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

particular aspects of their life history and the extreme Antarctic environment, Adélie penguins (*Pygoscelis adeliae*) are ideal for such a study. During the austral summer, Adélie penguins nest in distinct colonies in ice-free areas along a small proportion of the Antarctic coastline (Fig. 1). Colonies are characterized by high densities and high mortality (*6*). These factors have led to large deposits of subfossil bones that have been serially preserved in the cold Antarctic environment. The oldest reported subfossil Adélie penguin bone was dated at 7786 years before the present (yr B.P.) (*7*).

To measure evolutionary rates in Adélie penguins, we sequenced the mitochondrial HVRI from 96 ancient bone samples up to 6424 yr B.P. (*8*) and from 380 blood samples from birds at 13 Antarctic locations (*9*). We constructed median networks of the HVRI to display relationships between the ancient and modern sequences. Median networks provide a useful representation of intraspecies data that are characterised by a small number of base substitutions between sequences and high levels of homoplasy (parallel or convergent mutations). In contrast to standard tree representations, where only the tips of the tree are labeled, nodes in a median network represent either sampled haplotypes or inferred intermediates.

Thirteen mutually compatible nucleotide sites were used to define seven subgroups within the data (Fig. 2). The reduced median networks (*10*) for two representative subgroups are displayed in Fig. 2. Some sites and sequences were excluded from the analysis to avoid missing values. The networks contain a small number of common haplotypes surrounded by many haplotypes that are one or two point mutations distant. The networks also identify ancient haplotypes that are unlikely to be ancestral to the living population. Cycles within the networks display the many mutational pathways that may have occurred and suggest that it would be unwise to base a rate estimate on any single tree purported to describe the phylogenetic relationships between the samples.

A major feature of the HVRI sequences (n = 380) of living Adélie penguins is the presence of two mitochondrial DNA lineages that have distinct geographic distributions. Type A (Antarctica) is present at all locations around the conti-

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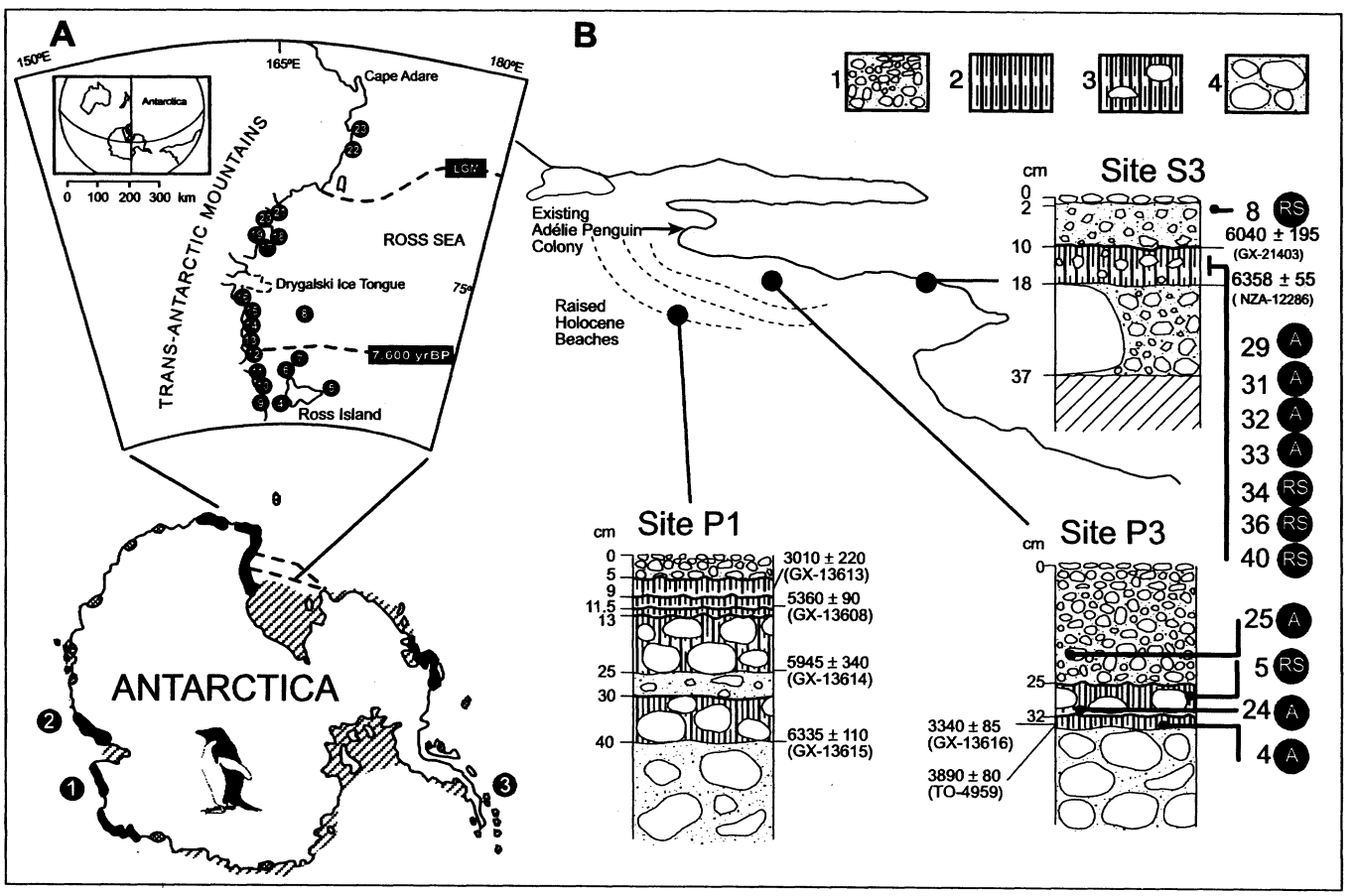

Fig. 1. Adélie penguin sampling locations. **(A)** Distribution of Adélie penguin breeding colonies during the austral summer in Antarctica. Black shading indicates areas of highest density of penguin colonies, and diagonal shading designates permanent ice shelves. The location of the grounding line of the ice shelf at the Last Glacial Maximum (LGM) and at 7600 yr B.P. is shown by dashed lines. Collection sites are given, together with the number of blood (b) and subfossil bone (sfb) samples analyzed from each, as follows: 1. Welch Island, b 21; 2. Gardner Island, b 21; 3. Torgersen Island, b 16; 4. Cape Royds, b 38; 5. Cape Crozier, b 29, sfb 14; 6. Cape Bird, b 122, sfb 9; 7. Beaufort Island, b 22, sfb 3; 8. Franklin Island, b 16; 9. Marble Point sfb 2; 10. Dunlop Island, sfb 6; 11. Cape Roberts sfb 1; 12. Cape Ross, sfb 10; 13. Depot Island, sfb 1; 14. Cape Day, sfb 1; 15.Cape Hickey, sfb 5; 16. Prior Island, sfb 9; 17. Inexpressible Island, b 27, sfb 19; 18. Adélie Cove, b 16, sfb 1; 19. Northern Foothills, sfb 8; 20. Gondwana Station, sfb 1; 21. Edmonson Point, b 13, sfb 3; 22. Cape Wheatstone, b 10; 23. Cape Hallett, b 29, sfb, 3. **(B)** Three soil profiles from abandoned Adélie penguin colonies on Inexpressible Island, Antarctica (collection site 17) from which subfossil bones were obtained. Details of stratigraphy are shown as follows: 1, surface horizon composed of pebbles accumulated from penguin nests; 2, organic horizon of guano; 3, sandy gravel with guano; and 4, sandy gravel and boulders. ^{14}C dates from penguin remains are given at a range of depths, together with the position in the profile of bones and their sample numbers and mitochondrial lineages.

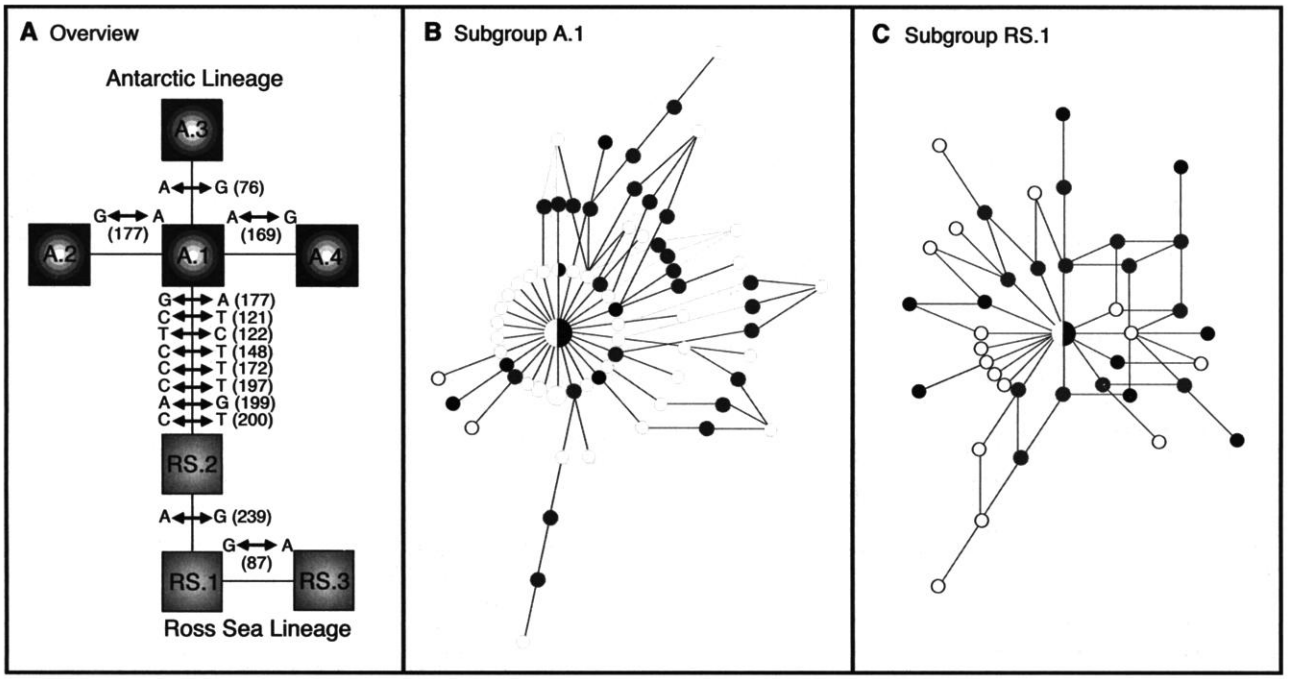

Fig. 2. Relationships among the HVRI mitochondrial DNA sequences of ancient and modern Adélie penguins. **(A)** Overall organization of subgroups. Double-headed arrows indicate a change at one of the 13 mutually compatible sites used to define the subgroups. **(B)** Minimum spanning network of the A.1 subgroup sequences. This subgroup is represented by a minimum spanning network, because it contains many mutually incompatible sites. **(C)** Median network of RS.1 sub-group sequences. In (B) and (C), modern samples are shown as white circles, subfossil bones as black circles, and hypothetical intermediates as gray circles. Parallel lines indicate a change at the same site. Small circles represent one to five sequences, medium circles 6 to 20 sequences, and large circles 20+ sequences. The points of connection between the subgroups are indicated by the larger circles.

nent that we have sampled, whereas the RS (Ross Sea) lineage appears to be restricted to the Ross Sea coast. These lineages each have high haplotype diversity (h_A = 0.995 and h_{RS} = 0.995), average within-lineage sequence difference of 2.1% (A type) and 2.5% (RS type), and an average of 8.3% sequence divergence between them.

The two lineages (A and RS) were recorded among the 96 subfossil bones of Adélie penguins preserved within ornithogenic soils (*11*, *12*) below extant and extinct penguin colonies. Soil horizons are composed of droppings, feathers, egg fragments, and other penguin remains mixed with sand, gravel, and pebbles (Fig. 1). Abandoned penguin nesting sites are common landscape features along the Antarctic coasts (*11*). For example, along the Ross Sea coast, 15 relict colonies are known (*7*). In this study, penguin guano or other remains, from both occupied and abandoned colonies, were radiocarbon-dated. Ages were assigned to nucleotide sequences from bones, either because the bones themselves were directly dated or strata from which they were isolated were dated [see the supplemental material (*12*)]. The radiocarbon ages of Adélie penguin bones demonstrate that both mitochondrial lineages were present in the Ross Sea area at least 6082 years ago (Fig. 1).

The ancient DNA extracted from the frozen Adélie penguin bones was of high quality. Polymerase chain reaction (PCR) enabled amplification of a 1600–base pair (bp) sequence from the mitochondrial control region of a 523–yr B.P. bone. In addition, a 390-bp fragment could be sequenced from 66% of all subfossil bones examined, including 45% of those older than 2000 years. From 35% of bones younger than 2000 years, longer sequences (663 to 1042 bp) could be amplified. Moreover, single-copy nuclear loci were routinely amplified from bone samples, also suggesting DNA of high quality.

As shown by the median networks, there is a high level of homoplasy in the HVRI sequences of living and ancient Adélie penguins, and consequently a high level of uncertainty about the genealogy, beyond the major split between A and RS. Hence, we employed an approach to estimate the rate of change of the HVRI that used Markov chain Monte Carlo (MCMC) (*13*, *14*) integration to allow incorporation of a large number of plausible trees into the analysis (*15*, *16*). Bayesian statistical inference using MCMC integration weights trees in proportion to their posterior probability, given the data, under the chosen model of evolution. Two models of population dynamics (constant population size and exponential growth) were compared to assess the robustness of the evolutionary rate estimate to these assumptions. Independent replicates of analyses under each of the two models were performed as part of convergence testing. All 96 ancient sequences, ranging in age from 88 to 6424 years old, were used in the analysis, together with modern sequences from each of the two lineages. The mean estimates of evolutionary rates were 0.96 s/s/Myr [95% highest posterior density (HPD) interval 0.53 to 1.43] and 0.93 (95% HPD interval 0.39 to 1.44), respectively, for constant and exponential growth models of population dynamics. Both estimates were very similar, showing that the estimated rate was not sensitive to model assumptions about the past demographic patterns of Adélie penguins. Furthermore, upper and lower boundaries on mutation rate and population size priors (*16*) were never impinged on, suggesting that the data were highly informative about the parameters of interest. Figure 3 shows the full posterior probability densities of the evolutionary rate under both models of population dynamics.

We have demonstrated sequence evolution over a significant geological time frame, using ancient DNA. Using the resulting densities of the rate of evolution, it is possible to calculate the probability that the evolutionary rate in the HVRI is greater than the phylogenetically derived rate of 0.208 s/s/Myr. Under both models of population dynamics, most of the estimated rate distribution was above the phylogenetically derived rate (99.9% for constant growth and 99.8% for exponential growth). In a Bayesian inference scheme, these values can be simply interpreted as the probability, given the data, that the true rate is higher than 0.208 s/s/Myr. Hence, the phylogenetically derived rate is an underestimate of the actual evolutionary rate in our samples.

For verification, a second approach, which does not rely on a tree, was used. This method (*17*) employs a general regression of the number

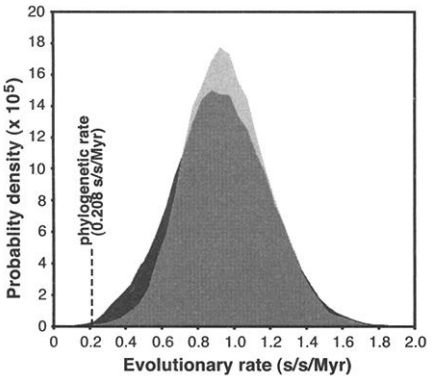

Fig. 3. Posterior probability densities of the evolutionary rate (s/s/Myr) under two models of population dynamics: constant population size (lighter distribution) and exponential growth (darker distribution).

of substitutions per nucleotide site against the time between serially preserved Adélie penguin samples. The regression estimated the rate of HVRI evolution to be 0.676 s/s/Myr; using a parametric bootstrap of 1000 replicates, the 95% confidence intervals were 0 to 2.04 s/s/Myr. The point estimate obtained from this analysis lies well within the two probability distributions obtained from the MCMC analyses. However, the wider confidence interval, which is expected because the method uses only summary distance information and ignores specific site patterns (*18*), does not exclude the phylogenetically derived estimate.

Mitochondrial HVRI sequences from Adélie penguins are evolving in a clock-like manner in that 89% of all samples belonging to the A and RS lineages passed a relative rate test (*19*) and a likelihood ratio test (*20*) ($P > 0.05$) [see the supplemental material (*12*)]. Estimates of the time of divergence of the A and RS lineages were produced by the MCMC analysis. The mean divergence times were 62,000 years (95% HPD interval 32,000 to 95,000) and 53,000 years (95% HPD interval 26,000 to 90,000) for constant and exponential growth, respectively. Both our point estimates and the 95% intervals indicate that the two lineages diverged during the last glacial cycle (*21*, *22*). This is consistent with the fact that at the Last Glacial Maximum, there were few, if any, ice-free areas in the Ross Sea, and Adélie penguins are likely to have been restricted to refugia.

Although other studies have used ancient DNA to document changes in animal populations over time (*23*, *24*), these data sets have not been used to estimate evolutionary rates. The fast evolutionary rate reported here of two to seven times that of the phylogenetic rate is concordant with the high rate of HVRI mutation found recently in humans (*25*). We suggest that an evolutionary rate of the mitochondrial HVRI of 0.4 to 1.4 s/s/Myr is more realistic than previous slower phylogenetic estimates, particularly for intraspecific studies and studies of closely related species. The fact that we have been able to use ancient DNA to measure the tempo of evolution illustrates the importance of these unique Adélie penguin bone deposits.

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A Common Rule for the Scaling of Carnivore Density

Chris Carbone[1]* and John L. Gittleman[2]

Population density in plants and animals is thought to scale with size as a result of mass-related energy requirements. Variation in resources, however, naturally limits population density and may alter expected scaling patterns. We develop and test a general model for variation within and between species in population density across the order Carnivora. We find that 10,000 kilograms of prey supports about 90 kilograms of a given species of carnivore, irrespective of body mass, and that the ratio of carnivore number to prey biomass scales to the reciprocal of carnivore mass. Using mass-specific equations of prey productivity, we show that carnivore number per unit prey productivity scales to carnivore mass near −0.75, and that the scaling rule can predict population density across more than three orders of magnitude. The relationship provides a basis for identifying declining carnivore species that require conservation measures.

Across communities in plants and animals, there is an inverse relationship between population density and body size, such that resource use and availability are driving consistent statistical patterns (*1*–*5*). The critical factor is the individual species' rate of resource use. Typically, resource use is identified in general metabolic or physiological terms, as these represent the invariant properties of all biological systems at different levels. The precise measure and form of resource use have only been described indirectly (*6*–*9*).

We developed a general model (*10*) to predict carnivore density relative to resourc-

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