GENETIC VARIATION OF MICROSATELLITE DNA IN MOOSE IN QUÉBEC

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ABSTRACT: We assessed genetic variation at 5 microsatellite DNA loci in 57 moose (*Alces alces*) from 3 populations in Québec. The 5 loci are linked to functional genes (opiod binding and cell adhesion molecule, corticotrophin releasing factor, interphotoreceptor retinal binding protein, kappa-casein, insulin-like growth factor-1) in cattle. The mean number of alleles per locus varied from 2.0 to 2.2 and the mean observed heterozygosity varied from 0.343 to 0.363 among the 3 Québec populations. Variation at these 5 microsatellite loci in moose is relatively low, but within the range observed for these loci and other microsatellites in cervids and bovids. Little genetic differentiation was observed among the 3 Québec populations ($F_{st} = 0.025$). There were no substantial differences in the numbers of alleles or the levels of heterozygosity among the 3 Québec populations, 1 of which has been heavily hunted by humans, 1 exposed to light hunting, and 1 not hunted.

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Genetic studies of moose (*Alces alces*, Cervidae, Artiodactyla) have included assessments of variation (Ryman et al. 1980), phylogenetic relationships (Cronin 1991, Cronin et al. 1996), population structure (Chesser et al. 1982), and potential effects of hunting (Ryman et al. 1981). Some moose populations exhibit considerable genetic variation at allozyme, minisatellite, and microsatellite loci (Ryman et al. 1980, Ellegren et al. 1991, Hundertmark et al. 1992, Røed and Midthjell 1998). There is enough allelic variation at allozyme loci in moose populations in Scandinavia to reveal patterns of genetic differentiation over relatively small geographical distances (i.e., a few hundred kilometers; Gyllensten et al. 1980, Ryman et al. 1980, Chesser et al. 1982). However, moose as a species may have less genetic variation than other cervids (Braend 1962, Ryman et al. 1977, Wilhelmson et al. 1978, Baccus et al. 1983, Smith et al. 1990). For example, Cronin (1992) found no variation in mitchondrial DNA (mtDNA) in moose but abundant mtDNA variation in white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*), and caribou (*Rangifer tarandus*). Moose are hunted extensively by humans across their distribution in North America and Europe. Ryman et al. (1981) used population genetic theory to conclude that genetic variation (i.e., heterozygosity) in moose can be reduced within short periods of time under certain hunting regimes.

The study of genetic variation in moose

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and other wildlife is of interest because there can be a relationship between genetic variation and fitness in natural, domestic, and laboratory populations (reviewed by Lerner 1954, Mitton and Grant 1984, Avise 1994, Powell 1997). One measure of genetic variation, heterozygosity, is particularly important because heterozygotes may have higher fitness than homozygotes (Mitton and Grant 1984). Positive associations of heterozygosity and traits that affect fitness have been described for several species (Cothran et al. 1983, Allendorf and Leary 1986, O'Brien and Evermann 1988, Scribner et al. 1989, Teska et al. 1990, Hartl et al. 1991, Hughes 1991, Gulland et al. 1993, Soulé and Zegers 1996, Coulson et al. 1998, Coltman et al. 1999). In addition, inbreeding, which can result in decreased heterozygosity, has been shown to decrease fertility or increase juvenile mortality in ungulates (Ballou and Ralls 1982). However, the relationship between heterozygosity and fitness is complex and not always positive (Pemberton et al. 1988, 1991; Leberg et al. 1990). Populations with small effective population sizes (i.e., small numbers of breeding individuals) can lose genetic variation through random genetic drift or inbreeding (Harris and Allendorf 1989). This is a concern for small, isolated populations and those that have experienced drastic reductions (i.e., a population bottleneck) or originated from a small number of founders (e.g., O'Brien et al. 1983, 1985; Paetkau et al. 1998). Hunting by humans might also alter effective population sizes and result in a loss of genetic variation (Ryman et al. 1981, Scribner 1993).

Our primary objective was to quantify genetic variation at 5 microsatellite loci in moose from Québec, including comparisons of hunted and non-hunted populations. There have been several assessments of microsatellite DNA variation in other cervids (DeWoody et al. 1995, Engel et al. 1996, Kuhn et al. 1996, Talbot et al. 1996, Wilson et al. 1997, Coulson et al. 1998), but only limited study of moose from Norway (Røed and Midthjell 1998). The 5 loci we studied are linked to functional genes (opiod binding and cell adhesion molecule, corticotrophin releasing factor, interphotoreceptor retinal binding protein, kappa-casein, and insulin-like growth factor-1) in cattle. Microsatellite DNA consists of short tandem repeats of 2-6 nucleotides, and tends to be quite variable in terms of the number of alleles and degree of heterozygosity at individual loci (Tautz 1989, Weber and May 1989). Microsatellite loci are used in population genetics, gene mapping, pedigree analysis (e.g., Fries et al. 1993, Bishop et al. 1994, DeWoody et al. 1995, Engel et al. 1996, Kuhn et al. 1996, Talbot et al. 1996), and for assessing levels of heterozygosity (e.g., Scribner et al. 1994, Patton et al. 1997, Coulson et al. 1998, Paetkau et al. 1998, Cronin et al. 1999, Rooney et al. 1999).

METHODS

In March 1995, 1996, and 1997, blood samples were taken by jugular veinipuncture from immobilized moose in 3 study sites in Québec: the Zecs, Park Jacques-Cartier, and Côte-Nord. Tongue tissue samples (200-250 g) were also collected from moose in the fall of 1996 from hunter-killed moose at the Côte-Nord site. The Zecs and the Park Jacques Cartier sites are located about 50 km north of Québec City in a transition zone between mixed deciduous and coniferous forests. In the Zecs prior to 1964, only male moose were harvested and the numbers were low. Hunting was intense in the Zecs between 1964-1994, and harvests of any sex and age moose resulted in low population density (about 1.0 moose/10 km^2) and a skewed sex-ratio (about 30% males among adults). Harvest rates have averaged about 20% of the total population in the Zecs during the last 2 decades. The number

of moose in the Zecs has grown from 93 animals in 1995 to 177 animals in 1998, an increase of 90% in 3 years.

Since its establishment in 1981, hunting has been prohibited in Park Jacques-Cartier. Moose density has exceeded 3.0/10 km^2 in most years with parity in the sex-ratio (48% males among adults). The number of moose in the Park has grown at the same rate (89% in 3 years) as in the Zecs population, from 169 moose in 1995 to 319 moose in 1998. Only 15-20 km separate the Zecs and Park Jacques-Cartier and moose could move between the areas, although telemetry studies have not documented this (R. Courtois, unpublished data).

The third study site (Côte-Nord) lies 700 km east of the other 2 sites on the north shore of the St. Lawrence River, about 50 km north of Sept-Îles. Black spruce (*Picea mariana*) dominates the vegetation here due to a harsher climate than at the other 2 sites. The Côte-Nord site supports a low density (0.3 moose/10 km^2) population as a result of low forage production and weather conditions (Crête and Courtois 1997). Although there are no restrictions on the sex and age of the moose harvested, the harvest rate has been moderate (14%) because of limited access, and the sex ratio is balanced (46% males in the adult population).

DNA was extracted from blood and tissues with standard methods involving incubation in SDS-proteinase-K, and extraction with phenol/chloroform (Maniatis et al. 1982). Methods for analysis of microsatellite loci developed in cattle with the polymerase chain reaction (PCR) (Saiki et al. 1988) were used. We chose 5 microsatellite loci that are linked to functional genes in cattle and for which the cattle PCR primers resulted in successful DNA amplification in moose. Several other researchers have successfully used bovine and ovine genetic markers in cervids (e.g., Cronin et al. 1995, Engel et al. 1996, Kuhn et al. 1996, Talbot et al. 1996, Slate et al. 1998). The 5 microsatellites we used are:

Opiod binding and cell adhesion molecule (OBCAM, Moore et al. 1992):
Forward primer;
CCTGACTATAATGTACAGATCCCTC
Reverse primer;
GCAGAATGACTAGGAAGGATGGCA
Corticotrophin releasing factor (CRFA, Moore et al. 1992):
Forward primer;
CTCGCTCACCTGCAGAAGCACC
Reverse primer;
GCTGAGCAGCCGTCTAAGTTGC
Interphotoreceptor retinal binding protein (IRBP, Moore et al. 1992):
Forward primer;
GCTATGATCACCTTCTATGCTTCC
Reverse primer;
CCCTAAATACTACCATCTAGAAG
and (IRBP-LGL, this study)
Forward primer;
TGTATGATCACCTTCTATGCTTC
Reverse primer;
GCTTTAGGTAATCATCAGATAGC
Kappa-casein (KCSN, Bishop et al. 1994):
Forward primer;
ATGCACCCTTAACCTAATCCC
Reverse primer;
GCACTTTATAAGCACCACAGC
Insulin-like growth factor-1 (IGF-1, Kirkpatrick 1992):
Forward primer;
GAGGGTATTGCTAGCCAGCTG
Reverse primer;
CATATTTTTCTGCATAACTTGAACCT

We used 2 primer sets for IRBP. The primers from Moore et al. (1992) result in amplification of alleles 177-181 base pairs (bp) in length, and the primers designed in this study result in amplification of alleles 141-145 bp. We designed the IRBP-LGL primers to allow discrimination of IRBP from OBCAM and KCSN (which have alleles 189-195 bp) when these loci were

Table 1. Allele frequencies and genetic variation measures for 5 microsatellite loci in 3 moose populations in Québec.

Locus/sample size/allele	Population		
	Zecs	Park Jacques-Cartier	Côte-Nord
OBCAM			
n	18	18	21
189 allele	0.389	0.444	0.452
195 allele	0.611	0.556	0.548
H_{obs}/H_{exp} [1]	0.667/0.475	0.667/0.494	0.524/0.495
IGF-1			
n	18	18	21
110 allele	0.694	0.778	0.786
112 allele	0.306	0.222	0.214
H_{obs}/H_{exp} [1]	0.278/0.424	0.333/0.346	0.238/0.337
CRFA			
n	18	18	21
255 allele	0.889	0.917	0.857
253 allele	0.111	0.083	0.143
H_{obs}/H_{exp} [1]	0.222/0.198	0.167/0.153	0.190/0.245
KCSN			
n	17	18	21
190 allele	0.706	0.306	0.476
192 allele	0.294	0.694	0.524
H_{obs}/H_{exp} [1]	0.588/0.415	0.389/0.424	0.476/0.499
IRBP			
n	17	18	21
143 (179) allele	0.971	0.917	0.857
145 (181) allele	0.029	0.083	0.071
141 (177) allele	0.000	0.000	0.071
H_{obs}/H_{exp} [1]	0.059/0.057	0.167/0.153	0.286/0.255
Total for 5 loci			
H_{obs} [1] (SE)	0.363 (0.115)	0.344 (0.092)	0.343 (0.066)
H_{exp} [1] (SE)	0.314 (0.080)	0.314 (0.070)	0.366 (0.056)
Mean number alleles/locus (SE)	2.0 (0.0)	2.0 (0.0)	2.2 (0.2)

[1] H_{obs} = Direct count heterozygosity, H_{exp} = Hardy-Weinberg expected heterozygosity.

run together in the same lane of electrophoretic gels. The same IRBP alleles, which differ by 2 bp (Table 1), are identified with either primer set.

PCR reactions (50 µL) contained 5-50 ng genomic DNA in 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1µM of each of the 2 primers, and 1.25 units of Amplitaq DNA polymerase (Perkin Elmer, Norwalk, CT, USA). Reactions were heated to 95°C for 5 minutes followed by 32 cycles of amplification. Each cycle consisted of 45 seconds at 95°C, 40 seconds at 50°C and 2.5 minutes at 70°C. Amplification products were separated on polyacrylamide gels on an ABI 373A autosequencer (ABI, Foster City, CA, USA). Genotypes were determined from the chromatographs derived from the gels, and verified with GeneScanTM and GenotyperTM 1.1 software (ABI, Foster City, CA, USA).

We used the BIOSYS-1 release 1.7 computer program (Swofford and Selander 1981) to assess genetic variation within each population, including the numbers of alleles per locus and heterozygosity (direct count heterozygosity [H_{obs}], and Hardy-Weinberg expected heterozygosity [H_{exp}]). H_{obs} reflects the actual heterozygosities observed and H_{exp} the heterozygosity expected under random mating in the population with the allele frequencies observed. We compared the H_{obs} and H_{exp} between each pair of populations with chi-square contingency tests. We also quantified the degree of genetic differentiation and gene flow among populations with estimates of Nei's (1978) genetic distance and Wright's (1978) F_{st} and Nm (where N = effective population size and m = rate of immigration/emigration). F_{st} and Nm are related by the equation $F_{st} = 1/(1 + 4Nm)$. Significant genetic differentiation can result from genetic drift alone if $Nm < 1$, but not if $Nm > 1$ (Slatkin 1987).

RESULTS

We observed 2 alleles at each of 4 loci (OBCAM, KCSN, IGF-1, CRFA), and 3 alleles at 1 locus (IRBP) in moose. The alleles differed in size by multiples of 2 bp, as expected for dinucleotide repeats. The allele sizes for each locus were: OBCAM 189, 195; KCSN 190, 192; IGF-1 110, 112; CRFA 253, 255; IRBP 141 (or 177), 143 (or 179), 145 (or 181) (Table 1). As described in the methods, there were 2 sizes for each IRBP allele depending on which primers were used.

The levels of heterozygosity were similar in the 3 Québec populations whether expressed as H_{obs} or H_{exp} (Table 1). H_{obs} ranged from 0.059 (IRBP in the Zecs population) to 0.667 (OBCAM in the Zecs and Park Jacques-Cartier populations). The only significant inter-population difference in H_{obs} was for the IRBP locus between the Côte-Nord and Zecs populations ($P = 0.03$). There were no significant inter-population differences in H_{exp} between populations for any locus ($P > 0.06$). The Zecs population had the highest H_{obs} for 3 loci, the Park Jacques-Cartier population had the highest H_{obs} for 2 loci, and the Côte-Nord population had the highest H_{obs} for 1 locus. The heavily-hunted Zecs population had the highest 5-locus mean H_{obs} (0.363), followed by the unhunted Park Jacques-Cartier population (0.344) and lightly-hunted Côte-Nord population (0.343). The 5-locus mean H_{exp} were the same in the Zecs and Park Jacques-Cartier populations (0.314) and slightly higher in the Côte-Nord population (0.366). The mean H_{obs} for the 3 populations were within 1 standard error of each other, as were the mean H_{exp} (Table 1). The numbers of alleles per locus were similar in each of the Québec moose populations, with an average of 2 alleles per locus for the Zecs and Park Jacques-Cartier populations, and 2.2 alleles per locus for the Côte-Nord

population (Table 1). Genotypes did not deviate from expected Hardy-Weinberg distributions for any of the 3 populations for any of the loci ($P > 0.08$).

There was little genetic differentiation among the 3 Québec moose populations. There were 2 common alleles for each locus in each population. For 4 of the loci (IRBP, OBCAM, IGF-1, CRFA) the same alleles predominated (i.e., allele frequencies > 0.5) in all 3 populations (Table 1). For KCSN, the 190 allele was most common in Zecs and the 192 allele was most common in Park Jacques-Cartier and Côte-Nord. The IRBP 141 allele occurred only in the Côte-Nord population in Québec (Table 1). Genetic distances (Nei 1978) between populations were 0.038 (Zecs-Park Jacques-Cartier), 0.009 (Zecs-Côte-Nord), and 0.000 (Park Jacques-Cartier-Côte-Nord). The 5-locus average F_{st} estimate among the 3 populations was 0.025, with a corresponding estimate of $Nm = 9.750$. F_{st} estimates were low for 4 of the 5 loci; 0.000 for OBCAM, IGF-1, and CRFA, and 0.005 for IRBP. Chi-square contingency tests showed non-significant differences of allele frequencies among the 3 populations for these 4 loci ($P > 0.187$). The F_{st} estimate for KCSN was moderate, 0.084 ($Nm = 2.736$), and there was a significant difference in allele frequencies among the 3 populations at this locus ($\chi^2 = 11.272$, 2 df, $P = 0.004$).

Moose from Québec have microsatellite variation (i.e., number of alleles and heterozygosity) within the range observed across cervids and bovids (including moose from Norway; Røed and Midthjell 1998), although the values are relatively low (Table 2). This is also the case for allozyme loci (Smith et al. 1990). The numbers of animals and geographic range sampled vary in the studies referenced in Table 2, so the levels of variation reported may not reflect that of each species in general. In addition, these studies used different microsatellite loci than ours, and levels of variation may differ among loci. Of the loci we analyzed, IGF-1 has been analyzed in several other cervids as well as bovids. Moose from Québec have fewer alleles or lower heterozygosity at IGF-1 compared to white-tailed deer, mule deer, caribou, and elk (*Cervus elaphus*), but values similar to those for reindeer (*Rangifer tarandus*), cattle, and the mean of 10 bovid species (Kirkpatrick 1992, Engel et al. 1996, Moody et al. 1996). The other 4 loci we studied have comparable levels of heterozygosity in cattle (Moore et al. 1992, Lien and Rogne 1993, Barendse et al. 1994).

DISCUSSION

Variation at microsatellite loci might not reflect genetic variation over the entire genome because different types of loci can exhibit more or less variation. For example, no variation was detected in mtDNA of moose (Cronin 1992) while considerable allozyme and repetitive DNA variation exists in this species (Ryman et al. 1980, Ellegren et al. 1991, Hundertmark et al. 1992). Our results also show considerable microsatellite DNA variation in moose, although the numbers of alleles per locus are lower than for microsatellites in some other species of deer (Table 2). It has been suggested that the relatively low level of genetic variation in some moose populations may have resulted from population bottlenecks during glacial periods (Cronin 1992, Hundertmark et al. 1992, Gaines et al. 1997), as for other species (Sage and Wolff 1986). The low mtDNA variation relative to nuclear genetic variation in moose is consistent with the higher probability of loss of mtDNA variation during bottlenecks. This is due to a smaller effective gene number resulting from the maternal, clonal mode of inheritance of mtDNA (Birky et al. 1983).

Microsatellite loci, like the ones we

Table 2. Comparison of microsatellite variation in cervids and bovids.

Species	Number of loci[1]	Alleles per locus	Heterozygosity	Reference
Moose	5 loci	2.10[2]	0.35[2]	This study
Moose	5 loci	2.80[3]	0.50[3]	Røed & Midthjell (1998)
Elk	14 loci	4.93[3]	0.50[3]	Talbot et al. (1996)
Caribou	17 loci	5.94[3]	0.64[3]	Røed & Midthjell (1998)
Roe deer	7 loci	3.86[3]	0.56[3]	Røed & Midthjell (1998)
Red Deer	6 loci	3.67[3]	0.51[3]	Røed & Midthjell (1998)
White-tailed deer	5 loci	7.60[3]	0.62[3]	DeWoody et al. (1995)
Elk	12 loci	2.33[3]	0.27[3]	Engel et al. (1996)
Red deer	9 loci	2.22[3]	0.34[3]	Engel et al. (1996)
Mule deer	13 loci	4.92[3]	0.48[3]	Engel et al. (1996)
White-tailed deer	13 loci	7.46[3]	0.68[3]	Engel et al. (1996)
Caribou	13 loci	4.69[3]	0.52[3]	Engel et al. (1996)
Reindeer	13 loci	2.89[3]	0.43[3]	Engel et al. (1996)
6 cervid species	9-13 loci	4.09[3]	0.45[3]	Engel et al. (1996)
10 bovid species	20 loci	2.70[3]	0.31[3]	Engel et al. (1996)

[1] Values for variable loci only.

[2] Mean values of 3 Québec populations.

[3] Mean values for several loci.

studied, may be linked to functional genes on which selection acts (Slatkin 1995, Coulson et al. 1998, Paterson 1998) and this could affect levels of variation. In other studies of artiodactyls, microsatellite alleles and genotypes have been associated with fitness differences and may be subject to selection (Bancroft et al. 1995, Moody et al. 1996, Pemberton et al. 1996, Coulson et al. 1998, Paterson 1998). Such analyses of microsatellites in moose and other cervids will be facilitated by the availability of many loci mapped in cattle (Fries et al. 1993, Bishop et al. 1994, Slate et al. 1998). This will allow more detailed analysis of genetic variation and population structure in moose, as has been done for other large mammals (e.g., Bancroft et al. 1995, Craighead et al. 1995, Pemberton et al. 1996, Paetkau et al. 1998).

Comparison of the hunted and unhunted moose populations in Québec is preliminary because of the small number of loci we used, and the short time period of intense hunting in the Zecs population (about 35 years). However, our data provide some of the only empirical data available regarding the postulated effects of hunting on genetic variation (Ryman et al. 1981) or population fitness (Scribner et al. 1989, Ginsberg and Milner-Gulland 1994, Williams et al. 1994, Hartl et al. 1995, Lukefahr and Jacobson 1998). It appears that intensive hunting for 35 years in the Zecs population resulted in a

low population density and a skewed sex ratio (Laurian et al. 2000), but not a reduction of genetic variation. This population has considerable heterozygosity at each of 5 microsatellite loci, and the highest mean heterozygosity of the 3 Québec populations. This suggests that the effective population has been large enough to maintain genetic variation. Cameron and Vyse (1978) made similar observations for elk (*Cervus elaphus*) which experienced historical population declines in North America. The lack of significant genetic differentiation among the 3 Québec moose populations suggests that immigration and emigration (with gene flow), has contributed to the maintenance of effective population size and genetic variation. The *Nm* estimates from our data were > 1, suggesting gene flow prevents genetic differentiation due to genetic drift. Assuming an effective population size of 100 (which is within the range of the size of the Québec moose populations) our data suggest there are about 3 - 10 immigrants entering populations each generation. Other areas that border the Zecs (the Laurentides and Portneuf wildlife reserves) are not heavily hunted and may provide immigrants into the Zecs. Although moose usually disperse < 15 km, some may disperse > 100 km which results in gene flow among populations. Labonté et al. (1998) suggested that immigration from reserves into harvested moose populations might augment population numbers and maintain genetic variation over larger areas.

The Zecs population grew at a rate similar to the Park Jacques-Cartier population (an annual rate of about 25%) between 1995 and 1998. This suggests there are no reproductive problems in the Zecs population as have been postulated for hunted populations (Ginsberg and Milner-Gulland 1994). Both the genetic and demographic data indicate the hunted population was large enough, with enough males, to maintain genetic variation and population growth similar to populations with little or no hunting. Moose have characteristics, (e.g., long-distance movements) that allow populations to persist at low densities in marginal habitat and maintain genetic variation (e.g., Crête and Courtois 1997). Our results support the contention that, in many managed populations, demographic factors will exert a greater influence on population fitness and survival than genetic factors (Lande 1988, Caro and Laurenson 1994, Gaines et al. 1997).

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