

Withering Away—25,000 Years of Genetic Decline Preceded Cave Bear Extinction

Mathias Stiller,^{*,1} Gennady Baryshnikov,² Hervé Bocherens,³ Aurora Grandal d'Anglade,⁴ Brigitte Hilpert,⁵ Susanne C. Münzel,⁶ Ron Pinhasi,⁷ Gernot Rabeder,^{8,9} Wilfried Rosendahl,¹⁰ Erik Trinkaus,¹¹ Michael Hofreiter,^{†,1} and Michael Knapp^{*,‡,1}

¹Research Group Molecular Ecology, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

²Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia

³Institut für Geowissenschaften, Biogeologie, Universität Tübingen, Tübingen, Germany

⁴Instituto Universitario de Xeoloxía, Universidade da Coruña, A Coruña, Spain

⁵Institut für Paläontologie, Universität Erlangen, Erlangen, Germany

⁶Institut für Ur- und Frühgeschichte, Universität Tübingen, Tübingen, Germany

⁷Department of Archaeology, University College Cork, Cork, Ireland

⁸Department of Paleontology, University of Vienna, Vienna, Austria

⁹Fossil Animals of Caves in Europe project of the Austria-Forschungszentrum, Austrian Academy of Sciences, Lunz/See, Austria

¹⁰Department "World Cultures and Environment", Reiss-Engelhorn-Museen, Mannheim, Germany

¹¹Department of Anthropology, Washington University

[†]Present address: Department of Biology, University of York, York, United Kingdom.

[‡]Present address: Allan Wilson Centre for Molecular Ecology and Evolution, Department of Anatomy and Structural Biology, University of Otago, 270 Great King Street, 9016 Dunedin, New Zealand.

***Corresponding author:** E-mail: michael.knapp@anatomy.otago.ac.nz.

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Abstract

The causes of the late Pleistocene megafaunal extinctions are still enigmatic. Although the fossil record can provide approximations for when a species went extinct, the timing of its disappearance alone cannot resolve the causes and mode of the decline preceding its extinction. However, ancient DNA analyses can reveal population size changes over time and narrow down potential causes of extinction. Here, we present an ancient DNA study comparing late Pleistocene population dynamics of two closely related species, cave and brown bears. We found that the decline of cave bears started approximately 25,000 years before their extinction, whereas brown bear population size remained constant. We conclude that neither the effects of climate change nor human hunting alone can be responsible for the decline of the cave bear and suggest that a complex of factors including human competition for cave sites lead to the cave bear's extinction.

Key words: ancient DNA, megafauna extinction, Bayesian skyline plot, cave bear, population dynamics.

Analyses of ancient DNA samples are a powerful tool for reconstructing past population size changes in extant and extinct species (Drummond et al. 2005). They can identify demographic events invisible in the fossil record and are therefore highly informative for testing hypothesis on the causes of the late Pleistocene megafaunal extinctions (Shapiro et al. 2004; Drummond et al. 2005). In order to isolate the cause(s) of the extinction of a species, it is particularly informative to compare its population dynamics to that of a closely related surviving species. The cave bear, which is extinct, and the brown bear, which is extant, are an ideal pair of species for such a comparison, as they are closely related (1.2–1.6 million years apart; Rustioni and Mazza 1992; Loreille et al. 2001; Rabeder and Withalm 2006; Sala and Masini 2007), similar in morphology and ecology, and shared the same habitat.

We have compared the late Pleistocene trajectories of the effective female population sizes (N_e) of European cave

and brown bears, using mitochondrial D-loop sequences from 59 temporally spaced cave bear samples and 40 temporally spaced brown bear samples. Estimates for the two species were individually derived from the respective inferred lineage coalescent rate through time and visualized using Bayesian skyline plots (BSPs) (Drummond et al. 2005). Our population size reconstructions show a constant population size for brown bear populations during the late Pleistocene but a drastic decline for cave bear populations, starting about 50,000 radiocarbon years before present (yBP), around the Middle-to-Upper Palaeolithic transition, and persisting until their extinction approximately 24,000 yBP (Pacher and Stuart 2009) (fig. 1). The slow demise of cave bears over approximately 25,000 years suggests that one or more environmental factors must have subtly increased their mortality and/or decreased their reproduction rate while leaving brown bear populations unaffected.

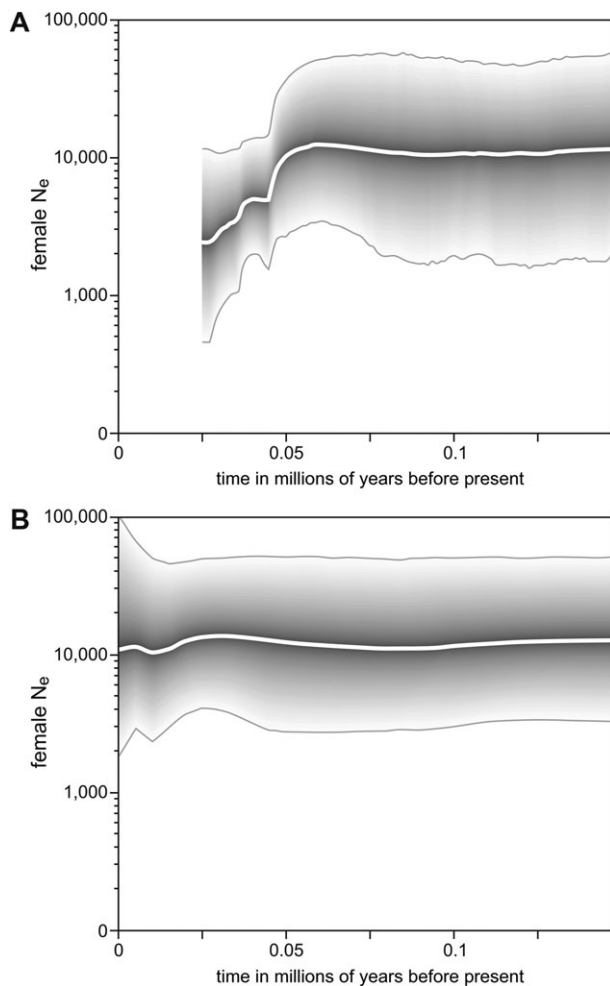


FIG. 1. Effective female population sizes (N_e) of cave bears (A) and brown bears (B). x axis: time in million years before present; y axis: female N_e ; center line: median N_e (assuming a generation time of ten years for both species; Tallmon et al. 2004); upper and lower lines: limits of 95% highest posterior density intervals.

Proposed differences between cave and brown bears include diet, geographical range, habitat preference, hibernation strategies, and predation by humans (Pacher and Stuart 2009). The question is which one of these factors, or which combination, influenced survival differently between the two species. Cave bears were long thought to lack brown bears' ability to survive in continental climates. However, it has recently been shown that the geographic range of cave bears extended to Siberia and beyond the Arctic Circle, suggesting a habitat range similar to that of brown bears (Knapp et al. 2009). Cave bears may also have been more vegetarian than brown bears (Pacher and Stuart 2009), making them potentially more susceptible to vegetation changes resulting from climate change. However, the start of the cave bear population decline is not correlated with any climate or vegetation changes more severe than those preceding the decline. Cooling climate and subsequent vegetation changes of the last glacial maximum (LGM) did not start before about 30,000 yBP (van Andel 2003), approximately 20,000 years after the beginning of the cave bear population decline.

Finally, it has been argued that humans were responsible for the decline of cave bears, either due to direct hunting (Münzel and Conard 2004) or due to competition for resources (Grayson and Delpech 2003). However, although for some regions, such as Australia and New Zealand, modern humans have been implicated as the major or even exclusive cause of megafaunal extinctions, the situation is much less clear in Eurasia (Barnosky et al. 2004). As there is rare evidence of both Neanderthals and early modern humans hunting brown and cave bears (Auguste 1995; Münzel and Conard 2004), the fossil record cannot reject human influence on the early cave bear decline. However, it appears unclear why hunting would have affected cave bears more than brown bears. Rather, different hibernation strategies of cave and brown bears might have made a difference. Judging from the relative amounts of cave bear and brown bear remains in European caves, cave bears were more dependent on caves for hibernation (Kurtén 1976; Rabeder et al. 2000). Both modern humans and Neanderthals would have been strong competitors for these caves and might have forced cave bears into less suitable sites for hibernation (Grayson and Delpech 2003). Even with low human population density, this might have increased cave bear mortality by a small but steady and eventually fatal degree. This scenario fits the continuous but slow decline, especially as there is evidence for a reduction in the amount of cave bear bones in caves with the emergence of the Upper Paleolithic and probable increases in human population size (Grayson and Delpech 2003). As suggested by Grayson and Delpech (2003), competition might have arisen not only from increases in human group sizes but also from increased human residence times. The negative effect on cave bear populations would have been enhanced by the cooling climate preceding cave bear extinction about 24,000 yBP during the height of the LGM.

Our study provides strong evidence that the extinction of cave bears cannot solely be blamed on the effects of the severe climate changes associated with the LGM. It also shows that the reconstruction of the population dynamics on the faunal community rather than the species level might be necessary to understand potential causes of the late Pleistocene megafaunal extinctions.

Materials and Methods

Samples and DNA Extraction

We obtained 17 cave bear samples ranging in age from approximately 24,000 to 60,000 yBP. Samples originated from eight geographical locations from all over Europe. DNA was extracted from bone or tooth sample material (100–500 mg) following the protocols described in Hofreiter et al. (2004) and Rohland and Hofreiter (2007). Further cave bear sequences of 251 bp in length, as well as all brown bear sequences, 177 bp in length and ranging from 0 to 80,000 years of age, were obtained from GenBank. In total, the datasets consisted of 40 brown bear and 59 cave bear samples (supplementary table 2, Supplementary Material online).

DNA Amplification and Sequencing

For all samples, we attempted to amplify an approximately 285-bp long fragment of the mitochondrial D-loop (Hofreiter et al. 2002). We used primers from Hofreiter et al. (2002) as well as primers that amplify shorter fragments (Hofreiter et al. 2004; Knapp et al. 2009). Amplifications were performed using either standard polymerase chain reaction (PCR) or multiplex PCR (Römler et al. 2006). Amplification conditions and annealing temperatures were adopted from Hofreiter et al. (2002). Amplification products were cloned into the pCR2.1-TOPO vector (Invitrogen) following the supplier's instructions. A minimum of three clones per sample were sequenced on an ABI 3730 sequencer using the BigDye Terminator v1.1 Cycle Sequencing Kit and M13 universal primers. Complete sequences were obtained from 17 samples and visually aligned using the program package BioEdit (Hall 1999). Each sequence position was determined from two independent amplifications to avoid sequence errors due to changes in the sequences caused by template damage (Hofreiter et al. 2001). Characteristic C to T and G to A changes resulting from cytosine deamination were observed, but no consistent nucleotide differences between two independent amplifications were found.

Population Genetic Analyses

Past population size trajectories for cave bears and brown bears were inferred using a Bayesian coalescent approach as implemented in BEAST 1.4.8. Sequence data obtained from cave bear (251-bp alignment) and brown bear samples (177-bp alignment) were used to reconstruct genealogies for both species. All genealogies were reconstructed under the HKY85+ Γ model of nucleotide substitution, which was for all datasets identified as the best fitting model by all three decision criteria implemented in Modeltest 3.7 (Posada and Buckley 2004) (i.e., hierarchical likelihood ratio tests, the Akaike Information Criterion, and the Bayesian Information Criterion). N_e of both species were then derived from the respective lineage coalescent rate through time and visualized using BSPs (Drummond et al. 2005). The timing of coalescence events was estimated using the age of the samples as calibration (supplementary table 2, Supplementary Material online). Temporal smoothing of the derived N_e trajectories was achieved by summarizing coalescence intervals across the complete genealogy into ten groups and estimating N_e for each of these groups. Posterior genealogies and all associated factors were estimated with three Markov chain Monte Carlo runs of 50,000,000 steps each, sampling every 5,000th step after a discarded burn-in of 5,000,000. Convergence of the chains and effective sample sizes were verified, and results from all three chains were combined using the program TRACER 1.5. To test the cave bear population decline hypothesis against the alternative hypothesis of a constant population size, we reconstructed the cave bear genealogy assuming a constant population size and used Bayes factors (Suchard et al. 2001) to compare it with a BSP reconstruction. The BSP model fitted the data decisively better (\log_{10} Bayes Factor = 5.804).

Further factors that can affect N_e reconstructions over time include population structure, non-random sampling, imprecise age estimates of samples, lack of information in the alignment, and natural selection. To test whether our results were influenced by any of these factors, we conducted extensive randomization and resampling experiments and used a wide range of different parameter settings (supplementary material, Supplementary Material online). All additional analyses confirmed our results.

Supplementary Material

Supplementary tables S1 and S2, figures S1 and S2, and material are available at *Molecular Biology and Evolution* online (<http://www.mbe.oxfordjournals.org/>).

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