ORIGINAL ARTICLE

Ancient diversification in glacial refugia leads to intraspecific diversity in a Holarctic mammal

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ABSTRACT

Aim Glacial-interglacial cycles influenced the contemporary genetic structure of many North American species. While phylogeographical lineage divergence among Pleistocene refugia has been proposed as a significant driver of subspecific and ecotypic differentiation, emerging evidence highlights the role of diversification within refugia in producing post-glacial variation. Caribou (*Rangifer tarandus*) exhibit significant morphological, ecological and behavioural phenotypic variation and occurred within Beringian and sub-Laurentide refugia. More specifically, the boreal ecotype of woodland caribou ranges from the southern regions of Canada to the Northwest Territories (NWT). Woodland caribou are generally accepted to have evolved south of the glacial extent, but the boreal ecotype in the northern part of their range co-occurs with caribou that have a Beringian origin. This proximity provides an opportunity to test whether woodland caribou colonized boreal habitats from a single southern refugial source or if independent evolution to a common ecotype resulted from diversification within refugia.

Location Northwestern Canada.

Methods We used approximate Bayesian computation to discriminate between alternate evolutionary histories of caribou belonging to boreal, northern mountain and barren-ground ecotypes using microsatellite and mtDNA markers.

Results Our analysis indicates that unlike the southern-evolved boreal ecotype, the boreal ecotype of central NWT has Beringian origins and arose from a common lineage with barren-ground and mountain caribou. Importantly, the divergence of the lineage resulting in the boreal ecotype of central NWT significantly pre-dates the Last Glacial Maximum.

Main conclusions We demonstrate that independent evolutionary trajectories can converge on a similar phenotype and for the first time show that the boreal ecotype of caribou in North America contains two phylogeographical assemblages. The ancient divergence suggests that diversification within Beringia could have resulted in ecological specialization. An eco-evolutionary focus will be essential to designing biodiversity conservation strategies for caribou that maximize genetic diversity and preserve adaptive potential in this intraspecifically diverse species.

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INTRODUCTION

Intraspecific variation is recognized as a significant driver in the establishment and function of ecological dynamics including population persistence, competition and responses to environmental change (Bolnick *et al.*, 2011). However, the evolutionary processes that lead to the development and persistence of intraspecific variation, especially for vagile species

in continuous habitats, can be difficult to identify (Fitzpatrick *et al.*, 2015; Puckett *et al.*, 2015). Glacial cycles during the Pleistocene have had a significant impact on species distributions and genetic diversity (Hewitt, 2000). In North America, vicariant divergence associated with the North American Laurentide and Cordilleran ice sheets facilitated phylogeographical lineage diversification in several species (Dyke, 2004; Weksler *et al.*, 2010 and references therein). Subsequent isolation and divergent selection pressures in the physiographic conditions of refugia are commonly considered to influence intraspecific diversification through genetic drift and adaptive evolution (Richardson *et al.*, 2014). However, recent research also points to the importance of divergence within single large refugia as a source of contemporary genetic variation and structure (Galbreath *et al.*, 2011; Lanier *et al.*, 2015).

Northern cold-adapted species experienced extensive range expansions, and in some cases increased population sizes during glacial periods (Flagstad & Røed, 2003; Lorenzen *et al.*, 2011). The extensive Beringian refugium, that stretched from eastern Siberia across the land bridge to Alaska and into the Yukon, fostered considerable genetic diversity and endemism (Weksler *et al.*, 2010; Galbreath *et al.*, 2011). Following glacial retreats, the reunification of divergent populations may have increased adaptive evolution through introgression, or alternatively, disrupted local adaptation and caused replacement or extinction of genealogical lineages (Lanier *et al.*, 2015; Klütsch *et al.*, 2016). During warm interglacial periods, the ranges of cold-adapted species contracted as viable tundra and boreal habitat were redistributed, which contrasts with the pattern of expansion out of refugia displayed by many temperate species (Stewart *et al.*, 2010). Molecular techniques provide an opportunity to reconstruct the population dynamics of cold-adapted species and predict how phylogeographical patterns influence the contemporary population structure (Stewart *et al.*, 2010; Galbreath *et al.*, 2011; Espíndola *et al.*, 2012).

In North America, caribou (*Rangifer tarandus*) persisted in both high- and low-latitude habitats over the course of the Pleistocene glaciations. The series of range oscillations and repeated demographic fluctuations associated with the expansion and retraction of continental glaciers produced conspecific populations with distinct morphological, ecological and behavioural traits (Flagstad & Røed, 2003). The diverse spatial-temporal evolutionary histories that characterize caribou have made taxonomic clarity within the species challenging and are evident in extensive intraspecific genetic structure (Serrouya *et al.*, 2012; Weckworth *et al.*, 2012; Klütsch *et al.*, 2016; Polfus *et al.*, 2016). Genetic evaluations have attributed the most pronounced intraspecific split (first formally described as subspecies by Banfield in 1961) to two distinct mitochondrial DNA (mtDNA) phylogeographical lineages that originated south of the ice sheets (North American lineage; NAL) and north of the ice sheets (Beringian–Eurasian lineage; BEL, Flagstad & Røed, 2003; Cronin *et al.*, 2005; McDevitt *et al.*, 2009; Klütsch *et al.*, 2012; Weckworth

et al., 2012; Yannic *et al.*, 2014). Finer-scale subdivisions further classify North American caribou into ecotypes based on geography and natural history traits (regardless of genealogical relationships), however, naming conventions do not always correspond between jurisdictions and ecotype identification can be ambiguous (COSEWIC, 2011; Pond *et al.*, 2016).

Woodland caribou (*R. t. caribou*) belong predominantly to the NAL and were isolated in habitats south of the Laurentide ice sheet during the Last Glacial Maximum (LGM: 26.5–19 thousand calendar years before present; kyr BP (Dyke, 2004)). Specifically, the boreal ecotype of woodland caribou are forest-dwelling animals known for their sedentary behaviour, dark pelage, large body and long legs, small group-size and low-population densities across their current range within the Canadian boreal zone (Fig. 1). The boreal ecotype is considered a Designatable Unit (DU; COSEWIC, 2011) and is listed as threatened by the Canadian Species at Risk Act as a result of population declines that are generally attributed to extensive habitat loss and fragmentation (Environment Canada, 2012).

In north-western Canada, at their northern range margin, the boreal ecotype co-occurs with barren-ground caribou (*R. t. groenlandicus*) that aggregate in large numbers to calve on the tundra and migrate to the boreal forest during the winter (Nagy *et al.*, 2011) and the northern mountain ecotype (*R. t. caribou*) that occur throughout the mountains of the Northwest Territories (NWT), northern British Columbia and Yukon Territory (COSEWIC, 2011). However, even in the face of range overlaps and known mixing between the types, recent genetic analysis has shown that in central NWT, the boreal ecotype can be differentiated (Polfus *et al.*, 2016). Likewise, indigenous Dene First Nation and Métis people of central NWT classify ţǫdzı 'boreal woodland caribou' based on identifiable physical features and behavioural traits, further supporting the boreal ecotype as a distinctive group in the northern extent of their range (Polfus *et al.*, 2016).

Interestingly, the boreal ecotype in central NWT (hereafter NWT boreal) assigns predominately to the BEL based on mitochondrial patterns (Polfus *et al.*, 2016), similar to sympatric barren-ground and northern mountain animals (Weckworth *et al.*, 2012), and unlike the boreal ecotype from southern provinces that assign to the NAL (Klütsch *et al.*, 2012). This proximity provides an ideal opportunity to test competing refugial hypotheses. If the boreal phenotype arose independently from distinct evolutionary lineages as a result of parallel phenotypic evolution, it would suggest that natural selection plays an important role in caribou intraspecific variation (Schluter *et al.*, 2004; Elmer & Meyer, 2011). In particular, ecological traits may be expected to converge in closely related genomes when certain environmental conditions strongly favour particular evolutionary outcomes (Rosenblum *et al.*, 2014). Alternatively, the boreal phenotype in central NWT may be a result of shared ancestry or historic introgression with NAL animals. Genetic drift may also be an important mechanism causing intraspecific
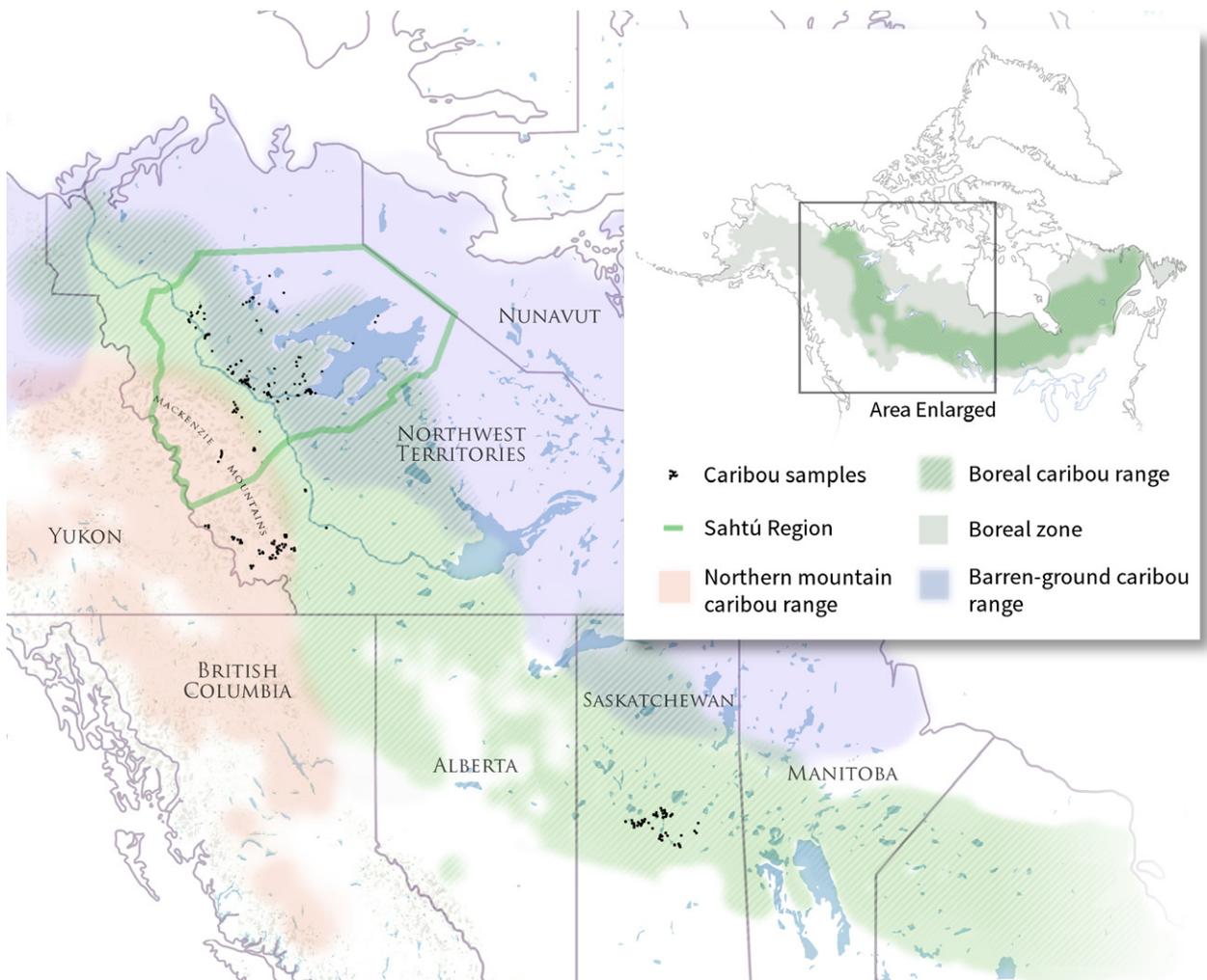
Figure 1 The range of the boreal ecotype of woodland caribou occurs within the boreal zone in Canada from the Northwest Territories to eastern Labrador (Brandt, 2009; COSEWIC, 2011; Environment Canada, 2012). Small black dots represent locations of caribou faecal, tissue and blood strip samples collected in the Mackenzie Mountains (within the range of the northern mountain ecotype), the boreal forest of the Sahtú region, central Northwest Territories (within the overlapping ranges of the boreal ecotype and barren-ground caribou) and the boreal forest of central Saskatchewan (Smoothstone–Wapeweka population of boreal ecotype).

differentiation in caribou, especially in small isolated populations (Serrouya *et al.*, 2012; Mager *et al.*, 2014).

Given signatures of significant BEL ancestry in central NWT caribou (Polfus *et al.*, 2016), our goal was to test alternative evolutionary models to assess the origin of the boreal ecotype at the northern range margin. Specifically, we tested the following two alternative hypotheses: (1) the NWT boreal ecotype diverged from the BEL and converged to a boreal phenotype within Beringia; (2) the NWT boreal ecotype represents NAL woodland caribou that subsequently colonized the northern boreal zone following retraction of the ice sheets and experienced some level of introgression from BEL caribou at the northern range margin. To discriminate between these alternate evolutionary histories, we applied approximate Bayesian computation (ABC) of nuclear and mitochondrial genetic markers in contemporary caribou populations representing the boreal ecotype in central NWT,

the barren-ground subspecies, the northern mountain ecotype, and the nearest population of boreal ecotype with NAL origins and little evidence of introgression from BEL. We also evaluated whether estimated divergence times coincided with significant glacial events. Ultimately, we tested whether the boreal ecotype of woodland caribou evolved from a single refugial lineage or independently from two refugial lineages.

MATERIALS AND METHODS

Study area and sample collection

A description of the central NWT study area and sample collection can be found in Polfus *et al.* (2016) and a description of central Saskatchewan (SK) can be found in Galpern *et al.* (2012a; Fig. 1). We assembled a dataset of caribou faecal and

tissue samples from animals belonging to four major groups: (1) barren-ground caribou from the Bluenose East and Bluenose West herds of central NWT; (2) northern mountain ecotype from the Mackenzie Mountains, NWT; and two populations of boreal ecotype from (3) central NWT and (4) the Smoothstone–Wapeweka population, SK.

Microsatellite DNA genotyping

We followed protocols for microsatellite DNA extraction, amplification and genotyping that were developed as part of a long-term caribou genetics database (Galpern *et al.*, 2012a; Klütsch *et al.*, 2012, 2016). We genotyped a panel of nine microsatellite loci (BM848, BM888, MAP2C, RT5, RT6, RT7, RT9, RT24 and RT30; Bishop *et al.*, 1994; Wilson *et al.*, 1997). We used GENEMARKER 1.9.1 (SoftGenetics, LLC) to determine allele size. Two people evaluated all electropherograms and scores were compared on an online server. We used ALLELEMATCH 2.5 (Galpern *et al.*, 2012b) to check for genotyping errors, remove duplicate profiles and identify individuals. Samples included in the final dataset had a minimum of eight successfully amplified loci.

Mitochondrial DNA sequencing

We amplified and sequenced 429 bp of the mtDNA control region using the primers L15394 and H15947 (Flagstad & Røed, 2003) following Klütsch *et al.* (2012, 2016). We used BIOEDIT 7.2.5 (Hall, 1999) to check and align sequences and DNASP 5 (Librado & Rozas, 2009) to distinguish haplotypes.

Statistical data analysis

We tested each locus and population for significant deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP 4.2 (Rousset, 2008). We used STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) to identify population clusters (K) for $K = 1$ through $K = 15$ under the admixture model with correlated allele frequencies. We conducted five iterations for each K with 1,000,000 burn-ins and 10,000,000 Markov chain Monte Carlo repetitions on a high-performance computing cluster (www.sharcnet.ca). We summarized run statistics using STRUCTURE HARVESTER 0.6.94 (Earl & vonHoldt, 2012). We used SPAGEDI 1.5 (Hardy & Vekemans, 2002) to test microsatellite pairwise differentiation.

Approximate Bayesian computation

We used ABC simulations to test competing evolutionary models. ABC analysis allows rapid tests of different scenarios by calculating summary statistics rather than exact likelihoods (Csilléry *et al.*, 2010). Deviations between the simulated and observed summary statistics are evaluated to measure fit for each model investigated (Lopes & Boessenkool, 2010). We used the software DIYABC 2.0.4 (Cornuet *et al.*, 2014) to explore whether the NWT boreal ecotype diverged from the BEL or the NAL. Alternative scenarios tested also included admixture between populations at various time-scales. We divided the evolutionary scenarios into two major groups: (1) admixture models with divergence and admixture events (Fig. 2; scenarios 1–3) and, (2) split models with no admixture events (scenarios 4–5). First, we tested a set of split scenarios to identify the most likely candidates. The top three split models were added to a series of preliminary runs that included admixture models. We narrowed down the supported models to the top five (Fig. 2) and included them in a final run to test support with three datasets: microsatellites, mtDNA and a combined dataset.

We initially set the mutation model parameters in DIYABC to a stepwise mutation model as identified by Klütsch *et al.* (2016) and then fine-tuned the parameters to the dataset. We set the prior range for the split between the two phylogeographical lineages to $t_4 = 10$–25,000 generations. The prior range of the divergence events were set to $t_3 = 100$–17,000 and $t_2 = 10$–10,000 generations. The youngest event had a prior range of $t_1 = 10$–3000 generations for the combined dataset. To convert time estimates to years we assumed a generation time of 7 years for female caribou. We chose summary statistics (i.e. mean number of alleles, mean size variance of alleles, mean number of haplotypes, etc.) based on their success in previous analyses on caribou (see Klütsch *et al.*, 2016). Approximately 3 million simulations were used to test scenarios on a high-performance computing cluster. We compared simulations through logistic regression and linear discriminant analysis in DIYABC. We used the model-checking option to assess the goodness-of-fit of model parameter posterior combination (Appendix S1 of the Supporting Information, Figs S1.1 & S1.2).

RESULTS

Population structure and diversity

We amplified 655 samples (Appendix S1, Table S1) from individual caribou. There was no evidence that certain loci deviated from HWE (6/36 cases significant after Bonferroni correction) or expressed LD (1/144 cases significant after Bonferroni correction). STRUCTURE analysis revealed $K = 2$ (ΔK criterion) that corresponded to a NWT group and SK group. The mean likelihood also supported additional substructure at $K = 4$ (all four groups showing differentiation; Appendix S1, Figs S1.3–S1.5). Pairwise comparisons (F_{ST} and R_{ST}) supported divergence among groups with the strongest differentiation found between the NWT boreal and the SK boreal (Appendix S1, Table S1.2).

We sequenced 370 caribou at the mtDNA control region and found 79 haplotypes that fit into the well-resolved phylogeny of NAL and BEL (Klütsch *et al.*, 2012). Most haplotypes were non-overlapping between groups (Appendix S1, Fig. S1.6). We found only three NAL haplotypes (in 11 caribou; 3.9%) in the NWT dataset, and only one BEL haplotype
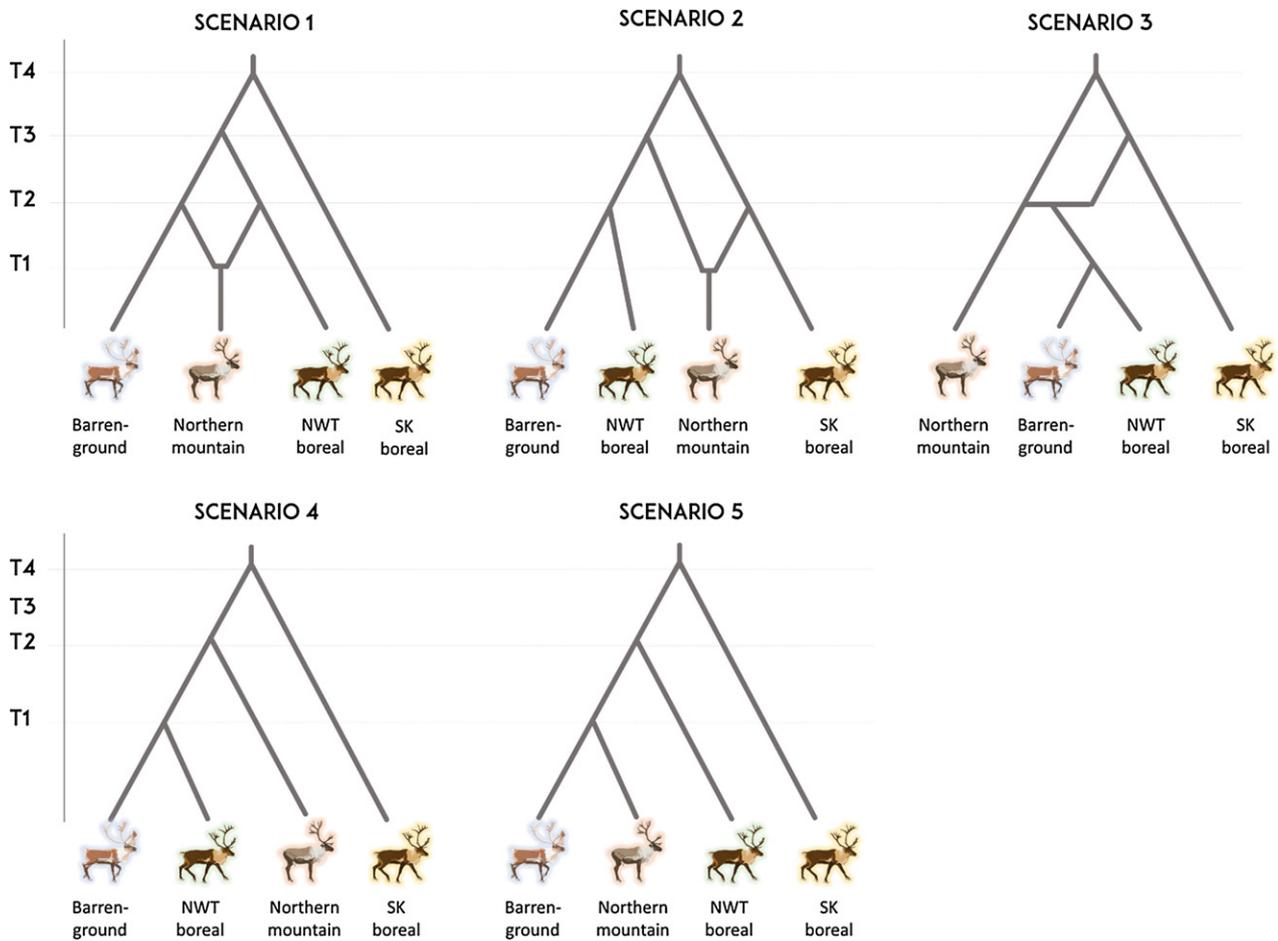
Figure 2 Top five approximate Bayesian computation scenarios tested with DIYABC that model the evolutionary history of four contemporary caribou (*Rangifer tarandus*) groups: barren-ground caribou, boreal ecotype of central Northwest Territories (NWT), northern mountain ecotype and boreal ecotype of central Saskatchewan (SK), Canada.

Table 1 Time estimates in calendar years before present for scenario 1 (found in Fig. 2) produced with approximate Bayesian computation for the combined dataset that includes caribou (*Rangifer tarandus*) microsatellite and mtDNA data from central Northwest Territories and central Saskatchewan, Canada.

Time point	Mean	Median	95% confidence interval	
t_1	4193	2688	206	16450
t_2	45920	46900	20790	66710
t_3	60550	59360	19460	109200
t_4	135800	141400	68600	173600

(in three caribou; 3.4%) in the SK dataset (Appendix S1, Table S1.3).

ABC analysis

All top models identified through ABC analysis suggested that the NWT boreal ecotype has a BEL origin (Fig. 2). Scenario 1 was identified as the most likely evolutionary model for the microsatellite and combined dataset based on the posterior probability values, credible intervals and logistic regression (Appendix S1, Table S1.4, Fig. S1.7). Scenario 1 suggests that the NWT boreal ecotype diverged from the BEL *c.* 60.5 kyr BP (CI: 19.5–109.2 kyr BP; combined dataset; Table 1). This model also estimates that the northern mountain ecotype arose relatively recently at *c.* 4.2 kyr BP (CI: 0.2–16.4 kyr BP; combined dataset) through admixture between two divergent populations that had initially split from the barren-ground and NWT boreal lineages of the BEL *c.* 45.9 kyr BP (CI: 20.8–66.7 kyr BP; combined dataset). Models that included divergence of the SK boreal ecotype from the NWT boreal ecotype (or *vice versa*) were not supported.

The most likely evolutionary model for the separate mtDNA dataset was scenario 4. This model suggests that both the northern mountain and the NWT boreal ecotypes diverged from the barren-ground lineage of the BEL at different time points. This result can be explained in part by the fact that NWT boreal caribou include primarily BEL haplotypes. In contrast, caribou mtDNA data from central Canada include more phylogenetically differentiated

haplogroups and therefore, more haplotypic diversity (Klütsch *et al.*, 2016). Since the majority of haplotypes in this analysis came from the BEL, the average number of mtDNA substitutions in this dataset was also lower than Klütsch *et al.* (2016), which could influence time estimates and model choice to a certain degree. Furthermore, replacement events may have resulted in the loss of ancient haplotypes.

DISCUSSION

The role of parallel evolution in intraspecific diversity

We show for the first time that multiple evolutionary routes can converge on a similar phenotype in an intraspecifically diverse Holarctic species. Our analysis points to the role of the Beringia refugium on genetic variation and structure in contemporary caribou populations. Boreal caribou of central NWT are specialized for survival in the boreal forest and are phenotypically and behaviourally similar to southern boreal ecotype animals (i.e. display sedentary behaviour, dark pelage and large body size; COSEWIC, 2011). However, ABC simulations and mtDNA lineage assignment support a BEL origin for the NWT boreal ecotype, distinct from the NAL of the boreal ecotype that diversified south of the ice sheets during the LGM. Because divergence between the Beringian-derived barren-ground and boreal lineages extends to *c.* 60.5 kyr BP, an alternative model is possible where the northern boreal lineage colonized southern habitats when the ice-free corridor between the Laurentide and Cordilleran first opened – *c.* 14–15 kyr BP (Dyke, 2004; Dixon, 2015) – or perhaps even predating the LGM. However, our results show that the representative southern boreal ecotype from SK diverged before the Beringian-derived barren-ground and boreal lineages. The order of divergence does not support a Pleistocene colonization model, but rather implies independent convergence to a similar boreal ecotype in separate refugia north and south of the ice sheets. Thus, caribou from distinct polyphyletic groups converged on a shared phenotype.

Our study suggests that natural selection has influenced the evolution of the boreal ecotype because a similar suite of traits evolved independently in association with the environmental pressures of the boreal forest. While we could not test the timing of ecological diversification compared to lineage divergence, we can infer that adaptation to Beringian microhabitat was likely an adaptive driver of this lineage. Furthermore, although genetic drift is suspected to play a role in genetic diversification in caribou (Serrouya *et al.*, 2012; Mager *et al.*, 2014), genetic drift would not be expected to produce parallel phenotypic traits in multiple lineages in correlation with specific environments (Schluter *et al.*, 2004). Thus, ecological variation and adaptive evolution may be significant drivers in caribou ecotype evolution to the extent that independent lineages converged to similar phenotypic outcomes.

Our results contrast with Banfield's classic *Rangifer* taxonomic interpretation, based largely on craniometrical measurements, that included western mountain and boreal ecotypes in the woodland subspecies (*R. t. caribou*) that originated in sub-Laurentide refugia. Rather, we show that the mountain and boreal ecotype of central NWT are distinct groups with BEL origins. Our results support the intuition of Geist (2007), who, using pelage characteristics and taxonomic inferences, suggested that the mountain and boreal woodland caribou north of 60° latitude were more likely "splinter populations of barren ground caribou, which have adapted to a more sessile life style, increased in body size and assumed some 'woodland mannerisms'".

In fact, the NWT boreal ecotype may be similar to sedentary caribou that occur in the boreal zone of Alaska. In general, Alaskan caribou belong to the BEL, but have behavioural strategies that have been classified into migratory and sedentary ecotypes (Hinkes *et al.*, 2005; Mager *et al.*, 2014). However, the sedentary Alaskan caribou display significantly less genetic structure than we found in the NWT boreal ecotype. Using 19 microsatellites from caribou across the Alaskan mainland, Mager *et al.* (2014) found little genetic differentiation between migratory or sedentary herds that also ranged greatly in population size and used both forest and tundra habitats. Thus, local behavioural strategies may be relatively plastic within Alaska (Hinkes *et al.*, 2005). Similarly, genetic evidence suggests that Eurasian forest reindeer (*R. t. fennicus*) arose from the large continuous population of BEL reindeer in the vast palaeo-tundra of Siberia and central Eurasia during the Pleistocene (Flagstad & Røed, 2003). Thus, it is possible that the forest reindeer, the NWT boreal ecotype, and the Alaskan sedentary ecotype may have arisen through similar processes of parallel phenotypic evolution.

Among ungulates, caribou and reindeer display high levels of microsatellite heterozygosity (Côté *et al.*, 2002; Boulet *et al.*, 2007). The extensive standing genetic variability in *Rangifer* may be essential to the evolution of convergent phenotypes (Barrett & Schluter, 2008; Elmer & Meyer, 2011). Understanding the source of variation (selection on new mutations or pre-existing genetic variation) can help explain how intraspecific variation is maintained in natural populations (Barrett & Schluter, 2008; Espíndola *et al.*, 2012). Parallel phenotypic evolution may be common in *Rangifer*. Genetic evidence suggests that Peary caribou (*R. t. pearyi*) and Svalbard reindeer (*R. t. platyrhynchus*) may have converged to a shared small-bodied, short-legged phenotype from two evolutionary lineages (Gravlund *et al.*, 1998). The high arctic islands represent a severe and unpredictable environment with selection pressures that could have independently produced the phenotypically divergent characteristics of the Peary and Svalbard animals (Flagstad & Røed, 2003).

Recent analysis suggests that mtDNA introgression (admixture of BEL and NAL) does not correspond to the presence of migratory behaviour in caribou (Klütsch *et al.*, 2016). If ecotypic adaptations to different environments are

a result of parallel phenotypic evolution then some behavioural traits, like migratory behaviour, may not match patterns of neutral marker genetic structure (Pond *et al.*, 2016). Furthermore, unique phenotypes and behavioural adaptations are likely to be influenced by behavioural plasticity, pleiotropy or interacting gene pathways (Réale *et al.*, 2003; Kopp, 2009). Cases of potential parallel evolution present an ideal opportunity for future genomic research to illuminate the genetic basis for adaptive traits (Elmer & Meyer, 2011). For example, grey wolves (*Canis lupus*), like caribou, are highly mobile and display divergent ecotypic adaptations (Carmichael *et al.*, 2001; Musiani *et al.*, 2007). Recently, Schweizer *et al.* (2016) used single-nucleotide polymorphisms to examine phenotypic diversity in wolves and found patterns of selection on morphological genes that were correlated with environmental gradients suggesting that local adaptation is important to ecotype divergence. Genomic research in non-model species holds the promise of exposing synergies among intraspecific diversity, local adaptations and population persistence; however, real-world conservation applications are still speculative (Shafer *et al.*, 2015).

Timing of divergence

Phylogeographical reconstructions provide context for current molecular patterns and allow for interpretation of the impact of past climatic cycles on caribou (Flagstad & Røed, 2003; Klütsch *et al.*, 2012, 2016; Røed *et al.*, 2014). Our ABC analysis suggests that the BEL and NAL split *c.* 135.8 kyr BP (CI: 68.6–173.6 kyr BP), which is comparable to Klütsch

et al. (2016) at 97.3 kyr BP (CI: 44.6–135.8; combined microsatellite and control region mtDNA). Our estimates are more recent than those predicted by Yannic *et al.* (2014) at 300 kyr BP (184–430 kyr BP) using cyt *b* sequences and significantly older than McDevitt *et al.* (2009) at 37.5 kyr BP (CI: 28.1–46.7 kyr BP) using mtDNA control region. However, the coalescence estimates reveal that it is important to consider multiple scales of cyclic climatic change, not just the LGM (Barnosky, 2008). The interstadial periods of warm climate between the early, middle and late Wisconsin glacial periods likely resulted in reunification and introgression between lineages (Fig. 3). In support of this assessment, an ancient caribou mtDNA sample dated to 29,775 ± 564 (IntCal09 years BP) recovered from the Yukon (Lorenzen *et al.*, 2011), is ancestral to the NAL (western clade *sensu* Klütsch *et al.*, 2012), and suggests that potential connections may have occurred prior to the LGM (Fig. 3).

Genetic diversification within refugia may be a source of post-glacial variation in cold-adapted species (Weksler *et al.*, 2010). The palaeoenvironment of Beringia included pockets of low-elevation spruce forests (especially during interglacials and interstadials) among the extensive steppe-tundra and grass-dominated ecosystem (Zazula *et al.*, 2007). The internal complexity of Beringia is thought to have influenced small mammal diversity (Weksler *et al.*, 2010; Galbreath *et al.*, 2011; Lanier *et al.*, 2015), and could also have facilitated ecological divergence of caribou. For example, our results reveal that the split between the NWT boreal ecotype and barren-ground caribou occurred prior to the LGM, which implies that genetic subdivision likely persisted within Beringia.
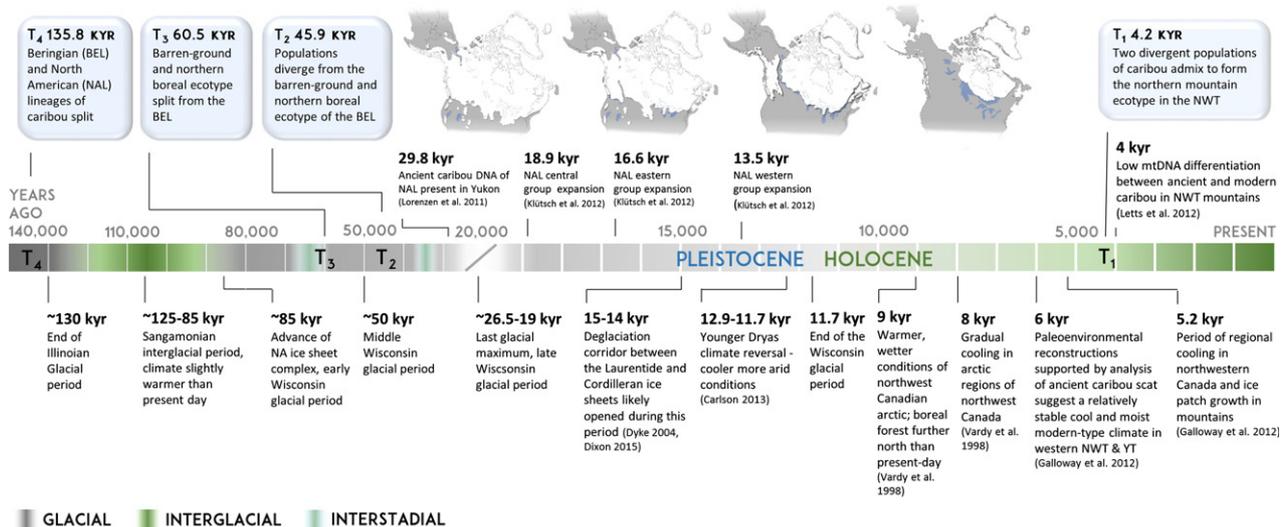
Figure 3 Timeline of last 140 thousand calendar years (kyr) before present. Blue bubbles represent the estimates (t_1–t_4) associated with the approximate Bayesian computation scenario 1 (found in Fig. 2). The timeline includes associated caribou (*Rangifer tarandus*) histories in Canada (Lorenzen *et al.*, 2011; Klütsch *et al.*, 2012; Letts *et al.*, 2012), palaeogeographical events (Carlson, 2013; Dixon, 2015), palaeoenvironmental reconstructions (Vardy *et al.*, 1998; Galloway *et al.*, 2012) and glacial maps (Dyke, 2004) for North America. The scale of the timeline shifts from 10-kyr increments to 1-kyr increments around the Last Glacial Maximum or at *c.* 20 kyr before present. BEL – Beringian–Eurasian lineage, NA – North America, NAL – North American lineage, NWT – Northwest Territories, YT – Yukon Territory.

While microgeographical adaptation to forested *versus* steppe-tundra habitats may have played a critical role in the development of caribou ecotypes during the Pleistocene, there is also the possibility that the ancient lineages of NWT boreal and barren-ground caribou experienced more pronounced geographical separation associated with the divide between the Eurasian and American landmasses.

The substantial sympatric phenotypic diversification in caribou suggests that some genetic signals can withstand contact zones. The Holocene has not been long enough for displacement or admixture to completely mask the genetic legacy of Pleistocene glacial vicariance in caribou. Interestingly, while overlapping ranges (Roffler *et al.*, 2012; Mager *et al.*, 2014) and large-scale merging between sedentary and migratory herds are common in Alaska (Hinkes *et al.*, 2005), population merging between the boreal ecotype and barren-ground caribou is not presently common in the western Canadian boreal zone (Nagy *et al.*, 2011). The genetic structure evident between barren-ground and the NWT boreal ecotype suggests that any mixing that does occur is not sufficient to prevent the perpetuation of distinct genetic signatures (Appendix S1, Figs S1.5 & S1.6).

The clear microsatellite genetic structure across fine spatial scales in central NWT are likely a result of ancestral genetic signals and current ecological adaptations or behavioural mechanisms that promote reproductive isolation (Rundle & Nosil, 2005). The relatively low genetic diversity in the modern NWT boreal ecotype may also suggest a recent expansion into the ice-free region of central NWT and potential founder effects. Likewise, the behaviours associated with the boreal ecotype likely confer increased fitness in the boreal forest, especially since similar phenotypes are expressed by Eurasian forest reindeer and Alaskan sedentary caribou. The genetic structure among neighbouring caribou types suggests that microgeographical adaptation and its driving mechanisms could promote the persistence of local diversification (Rundle & Nosil, 2005; Richardson *et al.*, 2014). Future research is needed to understand how long periods of isolation need to exist for genetic differentiation to arise and remain divergent when contact is re-established.

Similar to Weckworth *et al.* (2012) our results also contradict the inclusion of the northern mountain ecotype of western Canada in the woodland subspecies. Our analysis suggests that caribou in the Mackenzie Mountains arose *c*. 4000 years BP from ancient BEL populations. This corresponds to Letts *et al.* (2012) who found low mtDNA differentiation between ancient (up to 3790 years BP) and modern mountain caribou (Fig. 3). However, weak microsatellite structure between the barren-ground and the northern mountain ecotype implies that historic exchange or incomplete lineage sorting is influencing differentiation between the groups (Letts *et al.*, 2012; Polfus *et al.*, 2016). In northern Alberta, boreal ecotype caribou share BEL and NAL phylogeographical lineages (Weckworth *et al.*, 2012) as do both boreal and mountain ecotypes in the central Rockies which

suggest that zones of contact have occurred (McDevitt *et al.*, 2009). Future ancient DNA approaches may provide more insight into the history of post-glacial contact and illuminate geographical events that influenced population persistence at transitional periods during the late Pleistocene and early Holocene.

Conservation implications

We demonstrate that the boreal ecotype of caribou in North America contains two phylogeographical assemblages that compose an irreplaceable component of Canada's biodiversity. Importantly, our results also show that southern boreal ecotype animals belonging to the NAL represent an independent evolutionary unit of caribou. As was initially suggested by Geist (2007), protecting the 'true woodland caribou' becomes even more critical if the group includes only NAL animals along the southern edge of caribou distribution. The southern extent of the boreal forest also faces threats related to anthropogenic disturbance, fragmentation and shifting predatory–prey dynamics (Environment Canada, 2012). Because the contiguous habitat of the boreal forest and the dispersal capabilities of caribou are likely critical components to the long-term persistence of the boreal ecotype, the genetic variation in the boreal ecotype of central NWT could help prevent the extinction of behavioural adaptations in declining southern populations through evolutionary rescue (Bell & Gonzalez, 2009). Furthermore, as managers consider the re-introduction of programmes for declining caribou populations, our results indicate that attention must be paid to the evolutionary history of putative source populations.

Environmental change due to anthropogenic influence is an increasing threat to many species, especially cold-adapted species (Berteaux *et al.*, 2004; Post *et al.*, 2009). *Rangifer's* adaptation to a wide range of environments across the Holarctic and continuance through the glacial cycles of the Pleistocene suggests that a continuous geographical distribution and genetic mixing may be imperative to their success (Hinkes *et al.*, 2005; Boulet *et al.*, 2007; Lorenzen *et al.*, 2011). In particular, caribou show substantial adaptive capacity and potential phenotypic plasticity that seem to make the species as a whole especially tolerant of changing conditions, however, more information is needed to understand how caribou will respond to future environmental change (Yannic *et al.*, 2014). Understanding the synergies between ecology and evolution may facilitate the design of biodiversity conservation strategies for caribou that prepare for future responses to restrictions on current interglacial climate refugia (Stewart *et al.*, 2010). Dividing species into units (subspecies, ecotypes or DUs) that confine policies to particular groups in isolation, may misrepresent genetic histories and be an insufficient conservation approach. Rather, a focus on large-scale eco-evolutionary processes could provide a framework for maximizing genetic diversity and preserving adaptive potential.

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REFERENCES

Barnosky, A.D. (2008) Climatic change, refugia, and biodiversity: where do we go from here? An editorial comment. *Climatic Change*, **86**, 29–32.

Barrett, R.D. & Schluter, D. (2008) Adaptation from standing genetic variation. *Trends in Ecology and Evolution*, **23**, 38–44.

Bell, G. & Gonzalez, A. (2009) Evolutionary rescue can prevent extinction following environmental change. *Ecology Letters*, **12**, 942–948.

Berteaux, D., Réale, D., McAdam, A.G. & Boutin, S. (2004) Keeping pace with fast climate change: can arctic life count on evolution? *Integrative and Comparative Biology*, **44**, 140–151.

Bishop, M.D., Kappes, S.M., Keele, J.W., Stone, R.T., Sunden, S., Hawkins, G.A., Toldo, S.S., Fries, R., Grosz, M.D. & Yoo, J. (1994) A genetic linkage map for cattle. *Genetics*, **136**, 619–639.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution*, **26**, 183–192.

Boulet, M., Couturier, S., Côté, S.D., Otto, R.D. & Bernatchez, L. (2007) Integrative use of spatial, genetic, and demographic analyses for investigating genetic connectivity between migratory, montane, and sedentary caribou herds. *Molecular Ecology*, **16**, 4223–4240.

Brandt, J. (2009) The extent of the North American boreal zone. *Environmental Reviews*, **17**, 101–161.

Carlson, A. (2013) The younger Dryas climate event. *Encyclopedia of Quaternary Science*, **3**, 126–134.

Carmichael, L., Nagy, J., Larter, N. & Strobeck, C. (2001) Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. *Molecular Ecology*, **10**, 2787–2798.

Cornuet, J.-M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Marin, J.-M. & Estoup, A. (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism. DNA sequence and microsatellite data. *Bioinformatics*, **30**, 1187–1189.

COSEWIC (2011) *Designatable units for caribou (Rangifer tarandus) in Canada*. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario, Canada.

Côté, S., Dallas, J., Marshall, F., Irvine, R., Langvatn, R. & Albon, S. (2002) Microsatellite DNA evidence for genetic drift and philopatry in Svalbard reindeer. *Molecular Ecology*, **11**, 1923–1930.

Cronin, M.A., MacNeil, M.D. & Patton, J.C. (2005) Variation in mitochondrial DNA and microsatellite DNA in caribou (*Rangifer tarandus*) in North America. *Journal of Mammalogy*, **86**, 495–505.

Csilléry, K., Blum, M.G., Gaggiotti, O.E. & François, O. (2010) Approximate Bayesian computation (ABC) in practice. *Trends in Ecology and Evolution*, **25**, 410–418.

Dixon, E.J. (2015) Late Pleistocene colonization of North America from Northeast Asia: new insights from large-scale paleogeographic reconstructions. *Mobility and ancient society in Asia and the Americas* (ed. by M.D. Frachetti and R. Spengler III), pp. 169–184. Springer, New York, NY, USA.

Dyke, A.S. (2004) An outline of North American deglaciation with emphasis on central and northern Canada. *Quaternary Glaciations: Extent and Chronology*, **2**, 373–424.

Earl, D.A. & vonHoldt, B.M. (2012) Structure Harvester: a website and program for visualizing structure output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Elmer, K.R. & Meyer, A. (2011) Adaptation in the age of ecological genomics: insights from parallelism and convergence. *Trends in Ecology and Evolution*, **26**, 298–306.

Environment Canada (2012) *Recovery strategy for the woodland caribou (Rangifer tarandus caribou), boreal population, in Canada*. Species at Risk Act Recovery Strategy Series. Environment Canada, Ottawa, Ontario, Canada. xi + 138pp.

Espíndola, A., Pellissier, L., Maiorano, L., Hordijk, W., Guisan, A. & Alvarez, N. (2012) Predicting present and future intra-specific genetic structure through niche hindcasting across 24 millennia. *Ecology Letters*, **15**, 649–657.

Fitzpatrick, S., Gerberich, J., Kronenberger, J., Angeloni, L. & Funk, W. (2015) Locally adapted traits maintained in the face of high gene flow. *Ecology Letters*, **18**, 37–47.

Flagstad, Ø. & Røed, K.H. (2003) Refugial origins of reindeer (*Rangifer tarandus* L.) inferred from mitochondrial DNA sequences. *Evolution*, **57**, 658–670.

Galbreath, K.E., Cook, J.A., Eddingsaas, A.A. & DeChaine, E.G. (2011) Diversity and demography in Beringia: multilocus tests of paleodistribution models reveal the complex history of arctic ground squirrels. *Evolution*, **65**, 1879–1896.

Galloway, J.M., Adamczewski, J., Schock, D.M., Andrews, T.D., MacKay, G., Bowyer, V.E., Meulendyk, T., Moorman, B.J. & Kutz, S.J. (2012) Diet and habitat of mountain woodland caribou inferred from dung preserved in 5000-year-old alpine ice in the Selwyn Mountains, Northwest Territories, Canada. *Arctic*, **65**, 59–79.

Galpern, P., Manseau, M. & Wilson, P. (2012a) Grains of connectivity: analysis at multiple spatial scales in landscape genetics. *Molecular Ecology*, **21**, 3996–4009.

Galpern, P., Manseau, M., Hettinga, P., Wilson, P. & Smith, K. (2012b) ALLELEMATCH: an R package for identifying unique multilocus genotypes where genotyping error and missing data may be present. *Molecular Ecology Resources*, **12**, 771–778.

Geist, V. (2007) Defining subspecies, invalid taxonomic tools, and the fate of the woodland caribou. *Rangifer Special Issue*, **17**, 25–28.

Gravlund, P., Meldgaard, M., Pääbo, S. & Arctander, P. (1998) Polyphyletic origin of the small-bodied, high-arctic subspecies of tundra reindeer (*Rangifer tarandus*). *Molecular Phylogenetics and Evolution*, **10**, 151–159.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Hardy, O.J. & Vekemans, X. (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Hewitt, G. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hinkes, M.T., Collins, G.H., Van Daele, L.J., Kovach, S.D., Aderman, A.R., Woolington, J.D. & Seavoy, R.J. (2005) Influence of population growth on caribou herd identity, calving ground fidelity, and behavior. *Journal of Wildlife Management*, **69**, 1147–1162.

Klütsch, C.F.C., Manseau, M. & Wilson, P.J. (2012) Phylogeographical analysis of mtDNA data indicates postglacial expansion from multiple glacial refugia in woodland caribou (*Rangifer tarandus caribou*). *PLoS ONE*, **7**, e52661.

Klütsch, C.F.C., Manseau, M., Trim, V., Polfus, J.L. & Wilson, P.J. (2016) The eastern migratory caribou: the role of genetic introgression in ecotype evolution. *Royal Society Open Science*, **3**, 150469.

Kopp, A. (2009) Metamodels and phylogenetic replication: a systematic approach to the evolution of developmental pathways. *Evolution*, **63**, 2771–2789.

Lanier, H.C., Gunderson, A.M., Weksler, M., Fedorov, V.B. & Olson, L.E. (2015) Comparative phylogeography highlights the double-edged sword of climate change faced by arctic and alpine-adapted mammals. *PLoS ONE*, **10**, e0118396.

Letts, B., Fulton, T.L., Stiller, M., Andrews, T.D., MacKay, G., Popko, R. & Shapiro, B. (2012) Ancient DNA reveals genetic continuity in mountain woodland caribou of the Mackenzie and Selwyn Mountains, Northwest Territories, Canada. *Arctic*, **65**, 80–94.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Lopes, J.S. & Boessenkool, S. (2010) The use of approximate Bayesian computation in conservation genetics and its application in a case study on yellow-eyed penguins. *Conservation Genetics*, **11**, 421–433.

Lorenzen, E.D., Nogués-Bravo, D., Orlando, L., Weinstock, J., Binladen, J., Marske, K.A., Ugan, A., Borregaard, M.K., Gilbert, M.T.P. & Nielsen, R. (2011) Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature*, **479**, 359–364.

Mager, K.H., Colson, K.E., Groves, P. & Hundertmark, K.J. (2014) Population structure over a broad spatial scale driven by nonanthropogenic factors in a wide-ranging migratory mammal, Alaskan caribou. *Molecular Ecology*, **23**, 6045–6057.

McDevitt, A.D., Mariani, S., Hebblewhite, M., Decesare, N.J., Morgantini, L., Seip, D., Weckworth, B.V. & Musiani, M. (2009) Survival in the Rockies of an endangered hybrid swarm from diverged caribou (*Rangifer tarandus*) lineages. *Molecular Ecology*, **18**, 665–679.

Musiani, M., Leonard, J.A., Cluff, H., Gates, C.C., Mariani, S., Paquet, P.C., Vilà, C. & Wayne, R.K. (2007) Differentiation of tundra/taiga and boreal coniferous forest wolves: genetics, coat colour and association with migratory caribou. *Molecular Ecology*, **16**, 4149–4170.

Nagy, J.A., Johnson, D.L., Larter, N.C., Campbell, M.W., Derocher, A.E., Kelly, A., Dumond, M., Allaire, D. & Croft, B. (2011) Subpopulation structure of caribou (*Rangifer tarandus* L.) in arctic and subarctic Canada. *Ecological Applications*, **21**, 2334–2348.

Polfus, J.L., Manseau, M., Simmons, D., Neyelle, M., Bayha, W., Andrew, F., Andrew, L., Klütsch, C.F.C., Rice, K. & Wilson, P. (2016) Łeghágots'enetę (learning together): the importance of indigenous perspectives in the identification of biological variation. *Ecology and Society*, **21**(2), 18.

Pond, B.A., Brown, G.S., Wilson, K.S. & Schaefer, J.A. (2016) Drawing lines: spatial behaviours reveal two ecotypes of woodland caribou. *Biological Conservation*, **194**, 139–148.

Post, E., Forchhammer, M.C., Bret-Harte, M.S. *et al.* (2009) Ecological dynamics across the arctic associated with recent climate change. *Science*, **325**, 1355–1358.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Puckett, E.E., Etter, P.D., Johnson, E.A. & Eggert, L.S. (2015) Phylogeographic analyses of American black bears (*Ursus americanus*) suggest four glacial refugia and complex patterns of post-glacial admixture. *Molecular Biology and Evolution*, **32**, 2338–2350.

Réale, D., McAdam, A.G., Boutin, S. & Berteaux, D. (2003) Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 591–596.

Richardson, J.L., Urban, M.C., Bolnick, D.I. & Skelly, D.K. (2014) Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology and Evolution*, **29**, 165–176.

Røed, K.H., Bjørnstad, G., Flagstad, Ø., Haanes, H., Hufthammer, A.K., Jordhøy, P. & Rosvold, J. (2014) Ancient DNA reveals prehistoric habitat fragmentation and recent domestic introgression into native wild reindeer. *Conservation Genetics*, **15**, 1137–1149.

Roffler, G.H., Adams, L.G., Talbot, S.L., Sage, G.K. & Dale, B.W. (2012) Range overlap and individual movements during breeding season influence genetic relationships of caribou herds in south-central Alaska. *Journal of Mammalogy*, **93**, 1318–1330.

Rosenblum, E.B., Parent, C.E. & Brandt, E.E. (2014) The molecular basis of phenotypic convergence. *Annual Review of Ecology, Evolution, and Systematics*, **45**, 203–226.

Rousset, F. (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Rundle, H.D. & Nosil, P. (2005) Ecological speciation. *Ecology Letters*, **8**, 336–352.

Schluter, D., Clifford, E.A., Nemethy, M. & McKinnon, J.S. (2004) Parallel evolution and inheritance of quantitative traits. *The American Naturalist*, **163**, 809–822.

Schweizer, R.M., vonHoldt, B.M., Harrigan, R., Knowles, J.C., Musiani, M., Coltman, D., Novembre, J. & Wayne, R.K. (2016) Genetic subdivision and candidate genes under selection in North American grey wolves. *Molecular Ecology*, **25**, 380–402.

Serrouya, R., Paetkau, D., McLellan, B.N., Boutin, S., Campbell, M. & Jenkins, D.A. (2012) Population size and major valleys explain microsatellite variation better than taxonomic units for caribou in western Canada. *Molecular Ecology*, **21**, 2588–2601.

Shafer, A.B., Wolf, J.B., Alves, P.C., Bergström, L., Bruford, M.W., Brännström, I., Colling, G., Dalen, L., De Meester, L. & Ekblom, R. (2015) Genomics and the challenging translation into conservation practice. *Trends in Ecology and Evolution*, **30**, 78–87.

Stewart, J.R., Lister, A.M., Barnes, I. & Dalén, L. (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society of London B: Biological Sciences*, **277**, 661–671.

Vardy, S.R., Warner, B.G. & Aravena, R. (1998) Holocene climate and the development of a subarctic peatland near Inuvik, Northwest Territories, Canada. *Climatic Change*, **40**, 285–313.

Weckworth, B.V., Musiani, M., McDevitt, A.D., Hebblewhite, M. & Mariani, S. (2012) Reconstruction of caribou evolutionary history in western North America and its implications for conservation. *Molecular Ecology*, **21**, 3610–3624.

Weksler, M., Lanier, H.C. & Olson, L.E. (2010) Eastern Beringian biogeography: historical and spatial genetic structure of singing voles in Alaska. *Journal of Biogeography*, **37**, 1414–1431.

Wilson, G., Strobeck, C., Wu, L. & Coffin, J. (1997) Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. *Molecular Ecology*, **6**, 697–699.

Yannic, G., Pellissier, L., Ortego, J. *et al.* (2014) Genetic diversity in caribou linked to past and future climate change. *Nature Climate Change*, **4**, 132–137.

Zazula, G.D., Froese, D.G., Elias, S.A., Kuzmina, S. & Mathewes, R.W. (2007) Arctic ground squirrels of the mammoth-steppe: paleoecology of Late Pleistocene middens (∼ 24000–29450 14 C yr BP), Yukon Territory, Canada. *Quaternary Science Reviews*, **26**, 979–1003.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Population genetic summary statistics and ABC model evaluations.

Appendix S2 Full list of collaborators and contributors.

DATA ACCESSIBILITY

Genetic mtDNA data generated for this study are available on GenBank: KX463387–KX463407.

BIOSKETCH

The team is interested in developing collaborative applied research that integrates population genetics and ecological data to monitor and study the origin, genetic diversity, status and evolutionary history of wildlife populations. Their expertise includes the use of non-invasive methods, DNA markers and cross-cultural approaches in the management of wildlife and protected areas. The team's goals include developing new analytical methods, synthesizing knowledge across jurisdictional, cultural and political boundaries, and approaching conservation problems in a way that respects the lives and experiences of people that depend on natural resources for their livelihood. You can find more at http://lecol-ck.ca and http://srrb.nt.ca.

Author contributions: J.L.P. collected field samples, conducted research, contributed to research design, analysis and data interpretation, and wrote the first draft of the manuscript. M.M., P.J.W. and D.S. contributed to analysis approach, study design, and data interpretation. C.F.C.K. performed the ABC analysis and aided in data interpretation. All authors provided revisions to the manuscript and gave final approval.

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