

# Evidence for Reproductive Isolation between Cave Bear Populations

Michael Hofreiter,<sup>1,\*</sup> Gernot Rabeder,<sup>2</sup>  
Viviane Jaenicke-Després,<sup>1</sup> Gerhard Withalm,<sup>2</sup>  
Doris Nagel,<sup>2</sup> Maja Paunovic,<sup>3,4</sup>  
Gordana Jambrišić,<sup>3</sup> and Svante Pääbo<sup>1</sup>

<sup>1</sup>Max Planck Institute for Evolutionary Anthropology  
Deutscher Platz 6  
D-04103 Leipzig  
Germany

<sup>2</sup>Institute of Palaeontology  
University of Vienna  
Althanstr. 14  
A-1090 Vienna  
Austria

<sup>3</sup>Institute of Quaternary Palaeontology and Geology  
of the Croatian Academy of Sciences and Arts  
A. Kovacica 5/II  
HR-10000 Zagreb  
Croatia

## Summary

The European cave bear (*Ursus spelaeus*), which became extinct around 15,000 years ago, had several morphologically different forms. Most conspicuous of these were small Alpine cave bears found at elevations of 1,600 to 2,800 m [1–3]. Whereas some paleontologists have considered these bears a distinct form [4, 5], or even a distinct species [6], others have disputed this [7–9]. By a combination of morphological and genetic methods, we have analyzed a population of small cave bears from Ramesch Cave (2,000 m altitude) and one of larger cave bears from Gamssulzen Cave (1,300 m), situated approximately 10 km apart in the Austrian Alps (Figure 1A). We find no evidence of mitochondrial gene flow between these caves during the 15,000 years when they were both occupied by cave bears, although mitochondrial DNA sequences identical to those from Gamssulzen Cave could be recovered from a site located about 200 km to the south in Croatia. We also find no evidence that the morphology of the bears in the two caves changed to become more similar over time. We suggest that the two cave bear forms may have represented two reproductively isolated subspecies or species.

## Results

In order to establish the chronology of cave bear occupation of the two Austrian caves, we determined carbon dates for nine cave bear remains from Ramesch Cave and seven cave bear remains from Gamssulzen Cave. In addition, ten cave bear remains from Vindija Cave in Croatia (Figure 1A) were dated (Table S1 in the Supplemental Data available with this article online). Together with published carbon- and uranium-series dates [10–

12] (Figure 2), these dates show that cave bear populations in the three caves lived contemporaneously for at least 15,000 years.

We analyzed the morphology of about 2,000 teeth (695 from Ramesch, 944 from Gamssulzen, and 451 from Vindija), as well as about 2,200 metapodial bones of the anterior and posterior paws (944 from Ramesch, 542 from Gamssulzen, and 740 from Vindija) from the three caves. The values of the tooth measurements in Ramesch Cave are significantly smaller than the values in Gamssulzen Cave (two-tailed t test:  $p < 0.01$ , Figure S1, Table S2), and the teeth in Vindija are even larger than in Gamssulzen. Cave bears displayed a strong sexual dimorphism for canine size, with males carrying larger canines [5]. In accordance with this, the breadths of the canines are bimodally distributed in the two caves (Figure S2). Both modes are smaller for Ramesch Cave compared to Gamssulzen Cave, indicating that both males and females had smaller canines at Ramesch Cave.

Although the total lengths of metapodial bones in Ramesch Cave do not differ significantly from those in Gamssulzen Cave (Figure 3A, Table S2), the bones in Gamssulzen Cave are up to 8% thicker than the corresponding bones in Ramesch Cave (Figure 3B). This difference is statistically significant for nine of the ten different types of metapodial bones analyzed (Table S2). The Vindija metapodial bones are longer than the Gamssulzen bones but do not differ in thickness (Table S2).

The stratigraphy in Ramesch Cave allows one to identify different time periods during which the bones were deposited [11] and to investigate whether the morphology of the Ramesch bears changed over time. Despite the long time of occupation, there is no difference between layers for any of the morphological measurements analyzed (see Supplemental Data).

Thus, as it is typical for high-Alpine cave bears [13, 14], the “Ramesch” cave bears had smaller teeth and more slender extremities than the “Gamssulzen” cave bears. Moreover, there is no evidence for a morphological change in Ramesch Cave during 15,000 years of contemporaneous occupation of the two caves.

We extracted and amplified three short fragments of the mitochondrial control region from seven cave bears from Ramesch and Gamssulzen Caves, respectively, and from nine cave bears from Vindija Cave (Figure S3). The three fragments were chosen to cover all positions that have been found to vary in at least two samples among 18 cave bears for which a 285 bp fragment of the control region has been sequenced [15, 16]. Previously published mtDNA sequences from two bears from Ramesch and Vindija, respectively [15], were incorporated into the analyses. The eleven cave bears from Vindija yielded four distinct sequences, two of which were also found in Gamssulzen. All cave bears from Ramesch yielded one and the same sequence, which is different from the DNA sequences from the other two caves. Thus, the mtDNA sequences found in Ramesch and Gamssulzen show no evidence for female migration

\*Correspondence: hofreiter@eva.mpg.de

<sup>4</sup>Deceased.

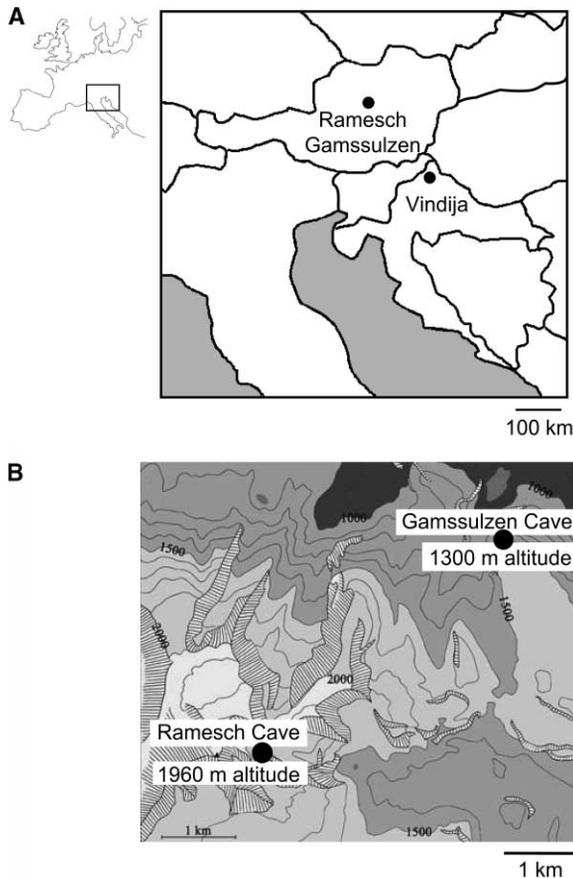


Figure 1. Caves from which Cave Bear Samples were Investigated Vindija Cave lies about 200 km southeast of the other two caves (1A), whereas the distance between Ramesch and Gamssulzen is less than 10 km (1B).

between the two caves over a time period of 15,000 years. This is remarkable; the two caves are geographically close and no physical barriers exist between them, as illustrated by the fact that a human can walk in less than 2 hr from Gamssulzen Cave to the higher situated Ramesch Cave.

### Discussion

Under the assumption that a single interbreeding cave bear population inhabited Ramesch Cave and Gamssulzen Cave, we can pool the mtDNA sequences determined from the period when the caves were inhabited contemporaneously (i.e., excluding the four samples from Ramesch Cave that exceed 50,000 years). Under this assumption, there is a 0.044% probability of recovering only one type of mtDNA sequence from five samples from one cave and only the other type of mtDNA sequence from the seven samples from the other cave. Thus, the most plausible interpretation of the data is that gene flow was restricted between the bears inhabiting the two caves. Nevertheless, there are several caveats to the interpretation of the data. First, mtDNA reflects only female gene flow. Second, relatively few mtDNA sequences have been retrieved, which causes

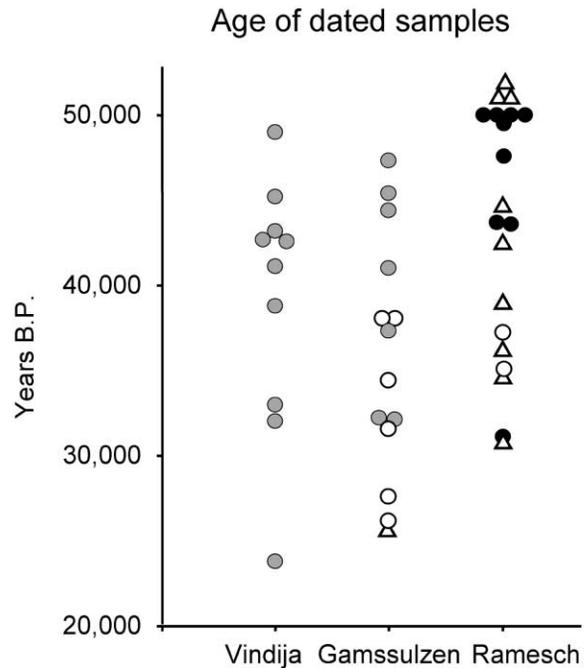


Figure 2. Absolute Dates for Cave Bear Remains from the Three Caves

The dots represent AMS dates done in this study, the open circles conventional radiocarbon dates from bone collagen, and the triangles uranium series dates taken from the literature [11]. MtDNA sequences were determined for samples represented by dots. Gray dots represent samples from which one of the four "Vindija sequences" was recovered; black dots represent samples from which the "Ramesch sequence" was recovered.

the genetic record, particularly in Ramesch Cave, to contain temporal gaps. It is possible that the dispersal capacity of cave bears was low [4, 5] and that mainly male bears dispersed, as is the case in extant brown bears [17]. However, the persistence of morphological differences argues against any substantial amount of gene flow between the two caves, even if it were predominantly mediated by males.

Although we cannot exclude that some gene flow occurred between the two caves, it is unlikely that the cave bears at Ramesch and Gamssulzen were part of a single panmictic population. The two populations could therefore either represent two phylogeographically distinct forms of bears that did not change their respective locations over thousands of years, even though no behavioral or other barriers to gene flow existed between them. Alternatively, they may have been reproductively isolated as a result of biological features inherent in the two forms of bears.

Although phylogeographic patterns in which groups of related mtDNA sequences found within a single species do not overlap geographically are commonly found in extant species (for reviews see [18, 19]), not much is known about the persistence of such patterns over time. However, Barnes et al. [20] showed that the geographical distribution of mtDNA clades within brown bears may shift over time and two major clades of mtDNA sequences that exist in brown bears have been found

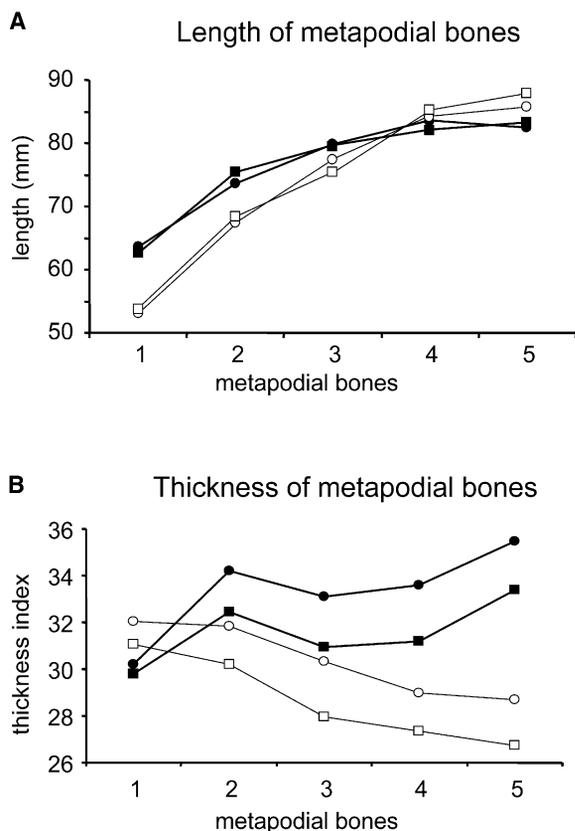


Figure 3. Length and Thickness of Metapodial Bones  
(A) Lengths of the ten metapodial bones (five each of the hand and the foot) from Ramesch Cave in comparison to Gamssulzen level. Closed circles: metacarpal bones Gamssulzen. Closed squares: metacarpal bones Ramesch. Open circles: metatarsal bones Gamssulzen. Open squares: metatarsal bones Ramesch. (B) Thickness index (= breadth of diaphysis/total length) of metapodials from Ramesch Cave and Gamssulzen Cave. Symbols are as in (A).

in the same populations in Romania [21]. Thus, phylogeographic patterns are not necessarily stable over long time in bears. Furthermore, the fact that the two mtDNA sequences in Gamssulzen Cave are identical to sequences recovered from Vindija Cave about 200 km to the south (Figure 1A) shows that female migration over long distances could take place. In addition, fluctuations of the climate between 30,000 and 50,000 year B.P. [22] can be expected to have contributed to movement of cave bears and admixture between the two populations. These considerations in conjunction with the physical difference between the two bear populations makes it likely that intrinsic barriers to gene flow existed between the cave bears in Ramesch Cave and Gamssulzen Cave. If this was the case, and if reproductive isolation in sympatry is taken as a species definition [23, 24], then these bears could have represented different species.

It is important to note that it is not the extent of DNA sequence differences between the two cave bear groups but the fact that no evidence for gene flow could be detected that leads us to suggest that they may have been distinct species. However, it might nevertheless be informative to compare the genetic distance between

the two cave bear forms to the genetic distance between brown and polar bears in order to judge if the time of separation may be compatible with the evolution of distinct biological adaptations. The mtDNA sequence found at Ramesch Cave is close to additional cave bear mtDNA sequences found elsewhere (Figure S4). The mean genetic distance between these sequences and those found at Gamssulzen and Vindija is 7.3% while the mtDNA distance for the same segment between polar bears and closely related brown bears is 5.4% [15]. It therefore seems that sufficient time for the evolution of morphological and behavioral differences restricting gene flow would have been available. As brown and polar bears produce fertile offspring in captivity, it is obvious that this does not mean that the Ramesch and Gamssulzen cave bears were incapable of producing offspring even if this did not occur at detectable levels in nature.

In summary, the situation at Ramesch and Gamssulzen Caves raises the possibility that two reproductively isolated forms of cave bears existed. More morphological and genetic data from additional securely dated cave bear populations will be necessary to obtain a more general overview over the variation and possible additional substructure among cave bears.

#### Supplemental Data

Supplemental Data are available with this article online at <http://www.current-biology.com/cgi/content/full/14/1/40/DC1/>.

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