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Genetic relationships of three Yukon caribou herds determined by DNA typing

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Abstract: In this paper we examine genetic relationships of caribou (*Rangifer tarandus caribou*) in the Aishihik, Chisana, and Wolf Lake herds in the Yukon using DNA fingerprinting. The assignment test used in this analysis showed that the caribou herds were distinct. This finding is consistent with movement data from radio-collared caribou which demonstrates home range fidelity. We found a high level of heterozygosity and a genetic basis for population boundaries. DNA fingerprinting may provide an effective means to compare ecological and genetic relationships.

Key words: DNA fingerprinting, home range, microsatellite, woodland caribou.

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Introduction

Comprehensive studies of caribou (*Rangifer tarandus caribou*) have been ongoing in the Yukon since 1980. The objective of these studies is to inventory all herds by identifying each population's total and seasonal ranges, secure reliable population size estimates, and monitor population trends using sex/age composition count surveys. From 1980 to 1998, 178 population counts were conducted on 17 of 22 herds, and 289 relocation flights were flown to provide relocation contacts for 485 radio-collared caribou (Farnell *et al.,* 1998). Since 1995, whole blood samples were collected from 228 caribou captured and handled for radio-telemetry studies of 13 herds. These samples allow the opportunity to examine the genetic relationships between herds.

Over the past 18 years, the data on movements have shown that caribou have strong fidelity to discrete home ranges (Fig. 1) (Farnell & Russell, 1984; Farnell & MacDonald, 1988; 1989; 1990; Farnell *et al.,* 1991; 1996; 1998; Kuzyk & Farnell, in prep). Caribou herds in the Yukon conform to typical patterns of distribution for woodland/mountain caribou, being highly dispersed during summer and clumped during winter. On the basis of these observations wildlife managers in Yukon have defined a caribou herd as those caribou sharing a common winter range (Farnell & Russell, 1984). Historic and present day distributions of migratory caribou are known to periodically overlap with sedentary caribou herds during winter (Boertje & Gardner, 1996; Fancy *et al.* 1994). Despite frequent overlap, radio-collared individuals have strong herd fidelity (Farnell & Russell, 1984). Consequently, caribou herds are managed as discrete populations. The geographic boundaries established from surveys have facilitated management planning on a herd basis but genetic relationships of these populations remain to be established.

Within this management framework there is concern for the viability of small caribou populations. There may be a genetic basement number below which a population cannot persist for very long because of the effects of inbreeding and loss of heterozygosity (Grumbine, 1992). This could reduce reproduction and survival capabilities of individuals and lead to extinction at the population (i.e. herd) level from demographic, environmental, and/or catastrophic uncertainties. To test this prediction, it is necessary to determine whether there is gene flow between adjacent populations or genetic variation within small populations.

The use of DNA fingerprinting using microsatellite loci allows measurement of variation within and among populations and can distinguish separate populations at the genetic level (Kushny *et al.,* 1996; Paetkau *et al.,* 1995). This could improve our understanding of potential recent and historical

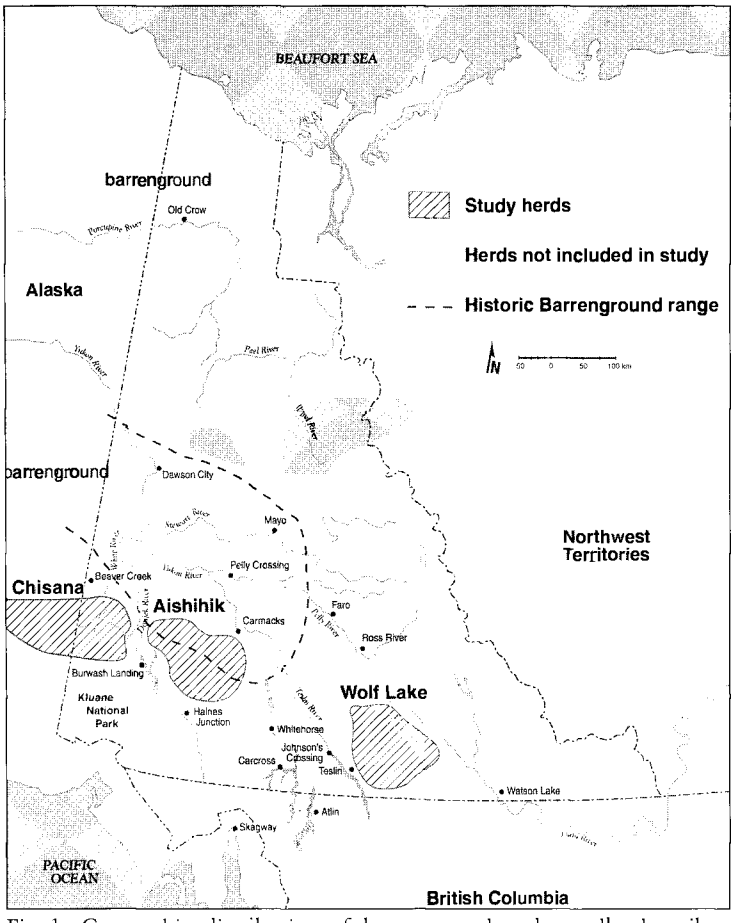

Fig. 1. Geographic distribution of barrenground and woodland caribou herds in Yukon as determined from inventory studies carried out between 1980 and 1998.

migrations between populations and therefore test our conceptual framework of population identity. DNA fingerprinting could furthermore provide a measure of individual herd heterozygosity and therefore provide insight into herd genetic variation and determination of minimum viable population sizes.

We describe the use of microsatellite markers to describe the genetic relationship between three woodland caribou herds that show varying degrees of geographic separation and population trends. Sample sizes were as follows: Aishihik (n=32), Chisana (n=22), Wolf Lake (n=31). All three study herds are located in southern Yukon, and their boundaries do not overlap (Fig. 1). No movement between herds has been documented, but there are other caribou herds located between them. The herds exhibited a variety of population trends. The Aishihik herd increased at r=0.12 (from 750 in 1993 to 1200 in 1997), the Chisana herd declined at r=-0.14 (from 2000 in 1989 to 500 in 1997), and the Wolf Lake herd remained relatively stable at r=0.03 (from 1200 in 1993 to 1400 in 1998) (data on file).

Materials and methods

Whole blood samples (approximately 10ml) were collected from caribou when they were radio-collared. The red blood cells were lysed by repeated washings with 1 X ACK (0.155 M NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA, pH 7.4). DNA was isolated from white blood cells using the QIAamp Blood protocol (QIAGEN Inc.). Each DNA sample was amplified at six microsatellite loci (RT6, RT7, RT9, RT13, RT24, and RT27; Wilson *et al.*, 1997) using the polymerase chain reaction (PCR) . PCR conditions for a Perkin-Elmer 9600 thermal cycler were 1 min at 94 °C, followed by three cycles of 30 s at 94 °C, 20 s at 54 °C and 5 s at 72 °C, followed by 33 cycles of 15 s at 94 °C, 20 s at 54 °C and 1 s at 72 °C, and then 30 s at 72 °C. One primer from each pair was fluorescently labelled. Allele sizes for each locus were determined by analysis of the PCR products after polyacrylamide gel electrophoresis on a model 373A Automated Sequencer (PE Biosystems), using "Genescan™ 2.0.2" and "Genotyper® 2.0" software.

The identified genotypes were used to measure the genetic diversity within herds and, subsequently, the relationships among herds. The genetic diversity within a herd was measured by the number of alleles per locus, the degree of heterozygosity, and the probability of identity. Heterozygosity is the proportion of a population exhibiting two different alleles at a given locus. The probability of identity is the probability that two randomly chosen individuals are genetically identical. Heterozygosity (H) and probability of identity at a single locus (P_{id}) were calculated by the following formulae:

$H = 1-\sum p_i^2$ and $P_{id} = \sum p_i^4 + \sum\sum(2p_ip_j)^2$, respectively, where p_i and p_j are the frequencies of the i^{th} and j^{th} allele. The probability of identity over all loci is the product of the probabilities of identity at each locus.

Genetic distances between herds were calculated by D_{LR} (as described by Paetkau *et al.,* 1997). In addition, an assignment test, which assigns each individual to the herd in which its genotype is most likely to occur, was performed to determine the distinctness of each herd. These data were then compared to herd home range boundaries determined from radio-collar movement data. The method for these calculations is available at the following website:
http://www.biology.ualberta.ca/jbrzusto/Doh.html.

Results

The measures of genetic diversity show that each herd exhibited a high degree of variation (Table 1). The number of alleles per locus ranged from 7.5 to 9.3. For the herds examined, heterozygosities ranged from approximately 74% to 82%. The probabilities of identity range from three in 10 million to one in 100 million.

Table 1. Measures of genetic diversity: heterozygosity, probability of identity and mean number of alleles per locus for three Yukon caribou herds.

Caribou Herd	Sample Size	Mean Number of Alleles	Hetero-zygosity %	Probability of Identity
Aishihik	32	7.50	74.1	3.8×10^{-7}
Chisana	22	8.00	81.6	1.1×10^{-8}
Wolf Lake	29	9.33	82.3	1.4×10^{-8}

Discussion

The genetic distances calculated by D_{LR} mean that, based on their genotypes, caribou from one population are that many times more likely to belong to their own population than to the other population. The genetic distances between the three caribou herds ranged from 2.2 to 3.2 (Table 2).

The genetic distances between the three herds were quite similar, despite their different geographic separation. The shortest genetic distance was between Chisana and Wolf Lake, which are the two most geographically distant herds. These results suggest that geographic distance between herds does not influence genetic relationships.

The results of the assignment test show that the three caribou herds are virtually distinct (Fig. 2). That is, almost all of the caribou assign to their respective herds; an average of only 8% of individuals were not assignable to their herds. Assignment tests have shown similar results in other species (Wasser & Strobeck, 1998).

The caribou that do not assign to the expected populations could be migrants or offspring of migrants. An alternative explanation may be that, originally, these herds were all related and they are not yet completely differentiated. Analysis of these samples at an increased number of loci may resolve this issue. Analyses of additional samples from the herds examined and from additional herds from the same area may provide a better understanding of caribou population structure in Yukon.

Conclusion

These data show that there is a genetic basis for population boundaries defined from seasonal movement data for adjacent and separate caribou herds, and so far justifies our management framework. Future sampling will provide more rigor to this analysis by examining genetic variability and possible patterns of gene flow between populations located at closer geographical proximity to each other.

At its present population level and trend the Chisana herd has a high level of heterozygosity and is presently not likely to be threatened by the detrimental effects of inbreeding (subject to lowered reproduction and survival as a result of lowered genetic variation). Further monitoring of genetic variability in the Chisana herd over time and increased sampling of other small caribou herds (<200 individuals) could provide insight into the potential level at which a genetic basement population may occur.

DNA fingerprinting using microsatellite analysis is an effective means of comparing ecological and genetic perspectives. These results constitute the first step needed to advance our understanding to the broader implications of genetic structure of cari-

Table 2. Genetic distances between three Yukon caribou herds.

	Aishihik	Chisana	Wolf Lake
Aishihik	0		
Chisana	2.9	0	
Wolf Lake	3.2	2.2	0

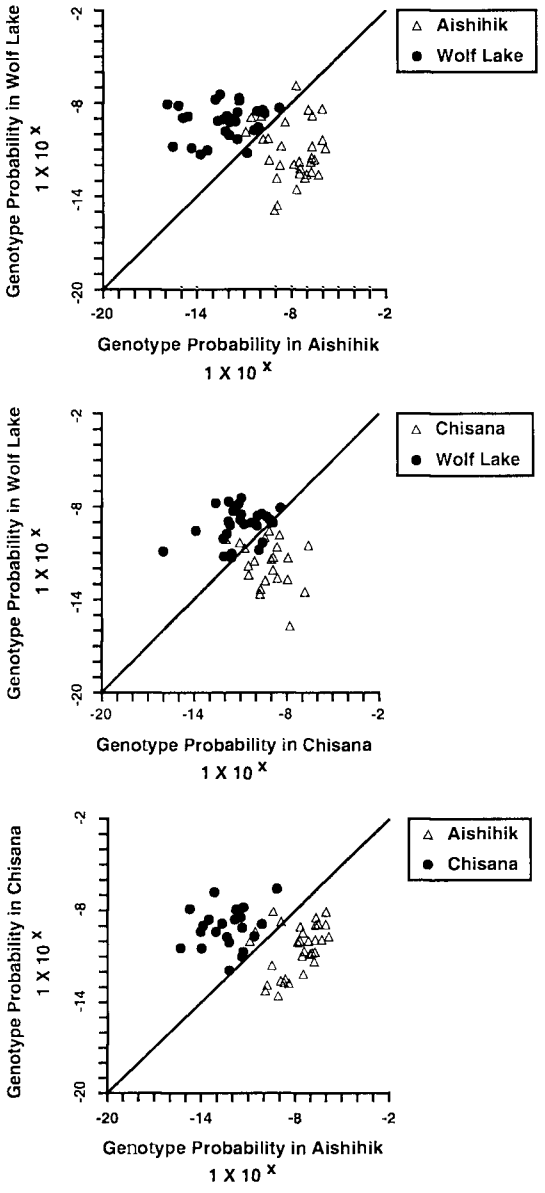

Fig. 2. Results of assignment tests for pairwise comparisons of each of three caribou herds. (An individual occurring on the diagonal has an equal probability of occurring in each population).

bou herds. With more data it may be possible to identify a "population of origin" for caribou samples.

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