

Genomic Evidence of Widespread Admixture from Polar Bears into Brown Bears during the Last Ice Age

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Abstract

Recent genomic analyses have provided substantial evidence for past periods of gene flow from polar bears (*Ursus maritimus*) into Alaskan brown bears (*Ursus arctos*), with some analyses suggesting a link between climate change and genomic introgression. However, because it has mainly been possible to sample bears from the present day, the timing, frequency, and evolutionary significance of this admixture remains unknown. Here, we analyze genomic DNA from three additional and geographically distinct brown bear populations, including two that lived temporally close to the peak of the last ice age. We find evidence of admixture in all three populations, suggesting that admixture between these species has been common in their recent evolutionary history. In addition, analyses of ten fossil bears from the now-extinct Irish population indicate that admixture peaked during the last ice age, whereas brown bear and polar bear ranges overlapped. Following this peak, the proportion of polar bear ancestry in Irish brown bears declined rapidly until their extinction. Our results support a model in which ice age climate change created geographically widespread conditions conducive to admixture between polar bears and brown bears, as is again occurring today. We postulate that this model will be informative for many admixing species pairs impacted by climate change. Our results highlight the power of paleogenomics to reveal patterns of evolutionary change that are otherwise masked in contemporary data.

Key words: genomics, hybridization, ancient DNA, ursus, paleogenomics, admixture.

Introduction

Postdivergence gene flow between species is increasingly understood to have been common in evolutionary history (Green et al. 2010; Dasmahapatra et al. 2012; Poelstra et al. 2014; Lamichhaney et al. 2015). Also known as admixture, this process most commonly occurs when two formerly geographically isolated species overlap in range and are

reproductively compatible. Genomic analyses across hybrid zones have revealed considerable variation among species pairs in both the spatial patterns and evolutionary consequences of admixture (Good et al. 2008; Dasmahapatra et al. 2012; Poelstra et al. 2014). In some cases, genomic incompatibilities lead to hybrid phenotypes that are less fit than

either parent species (Good et al. 2008). In other cases, new combinations of alleles may provide local adaptive advantages (Garroway et al. 2010). Hybridization may, therefore, be an important source of evolutionary novelty, for example, during periods of rapid climate change, when shifting habitats may form communities comprising previously isolated populations and species (Graham et al. 1996; Parmesan and Yohe 2003; Hoffmann and Sgrò 2011).

Polar bears and brown bears diverged <500 ka (Hailer et al. 2012; Cahill et al. 2013; Liu et al. 2014) but differ morphologically, physiologically, and behaviorally (Figueirido et al. 2009; Slater et al. 2010; Cahill et al. 2015; Liu et al. 2014). In recent years, whole genome sequencing has revealed that all North American brown bears derive between 3% and 8% of their genome from polar bear ancestry (Cahill et al. 2013, 2015; Liu et al. 2014). Polar bear ancestry is greatest among North American brown bears in Southeast Alaska's ABC (Admiralty, Baranof, and Chichagof) Islands (Liu et al. 2014; Cahill et al. 2015). Previously, we proposed a population conversion model of polar/brown bear admixture (Cahill et al. 2013), in which a warming climate at the end of the last ice age allowed brown bears to disperse into what had previously been polar bear range (today's ABC Islands), resulting in hybridization and the formation of a hybrid population (Cahill et al. 2013, 2015). Even after the climate stabilized during the Holocene, the ABC Islands continued to receive immigrants from the much larger and less admixed mainland population of brown bears, which gradually, further reduced the polar bear contribution in the population to the 6–8% observed in ABC Islands brown bears today (Cahill et al. 2015).

An alternative hypothesis has also been proposed. Liu et al. (2014) suggested that the admixture event occurred prior to the last ice age, and in fact may have ended as long ago as 150 ka with the proportion of polar bear ancestry in ABC Islands bears remaining relatively stable since that time. These models make very different predictions about the impact of admixture on the affected population's genetic diversity. The population conversion model suggests a stronger initial impact on the population's diversity and more dynamic change in the population's introgressed ancestry fraction than the alternative model, which posits that the admixed ancestry fractions in the present are similar to the introgressed ancestry at the time admixture occurred. Both models are consistent with some features of the nuclear genomic data from present-day individuals, and the absence of a direct measurement of polar bear ancestry in the past has prevented resolution of this question.

Here, we use a paleogenomic approach to directly explore the role of climate change in facilitating admixture between brown bears (*Ursus arctos*) and polar bears (*Ursus maritimus*; fig. 1). Focusing on a now-extinct population of brown bears from Ireland, we isolated genomic DNA from ten cave-preserved bones that were morphologically and isotopically identified as brown bears (Edwards et al. 2011) and that range in age from 37.5 to 3.9 thousand calibrated years before present (cal. ka BP). This interval spans the local peak of the last ice age circa 24.7 cal. ka BP (Peters et al. 2015), when polar bears' distribution would have been most proximate to

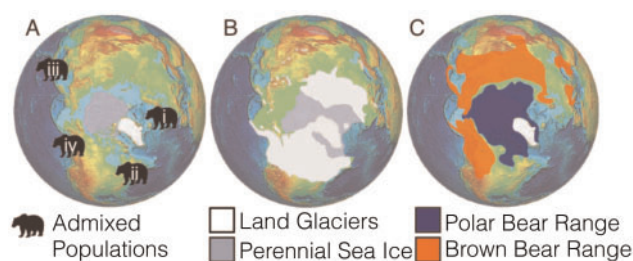


Fig. 1. (A) Geographic locations of brown bear populations identified here and in previous analyses (Cahill et al. 2013) as having some component of polar bear ancestry: (i) present-day Ireland; (ii) Chaplain Sea, Québec, Canada; (iii) Kunashir Island, Russia; (iv) Admiralty, Baranof, and Chichagof (ABC) Islands, Alaska, USA. Panel A shows the present day distribution of glaciers and sea ice. Details of samples used here are provided in table 1 and supplementary table S6, Supplementary Material online. Each of these admixed populations is located near the extent of sea and/or glacial ice at the last glacial maximum, circa 24 ka BP (Peters et al. 2015), which is depicted in panel B, but far from the present-day range of polar bears (Schliebe et al. 2008), as shown in panel C. Base image from (http://earthobservatory.nasa.gov/Features/BorealMigration/boreal_migration2.php; last accessed February 12, 2018).

present-day Ireland. Previously, mitochondrial DNA showed that some Irish brown bear fossils have polar bear-like mitochondrial haplotypes, which is consistent with admixture having occurred between polar bears and brown bears in Ireland (Edwards et al. 2011).

To explore the geographic extent of potential admixture between brown bears and polar bears, we also extracted and analyzed DNA from an 11.3 cal. ka BP (Harington et al. 2014) brown bear bone from the coast of the Champlain Sea in Québec, Canada, and from two brown bears from the present-day population of Kunashir Island, in eastern Russia (fig. 1). Both populations were located near perennial sea ice during the Last Glacial Maximum (LGM; Seki et al. 2004; Harington 2008), suggesting they may have been regions where polar bear and brown bear ranges overlapped. Interestingly, some Kunashir Island brown bears have partially white coats (Sato et al. 2011) and, while the possible reasons for this color variation are unknown, intermediate coat color is typical of polar/brown bear hybrids (Preuß et al. 2009). These samples, together with the Irish brown bears and the ABC islands brown bears, provide one population each from the east and west coasts of the Atlantic and Pacific, allowing us to test whether admixture between polar bears and brown bears was constrained to a small number of islands or was widespread throughout the Northern Temperate Zone.

Results

We used the D and \hat{f} statistics (Green et al. 2010; Durand et al. 2011), to estimate the amount of polar bear ancestry within each Irish bear genome (fig. 2, supplementary figs. S1, S2, supplementary tables S1, S2, Supplementary Material online). To assess statistical significance, we used the weighted block jackknife with 5 Mb blocks (Kunsch 1989). Z-scores are calculated by dividing the D or \hat{f} value by the weighted block jackknife standard error, and Z-scores >3 are considered

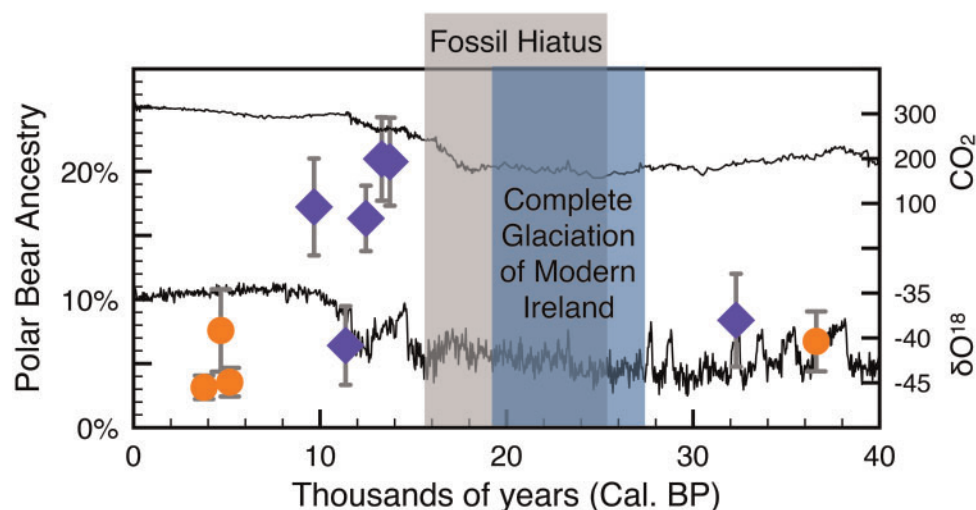


Fig. 2. The percentage of each Irish brown bear genome derived from polar bear ancestry, estimated using \hat{f} and plotted against its calibrated age (see Materials and Methods). Error bars show 95% confidence intervals estimated by weighted block jackknife (1.96 standard errors). Mitochondrial haplotype (Edwards et al. 2011) is indicated by color: polar bear like, clade 2 (blue diamonds) and brown bear like, clade 1 (orange dots). To show the correspondence between polar bear ancestry and climate, we show two climate proxies: $\delta^{18}\text{O}$ from NGRIP and CO_2 from Vostok; in both cases, values closer to the top of the figure indicate warmer temperatures. Glacial reconstructions indicate that all of modern Ireland was glaciated during the local peak of the last ice age from 27 to 19 ka (Clark et al. 2012), although radiocarbon dates indicate that some areas in the far south-east may have been ice free as late as 25 ka BP (Woodman et al. 1997). A general hiatus in the vertebrate fossil record is known in Ireland from the glacial peak until 15 ka BP (Woodman et al. 1997; Stuart et al. 2004). Brown bears occur in the Irish fossil record both before and after the glacial peak, but are absent during from 32 to 14 ka BP (Woodman et al. 1997; Edwards et al. 2011). The most recent preglacial and most ancient postglacial brown bear bones from the Irish fossil record are analyzed as part of this study.

significant evidence of admixture. We found significant polar bear ancestry in all ten Irish brown bears, ranging from 3% to 21.5% of their genomes (fig. 2, supplementary fig. S1; supplementary tables S1, S2, Supplementary Material online). Strikingly, the Irish brown bears with the largest proportion of polar bear ancestry lived temporally closest to the peak of the last ice age, with the most admixed bear, 21.5% polar bear ancestry ($Z = 11.7$), dating to circa 13 cal. ka BP. Observed polar bear ancestry in Irish brown bears declined between 13 cal. ka BP and 4 cal. ka BP (fig. 2).

We also found evidence for polar bear ancestry in the two other previously unstudied populations. The two brown bears sampled from Kunashir Island in eastern Russia have at least 4.0% ($Z = 6.1$) and 12.7% ($Z = 16.9$) polar bear ancestry (supplementary fig. S1; supplementary tables S1, S2, Supplementary Material online). Similarly, we found that the 11.3 cal. ka BP (Harington et al. 2014) brown bear bone recovered from Champlain Sea deposits in Québec, Canada (fig. 1) has at least 8.5% ($Z = 5.7$) polar bear ancestry (supplementary fig. S1; supplementary tables S1, S2, Supplementary Material online).

The D and \hat{f} statistics test for the presence of admixture but do not explicitly test whether gene flow was from polar bears into brown bears or the reverse (Green et al. 2010; Durand et al. 2011). In all three candidate hybrid populations (Ireland, Kunashir, and Québec), we tested the direction of gene flow by scanning the genome for regions where putative hybrids were excessively similar to polar bears and divergent from other brown bears. Such a pattern of diversity is expected if gene flow was from polar bear into brown bears but not if gene flow was from brown bears into polar bears

(supplementary material, Supplementary Material online, Green et al. 2010). To ascertain whether the putative hybrids were excessively enriched for these regions compared with a brown bear without detectable polar bear ancestry we compared the results from the putative hybrids to results from a Fennoscandian brown bear without detectable polar bear ancestry. Consistent with results in the previously studied ABC Islands brown bear population (Cahill et al. 2013; Liu et al. 2014), we found the candidate hybrids to be enriched for genomic regions of low polar bear divergence and high brown bear divergence relative to a Fennoscandian brown bear with no detectable polar bear ancestry (fig. 3). This provides additional support for polar bear introgression into the Ireland, Kunashir and Québec brown bear populations.

The marked decline in Irish brown bears' polar bear ancestry from 14,000 to 3,000 years ago could have been driven by: demographic processes, such as the immigration of unadmixed brown bears resulting in a decrease in the fraction of polar bear ancestry in the population; selection against polar bear alleles; or some combination of the two. The very small effective population size of polar bears (Miller et al. 2012) may have led to an overall higher genetic load compared with brown bears, which would exert selection against polar bear ancestry. To test whether selection against maladaptive polar bear alleles contributed to the postglacial decline in polar bear ancestry, we performed simulations of the impact of selection against introgressed loci due to polar bears' greater genetic load under a recently proposed population history model (Liu et al. 2014; see Materials and Methods). Our simulations show that polar bears are expected to have only a slightly higher genetic load than brown bears, resulting in a median decrease

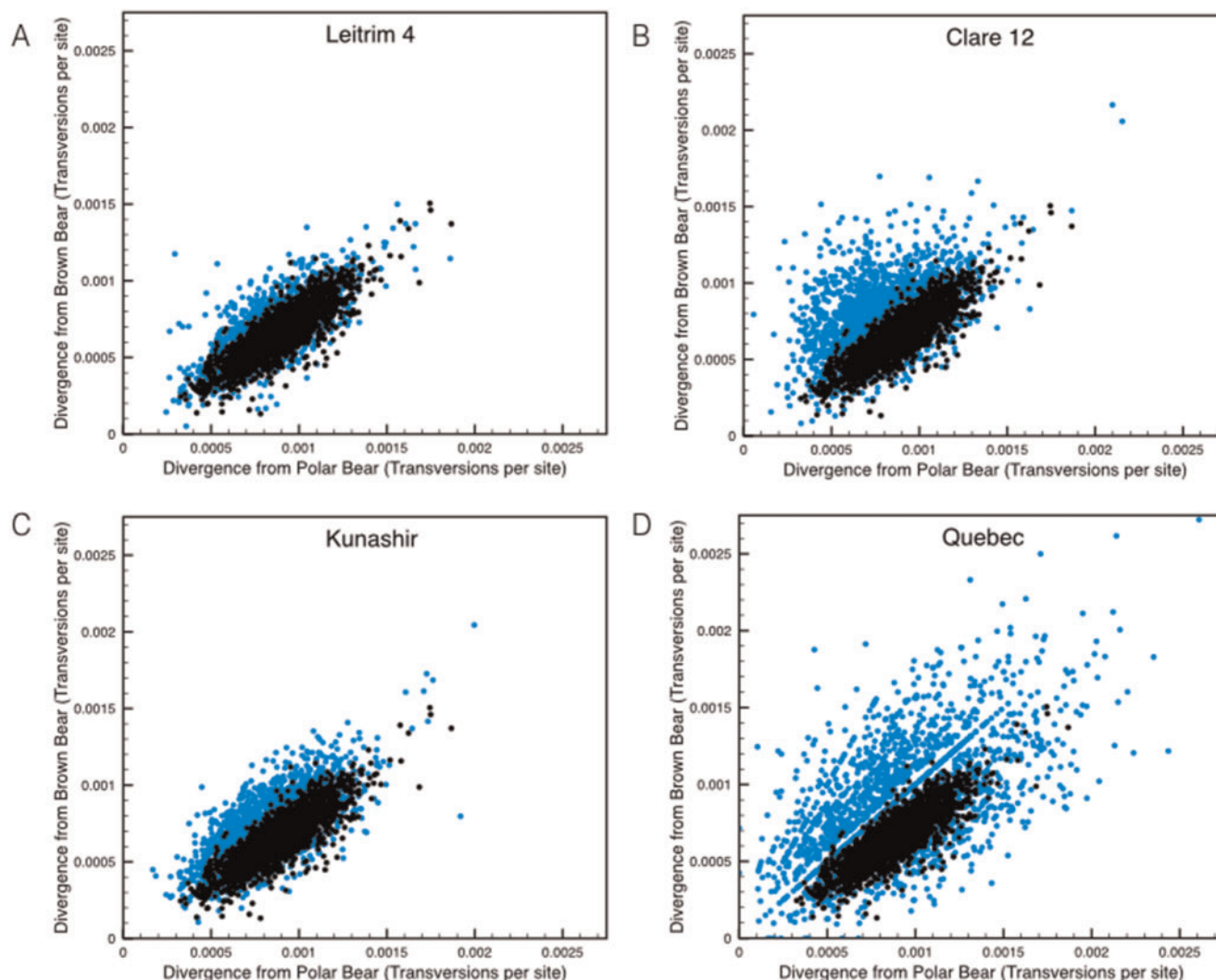


FIG. 3. Direction of gene flow. If the candidate hybrid brown bears are the recipients of introgression from polar bears, we would expect them to contain genomic regions of low polar bear divergence and higher brown bear divergence. Here, we show the distribution of divergence from polar bear and brown bear in 1 Mb bins (blue dots) for the two highest coverage Irish bears (A and B), the higher coverage Kunashir bear (C) and the Québec bear (D). We compared these to the result from the same analysis of a Finnish brown bear with no detectable polar bear ancestry (black dots). All three candidate hybrid populations have an excess of regions of lower polar bear divergence than that observed in the Finnish bear. The signal is much more pronounced in Clare-12 (B), which has the highest polar bear ancestry among these four bears. These results support brown bears as the recipients of polar bear introgression.

in fitness of 4% (supplementary fig. S3, Supplementary Material online). This resulted in a simulated decline in polar bear ancestry from a starting value of 25% to 22.4% before stabilizing (supplementary fig. S4, Supplementary Material online). The postLGM reduction in polar bear ancestry is, therefore, not primarily explained by genetic load or selection against polar bear alleles.

To examine where the samples fall within the diversity of extant bear populations we conducted a principal component analysis (PCA) of the samples (supplementary fig. S5, Supplementary Material online). We found the PCA from the high coverage individuals to mirror closely that from previous studies (Liu et al. 2014), with Principal Component 1 (PC1) broadly separating Eurasian and North American individuals. Ancient samples tended to fall toward the center of the PC space, possibly due to low data quality, but also conformed to

the continental pattern expected from PC1. Kunashir 2 is an outlier, and this, too, is probably a data quality artifact given that Kunashir 1 falls near the Irish bears. Interestingly, the distribution of postLGM Irish bears along PC2 mirrors their polar bear ancestry. However, as the ABC Islands bears' polar bear ancestry also distributes along PC2 (but in the opposite direction), simplistic interpretations of PC2 should be avoided.

Despite our selection of the best preserved Irish bear bones available for genomic analysis (table 1, supplementary table S3, Supplementary Material online), we were only able to generate low coverage genomic data sets from these bones. To determine whether the statistical analyses in this study are robust at this low coverage, we performed a simulation-based analysis to test for low coverage induced bias in D and \hat{f} statistics. Beginning with a multi-fold coverage modern

Table 1. Sample Information.

Sample ID	Museum ID	Tissue	Locality	mtDNA clade	Sample Age (Cal. yr BP)	Genomic CoverAge (×)
Leitrim-4	NMING: F21458	Tooth: molar	Poll na mBéar Cave, Glenade, Co. Leitrim, Ireland	1 (Edwards et al. 2011)	3,791 ± 50	0.2035
Sligo-5	NMING: F21439/85	Bone: metatarsal	Polldownin Cave, Co. Sligo, Ireland	1 (Edwards et al. 2011)	4,687 ± 94	0.0077
Leitrim-5	NMING: F21456/8	Bone: mandible	Poll na mBéar Cave, Glenade, Co. Leitrim, Ireland	1 (Edwards et al. 2011)	5,180 ± 93	0.1248
Limerick-10	NMING: F21749	Bone: calcaneum	Red Cellar Cave, Co. Limerick, Ireland	2 (Edwards et al. 2011)	9,702 ± 96	0.0079
Clare-11	NMING: F21752	Bone: vertebrae	Newhall Cave, Edenvale, Co. Clare, Ireland	2 (Edwards et al. 2011)	11,391 ± 125	0.0111
Clare-12	NMING: F21750	Bone: calcaneum	Newhall Cave, Edenvale, Co. Clare, Ireland	2 (Edwards et al. 2011)	12,393 ± 199	0.0527
Sligo-13	NMING: F21748	Bone: femur	Plunkett Cave, Kesh Corran, Co. Sligo, Ireland	2 (Edwards et al. 2011)	13,219 ± 105	0.0323
Sligo-14	NMING: F21119	Bone: humerus	Plunkett Cave, Kesh Corran, Co. Sligo, Ireland	2 (Edwards et al. 2011)	13,683 ± 182	0.0150
Waterford-33	NMING: F21753	Bone: astragalus	Shandon Cave, Dungarvan, Co. Waterford, Ireland	2 (Edwards et al. 2011)	33,067 ± 625	0.0071
Cork-38	NMING: F21751	Bone: humerus	Mammoth Cave, Castlepook, Co. Cork, Ireland	1 (Edwards et al. 2011)	37,970 ± 613	0.0162
Québec	MPEP 82.1	Bone: metatarsal	Saint-Nicolas, Québec, Canada	4 (Harington et al. 2014)	11,279 ± 30	0.0144
Kunashir1	N/A	Skin	Kunashir Island, Sakhalin, Russian Federation	3b	Historic (no date)	0.0718
Kunashir2	N/A	Skin	Kunashir Island, Sakhalin, Russian Federation	3b	Historic (no date)	0.0699

NOTE.—Sample information including: sample origin, age, and mitochondrial clade.

brown bear that we previously estimated to have 8.56% autosomal polar bear ancestry (Cahill et al. 2015; supplementary table S4, Supplementary Material online), we created subsampled data sets at coverage depths equivalent to what we recovered from our ancient bear samples. We ran ten simulations of 100 thousand, 1 million, and 10 million randomly sampled mapped reads under both a typical read mapping approach for modern reads and our ancient DNA optimized approach (supplementary material, Supplementary Material online). We found both methods result in overestimation of polar bear ancestry at low read numbers, but our ancient DNA optimized mapping approach mitigates this bias (fig. 4, supplementary table S4, Supplementary Material online).

This previously undocumented bias in D and \hat{f} statistic estimation should serve as a caution for future studies, in particular where admixture proportions are small. In this specific case, the proportion of polar bear ancestry tends to be overestimated at low coverage. We hypothesize that the overestimation is mainly due to biases caused by reference genome selection, because the reference genome is a polar

bear and we are testing for introgression from polar bears. These biases can, however, be mitigated by appropriate parameter selection during read mapping and by mapping to multiple reference genomes (supplementary material, Supplementary Material online; fig. 4). Although our results are therefore likely to be slightly overestimated, we note that the average overestimate of 0.48% polar bear ancestry for 1 million mapped reads (fig. 4) is much less than the total amount of observed admixture, suggesting our results are robust to this source of bias.

Discussion

We found that the proportion of polar bear ancestry in Irish brown bears was highest immediately after the local peak of the last ice age, followed by a decline toward the present day. This pattern corresponds closely to what is predicted by the population conversion model proposed to explain present-day polar bear ancestry in the brown bears of southeastern Alaska’s ABC Islands (Cahill et al. 2013). This is in contrast to the results of Liu et al (2014), who found that the length of haplotype blocks in sampled brown bears was too short to

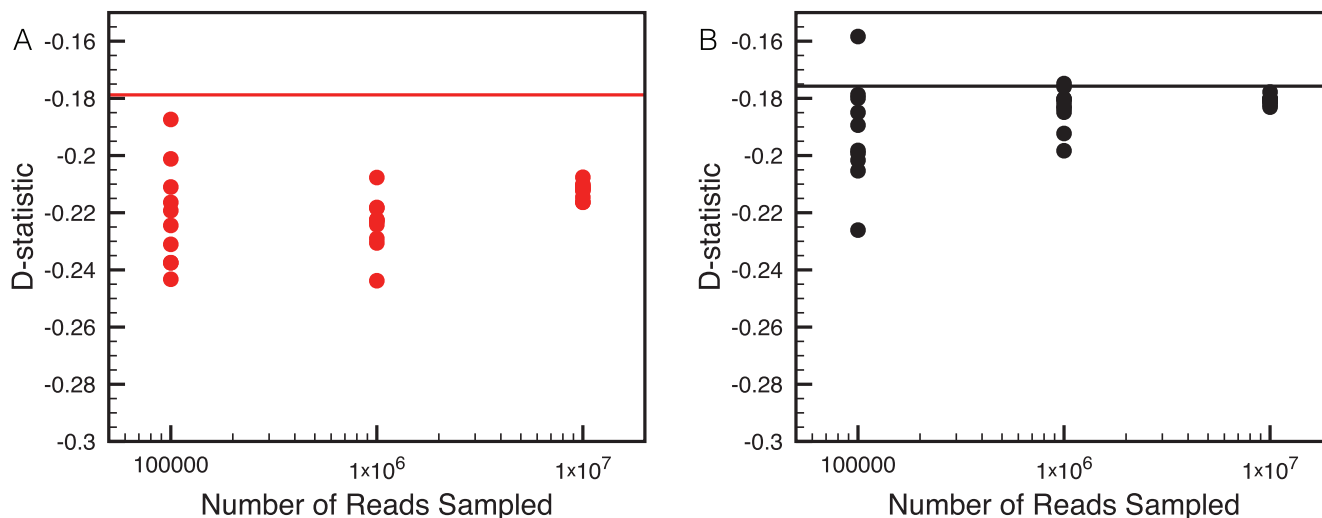


Fig. 4. *D*-statistic values from random downsampling. Here, we show the impact of decreasing the amount of available data on *D*-statistic analysis using three amounts of subsampled data (*x*-axes) and two different approaches to mapping reads to the polar bear reference genome. Dots indicate autosomal *D*-statistic values for each subsampling. The horizontal line indicates the expected value calculated from the entire data set. Using very low coverage samples can bias *D*-statistic analyses to overestimate the amount of introgressed ancestry. To test the impact of this bias on our data, we randomly sampled mapped reads from a multi-fold coverage contemporary brown bear to read counts comparable to our ancient samples (supplementary table S3, Supplementary Material online). Using BWA-aln, the traditional mapping approach used in contemporary DNA studies (Miller et al. 2012; Cahill et al. 2013, 2015), to map reads to the polar bear reference genome produces increasing overestimation of polar bear ancestry with smaller read counts (A). However, our optimized degraded DNA mapping approach results in only a very slight overestimation of polar bear ancestry (B).

support admixture having occurred within the last hundreds of generations. However, Liu et al (2014) also noted that their sample did not include brown bears from regions that were geographically proximate to polar bears, which would limit their ability to detect longer haplotype blocks, in particular if the recently admixed population did not contribute genetic material to any present-day population. The quality of the brown bear remains available in Ireland limit the precision with which we can estimate when admixture occurred. However, it seems reasonable to place the peak of admixture near to the peak of polar bear ancestry in the data, which is shortly after the LGM (fig. 2). The decline in polar bear ancestry among postLGM Irish brown bears indicates that the proportion of polar bear ancestry in this Irish population had not yet reached a stable state. Our data therefore provide strong support for the population conversion model occurring in Ireland. Although, unlike the ABC Islands where the polar bear mitochondrial haplotype is retained to the present day (Cronin et al. 1991) the later Irish brown bears have brown bear mitochondrial haplotypes (Edwards et al. 2011) consistent with a larger fraction of the immigrant brown bears being female in Ireland than in the ABC Islands. We further hypothesize that the population conversion model is operative in the ABC Islands and in other locations where the two species' ranges overlapped near the time of the LGM or overlap today.

In Ireland, we hypothesize that the observed instances of admixture between brown and polar bears were facilitated by a combination of local changes in habitat availability, lower sea levels, and species-specific natural histories. The paleoecological and fossil records of Ireland suggest that all or most of

the island was glaciated throughout much of the last ice age, leaving little to no habitat for brown bears (Clark et al. 2012; Ó Cofaigh et al. 2012; Edwards et al. 2014). At the same time, major tidewater glaciers on the western shelf and down the Irish Sea basin as well as offshore iceberg scouring of the sea floor suggest the possibility of productive sea ice habitat for polar bears along the Irish coast (Edwards et al. 2011; Clark et al. 2012; Ó Cofaigh et al. 2012; Peters et al. 2015). As resident brown bear populations declined during the approach of the LGM, this proximity in range probably led to admixture, as it can in present day populations of brown bears and polar bears whose ranges overlap (Stirling 2011). Although postLGM fossil record of bears in the region is sparse, it is thought that brown bears probably recolonized Ireland from mainland Europe or Great Britain (Edwards et al. 2014). These colonizing bears would have encountered and potentially hybridized with resident polar bears or hybrid bears. Continued dispersal of nonadmixed or less admixed brown bears into Ireland would reduce the proportion of polar bear ancestry in the Irish brown bear population, leading to the pattern observed in the Irish brown bear genomes (fig. 2).

The observation that the Kunashir and Québec populations also have polar bear ancestry provides further support for the conclusion that the ABC Islands and Ireland are part of a broader pattern of admixture and not isolated idiosyncratic events. Kunashir Island is the first Asian brown bear population shown to have polar bear ancestry. This population may include individuals with greater amounts of polar bear ancestry than the ABC Islands brown bears, as the Kunashir 2 individual's 12% polar bear ancestry exceeds the 8% that was the most polar bear ancestry previously observed in an

extant brown bear (Cahill et al. 2015). This may reflect a different demographic or selective regime in the Kunashir Islands than in the ABC Islands, which could be explored in future research. However, we suggest some caution in interpreting this result, because the Kunashir 2 value varies more than others according to the choice of bioinformatic approach, and because it is an outlier in PCA (supplementary fig. S5, Supplementary Material online). Nonetheless, all of our analyses support both Kunashir bears as having polar bear ancestry (supplementary tables S1, S2, Supplementary Material online).

Admixture between brown bears and polar bears has also been observed in the present-day Canadian Arctic (Doupé et al. 2007; Pongracz et al. 2017) and has been attributed to climate-induced overlap between the two species (Kelly et al. 2010). Together, these data reveal the ongoing and dynamic nature of gene flow between brown bears and polar bears, as well as the important role that consequent habitat redistribution plays in facilitating admixture. Intriguingly, the evolutionary consequences of this admixture appear to be mediated by ecological and behavioral differences between the two species, which maintain polar bears as a genetically distinct lineage in which brown bear introgression has not been detected (Cahill et al. 2015; Peacock et al. 2015). These results highlight the complicated nature of speciation, and suggest that *Ursus*, which includes brown bears and polar bears, may be a useful genus in which to explore the formation of incompatibilities between diverging lineages.

Conclusion

Admixture between polar bears and brown bears is geographically widespread and associated with fluctuations in climate around the last ice age and the present warming period. In Ireland, the proportion of polar bear ancestry in resident brown bears peaked after the last ice age and then declined until the population's extinction ~4 cal. ka BP (fig. 2). This pattern is consistent with the population conversion model of admixture previously suggested to explain the extant admixed population on Alaska's ABC Islands (Cahill et al. 2013). Our results do not preclude older admixture between these two lineages having occurred and, in fact, suggest that admixture is likely to have occurred whenever the ranges of these two species overlapped, which probably occurred several times since their initial divergence, coincident with glacial oscillations. The analysis of genomic data extracted directly from bones dating to the most recent ice age, however, provides clear evidence of admixture occurring during and/or near this time.

Correlation between recent climate change and admixture has been observed for several related species pairs, including trout (Muhlfeld et al. 2014), flying squirrels (Garroway et al. 2010), *Pachycladon* grasses (Becker et al. 2013), and damselflies (Sánchez-Guillén et al. 2014). Although the long-term evolutionary consequences to these species pairs are not yet known, preliminary evidence suggests a wide range of possible outcomes, from extinction via genetic replacement (Muhlfeld et al. 2014) to the creation of hybrid phenotypes

with higher fitness in the new habitat relative to the parental lineages (Becker et al. 2013). While it is tempting to consider these as localized examples, and therefore, unlikely to have widespread evolutionary consequences, introgressed DNA will, in many instances, spread to nonadmixed populations as individuals disperse. For example, introgressed polar bear DNA has been observed in mainland Alaskan brown bears, probably due to postglacial dispersal from the ABC Islands (Liu et al. 2014; Cahill et al. 2015). Thus, admixture resulting from climate-related habitat redistribution is likely to have long-term and widespread evolutionary consequences, and may be an important mechanism for generating and maintaining diversity.

Materials and Methods

DNA Extraction, Library Preparation, and Sequencing

All pre-amplification laboratory work on the ancient specimens was conducted in a dedicated clean lab facility at the UC Santa Cruz Paleogenomics Lab, following standard procedures for working with degraded DNA (Fulton 2012). We tested multiple extraction methods to optimize DNA recovery (supplementary material, Supplementary Material online). After DNA extraction, we converted the extracts into double-stranded, indexed sequencing libraries following Meyer and Kircher (2010), as modified by Heintzman et al. (2015). Then we pooled the libraries and sequenced them on the Illumina MiSeq and HiSeq 2500 platforms (supplementary material, Supplementary Material online).

Mapping and Reference Bias Correction

To identify optimal read-mapping parameters, we compared a range of parameters to an optimal read alignment (supplementary material, supplementary table S5, Supplementary Material online). This led us to select bowtie2 v2.1.0 (Langmead and Salzberg 2012), with the local alignment approach (-local flag), allowing a single mismatch allowed in the mapping seed (-N 1 flag) and a maximum mismatch penalty of 4 (-mp 4 flag) for use with the Ireland, Québec and Kunashir samples. We excluded read mappings with map quality scores <30 and removed duplicate reads with samtools v0.1.19 (Li et al. 2009). To test whether the samples exhibited patterns of cytosine deamination (C to T) damage consistent with ancient DNA and quantify the samples fragment lengths, we ran mapDamage v2.0.5 (Jónsson et al. 2013) for each Map Quality and PRC duplicate filtered bam file (supplementary figs. S6, S7, Supplementary Material online).

To describe and mitigate the impact of ascertainment bias from the reference genome, we mapped reads to the polar bear reference genome (Liu et al. 2014) and, because there is currently no brown bear de novo reference genome, to a consensus sequence of an unadmixed Swedish brown bear, SJS01 (Liu et al. 2014) which we refer to as a brown bear “pseudoreference” genome. As expected, a slightly different set of read mappings resulted from mapping to the polar bear reference compared with the brown bear pseudoreference (supplementary table S3, Supplementary Material online). Mappings to the polar bear reference results in greater

inferred polar bear introgression than mapping to the brown bear pseudoreference (supplementary fig. S1, Supplementary Material online), suggesting that both methods are somewhat biased toward their respective species. To minimize bias and capture as much of the admixed bears' genomes as possible, we combined the mappings to the polar bear and brown bear references and, for each read, retained the mapping coordinates with the highest map quality score using an in house script (supplementary material, Supplementary Material online). This two-reference approach recovered more reads than either single reference approach indicating that both references must have contributed unique mappings (supplementary table S3, Supplementary Material online) and produced intermediate estimates of polar bear ancestry (supplementary fig. S1, supplementary tables S1, S2, Supplementary Material online) which we consider to be minimally biased.

We note that the brown bear pseudoreference is less ideal than mapping to a de novo brown bear assembly. Because the brown bear genome was assembled by mapping resequencing data to the polar bear reference genome (Liu et al. 2014), this pseudoreference is likely to be somewhat biased toward polar bear. Because brown bears and polar bears are recently diverged, however, this bias is not likely to be extreme. While not ideal, comparison of the results of mapping reads to the polar bear reference and to the brown bear pseudoreference provides an additional test of the authenticity of identified polar bear introgression: reads resulting from introgression will disproportionately fail to map to the brown bear pseudoreference but not the polar bear reference, producing a signal of admixture that cannot be due to reference bias.

Finally, to test whether the giant panda might be a less biased reference we mapped each Irish bear to the giant panda reference genome (Li and Durbin 2010) using the same parameters as above. For *D*-statistic testing we mapped reads from a polar bear (WH2; Cahill et al. 2013), Swedish brown bear (Swe; Cahill et al. 2015), American black bear (Uam; Cahill et al. 2013), and spectacled bear (Kumar et al. 2017) with the bwa aln -n 0.01 method (supplementary table S5, Supplementary Material online). Because of the very low contiguity of the giant panda reference genome we decreased the minimum scaffold length from 1 Mb to 100 kb, however this still resulted in a reduction from 2.1 Gb to 1.4 Gb of analyzable sites. Using a more divergent reference genome is predicted to bias all of the samples toward that outgroup, that is, ancestral alleles will be more likely to map than derived alleles. For a given outgroup reference genome, the bias toward ancestral alleles will be greater in samples that are more difficult to map; for example ancient samples with short reads and high error rates. This in turn can potentially result in admixture underestimation in the ancient individual, as the ancient individual's excess of ancestral alleles results in a bias toward ABBA sites where the ancient sample matches the outgroup. We would expect this bias to be more severe in *D*-statistic analyses where the outgroup individual was more evolutionarily divergent.

To test whether using the giant panda reference introduces significant bias we calculated *D*(Irish brown, Swedish brown, Polar, American black) and *D*(Irish brown, Swedish brown, Polar, Spectacled) for each Irish bear and compared

the results (supplementary fig. S8, Supplementary Material online). We find a large difference between these tests (supplementary fig. S8, Supplementary Material online) indicating that the giant panda reference is introducing substantial ascertainment bias, and therefore, do not consider those results to be reflective of the actual admixture between polar bears and brown bears in Ireland.

Estimating the Proportion of Polar Bear Ancestry

We used the *D*-statistic (also known as the ABBA/BABA test) to test for the possibility of admixture between polar bears and the Ireland, Québec, and Kunashir Island brown bears (Green et al. 2010; Durand et al. 2011). For our comparisons we considered each of 30 polar bears (Miller et al. 2012; Cahill et al. 2013) and four Fennoscandian brown bears (Liu et al. 2014; Cahill et al. 2015), all of which had been previously shown to lack detectable introgressed ancestry (Liu et al. 2014; Cahill et al. 2015). To identify the ancestral state, we used an American black bear (*Ursus americanus*) and a giant panda (*Ailuropoda melanoleuca*) as outgroups. The two outgroups produced similar admixture estimates (supplementary fig. S2, Supplementary Material online) so we used the more closely related American black bear for all remaining analyses. To quantify the amount of admixture we used the *f* statistic which compares the observed derived allele sharing between a hybrid and a polar bear with the amount of derived allele sharing expected between two polar bears, conceptually a 100% introgressed hybrid (Green et al. 2010; Durand et al. 2011; supplementary material, Supplementary Material online). For both *D* and *f* we tested the impact of different reference genomes, and the inclusion or exclusion of transition sites which are susceptible to ancient DNA damage induced bias (supplementary fig. S1, Supplementary Material online; Hofreiter et al. 2001). We tested for significant evidence of admixture with the weighted block jackknife (Kunsch 1989) with 5 Mb nonoverlapping blocks, we consider *Z*-scores > 3 to be significant. We tested the direction of gene flow with the method of Green et al. (2010; fig. 3, supplementary material, Supplementary Material online), and tested for unexpected biases resulting from <1× coverage by randomly sampling reads from a high coverage modern brown bear with known polar bear ancestry (supplementary material, Supplementary Material online).

Influence of Selection against Polar Bear Alleles in Hybrids

To test whether the accumulation of weakly deleterious alleles in polar bears could be responsible for the decline in polar bear ancestry observed in the Irish brown bear population (fig. 2), we used the forward-simulation approach of Harris and Nielsen (2016), and the simulator SLiM (Messer 2013). For this analysis, we drew model parameters from a recent inference of polar bear demographic history (Liu et al. 2014; supplementary material, Supplementary Material online). To model the admixture occurring in Ireland we simulated a single 25% pulse of polar bear introgression into a brown bear population 15,000 years ago. We measured the expected difference in fitness between polar bears and brown

bears under the model at the time of the simulated admixture (supplementary fig. S3, Supplementary Material online) and the change in polar bear ancestry in the admixed population resulting from greater genetic load (supplementary fig. S4, Supplementary Material online).

PCA

As a general test of the relationship between these brown bears in this study, we conducted PCA. We calculated principal components from the divergence between well-preserved modern brown bears, using the princomp function in R. We then projected the low coverage samples onto that principal component space based on their divergence with each of the high coverage individuals.

Direction of Gene Flow

Neither the D nor the \hat{f} statistic explicitly tests for the direction of gene flow (Green et al. 2010; Durand et al. 2011). A significant D -statistic result indicates only an elevation in the frequency of shared derived alleles in conflict with the species tree expectation, it does not identify which individual was the recipient of introgression. To test whether our candidate hybrid populations were the recipients of gene flow, we tested for genomic regions in which the candidate hybrids exhibited low polar bear divergence and high brown bear divergence (Green et al. 2010). We subdivided the reference genome into 1 Mb nonoverlapping bins, and calculated for each bin the frequency of transversion differences between a candidate hybrid brown bear and nonadmixed representatives of each parent species: polar bear, SAMN01057676 (Miller et al. 2012), and Swedish brown bear, SAMN03252407 (Cahill et al. 2015). We restricted our analysis to bins with >10,000 informative sites, so as to minimize stochastic noise in the result. We compared the results for the candidate hybrids to an analysis testing for polar bear introgression into a Finnish brown bear, SAMN02256315 (Liu et al. 2014) that does not have detectable polar bear ancestry (fig. 3).

Mitochondrial Genome Haplotyping of the Kunashir Island Bears

To determine the mitochondrial haplotypes of the Kunashir Island brown bears, which were the only samples used herein that have not been typed previously, we used *mia* (<https://github.com/mpieva/mapping-iterative-assembler>; Briggs et al. 2009) to map SeqPrep-merged reads to a polar bear mitochondrial reference genome (NC_003428.1; Delisle and Strobeck 2002). We called the consensus sequence for sites with at least 3× coverage and masked all other sites. We performed a global alignment of each Kunashir mitochondrial haplotype to 39 previously published polar bear and brown bear mitochondrial haplotypes (Hirata et al. 2013), including at least one individual from each major mitochondrial clade of polar bears and brown bears, with MAFFT online version 7.245 (Katoh and Standley 2013) using default parameters. We constructed a neighbor joining tree (Saitou and Nei 1987) within MAFFT from all conserved sites (16,288 bp for Kunashir 1 and 11,533 bp for Kunashir 2) where all sequences were represented under a Jukes–Cantor mutation model

(Jukes and Cantor 1969). The Kunashir samples both fall into clade 3b, with previously published Kunashir brown bear mitochondrial sequences (Hirata et al. 2013).

Supplementary Material

Supplementary data are available at *Molecular Biology and Evolution* online.

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