POPULATION GENETICS OF WOODLAND CARIBOU (Rangifer tarandus caribou) ON THE ISLAND OF NEWFOUNDLAND

CORINNE D. WILKERSON

Your file Votre référence
ISBN: 978-0-494-64774-5
Our file Notre référence
ISBN: 978-0-494-64774-5

POPULATION GENETICS OF WOODLAND CARIBOU (*Rangifer tarandus caribou*) ON THE ISLAND OF NEWFOUNDLAND

by

© Corinne D. Wilkerson

A thesis submitted to the

School of Graduate Studies

in partial fulfillment of the

requirements for the degree of

Master of Science

Department of Biology, Faculty of Science

Memorial University of Newfoundland

January 2010

St. John's Newfoundland and Labrador

Abstract

The purpose of this study was to characterize the genetic variation and genetic structure of caribou herds on the island of Newfoundland. A 2223 bp sequence of mitochondrial DNA from the Control Region and the cytochrome *b* gene was sequenced for 233 Newfoundland caribou (*Rangifer tarandus caribou*). Phylogenetic analysis identified 32 mtDNA haplotypes structured into four clades, labeled A, B, C and D. The hierarchical AMOVA revealed there is little genetic differentiation among defined herds, although there is some regional differentiation of the Avalon Peninsula. Avalon Peninsula caribou are genetically depauperate and distinct, possibly as a result of founder effects. The Nested Clade Analysis identified significant phylogeographic associations possibly due to restricted gene flow with isolation by distance, contiguous range expansion and long distance colonization possibly coupled with subsequent fragmentation or past fragmentation followed by range expansion. Most Newfoundland caribou show close relationships to woodland caribou in Quebec, and a small subset show close relationships to woodland caribou in Labrador. There is some genetic evidence to suggest that caribou populated Newfoundland by way of the Straight of Belle Isle as opposed to coastal refugia. Newfoundland caribou are genetically distinct from Eurasian reindeer (*Rangifer tarandus tarandus*) and there is no evidence to support successful interbreeding with reindeer introduced from Norway. In anticipation of continuing population genomic studies of Newfoundland caribou, the entire mtDNA genome for one Newfoundland caribou was sequenced (16,359 bp).

Acknowledgements

I would like to thank my supervisor, Dr. Steven Carr, for his patience, guidance and mentorship throughout this project. I would like to thank to thank Dr. H. Dawn Marshall, for her invaluable technical expertise and advice. Special thanks to all members of Dr. Carr's lab who have assisted me throughout this project, including, Kimberly Johnstone, Stephanie Royston, Angela Pope, Sarah Flynn, Ana Duggan, and the WISE summer students from 2004-2006.

I would like to thank the Institute for Biodiversity and Ecosystem Studies (Province of Newfoundland and Labrador) for financial support. I would like to thank Shane Mahoney of the Department of Sustainable Development, for his guidance and extensive knowledge of Newfoundland caribou herds. I am grateful to a number of Provincial Wildlife staff for technical assistance, including, Marlene Dredge, Rick Curran, Barry Adams, and Frank Norman.

I would like to thank Dr. R.L. Haedrich for his assistance with this manuscript. I would like to thank Dr. Brian Staveley and members of the Staveley lab who always kept things fun and interesting. I am grateful to the staff of the Biology department for all their assistance. I would also like to thank Memorial University of Newfoundland for providing me with this opportunity and providing financial support.

Most of all I would like to thank my partner, Eric Haapala, for all his love and support.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ATPase – ATP synthase subunit, mitochondrial genome

bp – base pairs

BV – Bootstrap Value

COSEWIC – Committee on the Status of Endangered Wildlife in Canada

COX – Cytochrome oxidase gene subunit, mitochondrial genome

CR – Control Region

CSE - Cerebrospinal Elaphostrongylosis

DNA – Deoxyribonucleic Acid

DNA nucleotides (A=adenine, G=guanine, C=cytosine, T=thymine)

MP – Maximum Parsimony

MSN – Minimum Spanning Network

mtDNA – mitochondrial DNA

mybp – million years before present

NCA – Nested Clade Analysis

ND - NADH dehydrogenase subunit, mitochondrial genome

PAUP – Phylogenetic Analysis Using Parsimony

PCR – Polymerase Chain Reaction

RNA – Ribonucleic Acid

rpm – revolutions per minute

SNP – Single Nucleotide Polymorphism

UPGMA – Unweighted Pair Group Method with Arithmetic mean

List of Appendices

1.0 Introduction

1.1 Nature of The Problem

Woodland caribou *Rangifer tarandus caribou* populations are declining across most of Canada, due to factors such as, increased predation and habitat loss, and as a result most populations have been designated to be at risk (COSEWIC 2002). The Newfoundland population of woodland caribou, which is the focus of this study, remains at relatively high numbers (ca. 80,000 animals) and therefore has not been designated at risk. However, in recent years, signs of a population decline have been observed and numbers are predicted to decrease dramatically in the near future (Mahoney and Schaefer 2002, S. Mahoney pers. com.). Because the population is hunted annually for sport and as a food source for local residents it must be carefully managed in light of the predicted decline.

Preservation of genetic diversity is a key component to consider in the management of any species. Genetic diversity occurs at four levels of organization: among species, among populations, within populations and among individuals (Hunter 1996). It is generally accepted that a high level of genetic diversity allows a species to be adaptable to a range of conditions, thereby improving survivability, productivity, reproduction and physical condition. Isolated populations, such as the Newfoundland caribou, may have reduced genetic diversity and small gene pools associated with inbreeding that may result in poor environmental fitness, poor adaptability and increased risk of extinction in the face of environmental or other changes. Of course it is not

always the case that low levels of variation are manifested as reduced environmental fitness and in fact the opposite may be true, where homozygous genotypes may be positively selected as an adaptation to local conditions.

Little is known about the genetic diversity and genetic population structure of Newfoundland caribou herds or their relationships to caribou in North America and Eurasia. This lack of information limits our understanding of the viability of individual herds in the population, relationships with neighboring herds, and how caribou have populated and persisted on the island. This study aims to use mitochondrial DNA to characterize the genetic variability among and within herds on the island in order to evaluate the degree of gene flow among herds, and examine evolutionary relationships with caribou herds from mainland Canada and reindeer from Eurasia. The genetic information gathered from this study will greatly enhance our understanding of Newfoundland caribou population dynamics at the landscape level and will be integral in managing the caribou populations to preserve genetic diversity in light of the predicted population decline. Genetic comparisons with caribou populations from mainland Canada will help determine the degree of genetic differentiation from these populations and may give insight into the origins of the Newfoundland caribou population. Genetic comparisons with Eurasian reindeer (*Rangifer tarandus tarandus*) will help to verify whether interbreeding occurred with reindeer introduced to Newfoundland from Norway in the early 1900's and determine if any remnant populations of reindeer still exist.

1.2 Caribou as a Species

Caribou (*Rangifer tarandus* Linnaeus) are a holarctic species with extant populations in North America, Europe, Asia, Greenland, and Spitsbergen/Svalbard (Røed 2005; Kurtén and Anderson 1980; Banfield 1961). Caribou and reindeer are considered to be the same species, caribou refers to wild populations in North America and reindeer refers to wild and domesticated populations in Eurasia. Caribou are a member of the deer family (Cervidae), and are distinguished as being the only member of the family where both sexes have antlers (Kurtén and Anderson 1980; Banfield 1961). Caribou are a medium-to-large sized deer that possess a number of physical adaptations to arctic or sub-arctic environments (Banfield 1974; Banfield 1961), including a large, blunt and well-furred muzzle; short, broad and heavily furred ears; a short and well-furred tail; a compact body covered by long, thick pelage; and large feet with crescentic hooves that facilitate travel over snow-covered and boggy ground (Banfield 1974). In winter, the hooves grow longer and the hair between the toes forms tufts that cover and protect the pads from the snow and ice (Banfield 1974).

Modern deer evolved in the Old World (Eurasia) and are thought to have begun populating the New World (North America) sometime during the Pliocene ~3.5 mybp (Banfield 1961, Geist 1998, Guthrie and Matthews 1971, Kurtén 1968, Kurtén and Anderson 1980). The origin of the genus *Rangifer* is unknown, however, some suggest it originated in Alaska, Beringia or the mountains of northeastern Asia and later migrated into Western Europe (Banfield 1961, Guthrie and Matthews 1971, Martin and Klein 1984). The earliest record of *Rangifer* in North America is from a 1.6 million year old

tooth found in the Yukon Territory; other early records include 45,500-year-old cranial fragment from the Yukon and a 40,600-year-old antler from Quebec (Gordon 2003). The ancestral origins of caribou prior to the last glaciation (Wisconsin), which occurred approximately 80,000 to 10,000 years ago, are not well understood, however, during the last glaciation it is known that caribou were abundant and distributed in non-glaciated refugia both north and south of the Laurentide ice sheet (Banfield 1961, Martin and Klein 1984).

The eight subspecies of caribou currently recognized (Figure 1) are believed to have developed in three isolated non-glaciated refugia, designated Beringia, Eurasian and Southern, which were created as the continental ice sheets expanded and retreated throughout the Wisconsin (in North America) and Weichselian (in Eurasia) glaciations (Røed 2005; Banfield 1961). The Beringia refugium, extending through Eurasia, Alaska and Yukon, gave rise to all of the North American tundra forms (*Rangifer tarandus granti* and *Rangifer tarandus groenlandicus*), arctic forms (*Rangifer tarandus platyrhynchus*, *Rangifer tarandus pearyi*, and *Rangifer tarandus eogroenlandicus*), Eurasian tundra form (*Rangifer tarandus tarandus*) and the Eurasian forest form (*Rangifer tarandus fennicus*) (Røed 2005; Kurtén and Anderson 1980; Banfield 1961). The Eurasian refugium, small isolated refugium in Western Eurasia, also gave rise to *R. t. tarandus* and *R. t. fennicus*, suggesting a diphyletic origin for these subspecies (Røed 2005). The Southern refugium in North America, extending from New Jersey, Kentucky, Missouri, Illinois, and Iowa to the mountainous region of the southwest –New Mexico and Nevada, gave rise to the American woodland caribou subspecies (*Rangifer tarandus*

caribou) (Røed 2005, Kurtén and Anderson 1980; Banfield 1961). Evidence of cold, tundra-like conditions and fossils of tundra animals, including caribou have been found in the area defined as the Southern refugium (Pielou 1991). The caribou population in Newfoundland is currently assigned to the woodland caribou subspecies *(Rangifer tarandus caribou)*, however, it was at one time considered as a separate subspecies (*Rangifer tarandus terranovae*, Bangs). It should be noted that the subspecies designations of caribou (and use of the subspecies concept in general) are rather subjective, however, when applied properly, they do recognize potentially important geographic variation (Cronin 2006).

Woodland caribou are distributed throughout the taiga/boreal region from Newfoundland and Labrador and Quebec in the east, across to British Columbia, Yukon and Alaska in the west (Banfield 1961). They are adapted to live in forested areas, and are generally found in mature boreal forest, associated barrens, and bog-fen complexes (Banfield 1961). Woodland caribou tend to have larger body size, longer legs and a shorter distance between their antlers than caribou adapted to open tundra, mountain or arctic habitats (Røed 2005). Woodland caribou primarily depend on ground lichens (*Cladonia* spp.) and arboreal lichens for food (Banfield 1961, Bergerud 1972), however, they also eat significant amounts of sedges, fungi, deciduous and evergreen shrubs, and other plant material, depending on the season (Bergerud 1972). They tend to live in small groups and are mostly sedentary, however, some populations undertake local, seasonal migrations (Banfield 1961). The primary predator of woodland caribou in most areas is the wolf (*Canis lupus*), however, other predators are also known to feed on

caribou , including, grizzly bear (*Ursus arctos*), black bear (*Ursus americanus*), golden eagle (*Aquila chrysaetos*), bald eagle (*Haliaeetus leucocephalus*), wolverine (*Gulo gulo*), lynx (*Lynx canadensis*) and coyote (*Canis latrans*) (Bergerud 1971; Banfield 1974; Siep 1991).

At the close of the 19th century, the distribution of woodland caribou in North America followed the proximate distribution of the Boreal Forest (Banfield 1961). During the 1900's, woodland caribou populations across North America underwent significant declines (Bergerud 1974). Increased harvesting that resulted from the expansion of European immigrants into North America and increased wolf predation are cited as the main causes of the decline (Banfield 1961; Bergerud 1974; Seip 1991). Currently, several woodland caribou populations across Canada (Figure 2) are designated as Endangered (Atlantic-Gaspésie population), Threatened (Boreal population, Southern Mountain population) or Special Concern (Northern Mountain population) by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2002). The Newfoundland population is the only forest-dwelling population of woodland caribou that is not designated at risk.

1.3 Early Population History of Woodland Caribou Herds in Newfoundland

Caribou populated the island of Newfoundland sometime after the end of the Wisconsin Glaciation when the Laurentide ice sheet retreated. It is believed that caribou may have inhabited Newfoundland for as many as 8,000 years, likely originating from refugia located south of the Laurentide ice sheet, where it is thought all of the woodland

caribou subspecies originated (Banfield 1961; Kurtén and Anderson 1980; Røed et al. 1991; Røed 2005). There are different schools of thought as to how the founding populations of caribou populated the island of Newfoundland. One theory is that caribou came from Labrador over the ice on the Strait of Belle Isle to the northern tip of Newfoundland during an unusually cold winter (Hardy 1869). This would suggest that Newfoundland caribou are most closely related to caribou from Labrador and the Quebec North Shore. However, there here has been little documented movement of caribou between the island and Labrador/Quebec at the Strait of Belle Isle. The last documented event was made in the late 1800's by Captain C. Hardy based on information he gained from local fishermen (Hardy 1869). A second hypothesis is that caribou came from the eastern coastal plains refugia and/or various island refugia (Figure 3) located off the east coast of North America (Pielou 1991). Little is known of the inhabitants of these areas except that there were mastodons and mammoths on the southern parts of the coastal plains lands (Pielou 1991). It has been suggested that large active animals, such as caribou, may have journeyed frequently among the ring of refugia surrounding the Goldthwaite Sea and the islands of the coastal plains lands (Pielou 1991). Under this hypothesis, Newfoundland caribou would be most closely related to the caribou that once inhabited New England and the Atlantic provinces of Nova Scotia, New Brunswick and Prince Edward Island (Pielou 1991). Unfortunately, maritime caribou have been extinct since the late 1800's/early 1900's, and all that remains is a small population on the Gaspé Peninsula in Quebec (Figure 2), which is currently designated as Endangered (COSEWIC 2002). Whatever their origins, Newfoundland caribou are believed to have evolved

independently of mainland populations because of their long post-glacial isolation on the island. Under such circumstances, they may have developed unique behavioral and morphological characteristics, which may subsequently be due to unique alleles or genotypes.

Several significant events occurred over the past century that may have influenced the current genetic diversity and genetic population structure of caribou in Newfoundland. In the late 1800's a railroad was constructed across the center of the island in an east-west direction. The railroad intersected the path of fall caribou migration from the Northern Peninsula to southern parts of the island (Millais 1907). The railroad allowed easy access to thousands of caribou by hunters and resulted in substantial over-hunting until the government enforced game restrictions several years later (Millais 1907). In 1908, Sir Wilfred Grenfell brought 300 Norwegian reindeer to St. Anthony to provide a source of food for the locals (Millais 1907; Johnson 1967). The introduced reindeer herd flourished and rose to 1500 by 1913. However, when Grenfell left St. Anthony during the war most of the reindeer were killed by poachers (Johnson 1967). The remaining animals were relocated to Anticosti Island in the St. Lawrence River where they eventually died out (Bergerud and Mercer 1989; Johnson 1967).

During the reindeer introduction, a group of 50 reindeer were herded from St. Anthony, down the Northern Peninsula, through the Long Range Mountains, and east to Millertown (Johnson 1967). Along the way, these reindeer met with local caribou herds and likely introduced the nematode *Elaphostrongylus rangeriferi* (Scandinavian Brain Worm) to the Newfoundland caribou population (Lankester and Fong 1989). This

parasite causes the disease Cerebrospinal Elaphostrongylosis (CSE) and was first diagnosed in caribou of central Newfoundland during the mid 1970's (Lankester and Northcott 1979). The disease later spread to caribou herds on the isolated Avalon Peninsula around 1990, causing significant mortalities (Lankester and Fong 1998).

For reasons that are still not well understood, wolves were completely extirpated from Newfoundland in the early 1900's (Bergerud 1971; Mahoney and Virgl 2003). Wolf predation is considered the dominant, natural regulating factor in caribou populations (Seip 1991). The loss of this important predator from the Newfoundland caribou population may have had a significant effect on the population dynamics of caribou on the island.

Over the past century, caribou in Newfoundland have experienced dramatic population fluctuations that may have affected current levels of genetic variation. In the early 1900's caribou were abundant, with claims of population estimates ranging as high as 150,000 (Dugmore 1913) to 200,000 (Millais 1907) caribou. These estimates, however, were based largely on observations rather than a formal census. The population estimate for the interval of 1900-1910 was later revised to approximately 40,000 caribou based on estimates given in interviews by residents from across the island (Bergerud 1971). From about 1915 to 1925 the caribou population declined rapidly (Bergerud 1971) to such low numbers that there was concern that caribou would be extirpated from the island. This was around the same time that the wolf was extirpated from Newfoundland, and the decline of the caribou is cited as a possible reason for the wolf's extirpation. Due to the caribou population decline, the hunting season for caribou was

closed in 1925 (Bergerud 1971). It was believed there might have been as few as 200 caribou left on the island (Dugmore 1930); however other estimates suggested that the population declined to approximately 1,000 to 2,000 caribou (Bergerud 1971). At the time, the reason for the decline was largely attributed to over-hunting, which increased substantially when the railroad was built across the island in the late 1800's. However, it was later suggested that increased predation on caribou by lynx, which was brought on by a decline in their main prey base, the snowshoe hare, may have contributed significantly to the decline in caribou numbers (Bergerud 1971). Caribou in Newfoundland have been found to experience cyclical peaks and declines in their numbers every 60-90 years and this may have had more of an influence in their decline than over-hunting or lynx predation alone (S. Mahoney pers. com.).

By the early 1930's, caribou numbers began to increase and hunting was resumed in 1935 (Bergerud 1971). A peak population was reached around 1941, but declined again in the following few years (Bergerud 1971). By the mid-1940's, the population expanded and occupied much of its previous range (Bergerud 1971). A second peak of abundance was reached around 1951, after which caribou numbers declined yet again (Bergerud 1971). A census completed in 1957-58 estimated the population at approximately 6,500 caribou (Bergerud 1971). The population increased exponentially in subsequent years (Mahoney and Schaefer 2002; Mahoney et al.1991; Bergerud et al. 1983), numbering 23,000 to 44,000 in the mid-1980's (Williams and Heard 1986) and reaching a peak population of approximately 80,000 caribou by the mid-1990's.

1.4 Current Status of the Newfoundland Caribou Population

The current population is thought to be past its cyclical peak of abundance and declining (S. Mahoney pers. com.). Evidence of the decline has been indicated by long-term studies of caribou on the Buchans Plateau in central Newfoundland (Mahoney and Schaefer 2002). Signs of the decline have been observed in the form of lower annual survival of adults, decreased adult body size, decreased recruitment, low calf survival, late calving, and reduced pregnancy rates (Mahoney and Schaefer 2002). Some herds have experienced zero calf survival in recent years (S. Mahoney pers. com.). Other indications that the population may be past its peak of abundance include observations of early autumn migration and late spring migration which may be related to the herd's own depletion of its summer forage (Mahoney and Schaefer 2002).

A number of factors influence the survival of Newfoundland caribou. Although wolves have been extirpated from the island, caribou are subject to predation by black bear (Mahoney and Virgl 2003), coyote (S. Mahoney pers. com.), bald eagle (S. Mahoney pers. com.), and lynx (Bergerud 1971). The population also suffers from various diseases and parasites, including Scandinavian brainworm (*E. rangiferina)*, warble fly (*Hypoderma tarandi*); throat/ nose bot (*Cepehenemyia trompe*), and tapeworm cysts.

Previous studies on the population dynamics of Newfoundland caribou suggest the entire population comprises four broadly defined yet discrete herds, the Interior Herd, the Humber River Herd, the Avalon Peninsula Herd, and the Northern Peninsula Herd (Bergerud 1971). However, for current management purposes by the province of

Newfoundland and Labrador, the population of caribou on the island is subdivided into 19 Caribou Management Units (or herds), which are based on calving ranges (Figure 4). These caribou herds are hunted between September and December of each year, with the exception of 4 herds (Avalon Peninsula, Burin Knee, Burin Foot and Blow Me Down Mountains), which are closed to hunting. Three of the herds occur on islands, (Fogo Island, Grey Islands and Merasheen Island) and have a limited hunting season. From 1961 to 1982, 384 caribou were taken from native Newfoundland ranges and introduced to 22 sites in Newfoundland unoccupied by caribou, including several small islands (Bergerud and Mercer 1989). A number of the introductions were successful, including three herds that are included in this study, St. Anthony, Merasheen Island, and Cape Shore. The St. Anthony herd likely originated from the central herds, however, information on the source of this introduction is not clear. The caribou on Merasheen Island were introduced from the Buchans Plateau in the 1970's (Anonymous 1973). Caribou in the Cape Shore herd were introduced from the Avalon Peninsula herd (S. Mahoney pers. com.).

This study defines the 19 Caribou Management Areas (CMU's) as separate herds and focuses on a sub-sample of 14 of theses herds. The study samples come from 13 hunted herds from across the island and one non-hunted herd. The hunted herds include; Lapoile, Buchans, Grey River, Gaff Topsails, Pot Hill, Mount Peyton, Middle Ridge, Hampden Downs, Adies Lake, Northern Peninsula, St. Anthony, Cape Shore, and one island herd, which is located on Merasheen Island (Figure 4). The one non-hunted herd included in the study is located on the Avalon Peninsula (Figure 4). The 1070 km^2

Avalon Wilderness Reserve protects the range of this herd from habitat loss and hunting. The Avalon herd was severely depleted in the 1960's but increased steadily thereafter (Bergerud et al. 1983) until they were exposed to Scandinavian Brain Worm in the 1990's and faced significant reductions. The Middle Ridge herd is one of the largest herds on the island, numbering approximately 15,000 caribou. Although this herd is hunted, much of its winter habitat is protected by the 2895 km^2 Bay du Nord Wilderness Reserve and the adjacent Middle Ridge Wildlife Reserve.

1.5 Mitochondrial DNA as a Marker and Caribou Genetic Studies

A number of studies evaluating the phylogenetic relationships of mammal populations have used mitochondrial DNA (mtDNA) as a molecular marker. The advantages of using mtDNA for phylogenetic studies are well-known and numerous. Mammal cells contain thousands of copies of mtDNA, which provide a high number of starting copies for amplification by Polymerase Chain Reaction (PCR) (Palumbi 1996). Mitochondrial DNA evolves 5 to 10 times faster than single copy nuclear DNA so it is particularly useful in assessing genetic relationships among closely related individuals in populations which have more recent divergence times (Avise 2004, Brown et al. 1979,). Mitochondrial DNA is maternally inherited and non-recombining; therefore it gives a direct history of maternal inheritance throughout time (Avise 2004, Wilson et al. 1985). Gene phylogenies and haplotype frequencies produced from mtDNA sequence information can give estimates of gene flow among populations (Hudson et al. 1992; Slatkin and Madison 1989, 1990) and when combined with geographic information can

be used to evaluate the genetic structure of a population (Avise et al. 1987, Avise 2000, 2004).

Genetic relationships of caribou populations in North America and Europe have been studied using a variety of molecular markers, including, the major histocompatibility complex (Olsaker and Røed 1990; Cronin et al. 1995); *k*-casein (Cronin et al.1995); serum transferrin (Røed 1991; Røed 2005; Røed & Thomas 1990; Røed et al. 1991); microsatellite loci (Côté et al. 2002; Courtois et al. 2003; Cronin et. al 2003; Cronin et al. 2005; Cronin et al. 2006; Jepsen et al. 2002; McLoughlan et al. 2004; Røed 2005; Røed & Midthjell 1998; Valkenburg et al. 2000; Wilson et al. 1997; Zittlau et al. 2000); restriction enzyme analysis of mitochondrial DNA (Cronin 1992); mitochondrial DNA cytochrome *b* gene (Byun et al. 2002; Cronin et al. 2005; Cronin et al. 2006); and mitochondrial DNA Control Region (Cronin et al. 1995; Flagstad & Røed 2003; Gravlund et al. 1998; Røed 2005). A number of these studies have included caribou from the Newfoundland population (Cronin 1992; Cronin et al. 2003; Cronin et al. 2005; Røed et al. 1991). A study of transferrin alleles in North American caribou identified caribou from Brunette Island, Newfoundland as more closely related to woodland caribou (*R. t. caribou*) from Manitoba, Ontario and the Gaspé Peninsula than to barren-ground caribou (*R. t. groenlandicus*) from the North West Territories (Røed et al. 1991). A study of the cytochrome *b* gene of mitochondrial DNA identified two unique genotypes in Newfoundland caribou, which were also identified as most closely related to other woodland caribou (*R. t. caribou*) from Labrador, Quebec, and Alberta rather than barren-ground caribou (*R.t groenlandicus* and *R. t. granti*) from northern Canada and

Alaska (Cronin et. al 2005). Both of these studies (Røed et al. 1991; Cronin et al. 2005) and others (Flagstad & Røed 2003; Gravlund et al. 1998) identified a high degree of genetic separation between *R.t caribou* and other caribou subspecies, which suggest that the ancestral populations of *R. t. caribou* likely survived the Wisconsin glaciation in separate refugia located south of the continental ice sheet, while other *Rangifer tarandus* subspecies survived north of the ice sheet.

1.6 Mitochondrial Genomic Studies and their use for Newfoundland Caribou

A limitation of most population genetic studies is the lack of resolution attained when only a few loci are examined. In mitochondrial DNA studies, a particular gene or region of the genome is chosen for examination based on some previous knowledge of the degree of variation in that area with the expectation that it will provide enough resolution to decipher the genetic relationships accurately. Ideally, the best resolution would be attained using the entire mitochondrial genome (16 Kbp). Traditional sequencing methods are too laborious for such large-scale population genomic studies. A new biotechnology called iterative DNA "re-sequencing", uses a DNA microarray to generate >30Kbp of sequence data in a single experiment (Carr et al. 2008). Experiments with a first-generation microarray designed for mtDNA have shown it to be an accurate, efficient, time and cost effective method for DNA sequencing (Carr et al.2008). An experimental design has been developed to use the mtDNA microarray, known as the "ArkChip", to sequence multiple distantly related vertebrate species of interest in one experiment (Carr et. al 2008). In order to sequence the whole mtDNA genome for a

particular species of interest a species-specific microarray must be designed. The design of the microarray is expensive so it is designed for a high-profile species of interest, such as, the Atlantic Cod (*Gadus morhua*). Using the microarray designed to sequence the Atlantic Cod mtDNA genome, additional complete mtDNA genomes of homologous mtDNA from other species of interest, including, the Newfoundland caribou (*R. t. caribou*), are sequenced in the same experiment (Carr et al. 2008). The use of this technology will be beneficial for wildlife population genomic studies, as it will enable a standardized, co-ordinated investigation of multiple species of interest to Species-At-Risk agencies, managers and recovery teams (Carr et al. 2008).

Sequencing the mtDNA genome using this approach still requires at least one copy of the mtDNA genome for the species of interest as a basis of reference and comparison. In some cases, whole mtDNA genomes can be accessed from public databases (GenBank http://www.ncbi.nlm.nih.gov/Genbank/index.html), however, they are not readily available for all species. In anticipation of continuing population genomic studies of Newfoundland caribou using the "ArkChip" design, the whole mtDNA genome will be sequenced using traditional sequencing methods. This sequence will serve as a measure of accuracy for the "ArkChip" design and to provide a basis of comparison for additional samples. At the time this study began, the mtDNA genome for caribou had not yet been published, however, in January 2006 the mtDNA genome for reindeer (*R. t. tarandus,* 16,362 bp) was published on GenBank http://www.ncbi.nlm.nih.gov/Genbank/index.html (Wada et al., Tokyo University of Agriculture, unpublished). The availability of the reindeer mtDNA genome presents an

opportunity to compare the whole mtDNA genomes for two subspecies of caribou, reindeer of Eurasia (*R. t. tarandus*) and woodland caribou of Newfoundland (*R. t. caribou*).

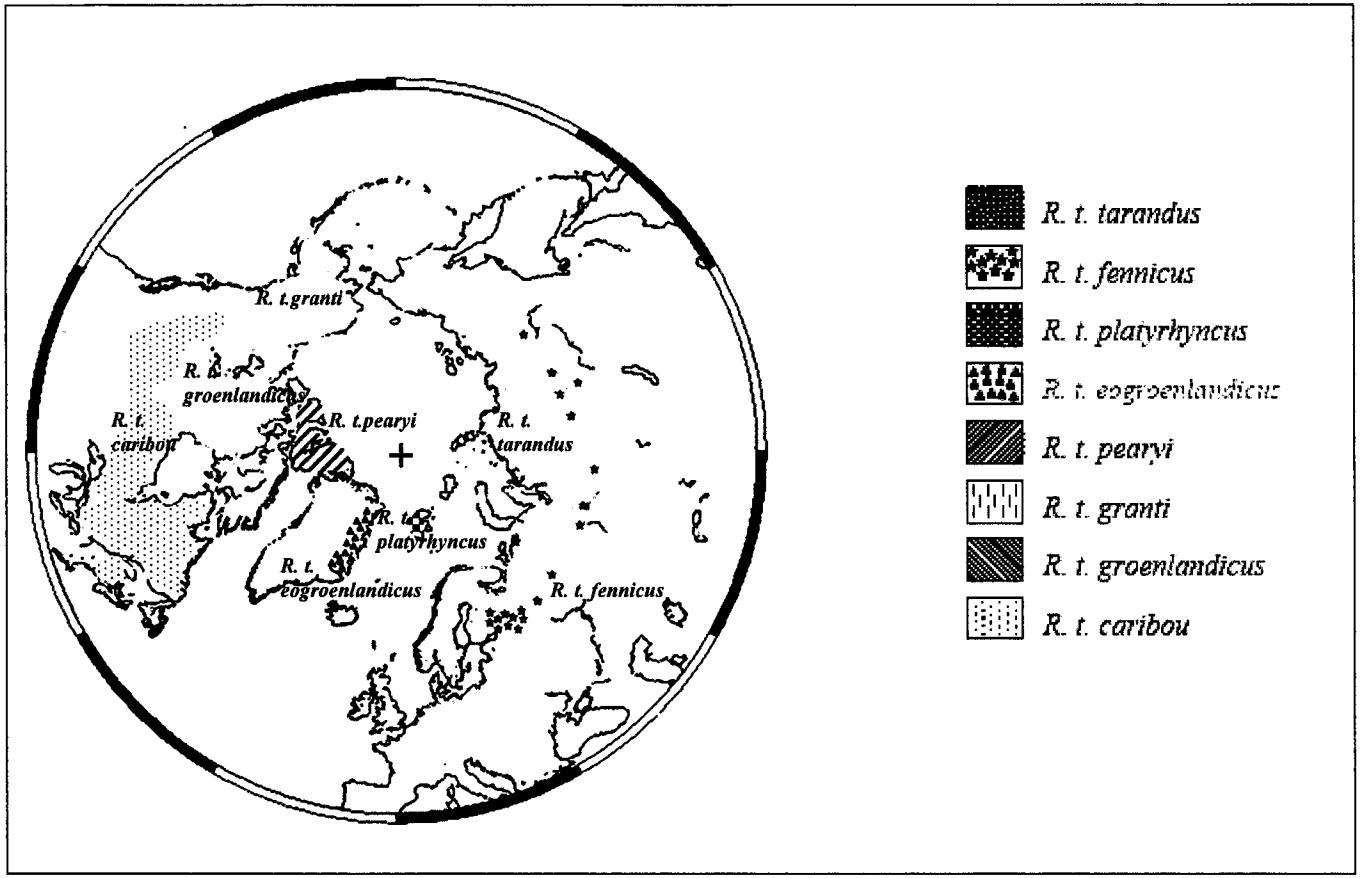


Figure 1. Global Distribution of eight extant subspecies of caribou. North American boreal form (*Rangifer tarandus caribou*), tundra forms (*Rangifer tarandus granti* and *Rangifer tarandus groenlandicus*), arctic forms (*Rangifer tarandus platyrhynchus*, *Rangifer tarandus pearyi*, and *Rangifer tarandus eogroenlandicus*), Eurasian tundra form (*Rangifer tarandus tarandus*) and the Eurasian forest form (*Rangifer tarandus fennicus*) [From Røed 2005].

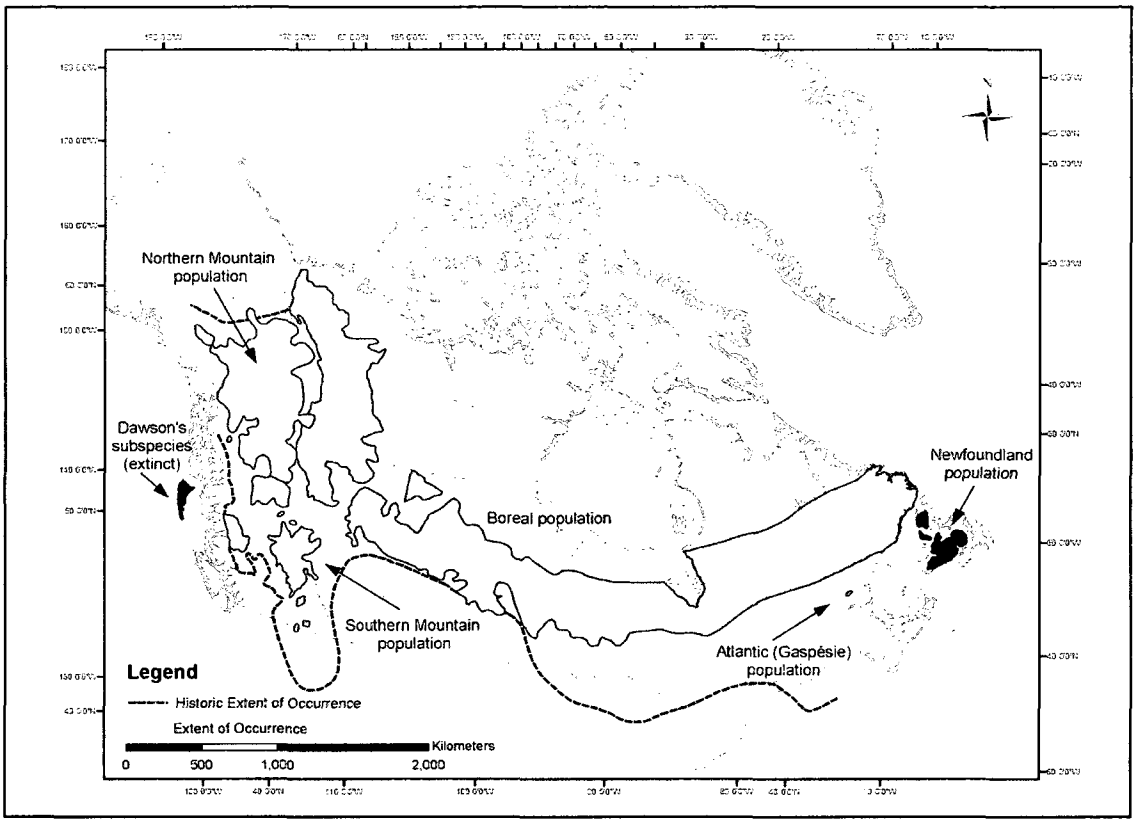


Figure 2. Extent of occurrence of forest-dwelling woodland caribou (*Rangifer tarandus caribou*) in North America in 2001. The subspecies is divided into the following populations: Newfoundland, Atlantic (Gaspesie), Boreal, Southern Mountain, Northern Mountain, and Dawson's (extinct). The current range is indicated by solid lines and the southern limit of historical range is indicated by the dashed line [from COSEWIC Status Report 2002].

(a) from Pielou 1991 (Fig 6.4) | (b) from Pielou 1991 (Fig. 6.5)

Figure 3. Locations of coastal refugia along the eastern coast of North America during the Wisconsin glaciation [from Pielou 1991]. (a). Patterns of land, sea and ice at glacial maximum (shaded areas = land). (b). Map outlining submarine banks that correspond to refugia during Wisconsin glaciation (GB=Grand Banks, SI=Sable Island, CC=Cape Cod).

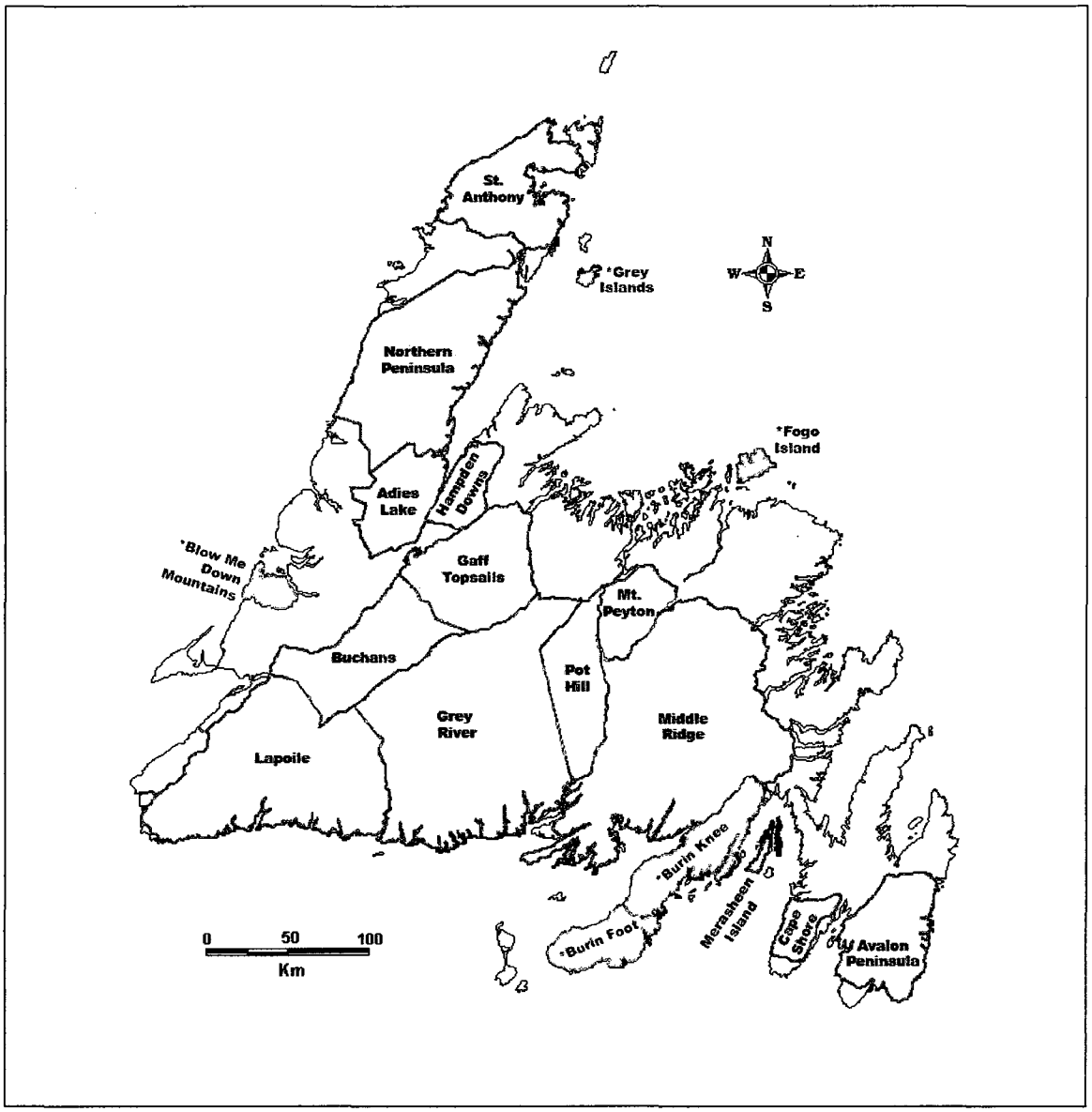


Figure 4. Distribution of Caribou Management Units (herds) across the island of Newfoundland. Grey shaded areas represent the 14 herds used in the present study. Of the 14 herds used in the study, 3 were introduced from other parts of Newfoundland; St. Anthony, Cape Shore, and Merasheen Island. Areas marked with an asterisk (*) were not used in the present study (Blow Me Down Mountains, Grey Islands, Fogo Island, Burin Foot, Burin Knee). [Map from Government of Newfoundland and Labrador].

2.0 Material and Methods

2.1 Samples of Caribou DNA

A total of 267 tissue samples were taken from individual caribou from across the island of Newfoundland, 157 from male caribou, 64 from female caribou and 46 from individuals of unknown sex (Table 1). Tissue samples were obtained during the fall hunting season (Sept. to Dec.) from caribou harvested in 14 of 19 defined Caribou Management Units (herds) located across the island (Figure 4) between 1999 and 2003. For each Caribou Management Unit (herd), 20 samples were collected, with the exception of Hampden Downs where 7 samples were collected. Most tissue samples were muscle remains (dried or fresh) taken from the jawbones of hunted caribou, which were submitted by local hunters to the Government of Newfoundland and Labrador. Eight samples from the Cape Shore herd were kidney tissue collected by provincial biologists. All samples were catalogued by government staff and immediately frozen at minus 20°C.

2.2 DNA Extraction

DNA was extracted from the frozen muscle or kidney tissue according to the tissue protocol as described in the QIAamp ® DNA Mini Kit Tissue Protocol (Qiagen Inc.). Approximately 25 mg of tissue was combined with 180 µl of Buffer ATL (Qiagen Inc.) and 20 µl of proteinase K, and then incubated overnight at 56°C until the tissue was

completely lysed. After lysis, 200 µl Buffer AL (Qiagen Inc.) was added to the mixture and incubated at 70°C for 10 minutes. The mixture was combined with 200 µl of 100% Ethanol, transferred to a QIAamp Spin Column and purified according to the manufacturer's protocol (Qiagen, Inc). The purified DNA was eluted with 200 µl AE Buffer (Qiagen Inc.) and subsequently stored at minus 20°C.

DNA samples were viewed on a 2% agarose gel containing ethidium bromide. The gel was loaded with 5µl of purified DNA, combined with 2 µl of the running dye 5X Stop Buffer (bromophenol blue in glycerol). As a standard, 2 µl of the molecular ladder, ΦX174, RF *Hae*III digest, was loaded into each gel to estimate the quality and size of the DNA sample. The gel was electrophoresed at 120 Volts for 30 minutes.

2.3 PCR Amplification of the Cytochrome b Gene and the Control Region

Two regions of mitochondrial DNA, the cytochrome *b* gene (1160 bp) and the Control Region (1063 bp) were amplified using the polymerase chain reaction (PCR). A number of primer pairs were tested throughout the experiment to gain the greatest number of successful amplifications (Table 2). A 401 bp region of the cytochrome *b* gene was amplified using the primers L14724 (5'-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3') described by Irwin et al. (1991) and H15149 (5'-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3') described by Kocher et al. (1989). There was no genetic variation detected in this region; therefore to increase resolution, the entire cytochrome *b* gene (1160 bp) was amplified using the primers L14724 and Cytb811R (5'-CTC CAT TTT TGG TTT ACA AGA C-3'), which was designed for use

on seals (H.D. Marshall pers. com.). The Control Region was amplified using several different primer pairs (Table 2), depending on which resulted in a successful amplification. A 1063 bp region was amplified using the primer pair L15926 (5'-TCA AAG CTT ACA CCA GTC TTG TAA ACC-3') and H00651 (5'-TAA CTG CAG AAG GCT AGG ACC AAA CCT-3') as described by Kocher et al. (1989). A 980 bp region was amplified using the primer pair Pro-5 (5'-CTA CCT CCA ACT CCC AAA GC-3') and Phe-3 (5'-TCT TCT AGG CAT TTT CAG TC -3') as described by Palumbi (1996). The L15926/H00651 primer pair yielded some successful amplifications, however, many samples did not amplify. The Pro-5/Phe-3 primer pair yielded some successful amplifications, however, the sequence data produced did not match the Control Region reference data so the primers were not used again. In order to increase the success of amplifications, caribou specific primers were designed. Based on the primers Pro-5 and Phe-3 (Palumbi 1996), caribou specific primers Rta-pro (5'-AGC TAT AGC CCC ACT ATC AAC ACC CAA AGC-3') and Rta-phe (5'-TGG AGT TAA TAT ACT CAT CTA GGC ATT TTC AGT GC-3') were designed and used successfully for many amplifications, however, they had some difficulties and did not always amplify successfully. Based on the primers L15926 and H00651 (Kocher et al. 1989), caribou specific primers Rta-L15926 (5'-GTA GTA CAC TTA ATA CAC TGG TCT TGT AAA CC-3') and Rta-H00651 (5'-GGG TTA ATA GGA AGG CTG GGA CCA AAC CTG TG-3') were designed to amplify a 1063 bp region. The Rta-L15926/Rta-H00651 primer pair was used for the majority of the amplifications since the results were consistently successful.

PCR amplifications were carried out in 25 µl volumes containing the following reagents: 2.5 µl of 10x PCR Buffer (Qiagen Inc.), 0.5 µl of 10µM dNTP mix (2.5 mM each of dATP, dCTP, dGTP, and dTTP), 1.0 µl of a 10µM stock solution of each primer (Operon Technologies, Inc.), 0.2 µl of Hot StarTaq DNA Polymerase (Qiagen, Inc), 19 µl of ddH_2O, and 1 µl of DNA template. A control reaction containing all of the reagents except the DNA template was included with all of the experiments to ensure there were no false positives caused by contamination. When false positives occurred, all reagents were discarded and the reaction was repeated. In an effort to keep contaminants out of the PCR, pipettes, tubes and the reagent mixture (containing all reagents except HotStarTaq and the DNA template), were sterilized by exposure to UV radiation for 15 to 20 minutes.

Polymerase Chain Reactions were carried out on an Eppendorf Mastercycler and an Eppendorf Mastercycler gradient thermal cycler (Eppendorf North America, Inc., Westbury, NY). Reactions started with an initial activation step at 95°C for 15 minutes. The amplification cycle consisted of denaturation at 93°C for 30 seconds, annealing at 50-55°C (depending on primer pair) for 30 seconds, and extension at 72°C for 1 minute. This cycle was repeated 40 times, and then followed by a final extension step at 72°C for 5 minutes. After amplification, samples were stored temporarily at 5°C, or longer term at minus 20°C.

PCR products were viewed on a 2% Agarose gel containing ethidium bromide. The gel was loaded with 5µl of the PCR product and combined with 2 µl of the running dye, 5X Stop Buffer (bromophenol blue in glycerol). As a standard, 2 µl of the molecular

ladder, ΦX174, RF HaeIII digest, was loaded into each gel to estimate the quality and size of the PCR product. The gel was electrophoresed at 120 Volts for 30 minutes.

Successful amplifications were purified using Spin Columns from the QIAquick PCR Purification Kit (Qiagen Inc.) as per the manufacturer's protocol. The purified DNA fragments were eluted in 30 µl of ddH_20 and stored at minus 20°C. In later experiments, PCR products were purified using the Millipore Multiscreen PCR_{96} Filter Plates following the manufacturers instructions, then eluted in 50 µl of ddH_20.

2.4 DNA Sequencing

PCR products were prepared for sequencing using ABI Prism® Big Dye™ Terminator chemistry (Applied Biosystems, Inc.). Each sequencing reaction contained 5 µl of PCR product that was dried by either leaving in an open tube overnight or by placing in an Eppendorf Vacufuge for 15 to 30 minutes. Sequencing reactions were carried out in 5.8 µl volumes containing the following: 5.0 µl PCR product (dried), 3.48 µl of ddH_20, 2.0 µl of Big Dye™ Terminator, and 0.32 µl of a 10µM stock solution of the same primer used in PCR amplification (forward or reverse). Sequencing reactions were carried out in both the forward and reverse direction and were run on a GeneAmp PCR System 9600 (Perkin Elmer), an Eppendorf Mastercycler or an Eppendorf Mastercycler gradient thermal cycler (Eppendorf North America, Inc., Westbury, NY). The sequencing cycle included an initial heating step at 96°C for 2 minutes, then, 35-50 cycles of the following: denaturation at 96°C for 30 seconds, annealing at 50°C for 15

seconds, and extension at 60°C for 4 minutes. Samples were stored at 5°C until they were ready to be purified.

Sequencing reactions were purified using isopropanol precipitation. This method involved adding 40 µl of 75% isopropanol to the sample and allowing precipitation at room temperature for 20 minutes. Samples were placed in a high-speed refrigerated centrifuge (15°C) for 20 minutes at a speed of 13,000 rpm (either the Eppendorf Centrifuge 5804R or the TOMY High Speed Micro Refrigerated Centrifuge MTX-150). All liquid was aspirated immediately after centrifugation and another 250 µl of 75% isopropanol was added to the sample. The mixture was allowed to precipitate for 10 minutes then placed in the high-speed refrigerated centrifuge (15°C) for 10 minutes at 13,000 rpm. The liquid was removed immediately by aspiration and the sample was left to dry by air (1 hour) or by placing in an Eppendorf Vacufuge (5 minutes). The samples were re-suspended in 5 µl of formamide EDTA Buffer (5 parts deionized formamide, 1 part 25 µM Na_2EDTA with 50 mg/ml blue dextran dye). Samples were snap-cooled in a thermal cycler by heating to 95°C for 2 minutes, followed by immediate cooling to 5°C. Sequencing samples were loaded onto a sequencing comb (1.2 µL) and run for 9 hours on a polyacrylamide gel using the ABIPrism 377™ Automated DNA Sequencer (Applied Biosystems, Inc.). The polyacrylamide gel was prepared by combining 9 g urea, 12.5 mL NANOpure water™, 2.5 mL 10X TBE Buffer, 3.0 mL PAGE-PLUS ™, and 125 µL 10% APS (Ammonium Persulfate) solution. The mixture was prepared in a beaker, placed on a heating block at a low heat and stirred until the urea was dissolved. Just prior to pouring the gel mixture, 12 µL of TEMED (Tetramethylethylenediamine) was added to

the beaker and gently stirred. The gel mixture was slowly poured between two glass sheets, carefully sliding one glass sheet over the other to allow for even distribution of the gel with no air pockets. The gel was allowed to polymerize for at least 1 hour, then the glass sheets containing the polyacrylamide gel were carefully cleaned, allowed to dry, and placed in the sequencer. A plate check was run on the sequencer to test for impurities in the gel. A 20% Ficoll solution was added to the comb area using a syringe and the sequencing comb containing the samples was loaded into the gel. The upper and lower buffer chambers were filled with 1X TBE Buffer and the sequencer was run for two minutes. The sequencing comb was removed and the comb area was flushed thoroughly with a syringe containing 1X TBE Buffer to remove the Ficoll. The sequencer was then run for 9 hours.

2.5 Analysis of Sequence Data for Newfoundland Population Data Set

Sequences were aligned and edited using Sequencher™ 4.1.2 (Gene Codes, Corp.) A consensus sequence for the Control Region and the cytochrome *b* gene was constructed for each sample using the forward and reverse strands. The consensus sequences were exported and used for all subsequent phylogenetic analysis. Samples that were missing sequence data were excluded from further analysis.

A phylogenetic tree was constructed in PAUP 4.0b (Swofford 2002) using a UPGMA (Unweighted Pair Group Method Using Arithmetic Averages) distance analysis. Haplotypes were determined by examining the UPGMA phylogram in PAUP 4.0b (Swofford 2002) and later confirmed using the program DnaSP Version 4.10.4 (Rozas et

al. 2005). Phylogenetic relationships of haplotypes were determined using Maximum Parsimony in PAUP 4.0b (Swofford, 2002) and by Bayesian inference using MR. BAYES 3.1.2 (Ronquist and Huelsenbeck 2003). In addition to the phylogenetic analysis, a minimum spanning network of haplotypes was constructed in the computer program TCS 1.21 (Clement et al. 2000) using the statistical parsimony approach of Templeton et al. (1992), which estimates the 95% plausible set for all haplotype linkages. The program TCS 1.21 (Clement et al. 2000) was also used to calculate haplotype outgroup probabilities (based on haplotype frequencies), which correspond to haplotype age, to determine the root of the minimum spanning network.

Estimates of genetic variation among herds, values of haplotypic diversity (Hd), average number of differences (K), and Nucleotide diversity (π) were calculated in DnaSP Version 4.10.4 (Rozas et al. 2005). Analysis of heterogeneity of haplotype distributions and haplogroup (clade) distributions using Monte Carlo simulations were completed in the program REAP 4.0 (McElroy et al. 1991) and DnaSp Version 4.10.4 (Rozas et al. 2005).

Population genetic structure was evaluated using Analysis of Molecular Variance (AMOVA) indices in the program Arlequin 3.01 (Excoffier et al. 2005). The AMOVA determined how genetic variation (haplotype diversity) was partitioned in the population at different hierarchical levels; the regional level (among defined geographic areas), population level (among identified herds within a particular geographic region), and individual level (within herds, amongst individuals with no regard to original herd identity or geographic region). The AMOVA estimated variance components and Φ-

statistics (analogous to F-statistics), which were correlated to haplotype diversity at different the hierarchical levels (Excoffier et al. 1992). Six scenarios of geographical structuring were tested where each individual was assigned to the herd they were sampled from then assigned to a broader geographical region.

The geographical regions for the AMOVA were defined by four different factors, including, (1) east-west differences in caribou herds, (2) north-south migration patterns, (3) geographical barriers to movement, and (4) previously defined regional herd groupings by Bergerud (1971). The distributions of genetic haplotypes across the island indicate there may be some factor that separates eastern herds from western herds between Pot Hill and Grey River. Scenarios 1, 2 and 5 tested different east-west separations (Figure 14). Scenario 1 includes five geographical groupings, which separate the interior herds from east to west between Pot Hill and Grey River. Scenario 1 also separates the Avalon Peninsula herds (Avalon and Cape Shore), herds in the north/west (Adies Lake and Hampden Downs) and herds in the north (Northern Peninsula and St. Anthony). Scenario 2 includes four geographical groupings, which also separates the interior herds east to west between Pot Hill and Grey River. Scenario 2 also separates the Avalon Peninsula herds, however, herds in the north/west and north are grouped together (Adies Lake, Hampden Downs, Northern Peninsula and St. Anthony). Scenario 5 includes two geographical groupings, which separates all herds in the east from all herds in the west between Pot Hill and Grey River. 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 specific dividing point

between central/interior herds and the northern peninsula herds is not clear, therefore, a number of north-west separations were tested in Scenarios 1 through 4 (Figure 14). There are two potential geographical barriers of movement for caribou on the island, these include: the narrow strip of land joining the Avalon Peninsula to the rest of the island, and the Main River on the Northern Peninsula (north end of the Adies Lake herd). The influence of these geographical barriers on mtDNA variance was tested in Scenarios 4 and 6 (Figure 14). Bergerud (1971) divided Newfoundland caribou into four discrete herds, defined as Interior, Humber River (Adies Lake herd), Avalon and Northern Peninsula, based on intensive studies on population dynamics. MtDNA variance among these herds is tested for in Scenario 3 (Figure 14).

A Nested Clade Analysis (NCA) of geographical distances was performed to test for geographical associations among haplotypes and to determine whether significant geographic associations were the result of population structure (restricted gene flow) or population history (fragmentation, colonization, range expansion) (Templeton 1998; Templeton et al.1995). A nested statistical design was applied to the minimum spanning network of haplotypes calculated in TCS 1.21 (Clement et al. 2000) according to the nesting rules described in Templeton et al. (1987), Templeton et al. (1995), and Templeton (1998). Decimal degree coordinates of the geographical centers of each Caribou Management Unit (herd) were used to calculate the geographic distances in the analysis. Merasheen Island was assigned the same geographic coordinates as Buchans since it is known that caribou occupying the island were recently introduced from Buchans. The NCA was carried out using the program GEODIS 2.4 (Posada et al. 2000)

and the results of the analysis were interpreted using the inference key (dated November 11, 2005) supplied with GEODIS 2.4 (Posada et al. 2000).

2.6 Analysis of Newfoundland caribou with North American caribou and Eurasian reindeer

Sequences for other North American caribou and Eurasian caribou were acquired from the GenBank database, (http://www.ncbi.nlm.nih.gov/Genbank/index.html), and directly from the manuscript by Cronin and Patton (2002). A 470 bp region of the mtDNA Control Region for 21 Eurasian reindeer (*R. t. fennicus*, *R. t. platyrhynhcus*, *R. t. tarandus*) and 42 North American caribou (*R. t. caribou*, *R. t. granti*, *R. t. groenlandicus*, and *R. t. pearyi*) was obtained from a study by Flagstad and Røed (2003; GenBank Accession No. AY178669 to AY178731). A 1194 bp sequence of the mitochondrial Cytochome *b* gene for 27 sequences of North American caribou (*R. t. caribou*, *R. t. granti*, and *R. t. groenlandicus*) was obtained from a study by Cronin et al. (2005; GenBank Accession No. AY726730 to AY726672). A 1194 bp sequence of the mitochondrial cytochrome *b* gene for 14 samples of Eurasian reindeer (*R. t. tarandus* and *R. t. platyrhynchus*) was obtained from a study by Cronin et al. 2006 (GenBank Accession No. DQ673122 to DQ673135). A 1212 bp sequence of the mitochondrial cytochrome *b* gene for 19 sequences of North American caribou (*R. t. caribou* and *R. t. groenlandicus*) was obtained directly from a manuscript by Cronin and Patton (2002).

Sequences were aligned with the consensus sequences of the 32 Newfoundland caribou haplotypes using Sequencher™ 4.1.2 (Gene Codes, Corp.). For the cytochrome *b*

sequences, some bases were removed on either end of the GenBank sequences to create an even data matrix. There were no substitutions in the removed regions. Five separate phylogenetic analyses were completed that compared; (1) Control Region sequences for Newfoundland and North American caribou (470 bp), (2) Control Region sequences for Newfoundland caribou and Eurasian reindeer (470 bp), (3) cytochrome *b* sequences for Newfoundland and North American caribou (1143 bp), (4) cytochrome *b* sequences for Newfoundland and woodland caribou (1143 bp), and (5) cytochrome *b* sequences for Newfoundland caribou and Eurasian reindeer (1111bp). A Neighbor Joining distance analysis was completed in PAUP 4.0b10 (Swofford 2001) to construct a phylogenetic tree for each of the data comparisons. Confidence of the Neighbor Joining Tree was estimated using the Bootstrap Method in PAUP 4.0b10 (Swofford 2001) and a 50% majority rule consensus tree was constructed (number of repetitions = 2000, confidence level = 50%, random tie breaks, distance = uncorrected "p"). Phylogenetic trees were rooted with a sequence for moose (*Alces alces*) obtained from GenBank (Control Region - Accession No. AF01695, Polziehn and Strobeck 1998 and Cytochrome *b* - Accession No. AJ000026, Randi et al. 1998).

2.7 Amplification and Sequencing of the Whole MtDNA Genome

The whole mitochondrial genome was sequenced for one male Newfoundland caribou aged 5-6 years from the Middle Ridge herd (Sample No. C-066, fresh muscle tissue). The mtDNA genome was amplified in two fragments of 10 Kbp and 7 Kbp in size (Table 3, Figure 5) with the Triple Master PCR System, High Fidelity PCR protocol

(Eppendorf). Fragment 1 was amplified with the Control Region primer Rtapro (designed from earlier experiments in this study) and the primer Rtacox1R1 (5'-TGA TGT AAA ATA GGC TCG TGT GTC AAC GTC-3'), which was designed from caribou sequence data in the cytochrome oxidase I gene (provided by S.M. Carr). Initial experiments also amplified Fragment 1 with Rtacox1R2 (5'-GCC TGA AAA TAG TGG AAA TCA GTG AAC AAA TCC-3') as the reverse primer; however, it did not prove to be as successful. Fragment 2 was amplified with the Control Region primer Rtaphe (designed from earlier experiments in this study) and the primer Rtacox1F1 (5'-CTA CTA CTC GGG AAA AAA AGA ACC ATT TGG-3'), which was designed from caribou sequence data in the cytochrome oxidase I gene (provided by S.M. Carr).

High Fidelity PCR amplifications were prepared as two Master Mixes of 10µl each and kept on ice. Master Mix 1 contained 8.4 µl of ddH_20, 0.3 µl of a 10 µM stock solution of each primer (Operon Technology, Inc.), and 1.0 µl of template DNA. Master Mix 2 contained 7.4 µl of ddH_20, 2.0 µl of 10x High Fidelity Buffer with Mg^{2+} (Eppendorf), 0.4 µl of 40 µM dNTP mix (containing 10 mM each of dATP, dCTP, dGTP, and dTTP), and 0.2 µl of Triple Master Polymerase Mix (Eppendorf). The two Master Mixes were combined just before thermal cycling.

The High Fidelity PCR was carried out on an Eppendorf Mastercycler or Eppendorf Mastercycler gradient thermal cycler (Eppendorf North America, Inc., Westbury, NY). The thermal cycler was first pre-heated to 94°C. Reactions started with an initial template denaturation step at 94°C for 2 minutes. The amplification cycle consisted of template denaturation at 94°C for 20 seconds, primer annealing at 50°C for

15 seconds, and extension/elongation at 68°C for 8 minutes. This cycle was repeated 35 times and samples were held at 5°C until removal.

PCR products were viewed on a 1% Agarose gel containing ethidium bromide. The gel was loaded with 4µl of the PCR product, which was combined with 2 µl of the running dye, 5X Stop Buffer (bromophenol blue in glycerol). In order to estimate the quality and size of the large PCR fragments, 2.5 µl of supercoiled DNA Ladder (Invitrogen), which measures fragments of 2 to 16 Kbp, was loaded into each gel. The gel was electrophoresed at 60 Volts for 6 hours. Successful amplifications were purified using Spin Columns from the QIAquick PCR Purification Kit (Qiagen Inc.) as per the manufacturer's protocol. The purified DNA fragments were eluted in 30 µl of ddH_20 and stored at minus 20°C.

PCR products were prepared for sequencing following the same procedure outlined in Section 2.4 (DNA Sequencing). Each of the large fragments was initially sequenced using the primers from the high fidelity PCR amplification. The sequences were read as far as possible from each end of the fragment, then new sequencing primers were designed to sequence the next segment in the mtDNA fragment. The method followed a "leap frog" strategy. Forward primers were designed at the 3' end of the forward sequences and reverse primers were designed at the 5' end of the reverse sequences. After each new segment was sequenced, another set of primers was designed and the next segment was sequenced. The cycle continued until the entire fragment was sequenced (Tables 4 and 5 show the primers that were designed to sequence the two PCR fragments). The sequences were aligned and edited using the computer program

Sequencher™ 4.1.2 (Gene Codes, Corp.). The mtDNA sequence of *Cervus nippon yesoensis* (Hokkaido Sika Deer) was obtained from GenBank (Accession No. NC_006973 Wada et al. 2007) and used as a reference for aligning the sequences. The mtDNA sequence of *Rangifer tarandus tarandus* (reindeer) was published in GenBank (Accession No. NC_007703 Wada et al. 2006) by the end of the experiments and was used as a reference in later alignments. DnaSP Version 4.10.4 (Rozas et al. 2005) was used to identify single nucleotide polymorphisms (SNPs) in the Newfoundland caribou and reindeer mtDNA genomes.

Table 1. Samples of caribou collected for genetic analysis. The actual number of samples used in the final genetic analysis is given in parenthesis. See Figure 4 for distribution of Caribou Management Units (herds) across Newfoundland.

Caribou Management Unit	Number of Samples	Male	Female	Unknown
61 - Lapoile	20 (18)	19 (17)	1 (1)	0 (0)
62 - Buchans	20 (17)	10 (8)	5 (5)	5 (4)
63 - Grey River	20 (19)	13 (13)	4 (3)	3 (3)
64 - Middle Ridge	20 (18)	8 (7)	7 (6)	5 (5)
65 - Avalon Peninsula	20 (18)	4 (3)	13 (13)	3 (2)
66 - Gaff Topsails	20 (20)	15 (15)	3 (3)	2 (2)
67 - Pot Hill	20 (18)	12 (10)	5 (5)	3 (3)
68 - Mount Peyton	20 (14)	13 (11)	6 (3)	1 (0)
69 - Northern Peninsula	20 (18)	14 (12)	6 (6)	0 (0)
70 - Merasheen Island	20 (20)	5 (5)	2 (2)	13 (13)
71 - Grey Islands	0	0	0	0
72 - Fogo Island	0	0	0	0
73 - Burin Knee	0	0	0	0
74 - Burin Foot	0	0	0	0
75 - Blow Me Down Mountains	0	0	0	0
76 - St. Anthony	20 (17)	16 (14)	3 (3)	1 (0)
77 - Cape Shore	20 (12)	7 (5)	4 (4)	9 (3)
78 - Hampden Downs	7 (6)	7 (6)	0	0
79 - Adies Lake	20 (18)	14 (13)	5 (4)	1 (1)
Total	**267 (233)**	**157 (139)**	**64 (58)**	**46 (36)**

Table 2. Primers used to amplify the cytochrome *b* gene and the Control Region for Newfoundland caribou samples. Forward (F) and Reverse (R) primers are indicated. Best results were achieved with the L14724/Cytb811R primer pair (cytochrome *b*) and Rta-L15926/Rta-H00651 primer pair (Control Region).

Region and Primer Name	Primer Sequence 5' to 3'	Reference
Cytochrome *b* (401 bp)		
L14724 (F)	CGAAGCTTGATATGAAAAACCATCGTTG (28-mer)	Irwin et al. 1991
H15149 (R)	AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA (33-mer)	Kocher et al. 1989
Cytochrome *b* (1170 bp)		
L14724 (F)	CGAAGCTTGATATGAAAAACCATCGTTG (28-mer)	Irwin et al. 1991
Cytb811R (R)	CTCCATTTTTGGTTTACAAGAC (22-mer)	Marshall (pers. com.) [1]
Control Region (1063 bp)		
L15926 (F)	TCAAAGCTTACACCAGTCTTGTAAACC (27-mer)	Kocher et al. 1989
H00651 (R)	TAACTGCAGAAGGCTAGGACCAAACCT (27-mer)	Kocher et al. 1989
Rta-L15926 (F)	GTAGTACACTTAATACACTGGTCTTGTAAACC (32-mer)	designed from Kocher et al.
Rta-H00651 (R)	GGGTTAATAGGAAGGCTGGGACCAAACCTGTG (32-mer)	designed from Kocher et al.
Control Region (980 bp)		
Pro-5 (F)	CTACCTCCAACTCCCAAAGC (20-mer)	Palumbi 1996
Phe-3 (R)	TCTTCTAGGCATTTTCAGTC (20-mer)	Palumbi 1996
RtaPro (F)	AGCTATAGCCCCACTATCAACACCCAAAGC (30-mer)	designed from Palumbi 1996
RtaPhe (R)	TGGAGTTAATATACTCATCTAGGCATTTTCAGTGC (35-mer)	designed from Palumbi 1996

[1] primer cytb811R was designed by H.D. Marshall to amplify the cytochrome *b* region in seals.

Table 3. Primers used to amplify Fragments 1 and 2 of caribou mtDNA genome using High Fidelity PCR. Forward (F) and Reverse (R) primers are indicated.

Fragment and Primer Name	Primer Sequence 5' to 3'
Fragment 1 (~ 7 Kbp)	
Rtapro (F) [1]	AGCTATAGCCCCACTATCAACACCCAAAGC (30-mer)
Rtacox1R1 (R) [2]	TGATGTAAAATAGGCTCGTGTGTCAACGTC (30-mer)
Rtacox1R2 (R)	GCCTGAAAATAGTGGAAATCAGTGAACAAATCC (33-mer)
Fragment 2 (~ 10 Kbp)	
Rtacox1F1 (F)	CTACTACTCGGGAAAAAAAGAACCATTTGG (30-mer)
Rtaphe (R) [1]	TGGAGTTAATATACTCATCTAGGCATTTTCAGTGC (35-mer)

[1] designed from Palumbi 1996
[2] primer Rtacox1R1 was more successful than Rtacox1R2 in PCR amplifications

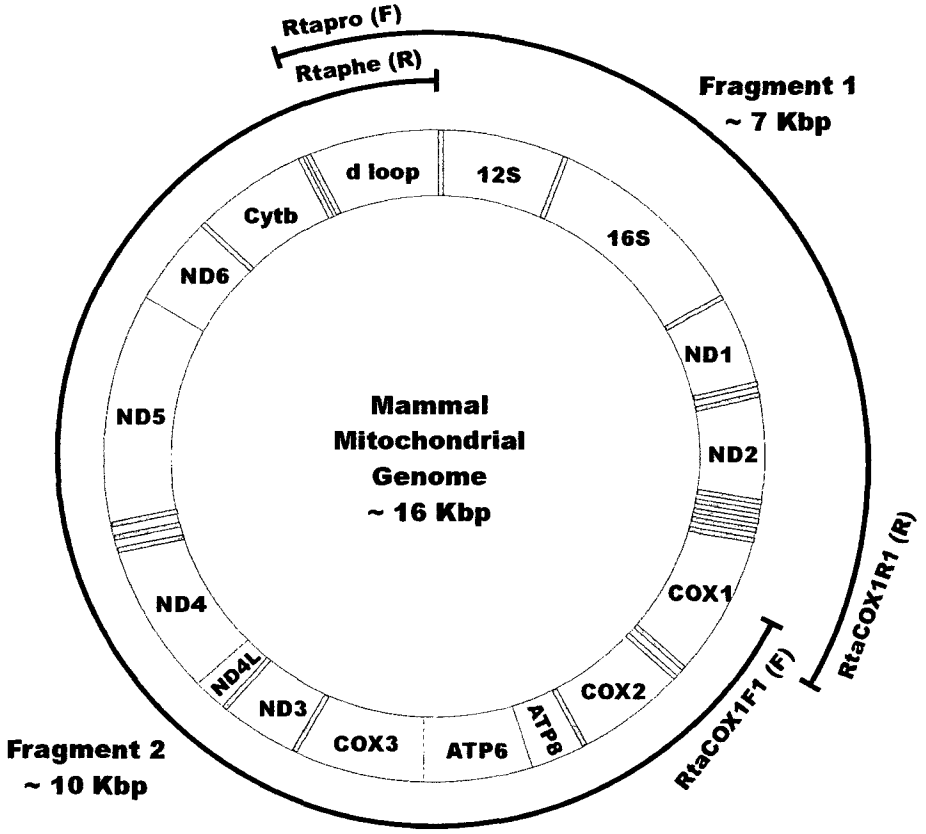


Figure 5. Diagram of circular mammal mitochondrial genome indicating the two fragments that were amplified by high fidelity PCR. Fragment 1 was approximately 7 Kbp in size and was amplified using the primer pair Rtapro (forward) and Rtacox1R1 (reverse). Fragment 2 was approximately 10 Kbp in size and was amplified using the primer pair Rtacox1F1 (forward) and Rtaphe (reverse).

Table 4. Primers used to sequence Fragment 1 of the caribou mtDNA genome. Forward (F) and Reverse (R) primers are indicated. PCR amplification primers are indicated in bold.

Primer Name	Primer Sequence 5' to 3'
Rtapro (F)	AGCTATAGCCCCACTATCAACACCCAAAGC (30-mer)
Rtaphe (R)	TGGAGTTAATATACTCATCTAGGCATTTTCAGTGC (35-mer)
RproF1 (F)	ACATTTTCAATACTCAAATAGCACTCCAGG (30-mer)
RProF2 (F)	CTCCAGGATAAGGTAAGTATATAAGCGCC (29-mer)
R12SF1 (F)	CCCAAGTTAATAGGCCTACGGCGTAAAGCG (30-mer)
R12SF2 (F)	CTATTCGCCAGAGTACTACCGGCAATAGC (29-mer)
R12SF3 (F)	CTATTCGCCAGAGTACTACCGGCAATAGC (29-mer)
27' (F) [2]	TATACCGCCATCTTCAGCAAAC (22-mer)
R16SF1 (F)	CATGGCGCTATAGAGAAAGTACCG (24-mer)
R16SF2 (F)	CACAAACATAATCCGCCCTAGAAAAGGGC (29-mer)
R16SF3 (F)	GCCTGGTGATAGCTGGTTGTCCAGGAAATG (30-mer)
R16SF4 (F)	CTCGGCAAACATTAAACCCCGCCTGTTTACC (31-mer)
R16SF5 (F)	AGCTTTGGTTGGGGTGACCTCGGAGAACAG (30-mer)
R16SF6 (F) [1]	CAGACCGGAGTAATCCAGGTCGGTTTCTATC (31-mer)
S1 (R) [2]	TGGCAGATCTCGGTAATTGCATAA (24-mer)
RND1F1 (F)	CGAGCCCCATTTGATCTCACTGAAGGTGAATC (32-mer)
RND1F2 (F)	TTCTTTTGAATCCGAGCATCATATCCTCGA (30-mer)
RND2R3 (R)	TGGTGTAATTGGGAGCACGAAGAGTTTTGG (30-mer)
RND2R2 (R)	GATAAAAGGGTAACGAGGATTAGAACAGC (29-mer)
RND2R1 (R)	GTATAAATTTAGTAGGGCTGTAATTGC (27-mer)
Rco1R6 (R)	CAGCGGTTAATGAACATAGGTAAAATGGC (29-mer)
Rco1R5 (R)	CTGGGGCACCAATTATCAGAGGGACAAG (28-mer)
Rco1R4 (R)	GGGATCCCCACCTCCTGCTGGGTCGAAGAAAG (32-mer)
Rco1R3 (R)	CAGGGTGTCCAAAGAATCAGAATAAGTG (28-mer)
Rtacox1R2 (R)	GCCTGAAAATAGTGGAAATCAGTGAACAAATCC (33-mer)
Rtacox1R1 (R)	TGATGTAAAATAGGCTCGTGTGTCAACGTC (30-mer)

[1] No sequence was produced from this primer

[2] primers for 16S rDNA from Hassanin and Douzery 2003

Table 5. Primers used to sequence Fragment 2 of the caribou mtDNA genome. Forward (F) and Reverse (R) primers are indicated. PCR amplification primers are indicated in bold.

Primer Name	Primer Sequence 5' to 3'
Rtacox1F1 (F)	CTACTACTCGGGAAAAAAAGAACCATTTGG (30-mer)
Rtacox1F2 (F)	GGGCTTTATTTTCCTTTTTACAGTTGGAGG (30-mer)
Rco1F3 (F)	GTTACAGGTGAAAACCCCGTACACCTCATATGGC (34-mer)
Rco1F4 (F)	CAACTAGGCTTCCAAGATGCAACATCACC (29-mer)
Rco2F2 (F)	GCTCCGAAATCTGCGGATCAAACCATAGC (29-mer)
Rco2F3 (F)	CAATACTCTCCTTAATGGTATGCCGCACC (29-mer)
Rco2F4 (F)	CCCAACTATCAATAAACTTAGGTATAGCC (29-mer)
Rco2F5 (F)	CTACTCACAAGTCCTTGGAATTCGCAGTAGCC (32-mer)
Rco3F1 (F)	TGTCTTACTGGCCTCAGGAGTCTCTATCAC (30-mer)
Rco3F2 (F)	TTGGCTTTGAAGCTGCCGCCTGATACTGAC (30-mer)
RND3F1 (F)	GCACTCCTCTTACCACTGCCATGAGCCTGTCA (32-mer)
RND3F2 (F)	ATTACTAGCTGCAAGTCTAGCTTATGAATG (30-mer)
RND4F1 (F)	CCTAATGTCCTCACTCTTATGCCTAGAAGGAA (32-mer)
RND4F2 (F)	CGACTCATTAGACTGTGATTAAACTCACAAC (31-mer)
RND4F3 (F)	TATCAAATACATACGGCACTGACTATGTTC (30-mer)
RND4F4 (F)	TCTGATTCTCTATCAACACCACTACTAATC (30-mer)
RND4F5 (F)	TTTAATTTGAAGCAACACTAGTCCCAACTC (30-mer)
RND4R3 (R)	ACTTCCAGGTGTCTGAATAAGAATAGCTAC (30-mer)
RND4R2 (R) [1]	CTACAAATAGTTCTCCAATTAGGTTAATTG (30-mer)
RND4R1 (R)	CGGCAAATAAGGAGATACAAGCTCCTATAATCAG (34-mer)
RND5R6 (R)	TAGGTTTTGTAGTTATCGGAGCTCGTGGTT (30-mer)
RND5R7 (R) [1]	CGAAAAAGCCATGTTGTTATACATGGGAGCATAGA (35-mer)
RND5R5 (R)	GCAGCTGTGTTTGCATCTGCTCGCCCATATC (31-mer)
RND5R4 (R)	CTGTTAGTGGGTGGAAGCGAATTAGTAGG (29-mer)
RND5R3 (R)	GGCAATTAATGTTATTAAGAGGGCTCAGGCGTTGG (35-mer)
RND5R2 (R)	GGAGTTAGGCGGTGTATAATTGTGGGAAAATATCC (35-mer)
RND5R1 (R)	CTGGGTGATCTTTGTTAATTGGTGTAGTAG (30-mer)
RND6R2 (R)	GGTTATACAACGGCTATGGCTACAGAACAGTATCC (35-mer)
RND6R1 (R)	CTTCAAAACCTTCGCCTATTTATGGGGGATTAGG (34-mer)

RcytbR2 (R)	GTTTGATGGGGCTGGGAGGTCAATAAATGCG (31-mer)
RcytbR1 (R)	CCTAGTAGAGAGCCAAAATTTCATCATG (28-mer)
L14724 (F) [2]	CGAAGCTTGATATGAAAAACCATCGTTG (28-mer)
cytb811R (R)	CTCCATTTTTGGTTTACAAGAC (22-mer)
RpheR2 (R)	TTTGTGGAGCTATATTAATATACGCCAGGG (30-mer)
RpheR1 (R)	GGATATAATATGGCTATTGAGTGCAGAAC (29-mer)
RcytbF1 (F)	CACTCACATGAATTGGAGGACAACCAGTTG (30-mer)
RcytbF2 (F)	GCACAATCGAAAATAATCTCCTAAAATGAGG (31-mer)
Rtaphe (R)	TGGAGTTAATATACTCATCTAGGCATTTTCAGTGC (35-mer)

[1] No sequence was produced from this primer

[2] Irwin et al. 1991

3.0 Results

3.1 DNA Sequences of Sampled Caribou

A total of 2223 base pairs of mitochondrial DNA were sequenced for 233 individual caribou. Sequences comprised the complete Control Region including portions of flanking tRNA-Thr, tRNA-Pro and tRNA-Phe genes (1063 bp), and the complete cytochrome *b* gene including a portion of the tRNa-Thr gene (1160 bp). Partial sequence data was obtained for an additional 18 individuals; however, since the data set was not complete, these individuals were removed from the analysis. No sequence data was obtained for 16 individuals, likely due to tissue degradation. This information is summarized in Table 6.

A total of 32 variable sites were found in the 2223 bp sequence data set, all of which were transitions. There were 21 substitutions between pyrimidines (C and T) and 11 substitutions between purines (A and G). The Control Region contained 18 variable sites (Table 7), 16 of which were parsimony informative (give information about relationships) and two of which were uninformative (do not provide information about relationships). The Control Region had five homoplastic sites (parallel or convergent mutations), which are indicated in Table 7. The Control Region is non-coding so there were no amino acid substitutions caused by the variable sites. The Control Region comprised 11 substitutions between pyrimidines and seven substitutions between purines. The Control Region also contained a run of four to five T's followed by a single base insertion/deletion (indel) and then a run of six to eight C's. This area of 9 to 14 bases

was extremely variable even within the same individual and reliable base calling was very difficult. As a result, three bases at positions 852, 853 and 854 in the Control Region were removed from the sequence data and left out of further analysis.

The cytochrome *b* gene contained 14 variable sites (Table 8, Table 9), eight of which were parsimony informative and six of which were uninformative. The cytochrome *b* gene had two homoplastic sites (parallel or convergent mutations), which are indicated in Table 8. There were ten substitutions between pyrimidines and four substitutions between purines. Nine variable sites were at the third codon position and did not result in amino acid substitutions (synonymous substitutions). Five variable sites resulted in amino acid substitutions (nonsynonymous substitutions), three were at the first codon position and two were at the second codon position. Interestingly, all of the amino acid substitutions involved the amino acid threonine, which may provide an opportunity for further research.

3.2 Phylogenetic Relationships of Newfoundland Caribou mtDNA Haplotypes

The 32 variable positions in the DNA sequence data define 32 mtDNA haplotypes. The full 2223 bp sequence for each haplotype can be seen in Appendix A. The UPGMA phylogram of all 233 samples of Newfoundland caribou and their associated haplotypes can be seen in Appendix B. The numbers of males versus females in each of the haplotypes is given in Table 10.

Phylogenetic analyses by Maximum Parsimony (Figure 6) and Bayesian inference (Figure 7) indicate the 32 mtDNA haplotypes are structured into four well supported

mtDNA clades or mtDNA haplogroups designated as **A**, **B**, **C**, and **D**. Clade **A** is the least abundant and includes three individuals with three haplotypes (**Aa**, **Ab** and **Ac**). Clade **B** includes 49 individuals that comprise eight haplotypes (**Ba** through **Bh**). All haplotypes in clade **B** are separated by one mutational change from the most common haplotype, **Ba**, which includes 32 individuals. Clade **C** is abundant but is the least diverse clade, including 60 individuals with only two haplotypes (**Ca** and **Cb**). Haplotype **Cb** is separated by one mutational change from the more common haplotype, **Ca**, which includes 51 individuals. Clade **D** is the most abundant and diverse clade, and includes 121 individuals comprising 19 haplotypes (**Da** through **Ds**).

Clade **D** has a number of common haplotypes of which **Da** is the most common with 33 individuals, followed by **Db** (22 individuals), **Dc** (17 individuals) and **Dd** (12 individuals). The 19 haplotypes within clade **D** are highly structured with only a few strongly supported groupings (Maximum Parsimony (MP) > 50%, Bootstrap Value (BV) > 0.5) and many unsupported groupings (MP<50%, BV<0.5). Two groups of haplotypes are strongly supported, these include: **Dg** with **Dq** (MP=100%, BV=0.82), and **Dj** with **Dp** (MP=100%, BV=0.82). Three groups of haplotypes were moderately to weakly supported, these include: **Dk** and **Dn** with **Da** (MP=73%, BV=0.57); **Dr** and **Ds** with **Dc** (MP=69%, BV=0.54); and the grouping of **De**, **Dh**, **Di** and **Dm** (MP=56%, BV=0.62), the configuration of which varies between the Maximum Parsimony (Figure 6) and Bayesian phylogenies (Figure 7). Haplotype **Do** is grouped with haplotypes **Dc**, **Dr**, and **Ds** with weak support in the Maximum Parsimony phylogeny (MP=59%); however, in the Bayesian phylogeny it remains ungrouped. The haplotypes **Db**, **Dd**, **Df**, and **Dl** are

ungrouped within the clade in the Maximum Parsimony phylogeny, however, in the Bayesian phylogeny haplotypes **Db** and **Dd** are identified as the ancestral haplotypes (shorter branch lengths).

The Minimum Spanning Network (MSN) also identifies 32 mtDNA haplotypes structured in four mtDNA clades (Figure 8). The network roots from haplotype **Dd**, which has the highest outgroup probability (p=0.16, Table 11). Clade **D** is made up of a network of many cross-linked haplotypes that connect within clade **D** as well as to clades **B** and **C**. There are seven homoplastic sites (convergent or parallel mutations) in the sequence data set (five in the Control Region, two in the cytochrome *b* gene) that complicate the minimum spanning network, particularly with respect to clade **D** (see Tables 7 and 8). The more likely connections (the black lines in Figure 8) were determined by an examination of outgroup weights from the MSN (Table 11) and the grouping of haplotypes in the phylogenetic trees produced from Maximum Parsimony and Bayesian analysis. The other three clades are more clearly defined in the MSN. Clade **A** is distinct and distant from other clades by at least nine mutational changes. Clade **B**, which is distinct from the other clades by at least five mutational changes, shows the common haplotype **Ba** as the central haplotype with the seven other haplotypes (**Bb** through **Bh**) as descendants. Clade **C**, which is distinct from other clades by at least six mutational changes, shows haplotype **Ca** as the common haplotype with haplotype **Cb** as descendant.

3.3 Distribution of mtDNA Clades Among Newfoundland Caribou Herds

The Monte Carlo χ^2 test indicates that the distribution of mtDNA clades among herds is heterogeneous (χ^2 = 113.12, p=0.0000±0.0000). Table 12 and Figure 9 show the distribution of clades among herds. Clade **A** (n=3), the smallest clade, appears only in western herds (St. Anthony, Hampden Downs and Buchans). Clade **B** (n=49), is widespread and abundant, appearing in all herds throughout the main island of Newfoundland and Merasheen Island but remaining absent from the Avalon Peninsula (Avalon and Cape Shore herds). Clade **C** (n=60), is also widespread and abundant, however, it is more prevalent in western and central herds. The frequency of clade **C** declines in herds further east, (Pot Hill and Mount Peyton) and it is absent altogether from the Middle Ridge herd. Clade **C** is the dominant clade in the Merasheen Island herd, which is a herd that was introduced from Buchans. Clade **D** (n=121) is the most widespread and abundant clade and it occurs in all herds across the island. Clade **D** is also the only clade that occurs in herds on the Avalon Peninsula (Avalon and Cape Shore herds).

3.4 Distribution of mtDNA Haplotypes Among Newfoundland Caribou Herds

Haplotype distributions vary among herds (Table 13, Table 14). The Monte Carlo χ^2 test indicates that the distribution of haplotypes is heterogeneous (χ^2 = 762.10, p=0.0000±0.0000). The haplotypes are widespread across the island with many caribou herds represented in each haplotype (Figure 10). Two notable exceptions are haplotype

Db (n=22), which is found only in the Avalon and Cape Shore herds (Figure 11) and haplotype **De** (n=7), which is found only in the Avalon herd (Figure 12).

There are six common haplotypes present in the population, these are considered as those that occurred in greater than ten individuals in the population sample (Figure 11). The most common haplotype, **Ca** (n=51), is most frequent in herds located in the central, west and southwest parts of Newfoundland. Haplotype **Ca** is frequent among adjacent herds located at Lapoile, Buchans, Grey River, Adies Lake and Gaff Topsails. Haplotype **Ca** is also frequent in St. Anthony although less frequent in Northern Peninsula, which is located just to the south. The highest frequency of haplotype **Ca** occurs at Merasheen Island, which is a population that was introduced from the Buchans Plateau in the 1970's. Haplotype **Ca** appears to be less frequent in eastern Newfoundland and is not detected in Middle Ridge, Avalon and Cape Shore. The second most common haplotype, **Da** (n=33), appears to be dispersed throughout most of the island, occurring in high frequencies in Northern Peninsula, Adies Lake, Gaff Topsails and Middle Ridge and at lower frequencies in St. Anthony, Hampden Downs, Buchans, Lapoile, Grey River, and Pot Hill. Haplotype **Da** is not detected on Merasheen Island, Mt. Peyton, Avalon and Cape Shore. Another common haplotype, **Ba** (n=32), also appears to be dispersed throughout most of the island, occurring at highest frequencies in the Northern Peninsula, Adies Lake and Gaff Topsails and at lower frequencies in St. Anthony, Hampden Downs, Buchans, Lapoile, Pot Hill, Mt. Peyton, and Middle Ridge. Haplotype **Ba** is not detected in Grey River, Merasheen Island, Avalon or Cape Shore. Haplotype **Db** (n=22) is also common, and appears to be restricted to the Avalon and Cape Shore herds. Haplotype **Dc**

(n=17) occurs at low frequency among adjacent herds in central Newfoundland, including, Gaff Topsails, Buchans, Merasheen Is., Lapoile, Grey River, Pot Hill, Mt. Peyton and Middle Ridge. Haplotype **Dc** also occurs at low frequency in St. Anthony but is not detected in Northern Peninsula, Adies Lake, Hampden Downs, Avalon or Cape Shore. Haplotype **Dd** (n=12) is more frequent in west-central herds, including, St. Anthony, Hampden Downs, Gaff Topsails, Buchans, Lapoile, and Pot Hill. Haplotype **Dd** is not detected in Northern Peninsula, Adies Lake, Merasheen Island, Grey River, Mt. Peyton, Middle Ridge, Avalon and Cape Shore.

There are 11 less common haplotypes present in the population; these include haplotypes that occur in less than 10 individuals, but more than one individual (Figure 12). Haplotype **Cb** (n=9) occurs in some eastern herds, Northern Peninsula, Adies Lake, Gaff Topsails, and Merasheen Island. Haplotype **De** (n=7) occurs only in the Avalon herd. Haplotype **Df** (n=7) occurs in the adjacent central herds Lapoile, Grey River, Pot Hill and Mt. Peyton. Haplotype **Dg** (n=6) occurs in the adjacent central herds Grey River, Pot Hill and Mt. Peyton. Haplotype **Bb** (n=6) is widespread, occurring in St. Anthony, Lapoile, Merasheen Island, Pot Hill and Mt. Peyton. Haplotype **Bc** (n=4) occurs in Mt. Peyton and Middle Ridge. Haplotype **Bd** (n=3) occurs in adjacent central herds Lapoile, Grey River and Pot Hill. Haplotype **Dh** (n=3) occurs in Middle Ridge and Avalon. Haplotype **Di** (n=2) occurs in adjacent herds Mt. Peyton and Middle Ridge. Haplotype **Dj** (n=2) occurs in central herds Lapoile and Mt. Peyton. Haplotype **Dk** (n=2) occurs in adjacent herds Pot Hill and Middle Ridge.

There are 15 rare haplotypes present in the population; these include haplotypes that occur in only one individual in the sample (Figure 13). The most notable of these rare haplotypes are **Aa**, **Ab** and **Ac**. These three haplotypes are distinct and genetically distant from all other haplotypes found on the island. These haplotypes are present in northwestern Newfoundland; haplotype **Aa** occurs in Buchans, haplotype **Ab** occurs in St. Anthony and haplotype **Ac** occurs in Hampden Downs. The remaining rare haplotypes are spread throughout central Newfoundland. There are no rare haplotypes detected in Adies Lake, Gaff Topsails, Merasheen Island or the Avalon Peninsula.

From the patterns of distribution of haplotypes among herds one clear association was observed between haplotype and herd. As mentioned earlier, haplotypes **Db** and **De** are only found in herds occupying the Avalon Peninsula (Avalon and Cape Shore). It is difficult to identify specific associations of haplotypes to other herds, however, there does appear to be some regional associations. These are assessed further with the hierarchical analysis of the AMOVA and the Nested Clade Analysis.

3.5 Haplotype Diversity Within Newfoundland Caribou Herds

Within the overall population (n=233), the haplotype diversity (Hd) is 0.89, the average number of differences (K) is 4.25 and the nucleotide diversity (π) is 0.0022 (Table 15). The lowest haplotype diversity, average number of differences, and nucleotide diversity is found in the Cape Shore herd, which has only one haplotype (Hd=0.00, K=0.00, π =0.00) among 12 individuals sampled. The next least variable populations are the Merasheen Island herd (Hd=0.56, K=2.25, π =0.0012), which has four

haplotypes among 20 individuals sampled, and the Avalon Peninsula herd (Hd=0.57, K=1.03, π =0.00052), which has three haplotypes among 18 individuals sampled. The Northern Peninsula herd has a lower than average haplotype diversity (Hd=0.67, K=4.12, π =0.0021), with five haplotypes among 18 sampled individuals. The highest haplotype diversity is found in the Pot Hill (Hd=0.95, K=4.87, π =0.0025) and Mt. Peyton herds (Hd=0.95, K=4.90, π =0.0025), which have 13 haplotypes among 18 individuals sampled and ten haplotypes among 14 individuals sampled, respectively. The highest nucleotide diversity and the next highest haplotype diversity (Hd=0.93, K=6.73, π =0.0034) are found in the Hampden Downs herd, which has five haplotypes and 15 segregating sites among six individuals sampled.

3.6 Analysis of Molecular Variance

For the Analysis of Molecular Variance (AMOVA), six scenarios of regional geographical structuring were tested (Figure 14). The geographical regions were defined by four different factors, including, (1) east-west differences in caribou herds, (2) north-south migration patterns, (3) geographical barriers to movement, and (4) previously defined regional herd groupings by Bergerud (1971).

In all six scenarios of geographical structuring, genetic variance in Newfoundland caribou is found mostly at the individual level (77.09% to 86.52%) and the amount of variance at the population (herd) level is small (2.60% to 7.37%). The amount of variance at the regional level (8.23% to 16.64%) is small, except when the Avalon Region is distinguished from the rest of the island as a whole (Scenario 6). Figure 14

outlines the geographical regions defined in each scenario. Table 16 presents the results of the hierarchical AMOVA for each scenario.

In Scenario 1, five geographical regions are defined (Figure 14); Avalon in pink (Avalon Peninsula and Cape Shore), Eastern Newfoundland in green (Middle Ridge, Mt. Peyton, Pot Hill), West/Central Newfoundland in yellow (Merasheen Island, Lapoile, Buchans, Gaff Topsails, Grey River), North/West Newfoundland in purple (Adies Lake, Hampden Downs), and Northern Newfoundland in blue (Northern Peninsula, St. Anthony). The "within herd" variance component is large (86.52%, Φ_{ST}=0.13478), compared with the lower "among regions" component (9.58%, Φ_{CT}=0.09583) and the "among herds/regions" component (3.90%, Φ_{SC}=0.04308) (Table 16). The results are significant for all three variance components ($p<0.05$).

In Scenario 2, four geographical regions are defined (Figure 14). The groupings for Avalon (pink), Eastern Newfoundland (green) , and West/Central Newfoundland (yellow) remain the same as Scenario 1, however, the North/West Newfoundland (Adies Lake, Hampden Downs), and Northern Newfoundland (Northern Peninsula, St. Anthony) regions are combined into one group shown in blue (North/West and Northern Newfoundland). The "within herd" variance component is still large but slightly lower (86.02%, Φ_{ST}=0.13977) than in the first scenario, the "among regions" component is slightly higher (10.58%, Φ_{CT}=0.10576) and the "among herds/regions" component is slightly lower (3.40%, Φ_{SC}=0.03803) (Table 16). The results are significant for all three variance components ($p<0.05$).

In Scenario 3, four geographical regions are defined (Figure 14) that roughly correspond to the regional populations described by Bergerud (1971). The groupings include the Avalon herds in pink (Avalon and Cape Shore), the Interior herds in yellow (Middle Ridge, Mount Peyton, Pot Hill, Merasheen Island, Lapoile, Buchans, Grey River, Gaff Topsails, Hampden Downs and St Anthony), the Humber herd in purple (Adies Lake) and the Northern Peninsula herd in blue (Northern Peninsula). Since the time of Bergerud's study, caribou from the Avalon were introduced to the Cape Shore area, and caribou from the Interior were introduced to Merasheen Island and St. Anthony, therefore the introduced herds were included with their source groupings. Bergerud (1971) suggested that the Humber herd and the Northern Peninsula herd were separated by the Main River and did not interact. For these groupings, the "within herd" variance component is still large but slightly lower (84.23%, Φ_{ST}=0.15774) than in the first and second scenarios, the "among regions" component is slightly lower (8.89%, Φ_{CT}=0..08891), and the "among herds/regions" component is slightly higher (6.88%, Φ_{SC}=0.07554) (Table 16). The results are significant for all three variance components ($p<0.05$).

In Scenario 4, three broad geographical areas are defined (Figure 14), Avalon in pink (Avalon Peninsula and Cape Shore), Central in green (Middle Ridge, Mt. Peyton, Pot Hill, Merasheen Island, Lapoile, Buchans, Grey River, Gaff Topsails, Adies Lake, Hampden Downs), and Northern in blue (Northern Peninsula and St. Anthony). The "within herd" variance component is still large but slightly lower (84.40%, Φ_{ST}=0.15599) than in the first and second and third scenarios, the "among regions" component is lower

(8.23%, Φ_{CT}=0.08230) and the "among herds/regions" component is much higher (7.37%, Φ_{SC}=0.08029) (Table 16). The results are significant for all three variance components ($p<0.05$).

In Scenario 5, two broad geographical areas are defined (Figure 14), Eastern Newfoundland in pink (Avalon Peninsula, Cape Shore, Middle Ridge, Pot Hill, Mt. Peyton) and Western Newfoundland in yellow (St. Anthony, Northern Peninsula, Adies Lake, Hampden Downs, Gaff Topsails, Buchans, Lapoile, Grey River, Merasheen Island). The results were similar to those in the fourth scenario where three geographical areas were defined, Avalon, Central and Northern. The "within herd" variance component is still large but slightly lower (84.44%, Φ_{ST}=0.15557) than in the first and second and third scenarios, the "among regions" component is lower (8.31%, Φ_{CT}=0.08310) and the "among herds/regions" component is much higher (7.25%, Φ_{SC}=0.07903) (Table 16). The results are significant for all three variance components ($p<0.05$).

In the Scenario 6, two broad geographical regions are defined (Figure 14), Avalon in pink (Avalon Peninsula, Cape Shore) and the Rest of Newfoundland in yellow (St. Anthony, N. Peninsula, Adies Lake, Hampden Downs, Gaff Topsails, Buchans, Lapoile, Grey River, Merasheen Is., Middle Ridge, Pot Hill, Mt. Peyton). The "within herd" variance component is still large (77.09%, Φ_{ST}=0.22915) but much lower than in all other scenarios, the "among regions" component is higher (16.64%, Φ_{CT}=0.16636) than in all other scenarios, and the "among herds/regions" component is higher (6.28%,

Φ_{SC}=0.07531) than the first thee scenarios but lower than the fourth and fifth scenarios (Table 16). The results are significant for all three variance components ($p<0.05$).

3.7 Nested Clade Analysis (NCA)

The application of the nested clade design to the haplotype network for Newfoundland caribou is shown in Figure 15. There are twenty nested clades, including, eleven 1-step Clades (1-1 to 1-11), which are nested together into five 2-step Clades (2-1 to 2-5). The 2-step Clades are nested together into four 3-step Clades (3-1 to 3-4). Each Nested Clade is assigned as being an "interior" clade or "tip" clade depending on its position in the haplotype network. Generally, the "interior" clade is the ancestral clade and the "tip" clade is the descendent clade. The geographical centers of the herds (latitude and longitude) were used in the NCA as a rough estimate of herd location to compare with the geographical centers of the clades (latitude and longitude) calculated in the NCA (these locations are given in Tables 17 and 18, respectively). The Null hypothesis of the Nested Clade Analysis is that there is no phylogeographic association of haplotypes (associations are random), thus, there are no restrictions to gene flow and the population is panmictic. Nested Clades in which the null hypothesis is accepted include, Clade 1-1, Clade 1-11, Clade 2-1, Clade 1-6, Clade 1-7, Clade 1-8, Clade 1-9 and Clade 1-10. There are seven Nested Clades in which the null hypothesis is rejected and thus have significant phylogeographic associations, these include, Clade 1-2, Clade 1-3, Clade 1-4, Clade 1-5, Clade 2-2, Clade 2-3, and Clade 3-1. Figure 16 presents the results of the Nested Clade Analysis for the 32 Newfoundland caribou mtDNA

haplotypes. Table 19 outlines the chain of inference followed using the inference key to interpret the results. Figure 17 (a through g) shows the distribution of haplotypes/sub-clades for each of the clades with significant phylogeographic associations.

Phylogeographic associations in three Nested Clades, Clades 1-3, 1-4, and 2-3, may be due to population structure as a result of restricted gene flow by isolation by distance (Table 19). Nested Clade 1-3, which represents Clade **C** (as described previously), includes two haplotypes that occur in 11 herds. The structure of this nested clade includes one interior haplotype (**Ca**) and one tip haplotype (**Cb**). The interior haplotype (**Ca**) occurs in 11 contiguous herds in central and western Newfoundland, occurring mainly in Lapoile, Buchans, Grey River, Gaff Topsails, Adies Lake and St. Anthony (Figure 17a). Haplotype **Ca** is also the most prevalent haplotype on Merasheen Island and is absent in herds east of Mount Peyton and Pot Hill (Figure 17a). The tip haplotype (**Cb**) occurs in a subset of herds, including, Adies Lake, Gaff Topsails, Northern Peninsula and Merasheen Island (Figure 17a).

Nested Clade 1-4 includes four haplotypes (**De, Dh, Di, Dm**) that occur in four herds (Avalon, Middle Ridge, Mt. Peyton, Buchans). The structure of this nested clade includes one interior haplotype (**Dh**) with 3 tip haplotypes (**De, Di, Dm**). The interior haplotype (**Dh**) occurs in low numbers the Middle Ridge and Avalon herds (Figure 17b). Tip haplotype (**De**) is confined to the Avalon herd while the other two tip haplotypes occur to the west, **Di** in Mount Peyton (which is adjacent to Middle Ridge) and **Dm** far to the west in Buchans (Figure 17b).

Nested Clade 2-3 includes five sub-clades, Nested Clade 1-7 (**Dd, Df**) as the interior clade and Nested Clades 1-6 (**Da, Dk, Dn**), 1-8 (**Dg, Dq**), 1-9 (**Dp, Dj**) and 1-10 (**Dc, Dr, Ds**) as the tip clades. Nested Clade 2-3 is represented by 12 haplotypes occurring in 12 herds (all herds except Avalon and Cape Shore). The structure of the sub-clades within Nested Clade 2-3 is diverse and complex. The interior clade (1-7) is centered among the Lapoile, Buchans, Grey River and Gaff Topsails herds but also extends north to Hampden Downs and St. Anthony, and east to Mount Peyton and Pot Hill (Figure 17c). The interior clade (1-7) is not detected in Northern Peninsula, Adies Lake, Middle Ridge or further east on the Avalon Peninsula; also, it does not occur on Merasheen Island (Figure 17c). Tip clade (1-6) is very widespread and abundant, occurring in all herds on the main part of the island except Mount Peyton. Tip clade (1-6) is not detected on Merasheen Island or the Avalon Peninsula (Figure 17c). Tip clade (1-8) is not widespread and occurs only in the adjacent herds of Grey River, Pot Hill and Mount Peyton (Figure 17c). Tip clade (1-9) is rare and occurs in the adjacent herds of Pot Hill and Mount Peyton, it also occurs far to the west in Lapoile. Tip clade (1-10) is widespread in the central herds and somewhat centered on Middle Ridge, Pot Hill and Grey River (Figure 17c). It also occurs in low numbers in herds to the west including, Mount Peyton, Gaff Topsails, Lapoile and Buchans (Figure 17c). Clade 1-10 is not detected in the Hampden Downs, Adies Lake or Northern Peninsula herds, however it is detected in St. Anthony (Figure 17c). Clade 1-10 is the only sub-clade in Clade 2-3 that is detected on Merasheen Island (Figure 17c).

Phylogeographic associations in three clades, Nested Clades 1-2, 1-5, and 2-2, may be due to contiguous range expansion (short-distance dispersal events by individuals), indicating that population history rather than current population structure has influenced these clades (Table 19). Nested Clade 1-2, which represents Clade **B** (as described previously), includes eight haplotypes that occur in 12 herds. The structure of this clade includes seven tip haplotypes (**Bb** through **Bh**) radiating from the interior haplotype (**Ba**) by one mutational step. The interior haplotype **Ba** is centered among Gaff Topsails, Adies Lake and Northern Peninsula, but also occurs throughout the main part of the island in all herds except Grey River; **Ba** is absent from Merasheen Island and the Avalon Peninsula (Figure 17d). Tip haplotype **Bb** is rare and far-reaching, occurring in adjacent herds Middle Ridge and Pot Hill, and distant herds Lapoile and Northern Peninsula (Figure 17d). Tip haplotype **Bc** occurs only in adjacent herds Middle Ridge and Mount Peyton. Tip haplotype **Bd** occurs in adjacent herds Pot Hill, Grey River and Lapoile (Figure 17d). Singleton haplotype **Be** occurs in the Northern Peninsula herd; singleton haplotypes **Bf** and **Bg** occur in Pot Hill, and singleton haplotype **Bh** occurs in Grey River (Figure 17d).

Nested Clade 1-5 includes three haplotypes (**Db**, **Dl**, **Do**) that occur in four herds (Avalon, Cape Shore, Mt. Peyton, Lapoile). The structure of this clade includes one interior haplotype (**Db**) with two tip haplotypes (**Dl**, **Do**). The interior haplotype (**Db**) is confined to herds on the Avalon Peninsula (Avalon and Cape Shore), while the two singleton tip haplotypes occur a long distance away to the west, **Dl** in Lapoile and **Do** in Mt. Peyton (Figure 17e).

Nested Clade 2-2 includes two sub-clades, Nested Clade 1-5 (**Db, Dl, Do**) as the interior clade and Nested Clade 1-4 (**De, Dh, Di, Dm**) as the tip clade. Nested Clade 2-2 is represented by seven haplotypes occurring in six herds (Cape Shore, Avalon, Middle Ridge, Mt. Peyton, Lapoile, Buchans). The interior clade (1-5) mainly occurs in the Avalon and Cape Shore herds but also appears to the west in Mt. Peyton and Lapoile (Figure 17f). The tip clade (1-4) has a similar range, it mainly occurs in the Avalon herd but is also found in adjacent herds Mt. Peyton and Middle Ridge and it also appears far to the west in Buchans (Figure 17f).

The phylogeographic associations in Nested Clade 3-1 may have a historical explanation, resulting from long distance colonization possibly coupled with subsequent fragmentation or past fragmentation followed by range expansion (Table 19). Nested Clade 3-1 includes two sub-clades, Nested Clade 2-2 as the interior clade and Nested Clade 2-3 as the tip clade. Both of these sub-clades were described in detail in the previous two paragraphs. Nested Clade 3-1 is represented by 19 haplotypes occurring in all 14 of the sampled herds. The interior clade (2-2) is centered among the Avalon and Cape Shore herds with some occurrence off the Avalon Peninsula into Middle Ridge and Mount Peyton (Figure 17g). There is also some detection of Nested Clade 2-2 all the way west to Buchans and Lapoile (Figure 17g). The tip clade (2-3), on the other hand, is widespread across the main part of the island but is absent altogether from the Avalon Peninsula (Figure 17g).

3.8 Comparisons of Newfoundland Caribou with Other North American Caribou

Figures 18, 19 and 20 illustrate the phylogenetic relationships of Newfoundland caribou with other North American caribou subspecies. Phylogenetic analyses of 470 bp mitochondrial DNA Control Region sequences using the Neighbor Joining method (Figure 18) group Newfoundland caribou with woodland caribou from Quebec (80.3% majority rule consensus), however, this tree is poorly resolved. Caribou subspecies (*R. t. groenlandicus, R. t. granti, R. t. pearyi*) from northern Canada (North West Territories and Peary Island) and Alaska are not included in this group. Two woodland caribou from Quebec are also not included in this group.

Phylogenetic analysis of mitochondrial DNA cytochrome *b* gene sequences by the Neighbor Joining method (Figure 19), indicate Newfoundland caribou are more closely related to the woodland caribou subspecies (*R. t. caribou*) than to other North American caribou subspecies (70.7% majority rule consensus). Newfoundland caribou belonging to clades **B**, **C**, and **D** are grouped with caribou from Quebec (Rtcar_06, Rtcar_07) and other caribou samples taken from Newfoundland (Rtcar_02, Rtcar_01, Nfnd_1787, Nfnd_2988, Nfnd_5588, Nfnd_7388) with 81.1% majority rule consensus. The Quebec sample Rtcar_06 has a close relationship with haplotype **Db** from the Avalon (55.0% majority rule consensus). The other Quebec sample (Rtcar_07) has a close relationship with haplotype **Bb** (60.0% majority rule consensus). Newfoundland caribou belonging to Clade **A** have close relationship with caribou from Labrador (Rtcar_03, Rtcar_04, Lab_166, Lab_193, Lab_198, Lab_220, LC_191) and Alberta (Rtcar_05) with 65.2% majority rule consensus, and may share a common ancestor. The common ancestry is

only weakly supported by the analysis and should be further explored. Caribou belonging to Clade A form a group that is separate (63.0% majority rule consensus) from the Labrador and Alberta caribou (59.0% majority rule consensus). A separate group of woodland caribou from Labrador (Lab_074, LC_236, Rtcar_C10) is more closely related to the tundra subspecies (*R. t. groenlandicus*) in the North West Territories (86.5% majority rule consensus).

Figure 20 shows the phylogenetic relationships of Newfoundland caribou with only woodland caribou subspecies (*R. t. caribou*). The relationships are similar to those described in the previous paragraph except that the percent occurrence of consensus trees are slightly different. Newfoundland caribou belonging to clades **B**, **C**, and **D** are grouped with caribou from Quebec (Rtcar_06, Rtcar_07) and other caribou samples taken from Newfoundland (Rtcar_02, Rtcar_01, Nfnd_1787, Nfnd_2988, Nfnd_5588) with 82.5% majority rule consensus. Newfoundland caribou belonging to clade **A** are grouped with caribou from Labrador (Rtcar_03, Rtcar_04, Lab_166, Lab_193, Lab_198, Lab_220, LC_191) and Alberta (Rtcar_05) with 68.9% majority rule consensus. Clade **A** caribou form a group which is separate (64.0% majority rule consensus) from the Labrador and Alberta caribou (75.2% majority rule consensus).

3.9 Comparisons of Newfoundland Caribou with Eurasian Reindeer

Figures 21 and 22 illustrate the phylogenetic relationships of Newfoundland caribou with Eurasian caribou subspecies. Phylogenetic analyses by the Neighbor Joining method (Figures 21 and 22) indicate Newfoundland caribou are clearly

distinguished from Eurasian reindeer. The analysis of mtDNA Control Region sequences (Figure 21) show all Newfoundland caribou haplotypes grouped in a distinct clade with 91.7% majority rule consensus. In Figure 21, the Eurasian haplotypes arise from the same node, indicating there is uncertainty in the branching order based on the available data. The analysis of the mtDNA cytochrome *b* sequences (Figure 22) show two distinct clades, one including the Newfoundland caribou haplotypes (75.4% majority rule consensus), the other including the Eurasian reindeer haplotypes (90.2% majority rule consensus).

3.10 Complete Newfoundland Caribou Mitochondrial Genome

The mitochondrial genome was sequenced for one male Newfoundland caribou, (sample C-066) from Middle Ridge (sequence is given in Appendix C). The individual belongs to clade D, and possesses the haplotype Dc. The mitochondrial genome for this Newfoundland caribou sample is 16,359 bases in length and the base composition is as follows: T(U) = 30.1%, C = 23.1%, A = 33.7%, G = 13.2%. The G-C content and A-T(U) content is 36.3% and 63.8%, respectively. The mitochondrial genome was sequenced in two fragments, Fragment 1, which is 7,171bp in length and Fragment 2, which is 10,252 bp in length (see Figure 5). The two fragments overlapped in two regions, the COX1 gene and the Control Region. In the COX1 gene there is an 83 bp overlapping section and in the Control Region there is a 981 bp overlapping section. Figure 23 shows the sequence contig of individual sequences that were aligned to create the complete mitochondrial genome sequence.

The mtDNA genome for the Newfoundland sample was compared with the mtDNA genome for reindeer (*Rangifer tarandus tarandus*), which is available from Genbank (Accession No. NC_007703 Wada et al. 2006). The Newfoundland mtDNA genome (16,359 bp) is 3 bases shorter than the published reindeer mtDNA genome (16,362 bp). There are 165 variable sites between the Newfoundland caribou and the reindeer mtDNA genome and 9 sites with alignment gaps (indels). Table 20 summarizes the occurrence and density of single nucleotide polymorphisms (SNPs) in genes throughout the genome. The Control Region has the highest SNP density (2.80 SNPs/100 bases). The total SNP density for the mtDNA genome is 16.04 SNPs/100 bases.

Table 6. Sequences obtained from each caribou herd for the Control Region, cytochrome *b* gene and both Regions combined. Complete sequences covered the entire target region in both the forward and reverse directions. Partial sequences covered a portion of the region or were in one direction. Some samples provided no sequence information, likely due to tissue degradation. For each sample, sequence data from both the Control Region and the cytochrome *b* gene were combined. Samples with complete sequences for both regions were used for phylogenetic analysis (n=233).

Caribou Management Unit (Herd)	# of Samples	Number of Individuals Sequenced								
		Control Region (1063bp)			Cytochrome *b* (1160bp)			Combined (2223 bp)		
		Complete	Partial	None	Complete	Partial	None	Complete	Partial	None
61 Lapoile	20	**18**	0	2	**18**	0	2	**18**	0	2
62 Buchans	20	**17**	0	3	**17**	0	3	**17**	0	3
63 Grey River	20	**19**	1	0	**19**	0	1	**19**	1	0
64 Middle Ridge	20	**20**	0	0	**18**	0	2	**18**	2	0
65 Avalon Peninsula	20	**19**	1	0	**19**	1	0	**18**	2	0
66 Gaff Topsails	20	**20**	0	0	**20**	0	0	**20**	0	0
67 Pot Hill	20	**20**	0	0	**18**	0	2	**18**	2	0
68 Mount Peyton	20	**14**	2	4	**16**	0	4	**14**	2	4
69 Northern Peninsula	20	**18**	1	1	**18**	1	1	**18**	1	1
70 Merasheen Island	20	**20**	0	0	**20**	0	0	**20**	0	0
76 St. Anthony	20	**17**	1	2	**18**	1	1	**17**	2	1
77 Cape Shore	20	**17**	0	3	**12**	0	8	**12**	5	3
78 Hampden Downs	7	**6**	0	1	**6**	0	1	**6**	0	1
79 Adies Lake	20	**19**	0	1	**18**	0	2	**18**	1	1
Totals	**267**	**244**	**6**	**17**	**237**	**3**	**27**	**233**	**18**	16

Table 7. Summary of the 18 variable sites found in the Control Region. The 32 haplotypes (Hap) are indicated in the left hand column and the numbers of individuals (N) with the haplotype are indicated in the adjacent column. The base position refers to the location in the sequence given in Appendix A. The five variable sites exhibiting homoplasies are indicated in bold (335, 378, 386, 509, 727). The two uninformative sites are marked with an asterisk* (404, 507).

		Base Position																	
Hap	N	247	280	308	**335**	352	360	**378**	**386**	401	404*	442	507*	**509**	533	576	**727**	795	841
Aa	1	T	T	G	T	G	A	G	A	C	T	C	T	T	C	G	C	A	C
Ab	1	T	T	G	T	G	A	G	A	C	T	C	T	T	C	G	C	G	C
Ac	1	T	T	G	T	G	A	G	A	C	T	C	T	T	C	G	C	A	C
Ba	32	T	T	G	T	G	A	A	G	T	T	T	T	T	C	A	C	G	C
Bb	6	C	T	G	T	G	A	A	G	T	T	T	T	T	C	A	C	G	C
Bc	4	T	T	G	T	G	A	A	G	T	T	T	T	T	C	A	C	G	C
Bd	3	T	T	G	C	G	A	A	G	T	T	T	T	T	C	A	C	G	C
Be	1	T	T	G	T	G	A	A	G	T	T	T	T	T	C	A	T	G	C
Bf	1	T	T	G	T	G	A	A	G	T	T	T	T	T	C	A	C	G	C
Bg	1	T	T	G	T	G	A	A	G	T	T	T	T	T	C	A	C	G	C
Bh	1	T	T	G	T	G	A	A	G	T	T	T	T	T	C	A	C	G	C
Ca	51	T	C	G	C	G	A	G	G	C	T	C	T	C	C	A	C	G	C
Cb	9	T	C	G	C	G	A	G	G	C	T	C	T	C	C	A	C	G	T
Da	33	T	C	A	T	G	A	A	A	C	T	C	T	C	C	A	T	G	C
Db	22	T	C	A	T	G	A	A	G	C	T	C	T	C	C	A	C	G	C
Dc	17	T	C	A	T	G	A	A	G	C	T	C	T	C	C	A	T	G	C
Dd	12	T	C	A	T	G	A	A	G	C	T	C	T	C	C	A	T	G	C
De	7	T	C	A	T	G	A	A	G	C	T	C	T	T	C	A	C	G	C
Df	7	T	C	A	T	A	A	A	G	C	T	C	T	C	C	A	T	G	C
Dg	6	T	C	A	T	G	A	A	G	C	T	C	T	C	T	A	T	G	C
Dh	3	T	C	A	T	G	A	A	G	C	T	C	T	C	C	A	C	G	C
Di	2	T	C	A	C	G	A	A	G	C	T	C	T	C	C	A	C	G	C
Dj	2	T	C	A	T	G	A	A	G	C	T	C	T	C	C	A	T	G	C
Dk	2	T	C	A	T	G	G	A	A	C	T	C	T	C	C	A	T	G	C
Dl	1	T	C	A	T	G	A	A	A	C	T	C	T	C	C	A	C	G	C
Dm	1	T	C	A	T	G	A	A	A	C	T	C	T	C	C	A	C	G	C
Dn	1	T	C	A	C	G	A	A	A	C	T	C	T	C	C	A	T	G	C
Do	1	T	C	A	T	G	A	A	G	C	T	C	T	C	C	A	C	G	C
Dp	1	T	C	A	T	G	A	A	G	C	T	C	T	C	C	A	T	G	C
Dq	1	T	C	A	T	G	A	A	G	C	T	C	T	C	T	A	T	G	C
Dr	1	T	C	A	T	G	A	A	G	C	T	C	C	C	C	A	T	G	C
Ds	1	T	C	A	T	G	A	A	G	C	C	C	T	C	C	A	T	G	C

Table 8. Summary of the 14 variable sites found in the cytochrome *b* gene. The 32 haplotypes (Hap) are indicated in the left hand column and the numbers of individuals (N) with the haplotype are indicated in the adjacent column. The base position refers to the location in the sequence given in Appendix A. The two variable sites exhibiting homoplasies are indicated in bold (2107, 2110). The six uninformative sites are marked with an asterisk* (1112, 1252, 1267, 1749, 1833, 1897).

		Base Position													
Hap	N	1112*	1130	1207	1252*	1267*	1681	1749*	1799	1833*	1897*	2101	**2107**	**2110**	2185
Aa	1	G	G	T	C	C	C	C	G	C	C	C	T	G	C
Ab	1	G	G	T	C	C	C	T	G	C	C	C	T	G	C
Ac	1	G	G	T	C	C	C	T	G	C	C	C	T	G	C
Ba	32	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Bb	6	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Bc	4	G	A	C	C	C	C	T	A	C	C	T	T	G	T
Bd	3	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Be	1	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Bf	1	A	G	C	C	C	C	T	A	C	C	T	T	G	T
Bg	1	G	G	C	C	C	C	T	A	C	T	T	T	G	T
Bh	1	G	G	C	T	C	C	T	A	C	C	T	T	G	T
Ca	51	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Cb	9	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Da	33	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Db	22	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Dc	17	G	G	C	C	C	C	T	A	C	C	T	C	G	T
Dd	12	G	G	C	C	C	C	T	A	C	C	T	T	G	T
De	7	G	G	C	C	C	C	T	A	C	C	T	T	A	T
Df	7	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Dg	6	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Dh	3	G	G	C	C	C	C	T	A	C	C	T	T	A	T
Di	2	G	G	C	C	C	C	T	A	C	C	T	T	A	T
Dj	2	G	G	C	C	C	T	T	A	C	C	T	T	G	T
Dk	2	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Dl	1	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Dm	1	G	G	C	C	C	C	T	A	C	C	T	T	A	T
Dn	1	G	G	C	C	C	C	T	A	C	C	T	T	G	T
Do	1	G	G	C	C	C	C	T	A	C	C	T	C	G	T
Dp	1	G	G	C	C	T	T	T	A	C	C	T	T	G	T
Dq	1	G	G	C	C	C	C	T	A	T	C	T	T	G	T
Dr	1	G	G	C	C	C	C	T	A	C	C	T	C	G	T
Ds	1	G	G	C	C	C	C	T	A	C	C	T	C	G	T

Table 9. Summary of the 14 variable sites in the cytochrome *b* gene and the associated amino acid substitutions. Base position numbers refer to position along cytochrome *b* gene itself. Base position in brackets refers to the position in the 2223 bp data set used in the analysis (Appendix A).

Cytochrome *b* (1170bp)			
Base Position	**Base Change**	**Codon Position**	**Amino Acid Substitution**
49 (1112)	***g***ca ↔***a***ca	1st	*alanine* ↔ *threonine*
67 (1130)	***g***cc↔***a***cc	1st	*alanine* ↔ *threonine*
144 (1207)	gg***c***↔gg***t***	3rd	*glycine* (no change)
189 (1252)	tt***c***↔tt***t***	3rd	*phenylalanine* (no change)
204 (1267)	ca***c***↔ca***t***	3rd	*histidine* (no change)
618 (1681)	aa***c***↔aa***t***	3rd	*aspargine* (no change)
686 (1749)	a***t***t↔a***c***t	2nd	*isoleucine* ↔ *threonine*
736 (1799)	***a***ca↔***g***ca	1st	*threonine* ↔ *alanine*
770 (1833)	a***c***c↔a***t***c	2nd	*threonine* ↔*isoleucine*
834 (1897)	ta***c***↔ta***t***	3rd	*tyrosine* (no change)
1038 (2101)	cc***t***↔ccc	3rd	*proline* (no change)
1044 (2107)	at***t***↔at***c***	3rd	*isoleucine* (no change)
1047 (2110)	ac***g***↔ac***a***	3rd	*threonine* (no change)
1122 (2185)	aa***t***↔aa***c***	3rd	*aspargine* (no change)

Table 10. Number of male and female caribou in each of the 32 Newfoundland caribou haplotypes. Zero values indicated with a dot.

Haplotype	*N*	Males	Females	Unknown
Aa	1	1	.	.
Ab	1	1	.	.
Ac	1	1	.	.
Ba	32	25	7	.
Bb	6	3	1	2
Bc	4	3	1	.
Bd	3	3	.	.
Be	1	.	1	.
Bf	1	.	1	.
Bg	1	.	1	.
Bh	1	1		.
Ca	51	31	9	11
Cb	9	6	1	2
Da	33	20	11	2
Db	22	7	11	14
Dc	17	11	2	4
Dd	12	8	1	3
De	7	1	5	1
Df	7	4	1	2
Dg	6	4	.	2
Dh	3	1	2	.
Di	2	1	1	.
Dj	2	2	.	.
Dk	2	.	1	1
Dl	1	1	.	.
Dm	1	.	.	1
Dn	1	1	.	.
Do	1	1	.	.
Dp	1	.	1	.
Dq	1	1	.	.
Dr	1	.	.	1
Ds	1	1	.	.
Total	**233**	**139**	**58**	**36**

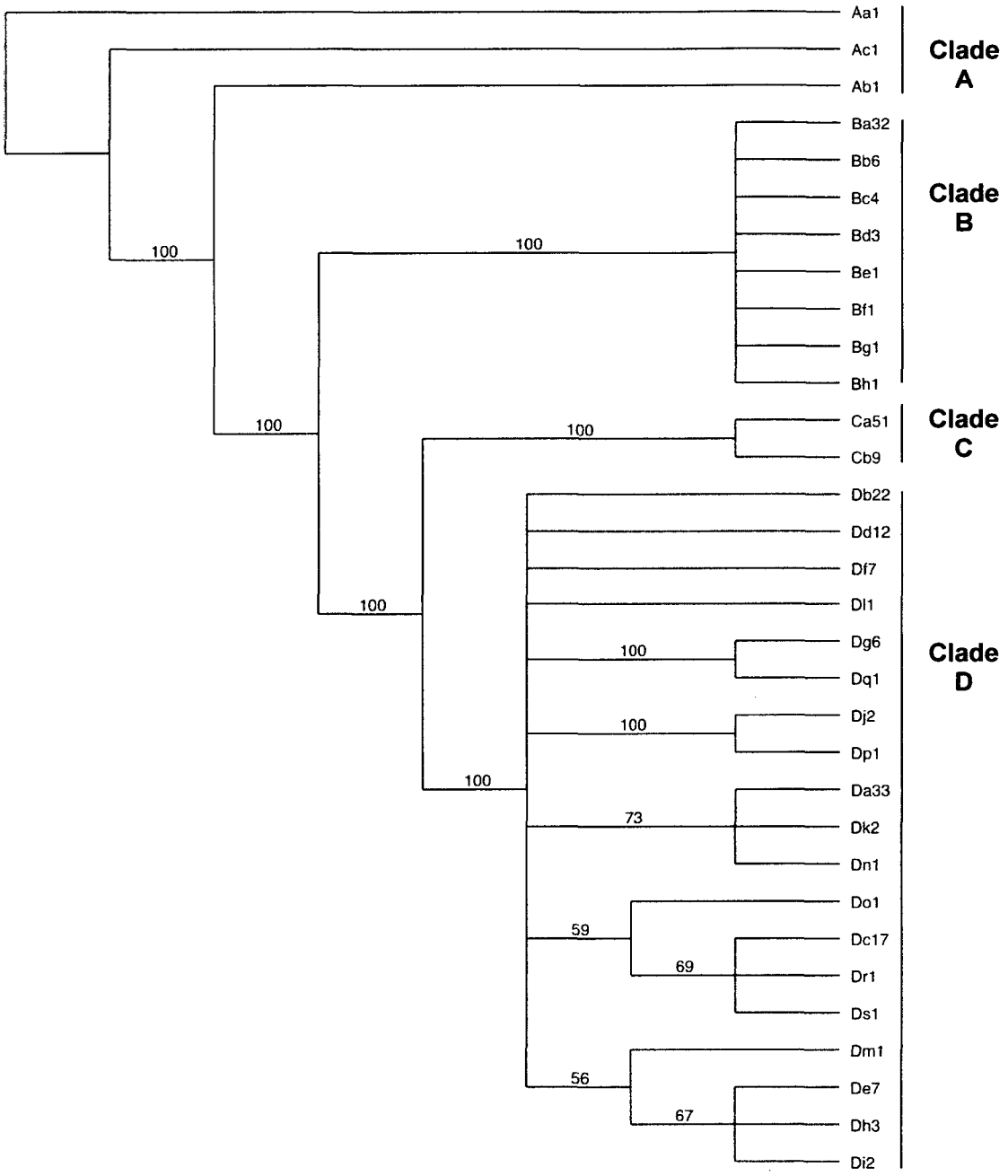


Figure 6. Maximum Parsimony 50% majority rule consensus tree of mtDNA haplotypes in Newfoundland caribou *Rangifer tarandus caribou*. The numbers at the nodes are the percentage of equally parsimonious trees from a total of 135 trees (values of <50% are not shown). The letters represent the mtDNA clade designation (A ,B, C, or D), mtDNA haplotype (a through t), and the numbers indicate the number of individuals in the population with that haplotype.

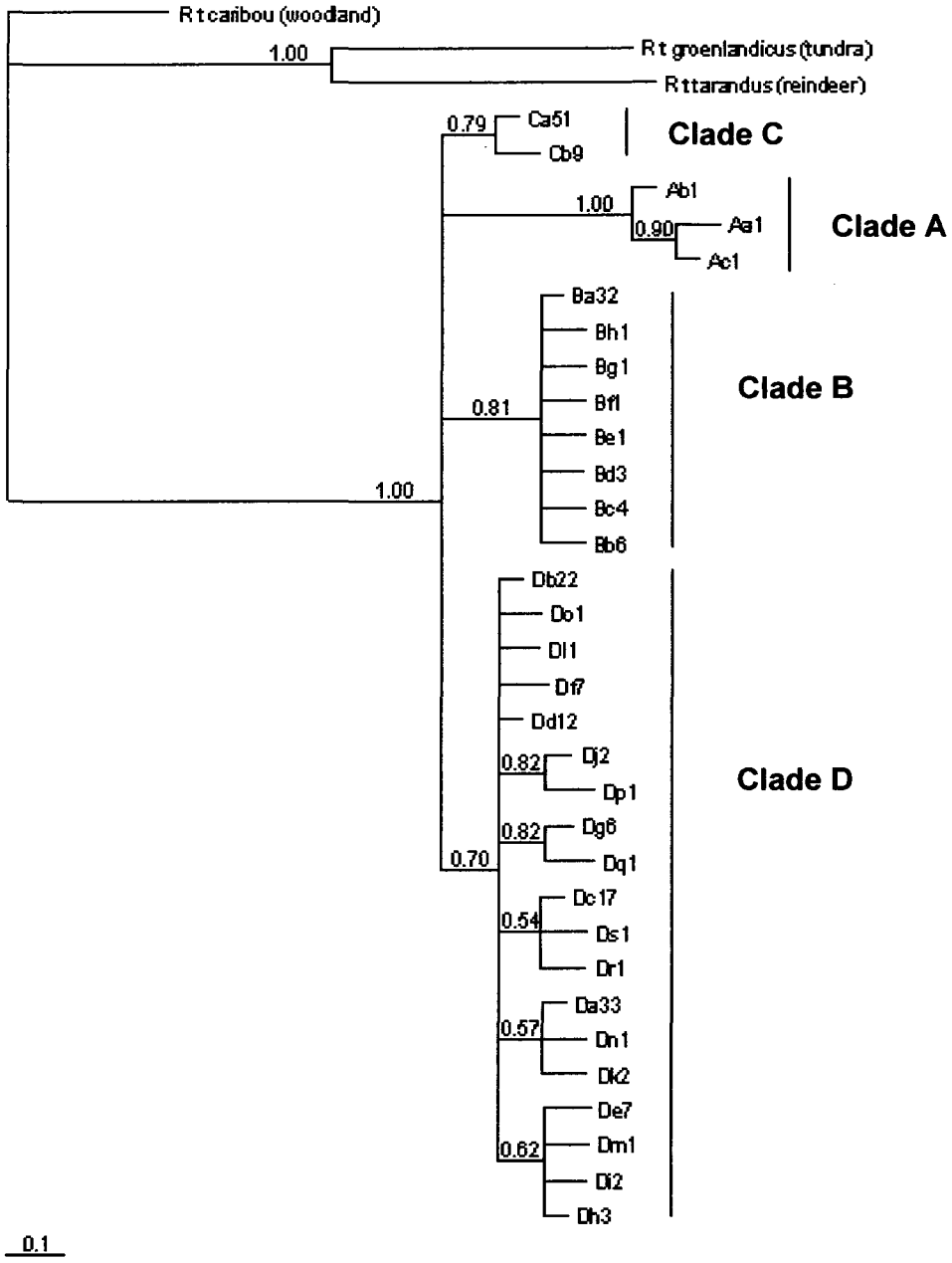


Figure 7. Bayesian phylogeny of 32 mtDNA haplotypes in Newfoundland caribou *Rangifer tarandus caribou* and 3 other caribou subspecies of unknown origins for comparison (sequences from Genbank). The numbers at the nodes represent bootstrap values (values of <0.5 are not shown). Shorter branch lengths indicate ancestral haplotypes within groupings.

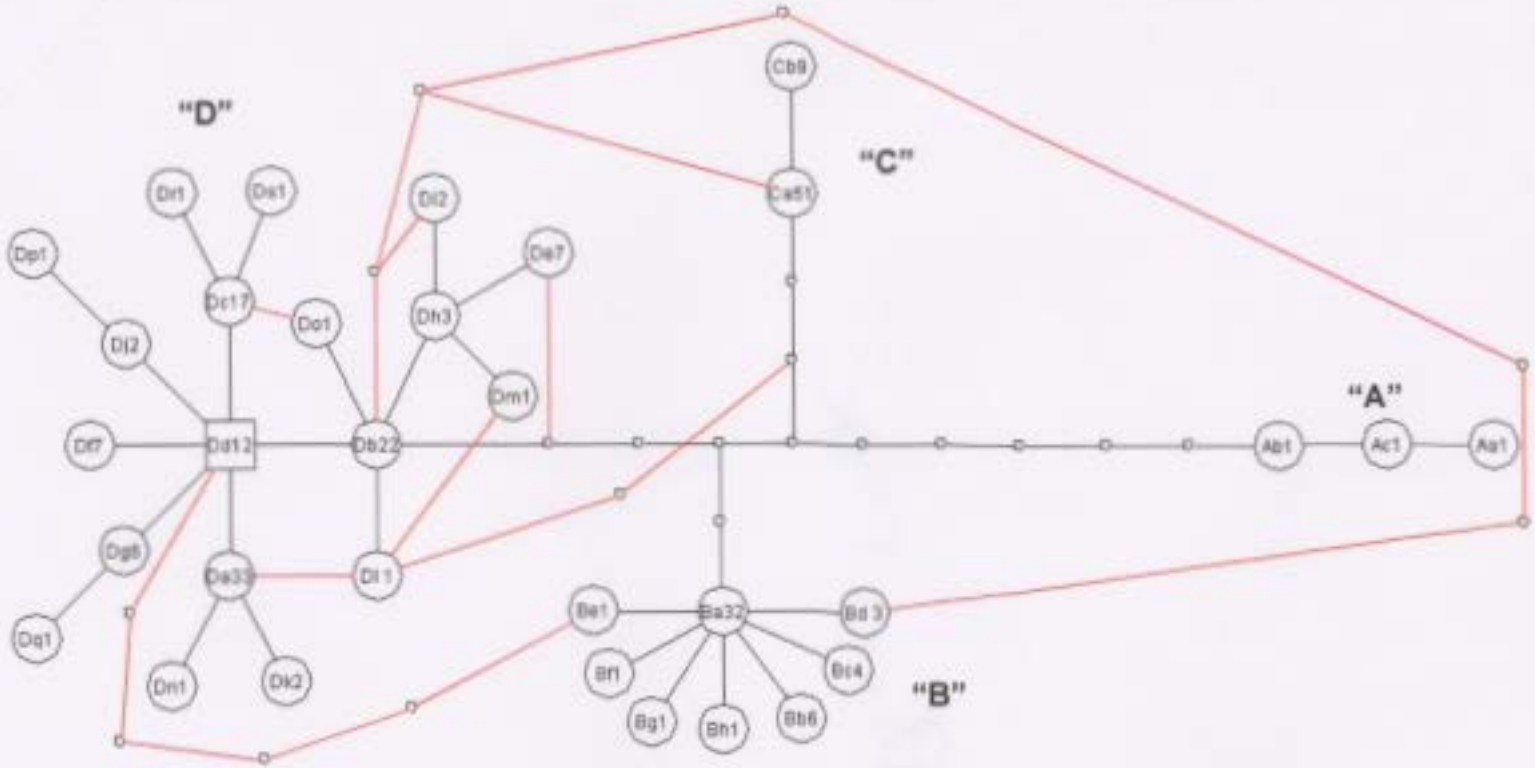


Figure 8. Minimum Spanning Network of 32 Newfoundland caribou mtDNA haplotypes. Each line represents a mutational change. Haplotype Dd is identified as the root (outgroup weight 0.16). Clades A, B, and C are distinct and well-resolved. Clade D is a complicated network of cross-links between haplotypes and between clades. Black lines represent connections between haplotypes that are supported by Maximum Parsimony and Bayesian phylogenies. Red lines represent other possible connections calculated by statistical parsimony. The number after each haplotype designation represents the number of individuals with that haplotype.

Table 11. MtDNA haplotype outgroup probabilities calculated from the Minimum Spanning Network in the program TCS 1.21 (Clement et al. 2000).

Haplotype	Outgroup Weight	Haplotype	Outgroup Weight
Aa	0	**Dd**	**0.16**
Ab	0	De	0.02
Ac	0	Df	0.01
Ba	0.08	Dg	0.03
Bb	0	Dh	0.06
Bc	0	Di	0.01
Bd	0.06	Dj	0.02
Be	0.05	Dk	0.00
Bf	0	Dl	0.09
Bg	0	Dm	0.01
Bh	0	Dn	0
Ca	0.10	Do	0.07
Cb	0.01	Dp	0
Da	0.08	Dq	0
Db	0.06	Dr	0
Dc	0.05	Ds	0

Table 12. ***Rangifer tarandus caribou.*** **Distribution of mtDNA clades among 14 herds located across the island of Newfoundland. The percentage of each clade in entire sample (n/233) is given in parentheses. Zero values indicated with a dot.**

		mtDNA Clades			
Herd	***N***	*A*	*B*	*C*	*D*
St. Anthony	*17*	1 (0.43)	4 (1.72)	7 (3.00)	5 (2.15)
N. Peninsula	*18*	.	7 (3.00)	2 (0.86)	9 (3.86)
Adies Lake	*18*	.	6 (2.58)	8 (3.43)	4 (1.72)
Hampden Downs	*6*	1 (0.43)	2 (0.86)	1 (0.43)	2 (0.86)
Gaff Topsails	*20*	.	6 (2.58)	6 (2.58)	8 (3.43)
Buchans	*17*	1 (0.43)	2 (0.86)	6 (2.58)	8 (3.43)
Merasheen Is.	*20*	.	1 (0.43)	16 (6.87)	3 (1.29)
Lapoile	*18*	.	3 (1.29)	6 (2.58)	9 (3.86)
Grey River	*19*	.	2 (0.86)	6 (2.58)	11 (4.72)
Pot Hill	*18*	.	7 (3.00)	1 (0.43)	10 (4.29)
Mt. Peyton	*14*	.	5 (2.15)	1 (0.43)	8 (3.43)
Middle Ridge	*18*	.	4 (1.72)	.	14 (6.00)
Avalon	*18*	.	.	.	18 (7.73)
Cape Shore	*12*	.	.	.	12 (5.15)
All Herds	***233***	**3 (1.29)**	**49 (21.0)**	**60 (25.8)**	**121 (51.9)**

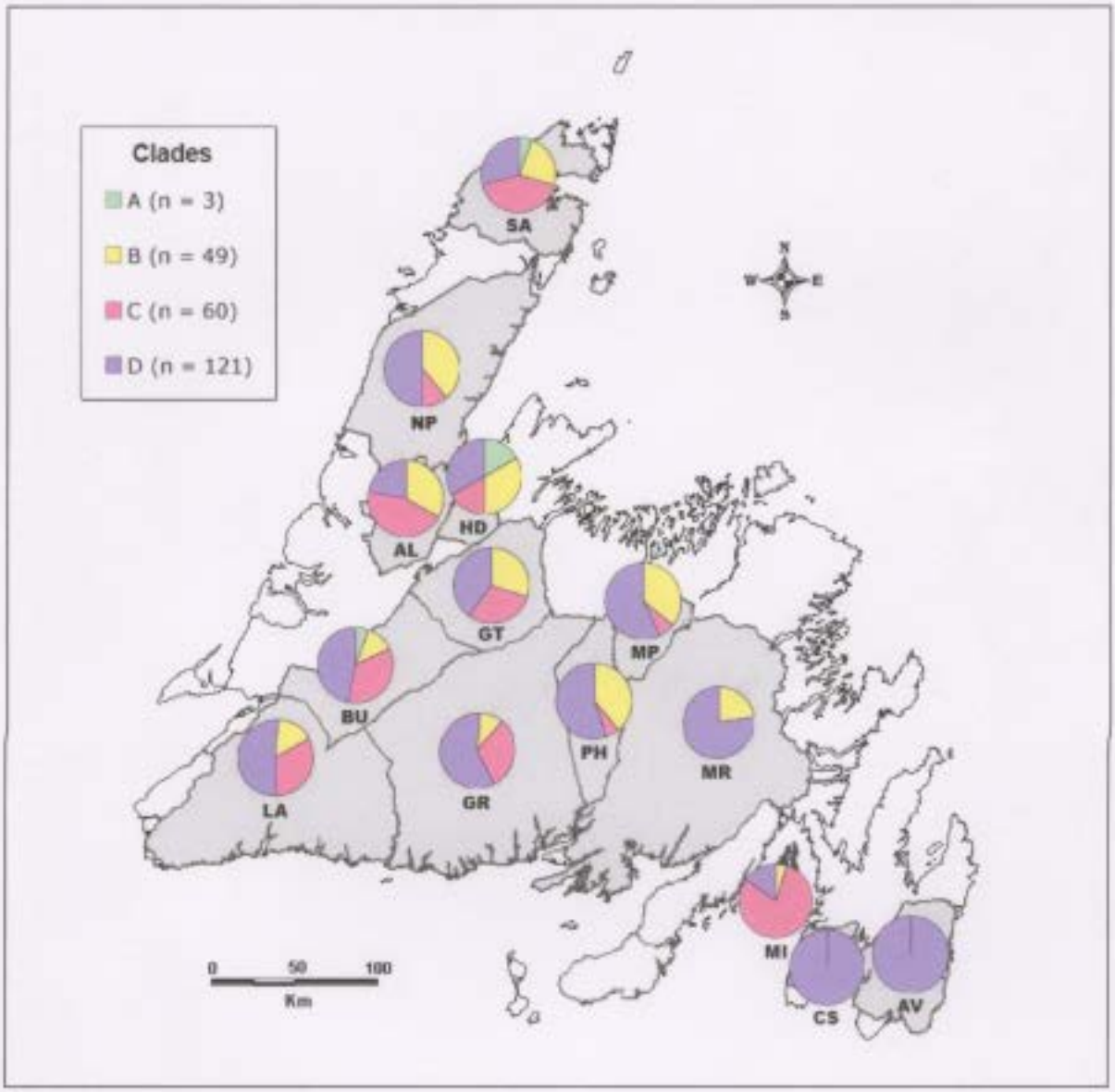


Figure 9. Distribution of four mtDNA clades across the island of Newfoundland. Herds are identified by 2-letter codes (SA = St. Anthony, NP = Northern Peninsula, HD = Hampden Downs, AL = Adies Lake, GT = Gaff Topsails, BU = Buchans, LA = Lapoile, GR = Grey River, PH = Pot Hill, MP = Mt. Peyton, MR = Middle Ridge, MI = Merasheen Island, CS = Cape Shore, AV = Avalon Peninsula). See Table 12 for actual frequencies.

Table 13. ***Rangifer tarandus caribou.*** **Distribution of mtDNA haplotypes among 14 herds located across the island of Newfoundland. Common haplotypes include, Ba, Ca, Da, Db, Dc and Dd. Zero values indicated with a dot.**

		mtDNA Haplotypes																																		
		Clade A					*Clade B*							*Clade C*						*Clade D*																	
Herd	**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	.	.	.	.	.
N. 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 Is.	20	.	.	.	.	1	.	.	.	.	.	.	13	3	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lapoile	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	.
Avalon	18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10	.	.	7	.	.	1	.	.	.	.	.	.	.	.	.	.	.
Cape Shore	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
All Herds	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

Table 14. Occurrence of herds in each of the 32 observed mtDNA haplotypes (Hap). The number of individuals with the haplotype for each herd is given in parentheses.

Hap	N	# of Herds	Names of Herds Present
Aa	1	1	*Buchans (1)*
Ab	1	1	*St. Anthony (1)*
Ac	1	1	*Hampden Downs (1)*
Ba	32	10	*Gaff Topsails (6), N. Peninsula (6), Adies Lake (6), Pot Hill (3), Mt. Peyton (3), St. Anthony (2), Hampden Downs (2), Lapoile (1), Buchans (2), Middle Ridge (1)*
Bb	6	5	*St. Anthony (2), Lapoile (1), Middle Ridge (1), Pot Hill (1), Merasheen Is. (1)*
Bc	4	2	*Middle Ridge (2), Mt. Peyton (2)*
Bd	3	3	*Lapoile (1), Grey River (1), Pot Hill (1)*
Be	1	1	*N. Peninsula (1)*
Bf	1	1	*Pot Hill (1)*
Bg	1	1	*Pot Hill (1)*
Bh	1	1	*Grey River (1)*
Ca	51	11	*Merasheen Is. (13), St. Anthony (7), Lapoile (6), Buchans (6), Grey River (6), Adies Lake (5), Gaff, Topsails (4), Pot Hill (1), Mt. Peyton (1), N. Peninsula (1), Hampden Downs (1)*
Cb	9	4	*Merasheen Is. (3), Adies Lake (3), Gaff Topsails (2), N. Peninsula (1)*
Da	33	10	*N. Peninsula (9), Middle Ridge (5), Gaff Topsails (4), Adies Lake (4), Buchans (3), Grey River (3), Lapoile (2), Pot Hill (1), St. Anthony (1), Hampden Downs (1)*
Db	22	2	*Cape Shore (12), Avalon (10)*
Dc	17	9	*Middle Ridge (4), Pot Hill (3), Merasheen Is. (3), Grey River (2), Lapoile (1), Buchans (1), Gaff Topsails (1), Mt. Peyton (1), St. Anthony (1)*
Dd	12	6	*Buchans (3), Gaff Topsails (3), Lapoile (2), St. Anthony (2), Pot Hill (1), Hampden Downs (1)*
De	7	1	*Avalon (7)*
Df	7	4	*Grey River (3), Lapoile (2), Pot Hill (1), Mt. Peyton (1)*
Dg	6	3	*Grey River (2), Pot Hill (2), Mt. Peyton (2)*
Dh	3	2	*Middle Ridge (2), Avalon (1)*
Di	2	2	*Middle Ridge (1), Mt.Peyton (1)*
Dj	2	2	*Lapoile (1), Mt. Peyton (1)*
Dk	2	2	*Middle Ridge (1), Pot Hill (1)*
Dl	1	1	*Lapoile (1)*
Dm	1	1	*Buchans (1)*
Dn	1	1	*St. Anthony (1)*
Do	1	1	*Mt.Peyton (1)*
Dp	1	1	*Pot Hill (1)*
Dq	1	1	*Mt. Peyton (1)*
Dr	1	1	*Middle Ridge (1)*
Ds	1	1	*Grey River (1)*

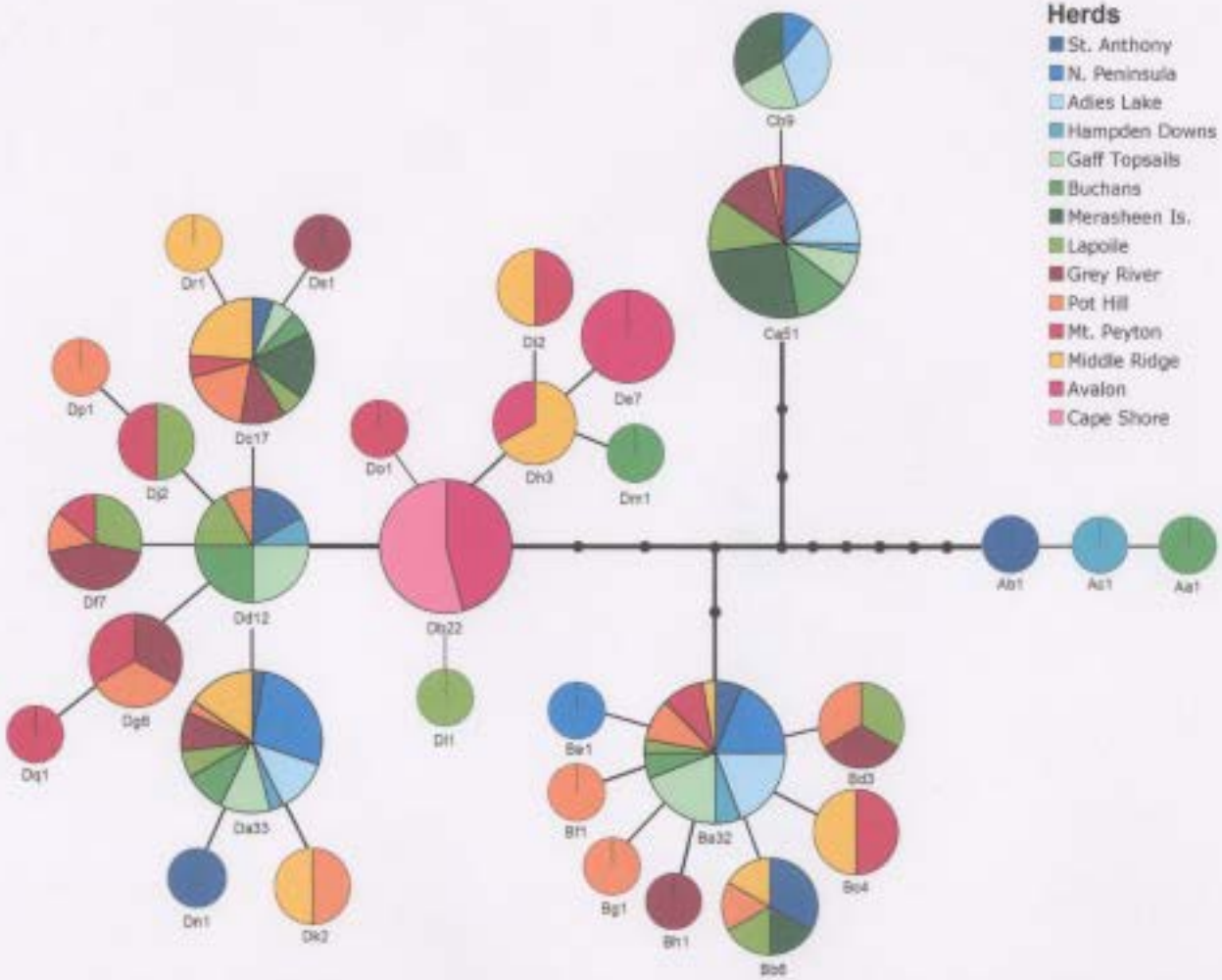


Figure 10. Network of 32 mtDNA haplotypes including distribution of herds for each haplotype. Network follows the most probable haplotype connections based on statistical parsimony and phylogenetic analyses. Each line between haplotypes, or nodes (black circles), represents a mutational change.

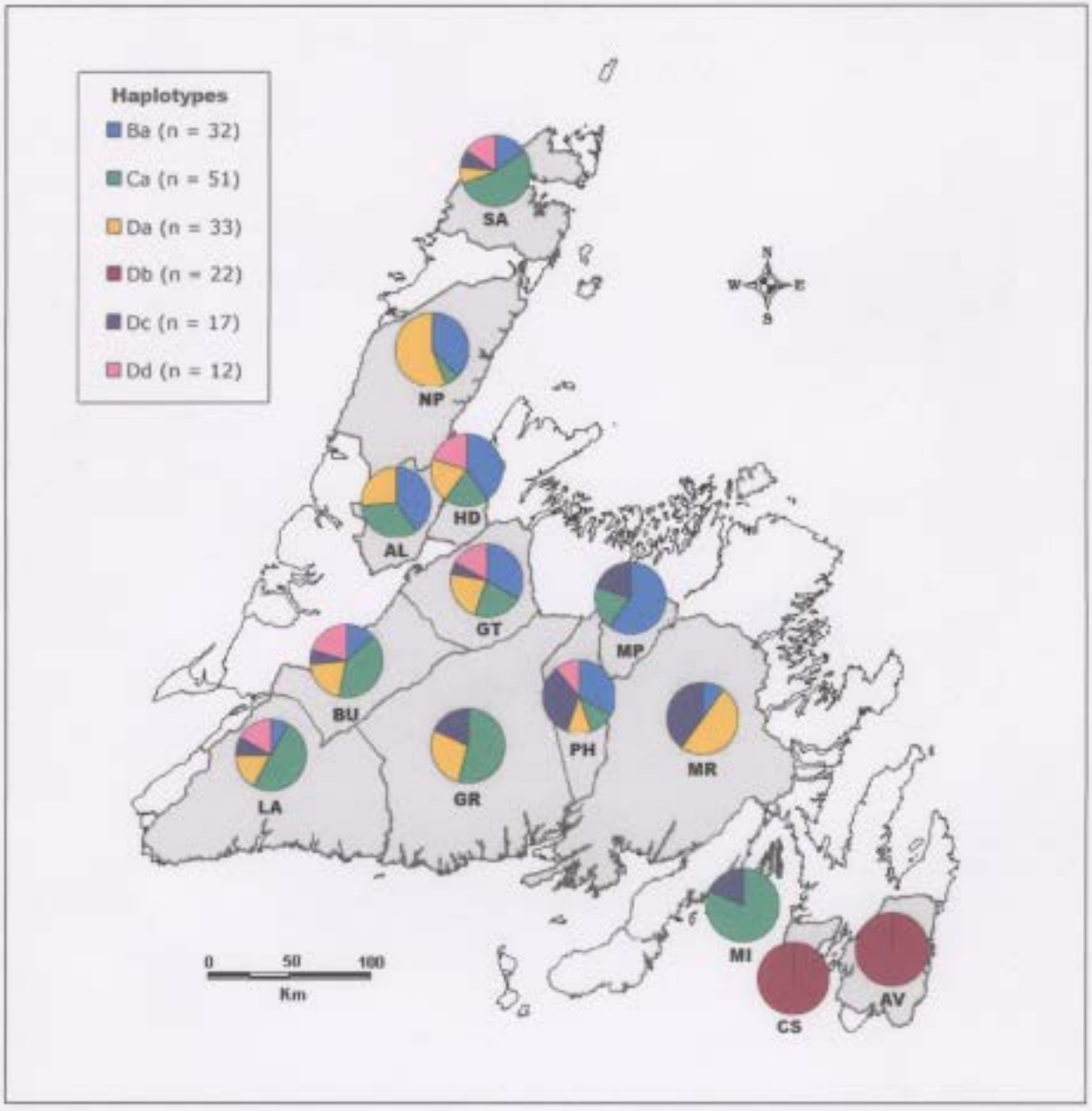


Figure 11. Distribution of 6 common haplotypes (occur in >10 individuals) across the island of Newfoundland. Herds are identified by 2-letter codes (SA = St. Anthony, NP = Northern Peninsula, HD = Hampden Downs, AL = Adies Lake, GT = Gaff Topsails, BU = Buchans, LA = Lapoile, GR = Grey River, PH = Pot Hill, MP = Mt. Peyton, MR = Middle Ridge, MI = Merasheen Island, CS = Cape Shore, AV = Avalon Peninsula). See Table 13 for actual frequencies.

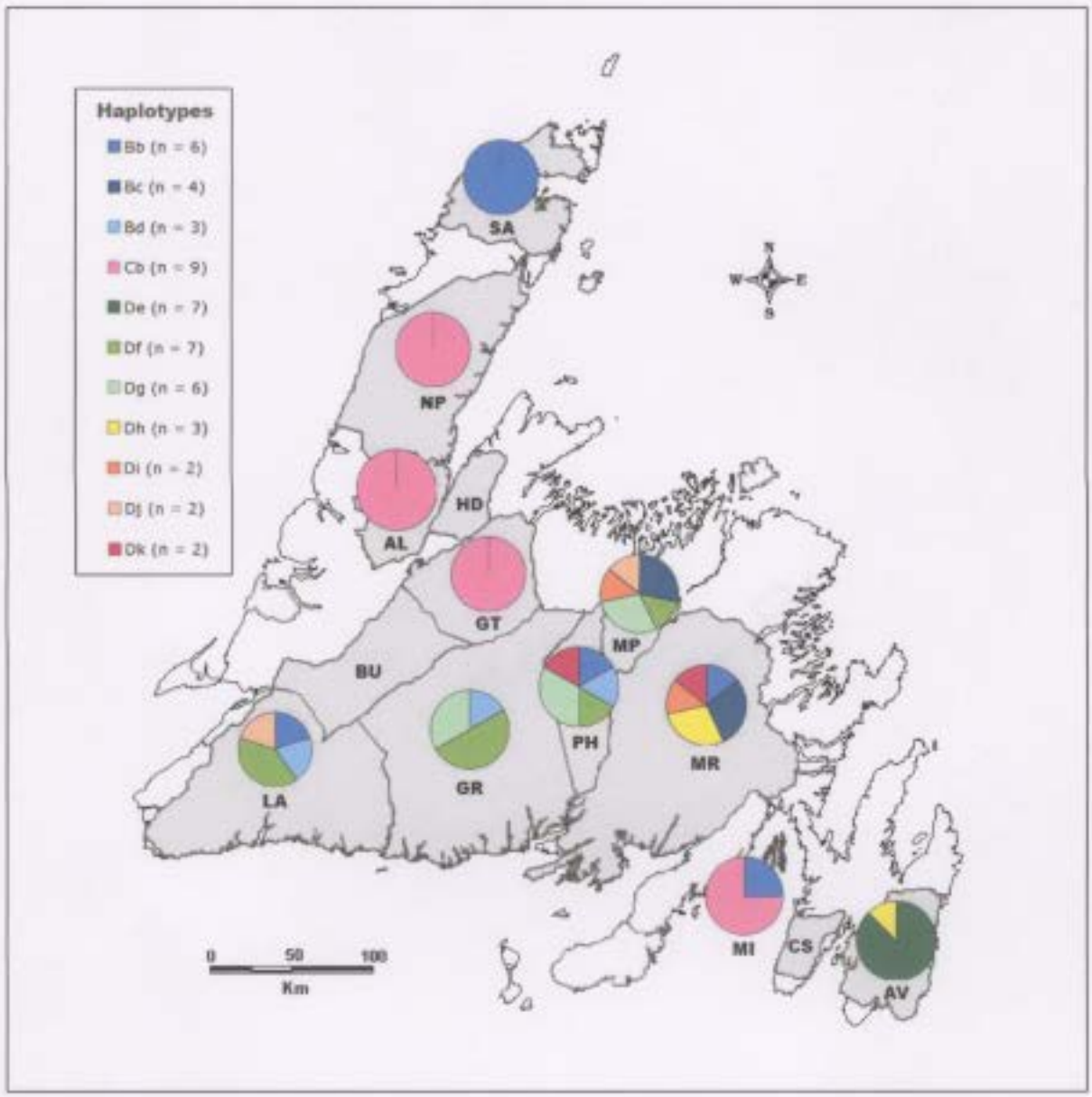


Figure 12. Distribution of 11 less common mtDNA haplotypes (occur in >1 and <10 individuals) across the island of Newfoundland. Herds are identified by 2-letter codes (SA = St. Anthony, NP = Northern Peninsula, HD = Hampden Downs, AL = Adies Lake, GT = Gaff Topsails, BU = Buchans, LA = Lapoile, GR = Grey River, PH = Pot Hill, MP = Mt. Peyton, MR = Middle Ridge, MI = Merasheen Island, CS = Cape Shore, AV = Avalon Peninsula). Herds with no representative pie diagram did not have any of the haplotypes listed. See Table 13 for actual frequencies.

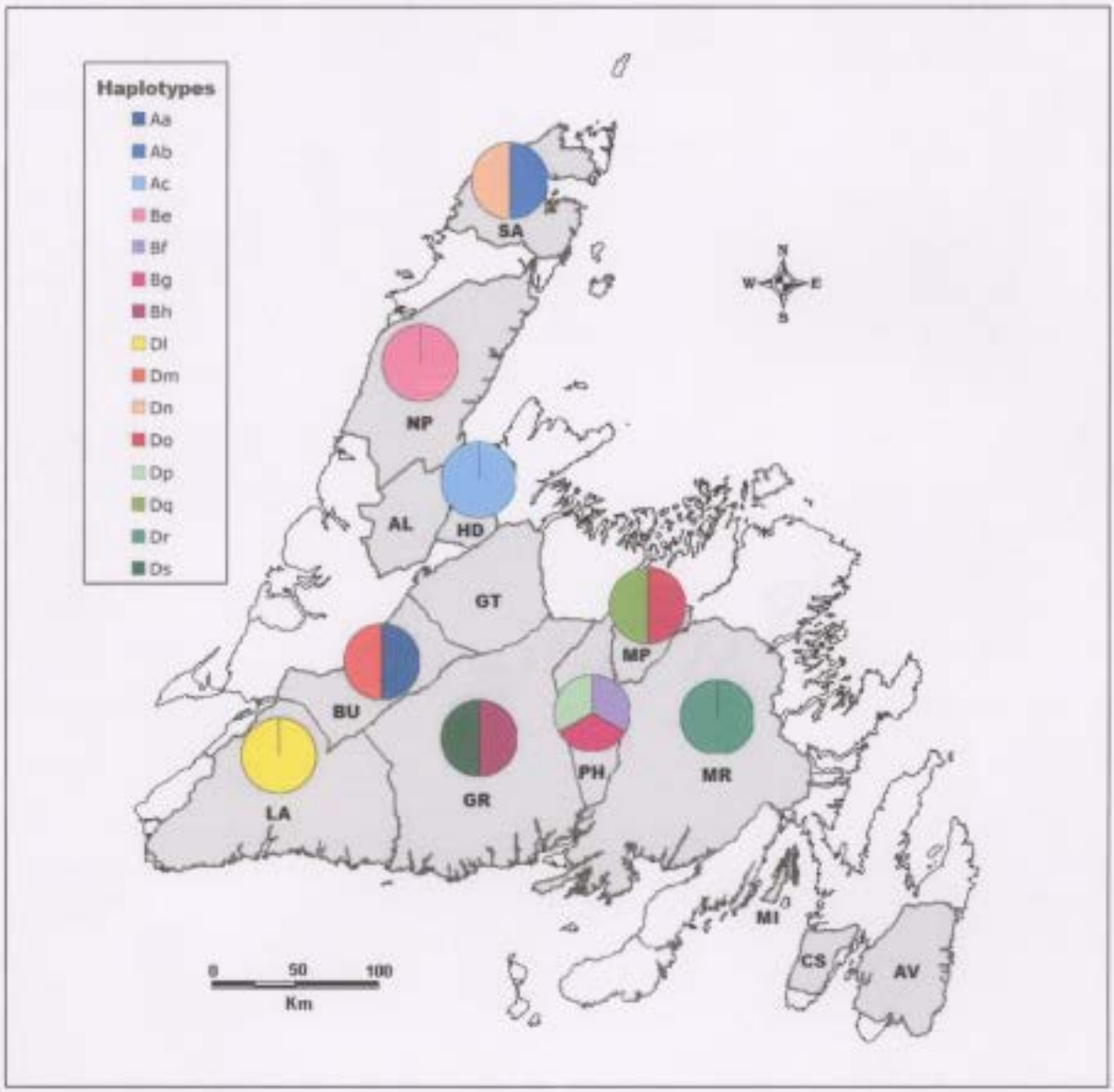


Figure 13. Distribution of 15 rare mtDNA haplotypes (occur in only 1 individual) across the island of Newfoundland. Herds are identified by 2-letter codes (SA = St. Anthony, NP = Northern Peninsula, HD = Hampden Downs, AL = Adies Lake, GT = Gaff Topsails, BU = Buchans, LA = Lapoile, GR = Grey River, PH = Pot Hill, MP = Mt. Peyton, MR = Middle Ridge, MI = Merasheen Island, CS = Cape Shore, AV = Avalon Peninsula). Herds with no representative pie diagram did not have any of the haplotypes listed. See Table 13 for actual frequencies.

Table 15. Indices of genetic mtDNA variation among the 14 herds studied. Values of haplotypic diversity (Hd), average number of differences (K), and nucleotide diversity (π).

Herd	N	# of Segregating Sites	# of Haplotypes	Hd	K	π
St. Anthony	17	16	8	0.82353	4.82353	0.00245
N. Peninsula	18	10	5	0.66667	4.12418	0.00210
Adies Lake	18	10	4	0.77778	3.32026	0.00220
Hampden Downs	6	15	5	0.93333	6.73333	0.00343
Gaff Topsails	20	11	6	0.83684	4.28947	0.00219
Buchans	17	18	7	0.83824	4.60294	0.00234
Merasheen Is.	20	11	4	0.55789	2.24737	0.00115
Lapoile	18	13	10	0.88235	4.03922	0.00206
Grey River	19	14	8	0.86555	4.02339	0.00205
Pot Hill	18	18	13	0.95425	4.86928	0.00248
Mt. Peyton	14	15	10	0.94505	4.90110	0.00249
Middle Ridge	18	14	9	0.88235	4.17647	0.00213
Avalon	18	2	3	0.56863	1.02614	0.00052
Cape Shore	12	0	1	0.00000	0.00000	0.00000
Values for Population	**233**	**32**	**32**	**0.89437**	**4.25277**	**0.00216**

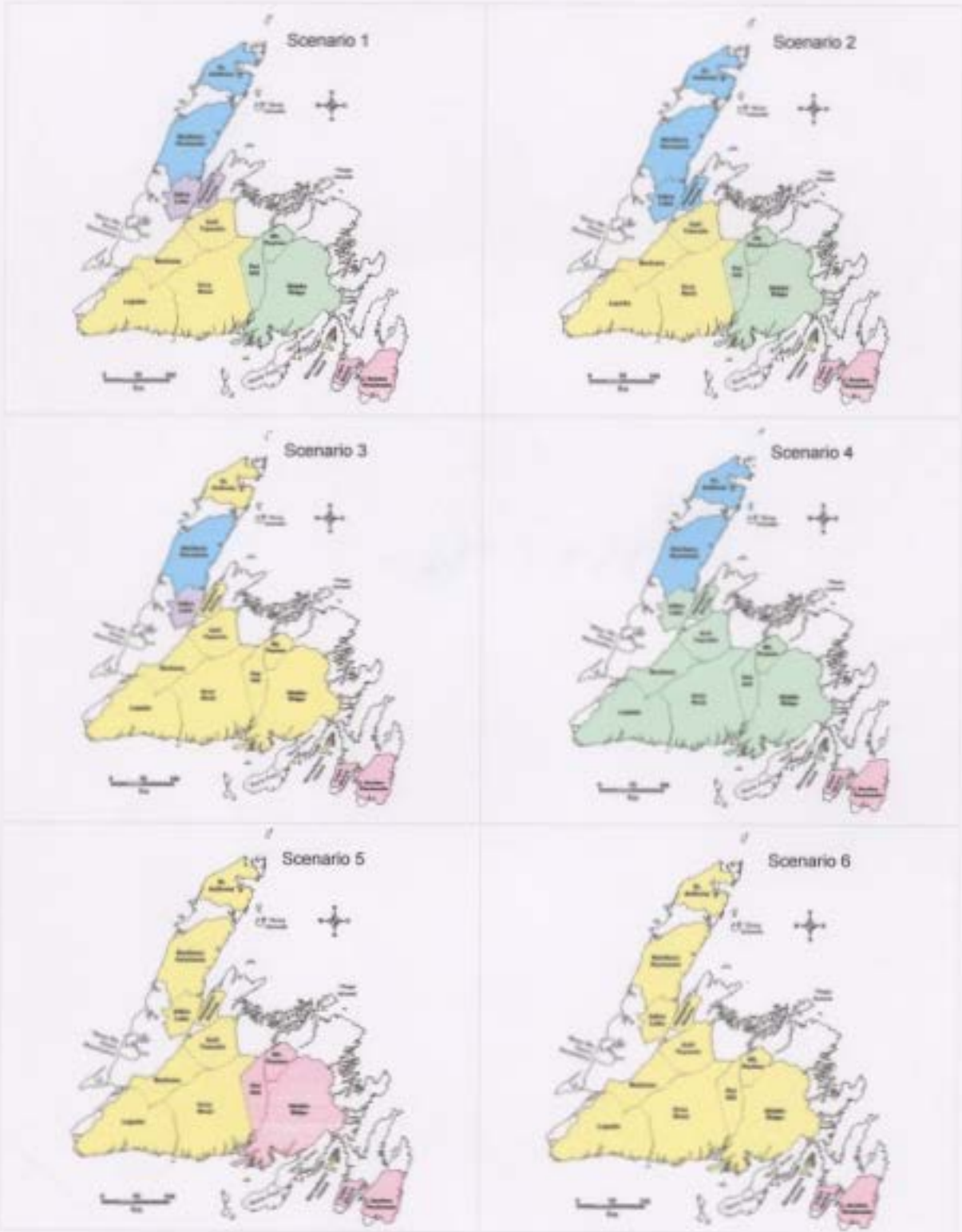


Figure 14. Geographical regions defined for six scenarios used in hierarchical AMOVA analysis. Scenario 3 tests regional groupings suggested by Bergerud 1971.

Table 16. Analysis of Molecular Variance (AMOVA) for different hierarchical groupings of mtDNA haplotypes. Haplotypes are grouped first by herd, then by another regional level, which can be seen in Figure 14. Most of the variation occurs at the individual level within herds in all grouping scenarios. Scenario 3 tests regional groupings suggested by Bergerud 1971.

SCENARIO 1 - Five Geographical Regions

Avalon (Avalon Peninsula, Cape Shore); Eastern (Middle Ridge, Mt. Peyton, Pot Hill); West/Central (Merasheen Is., Lapoile, Buchans, Grey River, Gaff Topsails); North/West (Adies Lake, Hampden Downs); Northern (N. Peninsula, St. Anthony)

		Observed Partition			
Variance Component		Variance	% Total	p[a]	Φ-statistics
Among Regions	σ^2_a	0.20908	9.58	<0.00000	Φ_{CT} = 0.09583
Among Herds/Regions	σ^2_b	0.08499	3.90	<0.00000	Φ_{SC} = 0.04308
Within Herds	σ^2_c	1.88773	86.52	<0.00000	Φ_{ST} = 0.13478

SCENARIO 2 - Four Geographical Regions

Avalon (Avalon Peninsula, Cape Shore); Eastern (Middle Ridge, Mt. Peyton, Pot Hill); West/Central (Merasheen Is., Lapoile, Buchans, Grey River, Gaff Topsails); North/West and Northern (Adies Lake, Hampden Downs, N. Peninsula, St. Anthony)

		Observed Partition			
Variance Component		Variance	% Total	p[a]	Φ-statistics
Among Regions	σ^2_a	0.23250	10.58	<0.00000	Φ_{CT} = 0.10576
Among Herds/Regions	σ^2_b	0.07475	3.40	<0.00000	Φ_{SC} = 0.03803
Within Herds	σ^2_c	1.89103	86.02	<0.00000	Φ_{ST} = 0.13977

SCENARIO 3 - Four Geographical Regions

Avalon (Avalon Peninsula, Cape Shore); Interior (Middle Ridge, Mt. Peyton, Pot Hill, Merasheen Is., Lapoile, Buchans, Grey River, Gaff Topsails, Hampden Downs, St. Anthony); Northern Peninsula (N. Peninsula,), Humber River (Adies Lake)

		Observed Partition			
Variance Component		Variance	% Total	p[a]	Φ-statistics
Among Regions	σ^2_a	0.19962	8.89	<0.00000 - 0.02835	Φ_{CT} = 0.08891
Among Herds/Regions	σ^2_b	0.15452	6.88	<0.00000	Φ_{SC} = 0.07554
Within Herds	σ^2_c	1.89103	84.23	<0.00000	Φ_{ST} = 0.15774

SCENARIO 4 - Three Geographical Regions

Avalon (Avalon Peninsula, Cape Shore); Central (Middle Ridge, Mt. Peyton, Pot Hill, Merasheen Is., Lapoile, Buchans, Grey River, Gaff Topsails, Adies Lake, Hampden Downs); Northern (N. Peninsula, St. Anthony)

		Observed Partition			
Variance Component		Variance	% Total	p[a]	Φ-statistics
Among Regions	σ^2_a	0.18407	8.23	<0.00000 - 0.01955	Φ_{CT} = 0.08230
Among Herds/Regions	σ^2_b	0.16481	7.37	<0.00000	Φ_{SC} = 0.08029
Within Herds	σ^2_c	1.88773	84.40	<0.00000	Φ_{ST} = 0.15599

SCENARIO 5 - Two Geographical Regions

East (Avalon Peninsula, Cape Shore, Middle Ridge, Pot Hill, Mt. Peyton);
West (St. Anthony, N. Peninsula, Adies Lake, Hampden Downs, Gaff Topsails, Buchans, Lapoile, Grey River, Merasheen Is.)

		Observed Partition			
Variance Component		Variance	% Total	p[a]	Φ-statistics
Among Regions	σ^2_a	0.18578	8.31	<0.00000	Φ_{CT} = 0.08310
Among Herds/Regions	σ^2_b	0.16200	7.25	<0.00000	Φ_{SC} = 0.07903
Within Herds	σ^2_c	1.88773	84.44	<0.00000	Φ_{ST} = 0.15557

SCENARIO 6 - Two Geographical Regions

Avalon (Avalon Peninsula, Cape Shore); Rest of Island (St. Anthony, N. Peninsula, Adies Lake, Hampden Downs, Gaff Topsails, Buchans, Lapoile, Grey River, Merasheen Is., Middle Ridge, Pot Hill, Mt. Peyton)

		Observed Partition			
Variance Component		Variance	% Total	p[a]	Φ-statistics
Among Regions	σ^2_a	0.40741	16.64	<0.00000 - 0.01466	Φ_{CT} = 0.16636
Among Herds/Regions	σ^2_b	0.15375	6.28	<0.00000	Φ_{SC} = 0.07531
Within Herds	σ^2_c	1.88773	77.09	<0.00000	Φ_{ST} = 0.22915

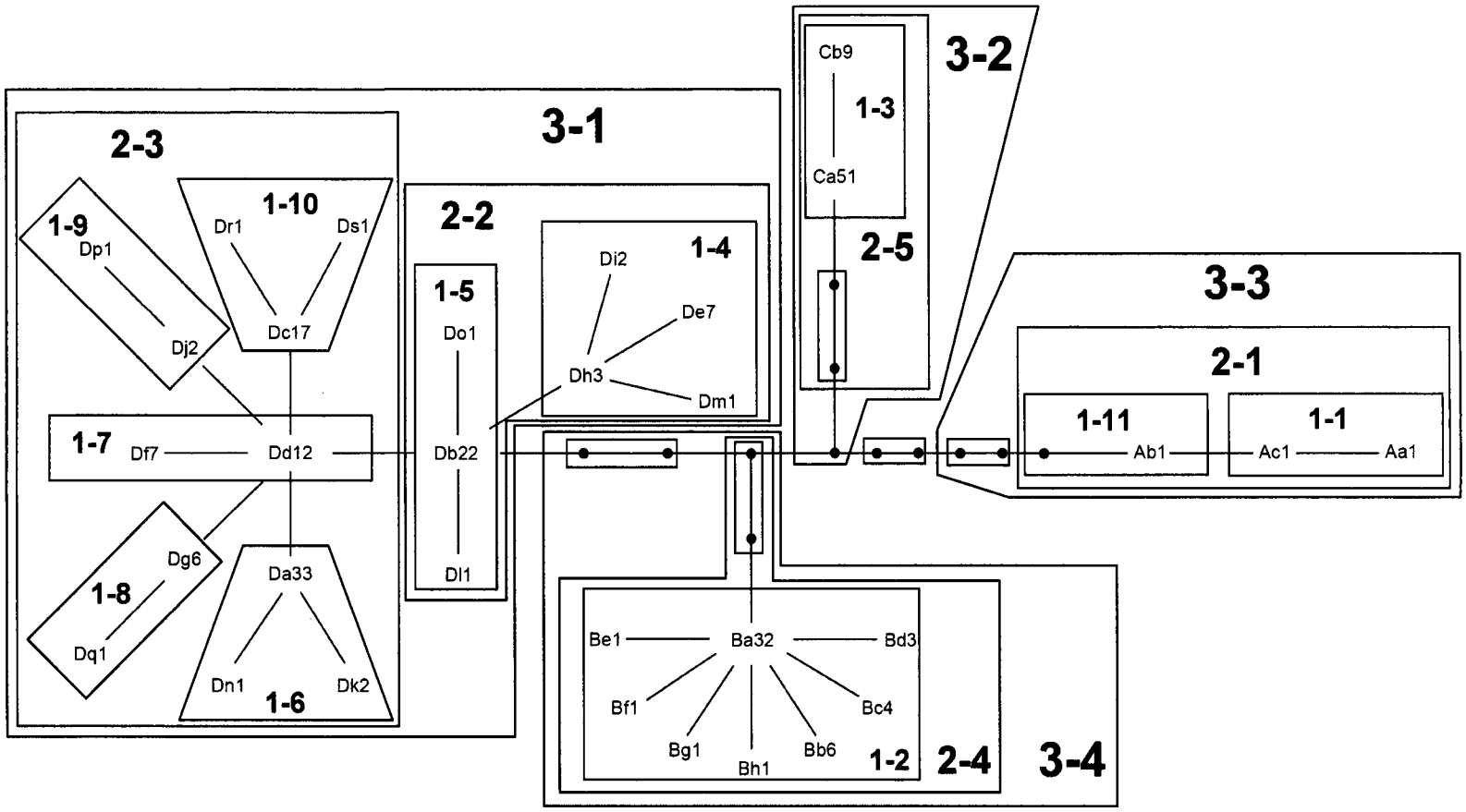


Figure 15. Nesting design for Newfoundland caribou mtDNA haplotypes used in the Nested Clade Analysis. Each line between haplotypes or nodes (black circles) represents a mutational change. The nested design includes eleven 1-step Clades (1-1 to 1-11). The 1-step Clades are nested together into five 2-step Clades (2-1 to 2-5). The 2-step Clades are nested together into four 3-step Clades (3-1 to 3-4).

Table 17. Coordinates of geographical centres of herds used in Nested Clade Analysis. West Longitude (-) coordinates and North Latitude (+) coordinates.

Herd	N	Decimal Degree (West, North)	Deg-Min-Sec (West, North)
St. Anthony	17	(-56.24, 51.27)	(-56° 14' 10", 51° 15' 58")
N. Peninsula	18	(-56.94, 50.26)	(-56° 56' 36", 50° 15' 44")
Adies Lake	18	(-57.20, 49.50)	(-57° 12' 1", 49° 29' 51")
Hampden Downs	6	(-56.65, 49.58)	(-56° 38' 59, 49° 34' 48")
Gaff Topsails	20	(-56.62, 49.12)	(-56° 37' 6", 49° 7' 16")
Buchans	17	(-57.44, 48.54)	(-57° 26' 6", 48° 32' 22")
Merasheen Is.*	20	(-57.44, 48.54)	(-57° 26' 6", 48° 32' 22")
Lapoile	18	(-58.30, 48.05)	(-58° 18' 0", 48° 2' 53")
Grey River	19	(-56.52, 48.25)	(-56° 31' 8", 48° 14' 57")
Pot Hill	18	(-55.75, 48.47)	(-55° 45' 1", 48° 28' 19")
Mount Peyton	14	(-55.20, 48.85)	(-55° 11' 55", 48° 51' 6")
Middle Ridge	18	(-55.06, 48.17)	(-55° 3' 30", 48° 9' 55")
Avalon	18	(-52.79, 47.33)	(-53° 14' 1", 47° 2' 22")
Cape Shore	12	(-53.89, 47.06)	(-53° 53' 9", 47° 3' 47")

* The geographical centre of Buchans was used in place of the actual coordinates of Merasheen Island since the Merasheen Island population was recently introduced from Buchans. The actual coordinates for Merasheen Island are Dec. Deg (-54.19, 47.53), Deg-Min-Sec (-54° 11' 37", 47° 31' 49").

Table 18. Geographical centres of clades calculated in the Nested Clade Analysis (Decimal Degrees). Distances between centres are used in the geographic distance analysis. Clades with significant phylogeographic associations are highlighted in bold.

Clade	Subclade	West	North
1-1		-56.8561	49.3087
	Aa	-57.4400	48.5400
	Ac	-56.6500	49.5800
1-2		-56.4479	49.2285
	Ba	-56.5843	49.4027
	Bb	-56.5084	49.3660
	Bc	-55.1424	48.5700
	Bd	-56.8627	48.2568
	Be	-56.9400	50.2600
	Bf	-55.7500	48.4700
	Bg	-55.7500	48.4700
	Bh	-56.5200	48.2500
1-3		-57.0559	49.0909
	Ca	-57.0450	49.0724
	Cb	-57.1228	49.2040
1-4		-53.8748	47.7519
	De	-52.7900	47.3300
	Dh	-54.2493	47.8700
	Di	-55.1424	48.5700
	Dm	-57.4400	48.5400
1-5		-53.7280	47.2578
	Db	-53.4971	47.1564
	Dl	-58.3000	48.0500
	Do	-55.2000	48.8500
1-6		-56.6588	49.2768
	Da	-56.6984	49.2387
	Dk	-55.7500	48.4700
	Dn	-56.2400	51.2700
1-7		-56.8454	48.9160
	Dd	-56.9330	49.2129
	Df	-56.6733	48.3330
1-8		-55.6645	48.6053
	Dg	-55.7569	48.5567
	Dq	-55.2000	48.8500
1-9		-56.3109	48.4909
	Dj	-56.5563	48.5000
	Dp	-55.7500	48.4700
1-10		-56.2177	48.5739
	Dc	-56.2635	48.6145
	Dr	-55.0600	48.1700
	Ds	-56.5200	48.2500
2-1		-56.7268	49.7145
	1-1	-56.8561	49.3087
	1-11	-56.2400	51.2700
2-2		-53.7722	47.4066
	1-4	-53.8748	47.7519
	1-5	-53.7280	47.2578
2-3		-56.4940	48.9425
	1-6	-56.6200	49.2500
	1-7	-56.8454	48.9160
	1-8	-55.6645	48.6053
	1-9	-56.3109	48.4909
	1-10	-56.2177	48.5739
3-1		-55.5935	48.4344
	2-2	-53.7722	47.4066
	2-3	-56.4940	48.9425

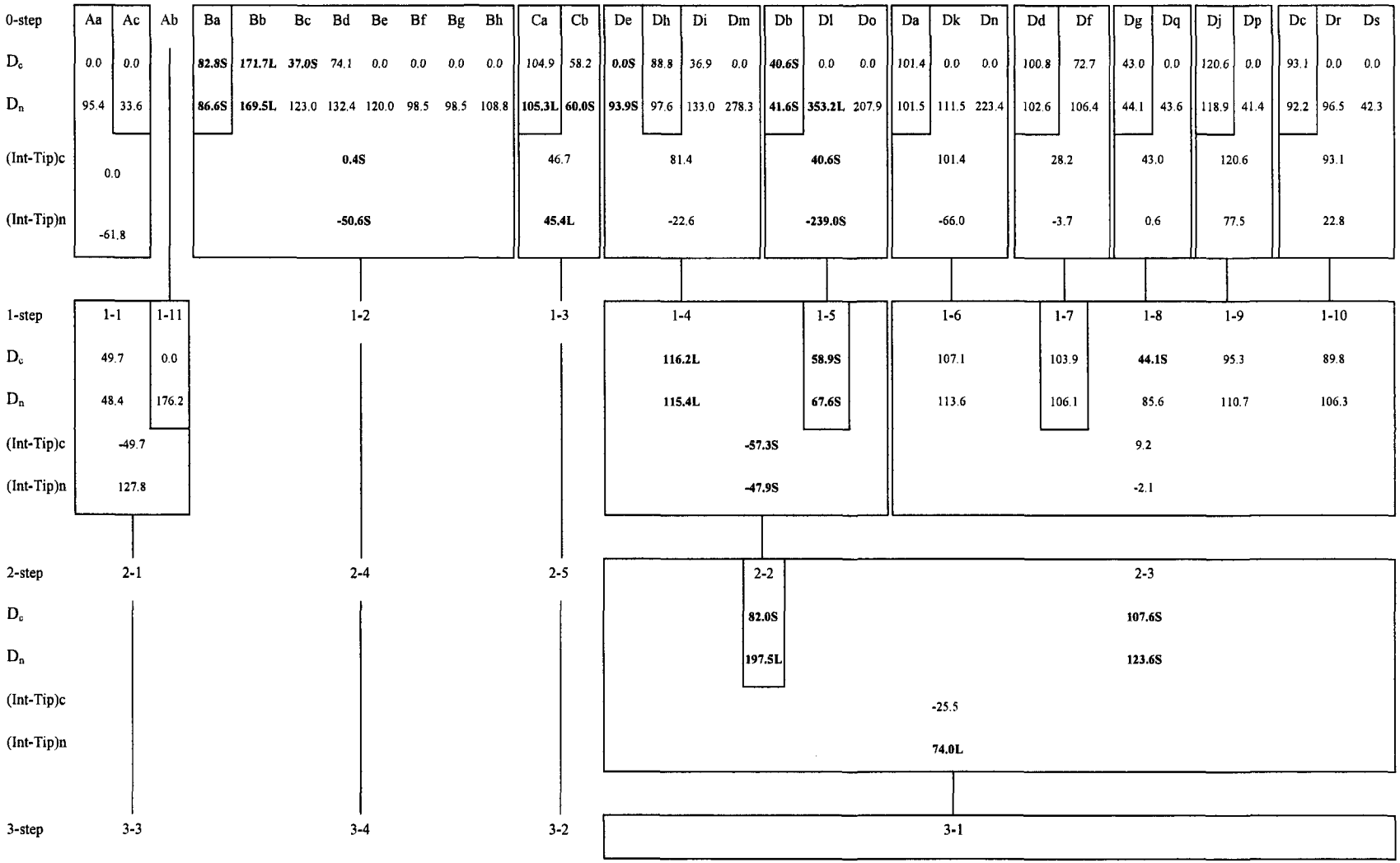

0-step
Aa Ac Ab Ba Bb Bc Bd Be Bf Bg Bh Ca Cb De Dh Di Dm Db Dl Do Da Dk Dn Dd Df Dg Dq Dj Dp Dc Dr Ds
Dc
0.0 0.0 82.8S 171.7L 37.0S 74.1 0.0 0.0 0.0 0.0 104.9 58.2 0.0S 88.8 36.9 0.0 40.6S 0.0 0.0 101.4 0.0 0.0 100.8 72.7 43.0 0.0 120.6 0.0 93.1 0.0 0.0
Dn
95.4 33.6 86.6S 169.5L 123.0 132.4 120.0 98.5 98.5 108.8 105.3L 60.0S 93.9S 97.6 133.0 278.3 41.6S 353.2L 207.9 101.5 111.5 223.4 102.6 106.4 44.1 43.6 118.9 41.4 92.2 96.5 42.3
(Int-Tip)c
0.0 0.4S 46.7 81.4 40.6S 101.4 28.2 43.0 120.6 93.1
(Int-Tip)n
-61.8 -50.6S 45.4L -22.6 -239.0S -66.0 -3.7 0.6 77.5 22.8
1-step
1-1 1-11 1-2 1-3 1-4 1-5 1-6 1-7 1-8 1-9 1-10
Dc
49.7 0.0 116.2L 58.9S 107.1 103.9 44.1S 95.3 89.8
Dn
48.4 176.2 115.4L 67.6S 113.6 106.1 85.6 110.7 106.3
(Int-Tip)c
-49.7 -57.3S 9.2
(Int-Tip)n
127.8 -47.9S -2.1
2-step
2-1 2-4 2-5 2-2 2-3
Dc
82.0S 107.6S
Dn
197.5L 123.6S
(Int-Tip)c
-25.5
(Int-Tip)n
74.0L
3-step
3-3 3-4 3-2 3-1

Figure 16. Results of the Nested Clade Analysis for mtDNA haplotypes of Newfoundland caribou. Haplotypes are listed across the top and are boxed together to reflect the 1-step nesting design as shown in Figure 15. Groupings further down reflect the nesting structure of the higher clades. The D_c and D_n values are the clade and nested clade distances, respectively. Interior clades are outlined with a box, while tip clades are left blank. The $(Int\text{-}Tip)_c$ and the $(Int\text{-}Tip)_n$ are the average distances between interior clades and tip clades within the nested group for clade distances, and nested clade distances, respectively. Significant values ($p < 0.05$) are highlighted in bold, the "S" designation indicates the distance is significantly small at the 5% level and the "L" designation indicates that the distance is significantly large.

Table 19. Chain of Inference followed using the Inference Key and the results of the Nested Clade Analysis, shown in Figure 16.

Clade	Chain of Inference	Inference
1-2 (B)	1No-2No-11Yes(a,b,c)-12No	Contiguous range expansion
1-3 (C)	1No-2No-11No-17Yes(a,b,c)-4No	Restricted gene flow with isolation by distance
1-4 (D)	1No-2Yes(a)-3No-4No	Restricted gene flow with isolation by distance
1-5 (D)	1Yes-19Yes-20Yes-2No-11Yes-12No	Contiguous range expansion
2-2 (D)	1No-2No-11Yes(a,b,c)-12No	Contiguous range expansion
2-3 (D)	1No-2Yes(a)-3No-4No	Restricted gene flow with isolation by distance
3-1 (D)	1No-2No-11Yes(b)-12Yes-13Yes	Long distance colonization possibly coupled with subsequent fragmentation or Past fragmentation followed by range expansion

Figure 17 (a). Distribution of haplotypes Ca and Cb in Clade 1-3. Population structure may be due to restricted gene flow with isolation by distance. Black = interior haplotypes, colour = tip haplotypes.

Figure 17 (b). Distribution of haplotypes De, Dh, Di, Dm in Clade 1-4. Population structure may be due to restricted gene flow with isolation by distance. Black = interior haplotypes, colour = tip haplotypes.

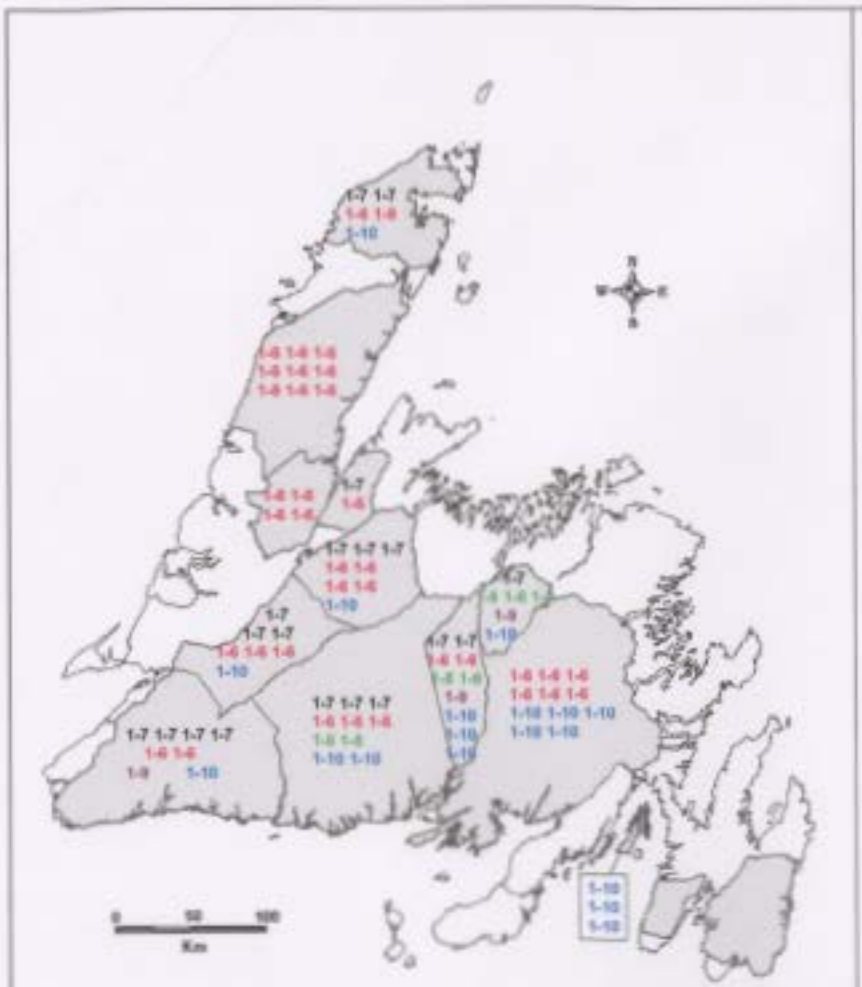


Figure 17 (c). Distribution of sub-clades 1-6, 1-7, 1-8, 1-9, and 1-10 in Clade 2-3. Population structure may be due to restricted gene flow with isolation by distance. Black = interior sub-clades, colour = tip sub-clades.

Figure 17 (d). Distribution of haplotypes Ba through Bh in Clade 1-2. Significant phylogeographic associations may be due to contiguous range expansion. Black = interior haplotypes, colour = tip haplotypes.

Figure 17 (e). Distribution of haplotypes Db, Do and Dl in Clade 1-5. Significant phylogeographic associations may be due to contiguous range expansion. Black = interior haplotypes, colour = tip haplotypes.

Figure 17 (f). Distribution of sub-clades 1-4 and 1-5 in Clade 2-2. Significant phylogeographic associations may be due to contiguous range expansion. Black = interior sub-clades, colour = tip sub-clades.

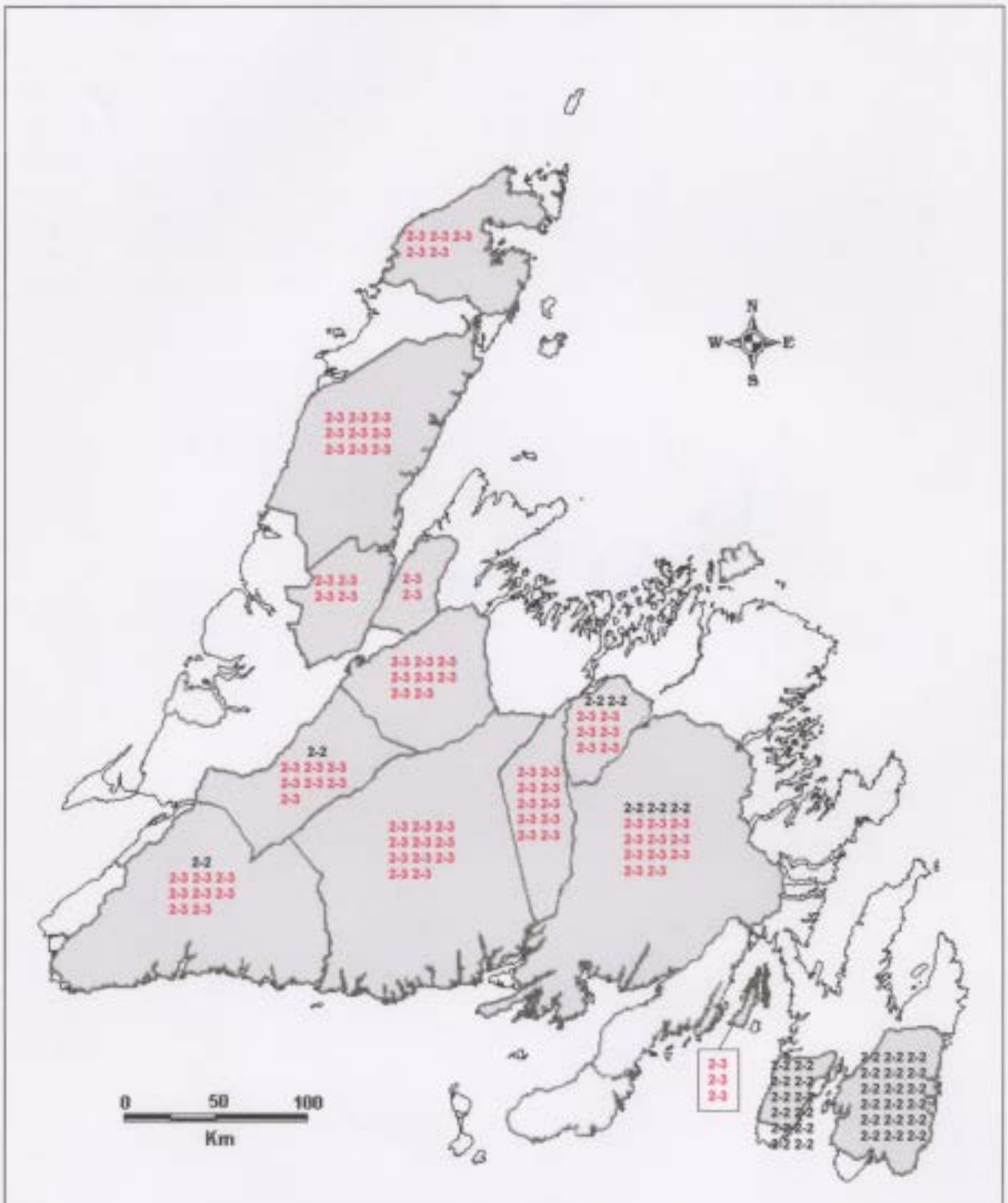


Figure 17 (g). Distribution of sub-clades 2-2 and 2-3 in Clade 3-1. Significant phylogeographic associations may be due to long distance colonization possibly coupled with subsequent fragmentation or past fragmentation followed by range expansion. Black = interior sub-clades, colour = tip sub-clades.

Figure 17. Distribution of haplotypes/sub-clades across the island of Newfoundland. Figures a, b and c, representing Clades 1-3, 1-4 and 2-3, respectively have population structure which may be due to restricted gene flow with isolation by distance. Figures d, e, and f, representing Clades 1-2, 1-5, and 2-2, respectively, have significant phylogeographic associations, which may be due to contiguous range expansion. Figure g, representing Clade 3-1, has significant phylogeographic associations, which may due to long distance colonization possibly coupled with subsequent fragmentation or past fragmentation followed by range expansion.

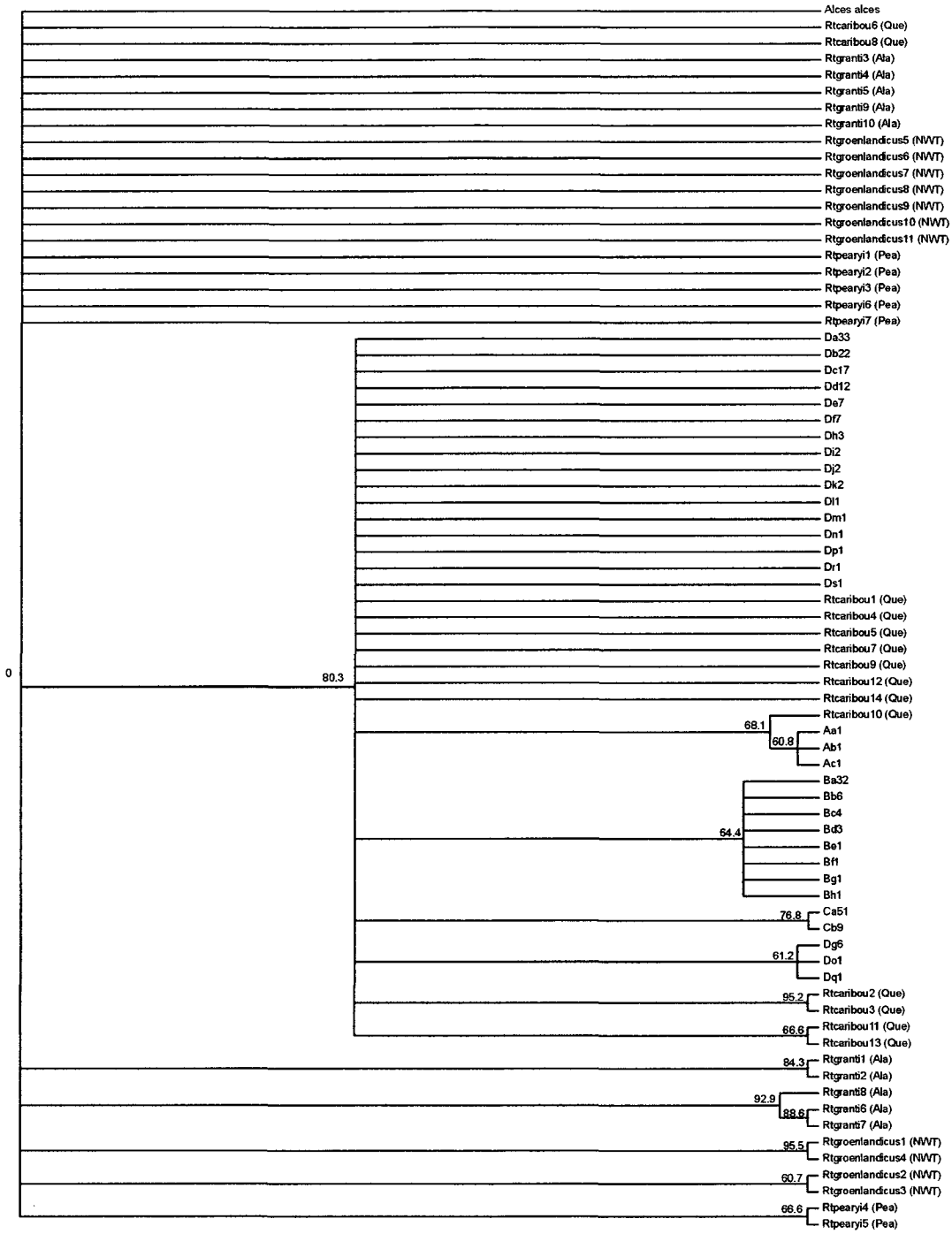

Alces alces
Rtcaribou6 (Que)
Rtcaribou8 (Que)
Rtgranti3 (Ala)
Rtgranti4 (Ala)
Rtgranti5 (Ala)
Rtgranti9 (Ala)
Rtgranti10 (Ala)
Rtgroenlandicus5 (NWT)
Rtgroenlandicus6 (NWT)
Rtgroenlandicus7 (NWT)
Rtgroenlandicus8 (NWT)
Rtgroenlandicus9 (NWT)
Rtgroenlandicus10 (NWT)
Rtgroenlandicus11 (NWT)
Rtpearyi1 (Pea)
Rtpearyi2 (Pea)
Rtpearyi3 (Pea)
Rtpearyi6 (Pea)
Rtpearyi7 (Pea)
Da33
Db22
Dc17
Dd12
De7
Df7
Dh3
Di2
Dj2
Dk2
Dl1
Dm1
Dn1
Dp1
Dr1
Ds1
Rtcaribou1 (Que)
Rtcaribou4 (Que)
Rtcaribou5 (Que)
Rtcaribou7 (Que)
Rtcaribou9 (Que)
Rtcaribou12 (Que)
Rtcaribou14 (Que)
Rtcaribou10 (Que)
Aa1
Ab1
Ac1
Ba32
Bb6
Bc4
Bd3
Be1
Bf1
Bg1
Bh1
Ca51
Cb9
Dg6
Do1
Dq1
Rtcaribou2 (Que)
Rtcaribou3 (Que)
Rtcaribou11 (Que)
Rtcaribou13 (Que)
Rtgranti1 (Ala)
Rtgranti2 (Ala)
Rtgranti8 (Ala)
Rtgranti6 (Ala)
Rtgranti7 (Ala)
Rtgroenlandicus1 (NWT)
Rtgroenlandicus4 (NWT)
Rtgroenlandicus2 (NWT)
Rtgroenlandicus3 (NWT)
Rtpearyi4 (Pea)
Rtpearyi5 (Pea)
0
80.3
68.1
60.8
64.4
76.8
61.2
95.2
66.6
84.3
92.9
88.6
95.5
60.7
66.6

Figure 18. Neighbour Joining 50% majority rule consensus tree of 32 Newfoundland caribou mtDNA haplotypes and 42 selected mtDNA haplotypes of North American caribou (from Flagstad and Røed 2003). Tree is constructed from 470 bp of sequence data from the Control Region of the mitochondrial genome. Haplotypes Aa through Ds represent the 32 Newfoundland haplotypes from the present study. Haplotypes from Flagstad and Røed (2003) include 4 caribou subspecies from 4 locales; *R. t. caribou* from Quebec (Que), *R. t. granti* from Alaska (Ala), *R. t. groenlandicus* from North West Territories (NWT), and *R. t. pearyi* from Peary Islands, Canadian Archipelago (Pea). Moose (*Alces alces*) is included in the analysis as an outgroup.

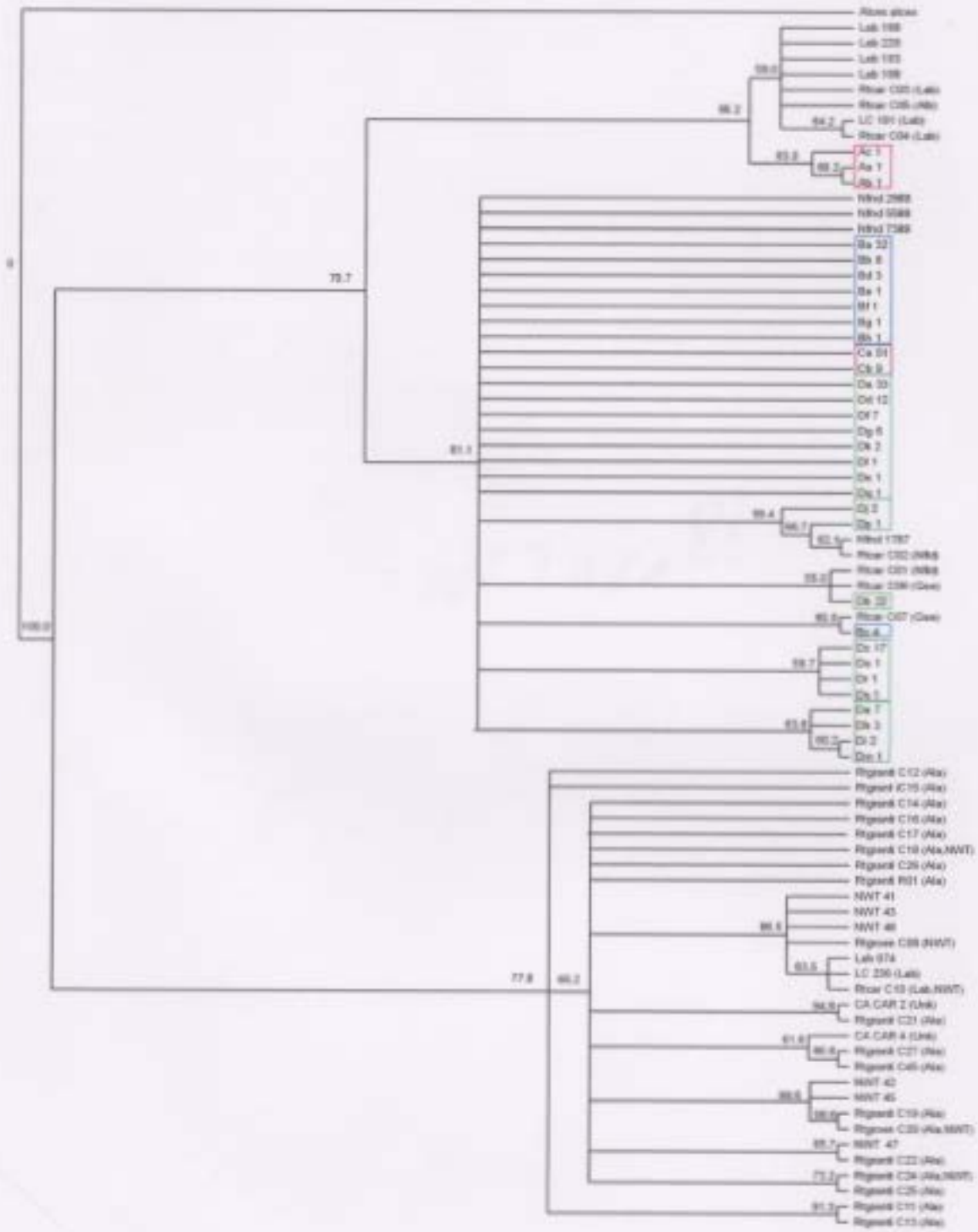


Figure 19. Neighbour Joining 50% majority rule consensus tree of 32 Newfoundland caribou mtDNA haplotypes and 46 selected mtDNA haplotypes of North American caribou from Cronin et al. (2005) and Cronin and Patton (2002). Tree is constructed from 1143 bp of sequence data from the cytochrome *b* gene in the mitochondrial genome. Haplotypes Aa through Ds represent the 32 Newfoundland haplotypes from the present study. Haplotypes from Cronin et al. (2005) include 3 caribou subspecies, Rtcar (*R. t. caribou)*, Rtgranti (*R. t. granti)*, and Rtgroen *(R. t. groenlandicus)*, from 6 locales; George River, Labrador (Lab), Newfoundland (Nfld), Alberta (Alb), Val d'Or, Quebec (Que), Alaska (Ala) and the North West Territories (NWT). Haplotypes from Cronin and Patton (2002) include: woodland caribou (*R. t. caribou*) from Newfoundland (Nfnd) and Labrador (Lab, LC); and tundra caribou (*R. t. groenlandicus*) from the North West Territories (NWT) and unknown parts of northern Canada (CA CAR Unk). Moose (*Alces alces*) is included in the analysis as an outgroup.

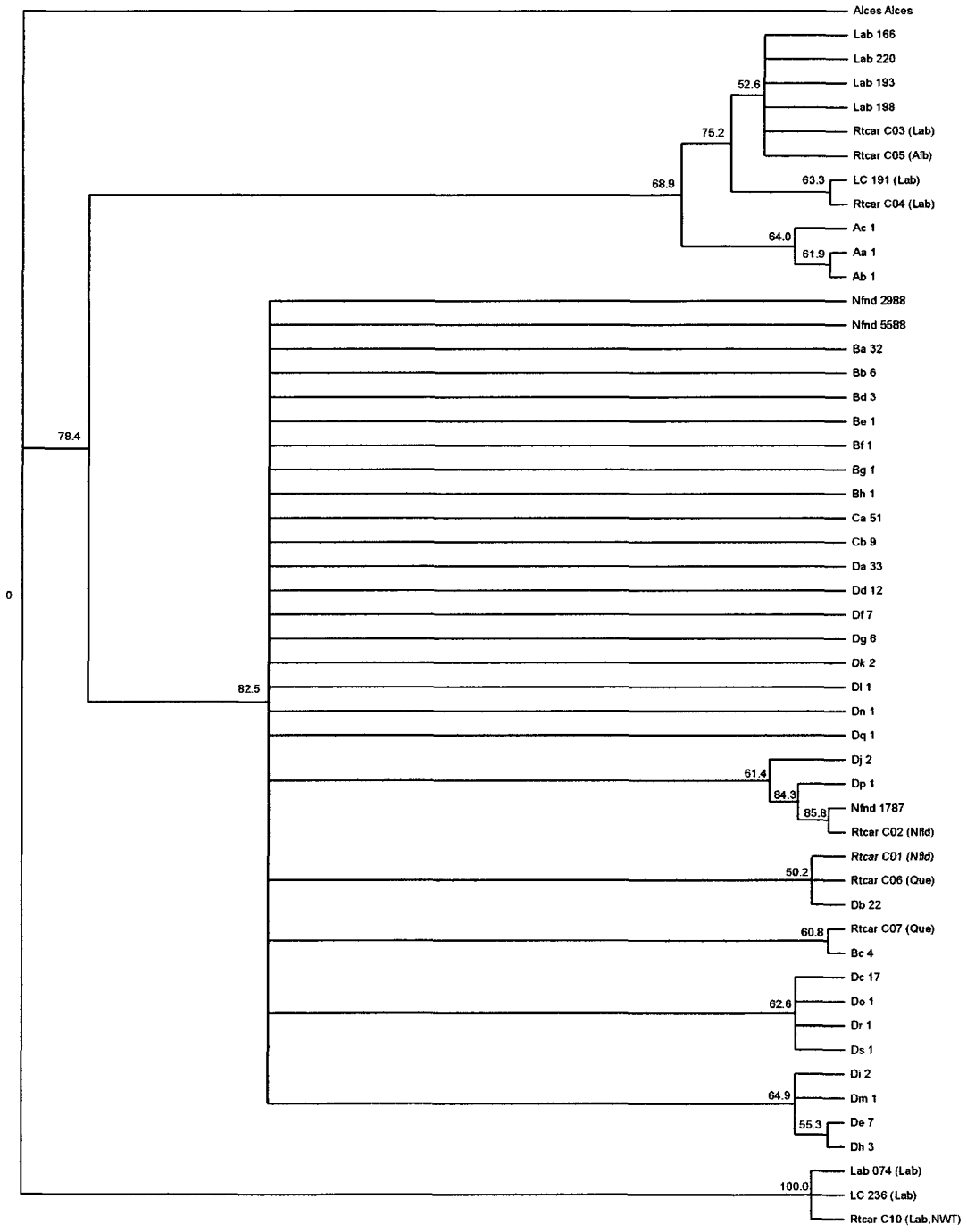

Alces Alces
Lab 166
Lab 220
52.6
Lab 193
Lab 198
75.2
Rtcar C03 (Lab)
Rtcar C05 (Alb)
68.9
63.3
LC 191 (Lab)
Rtcar C04 (Lab)
Ac 1
64.0
61.9
Aa 1
Ab 1
Nfnd 2988
Nfnd 5588
Ba 32
Bb 6
Bd 3
Be 1
78.4
Bf 1
Bg 1
Bh 1
Ca 51
Cb 9
Da 33
0
Dd 12
Df 7
Dg 6
Dk 2
82.5
Dl 1
Dn 1
Dq 1
Dj 2
61.4
Dp 1
84.3
85.8
Nfnd 1787
Rtcar C02 (Nfld)
Rtcar C01 (Nfld)
50.2
Rtcar C06 (Que)
Db 22
60.8
Rtcar C07 (Que)
Bc 4
Dc 17
62.6
Do 1
Dr 1
Ds 1
Di 2
64.9
Dm 1
55.3
De 7
Dh 3
Lab 074 (Lab)
100.0
LC 236 (Lab)
Rtcar C10 (Lab,NWT)

Figure 20. Neighbour Joining 50% majority rule consensus tree of 32 Newfoundland caribou mtDNA haplotypes and 18 selected mtDNA haplotypes of North American woodland caribou (*R. t. tarandus*) from Cronin et al. (2005) and Cronin and Patton (2002). Tree is constructed from 1143 bp of sequence data from the cytochrome *b* gene in the mitochondrial genome. Haplotypes Aa through Ds represent the 32 Newfoundland haplotypes from the present study. Haplotypes from Cronin et al. (2005) include 8 woodland caribou from 4 locales; George River, Labrador (Lab), Newfoundland (Nfld), Alberta (Alb), Val d'Or, Quebec (Que). Haplotypes from Cronin and Patton (2002) include: 10 woodland caribou (*R. t. caribou*) from Newfoundland (Nfnd) and Labrador (Lab, LC). Moose (*Alces alces*) is included in the analysis as an outgroup.

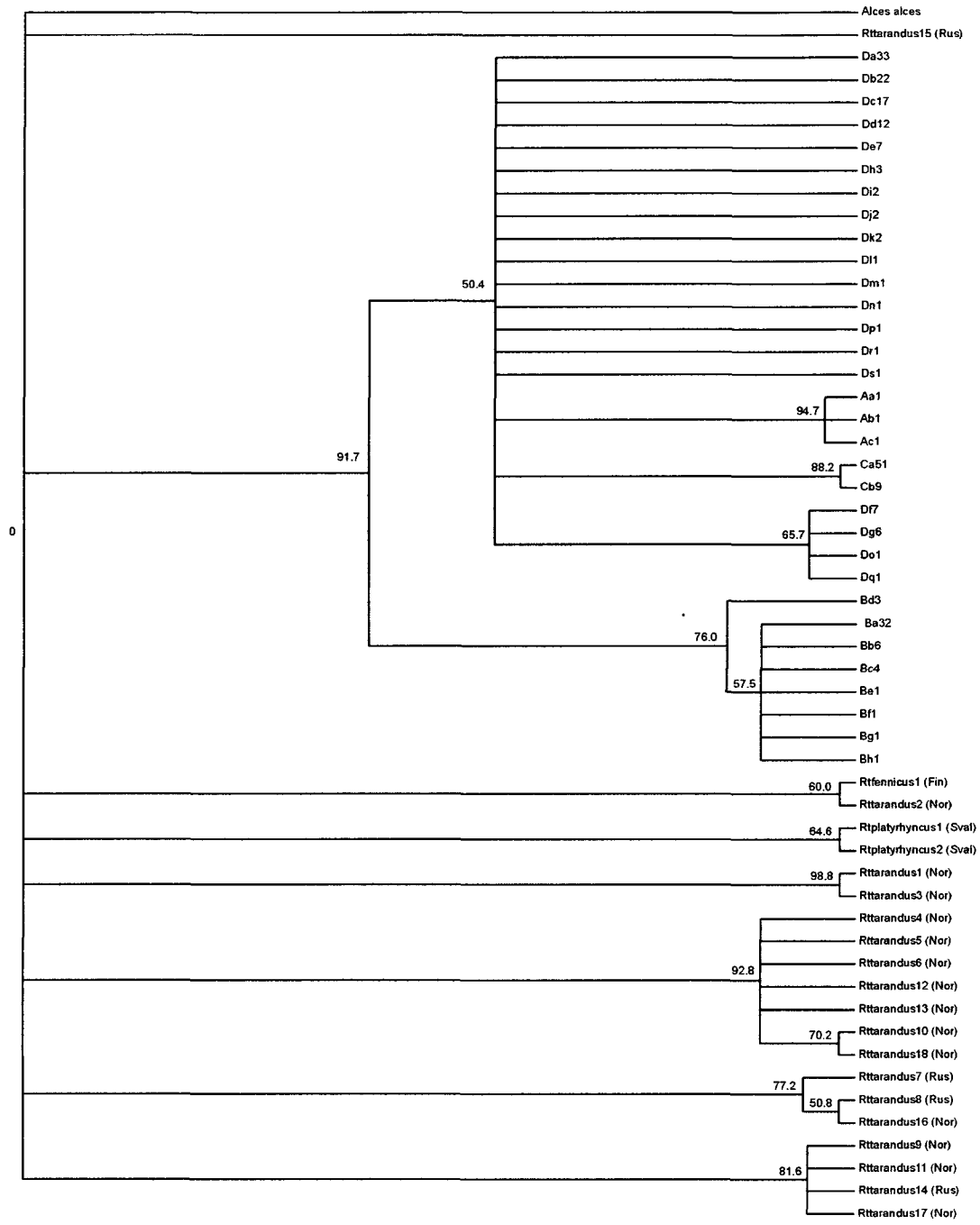

Alces alces
Rttarandus15 (Rus)
Da33
Db22
Dc17
Dd12
De7
Dh3
Di2
Dj2
Dk2
Dl1
Dm1
Dn1
Dp1
Dr1
Ds1
Aa1
Ab1
Ac1
Ca51
Cb9
Df7
Dg6
Do1
Dq1
Bd3
Ba32
Bb6
Bc4
Be1
Bf1
Bg1
Bh1
Rtfennicus1 (Fin)
Rttarandus2 (Nor)
Rtplatyrhyncus1 (Sval)
Rtplatyrhyncus2 (Sval)
Rttarandus1 (Nor)
Rttarandus3 (Nor)
Rttarandus4 (Nor)
Rttarandus5 (Nor)
Rttarandus6 (Nor)
Rttarandus12 (Nor)
Rttarandus13 (Nor)
Rttarandus10 (Nor)
Rttarandus18 (Nor)
Rttarandus7 (Rus)
Rttarandus8 (Rus)
Rttarandus16 (Nor)
Rttarandus9 (Nor)
Rttarandus11 (Nor)
Rttarandus14 (Rus)
Rttarandus17 (Nor)
0
91.7
50.4
94.7
88.2
65.7
76.0
57.5
60.0
64.6
98.8
92.8
70.2
77.2
50.8
81.6

Figure 21. Neighbour Joining 50% majority rule consensus tree of 32 Newfoundland caribou mtDNA haplotypes and 21 selected mtDNA haplotypes of Eurasian reindeer (from Flagstad and Røed 2003). Tree is constructed from 470 bp of sequence data from the Control Region of the mitochondrial genome. Haplotypes Aa through Ds represent the 32 Newfoundland haplotypes from the present study. Haplotypes from Flagstad and Røed (2003) include 3 reindeer subspecies from 4 locales; *R. t. tarandus* from Norway (Nor) and Russia (Rus), *R. t. platyrhynchus* from Svalbard (Sval), and *R. t. fennicus* from Southeastern Finland (Fin). Moose (*Alces alces*) is included in the analysis as an outgroup.

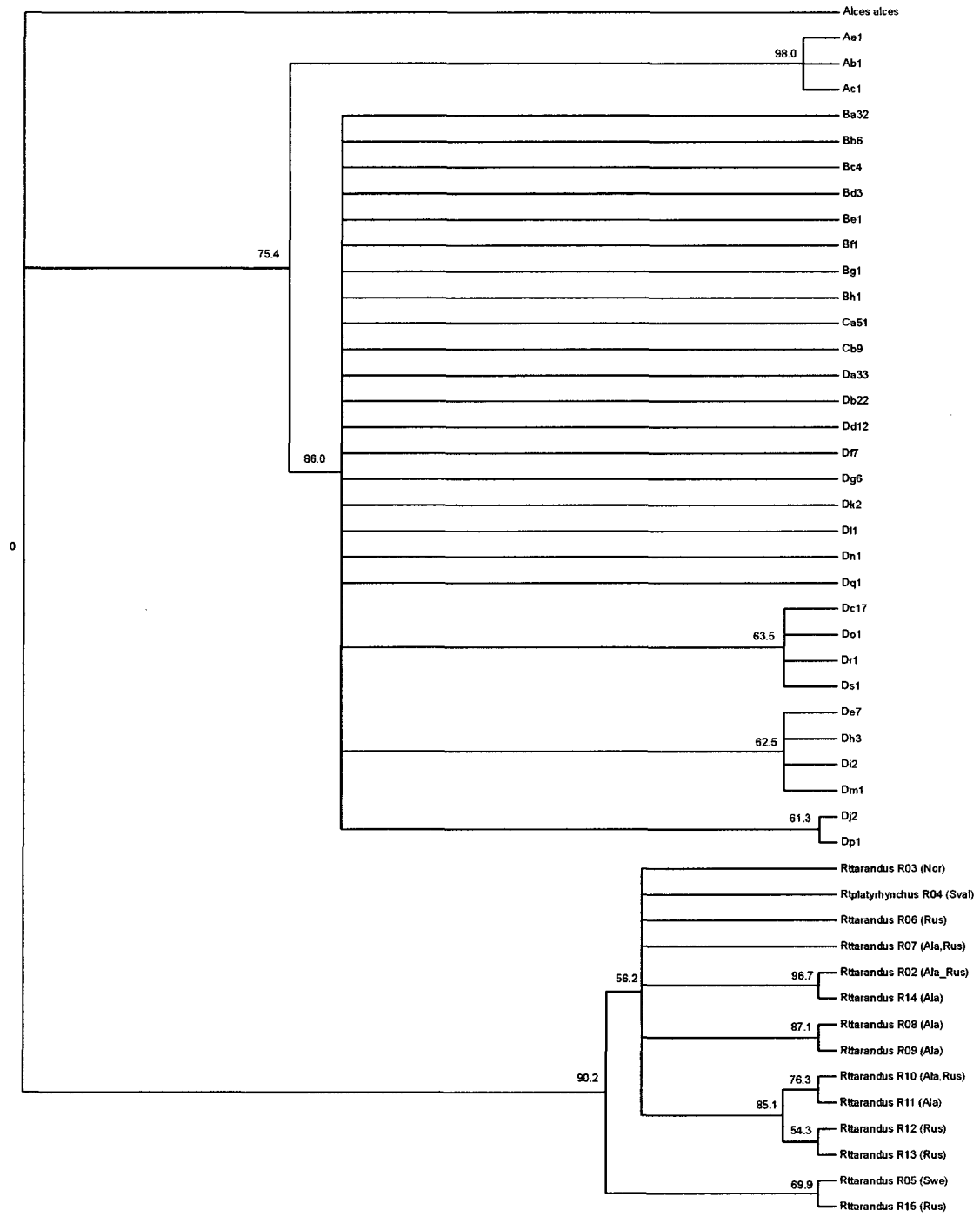

Alces alces
Aa1
98.0
Ab1
Ac1
Ba32
Bb6
Bc4
Bd3
Be1
Bf1
75.4
Bg1
Bh1
Ca51
Cb9
Da33
Db22
Dd12
86.0
Df7
Dg6
Dk2
Dl1
0
Dn1
Dq1
Dc17
63.5
Do1
Dr1
Ds1
De7
62.5
Dh3
Di2
Dm1
61.3
Dj2
Dp1
Rttarandus R03 (Nor)
Rtplatyrhynchus R04 (Sval)
Rttarandus R06 (Rus)
Rttarandus R07 (Ala,Rus)
56.2
96.7
Rttarandus R02 (Ala_Rus)
Rttarandus R14 (Ala)
87.1
Rttarandus R08 (Ala)
Rttarandus R09 (Ala)
90.2
76.3
Rttarandus R10 (Ala,Rus)
85.1
Rttarandus R11 (Ala)
54.3
Rttarandus R12 (Rus)
Rttarandus R13 (Rus)
69.9
Rttarandus R05 (Swe)
Rttarandus R15 (Rus)

Figure 22. Neighbour Joining 50% majority rule consensus tree of 32 Newfoundland caribou mtDNA haplotypes and 14 selected mtDNA haplotypes of Eurasian reindeer (from Cronin et al. 2006). Tree is constructed from 1111 bp of sequence data from the cytochrome *b* gene in the mitochondrial genome. Haplotypes Aa through Ds represent the 32 Newfoundland haplotypes from the present study. Haplotypes from Cronin et al. (2006) include 2 reindeer subspecies from 5 locales, *Rangifer tarandus tarandus* from Alaska (Ala), Russia (Rus) and Norway (Nor) and Sweden (Swe), and *R. t. platyrhynchus* from Svalbard Island. Alaskan reindeer were introduced from Siberia, Russia. Moose (*Alces alces*) is included in the analysis as an outgroup.

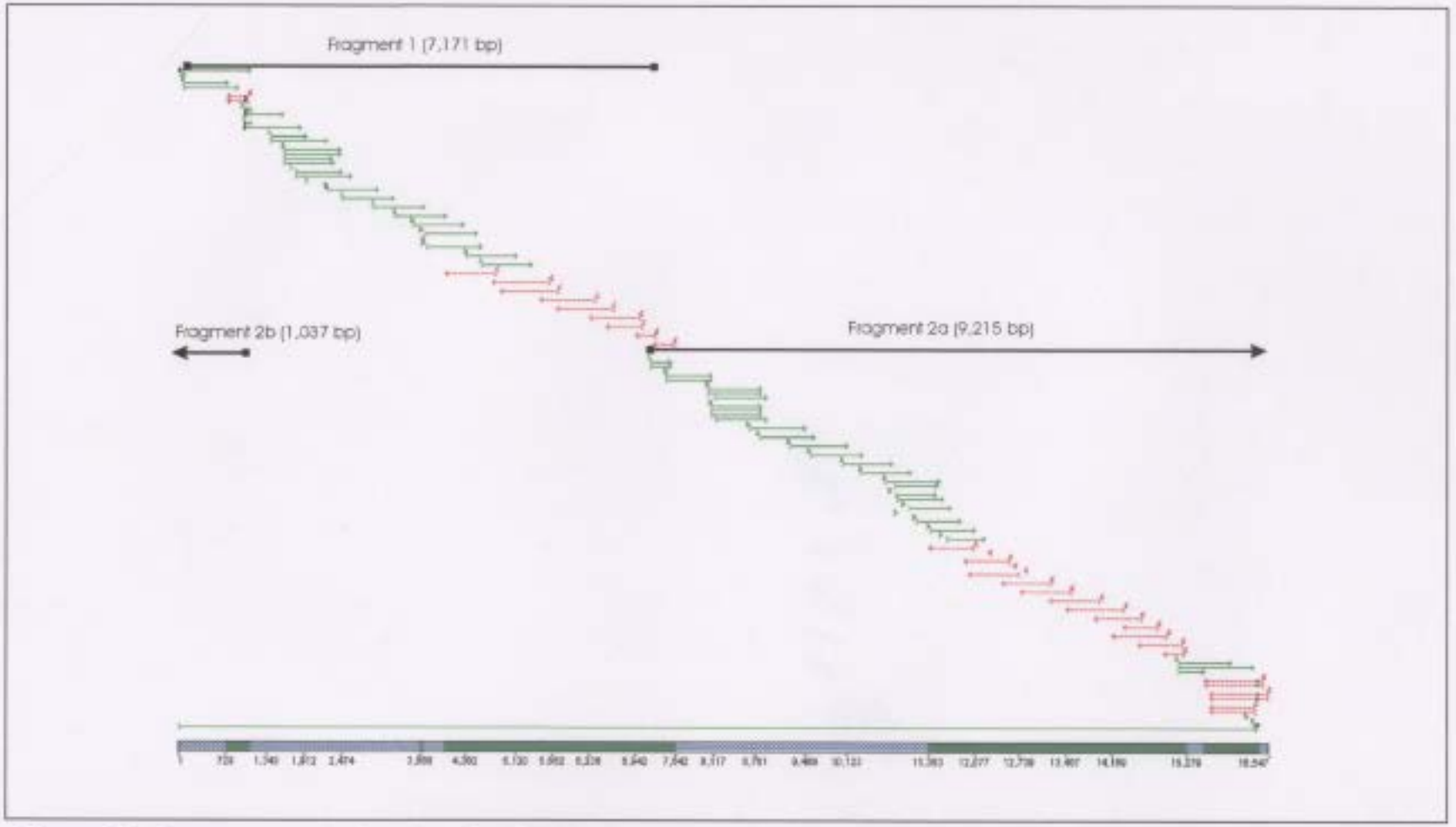


Figure 23. Sequence contig showing the individual sequences that were aligned to obtain the whole mtDNA genome for Newfoundland caribou. Green forward facing arrows represent forward sequences; red backward facing arrows represent reverse sequences. In the figure, the mtDNA genome starts with tRNA-Pro and then the Control Region, it ends with the cytochrome *b* gene and tRNA-Thr. Fragment 1 (7,171 bp) and Fragment 2 (10,252 bp) are shown.

Table 20. Summary of genes, gene length, single nucleotide polymorphism (SNP) occurrence and SNP density (Number of SNPs per 100 bp) in the mitochondrial genomes for Newfoundland woodland caribou (*Rangifer tarandus caribou*) and reindeer (*Rangifer tarandus tarandus*).

Gene	Length (bp)	SNP Occurrence	SNP Density
12sRNA	955	1	0.10
16sRNA	1570	4	0.25
ND1	957	9	0.94
ND2	1044	15	1.44
COX1	1545	14	0.91
COX2	684	5	0.73
ATPase8	201	1	0.50
ATPase6	681	8	1.17
COX3	785	10	1.27
ND3	346	1	0.29
ND4L	297	1	0.34
ND4	1378	21	1.52
ND5	1821	15	0.82
ND6	528	3	0.57
Cytb	1142	15	1.31
CR	927	26	2.80
tRNAs	1504	16	1.06
Total	**16365**	**165**	**16.04**

4.0 Discussion

4.1 Distinct mtDNA Clades Present in the Newfoundland Caribou Population

The Newfoundland caribou population is genetically variable, comprising 32 mtDNA haplotypes structured into 4 distinct clades or haplogroups of differing ages and complexity. Clade D is the most numerous, diverse and complex clade, containing the greatest number of individuals (n=121), the greatest number of haplotypes (n=19) and the most complex structure. Accordingly, clade D is also geographically widespread in Newfoundland, and is detected in all 14 herds sampled in the study. The patterns indicate that caribou that are members of clade D have ancient matrilineal subdivisions and may have dispersed throughout Newfoundland through variety of methods, including; restricted gene flow with isolation by distance, contiguous range expansion, and long distance colonization possibly coupled with subsequent fragmentation of past fragmentation followed by range expansion (Nested Clade Analysis, Table 19).

Clades B and C are less abundant than clade D. Based on the phylogenies presented it is difficult to determine the relative ages of the three clades. Clade B is fairly numerous (n=49), and has 7 closely related haplotypes that radiate in a star pattern from one common haplotype (Ba). Clade B is relatively widespread throughout Newfoundland, occurring in all herds except those on the Avalon Peninsula. The close matrilineal relationships of the haplotypes suggest that caribou that are members of clade B may have maintained strong genetic contact in recent time and may have dispersed by means of continuous range expansion (Nested Clade Analysis, Table 19), but have not

expanded to the Avalon Peninsula. Clade C is also numerous (n=60), however, it is less diverse containing only two closely related haplotypes in which most of the individuals belong to the common haplotype (Ca). Clade C is widespread throughout western Newfoundland but declines further east toward Mount Peyton and Pot Hill, and is not detected at all in Middle Ridge or on the Avalon Peninsula. The close matrilineal relationships of caribou that belong to clade C indicate that individuals may have maintained strong genetic contact in recent time and may have dispersed by way of restricted gene flow with isolation by distance (Nested Clade Analysis, Table 19).

Clade A is genetically distant and distinct from clades B, C, and D. It includes only three individuals each with a different haplotype (n=3 haplotypes) and has a limited range among western Newfoundland herds, including, St. Anthony, Hampden Downs and Buchans. The three haplotypes are closely related to each other and yet are genetically distant from the 29 other haplotypes in the population. The presence of this rare clade is unusual and may represent a remnant lineage or a recent immigrant from a mainland population, possibly Labrador (Figures 19 and 20).

4.2 Insights into the Founding Population of Newfoundland Caribou

The mtDNA clade structure described above provides a number of insights into how caribou came to populate Newfoundland. Genetic comparisons with other North American caribou are consistent with the Newfoundland caribou being of the woodland caribou subspecies, *Rangifer tarandus caribou* and thus have maternal mtDNA origins in refugia that existed south of the Laurentide ice sheet during the last glaciation (as

explained in Section 1.2). What is not clear is where and when caribou populated the island of Newfoundland. It appears that there may have been two separate events that brought caribou to Newfoundland; one that brought caribou belonging to clades B, C and D and another that brought caribou belonging to clade A. It is also possible that one herd containing all four clades may have colonized the island.

There are two proposed routes by which caribou belonging to clades B, C, and D may have populated the island of Newfoundland; a Southern Route from glacial refugia that existed off the east coast of North America during the last ice age, or a Northern Route from Labrador coming across the Strait of Belle Isle (Figure 24). It is known that the woodland caribou subspecies originated from the Southern Refugium, located south of the Laurentide ice sheet, spanning from New Jersey, Kentucky, Missouri, Illinois, and Iowa to the mountainous region of the southwest –New Mexico and Nevada (Røed 2005, Kurtén and Anderson 1980; Banfield 1961). It is possible that caribou in the Southern Refugia occupied some of the eastern coastal plains refugia and/or various island refugia (Figure 3, Figure 24) located off the east coast of North America and then populated the island of Newfoundland and other Atlantic provinces, such as Nova Scotia, New Brunswick and Prince Edward Island, as the ice sheet retreated (Pielou 1991). Under this hypothesis, caribou in Newfoundland would be most closely related to caribou from New England and the Atlantic Provinces. Unfortunately this study did not include any samples from theses areas, therefore it is difficult to accept or reject the Southern route hypothesis at this point. However, this does provide an opportunity for additional examination. Caribou were extirpated from the Atlantic Provinces sometime before 1939

(Tufts 1939). Samples for comparison would need to be sought from caribou skins, bones or teeth, which may be accessed from local museums. DNA may be isolated from the museum specimens and genetic comparisons could be made to the samples already obtained from Newfoundland caribou.

The Northern Route, coming from Labrador, would suggest that caribou occupying the Southern Refugium gradually moved north as the ice sheet retreated, eventually reaching Quebec and Labrador (Figure 24). Along the way some caribou may have populated the Atlantic Provinces, while others continued north, crossing the St. Lawrence River into Quebec and continuing on to central Labrador. Some of the caribou may have moved south and crossed from the Labrador Peninsula onto the island of Newfoundland at the Strait of Belle Isle, which is approximately 18 km across and remains frozen with sea ice for much of the winter. The difficulty with this hypothesis is that there is little documented evidence of caribou making any significant movements across the Strait of Belle Isle; therefore it would seem unlikely that caribou would be able to populate the entire island of Newfoundland from that direction. Under this hypothesis, caribou in Newfoundland would be most closely related to caribou from Labrador and adjacent Quebec. The comparisons of Newfoundland caribou to other North American caribou (Figures 18 and 19) indicate that Newfoundland caribou cannot be genetically differentiated from other woodland caribou in North America. Based on the samples of woodland caribou available in Genbank, there appear to be close genetic relationships between Newfoundland caribou from clades B, C, and D and woodland caribou from Quebec, which potentially supports the Northern Route hypothesis. In addition, the

samples obtained from Genbank for Labrador caribou indicate there is genetic separation of Labrador (and Alberta) caribou from Newfoundland caribou that are members of clades B, C, and D. This result suggests that perhaps caribou migrated as far north as Quebec as the ice sheet retreated, came east along the Quebec north shore and then crossed into Newfoundland at the Strait of Belle Isle (Figure 24, Northern Route 2). Meanwhile, other caribou continued north into central Labrador. This would support a revised hypothesis that, caribou populated the island of Newfoundland from Quebec by way of the Strait of Belle Isle. In this case, Newfoundland caribou would be more closely related to caribou in Quebec (particularly the population along the north shore of the St. Lawrence River). Perhaps a better selection of samples, which includes the Quebec North Shore, central Labrador, southern Labrador, and the Atlantic Provinces could be used for further interpretation and clarification of this hypothesis.

The founding event that brought caribou belonging to clade A to Newfoundland may be a secondary event unrelated to the event that brought clades B, C, and D. Caribou from clade A are genetically distinct and genetically distant from caribou belonging to clades B, C and D, which indicates that clade A may have appeared in Newfoundland separately. The small number of individual caribou detected also supports the idea that clade A may have come from a small secondary colonization. The presence of clade A caribou in northern/western Newfoundland and their close genetic relationship to Labrador caribou (Figure 19) indicate that the colonization may have come from Labrador. It is possible to speculate that as the ice retreated some caribou migrated north to Labrador and over time they became genetically differentiated from their southern

counterparts. At some point, it is likely that a group of caribou left the Labrador herd, traveled south and crossed the Strait of Belle Isle into Newfoundland and over time became differentiated from their Labrador counterparts (Figure 24, Northern Route 1).

4.3 Genetic Variation Among Caribou Herds in Newfoundland

The hierarchical analysis of molecular variance (AMOVA) revealed that some genetic variation is partitioned among herds (2.60% to 7.37%) and among regions (8.23% to 16.64%), however, most of the genetic variation (77.09% to 86.52%) is attributed to the individual level within herds (Table 16). The lack of genetic differentiation among geographical regions or among herds suggests there is a large amount of female mediated genetic interchange among most herds in Newfoundland, particularly in central Newfoundland.

There is one exception to this finding, which is indicated in the AMOVA, when the Avalon Region was distinguished from the rest of the island as a whole (Figure 14, Scenario 6). Under this regional grouping scenario, there is decreased genetic variation partitioned at the individual level and increased genetic variation partitioned at the regional level, indicating caribou on the Avalon Peninsula are somewhat differentiated from caribou on the rest of the island. This finding is also supported by the presence of two unique haplotypes in the Avalon Peninsula herds. These haplotypes are not detected anywhere else in Newfoundland and are the prevalent haplotypes present on the Avalon Peninsula. The genetic differentiation and presence of unique haplotypes suggests there is little genetic exchange between the Avalon/Cape Shore herds and the rest of

Newfoundland. The isthmus joining the Avalon Peninsula to the rest of Newfoundland seems to act as a barrier to movement of caribou. This supports Bergerud's (1971) suggestion that caribou on the Avalon Peninsula formed a herd distinct from other herds in Newfoundland.

4.4 Genetic Variation Within Caribou Herds in Newfoundland

The haplotype diversity within caribou herds in Newfoundland is relatively high for most herds. Generally, herds in central Newfoundland have high levels of haplotype diversity, ranging from Hd=0.82 to Hd=0.95 (Table 15). The Northern Peninsula and Adies Lake herds have lower haplotype diversity (Hd=0.66 and Hd=0.78, respectively) than the central herds. The Northern Peninsula and Adies Lake herds did not contain any unique haplotypes but rather contained a subset of haplotypes found in the central herds. This may be an indication that there is some isolation that prevents complete genetic interchange among the herds. Bergerud (1971) indicated the Northern Peninsula herd and the Humber River herd (which corresponds to Adies Lake) were each a discreet population separate from each other and the Interior (Central) herds.

The haplotype diversity and distribution of haplotypes in the St. Anthony herd is similar to that of central herds, rather than its closest neighbor, the Northern Peninsula herd. The St. Anthony herd was introduced to the area in the 1970's from Newfoundland caribou source stock. It not clear from the records of the introductions exactly where the source population originated. The genetic information suggests that the central herds were the likely source.

The Merasheen Island herd has a lower level of haplotype diversity than the central herds (Hd=0.56, Table 15) and contains a subset of haplotypes present in the central herds. The low haplotype diversity is not surprising since it is a population that was introduced (from Buchans) and thus founded by only a few individuals with a limited number of haplotypes. Since the herd exists on an island, the population is essentially closed to immigration/emigration of caribou.

The Avalon herd also has lower haplotype diversity than the central herds (Hd=0.57, Table 15) but in this case the haplotypes are unique from those detected in the central herds. The low haplotype diversity in the Avalon herds can be interpreted in a number of ways; caribou in the Avalon herd have experienced little genetic differentiation over time, or haplotypes in the Avalon herd have been lost over time due to population declines with the result that caribou with haplotypes Da and Dd have established the current population.

The Cape Shore herd had no haplotype diversity (Hd=0, Table 13), because it contained only one haplotype. This is not surprising since the Cape Shore herd was founded by a small number of individuals introduced from the Avalon herd. The founding animals for the Cape Shore herd likely all had the Db haplotype, which is the dominant haplotype in the source herd (Avalon herd).

4.5 Separating Population Structure From Population History in Newfoundland Caribou Herds

The complex population history of Newfoundland caribou makes it difficult to distinguish whether phylogeographic associations among haplotypes are due to population structure (recurrent but restricted matrilineal gene flow) or due to historical events acting at the population level (fragmentation, range expansion, colonization events) (Templeton 1998; Templeton et al. 1995). According to the NCA, the current phylogeographic associations among Newfoundland caribou may be the result of a combination of restricted gene flow with isolation by distance, contiguous range expansion and long distance colonization possibly coupled with subsequent fragmentation (or past fragmentation followed by range expansion).

The NCA indicates that Nested Clades 1-3, 1-4 and 2-3 may have phylogeographic associations that are due to population structure as a result of restricted gene flow with isolation by distance. Under restricted gene flow there are a number of predictions made with respect to haplotype/clade relationships in the nested clade design: older haplotypes/clades are more widespread than younger haplotypes/clades; and tip clades are younger than the interior clades to which they are connected (Templeton 1998; Templeton et al. 1995).

The structure of Nested Clade 1-3, which is representative of haplotypes Ca and Cb from clade C, demonstrates the patterns predicted under restricted gene flow (Figure 17a). The interior haplotype (Ca) is fairly widespread in herds west of Pot Hill and Mount Peyton. The tip haplotype (Cb) is less widespread and found in a subset of herds

represented by the interior haplotype (Ca), including, Gaff Topsails, Adies Lake and Northern Peninsula. There is a low frequency of haplotype Ca in the Northern Peninsula herd, which may support the suggestion made in the Section 4.4 that there is some barrier to movement of caribou from central herds to the Northern Peninsula. The high frequency of haplotype Ca in St. Anthony herd supports the idea (also made in the Section 4.4) that it was introduced from central herds, most likely Buchans, Grey River or Lapoile. The structure in Nested Clade 1-3 suggests that caribou in this clade move mainly among the west-central herds (Lapoile, Buchans, Grey River, and Gaff Topsails and Adies Lake) and there is only infrequent movement further north to the Northern Peninsula and east to Pot Hill and Mount Peyton. Caribou in this clade do not appear to move east as far as Middle Ridge or onto the Avalon Peninsula. Another interesting pattern apparent in this clade is that haplotypes Ca and Cb both occur on Merasheen Island. The prevalence of haplotype Ca on Merasheen Island indicates that many of the animals introduced from Buchans carried haplotype Ca. Haplotype Cb is present on Merasheen Island but not present in the original sample from Buchans. There are a number of explanations for this; the introduced animals came from a wider area that included Gaff Topsails (where Cb is detected), caribou with haplotype Cb moved away from Buchans at the time of sampling, haplotype Cb was lost from the Buchans herd, or haplotype Cb in Buchans went undetected during the sampling.

The phylogenetic relationships of Nested Clade 1-4, which represents haplotypes De, Dh, Di and Dm from clade D, may be attributed to restricted gene flow with isolation by distance (Figure 17b). The interior haplotype (Dh) is only present in two herds,

Middle Ridge and Avalon. The presence of Dh in these two herds indicates there may have been some movement of caribou between the Avalon Peninsula and herds in Middle Ridge. It is difficult to say whether Dh, which is indicated as the ancestral haplotype, originated in Middle Ridge and moved to the Avalon or originated in the Avalon and moved to Middle Ridge. The tip haplotypes (De, Di, Dm), which are descended from caribou with haplotype Dh, are clearly isolated from one another. Haplotype De in particular is confined to the Avalon herd, which suggests that there is only limited movement from the Avalon to other areas. The presence of haplotype Di in Middle Ridge and Mount Peyton suggests there is some movement of caribou between these two herds. The presence of haplotype Dm in Buchans may suggest long distance movement of caribou from Middle Ridge, however, since this is a rare haplotype it is difficult to make any clear determination. Given the complex nature of clade D and the presence of homoplasies (see Tables 7 and 8), there is a possibility that haplotype Dm was assigned to the wrong clade.

Nested Clade 2-3 is a large and complex clade, which is representative of haplotypes Da through Ds in clade D. The phylogenetic relationships in this clade may be attributed to restricted gene flow with isolation by distance (Figure 17c). The interior clade, Nested Clade 1-7, has a similar distribution to haplotype Ca as described above; it occurs predominantly in the central herds, it does not occur in Adies Lake or the Northern Peninsula herds but does occur in St Anthony, also it does not occur in herds east of Pot Hill and Mount Peyton. Conversely, Nested Clade 1-6 (tip) occurs in nearly every herd on the main part of the island with the exception of Mount Peyton. Nested Clade 1-10,

which is also a tip clade, is found throughout central Newfoundland but is more predominant in the east around Middle Ridge. Nested Clades 1-8 and 1-9 have distributions more typical of tip clades, occurring in a subset of herds represented by the interior clade (Nested Clade 1-7), including, Mount Peyton, Pot Hill, Grey River and Lapoile. The absence of all clades except Nested Clade 1-6 in the Northern Peninsula and Adies Lake herds supports the previously mentioned idea that there may be a barrier to movement for some caribou from central herds to the Northern Peninsula and possibly Adies Lake. The presence of Nested Clades 1-6, 1-7 and 1-10 in St. Anthony and central herds (Lapoile, Buchans, Gaff Topsails, and Grey River) supports the idea that the St. Anthony herd was introduced from herds in central Newfoundland.

The phylogenetic relationships of Nested Clade 1-2, which represents haplotypes Ba through Bh from clade B, may be attributed to contiguous range expansion by short-distance dispersal events by individuals (Figure 17d). Some interesting patterns emerge when looking at the distribution of this clade throughout Newfoundland: the interior haplotype (Ba) is centered on the Gaff Topsails, Adies Lake and Northern Peninsula herds then radiates out to the north, east and south; the tip haplotypes do not occur in the same central area but are found further to the north, east and south; most the of the tip haplotypes (Bb through Bh) are found to the east while only haplotypes (Bb and Be) are found to the north and south. These patterns suggest that female caribou in this clade move frequently among the adjacent herds Northern Peninsula, Adies Lake and Gaff Topsails and make some movements southward and eastward. Interestingly, the interior haplotype (Ba) does not occur in Grey River or on Merasheen Island. The absence of

haplotype Ba in Grey River is surprising since it occurs in all adjacent herds, including, Lapoile, Buchans, Gaff Topsails, and Pot Hill. A number of possibilities could explain the absence of haplotype Ba in Grey River; caribou with the Ba haplotype never expanded into Grey River; caribou with the Ba haplotype expanded into Grey River but the animals had moved away from the area at the time of sampling, or the haplotype is present in Grey River but it was not detected with the sampling regime used in this study. The absence of haplotype Ba in Merasheen Island is not unexpected since this population was introduced from the Buchans Plateau and thus it is possible that haplotype Ba was not brought into the population with the introduced animals. The presence of haplotypes Ba and Bb in St. Anthony and some of the central herds again supports the idea that the St. Anthony herd was introduced from herds in central Newfoundland.

The phylogenetic relationships in Nested Clade 1-5, which represents haplotypes Db, Dl and Do may be attributed to contiguous range expansion by short-distance dispersal events by individuals (Figure 17e). The interior haplotype (Db) is completely confined to the Avalon Peninsula and the two tip haplotypes occur a long distance away in Mount Peyton (Do) and Lapoile (Dl). There is a difficulty with this finding because it is known that the Cape Shore herd was introduced from the Avalon herd and so it does not actually have contiguous range expansion. For the NCA, the Cape Shore herd should have been assigned the same geographical center as the Avalon herd, as was done with Merasheen Island and Buchans. The presence of the tip haplotypes in herds such a long distance away from the interior haplotypes is difficult to interpret for two reasons: because of the small sample size (n=1 for both Dl and Do) and because the presence of

homoplasies in the haplotype network makes the placement of these haplotypes uncertain (see Tables 7 and 8).

The phylogenetic relationships in Nested Clade 2-2, which represents Nested Clades 1-4 (De, Dh, Di, Dm) and 1-5 (Db, Dl, Do), may be attributed to contiguous range expansion by short-distance dispersal events by individuals (Figure 17f). The interior clade (1-5) is mostly confined to the Avalon Peninsula, while the tip clade (1-4) is more widespread occurring on the Avalon Peninsula and in the Middle Ridge, Mount Peyton and Buchans herds. The patterns suggest that caribou in the ancestral clade (1-5) rarely move off the Avalon Peninsula, whereas, caribou in the tip clade have made some movements off the Avalon, expanding into Middle Ridge and Mount Peyton. As mentioned in the previous paragraph, it is difficult to interpret the presence of rare tip clades in herds such a long distance away from interior clades because of the small sample size and because the presence of homoplasies in the haplotype network (see Tables 7 and 8).

The phylogenetic relationships in Nested Clade 3-1, which represents Nested Clades 2-2 and 2-3, are attributed to long distance colonization possibly coupled with subsequent fragmentation or past fragmentation followed by range expansion (Figure 17g). Nested Clades 2-2 and 2-3 are clearly genetically and geographically distinct, the interior clade (2-2) occurs mainly on the Avalon Peninsula but also occurs in Middle Ridge, Mount Peyton and Buchans, the tip clade (2-2) is widespread throughout Newfoundland occurring in every herd except those on the Avalon Peninsula. The patterns suggest there is very little movement of caribou between the Avalon Peninsula

and the main part of the island and that the movement of caribou that does occur between these areas is unidirectional: from the Avalon to the main part of the island. It is also interesting that the interior/ancestral clade is confined to the Avalon rather than being the more widespread clade. There are a number of explanations for this, which are discussed further the next section.

4.6 Low Genetic Diversity of the Avalon Peninsula Herd

The low mtDNA variation in the Avalon herd is of great importance when considering the management of the Newfoundland caribou population. The herd may have limited ability to deal with environmental change and disturbances, such as extreme weather or parasitic infestations. In addition, the mtDNA haplotypes present on the Avalon Peninsula are unique to the area, therefore, the preservation of these haplotypes should be considered when making management decisions. Conversely, it is important to note that this study does not consider nuclear genetic variation, which may be higher than the mtDNA variation shown here. Further examination of the Avalon herds should include a study of nuclear genes so that both male and female genetic variation is considered.

There are several possible reasons why the mtDNA of the Avalon herd is genetically depauperate and genetically distinct compared with the rest of the herds in Newfoundland. These reasons are presented as three hypotheses that could be tested in further studies.

Hypothesis one: Variation was lost when caribou moved away from the Avalon Peninsula and became geographically isolated. When caribou populated Newfoundland, they may have populated the Avalon Peninsula first then moved on to the main part of the island. The small number of caribou that stayed on the Avalon had little subsequent genetic exchange and became isolated from the rest of the island, a result similar to a founder effect or genetic drift.

Hypothesis two: Variation was lost when caribou moved to the Avalon Peninsula and became geographically isolated. When caribou populated Newfoundland, they may have populated the main part of the island first, and then a small number of caribou populated the Avalon Peninsula, likely from the Middle Ridge herd. The founding individuals brought only a small fraction of haplotypes to the Avalon (possibly only haplotype Db), and then were geographically isolated from caribou on the rest of the island, resulting in a founder effect.

Hypothesis Three: Variation was lost through a population bottleneck and resulted in a founder effect. Under this hypothesis it does not matter how caribou populated the Avalon Peninsula. The herds on the Avalon have experienced significant population crashes in which significant levels of genetic diversity may have been lost, resulting in population bottlenecks. Subsequent expansions of the population were from a small number of individuals, resulting in a founder effect.

4.7 Evidence of Founder Effects in the Newfoundland Caribou Population

Founder effects are observed in the Newfoundland caribou population resulting from both historical natural events and from recent population introductions. As described in the previous section, the Avalon Peninsula herd is thought to have experienced founder effects resulting from a small founding population, genetic drift, or a population bottleneck. Other founder effects are observed with herds purposely introduced to areas previously unpopulated with caribou. Provincial wildlife managers did these introductions over the past 30 to 40 years. The high frequency of haplotype Ca on Merasheen Island is indicative of a founder effect. The Merasheen Island herd was founded by a small number of individuals from the Buchans herd, where haplotype Ca is common. The small number of founding individuals has expanded the population and haplotype Ca has become dominant. The high frequency of haplotype Db in the Cape Shore herd is another example of a founder effect. The Cape Shore herd was founded by a small number of individuals introduced from the Avalon herd, where haplotype Db is common. All of the caribou introduced to Cape Shore likely carried the Db haplotype, resulting in a genetically homogenous herd.

4.8 Relationships of Newfoundland Caribou to Quebec and Labrador

The comparisons of Newfoundland caribou with caribou from other parts of North America show there is a clear distinction between caribou belonging to the woodland caribou subspecies (*R. t. caribou*) and the tundra forms (*R. t. groenlandicus, R. t. pearyi, R. t. granti*). Although it should be noted that Cronin et al. (2005) found

evidence of intermixing of the two subspecies in Labrador, which causes the subspecies designations to be unclear. According to the mtDNA phylogenies considered here, caribou in Newfoundland clearly belong to the woodland caribou subspecies and caribou belonging to clades B, C and D are closely related to woodland caribou in Quebec. There are particularly close relationships of woodland caribou from Quebec to Newfoundland haplotypes Bc and Db. The lack of mtDNA separation between Newfoundland caribou and woodland caribou from Quebec is unexpected since it is believed that caribou in Newfoundland have been geographically isolated from herds occupying mainland Canada for approximately 8000 years. There may be a remote possibility that the Quebec samples obtained from Genbank represent remnant populations of Newfoundland caribou which were introduced to Laurentide Park in Quebec between 1966 and 1972 (see Bergerud and Mercer 1989) or some other unknown introduction of Newfoundland caribou. However, given the number of samples examined, 14 Quebec samples for the Control Region and two Quebec samples for the cytochrome *b* gene, this seems unlikely. This finding warrants further examination, which should include additional samples from Quebec and possibly other woodland caribou herds in Ontario, Manitoba, Saskatchewan and Alberta.

Newfoundland caribou belonging to clade A are in a mtDNA lineage more closely related to those of caribou in Labrador (and possibly Alberta) than to most caribou in Newfoundland. As mentioned previously, caribou in clade A are genetically distinct and genetically distant from other caribou in Newfoundland. The close relationship of this clade to caribou in Labrador and the rarity of this clade in Newfoundland may suggest

that clade A appeared on the island separately from the caribou belonging to clades B, C and D. Although the mtDNA of caribou in clade A are more closely related to caribou in Labrador, they still have different haplotype which separate them from Labrador caribou. This genetic differentiation suggests that the crossing of caribou into Newfoundland from Labrador was not recent and may have occurred soon after caribou occupied Labrador during the glacial retreat.

4.9 No Evidence of Interbreeding with Eurasian Reindeer

There is clear genetic distinction between Newfoundland caribou and Eurasian reindeer, which confirms that there has been little to no genetic interchange with Norwegian reindeer introduced to Newfoundland in the early 1900's. Historical records suggest that the reindeer brought in by Sir Wilfred Grenfell either died off, were shot or were relocated to Anticosti Island in Quebec. Despite this, there was still a remote possibility that some of the reindeer survived and integrated into the native Newfoundland caribou population. If integration did occur and the female reindeer interbred with Newfoundland caribou, haplotypes would occur in the population that would show close relationships to Eurasian reindeer, rather than clear separation.

Even if a handful of reindeer did survive, there is little evidence to support successful interbreeding between native caribou herds and introduced reindeer herds. Studies completed in Alaska, where there are large populations of both native caribou and introduced reindeer, have indicated that there is little evidence of successful interbreeding even when the two populations experience extensive mixing (Cronin et al. 1995; Cronin

et al. 2003; Cronin et al. 2006). The reasons for this are cited as: possible selection against hybrid progeny, relatively lower fitness of reindeer (smaller size, different foraging habits and less caution for predators), the difference in breeding season (it is 2-4 weeks earlier for reindeer) and their lower tendency to migrate (Cronin et al. 1995; Cronin et al. 2003; Cronin et al. 2006).

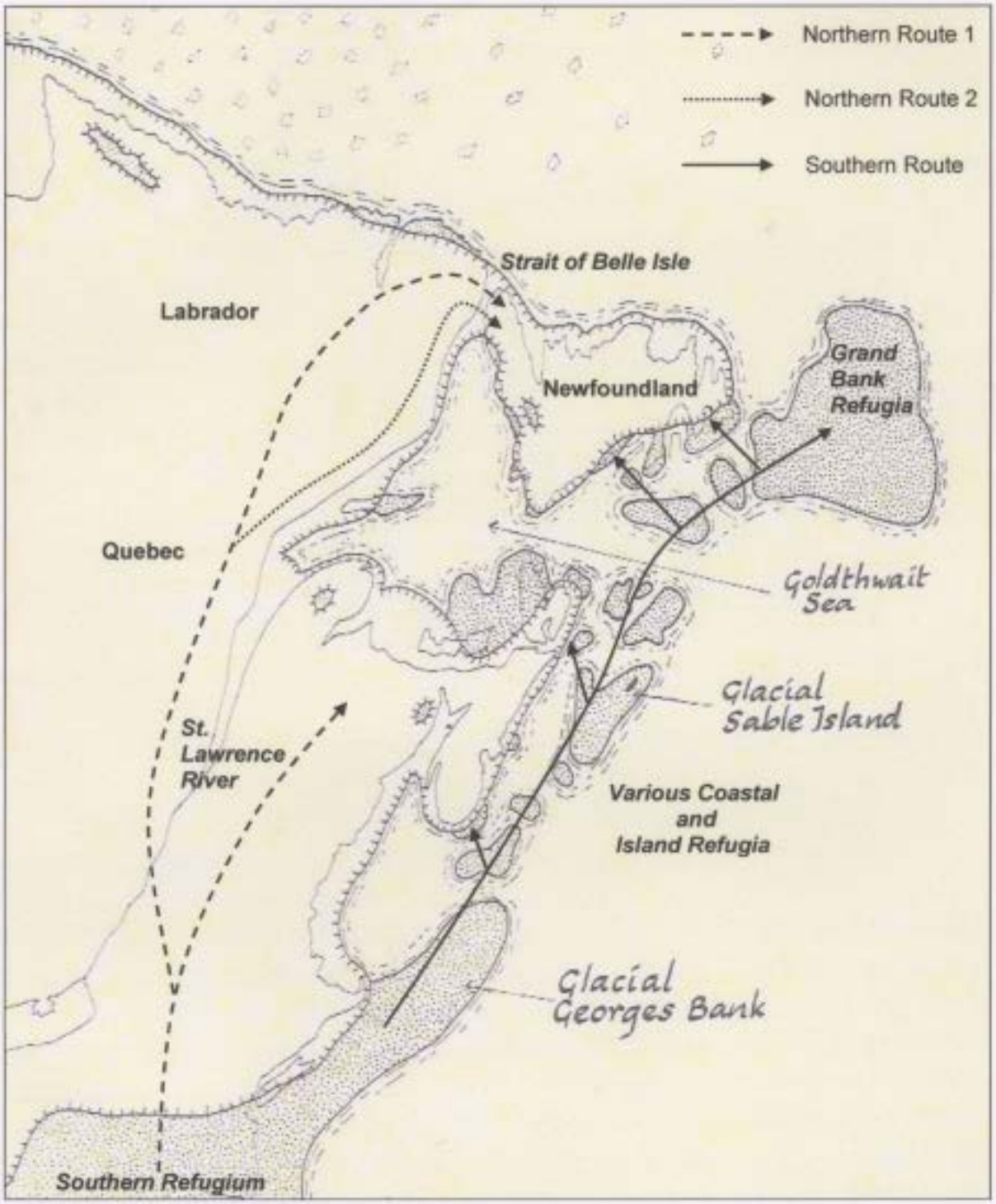


Figure 24. Possible migration routes of founding Newfoundland Caribou from the Southern Refugium after glacial retreat. Solid white areas with hatched border indicate the glacial ice margin. Speckled areas indicate land refugia. Map adapted from Figure 6.4 in Pielou 1991.

5.0 Conclusion

The Newfoundland caribou population is clearly structured into four mtDNA clades, comprising 32 haplotypes. The majority of the genetic variance in the population is at the individual level, although there is some regional differentiation of the Avalon caribou from other caribou in Newfoundland. The current genetic structure in Newfoundland caribou herds appears to be the result of a combination of founder effects, genetic drift, restricted gene flow with isolation by distance, contiguous range expansion and long distance colonization, possibly coupled with subsequent fragmentation or past fragmentation followed by range expansion.

The data suggest that mtDNA clade A is ancestral to mtDNA clades B, C and D, however, the branching order of these three clades is unclear. Further research is warranted to help resolve the placement of clades B, C, and D. This may be achieved through a whole genome approach, utilizing the Newfoundland caribou mitochondrial genome sequenced here with the DNA re-sequencing technique proposed by Carr et al. (2008). A number of hypotheses should be considered if further study is undertaken: Hypothesis 1: clade D is ancestral to clades B and C, based on the relatively higher diversity, abundance and widespread haplotypes in clade D; Hypothesis 2: clade B is older than clade C, based on the diversity and widespread haplotypes in clade B; Hypothesis 3: clade A comes from a separate founding population than clades, B, C and D, based on the significant genetic separation of these clades.

Caribou on the Avalon Peninsula have distinct mtDNA from other caribou in Newfoundland and they are also genetically homogenous. There are a number of explanations for the genetic differentiation and the lack of mtDNA variation in the Avalon herd, which should be considered if there is any further examination of this herd. Hypothesis 1: Newfoundland caribou herds originated on the Avalon and then moved off to main part of island, taking most of the genetic variation with them; Hypothesis 2: Newfoundland caribou herds originated on the main part of the island, then a few individuals moved to the Avalon and founded the population. Genetic variation was subsequently lost through a founder effect and associated geographic isolation; Hypothesis 3: Genetic variation was lost through a population bottleneck resulting from severe population crashes.

The question concerning the origins of the founding population of Newfoundland caribou remains unresolved; however, there are some insights that would benefit from further study. The close genetic relationships of Newfoundland caribou to Quebec caribou suggest that caribou may have populated Newfoundland across the Strait of Belle Isle. The lack of genetic diversity on the Avalon Peninsula may suggest that a founding population from coastal refugia is unlikely. Genetic comparisons with additional samples from areas such as, the Atlantic provinces, Labrador and Quebec, may help resolve the question of the founding population. Also, the incorporation of the DNA re-sequencing technique into the data set, as proposed by Carr et.al. (2008), would help achieve the greatest possible resolution. If further study is undertaken, the following two hypotheses should be considered: Hypothesis 1: clades B, C, and D, originated from

Quebec/Labrador (came from the north) or Hypothesis 2: clades B, C, and D originated from coastal and island refugia, such as the Grand Bank refugia (came from the south, southwest).

The complete mtDNA genome for Newfoundland caribou (*Rangifer tarandus caribou*) was successfully sequenced as a part of this study and was compared with that of the reindeer subspecies (*Rangifer tarandus tarandus*). There were 165 variable sites between the Newfoundland caribou and the reindeer mtDNA genome and 9 sites with alignment gaps (indels). The mtDNA genome for Newfoundland caribou will serve as a reference sequence for further mtDNA studies of the Newfoundland caribou which use the DNA re-sequencing technique of Carr et. al (2008).

Literature Cited

Anonymous. 1973. Capture and introduction of Newfoundland caribou to various sites across the province. Document obtained from the Centre for Newfoundland Studies, St. John's, Newfoundland and Labrador.

Avise, J.C. 2000. *Phylogeography, The History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts.

Avise, J.C. 2004. *Molecular Markers, Natural History and Evolution, Second Edition.* Sinauer Associates, Inc., Sunderland, Massachusetts.

Avise, J.C., I. Arnold, R.M. Ball, E. Bermingham, T. Lamb, I.B. Neigel, C.A. Reeb, and N.C. Saunders. 1987. Interspecific phylogeography: the mitochondrial DNA bridge between populations genetics and systematics. *Annual Review of Ecology and Systematics* 18: 489-522.

Banfield, A.W.F. 1961. A revision of the reindeer and caribou, genus *Rangifer*. *Bulletin of the National Museum of Canada*, No. 177: 1-137.

Banfield, A.W.F. 1974. The Mammals of Canada. University of Toronto Press, Toronto, Ontario.

Bergerud, A.T. 1971. The Population Dynamics of Newfoundland Caribou. *Wildlife Monographs*, No. 25. 55 pp.

Bergerud, A.T. 1972. Food Habits of Newfoundland Caribou. *Journal of Wildlife Management* 36 (3): 913-923.

Bergerud, A.T. 1974. Decline of caribou in North America following settlement. *Journal of Wildlife Management* 38: 757-770.

Bergerud, A.T., M.J. Nolan, K. Curnew, W.E. Mercer. 1983. Growth of the Avalon Peninsula, Newfoundland caribou herd. *Journal of Wildlife Management* 47(4): 989-998.

Bergerud, A.T. and W.E. Mercer. 1989. Caribou Introductions in Eastern North America. *Wildlife Society Bulletin* 17: 111-120.

Brown, W.M., M. George, Jr., and A.C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences*, USA 76 (4) 1967-1971.

Byun, S.A., B.F. Koop, and T.E. Reimchen. 2002. Evolution of the Dawson caribou (*Rangifer tarandus dawsoni*). *Canadian Journal of Zoology* 80: 956-960.

Carr, S.M., H.D. Marshall, A.T. Duggan, S.M.C. Flynn, K.A. Johnstone, A.M. Pope, C.D. Wilkerson. 2008. Phylogeographic genomics of mitochondrial DNA: Highly-resolved patterns of intraspecific evolution and a multi-species, microarray-based DNA sequencing strategy for biodiversity studies. *Comparative Biochemistry and Physiology, Part D 3:1-11.*

Clement, M., D. Posada and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9(10): 1657-1660.

COSEWIC 2002. COSEWIC assessment and status report on the woodland caribou *Rangifer tarandus caribou* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xi + 98 pp.

Côté, S.D., J.F. Dallas, F. Marshall, R.J. Irvine, R. Langvatn, and S.D. Albon. 2002. Microsatellite DNA evidence for genetic drift and philopatry in Svalbard reindeer. *Molecular Ecology* 11: 1923-1930.

Courtois, R., L. Bernatchez, J-P. Ouellet, and L. Breton. 2003. Significance of caribou (*Rangifer tarandus*) ecotypes from a molecular genetics viewpoint. *Conservation Genetics* 4: 393-404.

Cronin, M.A. 1992. Intraspecific variation in mitochondrial DNA of North American cervids. Journal of Mammalogy 73(1): 70-82.

Cronin, M.A. 2006. A proposal to eliminate redundant terminology for intra-species groups. *Wildlife Society Bulletin* 34(1): 237-241.

Cronin, M.A, L. Renecker, B.J. Pierson, and J.C. Patton. 1995. Genetic variation in domestic reindeer and wild caribou in Alaska. *Animal Genetics* 26: 427-434.

Cronin, M.A. and J.C. Patton. 2002. Caribou and reindeer (*Rangifer tarandus*) genetic variation and herd structure in Northern Alaska. *In* LGL Report P587, Chapter 5, for BP Exploration (Alaska) Inc., Environmental Studies Group, Anchorage, Alaska.

Cronin, M.A., J.C. Patton, N. Balmysheva, and M.D. MacNeil. 2003. Genetic variation in caribou and reindeer (*Rangifer tarandus*). *Animal Genetics* 34: 33-41.

Cronin, M.A., M.D. MacNeil, and J.C. Patton. 2005. Variation in mitochondrial DNA and microsatellite DNA in caribou (*Rangifer tarandus*) in North America. *Journal of Mammalogy* 86(3): 495-505.

Cronin, M.A., M.D. MacNeil, and J.C. Patton. 2006. Mitochondrial DNA and microsatellite DNA variation in domestic reindeer (*Rangifer tarandus tarandus*) and relationships with wild caribou (*Rangifer tarandus granti*, *Rangifer tarandus groendlandicus*, and *Rangifer tarandus caribou*). *Journal of Heredity* 97(5): 525-530.

Dugmore, A.A.R. 1913. The Romance of the Newfoundland Caribou. J. P. Lippincott Co., Philadelphia, Pennsylvania, USA. 186 pp.

Dugmore, A.A.R. 1930. In the Heart of the Northern Forests. Chatto and Windus, London, England. 243 pp.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47-50.

Excoffier, L., P.E. Smouse, J.M. Quattro. 1992. Analysis of Molecular Variance Inferrred from Metric Distances among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. *Genetics* 131: 479-491.

Flagstad, Ø., and K. H. Røed. 2003. Refugial origins of reindeer (*Rangifer tarandus* L.) inferred from mitochondrial DNA sequences. *Evolution* 57(3): 658-670.

Geist, V. 1998. Deer of the World: Their Evolution, Behavior and Ecology, 1st edition. Stackpole Books, Mechanicsburg, Pennsylvania. 421 pp.

Gordon, B. 2003. *Rangifer* and man: An ancient relationship. *Rangifer*, Special Issue No.14, 15-28. Proceedings of the Ninth North American Caribou Workshop, Kuujjuaq, Québec, Canada.

Gravlund, P., M. Meldgaard, S. Pääbo, and P. Arctander. 1998. Polyphyletic origin of the small-bodied, high-arctic subspecies of tundra reindeer (*Rangifer tarandus*). *Molecular Phylogenetics and Evolution* 10 (2): 151-159.

Guthrie, R.D. and J.V. Mathews. 1971. The cape deceit fauna - early Pleistocene mammalian assemblage from the Alaskan Arctic. *Quaternary Research* 1(4): 474-510.

Hardy, C.C. 1869. Forest Life in Acadie. Chapman and Hall, London, England. 371 pp.

Hudson, R.R., M. Slatkin, and W.P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. *Genetics* 132: 583-589.

Hunter, M.L. Jr. 1996. *Fundamentals of Conservation Biology*. Blackwell Science, Oxford University Press, Ontario, Canada.

Iwrin, D.M., T.D. Kocher, and A.C. Wilson. 1991. Evolution of the Cytochrome *b* gene of mammals. *Journal of Molecular Evolution* 32: 128-144.

Jepsen, B.I., H.R. Siegismund, and M, Fredholm. 2002. Population genetics of the native caribou (*Rangifer tarandus groenlandicus*) and the semi-domestic reindeer (*Rangifer tarandus tarandus*) in Southwestern Greenland: Evidence of introgression. *Conservation Genetics* 3: 401-409.

Johnson, A.B. 1967. Hugh Cole's 400-mile Trek with Reindeer. *In* Smallwood, J.R. (ed.). The Book of Newfoundland, Volume 3. Newfoundland Book Publishers, St. John's, Newfoundland, Canada.

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Pääbo, F.X. Villablanca, and A.C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA*. 86: 6196-6200.

Kumar S, Tamura K & Nei M. 2004. MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment Briefings in *Bioinformatics* 5:150-163.

Kurtén, B. 1968. *Pleistocene mammals of Europe*. Weidenfeld and Nicolson, London, England.

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

Lankester, M.W. and T.H. Northcott. 1979. *Elaphostrongylus cervi* Cameron 1931 (Nematoda: Metastrongyloidea) in caribou (*Rangifer tarandus caribou*) of Newfoundland. *Canadian Journal of Zoology* 57: 1384-1392.

Lankester, M.W. and D. Fong. 1989. Distribution of elaphostrongyline nematodes (Metastrongyloidea: Protostrongylidae) in cervidae and possible effects of moving *Rangifer* spp. into and within North America. *Alces* 25: 133-145.

Lankester, M.W. and D. Fong. 1998. Protostrongylid nematodes in caribou (*Rangifer tarandus caribou*) and moose (*Alces alces*) of Newfoundland. *Rangifer*, Special Issue No. 10: 73-83. The Seventh North American Caribou Conference, Thunder Bay, Ontario, Canada.

Mahoney, S.P. and J.A. Virgl. 2003. Habitat selection and demography of a nonmigratory woodland caribou population in Newfoundland. *Canadian Journal of Zoology* 81: 321-334.

Mahoney, S.P. and J.A. Schaefer. 2002. Long-term changes in demography and migration of Newfoundland caribou. *Journal of Mammalogy* 83(4): 957-963.

Mahoney, S.P., B.J. Tucker, S.H Ferguson, B. Greene, E. Menchenton, and L.H. Russell. 1991. Impact of the Hope Brook Gold Mine on the Lapoile caribou herd. *In* Butler, C.E. and S.P Mahoney (eds.). Proceedings of the 4th North American Caribou Workshop, St. John's, Newfoundland and Labrador.

Martin, P.S. and R.G. Klein (eds.). 1984. *Quaternary Extinctions: A Prehistoric Revolution.* The University of Arizona Press, Tucson, Arizona.

McElroy, D., P. Moran, E. Bermingham, and I. Kornfield. 1991. REAP: the Restriction Enzyme Analysis Package, Version 4.0 edition. University of Main, Orono.

McLoughlin, P.D., D. Paetkau, M. Duda, S. Boutin. 2004. Genetic Diversity and relatedness of boreal caribou populations in western Canada. *Biological Conservation* 118: 593-598.

Millais, J.G. 1907. Newfoundland and its Untrodden Ways. Longmans, Green and Co., London, England. 340 pp. Reprinted in 2005 by Boulder Publications Ltd., Newfoundland and Labrador, Canada.

Olsaker I. and K. H. Røed. 1990. The major histocompatibility complex of reindeer. *Rangifer*, Special Issue No. 3: 369-372. Proceedings of the 5th International Reindeer/Caribou Symposium, Arvidsjaur, Sweden.

Palumbi, S.R. 1996. Nucleic acids II: The Polymerase Chain Reaction. *In* Molecular Systematics, 2nd ed., Hillis, D.M., Moritz, C. & Mable, B.K. (eds.). pp. 205-247. Sinauer Associates Inc., Sunderland, MA.

Pielou, E. C. 1991. *After the Ice Age, The Return of Life to Glaciated North America.* The University of Chicago Press, Chicago and London.

Polziehn, R.O. and C. Strobeck. 1998. Phylogeny of wapiti, red deer, sika deer, and other North American cervids as determined from mitochondrial DNA. *Molecular Phylogenetics and Evolution* 10(2): 249-258.

Posada D., K.A. Crandall and A.R. Templeton AR. 2000. GeoDis: A program for the Cladistic Nested Analysis of the Geographical Distribution of Genetic Haplotypes. *Molecular Ecology* 9(4):487-488.

Randi. E., N. Mucci, M. Pierpaoli, E. Douzery. 1998. New phylogenetic perspectives on the Cervidae (Artiodactyla) are provided by the mitochondrial cytochrome *b* gene. *Proceedings of the Royal Society of London, Series B, Biological Sciences* 265: 793-801.

Røed, K.H. 1991. Genetic differentiation and evolution of tundra reindeer and caribou. *In* Butler, C.E. and S.P. Mahoney (eds.). Proceedings of the 4th North American Caribou Workshop, St. John's, Newfoundland. pp. 64-76

Røed, K.H. 2005. Refugial origin and postglacial colonization of holarctic reindeer and caribou. *Rangifer* 25(1): 19-30.

Røed, K.H., and L. Midthjell. 1998. Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids. *Molecular Ecology* 7: 1771-1788.

Røed, K.H. and D. Thomas. 1990. Transferrin variation and evolution of Canadian barren-ground caribou. *Rangifer*, Special Issue No. 3: 385-389. Proceedings of the 5th International Reindeer/Caribou Symposium. Arvidsjaur, Sweden.

Røed, K.H., M.A.D. Ferguson, M. Crête, and T.A. Bergerud. 1991. Genetic variation in transferrin as a predictor for differentiation and evolution of caribou from eastern Canada. *Rangifer* 11(2): 65-74.

Ronquist, F. and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574.

Rozas, J. C., Sánchez-DelBarrio, X. Messeguer and R. Rozas. 2005. DnaSP version 4.10.4: an integrated program for molecular population genetics and molecular evolution analysis. Universitat de Barcelona.

Seip, D.R. 1991. Predation and caribou populations. *Rangifer*, Special Issue No. 7: 46-52. Proceedings of the Fifth North American Caribou Workshop, Yellowknife, NWT, Canada.

Slatkin, M, and W.P Madison. 1989. A cladisitic measure of gene flow inferred from the phylogenies of alleles. *Genetics* 123: 603-613.

Slatkin, M., and W.P. Maddison. 1990. Detection of isolation by distance using phylogenies of genes. *Genetics* 126: 249-260.

Swofford, D. 2002. Phylogenetic Analysis Using Parsimony, Version 4.0b for Macintosh. Computer program published by Sinauer Associates Inc., Sunderland, Massachusetts.

Swofford, D. 2001. Phylogenetic Analysis Using Parsimony, Version 4.0b10 for 32-bit Microsoft Windows. Computer program published by Sinauer Associates Inc., Sunderland, Massachusetts.

Templeton, A.R. 1998. Nested Clade Analysis of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* 7: 381-397.

Templeton, A.R., E. Routman, and C.A. Phillips. 1995. Separating population structure form population history: A cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. *Genetics* 140: 767-782.

Templeton, A.R., K.A. Crandall, and C.F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram Estimation. *Genetics* 132: 618-633.

Templeton, A.R., E. Boerwinkle, and C.F. Sing. 1987. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in drosophila. *Genetics* 117: 343-351.

Tufts, R.W. 1939. Newfoundland caribou liberated in Nova Scotia. *The Canadian Field-Naturalist* Vol. LIII, p. 123.

Valkenburg, P., B.W. Dale, R.W. Tobey, and R.A. Sellers. 2000. Investigation of Regulating and Limiting Factors in the Delta Caribou Herd, 1 July 1999 – 30 June

2000. Alaska Department of Fish and Game. Federal aid in wildlife restoration research performance report, grant W-27-3, study 1.52, Juneau, Alaska.

Wada, K., M. Nakamura, M. Nishibori, and M. Yokohama. 2006. The complete nucleotide sequence of mitochondrial genome in the reindeer (*Rangifer tarandus*) and red deer (*Cervus elaphus*). Unpublished. Direct submission to GenBank (Accession No. NC_007703).

Wada, K. M. Nishibori, M. Yokohama. 2007. The complete nucleotide sequence of the mitochondrial genome in the Japanese Sika deer (*Cervus nippon*) and a phylogenetic analysis between Cervidae and Bovidae. *Small Ruminant Research* 69: 46-54.

Williams, T.M. and D.C. Heard. 1986. World status of wild *Rangifer tarandus* populations. *Rangifer*, Special Issue No. 1: 19-28. Proceedings of the 4th International Reindeer/Caribou Symposium. Whitehorse, Canada.

Wilson, A.C., R.L. Cann, S.M. Carr, M. George, Jr., U.B. Gyllensten, K. Helm-Bychowski, R.C. Higuchi, S.R. Palumbi, E.M. Prager, R.D. Sage, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives of evolutionary genetics. *Biological Journal of the Linnaean Society* 26: 375-400.

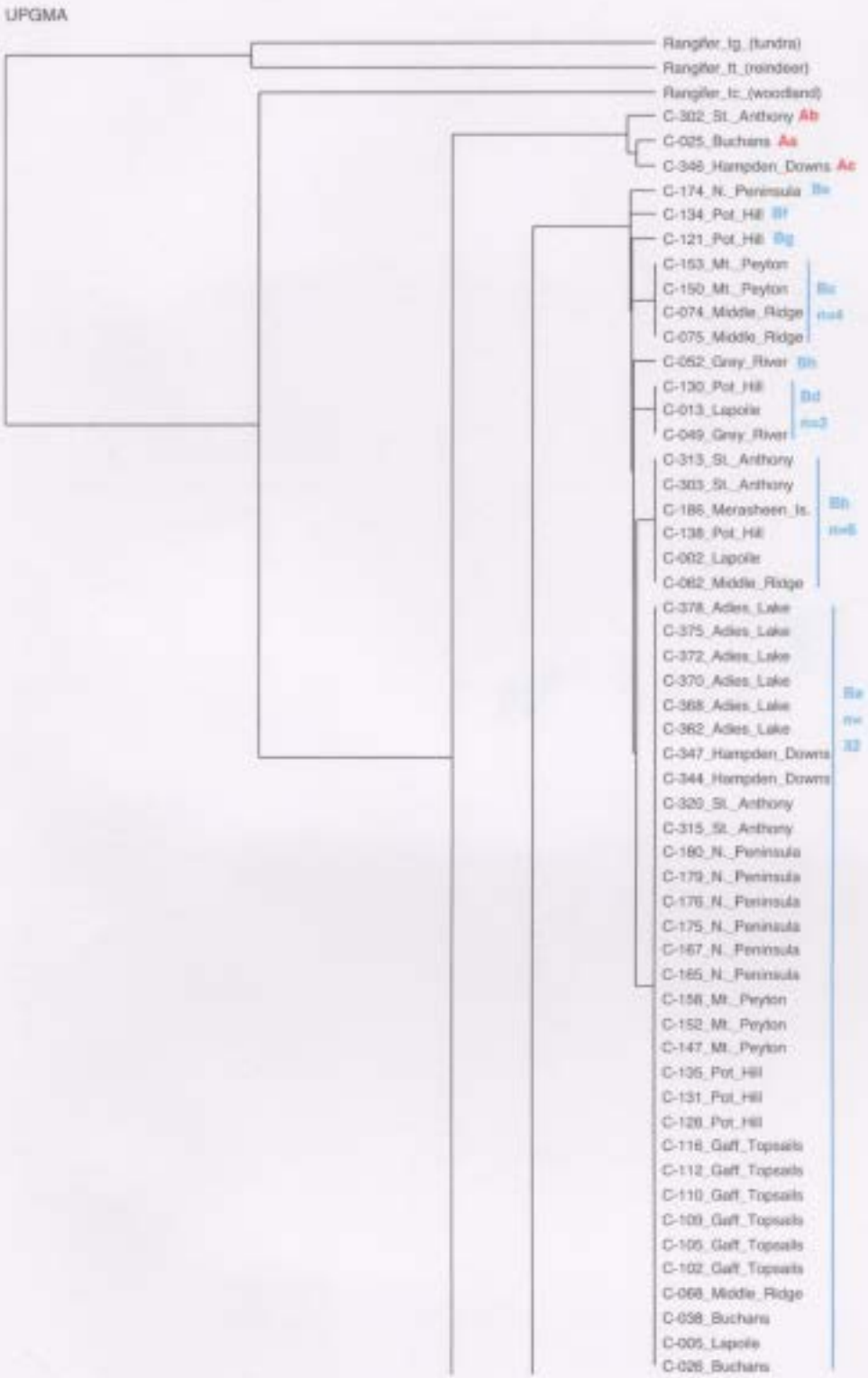
Wilson, G.A., C. Strobeck, L. Wu, and J.W. Coffin. 1997. Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. *Molecular Ecology* 6: 697-699.

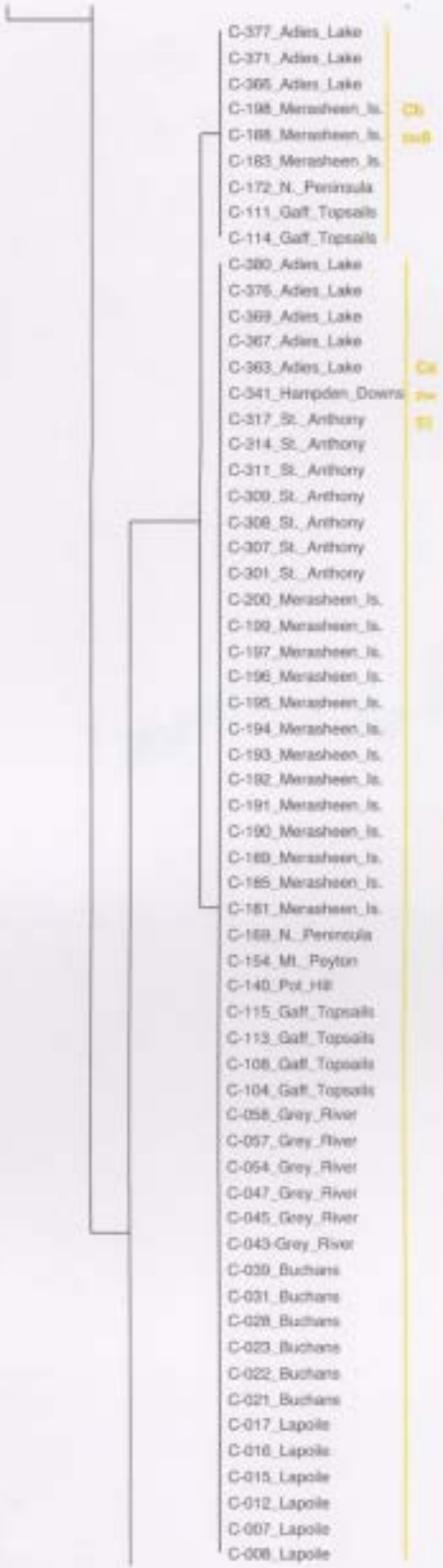
Zhang, Ai-Bing, Tan, S., Sota, T. 2006. Autoinfer1.0: a computer program to infer biogeographical events automatically. *Molecular Ecology Notes* 6(3): 597–599.

Zittlau, K, J. Coffin, R. Farnell, G. Kuzyk, and C. Strobeck. 2000. Genetic relationships of three Yukon caribou herds determined by DNA typing. *Rangifer*, Special Issue No. 12: 59-62. The Eight North American Caribou Workshop, Whitehorse, Yukon, Canada.

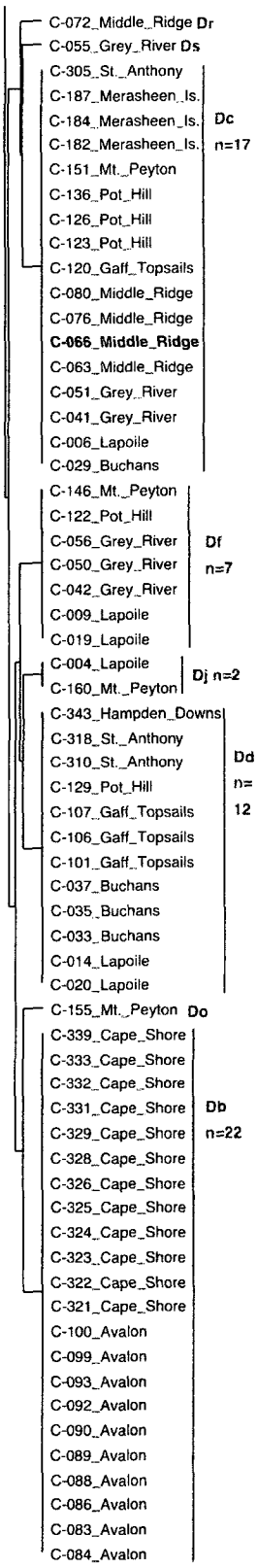
Appendix B

UPGMA Phylogram of all 233 Newfoundland caribou samples based on 2222 bp (site 853 excluded from analysis). UPGMA Phylogram was created in the program PAUP 4.0b (Swofford 2002) and used to identify haplotype groupings. Haplotypes Aa through Ds are identified to the right of the sample name.


UPGMA
Rangifer_tg_(tundra)
Rangifer_tt_(reindeer)
Rangifer_tc_(woodland)
C-302_St._Anthony Ab
C-025_Buchans Aa
C-346_Hampden_Downs Ac
C-174_N._Peninsula Be
C-134_Pot_Hill Bf
C-121_Pot_Hill Bg
C-153_Mt._Peyton
C-150_Mt._Peyton
C-074_Middle_Ridge
C-075_Middle_Ridge
Bc
n=4
C-052_Grey_River Bh
C-130_Pot_Hill
C-013_Lapoile
C-049_Grey_River
Bd
n=3
C-313_St._Anthony
C-303_St._Anthony
C-186_Merasheen_Is.
C-138_Pot_Hill
C-002_Lapoile
C-062_Middle_Ridge
Bh
n=6
C-378_Adies_Lake
C-375_Adies_Lake
C-372_Adies_Lake
C-370_Adies_Lake
C-368_Adies_Lake
C-362_Adies_Lake
C-347_Hampden_Downs
C-344_Hampden_Downs
C-320_St._Anthony
C-315_St._Anthony
C-180_N._Peninsula
C-179_N._Peninsula
C-176_N._Peninsula
C-175_N._Peninsula
C-167_N._Peninsula
C-165_N._Peninsula
C-156_Mt._Peyton
C-152_Mt._Peyton
C-147_Mt._Peyton
C-135_Pot_Hill
C-131_Pot_Hill
C-128_Pot_Hill
C-116_Gaff_Topsails
C-112_Gaff_Topsails
C-110_Gaff_Topsails
C-109_Gaff_Topsails
C-105_Gaff_Topsails
C-102_Gaff_Topsails
C-066_Middle_Ridge
C-038_Buchans
C-005_Lapoile
C-026_Buchans
Ba
n=
32


C-377_Adies_Lake
C-371_Adies_Lake
C-366_Adies_Lake
C-198_Merasheen_Is.
C-188_Merasheen_Is.
C-183_Merasheen_Is.
C-172_N._Peninsula
C-111_Gaff_Topsails
C-114_Gaff_Topsails
C-380_Adies_Lake
C-376_Adies_Lake
C-369_Adies_Lake
C-367_Adies_Lake
C-363_Adies_Lake
C-341_Hampden_Downs
C-317_St._Anthony
C-314_St._Anthony
C-311_St._Anthony
C-309_St._Anthony
C-308_St._Anthony
C-307_St._Anthony
C-301_St._Anthony
C-200_Merasheen_Is.
C-199_Merasheen_Is.
C-197_Merasheen_Is.
C-196_Merasheen_Is.
C-195_Merasheen_Is.
C-194_Merasheen_Is.
C-193_Merasheen_Is.
C-192_Merasheen_Is.
C-191_Merasheen_Is.
C-190_Merasheen_Is.
C-189_Merasheen_Is.
C-185_Merasheen_Is.
C-181_Merasheen_Is.
C-169_N._Peninsula
C-154_Mt._Peyton
C-140_Pot_Hill
C-115_Gaff_Topsails
C-113_Gaff_Topsails
C-108_Gaff_Topsails
C-104_Gaff_Topsails
C-058_Grey_River
C-057_Grey_River
C-054_Grey_River
C-047_Grey_River
C-045_Grey_River
C-043-Grey_River
C-039_Buchans
C-031_Buchans
C-028_Buchans
C-023_Buchans
C-022_Buchans
C-021_Buchans
C-017_Lapoile
C-016_Lapoile
C-015_Lapoile
C-012_Lapoile
C-007_Lapoile
C-006_Lapoile


C-079_Middle_Ridge
C-148_Mt._Peyton
Di n=2
C-032_Buchans Dm
C-091_Avalon
C-063_Middle_Ridge
C-071_Middle_Ridge
Dh n=3
C-098_Avalon
C-096_Avalon
C-095_Avalon
C-094_Avalon
C-087_Avalon
C-082_Avalon
C-085_Avalon
De n=7
C-127_Pot_Hill Dp
C-157_Mt._Peyton Dq
C-144_Mt._Peyton
C-141_Mt._Peyton
C-132_Pot_Hill
C-125_Pot_Hill
C-053_Grey_River
C-050_Grey_River
Dg n=6
C-304_St._Anthony Dn
C-069_Middle_Ridge
C-137_Pot_Hill
Dk n=2
C-001_Lapoile Dl
C-373_Ades_Lake
C-365_Ades_Lake
C-364_Ades_Lake
C-351_Ades_Lake
C-342_Hampden_Downs
C-306_St._Anthony
C-178_N._Peninsula
C-173_N._Peninsula
C-171_N._Peninsula
C-170_N._Peninsula
C-168_N._Peninsula
C-164_N._Peninsula
C-163_N._Peninsula
C-162_N._Peninsula
C-161_N._Peninsula
C-124_Pot_Hill
C-119_Gaff_Topsails
C-118_Gaff_Topsails
C-117_Gaff_Topsails
C-103_Gaff_Topsails
C-078_Middle_Ridge
C-077_Middle_Ridge
C-073_Middle_Ridge
C-070_Middle_Ridge
C-067_Middle_Ridge
C-059_Grey_River
C-048_Grey_River
C-046_Grey_River
C-038_Buchans
C-030_Buchans
C-027_Buchans
C-011_Lapoile
C-018_Lapoile
Da n= 33


C-072_Middle_Ridge Dr
C-055_Grey_River Ds
C-305_St._Anthony
C-187_Merasheen_Is.
C-184_Merasheen_Is.
C-182_Merasheen_Is.
Dc
n=17
C-151_Mt._Peyton
C-136_Pot_Hill
C-126_Pot_Hill
C-123_Pot_Hill
C-120_Gaff_Topsails
C-080_Middle_Ridge
C-076_Middle_Ridge
C-066_Middle_Ridge
C-063_Middle_Ridge
C-051_Grey_River
C-041_Grey_River
C-006_Lapoile
C-029_Buchans
C-146_Mt._Peyton
C-122_Pot_Hill
C-056_Grey_River
C-050_Grey_River
C-042_Grey_River
C-009_Lapoile
C-019_Lapoile
Df
n=7
C-004_Lapoile
C-160_Mt._Peyton
Dj n=2
C-343_Hampden_Downs
C-318_St._Anthony
C-310_St._Anthony
C-129_Pot_Hill
C-107_Gaff_Topsails
C-106_Gaff_Topsails
C-101_Gaff_Topsails
C-037_Buchans
C-035_Buchans
C-033_Buchans
C-014_Lapoile
C-020_Lapoile
Dd
n=
12
C-155_Mt._Peyton Do
C-339_Cape_Shore
C-333_Cape_Shore
C-332_Cape_Shore
C-331_Cape_Shore
C-329_Cape_Shore
C-328_Cape_Shore
C-326_Cape_Shore
C-325_Cape_Shore
C-324_Cape_Shore
C-323_Cape_Shore
C-322_Cape_Shore
C-321_Cape_Shore
C-100_Avalon
C-099_Avalon
C-093_Avalon
C-092_Avalon
C-090_Avalon
C-089_Avalon
C-088_Avalon
C-086_Avalon
C-083_Avalon
C-084_Avalon
Db
n=22
— 0.5 changes

Appendix B