Ancient DNA Reveals Genetic Continuity in Mountain Woodland Caribou of the Mackenzie and Selwyn Mountains, Northwest Territories, Canada

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ABSTRACT. We examine the mitochondrial genetic stability of mountain woodland caribou (*Rangifer tarandus caribou*) in the Mackenzie and Selwyn mountains, Northwest Territories, over the last 4000 years. Unlike caribou populations in the Yukon, populations in the Northwest Territories show no evidence for mitochondrial genetic turnover during that period, which indicates that they were not adversely affected by the widespread deposition of the White River tephra around 1200 years ago. We detect moderate genetic differentiation between mountain woodland and barren-ground caribou in both territories, lending support to the current subspecies designations. In addition, we identify moderate genetic differentiation between Northwest Territories and western Yukon mountain woodland caribou, suggesting that there has been minimal mixing of matrilines between these herds.

Key words: ice patch, Redstone herd, ecotype, White River tephra, mitochondrial, barren-ground

RÉSUMÉ. Nous examinons la stabilité génétique mitochondriale des caribous des bois des montagnes (Rangifer tarandus caribou) qui ont évolué dans les monts Mackenzie et dans la chaîne de Selwyn, Territoires du Nord-Ouest, ces 4 000 dernières années. Contrairement aux populations de caribou du Yukon, les populations de caribou des Territoires du Nord-Ouest ne montrent aucun signe de rotation génétique mitochondriale pendant cette période, ce qui indique qu'ils n'ont pas été affectés de manière défavorable par le dépôt à grande échelle du téphra de la rivière White, il y a environ 1 200 ans. Nous détectons une différentiation génétique modérée entre le caribou des bois des montagnes et le caribou de la toundra dans les deux territoires, ce qui vient appuyer les désignations actuelles de sous-espèces. Par ailleurs, nous avons dénoté une différenciation génétique modérée entre le caribou des bois des montagnes des Territoires du Nord-Ouest et celui de l'ouest du Yukon, ce qui laisse croire qu'il y aurait eu peu de mélanges matrilinéaires entre ces troupeaux.

Mots clés : plaque de glace, troupeau de Redstone, écotype, téphra de la Rivière White, mitochondrial, toundra

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INTRODUCTION

Caribou (*Rangifer tarandus*) have inhabited the Arctic since the Pleistocene (Flagstad and Røed, 2003). After the last glacial maximum, the barren-ground and woodland caribou of Alaska and Canada expanded out of Beringia into their current ranges (Zittlau, 2004). Anthropogenic habitat fragmentation is now placing unprecedented pressure on the mountain woodland caribou, leading the Canadian government to classify the southern mountain caribou as threatened and the northern mountain caribou as a species of special concern (Bergerud, 1974; COSEWIC, 2002; McLoughlin et al., 2003).

Caribou are holarctic in distribution, ranging across North America, Greenland, Europe, and Siberia. They form herds of dozens to hundreds of thousands of individuals,

depending on ecotype. In the Canadian Yukon Territory (YT) and Northwest Territories (NWT), three subspecies are recognized (Flagstad and Røed, 2003). The barren-ground caribou (*Rangifer tarandus groenlandicus* in the NWT; *R. t. granti* in the YT and Alaska) inhabit the open tundra, sometimes forming herds of hundreds of thousands of individuals. These herds are known to migrate as far as 4000 km between seasonal ranges (Zittlau, 2004). In contrast, woodland caribou (*R. t. caribou*) form smaller herds that inhabit the boreal forests. Depending on their habitat, these herds are considered either mountain woodland or boreal woodland caribou (Olsen et al., 2001).

Along the YT-NWT border, several mountain woodland caribou herds inhabit the areas around and within the Mackenzie and Selwyn mountains (Fig. 1; Yukon Geomatics, 2008). During calving season, these herds are largely

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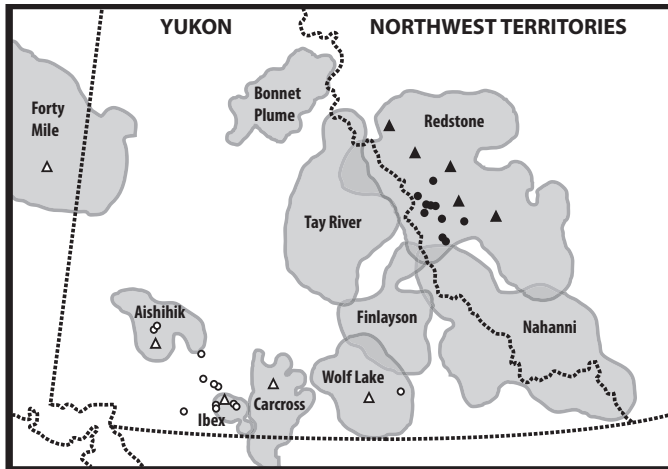[4] Corresponding author: beth.shapiro@gmail.com

FIG. 1. Ranges of the caribou herds represented in this study. Mitochondrial DNA is available from the Redstone, Wolf Lake, Ibex, Aishihik, and Forty-mile herds (Table 1). Ranges for the neighbouring herds of Bonnet Plume, Nahanni, Finlayson, and Tay River are also shown. The circles represent ancient ice patch specimens, and the triangles represent modern specimens. Closed symbols (▲,●) represent the locations of new specimens, while open symbols (△,○) represent those of sequences acquired in previous studies.

isolated from one another. However, genetic analyses suggest that these herds may actually function as a single large herd, or metapopulation (Zittlau, 2004). Understanding the relationships between these herds has important consequences, as long-term caribou conservation policy should be based on metapopulation dynamics rather than herd dynamics (Hinkes et al., 2005).

During the summer months, caribou are known to use perennial alpine ice patches to keep cool and escape insect harassment (Ion and Kershaw, 1989). These ice patches are continuously replenished by snowfall during the winter and are effectively permanent, although recent surveys have shown they are shrinking in the Yukon (Farnell et al., 2004). Numerous ice patches have been discovered within the current caribou range in the YT and NWT, including several in the Mackenzie and Selwyn mountains (Andrews et al., 2012). These ice patches preserve a record of local caribou occupation over thousands of years in the form of dung and bones incorporated into the ice layers (Farnell et al., 2004; Andrews et al., 2012). Radiocarbon dating of fecal material from the NWT ice patches revealed the continuous presence of caribou in the region over the last 4500 years (Andrews et al., 2012).

Recently, melt-out of the ice patches released bones that had been frozen since deposition, providing the opportunity to use ancient DNA (aDNA) techniques to examine the population dynamics of caribou in this region through time. Ancient DNA can permit the direct observation of how populations responded to past periods of environmental change (Leonard, 2008). Such information can provide a crucial temporal perspective, which has been shown to facilitate the most effective conservation strategies (Willis and Birks, 2006).

We use ancient mitochondrial DNA isolated from bones recovered from the NWT ice patches to infer the recent

evolutionary history of the caribou populations from these regions. Mitochondrial DNA is a maternally inherited genetic locus that is favoured in ancient DNA analyses because (1) it is at a much higher copy-number per cell than is nuclear genetic DNA, and therefore it is much more likely to be preserved through time (Willerslev and Cooper, 2005); and (2) it does not recombine, and therefore the evolutionary history of this locus is relatively simple to infer compared to that of nuclear genetic loci (Rokas et al., 2003).

We address four questions about the recent population dynamics of caribou in the Mackenzie and Selwyn mountains. First, we assess whether the mountain woodland caribou in the central Mackenzie and Selwyn mountains, NWT, comprise a single, large herd: the Redstone herd (Fig. 1). If this is the case, we expect to find a single, homogenous population. Alternatively, the traditional Redstone herd could be a metapopulation composed of several herds sharing a common range (Olsen et al., 2001). In this case, we expect to see distinct genetic clusters related to the geographic proximity of the individuals.

Second, we address the temporal stability of the NWT mountain woodland caribou populations. We propose that ancient caribou using the NWT ice patches are the ancestors of the modern Redstone herd. This assumes that the deposition of the White River tephra (volcanic ashfall) around 1200 years ago (Froese et al., 2007) did not force caribou to move out of the area, as has been shown for caribou living in the Southern Lakes region, YT, where more ash was deposited than in the NWT (Lerbekmo, 2008; Kuhn et al., 2010).

Third, we explore whether or not the mountain woodland caribou of the NWT and of YT are genetically distinct from one another. It has been proposed (Kuhn et al., 2010) that the ancient caribou populations that previously resided in the southwestern YT (Fig. 1) are genetically distinct from the populations currently residing in the same area of the YT. If the ancient NWT mountain woodland caribou are genetically distinct from the ancient YT caribou, but not from the modern YT caribou, then the NWT mountain woodland caribou could represent a potential source population for the modern YT caribou.

Fourth, we test for genetic differentiation corresponding to ecotype within the region—mountain woodland form (*R. t. caribou*) vs. continental tundra form (*R. t. groenlandicus*, Canadian barren-ground; *R. t. granti*, Alaska caribou)—and between subspecies within the NWT (*R. t. groenlandicus* v. *R. t. caribou*). We expect to see no genetic differentiation, as previous studies have failed to identify mitochondrial differentiation between these ecotypes or subspecies (Flagstad and Røed, 2003; Kuhn et al., 2010). We propose that this lack of differentiation is not a modern phenomenon, and that the two ecotypes either shared a very recent common ancestor or have undergone considerable recent admixture. However, genetic differentiation may exist, as herds belonging to each of the two ecotypes are unlikely to experience significant mixing.

MATERIALS AND METHODS

Sample Collection

Between 2005 and 2008, 17 caribou bones and teeth were collected from 11 ice patches in the Mackenzie and Selwyn mountains of the western NWT, Canada, along the border with the YT (Andrews et al., 2012). A modern antler was also collected from an ice patch and sampled for DNA. Radiocarbon (^{14}C) dates on ancient specimens yielded ages ranging from a few hundred to almost 4000 years (Table 1). Dates are reported as radiocarbon years before present (BP), calibrated with the IntCal09 curve (Reimer et al., 2009) using CALIB 6.0 (Stuiver and Reimer, 1993).

To assess changes in the mitochondrial genetic diversity of caribou living in the region over time, we obtained 38 hair samples from the Northwest Territories Department of Environment and Natural Resources that had been collected from caribou killed in defined outfitter (hunting) zones (Fig. 2). Although none of the hair specimens we obtained were attributed to any particular herd, they likely belonged to one of three: the mountain woodland Redstone herd, whose range overlaps all outfitter zones and ice patches from which samples were obtained; the Bonnet Plume herd, which is present in the western part of outfitter zone S/OT/01; and the Carcajou herd, which may overlap the eastern part outfitter zone S/OT/01.

The ice patches yielding ancient caribou remains fall mainly within the Ram Head outfitter zone S/OT/03, from which we obtained 10 modern samples. The study area over which ice patches have been discovered also includes the Mackenzie Mountain outfitter zone S/OT/02 (represented in our study by 9 modern samples) in the north, and the NWT outfitter zone S/OT/04 (represented by 9 modern samples) in the south. The Gana River outfitter zone S/OT/01 (represented by 4 modern samples) borders the study area to the north, while the Redstone outfitter zone S/OT/05 (represented by 4 modern samples) borders the study area to the southeast. No samples from the Nahanni Butte outfitter zone (D/OT/02) or the South Nahanni outfitter zone (D/OT/01) were available.

Sample Processing

DNA Extraction from Ancient Specimens: We processed all ancient samples following standard protocols for working with ancient DNA (Cooper and Poinar, 2000). For example, we performed all DNA extraction and pre-amplification steps in a sterile, dedicated laboratory that is spatially isolated from other molecular biology research at the Pennsylvania State University. We used multiple negative controls during both the DNA extraction and PCR phases, and we cloned samples to assess both DNA damage and contamination by exogenous sources.

We prepared powder samples from ancient bones and teeth using a Dremel tool equipped with a cutting disk and a Mikrodismembrator (B. Braun Biotech International) for pulverization. We extracted DNA following a silica-based protocol (Rohland et al., 2009), using no more than 500 mg of bone powder from each sample. We eluted the aDNA extracts in a final concentration of 50μl of TE buffer.

DNA Extraction from Modern Specimens: We processed the hair sampled from living caribou in the modern molecular biology facility after all ancient caribou work had been completed, so as to avoid the possibility of contamination of ancient extracts with modern caribou DNA. We use the Qiagen DNeasy Blood and Tissue kit (Qiagen, Inc.) with several modifications to more efficiently digest hair: we doubled the amount of lysis buffer ATL, increased the amount of proteinase K from 20 μl to 30 μl, and added 20 μl of 1M DTT to promote lysis of the hair shafts. Incubation was carried out overnight at 56°C. Because of the increased volume of the extraction mixture, it was also necessary to double the amount of buffer AL and ethanol during the silica-binding and wash phases. Samples were eluted in 150 μl of buffer TE.

DNA Amplification from Ancient Specimens: We amplified the entire 564 bp (base pair) fragment of caribou mitochondrial control region hypervariable segment 1 using a single primer pair (CBF1/CB5R; Kuhn et al., 2010). Prior to amplification, we added 2 μl of Tween 20 (Sigma-Aldrich) to each ~50 μl extract. We performed PCR in 25 μl reactions using Platinum Taq High Fidelity DNA polymerase (Invitrogen), with the following components and final concentrations: 2 mM MgSO$_4$, 250 μM dNTPs, 1 μM primers, 2 mg/ml rabbit serum albumin (Sigma-Aldrich). Cycling conditions were 94°C for 60 s, followed by 55 cycles of 94°C for 30 s, 63°C for 45 s, and 68°C for 45 s. PCR products were resolved on a 2% agarose gel and visualized using ethidium bromide and UV transillumination. Amplification was successful for all extracts, and no PCR products were observed in the negative extraction or amplification controls.

To assess DNA damage and screen ancient specimens for contamination, we cloned all but one sample using the TOPO TA cloning kit (Invitrogen) according to manufacturers' instructions. Six clones from each sample were sequenced using the M13F(-20) primer (TOPO manual).

DNA Amplification from Modern Specimens: For the modern specimens, we amplified the same 564 bp fragment used in the aDNA analysis. Reactions were performed in 25 μl reactions using GoTaq Flexi DNA polymerase (Promega), with the following components and final concentrations: 2 mM MgCl2, 250 μM dNTPs, 1 μM primers. Cycling conditions were 94°C for 60 s, followed by 35 cycles of 94°C for 1 min., 63°C for 1 min., 72°C for 2 min, and a final extension of 72°C for 10 min. Sequencing of amplified products was performed using CB1F and two nested sequencing primers CARseqF (5' TAAAC-TATTCCCTGGCG 3') and CARseqR (5' AATAGCTAC-CCCCACAG 3'). All sequencing reactions were performed using the BigDye v3.1 chemistry (Applied Biosystems) and resolved on an ABI 3730xl capillary sequencer at the Penn State Genomics Core Facility (University Park, PA).

TABLE 1. Samples from ancient and modern caribou of the Yukon, Northwest Territories, and Alaska used for genetic analysis. Specific location indicates ice patch (IP) or outfitter zone (OZ). The element sampled is provided only for data generated as part of this study. Ancient specimens are assigned to the herd that presently occupies their geographic location or to the closest neighbouring herd(s).

Subspecies	Sample name	Haplotype	Collection ID	Herd	Specific location	Sample type	C¹⁴ BP	Cal C¹⁴ BP	Genbank
R. t. caribou (NWT)	BL125	Rt014	7T9-Z2	Redstone	7T9 (IP)	mandible (left)	570 ± 40	615.5 ± 35.5	JF894172[1]
	BL127	Rt047	KfTe-1	Redstone	KfTe-1 (IP)	vertebra (thoracic)	2490 ± 40	2582 ± 147	JF894173[1]
	BL128	Rt048	KfTd-1	Redstone	KfTd-1 (IP)	mandible (right)	330 ± 40	394.5 ± 89.5	JF894174[1]
	BL129	Rt052	KhTe-2	Redstone	KhTe-2 (IP)	phalange (1st)	880 ± 40	820 ± 95	JF894175[1]
	BL131	Rt048	KhTe-2	Redstone	KhTe-2 (IP)	rib (left)	990 ± 40	879.5 ± 84.5	JF894176[1]
	BL132	Rt007	7T10-Z20	Redstone	7T10 (IP)	vertebra (thoracic)	910 ± 40	829 ± 89	JF894177[1]
	BL133	Rt049	KhTe-2	Redstone	KhTe-2 (IP)	axis	1140 ± 40	1056 ± 93.5	JF894178[1]
	BL134	Rt050	7T1-Z1	Redstone	7T1 (IP)	scapula	2520 ± 40	2605.5 ± 139.5	JF894179[1]
	BL136	Rt011	7T9-Z1	Redstone	7T9 (IP)	antler	3510 ± 40	3790 ± 102	JF894180[1]
	BL139	Rt038	KiTf-1-Z6	Redstone	KiTf-1 (IP)	vertebra (lumbar)	370 ± 40	462 ± 42	JF894181[1]
	BL140	Rt032	7T2-Z6	Redstone	7T2 (IP)	innominate (left)	1830 ± 40	1782 ± 89	JF894182[1]
	BL141	Rt052	8T3-ZI	Redstone	8T3 (IP)	calcaneous (right)	2950 ± 40	3111 ± 136	JF894183[1]
	BL142	Rt042	KfTe-1	Redstone	KfTe-1 (IP)	vertebra (lumbar)	2530 ± 40	2616 ± 130.5	JF894184[1]
	BL143	Rt053	7T1-Z8	Redstone	7T1 (IP)	mandible (right)	520 ± 40	532.5 ± 29.5	JF894185[1]
	BL144	Rt007	7T10-Z3	Redstone	7T10 (IP)	vertebra (lumbar)	1080 ± 40	995 ± 66	JF894186[1]
	BL145	Rt049	KhTe-2	Redstone	KhTe-2 (IP)	mandible (left)	800 ± 40	727.5 ± 55.5	JF894187[1]
	BL155	Rt007	7T10-Z2	Redstone	7T10 (IP)	innominate	1050 ± 40	987 ± 71	JF894188[1]
	BL157	Rt054	KiTf-1-Z8	Redstone	KiTf-1 (IP)	antler	modern	modern	JF894189[1]
	BL160	Rt054	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894190[1]
	BL161	Rt049	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894191[1]
	BL162	Rt055	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894192[1]
	BL163	Rt054	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894193[1]
	BL164	Rt063	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894194[1]
	BL165	Gra907	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894195[1]
	BL166	Rt054	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894196[1]
	BL167	Rt049	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894197[1]
	BL168	Rt049	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894198[1]
	BL169	Rt056	2002	Redstone	Mackenzie Mountains OZ	hair	modern	modern	JF894199[1]
	BL170	Rt037	2002	Redstone	Ramhead OZ	hair	modern	modern	JF894200[1]
	BL171	Rt056	2002	Redstone	Ramhead OZ	hair	modern	modern	JF894201[1]
	BL172	Rt037	2002	Redstone	Ramhead OZ	hair	modern	modern	JF894202[1]
	BL173	Rt057	2002	Redstone	Ramhead OZ	hair	modern	modern	JF894203[1]
	BL174	Rt049	2002	Redstone	Ramhead OZ	hair	modern	modern	JF894204[1]
	BL175	Rt058	2002	Redstone	Ramhead OZ	hair	modern	modern	JF894205[1]
	BL176	Rt059	2002	Redstone	Ramhead OZ	hair	modern	modern	JF894206[1]
	BL177	Gra101 (Rt030)	2003	Redstone	Ramhead OZ	hair	modern	modern	JF894207[1]
	BL178	Rt060	2003	Redstone	Ramhead OZ	hair	modern	modern	JF894208[1]
	BL179	Rt061	2003	Redstone	Ramhead OZ	hair	modern	modern	JF894209[1]
	BL181	Rt049	2002	Redstone	Northwest Territories OZ	hair	modern	modern	JF894210[1]
	BL182	Rt061	2002	Redstone	Northwest Territories OZ	hair	modern	modern	JF894211[1]
	BL183	Rt062	2002	Redstone	Northwest Territories OZ	hair	modern	modern	JF894212[1]
	BL184	Rt037	2002	Redstone	Northwest Territories OZ	hair	modern	modern	JF894213[1]
	BL185	Rt063	2002	Redstone	Northwest Territories OZ	hair	modern	modern	JF894214[1]
	BL186	Rt037	2002	Redstone	Northwest Territories OZ	hair	modern	modern	JF894215[1]
	BL187	Rt037	2002	Redstone	Northwest Territories OZ	hair	modern	modern	JF894216[1]
	BL188	Rt064	2002	Redstone	Northwest Territories OZ	hair	modern	modern	JF894217[1]
	BL189	Rt037	2003	Redstone	Northwest Territories OZ	hair	modern	modern	JF894218[1]
	BL190	Rt056	2002	Redstone	Redstone OZ	hair	modern	modern	JF894219[1]
	BL191	Rt038	2002	Redstone	Redstone OZ	hair	modern	modern	JF894220[1]
	BL192	Rt061	2002	Redstone	Redstone OZ	hair	modern	modern	JF894221[1]

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Subspecies	Sample name	Haplotype	Collection ID	Herd	Specific location	Sample type	C[14] BP	Cal C[14] BP	Genbank
	BL194	Rt061	2002	Redstone	Redstone OZ	hair	modern	modern	JF894222[1]
	BL195	Rt054	2002	Redstone/Bonnet Plume/Carcajou	Gana River OZ	hair	modern	modern	JF894223[1]
	BL196	Rt064	2002	Redstone/Bonnet Plume/Carcajou	Gana River OZ	hair	modern	modern	JF894224[1]
	BL197	Rt011	2002	Redstone/Bonnet Plume/Carcajou	Gana River OZ	hair	modern	modern	JF894225[1]
	BL198	Rt049	2002	Redstone/Bonnet Plume/Carcajou	Gana River OZ	hair	modern	modern	JF894226[1]
	BL199	Rt056	2002	Redstone/Bonnet Plume/Carcajou	Gana River OZ	hair	modern	modern	JF894227[1]
R. t. caribou (YT)	TK11	Rt001	IP36-01-87	Aishihik	Gladstone (IP)		3790 ± 40	4185 ± 110.5	GU32754[2]
	TK12	Rt002	IP36-01-89	Aishihik	Gladstone (IP)		3820 ±	4228.5 ± 88.5	GU327543[2]
	TK15	Gra102 (Rt003)	IP54-03-04	Ibex	Sandpiper (IP)		90 ± 40	80 ± 68	GU327544[2]
	TK46	Gra102 (Rt003)	GL-03-08	Aishihik	Gladstone (IP)		5000	5709 ± 65	GU327545[2]
	TK13	Gra102 (Rt003)	IP54-03-03	Ibex	Sandpiper (IP)		120 ± 40	80 ± 71	GU327546[2]
	TK18	Rt004	FRI-99-205	Ibex	Friday Creek (IP)		2500	2596.5 ± 142.5	GU327547[2]
	TK19	Rt005	THA-98-10	Aishihik/Ibex	Thandlat (IP)		3000	3203.5 ± 131.5	GU327548[2]
	TK20	Rt006	FRI-99-232	Ibex	Friday Creek (IP)		2500	2596.5 ± 142.5	GU327549[2]
	TK21	Rt006	BRA-98-5	Aishihik/Ibex	Bratnober (IP)		5000	5709 ± 65	GU327550[2]
	TK54	Rt007	IP38-99-04	Ibex	Alligator (IP)		2340 ± 40	2397 ± 91	GU327551[2]
	TK43	Rt008	FRI-99-237	Ibex	Friday Creek (IP)		2500	2596.5 ± 142.5	GU327552[2]
	TK44	Rt009	GL-03-10	Aishihik	Gladstone (IP)		5000	5709 ± 65	GU327553[2]
	TK48	Rt010	GL-03-09	Aishihik	Gladstone (IP)		5000	5709 ± 65	GU327554[2]
	TK50	Rt011	IP37-99-06	Ibex	Friday Creek (IP)		3760 ± 40	4150.5 ± 90.5	GU327555[2]
	TK51	Rt012	IP37-99-11	Ibex	Friday Creek (IP)		3890 ± 40	4324.5 ± 97.5	GU327556[2]
	TK52	Rt013	IP37-99-07	Ibex	Friday Creek (IP)		2510 ± 40	2600 ± 142.5	GU327557[2]
	TK53	Rt012	IP29-99-04	Ibex	Sandpiper (IP)		5710 ± 40	6489 ± 142	GU327558[2]
	TK55	Rt014	IP37-99-10	Ibex	Friday Creek (IP)		3150 ± 40	3386.5 ± 67.5	GU327559[2]
	TK61	Rt014	IP37-00-20	Ibex	Friday Creek (IP)		3220 ± 40	3428 ± 62	GU327560[2]
	TK60	Rt014	IP1-98-03	Aishihik/Ibex	Thandlat (IP)		3140 ± 40	3382.5 ± 65.5	GU327561[2]
	TK62	Rt015	IP38-00-14	Ibex	Alligator (IP)		1000	885 ± 90	GU327562[2]
	TK64	Rt016	IP52-00-03A	Ibex	Texas Gulch (IP)		6320 ± 40	7243.5 ± 80.5	GU327563[2]
	TK47	Rt010	GL-03-11	Aishihik	Gladstone (IP)		5000	5709 ± 65	GU327564[2]
	TK23	Rt017	FRI-99-251	Ibex	Friday Creek (IP)		2500	2596.5 ± 142.5	GU327565[2]
	TK45	Rt006	GL-03-07	Aishihik	Gladstone (IP)		5000	5709 ± 65	GU327566[2]
	IP02	Rt018	IP1-98-01	Aishihik/Ibex	Thandlat (IP)		4760 ± 40	5519 ± 70	GU327567[2]
	IP04	Rt019	IP31-00-02	Aishihik/Ibex	Upper Jo Jo (IP)		790 ± 40	722.5 ± 53.5	GU327568[2]
	IP06	Rt020	IP34-04-02	Aishihik/Ibex	Vand Creek (IP)		2700 ± 40	2808.5 ± 59.5	GU327569[2]
	IP07	Rt021	IP35-04-01	Aishihik	L. Gladstone (IP)		4190 ± 40	4688.5 ± 79.5	GU327570[2]
	IP11	Rt006	IP36-00-18	Aishihik	Gladstone (IP)		4830 ± 40	5512.5 ± 39.5	GU327571[2]
	IP18	Rt006	IP36-04-56	Aishihik	Gladstone (IP)		3480 ± 40	3743.5 ± 103.5	GU327572[2]
	IP19	Rt022	IP37-00-19	Ibex	Friday Creek (IP)		3740 ± 40	4080.5 ± 101.5	GU327573[2]
	IP28	Rt023	IP37-99-08	Ibex	Friday Creek (IP)		2320 ± 40	2381 ± 79	GU327574[2]
	IP29	Rt008	IP37-99-09	Ibex	Friday Creek (IP)		1940 ± 40	1904 ± 89	GU327575[2]
	IP33	Rt024	IP37-99-13	Ibex	Friday Creek (IP)		2550 ± 40	2566 ± 77	GU327576[2]
	IP34	Rt025	IP38-00-15	Ibex	Alligator (IP)		360 ± 40	364.5 ± 49.5	GU327577[2]
	IP36	Rt026	IP50-99-01	Aishihik	E. Thulsoo (IP)		190 ± 40	180.5 ± 48.5	GU327578[2]
	IP42	Rt018	IP80-03-01B	Wolf Lake	Irvine (IP)		190 ± 40	180.5 ± 48.5	GU327579[2]
	IP43	Rt014	JBVB-1:11	Ibex	Texas Gulch (IP)		5000 ± 40	5709 ± 65	GU327580[2]
	IP44	Rt027	JCUU-2:20	Ibex	Alligator (IP)		3720 ± 40	4061 ± 97	GU327581[2]
	AF096444	Rt018		Wolf Lake			modern	modern	AF096444[4]
	TK72	Gra102 (Rt003)	ACH/95F-B4	Aishihik			modern	modern	GU327582[2]
	TK78	Gra102 (Rt003)	ACH/96F-Y16	Aishihik			modern	modern	GU327582[2]
	TK91	Gra102 (Rt003)	104817	Fortymile			modern	modern	GU327582[2]

TABLE 1. Samples from ancient and modern caribou of the Yukon, Northwest Territories, and Alaska used for genetic analysis. Specific location indicates ice patch (IP) or outfitter zone (OZ). The element sampled is provided only for data generated as part of this study. Ancient specimens are assigned to the herd that presently occupies their geographic location or to the closest neighbouring herd(s) – *continued:*

Subspecies	Sample name	Haplotype	Collection ID	Herd	Specific location	Sample type	C¹⁴ BP	Cal C¹⁴ BP	Genbank
	TK93	Gra102 (Rt003)	104820	Fortymile			modern	modern	GU327582[2]
	TK31	Rt028	10349	Ibex			modern	modern	GU327583[2]
	TK34	Rt028	10343	Ibex			modern	modern	GU327583[2]
	TK105	Rt028	1.162	Ibex			modern	modern	GU327583[2]
	TK106	Rt028	1.832	Ibex			modern	modern	GU327583[2]
	TK108	Rt028	ET282	Ibex			modern	modern	GU327583[2]
	TK111	Rt028	ICH/95F-B26	Ibex			modern	modern	GU327583[2]
	TK113	Rt028	ICH/95F-B48	Ibex			modern	modern	GU327583[2]
	TK32	Rt007	10351	Aishihik			modern	modern	GU327584[2]
	TK33	Rt007	10348	Aishihik			modern	modern	GU327584[2]
	TK71	Rt007	ACH/95F-B3	Aishihik			modern	modern	GU327584[2]
	TK73	Rt007	ACH/95F-B73	Aishihik			modern	modern	GU327584[2]
	TK74	Rt007	ACH/95F-B82	Aishihik			modern	modern	GU327584[2]
	TK75	Rt007	ACH/95F-B84	Aishihik			modern	modern	GU327584[2]
	TK76	Rt007	ACH/95F-F1	Aishihik			modern	modern	GU327584[2]
	TK77	Rt007	ACH/95F-F3	Aishihik			modern	modern	GU327584[2]
	TK79	Rt007	ACH/96F-Y19	Aishihik			modern	modern	GU327584[2]
	TK80	Rt007	ACH/96F-Y34	Aishihik			modern	modern	GU327584[2]
	TK102	Rt007	105215	Fortymile			modern	modern	GU327585[2]
	TK97	Rt011	104824	Fortymile			modern	modern	GU327585[2]
	TK85	Gra951 (Rt029)	104811	Fortymile			modern	modern	GU327586[2]
	TK95	Gra951 (Rt029)	104822	Fortymile			modern	modern	GU327586[2]
	TK100	Gra951 (Rt029)	104831	Fortymile			modern	modern	GU327586[2]
	TK92	Gra101 (Rt030)	104819	Fortymile			modern	modern	GU327587[2]
	TK86	Gra905 (Rt031)	104812	Fortymile			modern	modern	GU327588[2]
	TK101	Gra905 (Rt031)	104842	Fortymile			modern	modern	GU327588[2]
	TK107	Rt032	1.87	Ibex			modern	modern	GU327589[2]
	TK87	Rt033	104813	Fortymile			modern	modern	GU327590[2]
	TK94	Rt034	104821	Fortymile			modern	modern	GU327591[2]
	TK81	Rt035	CCH/95F-B51	Carcross			modern	modern	GU327592[2]
	TK88	Rt036	104814	Fortymile			modern	modern	GU327593[2]
	TK109	Rt037	ET284	Ibex			modern	modern	GU327594[2]
	TK110	Rt037	ICH/95F-B19	Ibex			modern	modern	GU327594[2]
	TK89	Rt026	104815	Fortymile			modern	modern	GU327595[2]
	TK90	Rt038	104816	Fortymile			modern	modern	GU327596[2]
	TK96	Rt038	104823	Fortymile			modern	modern	GU327596[2]
	TK103	Rt038	105216	Fortymile			modern	modern	GU327596[2]
	TK104	Rt039	0.671	Ibex			modern	modern	GU327597[2]
	TK84	Rt040	104810	Fortymile			modern	modern	GU327598[2]
R. t. granti (Alaska)	AY178675	Gra102 (Rt003)		unknown			modern	modern	AY178675[3]
	AY178675.2	Gra102 (Rt003)		unknown			modern	modern	AY178675[3]
	AY178676	Gra104		unknown			modern	modern	AY178676[3]
	AY178676.2	Gra104		unknown			modern	modern	AY178676[3]
	AY178677	Gra101 (Rt030)		unknown			modern	modern	AY178677[3]
	AY178678	Gra103		unknown			modern	modern	AY178678[3]
	AY178715	Gra899		unknown			modern	modern	AY178715[3]
	AY178716	Gra903		unknown			modern	modern	AY178716[3]
	AY178717	Gra904		unknown			modern	modern	AY178717[3]
	AY178718	Gra905 (Rt031)		unknown			modern	modern	AY178718[3]

TABLE 1. Samples from ancient and modern caribou of the Yukon, Northwest Territories, and Alaska used for genetic analysis. Specific location indicates ice patch (IP) or outfitter zone (OZ). The element sampled is provided only for data generated as part of this study. Ancient specimens are assigned to the herd that presently occupies their geographic location or to the closest neighbouring herd(s).

Subspecies	Sample name	Haplotype	Collection ID	Herd	Specific location	Sample type	C^{14} BP	Cal C^{14} BP	Genbank
R. t. groenlandicus (NWT)	AY178720	Gra907		unknown			modern	modern	AY178720[3]
	AY178721	Gra951 (Rt029)		unknown			modern	modern	AY178721[3]
	AY178706	Gro20		unknown			modern	modern	AY178706[3]
	AY178707	Gro23		unknown			modern	modern	AY178707[3]
	AY178708	Gro25		unknown			modern	modern	AY178708[3]
	AY178709	Gro26		unknown			modern	modern	AY178709[3]
	AY178700	Gro55		unknown			modern	modern	AY178700[3]
	AY178701	Gro56		unknown			modern	modern	AY178701[3]
	AY178696	Gro59		unknown			modern	modern	AY178696[3]
	AY178699	Gro64		unknown			modern	modern	AY178699[3]
	AY178697	Gro67		unknown			modern	modern	AY178697[3]
	AY178698	Gro68		unknown			modern	modern	AY178698[3]
	AY178710	Gro71		unknown			modern	modern	AY178710[3]

[1] This study.
[2] Kuhn et al. (2010).
[3] Flagstad and Roed (2003).
[4] Unpublished.

Genetic Analysis

We used the Lasergene Suite (DNAStar) software to align sequences and checked the alignments by eye in Se-Al version 2.0a11 (Rambaut, 2002). To expand the geographic range of our data set, we obtained 106 additional, previously published caribou sequences from Genbank, including 11 NWT barren-ground caribou, 30 YT barren-ground caribou, and 65 modern and ancient YT northern mountain woodland caribou (Table 1) corresponding to five recognized herds (Table 2). The final alignment length was 503 bp.

To identify population structure, we constructed a median-joining network using Network v4.6.0.0 (Bandelt et al., 1999). Twelve sequences (TK31, TK34, TK105, TK106, TK108, TK111, TK113, TK45, TK61, AY178708, AY178701, AY178700) containing ambiguous nucleotides were not included in the analysis, as recommended by the Network user manual. In addition, to achieve less than 5% missing data for each nucleotide position in the alignment, while maintaining as much data (alignment length) and as many individuals as possible, seven modern NWT individuals (BL164, BL170, BL172, BL178, BL184, BL189, BL196) were removed.

To incorporate the spatial and temporal aspect of our data, we used BEAST v1.6.1 (Drummond and Rambaut, 2007), which allows the explicit use of sample dates in a coalescent-based genealogical inference framework. We selected the best-fitting evolutionary model using jModeltest v0.1.1 (Guindon and Gascuel, 2003; Posada, 2008). BEAST analyses were performed using the HKY model of nucleotide substitution (Hasegawa et al., 1985) with gamma distributed rate heterogeneity and assuming a strict molecular clock and constant population size coalescent prior. We simulated one MCMC run for 50 million iterations, drawing samples from the posterior every 5000 iterations. We used Tracer v1.5 (Drummond and Rambaut, 2007) to check that estimated sample sizes of all parameters were sufficient (ESS > 200) and to verify that the parameter estimates had reached stationarity. The maximum clade credibility (MCC) tree was calculated using treeAnnotator, following removal of the initial 10% of samples as burn-in.

Finally, to measure genetic differentiation between predefined groups, we used Arlequin v3.5.1.2 (Excoffier and Lischer, 2010) to calculate FST values and to perform a standard AMOVA (Analysis of MOlecular Variance; Weir and Cockerham, 1984), which calculates the degree of genetic variation attributable to among-group differences and within-group differences. Significance of the AMOVA was determined using a null distribution obtained from 1023 nonparametric permutations. A Bonferroni correction for multiple tests was applied. Summary statistics including nucleotide diversity (π) and haplotype diversity (h) were also calculated for many of the groups in Arlequin.
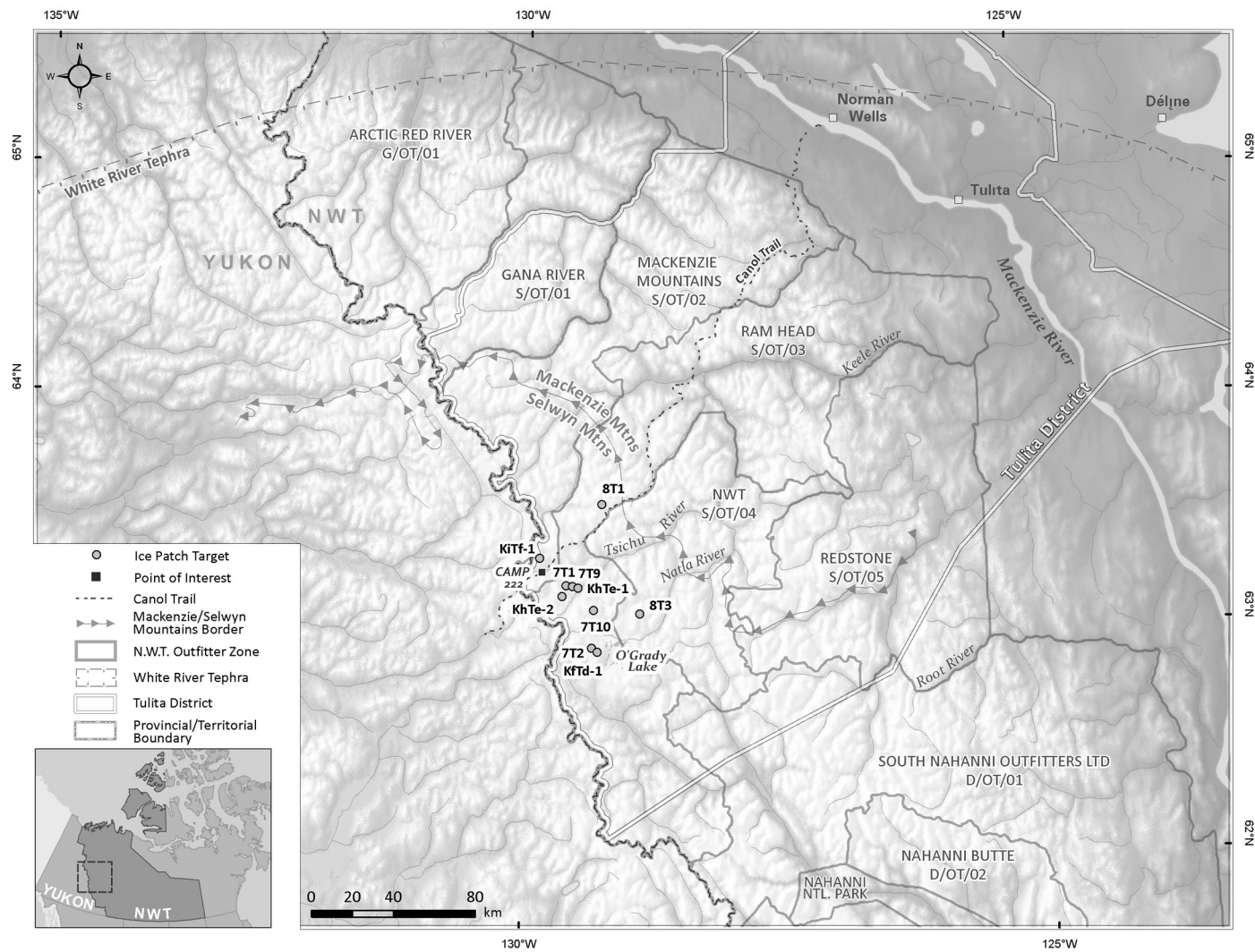
FIG. 2. The study area in the Mackenzie and Selwyn mountains, NWT, indicating the locations of ice patches, the NWT outfitter zones, and the margins of the White River tephra (Lerbekmo, 2008).

TABLE 2. Summary of samples analyzed from each herd. Numbers shown below include sequences generated as part of this study and previously published sequences obtained from GenBank (Table 1).

Subspecies	Territory or state	Herd (modern)	Ecotype (modern)	Migratory (modern)	N (modern)	N (ancient)
R. t. caribou	NWT	Redstone	woodland	yes	39	17
	YT	Wolf Lake	woodland	no	1	1
	YT	Aishihik	woodland	no	12	11
	YT	Ibex	woodland	no	11	22
	YT	Aishihik/Ibex	woodland	no	0	6
	YT	Carcross	woodland	no	1	0
R. t. groenlandicus	NWT	unknown	barrenground	yes	11	0
R. t. granti	YT and AK	Fortymile	barrenground	yes	18	0
	AK	unknown	barrenground	yes	12	0

RESULTS

DNA Sequencing Results and Ancient DNA Damage

All new sequences have been deposited in Gen-Bank under the accessions JF894172–JF894227 (Table 1). No non-caribou sequences were obtained in any cloning reactions, indicating the lack of PCR contamination.

In one case, a nuclear mitochondrial insertion (numt) was observed. The closest match to this sequence retrieved by BLAST was an imperfect alignment to white-tailed deer, and as no deer had previously been handled in this lab, the sequence was assumed to be a caribou numt. In no case was the sequence found in other caribou amplifications. Cloning revealed varying degrees of DNA damage in all specimens, with an excess of C to T substitutions, as expected from
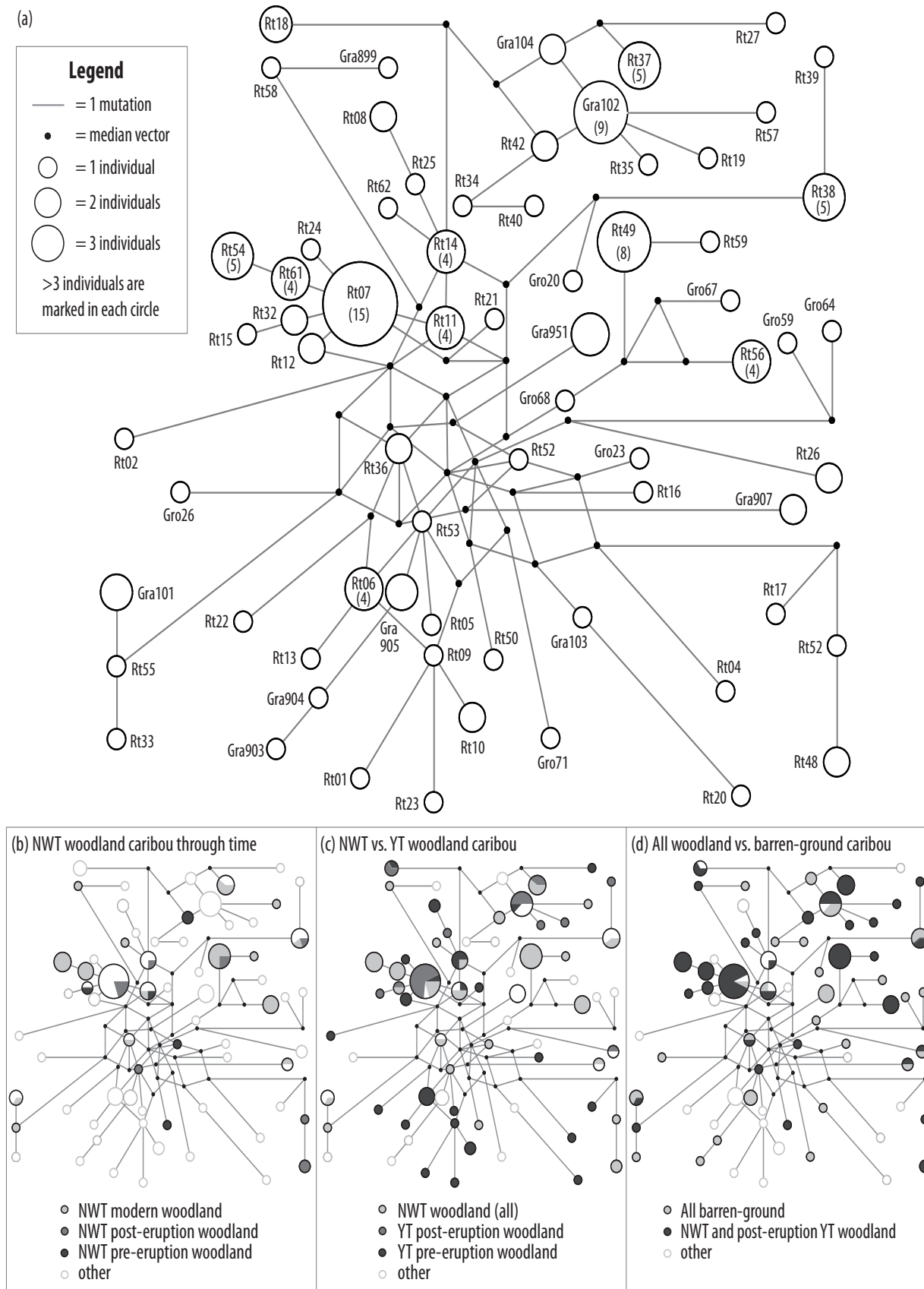
FIG. 3. Median-joining network of caribou mitochondrial haplotypes included in this study. (a) Unweighted median-joining network for all individuals included in this study that did not contain ambiguous bases or excess levels of missing data. Haplotype numbers (Table 1) are indicated for each circle, and circle size indicates the number of observations of each haplotype. (b–d) The network in (a) is shaded to highlight individuals relevant to three questions addressed in the text.

cytosine deamination in ancient DNA molecules (Hofreiter et al., 2001; Gilbert et al., 2006).

Genetic Analysis

To test whether the Redstone herd is actually composed of multiple herds with overlapping ranges, we examined the relationships among caribou haplotypes identified by Network and BEAST. Well-defined clades could be indicative of herd fidelity. The median-joining network (Fig. 3) and the MCC tree (Fig. 4) revealed very little genetic structure in YT and NWT caribou.

To test the next three hypotheses, we performed FST and AMOVA calculations to examine the degree of genetic differentiation between predefined groups (Table 3). To assess whether ancient caribou from the ice patches are the ancestors of the modern Redstone herd, we compared all ancient (ice patch samples older than modern) NWT caribou to all modern NWT mountain woodland caribou. An FST score of 0.042 indicated little differentiation between the two groups. The AMOVA suggested that ancient and modern NWT caribou were not significantly differentiated ($p = 0.0645$) and that the majority of variation was due to within-group diversity (96%), rather than to between-group differentiation. Furthermore, haplotype diversity (h) in the NWT mountain woodland caribou did not change through time (ancient $h = 0.923$, modern $h = 0.963$, Table 4). On the basis of these results indicating that the NWT population is not significantly differentiated through time, all NWT mountain woodland caribou were treated as a single population for all subsequent analyses.

To test whether NWT caribou were affected by the deposition of the White River tephra, we again assumed two subpopulations, representing caribou living in the region before and after the deposition event around 1200 years ago. No genetic difference was detected between these two groups using either FST (0.0) or AMOVA ($p = 0.4106$). To compare our results with those of Kuhn et al. (2010), we performed a similar set of tests using the previously published data set of Yukon caribou. In agreement with their results, we found an FST value of 0.169, indicating moderate differentiation (Wright, 1978) between caribou inhabiting the Yukon before and after the eruption. An AMOVA showed that 17% of the total genetic variation was explained by differences between the pre- and post-eruption groups and was highly significant ($p = 0.0$). Haplotype diversity (Table 4) in the caribou inhabiting the area of the ice patches after the eruption ($h = 0.851$) was lower than that in the population inhabiting the area before the eruption ($h = 0.977$). In addition to these results, we found significant differentiation (FST = 0.108 – 0.138, AMOVA $p = 0.0$, Table 2) between mountain woodland caribou in the NWT and both pre- and post-eruption YT populations.

To test whether barren-ground and mountain woodland caribou ecotypes are genetically distinct from each other in the NWT, we compared modern barren-ground caribou to ancient and modern mountain woodland caribou from the study area. Ancient caribou from the ice patches were assumed to be woodland, as only mountain woodland caribou use the ice patches during summer months. The two ecotypes appear significantly differentiated in the NWT (FST = 0.082, AMOVA $p = 0.0068$). A similar result (FST = 0.041, AMOVA $p = 0.0020$) was obtained when all individuals in the study were included except the pre-eruption YT caribou, which appear significantly genetically differentiated from all modern caribou in our study. Both mountain woodland vs. barren-ground comparisons are significant at $p = 0.05$ when Bonferroni correction for 7 tests is applied ($p < 0.00714$).

DISCUSSION

Redstone Herd Identity

Miller (1982) described a herd as an aggregation of caribou that remained together for at least a major portion of the year, and in which all breeding females shared an affinity for specific calving grounds not used by females from other herds. Three mountain woodland herds are defined in the Mackenzie Mountains of the NWT (Lortie, 1982). From north to south, they are the Bonnet Plume herd, the Redstone herd, and the South Nahanni herd (Fig. 1). The Redstone caribou herd is one of the largest woodland herds in Canada, numbering 5000 to 10 000 individuals (Olsen et al., 2001). The Redstone herd occupies a summer range along the border with the Yukon near MacMillan Pass. During the winter, the herd ranges from the eastern slopes of the Mackenzie Mountains to the Redstone, Keele, and Moose Horn river basins. However, groups of several thousand animals have also been observed in this winter range during July and August (Veitch et al., 2000), which could suggest that more than one herd is living within the Redstone herd range. We do not observe any distinct clusters in either the Network (Fig. 3) or BEAST (Fig. 4) analyses, indicating that the Redstone herd likely represents a single large, diverse population. Although no groupings corresponding to outfitter zones are seen, using such large areas as kill localities may mask the observation of smaller geographic groupings. In the future, it will be important to record more specific kill locations, when feasible, in order to determine if any genetic structure exists within the Redstone range.

The White River Tephra and Caribou Displacement

The White River tephra is an ash layer resulting from the second Holocene eruption of the Bona-Churchill volcanic complex in southern Alaska around 1200 years ago (West and Donaldson, 2002; Froese et al., 2007; Lerbekmo, 2008). The eruption resulted in an ashfall over as much as one million km^2 of land in Alaska, the Yukon, British Columbia, and the NWT (Lerbekmo, 2008). The thickness of the ash ranged from dozens of meters in Alaska to just a few millimeters at its farthest extent near Great Slave Lake, NWT (Lerbekmo, 2008).
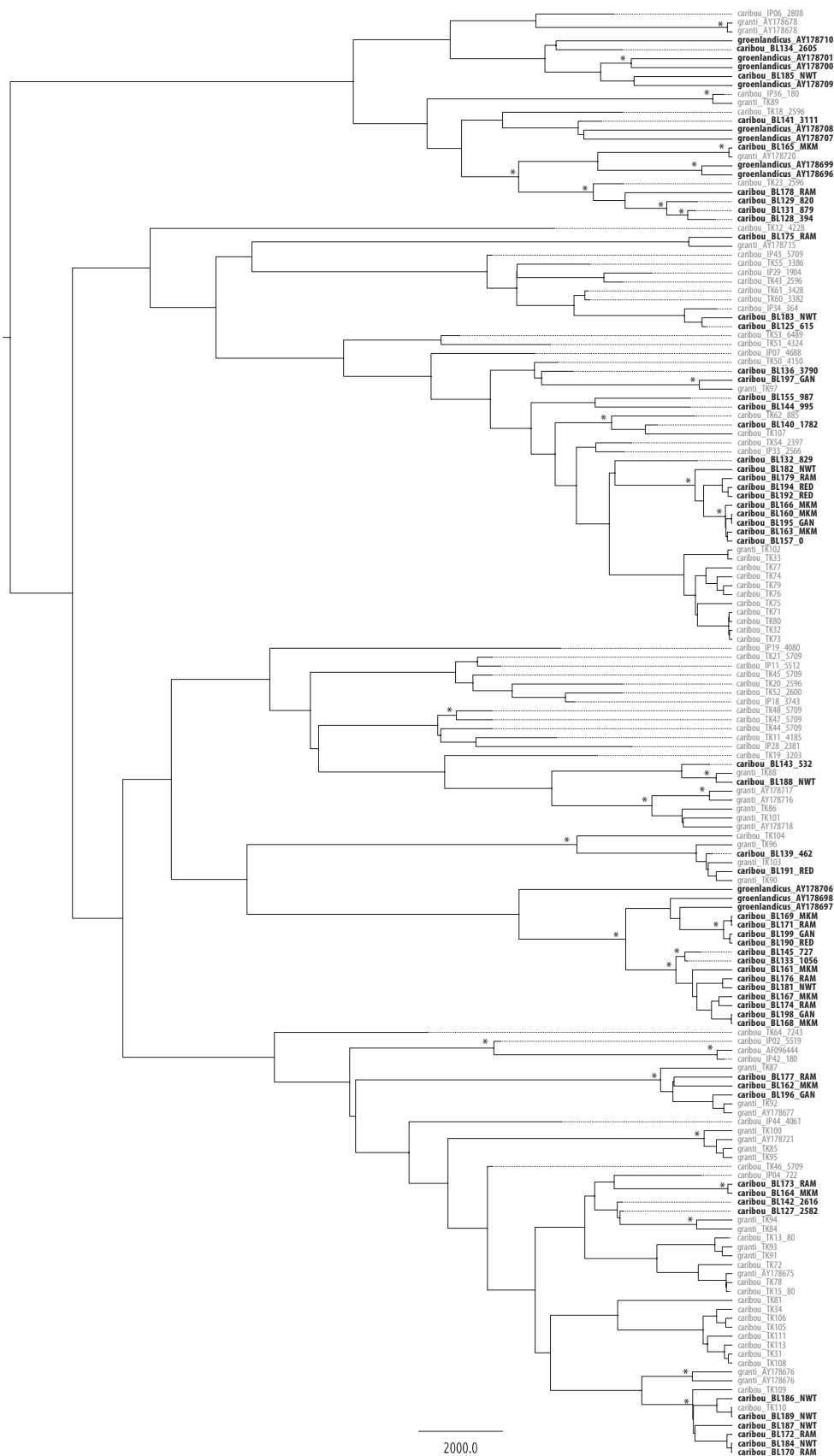
FIG. 4. Maximum Clade Credibility (MCC) tree from the BEAST analysis described in the text. Stars indicate posterior support values equal to 0.90 or greater. Individuals in black originated in the NWT, while those in gray originated in the YT or Alaska. Tips are labeled with subspecies, sample name, and age (years BP) of ancient samples, or with outfitter zone for modern NWT samples. Abbreviations for outfitter zones: RED = Redstone, RAM = Ram Head, MKM = Mackenzie Mountains, NWT = Northwest Territories, and GAN = Gana River.

TABLE 3. Analysis of Molecular Variance (AMOVA) results for four tests of North American caribou populations. Asterisks indicate significant values (* = $p < 0.05$, ** = $p < 0.01$, as determined after Bonferroni correction).

Test	N per group	FST	AMOVA % of variation explained by diversity among-group	within-group	p-value
Is the NWT woodland caribou population stable through time?					
Pre vs. Post Eruption (~~1147~~ ka)	6/43	0.000	0	100	0.4106 ± 0.0139
Ancient (330–3790 ka) vs. Modern	32/17	0.042	4	96	0.0645 ± 0.0074
Is the YT woodland caribou population stable through time?					
Pre vs. Post Eruption (~~1147~~ ka)	33/32	0.169	17	83	0.0000 ± 0.000**
Are the NWT and YT woodland caribou genetically distinct?					
(i.e., Are the NWT woodland caribou a potential source population of the modern YT woodland caribou?)					
NWT vs. Post-Eruption YT	49/32	0.138	14	86	0.0000 ± 0.000**
NWT vs. Pre-Eruption YT	49/33	0.108	11	89	0.0000 ± 0.000**
Are the barren-ground and woodland caribou genetically distinct?					
All (NWT & < ~~1147~~ ka YT/Alaska) barrenground vs. woodland	42/82	0.041	4	96	0.0020 ± 0.0014*
NWT barren-ground vs. NWT woodland	11/49	0.082	8	92	0.0068 ± 0.0027*

TABLE 4. Diversity statistics for caribou groups discussed in text. Pre- and post-eruption refer to the White River ashfall around 1200 years ago (ya).

Subspecies	Province	Group	Age range (ya)	N[1]	No. haplotypes	Haplotype diversity (h)	Nucleotide diversity (pi)
R. t. caribou	NWT	modern	0	32	16	0.923	0.017
		ancient	3790–394	17	13	0.963	0.016
R. t. groenlandicus	NWT	all	0	11	11	1.000	0.020
R. t. caribou	YT	pre-eruption	6489–1904	33	25	0.977	0.013
		post-eruption	885-0	32	12	0.851	0.014
R. t. granti	YT	all	0	30	19	0.961	0.018

[1] Total is lower than in Table 2 because some individuals with missing data were removed.

An analysis of caribou remains from ice patches in the southern YT suggested that the ashfall may have displaced caribou from the Southern Lakes region (Kuhn et al., 2010). After the ashfall, caribou are not found in the Southern Lakes region for 400 years, after which a genetically distinct population of caribou appears (Table 3). Like the Yukon ice patches, NWT ice patches were within the depositional range of the White River tephra (Fig. 2). Our results indicate, however, that the ashfall had no effect on the sampled NWT caribou, or, if it did, the population recovered quickly with no introgression from outside caribou herds or loss of genetic variation (Tables 3, 4). This result is perhaps not surprising, as caribou are known to dig down or "crater" for food in snow depths of up to 120 cm (Johnson et al., 2001). As the layers of ash deposited around the NWT ice patches were less than 5 cm thick, and the ashfall occurred when there was already significant snow in the region (West and Donaldson, 2002), the deposition may have gone largely unnoticed by these caribou.

Genetic Differentiation between NWT and YT Caribou

Mountain woodland caribou are strongly tied to their home ranges, and mixing between herds is unlikely without range overlap (Zittlau, 2004). Studies have also shown that mountains (Zittlau, 2004) and rivers (McLoughlin et al., 2004) reduce gene flow in caribou. We therefore expected to see genetic differences between caribou herds on opposite sides of the Mackenzie and Selwyn mountains. Our results comparing populations in the YT and NWT indicate significant differentiation at a moderate level between caribou in the two territories (Table 3). The observed divergence could be the result of the Mackenzie and Selwyn mountains acting as a barrier to gene flow. However, because the Redstone herd crosses the mountains each year (Olsen et al., 2001), it is more likely that the genetic differentiation is a product of the geographic distance between the caribou herds analyzed here. These herds do not overlap in range, and in some cases they are separated by large distances (Fig. 1). Data from the Tay River and Finlayson herds, whose ranges overlap that of the NWT Redstone herd (Fig. 1), are needed for a more robust assessment of whether the mountains act as a genetic barrier for caribou.

We were also interested in determining whether or not the NWT mountain woodland caribou could represent a potential source population for the modern YT northern mountain woodland caribou that moved into the southwest YT after the White River ashfall. The NWT mountain woodland caribou are significantly different from pre- and post-eruption YT populations occupying the modern

Aishihik and Ibex herd region (Table 3), making them an unlikely source population for the modern YT caribou in the those herds.

Genetic Differentiation Based on Ecotype

The distinction between barren-ground and woodland caribou is based on lifestyle and morphology (Banfield, 1961). Today, morphology is a controversial means of classification, as characters such as antler size and shape can be influenced by factors like variation in nutrition (Geist, 2007). The most obvious difference between the ecotypes is their lifestyle, with barren-ground caribou generally congregating in massive, migratory herds on the open tundra, and woodland caribou generally in smaller, sedentary herds within the boreal coniferous forests.

Three broad mitochondrial haplogroups have been identified across the holarctic range of the seven *Rangifer tarandus* subspecies (caribou and reindeer), hypothesized to correspond to three refugia (Flagstad and Røed, 2003). Haplogroup I comprises primarily southern woodland caribou (*R. t. caribou*), likely stemming from a southern North American refugium. Haplogroup II contained some European tundra reindeer (*R. t. tarandus*), possibly from a western Eurasian refugium. ~~And the~~ widespread haplogroup III included individuals of all subspecies, but its primarily northern North American composition indicates a recent Beringian origin (Flagstad and Røed, 2003). All of the northern woodland haplotypes recovered in this study and by Kuhn et al. (2010) fall within haplogroup III. Interestingly, this ~~fact~~ illustrates that the northern and southern woodland caribou (*R. t. caribou*) are quite genetically distinct, as the northern woodland caribou appear to have originated from a Beringian refugium, along with the continental tundra forms (*R. t. tarandus*, *granti*, and *groenlandicus*) and Arctic forms (Svalbard reindeer, *R. t. platyrhynchus*, and Peary caribou, *pearyi*). The genetic distinction between northern and southern woodland caribou provides further evidence that the present species and subspecies-level designations are not supported by mitochondrial data on a holarctic scale. However, mitochondrial DNA is only maternally inherited, and bi-parentally inherited nuclear markers may shed more light on the degree of genetic differentiation between groups.

In contrast, our more focused and extensive sampling within a single geographic location showed that barren-ground and mountain woodland caribou populations of the NWT are significantly genetically differentiated from one another (Table 3). This finding is consistent with previously identified allele frequency differences between the two ecotypes in North America (Cronin et al., 2005). Yet, there remains a very high degree of mitochondrial haplotype similarity between the two ecotypes (Figs. 3, 4), suggesting that insufficient time has lapsed for complete lineage sorting to occur within haplogroup III (Flagstad and Røed, 2003).

A low level of mixing between barren-ground and mountain woodland caribou cannot be discounted to explain the degree of haplotype similarity observed here. Barren-ground caribou generally calve on the open tundra and migrate below the tree line into the boreal forest during the winter. Given the geographic distance from our study area to the tree line, which has been east of Great Slave Lake and Great Bear Lake during the entire period of our study (Moser and Macdonald, 1990) and north of the Richardson Mountains, there is little chance of contact between the mountain woodland caribou in our sampling area and the barren-ground caribou herds from north of the tree line. Despite this low probability of contact, stray barren-ground caribou venturing into the boreal forest as far as our study region could be responsible for the incomplete differentiation between the ecotypes analyzed here.

CONCLUSIONS

Using ancient and modern DNA isolated from samples collected from alpine ice patches, we explore the recent history of the mountain woodland caribou inhabiting the central Mackenzie and Selwyn mountains of the NWT, Canada. Our results suggest that the Redstone herd has been robust to environmental change over the past 4000 years, having withstood Holocene environmental events without the loss of genetic variation. We find significant genetic differentiation between mountain woodland and barren-ground ecotypes in our study area, and between mountain woodland caribou in the NWT and YT. It appears that these ecotypes share a relatively recent common ancestor that is distinct from southern woodland caribou, but they have long lived separately, which has led to population genetic and ecological differentiation. Our mitochondrial results suggest that the mountain woodland caribou of the NWT and YT may represent a unique group within *Rangifer tarandus*, but further research incorporating more quickly evolving nuclear DNA markers is required to determine the extent and significance of this genetic differentiation.

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