

Sudden replacement of cave bear mitochondrial DNA in the late Pleistocene

Michael Hofreiter^{1*}, Susanne Münzel², Nicholas J. Conard², Joshua Pollack³, Montgomery Slatkin³, Gunter Weiss^{1,4} and Svante Pääbo¹

In the absence of interaction with genetically distinct populations, changes in frequencies of distinct mitochondrial DNA (mtDNA) sequences (haplotypes) within a population are caused by mutations and genetic drift, both of which are comparatively slow processes. By contrast, interactions between populations may lead to rapid changes in haplotype frequencies [1–3], for instance through extinction and recolonisation. So far, the only case of a direct replacement of mtDNA sequences within a continuous population has been described in historical mouse populations [2]. To investigate the genetic stability of a Pleistocene population over time, we have investigated the mtDNA haplotype spectrum of cave bears from a location in southern Germany, continuously inhabited for thousands of years.

We extracted 29 cave bear teeth from three geographically close caves from the Ach Valley (Supplemental data), 20 of which yielded amplifiable mtDNA. Altogether, we found five mtDNA haplotypes, four of which are closely related, differing by one to three substitutions from each other, whereas the fifth one differs from the other four by ten to thirteen substitutions (Supplemental data). All samples that yielded DNA were radiocarbon dated by accelerator mass spectrometry from purified bone collagen, either at BETA Analytic Inc. (17 samples) or at the Oxford dating unit (three samples). The dates range from 38,000 years

before present (B.P.) to ~25,500 years B.P. (Figure 1; Supplemental data).

Interestingly, the four closely related haplotypes all originate from bears dated to between 28,000 and 38,000 years, whereas the fifth, highly divergent haplotype was found exclusively in bears about 28,000 years old or younger (Figure 1; Supplemental data). Due to the confidence intervals of radiocarbon dates, it is not possible to decide from these ages whether there was any temporal overlap between the two types of sequences. However, while the ages of several samples belonging to the different haplogroups overlap when standard deviations are taken into account (Supplemental data), only one of the bears carrying the single divergent haplotype shows an older point estimated carbon age (by 30 years) than the youngest one carrying one of the other four haplotypes. To investigate whether the observed pattern could also be detected when the different haplotypes were randomly distributed across time within the cave bear population we performed randomization simulations. The results show that it is highly unlikely ($p < 0.001$) to obtain the observed pattern if the haplotypes were in reality randomly distributed across time. Although we cannot exclude that the minority haplotypes existed

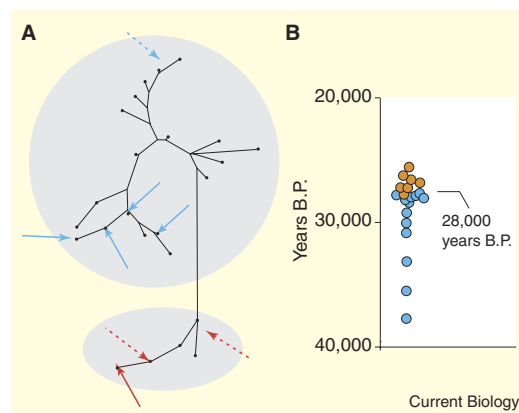
at low frequencies outside the time range within which we could detect them (Supplemental data), we think that the most likely explanation for the observed pattern (Figure 1) is a replacement of one group of sequences by the second.

In previous studies of ancient mtDNAs spanning several thousand years [3–6], genetic continuity [5], introgression [3] and in one case also a mtDNA sequence replacement have been observed [6]. However, in the latter case, this was accompanied by local extinction of the species in question for several thousand years. Our data thus document the first case of a Pleistocene mtDNA sequence replacement within a population without evidence for temporary extinction. This result differs strongly from three other cave bear populations studied to date where genetic stability over 15,000 to 20,000 years has been observed [5].

We estimated the most recent common ancestor (MRCA) of the four closely related haplotypes using a Bayesian approach [7], resulting in an age of 130,000 years (95% credibility interval 40,000 to 390,000 years). As each of these four haplotypes differs from the closest different one by a single substitution (Supplemental data), none of the haplotypes has been lost since they shared a common ancestor. Thus, this cave bear population

Figure 1. Phylogenetic tree of 26 published cave bear haplotypes and temporal relationship of the two haplogroups in the Ach Valley.

(A) Phylogenetic positions of the sequences from two reproductively isolated cave bear populations from Austria (dotted arrows) and from the Ach Valley (normal arrows). Haplotypes from the older sequence group from the Ach Valley fall within the same clade as those from the higher elevation site (2,000 m) in Austria (blue arrows), while the younger haplotype from the Ach Valley clusters with haplotypes from the lower elevation site (1,300 m above sea level) in Austria (red arrows). (B) Radiocarbon dates for the fossil teeth from the Ach Valley. The different sequence groups are shown in the same colors as in (A).



must have been genetically and demographically stable for at least 40,000 years, but probably more than 100,000 years before the mtDNA sequence replacement occurred some 28,000 years ago. If the cave bear population had not been continuously present in the region of the Ach Valley since the MRCA of the four haplotypes, it is likely that one or more haplotypes had been lost. Intriguingly, if the average age of the samples of about 30,000 years is added to the estimate for the MRCA for the four haplotypes, the date becomes 160,000 years B.P. close to the penultimate glacial maximum [8]. Thus, it is likely that a population either survived the penultimate glacial maximum locally or immigrated afterwards and lived in the Ach Valley for about 130,000 years without major demographic changes. In light of this long stability, the sudden change in the mtDNA sequences we observe is remarkable.

The two groups of sequences are closely related to cave bear sequences from two geographically close Austrian sites, which show no evidence for gene flow between the two populations despite 15,000 years of coexistence [5]. The younger sequence group from the Ach Valley is closely related to the sequences that were found in the lower elevation cave in Austria, whereas the older haplotypes are related to the higher elevation cave from Austria (Figure 1A). As no evidence for gene flow was detected between the Alpine caves, it is likely that the genetic replacement in the Ach valley occurred by population replacement.

Given its long-term stability, external factors may have caused a reduction of the Ach valley population around 28,000 years B.P. One possible cause could be climate change. However, Pleistocene climate was variable from about 75,000 year B.P. onwards and the beginning of the last glacial maximum had not yet started around 28,000 years B.P. [9]. Moreover, neither the floral nor the faunal data from the Ach valley provide evidence

for marked climate changes around 30,000 years ago and cave bears had survived numerous previous climatic shifts during the Pleistocene.

Alternatively, the arrival of modern humans in the Ach Valley by 35,000 years B.P. [10] could have caused a reduction of cave bear populations. Interestingly, there is evidence for higher population size and increased hunting pressure on cave bears with the advent of modern humans in the Ach valley [11]. Strikingly, a cave bear vertebra carrying a spear point was dated to 27.830 ± 145 years B.P. – almost exactly the time of the haplotype replacement described here [12]. Thus, human impact in the form of both hunting and competition for caves may have led to the replacement in the cave bear population about 28,000 years B.P.

This raises the question why cave bears should have recolonized the Ach valley at all. Two points are worth noting in this context. First, the younger cave bears could have been less naïve with regard to humans if they had encountered modern humans before colonizing the Ach valley. This is possible, as the younger haplogroup tends to be frequent in the east of Europe [13] where the first modern humans have been recorded [14]. Second, these bears colonized the Ach valley for only about 2,000 years before they became extinct in this region. Thus, also the new cave bear group resisted human impact for only a relatively short time.

Supplemental data

Supplemental data including experimental procedures, tables and figures can be found at <http://www.current-biology.com/cgi/content/full/17/4/R122/DC1>.

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¹Max-Planck-Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany.

²Institut für Ur- und Frühgeschichte, Eberhard-Karls-Universität Tübingen, Schloß Hohentübingen, Burgsteige 11, D-72070 Tübingen, Germany.

³Department of Integrative Biology, University of California, Berkeley, CA 94720, USA

⁴Present address: Institut für Bioinformatik, Heinrich-Heine-Universität Düsseldorf Universitätsstr. 1, D-40225 Düsseldorf, Germany.

*E-mail: hofreiter@eva.mpg.de