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ARTICLE

Post-glacial recolonization of insular Newfoundland across the Strait of Belle Isle gave rise to an endemic subspecies of woodland caribou, *Rangifer tarandus terranovae* (Bangs, 1896): evidence from mtDNA haplotypes

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Abstract: Post-glacial origins of woodland caribou (*Rangifer tarandus* subsp.) on the island of Newfoundland and their relationship to mainland populations have been uncertain. Sequence analysis of 2223 bp of the mitochondrial DNA control region and cytochrome *b* gene from 233 Newfoundland caribou identified 32 haplotypes in four major clades. Comparison with other Nearctic caribou confirms a closer affinity of the basal Clade A with animals from the mainland, and as an outgroup to Clades B, C, and D that are endemic to the island. This indicates re-entry of caribou to post-glacial Newfoundland across the Strait of Belle Isle from Labrador, rather than from southern coastal refugia. Newfoundland caribou are a distinct subspecies, *Rangifer tarandus terranovae* (Bangs, 1896). Hierarchical AMOVA shows significant clinal differentiation of the major clades from northwest to southeast across the island. The isolated Avalon Peninsula population in the extreme southeast is genetically depauperate. Founder effects are evident in herds introduced to previously unoccupied areas by wildlife managers over the past 40–50 years. Reindeer introduced in the early 20th century have not contributed to mtDNA diversity in Newfoundland caribou.

Key words: Rangifer, insular subspecies, post-glacial recolonization, founder and bottleneck effects.

Résumé : Les origines postglaciaires du caribou forestier (*Rangifer tarandus* subsp.) sur l'île de Terre-Neuve et sa relation avec les populations continentales demeurent incertaines. Le séquençage et l'analyse d'un segment de 2223 pb de la région de contrôle mitochondriale et du gène codant pour le cytochrome *b* chez 233 caribous de Terre-Neuve a permis d'identifier 32 haplotypes formant quatre clades majeurs. Une comparaison avec d'autres caribous néarctiques a confirmé une parenté plus grande entre les animaux appartenant au Clade A (basal) et ceux du continent, et le fait que ceux-ci forment un groupe extérieur par rapport aux Clades B, C et D qui regroupent des animaux endémiques de l'île. Ceci indique une réintroduction postglaciaire du caribou à Terre-Neuve via une traversée du détroit de Belle-Isle à partir du Labrador, plutôt qu'en provenance de refuges côtiers méridionaux. Les caribous de Terre-Neuve sont ainsi une sous-espèce distincte, *Rangifer tarandus terranovae* (Bangs, 1896). Une AMOVA hiérarchique a montré une différenciation significative des clades principaux le long d'un cline qui traverse l'île du nord-ouest au sud-est. La population isolée de la péninsule d'Avalon dans l'extrémité sud-est est appauvrie sur le plan génétique. Des effets fondateurs sont évidents au sein des hardes introduites au cours des 40–50 dernières par des gestionnaires de la faune dans des régions inoccupées jusqu'alors. Les rennes introduits au début du 20ième siècle n'ont pas contribué à la diversité de l'ADN mitochondrial chez les caribous de Terre-Neuve. [Traduit par la Rédaction]

Mots-clés : Rangifer, sous-espèces insulaires, phylogéographie postglaciaire, effets fondateurs et de goulot.

Introduction

Populations of woodland caribou (*Rangifer tarandus* subsp.) have declined across most of Canada, and most Canadian subspecies and (or) populations have been formally designated to be at some risk of extinction (COSEWIC 2002). Increased hunting and wolf predation are cited as the main causes (Banfield 1961; Bergerud 1974; Seip 1991). The insular Newfoundland population had relatively high and constant numbers, and was therefore assessed as Not At Risk under the *Canadian Species At Risk Act* (SARA) in 2002. Since then, the population has declined dramatically, and the question of its status and relationships has again become important. In assessing the species as of Special Concern, COSEWIC (2014) noted, "The present decline appears to be part of natural population fluctuations and recently several indices on health and calf survival suggest that the population will increase."

The insular Newfoundland population has undergone dramatic fluctuations in population size throughout the past century. From a peak of 40 000 – 100 000 individuals in the early 1900s, counts bottomed out to 2000 animals in 1925, rose in the 1930s and 1940s to 10 000 – 15 000 individuals, then declined again in the late 1950s to ~6500 animals (Dugmore 1913; Bergerud 1971; Williams and Heard 1986; Mahoney et al. 1991; Bergerud et al. 1983). The Amulree Commission in 1933 attributed the decline to extensive overhunting by Newfoundlanders, and gave it as one of the reasons for withdrawal of Responsible Government from Newfoundland by

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the British crown in 1934 (Newfoundland Royal Commission 1933). Population fluctuations were also attributed to the absence of wolf predators after ~1900, and a new predator–prey dynamic with lynx and snowshoe hares (Seip 1991; Mahoney and Virgl 2003).

A second peak of 80 000 – 94 000 individuals was reached in 1996, but the population again declined to 68 000 individuals by 2002, and to 32 000 by 2013, for a 66% decline over 17 years (approximately three-generations), just short of the 70% criterion for Threatened status under the SARA. Long-term study of caribou on the Buchans Plateau indicated that the population began to decline in the early 2000s. Signs included lower annual survival of adults, decreased adult body size, decreased recruitment, low calf survival, late calving, and reduced pregnancy rates. Also, earlier autumn and later spring migrations, suggesting a depletion of summer forage (Mahoney and Schaefer 2002). The recent decline has been attributed to limited forage that reduced juvenile productivity and survival, over-hunting, and possible increased predation following the introduction of coyotes (Weir et al. 2014).

Rangifer tarandus L., 1758 is a Holarctic deer species that extends across North America, Europe, Asia, Greenland, and the Spitsbergen and Svalbard Islands (Røed 2005; Kurtén and Anderson 1980; Banfield 1961). The name Caribou refers to wild populations in North America, and Reindeer refers to the wild and (or) domesticated populations in Eurasia. During the most recent Nearctic glaciation, caribou were abundant and distributed in non-glaciated refugia both north and south of the Laurentide ice sheet (Banfield 1961). The Beringian refugium extended from northeastern Eurasia across to northwestern North America, and was the source of the North American tundra and arctic forms. The Southern refugium extended from the Atlantic across the central Midwest to the mountainous region of the southwest, and was the source of the widespread woodland caribou form (*Rangifer tarandus caribou*) (Røed 2005; Kurtén and Anderson 1980; Banfield 1961).

Woodland caribou are currently distributed throughout the taiga and boreal region from the island of Newfoundland and mainland Labrador in the east, across to British Columbia, Yukon, and Alaska in the west (Banfield 1961) (supplementary data, File S1[1]). They are found in mature boreal forest, associated barrens, and bog–fen complexes, where they live in small, sedentary groups, although some populations undertake local, seasonal migrations (Banfield 1961). Insular Newfoundland caribou are often considered con-subspecific with woodland caribou; however, they were originally described as a separate subspecies, *Rangifer tarandus terranovae* (Bangs, 1896) and are notably more ecologically diverse than mainland forms (Mahoney et al. 1991; Geist 2007). They tend to have larger body size, longer legs, and a narrower antler span than other subspecies (Røed 2005), a possible reflection of "Foster's Rule" of island gigantism, in the absence of their usual predators (Foster 1965).

Caribou are believed to have re-populated insular Newfoundland ca. 8000 years ago from refugia south of the Laurentide ice sheet (Banfield 1961; Kurtén and Anderson 1980; Røed et al. 1991; Røed 2005). Alternative routes are from Labrador across the Strait of Belle Isle to the Northern Peninsula of Newfoundland, or from eastern coastal plains and (or) island refugia, including the Grand Banks (Pielou 1991; File S2[1]). Survival of large ungulates in these refugia is well documented, and they may have moved among the ring of refugia surrounding the Goldthwait Sea (Gulf of St. Lawrence) and the islands of the coastal plains (Pielou 1991). Affinities of historical maritime caribou populations are unknown, as all were extirpated by the early 1900s, except for a small Endangered population on the Gaspé Peninsula (COSEWIC 2002).

Long-term study of population dynamics indicate that the Newfoundland population comprises four relatively discrete herds: Northern Peninsula, Humber River, Interior, and Avalon Peninsula (also called Northern, Western, Interior, and Avalon) (Bergerud 1971). Based on calving ranges, caribou within these herds are assigned to 19 Wildlife Management Units (WMUs) (File S3[1]). Hereinafter, we confine use of herd to refer to the four geographic entities described above. Between 1961 and 1982, caribou were transplanted from their native ranges to previously unoccupied sites: successful introductions include those at St. Anthony (transplanted from the Interior Herd), Merasheen Island (from the Buchans Plateau WMU), and the Cape Shore (from the Avalon Peninsula WMU).

Mitochondrial DNA sequence data have been widely used in the elucidation of among- and within-species phylogenetics (Wilson et al. 1985). Though the included genes and SNPs segregates as a single linkage group, its success in tracing maternal lineages is unquestioned. There is extensive prior work on genetic variation in *Rangifer*, including both Old and New World types; however, these have included only a few animals from insular Newfoundland. Phylogenetic analyses of transferrin allozyme alleles (Røed et al. 1991) and short mtDNA cytochrome *b* or control region sequences (Cronin and Patton 2002; Cronin et al. 2003, 2005, 2006) suggest that Newfoundland caribou are more closely related to woodland caribou from central and eastern Canada than to more northerly barren-ground caribou but have not provided clear evidence on their sub-specific status.

We provide here the first extensive data and analysis of mtDNA genetic variation in caribou from insular Newfoundland. Our aim is to understand their relationships to mainland caribou with respect to hypotheses of post-glacial recolonization (by southern or northern routes?), phylogenetic relationships of insular animals to other *Rangifer* subspecies (is *R. t. terranovae* a distinct subspecies?), and landscape-level herd and WMU structures with respect to previous and present management concerns during a period of population decline (is Bergerud's (1971) four-herd hypothesis accurate?).

Material and methods

Caribou samples

A total of 233 tissue samples were analyzed from individual caribou from across the island of Newfoundland. Tissue samples were obtained from caribou in 14 of the 19 provincially defined WMUs between 1999 and 2003 during the fall (September–December) hunting season. Tissue samples were typically fresh or dried muscle from the jawbones of hunted caribou submitted by local hunters to the Government of Newfoundland and Labrador. No animals were shot specifically for this study. All samples were catalogued by government staff, and immediately frozen at –20 °C.

DNA preparation

DNA was extracted from the frozen muscle or kidney tissue with the QIAamp® DNA Mini Kit Tissue Protocol (Qiagen Inc.) according to the manufacturer's protocol. Two regions of mitochondrial DNA, including the control region and the cytochrome *b* gene, were amplified by the polymerase chain reaction (PCR). Amplicons included the complete control region and portions of the flanking tRNA-Thr, tRNA-Pro, and tRNA-Phe genes, and the complete cytochrome *b* gene and a portion of the tRNA-Thr gene. The delimited regions are referred to hereinafter as CR (1063 bp) and CYTB (1170 bp), respectively. A complete list of amplification and sequencing primers is given in the supplementary data (File S4[1]).

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2017-0199.

PCR amplifications were carried out with Hot StarTaq DNA Polymerase (Qiagen, Inc.) on an Eppendorf Mastercycler and an Eppendorf Mastercycler gradient thermal cycler (Eppendorf North America, Inc., Westbury, NY). Amplification products were purified with Spin Columns from the QIAquick PCR Purification Kit (Qiagen Inc.) according to the manufacturer's protocol. PCR products were sequenced with the ABI Prism® Big Dye™ Terminator chemistry on an ABIPrism 377™ Automated DNA Sequencer (Applied Biosystems, Inc.).

Sequence analysis

Sequences were aligned and edited with Sequencher™ 4.1.2 (Gene Codes). Phylogenetic relationships of haplotypes were determined by Bayesian inference with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with a General Time Reversible, linearized model. A network of haplotypes was constructed with TCS 1.21 (Clement et al. 2000) by the statistical parsimony approach of Templeton et al. (1992), which estimates the 95% plausible set for all haplotype linkages. TCS 1.21 was also used to calculate haplotype outgroup probabilities based on frequencies, which correspond to haplotype age, to determine the root of the statistical parsimony network.

Estimates of genetic variation among WMUs, values of haplotypic diversity (Hd), average number of differences (K), and nucleotide diversity (π) were calculated with DnaSP Version 4.10.4 (Rozas et al. 2003). Analyses of heterogeneity of haplogroup and haplotype distributions were calculated with the Monte Carlo chi-square procedure in REAP 4.0 (McElroy et al. 1991).

Population genetic structure was evaluated by analysis of molecular variance (AMOVA) indices in the program Arlequin 3.01 (Excoffier et al. 2005). Here, the AMOVA partitions mtDNA haplotype diversity among hierarchies at individual, population (WMU), and regional (herd) levels. We evaluated five a priori scenarios of biogeographic structure. These hypotheses were suggested in the first instance by Bergerud's (1971) herds, as well as recognized north–south migration patterns and geographical barriers to movement. Among the central/interior herds, caribou tend to migrate north–south in spring and fall. However, it is not clear whether the Northern Peninsula Herd participates in this migration. The major geographical barriers to free movement are Main River on the Northern Peninsula at the north end of Adies Lake, and the isthmus joining the Avalon Peninsula to the rest of the island. A striking natural history feature of the isthmus is the presence of abundant lichen colonies at least several decades old, clear evidence of lack of grazing by caribou (S. Mahoney, pers. obs.).

Scenarios are ordered by number of component groups. Scenario 3 tests the historic four-herd hypothesis proposed by Bergerud (1971), based on long-term studies on population dynamics, plus populations introduced as more recent transplantations. These are the Northern Peninsula Herd (Northern Peninsula WMU), Humber Herd (Adies Lake WMU), the Avalon Herd (Avalon WMU, plus introduced Cape Shore WMU), and the remaining WMUs in the Interior Herd (Middle Ridge, Mount Peyton, Pot Hill, Lapoile, Buchans, Grey River, Gaff Topsails, Hampden Downs), and introduced populations at Merasheen Island, Buchans, and St. Anthony.

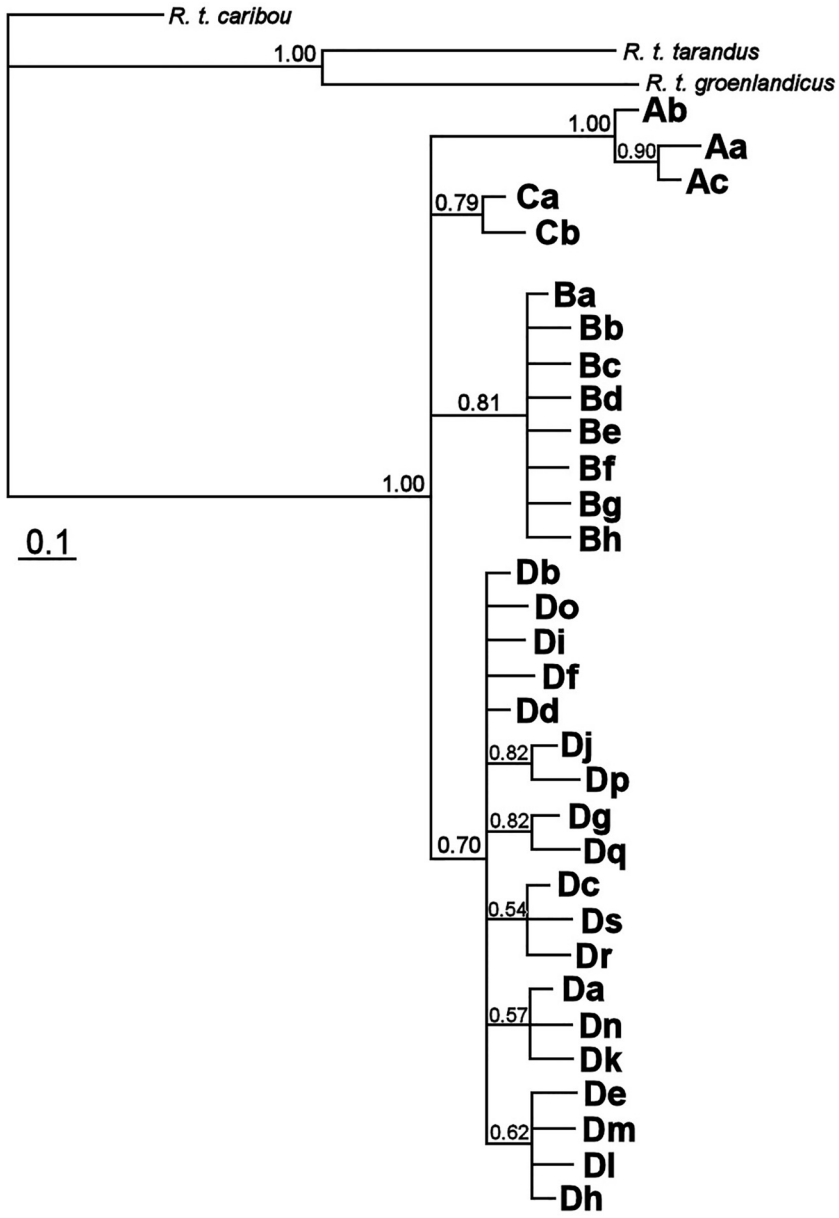
Scenario 1 defines five geographical groups by separating the Interior Herd as (1) Western versus (2) Eastern herds separated between the Pot Hill and Grey River WMUs, (3) the Avalon Herd, and separation of the Humber Herd as (4) northern WMU pairs as Adies Lake and Hampden Downs versus (5) Northern Peninsula and St. Anthony. Scenario 2 simplifies Scenario 1 to four groups, where (1), (2), and (3) are as in Scenario 1, and (4) groups the four northwestern and northern WMUs as a single entity. Scenario 5 further simplifies this to just the Western and Eastern groups as Scenarios 1 and 2, with all other WMUs included in one of these

Table 1. Distribution of mtDNA haplotypes among 14 Wildlife Management Units (WMUs).

		Clade A			Clade B							Clade C	Clade D																
Wildlife Management Unit	*N*	Aa	Ab	Ac	Ba	Bb	Bc	Bd	Be	Bf	Bg	Bh	Ca	Cb	Da	Db	Dc	Dd	De	Df	Dg	Dh	Di	Dj	Dk	Dl	Dm	Dn	Do	Dp	Dq	Dr	Ds
St. Anthony	17	—	1	—	2	2	—	—	—	—	—	—	7		1	—	1	2	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
Northern Peninsula	18	—	—	—	6	—	—	—	1	—	—	—	1	1	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Adies Lake	18	—	—	—	6		—	—	—	—	—	—	5	3	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hampden Downs	6	—	—	1	2		—	—	—	—	—	—	1		1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gaff Topsails	20	—	—	—	6		—	—	—	—	—	—	4	2	4	—	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Buchans	17	1	—	—	2		—	—	—	—	—	—	6		3	—	1	3	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
Merasheen Island	20	—	—	—	—	1	—	—	—	—	—	—	13	3	—	—	3		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lapoille	18	—	—	—	1	1	—	1	—	—	—	—	6		2	—	1	2	—	2		—	—	1		1	—	—	—	—	—	—	—
Grey River	19	—	—	—	—	—	—	1	—	—	—	1	6		3	—	2	—	—	3	2	—	—	—	—	—	—	—	—	—	—	—	1
Pot Hill	18	—	—	—	3	1	—	1	—	1	1		1		1	—	3	1	—	1	2	—	—	—	1	—	—	—	1	—	—	—	—
Mt. Peyton	14	—	—	—	3	—	2	—	—	—	—	—	1		—	—	1		—	1	2		1	1	—	—	—	—	1	—	1	—	—
Middle Ridge	18	—	—	—	1	1	2	—	—	—	—	—			5	—	4	—	—	—	—	2	1	—	1	—	—	—	1	—	—	1	
Avalon	18	—	—	—	—	—	—	—	—	—	—	—			—	10	—	—	7	—	—	1	—	—	—	—	—	—	—	—	—	—	—
Cape Shore	12	—	—	—	—	—	—	—	—	—	—	—			—	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Summary	233	1	1	1	32	6	4	3	1	1	1	1	51	9	33	22	17	12	7	7	6	3	2	2	2	1	1	1	1	1	1	1	1

Note: Absent haplotypes are indicated by dashes.

Fig. 1. Bayesian phylogeny of 32 mtDNA haplotypes in Newfoundland caribou with respect to three other caribou subspecies. Values at the nodes are posterior probabilities >0.5.



two groups according to west versus east geography. Scenario 4 contrasts the geographic extremes of the two Avalon Peninsula WMUs, the two northern-most Northern Peninsula and St. Anthony WMUs versus the remainder of the insular WMUs.

Comparison with other North American caribou subspecies and Eurasian reindeer

Homologous 1212 bp CYTB sequences were obtained for 59 North American caribou (*R. t. caribou*, *R. t. granti*, and *R. t. groenlandicus*) from Cronin et al. (2005) (GenBank AY726672–AY726730). These sequences were aligned over the homologous sequences from the 32 Newfoundland caribou haplotypes with Sequencher™ 4.1.2 (Gene Codes, Corp.). A Maximum Likelihood analysis was made with MEGA7 (Kumar et al. 2016). The analysis includes a homologous sequence from a complete reindeer mitogenome (*R. t. tarandus*: GenBank NC007703). Confidence in branches of the tree was estimated by 3000 bootstrap replications.

Results

DNA sequence variation

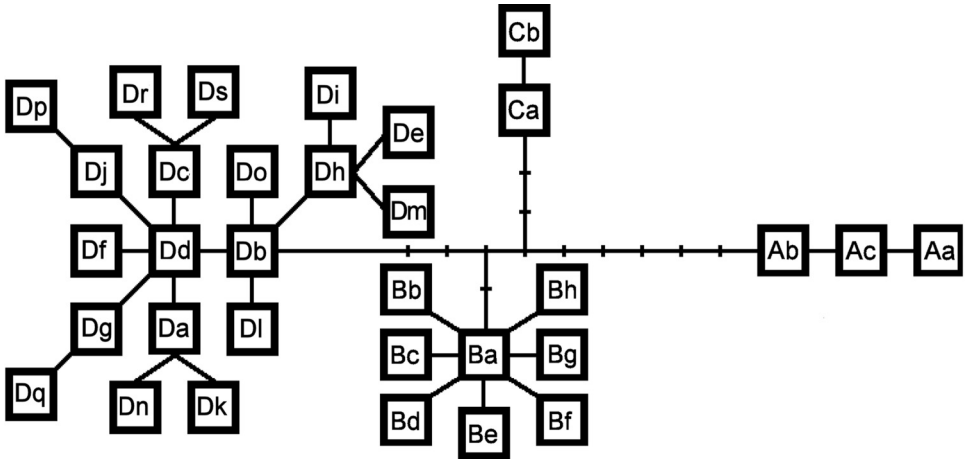
The combined 2223 bp CR and CYTB sequences for 233 individuals contained 18 and 14 SNP sites, respectively (File S5[1]). One region of the CR comprised a run of 4–5 Ts followed by 6–8 Cs: because of the difficulty of alignment in this region, variation at positions 852–854 was excluded from the analysis.

Phylogenetic relationships of haplotypes

The 32 variable positions in the DNA sequence data define 32 distinct sequence haplotypes (Table 1). All sequences have been submitted to GenBank, and assigned accession numbers MH266663–MH266694 and MH266631–MH266662 for the control and cytochrome *b* regions, respectively.

Phylogenetic analyses by Bayesian inference (Fig. 1) allocated the 32 haplotypes to one of four haplogroups, herein referred to as

Fig. 2. Statistical parsimony network of 32 mtDNA haplotypes. Haplotype designations as in Table 2. Connections are consistent with those in the Bayesian analysis (Fig. 1). Haplotypes are separated by single SNPs, except where cross-bars indicate multiple events.



Clades A, B, C, and D. These comprise 3, 8, 2, and 19 haplotypes among n = 3, 49, 60, and 121 individuals, respectively. The statistical parsimony network also identifies the same four clades (Fig. 2), and assigns haplotype Dd as that with the highest outgroup probability (p = 0.16). Clades A, B, and C are then defined by 9, 5, and 6 apomorphic SNPs, respectively (File S6[1]). Resolution of relationships within Clade D and with respect to Clades B and C are complicated by several homoplasious sites. Figure 2 shows the most likely connections as determined by an examination of outgroup weights as calculated in TCS 1.21 (File S7[1]) and the groupings of haplotypes from the phylogenetic analysis.

To ascertain the phylogenetic affinities of Clades A, B, C, and D with respect to other geographic samples and subspecies of caribou, we made an initial analysis of the overlapping 1170 bp among the 32 haplotypes in this study and the fifty-nine 1212 bp haplotypes in Cronin et al. 2005. Based on the topology of that analysis, we reduced the data set by retaining (*i*) the eight most common haplotypes in Clades B, C, and D (Table 1: Ba, Bd; Ca; Da, Db, Dc, Dd, De) plus Aa and Ab as outgroup; (*ii*) all of the *R. t. groenlandicus* and *R. t. caribou* sequences from Cronin et al. 2005; and (*iii*) the first sister sequence among *R. t. granti* to any of these. We also included (*iv*) the homologous sequence from the single reindeer mitogenome (GenBank NC007703). Figure 3 shows the Maximum Likelihood result for these n = 33 haplotypes.

Distribution of clades and haplotypes among WMUs

The distribution of clades and haplotypes across WMUs (Tables 1 and 2; Fig. 4) are both significantly non-random (Monte Carlo χ^2 = 113.12, df = 39, $p \ll 0.001$, and χ^2 = 762.10, df = 403, $p \ll 0.001$, respectively). The overall haplotype diversity (Hd) is 0.89, the average number of pairwise differences (K) is 4.25, and the nucleotide diversity (π) is 0.0022 (Table 2). The least diverse WMUs are the those isolated on the Avalon Peninsula and Merasheen Island (Hd < 0.56, Table 2). The most diverse WMUs with Hd > 0.93 occur in Pot Hill, Mount Peyton, and Hampden Downs (Table 2).

Analysis of molecular variance among regional partitions of WMUs

In all five scenarios of geographical structure tested, the variance components at all hierarchal levels are significant ($p < 0.05$). The fraction of genetic variance is consistently high among individuals within WMUs (0.842–0.865), small to moderate among WMUs within regions (0.0340–0.0737), and moderate among regions (0.0823–0.1058) (see File S8[1]). The four-region Scenario 2 explains marginally more of the among-region variance, consistent with a unified Northern region, than any of the other scenarios, including Bergerud's (1971) four-herd hypothesis (Scenario 3) (0.1058 versus 0.0889).

Discussion

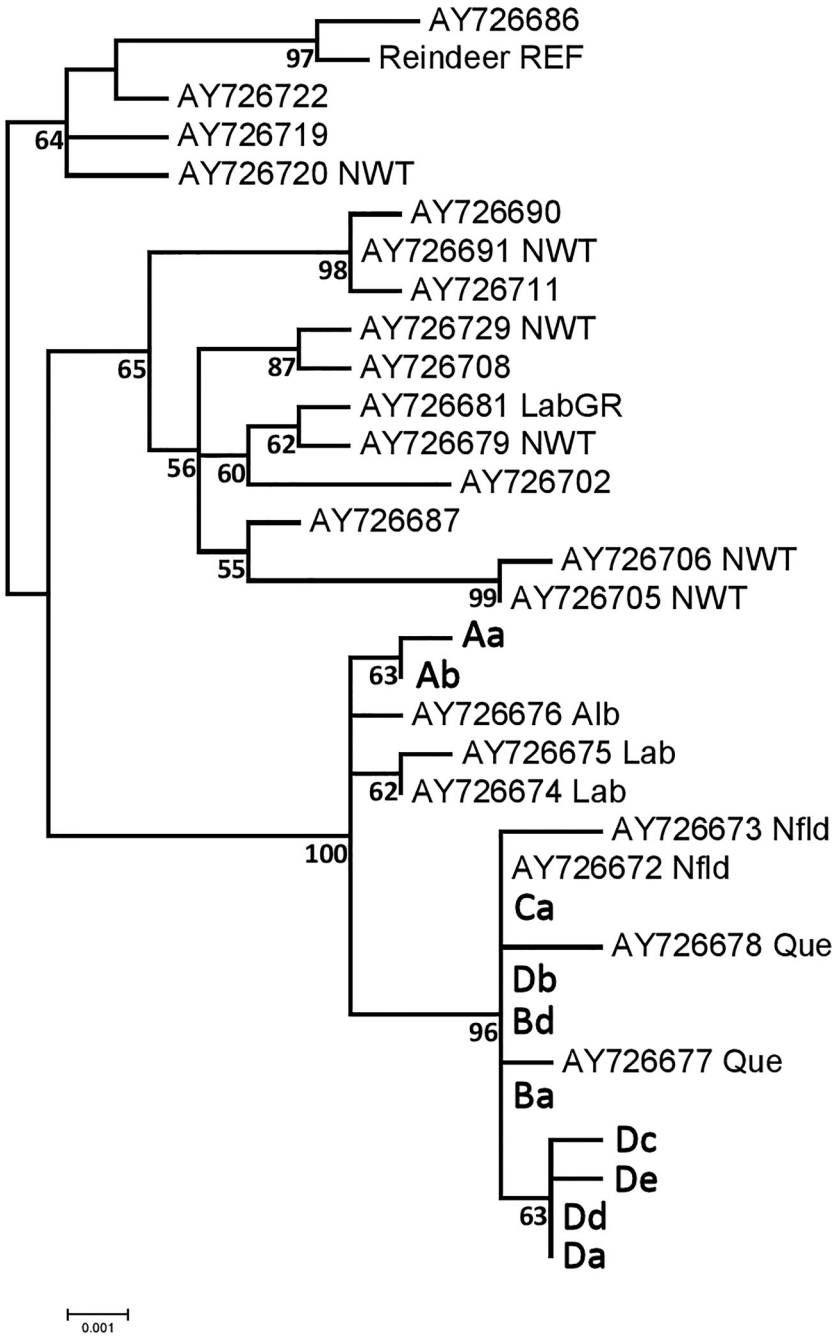
Genetic organization of Newfoundland caribou

The Newfoundland caribou population comprises 32 haplotypes in four distinguishable separate clades of differing size, distribution, and complexity (Figs. 1 and 2; Tables 1 and 2). The distribution of the clades across Newfoundland is illustrated in Fig. 4. Clade D is the most numerous, diverse, and complex, and is found in all 14 WMUs examined. Clade B is a "star" phylogeny predominated by haplotype Ba, and occurs in all WMUs except those on the Avalon Peninsula. Clade C is less diverse, with only two closely related haplotypes. It is widespread throughout western Newfoundland but declines eastward, and is not found in the Middle Ridge WMU or on the Avalon Peninsula. Clade A comprises three individuals found only on the Northern Peninsula and the Buchans WMU in western Newfoundland, and differs by 9 or 10 SNPs from the 29 other haplotypes in the population. Clades A and D occur at opposite ends of an unrooted phylogenetic network, with Clades B and C intermediate but more similar to D. Comparison of complete mtDNA genome sequences from representative Aa, Ba, Ca, and Dd haplotypes confirms clade B as sister to clades C + D, when rooted with Clade A as the outgroup (S. Carr, work in progress).

Genetic variation within and among caribou WMUs

Hierarchical AMOVA consistently partitions a relatively small fraction of the genetic variance among WMUs (0.0340–0.0737) or among regional models variously defined (0.0823–0.1058), with the largest fraction among individuals (0.7709–0.8652) (Fig. 5). This suggests ongoing genetic interchange among most WMUs, particularly in central Newfoundland. Bergerud (1971) proposed that the Northern Peninsula Herd (including Northern Peninsula WMU) and the Humber River Herd (including Adies Lake WMU) were discreet herds, each separated from the other and the Interior Herd (Scenario 3). Here, central WMUs have higher haplotype diversity (Hd = 0.82–0.95) than do the Northern Peninsula and Adies Lake WMUs (Hd = 0.66 and 0.78, respectively) (Table 2). Neither the Northern Peninsula nor Adies Lake WMUs include any unique haplotypes, and instead they include a subset of haplotypes found in the central WMUs. This may indicate some physical isolation between these areas, as Bergerud believed. Reallocation of the Adies Lake and Hampden Downs WMUs to the Northern Peninsula Herd, and separation of the Pot Hill, Mount Peyton, and

Fig. 3. Maximum Likelihood tree of 1212 bp CYTB from representative Newfoundland and North American caribou. The eight most common Newfoundland haplotypes (Ba, Bd, Ca, Da, Db, Dc, Dd, and De) plus Aa and Ab are included and shown in bold. GenBank haplotypes are taken from (Cronin et al. 2005), and include three caribou subspecies, *Rangifer tarandus granti* (unlabelled: central Alaska herd), *Rangifer tarandus groenlandicus* (North West Territory: NWT), and *Rangifer tarandus caribou* from six locations: Alberta (Alb), Val d'Or, Quebec (Que), Labrador (Lab), and insular Newfoundland (Nfld). The reindeer sequence is from GenBank (NC007703).



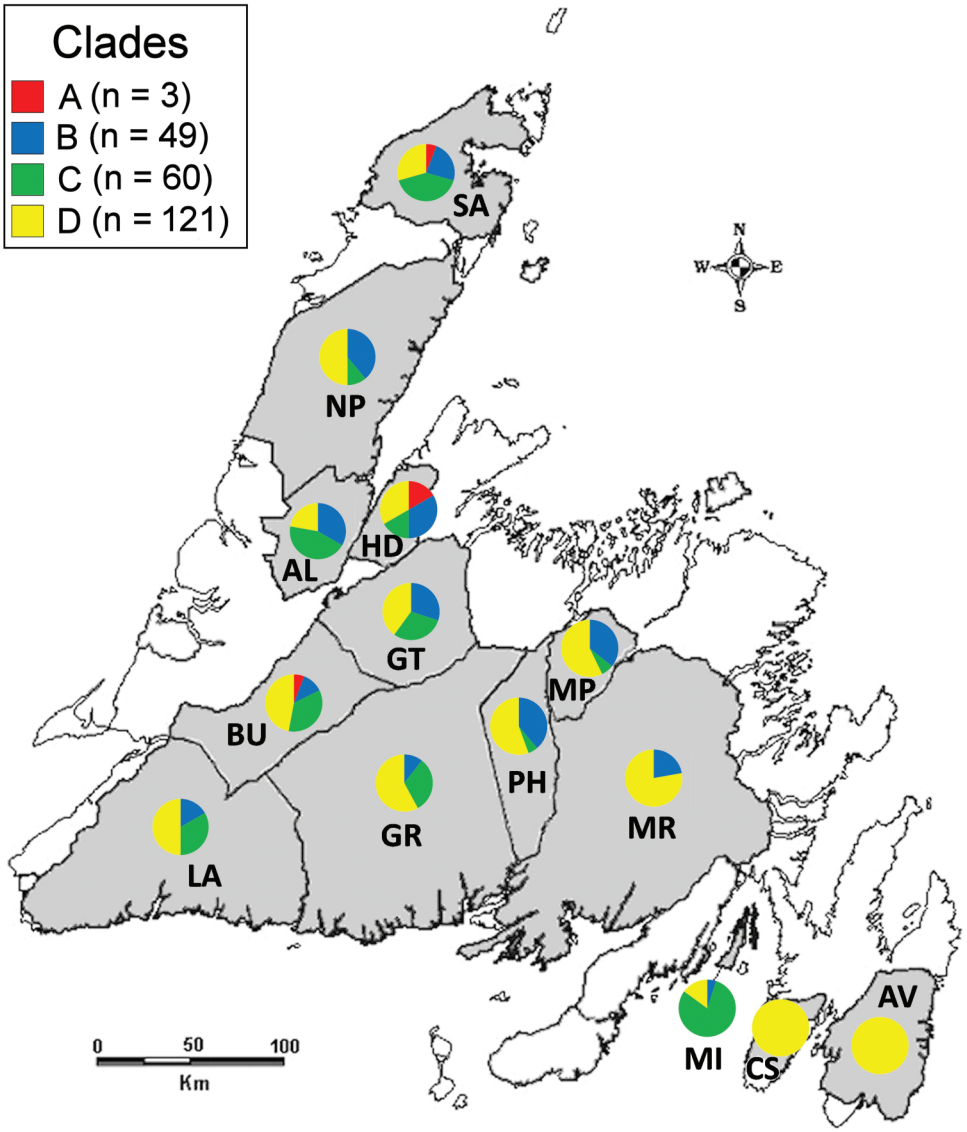
Middle Ridge WMUs as a distinctive Eastern Herd (Fig. 5, Scenario 2) would explain the partition of genetic variance somewhat better ($\sigma^2 = \Phi_{CT} = 0.1058$ versus 0.0889) than Bergerud's (1971) four-herd hypothesis (Fig. 5, Scenario 3).

Cladistic insights into the biogeographic origin of Newfoundland caribou: two hypotheses

The mtDNA clade structure of Newfoundland caribou provides insight into the post-glacial re-population of Newfoundland. Historically, two recolonization routes have been proposed: a southern route from southeasterly coastal or island refugia, or a northern route across the Strait of Belle Isle (Fig. 6).

Caribou are known to have occupied easterly refugia on the coastal plains of what are now the New England and the Atlantic Provinces, and it has been suggested that they may have occupied island refugia such as the Grand Bank to the south and east of Newfoundland. These populations might have re-populated the island of Newfoundland and the rest of Atlantic Canada from the south as the ice sheet retreated (Pielou 1991). Under this hypothesis, caribou would first have entered Newfoundland from its

Fig. 4. Distribution of Clades A, B, C, and D across 14 Wildlife Management Units (WMUs). Note that Clade A is confined to two WMUs on the Great Northern Peninsula and one slightly south at Buchans, that Clade C declines from west to east except for the introduced population on Merasheen Island, and that the Avalon Peninsula populations in the extreme southeast are fixed for Clade D (cf. Table 2).



southeast coast, and would be most closely related to the southeasterly coastal and (or) island populations. However, as these populations were extirpated prior to 1939 (Tufts 1939), it is difficult to test this hypothesis directly.

The alternative hypothesis is that woodland caribou were pushed into central continental refugia well south of the Laurentide ice sheet, as far west as the mountainous region of the southwest. As the ice sheet retreated ca. 10 kya, they returned northeastward along the St Lawrence River valley into the Ungava Peninsula, and thence across the Strait of Belle Isle (Banfield 1961; Kurtén and Anderson 1980; Røed 2005). Figure 3 shows that Newfoundland Clades B, C, and D are a sister group to caribou from Labrador and Quebec, which supports the northern route hypothesis (Fig. 6, Northern Route 2).

Clade A seems to have arrived in Newfoundland by an independent later event. The clade includes only a few individuals, and is confined to the northwestern part of the island across from the Strait of Belle Isle. Figure 3 indicates that Clade A is more closely related to mainland animals from Labrador, and is distinct from the other island animals in Clades B, C, and D. The Belle Isle crossing is now a swim of approximately 18 km across sea ice that remains frozen for much of the winter. Although there are no reliable contemporary observations of such crossings, the phylogenetic relationship reinforces the dispersal biogeography required by the northern route hypothesis (Fig. 6).

Low genetic diversity of the Avalon Peninsula Herd

The Avalon WMU has only three haplotypes (Hd = 0.569), one of which (Db) is unique to the Avalon Peninsula and shared with the Cape Shore WMU, where it is fixed (Hd = 0.0) (Table 2). An intuitive explanation for this low haplotypic diversity would be that this WMU is the endpoint of a northwest to southeast movement across the island from the Northern Peninsula, via the Isthmus of Avalon that connects it to the rest of the island, with consequently restricted initial colonization numbers and population genetic bottleneck. In Fig. 2, haplotype Db is basal, rather than terminal, to the phylogenetic radiation of Clade D. Considered in isolation, this might suggest an initial colonization of the Avalon by the

Table 2. Variation of mtDNA clades among 14 Wildlife Management Units (WMUs).

Wildlife Management Unit	*N*	Total				Fraction				Segregating sites	No. of haplogroups	Hd	*K*	π
		A	B	C	D	A	B	C	D					
St. Anthony	17	1	4	7	5	0.06	0.24	0.41	0.29	16	8	0.824	4.82	0.00245
Northern Peninsula	18	—	7	2	9	—	0.39	0.11	0.50	10	5	0.667	4.12	0.00210
Adies Lake	18	—	6	8	4	—	0.33	0.44	0.22	10	4	0.778	3.32	0.00220
Hampden Downs	6	1	2	1	2	0.17	0.33	0.17	0.33	15	5	0.933	6.73	0.00343
Gaff Topsails	20	—	6	6	8	—	0.30	0.30	0.40	11	6	0.837	4.29	0.00219
Buchans	17	1	2	6	8	0.06	0.12	0.35	0.47	18	7	0.838	4.60	0.00234
Merasheen Island	20	—	1	16	3	—	0.05	0.80	0.15	11	4	0.558	2.25	0.00115
Lapoille	18	—	3	6	9	—	0.17	0.33	0.50	13	10	0.882	4.04	0.00206
Grey River	19	—	2	6	11	—	0.11	0.32	0.58	14	8	0.866	4.02	0.00205
Pot Hill	18	—	7	1	10	—	0.39	0.06	0.56	18	13	0.954	4.87	0.00248
Mt. Peyton	14	—	5	1	8	—	0.36	0.07	0.57	15	10	0.945	4.90	0.00249
Middle Ridge	18	—	4	—	14	—	0.22	—	0.78	14	9	0.882	4.18	0.00213
Avalon	18	—	—	—	18	—	—	—	1.00	2	3	0.569	1.03	0.00052
Cape Shore	12	—	—	—	12	—	—	—	1.00	0	1	0.000	0.00	0.00000
Summary	233	3	49	60	121	0.013	0.210	0.258	0.519	32	32	0.894	4.25	0.00216

Note: For each WMU, the table gives the count of each major clade, fractional contribution of that count to the total for the WMU, number of segregating sites, number of haplotypes, haplotypic diversity (Hd), average number of pairwise differences (*K*), and nucleotide diversity (π).

Fig. 5. Geographical regions as defined for five scenarios in the hierarchical AMOVA analysis. Panel 1 shows the names of the Wildlife Management Units (WMUs). Scenario 3 is the a priori four-herd model of Bergerud (1971).

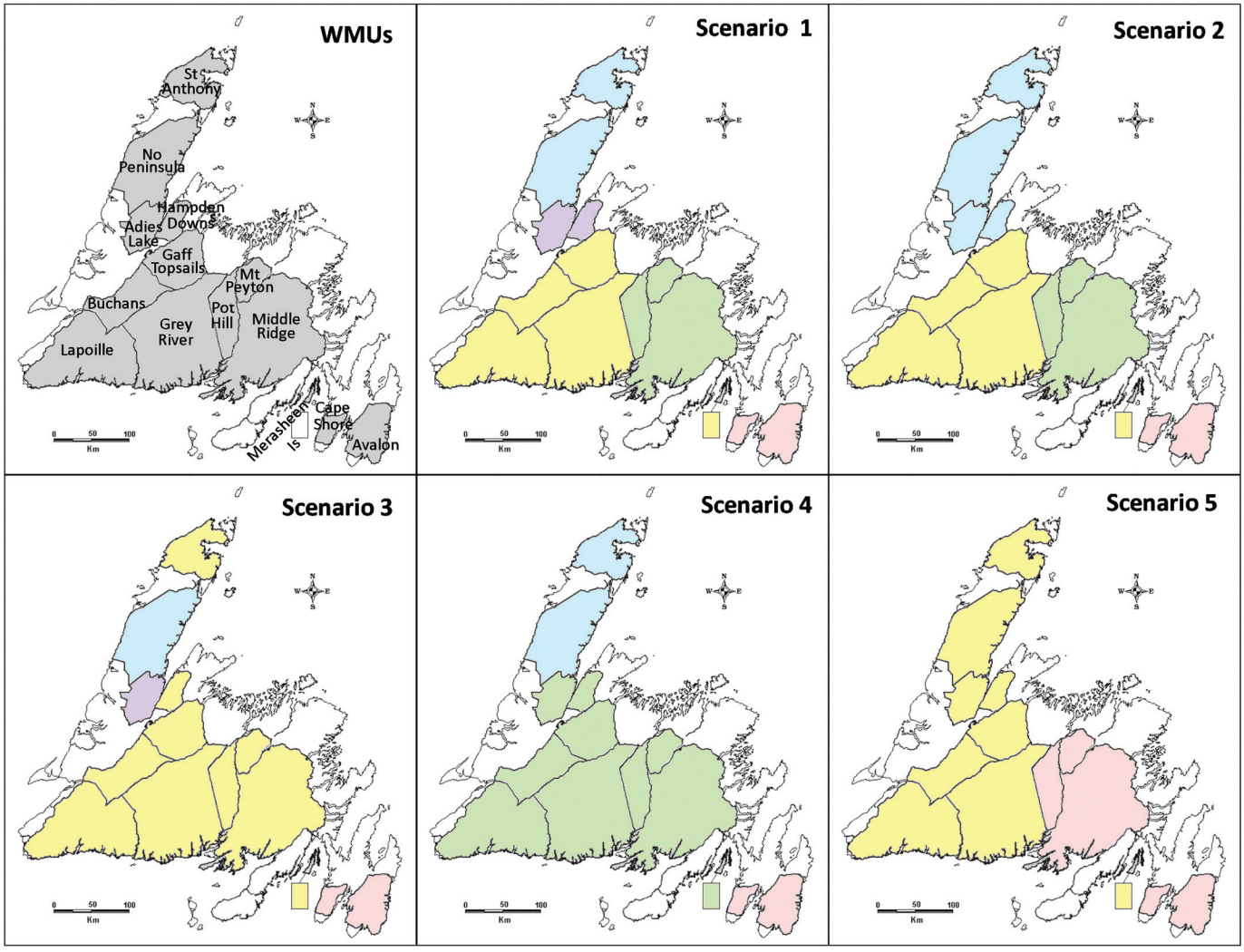
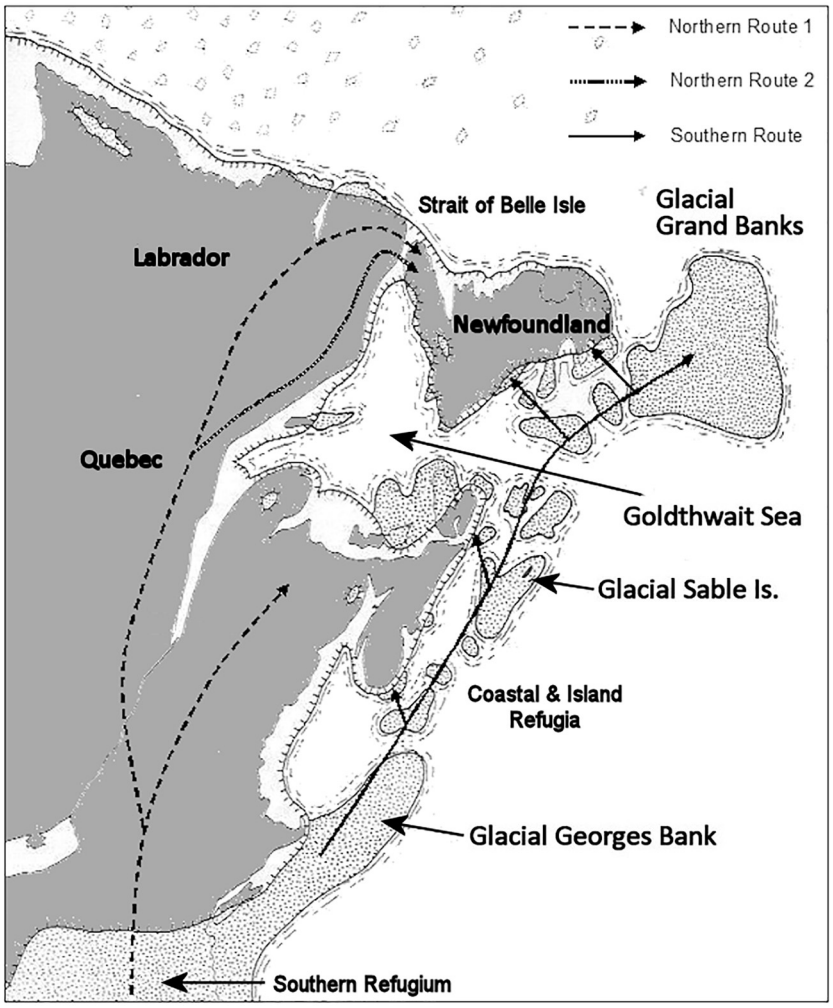


Fig. 6. Hypothetical recolonization routes of caribou returning to insular Newfoundland from Continental refugia after retreat of the Laurentide ice sheet. Solid white areas with hatched border indicate the glacial ice margin. Speckled areas indicate land refugia (Adapted from Pielou 1991).



alternative southern route colonization via an island refugium, and expansion northwestward. However, Clade D is part of the BCD lineage rooted to Clade A with affinities to lineages on the Labrador mainland. The placement of Db may also reflect incomplete resolution of haplotypes within Clade D. Alternative explanations include that the geographically isolated Avalon population experienced significant population fluctuations, during which haplotypic diversity was lost due to genetic drift. High mortality due to parasitic brain worms (see below) may also have contributed to a post-colonization reduction in population numbers.

Founder effects in introduced populations

Founder effects are evident in herds introduced to previously unoccupied areas by wildlife managers over the past 40–50 years. The Merasheen Island WMU was founded by a small number of individuals from the Buchans WMU, where the predominant haplotype Ca is less than one-third of the total. That proportion has increased to more than two-thirds in the post-founder population. The Cape Shore WMU was founded by a small number of individuals introduced from the Avalon WMU, where the private haplotype Db is predominant: the WMU is now homogeneous for that haplotype. The St. Anthony WMU was established in the 1970s from the central Newfoundland population: its haplotype composition is similar to central populations rather than to the adjacent Northern Peninsula WMU.

Taxonomic status of Newfoundland caribou with respect to other Nearctic caribou

Data sets exist for various *Rangifer* subspecies and geographic variants for portions of both the cytochrome *b* and control regions, however most of these do not include both regions and (or) major overlaps within regions. Figure 3 retains the most common haplotypes from this study (Table 1), all of the *R. t. groenlandicus* and *R. t. caribou* from Cronin et al. 2005, and the first sister sequence among *R. t. granti* to any of these. Newfoundland Clade A sequences are included in the outgroup to all sequences in Clades B, C, and D, which includes two insular Newfoundland sequences from Cronin et al. (2005). The basal Clade A-inclusive group includes two sequences from Labrador, which is assigned to *R. t. caribou*. The magnitude of the differences among the outgroup sequences are on the same order as those among Clade B, C, and D haplotypes. The GenBank reindeer sequence (*R. t. tarandus*) clusters with a *R. t. granti* caribou sequences from Alaska.

We conclude that insular Newfoundland caribou are a distinct phylogenetic clade, *Rangifer tarandus terranovae* (Bangs, 1896), except that Clade A is likely a recent introduction from the mainland, as it is more closely related to animals on the mainland than to Clades B, C, and D (Fig. 6).

No evidence of introduced Eurasian reindeer mtDNA

Norwegian reindeer (*R. t. tarandus*) were introduced in 1908 to the Northern Peninsula at St. Anthony as a resource for subsis-

tence hunting. Reindeer were herded southward from St. Anthony, down the Northern Peninsula, and as far east as Millertown (Johnson 1967). The reindeer were heavily hunted by poachers during the Great War, and survivors removed from the island shortly thereafter (Millais 1907; Johnson 1967). Contact with reindeer did however introduce the Scandinavian Brain Worm (*Elaphostrongylus rangeriferi*), the causative parasite for cerebrospinal elaphostrongylosis. The brain worm was not diagnosed in caribou until decades later, and by 1990 it had become a significant source of mortality in the Avalon WMU (Lankester and Northcott 1979; Lankester and Fong 1998).

We found no evidence of introduced reindeer mtDNA sequences in any of more than 200 caribou examined. Persistence of introduced reindeer mtDNA in native caribou would require mating of female reindeer with male caribou, production and survival of female F_1s, and subsequent backcrosses to caribou to maintain reindeer mother–daughter lineages (cf. Carr et al. 1986). In Alaska, where native caribou mix with introduced reindeer, there is little if any successful interbreeding, even in mixed herds. Reindeer are smaller, less migratory, and breed several weeks earlier than caribou (Cronin et al. 1995, 2003, 2006). The persistence of reindeer nuclear alleles cannot be ruled out by the current data.

Conclusions

There are four insular clades, [A + [B + [C + D]]], with A as outgroup to others. Clade A may be either a remnant of first colonists, or perhaps more likely given its phylogenetic relationships, a secondary introduction from the mainland. If the latter, then movement across the Strait of Belle Isle is ongoing. Clades B, C, and D reestablished caribou on the island by northern route 1 or 2 (Fig. 6), spreading northwest to southeast. Additional genetic comparisons with Quebec and Labrador caribou and museum skins of extinct Atlantic caribou would be beneficial to the understanding of postglacial movements and re-colonization. Outgroup relationships with mainland caribou clearly support insular Newfoundland caribou as a separate subspecies, *Rangifer tarandus terranovae* (Bangs, 1896).

Genetic variation in Newfoundland caribou occurs primarily at the individual, rather than herd or regional level, which suggests ongoing genetic interchange among most WMUs, particularly in central Newfoundland. Partitioning of regional genetic variance is somewhat better explained with Scenario 2 (Fig. 5) than Bergerud's four-herd hypothesis (1971). Scenario 2 describes a unified Northern Herd (including Adies Lake and Hampden Downs WMUs), distinctive East and West Herds, and the Avalon Herd. Further examination of the movement of caribou in central Newfoundland could assist in east–west herd delineation. Despite their widespread introduction in Newfoundland, reindeer have not contributed to mtDNA diversity in caribou.

Founder effects are evident in the introduced populations in Merasheen Island, St. Anthony, and Cape Shore WMUs. Merasheen Island and Cape Shore WMUs both exhibit low haplotype diversity, possibly due to their relative isolation from other caribou herds. The Avalon WMU is genetically depauperate not because of founder effect, but because of subsequent genetic drift and loss of haplotypes, possibly exacerbated by excess mortality from brain worms. Implications for low genetic diversity are the limited ability of the herd to deal with environmental change and disturbances, such as extreme weather or parasitic infestations.

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