

# Reconstruction of caribou evolutionary history in Western North America and its implications for conservation

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## Abstract

The role of Beringia as a refugium and route for trans-continental exchange of fauna during glacial cycles of the past 2 million years are well documented; less apparent is its contribution as a significant reservoir of genetic diversity. Using mitochondrial DNA sequences and 14 microsatellite loci, we investigate the phylogeographic history of caribou (*Rangifer tarandus*) in western North America. Patterns of genetic diversity reveal two distinct groups of caribou. Caribou classified as a Northern group, of Beringian origin, exhibited greater number and variability in mtDNA haplotypes compared to a Southern group originating from refugia south of glacial ice. Results indicate that subspecies *R. t. granti* of Alaska and *R. t. groenlandicus* of northern Canada do not constitute distinguishable units at mtDNA or microsatellites, belying their current status as separate subspecies. Additionally, the Northern Mountain ecotype of woodland caribou (presently *R. t. caribou*) has closer kinship to caribou classified as *granti* or *groenlandicus*. Comparisons of mtDNA and microsatellite data suggest that behavioural and ecological specialization is a more recently derived life history characteristic. Notably, microsatellite differentiation among Southern herds is significantly greater, most likely as a result of human-induced landscape fragmentation and genetic drift due to smaller population sizes. These results not only provide important insight into the evolutionary history of northern species such as caribou, but also are important indicators for managers evaluating conservation measures for this threatened species.

**Keywords:** ecotypes, endangered species, genetic markers, phylogeography, *Rangifer tarandus*, subspecies

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## Introduction

The biogeographical histories of North American biota are intertwined with late Quaternary climate change. Glaciation was particularly pervasive at high latitudes during the last glacial maximum (LGM, ~21 BP, Clark & Mix 2002; Dyke *et al.* 2002) covering northern North America. However, glacial refugia have been proposed

based on biogeographical and fossil evidence, particularly in Beringia, the ice-free refugium stretching from eastern Siberia to the Northwest Territories of Canada (Abbott & Brochmann 2003). Following deglaciation, recolonization likely occurred from several refugia, including Beringia, unglaciated regions of the Canadian high arctic (Waltari & Cook 2005) and south of the ice sheets (Runck & Cook 2005; Weckworth *et al.* 2010). Post glacial expansion dynamics are suggested as an important basis for the geographic distribution of contemporary genomes (Avice 2000; Hewitt 2000; Lessa

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et al. 2003). Therefore, understanding the relationship between climate changes and phylogeography is necessary to describe intra- and inter-species diversity, current population genetic structure and the potential for local adaptation (Dynesius & Jansson 2000).

The caribou (*Rangifer tarandus*; known as reindeer in Eurasia) is a circumpolar New World ungulate (family Cervidae), with the oldest fossils found in eastern Beringia (Fort Selkirk, Yukon) and dated to 1.6 Ma (Harington 1999), implying a Beringian origin of the genus. In a statistically rigorous review of *Rangifer* taxonomy in North America (Banfield 1961) results from morphological analysis indicated two distinct groups, tundra and forest caribou, with three extant subspecies in western North America (Fig. 1). The tundra form is represented by the Alaskan caribou (*Rangifer tarandus granti*) and the Canadian barren-ground caribou (*R. t. groenlandicus*), and the forest form includes the remaining North American woodland caribou (*R. t. caribou*). Genetic evaluations have generally supported the ice age isolation and significant divergence of the tundra subspecies, *R. t. granti* and *R. t. groenlandicus* from the woodland *R. t. caribou* (Flagstad & Røed 2003). McDevitt et al. (2009) confirmed tundra versus woodland split, but also demonstrated

historic interbreeding of the two clades along the eastern front of the Canadian Rockies. However, the pattern of expansion into deglaciated regions and the phylogenetic backdrop of contemporary populations remain uncertain.

In western Canada, federal classification of woodland caribou further divides them into three ecotypes based upon geography and life history characteristics rather than morphology or genetics (Fig. 1; COSEWIC 2002). The federal listing of these ecotypes by COSEWIC and the Species at Risk Act (SARA) identify the Southern Mountain and Boreal as threatened and the Northern Mountain as a population of concern (COSEWIC 2002). Many provinces have also listed woodland caribou ecotypes and have specific mandates for their recovery. However, provincial ecotype designations do not necessarily match, in name or criteria, those described federally or in neighbouring provinces. Little to no genetic analysis has been carried out to support ecotype designations of western caribou populations (McDevitt et al. 2009).

Ecotypes are defined as populations within a species that have evolved particular life history and behavioural adaptations in response to specific environmental

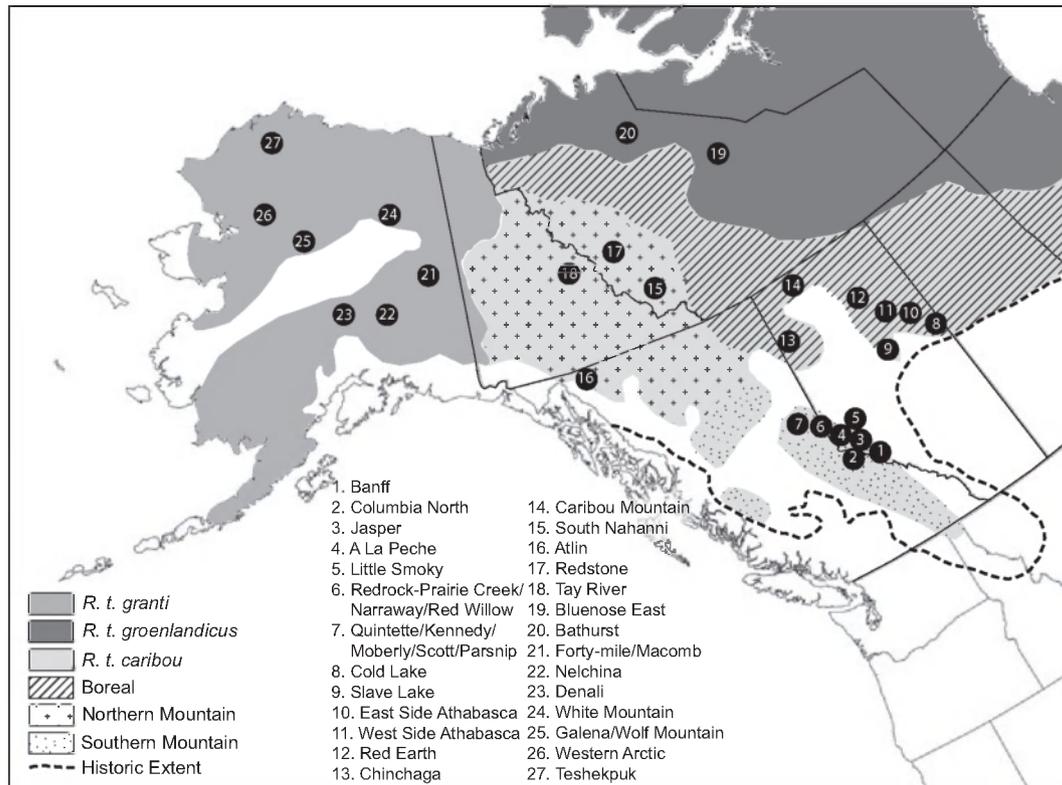


Fig. 1 Map showing sampling design. Black numbered circles indicate sample locations. The grey-scale polygons show a generalized distribution for each subspecies; dark grey represents the barren-ground subspecies (*Rangifer tarandus groenlandicus*), medium-grey indicates Alaska subspecies (*R. t. granti*) and light-grey shows the woodland subspecies (*R. t. caribou*). The three different federally designated ecotypes of woodland caribou are indicated with patterned polygons as indicated.

constraints (Courtois *et al.* 2003). This definition does not associate concordant genealogical relationships, yet abrupt transitions from one habitat type to another have been implicated as a mechanism of genetic divergence in otherwise continuously distributed species (e.g. Munoz-Fuentes *et al.* 2009; Weckworth *et al.* 2011). In caribou, Courtois *et al.* (2003) found genetic concordance of eastern Canadian mountain, boreal and barren-ground ecotypes. An understanding of the genetic dynamics of ecotypes has implications for species of conservation concern to aid in recognizing appropriate legal status and range management (Courtois *et al.* 2003).

The threatened status of most woodland caribou reflects a general decline in their numbers since the early 20th century (Bergerud 1974). The reasons for their decline are attributed to the fragmentation and alteration of their primary habitat and ecosystem function through human land-use activities, including natural resource extraction (Bergerud 1974; James & Stuart-Smith 2000). Their threatened conservation status has resulted in much ecological research aimed at improving recovery planning (McLoughlin *et al.* 2003; Weclaw & Hudson 2004; Whittington *et al.* 2011). While ecological information is important for identifying how and why caribou populations are in decline on a contemporary scale, molecular data are essential for elucidating evolutionary history, cryptic population structure, genetic parameters and conservation units important for long-term survival.

Here, we employ mtDNA sequencing and microsatellite genotyping to provide a large-scale assessment of genetic variability of caribou in western North America. We synthesize, using the largest caribou molecular data set to date, a comprehensive molecular perspective on the evolutionary relationships of caribou in western North America. We test the null hypotheses that a priori subspecies and ecotypes currently accepted reflect evolutionary patterns of genetic diversity in caribou. We then explore alternative hypotheses, a posteriori, that reconstruct the evolutionary history of caribou as presented by mtDNA and microsatellites. These results have the potential for far-reaching impacts on current caribou management policy. Furthermore, we provide insight into the post glacial patterns of expansion from ice age refugia that laid the foundation for current patterns of biodiversity at northern latitudes.

## Methods

### Sampling

Archived samples of blood, muscle, skin or hair were obtained for 854 individual caribou from capture and

hunting activities across western North America over the past decade (Table 1; Fig. 1). Of the 854, 223 were from individuals previously analysed in McDevitt *et al.* (2009). Furthermore, we added 34 barren-ground and woodland caribou sequences from GenBank to augment the geographic distribution of our sampling (Supporting Information). Sequences from GenBank were used only in phylogenetic analyses, but not in population-level analysis.

Our samples include caribou that represent a variety of currently recognized taxonomic identities. Individuals from Alaska represent the subspecies *Rangifer tarandus granti*. Samples from eastern Northwest Territories are classified as the barren-ground subspecies *R. t. groenlandicus*. Finally, samples from British Columbia, Alberta, Yukon Territories, western Northwest Territories and GenBank sequences from central and eastern Canada fall into the woodland subspecies *R. t. caribou*. However, woodland caribou have been further classified into Northern Mountain, Southern Mountain and Boreal ecotypes (Fig. 1). Given the complexity and lack of understanding of these taxonomic labels, we will simply refer to the five different 'types' of caribou examined as: Alaska, Barren-ground, Boreal, Northern Mountain and Southern Mountain.

### Genetic analysis

DNA extractions from blood were carried out using the DNeasy Blood and Tissue Kit (QIAGEN) following the manufacturer's protocol or using a standard salt extraction protocol (Miller *et al.* 1988).

We examined a ~496-bp fragment of maternally inherited mtDNA control region. This fragment was amplified in 711 individuals using the primer pair L15394 and H15947 following Flagstad & Røed (2003). Sequences were created by Macrogen Inc. (Seoul, Korea). Chromatogram images were assembled and aligned manually using BioEDIT v7.0.5. All individuals were also PCR amplified and genotyped at 14 polymorphic microsatellite loci, which include 10 markers used by McDevitt *et al.* (2009), RT1, RT5, RT6, RT9, RT13, RT24, RT27 (Wilson *et al.* 1997), BL42, BM4513 and BM6506 (Bishop *et al.* 1994); and four additional unguulate dinucleotide loci which were found to be polymorphic in caribou, BM757 and BM848 (Bishop *et al.* 1994), Eth152 (Steffen *et al.* 1993) and Oar193 (Buchanan & Crawford 1993). PCR amplifications were carried out in a final volume of 10- $\mu$ L in multiplex reactions using 1  $\mu$ L of DNA extract and 1 $\times$  Multiplex PCR Mastermix (QIAGEN). Primer concentrations ranged from 0.15 to 0.5  $\mu$ M. Amplification reactions typically began with 95 °C for 15 min; 35 cycles of 94 °C for 45 s, 54–58 °C for 90 s, 72 °C for 1 min and a final extension at 72 °C

**Table 1** Descriptive statistics for *Rangifer tarandus* herds and types in western North America

#	Populations	Abbr.	<i>n</i>	Alleles	Richness	$H_E$	$H_O$	Haps	<i>h</i>	$\pi$
Southern Mountain			190/247	12.36	9.98	0.83	0.76	21	0.790	0.0156
1	Banff National Park	BNP	5/5	2.29	1.98	0.45	0.60	1	0.000	0.0000
2	Columbia North	CN	24/24	7.71	2.97	0.78	0.75	6	0.761	0.0159
3	Jasper National Park	JNP	28/29	6.14	2.72	0.72	0.70	3	0.593	0.0130
4	A La Peche	ALP	27/34	8.57	3.04	0.80	0.82	4	0.604	0.0149
6	Narraway	NAR	26/46	9.00	3.04	0.80	0.76	6	0.354	0.0068
6	Redrock-Prairie Creek	RPC	28/55	8.93	3.04	0.80	0.76	9	0.849	0.0173
6	Red Willow	RWR	2/2	2.86	2.86	0.56	0.75	1	0.000	0.0000
7	Kennedy	KEN	15/18	8.36	3.10	0.80	0.81	4	0.657	0.0053
7	Moberly	MOB	3/3	4.07	3.12	0.69	0.76	3	1.000	0.0029
7	Parsnip	PAR	16/17	7.93	3.02	0.78	0.75	6	0.808	0.0065
7	Quintette	QUI	11/9	6.57	3.15	0.79	0.74	5	0.764	0.0192
7	Scott	SC	5/5	5.00	3.00	0.72	0.81	3	0.800	0.0177
Boreal			194/213	14.14	10.86	0.80	0.76	18	0.900	0.0171
5	Little Smoky	LSM	25/38	7.00	2.77	0.73	0.75	2	0.500	0.0151
8	Cold Lake	CL	26/26	9.21	3.08	0.80	0.81	7	0.840	0.0156
9	Slave Lake	SL	10/11	3.71	2.35	0.59	0.60	2	0.200	0.0056
10	East-side Athabasca	ESAR	17/17	6.93	2.87	0.74	0.79	5	0.772	0.0120
11	West-side Athabasca	WSAR	28/32	7.93	2.86	0.75	0.75	6	0.799	0.0031
12	Red Earth	RE	30/30	7.43	2.77	0.73	0.75	5	0.800	0.0026
13	Chinchaga	CH	30/31	8.57	2.95	0.77	0.78	4	0.717	0.0096
14	Caribou Mountain	CM	28/28	10.43	3.12	0.81	0.80	13	0.929	0.0199
Northern Mountain			141/151	15.50	12.48	0.86	0.82	34	0.873	0.0088
15	South Nahanni	SNH	22/22	10.36	3.22	0.83	0.81	8	0.853	0.0181
16	Atlin	AT	28/30	8.50	3.08	0.81	0.78	4	0.643	0.0124
17	Redstone	RED	86/93	14.86	3.29	0.86	0.83	30	0.917	0.0181
18	Tay River	TR	5/6	6.21	3.19	0.77	0.82	2	0.400	0.0097
Barren-ground			40/40	13.79	13.73	0.87	0.84	30	0.981	0.0135
19	Bluenose East	BE	20/20	11.21	3.31	0.85	0.83	16	0.974	0.0121
20	Bathurst	BA	20/20	12.21	3.39	0.86	0.86	17	0.984	0.0148
Alaska			144/151	16.14	13.10	0.87	0.85	66	0.975	0.0182
21	Forty-mile	FM	34/34	12.57	3.34	0.86	0.87	21	0.961	0.0174
21	Macomb	MB6/7		6.71	3.25	0.80	0.84	5	0.933	0.0090
22	Nelchina	NEL	19/19	10.07	3.26	0.84	0.87	12	0.942	0.0161
23	Denali	DEN	22/23	10.29	3.26	0.84	0.85	12	0.948	0.0161
24	White Mountains	WhM	5/5	5.86	3.26	0.77	0.84	3	0.700	0.0101
25	Galena Mountain	GM	6/8	5.21	2.68	0.66	0.68	3	0.600	0.0110
25	Wolf Mountains	WoM	8/9	6.71	3.07	0.77	0.82	4	0.643	0.0119
26	Western Arctic	WA	24/26	12.36	3.35	0.86	0.86	18	0.971	0.0193
27	Teshkepuk	TK	20/20	11.14	3.34	0.86	0.86	17	0.984	0.0210
All			709/802	20.14	20.13	0.86	0.79	151	0.959	0.0237

#, Herd location on Fig. 1; Abbr., abbreviation; *n*, sample size for mtDNA/microsatellites; Alleles, mean number of alleles per locus; richness, allelic richness;  $H_E$ , expected heterozygosity;  $H_O$ , observed heterozygosity; Haps, number of haplotypes; *h*, haplotype diversity;  $\pi$ , nucleotide diversity.

for 45 min. PCR products were the run on an ABI 3130xl Genetic Analyser 16 capillary system (Applied Biosystems) and sized with internal lane standard (600 LIZ; Applied Biosystems) using the program GeneMapper v4.0 (Applied Biosystems). An average of 114 individuals per locus were randomly selected and independently re-amplified. The resulting error rate due to allelic drop-out and scoring errors was 0.38% per allele, which is considered negligible in studies evaluating population structure (Bonin *et al.* 2004). The

program Micro-Checker (van Oosterhout *et al.* 2004) was used to check all herds for the presence of null alleles, large allelic dropout and possible scoring errors.

#### Mitochondrial DNA phylogenetic analysis

Phylogenetic relationships among mitochondrial haplotypes were examined using Bayesian methods. The program JMODELTEST 0.1.1 (Guindon & Gascuel 2003; Posada 2008) identified TPM1uf + I + G as the best model

according to the Bayesian Information Criterion. TPM1uf is the Kimura 3-parameter model (K81, Kimura 1981) with unequal base frequencies (uf), invariant sites (I) and gamma distributed rates (G). Parameters from this model were used in subsequent analyses where such inputs are permitted. Bayesian trees were examined using MrBayes v.3.1 (Ronquist & Huelsenbeck 2003); initiated with a random tree, three independent chains were run for 25 million iterations sampled every 5000 generations. Log-likelihood values for each sampled generation were evaluated, and those preceding stationarity (plotting  $-\ln L$  over generation time) was discarded. Data collected post stationarity were used to estimate posterior probability nodal support. Another Cervid species, moose (*Alces alces*), was used as an out-group. Net divergence ( $D_a$ ) between the mtDNA lineages in the study area was calculated in MEGA version 5 (Tamura *et al.* 2011).

ARLEQUIN 3.5 (Excoffier & Lischer 2010) was used to calculate haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ), as well as to calculate population pairwise estimates of  $\Phi_{ST}$  values, taking into consideration differences in both haplotype frequency and differences in sequences. In mtDNA,  $\Phi_{ST}$  uses information on allelic relationships as a measurement of how isolation among populations increases time of coalescence for genes drawn randomly from different populations relative to genes within a single population (Slatkin 1991). The resulting matrix of pairwise  $\Phi_{ST}$  estimates was then analysed using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA) to create a multidimensional scaling plot (MDS) to visualize the genetic relationships among populations, and a corresponding stress value for the MDS that evaluates the fit on two dimensions. To test for a pattern of isolation by distance, we compared  $\Phi_{ST}$  values against straight-line geographic distance among sampled populations (using centre point of each population's home range). We assessed the significance of this comparison using a Mantel test as implemented in IBD (Bohonak 2002).

To evaluate significant geographic divisions of hypothesized a priori subspecies and ecotype groups, we used hierarchical analyses of molecular variance

(AMOVA, Excoffier *et al.* 1992) in ARLEQUIN (Excoffier & Lischer 2010). This analysis divides total variance into variance components via differences among groups ( $\Phi_{CT}$ ), among populations within groups ( $\Phi_{SC}$ ) and within populations ( $\Phi_{ST}$ ). Spatial AMOVA designs based on a priori subspecies and ecotype hypotheses were contrasted to various a posteriori groups implied from the results of the above phylogenetic analyses (Table 2). We assumed that the best geographic subdivisions were significantly different from random distributions and had maximum among group variance ( $\Phi_{CT}$  values). We expected that the optimal genetic subdivisions of subspecies/ecotypes will maximize the between-group variance ( $\Phi_{CT}$ ) compared to the within-group component ( $\Phi_{SC}$ ).

Finally, population expansion was examined using multiple methods. Demographic events such as rapid population expansion and growth leads to low levels of diversity among haplotypes over large areas and results in phylogenies that are diverse at external nodes and compressed near the root (i.e. star-like phylogenies; Avise 2000). Examining mtDNA genealogies (Avise 2000) and analysis of the raggedness index of mismatch distributions (Rogers & Harpending 1992) provide a method for inferring such demographic events. ARLEQUIN was used to calculate mismatch distribution of observed differences between pairs of haplotypes in the two genetic groups (Northern and Southern) identified in above phylogenetic analyses. We also calculated in ARLEQUIN Tajima's  $D$  (Tajima 1989) and Fu's  $F_S$  (Fu 1997) tests of neutrality, where significantly negative values may indicate recent population expansion. Fu & Li's (1993)  $D^*$  and  $F^*$  statistics (calculated in DnaSP 5.1; Librado & Rozas 2009) help distinguish background selection from population growth or range expansion when compared with Fu's  $F_S$ . If  $F_S$  is significant and  $D^*$  and  $F^*$  statistics are not, population growth or range expansion is supported. The reverse combination suggests selection (Fu 1997).

#### Microsatellite population genetic analysis

Hardy-Weinberg equilibrium (HWE) and microsatellite allele number and heterozygosity (observed and

**Table 2** Analysis of molecular variance for five models of groupings according to different subspecies and ecotype designations based on morphology and natural history characteristics

Model	Hypothesized groupings	$\Phi_{SC}$	$\Phi_{ST}$	$\Phi_{CT}$	% Among groups	$P \Phi_{CT}$
A	[Southern Mountain] [Boreal] [Northern Mountain] [Barren-ground] [Alaska]	0.217	0.451	0.300	29.95	<0.0001
B	[Southern Mountain, Boreal, Northern Mountain] [Barren-ground] [Alaska]	0.355	0.478	0.191	19.08	0.00293
C	[Southern Mountain, Boreal, Northern Mountain] [Barren-ground, Alaska]	0.353	0.486	0.206	20.58	0.00293
D	[Southern Mountain, Boreal] [Northern Mountain, Barren-ground, Alaska]	0.227	0.521	0.380	38.02	<0.0001
E	[Southern Mountain] [Boreal] [Northern Mountain, Barren-ground, Alaska]	0.230	0.474	0.317	31.70	<0.0001

expected) for each locus across populations were calculated using GENALEX 6.4 (Peakall & Smouse 2006). FSTAT 2.9.3 (Goudet 2001) was used to calculate Allelic richness and linkage disequilibrium (LD). Deviations from HWE and LD were tested per locus and between each pair of loci for each population, respectively. Estimated  $P$  values were calculated under a Markov chain method using 10 000 simulations.  $P$  values for tests were corrected using a strict Bonferroni adjustment (initial  $\alpha = 0.01$ ) for multiple comparisons.

We analysed population structure at two distinct levels. First, the Bayesian-clustering program, STRUCTURE v2.3 was used to conduct admixture and assignment tests to evaluate inferred population clusters based on multi-locus genotype data (Pritchard *et al.* 2000; Falush *et al.* 2003). Five independent runs were performed for each  $K$  value (1–20) using 2 million iterations with a burn-in period of 500 000. Assignment tests were run under the default settings with the admixture model. The standard procedure to determine the probability of how the data best fit into each number of  $K$  clusters is by calculating the mean posterior probability of the data [ $\log$  probability of data;  $L(K)$ ]. Individuals are assigned probabilistically to a single cluster or to multiple clusters if their genotypic profile indicates admixture. Evanno *et al.* (2005) used an ad hoc quantity based on the second order rate of change of the likelihood function with respect to  $K$  ( $\Delta K$ ). Evaluation of assignment test functions (Waples & Gaggiotti 2006) indicates that both methods work well when genetic differentiation is strong, but under moderate genetic differentiation the standard procedure performed better. Here, we apply both approaches to allow not only for the identification of the uppermost level of population hierarchy, but also clusters that may indicate sublevels of structuring (Evanno *et al.* 2005).

Finally, we used conventional  $F_{ST}$  population pairwise comparisons (Weir & Cockerham 1984) to assess the genetic relationships among currently recognized herds and their associated ecotype designations. MSA 3.0 (Dieringer & Schlötterer 2003) was used to estimate these  $F_{ST}$  values and significance was calculated using 10 000 randomizations, correcting for type I errors using sequential Bonferroni adjustment. The resulting distance matrix was visualized, similar to mtDNA data, through an MDS plot. As with mtDNA, we assessed the significance of isolation by distance using IBD (Bohonak 2002).

## Results

### Mitochondrial DNA

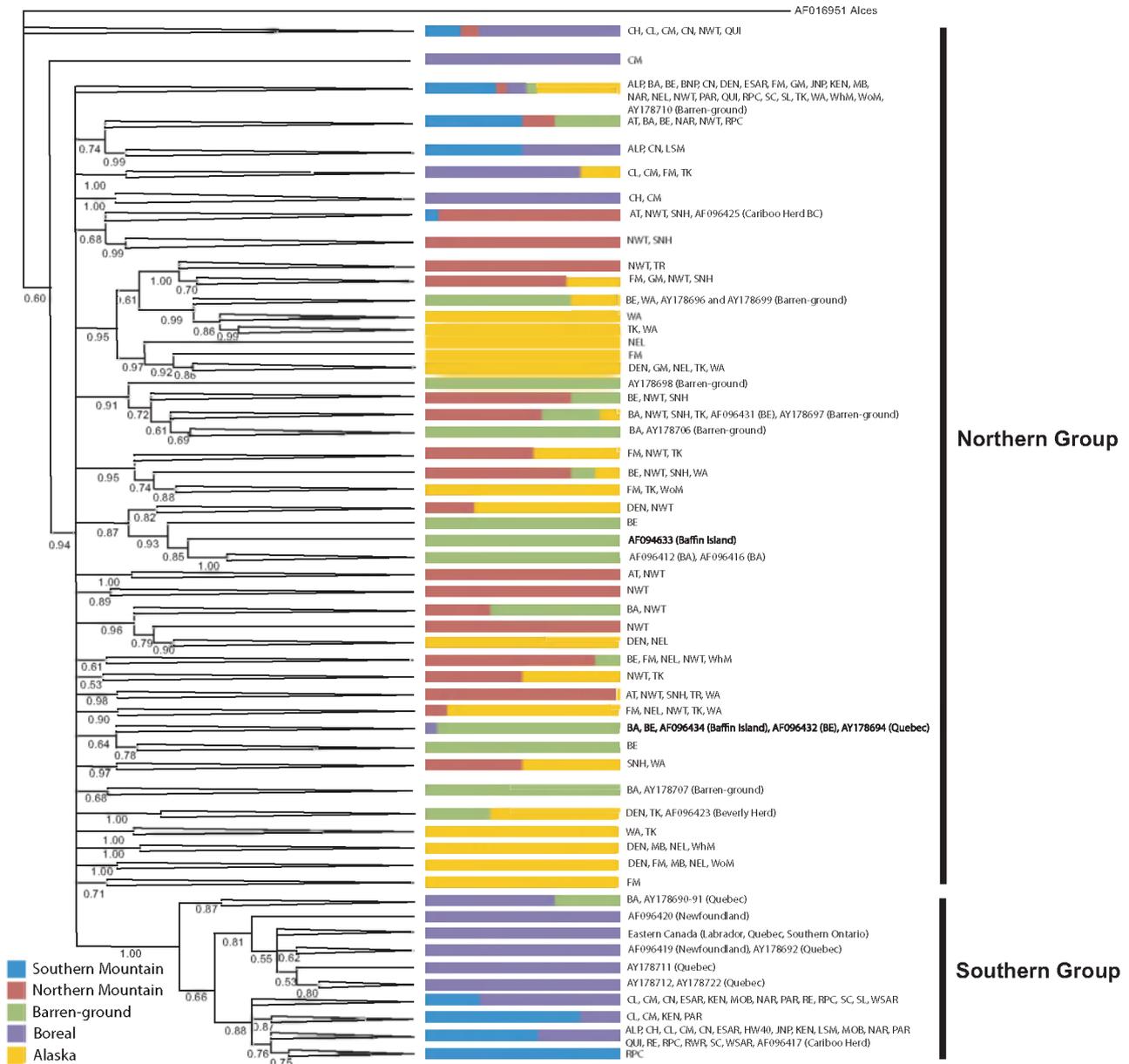
A total of 709 individuals were sequenced for mtDNA control region. This yielded 85 polymorphic sites that

included 89 different mutations which totalled 151 haplotypes (17 from McDevitt *et al.* 2009, GenBank accession numbers EU915191–EU915207; and 134 unique haplotypes, GenBank accession numbers JQ743347–JQ743480). There was a tendency for most haplotypes to be endemic to one of the specific caribou types; 15, 10, 24, 29 and 56 haplotypes were unique to the Southern Mountain, Boreal, Northern Mountain, Barren-ground and Alaska caribou types, respectively. The remaining 17 haplotypes were shared by at least two caribou types, but no haplotype was found among more than three types.

The haplotype phylogeny (Fig. 2) reflects a high level of variation in caribou of North America. As in previous studies, the phylogeny can be organized approximately into two primary groups (net divergence between groups was 2.5%, 95% CI: 1.8–3.2%); a Southern group consisting of mostly woodland caribou that presumably originated from southern refugia (net divergence within South was 0.8%, 95% CI: 0.6–1.0%), and a Northern group that has a Beringian origin (net divergence within North was 2.1%, 95% CI: 1.7–2.5%). The Southern group is almost entirely made up of Southern Mountain and Boreal individuals, while the Northern group includes Northern Mountain, Barren-ground and Alaska individuals. As described in McDevitt *et al.* (2009), a number of woodland caribou individuals from Boreal and Southern Mountain herds have haplotypes that fall within the Northern group, conversely only a single haplotype from the Northern Mountain, Barren-ground or Alaska herds (rt105, from BA) was found in the Southern group.

Population pairwise  $\Phi_{ST}$  comparisons tended to support significant differentiation between herds (Table S1, Supporting information). Most non significant relationships were in intra-type comparisons and between Boreal and Southern Mountain types, a pattern further supported by a significant correlation of pairwise  $\Phi_{ST}$  and geographic distance ( $P = 0.001$ ; Fig. S1, Supporting information). The MDS plot of the  $\Phi_{ST}$  distance matrix (Fig. 3) distinguishes two primary clusters, one containing herds from Boreal and Southern Mountain types (Southern Group) and the other from Northern Mountain, Barren-ground and Alaska types (Northern Group). Within the Southern Group, Boreal and Southern Mountain types scatter indiscriminately, while in the Northern Group, the three different types segregate from one another. The low measure of stress (0.019) indicated a good fit of the data in two dimensions.

When populations are organized according to three a priori (as currently accepted) and two a posteriori (as for our study) hypothesized models of hierarchical grouping, AMOVA results support better performance of a posteriori models (Table 2). Model A tests the five



**Fig. 2** Bayesian phylogeny of 151 caribou haplotypes and 34 GenBank sequences. Outgroup is moose (*Alces alces*). Support values >0.50 are provided below branches. Triangles at terminal nodes indicate where structure <0.50 and unresolved polytomies were collapsed into a single branch for clarity. At the end of each branch a coloured bar represents proportion of individuals of each caribou ‘type’ that had haplotype(s) at that branch (colour legend in lower left). Also indicated are from which herds those individuals originated (abbreviations as in Table 1).

hypothesized caribou types, and indicates this level of organization explains 30.0% of all genetic variation. Model B tests the subspecies designations currently accepted (i.e. *R. t. granti*, *R. t. groenlandicus* and *R. t. caribou*) and Model C tests the Beringian group that combines Alaska and Barren-ground and a Southern group that includes all herds currently classified as *R. t. caribou* (Boreal, Northern Mountain and Southern Mountain ecotypes). Although both groupings are significant, they do not perform as well as Model A,

explaining 19.1% and 20.6% of genetic variability, respectively. Model D tests the Northern and Southern groups as identified in Fig. 3, which reassigns the Northern Mountain ecotype of woodland caribou as having a Beringian origin, and explains 38.0% of between-group variation. Model E tests the new Northern group and separates Boreal and Southern Mountain ecotypes into distinct groups, explaining 31.7% of group variance. Consequently, our analysis supports currently accepted ‘ecotypes’, but not subspecies.

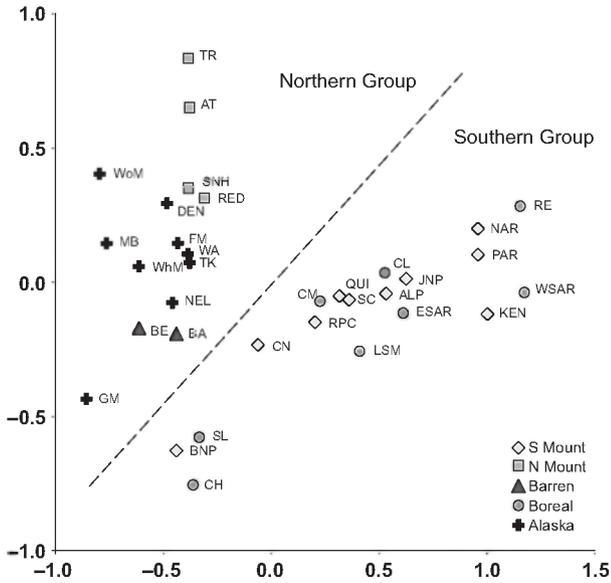


Fig. 3 The multi-dimensional scaling (MDS) plot visualizing, in two-dimensional space, a population pairwise  $\Phi_{ST}$  distance matrix (Table S1, Supporting information) that reflects evolutionary relationships between populations. All herds with less than five individuals have been dropped from the analysis.

Tajima's  $D$  was not significantly negative for either the North or South group ( $D = -1.11$ ,  $P = 0.13$ ;  $D = 0.60$ ,  $P = 0.79$ , respectively), however significant Fu's  $F_S$  values ( $F_S = -24.43$ ,  $P < 0.01$ ;  $F_S = -24.72$ ,  $P < 0.01$ , respectively) and non-significant  $D^*$  and  $F^*$  ( $P > 0.05$ ) for both North and South groups indicate demographic expansion. Additionally, non significant ( $\alpha = 0.01$ ) raggedness indices from mismatch distributions for both North and South groups ( $r = 0.007$ ,  $P = 0.049$ ;  $r = 0.024$ ,  $P = 0.207$ , respectively) fail to reject the null model of historically expanding populations (Rogers & Harpending 1992). In aggregate, tests for population expansion support expansion in both the North and South groups.

Microsatellites

A total of 802 individuals provided reliable microsatellite amplification (Table 1). Each locus, except for BM6506, deviated from HWE in one or more herds, but no locus deviated significantly from HWE in all herds. Similarly, every herd showed LD at one or more pairs of loci, but none of the loci had a significant pattern of LD at all pairs of loci. There was no evidence of systematic allelic dropout, null alleles or possible scoring errors. At the coarse scale, different indices of microsatellite variability revealed some differences across caribou groups. Allelic richness per population varied from 1.98 to 3.39, and per caribou type from 9.98 to 13.73,

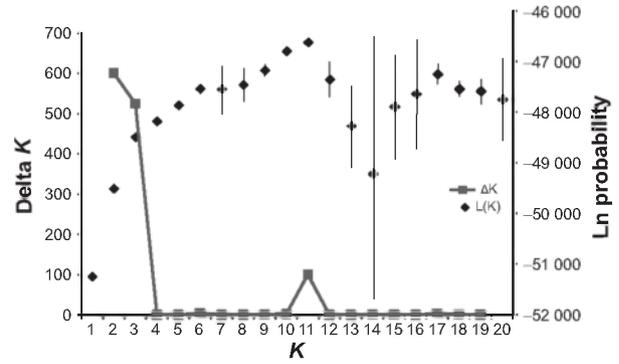
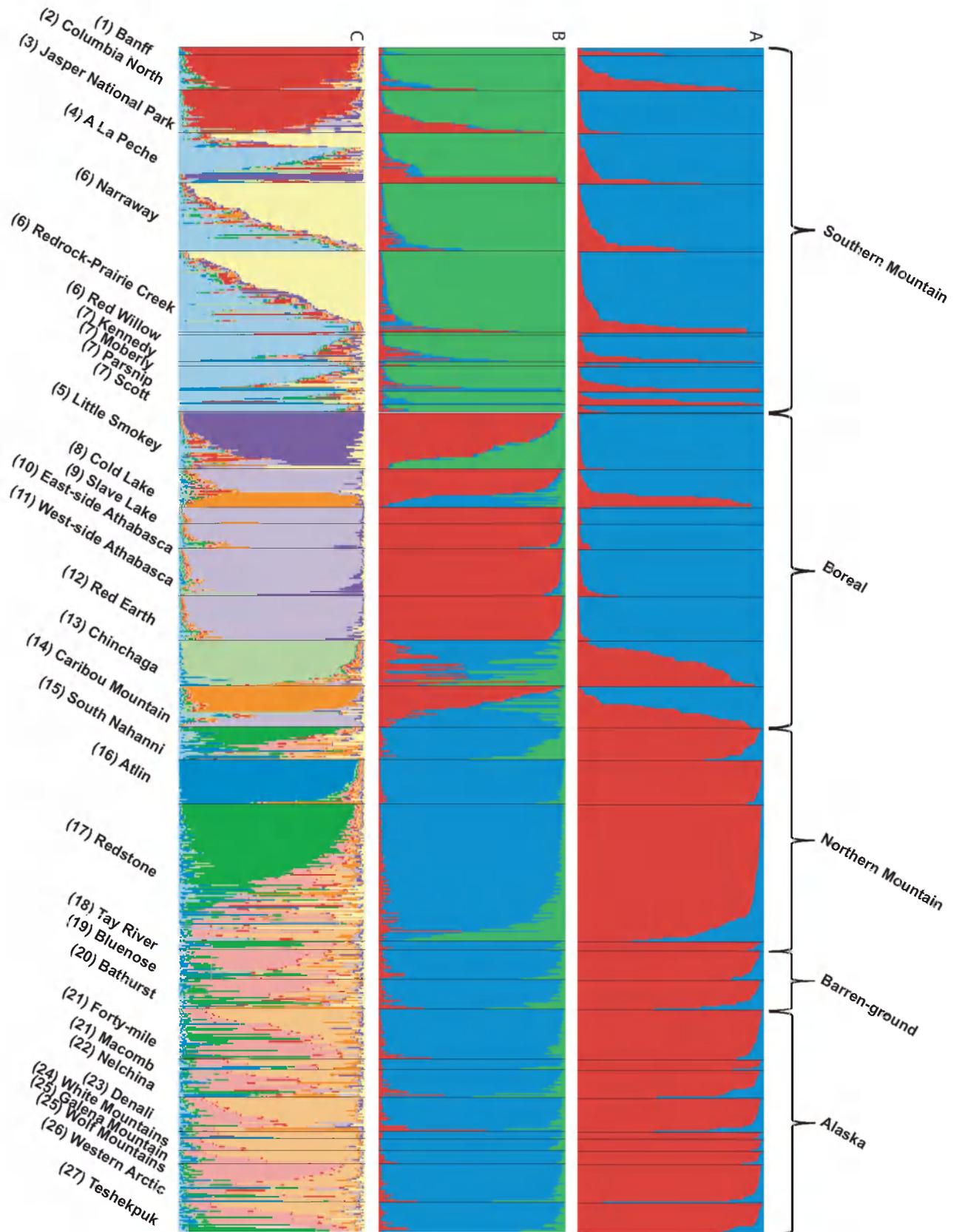


Fig. 4 Bayesian clustering analysis for 808 individuals analysed at 14 microsatellite loci using STRUCTURE (Pritchard *et al.* 2000). Ln probability (right y-axis; Pritchard *et al.* 2000) and Delta K (left y-axis; Evanno *et al.* 2005) indicate the most probable number of distinct population clusters ( $K$ , x-axis).

and was marginally significantly different among the five caribou types ( $P = 0.043$ ). The Northern Group (comprising Northern Mountain, Barren-ground and Alaska types, see above) exhibited significantly greater allelic richness and observed heterozygosity ( $P = 0.001$  for both) from the Southern Group (comprising Southern Mountain and Boreal, see above), while the Southern Group had significantly higher  $F_{ST}$  ( $P = 0.002$ ) from the Northern Group.

Calculation of  $L(K)$  and  $\Delta K$  revealed some discordant results (Fig. 4). Using the standard procedure (Pritchard *et al.* 2000) of calculating  $L(K)$ , results support  $K = 11$  (Fig. 4). Following the Evanno method (Evanno *et al.* 2005),  $K$  was optimized at three values (2, 3 and 11). A histogram of individual assignment at  $K = 2$  (Fig. 5A) displays a clear delineation of the same Southern and Northern groups defined in the mtDNA MDS plot (Fig. 4). The histogram of  $K = 3$  (Fig. 5B) further distinguishes the Southern Group into Boreal and Southern Mountain types, whereas the Northern Group retains fidelity to assignment within a single group. Notably, admixture of Boreal and Southern Mountain individual genotypic signatures is apparent in a number of herds that are at the boundary between different ecotypes, such as ALP and LSM. Other herds (i.e. CL, CH and CM) are also admixed, indicating either gene flow in the recent past, or the lack of precision at  $K = 3$  to fully describe the genetic variability in all herds. Finally, the histogram at  $K = 11$  (Fig. 5C) describes the fine scale relationships among herds. Here BNP, JNP and CN herds form a single group in the central Canadian Rockies whereas other Southern Mountain herds appear to be admixtures of two different genetic signatures. Boreal herds tend to separate into four groups with the LSM, CH and CM herds largely showing discrete signatures and the remaining Boreal herds grouping into a single



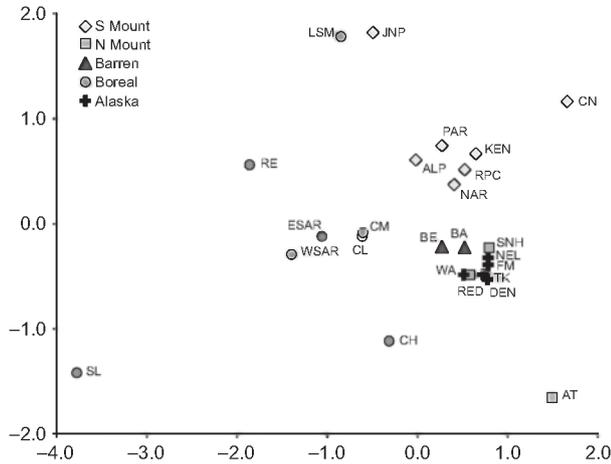


Fig. 6 Multi-dimensional scaling (MDS) of microsatellite population  $F_{ST}$  distances (Table S2, Supporting information). Populations with fewer than 10 individuals have been dropped from this analysis.

cluster. AT and RED herds of the Northern Mountain type also show relatively endemic assignment, whereas the rest of the Northern Mountain and Alaska herds show no clear delineation of clusters, but rather an admixture of 2–3 different genotypic signatures.

$F_{ST}$  values between caribou herds ranged from 0.01 to 0.32 (Table S2, Supporting information). As with the mtDNA pairwise  $\Phi_{ST}$  comparisons, most significant genetic distances were in inter-type comparisons. Controlling for the number of herds analysed, a Mantel test shows significant correlation between the  $F_{ST}$  and  $\Phi_{ST}$  matrices ( $P < 0.0001$ ). Notably, with just one exception, no comparisons between two Barren-ground and the five Alaska herds analysed showed significant differentiation. The MDS plot (Fig. 6) demonstrates a tightly clustered, often overlapping, relationship among the Barren-ground, Alaska and most Northern Mountain herds (except geographically distant AT). In contrast to what was observed for mtDNA, microsatellite data unambiguously distinguish Boreal and Southern Mountain types from each other, as well as detect strong divergence within these types (Fig. 6). Pairwise  $F_{ST}$  distances were not significantly correlated with geographic distance ( $P = 0.199$ ; Fig. S1, Supporting information).

Discussion

Phylogeography and evolutionary history

Isolation and expansion from refugia during Pleistocene glacial cycles have left a genetic legacy across many taxa (Fontanella *et al.* 2008; Latch *et al.* 2009) and is often apparent in the phylogenetic discontinuities sometimes reflected in subspecific taxonomic classifications. These patterns are evident even in large, vagile mammals including wolves (Weckworth *et al.* 2010), black bears (Peacock *et al.* 2007) and mule deer (Latch *et al.* 2009). A similar pattern appears to also describe the phylogeographic history of caribou. Phylogenetic patterns in mtDNA diversity followed previous work (Flagstad & Røed 2003; Cronin *et al.* 2005; McDevitt *et al.* 2009) by revealing that two distinct phylogenetic assemblages of caribou are diverged into Northern and Southern groups. However, here we demonstrate for the first time that the Northern Mountain ecotype of woodland caribou shows a clear genealogical heritage with that of the Northern group (Figs 2 and 3) and as such its taxonomical status has up until now been grossly misperceived.

Generally, herds identified as belonging to the Northern group show genetic levels of variability and haplotype divergence that would be expected under a model of repeated demographic expansion and contraction from Beringian refugia through multiple glacial-interglacial cycles. The polytomy of the Northern group mtDNA phylogeny (Fig. 2) corresponds to the extensive geographic range to which Beringian caribou have subsequently expanded to following the LGM. It is possible that we were unable to resolve the polytomy due to incomplete lineage sorting, or due to insufficient data with a 497 bp fragment of mtDNA. However, this is consistent with other studies on caribou (Flagstad & Røed 2003; McDevitt *et al.* 2009). Additionally, tests of expansion for the Northern group appear to corroborate a pattern of rapid population expansion. Herds in the Northern group tended to demonstrate greater number and variability in mtDNA haplotypes as compared to the Southern group (Table 1), strengthening the view that caribou originated in eastern Beringia during the

Fig. 5 Taxonomic subdivision and individual assignment test of 808 individuals using microsatellites in the program STRUCTURE (Pritchard *et al.* 2000). Results illustrate each individual’s herd (below figure) with its putative ecotype (above figure). Each bar represents an individual’s assignment into  $K$  clusters. Statistical results based on both the standard calculation of  $L(K)$  and Evanno’s  $\Delta K$  indicate that the best  $K$  is 2, 3 and/or 11. At  $K = 2$  (A), this analysis demonstrates a test of the hypothesis of the Northern Group (red), of Beringian origin, and the Southern Group (blue), of conterminous US origin. (B) Assignment of individuals at  $K = 3$ , and can be evaluated as a test of the hypothesis of three distinct subspecies; *Rangifer tarandus granti* (Alaska), *R. t. groenlandicus* (Barren-ground) and *R. t. caribou* (woodland; Southern Mountain, Northern Mountain and Boreal). (C) Assignment of individuals at  $K = 11$ , and likely reflects contemporary patterns of gene flow among the current herd designations. Numbers adjacent to herd names coincide with herd location on Fig. 1.

early Pleistocene (~1.6 Ma) as species diversity tends to be the greatest in regions of their origin (Tishkoff *et al.* 2009). That introgression of haplotypes is not reciprocal, with only Northern haplotypes found in Southern populations, suggests that population expansion and admixture were fostered from a north to south direction. Although analysis also tended to support demographic expansion in the Southern group, the life history characteristics of woodland caribou may have limited the extent of their expansion to tracking mature forests as it became available post glaciation. The lack of distinction between Southern Mountain and Boreal herds at the phylogenetic level of mtDNA (Figs 2 and 3) versus their divergence at microsatellites (Figs 5b and 6), suggests that specialization into mountain vs. boreal habitats is a more recent life history characteristic.

Microsatellite assignment tests also reflect the geographic split of the Northern and Southern groups (Fig. 5a). At  $K = 2$  there is still a very clear distinction of the two groups identified with mtDNA sequences. This suggests limited contemporary gene flow between herds of the two groups, in spite of contact zones across their distributions. This apparent lack of genetic connectivity may be related to landscape features that discourage or prevent caribou dispersal, or to behavioural and/or ecological mechanisms that reinforce divergence and reproductive isolation (e.g. migratory vs. non migratory behaviour). Alternatively, contact zones may exist where intermixing between groups produces individuals with mixed microsatellite genotypic signatures, and we have simply not sampled them in this study. Most herds in Fig. 5a demonstrate some admixture. In most cases, a direct migrant is unlikely due to the geographic distance that would be required. In herds with only a few admixed or miss-assigned individuals, this may reflect a signature of historic gene flow, an idea supported in a previous study that has identified an apparent hybrid swarm of the Northern and Southern groups in Alberta (McDevitt *et al.* 2009). Conversely, the apparent admixture may simply reflect a level of genetic variability of individuals that cannot be clearly distinguished at the coarse scale imposed at  $K = 2$ .

Phylogenetic diversity among populations in the Southern group is much lower than in the Northern group (Table 1; Fig. 2). In our study, the major phylogenetic pattern in the Southern group is an apparent divergence of western and eastern North American haplotypes (Fig. 2). This suggests that post glacial expansion of woodland caribou may have been initiated from two distinct refugial sources. Given the ecological variability within Beringia (Lozhkin *et al.* 2011), the relative distinction of the three different types of caribou in the Northern group (i.e. Northern Mountain, Barren-ground and Alaska) may indicate differentiation of

caribou during glacial maxima, and/or may reflect different waves of expansion during interglacials (Waltari *et al.* 2007). The latest expansion following the LGM appears to have also resulted in the mixing of the two clades of woodland and tundra refugial groups within Alberta. This region was some of the first terrestrial habitat to be vacated by glacial ice as the Cordilleran and Laurentide ice sheets began to recede (Catto *et al.* 1996; Shapiro *et al.* 2004). Our findings confirm the previously detailed hybrid swarm within the Canadian Rockies of Alberta (McDevitt *et al.* 2009), but here extended sampling also reveals boreal herds in Alberta with similarly mixed origins (Fig. 2).

#### *Population structure and management implications*

Large mammals are often typified by high dispersal capability and having distributions that range across multiple habitat types. If these species are also structured genetically as a result of different ecological specializations, then calculating the degree to which ecological variation drives genetic differentiation is important. A number of studies have begun to emerge that demonstrate an association of genetic differentiation of populations and ecological differences. These include studies of other mammals with similar distributions as caribou, such as wolves (*Canis lupus*; Musiani *et al.* 2007; Munoz-Fuentes *et al.* 2009) and lynx (*Lynx lynx*; Rueness *et al.* 2003).

In the caribou populations analysed here, microsatellite analyses clearly separate Boreal and Southern Mountain 'ecotypes'. These two groups are primarily distinguished based on different migratory (Southern Mountain) vs. non migratory (Boreal) behaviours (COSEWIC 2002). Our sampling scheme does not provide for the comparison of geographically contiguous populations of different behavioural types, with the exception of Little Smoky (Boreal) and A La Peche (Southern Mountain). These herds are directly adjacent to each other on the landscape (Fig. 1) and appear to exchange some migrants (Fig. 5c). Nonetheless, gene flow between the two herds does not appear to be sufficient to prevent distinctive genotypic signatures. This suggests that Boreal and Southern Mountain caribou may have reached a stage whereby interbreeding would be disfavoured. However, it remains unclear as to whether this level of genetic distinction parallels other ecological, behavioural and life history characteristics, and whether adaptive genomic regions also reveal distinctiveness between groups. Additional analysis, emphasizing sampling of geographically adjacent populations of different putative ecotypes and the use of genome-wide approaches, will provide the framework to address these outstanding questions.

The premise of conservation efforts on individual species is to not only preserve the species itself, but also the evolutionary potential and processes that have created that diversity (Moritz 2002). A prerequisite to this is a clear understanding of the distribution and patterns of diversity across a species' distribution. Our results develop a new understanding of caribou diversity and call for a restructuring of current caribou classification that acknowledges the division of two main clades in western North America and the distinction of Northern Mountain woodland caribou from Boreal and Southern types. This revised classification should similarly be reflected in both federal and provincial jurisdiction recovery plans as well as COSEWIC's re-evaluation of caribou designatable units.

Our results also have important implications for recovery planning within the subspecies level. For example, in the draft boreal woodland caribou recovery plan (Environment Canada 2011), the functional conservation unit is considered the individual local population (i.e. herd) with little consideration of meta-population genetic structure at a higher level than the local population. Our results suggest that indeed, some intermediate level of meta-population structure may be worthy of consideration as a conservation unit rather than or in addition to the local population level. The seeming correspondence between some of the potential conservation units (e.g., Alberta boreal) and ecozones (Boreal plain; Ecological Stratification Working Group 1995) is striking, and suggests the hypothesis that caribou meta-populations may be a functional conservation unit. In other cases, however, specific local populations may be the functional conservation unit (e.g. Little Smoky). However, interpretation of microsatellite data warrants some caution because of the lag-time in genetic signatures and the confounding patterns of modern habitat fragmentation, and because of the greater potential for genetic drift in small populations (Landguth *et al.* 2010). For example, although Fig. 5c could exemplify meta-population dynamics in woodland caribou, these genetic patterns may largely be reflective of long-term population dynamics established in caribou generations of the recent past, but not necessarily current levels of gene flow. This is exemplified in comparing van Oort *et al.* (2011), who show little successful dispersal of radiocollared individuals among Southern Mountain caribou herds, to Serrouya *et al.* (2012), who show genetic clustering of the same herds using microsatellite data.

The disparity in telemetry data versus patterns of genetic variability is paramount towards conservation and management policy. Figure 6 shows threatened Boreal and Southern Mountain herds scattered across the graph in contrast to the tight clustering of herds

from the Northern Group. This pattern likely reflects the declining numbers of the southern herds and the subsequent genetic isolation and drift. A lack of dispersal accompanied by small population size can lead to rapid genetic drift (i.e. phenomena documented for caribou in this region; van Oort *et al.* 2011; Serrouya *et al.* 2012). As a result, the fragmentation of the meta-populations that we described might preclude gene flow or a rescue effect for locally extirpated populations as predicted in meta-population theory (Pannell & Charlesworth 2000; Hanski & Gaggiotti 2004). This alarming effect is most clearly illustrated with the Banff herd of the Canadian Rockies, with their persistent bottleneck over the past several decades (and demonstrated low genetic variability; Table 1) and their eventual extirpation in an avalanche in 2009 (Hebblewhite *et al.* 2010). Clearly, conservation and management strategies should emphasize fostering connectivity among caribou populations and maintaining demographically viable population sizes.

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B.W.'s research focuses on using molecular ecology and phylogenetics tools to inform policy and management efforts on species of conservation concern. M.M. is a biologist who uses both spatial and molecular ecology methods to address species evolution, management and conservation issues internationally. A.D.M. is interested in applying genetic techniques to examine dispersal, range expansions and colonization histories of mammals. M.H. studies the interrelationships between habitat, populations, and conservation of terrestrial carnivores and ungulates. S.M. is a population biologist interested in the application of evolutionary theory and interdisciplinary approaches to resolve practical issues relevant to conservation and exploitation management.

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### Data accessibility

DNA sequences: Genbank accessions JQ743347–JQ743480.

Microsatellite data: DRYAD entry doi:10.5061/dryad.gn22271h.

### Supporting information

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

**Data S1** Methods.

**Table S1** Pairwise estimates of mtDNA  $\Phi_{ST}$  for all population pairs for herds with more than four individuals sampled.

**Table S2** Pairwise estimates of nuclear microsatellite  $F_{ST}$  values for all populations pairs for herds with more than 10 individuals sampled.

**Fig. S1** Relationships between pairwise geographic distances and pairwise  $F_{ST}$  (triangles) and  $\Phi_{ST}$  (squares) comparisons.

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