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wide range of Aβ and amyloid deposition that occurs with age in both humans and PDAPP mice, all PDAPP mice overproduce human Aβ and, unlike humans, all mice will eventually develop Aβ and amyloid deposition in the brain. In quantitative terms, this contrasts with what is observed in the aging human brain. Whereas cortical amyloid plaque burden in humans with pre-clinical and clinical AD are similar to each other and to that observed in the PDAPP mice we studied with high Aβ burden, studies have shown that most cognitively normal elderly humans (~70% by age 75) have either no or only very small amounts of cortical Aβ deposition (*3*, *20*). The latter human group would be analogous to the mice in our study with little to no Aβ deposition (lowest quartile). This dichotomy in amyloid plaque burden observed in the aging human brain suggests, therefore, that measuring plasma Aβ after administration of antibody to Aβ may be able to clearly distinguish such individuals. Thus, the use of a monoclonal antibody with characteristics similar to m266 but developed for humans may provide a means to develop a facile diagnostic test to quantify amyloid burden in persons with pre-clinical AD, as well as to assist in the differential diagnosis of clinical AD. Such a test may also have utility for monitoring the response to anti-amyloid therapy.

The highly significant correlations between plasma Aβ and both brain Aβ and amyloid burden strongly suggest that the presence of m266 in the peripheral circulation directly facilitated net Aβ efflux from the brain, acting as a "peripheral sink." Further supporting this model is that significant correlations were observed within 5 min after peripheral injection of m266. By increasing Aβ efflux from brain, it appears that the presence of m266 in plasma can also reveal quantitative differences in brain Aβ deposition, presumably by facilitating efflux of soluble Aβ from brain. Taken together, our data suggest that brain Aβ clearance is a dynamic process and that modifying this process may be useful in both diagnosing and treating AD.

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Dynamics of Pleistocene Population Extinctions in Beringian Brown Bears

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The climatic and environmental changes associated with the last glaciation (90,000 to 10,000 years before the present; 90 to 10 ka B.P.) are an important example of the effects of global climate change on biological diversity. These effects were particularly marked in Beringia (northeastern Siberia, northwestern North America, and the exposed Bering Strait) during the late Pleistocene. To investigate the evolutionary impact of these events, we studied genetic change in the brown bear, *Ursus arctos*, in eastern Beringia over the past 60,000 years using DNA preserved in permafrost remains. A marked degree of genetic structure is observed in populations throughout this period despite local extinctions, reinvasions, and potential interspecies competition with the short-faced bear, *Arctodus simus*. The major phylogeographic changes occurred 35 to 21 ka B.P., before the glacial maximum, and little change is observed after this time. Late Pleistocene histories of mammalian taxa may be more complex than those that might be inferred from the fossil record or contemporary DNA sequences alone.

Throughout the late Pleistocene Beringia formed a largely ice-free subcontinent connecting the Old and New Worlds. This period saw a number of major events including global climatic change, the movement of humans into the New World (~13 ka B.P.), and a large-scale extinction of megafauna (~12 to 10 ka B.P.). Preliminary studies of mammal bones preserved in permafrost deposits have shown that genetic information can be retrieved from material aged more than 60 ka B.P., beyond the limit of radiocarbon dating (*1*–*4*). To study genetic change in large-mammal populations throughout this period, we examined 71 brown bears preserved in east Beringian permafrost and cave deposits, comprising all suitable specimens available in museums. Brown bears were chosen because their extensive modern distribution in Europe, Asia, and North America shows strong phylogeographic structuring (*5*–*7*), and they are thought to have entered Eastern Beringia early in the last (Wisconsinan) glaciation (*8*). In North America, three genetically and geographically distinct clades of brown bears (2, 3, and 4) are currently recognized (Figs. 1 and 2D) as well as several subclades (3a, 3b, 2a), one of which consists of the polar bear [*U. maritimus*, 2b (*7*)]. Studies suggest that much of this structure may have resulted from expansions following glacial population bottlenecks, and that late Pleistocene populations were considerably more diverse genetically (*2*).

DNA was obtained from cortical bone samples (~0.5 g) of 36 specimens by established ancient-DNA techniques (*9*). Two sections of the mitochondrial (mt) control region, 135 and 60 base pairs (bp), respectively, were amplified by polymerase chain reaction (PCR). Primer pairs were chosen to amplify short yet highly variable regions of mtDNA, to maximize ancient-DNA recovery while allowing the detection of population turnover through time. The phylogenetic relationships of brown bear clades have been previously established with the use of longer sequences (*7*). Stratigraphic control is often lacking for permafrost bones, so radiocar-

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bon dates were obtained for 30 specimens that contained DNA (*10*), along with carbon and nitrogen stable-isotope values [see supplementary material (*11*)].

The genetic and radiometric data (Figs. 1 and 3A) record a dynamic population history in eastern Beringia over the past 60 ka B.P. and reveal genetic continuity within each of three periods: pre–35 ka B.P., 21 to 10 ka B.P., and at present. In the oldest period (Fig. 2), the fossil sequences reveal the presence of extinct subclades in both interior Alaska (3c) and Yukon/northern Alaska/Siberia (2c). No brown bear fossils dating between 35 and 21 ka B.P. have been found in east Beringia, but after this time the modern subclade 3b, which does not seem to be directly derived from 3c, appears in the Fairbanks area. This population remained in place until at least 10 ka B.P., some point after which it was replaced by subclade 3a, and it is now present only in the eastern and southeastern margins of Alaska. The extinct subclade 2c indicates that clade 2 was formerly more diverse and widespread across eastern Beringia, and that the current restriction to the Alexander/Baranof/Chicaghof (ABC) Islands (2a) and polar bears (2b) is a recent pattern. A contraction in range is supported by the finding that ABC Island bear sequences occurred farther south, on Prince of Wales Island, in the terminal Pleistocene. The finding of a polar bear sequence (2b) in Fairbanks is particularly unexpected, even at 19 ka B.P. during the height of the glaciation, and although stable-isotope data support this identification (Fig. 3B), inaccurate provenance data may be a more likely explanation [see supplementary material (*11*)].

Within each of the three periods identified in the data, bear populations covering quite broad geographic areas appear reciprocally monophyletic for mt clades (Fig. 2, A to D). This suggests that, despite several population extinctions and replacements, east Beringian bear populations have maintained a large degree of genetic structure over time and space. Well-sampled groups such as 3b (n = 20) indicate that the earliest members of the colonizing population were already monophyletic for the subclade (Fig. 3A), and therefore that the monophyletic pattern was not the product of genetic drift within a diverse founder population. Furthermore, the sequence diversity within fossil 3b specimens is far greater than would be expected from mutation alone following an initial appearance at 21 ka B.P., assuming a mutation rate of 11 to 14% per million years (*7*). Therefore, the founding population at 21 ka B.P. appears to have contained considerable amounts of existing, but already monophyletic, mt diversity. This is not consistent with a very small founding population, but rather indicates a larger group that had been drawn from a more diverse, but primarily monophyletic, population elsewhere.

Few specimens are available from pre–35 ka B.P. populations, but within this group only the Yukon Territory population (Sixtymile) appears polyphyletic (4 and 2c, n = 2). In contrast, the bear population around Fairbanks at the same time appears monophyletic for subclade 3c (n = 8). This geographic division between bear populations in interior Alaska and the Yukon Territory is similar to that between modern 3a/3b populations, indicating that the geographic location of population barriers (but not the populations themselves) may be relatively constant through time. Such barriers are presumably ecological or physiographic (e.g., the Yukon-Tanana Uplands, Olgilvie Mountains, Brooks Range) (Fig. 2A).

Currently, mt subdivisions in extant brown bear populations are allopatric, and this pattern is assumed to result from barriers to gene flow and low dispersal rates maintained by maternal philopatry and population isolation (*12*). However, our data suggest that marked phylogeographic structure has existed for long periods, even during large-scale phases of dispersal, extinction, and replacement. Furthermore, the implicit diversity of the founding members of clade 3b suggests that the monophyletic populations detected in this study may have been drawn in turn from other primarily monophyletic, diverse populations elsewhere, indicating that the pattern may hold on a greater Holarctic scale. It is not obvious how the strict, widespread genetic partitioning observed here could have been maintained under such dynamic conditions for over 60,000 years. It is possible that rapid climatic shifts, combined with the strong environmental regionalism identified in Beringian climatic and palynological records (*13*, *14*), may have caused repeated phases of population isolation and localized extinctions, promoting monophyletic mtDNA population structures (*15*).

A hiatus in the fossil record, such as the absence of brown bear ~35 to 21 ka B.P., may represent either true species absence, taphonomic biases, or random sampling error. Taphonomic exclusion of brown bear fossils seems unlikely because hundreds of radiocarbon dates have been generated for late Quaternary mammals in eastern Beringia, and there are no general hiatuses in the overall record (*16*). To test the likelihood of random sampling error, we generated 100 random subsamples of 31 dates from a large (n = 188) database of radiocarbon-dated bison (*Bison bison*) and caribou (*Rangifer tarandus*) from eastern Beringia (there are 31 brown bear dates from easternmost Beringia in our data set). In no case did random hiatuses between consecutive dates approach the size of that found in the brown bear data set (95% of gaps ≤ 9700 years, mean 6264 years). We therefore conclude that if brown bears were

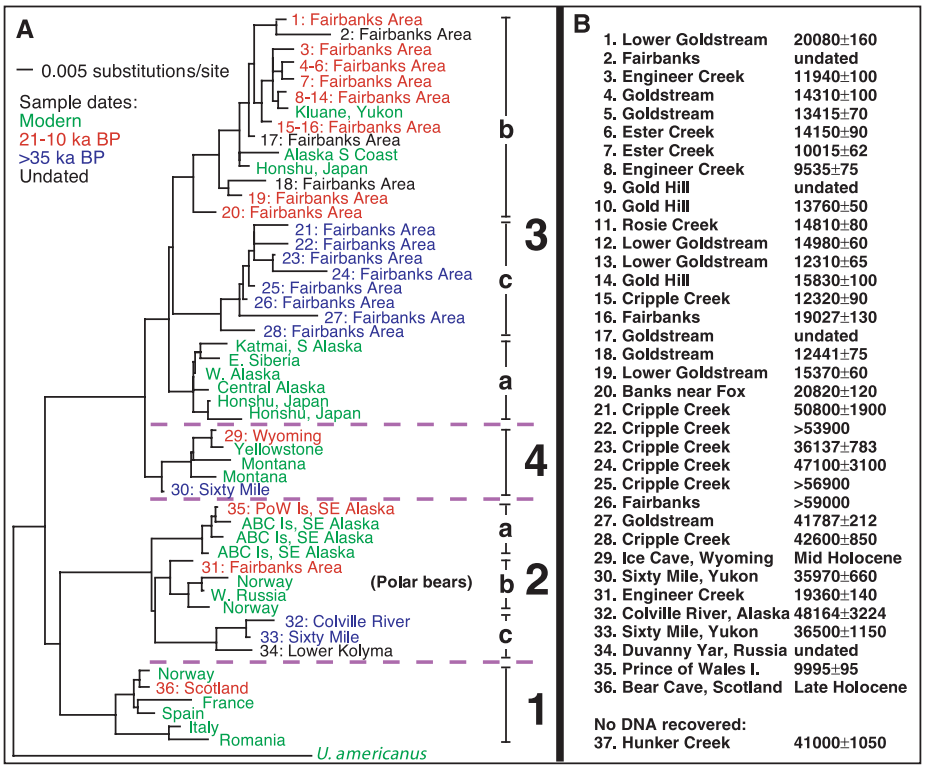

Fig. 1. Phylogeny and sample details for brown bear (*U. arctos*) specimens. (**A**) Phylogenetic tree constructed with two fragments (135 and 60 bp) of the control region and neighbor-joining (HKY model) showing clades (1 to 4) and subclades (a to c). The topology is in agreement with that of other, larger data sets (*7*). (**B**) Radiocarbon dates and specific locations of the specimens. Additional specimen data are provided in the supplementary material (*11*).

distributed through time in the same way as these other large-mammal taxa, the gap in radiocarbon dates represents a genuine local extinction of brown bears in eastern Beringia. This interpretation is further supported by the different haplotypes observed on either side of the hiatus.

Additional radiocarbon studies may modify the absolute dates of this hiatus, but the current broad pattern of extinction and subsequent recolonization appears difficult to explain solely from paleoclimatic and paleoecological data, which define four broad periods in late Quaternary Beringia. Toward the end of the Wisconsinan glaciation, the interstadial [marine isotope stage (MIS) 3, ~60 to 28 ka B.P.] was a period of climatic amelioration, which deteriorated into MIS 2 (28 to 10 ka B.P.) and the last glacial maximum (LGM), characterized by dry tundra and steppe herb-dominated communities. The early postglacial period in eastern and central Beringia featured the development of a shrub tundra (the "birch rise") ~13.5 ka B.P., which was succeeded by the onset of relatively modern conditions early in the Holocene (MIS 1, 10 to 0 ka B.P.) (*17*). Therefore, the 35 to 21 ka B.P. hiatus spans a range of late Quaternary climatic and environmental conditions, from the last interstadial through to the LGM. Although some interstadial sites in eastern Beringia suggest a moderate warming and increase in summer precipitation around 35 to 30 ka B.P., other sites do not (*18*). Furthermore, the brown bear is ecologically plastic, and it seems unreasonable to attribute the extinction in eastern Beringia to minor climate changes. The recolonization around 21 ka B.P. is particularly surprising because this period marks the beginning of the LGM.

Because no obvious climatic or environmental events appear to explain the extinction and recolonization of brown bears in eastern Beringia, alternative explanations need to be considered. There is a marked inverse correlation between the chronology of brown bears and the much larger, hypercarnivorous, short-faced bears in eastern Beringia (Fig. 3A). Although the two species coexisted for at least 10,000 years (~45 to 35 ka B.P.) during the interstadial, short-faced bear fossil dates are concentrated between 35 to 21 ka B.P. when brown bears were absent. Furthermore, brown bear recolonization (~21 ka B.P.) is precisely coincident with the last record of short-faced bears in Beringia.

Stable-isotope data (Fig. 3B) suggest that the diets of the two bear species differed substantially while they were contemporaneous. Enriched levels of ^{15}N show that short-faced bears were carnivorous, whereas brown bears were variably omnivorous and herbivorous, similar to most noncoastal bears today (Fig. 3B) (*8*, *19*). In contrast, during the period 21 to 10 ka B.P. following the apparent extinction of short-faced bears in Beringia, brown bears also show an enriched mean ^{15}N signal relative to both the pre–35 ka B.P. and modern populations. However, competitive interaction is extremely difficult to infer from the paleo-record, and several environmental factors can affect isotopic ratios. In addition, much taxonomic turnover would be expected to occur around 21 ka B.P. during the environmental changes of the early LGM. If the enriched signal does indeed reflect a higher trophic level, then it may simply indicate an increased carcass biomass availability 21 to 10 ka B.P., which presumably disappeared following the extinction of many large-mammal taxa in the terminal Pleistocene.

Although many studies have used the distributions of genetic markers in modern popula-

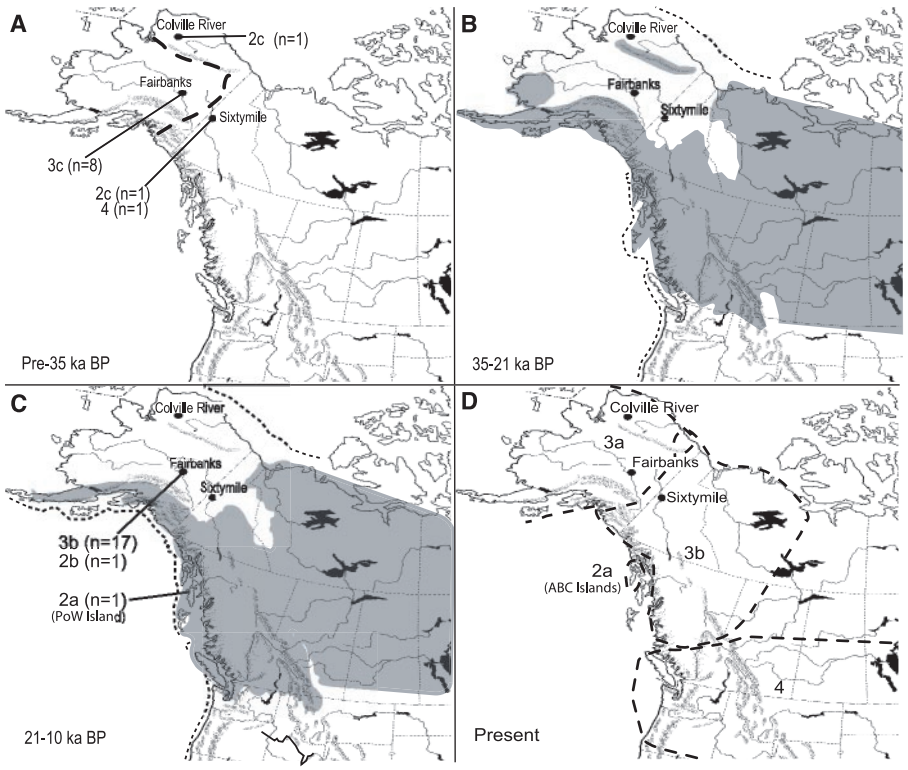

Fig. 2. (**A** to **D**) Map showing the location of brown bear clades at four time categories in eastern Beringia. The dashed line in Fig. 2A indicates a possible boundary to migration (see text). The dashed lines in Fig. 2, B and C, indicate the extent of coastline, and the gray area indicates the extent of glaciation at 21 to 18 ka B.P. and 18 ka B.P., respectively (*28*). The dashed lines in Fig. 2D refer to the approximate distributions of modern brown bear clades after (*7*, *29*).

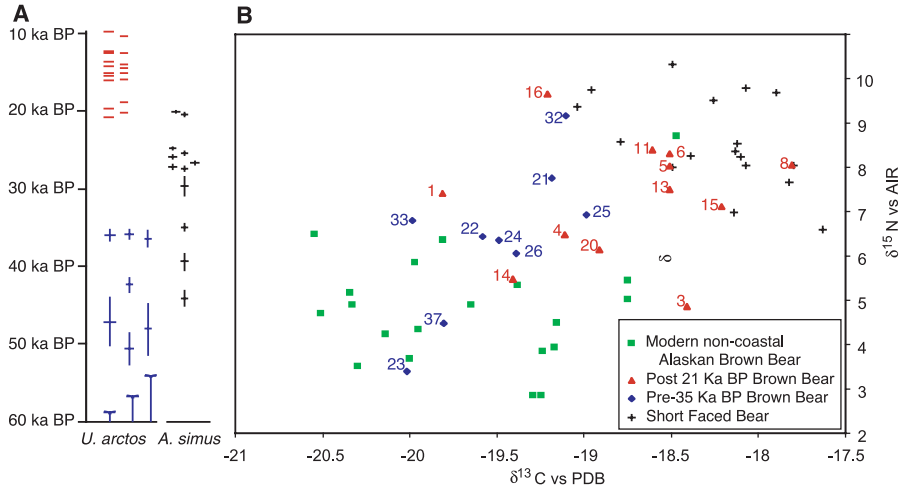

Fig. 3. Radiocarbon and stable-isotope data. (**A**) Timeline of radiocarbon dates for east Beringian brown (*U. arctos*) and short-faced (*A. simus*) bears. Dates are also shown with one standard error. (**B**) $\delta^{13}C$ and $\delta^{15}N$ data for the same species, including data from (*8*, *19*, *30*). Numbering as in Fig. 1. Values for two samples with highly enriched ^{15}N values [sample 31 (polar bear): $\delta^{15}N = 21.7$, $\delta^{13}C = -12.3$.; sample 7: $\delta^{15}N = 12.9$, $\delta^{13}C = -16.8$] are not plotted. PDB, Pee Dee belemnite standard.

tions to infer the timing and pattern of colonization of a region (*20*–*22*), in this case simple interpretations would be incorrect. The pronounced phylogeographic structure of modern Alaskan bears is unrelated to the separation of clades 3a and 3b [245 to 310 ka B.P. (*7*)] or the subsequent expansion of clade 3b (79 to 100 ka B.P.), which probably long preceded the colonization events in east Beringia (Fig. 2). This finding is supported by the presence of both clades in Japan (*5*). Furthermore, the phylogeographic pattern is not directly attributable to a post-LGM expansion (*2*, *7*, *23*, *24*) because clade 3b is present in the Fairbanks region at 21 ka B.P., coincident with the LGM, and 3a is unrecorded until after 10 ka B.P. By combining a large number of ancient DNA sequences with radiocarbon, stable-isotope, and palaeoclimatic data, we have been able to directly study phylogeographic change in late Pleistocene populations. This record shows that the most important changes occurred before the LGM, human entry to the New World, or the megafaunal extinction.

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9. Samples were obtained from the Canadian Museum of Nature, Ottawa; American Museum of Natural History, New York; Royal Museum, Edinburgh; Kansas University, Lawrence; and field sites. Most samples came from a 30-km^2 area surrounding Fairbanks. Full details are given in the supplementary material (*11*). DNA extraction was performed as in (*2*), with the exception that a Braun Mikrodismembrator was used to grind samples. Grinding equipment (stainless-steel balls and cups, rubber washers) was thoroughly bleached between each use. Primer sequences are as follows: short fragment: L16030 (CTATTCCCTGGTACATAC) and H16091 (GGGGGTATTCGAGGACATAC); long fragment: L16164 and H16299 (*25*). PCR amplifications were performed with AmpliTaq Gold (Perkin-Elmer) or Platinum HiFi (Gibco-BRL), and reaction conditions were as described in (*2*, *26*). PCR products were directly sequenced with the ABI Big Dye Terminator chemistry and resolved on ABI 377 and 310 automated sequencers (Perkin-Elmer). A subset of PCR products from four samples was cloned with a pGem-T vector cloning kit (Promega), and 5 to 10 clones were sequenced to examine the extent of damage and confirm the absence of numts (nuclear copies of mitochondrial sequences) (*3*, *26*) [supplementary material (*11*)]. Sequence data were aligned manually and analyzed with PAUP*4.0b8 (*27*). The authenticity of sequences is supported by a number of factors. All DNA extraction and PCR setup took place in an ancient-DNA laboratory in the Oxford University Museum, where no other molecular research occurs. Post-PCR procedures were conducted in the physically distant Zoology Department. Contamination was monitored through the use of multiple extraction and PCR control blanks, and consistent results were obtained for repeated extractions and amplifications. Some samples consistently yielded only the smaller PCR product (data from these samples were not used), suggesting that DNA damage was more extensive in this material; however, no brown bear samples gave only the large fragment. The cloning experiments showed limited amounts of sequence variation characteristic of damaged DNA. Sequences from six specimens obtained by J. Leonard (*2*) were independently replicated in Oxford by using blind tests and different samples of the same specimen. Collagen levels in samples submitted for radiocarbon dating were consistently high, suggesting excellent macromolecular preservation. Lastly, haplotypes that could be predicted on the basis of geographic origin (e.g., clade 1 in Europe) were found to fulfill those predictions. Sequences were deposited in GenBank with accession numbers AY082810 to AY082881.
10. Analyses for most fossil brown bear samples were carried out by the Oxford Radiocarbon Accelerator Unit (ORAU) with a 0.2- to 0.5-g sample of bone adjacent to the sample used for DNA extraction. Total bone collagen was extracted, graphitized, and dated by accelerator mass spectrometry. The dates used are uncalibrated. Collagen extractions and stable-isotope analyses of specimens 7, 16, 23, and 32 and the modern and short-faced bears were performed at the University of Alaska Fossil Bone and Stable Isotope Facilities as described (*8*). Radiocarbon dating of these specimens was performed by the NSF-Arizona AMS Laboratory.
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Rates of Evolution in Ancient DNA from Adélie Penguins

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Well-preserved subfossil bones of Adélie penguins, *Pygoscelis adeliae*, underlie existing and abandoned nesting colonies in Antarctica. These bones, dating back to more than 7000 years before the present, harbor some of the best-preserved ancient DNA yet discovered. From 96 radiocarbon-aged bones, we report large numbers of mitochondrial haplotypes, some of which appear to be extinct, given the 380 living birds sampled. We demonstrate DNA sequence evolution through time and estimate the rate of evolution of the hypervariable region I using a Markov chain Monte Carlo integration and a least-squares regression analysis. Our calculated rates of evolution are approximately two to seven times higher than previous indirect phylogenetic estimates.

Most estimates of rates of nucleotide sequence evolution have been derived from comparative approaches among living taxa, where sequence divergence is calibrated against geological estimates of divergence time (*1*). Shields and Wilson (*2*) estimated that the entire avian mitochondrial genome evolves at a rate of approximately 2% per million years, which is similar to the value commonly accepted for mammals (*3*). This value of 0.02 substitutions per site per million years (s/s/Myr) was then used to calculate the rate of substitution for a portion of the hypervariable region I (HVRI), estimated at 0.208 s/s/Myr, on the basis that it evolves 10.4 times faster than the entire mitochondrial genome (*4*). Ancient DNA technology (*5*), in principle, offers an opportunity to estimate more directly the rate of nucleotide evolution of a population, using analyses of individuals from different times. However, it is usually difficult to obtain a sufficient number and distribution of ancient samples of known ages. Because of the