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Modern and ancient DNA reveal recent partial replacement of caribou in the southwest Yukon

TYLER S. KUHN,*† KERI A. MCFARLANE,‡ PAMELA GROVES,§ ARNE Ø. MOOERS* and BETH SHAPIRO†¶

**Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia, Canada V5A 1S6, †Henry Wellcome Ancient Biomolecules Centre, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK, ‡The King's University College, 9125-50 St. NW, Edmonton, Alberta, Canada T6B 2H3, §Institute of Arctic Biology, University of Alaska Fairbanks, PO Box 757000, Fairbanks, AK 99775, USA, ¶Department of Biology, The Pennsylvania State University, 208 Mueller Laboratory, University Park, PA 16802, USA*

Abstract

The long-term persistence of forest-dwelling caribou (*Rangifer tarandus caribou*) will probably be determined by management and conservation decisions. Understanding the evolutionary relationships between modern caribou herds, and how these relationships have changed through time will provide key information for the design of appropriate management strategies. To explore these relationships, we amplified microsatellite and mitochondrial markers from modern caribou from across the Southern Yukon, Canada, as well as mitochondrial DNA from Holocene specimens recovered from alpine ice patches in the same region. Our analyses identify a genetically distinct group of caribou composed of herds from the Southern Lakes region that may warrant special management consideration. We also identify a partial genetic replacement event occurring 1000 years before present, coincident with the deposition of the White River tephra and the Medieval Warm Period. These results suggest that, in the face of increasing anthropogenic pressures and climate variability, maintaining the ability of caribou herds to expand in numbers and range may be more important than protecting the survival of any individual, isolated sedentary forest-dwelling herd.

Keywords: ancient DNA, caribou, conservation genetics, microsatellite

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Introduction

Conservation and management are often focused on species or populations that have small population sizes or have been significantly impacted by recent events, both of which are known to produce biases in estimates of modern genetic diversity and its inferred ancestry (Leonard 2008). Utilizing the historical perspective afforded by ancient DNA (aDNA) can contribute significantly to the accurate characterization of historic population sizes, levels of gene flow and interpopulation relationships (Ramakrishran *et al.* 2005) – contributions which in turn are critical to appropriate conservation and management decisions. Here, we utilize combined modern microsatellite (short tandem repeats, STR) as well as modern mitochondrial DNA (mtDNA) markers and ancient mtDNA recovered from alpine ice patches to investigate historic interpopulation relationships and levels of gene flow for caribou (*Rangifer tarandus*) in northern Canada.

Caribou (*R. tarandus*) are an iconic holarctic species, representing one of the last remnants of the Beringian megafauna (Kurtén & Anderson 1980; Anderson 1984), and the last remaining large-scale ungulate migration in the northern hemisphere (Berger 2004). The continued presence of caribou throughout their range is under threat; Vors & Boyce (2009) describe a holarctic synchronous population decline of nearly 60% from recent observed population maxima in 34 of 43 major caribou

Correspondence: Arne Ø. Mooers, Fax: (778) 782 3496;
E-mail: amooers@sfu.ca

populations for which data are available. The authors attribute this unprecedented *synchronous* decline to anthropogenic habitat modification and climate warming, demonstrating the fragility of many of these large caribou populations. In North America, the outlook for caribou is grim, in particular for the forest-dwelling woodland caribou, *R. tarandus caribou* (Webber & Flanigan 1997; James & Stuart-Smith 2000; COSEWIC 2002; McLoughlin *et al*. 2003; Schaefer 2003; James *et al*. 2004; Wittmer *et al*. 2005; Hebblewhite *et al*. 2007; Post & Forchammer 2008; McDevitt *et al*. 2009).

Forest-dwelling woodland caribou, hereafter referred to as woodland caribou, are a paraphyletic subspecies of *R. tarandus*. They are almost exclusively found in Canada, as local extirpations have all but removed them from the southern extreme of their range in the United States. In Canada, woodland caribou are further classified into one of four federally recognized and at-risk ecotypes: Atlantic, boreal, southern mountain and northern mountain (Thomas & Gray 2002). Northern and southern mountain caribou are distinguished based on their winter diets (terrestrial vs. arboreal lichens, respectively; Bergerud 2000; Thomas & Gray 2002). The relict Atlantic caribou populations are listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2002), whereas both southern mountain and boreal caribou populations are listed as threatened. Northern mountain caribou are classified as being of special concern (COSEWIC 2002), with many populations appearing to be stable or increasing (e.g., Farnell *et al*. 1998, 2004; COSEWIC 2002; Farnell & Gardner 2003). Northern mountain herds exist as small, isolated, sedentary populations. Within our study region in the southern Yukon, northern mountain herds range in size from 235 to 3750 individuals for the Kluane and Tay River herds, respectively (Yukon Environment Geomatics 2008), with the majority of herds within our study region having populations of less than 1500 (10 of 16 herds, Yukon Environment Geomatics 2008). These small population sizes coupled with the remoteness of their habitat, and several decades of active management (Farnell & Russell 1984; Farnell & MacDonald 1988, 1989; Southern Lakes caribou recovery program 1996; Farnell *et al*. 1998; Boertje & Gardner 2000; Thomas & Gray 2002; Farnell & Gardner 2003) probably underlie the observed stabilizing trend; many herds were in decline prior to management intervention. Notwithstanding current trends, recent work has suggested that these isolated northern mountain populations may be particularly vulnerable to the rapidly changing arctic climate (Webber & Flanigan 1997; Schaefer 2003; Weladji *et al*. 2003; Post & Forchammer 2008; Vors & Boyce 2009).

Caribou conservation and management practitioners have called for research on the temporal persistence of caribou herds, which may provide key information to ensure their long-term survival (Farnell *et al*. 2004). A temporal perspective is also crucial for dealing with the large natural fluctuations in population size that characterize many caribou herds (Kuzyk *et al*. 1999; Boertje & Gardner 2000). Here, we address these questions using mtDNA isolated from caribou that lived in the southwest Yukon over the last 6000 years, in combination with eight microsatellite (STR) loci isolated from modern southern Yukon caribou herds. This combination of genetic markers makes it possible to investigate both short- and medium-term changes in the genetic structure and diversity of northern mountain caribou in the southern Yukon.

Materials and methods

Sample collection

Figure 1 outlines the study area and the locations of the relevant herds sampled for this study. In 1997, researchers observed extremely high concentrations of caribou faecal material preserved in the alpine Thandlät ice patch in the southwest Yukon (Fig. 1; Kuzyk *et al*. 1999). Faecal material from the base of a 1.6-m ice core was radiometrically dated at 2450 ± 50 years before present (BP), opening up the possibility of recovering genetic material from ancient caribou herds (Kuzyk *et al*. 1999). Since 1997, 72 ice patches in the southern Yukon have been surveyed by air and ground, with 35 yielding significant biological and archaeological remains (Farnell *et al*. 2004).

We sampled 40 caribou bone and teeth samples from 13 of these ice patches for control region mtDNA (names in italics in Fig. 1, Appendix S1, Supporting information). These 13 ice patches are located close to or within the present-day range of the Aishihik caribou herd, the Southern Lakes caribou herds (Ibex, Carcross and Atlin herds) and the Wolf Lake caribou herd. Twenty-eight of the caribou remains were radiometrically dated as part of an independent study on caribou heavy metal contaminants. Ages for the remaining twelve samples were estimated using the distribution of dated caribou samples from the relevant ice patches (G. Hare, personal communications; Appendix S1, Supporting information). All dates are reported as ^{14}C BP.

To explore the phylogeographical patterns of modern caribou herds that overlap with the sampled ice patches, genotypes at eight STR loci (BM4513 from Bishop *et al*. 1994, and RT1, RT5, RT6, RT7, RT9, RT24 and RT27 from Wilson *et al*. 1997) were obtained for 395 caribou specimens from 16 herds across southern

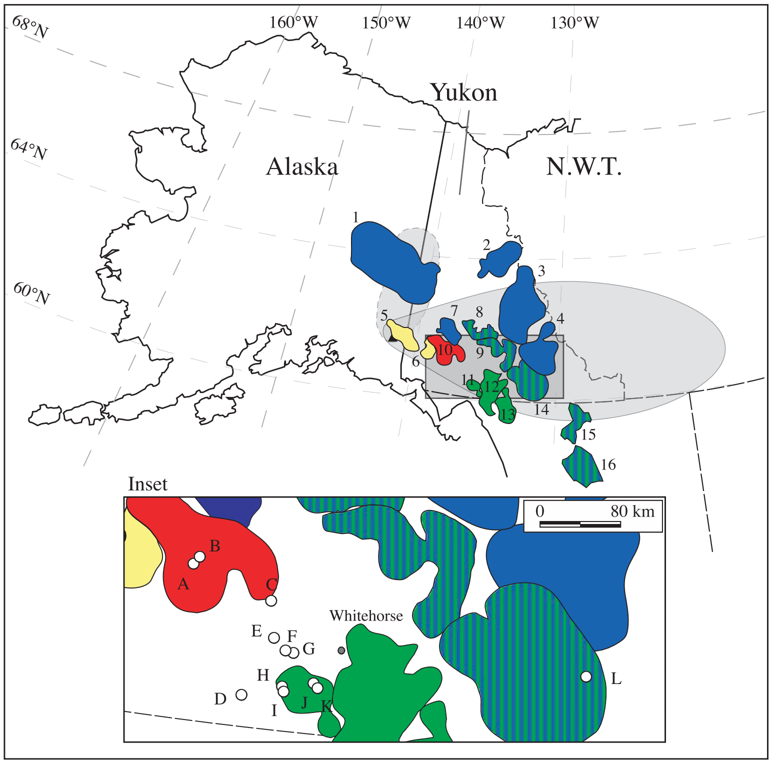

Fig. 1 Location map of Yukon and Alaskan caribou herds showing herd ranges estimated in 2008 (adapted from Zittlau 2004; Yukon Environment Geomatics 2008). Only herds referred to within the present study are shown: (1) Fortymile, (2) Bonnet Plume, (3) Tay River, (4) Finlayson, (5) Chisana, (6) Kluane, (7) Klaza, (8) Tatchun, (9) Pelly, (10) Aishihik, (11) Ibex, (12) Carcross, (13) Atlin, (14) Wolf Lake, (15) Cassiar and (16) Chase caribou herds. Colours represent region scale structure identified using 8 microsatellite loci: (green) Cluster 1 – Southern Lakes caribou herds; (red) Cluster 2 – Aishihik herd; (blue) Cluster 3 – Fortymile, Pelly, Tay River, Wolf Lake herds; and (yellow) Cluster 4 – Chisana and Kluane herds. Herds colour coded with blue–green strips represent herds that were not assigned to a single cluster, but equally to Clusters 1 and 3. Inset shows detailed view of ice patch locations for samples used in this study: (A) *Gladstone (IP36)*, (B) *Lower Gladstone (IP35)*, (C) *East Thulsoo (IP50)*, (D) *Vand Creek (IP34)*, (E) *Bratnober (IP18)*, (F) *Upper Jo Jo (IP31)*, (G) *Thandlät (IP1)*, (H) *Texas Gulch (IP52)*, (I) *Sandpiper (IP29)*, (J) *Friday Creek (IP37)*, (K) *Alligator (IP38)*, (L) *Irvine (IP80)*. The solid line, greyed area represents the observed extent of the eastern lobe of the White River tephra laid down during the 1200 BP eruption. The dashed, greyed area represents the observed extent of the smaller northern lobe, deposited between 1900 and 1500 BP. Data modified from Robinson (2001).

Yukon, northern BC and eastern Alaska. These samples, collected during the 1990s, can be used to infer recent genetic relationships among caribou across the study area. The herds are (refer to Fig. 1) Aishihik ($n = 42$), Atlin ($n = 37$), Bonnet Plume ($n = 19$), Carcross ($n = 18$), Cassiar ($n = 9$), Chase ($n = 25$), Chisana ($n = 51$), Finlayson ($n = 20$), Fortymile ($n = 20$), Ibex ($n = 20$), Klaza ($n = 10$), Kluane ($n = 21$), Pelly ($n = 36$), Tatchun ($n = 18$), Tay River ($n = 20$) and Wolf Lake ($n = 29$).

Mitochondrial DNA made use of 42 samples from five of these modern caribou herds (Fortymile, $n = 20$; Aishihik, $n = 12$; Ibex, $n = 11$ and Carcross, $n = 1$) for comparison with ancient materials. These 42 samples were chosen because they represent the only available samples from the most likely modern relatives of the ice patch remains (based upon the results of STR analysis detailed below). Modern samples were obtained as DNA extracts, originally isolated from caribou tissue samples, from the Parks Canada DNA Repository (PCDNAR), University of Alberta, Edmonton, Alberta (Appendix S1, Supporting information). To augment this data set, nine additional geographically appropriate control region sequences from the Wolf Lake ($n = 1$) and Fortymile ($n = 8$) herds were obtained from GenBank (Appendix S1, Supporting information).

Molecular methods

Modern STR extractions and amplifications were done by Zittlau (2004). DNA was isolated from both whole blood and tissue samples. Following lysis of red blood cells using repeated 1× ACK (0.155 M NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA, pH 7.4) washes, DNA was extracted from white blood cells using the QIAamp Blood protocol (QIAGEN Inc.). Twenty-five milligram tissue samples where lysed using QIAGEN lysis buffers and proteinase K (QIAGEN Inc.). DNA was then extracted using QIAamp spin columns according to the DNeasy DNA extraction protocol (QIAGEN Inc.). Polymerase chain reaction (PCR) amplification of eight microsatellite loci (BM4513 from Bishop *et al.* 1994, and RT1, RT5, RT6, RT7, RT9, RT24 and RT27 from Wilson *et al.* 1997) from both sample types was done as described in (Zittlau 2004). PCR products were separated using ABI Prism 373A and 377 Automated DNA Sequencers (PE Biosystems). Genotypes were scored using GeneScan Analysis 2.0.2 and Genotyper 2.0 software (ABI).

Ancient caribou remains used in this study have been frozen within the ice patches from the time of deposition to the time of sampling, and as such are exceptionally well preserved, with the physical appearance and colour very similar to modern material. This exceptional preservation is further supported by the recovery of well-preserved soft tissues in both faunal and archaeological remains and by the recovery of STR loci from several ancient samples (TSK, PG and BS, unpublished data; Farnell *et al.* 2004). However, as with all aDNA research, extreme care is required to avoid contamination by modern DNA and/or the potentially complicating effect of DNA damage-induced postmortem changes (Cooper & Poinar 2000). To minimize such problems, we did the following. Extraction and amplification of ancient mtDNA was carried out either in the physically isolated aDNA facility at the Henry Wellcome Ancient Biomolecules Centre (ABC), University of Oxford, or at a dedicated laboratory at the University of Alaska Museum of the North (MotN) (Appendix S1, Supporting information). These extractions where completed prior to work on any modern samples. All extractions were analysed with appropriate extraction and PCR negative controls inserted after every sixth sample, all of which were sequenced. Seven samples were separately extracted and amplified at both the University of Oxford and University of Alaska Fairbanks facilities, using separate primer sets, and then compared. As a final precaution, four ancient samples, including two samples extracted at both facilities, were examined using bacterial cloning techniques.

Samples extracted at the ABC used a phenol–chloroform protocol as described in Shapiro *et al.* (2004). Samples extracted at MotN used a Qiagen QIAquick Nucleotide Removal Kit^{TM} (see Appendix S2, Supporting information for more detail). Multiple overlapping primers (four pairs at ABC and five different pairs at MotN; Table S1) were used to amplify a 454-bp fragment of mtDNA control region. Sequencing was performed on both the forward and reverse strands using PRISM BigDye Terminator version 3.0 (Applied Biosystems) and sequenced following ethanol precipitation (ABC) or G-50 Sephadex protocol (MotN; GE Healthcare) according to manufacturer's instructions. To investigate sequence fidelity and DNA damage, four ancient extracts, representing the temporal range of specimens, were cloned using the Invitrogen (UK Ltd.) Topo-TA bacterial cloning kit according to manufacturer's instructions (Appendix S1, Supporting information). From each PCR product, 10 bacterial colonies were selected for sequencing (as above) and results compared with those from previously recovered direct sequencing.

Genetic analysis

STR analyses. The eight STR loci are known to be in Hardy–Weinberg Equilibrium and not to be in linkage disequilibrium for the caribou herds used in this study (Zittlau *et al.* 2000; Zittlau 2004). Comparative measures of genetic diversity are shown for each herd (Table 1). To examine population substructure among present-day caribou herds across the study area, we employed a Bayesian clustering method (Structure version 2.2; Pritchard *et al.* 2000; Falush *et al.* 2003) on the STR data to estimate the number of genetic clusters that occur within the set of sampled individuals. We used an iterative approach to select the best Structure run. Each chain was run for 500 000 iterations with a burn-in of

Table 1 Comparative measures of genetic diversity

	Microsatellite analysis		
Herd	*N*	*A*	H_E
Atlin	37	7.3	0.833
Carcross	18	7.4	0.791
Ibex	20	7.3	0.793
Aishihik	42	7.9	0.743
Bonnet Plume	19	9.9	0.857
Finlayson	20	9.8	0.851
Fortymile	20	10	0.866
Klaza	10	5.8	0.755
Tay River	20	8.9	0.833
Chisana	51	9.4	0.822
Kluane	21	6.4	0.748
Pelly	36	10	0.845
Tatchun	18	7.5	0.811
Wolf Lake	29	9.5	0.835
Cassiar	9	7.6	0.848
Chase	25	9.4	0.826

N, sample size; *A*, mean number of alleles per locus; H_E, expected heterozygosity.

30 000. No prior information about an individual's source herd was used. We assumed an admixture model for the ancestry of individuals and correlated allele frequencies. We then calculated *K* according to Evanno *et al.* (2005) to determine the highest level of population structure.

First, as a broad-scale analysis, we examined the whole data set of 16 caribou herds (Aishihik, Atlin, Bonnet Plume, Carcross, Cassiar, Chase, Chisana, Finlayson, Fortymile, Ibex, Klaza, Kluane, Pelly, Tatchun, Tay River and Wolf Lake) and estimated the number of distinct genetic clusters by performing three independent runs each for *K* = 1–16.

Next, we performed a finer-scale analysis by examining population substructure among the three Southern Lakes herds (Atlin, Carcross and Ibex) and between those herds and their neighbouring populations. To do this, we performed two different analyses with different sets of populations: (i) we examined the Southern Lakes caribou and the three present-day herds that occur on the range of ice patch samples (Aishihik, Fortymile and Wolf Lake); and (ii) we examined Southern Lakes caribou and the herds immediately adjacent to them (Pelly, Tatchun, Tay River and Wolf Lake). For each of these analyses, the number of distinct genetic clusters was estimated by performing three independent runs each for *K* = 1–7.

Modern and ancient mitochondrial sequences. Modern and ancient mtDNA sequences were aligned manually in Se-Al version 2.0a11 (Rambaut 1996). We first created a descriptive median-joining network using Network version 4.5 (Bandelt *et al.* 1999; Fluxus Engineering 2008). We ran a Maximum Parsimony postprocessing step (Polzin & Daneschmand 2003), and down-weighted the highly variable mutational positions 183, 217, 268, 315 and 444 by 50%, as suggested in the Network user's guide. To identify maximally differentiated, but geographically homogenous population structure without conditioning the analysis on *a priori* population structures (groupings of one or more populations), we employed the program Spatial Analysis of MOlecular Variance, SAMOVA version 1.0 (Dupanloup *et al.* 2002; http://web.unife.it/progetti/genetica/Isabelle/samova.html). The SAMOVA analysis was run using 1000 initial states and geographic groupings from *K* = 2 to 6. We selected *K* by identifying a plateau in the decrease of the total genetic variance that could be explained by differences among populations within groups. *Alligator* and *Sandpiper* ice patches (Fig. 1; Appendix S1, Supporting information) contain ancient samples spanning a large time period (2000 years and ~5500 years, respectively) and have bimodal age distributions. We therefore ran the SAMOVA analysis twice: first considering them a single 'population' and second with the two ice patches each subdivided into 'recent' and 'ancient' samples (recent ice patch samples are denoted with an '*R*', e.g. *SandpiperR*). For both populations, recent samples represent those <1000 BP, with ancient samples ≫1000 BP as identified by the bimodal age distribution. As each population within SAMOVA must have a unique geographic location, the coordinates of recent samples from *Alligator* and *Sandpiper* ice patches were shifted <0.05° north and west of the locations used for ancient *Alligator* and *Sandpiper* ice patch samples.

Although SAMOVA is not preconditioned on population structure (predefined groups of populations), individual samples must be placed within individual populations *a priori*. Defining these populations *a priori* and not explicitly incorporating information about variation in population range locations through time, sample age or evolutionary history may influence the optimal SAMOVA groupings. To properly incorporate these factors into our investigation, we utilized Bayesian phylogenetic reconstruction approach using the Bayesian genealogical inference package BEAST version 1.4.8 (Drummond & Rambaut 2007). A major advantage of BEAST is that it allows the explicit incorporation of noncontemporaneous DNA sequences in a coalescent-based inference framework. Each BEAST analysis was run for 30 000 000 iterations, sampling every 3000, with the first 3 000 000 steps discarded as burn-in. We selected the optimal evolutionary model using the software ModelGenerator version 0.85 (available from http://bioinf.may.ie/software/modelgenerator/). For all BEAST analyses, we used an Hasegawa–Kishino–Yano model of nucleotide substitution with gamma-distributed rate heterogeneity (Hasegawa *et al.* 1985) assuming a strict molecular clock and a constant population size. We also tested a more generalized model allowing for dynamic population changes (the Bayesian Skyline plot; Drummond *et al.* 2005), however, Bayes Factor analysis showed stronger support for the simpler constant population size model (results not shown). Estimated sample sizes for each parameter in each independent chain were examined using Tracer version 1.4 (Rambaut & Drummond 2007) to evaluate mixing, and plots of posterior samples were evaluated by eye to ensure each run reached stationarity.

Results

Sequence authenticity

Consistent with the level of preservation of the ancient remains used in this study, we observed no evidence of contamination or DNA damage. Cloned reagent and PCR negative controls produced no recoverable DNA. The four extracts selected for bacterial cloning produced identical sequences to those previously recovered from direct sequencing of these same extracts. Finally, each of the seven sequences that were extracted and sequenced at independent facilities, using different extraction and amplification protocols, resulted in identical sequences. This exceptional preservation is further demonstrated by recovery of STR loci from several ancient samples (TSK, PG and BS, unpublished data; J. Haile, personal communication). However, because of the limitations of the available ancient samples, detailed STR analysis of these ancient samples could not be performed.

Modern microsatellite DNA analyses

The clustering analysis of Structure revealed four distinct genetic clusters among the 16 caribou herds: Cluster 1 included the Southern Lakes herds (Atlin, Carcross and Ibex); Cluster 2 included the Aishihik herd; Cluster 3 included the Bonnet Plume, Finlayson, Fortymile, Klaza and Tay River herds; and Cluster 4 was composed of the Chisana and Kluane herds (Fig. 2a). Assignment probabilities were highest among individuals belonging to Cluster 2 (Aishihik). Caribou from Pelly, Tatchun and Wolf Lake assigned almost equally between Clusters 1 and 3. The remaining herds could not be strongly assigned to any one cluster.

At the finer scale, analyses examining substructure among the Southern Lakes caribou and the present-day herds that occupy the range of ice patch samples (Aishihik, Fortymile and Wolf Lake) revealed three distinct genetic clusters similar to those identified at the broad-scale analysis: Cluster 1 contained the Southern Lakes caribou; Cluster 2 corresponded to Aishihik caribou; and Cluster 3 corresponded to Fortymile caribou (Fig. 2b). Again, assignment probabilities were highest for Cluster 2. Wolf Lake caribou were equally likely to be assigned to Clusters 1 and 3, suggesting that the caribou from the Wolf Lake herd may represent an admixture of both Fortymile and Southern Lakes herds or be derived from populations ancestral to both the Fortymile and the Southern Lakes herds.

To estimate the spatial extent of Cluster 1 (Southern Lakes caribou), we analysed only the three Southern Lakes caribou (Atlin, Carcross and Ibex) and immediately adjacent herds (Pelly, Tatchun, Tay River and Wolf Lake herds; herds that did not strongly associate with any one cluster in the regional scale analysis). At this spatial scale, the Atlin, Carcross and Ibex herds form a weak genetic cluster, observed when $K = 2$ (mean ln likelihood = −6464). However, the estimated ln likelihood of the data was largest and most strongly supported for $K = 1$ (mean ln likelihood = −5906), suggesting a single population.

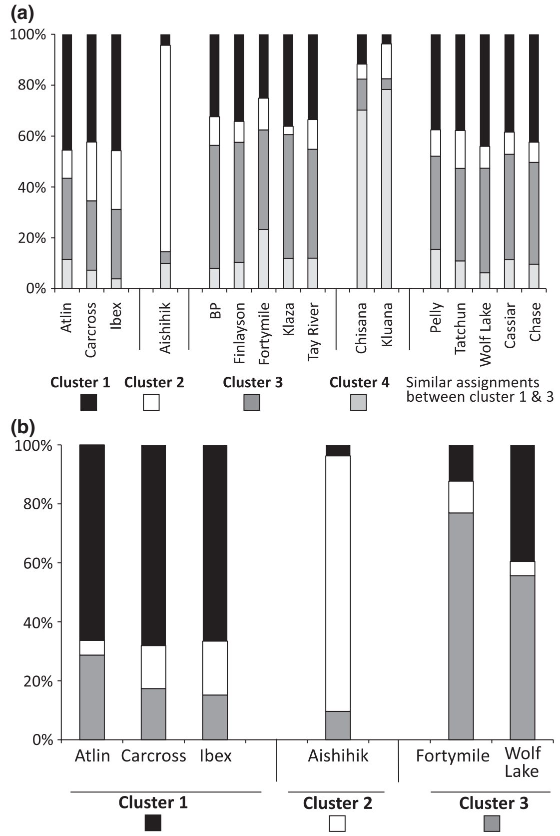

Fig. 2 Mean proportion of each caribou herd assigned to genetic Cluster 1, 2, 3 or 4, based on Bayesian clustering analyses of individual genotypes at eight STR loci. (a) Broad-scale analysis of the whole STR data set of 16 caribou herds; (b) finer-scale analysis of Southern Lakes caribou and the herds covering the range of ice patch samples.

Modern plus ancient mtDNA analyses

All ancient and modern haplotypes included herein are representatives of the Beringian-Eurasian Lineage (as defined by Dueck 1998; Flagstad & Røed 2003; and McDevitt *et al.* 2009; analysis and results not shown). As a result, we felt it inappropriate to include many previously published modern haplotypes that are representatives of the North American Lineage as the very large genetic distance between these two groups would probably have diminished our ability to assess regional phylogeographic patterns.

The median-joining network shows considerable sequence diversity within the caribou mtDNA control region (Fig. 3). Two high-frequency haplotypes are centrally located within the network (Fig. 3; haplotypes Rt003 and Rt007), and correspond to the only two haplotypes observed in the Aishihik herd. The haplotypes

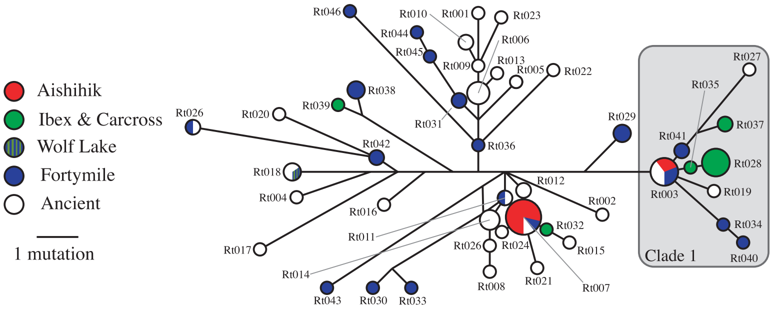

Fig. 3 Median-joining network of all ancient and modern haplotypes. Appendix S1 (Supporting information) provides further information on sampling locations, ages and haplotype frequencies (haplotype labels identify unique haplotypes only). Colors indicate clusterings identified using eight STR loci. Clade 1, outlined in grey, is the only visually distinct group within the diverse network, and its member haplotypes corresponds closely with haplotypes found in populations identified using Spatial Analysis of MOlecular Variance, SAMOVA (Table 2), and results from BEAST (Fig. 4).

found in the Carcross and Ibex herds tend to be on short branches related to these two common sequences. In contrast, haplotypes observed in the larger Fortymile herd (haplotypes in blue in Fig. 3) are distributed throughout the network, but tend to be on longer branches arising from the central portion of the network. Ancient haplotypes show two similar patterns: short branches generally arising from a central ancient haplotype, or long branches similar to the Fortymile haplotypes. The network identifies one small grouping of haplotypes (Clade 1, Fig. 3) that appear closely related, with the remaining haplotypes forming a diverse cluster with no clear internal structures (Fig. 3). Clade 1 is composed of the dominant haplotypes from two of the Southern Lakes herds (Ibex and Carcross), one of the two haplotypes observed in the Aishihik herd and several ancient haplotypes, all more recent than ~800 BP. Clade 1 also contains a number of haplotypes found only in the Fortymile herd. The herds that represent haplotypes identified within Clade 1 correspond to those herds indentified within Cluster 1 of the STR analysis, with one key difference: Clade 1 also includes several Aishihik and Fortymile herd haplotypes. For this reason, we distinguish Clade 1 as representing *mtDNA* genetic structure and Cluster 1 as representing the corresponding *STR* genetic structure.

The mtDNA SAMOVA results for the multiple K values examined for mtDNA data are summarized in Table 2. These results suggest that three clades ($K = 3$) account for the optimal genetic/geographic structure, accounting for 25.5% of variation at the Among Groups level (F_{ct} = 0.255, $P \ll 0.0001$). The three groups are: (i) Carcross, Ibex, *SandpiperR, Upper Jo Jo*; (ii) Fortymile, Aishihik, Wolf Lake, and all Ancient samples excluding *SandpiperR* and *Upper Jo Jo*; and (iii) *Thulsoo*. Ignoring the age dichotomies in *Alligator* and *Sandpiper* ice patches produced a similar but less optimal structure ($K = 3$, 22.6% of variation at the Among Groups level, $F_{ct} = 0.226$, $P \ll 0.0001$). Geographic position was found to have no effect on the ability of SAMOVA to recover the optimal outcome (results not shown). Excluding the *Thulsoo* sample from the analysis returned an optimal K-value of $K = 2$, with the same clades described above (results not shown).

The Bayesian reconstructions, which were performed so as to incorporate explicitly the temporal component of the data, reveal a single well-supported clade (posterior support of 0.93; Fig. 4) that includes the same haplotypes isolated in Clade 1 identified in the network analysis (Fig. 3). The corresponding herds which contained these Clade 1 haplotypes mirror the SAMOVA grouping of the modern Ibex and Carcross herds, plus recent (<1000 BP) *Sandpiper* and *Upper Jo Jo* ice patch samples.

Discussion

Our analyses reveal a dynamic recent history for caribou in southern Yukon. The rapidly evolving STR loci identify strong interherd genetic structure at broad geographic scales. More slowly evolving maternally inherited mtDNA supports some of these multi-herd genetic groupings (specifically a clade encompassing herds from the Southern Lakes region), and when coupled with the STR data, identify past genetic mixing and possible founder effects (specifically the lack of correlation between STR and mtDNA markers for the Aishihik herd). Finally, the addition of data from ancient caribou allow novel inference about the medium-term geographic and genetic stability of caribou populations, and recover an entirely unforeseen partial replacement event in the southern-most part of the Yukon around 1000 BP.

In a recent analysis of caribou diet, Farnell *et al.* (2004) analysed faecal material collected at the *Friday Creek* ice patch, and found that ice patch caribou had a diet simi-

Table 2 Results from Spatial Analysis of MOlecular Variance (SAMOVA) for K = 2–6

K	F_{st} (%var)	F_{sc} (%var)	F_{ct} (%var)	Structure
2	0.40515 (59.48)*	0.21069 (15.88)*	0.24637 (24.64)**	(*Thulsoo*) (Remainder)
3	**0.34865 (65.13)***	**0.12612 (9.40)***	**0.25465 (25.46)***	(*Thulsoo*) (Carcross, Ibex, *SandpiperR*, *Ujo*) (Remainder)
4	0.34579 (65.42)*	0.10920 (8.02)*	0.26559 (26.56)*	(*Thulsoo*) (Carcross, Ibex, *SandpiperR*, *Ujo*) (Wolf, Irvine) (Remainder)
5	0.34419 (65.58)*	0.10023 (7.31)*	0.27113 (27.11)*	(*Thulsoo*) (Carcross, Ibex, *SandpiperR*, *Ujo*) (Wolf, *Irvine*) (*Vand*) (Remainder)
6	0.30317 (76.82)*	−0.10242 (−7.14)*	0.30317 (30.32)*	(*Thulsoo*, *Gladstone*, *Bratnober*) (Carcross, Ibex, *SandpiperR*, *Ujo*) (Wolf, *Irvine*) (*Vand*, *Friday*, *Thandlat*, *AlligatorR*, *Texas*) (Aishihik, *AlligatorA*, *SandpiperA*) (Fortymile)

*$P \leqslant 0.005$; **$P = 0.054$. Optimal grouping is $K = 3$ (bold), as increased K values beyond $K = 3$ result in minimal increase to Fct. The analysis was also run excluding the *Thulsoo* ice patch sample (not shown), which produced similar results to those reported here and an optimal K of $K = 2$. SAMOVA consistently identifies the Southern Lakes caribou herds as distinct from surrounding herds at all K values.

lar to the small sedentary northern mountain caribou herds that inhabit the Southern Lakes region today (Ibex, Carcross and Atlin herds; STR Cluster 1). This distinguishes these ancient caribou from the large migratory Fortymile herd whose historic range encompassed this ice patch as recently as 100 BP (Boertje & Gardner 2000). This dietary analysis is consistent with modern caribou behaviour and habitat usage, where northern mountain caribou undergo modest altitudinal migrations from their winter range in valley bottoms to higher elevations during the summer. At higher elevations, these caribou are known to aggregate on alpine ice patches to avoid insect harassment (Weladji *et al.* 2003). In contrast, the migratory Fortymile herd would have occupied its southern range limits during the winter months, when individuals are most dispersed, the ice patches are covered in deep winter snow and suitable habitat is only found in valley bottoms. In support of these divergent life histories, we find little or no gene flow between the Fortymile herd and the herds of the Southern Lakes region. These results are in contrast to observations from Alaska, where an expanding migratory herd introgressed with the resident sedentary herds to such an extent that individual herd identification became impossible (Hinkes *et al.* 2005). Interestingly, sedentary northern mountain caribou herds that surround the Southern Lakes region (Bonnet Plume, Finlayson, Klaza and Tay River herds; members of STR Cluster 3), which are behaviourally similar to the Southern Lakes herds, are genetically indistinguishable, using fast evolving STRs, from the migratory Fortymile herd. This close genetic history between migratory and sedentary ecotypes suggests that significant gene flow still exists between these geographically dispersed herds, a result that is at odds with present management frameworks.

Short tandem repeats and mtDNA data provide differing results with regards to the evolutionary relationships among the sedentary herds. In the STR analysis, the Aishihik herd forms a distinct cluster (STR cluster 2), suggesting strong isolation from surrounding herds. Had this herd been isolated from surrounding herds for several 1000 years, we would have expected that Aishihik caribou and the ancient caribou recovered from ice patches within their geographic range (*Gladstone*, *Lower Gladstone* and *Thulsoo* ice patches) would form a distinct clade. However, all of the modern and ancient mtDNA haplotypes from this herd are shared with the surrounding herds. Unfortunately, we were unable to isolate useable STRs from these ice patch remains. The distinctiveness of the Aishihik herd observed using STR markers is then best explained as the result of recent founder effects or population bottleneck coupled with short-term isolation. Population estimates for the Aishihik caribou herd document a 50% reduction in herd size between 1981 and 1993, which precipitated an extensive predator control and management strategy designed to stabilize this economically and culturally important herd (Hayes *et al.* 2003).

In contrast to the Aishihik herd, both STR and mtDNA results provide evidence for the distinctiveness of the Ibex, Carcross and Atlin herds of the Southern Lakes region (mtDNA Clade 1; STR Cluster 1). If these herds had been isolated from others for the last several 1000 years, caribou recovered from *Alligator, Bratnober, Friday Creek, Texas Gulch, Thandlät, Sandpiper* and *Vand Creek* ice patches should have fallen within this group. However, while many recent (<1000 BP) ice patch samples from the Southern Lakes region show close genetic affinity with the herds present on the landscape today, our results identify a distinct transi-

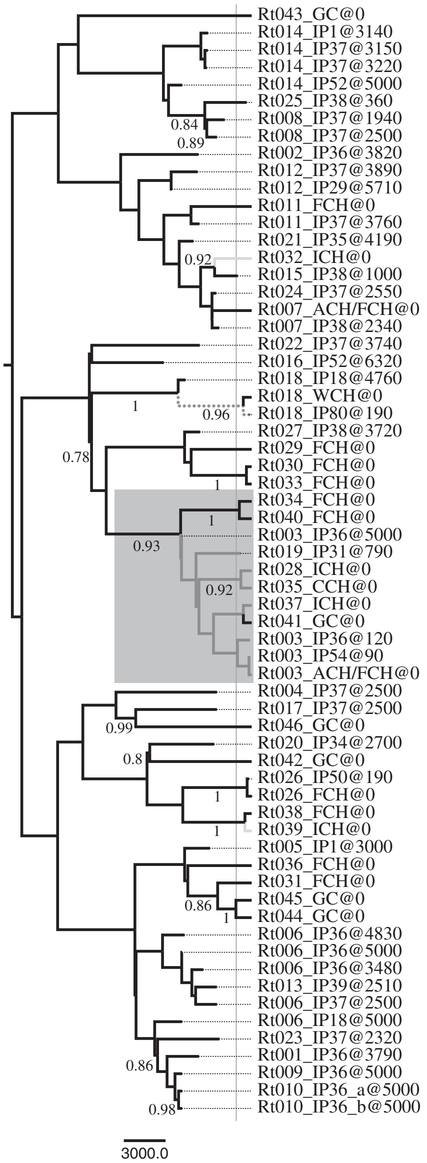

Fig. 4 Maximum Clade Credibility genealogy summarized from the BEAST analysis described in the main text. Posterior support values greater than 0.75 are shown adjacent to each node. Line styles indicate structure obtained from Spatial Analysis of MOlecular Variance (SAMOVA) (Table 2). Branch lengths are measured in radiocarbon years before present, with the rate estimated from dated sequences. The only well-supported clade comprises the same samples that are identified as Clade 1 in the median-joining network. Similar to the tree topology, the optimal SAMOVA structure includes the Southern Lakes herds as a separate group (mtDNA Clade 1), however, as the SAMOVA approach requires samples to be assigned to an individual population *a priori*, several individual haplotypes appear to represent recent migrants to or from the Southern Lakes mtDNA Clade 1. All but one ancient samples dated before ~1000 BP (indicated by the thin grey vertical line) group outside the well-supported Clade 1, indicating that ancient caribou from the Southern Lakes region do not represent the ancestral source of present-day Southern Lakes herds.

tion occurring ~1000 BP. Caribou samples older than this invariably fell outside the well-supported Clade 1, and instead were indistinguishable from modern Aishihik and Fortymile samples and ice patch samples from the Aishihik range.

While the proposed replacement event explains most of the differences in caribou genetic diversity observed before and after 1000 BP, there are several exceptions. For example, the small but diverse Ibex caribou herd contains mtDNA haplotypes found both within and outside Clade 1. These haplotypes are not shared with any of the herds tested here, or any of the previously published sequences (broad-scale analysis results not shown). Similarly, a small number of Fortymile haplotypes are found within Clade 1. These exceptions may represent recent dispersal and gene flow within the Southern Lakes region or ancestral haplotypes that survived the replacement event. Given this, we adopt a conservative interpretation of partial replacement to explain the 1000 BP replacement event.

The timing of the proposed partial replacement event is consistent with two large-scale environmental disturbances within the southern Yukon: (i) the eruption and deposition of the White River tephra, and (ii) the global climate warming of the Medieval Warm Period (MWP).

The White River tephra was deposited in two lobes: the first, smaller, northern lobe between 1900 and 1500 BP, and the much larger, eastern lobe ~1200 BP (Fig. 1; Clague *et al.* 1995; Robinson 2001). The eastern lobe represents the largest known Holocene stratovolcano (Lerbekmo 2008), with an estimated 47 km^3 of volcanic ash deposited across *c.* 540 000 km^2 (Robinson 2001; Lakeman *et al.* 2008). Ash thicknesses of up to 30 cm having been recorded more than 300 km from the source volcano (Bostock 1952). Perhaps unsurprisingly, the deposition of this lobe has been linked to extensive archaeological upheaval in the southern Yukon (Workman 1979; Moodie *et al.* 1992; Robinson 2001; Hare *et al.* 2005).

The detrimental effects of ash fall on livestock and caribou have been observed from 2.5–10 cm of ash deposited from the 1912 Mount Katmai eruption in Alaska (Jagger 1945) and as little as 1.9 cm of ash following the 1947 eruption of Hekla, in Iceland (Malde 1964). Given that much of the Southern Lakes region falls within the 5 cm isopach (ash depths of 5 cm or greater) of the eastern lobe of the White River tephra (Fig. 1), it seems probably that this cataclysmic event had a noticeable effect on the distribution of caribou populations living within the region at the time.

The appearance of Clade 1 in the Southern Lakes region at ~1000 BP follows a 400 year period during which no remains were preserved within the sampled ice patches (1440–1030 BP, Farnell *et al.* 2004). This hiatus is coincident with the increase in global temperatures associated with the MWP (Farnell *et al.* 2004; Helama *et al.* 2009). An increase in mean July temperatures is also inferred from both pollen and chironomid accumulation rates within our study region (Bunbury & Gajewski 2009). Warmer temperatures mean more frequent periods of winter thawing, increased winter snowfall, increased insect harassment and loss of perennial snow patches (Thomas & Gray 2002; Weladji *et al.* 2003), all of which have detrimental effects on caribou. It is therefore possible, even probable, that the MWP also played a role in the extirpation of caribou from the Southern Lakes region, with subsequent recolonization occurring as temperatures cooled during the Little Ice Age. Interestingly, and as a result of warmer summer temperatures observed in recent years within our study region, the vast majority of sampled ice patches, which had persisted for upwards of 7000 years, no longer exist.

Our combined analysis of ancient and modern caribou provides several insights for caribou management and conservation. First, combined STR and mtDNA analyses demonstrate that the Southern Lakes caribou herds are distinguishable from other caribou herds, but that recent shared ancestry or gene flow has blurred this genetic division on smaller spatial scales. Our results support management of these three herds (Atlin, Carcross and Ibex) as a single management unit, and provide genetic evidence in support of active management practices designed to ensure their long-term persistence.

Interestingly, however, the most distinctive herd based on STR data, the sedentary Aishihik herd, is indistinguishable from the adjacent migratory Fortymile herd when using mtDNA. However, a dichotomy between STR and mtDNA analysis is not unsurprising given observations for woodland caribou from the Central Rocky Mountains of British Columbia and Alberta (McDevitt *et al.* 2009). In contrast, where McDevitt *et al.* (2009) observed a dichotomy between markers for herds of the same ecotype with recent mixing of two divergent mitochondrial lineages, the dichotomy observed in southern Yukon is between different ecotypes, but where we see isolation of a formerly uniform mitochondrial lineage. The unique genetic information contained within the Aishihik herd is then a very recent phenomenon. Coupled with the observed genetic relationships between woodland herds of the central Yukon, we suggest that the total unique genetic diversity contained within these herds remains very small and of recent origin. As a result, the loss of any one herd would not have a significant effect on the overall genetic diversity of caribou within the region. It is, however, important to acknowledge the economic and cultural importance of each individual herd within its region. Our results underscore the importance of using multiple lines of evidence when making appropriate management decisions, as analysis of STR markers alone could have lead to different management priorities regarding the Aishihik herd.

Finally, by adding a temporal perspective to our analysis, we identify a recent, partial replacement event that is not discernible from modern data alone. This replacement event was most probably caused by changes in caribou habitat as a result of either the MWP or the deposition of the White River tephra. At present, we cannot distinguish between these two closely timed events, however, increased sampling and stratigraphic coverage of the 1500–500 BP period may be sufficient to identify a single causal mechanism. Whether this replacement event was caused by a large volcanic eruption or increased snowfall and warmer temperatures, caribou were probably able to recolonize the large region in the southern Yukon as a result of their ability to expand in numbers and migrate into newly available habitats as cooler temperatures of Little Ice Age prevailed (Viau *et al.* 2006; Bunbury & Gajewski 2009).

In the face of current climate change, with temperature increases predicted to be an order of magnitude larger than that observed during the Holocene (Viau *et al.* 2006), preserving connectivity between isolated patches of caribou habitat may become more important than the individual preservation of small, isolated sedentary herds. With ongoing research into the recoverability and usability of STR markers from ancient samples, we hope to further test these hypotheses.

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References

Anderson E (1984) Who's who in the Pleistocene: a mammalian bestiary. In: *Quaternary Extinctions: A Prehistoric*

Revolution (eds Martin PS, Klein RG), pp. 40–84. The University of Arizona Press, Tucson, Arizona.

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Berger J (2004) The last mile: how to sustain long-distance migrations in mammals. *Conservation Biology*, **18**, 320–331.

Bergerud AT (2000) Caribou. In: *Ecology and Management of Large Mammals in North America* (eds Demarais S, Krausman PR), pp. 658–693. Prentice Hall, Upper Saddle River, New Jersey.

Bishop MD, Kappes SM, Keele JW *et al.* (1994) A genetic linkage map for cattle. *Genetics*, **136**, 619–639.

Boertje RD, Gardner CL (2000) The Fortymile caribou herd: novel proposed management and relevant biology 1992-1997. *Rangifer*, Special Issue No. 12, 17–38.

Bostock HS (1952) Geology of northwest Shakwak Valley, Yukon Territory, *Geological Survey of Canada Memoir*, **267**.

Bunbury J, Gajewski K (2009) Postglacial climates inferred from a lake at treeline, southwest Yukon Territory, Canada. *Quaternary Science Reviews*, **28**, 354–369.

Clague JJ, Evans SG, Rampton VN, Woodsworth GJ (1995) Improved age estimates for the White River and Bridge River tephras, Western Canada. *Canadian Journal of Earth Sciences*, **32**, 1172–1179.

Cooper A, Poinar HN (2000) Ancient DNA: do it right or not at all. *Science*, **289**, 1139–1139.

COSEWIC (2002) COSEWIC assessment and update status report on the woodland caribou Rangifer tarandus caribou in Canada. Committee on the Status of Endangered Wildlife, Canada. p. xi +98.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, **22**, 1185–1192.

Dueck GS (1998) *Genetic Relations and Phylogeography of Woodland and Barrenground Caribou*. University of Alberta, Edmonton, Alberta.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, **11**, 2571–2581.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure: extensions to linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Farnell RS, Gardner CL (2003) *Status Report on the Chisana Caribou Herd – 2002*. Report TR-03-01. Yukon Fish and Wildlife Branch, Whitehorse, Yukon Territory.

Farnell RS, MacDonald J (1988) *The Demography of the Yukon Finlayson Caribou Herd 1982–1987*. Interim Report. Yukon Fish and Wildlife Branch, Whitehorse, Yukon Territory, p. 54.

Farnell RS, MacDonald J (1989) *Inventory of the Wolf Lake Caribou Herd*. Final Report. Yukon Department of Renewable Resources, Yukon Territory, p. 70.

Farnell RS, Russell DE (1984) *Wernecke Mountain Caribou Studies*. Final Report. Yukon Fish and Wildlife Branch, Whitehorse, Yukon Territory.

Farnell RS, Florkiewicz R, Kuzyk G, Egli K (1998) The status of *Rangifer tarandus caribou* in Yukon, Canada. *Rangifer*, Special Issue No. 10, 131–137.

Farnell RS, Hare PG, Blake E *et al.* (2004) Multidisciplinary investigations of alpine ice patches in the Southwest Yukon, Canada: paleoenvironmental and paleobiological investigations. *Arctic*, **57**, 247–259.

Flagstad Ø, Røed KH (2003) Refugial origins of reindeer (*Rangifer tarandus* L.) inferred from mitochondrial DNA sequences. *Evolution*, **57**, 247–259.

Fluxus Engineering (2008) *Network v.4.5*. Available from http://www.fluxus-engineering.com.

Hare PG, Greer S, Gotthardt RM, Strand D (2005) The effect of the White River Ash on the archaeological record - a view from the Yukon alpine.

Hasegawa M, Kishino K, Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160–174.

Hayes RD, Farnell RS, Ward RMP *et al.* (2003) Experimental reduction of wolves in the Yukon: ungulate responses and management implications. *Wildlife Monographs*, (152), 1–35.

Hebblewhite M, Whittington J, Bradley M *et al.* (2007) Conditions for caribou persistence in the wolf-elk-caribou systems of the Canadian Rockies. *Rangifer*, Special Issue No. 17, 79–91.

Helama S, Timonen M, Holopainen J *et al.* (2009) Summer temperature variations in Lapland during the Medieval Warm Period and the LIttle Ice Age relative to natural instability of thermohaline circulation on multi-decadal and multi-centennial scales. *Journal of Quaternary Science*, **24**, 450–456.

Hinkes MT, Collins GH, Van Daele LJ *et al.* (2005) Influence of population growth on caribou herd identity, calving ground fidelity, and behavior. *Journal of Wildlife Management*, **69**, 1147–1162.

Jagger TA (1945) Volcanoes declare war. *Logistics and Strategy of Pacific Volcano Science*. Paradise of the Pacific Ltd, Honolulu, USA.

James ARC, Stuart-Smith AK (2000) Distribution of caribou and wolves in relation to linear corridors. *Journal of Wildlife Management*, **64**, 154–159.

James ARC, Boutin S, Hebert DM, Rippin AB (2004) Spatial separation of caribou from moose and its relation to predation by wolves. *Journal of Wildlife Management*, **68**, 799–809.

Kurtén B, Anderson E (1980) *Pleistocene Mammals of North America*. Columbia University Press, New York.

Kuzyk GW, Russell DE, Farnell RS *et al.* (1999) In pursuit of prehistoric caribou on Thandlät, Southern Yukon. *Arctic*, **52**, 214–219.

Lakeman TR, Clague JJ, Menounos B *et al.* (2008) Holocene tephras in lake cores from northern British Columbia, Canada. *Canadian Journal of Earth Sciences*, **45**, 935–947.

Leonard JA (2008) Ancient DNA applications for wildlife conservation. *Molecular Ecology*, **17**, 4186–4196.

Lerbekmo JF (2008) The White River Ash: largest Holocene Plinian tephra. *Canadian Journal of Earth Sciences*, **45**, 693–700.

McDevitt AD, Mariani S, Hebblewhite M *et al.* (2009) Survival in the Rockies of an endangered hybrid swarm from diverged caribou (*Rangifer tarandus*) lineages. *Molecular Ecology*, **18**, 665–679.

McLoughlin PD, Dzus E, Wynes B, Boutin S (2003) Declines in populations of woodland caribou. *Journal of Wildlife Management*, **67**, 755–761.

Malde HE (1964) The ecological significance of some unfamiliar geological processes. In: *The reconstruction of past environments. Proceedings of the Fort Burgwin Research Center on Paleoecology 1962*. Fort Burgwin Research Center, Fort Burgwin, USA.

Moodie DW, Catchpole AJW, Abel K (1992) Northern Athapaskan oral traditions and the White River volcano. *Ethnohistory*, **39**, 169–180.

Polzin T, Daneschmand SV (2003) On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters*, **31**, 12–20.

Post E, Forchammer MC (2008) Climate change reduces reproductive success of an Arctic herbivore through trophic mismatch. *Philosophical Transactions of the Royal Society of London, B*, **363**, 817–818.

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

Ramakrishran U, Hadly EA, Mountain JL (2005) Detecting past population bottlenecks using temporal genetic data. *Molecular Ecology*, **14**, 2915–2922.

Rambaut A (1996) *Se-Al: Sequence Alignment Editor, v2.0a11*. Available from http://tree.bio.ed.ac.uk/software/seal/.

Rambaut A, Drummond AJ (2007) *Tracer v1.4*. Available from http://beast.bio.ed.ac.uk/Tracer.

Robinson SD (2001) Extending the Late Holocene White River Ash distribution, Northwestern Canada. *Arctic*, **54**, 157–161.

Schaefer JA (2003) Long-term range recession and the persistence of caribou in the Taiga. *Conservation Biology*, **17**, 1435–1439.

Shapiro B, Drummond AJ, Rambaut A *et al.* (2004) Rise and Fall of the Beringian Steppe Bison. *Science*, **306**, 1561–1565.

Southern Lakes caribou recovery program (1996) *Progress Report: 1992–1996*. Yukon Fish and Wildlife Branch, Whitehorse, Yukon Territory.

Thomas DC, Gray DR (2002) Update COSEWIC status report on the woodland caribou *Rangifer tarandus caribou* in Canada. In: *COSEWIC assessment and update status report on the Woodland Caribou Rangifer tarandus caribou in Canada* (eds Thomas DC, Gray DR), pp. 1–98. Committee on the Status of Endangered Wildlife in Canada, Ottawa.

Viau AE, Gajewski K, Sawada MC, Fines P (2006) Millennial-scale temperature variations in North America during the Holocene. *Journal of Geophysical Research - Atmosphere*, **111**, D09102.

Vors LS, Boyce MS (2009) Global declines of caribou and reindeer. *Global Change Biology*, **15**, 2626–2633.

Webber MG, Flanigan MD (1997) Canadian boreal forest ecosystem structure and function in a changing climate: impact on fire regimes. *Environmental Reviews*, **5**, 145–166.

Weladji RB, Holand Ø, Almøy T (2003) Use of climatic data to assess the effect of insect harassment on the autumn weight of reindeer (*Rangifer tarandus*) calves. *Journal of Zoology*, **260**, 79–85.

Wilson GA, Strobeck C, Coffin JW (1997) Characterization of microsatellite loci in caribou, *Rangifer tarandus*, and their use in other artiodactyls. *Molecular Ecology*, **6**, 667–699.

Wittmer HU, Sinclair ARE, McLellan BN (2005) The role of predation in the decline and extirpation of woodland caribou. *Oecologia*, **144**, 257–267.

Workman WB (1979) The significance of volcanism in the prehistory of subarctic North America. In: *Volcanic Activity and Human Ecology* (eds Sheets PD, Grayson DK), pp. 339–371. Academic Press, New York.

Yukon Environment Geomatics (2008) *Caribou Herd Ranges 2008, Yukon Territory*. Department of Environment, Yukon Territorial Government, Whitehorse, Yukon.

Zittlau KA (2004) *Population Genetic Analyses of North American Caribou (Rangifer Tarandus)*. University of Alberta, Edmonton, Alberta.

Zittlau KA, Coffin J, Farnell RS, Kuzyk GW, Strobeck C (2000) Genetic relationships of three Yukon caribou herds determined by DNA typing. *Rangifer*, Special Issue No. 12, 59–62.

T.S.K. is completing his MSc at SFU under the supervision of A.Ø.M., with an interest centred on the belief that palaeontology can provide crucial information needed to address current conservation problems. A.Ø.M.'s work concerns explaining patterns of biodiversity, and in applying phylogenetic data to conservation problems. K.A.M. is a conservation geneticist interested in improving management of large ungulates in North America. P.G.'s research focuses on Arctic ungulate evolution, in particular through the use of aDNA analysis. B.S. is an aDNA specialist whose broad research interests include a focus on the phylogeography and population genetics of Beringian megafauna.

Supporting Information

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

Table S1 mtDNA primer information

Appendix S1 Sample information.

Appendix S2 Detailed mtDNA methodology.