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Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears

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Abstract

Polar bears are an arctic, marine adapted species that is closely related to brown bears. Genome analyses have shown that polar bears are distinct and genetically homogeneous in comparison to brown bears. However, these analyses have also revealed a remarkable episode of polar bear gene flow into the population of brown bears that colonized the Admiralty, Baranof and Chichagof islands (ABC islands) of Alaska. Here, we present an analysis of data from a large panel of polar bear and brown bear genomes that includes brown bears from the ABC islands, the Alaskan mainland and Europe. Our results provide clear evidence that gene flow between the two species had a geographically wide impact, with polar bear DNA found within the genomes of brown bears living both on the ABC islands and in the Alaskan mainland. Intriguingly, while brown bear genomes contain up to 8.8% polar bear ancestry, polar bear genomes appear to be devoid of brown bear ancestry, suggesting the presence of a barrier to gene flow in that direction.

Keywords: brown bear, ecological genetics, genomics, hybridization, polar bear, *Ursus*

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Introduction

Polar bears (*Ursus maritimus*) have evolved numerous morphological, behavioural and physiological specializations for their arctic habitat, including white coat colour, a reduced hibernation regime, and a strictly carnivorous diet with corresponding changes in tooth morphology and cranial structure (Sacco & Van Valkenburgh 2004; Slater *et al.* 2010). These adaptations distinguish polar bears from their closely related sister taxon, brown bears (*U. arctos*), who have a far more diverse morphology, ecology and geographic range than do polar bears. Brown bears vary widely in size, coloration and diet regimes that range from primarily herbivorous to populations that are largely dependent on salmon. The historical range of brown bears includes most of northern Eurasia and western North America, while polar bears are found in the continental shelf sea ice regions of the north Arctic (Fig. 1).

Despite the substantial morphological, behavioural and ecological differences between brown bears and polar bears, the two species share a very close evolutionary relationship. Precisely how close, however, remains a subject of substantial ongoing debate. At many loci, lineages have not yet sorted between the two species (Hailer *et al.* 2012, 2013; Cahill *et al.* 2013), which complicates estimates of when the two species diverged. In addition, polar bears and brown bears produce viable and fertile hybrids both in the wild and in captivity (Preuß *et al.* 2009; Stirling 2011), suggesting a recent divergence between the two lineages.

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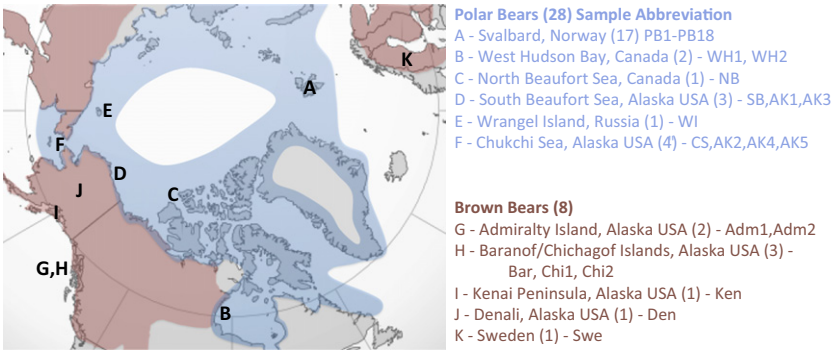

Fig. 1 Sample Map. Map of the present-day geographic range of brown bears (red) and polar bears (blue). Letters indicate location from which bears were sampled.

Published estimates of the time of divergence between brown bears and polar bears using genetic data range from 340 thousand to 4–5 million years ago (Hailer *et al.* 2012; Miller *et al.* 2012; Cahill *et al.* 2013; Cronin *et al.* 2014; Liu *et al.* 2014). Not all of these estimates are directly comparable, however. Divergence estimates based on the molecular clock assume limited or no gene flow and range from ~600 thousand to 3 million years ago, depending largely on how the molecular clock is calibrated (Hailer *et al.* 2012; Cahill *et al.* 2013; Cronin *et al.* 2014). Importantly, these estimates are genomic divergence times and not population divergence times and will therefore predate the origin of polar bears as a distinct lineage.

Two studies, Miller *et al.* 2012 and Liu *et al.* 2014; have also attempted to estimate the time of population divergence from whole genome data using population-modelling frameworks. Liu *et al.* estimated population divergence to have occurred 343–479 thousand years ago with postdivergence gene flow from polar bears to brown bears (Liu *et al.* 2014). Miller *et al.* estimated a much earlier 4–5 million years ago divergence followed by bidirectional postdivergence gene flow (Miller *et al.* 2012). Given the greater concordance of the 343–479 thousand years ago population divergence with other genetic divergence estimates it appears to be a better supported.

Analyses of mitochondrial DNA further complicated interpretation of polar bear and brown bear history. Most brown bear mitochondrial haplotypes show strikingly strong geographic structure, likely resulting from female philopatry (Korsten *et al.* 2009; Davison *et al.* 2011; Edwards *et al.* 2011). However, the first genetic studies of bear mitochondrial DNA identified a strange exception to this rule: polar bear mitochondrial haplotypes fall within the range of diversity of brown bear mitochondrial haplotypes, rather than outside of brown bear mitochondrial diversity, as would be expected of separate species (Cronin *et al.* 1991; Talbot & Shields 1996; Waits *et al.* 1998; Lindqvist *et al.* 2010). The brown bear mitochondrial lineage that is most closely related to those of polar bears is found today only on Alaska's ABC islands (Fig. 1 Locations G and H). In fact, these brown bear mitochondria are more similar to the mitochondria of polar bears than they are to other brown bear mitochondrial lineages. This finding led to early speculation that the ABC islands population was a very ancient population of brown bears and therefore the most closely related population to polar bears (Talbot & Shields 1996).

Later surveys of bear mitochondrial DNA included geographically diverse samples of both living brown bears and extinct populations (Leonard *et al.* 2000; Barnes *et al.* 2002; Valdiosera *et al.* 2007; Edwards *et al.* 2011). These analyses further complicated the scenario by revealing three geographically and temporally distinct brown bear populations that had mitochondrial haplotypes that were very similar to those of polar bears (hereafter the brown/polar mtDNA clade). In addition to the ABC islands population, this includes an extinct population that lived on present-day Ireland until around 9000 years ago (Edwards *et al.* 2011) and another that lived in Pleistocene Beringia – the once-contiguous landmass that connected Alaska to north-eastern Siberia – more than 50 000 years ago (Barnes *et al.* 2002). These findings led to further speculation that the geographic distribution of mitochondrial haplotypes in the brown/polar mitochondrial clade was due not to long-term evolution, but instead to multiple instances of hybridization, during which the mitochondrial lineage was passed between the two species, eventually leading to the geographic pattern of the present day (Edwards *et al.* 2011).

More recently, analyses of nuclear genomic and Y chromosome data have provided additional insights into the evolutionary relationship between brown and polar bears. Consensus nuclear DNA phylogenies (Hailer *et al.* 2012; Cahill *et al.* 2013; Bidon *et al.* 2014; Cronin *et al.* 2014) and Y chromosome phylogenies (Bidon *et al.* 2014) indicate that, on average, polar bears and brown bears form two distinct lineages. However, many nuclear loci and the mitochondria deviate from this

pattern (Hailer *et al.* 2012, 2013; Cahill *et al.* 2013). This deviation from the average species tree topology is most likely due to the effects of incomplete lineage sorting and, possibly, admixture.

Nuclear genomic data also reveal a remarkable difference in the amount of diversity within the two bear lineages. Mirroring their respective levels of ecological and biological diversity, polar bears are much more genetically homogenous than are brown bears (Miller *et al.* 2012; Cahill *et al.* 2013). Two polar bear individuals differ at only about 0.03% of sites, while two brown bears from Alaska differed at 0.16% of sites (Cahill *et al.* 2013). On average, a polar bear differs from a brown bear at approximately 0.24% of sites in the genome (Cahill *et al.* 2013).

We recently proposed that the ABC islands brown bear population descends from an admixture event with polar bears (Cahill *et al.* 2013). We observed that ABC islands brown bears show evidence of increased polar bear ancestry relative to other brown bear populations throughout their autosomal genomes. However, polar bear ancestry is further elevated on ABC island brown bear X chromosomes compared to their autosomes (Cahill *et al.* 2013), and they contain mitochondria that are more similar to those of polar bears than to other brown bears.

These genetic observations and the natural history of the ABC islands are consistent with a model wherein ABC islands brown bears are the descendants of an original population of polar bears. Immigration of primarily or exclusively male brown bears gradually converted the phenotype and genotype of the ABC islands bears into those of brown bears. This male-biased gene flow did not convert the strictly maternal mitochondrial DNA and was less pronounced in converting X chromosome loci, but completely converted the paternal Y chromosome. This result overturns the previous hypothesis that polar bears received their mitochondrial haplotype via introgression from a population of brown bears closely related to the ABC island brown bears or ancient Irish brown bears (Edwards *et al.* 2011). Rather, the mitochondrial haplotype found in polar bears is of polar bear origin. Under this scenario, brown bears in the brown/polar mtDNA clade are the recipients of introgression from polar bears.

Previously, we measured the impact of admixture on the nuclear genomes of ABC bears using the *D*-statistic test for admixture (Green *et al.* 2010; Durand *et al.* 2011). We showed that a brown bear from the ABC islands carried more polar bear matching alleles than a brown bear from Denali National Park. While the result was statistically well supported, this framework – using a single, non-ABC islands brown bear for comparison – lacks the power to assess whether the mainland brown bear is devoid of polar bear ancestry or simply has less polar bear ancestry than does the ABC islands bear. We were therefore unable to explore the geographic extent to which this admixture event affected bear populations or to explore hypotheses about frequency of admixture between the two bear lineages. Recently, a study calculated *D*-statistics suggesting that other ABC islands brown bears and a brown bear from Glacier National Park in Montana, USA, possessed polar bear ancestry (Liu *et al.* 2014).

Here, we test the extent of polar bear ancestry within and beyond the ABC islands population by analysing genomewide data from a more diverse panel of brown bears (Fig. 1). Comparisons between bears in this larger panel reveal a more widespread pattern of polar bear admixture into brown bears. We find evidence of polar bear admixture in ABC islands brown bears and in brown bears from the Alaskan mainland. Within the ABC islands, we identify a geographic cline of admixture, with more retained polar bear ancestry in bears further from the mainland. Finally, we find no evidence of gene flow from brown bears into polar bears, in stark contrast to the widespread signal of gene flow in the other direction.

Methods

Assembling a large panel of brown bear and polar bear genome sequences

Previous analysis indicated that the genomes of ABC islands brown bears contain more polar bear ancestry than do those of mainland Alaskan brown bears. However, this analysis was performed using only a single genome representing each population. To more fully explore the spatial distribution of the signal of polar bear ancestry within the ABC islands, we collected a larger panel of brown and polar bear genomes. We sequenced three previously unpublished brown bears one from Sweden and two from Chichagof Island. We analysed these samples along with samples from two previously published data sets; two brown bears, seven polar bears and an American black bear published in Cahill *et al.* 2013 (Cahill *et al.* 2013) and three brown bears and twenty-three polar bears published in Miller *et al.* 2012 (Miller *et al.* 2012) (Fig. 1, Table S1, Supporting Information).

For many bear genomes, the depth of coverage was insufficient to call heterozygote sites reliably. We therefore used the strategy described previously (Green *et al.* 2010; Cahill *et al.* 2013) to sample one high-quality base at each genomic position from each bear, thereby creating a pseudo-haploid sequence. Two of the polar bear samples were unsuitable for this approach, and we

excluded these from further analysis (Figs S1 and S2, Supporting Information). As described previously, we partitioned the polar bear genome assembly (Li *et al.* 2011) to which all sequence data were mapped into scaffolds that are likely to be on autosomes and those likely to be X chromosomes (Cahill *et al.* 2013). No Y chromosome scaffolds were found to meet our minimum scaffold length filtering criteria. To infer the history of the Y chromosome, we therefore assessed separately only the largest Y chromosome scaffold (Supporting Information).

DNA extraction, library preparation and sequencing

We extracted DNA from the Chichagof Island brown bears and the Swedish brown bear using the DNeasy Blood & Tissue Kit (Qiagen) and the QIAmp Micro Kit (Qiagen) according to the manufacturer's specifications. We physically sheared the DNA of the three new brown bear samples using a Diagenode Bioruptor NGS instrument. Extracts were transferred into 1.5-mL tubes and exposed to seven rounds of sonication, using the energy setting 'HIGH' and an 'ON/OFF interval' of 30/30 s. We then purified and concentrated the extracts using the Agencourt AMPure XP PCR purification kit, according to manufacturer's instructions, and eluted in 20 μL of 1xTE, with 0.05% Tween20.

We prepared indexed Illumina libraries using 15 μL of each extract following the protocol described by Meyer & Kircher (Meyer & Kircher 2010), with reaction volumes scaled to total volume of 40 μL. To verify final DNA concentration and the distribution of insert sizes, we ran each library on an Agilent 2100 Bioanalyzer. We then sequenced each bear on half of a lane of an Illumina HiSeq 2000 instrument using 150-base pair (bp) paired-end chemistry at the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley.

Quality control, mapping and pseudo-haploidization

From the Illumina sequence data, we removed the index and adapter sequence and merged paired reads using a script provided by M. Kircher (Kircher 2012). We then trimmed each read to remove low-quality bases by trimming inward from the 3′ end of the read until detecting a base with quality score ≥13 (~95% confidence) (Lohse *et al.* 2012). We mapped the resulting data to the draft polar bear genome (Li *et al.* 2011) using BWA (Li & Durbin 2010). We removed duplicated reads created by PCR amplification using the rmdup program from samtools (Li *et al.* 2009).

For all individuals at each position in the genome, we chose a random, single allele from among reads that passed the following filtering criteria: (i) read map quality Phred-30 or greater; (ii) Illumina base quality Phred-30 or greater; (iii) genomic position is within a uniquely mappable 35-mers identified using GEM (Derrien *et al.* 2012); (iv) read coverage at that position is between the 5th and 95th percentiles genomewide identified using BEDTools coverageBed (Quinlan & Hall 2010); and (v) discarding scaffolds <1 MB in length. This approach is designed to have uniform power to detect rare alleles at all mappable positions in all individuals. In contrast, using a genotyping inference programme to call heterozygous sites would have greater power to detect rare, nonreference alleles in higher coverage individuals. This would, however, confound downstream analysis. The result of our approach is that a single base call, randomly selected from among the mapped reads, represents the individual at every site in the reference genome. Because bears are diploid, this single base call necessarily only represents one of the two alleles at sites where the bear is heterozygous.

Detecting admixture

We used the *D*-statistic framework (Green *et al.* 2010; Durand *et al.* 2011) to measure the relative amounts of polar bear ancestry between pairs of brown bears. The *D*-statistic is a comparison between four individuals: two conspecific individuals, P1 and P2, a candidate introgressor, P3, and an out-group, O. At each site in the genome, we test whether the relationship between these four individuals is inconsistent with the species tree topology. Sites that are considered inconsistent with the species tree are those at which P2 shares a derived allele with P3 but not P1 (ABBA sites) or sites where P1 shares a derived allele with P3 but not P2 (BABA sites). In the absence of ancestral population structure, processes other than admixture that produce loci inconsistent with the species tree, such as incomplete lineage sorting and error, are expected to produce an equal number of ABBA and BABA sites (Green *et al.* 2010; Durand *et al.* 2011). An excess of either ABBA or BABA sites is evidence of admixture. In this framework, admixture between P1 and P3, for example, is expected to produce an excess of BABA sites compared to ABBA sites. As described previously, we used the black bear genome to determine the ancestral state at each polymorphic genomic position (Cahill *et al.* 2013).

We performed analyses for all combinations of pairs of conspecific individuals and candidate admixers. This amounted to 720 comparisons for eight brown bears with 28 candidate introgressor polar bears (Table 1; Fig. 2; Table S2, Supporting Information) and 3360 comparisons for 28 polar bears with eight candidate introgressor brown bears (Table S3, Supporting Information). For separate analysis of the X chromosome, we used

Table 1 Polar bear ancestry in brown bear autosomes

	P2			
P1	Sweden	Kenai	Denali	% Polar Bear
Adm1	0.1258 (12.8)	0.0685 (5.9)	0.0160 (1.3)	5.99 (12.2)
Adm2	0.1231 (12.2)	0.0669 (6.1)	0.0139 (1.1)	5.88 (11.7)
Bar	0.1613 (14.7)	0.1091 (8.9)	0.0573 (4.3)	7.82 (13.9)
Chi1	0.1786 (17.7)	0.1278 (11.3)	0.0777 (6.4)	8.68 (16.0)
Chi2	0.1819 (18.3)	0.1323 (12.1)	0.0819 (6.7)	8.83 (16.5)
Den	0.1267 (14.3)	0.0571 (5.6)	N/A	5.38 (12.7)
Ken	0.0719 (9.6)	N/A	−0.0571 (5.6)	3.17 (9.2)

Average autosomal *D*-statistic values reflecting the amount of polar bear ancestry in each brown bear (P1) that results from tests in which the Swedish, Kenai or Denali brown bears (P2) are used as the polar bear-free baseline. For each *D*-statistic reported, the corresponding *Z* score (Green *et al.* 2010; Durand *et al.* 2011), estimated using a weighted block jackknife approach with 5 MB blocks (Green *et al.* 2010; Cahill *et al.* 2013, Materials and Methods), is indicated in parentheses. The final column shows the average proportion of polar bear ancestry in each brown bear autosomal genome ($\hat{f}$ estimator) and corresponding *Z* score. A summary of all *D*-statistic comparisons performed in this study is provided in Table S2 in Supporting Information.

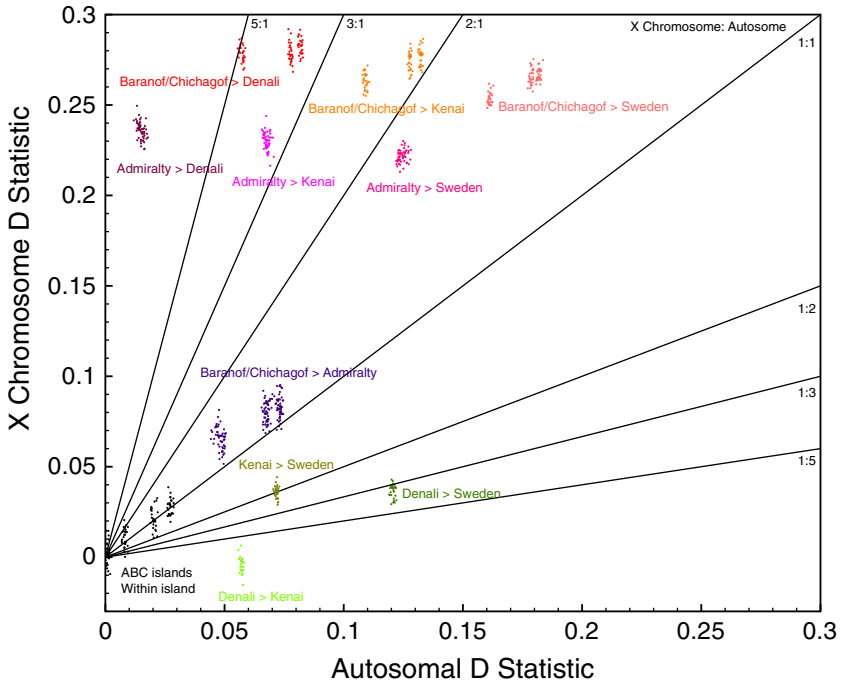

Fig. 2 *D*-statistic measure of admixture in brown bears. Distribution of *D*-statistic tests between two brown bears and a polar bear candidate introgressor with an American black bear out-group. Each dot represents an independent test with a different polar bear as the candidate introgressor. ABC islands bears, particularly those from Baranof and Chichagof islands, show the highest amount of polar bear introgression. Admiralty Island brown bears show the greatest bias toward polar bear ancestry on the X chromosome vs. the autosomes. The Denali brown bear shows the greatest bias toward polar bear ancestry on the autosomes relative to the X chromosome.

the X chromosome polar bear genome scaffolds identified in our previous study (Cahill *et al.* 2013). To be classified as an X chromosome scaffold, a scaffold must meet two criteria: differences in male vs. female shotgun sequence coverage and the presence of orthologs to X-linked genes from the dog genome (Lindblad-Toh *et al.* 2005).

The related $\hat{f}$ estimator (Green *et al.* 2010; Durand *et al.* 2011) was used to estimate the proportion of the genome derived from admixture. Ideally, this test requires two individuals of the candidate introgressor species that are not themselves admixed. This was not possible in all cases, however, because all of the brown bears except the Swedish brown bear were found to be

Table 2 Polar bear ancestry in brown bear X chromosomes

	P2			
P1	Sweden	Kenai	Denali	% Polar Bear
Adm1	0.2226 (1.9)	0.2285 (2.7)	0.2330 (4.5)	7.63 (1.8)
Adm2	0.221 (1.8)	0.2323 (2.9)	0.2388 (3.2)	7.53 (1.7)
Bar	0.2538 (1.8)	0.2632 (2.6)	0.2785 (2.7)	9.35 (1.5)
Chi1	0.2654 (2.2)	0.2736 (3.3)	0.2787 (4.0)	9.59 (1.9)
Chi2	0.2669 (2.6)	0.2769 (4.1)	0.2826 (3.9)	9.71 (2.3)
Den	0.0364 (0.4)	−0.0041 (0.1)	N/A	1.04 (0.3)
Ken	0.0360 (0.7)	N/A	0.0041 (0.1)	1.04 (0.7)

Average X chromosome *D*-statistic values reflecting the amount of polar bear ancestry in each brown bear (P1) that results from tests in which the Swedish, Kenai or Denali brown bears (P2) are used as the polar bear-free baseline. For each *D*-statistic reported, the corresponding *Z* score is reported as in Table 1. The final column shows the average proportion of polar bear ancestry in each brown bear X chromosome ($\hat{f}$ estimator) and corresponding *Z* score.

admixed with polar bears. To minimize bias, we used the two least admixed brown bears – the Swedish and Kenai individuals – to estimate the fraction of brown bear genomes that had introgressed from polar bears (Table S5, Supporting Information).

For both the *D*- and $\hat{f}$ statistics, we measure *D*-statistical significance using a weighted block jackknife using 5 Mb blocks (Green *et al*. 2010; Durand *et al*. 2011). The weighted block jackknife tests whether admixture signals are uniform across the genome and therefore reflect the same population history. The weighted block jackknife produces a standard error value. The number of standard errors by which the observed value of *D* or $\hat{f}$ differs from the null expectation of zero is the *Z* score. In keeping with Green *et al*. 2010 (Green *et al*. 2010), we define significant *D*- and $\hat{f}$ statistic results as having *Z* scores greater than three. Note that we do not perform multiple-test correction, as it is not clear how to correctly account for multiple tests in this exploratory framework in which most tests are not independent.

Results

Admixture analysis

D-statistic comparisons between pairs of brown bears from different populations revealed statistically significant differences in all comparisons (Fig. 2; Tables 1 and 2, Table S2, Supporting Information). Consistent with previous observations using only a single mainland brown bear, comparison between any ABC islands bear and any non-ABC islands brown bear showed an excess of polar bear ancestry in the ABC islands bear. Also as before, the excess of polar bear ancestry in ABC islands bears was greater on the X chromosomes than on the autosomes (Fig. 2, Table 1). The lower statistical significance of X chromosome results compared to autosomal results (Tables 1 and S2–S5, Supporting Information) is due to the much smaller size of the X chromosome and corresponding increased influence of removing 5 MB blocks.

The brown bear from Sweden had the lowest rate of matching polar bear alleles in all pairwise comparisons with other brown bears. The Kenai brown bear had the next lowest rate, with $\hat{f}$ estimated polar bear ancestry of 3.17% on the autosomes and 1.04% on the X chromosome. The Denali brown bear, which was the only mainland brown bear sample from our previous report, had the highest rate of polar bear allele matching among all non-ABC islands brown bears in this larger sample of brown bears (Fig. 2). Using $\hat{f}$, we estimate polar bear ancestry in the Denali bear to be at least 5.38% on the autosomes and 1.04% on the X chromosome.

D-statistic measurements of polar bear ancestry on the X chromosome vs. autosomes reveal a striking difference between ABC islands bears and non-ABC islands bears. The pattern of *enriched* polar bear ancestry on the X chromosome of ABC islands brown bears is *reversed* in non-ABC islands brown bears (Fig. 2, Table 1); that is, non-ABC island brown bears have less polar bear ancestry on the X chromosome relative to their autosomes.

Within the ABC islands, the brown bears from Baranof and Chichagof islands have more polar bear ancestry than the brown bears from Admiralty Island (Fig. 2, Tables 1 and 2). $\hat{f}$ estimates of polar bear ancestry in ABC islands brown bears ranges from 5.9% to 8.8% of the autosomes and 7.5 to 9.7% of the X chromosome. As noted above, all of the ABC islands brown bears' X chromosomes are enriched for polar bear ancestry compared to their autosomes. Within the ABC islands, X chromosome bias of polar bear ancestry increases as total polar bear ancestry decreases. The brown bears of

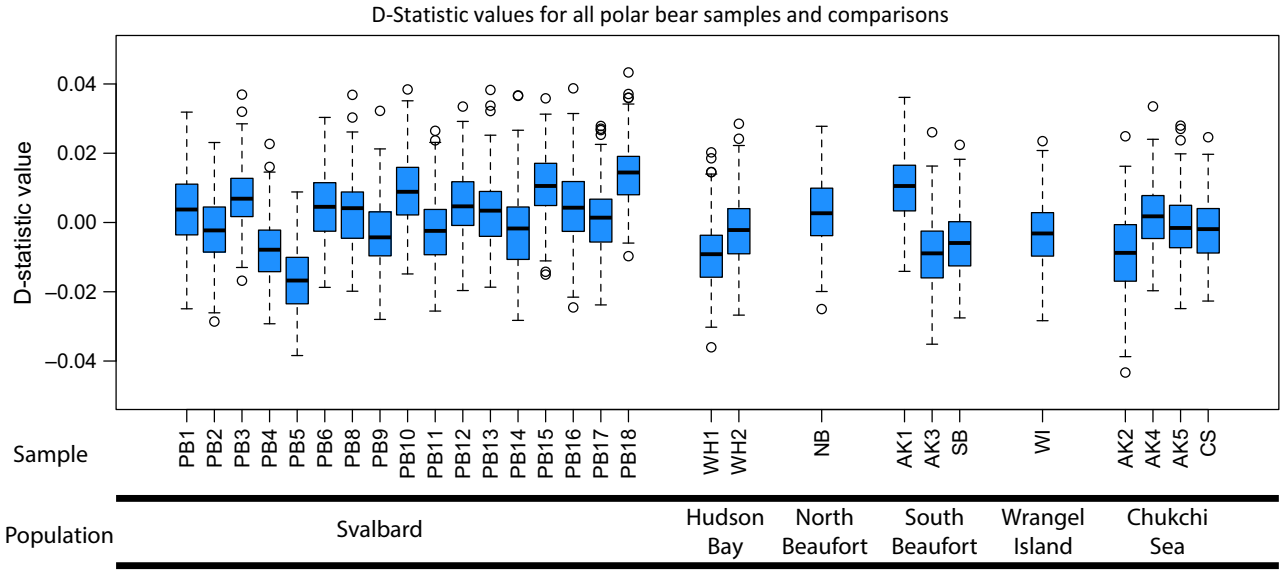

Fig. 3 *D*-statistic measure of admixture in polar bears. Box-and-whisker plots showing the range of *D*-statistic values for a single polar bear (Sample), arranged along the *x*-axis by geographic location (Population), compared to every other polar bear with every brown bear as a candidate introgressor. For each box-and-whisker-plot, boxes range from the 25th to 75th percentiles, whiskers are 1.5 times the distance from the 25th to 75th percentile, or the most extreme result if it is less than 1.5 times the distance from the 25th to 75th percentile. Circles indicate data that fall outside of 25th to 75th percentile (outliners). Statistically significant *D*-statistic values indicate that the subject polar bear shares an excess of derived alleles with brown bears. None of the comparisons, including the outliners, resulted in *D*-statistic values that differed significantly from zero ($Z > 3$).

Admiralty Island, the island closest to the mainland, have the least polar bear ancestry, and polar bear ancestry is the most X chromosome biased (Tables 1 and 2).

We also tested for a signal of admixture within polar bear genomes. *D*-statistic tests between pairs of polar bear genomes for unequal rates of matching derived brown bear alleles resulted in no *D*-statistics that differed statistically from zero (weighted block jackknife $Z > 3$) (Fig. 3; Table S3; Fig. S1, Supporting Information). $\hat{f}$ estimators also indicated an absence of detectable gene flow from brown bears into polar bears, with no comparisons deviating statistically from zero ($\hat{f}$ statistics with weighted block jackknife $Z > 3$).

Discussion

Uneven amounts of polar bear ancestry among brown bears

Our previous observations about polar bear ancestry within ABC islands bears relied on comparison to a single mainland brown bear from Denali National Park, Alaska. While the comparisons were consistently and strongly in the direction of excess polar bear ancestry in ABC islands bears, use of a single comparison genome is limiting in an important way. Quantifying the absolute amount of polar bear ancestry requires making an assumption about the amount of polar bear ancestry in the comparison brown bear. For our previous work, we made the assumption that the Denali bear was free of polar bear ancestry. Following this assumption, the excess polar bear ancestry in ABC islands brown bears was interpreted as a measure of the absolute amount of polar bear ancestry in the ABC islands brown bears.

Our new results indicate that this assumption was incorrect – the Denali brown bear is *not* free of polar bear ancestry. In fact, among the non-ABC islands brown bears analysed here, the Denali bear has the *greatest* polar bear ancestry: at least 5.38% of the autosomes and 1.04% of the X chromosome derives from polar bear. The Swedish brown bear has the least polar bear ancestry in all pairwise comparisons and thus establishes a new baseline for admixture-free brown bear.

A further unexpected result is that in contrast to the excess of polar bear ancestry on the X chromosomes relative to autosomes among ABC islands bears, we see the opposite pattern within non-ABC island brown bears; that is, these bears have lower levels of polar bear ancestry within their X chromosomes vs. their autosomes (Fig. 2).

These results have several important implications for understanding the admixture event on the ABC islands. Most importantly, we now estimate a much greater amount of polar bear ancestry in each ABC islands brown bear than previously reported. Recalculating the

absolute amount of polar bear ancestry for the five ABC islands bears using the Swedish bear as the nonadmixed standard results in higher estimated proportions of polar bear ancestry than when using the Denali bear (Table 1). Whereas we previously reported an absolute amount of 6.5% polar bear ancestry on the X chromosome and 0.5% polar bear ancestry on the autosomes of the bear from Admiralty Island, estimates based on the Swedish bear indicate that 7.6% of the X chromosomes of this bear are derived from polar bear ancestry, as is 6.0% of the autosomes. These results may explain the very high X: autosome ratio of polar bear ancestry that was estimated while using the Denali brown bear as standard, which fell outside of the distribution of ratios predicted by demographic simulation (Cahill *et al.* 2013). Because the Denali bear has a significant amount of polar bear ancestry on the autosomes, this led to an underestimate of the amount of polar bear ancestry on the autosomes of the ABC islands brown bear.

We observe a geographic signal wherein bears from the islands less accessible from the mainland – Baranof and Chichagof islands – have more polar bear ancestry than the bears of Admiralty Island (Fig. 2). A previous analysis of microsatellite data from a large sample of Alaskan brown bears similarly reported more gene flow between Admiralty Island and the Alaskan mainland than between the more distant Baranof and Chichagof islands and the Alaskan mainland, and very little gene flow between Baranof and Chichagof islands and Admiralty Island (Paetkau *et al.* 1998, 1999). These data support a model of brown bear dispersal from the Alaskan mainland that is limited mainly by long-distance water crossings (Fig. 2).

Notably, Baranof and Chichagof islands brown bears' polar bear ancestry is less X chromosome biased than Admiralty Island bears' polar bear ancestry (Fig. 2). This result is consistent with and extends the model we proposed previously wherein male-dominated gene flow from the mainland onto these islands carries brown bear genetic material into a population that was initially polar bears. More distal bears are further from the source of this gene flow and thus less impacted by migration from the mainland. In this way, the brown bears of Baranof and Chichagof islands retain more of their polar bear genetic ancestry, but exhibit comparatively less bias toward the X chromosome. Lending further support to this hypothesis of female-biased polar bear ancestry and male-biased brown bear ancestry, a recent study by Bidon and colleagues found Y chromosome ancestry on the ABC islands to be exclusively brown bear (Bidon *et al.* 2014). Similarly, we find no evidence of polar bear ancestry in any brown bears when analysing the largest Y chromosome scaffold in the polar bear assembly (Supporting Information).

Polar bear ancestry in non-ABC islands brown bears

For each of the non-ABC islands Alaskan brown bears we analysed, excess polar bear ancestry is observed on the autosomes over the X chromosome when compared to the Swedish brown bear. The opposite is observed in the ABC islands brown bears. This X chromosome *depletion* is more pronounced than the X chromosome enrichment on the ABC islands. There are multiple plausible hypotheses that could explain this result, both demographic and selective.

Demographically, brown bear dispersal is primarily male-mediated (McLellan & Reiner 1994; Støen *et al.* 2006). Populations that are located further from the site of hybridization would be expected therefore to have less polar bear ancestry on the female-biased X chromosome than on the autosomes. This behavioural process is supported by genetic evidence: while brown bear mitochondrial haplotypes, which are exclusively maternally inherited, show strong geographic structuring (Korsten *et al.* 2009; Davison *et al.* 2011; Edwards *et al.* 2011), Y chromosome haplotypes, which are exclusively paternally inherited, show no such geographic structure (Bidon *et al.* 2014). Thus, one hypothesis that is consistent with our data is that brown bears from the ABC islands, and perhaps other regions of polar bear admixture, will have their polar bear ancestry dispersed primarily by male brown bears. As males carry fewer X chromosomes than autosomes, polar bear ancestry will become increasingly less visible on the X chromosome than on autosomes as one samples brown bears farther from the site of admixture.

From a selective standpoint, it has been suggested that loci involved in hybrid incompatibility are overrepresented on the X chromosome (Masly & Presgraves 2007). This is because, in the heterogametic sex, the presence of only one copy of any incompatible allele prevents a homologous compatible allele from masking the incompatibility. In theory, this should lead to a reduction in introgressed ancestry on the X chromosome relative to the rest of the genome. Such an effect was recently observed in the case of Neandertal introgression into non-African humans, where Neandertal ancestry is almost absent on the X chromosome (Sankararaman *et al.* 2014). In that case, the authors' simulations appeared to reject a demographic explanation.

These processes are not mutually exclusive, and both biased dispersal and selection against polar bear ancestry on the X chromosome may play a role in explaining the lower polar bear ancestry on the X chromosome of non-ABC islands brown bears. At this stage, it seems likely that there is insufficient understanding of the demography of bears throughout Alaska and northern Canada to make a definitive assessment of the role of each.

Our results showing excess polar bear ancestry in every brown bear we sampled compared with the Swedish brown bear, whose own polar bear ancestry we cannot reliably estimate for the reasons noted above, suggest a higher rate of introgression of polar bear DNA into brown bear genomes than previously calculated. It may be that the epicentre of this introgression is in the ABC islands and the surrounding area. Following the initial introgression event, predominantly male migration could have then carried polar bear ancestry away from the Islands. This model is simple and not obviously contradictory to the data. However, further study will be required to refine the number, timing and geographic locations of polar bear admixture into Alaskan brown bears. One particularly intriguing possibility concerns the other brown bears that were found with a mitochondrial haplotype similar to the polar bear haplotype: some Beringian brown bears that lived more than 50 thousand years ago (Barnes *et al.* 2002) and a population of now extinct Irish brown bears (Edwards *et al.* 2011). It will be interesting to know whether these results are due to a similar process of male-mediated gene flow from brown bears into other polar bear populations in the past and, if so, whether there is any remaining polar bear ancestry from those introgression events in brown bear populations today.

Absence of detectable brown bear ancestry in polar bears

Admixture is more easily detected by the *D*-statistic when the population receiving gene flow is small (Durand *et al.* 2011). Given that the effective population size of polar bears is and likely has been small for many thousands of years (Miller *et al.* 2012), our observation that none of the 28 polar bear genomes used in this analysis have detectable brown bear ancestry is even more striking and has important implications for understanding both the relationship between the two species and for predicting the long-term consequences of hybridization.

Despite the widespread impact of admixture on brown bear genomes, the genetic data indicate no corresponding effect on polar bears. The absence of detectable gene flow into polar bears may therefore reflect an ecological barrier to admixed individuals surviving as polar bears, where any introduction of brown bear DNA into polar bears may be strongly deleterious (Schluter 2009). Within the polar bear lineage, there is evidence of powerful episodes of positive selection (Liu *et al.* 2014). One possibility is that the extremely specialized adaptations of polar bears may quickly place phenotypically intermediate hybrids at more of a disadvantage in the polar bear environment than the brown bear environment.

One simple example of this could be coat colour – a trait that would likely play a more severe role in decreasing fitness of F1 hybrids in the polar bear habitat than the brown bear habitat. Like other arctic predators hunting on snow or ice, such as arctic wolves (*Canis lupus arctos*) or arctic foxes (*Vulpes lagopus*) in winter, polar bears are uniformly white except for their eyes and nose. In contrast, hybrids have darker patches and sometimes overall coloration (Gray 1971; Stirling 2011). When a polar bear stalks a seal on the ice, it holds the head down low and walks in a straight line toward the intended prey (Stirling 1974), presumably because that minimizes contrasting dark spots that a seal may notice. An important time for polar bear feeding is late spring when the new crop of young ringed seals, with little experience with predators, is weaned (Stirling & Øristland 1995) and later in the spring, as the snow melts that covers breathing holes and birth lairs when a high proportion of seals are out on the ice basking and moulting (Kelly & Quakenbush 1990). Much of the hunting of these seals is performed by stalking (Stirling 1974). Hybrid bears with patches or darker pelage, or darker shades would be more visible to the seals and therefore less successful hunters. In contrast, variation in coloration may be less of a constraint for a hybrid bear feeding on brown bear food sources: vegetation, salmon or carrion. While this model – extreme reduction in F1 hybrid fitness in the polar bear ecological environment – is speculative and simplistic, it would suffice to explain the striking absence of brown bear genetic introgression into polar bear populations.

Another possible explanation for the observed imbalance in admixture proportions may be that brown bear DNA did introgress into polar bears, but that all polar bears have equivalent levels of brown bear ancestry. The *D*-statistic, which is a pairwise comparison method, has no power to detect admixture in this unique scenario. Such a scenario could manifest in two ways. First, all of the polar bears sampled here may have received an exactly equal amount of brown bear ancestry via introgression more recently than the time of the polar bear populations shared common ancestor. This scenario seems unlikely due to the size and geographic diversity of the panel of polar bears analysed here. Widespread brown bear into polar bear introgression might be expected to result in at least some variation in the amount of introgressed brown bear ancestry in one of these bears. Second, brown bear DNA may have introgressed into the polar bear population that was ancestral to all extant populations of polar bears. In this second scenario, all polar bears would have exactly the same brown bear ancestry, thereby masking the signal of admixture. Under this scenario, no living

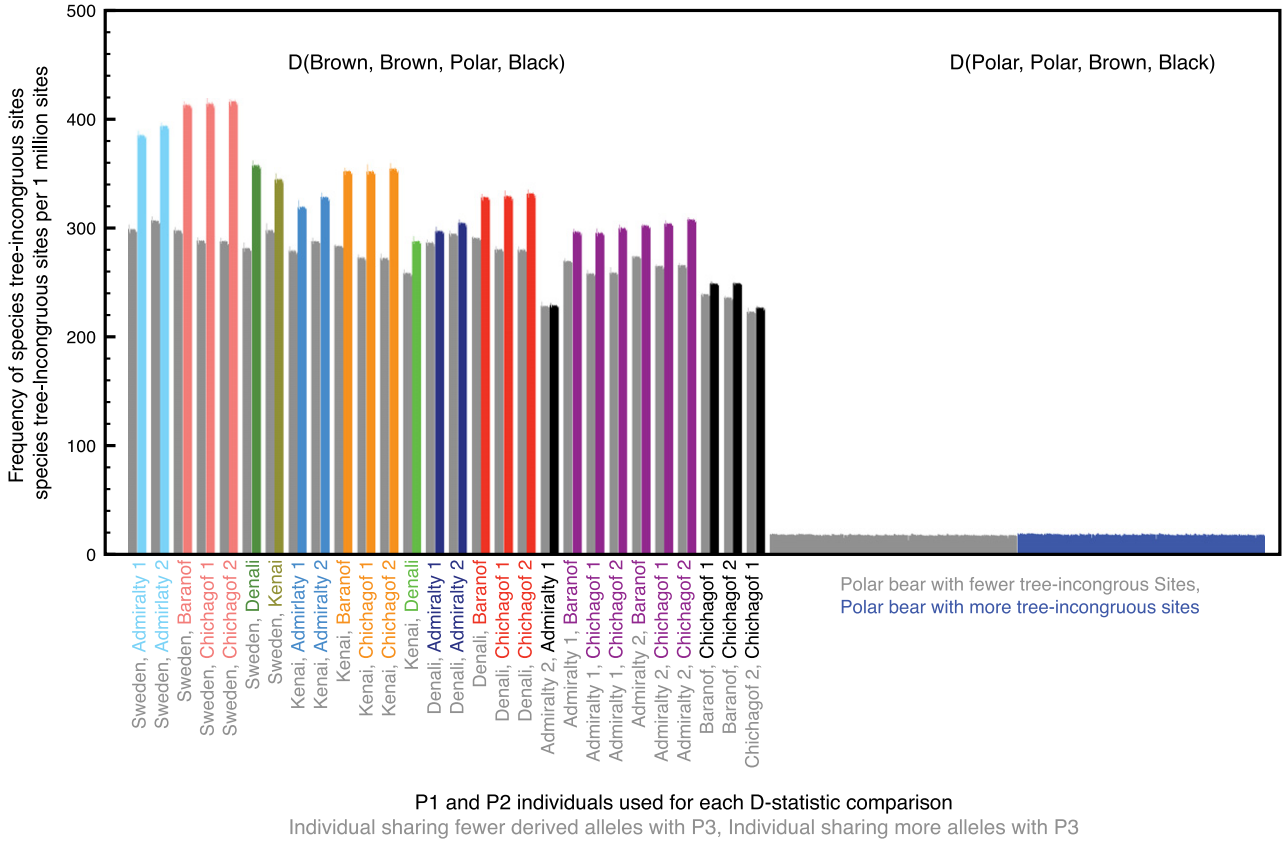

Fig. 4 Frequency of sites informative to the *D*-statistic. The frequency of ABBA sites (grey bars) and BABA sites (coloured bars) for each *D*-statistic comparison. Both ABBA and BABA sites are considered species tree-incongruent sites. Processes other than admixture, such as incomplete lineage sorting and sequencing error, are expected to produce an equal number of ABBA and BABA sites. Any difference between the number of ABBA and BABA sites – here, the difference between coloured and grey bars – is interpreted as evidence of admixture. Comparisons involving pairs of polar bears show very few tree-incongruent sites and no evidence of admixture from brown bears.

polar bear would have detectable excess brown bear ancestry.

One way to investigate this second scenario is to examine the number of *D*-statistic informative sites. *D*-statistic informative sites are incongruous with the species tree and can arise from a variety of processes including incomplete lineage sorting, sequencing errors, multiple mutations at a site and admixture. If two conspecific individuals, P1 and P2, received the same amount of introgression from P3, then the *D*-statistic for *D*(P1, P2, P3, O) would be zero. However, the number of species tree incongruous sites would be greater than if no introgression had occurred. In addition to *D*-statistics of zero, we observe very few sites that are incongruous with the species tree (Fig. 4) when comparing two polar bears for brown bear matching alleles. This suggests that the second scenario – brown bear admixture into the ancestral population of polar bears – is also unlikely.

If brown bear introgression were very ancient and took place prior to the most recent common ancestor of polar bears, then it would be impossible to detect this admixture event as all polar bears would carry these introgressed and fixed alleles. However, estimates of the timing of both the genetic time to most recent common ancestor between polar bears (130–650 thousand years ago; Cahill *et al.* 2013) and speciation between polar bears and brown bears (343–479 thousand years ago; Liu *et al.* 2014) indicate that these two events were nearly simultaneous, making this situation unlikely. While it is not possible to exclude the possibility of minimal, evenly distributed brown bear introgression into polar bears, any introgression that did occur must have been limited and makes no impact on the extant genetic diversity of polar bears.

Wider implications of asymmetric gene flow

Geneflow asymmetry is not an obvious or expected outcome of admixture. Nevertheless, evidence for asymmetric gene flow has been presented in other instances,

notably between modern humans and Neandertals (Green *et al.* 2010), between subspecies of house mouse (*Mus musculus*) (Good *et al.* 2008; Teeter *et al.* 2008) and among some Darwin's finches (Grant & Grant 2010). The asymmetric hybridization we observe between polar bears and brown bears differs in important ways from these examples. While the impact of human and Neandertal admixture was geographically widespread, it is currently not possible to know the extent of gene flow into Neandertal populations as population data from this extinct species are scarce. In any case, it is possible that the asymmetry observed thus far is due to demographic phenomenon – a growing, expanding human population entering and replacing a dwindling Neandertal population (Mellars & French 2011; Prüfer *et al.* 2014).

Hybridization between house mouse subspecies *M. m. musculus* and *M. m. domesticus* occurs in a large hybrid zone across central Europe, in which genes flow more readily from *M. m. domesticus* introgressing into *M. m. musculus* than they do in the reverse direction (Teeter *et al.* 2008; Wang *et al.* 2011; Staubach *et al.* 2012). However, unlike the strictly asymmetric gene flow from polar bears into brown bears, genes are also known to flow from *M. m. musculus* into *M. m. domesticus* (Teeter *et al.* 2008; Wang *et al.* 2011; Staubach *et al.* 2012). Thus, the asymmetry is a quantitative and not a qualitative phenomenon. Among Darwin's finches, F1 hybrids exhibit biased backcrossing into the paternal species, whichever that may be. This bias is believed to be mediated by imprinting of paternal song (Grant & Grant 1997) and in any case is not a strict barrier to gene flow in either direction (Grant *et al.* 2004).

Our observation of extreme asymmetry in gene flow between polar bears and brown bears suggests that the impacts of admixture may differ considerably among these closely related species. More generally, these results may present a new challenge to the concept of species. The model of past hybridization and gene flow that we present is consistent with a biological species definition of brown bears that includes polar bears, but *inconsistent* with a biological species definition of polar bears that includes brown bears. To our knowledge, there is no current species concept that fully accommodates an *asymmetric* definition of species.

Understanding *why* brown bear alleles do not introgress into polar bear populations may provide important insights into how polar bears survive in their extreme, arctic habitat. The consequences of this observation for conservation of polar bears are clear: polar bears have very little genetic diversity, and this pattern has persisted despite geographically widespread signals of admixture within brown bears. It seems unlikely therefore that hybridization or the paucity of genetic diversity among polar bears represents the principle threat to the long-term survival of polar bears. Rather, the rapid rate of recent climate change and consequent disappearance of their habitat (Stirling & Derocher 2012) remain the most proximate and serious threats to polar bears.

Conclusion

Hybridization between polar bears and brown bears has exerted a surprisingly large and asymmetrical influence on the genomes of polar bears and brown bears carrying polar bear genes into brown bears inhabiting a wide geographic area. Interestingly, while brown bears possess polar bear ancestry across significant portions of their genomes, brown bear ancestry appears absent from polar bears. This suggests that an as yet unidentified barrier to gene flow exists that prevents hybrid individuals from successfully backcrossing with the polar bear population. This one-way barrier to gene flow provides an interesting new framework for the study of the interactions between climate, ecology and speciation.

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J.C., R.E.G. and B.S. designed the experiment. J.C., I.S., R.E.G. and B.S. interpreted the results and wrote the manuscript. J.C. performed experiments. L.K. and M.S. generated sequencing libraries. E.E. extracted DNA from the Swedish bear. B.S. extracted DNA from the Chichagof bears. J.C. and R.S. prepared sequence data for analysis.

Data accessibility

Whole genome shotgun sequencing data produced for this study are available in the NCBI Short Read Archive as SRX795188, SRX796430 and SRX796442. Data from previously published studies are available at the NCBI Short Read Archive SRX155945–51, SRX155953–62, SRX156012–08, SRX156136, SRX156156–63, SRX265152, SRX265434–36, SRX265452–54, SRX265456, SRX265457, SRX265459.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1. *D*-statistic tests for brown bear admixture into individual polar bears.

Fig. S2. Tests for contamination of PB7 by Ken.

Fig. S3. Y chromosome pairwise difference between male bears.

Table S1. Sample collection details for each bear analyzed in this study.

Table S2. *D*-statistic results for tests of excess allele sharing between one of two brown bears and a polar bear.

Table S3. *D*-statistic results for tests of excess allele sharing between one of two polar bears and a brown bear.

Table S4. $\hat{f}$ estimates measuring the proportion of the each brown bear's genome resulting from polar bear ancestry.

Table S5. $\hat{f}$ estimates measuring the proportion of the each polar bear's genome resulting from brown bear ancestry.

Appendix S1. Methods and results indicating the unsuitability of the PB7 and LS samples for analysis by the methods described here.