

Survival in the Rockies of an endangered hybrid swarm from diverged caribou (*Rangifer tarandus*) lineages

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Abstract

In North America, caribou (*Rangifer tarandus*) experienced diversification in separate refugia before the last glacial maximum. Geographical isolation produced the barren-ground caribou (*Rangifer tarandus groenlandicus*) with its distinctive migratory habits, and the woodland caribou (*Rangifer tarandus caribou*), which has sedentary behaviour and is now in danger of extinction. Herein we report on the phylogenetics, population structure, and migratory habits of caribou in the Canadian Rockies, utilizing molecular and spatial data for 223 individuals. Mitochondrial DNA analyses show the occurrence of two highly diverged lineages; the Beringian–Eurasian and North American lineages, while microsatellite data reveal that present-day Rockies' caribou populations have resulted from interbreeding between these diverged lineages. An ice-free corridor at the end of the last glaciation likely allowed, for the first time, for barren-ground caribou to migrate from the North and overlap with woodland caribou expanding from the South. The lack of correlation between nuclear and mitochondrial data may indicate that different environmental forces, which might also include human-caused habitat loss and fragmentation, are currently reshaping the population structure of this postglacial hybrid swarm. Furthermore, spatial ecological data show evidence of pronounced migratory behaviour within the study area, and suggest that the probability of being migratory may be higher in individual caribou carrying a Beringian–Eurasian haplotype which is mainly associated with the barren-ground subspecies. Overall, our analyses reveal an intriguing example of postglacial mixing of diverged lineages. In a landscape that is changing due to climatic and human-mediated factors, an understanding of these dynamics, both past and present, is essential for management and conservation of these populations.

Keywords: adaptation, conservation, endangered species, hybridization, migration, phylogeography

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Introduction

Pleistocene biogeographical events have traditionally been seen as a major force in molding modern species diversity and distribution. Glacial–interglacial cycles, in particular, had a profound impact on the evolutionary histories of organisms in the northern latitudes (Webb & Bartlein 1992;

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Hewitt 1996), where species were forced to drift latitudinally, and experienced large-scale range contraction and expansion (Hewitt 2000; Lessa *et al.* 2003). During these cycles, the ability to migrate through large-scale contiguous habitat and corridors was a key adaptation for species survival (Hewitt 2000; Shapiro *et al.* 2004). In North America, one of the routes through which species travelled — including many still-extant large mammals — was the corridor that became ice-free approximately 14 000 BP, along the eastern slopes of the Rocky Mountains, connecting Beringia to regions of North America south of the ice sheets (Catto *et al.* 1996;

Shapiro *et al.* 2004). Caribou (*Rangifer tarandus*), in particular, during the late Pleistocene, were largely separated into populations north and south of the glacial ice (Dueck 1998; Geist 1998; Flagstad & Røed 2003); caribou south of the ice sheets colonized the boreal forest and mountains of western North America (the North American lineage; NAL), while caribou north of the ice sheets in Beringia specialized in the tundra environments of the arctic (the Beringian–Eurasian lineage; BEL). This resulted in the evolution of two of the three main subspecies considered today, woodland (*Rangifer tarandus caribou*) and barren-ground (*Rangifer tarandus groenlandicus*) caribou, respectively (Dueck 1998; Geist 1998; Flagstad & Røed 2003; Cronin *et al.* 2005). Current designation of caribou subspecies is based upon a variety of (and often conflicting) criteria in different studies including habitat, genetic, morphological, and behavioural differentiation (Banfield 1961; Geist 1998; Cronin *et al.* 2005).

In Canada, woodland caribou are listed as endangered or threatened by both national and provincial governments; of 61 known populations, ~25% are at immediate risk of extirpation, with two local extinctions in the last decade (Hatter *et al.* 2004; Wittmer *et al.* 2005a). The future for woodland caribou in North America is bleak due to increasing human-induced habitat loss and alterations of predator–prey dynamics (James & Stuart-Smith 2000; McLoughlin *et al.* 2003; James *et al.* 2004; Wittmer *et al.* 2005b; Hebblewhite *et al.* 2007a), as well as the potential impact of global warming (Weber & Flanigan 1997; Schaefer 2003; Post & Forchhammer 2007).

The management of these endangered populations is further complicated by the uncertainty over taxonomic status and distribution, and population delineation, all of which are the central premise of recent recovery strategies (Government of Canada 2002; Hatter *et al.* 2004; Alberta Woodland Caribou Recovery Team 2005; Wittmer *et al.* 2005a). Woodland caribou are divided into three further 'ecotypes' by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2002): boreal, southern mountain, and northern mountain. Unfortunately, provincial governments use different, noncomplementary classifications: Alberta recognizes two ecotypes, 'mountain' and 'boreal', which are classified according to migrant and nonmigrant behaviour, respectively. This classification approach highlights how woodland caribou may migrate too, which is not consistent with the sedentary behaviour generally described for this subspecies. British Columbia distinguishes 'northern' and 'mountain' ecotypes, which are defined according to whether they feed, respectively, on terrestrial or arboreal lichens.

Our primary objective was to examine the genetic structure of woodland caribou in the Rockies where two out of the three federally designated ecotypes occur and the provincial designations may be confounding. Using a combination of

mitochondrial DNA (mtDNA), microsatellites, migratory behaviour and spatial ecology, we addressed (i) what patterns of genetic substructure best represent the caribou populations in the studied area; (ii) whether mtDNA and microsatellites provide congruent patterns of diversification; (iii) what processes underlie the current patterns of genetic structure (e.g. glacial vicariance, current migratory dynamics, habitat fragmentation, etc.). The combined results provide an intriguing picture of caribou allopatric evolution and subsequent hybridization of diverged lineages in the Rockies. Our findings generate evidence that should be taken into account for future conservation strategies and are an example of species responses to long-term changes in climate and habitat.

Materials and methods

Study area

The ~50 000 km² study area in western Alberta and eastern British Columbia (Fig. 1) lies in the Central Rockies Ecosystem (White *et al.* 1995). Banff and Jasper National Parks form one of the world's largest World Heritage Sites including smaller adjoining protected areas such as Kakwa Provincial Parks and the Willmore Wilderness (Fig. 1). The study area also includes private and multi-use public lands. Long winters and short, dry summers characterize the climate, with precipitation increasing from east to west (Holland & Coen 1983). The topography is portrayed by flat valley bottoms surrounded by the steep slopes of the Rocky Mountains (400–3937 m). From east to west, habitat types include montane, subalpine, and alpine ecoregions which correspond to increasing elevation and decreasing annual productivity (Holland & Coen 1983). Roads, seismic exploration lines, and railways are present especially in the relatively more populated eastern part of the study area.

Spatial data

We investigated 12 different caribou herds as part of our study, where herd was defined usually by provincial wildlife agencies as a 'local population', typically according to the watershed frequented (Fig. 1). In total, our study area likely contains approximately 1000 caribou distributed in 12 local herds (Thomas & Gray 2002). At least three of these herds are known to be in decline (Table 1).

Caribou were captured via helicopter net-gunning and darting by trained personnel between 1 October and 31 March, 2001–2007, under approved university and government animal handling protocols. To monitor migration, seasonal home range overlap, herds overlap, and breeding locations, we outfitted adult (> 1.5 years old) female caribou ($n = 231$) with either very high frequency (VHF) or global positioning system (GPS) radiocollars (LOTEK). GPS collars were

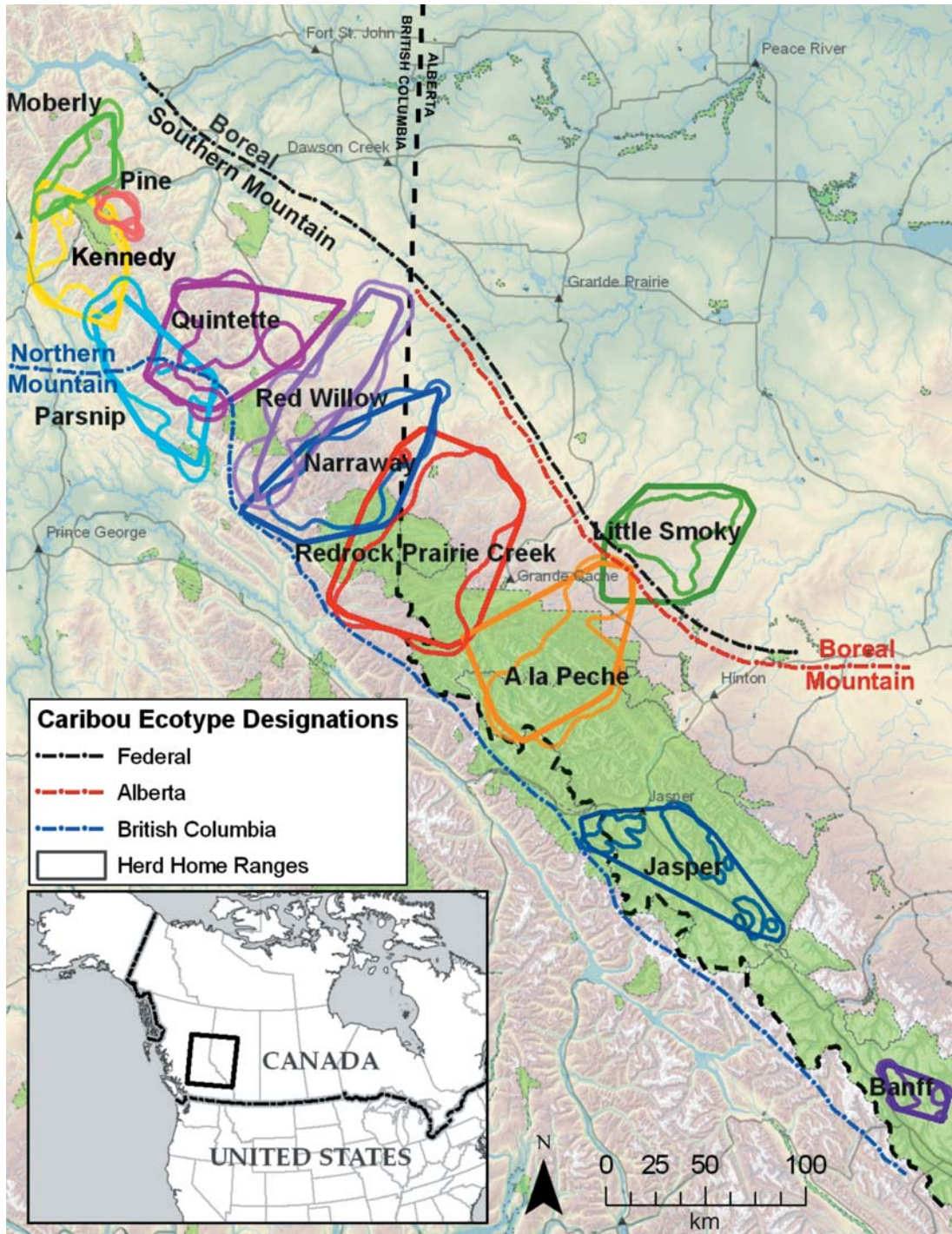


Fig. 1 Ranges of caribou herds analysed in this study (minimum convex polygons; straight lines on map) and core areas used by monitored individuals (kernel 95% probability polygons; curved lines) in the Canadian Rocky Mountains, Alberta (AB) and British Columbia (BC) provinces. Dotted lines delineate the boundaries between federal (marked as Federal) and provincial ecotype designations of herds (AB or BC). Green shading depicts national parks and provincial protected areas; topography and major roads are also indicated.

collected from 4 to 12 locations per caribou per day with an average fix location error of 33 m (Hebblewhite *et al.* 2007b). VHF-collared caribou were located from fixed-wing or rotary wing aircraft approximately once per month for an

average of 12–14 locations/year per caribou. Each individual caribou was monitored an average of 2.1 years. For subsequent genetic analyses, we designated a single, average location of those collected during the fall rutting period, from

mid-September to mid-November of each year (Dyer *et al.* 2001, 2002). Criteria for estimating average breeding location for each individual are detailed in Appendix S1, Supporting information.

We estimated the spatial overlap between caribou herds to be compared to their degree of genetic differentiation. We calculated the coefficient of overlap (Cole 1949; Walls & Kenward 2001; Wronski & Apio 2006) between ranges of caribou herds (95% kernel probability polygons):

$$O = 2 A_{12} / (A_1 + A_2)$$

where O is the coefficient of overlap, A_{12} is the area of overlap of the 95% kernel probability polygons of any two herds (herd 1 and herd 2), A_1 and A_2 are the 95% kernel probability polygons calculated for all sampled caribou in herd 1 and herd 2, respectively. Coefficient of overlap values range from zero (herds fully separate) to one (herds fully sympatric).

We characterized individual caribou as migratory or sedentary following Craighead *et al.*'s (1972) definition of ungulate migration as movement between seasonally nonoverlapping, allopatric ranges. We used the same coefficient of overlap also used for herds (see above) to assess overlap between summer ranges (1 July–15 September; Dyer *et al.* 2001, 2002) and winter ranges (1 December–30 April; Smith *et al.* 2000). A given caribou was considered as migratory if it showed nonoverlapping ranges (Fig. 2a), partially migratory if it migrated in some years but not in others, and as sedentary if seasonal ranges were overlapping (Fig. 2b).

Genetic data

Blood samples were taken from the 231 female caribou that were also spatially monitored (see above). DNA extractions were carried out using the DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacturer's protocol. We examined population structure and phylogeny using a 470-bp fragment of the mitochondrial control region that was amplified from 160 individuals using the primer pair L15394 and H15947 according to Flagstad & Røed (2003). Sequencing was carried out by MacroGen Inc.. Chromatogram contigs were assembled in Sequencher 4.2 (Gene Codes) and sequences were aligned manually using Se-Al 2.0 (Rambaut 1996).

Of the 231 individuals, 223 were reliably polymerase chain reaction (PCR) amplified and genotyped at 11 polymorphic microsatellite loci: RT1, RT5, RT6, RT7, RT9, RT13, RT24, RT27 (Wilson *et al.* 1997), BL42, BM4513 and BM6506 (Bishop *et al.* 1994). Individuals were genotyped in 10- μ L multiplex reactions containing 1 μ L of DNA extract and 1 \times Multiplex PCR MasterMix (QIAGEN). Primer concentrations ranged from 0.15 to 0.5 μ M. Amplification conditions

Table 1 Caribou herds, federal and provincial ecotype designation, and conservation status (COSEWIC 2002; Hatter *et al.* 2004; Alberta Woodland Caribou Recovery Team 2005; Wittmer *et al.* 2005a)

Herd	Ecotype designations			Status	N (ms/mt)	H_E	A_R	Percentage of partially migratory individuals	Percentage of migratory individuals	Percentage of individuals in BEL
	Code	Federal	Alberta							
Redrock-Prairie Creek	RPC	Southern mountain	Mountain	Northern	Stable	0.793	5.762	11	100	56
Narraway	NAR	Southern mountain	Mountain	Northern	Unknown	0.789	6.051	0	100	8
Little Smoky	LSM	Boreal	Boreal	—	Risk of extirpation	0.669	5.074	60	68	38
A La Peche	ALP	Southern mountain	Mountain	Northern	Stable	0.799	5.993	57	68	30
Parsnip	PAR	Southern mountain	—	Mountain	Increasing	0.804	6.141	18	18	6
Kennedy	KEN	Southern mountain	—	Northern	Stable	0.795	5.972	55	73	7
Jasper	JNP	Southern mountain	Mountain	Northern	In decline	0.751	5.198	20	70	42
Quintette	QUI	Southern mountain	—	Northern	Stable	0.837	6.545	55	64	45
Moberly	MOB	Southern mountain	—	Northern	Stable	—	—	67	67	0
Pine	PIN	Southern mountain	—	Northern	Stable	—	—	0	0	0
Red Willow	RWR	Southern mountain	—	Northern	Unknown	—	—	0	100	0
Banff	BAN	Southern mountain	Mountain	Northern	Risk of extirpation	—	—	0	50	100

Sample sizes for telemetry (N) and genetic analyses (ms, individuals genotyped at 11 microsatellite loci and mt, individuals sequenced for mtDNA), genetic diversity indices from microsatellite markers (H_E , expected heterozygosity; A_R , allelic richness), proportion of partially migratory and migratory individuals in each herd, and percentage of individuals per herd in the mtDNA Beringian–Eurasian lineage (BEL), Alberta and British Columbia, Canada, 2001–2007.

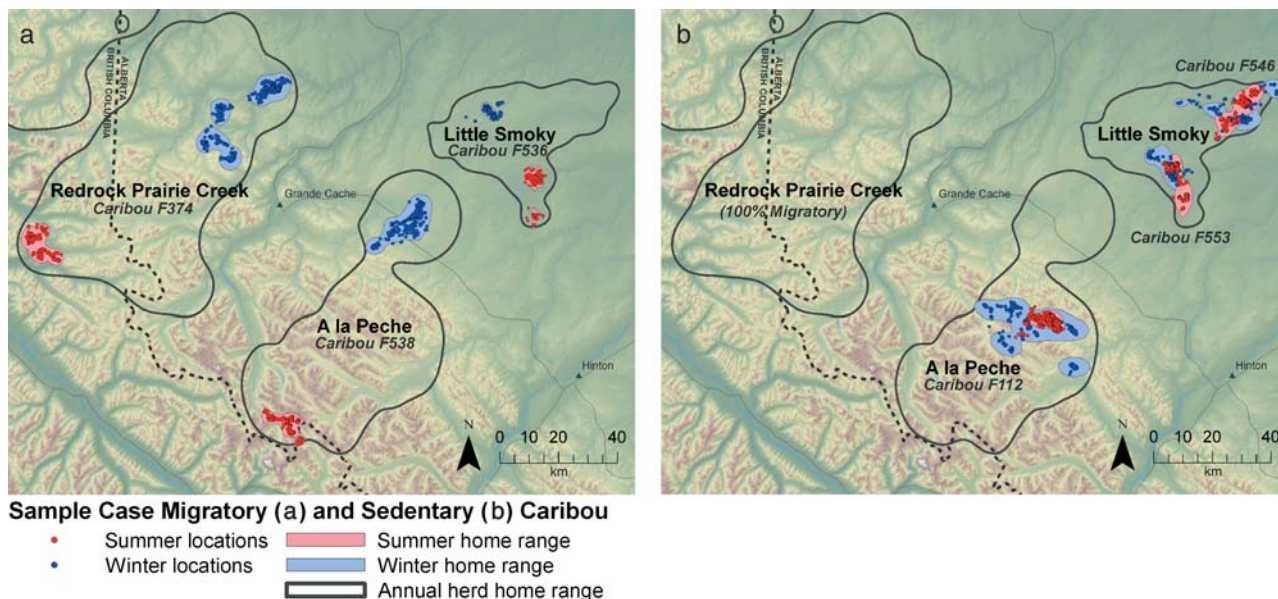


Fig. 2 Sample case of summer and winter global positioning system locations and home ranges (95% probability kernels; in red and blue tones, respectively) depicting migratory and sedentary behaviours of caribou (panels a and b, respectively) in Alberta and British Columbia, Canada. Summer and winter seasons were defined according to regional caribou behaviour (1 July–15 September, Dyer *et al.* 2001, 2002; 1 December–30 April, Smith *et al.* 2000). The annual home ranges for other caribou monitored in each herd are also indicated (named as in Fig. 1). Sedentary behaviours were not observed in the Redrock Prairie Creek herd, which included migratory or partially migratory individuals only (see Table 1).

were as follows: 95 °C for 15 min; 35 cycles of 94 °C for 45 s, 54 °C for 90 s, 72 °C for 1 min and a final extension at 72 °C for 45 min. All PCR products were 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 version 4.0 (Applied Biosystems). Thirty individuals (13.1%) chosen randomly from the data set were re-extracted and re-amplified for the 11 loci. This resulted in two allelic dropouts being observed which translates into a genotyping error rate of 0.3% per allele. This rate is considered low enough not to affect the results significantly in studies of population substructure (Bonin *et al.* 2004; Frantz *et al.* 2006). The software Micro-Checker (van Oosterhout *et al.* 2004) was used to check for the presence of null alleles, large allele dropout and possible scoring errors in each herd comprising at least eight individuals.

Mitochondrial DNA analysis

Control region haplotypes were identified using DAMBE 4.5.50 (Xia & Xie 2001). Nucleotide (π) and haplotype (h) diversity were calculated using DnaSP 4.10.9 (Rozas *et al.* 2003). Neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) phylogenetic trees of control region haplotypes were constructed in PAUP* 4.0b10 (Swofford 1998). A web BLAST search revealed that both the NAL (mostly *Rangifer tarandus caribou*) and the BEL (mostly

Rangifer tarandus groenlandicus) control region haplotypes were present in our samples (Dueck 1998; Flagstad & Røed 2003). Therefore, 35 extra control region sequences from these two lineages (Dueck 1998; Flagstad & Røed 2003) were downloaded from GenBank and included in the phylogenetic analyses. NJ and ML trees were constructed using the HKY85 + I + G (Hasegawa *et al.* 1985) model ($I = 0.6236$; $\alpha = 0.4758$) selected by the Akaike information criterion (AIC, Burnham & Anderson 1998) in ModelTest 3.06 (Posada & Crandall 1998). Bootstrap support was calculated from 1000 NJ, MP and ML parametric replicates, respectively. Bayesian posterior probabilities were estimated in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) from four chains over 1 000 000 generations sampled every 1000th generation excluding a burn-in of 100 000 steps. Elk (*Cervus elaphus*) and moose (*Alces alces*) were used as outgroups. Median-joining networks of the lineages identified by phylogenetic analysis were also constructed using Network 4.5 (Bandelt *et al.* 1999) to graphically illustrate relationships among haplotypes within lineages in the Rockies.

Net divergence (D_n) between the mtDNA lineages found in the study area was calculated in MEGA version 4.0 (Tamura *et al.* 2007) using Kimura 2-parameter distances (Kimura 1980). Standard errors (SE) were calculated by bootstrapping with 10 000 replicates. The genetic distance between the lineages identified by phylogenetic analysis was corrected for ancestral mtDNA polymorphism (Edwards 1997). Estimates for divergence time (T) between lineages

for control region data were calculated by $T = Da/2\mu$, where 2μ is the evolutionary rate. An evolutionary rate of 58.9%/million years based on the bovine control region (Ho *et al.* 2008) was chosen because Ho *et al.* (2005, 2007) have argued that evolutionary rates are vastly underestimated at the intraspecific level and therefore population expansions and divergences have often been overestimated. Therefore, the rate originally used for caribou (16%/million years; Flagstad & Røed 2003) will greatly overestimate divergence and expansion times. Mismatch distributions of pairwise nucleotide differences of each lineage were calculated and compared with expected values for a population/lineage undergoing a sudden demographic expansion (Rogers & Harpending 1992) using tests for goodness-of-fit statistics based on the sum of square deviations (SSD) for a model of sudden expansion in Arlequin version 3.11 (Excoffier *et al.* 2005). Tests for goodness-of-fit were generated using parametric bootstrapping with 10 000 replicates. Expansion times for lineages were estimated from the mismatch distribution (τ) of each lineage, using the formula $\tau = 2\mu t$ (where τ is the time since expansion in units of $1/2\mu$ generations), using a generalized least squares approach adapted from Rogers (1995), as described in Schneider & Excoffier (1999). Generation time was assumed to be an average of 4 years (Adams & Dale 1998; Boulet *et al.* 2007).

A spatial analysis of molecular variance (SAMOVA) based on F -statistics was performed with SAMOVA version 1.0 (Dupanloup *et al.* 2002) to investigate the distribution of genetic diversity over the whole data set. Significance of F -statistics was tested by 100 000 permutations. The software SAMOVA version 1.0 implements a method to define groups of populations that are geographically homogeneous and maximally differentiated: the groupings that maximized F_{CT} values (among-group variance) and minimized F_{SC} values (among populations, within-group variance) were assumed to be the most probable geographical subdivisions. Herds with two to three individuals (see AMOVA analysis) were not included. A Mantel test was also performed to check if there was any association between mtDNA- and microsatellite-based pairwise matrices of 'herd' F_{ST} differentiation. Significance was tested using 1000 randomizations (Mantel 2.0, A. Liedloff © 1999).

Microsatellite analysis

We tested for population substructure using three analysis frameworks for microsatellite loci. Initially we made no assumptions as to the number or the spatial localization of samples, and applied the clustering algorithm implemented in Structure 2.2 (Pritchard *et al.* 2000; Falush *et al.* 2003) using all 223 successfully genotyped individuals. Ten independent runs were performed for each K value (1–10) using 500 000 iterations with a burn-in period of 50 000. Structure was run using the default settings (Pritchard *et al.*

2000; Falush *et al.* 2003) with the admixture model. The number of clusters (K) was calculated by obtaining the mean posterior probability of the data (log probability of data; $L(K)$) over the 10 independent runs. Individuals were assigned to clusters based on their highest membership coefficient to a particular cluster averaged over the 10 independent runs.

The program Structure has known limitations and its results may depend upon sampling protocols and distribution of samples (Schwartz & McKelvey 2008). In our second population substructure analysis, we incorporated the average breeding season location (described in Appendix S1) for each caribou, using the spatially explicit software TESS 1.2 (Chen *et al.* 2007). TESS was run using the Markov chain Monte Carlo algorithm with the admixture model. We first used three initial starting values for the interaction parameter, which represents spatial interactions ($\theta = 0.6$; 0.75 and 0.9) to select the optimal interaction parameter using likelihood ($\theta = 0.6$). Next, 10 independent simulations were performed for each K value (1–10) using 50 000 iterations and a burn-in period of 10 000 iterations to identify which K values produced the highest likelihood runs (K_{max}). We then conducted 100 simulations for each K_{max} (Fedy *et al.* 2008), and estimated membership probabilities were calculated from the 20 highest likelihood simulations for each K_{max} (Chen *et al.* 2007; Fedy *et al.* 2008).

Finally, we used conventional F_{ST} analysis (Weir & Cockerham 1984) to compare the above results with those obtained by considering the currently accepted herd denominations. FSTAT 2.9.3 (Goudet 1995) was used for estimating pairwise F_{ST} values (among inferred clusters or known herds) and significance was calculated using 10 000 simulations. Pairwise comparisons were corrected for type I errors using sequential Bonferroni corrections (Rice 1989).

FSTAT 2.9.3 was also used to estimate the inbreeding coefficient, F_{IS} , for each locus in each herd (and each inferred cluster) with significance levels calculated by randomizing alleles among individuals within each herd and cluster. We then compared this to the observed data to determine deviations from Hardy–Weinberg equilibrium (HWE), using 10 000 simulations. Tests for linkage disequilibrium between all pairs of loci were carried out in FSTAT 2.9.3. Expected heterozygosity (H_E ; Nei 1978) and allelic richness (A_R) for each herd was calculated (Table 1). We used analysis of molecular variance (AMOVA) implemented in Arlequin (Excoffier *et al.* 2005) in order to test whether the existing federally and provincially designated 'ecotype' subdivisions (see Table 1) could represent biologically meaningful groupings for the studied herds. The AMOVA was performed at three levels: between herds within ecotypes (F_{SC}), between herds overall (F_{ST}), and among ecotypes (F_{CT}) using 10 000 permutations. Due to low samples sizes (two to three individuals, see Table 1) the Moberly, Pine, Red Willow and Banff herds were not included.

Integration of spatial ecology and genetic data

We tested the hypothesis that the BEL was nonrandomly distributed among herds using chi-squared tests. At the individual level, we then tested: (i) whether caribou that migrate have a higher probability of bearing the BEL; (ii) whether different herds differed in their baseline 'migration' level (i.e. individuals in different herds may have a different migratory tendency overall); (iii) whether or not individuals belonging to the BEL as a function of migratory behaviour differ between herds due to some unknown latent variable (habitat fragmentation, ecoregion) that reduces certain individual's migration; and (iv) the relationship between migration and the BEL within each possible population designation, namely management 'herd', Structure cluster, or TESS cluster. As the population clusters found with Structure and with TESS were correlated, Structure and TESS results were not included together in the models we tested. To test these hypotheses, we used generalized linear mixed-models (GLLAMM, see Hebblewhite & Merrill 2008) where the probability of an individual caribou belonging to BEL was modelled as a binomial ($1 = \text{BEL}, 0 = \text{NAL}$) logistic function of migratory tendency categories (0=no, 1=maybe, 2=yes), accounting for three different herd structures ('herd', Structure or TESS). The most parsimonious and best-fitting model was selected using AIC (Burnham & Anderson 1998). Details on the equation for our logit GLLAMM is provided in Appendix S1.

Results

Telemetry and migration in traditionally defined herds

We obtained > 500 000 VHF and GPS locations from 231 adult female caribou from 2001 to 2007 throughout the 12 caribou herds. Twenty-five out of 28 range overlap estimates were equal to 0 (herds fully separate; Table S1, Supporting information). The highest value (still relatively low: 0.128) was recorded in the North, between the 'Quintette' and the 'Parsnip' herds. A value of 0.076 was also recorded between 'Parsnip' and 'Kennedy'. A full suite of migratory behaviours from sedentary, to partially or fully migratory characterized each population (Table 1; Fig. 2) The proportion of migratory individuals in caribou herds ranged from 0.18 (in the Parsnip herd) to 1.00 in the Narraway, Red Rock Prairie Creek, and Red Willow herds (Table 1).

Mitochondrial DNA and phylogeography

Twenty-four polymorphic sites were identified in the control region of caribou in the study area which resulted in 17 unique haplotypes (GenBank Accession nos: EU915191–EU915207), neatly joined into the two lineages identified previously (Fig. 3; Dueck 1998; Flagstad & Røed 2003) as

the North American lineage and the Beringian–Eurasian lineage (Boulet *et al.* 2007). Both lineages were found throughout the study area (Fig. 4c). The net divergence between the lineages (corrected for ancestral polymorphism) was 2.21% (95% CI: 1.66–2.75%). This gave an estimate for time since divergence between these lineages at about 37 500 BP (95% CI: 28 100–46 700 BP). Mismatch distributions revealed that the NAL conformed to the sudden expansion model ($P = 0.153$) while the BEL did not ($P = 0.047$). These findings indicated that the NAL expanded from a smaller founder population while the BEL remained comparatively more constant in size. Estimation of time since expansion of the NAL based on Rogers's (1995) τ (1.111; 95% CI: 0.736–1.590) was 8000 BP (5300–11 500 BP).

The NAL (Fig. 4b) comprised of 11 haplotypes found in 113 individuals in our study area. Nucleotide diversity of this haplogroup was low ($\pi = 0.00215 \pm 0.00019$) and haplotype diversity was $h = 0.688 \pm 0.037$. The BEL (Fig. 4a) comprised of six haplotypes in 47 individuals. Nucleotide diversity was almost three times higher than NAL ($\pi = 0.00621 \pm 0.00058$) while haplotype diversity was similar ($h = 0.665 \pm 0.054$).

Population subdivision was evaluated based on mitochondrial DNA using spatial analysis of molecular variance (SAMOVA). The analysis showed that genetic differentiation among groups was significant ($0.167 < F_{CT} < 0.208, P < 0.039$) for two, three, four, five, six and seven groups. However, genetic differentiation between herds within such groups decreased only in the case of seven clusters ($F_{SC} = -0.024, P < 0.023$), which indicated that homogeneity within clusters was achieved at this point (Dupanloup *et al.* 2002). With the organization in seven groups, there was a tendency for the Narraway and Parsnip herds to form one cluster, whereas all other herds clustered separately ($F_{ST} = 0.189, P < 0.000001; F_{CT} = 0.208, P < 0.038$).

Microsatellites and population structure

No evidence of null alleles, large allele dropout or possible scoring errors was found. Tests for linkage disequilibrium over all herds revealed that no loci were linked in recombination. Clustering analysis in Structure revealed the presence of four distinct genetic clusters (Fig. S1, Supporting information). Cluster 2 largely corresponded to the herd in the Little Smoky area, cluster 1 was mostly confined to Narraway and Red Rock Prairie Creek, cluster 3 had a more southerly distribution but also had individuals present far north, while cluster 4 had a mainly northerly distribution (Fig. 5a). Assignment probabilities were highest among individuals belonging to clusters 1 and 2, and 3 (Fig. S2, Supporting information). When spatial data were incorporated, TESS also revealed that the most likely number of clusters was $K = 4$ (average log likelihood: -9162), yielding a picture that is largely in

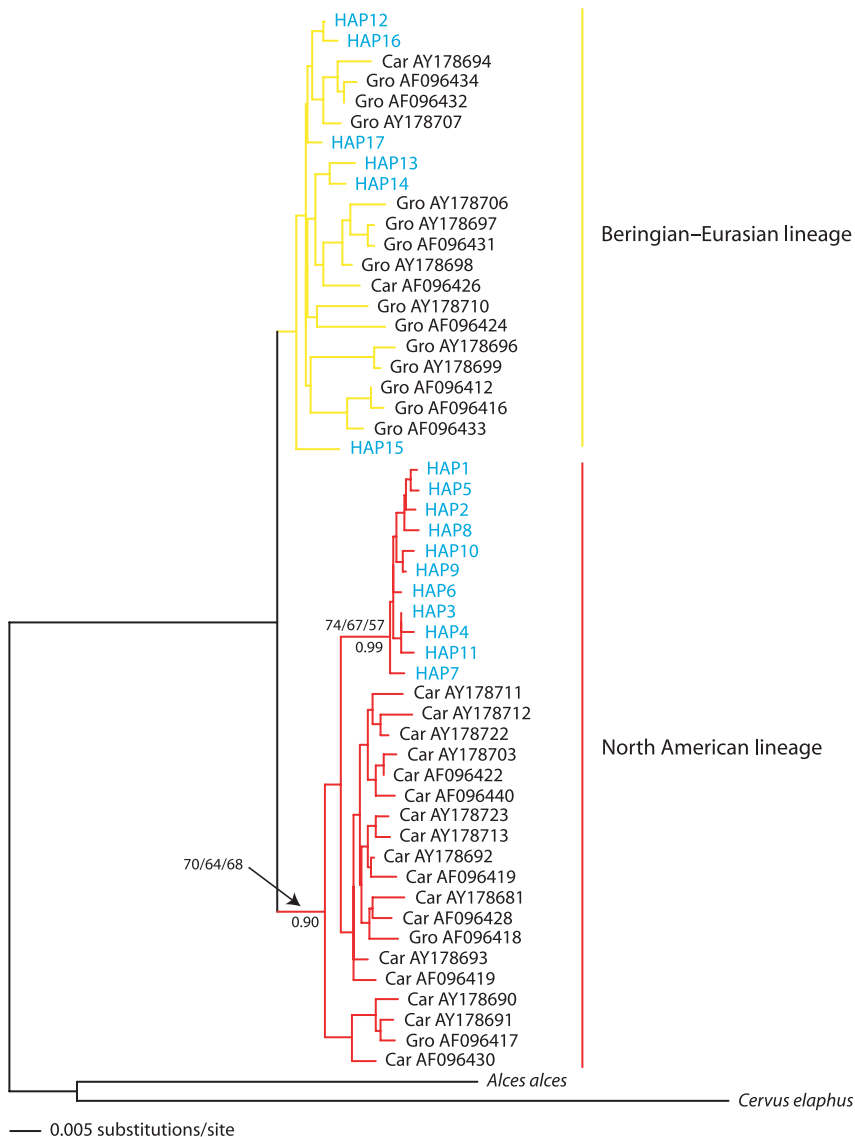


Fig. 3 Neighbour-joining tree of mtDNA control region haplotypes showing the Beringian–Eurasian and North American lineages (in yellow and red, respectively). Haplotypes sequenced in this study area are labelled in blue, whereas sequences from downloaded from GenBank are in black. Bootstrap values are shown on nodes when topology was supported at values > than 50 (NJ/MP/ML and Bayesian).

agreement with that obtained with Structure (Fig. 5b), yet providing a higher degree of detail, which the nonspatially explicit model of Structure cannot fully capture.

No significant departures from HWE were found in any herd or clusters identified by either Structure or TESS. Global F_{ST} values were similar and significant for all groupings tested (herds: 0.044; Structure: 0.048; TESS: 0.042; $P < 0.0001$). All pairwise comparisons of F_{ST} between clusters identified by Structure (pairwise F_{ST} values: 0.020–0.091) and TESS (pairwise F_{ST} values: 0.016–0.067) were significant. When caribou were grouped in herds, several pairwise comparisons were nonsignificant (Table 2). The Quintette herd was not significantly differentiated from the herds in Narraway, Red Rock Prairie Creek and Parsnip. The Parsnip herd was also not significantly differentiated from the herd in Kennedy (Table 2). This mirrors, to a good extent, the data on range overlap (Fig. 1).

Levels of genetic diversity (H_E and A_R) were moderately high among herds (Table 1) and comparable to other population level studies of caribou herds (McLoughlin *et al.* 2004; Zittlau 2004; Boulet *et al.* 2007). However, the Little Smoky herd had lower levels of diversity compared to the other herds (Table 1). Genetic differentiation between Federal ($F_{CT} = 0.031$; $P = 0.005$) and Alberta ‘ecotypes’ ($F_{CT} = 0.029$; $P = 0.015$) was significant but British Columbia ‘ecotypes’ were not significant ($F_{CT} = -0.010$; $P = 0.931$). Spatial autocorrelation analysis conducted on individual caribou found little evidence of genetic correlation for geographical distances greater than 40 km (Fig. S3, Supporting information). Correlation values were significantly different from zero only for pairwise comparisons less than 40 km apart. Consequently, geographical distance does not appear to strongly influence population or individual level differentiation calculated with autosomal microsatellite data.

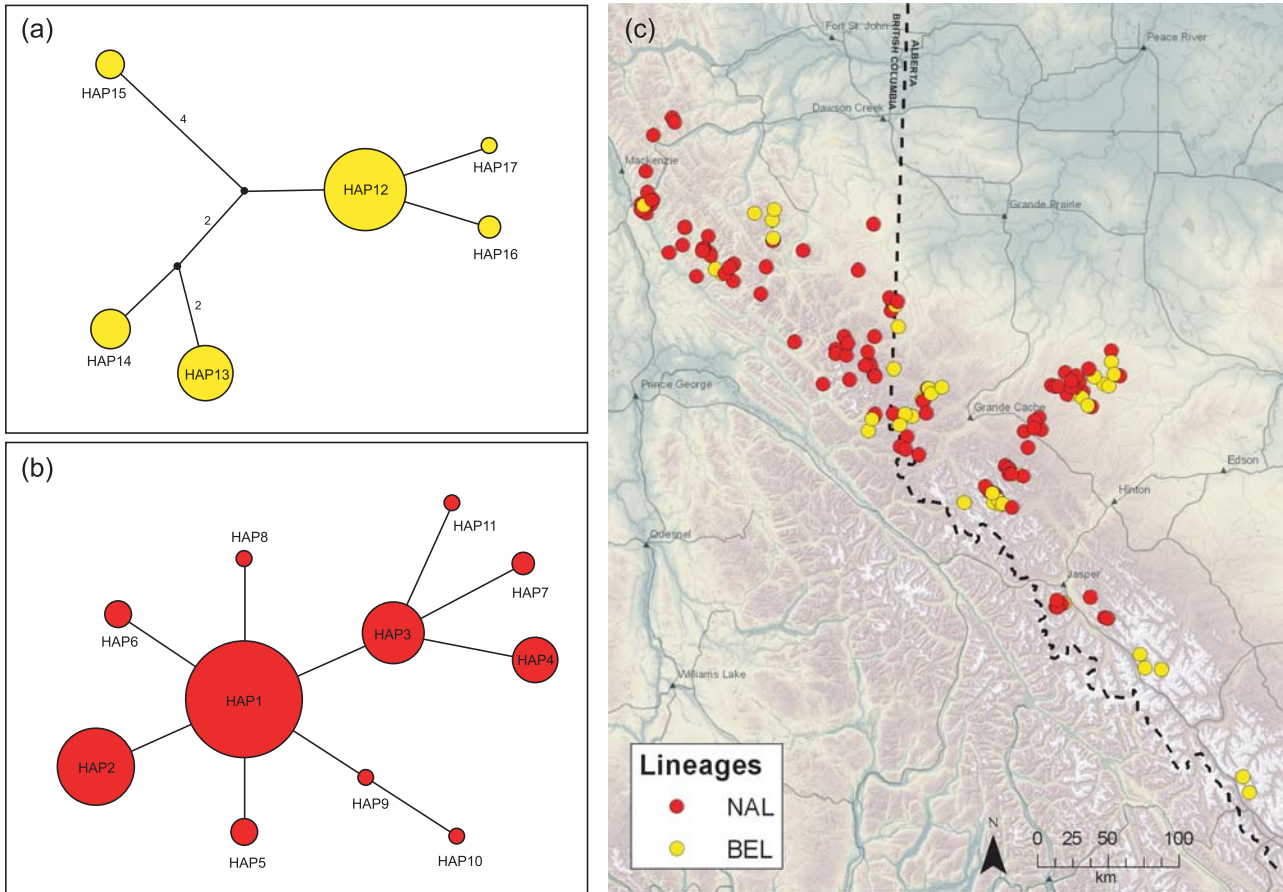


Fig. 4 Median-joining networks of (a) the Beringian–Eurasian and (b) the North American lineages. Numbers on branches indicate number of mutations if greater than one. The geographical distribution of both haplogroups is shown in (c) (red circles: NAL; yellow circles: BEL).

Table 2 Pairwise F_{ST} values between herds using mtDNA (upper diagonal) and microsatellites (lower diagonal).

Herd	RPC	NAR	LSM	ALP	PAR	KEN	JNP	QUI
RPC		0.3656*	0.3100*	0.1537*	0.1344*	0.1895*	0.0459	0.1255
NAR	0.0238*		0.5619*	0.0996	0.2030*	0.5078*	0.3419*	0.1842
LSM	0.0609*	0.0705*		0.3327*	0.2085*	0.3983*	0.3762*	0.3156*
ALP	0.0187*	0.0384*	0.0503*		0.0520	0.3193*	0.0779	0.0244
PAR	0.0191*	0.0339*	0.0749*	0.0232*		0.1449	0.1290	0.0180
KEN	0.0275*	0.0430*	0.0504*	0.0305*	0.0125		0.2704*	0.2563*
JNP	0.0558*	0.0761*	0.0955*	0.0558*	0.0442*	0.0626*		0.0368
QUI	0.0267	0.0252	0.0835*	0.0332*	0.0652	0.0392*	0.0652*	

*represents significance after Bonferroni correction (initial $\alpha = 0.0018$).

However, some differentiation was evidenced in relationship to short-range spatial isolation, which could be due to reproductive isolation of family groups, especially during the breeding season.

The pairwise matrices of F_{ST} differentiation between herds defined ‘a priori’ that were obtained with microsatellites and mtDNA were uncorrelated (Mantel test; $Z = 0.43$; $r = -0.06$; $P = 0.41$). Moreover, we found no patterns of

congruence between the current genetic structure identified by microsatellites and the spatial distribution of the BEL and NAL lineages ($\chi^2 = 0.04$ – 1.3 ; $P = 0.3$ – 0.8).

Association of migratory behaviour and the BEL

At the herd level, there was no association between the two mitochondrial lineages and proportions of ‘migratory’ vs.

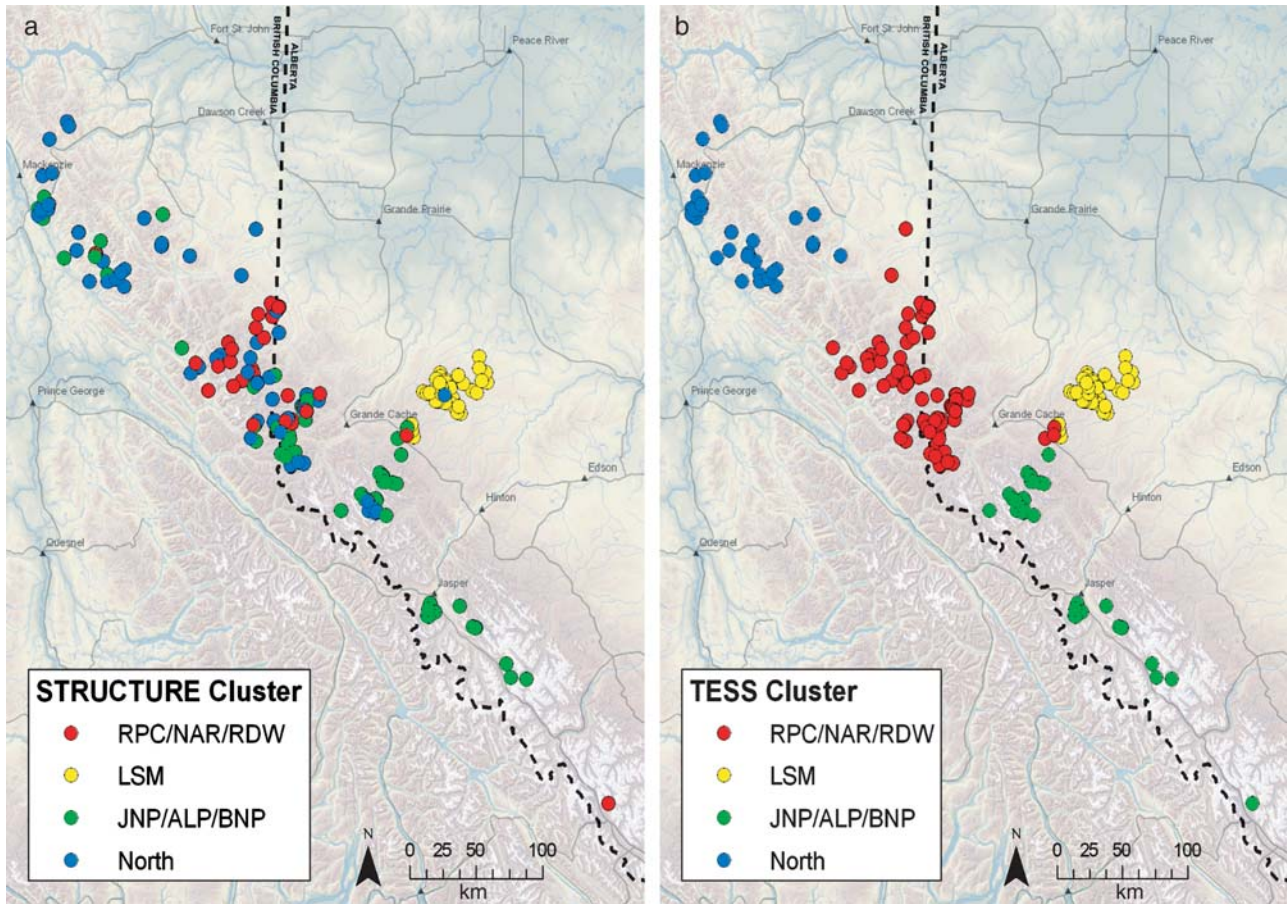


Fig. 5 Geographical distribution of inferred genetic clusters identified by Structure (a) and TESS (b). In both graphs, the red circles largely represent individuals from the Red Rock Prairie Creek and Narraway areas (RPC/NAR/RDW), individual caribou from the distinctive Little Smoky (LSM) area are identified by yellow circles, the green cluster essentially includes A la Peche and Jasper (JNP/ALP/BNP) area caribou and the blue cluster mainly contains northern individuals (north) which corresponds to five herds (QUI, MOB, PIN, PAR and KEN; see Fig. 1 and Table 1).

‘sedentary’ caribou ($\chi^2 = 0.04$; $P = 0.83$). However, at the individual level, there was a positive association between whether or not an individual caribou migrated and belonged to the BEL (Fig. 6). Results of model selection among different ‘herd’ designations (original management herds, Structure, and TESS) confirmed that the most parsimonious and best-supported population structure from the perspective of variation within herds in the relationship between migration and the BEL was the TESS structure (Table S2, Supporting information). The best-supported model was a simplified function that related the probability of belonging to the BEL to whether or not a caribou was migratory (‘yes’ or ‘no’, where ‘possible’ = yes) accounting for differences in the function between migration and BEL for different TESS four herd units (Fig. 6). Although the variance explained by this model was relatively low (pseudo $R^2 = 0.11$), the biological effects were still significant indicating a range of 4–25% increase in migratory probability for individual caribou if they belonged to the BEL lineage (Fig. 6).

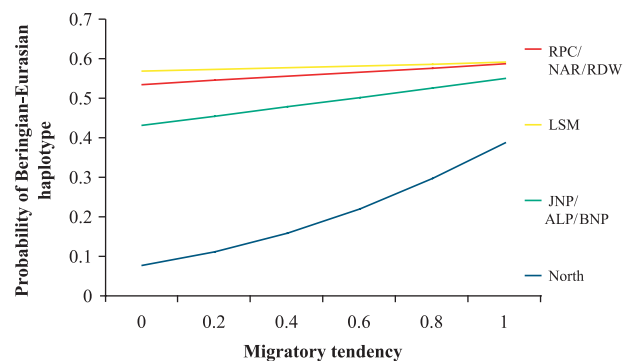


Fig. 6 Relationship between migratory tendency (classified as sedentary, 0, or partially migratory and migratory, 1) and the probability of an individual caribou to belong to the Beringian-Eurasian lineage, conditional on each of the main TESS genetic clusters we identified ($K = 4$, Fig. 5b). Genetic clusters are colour-coded as in Fig. 5(b). Conditional probabilities were derived from a generalized linear mixed model run on individual caribou ($n = 223$).

Discussion

Caribou in our study area have long been considered to belong to the woodland subspecies, with the barren-ground subspecies occurring in the tundra, hundreds of kilometres further north. Here we show unambiguously that the Beringian–Eurasian mitochondrial lineage associated with the barren-ground caribou is present in the Canadian Rocky Mountains (Fig. 4c). Even though the Beringian–Eurasian and North American lineages diverged before the onset of the last glacial maximum (23 000–19 000 BP), nuclear DNA and spatial data clearly demonstrate for the first time that the two lineages have extensively interbred, possibly since the end of the Wisconsin glaciations (~14 000 BP), producing a unique, mixed gene pool, structured into relatively independent, mountain and boreal (Little Smoky) population units. The ice-free corridor constituted by the eastern slopes of the Rockies at the end of the last glaciation (Catto *et al.* 1996; Shapiro *et al.* 2004) likely allowed for the barren-ground caribou to migrate from the North and overlap with woodland caribou populations expanding from the South. The woodland caribou's limited migratory ability and subsequent re-colonization would have been allied to the spread of forested habitat and therefore did not spread far north along this corridor. The expansion of the North American lineage occurred approximately 8000 years ago which is around the time when the spruce forests began to rapidly spread in Alberta 10 000 years ago (Catto *et al.* 1996). This would have allowed the North American lineage to expand, resulting in the 'hybrid zone' in the Rockies that we still observe today.

Major barriers to dispersal and, potentially, to gene flow are not present in the study area, or between the distribution ranges of the barren-ground and woodland caribou in North America (Miller 1982; Geist 1998). In addition, the habitats occupied by the two North American subspecies grade into each other. It is therefore possible that some gene flow is still ongoing in ecotonal areas. This view is consistent with earlier findings that the two subspecies are not completely sorted with regard to mitochondrial DNA haplotypes (Dueck 1998; Flagstad & Røed 2003; Cronin *et al.* 2005). However, the large-scale differences in occurrence of the two mitochondrial lineages in the subspecies indicate some levels of reproductive isolation, which are likely related to adaptation to drastically different environments — open tundra vs. boreal forest. Perhaps more importantly, previous studies could only detect the localized geographical overlap of the two lineages, but could not ascertain conclusively that such co-occurrence was as a result of effective interbreeding of the two lineages. Here, through the integrated use of nuclear and mitochondrial markers, we showed that the previously diverged lineages have interbred in more recent times. Behavioural data also seem to indicate adaptation to a unique mountain environment. Areas

occupied by the Rockies' caribou have mixed characteristics, including both tundra-like alpine and boreal forest areas. In the Rockies, alpine areas and forest areas occur at different altitudes — whereas at the subcontinental scale tundra and forest occur at different latitudes. The spatial and altitudinal range of caribou migration in the Rockies is unique when compared to northern caribou (Musiani *et al.* 2007). Finally, the fact that lineage mixing in our study area occurs relatively far from the obvious ecotone zones between the barren-ground and woodland ranges also suggests uniqueness of these populations.

The lack of correlation between the pattern of microsatellite variation and the spatial distribution of mitochondrial haplotypes indicates that recent and current forces shaping the population structure of caribou in the Rockies are remarkably different from the postglacial phenomena that allowed for the very existence of the studied populations. The signature of significant, spatial segregation of haplotypes in herds across the study area might also suggest some degree of female philopatry (Calef 1981; Miller 1982; Musiani *et al.* 2007) which was also confirmed by telemetry data. In fact, both the telemetry data and the data used for SAMOVA related to female and female lineages (mtDNA). Other studies on caribou spatial ecology and landscape genetics also used a disproportionate number of females: 90% and 83% in studies conducted by McLoughlin *et al.* (2004) and by Boulet *et al.* (2007), respectively. Noticeably, Boulet *et al.* (2007) studied sex biases in dispersal and gene flow and found no significant differences between females and males calculated from both telemetry and genetic data, although future studies using male samples are likely to illuminate aspects of caribou population structure in the study area.

The pattern of microsatellite structuring in our study was consistent with the information from range overlap. However, the almost complete spatial separation among 'herds' in our study area (25 out of 28 values = 0) partially masks the heterogeneous pattern of genetic differentiation observed between pairs of herds, not all of which proved fully genetically isolated. Future telemetry studies including males are likely to complete the picture. In more detail, the data reveal that populations along the northwest–southeast axis of the Rocky Mountains are less divergent than, for instance, the Little Smoky herd, east of Highway 40 (Fig. 1). This herd is spatially contiguous to the A la Pêche herd (Fig. 1), yet from a genetic point of view it constitutes a unique and separate cluster detectable using both mtDNA and microsatellite data and with or without the incorporation of spatially explicit data into analytical models (Table 2, Fig. 5a and 5b). The Little Smoky herd also happens to be the only of 'boreal' ecotype, according to both Federal and Provincial (Alberta) caribou designations, which was alarming in some respects because of its relative isolation from other boreal woodland populations (the

closest other one being > 100 km away and also at immediate risk of extirpation). Yet, the Little Smoky herd also shows a high proportion of barren-ground haplotypes, suggesting that despite having originated from the same postglacial event as the other populations, other factors – possibly anthropogenic – have contributed to its recent independent evolution.

Compared to typical woodland populations elsewhere in Canada, caribou in the Canadian Rockies exhibit a more pronounced tendency to migrate (Bergerud *et al.* 1990; Terry *et al.* 2000; Apps *et al.* 2001; Boulet *et al.* 2007). Our modelling revealed that within the mountain park and the Northern caribou populations (Fig. 6), the probability of being migratory increased if an individual caribou belonged to the Beringian–Eurasian lineage. In these environments, migratory ability may represent an important adaptive trait of these genetically unique ‘hybrid populations’, whose habitat presents the challenge of spatial and seasonal changes in forage quality. In fact, migratory caribou frequent the tundra-like alpine areas during the summer only (Fig. 2), that is, the period of high plant productivity (Shackleton 1999; Apps *et al.* 2001). Clearly the nonadaptive nature of the genetic markers employed here does not allow for the detection of any causal relationship between genetic and phenotypic (behavioural) traits; yet, the presence of a subtle association between what is arguably an adaptive trait and the phylogenetic signature inferred by mtDNA intriguingly echoes work on human mitochondrial variation and adaptation to climate (Mishmar *et al.* 2003; Ruiz-Pesini *et al.* 2004). Although we cannot fully comprehend the actual significance of the detected association, it is important to stress that migration has been an adaptive response to climate change in the past and is predicted to reduce the risk of extirpation given future climate change (Austin & Rehfisch 2005; Broenniman *et al.* 2006; Levinsky *et al.* 2007). Bioclimatic envelope modelling (Levinsky *et al.* 2007) shows that migration ability would reduce mammalian extinctions in Europe by 8% and reduce the risk of species becoming threatened by 30%. Similarly, in Africa, National Parks provide some means to buffer negative effects of climate change, but introduction of migration into the model reduced the number of species becoming critically endangered by 2080 by 30%. Additional research confirms that climatic impacts will be reduced for species with a behavioural predisposition for migration (Austin & Rehfisch 2005; Broenniman *et al.* 2006). Further investigations should evaluate the relationships between the presence of the two mitochondrial DNA lineages in our caribou populations, and the implications for individual fitness and for population survival of the interrelated sedentary and migratory life strategies.

Our study highlights that caribou of the Canadian Rockies illustrate an intriguing example of glacial vicariance creating diverged lineages, followed by a localized postglacial

sympatry, which promoted for the first time the mixing of gene pools that had been evolving independently for several thousand years. Recent studies on insects, fish, amphibians and birds (see Seehaussen 2004; Pfennig 2007; Reyer 2008) have argued that new adaptive genetic diversity can be generated by ‘hybrid swarming’, especially when a new habitat becomes available over a relatively short period of time. The Canadian Rockies caribou exhibit the genetic and phenotypic traits of an intraspecific hybrid swarm that has proved very successful in adapting to the forests and alpine tundra of the postglacial Rocky Mountains environment. In a landscape that is changing due to climatic and human-mediated factors, an understanding of these dynamics, both past and present, is essential for management and conservation of these populations.

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This paper resulted from an interdisciplinary collaboration among authors with expertise in ecology, genetics and conservation biology. ADM is a Postdoc at the University of Calgary (UofC). SM is a Lecturer at UCD Dublin (www.ucd.ie/marbee) and runs the lab where the genetic analyses were conducted. MH is an Assistant Professor at the University of Montana where he also supervises NJD's PhD. LM and DS are ecologists with Weyerhaeuser Company and the BC Government, respectively. BVW is a PhD student supervised by MM, an Assistant Professor at UofC and head of the lab where this research was conceived and organized.

Supporting information

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

Appendix S1 Materials and methods.

Fig. S1 Number of inferred genetic clusters estimated from the mean $L(K)$ (\pm SD) over 10 independent runs in Structure revealing the presence of four distinct population units (Pritchard *et al.* 2000).

Fig. S2 Geographical distribution of membership coefficients for individual caribou assigned to their cluster identified by Structure. Assignment probabilities were highest (> 0.75) among individuals belonging to clusters RPC/NAR/RDW, LSM, and JNP/ALP/BNP (see Fig. 5a).

Fig. S3 Correlograms showing the combined spatial correlation r as a function of distance (in class sizes of 10 km), 95% CI about the null hypothesis of a random distribution of caribou, and 95% confidence error bars about r as determined by bootstrapping. Distances between capture localities for individual caribou are included in the upper panel, whereas the lower panel accounts for distances between caribou breeding locations.

Table S1 Herd home range overlap using 95% fixed kernels and a random sample of 5000 points drawn from the pool of all points for the herd (to obtain equal sample size for all herds). As for the F_{ST} comparisons, herds with low samples sizes (Moberly, Pine, Red Willow and Banff) were not included.

Table S2 Model selection results for generalized linear logistic models relating ability to migrate (0,1) to the presence of the barren-ground haplotype, showing model structure, the log likelihood, the degrees of freedom, Δ AIC, and the Akaike weight (Burnham & Anderson 1998). Sample size = 223 for all models. A, model structure is logit(BEL) = migratory status (Y, yes; P, possible; Y2, yes + possible); random intercept (RI) and random coefficient (RC) for yes or yes2, and the specified grouping unit (TESS, Structure or herd). See equation 1 for details.

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