild progenitors are extinct cannot be enriched through periodic interbreeding, and change under artificial selection may be more limited. Consequently, the preservation of wild progenitors may be a critical issue in the continued evolution of domestic plants and animals.

## REFERENCES AND NOTES

1. J. Clutton-Brock, in *The Domestic Dog, Its Evolution, Behaviour and Interactions with People*, J. Serpell, Ed. (Cambridge Univ. Press, Cambridge, 1995), pp. 7-20.
2. S. J. Olsen and J. W. Olsen, *Science* **197**, 533 (1977).
3. J. Clutton-Brock, *A Natural History of Domesticated Mammals* (Cambridge Univ. Press, British Museum of Natural History, Cambridge, 1987).
4. F. E. Zeuner, *A History of Domesticated Animals* (Harper & Row, New York, 1963); H. Epstein, *The Origin of the Domestic Animals of Africa* (Africana Publishing, New York, 1971); I. L. Brisbin Jr., *Am. Kennel Gaz.* January 1976, p. 23.
5. C. Saccone, M. Attimonelli, E. Sbisà, *J. Mol. Evol.* **26**, 205 (1987).
6. DNA from blood, tissue, or hair was isolated by cell lysis followed by organic solvent purification. A 261-bp control region fragment was sequenced from a slightly larger (394 bp) fragment amplified with primers L15910 and H16498 [R. DeSalle, A. K. Williams, M. George, *Methods Enzymol.* **224**, 176 (1993)]. See polymerase chain reaction and sequencing conditions and protocols in J. E. Maldonado, F. O. Davila, B. S. Stewart, E. Geffen, R. K. Wayne, *Mar. Mamm. Sci.* **11**, 46 (1995).
7. A. P. Gray, *Mammalian Hybrids, a Check-list with Bibliography* (Farnham Royal, Bucks, Commonwealth Agricultural Bureaux, UK, 1954).

8. D. Gottelli *et al.*, *Mol. Ecol.* **3**, 301 (1994); J. Garcia-Moreno, M. D. Matocq, M. S. Roy, E. Geffen, R. K. Wayne, *Conserv. Biol.* **10**, 376 (1996); P. W. Hedrick, P. S. Miller, E. Geffen, R. K. Wayne, *Zoo Biol.* **16**, 47 (1997).
9. K. Strimmer and A. von Haessler, *Mol. Biol. Evol.* **13**, 964 (1996).
10. D. L. Swofford, *PAUP: Phylogenetic Analysis Using Parsimony, Version 3.0* (Illinois Natural History Survey, Champaign, IL, 1990).
11. L. Excoffier, P. E. Smouse, J. M. Quattro, *Genetics* **131**, 479 (1992).
12. A. R. Templeton, K. A. Crandall, C. F. Sing, *ibid.* **132**, 619 (1992).
13. The coyote was used as an outgroup on the basis of morphologic and mitochondrial DNA sequence analyses [R. M. Nowak, *North American Quaternary Canis* (Monograph of the Museum of Natural History, University of Kansas, N 6, Lawrence, KS, 1979); R. K. Wayne, *Trends Genet.* **6**, 218 (1993); C. Vilà *et al.*, unpublished data]. Trees retaining the four indicated clades resulted from neighbor-joining searches with Tamura-Nei, Kimura 2-parameter, Kimura 3-parameter, and HKY85 models of evolution; parsimony searches with equal, 2:1, and 10:1 weights of transversions to transitions; and maximum likelihood analyses with the HKY85 model of evolution, taking into account differences in nucleotide frequencies and transition and transversion biases.
14. R. Coppinger and R. Schneider, in *The Domestic Dog, Its Evolution, Behaviour and Interactions with People*, J. Serpell, Ed. (Cambridge Univ. Press, Cambridge, 1995), pp. 21-47.
15. We amplified the entire canid control region with primers in (6) and the following primers: L16462 (5'-CATACTAACGTGGGGTTAC-3'), H222 (5'-AACTATATGTCCTGAAACC-3'), L296 (5'-ATA-CAAAACCCCTTACC-3'), and H652 (5'-AAG-GCTAGGACCAACCT-3'). The primer numbering refers to their approximate location in the human mitochondrial genome [S. Anderson *et al.*, *Nature* **290**, 457 (1981)]. The 1030-bp sequence that we report includes the entire control region except for a hypervariable region close to the 3' end that encompasses an imperfect 20-bp repeat having a variable number of units [A. R. Hoelzel, J. V. Lopez, G. A. Dover, S. J. O'Brien, *J. Mol. Evol.* **39**, 191 (1994)].
16. A. R. Templeton, *Evolution* **37**, 221 (1983).
17. Sequence divergence values and trees were based on a gamma correction [K. Tamura and M. Nei, *Mol. Biol. Evol.* **10**, 513 (1993)] with the empirically determined parameter  $a = 0.18$  for the 261-bp sequences and  $a = 0.22$  for the 1030-bp sequences [S. Kumar, K. Tamura, M. Nei, *MEGA: Molecular Evolutionary Genetic Analysis, Version 1.01* (Pennsylvania State University, University Park, PA, 1993)].
18. R. K. Wayne, B. Van Valkenburgh, S. J. O'Brien, *Mol. Biol. Evol.* **8**, 297 (1991).
19. A relative rate test indicated that substitution rates were similar for all wolf and dog sequences [F. Tajima, *Genetics* **135**, 599 (1993)].
20. T. J. Parsons *et al.*, *Nature Genet.* **15**, 363 (1997).
21. G. Nobis, *Umshau* **19**, 610 (1979); S. J. Olsen, *Origins of the Domestic Dog* (University of Arizona Press, Tucson, AZ, 1985).
22. M. E. Thurston, *The Lost History of the Canine Race. Our 15,000-Year Love Affair with Dogs* (Andrews & McMeel, Kansas City, KS, 1996).
23. D. F. Morey, *Am. Sci.* **82**, 336 (1994).
24. We thank P. Taberlet for providing wolf sequences from France and Poland and J. Castroviejo, Grupo Lobo-Portugal, E. Geffen, and many others for their help in obtaining samples. C.V. was supported by a postdoctoral fellowship from the Spanish Ministerio de Educación y Ciencia, and I.R.A. was supported by a fellowship from Junta Nacional de Investigação Científica e Tecnológica, Portugal. This research was supported in part by NSF grants to R.L.H. (DEB-9208022) and R.K.W. (BSR-9020282) and by a Sloan Young Investigator Award to K.A.C.

10 February 1997; accepted 28 April 1997