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Genetic survey of caribou populations using microsatellite DNA

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Abstract: Microsatellite loci are highly variable regions of eukaryotic DNA that consist of tandemly repeated sequences of one to six nucleotides in length. The use of microsatellites and the Polymerase Chain Reaction (PCR) are powerful tools for quantifying genetic variation within and among individual populations. Recently, we have developed primers for caribou that amplify 4 microsatellite loci. These microsatellite loci were used to survey the genetic variation in populations of Barren-ground caribou (*Rangifer tarandus groenlandicus*), Peary caribou (*R.t. pearyi*) and Woodland caribou (*R.t. caribou*) of Canada. The four loci examined were all polymorphic, revealing high levels of heterozygosity (> 0.74) in all of the study populations.

Key words: microsatellites, DNA fingerprinting, population level analysis, *Rangifer tarandus*

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Introduction

DNA fingerprinting was first introduced in 1985 by Jeffreys *et al.*, which involved the analysis of highly variable regions of DNA (tandem-repetitive 'minisatellite' regions of 10-50 bases). Variation observed at these regions arises very rapidly relative to other types of DNA and the high levels of heterozygosity at these regions have been utilized to identify relatively unique genotypes in individuals, dubbed 'DNA fingerprints'. The use of DNA fingerprinting has provided valuable information in the analysis of genetic diseases, forensics and ecological genetics (Jarman & Wells, 1989). This technique has been applied to study genetic variation in wildlife populations. For example, Gilbert *et al.* (1990) were able to show population differentiation using DNA fingerprints among Channel Island fox (*Urocyon litoralis*) from isolated islands, and Gibbs et al. (1990) revealed multiple paternity in families of polygynous red-winged blackbirds (*Agelaius phoeniceus*). However, there are limitations to the standard methods of DNA fingerprinting and their application to population level analysis. For example, multi-locus minisatellite analysis may reveal bands of varying intensity and comparisons between gels may lead to subjective and possibly incorrect decisions about band identities. Similarly sized alleles from different loci make the estimate of genetic parameters much more difficult. Furthermore, slight variation in the experimental conditions can produce erratic results, such as samples that vary in the amount of DNA and gels run under slightly different conditions (Queller *et al.*, 1993).

In 1989, Weber & May introduced a new technique of DNA fingerprinting, known as microsatellite analysis, which was first developed to facilitate genetic mapping in humans. Microsatellites are simple sequence loci that are widely dispersed in eukaryotic genomes and are highly polymorphic due to variation in the number of repeat units (1 to 6 base pairs in length) . The large number of microsatellite loci combined with their high variability make them ideal markers for genetic analyses at the level of populations. Microsatellites provide many advantages over other methods of genetic analysis, including: single locus analysis - providing for ease of interpretation (Weber & May, 1989), comparisons may be made across gels for loci, the genotypes of 36 individuals at as many as 3 loci can be determined from a single gel (Edwards *et al.*, 1991), and microsatellites developed in one species can used in related taxa thereby saving time and effort in the development and screening of genomic libraries. For example, microsatellites developed for the long- finned pilot whale (*Globicephala melas:* Odontoceti) were found to be useful in the analysis of variability in other whale species, including all toothed and baleen whales (*Mysticeti*) (Schlötterer *et al.,* 1991). In addition, amplification of microsatellite loci through the Polymerase Chain Reaction

(PCR) allows for use of small samples of DNA. Large amounts of specific DNA fragments can be synthesized from a small amount of initial DNA template. The template is amplified by repeated cycles of heat denaturation, annealing of oligonucleotide primers (short specific sequences of single stranded DNA) to the complementary strands of the template DNA, and the synthesis of DNA (White *et al.*, 1989). A single copy of a nucleotide sequence of interest can be amplified over a million times in only a few hours. With the use of PCR it is possible to obtain DNA from single hairs (Higuchi *et al.*, 1988) and fecal samples (Höss *et al.*, 1992) for use in DNA fingerprint analyses.

Here we report data from the analysis of four microsatelhte loci in populations of Peary caribou (*Rangifer tarandus pearyi* - Western Queen Elizabeth Islands), Barren-ground caribou (*R.t. groenlandicus* - Beverly and Bathurst herds) and Woodland caribou (*R.t. caribou* - George River herd and Mount Revelstoke and Glacier National Parks).

Materials and methods

Caribou samples were obtained through the DNA Repository at the University of Alberta maintained by Parks Canada. The distribution of caribou populations used in this study is represented in Fig. 1. Caribou genomic DNA was isolated from blood or tissue samples using an Applied Biosystems Genepure 341 Nucleic Acids Purification System.

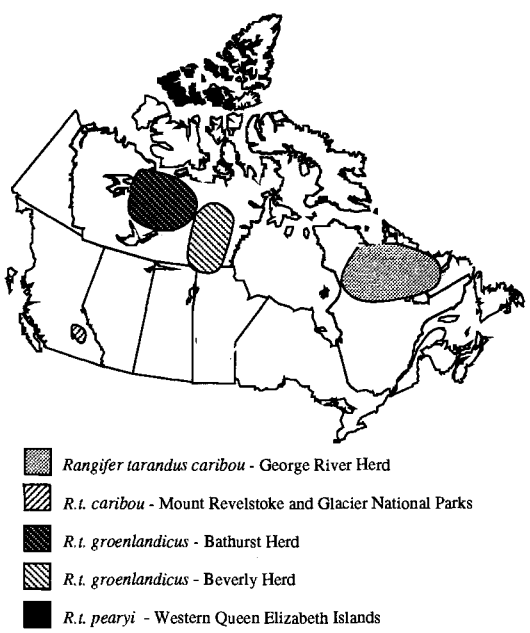

Fig. 1. Study populations of caribou (*Rangifer tarandus* sp.) in Canada.

Four microsatelhte loci were developed within our laboratory for caribou. Random segments of caribou genomic DNA were cloned into M13mp18, and the resulting library was screened with a biotin-labeled oligonucleotide which would hybridize to a microsatellite repeat [AC] . The nucleotide sequences of the positive clones were determined and primers that bound to the DNA flanking uninterrupted repeats were chosen (Coffin & Strobeck, unpubl. data). Subsequently, one primer for each clone was flourescently labeled by incorporation of FAM-amidite (Applied Biosystems) during synthesis.

Microsatellite loci were amplified using Taq polymerase and the fluorescently labeled primers in a Perkin-Elmer 9600 Thermal Cycler. Alleles of the four loci were separated by polyacrylamide gel electrophoresis using a Model 373A Automated DNA Sequencer (Applied Biosystems) and scored using 672 Genescan PCR Analysis software (version 1.2, Applied Biosystems). DNA size standards (Genescan 2500 ROX, Applied Biosystems) were run on all gels to provide a reproducible standard at every base position and to allow for comparisons to be made across gels.

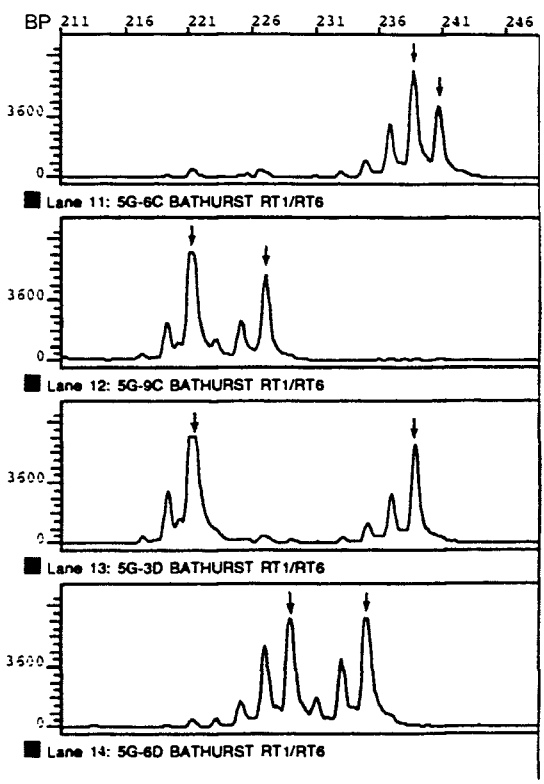

Fig. 2. Electrophoretogram revealing 6 alleles at microsatellite Locus RT 1 (resolution of alleles differing by as little as one base pair).

Heterozygosity and probability of identity were calculated using the formulae:

$$1 - \sum_i p_i^2, \text{ and } \sum_i p_i^4 + \sum_i \sum_{j>1} (2p_i p_j)^2$$

respectively, where p_i and p_j are the frequencies of the ith and jth allele.

Results and discussion

The 4 loci used to survey caribou were all highly polymorphic. Alleles were sized according to the length of nucleotide base pairs (Fig. 2), allowing for precise allele identification at all loci. A minimum of 5 alleles were identified for any given locus in all of the caribou populations (Table 1). A total of 12, 13, 12 and 11 alleles were identified for microsatellite loci RT 1, RT 6, RT 7 and RT 9, respectively. Loci reveal considerable variation of alleles within and among caribou populations. For example, locus RT 1 in *R.t. pearyi* (WQ) exhibits a relatively high frequency of alleles 1 and 6, and at locus RT 7 in *R.t. caribou* (MG), alleles 3 and 12 are observed with relatively high frequency in comparison to other caribou. Also, the George River herd (*R.t. caribou*) exhibits a high frequency of allele 11 which is not present in any other of the study populations. These data suggest that caribou populations have significant differences in allele frequency distributions at microsatellite loci. With the sensitivity exhibited at loci, it may be possible to determine from which population a caribou originated. An unknown caribou could be assigned to a population in which its genotype (across many loci) is most common, with a minimal margin of error.

Expected heterozygosity is a standard measure of genetic diversity that estimates the probability that an individual in a random mating population will be a heterozygote (two randomly chosen gametes are different). Heterozygosity indices of the 4 loci combined range from 75% in the Western Queen Elizabeth Islands herd (*R.t. pearyi*) to 82% in the Bathurst herd (*R.t. groenlandicus*) of the Northwest Territories (Table 2). Individual loci had a minimum heterozygosity of 66% (RT 7, WQ), indicating that microsatellite loci are highly variable in caribou. These results are consistent with the levels of heterozygosity found in other large mammal populations. For example, Amos et al. (1993) found 54 alleles at a single microsatellite locus in long-finned pilot whale (*G. melas*) pods. Also, Paetkau & Stobeck (1995) found a mean heterozygosity level of 60% in Canadian polar bear populations (*Ursus maritimus*), whereas previous genetic studies of such populations using allozymes and mitochondrial DNA sequence have consistently found little or no variation.

Probability of identity estimates the probability that two randomly chosen individuals in a population have identical genotypes (Paetkau & Strobeck, 1994). The probability of identity was approximately 1:25,000, 1:60,000, 1:132,000, 1:1,600 and 1:14,000 for QW, BE, BA,GR and MG caribou populations , respectively (Table 2). The ability to identify individuals in a given population has many applications for use in wildlife forensics and management. The low

Table 1. Allelic variation at microsatellite loci in caribou populations of Canada. WQ - *R.t. pearyi* - Western Queen Elizabeth Islands (n=10), BE - *R.t. groenlandicus* - Beverly herd (n=9), BA - *R.t. groenlandicus* - Bathurst herd (n=9),GR - *R.t. caribou* - George River herd (n=13), MG - *R.t. caribou* - Mount Revelstoke & Glacier National Park (n=18).

	Number of Alleles at Microsatellite Loci																			
	Locus RT 1					Locus RT 6					Locus RT 7					Locus RT 9				
Allele	WQ	BE	BA	GR	MG	WQ	BE	BA	GR	MG	WQ	BE	BA	GR	MG	WQ	BE	BA	GR	MG
1	4	–	1	1	–	–	–	1	–	–	–	–	1	–	–	–	–	1	–	–
2	–	3	4	–	–	1	–	2	–	–	–	2	–	–	–	–	1	–	–	–
3	–	–	–	–	1	1	–	–	1	–	1	6	7	1	14	–	1	1	–	–
4	2	2	–	9	3	1	1	–	1	–	2	1	3	1	1	–	1	1	–	3
5	–	–	2	1	–	–	–	–	1	–	1	1	1	–	1	6	5	1	–	5
6	3	–	1	–	–	1	–	–	–	1	3	–	–	1	–	4	2	4	–	11
7	–	–	–	1	8	3	1	4	1	6	11	3	4	8	7	–	1	–	–	7
8	1	1	1	1	3	7	4	3	1	7	–	2	1	6	7	1	4	1	–	2
9	2	6	2	–	9	2	5	4	5	13	1	2	1	2	–	3	1	4	–	5
10	6	3	2	2	–	2	6	1	11	3	1	1	–	–	–	2	2	4	–	1
11	–	2	4	5	–	–	1	1	5	1	–	–	–	6	–	–	–	1	–	–
12	–	1	1	6	12	–	–	2	–	1	–	–	–	1	6	–	–	–	–	–
13	–		–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 2. Heterozygosity and probability of identity indices for caribou populations.

	Heterozygosity					Probability of Identity				
Locus	WQ	BE	BA	GR	MG	WQ	BE	BA	GR	MG
RT 1	0.784	0.802	0.850	0.778	0.762	0.077	0.064	0.038	0.080	0.094
RT 6	0.815	0.753	0.840	0.740	0.740	0.045	0.101	0.046	0.101	0.104
RT7	0.655	0.815	0.760	0.787	0.744	0.123	0.055	0.090	0.076	0.105
RT 9	0.742	0.833	0.833	–	0.798	0.095	0.047	0.048	–	0.068
4Loci	0.749	0.801	0.821	0.768	0.761	$4.0x10^{-5}$	$1.7x10^{-5}$	$7.6X10^{-6}$	$6.1x10^{-4}$	$7.0x10^{-5}$

values for four loci combined in QW, BA and MG indicate that microsatellite analysis has a very high potential for identifying individuals in respective populations, considering recent caribou population estimates (Ferguson & Gauthier, 1992).

In conclusion, the high levels of variation observed at microsatellite loci in caribou could be potentially useful for analyzing caribou at the level of populations. The hyper-variability of fragments containing microsatellites make it possible to uniquely identify individuals in a population, useful for forensics and studies of paternity and reproductive success, as well as distinguishing populations that have only been recently separated. Spatial distributions of alleles can be used to study local gene flow and the recent history of large scale migrations. A temporal dimension could also be added by utilizing PCR to amplify ancient DNA that may be degraded (Thomas *et al.*, 1990). It may also be possible to obtain DNA from gut cells in fecal pellets (Höss *et al.*, 1992), allowing for non-intrusive sampling of caribou. We are presently refining the fecal analysis technique in caribou to make it a more efficient method of processing large sample sizes.

Microsatellite analysis will provide considerable resolution to the measurement of variation and population structure in caribou. Analyses of population differentiation and sub-structuring are currently under way in our laboratory.

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