



## Significance of caribou (*Rangifer tarandus*) ecotypes from a molecular genetics viewpoint

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### Abstract

Three caribou ecotypes are present in eastern North America: the mountain caribou which is found south of the St. Lawrence River, the barren-ground caribou which calves in the tundra, and in between, the forest-dwelling ecotype which lives all year long in the boreal forest. Blood and muscle samples were collected from seven populations and characterized at eight microsatellite loci to test the hypotheses that forest-dwelling and barren-ground ecotypes constitute a single metapopulation and that geographical isolation results in reduced genetic diversity. The mean number of alleles per locus, allelic richness, and observed and expected heterozygosity declined from north to south and were the smallest in isolated forest-dwelling populations. Correspondence analysis showed three groups of samples corresponding to the three ecotypes. Gene flow estimates were moderate or high among all forest-dwelling populations and particularly between those <200 km apart. Our results suggest that the three caribou ecotypes represent three distinct genetic entities and that the forest-dwelling populations in the continuous range form a metapopulation. Genetic diversity was lower in isolated populations but does not seem to be of immediate concern for conservation. We propose that management strategies should favor increase in caribou numbers in order to avoid extinction due to stochastic events and to maintain local biodiversity. In the continuous range, conservation strategies of caribou populations must be planned on a large scale to maintain occasional exchanges between populations, thus preserving genetic diversity.

### Introduction

Five subspecies of caribou are usually recognized in North America: the Grant's caribou in Alaska (*Rangifer tarandus granti*), the Canadian barren-ground caribou in Nunavut and Northwest Territories (*R. t. groenlandicus*), the Peary caribou in the Arctic (*R. t. pearyi*), the Queen Charlotte archipelago caribou (*R. t. dawsoni*), which has been extinct since 1910, and finally, the woodland caribou (*R. t. caribou*), found from Newfoundland and Labrador and northern Québec through British Columbia and southern Yukon (Banfield 1961). The first two subspecies could have descended from a population that survived the last

glaciation in the Beringia refugium in Alaska-Yukon or in northern Europe; *R. t. pearyi* could have survived in refugia north of the continental ice-sheets in arctic Canada or northern Greenland while woodland caribou are believed to have descended from populations that took refuge south of the continental ice-sheet, from New Jersey to Iowa and the mountainous region of New Mexico and Nevada (Banfield 1961; Roed et al. 1991).

Banfield's classification (1961), however, was based on craniometrical measurements and is not unanimously accepted. Geist (1991, 1998) has proposed the existence of seven North American subspecies using coat color, social behavior and

antler shape in large males during the rut. Roed et al. (1991), using variability in transferrin, provided evidence for important differences between woodland and barren-ground caribou. They also noticed substantial genetic heterogeneity among populations of woodland subspecies, subsequently corroborated by Van Staaden et al. (1995). On the other hand, Cronin (1992) identified many caribou genotypes using mitochondrial DNA without any apparent relationship with the current subspecies.

In the absence of a clear consensus on taxonomy, researchers came to empirically categorize caribou populations into ecotypes depending on habitat use and behavior (Edmonds 1988; Mallory and Hillis 1998). Each ecotype can consist of one or more populations. Three ecotypes of the woodland caribou thus exist in eastern North America: (1) the mountain ecotype comprising only one known population found in the Chic-Chocs mountains of southeastern Québec; (2) the barren-ground ecotype comprising two large populations occurring in the tundra and the taiga of the northern Québec-Labrador peninsula; and in-between, (3) the forest-dwelling ecotype formed by many small populations inhabiting the boreal forest. The extent of genetic exchange between caribou ecotypes is not known, nor has it been clearly established whether the three ecotypes are spatially structured. Genetic differentiation among ecotypes seems possible since the mountain ecotype is geographically isolated from other populations (Ouellet et al. 1996), and the barren-ground ecotype normally occurs far to the north of the range of the forest-dwelling ecotype during the rut in early fall. However, during periods of abundance, the barren-ground ecotype expands its distribution area to the south (Bergerud 1974; Geist 1998). In winter, the barren-ground range now overlaps that occupied by certain forest-dwelling populations (Schaefer et al. 2001) and some forest-dwelling females could follow the barren-ground caribou during spring migration (Paré 1987; Schaefer et al. 1999). Similarly, it cannot be ruled out that some barren-ground individuals establish home ranges in the area used by forest-dwelling caribou.

The impact of occasional genetic exchange among ecotypes is not known. On one hand, the presumed genetic distinctness of the forest-dwelling ecotype could disappear if exchange with barren-ground caribou has occurred during recent range expansion. On the other hand, small isolated populations in the south of the province of Québec could experience reduced genetic diversity, possibly associated

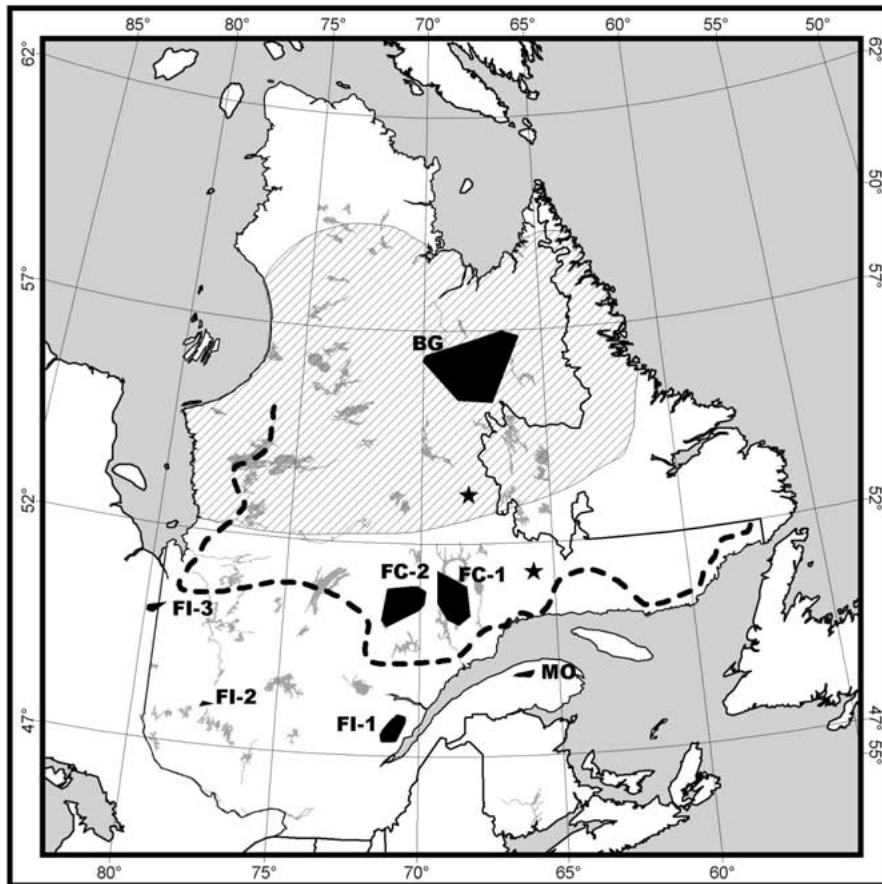
with inbreeding (Caughley 1994). This could lead to a reduction in environmental fitness and increased extinction risks associated with abiotic, biotic and stochastic factors (Levin 1995).

In this study, DNA analyses were used to compare one mountain, five forest-dwelling and one barren-ground populations of *R. t. caribou*. Considering the geographical isolation of the mountain population and the occasional range overlap of the forest-dwelling and barren-ground populations, we hypothesized that the latter two ecotypes constitute one metapopulation (sensu Lindenmayer and Lacy 1995) genetically distinct from the mountain caribou population. We also hypothesized that geographically isolated populations exhibit lower heterozygosity because of inbreeding. We predicted: (P1) that mean number of alleles per locus (A) and mean heterozygosity (H) would not differ between forest-dwelling and barren-ground populations, whose distributions partly overlap; (P2) that A and H would differ between the mountain and the other populations, the former being geographically separated by the St. Lawrence river; (P3) that the isolated forest-dwelling populations would exhibit a lower genetic diversity (A and H) than forest-dwelling populations in the continuous distribution area. Such information should help to evaluate the pertinence of current ecotype classifications and should allow for delineation of more accurate conservation strategies of caribou.

### Caribou ecotypes and study populations

In this paper, the term ecotype is used to designate populations of the same species that evolved different demographic and behavioral adaptations to cope with specific ecological (biotic and abiotic) constraints (parameters that tend to limit population growth) and releases (promoting population growth) (adapted from Mallory and Hillis 1996). A population refers to a group of conspecific individuals that is demographically, genetically, or spatially disjunct from other groups of individuals (Wells and Richmond 1995). A metapopulation represents a set of spatially disjunct populations, among which there is some immigration (Wells and Richmond 1995).

Genetic analyses were carried out on one mountain population (Gaspésie [MO]), two forest-dwelling populations of the continuous distribution area (Manicouagan [FC-1] and Pipmuacan [FC-2]), three isolated forest-dwelling populations (Charlevoix [FI-



*Figure 1.* Location of the seven studied caribou populations in which blood and muscle samples were collected, Québec. Samples were collected in all the area used by the mountain ecotype (MO). Lines delimit the distribution area of the two other study ecotypes: dotted line: southern limit of continuous distribution area of forest-dwelling ecotype (FC: forest-dwelling population within the continuous distribution area; FI: isolated forest-dwelling population); hatched lines: barren-ground ecotype (BG). Asterisks identify the origin of adult caribou used to reestablish the FI-1 population.

1], Val-d'Or [FI-2], La Sarre [FI-3]), and on the George River barren-ground population (BG; Figure 1).

Natural history, population dynamics and behavior differ between ecotypes and populations. The MO population is the only one known to pertain to the mountain ecotype in eastern North America. It has been geographically isolated from other populations for more than 150 years, and is actually the only caribou population that exists south of the St. Lawrence River (Ouellet et al. 1996). These caribou occupy a mountain range all year long (Moisan 1956). They make altitudinal movements between alpine tundra and boreal forest of the mountain slopes mostly above 700 m altitude (Ouellet et al. 1996). The area used by this population has been protected by various

statuses since 1937, and especially since the creation of a conservation park in 1981. Despite protection, their numbers have declined from approximately 700–1500 during the 1950s (Moisan 1957) to only 140–200 in recent years (Fournier and Faubert 2001).

The forest-dwelling ecotype is more widely distributed, being found discontinuously in many parts of the boreal forest. Forest-dwelling caribou aggregate in groups of a few dozen individuals in autumn and winter (Brown et al. 1986; Edmonds 1991), often in sites where they can find terrestrial lichens (Barrette and Vandal 1986). In spring, they move as individuals into the boreal forest and stay isolated from conspecifics during spring and summer (Brown et al. 1986; Edmonds 1991). They do not migrate, since distances traveled are modest (<50 km) and direc-

tion varies among animals. In the continuous distribution area, i.e., between the 49th and 54th parallels, many populations of a few thousand caribou were observed until the mid-1970s, but only reduced populations have persisted at densities of  $<2$  caribou /  $100 \text{ km}^2$  (Bergerud 1967, 1974; Mallory and Hillis 1998; Schaefer et al. 1999; Courtois et al. 2001). Despite geographic proximity ( $\approx 170 \text{ km}$ ), FC-1 and FC-2 seem to make up distinct populations according to telemetry and separation between track networks during winter aerial surveys (R. Courtois, unpublished data). FC-1 is found in old-growth coniferous forests while the habitat of FC-2 has been disturbed by a large forest fire and forest logging over the last 15 years. In 1999, these two populations comprised 216 and 127 caribou respectively.

South of the 49th parallel, only three small populations have persisted. The FI-1 population disappeared during the 1920s but was restored in the 1970s from 40 individuals captured in the continuous distribution area of forest-dwelling caribou (Jolicoeur 1993). Numbers increased over the years reaching 126 caribou in 1992. These caribou range in mostly undisturbed environments of a conservation park and adjacent areas where forest harvesting has occurred. FI-2 and FI-3 have persisted naturally and are located  $\approx 220 \text{ km}$  apart. They number approximately 45 and 370 caribou respectively (M. Paré, pers. comm.), with the former population living in and at the edge of large wetlands (Paré and Brassard 1994), while the latter population occupies harvested boreal forest.

BG caribou use the arctic tundra during calving and in summer (Couturier et al. 1996). During fall and winter, these caribou migrate in large groups  $>250 \text{ km}$  towards the south in order to use a northern section of taiga between the 52nd and 57th parallels, and into areas occupied by some forest-dwelling caribou populations. There is no forest harvesting in these areas but due to a huge population increase during the last 30 years ( $\approx 800,000$  caribou in 1993, one of the largest ungulate populations on earth, Couturier et al. 1996), grazing and trampling by caribou has led to deterioration of the summer range (Crête et al. 1996).

## Methods

### *Sampling and genetic analyses*

Most analyses were performed on blood samples (7 cc with EDTA; 199 samples) collected during collaring

of caribou (Figure 1; BG: Sept. 2000; FC-1 and FC-2: Feb.–March 1998, 1999 and 2000; FI-1: Oct. 1998–March 2000; MO: Nov.–Dec. 1998, Feb. 1999, March 2000; FI-2: March 2000; FI-3: Jan. 1998 and 1999). However, 16 muscle samples were taken from hunter kills during the hunting season in FC-1 and FC-2 (Sept.–Oct. 2000) and from carcasses of two dead animals in MO. Since animals disperse during summer and FC-1 and FC-2 are relatively close in proximity, these samples were retained only if they had been taken  $<10 \text{ km}$  from the wintering areas, which were delimited by the extent of track networks of each population.

Laboratory analyses focused on eight microsatellite loci of nuclear DNA, using methods described for caribou by Wilson et al. (1997). Loci RT1, RT5, RT6, RT7, RT9, RT24 and RT27 were retained (Wilson et al. 1997), as well as locus BM4513, first developed in cattle (Bishop et al. 1994). DNA extractions were made according to the phenol-chloroform protocol. Polymerase chain reaction (PCR) was performed using a Perkin Elmer 9600 thermal cycle GeneAmp PCR System in a final volume of  $15 \mu\text{l}$ , containing about 40 ng of nuclear DNA, between 1 and 3 pMol of each primer,  $120 \mu\text{M}$  desoxynucleic triphosphate (dNTP), 0.6 or 1.2 mM  $\text{MgCl}_2$  depending on the primer used, 0.3 units of *Taq* DNA polymerase and PCR buffer containing 10 mM Tris buffer, pH 8.8, including 1% Triton X100, 50 mM KCl and 0.16 mg/ml BSA. The PCR cycling conditions were 1 min 30 s at  $94^\circ\text{C}$ , followed by 3 cycles of 30 s at  $94^\circ\text{C}$ , 20 s at  $54^\circ\text{C}$  and 5 s at  $72^\circ\text{C}$ , then 33 cycles of 30 s at  $94^\circ\text{C}$ , 20 s at  $54^\circ\text{C}$  and 20 s at  $72^\circ\text{C}$  and finally 30 min at  $72^\circ\text{C}$ . Amplification products were separated by electrophoresis on a polyacrylamide gel, which was run on an automated ABI Prism 377 DNA Sequencer. Genotypes were interpreted from chromatographs derived from gels using GeneScan Analysis 2.1 and Genotyper 2.0 (ABI, Foster City, CA) softwares.

### *Data analyses*

Genetic diversity was assessed using mean number of alleles per locus (A), allelic richness (A[34]-1) estimated with the rarefaction method of Petit et al. (1998), observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ; Nei 1978) in each population. The rarefaction method of Petit et al. (1998) allows the estimation of allelic richness that would be obtained if all samples were of equal size (here  $n = 34$  haplo-

types). Analysis of variance (Proc Glim, SAS Institute 1989) was used to compare mean number of alleles per locus, allelic richness and mean heterozygosity among populations.

The Hardy-Weinberg (HW) equilibrium, per locus and by population, was tested using the score test (U test) of Raymond and Rousset (1995), while  $F_{IS}$  ( $f$  estimate of Weir and Cockerham 1984) allowed quantification of heterozygous deficiency. Significance of departures from HW expectations across loci or populations was tested using the Fisher method (Raymond and Rousset 1995).

The genetic and genotypic differentiation tests of Raymond and Rousset (1995) were used to compare allelic and genotypic frequency distributions between populations. The extent of pairwise genetic differentiation ( $F_{st}$ ) was estimated from  $\theta$  of Weir and Cockerham (1984). An approximation of gene flow, under the hypothesis of migration-drift equilibrium, was estimated using the equation  $Nm = (1 - F_{st})/4F_{st}$  (Prior et al. 1997). Genepop, version 3.1d (updated version of Raymond and Rousset 1995), and Arlequin 2.000 (Schneider et al. 2000) softwares were used for calculations and statistical comparisons. The Mantel (1967) test was used to examine the relationship between geographic distance and  $F_{st}/(1 - F_{st})$  (Genetix software, version 4.01, Belkhir et al. 2001).

Degree of population overlap was represented by positioning the samples on the axis of the greatest inertia established using factorial correspondence analyses (FCA, Belkhir et al. 2001). Principles of FCA have been described by Benzécri (1973) and application to genetic data can be found in She et al. (1987). In brief, FCA is based on the similarity of individuals in their allelic state for each allele which allows the projection of all individuals in a space defined by the components, the most similar individuals being represented close together. In all tests, the significance threshold was adjusted in order to take into account multiple comparisons (Bonferroni correction, Miller 1981).

## Results

The eight loci studied were all quite variable (Table 1). Loci each contained between 12 and 33 alleles, the maximum number being noted on locus BM4513. Mean number of alleles per locus differed among populations ( $F_{[6,42]} = 13.55$ ,  $P < 0.0001$ ). BG exhib-

ited a significantly higher mean number of alleles per locus than those of all other populations. FC-1, FC-2, FI-3 and FI-1 displayed intermediate values while mean number of alleles per locus was the lowest in MO and FI-2, the two most geographically isolated populations. Allelic richness was the highest in BG and FC-1, and the lowest in FI-2, MO and FI-1 ( $F_{[6,42]} = 9.24$ ,  $P < 0.0001$ ), other populations exhibiting intermediate values. Observed ( $F_{[6,42]} = 2.73$ ,  $P = 0.0250$ ) heterozygosity tended to be higher in BG and lower in MO and FI-2. Expected heterozygosity was the lowest in FI-2, intermediate in MO and FI-3 and higher in other populations ( $F_{[6,42]} = 4.07$ ,  $P = 0.0026$ ).

The Hardy-Weinberg equilibrium was not respected for locus RT1 in FI-3 ( $P = 0.001$ ) and locus RT5 ( $P \leq 0.001$ ) in FI-1, FC-1 and FC-2. Significant departures from HW expectations across loci within populations was observed in BG, which indicated a heterozygote deficiency at RT5 and an excess at RT1, RT7 and BM4513 (multi-loci;  $\chi^2 = \infty$ ,  $df = 16$ ,  $P < 0.001$ ). Allelic frequency distributions differed among samples for each locus ( $P < 0.001$ ), suggesting that they represented genetically distinct populations. Populations also differed in genotypic distributions on each locus ( $P < 0.001$ ). Extent of genetic differentiation between pairs of populations were all significant ( $P < 0.001$ ). Largest values were observed between BG paired with MO, FI-2, FI-1 and FI-3 ( $\theta \geq 0.113$ ) and between MO and all other populations ( $\theta \geq 0.103$ ; Table 2). Genetic distance was usually low among forest-dwelling populations except for two pairs including isolated populations (FI-1 vs. FI-2 and FI-2 vs. FC-2). FC-1 and FC-2 were the least genetically different populations ( $\theta = 0.016$ ). Gene flow estimates were particularly high between these two populations ( $Nm = 15.0$ ) and low between MO and BG and all other populations ( $Nm \leq 2.6$ ). Moderate gene flow estimates ( $Nm \geq 3.1$ ) were observed among all pairs of forest-dwelling populations, except three isolated pairs (FI-1 vs. FI-2 and FI-2 vs. FC-1 and FC-2). A pattern of genetic isolation by distance was revealed when the seven populations were considered simultaneously in the Mantel test ( $G = 2.018$ ;  $P = 0.025$ ;  $r = 0.64$ , Figure 2). Nevertheless, the relationship appeared to be mainly caused by the two most distant populations since the relationship was not significant when we excluded BG ( $G = 1.450$ ;  $P = 0.130$ ;  $r = 0.38$ ) or MO ( $G = 0.826$ ;  $P = 0.243$ ;  $r = 0.21$ ) from the analysis. Similarly, the relationship was not strong enough to be significant

Table 1. Number of alleles observed (A) and expected with a sample size of 34 haplotypes (A[34]-1; Petit et al. 1998), observed (Ho) and expected heterozygosity (He, corrected for sample bias according to Nei 1978) and heterozygous deficiency (F<sub>IS</sub>, estimated from *f* of Weir and Cockerham 1984) for seven caribou populations in Québec that belong to three ecotypes (MO = mountain; FI and FC = forest-dwelling ecotype (isolated or continuous distribution, respectively); BG = barren-ground)

Locus	MO (n = 33)	FI-1 (n = 29)	FI-2 (n = 18)	FI-3 (n = 30)	FC-2 (n = 28)	FC-1 (n = 32)	BG (n = 40)
RT1 A	6	5	4	7	9	20	
A[34]-1	4.230	3.986	3.000	5.772	5.671	6.122	13.059
Ho	0.73	0.76	0.67	0.70	0.75	0.78	0.90
He	0.68	0.74	0.58	0.81	0.75	0.80	0.88
F <sub>IS</sub>	-0.076	-0.026	-0.156	0.136*	-0.002	0.013	-0.018
RT5 A	4	7	4	8	13	13	13
A[34]-1	2.407	5.005	2.998	6.074	9.732	9.822	8.770
Ho	0.45	0.62	0.56	0.70	0.61	0.58	0.53
He	0.56	0.75	0.65	0.77	0.83	0.88	0.67
F <sub>IS</sub>	0.195	0.175*	0.148	0.098	0.273*	0.343*	0.219
RT6 A	7	6	5	7	6	7	9
A[34]-1	4.749	4.511	4.000	5.723	4.787	5.409	6.257
Ho	0.64	0.62	0.72	0.77	0.71	0.72	0.60
He	0.74	0.75	0.72	0.77	0.72	0.74	0.70
F <sub>IS</sub>	0.138	0.178	-0.005	0.001	0.013	0.027	0.142
RT7 A	7	7	6	5	7	12	13
A[34]-1	4.056	5.349	5.000	3.490	5.214	8.786	7.339
Ho	0.58	0.72	0.41	0.60	0.71	0.91	0.98
He	0.62	0.73	0.55	0.62	0.82	0.86	0.80
F <sub>IS</sub>	0.079	0.011	0.256	0.028	0.135	-0.060	-0.215
RT9 A	6	4	3	8	4	7	10
A[34]-1	3.980	2.419	2.000	5.436	2.925	4.860	6.474
Ho	0.64	0.66	0.67	0.70	0.75	0.78	0.80
He	0.73	0.55	0.60	0.70	0.62	0.69	0.76
F <sub>IS</sub>	0.131	-0.193	-0.109	0.002	-0.222	-0.132	-0.053
RT24 A	3	7	4	8	6	9	14
A[34]-1	1.769	5.755	2.998	6.303	4.382	6.446	9.798
Ho	0.48	0.86	0.56	0.60	0.57	0.56	0.78
He	0.49	0.79	0.58	0.71	0.56	0.61	0.84
F <sub>IS</sub>	0.012	-0.086	0.040	0.159	0.135	0.079	0.082
RT27 A	4	4	3	6	10	7	11
A[34]-1	2.568	2.998	2.000	4.158	7.810	4.948	7.139
Ho	0.56	0.68	0.71	0.64	0.81	0.67	0.68
He	0.56	0.72	0.68	0.59	0.82	0.76	0.80
F <sub>IS</sub>	0.001	0.057	-0.035	-0.090	0.006	0.127	0.159
BM4513 A	9	14	6	12	15	15	14
A[34]-1	6.739	10.869	4.887	8.529	11.584	10.928	8.379
Ho	0.91	0.79	0.67	0.83	0.86	0.78	0.95
He	0.84	0.90	0.71	0.82	0.90	0.89	0.78
F <sub>IS</sub>	-0.084	0.123	0.064	-0.013	0.048	0.119	-0.224

Table 1. Continued

Locus	MO (n = 33)	FI-1 (n = 29)	FI-2 (n = 18)	FI-3 (n = 30)	FC-2 (n = 28)	FC-1 (n = 32)	BG (n = 40)
Mean values							
A	5.8 <sup>de</sup>	6.8 <sup>cd</sup>	4.4 <sup>e</sup>	7.6 <sup>bcd</sup>	8.5 <sup>bc</sup>	9.9 <sup>b</sup>	13.0 <sup>a</sup>
A[34]-1	3.812 <sup>d</sup>	5.111 <sup>cd</sup>	3.360 <sup>d</sup>	5.686 <sup>bc</sup>	6.551 <sup>bc</sup>	7.165 <sup>ab</sup>	8.402 <sup>a</sup>
Ho	0.62 <sup>b</sup>	0.71 <sup>ab</sup>	0.62 <sup>b</sup>	0.69 <sup>ab</sup>	0.72 <sup>ab</sup>	0.72 <sup>ab</sup>	0.77 <sup>a</sup>
He	0.65 <sup>bc</sup>	0.74 <sup>a</sup>	0.63 <sup>c</sup>	0.72 <sup>ab</sup>	0.78 <sup>a</sup>	0.77 <sup>a</sup>	0.78 <sup>a</sup>

\*Significant difference from Hardy-Weinberg equilibrium at  $P < 0.007$  ( $\alpha = 0.05$ ;  $k = 7$ ).

<sup>abcd</sup>Population means with the same letter do not differ at  $P < 0.05$  (Duncan's multiple range test).

Table 2. Genetic differentiation ( $F_{ST}$ , estimated from  $\theta$  of Weir and Cockerham (1984), above the diagonal) and gene flow estimates ( $Nm$ ; below the diagonal) between seven caribou populations in Québec that belong to three ecotypes (MO = mountain; FI and FC = forest-dwelling ecotype, isolated or continuous distribution, respectively; BG = barren-ground)

	MO (n = 33)	FI-1 (n = 29)	FI-2 (n = 18)	FI-3 (n = 30)	FC-2 (n = 28)	FC-1 (n = 32)	BG (n = 40)
MO	—	0.117*	0.167*	0.104*	0.114*	0.103*	0.172*
FI-1	1.9	—	0.116*	0.050*	0.062*	0.051*	0.118*
FI-2	1.3	1.9	—	0.074*	0.111*	0.097*	0.172*
FI-3	2.2	4.8	3.1	—	0.065*	0.059*	0.113*
FC-2	2.0	3.8	2.0	3.6	—	0.016*	0.094*
FC-1	2.2	4.6	1.2	4.0	15.0	—	0.087*
BG	1.2	1.9	1.5	2.0	2.4	2.6	—

\*Significant difference at  $P < 0.002$  ( $\alpha = 0.05$ ;  $k = 21$ ).

for the five forest-dwelling populations alone ( $G = 1.793$ ;  $P = 0.108$ ;  $r = 0.49$ ).

Correspondence analysis underscored the uniqueness of MO and BG, which appeared completely separate from each other and the other populations (Figure 3a). An analysis restricted to the five forest-dwelling populations demonstrated three groups: FI-1, separated from the other populations according to the first and second axes, and two relatively compact groups separated according to axis I: FC-1 and FC-2 on one side and FI-2 and FI-3 on the other side (Figure 3b). However, all five populations partially overlapped.

## Discussion

### Ecotypes and populations

Adequate differentiation among taxonomic groups requires an examination of many genetic and biological characteristics such as natural history, morphological traits, geographic distribution and direct study of

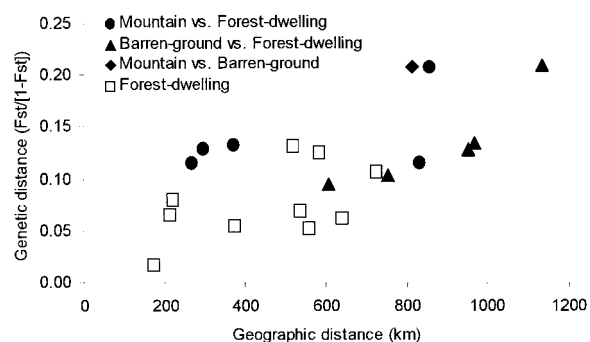


Figure 2. Relationship between geographic and genetic distance in seven caribou populations studied in Québec that belong to three ecotypes (MO = mountain; FI and FC = forest-dwelling ecotype (isolated or continuous distribution, respectively); BG = barren-ground).

individual movements (Geist 1991; Roed et al. 1991; Cronin 1992; Bossart and Prowell 1998). Based on geographic distribution and movements of caribou, we hypothesized that forest-dwelling and barren-ground ecotypes represent one metapopulation, distinct from mountain caribou. According to microsatellite loci, all

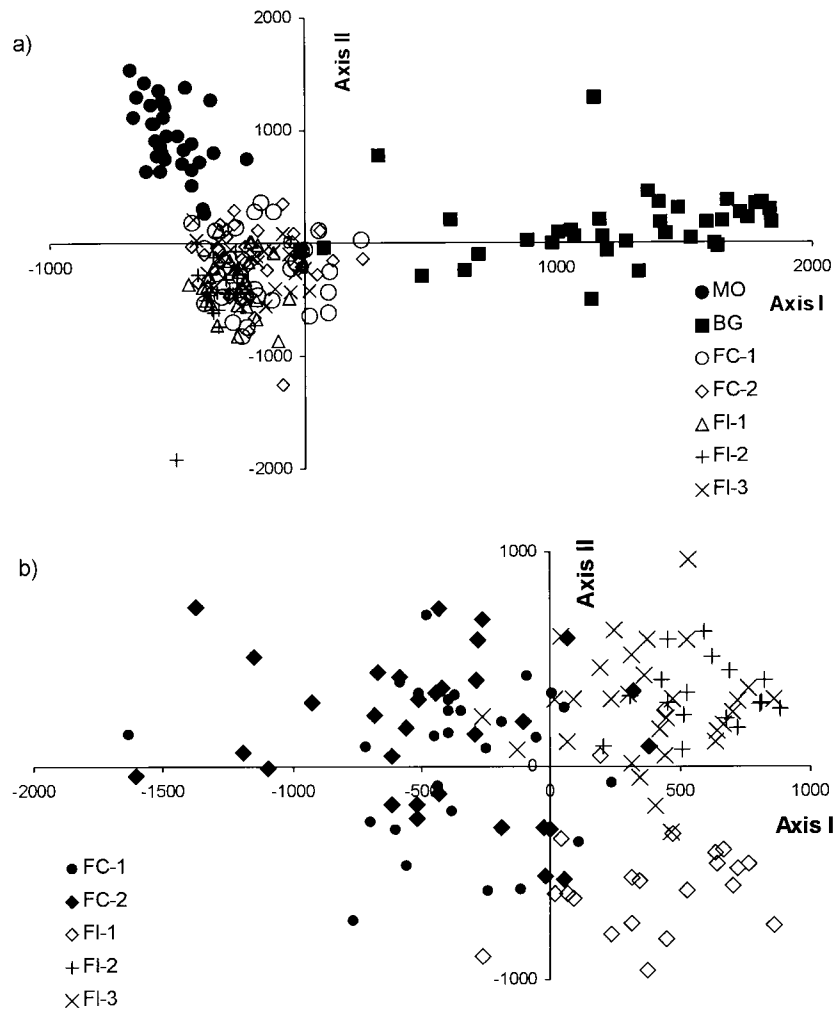


Figure 3. Projection of the caribou samples on the first two axes after correspondence analyses; (a) analysis including the seven populations studied: the mountain (MO) and the barren-ground (BG) caribou populations are clearly separate from each other and those of the forest-dwelling ecotype (FI and FC = isolated or continuous distribution, respectively); (b) analysis including only the forest-dwelling populations: these populations overlap, particularly those situated <200 km apart (FC-1 and FC-2; FI-2 and FI-3).

three ecotypes correspond to distinct genetic entities. All populations exhibited significant genetic differentiation, particularly the barren-ground and mountain populations. The low genetic flow among populations of different ecotypes and projection of individuals with correspondence analysis also suggest a distinction between the three caribou ecotypes. Contrary to our first prediction, the mean number of alleles per locus was higher for barren-ground than forest-dwelling populations. This could be partly related to different sample sizes since mean richness did not differ for BG and one forest-dwelling population. A north-south decline is suggested by the data with the lowest values noted in isolated populations.

Genetic exchanges between barren-ground and forest-dwelling ecotypes cannot be completely ruled out since our first prediction was only verified for the mean number of alleles per locus and not for allelic richness or heterozygosity. However, these two ecotypes are separated during the rut in early fall and there is no evidence that BG caribou have migrated as far south as the area actually frequented by FC-1 or FC-2 populations. If some exchange exists, as suggested by Schaefer et al. (1999), it occurs between the barren-ground and northern forest-dwelling populations, which in turn exchange individuals with those located farther south. However, the estimates of gene flow based on  $Nm$  values



derived from  $F_{st}$  in this study suggest that such genetic exchange is very limited. Actual  $N_m$  values can also be overestimated due to past events. For example, positive  $N_m$  values were observed between MO and other populations despite geographic isolation preventing exchange for at least 150 years. Exclusion between groups has also been observed in Alaska where caribou and reindeer populations that have been sympatric for a hundred years still remain genetically distinct (Cronin et al. 1995).

The various forest-dwelling populations resemble each other genetically, although some populations have been isolated for many decades. Movements between populations are potentially high because caribou can travel long distances and have large annual home ranges ( $\geq 200$ – $300$  km<sup>2</sup>, Stuart-Smith et al. 1997). Nevertheless, movement of forest-dwelling caribou are much shorter than that of barren-ground caribou and telemetry has shown a propensity for philopatry; forest-dwelling animals tend to be faithful to seasonal home ranges, including those used during the rutting period (Edmonds 1988; Cumming 1992; Brown et al. 1996). Exchange between populations is therefore more likely to result from natal dispersal. Among cervids, fawns usually follow their mother for up to one year but then disperse away their yearlings at the time of next parturition. Yearlings tend to establish themselves close to their mother, but some can travel tens of kilometers before settling down (Labonté et al. 1998), especially if habitat conditions do not impede movements (Nixon et al. 1991). This results in population structuring.

Furthermore, telemetry demonstrates that areas used by different forest-dwelling herds can overlap (Rettie and Messier 1998; Schaefer et al. 2001). Thus, it is probable that populations within the continuous distribution area constitute one metapopulation. Isolation by distance in forest-dwelling populations cannot be ruled out at this time. Genetic and geographic distances among forest-dwelling populations were not significantly correlated but a trend was suggested in Figure 2 ( $P = 0.108$ ). Conversely, several populations relatively close in proximity, such as FI-2 and FI-3 (220 km) or FI-1 and FC-2 (212 km), experienced only moderate gene flow which suggests that populations spaced  $>200$  km apart are isolated from each other when located outside the continuous distribution area.

The reasons why forest-dwelling caribou are not present throughout the entire boreal forest are not entirely known. Historically, caribou numbers were drastically reduced by hunting and predation

(Bergerud 1974) and the species was extirpated from large areas. In the southern part of the boreal forest, the remaining populations appear to be too small to settle new areas. Also, habitat modification could prevent dispersal (Lindenmayer and Lacy 1995). For example, forest harvesting and wildfires fragment the habitat, creating discontinuous islands of forests over the short term (Hanson et al. 1990; Caughley 1994). In certain cases, coniferous stands that caribou prefer are not restored or are replaced by succession species (Sirois 1997; Gagnon et al. 1998; Lavoie and Sirois 1998). Caribou avoid these disturbed habitats (Saperstein 1996), which could act as a barrier to dispersal of individuals.

### *Genetic diversity*

Many authors have stressed the importance of genetic diversity for adaptability (survival, productivity, reproduction and physical condition; Hundertmark et al. 1992; Cronin 1992; Caughley 1994; Levin 1995; Delany et al. 2000). However, the gradual evolution towards homozygosity is inevitable in closed populations. Nonetheless, the speed of this phenomenon is inversely proportional to effective population size (Wright 1951). In agreement with our second and third predictions, genetic diversity was lower in the geographically isolated populations, particularly MO, FI-1 and FI-2. As a comparison, 6.9 alleles per locus were found in the Selkirk caribou population in southeastern British Columbia (Wilson et al. 1997) while MO, FI-1 and FI-2 exhibited 4.1–5.7 alleles on the seven loci which were in common in this study and that of Wilson et al. (1997). The Selkirk population included only 25 to 35 caribou, but 60 individuals had been previously relocated there, and some of those individuals reproduced with the original caribou population (Simpson et al. 1997). This probably explains why the mean number of alleles found per locus was higher than in our isolated populations.

Locus by locus, expected levels of heterozygosity in our isolated populations were all lower than those of the Selkirk caribou, except for locus RT24 (Wilson et al. 1997). Nevertheless, the mean number of alleles per locus and expected heterozygosity of our isolated populations were still twice as high as those of insular Svalbard reindeer (Côté et al. 2002). The present situation does not seem to affect the population dynamic of caribou, judging from productivity (14–20% fawns in winter) which appears to be comparable in the

FI-2 population to values observed in other caribou populations (Paré and Brassard 1994).

Forest-dwelling populations located in the continuous distribution area and BG caribou exhibited substantial genetic diversity. Mean number of alleles per locus (8.5–13.0) was higher than those of Selkirk caribou and similar to that reported for caribou (4.7–5.9) and other species (2.1–7.6) for a comparable number of loci, i.e., 5–20 (Cronin et al. 2001). Likewise, heterozygosity rates (0.77–0.78) were similar to those of Selkirk caribou and higher than those of other cervid populations (caribou: 0.52–0.64; other species: 0.27–0.68; Cronin et al. 2001). The large size of the BG population, and genetic exchange between FC-1 and FC-2, has undoubtedly enabled these three populations to maintain an elevated heterozygosity rate.

Due to geographical isolation, it is probable that genetic diversity will decrease and genetic differentiation will increase in the isolated populations of MO, FI-1 and FI-2 and possibly FI-3. Nevertheless, the number of alleles and level of heterozygosity measured were still relatively elevated. Consequently, the increased risks for conservation of isolated caribou populations will likely be linked more to low numbers than to inbreeding. Indeed, it has been proposed that animal populations are more vulnerable to stochastic variation in population dynamics and to habitat loss than to consequences of inbreeding (Caughley 1994; Levin 1995). The recent loss of four of the seven collared females to predation in the small FI-2 population following six years without mortality (M. Paré, pers. comm.) stresses the demographic fragility of isolated populations.

#### *Implications for conservation*

Many taxonomic classifications have been previously proposed for caribou (see Banfield 1961 for review). Before Banfield (1961), the forest-dwelling and barren-ground ecotypes of the Québec-Labrador peninsula were considered distinct species (*R. caribou* and *R. caboti*, respectively, Moisan 1956; Banfield 1958, 1961) and Geist (1998) recently classified them as different subspecies (*R. t. caribou* and *R. t. caboti*).

The phylogeny of the three studied ecotypes could differ appreciably. Cameron (1958) suggested that terrestrial animals from eastern Canada originated from populations that survived the Wisconsin glaciation in the coastal regions of southeastern United States and were separated from the western popula-

tions by glaciers covering the Appalachian mountains. Furthermore, species that moved into what is now Ontario, northern Québec and Labrador including caribou (Banfield 1961) may have spent the last ice-period west of the Appalachian mountains (Cameron 1958). Finally, contrary to other ecotypes, the barren-ground caribou of the Québec-Labrador peninsula could have had some post ice-age contact with the subspecies *R. t. groenlandicus* (Roed et al. 1991).

A broad scale study including several populations of each ecotype would help to determine if the three studied caribou ecotypes could be considered as distinct subspecies. They meet guidelines suggested by Cronin (1992) as they have different natural histories, distributions (behavior, migration, habitat selection, dispersal; see introduction), and genetic traits (this study). In addition, they meet those of Avise and Ball (1990), i.e., groups of actually or potentially interbreeding populations; groups phylogenetically distinguishable from, but reproductively compatible with other such groups; groups identified from the examination of multiple, independent genetically based traits; and groups that are normally allopatric but that may meet in secondary hybrid zones.

Population and habitat management should be revised to adopt specific guidelines for each ecotype in terms of legal status, sport and native hunting, and range management. All known forest-dwelling caribou populations should be considered vulnerable in the Québec-Labrador peninsula given that an effective population size of 50 is necessary to keep inbreeding at a tolerable level and to secure short term survival, and that  $\geq 500$  individuals are needed to ensure long term persistence of a population (Frankel and Soulé 1981; Cumming and Beange 1993; Caughley 1994). In order to preserve biodiversity, appropriate management measures should be adopted to maintain and even increase local population abundance. At present, no artificial measures seem necessary to increase genetic diversity. However, if forest-dwelling caribou form a metapopulation, as our results suggest, an important strategy for stabilizing numbers and heterozygosity and to allow for colonization of vacant habitats would be to maintain connectivity between the units forming the metapopulation (Levin 1995; Hanski and Simberloff 1997). Forest management based on the preservation of preferred habitats linked by travel corridors (Cumming 1992) seems of prime concern for insuring the conservation of forest-dwelling caribou.

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