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Partial genomic survival of cave bears in living brown bears

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Supplementary Table 1. Details of samples used in this study

| Common name | Taxon | Sample code | Locality | Sex | Reference | Accession |
|---------------------|----------------------|-------------|-------------------------------|----------|--------------|------------|
| Cave bear | <i>eremus</i> | WK01 | Windischkopf, Austria | untested | This study | * |
| Cave bear | <i>ingressus</i> | GS136 | Gamssulzen Cave, Austria | untested | This study | * |
| Cave bear | <i>spelaeus</i> | E-VD-1838 | Eiros Cave, Spain | untested | This study | * |
| Cave bear | <i>kudarensis</i> | HV74 | Hovk Cave, Armenia | untested | This study | * |
| Brown bear | <i>U. arctos</i> | Uap | Winden Cave, Austria | untested | This study | * |
| Brown bear | <i>U. arctos</i> | Ge | Georgia, Great Caucasus | untested | This study | * |
| Brown bear | <i>U. arctos</i> | 191Y | Hrušica, Slovenia | untested | This study | * |
| Brown bear | <i>U. arctos</i> | 235 | Balakhtinsky District, Russia | untested | This study | * |
| Brown bear | <i>U. arctos</i> | Swe | Sweden | Female | ¹ | SRX796442 |
| Brown bear | <i>U. arctos</i> | LS039 | Spain | Male | ² | SRR5878347 |
| Brown bear | <i>U. arctos</i> | Adm1 | Alaska (Admiralty) | Female | ³ | SRX265457 |
| Brown bear | <i>U. arctos</i> | Den | Alaska (Denali) | Female | ³ | SRX265456 |
| Polar bear | <i>U. maritimus</i> | NB | North Beaufort Sea | Male | ³ | SRX265452 |
| Polar bear | <i>U. maritimus</i> | SB | South Beaufort Sea | Male | ³ | SRX265435 |
| Polar bear | <i>U. maritimus</i> | WH2 | West Hudson Bay | Male | ³ | SRX265434 |
| American black bear | <i>U. americanus</i> | Uam | Pennsylvania, USA | Female | ³ | SRX265459 |
| Asiatic black bear | <i>U. thibetanus</i> | ERS781634 | Zoo Madrid | Female | ⁴ | ERS781634 |
| Spectacled bear | <i>T. ornatus</i> | ERR946788 | Zoo Basel | Male | ⁴ | ERR946788 |

* European Nucleotide Archive run accessions ERR2678614–ERR2678640

Supplementary Table 2. Ancient sample radio-carbon ages and estimated endogenous contents

| Common name | Taxon | Sample code | ¹⁴ C age | Calibrated age BP | Lab No. MAMS | Endo % |
|-------------|-------------------|-------------|------------------------|-------------------|--------------|--------|
| Cave bear | <i>eremus</i> | WK01 | >49000 ⁵ | - | 23137 | 66.1 |
| Cave bear | <i>ingressus</i> | GS136 | 31026±500 ⁵ | 35062±966 | 23139 | 63.7 |
| Cave bear | <i>spelaeus</i> | E-VD-1838 | 30737±500 ⁵ | 34806±931 | 23140 | 74.4 |
| Cave bear | <i>kudarensis</i> | HV74 | >49000 ⁵ | - | 23142 | 66.0 |
| Brown bear | <i>U. arctos</i> | Uap | 36680±500 ⁵ | 41201±895 | 23141 | 65.7 |

Endogenous % is an estimate calculated by dividing the proportion of reads mapping to the polar bear reference genome by the proportion mapping from a high quality modern brown bear sample (Ge), which we determined to be approximately 87.9%. This procedure provides a degree of correction for false negatives due to an incomplete or misassembled reference genome.

Supplementary Table 3. Mapping results. Outcome of mapping ancient DNA sequences to the reference genome assembly of the giant panda.

| sample | unique mapped reads ^A | % uniquely mapping reads | Mean read depth ^B | Total mapped GB |
|-----------|----------------------------------|--------------------------|------------------------------|-----------------|
| WK01 | 148679922 | 44.6 | 3.49 | 6.26 |
| GS136 | 69053691 | 23.5 | 3.55 | 3.82 |
| E-VD-1838 | 116172796 | 50.5 | 2.85 | 4.69 |
| HV74 | 81320041 | 43.7 | 2.39 | 3.82 |
| Uap | 61074228 | 43.6 | 1.93 | 2.71 |

^A *unique mapped reads*: is the number of mapped reads with mapping quality Q30 or above, after removal of potential PCR duplicates using samtools⁷ rmdup

^B *Mean read depth*: is mean read depth per covered position of the reference

Supplementary Table 4. Evaluation of phylogenetic test of directional admixture using a well characterised system: gene flow from polar bears into brown bears. Shown are total counts of blocks returning all 15 possible rooted tree topologies for 2 different block sizes. The four taxa used for this investigation were: NB (Polar1), WH (Polar2), 191Y (EuroBrown), Adm1 (ABCbrown). Trees were rooted using the American black bear outgroup. The distribution of tree topologies is consistent with increased gene flow from polar bears into the ABC islands brown bears relative to European brown bears, and a lack of gene flow from brown bears into polar bears following the basal divergence of these polar bear populations.

| Topology | Description | Tree | 0.25MB | 0.1MB | Prop* |
|---------------|--------------|---------------------------------------|--------|-------|--------|
| 1 | species tree | ((Polar1,Polar2)(EuroBrown,ABCbrown)) | 3898 | 8692 | 0.6599 |
| 2 | symmetrical | ((EuroBrown,Polar2)(Polar1,ABCbrown)) | 0 | 2 | 0.0002 |
| 3 | symmetrical | ((ABCbrown,Polar2)(Polar1,EuroBrown)) | 0 | 1 | 0.0001 |
| 4 | Class 1 | ((Polar1,Polar2),ABCbrown),EuroBrown) | 855 | 2751 | 0.2089 |
| 5 | Class 1 | ((ABCbrown,Polar2),Polar1),EuroBrown) | 8 | 49 | 0.0037 |
| 6 | Class 1 | ((Polar1,ABCbrown),Polar2),EuroBrown) | 0 | 41 | 0.0031 |
| 7 | Class 2 | ((Polar1,Polar2),EuroBrown),ABCbrown) | 354 | 1590 | 0.1207 |
| 8 | Class 2 | ((EuroBrown,Polar1),Polar2),ABCbrown) | 0 | 5 | 0.0004 |
| 9 | Class 2 | ((EuroBrown,Polar2),Polar1),ABCbrown) | 1 | 4 | 0.0003 |
| 10 | Class 3 | ((EuroBrown,ABCbrown),Polar2),Polar1) | 2 | 20 | 0.0015 |
| 11 | Class 3 | ((EuroBrown,Polar2),ABCbrown),Polar1) | 0 | 1 | 0.0001 |
| 12 | Class 3 | ((ABCbrown,Polar2),EuroBrown),Polar1) | 0 | 2 | 0.0002 |
| 13 | Class 4 | ((EuroBrown,ABCbrown),Polar1),Polar2) | 1 | 11 | 0.0008 |
| 14 | Class 4 | ((EuroBrown,Polar1),ABCbrown),Polar2) | 0 | 0 | 0.0000 |
| 15 | Class 4 | ((Polar1,ABCbrown),EuroBrown),Polar2) | 4 | 3 | 0.0002 |
| Totals | | | 5123 | 13172 | 1 |

* “Prop” indicates proportion of blocks returning this topology at 0.1MB block size.

Supplementary Table 5. Observed length frequencies of topology Classes in brown bears, as measured by the number of contiguous 25Kbp blocks returning the same topology. Note that, although topologies are grouped here by topology Class, counts represent continuous genomic regions returning a single tree topology.

| Sample | Topology Class | 25 | 50 | 75 | 100 | 125 | 150 | 175 |
|--------|----------------|------|-----|-----|-----|-----|-----|-----|
| 191Y | Class 1 | 6014 | 806 | 152 | 26 | 8 | 2 | - |
| | Class 2 | 4931 | 620 | 89 | 14 | 3 | 2 | - |
| | Class 3 | 3165 | 199 | 24 | 3 | - | - | - |
| | Class 4 | 2862 | 173 | 22 | 1 | 1 | - | - |
| 235 | Class 1 | 5717 | 808 | 159 | 26 | 6 | 3 | - |
| | Class 2 | 4922 | 619 | 96 | 15 | 4 | 2 | - |
| | Class 3 | 3171 | 226 | 21 | 1 | 1 | - | - |
| | Class 4 | 2922 | 188 | 11 | 2 | 1 | - | - |
| Adm1 | Class 1 | 5304 | 702 | 116 | 18 | 6 | 1 | - |
| | Class 2 | 4582 | 516 | 87 | 13 | 1 | 1 | - |
| | Class 3 | 3203 | 247 | 27 | 5 | 1 | - | - |
| | Class 4 | 2917 | 199 | 24 | 3 | 1 | - | 1 |
| Ge | Class 1 | 6240 | 838 | 162 | 28 | 11 | 2 | - |
| | Class 2 | 4956 | 610 | 102 | 15 | 2 | 2 | - |
| | Class 3 | 3240 | 207 | 23 | - | - | - | - |
| | Class 4 | 2961 | 186 | 10 | 2 | 1 | - | - |
| Den | Class 1 | 5566 | 738 | 141 | 22 | 4 | 1 | 1 |
| | Class 2 | 4691 | 570 | 88 | 16 | 1 | 1 | - |
| | Class 3 | 3230 | 234 | 19 | 1 | - | - | - |
| | Class 4 | 2969 | 172 | 13 | 4 | 2 | - | - |
| Uap | Class 1 | 6756 | 987 | 201 | 52 | 8 | 1 | - |
| | Class 2 | 5000 | 588 | 99 | 14 | - | 1 | - |
| | Class 3 | 2798 | 170 | 18 | 4 | - | - | - |
| | Class 4 | 2693 | 148 | 6 | - | 1 | - | - |
| Swe | Class 1 | 6122 | 864 | 150 | 28 | 6 | 2 | - |
| | Class 2 | 5032 | 621 | 99 | 14 | 4 | 2 | - |
| | Class 3 | 3210 | 216 | 26 | 1 | - | - | - |
| | Class 4 | 2877 | 174 | 10 | 4 | 1 | - | - |
| LS039 | Class 1 | 6301 | 851 | 146 | 20 | 10 | 1 | - |
| | Class 2 | 5119 | 612 | 89 | 14 | 1 | 1 | - |
| | Class 3 | 3185 | 215 | 21 | 3 | - | - | - |
| | Class 4 | 2889 | 165 | 15 | 1 | 1 | - | - |

“-” indicates that this topology Class was not observed at this length, in this individual.

Supplementary Table 6. Summary of ancient DNA sequencing. The ancient data were generated over a series of experiments described elsewhere⁶.

| sample | library code | DNA extraction ^A | library ^B | primer ^C | platform | read length | read pairs |
|--------------|--------------|-----------------------------|----------------------|---------------------|----------|-------------|------------|
| WK01 | WK01_1 | Dab | SS | CL72 | MiSeq | 75 | 5272087 |
| | | | | | NextSeq | 75 | 494438691 |
| <i>total</i> | | | | | | | 499710778 |
| GS136 | GS136_1 | Dab | DS | standard | HiSeq | 100 | 25064683 |
| | | | | | HiSeq | 100 | 303706660 |
| | GS136_2 | Roh | DS | standard | HiSeq | 100 | 29871305 |
| <i>total</i> | | | | | | | 358642648 |
| E-VD-1838 | UD1838_1 | Dab | SS | CL72 | NextSeq | 75 | 9201899 |
| | | | | | NextSeq | 75 | 399915983 |
| <i>total</i> | | | | | | | 409117882 |
| HV74 | HV74_1 | Dab | DS | CL72* | MiSeq | 75 | 2056535 |
| | HV74_2 | com | DS | CL72* | MiSeq | 70 | 420279 |
| | HV74_3 | com | SS | CL72 | MiSeq | 70 | 209010 |
| | HV74_4 | Dab | DS | CL72* | MiSeq | 70 | 493704 |
| | HV74_5 | Dab | SS | CL72 | MiSeq | 70 | 734342 |
| | | | | | NextSeq | 75 | 238422118 |
| | HV74_6 | Roh | DS | CL72* | MiSeq | 70 | 412114 |
| HV74_7 | Roh | SS | CL72 | MiSeq | 70 | 712976 | |
| <i>total</i> | | | | | | | 243461078 |
| Uap | Uap_1 | Dab | DS | CL72* | MiSeq | 75 | 848886 |
| | | | | | NextSeq | 75 | 154158768 |
| | Uap_2 | com | DS | CL72* | MiSeq | 70 | 687177 |
| | | | | | NextSeq | 75 | 59016440 |
| | Uap_3 | com | SS | CL72 | MiSeq | 70 | 230543 |
| | Uap_4 | Dab | DS | CL72* | MiSeq | 70 | 666349 |
| | Uap_5 | Dab | SS | CL72 | MiSeq | 70 | 297363 |
| NextSeq | | | | | 75 | 154158768 | |
| Uap_6 | Roh | DS | CL72* | MiSeq | 70 | 639214 | |
| Uap_7 | Roh | SS | CL72 | MiSeq | 70 | 722978 | |
| <i>total</i> | | | | | | | 217267718 |

^A *DNA extraction*: “Dab” refers to the published method⁸, “Roh” refers to the published method⁹, and “com” refers to the combined protocol described in⁶.

^B *Library*: “DS” refers to the published method¹⁰, “SS” refers to the published method¹¹

^C *primer*: describes the R1 sequencing primer used for sequencing, either standard Illumina or the custom primer CL72¹¹

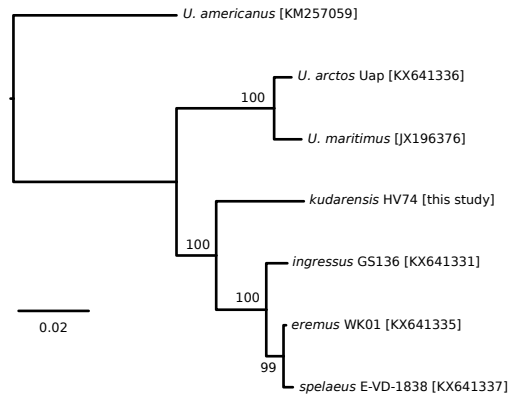
* DS libraries sequenced using the CL72 primer require removal of the first 5bp of R1 prior to data processing. This was accomplished using the software seqtk¹²

Supplementary Table 7. Block size and detection of alternative topologies. Shown are total counts of blocks returning all 15 possible rooted tree topologies for 6 different block sizes. 25Kbp was selected as an appropriate block size providing adequate sensitivity to detect incomplete lineage sorting and admixture. The four taxa used for this investigation were: NB (Polar), Uap (Brown), HV74 (Cave-), E-VD-1838 (Cave+).

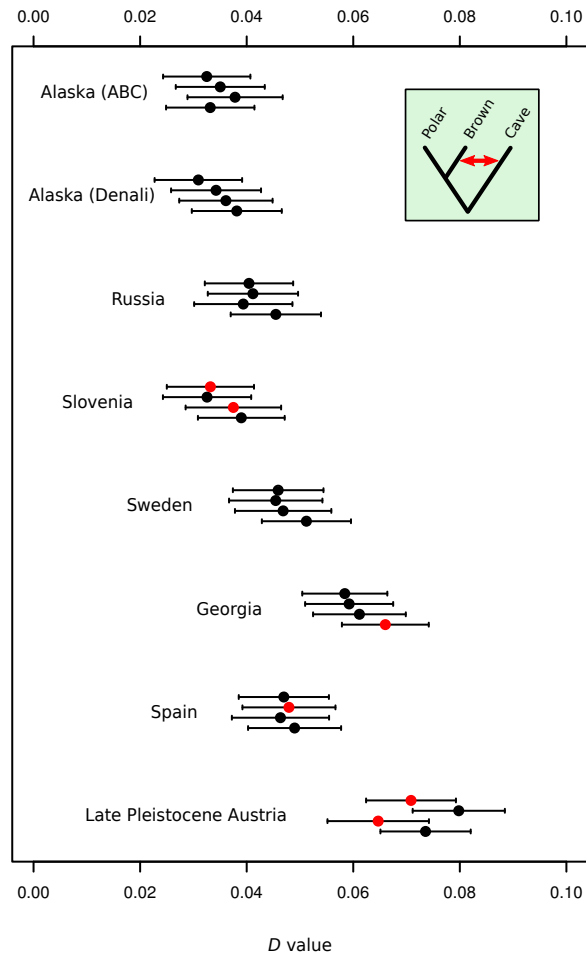
Trees were rooted using the black bear outgroup.

| Topol. | Description | Tree | 1MB | 0.5MB | 0.25MB | 100Kbp | 50Kbp | 25Kbp | Prop* |
|---------------|--------------------|------------------------------|------------|--------------|---------------|---------------|--------------|--------------|--------------|
| 1 | species tree | ((Polar,Brown)(Cave-,Cave+)) | 1051 | 2269 | 4413 | 9518 | 15714 | 23817 | 0.4919 |
| 2 | symmetrical | ((Cave-,Brown)(Polar,Cave+)) | | | | 11 | 77 | 298 | 0.0062 |
| 3 | symmetrical | ((Cave+,Brown)(Polar,Cave-)) | | | 1 | 16 | 75 | 347 | 0.0072 |
| 4 | Class 1 | ((Cave-,Cave+),Brown),Polar) | 3 | 65 | 366 | 1667 | 4039 | 8979 | 0.1854 |
| 5 | Class 1 | ((Cave-,Brown),Cave+),Polar) | | | | 49 | 192 | 692 | 0.0143 |
| 6 | Class 1 | ((Cave+,Brown),Cave-),Polar) | | | 1 | 36 | 208 | 1191 | 0.0246 |
| 7 | Class 2 | ((Cave-,Cave+),Polar),Brown) | 3 | 19 | 146 | 918 | 2487 | 5330 | 0.1101 |
| 8 | Class 2 | ((Cave-,Polar),Cave+),Brown) | | | 2 | 32 | 153 | 500 | 0.0103 |
| 9 | Class 2 | ((Polar,Cave+),Cave-),Brown) | | | 2 | 24 | 129 | 512 | 0.0106 |
| 10 | Class 3 | ((Polar,Brown),Cave+),Cave-) | | | 11 | 178 | 817 | 2839 | 0.0586 |
| 11 | Class 3 | ((Polar,Cave+),Brown),Cave-) | | | | 18 | 97 | 428 | 0.0088 |
| 12 | Class 3 | ((Cave+,Brown),Polar),Cave-) | | | | 19 | 117 | 532 | 0.0110 |
| 13 | Class 4 | ((Polar,Brown),Cave-),Cave+) | | 1 | 11 | 167 | 788 | 2123 | 0.0438 |
| 14 | Class 4 | ((Cave-,Polar),Brown),Cave+) | | | | 17 | 84 | 362 | 0.0075 |
| 15 | Class 4 | ((Cave-,Brown),Polar),Cave+) | | | 1 | 12 | 82 | 473 | 0.0098 |
| Totals | | | 1057 | 2354 | 4954 | 12682 | 25059 | 48423 | 1 |

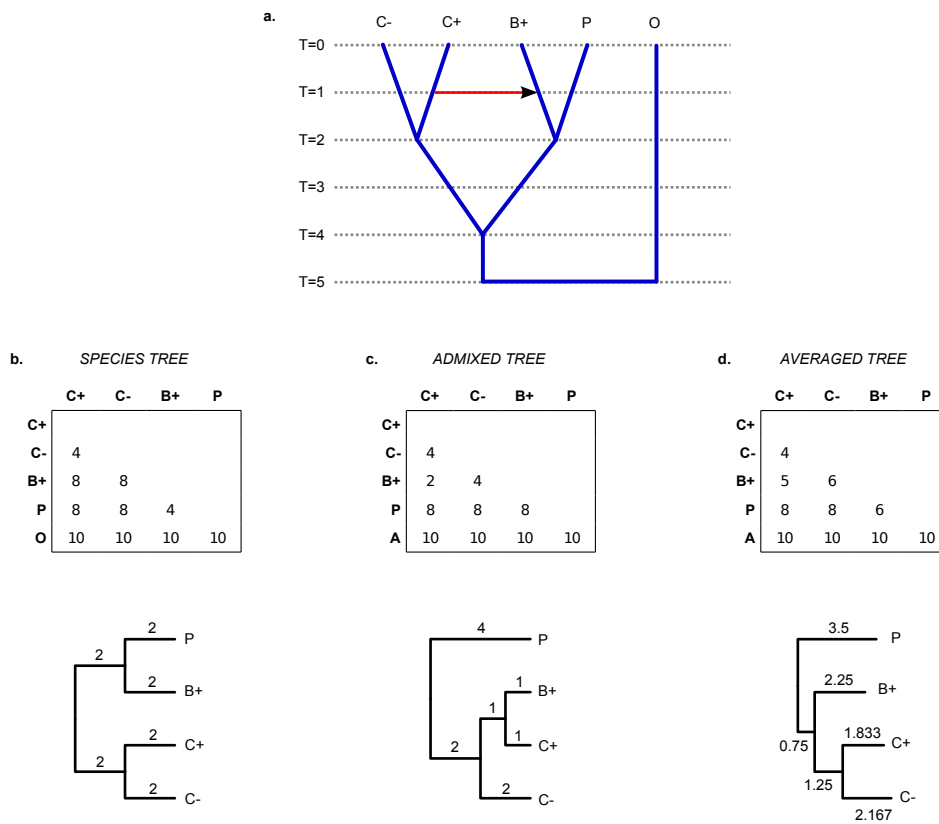
* "Prop" indicates proportion of blocks returning this topology at 25Kbp block size.



Supplementary Figure 1. Maximum-likelihood mitochondrial phylogeny of cave bears used in this study. The Pleistocene brown bear (*U. arctos* Uap) and a polar bear (*U. maritimus*) are included to show their respective phylogenetic positions, and the tree is rooted using the American black bear (*U. americanus*). Clade support values are bootstrap percentages, and the scale bar indicates substitutions per site. Genbank accession numbers for previously published sequences are shown inside square brackets.



Supplementary Figure 2. *D* statistic tests for admixture. Results are presented for eight brown bears (indicated by locality on the left) and a polar bear, with clusters of four points for each brown bear corresponding to four separate tests, each using a different cave bear as potential introgressor (ordered within each cluster from top to bottom: *eremus*, *spelaeus*, *ingressus*, *kudarensis*). X-axes indicate *D* values and bars indicate ± 1 weighted block jackknife standard error. Positive *D* values provide evidence of admixture (in either direction) between cave bears and brown bears following the divergence of brown bears and polar bears (inset top right). All *D* values are statistically significant ($Z > 3$ in all comparisons). Red points indicate comparisons involving geographically paired brown bears and cave bears. No obvious pattern of geographically localised admixture is apparent. Although tests of the Georgian brown bear returned a higher *D* value for the geographically paired Caucasus cave bear *kudarensis*, error estimates are widely overlapping with those for other cave bears precluding any conclusive interpretation of this pattern.

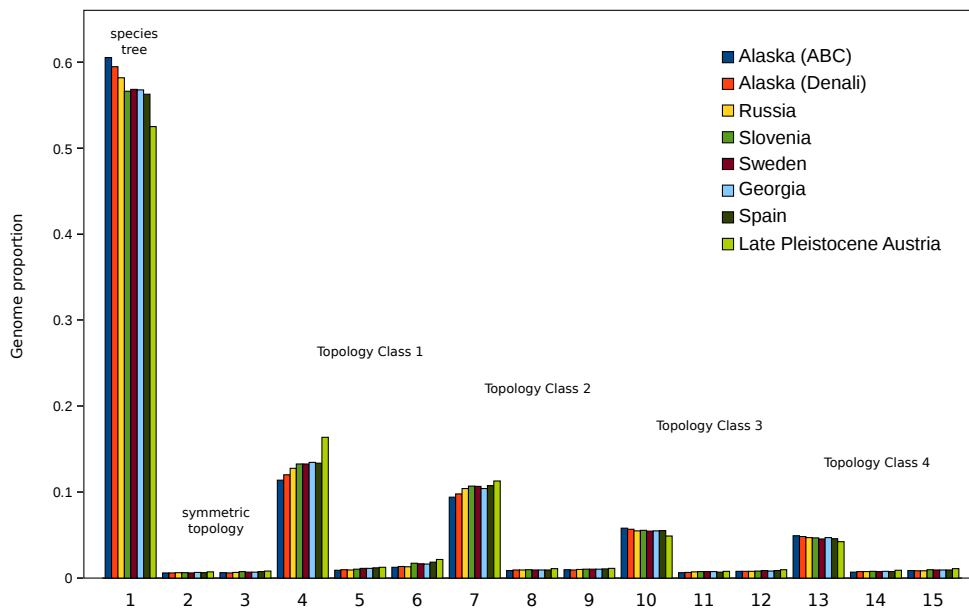


Supplementary Figure 3. Effect of pseudohaploid sequences, when an individual is heterozygous for admixed and unadmixed alleles. Consider an admixture scenario between cave bears and brown bears. We sample five individuals: an admixed cave bear (C+), an unadmixed cave bear (C-), an admixed brown bear (B+), a polar bear which is not admixed with cave bear (P), and an outgroup (O). a. shows the species tree with divergence times indicated by dashed lines. At T=1 gene flow from the admixed cave bear lineage to the brown bear lineage occurs. b.-c. Show distance matrices for alternative trees, proportional to divergence times indicated in a. Below each matrix is the calculated neighbour-joining tree, with branch lengths indicated. Pseudohaploidisation of a heterozygous region results in a matrix (d.) that is averaged across the species tree (b.) and an admixed tree (c.). The position of the admixed brown bear (B+) in the resulting averaged tree (d.) has shifted relative to the admixed tree (c.), but overall tree asymmetry has been preserved.

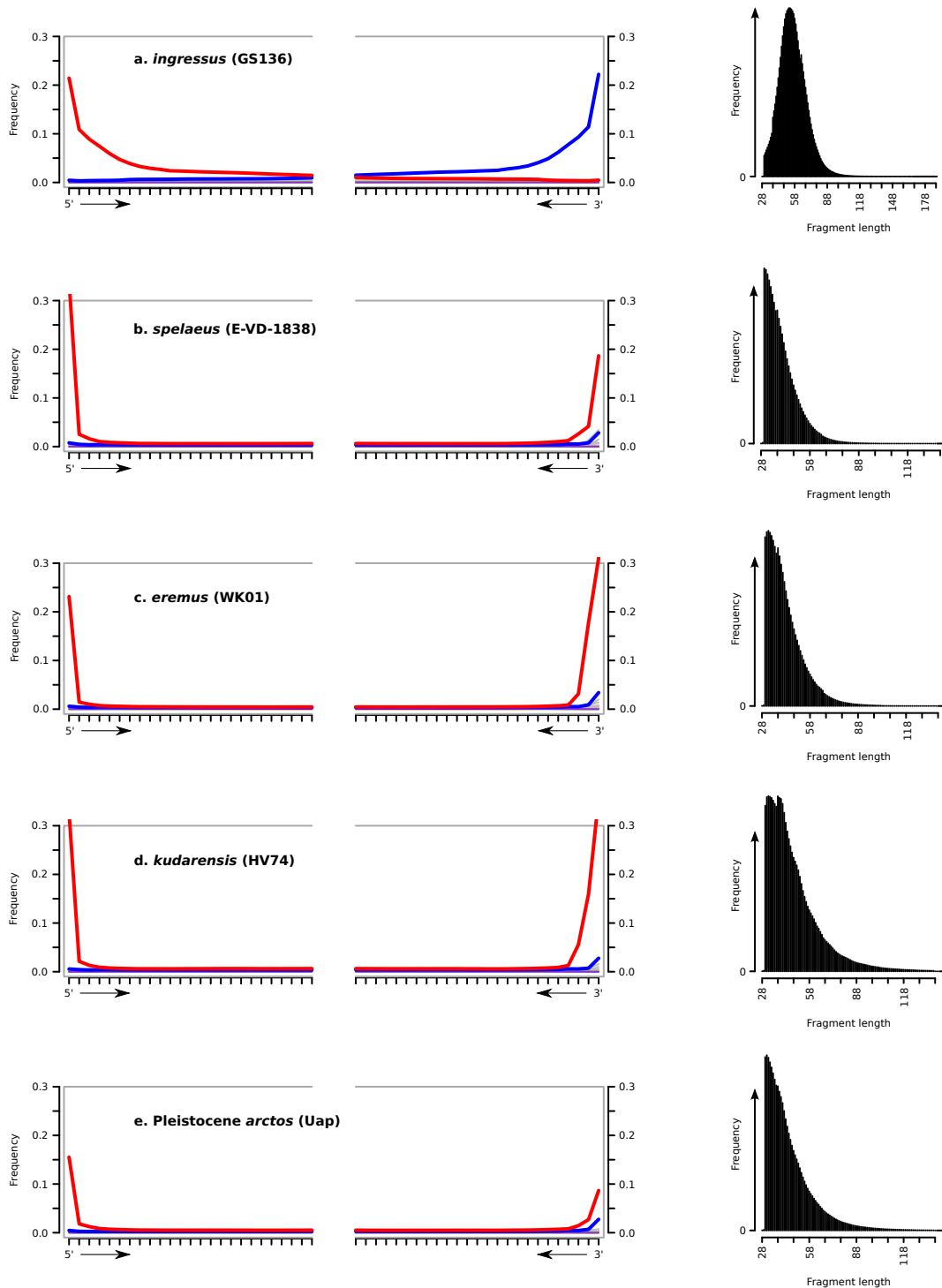
a

| Topology | Description | Tree | Alaska (Admiralty) | Alaska (Denali) | Russia | Slovenia | Sweden | Caucasus | Spain | Austria Pleistocene |
|--|--------------|------------------------------|-----------------------|--------------------|---------|----------|---------|----------|---------|------------------------|
| 1 | species tree | ((Polar,Brown)(Cave-,Cave+)) | 32133 | 31551 | 30868 | 29205 | 30010 | 30128 | 29484 | 25495 |
| 2 | symmetrical | ((Cave-,Brown)(Polar,Cave+)) | 302 | 307 | 326 | 313 | 313 | 346 | 319 | 346 |
| 3 | symmetrical | ((Cave+,Brown)(Polar,Cave-)) | 319 | 316 | 340 | 375 | 364 | 361 | 383 | 385 |
| 4 | Class 1 | ((Cave-,Cave+),Brown),Polar) | 6032 | 6370 | 6776 | 6835 | 6992 | 7131 | 7000 | 7940 |
| 5 | Class 1 | ((Cave-,Brown),Cave+),Polar) | 4981 | 5186 | 5525 | 5510 | 5627 | 5526 | 5636 | 5475 |
| 6 | Class 1 | ((Cave+,Brown),Cave-),Polar) | 455 | 490 | 490 | 491 | 496 | 493 | 487 | 526 |
| 7 | Class 2 | ((Cave-,Cave+),Polar),Brown) | 364 | 393 | 400 | 397 | 394 | 406 | 388 | 432 |
| 8 | Class 2 | ((Cave-,Polar),Cave+),Brown) | 450 | 435 | 448 | 488 | 486 | 482 | 486 | 529 |
| 9 | Class 2 | ((Polar,Cave+),Cave-),Brown) | 479 | 504 | 496 | 530 | 590 | 596 | 614 | 602 |
| 10 | Class 3 | ((Polar,Brown),Cave+),Cave-) | 502 | 494 | 525 | 520 | 536 | 545 | 554 | 534 |
| 11 | Class 3 | ((Polar,Cave+),Brown),Cave-) | 318 | 339 | 378 | 378 | 385 | 394 | 360 | 374 |
| 12 | Class 3 | ((Cave+,Brown),Polar),Cave-) | 2597 | 2550 | 2496 | 2398 | 2396 | 2488 | 2399 | 2051 |
| 13 | Class 4 | ((Polar,Brown),Cave-),Cave+) | 3078 | 3009 | 2907 | 2858 | 2881 | 2910 | 2887 | 2367 |
| 14 | Class 4 | ((Cave-,Polar),Brown),Cave+) | 407 | 411 | 410 | 411 | 458 | 419 | 443 | 467 |
| 15 | Class 4 | ((Cave-,Brown),Polar),Cave+) | 653 | 712 | 690 | 873 | 872 | 854 | 963 | 1045 |
| Total number of blocks | | | 53070 | 53067 | 53075 | 51582 | 52800 | 53079 | 52403 | 48568 |
| cave bear admixture proportion* | | | 0.01155 | 0.01334 | 0.01340 | 0.01664 | 0.01700 | 0.01900 | 0.01813 | 0.03142 |

b

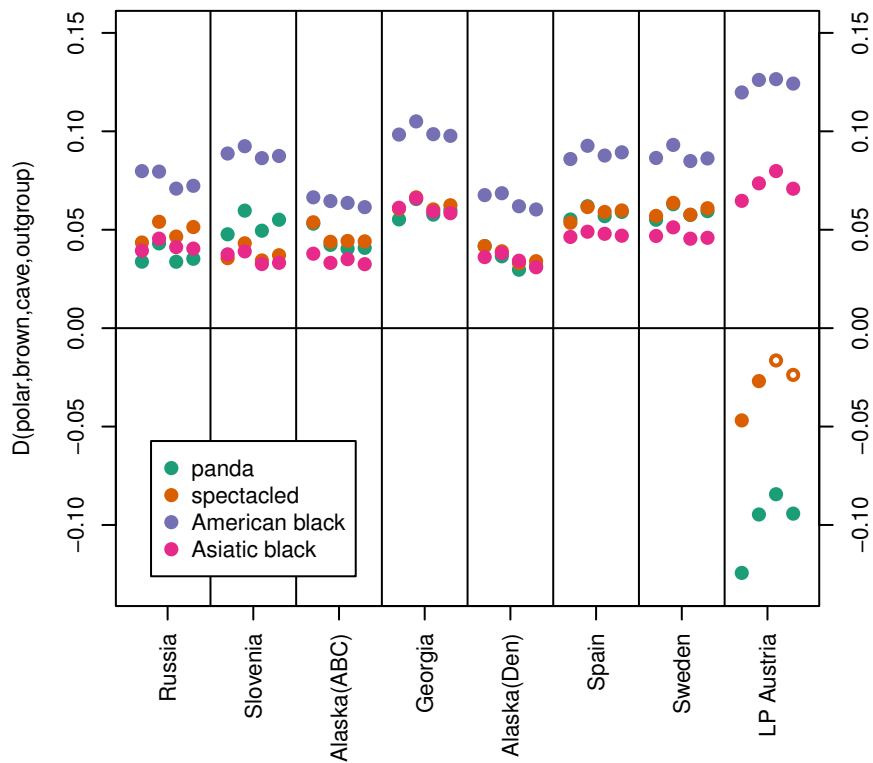


Supplementary Figure 4. a. Results of phylogenetic admixture tests, showing total counts of 25kbp non-overlapping genomic blocks returning 15 possible rooted tree topologies, for each brown bear investigated. The indicated tree topologies comprise polar bear (NB), *spelaeus* (Cave+), *kudarensis* (Cave-), and all brown bears investigated (right most eight columns, with locality indicated). The cave bear admixture proportion is estimated by subtracting the total number of 25Kbp blocks returning Class 2 from the total number returning Class 1, and dividing the resulting number by two times the total number of observed blocks. This method assumes that all introgressed blocks in brown bears are heterozygous for cave bear and brown bear haplotypes. It therefore represents an underestimate of the true admixture proportion, since some introgressed blocks appear to exist in a homozygous state (topologies 5 and 6). b. Bar charts showing the genomic representation of each topology for each investigated brown bear.



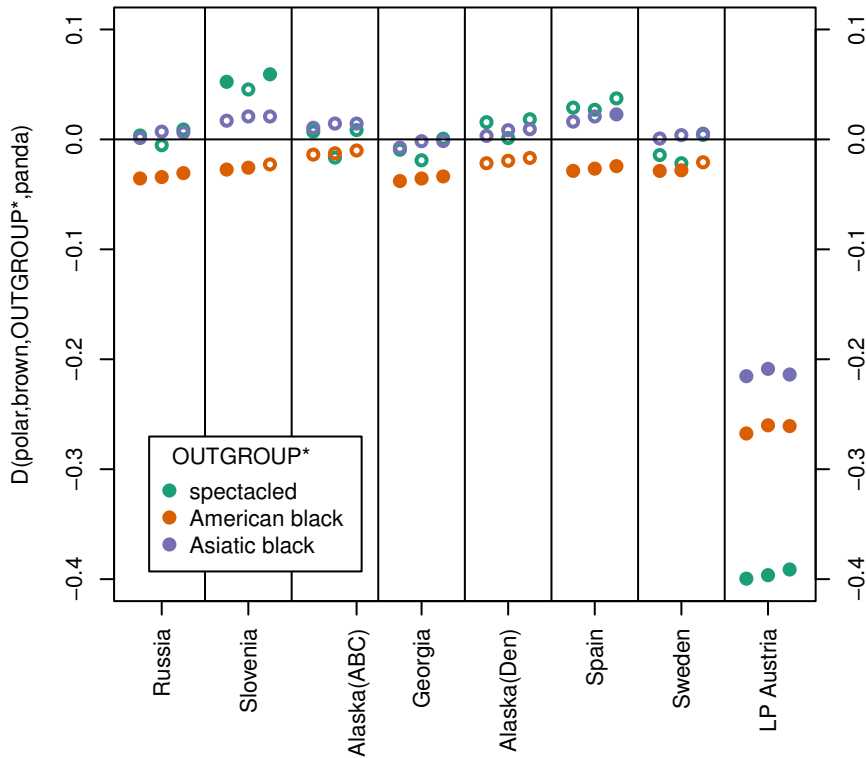
Supplementary Figure 5. Authentication of ancient DNA data from ancient bear samples (a.-e.). Plots on the left show cytosine deamination patterns. X axes indicate individual nucleotide positions of ancient DNA fragments, with 5' and 3' fragment ends indicated. Plots show the proportion of fragments possessing a T where the reference polar bear genome possesses a C (red plots), and proportion of fragments possessing an A where the reference possesses a G (blue plots). An overabundance of C->T substitutions at the fragment ends is indicative of cytosine deamination and ancient DNA damage. The overabundance in G->A substitutions at 3' read fragment ends in sample GS136 (a.) is an artefact of the double-stranded library procedure, and in reality represents C->T changes. Fragment length distributions for each sample are shown to the right, determined from the lengths of merged reads mapping to the polar bear reference. The minimum read length for mapping was 30bp, resulting in truncation of the distribution at this length. The double-stranded library preparation method used for sample GS136 (a.) is known to be associated with a loss of very short DNA fragments relative to the single-stranded methods (b.-e.), and likely explains the increase in fragment lengths observed in this dataset.

Effect of outgroup on D statistic

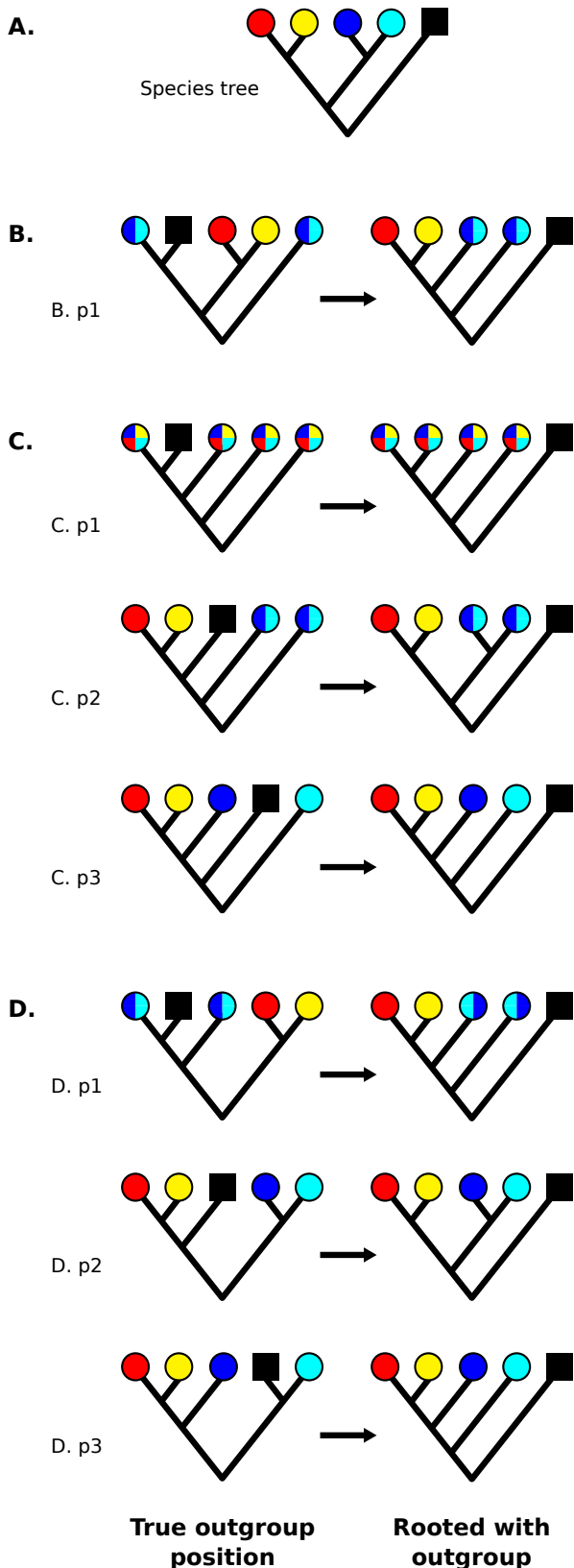


Supplementary Figure 6. Effect of outgroup selection on inferred patterns of admixture between brown bears and cave bears, following the divergence of brown bears and polar bears. The species tree is (((polar bear, brown bear),cave bear),outgroup), with American black bear, Asiatic black bear, spectacled bear and giant panda representing, respectively, increasingly divergent outgroups. We present D statistic (y-axis) results for 8 brown bears (x-axis) as points coloured according to the outgroup utilised, with sets of 4 points per brown bear/outgroup combination representing values calculated each using one of four cave bears. Open points indicate non-significant D values (absolute Z score < 3). For modern brown bears, all four outgroups produce positive D values for all tests, but we observe elevated D values when using American black bear, relative to other outgroups. Positive values were also produced for the ancient brown bear (LP Austria) when using the two least divergent outgroups (American and Asiatic black bears). However, negative values are produced when the two most divergent outgroups (spectacled bear and giant panda) are used, which are significant in most cases. We attribute this effect to accumulated errors in the ancient pseudohaploid sequences as a result of both sequencing error and spurious read mapping, both of which tend to occur at higher rates in ancient relative to modern DNA datasets. Specifically, at sites where the outgroup has a private allele, accumulated errors in an ancient sample occupying the P2 position would convert a proportion of these BBBA sites to BABA sites, causing that individual to appear unadmixed relative to P1. This effect is amplified with more divergent outgroups since they will possess more private alleles, relative to the ingroup.

Test of admixture with alternative outgroups



Supplementary Figure 7. Assessment of imbalance between polar bears and brown bears in derived allele sharing with three candidate outgroups. We present D statistic (y-axis) results for 8 brown bears (x-axis) as points coloured according the candidate outgroup utilised, with sets of 3 points per brown bear/outgroup combination representing values calculated each using a different polar bear. Open points indicate non-significant D values (absolute Z score < 3). The giant panda served as the P4 outgroup taxon for allele polarisation. We find a significant excess of derived alleles shared between polar bears and American black bear relative to modern brown bears in almost all comparisons. D values for Asiatic black bear are generally closest to zero and with the fewest significant values. All comparisons involving the ancient brown bear (LP Austria) produced large and significantly negative D values, which is attributable to accumulated errors in the ancient brown bear pseudohaploid sequence (see Supplementary Figure 6 for further explanation).



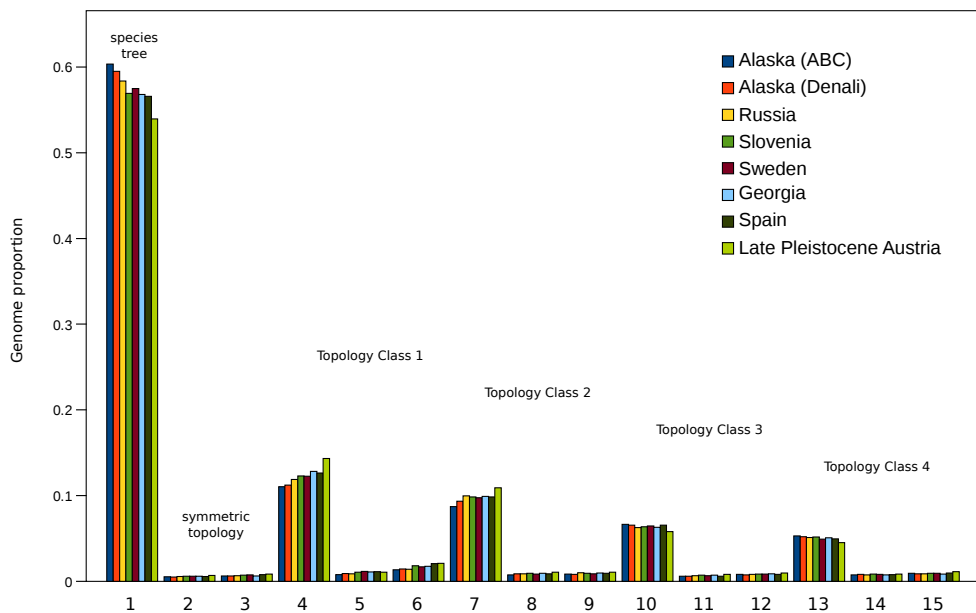
Supplementary Figure 8.

Effect of outgroup misspecification. We consider a rooted symmetrical four-species tree with a fifth outgroup taxon (A.) which is suited to admixture testing. We consider the effect of violating the assumed outgroup position. That is, the outgroup lineage in fact occupies an ingroup position for a given genomic block. A rooted five-taxon tree has 105 possible topologies representing three possible shapes (B., C., D.). For each of these tree shapes, the assumed outgroup taxon may occupy one (B. p1), three (C. p1–3) or three (D. p1–3) alternative ingroup positions, respectively. The three tree shapes with each possible outgroup position are shown on the left, and the trees resulting from rooting these trees using the assumed outgroup (black square) are shown on the right. For illustrative purposes, the four ingroups are represented by coloured circles, but it should be noted that this represents only a small subset of the total possible topologies. We can, however, consider the effect of outgroup misspecification across all topologies by reducing them to these seven combinations of tree shape and outgroup position. Where ingroup lineages have sorted with respect to the outgroup, these are shown as single-colour circles. Where lineages are incompletely sorted with respect to the outgroup, they are shown as multi-coloured circles, reflecting that under a model of random lineage sorting these lineages will be represented equally at each of these positions if a large number of loci are sampled. In some cases, incomplete sorting of one (C. p3, D. p3) or two (B. p1, D. p1) ingroup lineages is correctly identified, albeit with an incorrect outgroup position, but in none of these cases do we predict an imbalance in frequency by which two incompletely sorted lineages occupy, respectively, basal ingroup positions. When no two lineages have sorted with respect to the outgroup, a topology which is informative on admixture is artifactually produced (C. p1), but under random lineage sorting all possible topologies will occur at equal frequencies and equilibrium of admixture informative topology classes is maintained. Outgroup misspecification can also artifactually group two lineages that have not yet sorted, but the resulting tree has a symmetrical ingroup and does not contribute to admixture inference (C. p2). Any decreases in admixture informative topology counts resulting from this effect will be balanced, respectively, between the two incompletely sorted lineages, preserving equilibrium of topology classes. When both ingroup clades have sorted with respect to each other, but not with respect to the outgroup (D. p2), ingroup monophyly is incorrectly inferred but the resulting tree is symmetrical and does not contribute to admixture inference.

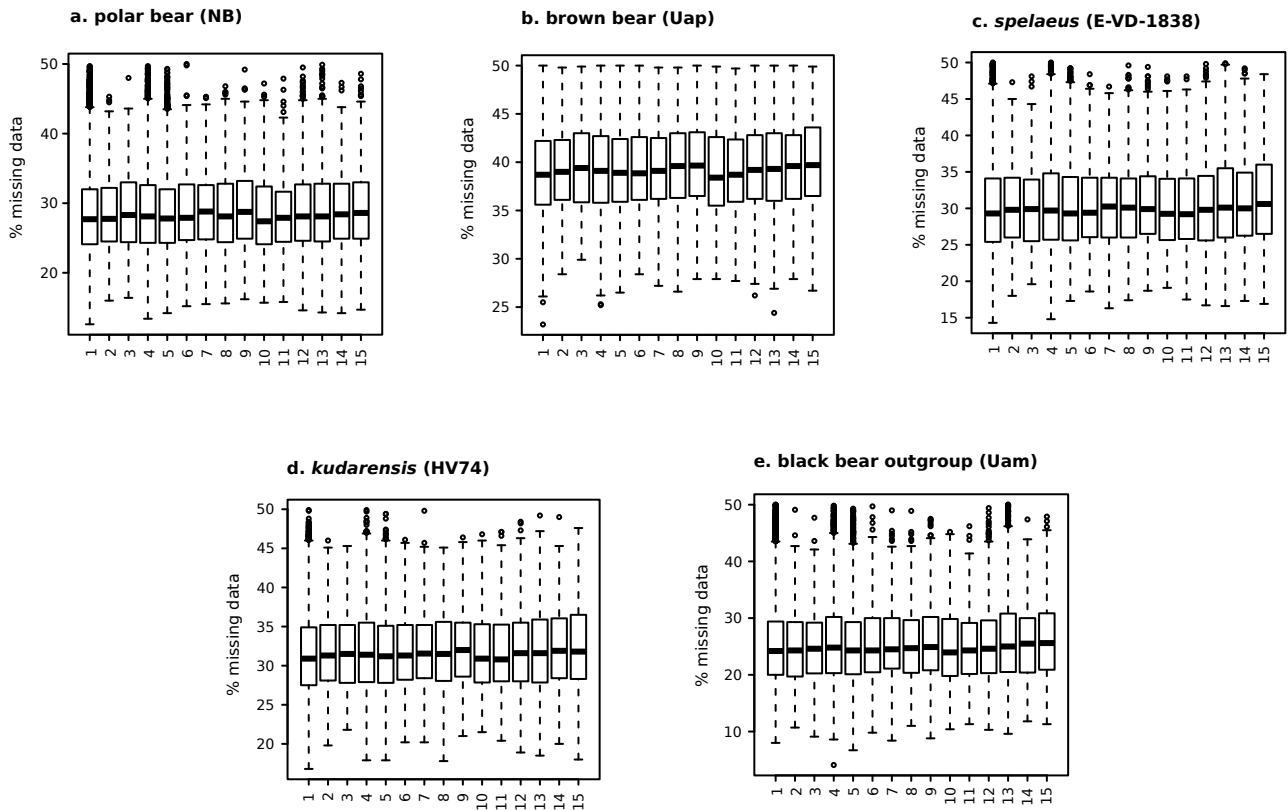
a

| Topology | Description | Tree | Admiralty (Admiralty) | Alaska (Denali) | Russia | Slovenia | Sweden | Caucasus | Spain | Austria Pleistocene |
|-------------------------------|--------------|--------------------------------|-----------------------|-----------------|--------|----------|--------|----------|-------|---------------------|
| 1 | species tree | ((Polar,Brown)(Cave-,Cave+)) | 14625 | 14393 | 14073 | 13353 | 13810 | 13816 | 13428 | 11213 |
| 2 | symmetrical | ((Cave-,Brown)(Polar,Cave+)) | 130 | 119 | 135 | 139 | 139 | 139 | 129 | 142 |
| 3 | symmetrical | ((Cave+,Brown)(Polar,Cave-)) | 154 | 153 | 157 | 166 | 176 | 149 | 183 | 174 |
| 4 | Class 1 | ((((Cave-,Cave+),Brown),Polar) | 2672 | 2716 | 2862 | 2885 | 2946 | 3116 | 2994 | 2978 |
| 5 | Class 1 | ((((Cave-,Brown),Cave+),Polar) | 188 | 221 | 214 | 245 | 279 | 263 | 264 | 217 |
| 6 | Class 1 | ((((Cave+,Brown),Cave-),Polar) | 321 | 350 | 335 | 425 | 403 | 428 | 489 | 434 |
| 7 | Class 2 | ((((Cave-,Cave+),Polar),Brown) | 2110 | 2259 | 2406 | 2310 | 2342 | 2407 | 2335 | 2271 |
| 8 | Class 2 | ((((Cave-,Polar),Cave+),Brown) | 184 | 207 | 208 | 221 | 201 | 231 | 209 | 220 |
| 9 | Class 2 | ((((Polar,Cave+),Cave-),Brown) | 205 | 192 | 238 | 223 | 212 | 236 | 225 | 217 |
| 10 | Class 3 | ((((Polar,Brown),Cave+),Cave-) | 1610 | 1587 | 1513 | 1491 | 1550 | 1532 | 1551 | 1206 |
| 11 | Class 3 | ((((Polar,Cave+),Brown),Cave-) | 146 | 145 | 160 | 169 | 160 | 176 | 142 | 166 |
| 12 | Class 3 | ((((Cave+,Brown),Polar),Cave-) | 192 | 181 | 191 | 194 | 201 | 208 | 192 | 202 |
| 13 | Class 4 | ((((Polar,Brown),Cave-),Cave+) | 1284 | 1260 | 1232 | 1215 | 1182 | 1235 | 1176 | 936 |
| 14 | Class 4 | ((((Cave-,Polar),Brown),Cave+) | 183 | 194 | 180 | 199 | 194 | 183 | 186 | 175 |
| 15 | Class 4 | ((((Cave-,Brown),Polar),Cave+) | 225 | 212 | 209 | 215 | 225 | 206 | 227 | 235 |
| Total number of blocks | | | 24229 | 24189 | 24113 | 23450 | 24020 | 24325 | 23730 | 20786 |

b



Supplementary Figure 9. a. Results of phylogenetic admixture tests after filtering for only those blocks where the ingroup is monophyletic with respect to the assumed outgroup (Asiatic black bear). b. Bar charts showing the genomic representation of each topology for each investigated brown bear. Annotation is identical to Supplementary Figure 4, which shows results for the complete dataset. Although absolute topology counts are reduced in the filtered dataset compared to the complete dataset, relative abundances are highly consistent and support identical admixture inferences.



Supplementary Figure 10. Effect of missing data on the phylogenetic test for admixture. Shown are results for the test involving the Late Pleistocene brown bear (Uap). This dataset has far lower coverage than for any modern brown bear, and is therefore most sensitive to any potential effect of missing data. Panels a-e. are boxplots showing the amounts of missing data across 25Kbp genomic blocks returning each of the 15 possible rooted tree topologies (x-axis, numbering corresponds with Supplementary Figure 4), for each of the five individuals used in the test (panels a-e., respectively). Specific tree topologies are not associated with any obvious increase or decrease in missing data for any of the five individuals, indicating that the inferred admixture patterns are not driven by sequencing coverage.

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