Genetic variation and population structure of moose (*Alces alces*) at neutral and functional DNA loci

Paul J. Wilson, Sonya Grewal, Art Rodgers, Rob Rempel, Jacques Saquet, Hank Hristienko, Frank Burrows, Rolf Peterson, and Bradley N. White

Abstract: Genetic variation was examined for moose (*Alces alces*) from Riding Mountain, Isle Royale, and Pukaskwa national parks; northwestern, Nipigon, northeastern, and central Ontario; New Brunswick; and Newfoundland. The national parks were identified as maintaining potentially different local selection pressures due to the absence of hunting and the presence or absence of the parasite *Parelaphostrongylus tenuis*. Genetic variation was estimated using neutral DNA markers, assessed by multilocus DNA fingerprinting and five microsatellite loci, and the functional antigen binding region (ARS) (exon 2) of the major histocompatibility complex (MHC) gene *DRB*. There was discordance in the allelic diversity observed at the neutral loci compared with the MHC *DRB* locus in a number of populations. Ontario populations demonstrated higher levels of variability at the neutral loci and relatively low levels at the *DRB* locus. Conversely, the Isle Royale population has the lowest genetic variability, consistent with a historic small founding event, at the neutral DNA markers and relatively high variability at the MHC gene. Relatively high levels of genetic variation at the *DRB* locus were observed in protected park populations concomitant with the absence of white-tailed deer (*Odocoileus virginianus*) or the parasite *P. tenuis* and an absence of hunting. Gene flow was observed among the neighboring geographic regions within Ontario, including Pukaskwa National Park, with evidence of isolation-by-distance among more distant regions within Ontario. The discordant patterns between DNA markers suggest that neutral DNA markers may not accurately reflect adaptive variation present at functional loci.

Résumé : Nous avons étudié la variation génétique chez les orignaux (*Alces alces*) des parcs nationaux de Riding Mountain, d'Isle Royale et de Pukaskwa, des régions nord-ouest, nord-est et centrale et de la région de Nipigon en Ontario, ainsi que du Nouveau-Brunswick et de Terre-Neuve. Les parcs nationaux présentent des conditions locales de pression sélective potentiellement différentes, car il n'y a pas de chasse et le parasite *Parelaphostrongylus tenuis* peut être présent ou absent. La variation génétique a pu être mesurée par génotypage de l'ADN et déterminée à des marqueurs neutres de l'ADN, ainsi qu'à cinq locus microsatellites et dans la région fonctionnelle de liaison des antigènes (ARS) (exon 2) du gène *DRB* du complexe majeur d'histocompatibilté (MHC). Il y a une divergence entre la diversité allélique observée aux locus neutres et celle du locus MHC *DRB* chez plusieurs populations. Les populations ontariennes présentent des niveaux élevés de variabilité au locus neutres et une variabilié relativement faible au locus *DRB*. En revanche, la population de l'Isle Royale possède la variabilité génétique la plus faible dans les marqueurs neutres de l'ADN, ce qui est en accord avec son épisode de fondation restreint et la variabilité relativement élevée du gène MHC. Des niveaux relativement élevés de variation génétique au locus *DRB* existent chez les populations protégées de parcs où il n'y a pas de chasse et d'où le cerf de Virginie (*Odocoileus virginianus*) ou le parasite *P. tenuis* sont absents. Le flux génétique s'observe entre les régions géographiques adjacentes de l'Ontario y compris le parc national de Pukaskwa; il y a cependant des indices d'un isolement par la distance dans les régions plus éloignées de l'Ontario. Les patterns divergents entre les marqueurs de l'ADN indiquent que les marqueurs neutres peuvent ne pas refléter de façon précise la variation adaptative présente aux locus fonctionnels.

[Traduit par la Rédaction]

Received 12 March 2002. Accepted 29 January 2003. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 2 May 2003.

P.J. Wilson,[1] S. Grewal, and B.N. White. Natural Resources DNA Profiling and Forensic Centre, Biology Department, Trent University, Peterborough, ON K9J 7B8, Canada.
A. Rodgers and R. Rempel. Centre for Northern Forest Ecosystem Research, Ontario Ministry of Natural Resources, 955 Oliver Road, Thunder Bay, ON P7B 5E1, Canada.
J. Saquet. Riding Mountain National Park, MB R0J 1B0, Canada.
H. Hristienko. Manitoba Department of Natural Resources, Box 24, 1495 St. James Street, Winnipeg, MB R3H 0W9, Canada.
F. Burrows. Bruce Peninsula National Park – Fathom Five Marine Park, Box 189, Tobermory, ON N0H 2R0, Canada.
R. Peterson. School of Forestry and Wood Products, Michigan Technological University, Houghton, MI 49931, U.S.A.

[1]Corresponding author (e-mail: paul.wilson@nrdpfc.ca).

 doi: 10.1139/Z03-030

Introduction

Maintaining genetic variation is an important factor in protecting the evolutionary potential and promoting the long-term persistence of populations (Leberg 1992; Frankham 1995; Lacy 1997). The movement of animals and subsequent gene flow within a metapopulation structure can maintain genetic variation among local subpopulations (Harrison and Hastings 1996). Low levels of genetic variation within subpopulations of a larger metapopulation were identified as a causal factor in increasing the probability of extinction within local populations in greater prairie chickens (Westemeier et al. 1998) and the Glanville frittiliary butterfly (*Melitaea cinixia*) (Saccheri et al. 1998). Neutral DNA markers have been used extensively in studying genetic variation and population structure in natural populations (Avise 1994; Ferris and Palumbi 1996; Carvalho 1998; Estoup and Angers 1998).

The application of neutral DNA markers in assessing population structure and levels of genetic variation assumes that these loci reflect adaptive variation within populations (King and Burke 1999). However, local selection pressures within a metapopulation framework affecting the levels of genetic variation in subpopulations at adaptively important loci may not be readily detected using neutral DNA marker systems (Hedrick 1994) and, conversely, functional loci may not accurately reflect gene flow among different geographic regions with different selection pressures. Despite this concern, few studies have directly compared neutral genetic variation with adaptive variation at the intraspecific level (Carvalho 1998; Crandall et al. 2000). In this study, we present results on genetic variation and population structure in moose (*Alces alces*) populations using neutral mini- and micro-satellite loci and a major histocompatibility complex (MHC) gene.

Neutral DNA markers such as minisatellite loci and microsatellites have identified levels of genetic variation as representative of overall levels of genome variation (Lacy 1997). Minisatellite loci, as detected with multilocus DNA fingerprinting, have had limited application in assessing population differentiation (Schenk et al. 1998; Wilson et al. 2000), owing to technical limitations (Estoup and Angers 1998), while microsatellites have proven more effective at assessing the population structure in natural wildlife populations, such as black bears (*Ursus americanus*) (Paetkau and Strobeck 1994), wolves (*Canis lupus*) (Roy et al. 1994), African water buffalo (*Syncerus caffer*) (Simonsen et al. 1998), and moose (Broders et al. 1999).

The MHC is the most commonly used DNA marker system for assessing genetic variation at a functional gene locus (Hedrick 1994; Murray et al. 1995; Murray and White 1998; Wenink et al. 1998; Murray et al. 1999). MHC proteins bind pathogen-derived foreign peptides to T-cells to initiate an immune response. As a result of its role in disease resistance, MHC genes can be highly polymorphic, and genetic variation is proposed to be adaptive to the large numbers of pathogens for which natural populations are exposed (O'Brien and Evermann 1988). One mechanism proposed for maintaining high levels of variation at MHC genes is balancing selection (Hedrick and Thomson 1983; Hughes and Nei 1988; Yuhki and O'Brien 1990; Hughes 1991; Hedrick 1994; Hughes and Yeager 1998) through overdominance or negative frequency dependence. Overdominance, or heterozygote advantage, increases the range of pathogens recognized by the MHC proteins, while negative frequency dependent selection is based on host–parasite interactions, causing an increase of low-frequency alleles followed by a shift in the composition of the parasite population (reviewed in Potts and Wakeland 1993; Hedrick 1994; Hughes and Yeager 1998). The importance of MHC genes in a conservation context has been identified for captive-breeding (Hughes 1991) and isolated (Yuhki and O'Brien 1990) populations, indicating that low variation at these loci may result in increased disease susceptibility and potential local extinction.

Moose are the largest land mammals in the circumpolar boreal forests of Canada and Eurasia (Telfer 1984). Despite the importance of moose as a game species within most provinces in Canada and a number of states in the U.S.A. (Cumming 1974; Ritchey 1974; Timmerman and Buss 1998), there is uncertainty about the movement patterns among moose populations. Moose movements have been classified as either dispersal or migration; dispersal is defined as the movement of a moose from a natal range to an area where it will potentially breed and migration is defined as the seasonal movement of moose between mating and nonmating ranges (Hundertmark 1998). Previous studies of moose populations have indicated differential amounts of moose migration (LeResche 1974; Telfer 1994). Limited dispersal has been described for moose (Hundertmark 1998), suggesting that the amount of dispersal may be inadequate to effectively influence neighboring populations, and the recovery of moose populations in heavily hunted regions in Ontario was attributed to reproduction and not to the immigration of moose from adjacent areas (Goddard 1970). Moose populations have also been proposed to be composed of both migratory and nonmigratory animals (LeResche 1974), although exclusively "island" populations of nonmigratory moose have been described in the prairie provinces of Canada (Karns 1998).

Previous genetic studies of moose have left the question of local movement patterns and gene flow unresolved. A genetic survey of North American moose (Cronin 1992) could not distinguish among proposed *A. alces* subspecies with restriction fragment length polymorphisms (RFLP) of the mitochondrial DNA (mtDNA), suggesting that the high potential for dispersal in moose has caused a genetic homogenization among moose populations. However, only one mtDNA haplotype was detected in this survey, likely owing to the resolution of RFLP analysis in detecting variation. Population structure was identified among Canadian moose representing populations from different provinces (Broders et al. 1999) using microsatellite loci, although this geographic scale could not address levels of migration and dispersal in a range consistent with moose biology (LeResche 1974; Hundertmark 1998). Other evidence in European moose populations suggests limited home ranges and site fidelity to the natal home range (Cederlund et al. 1987), and population structuring in Scandinavian moose was detected using protein polymorphisms over geographic distances of approximately 50 km (Ryman et al. 1980; Chesser et al. 1982).

In addition to the amount of gene flow through dispersal and migration, local selection pressures such as disease and hunting further impact levels of genetic variation within wildlife populations. The impact of disease on MHC variation in moose populations may be of particular importance

in Ontario, given the distribution of white-tailed deer (*Odocoileus virginianus*) and the incidence of *Parelaphostrongylus tenuis* throughout the province (Whitlaw and Lankester 1994). Furthermore, computer simulations of different hunting regimes of moose and white-tailed deer populations modeled a loss of genetic variability and a decrease in the ratio of effective population size to census population size over time (Ryman et al. 1981). The amount of inbreeding under the most severe hunting regime, that is, random hunting of any age and sex, was modeled to be the equivalent of full-sibling matings after 50 years.

The potential impact of disease and hunting on the genetic variation of game species makes protected areas such as parks potentially important reservoirs for maintaining genetic diversity within a metapopulation structure. However, the role of parks as a source of genetic variation will be effective in a larger metapopulation only if the genes associated within these protected areas can be exchanged among adjacent populations through animal movements and gene flow. An assessment of Canadian national and provincial parks showed that parks surrounded by human-altered landscapes reduced the immigration of mammals into park systems (Gurd and Nudds 1999), demonstrating the potential for isolation. As a result, it is important to determine the level of "genetic connectivity" between protected and nonprotected areas and the amount of genetic variation within park populations in comparison with neighboring geographic regions.

We have performed genetic analyses using minisatellite loci (detected using multilocus DNA fingerprinting), microsatellite loci, and the functional antigen-recognition binding region (ARS), exon 2, of the MHC gene *DRB* on moose populations from islands, continuous geographic areas, and three national parks. The moose populations in this study have different histories, ranging from continuous geographic ranges to island and isolated populations originating from small founder events. Furthermore, these regions have potentially different local selection pressures, based on the presence or absence of hunting and the parasite *P. tenuis*, corresponding to park and nonpark environments. The objective of this study was to assess the genetic variation and population structure at neutral and functional genetic loci in moose within the context of different historic factors, ecological factors, and human impacts, specifically the degree of isolation, the presence or absence of *P. tenuis*, and the presence or absence of hunting, respectively.

Materials and methods

Sample collection

Tissue samples were collected from moose representing nine geographic regions (Table 1, Fig. 1). Riding Mountain National Park, Manitoba, represents a potentially isolated population, based on the difference in habitat within and outside (highly developed agricultural areas) the park boundaries (Karns 1998). Various regions within Ontario were sampled and categorized on a provincial scale. Moose were analysed from northwestern Ontario, representing Red Lake and Sioux Lookout; the Nipigon region, which included Thunder Bay; northeastern Ontario, which included Kirkland Lake north to Moosonee; central Ontario, representing areas surrounding North Bay, Sault Ste. Marie, and Sudbury; and Pukaskwa National Park. Isle Royale, Michigan, was the only U.S. population included in the study representing a U.S. national park. The Isle Royale population was proposed to have been founded in the early 1900s by several moose. The population increased in size to about 200 in 1915 and expanded rapidly in the late 1920s to 5000 animals, followed by a population crash in the early 1940s. Levels remained at approximately 1000 moose upon introduction of wolves to the island in the late 1940s (Mech 1966). Newfoundland moose were included, as they represent a population established by a small founding event (Broders et al. 1999). Two moose were introduced to Newfoundland in the 1870s from Nova Scotia and four in the early 1900s from New Brunswick (Pimlott 1953; Broders et al. 1999). New Brunswick moose from the north shore of the province were also included in this study, as one of the sources of the Newfoundland population.

DNA profiling

DNA from the moose samples in this study was extracted according to Guglich et al. (1993). Multilocus DNA fingerprints were generated for samples within several of the populations we examined also according to Guglich et al. (1993). Polymorphic microsatellite loci were identified in moose from bovine (BM4513, BM1225, IGF-1; Bovine Genome, Research Genetics Inc.) or white-tailed deer (Cervid 2, Cervid 14; DeWoody et al. 1995) primer sets.

Microsatellite loci were analysed from nuclear DNA using a primer end labeled with [λ-^{33}P]ATP and a T4 polynucleotide kinase reaction (Boehringer-Mannheim). A total reaction volume of 10 μL per tube was used, containing 25–50 ng of genomic DNA, 200 μM dNTPs, 10× buffer, 2 mM $MgCl_2$, unlabelled primer (0.2 mM), labeled primer (4.6 pmol), 1.0 μg of bovine serum albumin (BSA) (BRL), and 0.5 U of Taq polymerase (BRL). The reaction conditions were 95°C for 15 s, annealing at 55–60°C for 15 s, and extension at 72°C for 30 s (Perkin Elmer). Products were mixed with a 0.4 volume of formamide loading buffer and were heated at 95°C for 5 min before loading onto a 6% sequencing gel containing 50% (w/v) urea. A control sequencing reaction of phage M13 DNA was run adjacent to the samples, to produce size markers for the microsatellite alleles.

The MHC *DRB* gene was amplified, cloned, and sequenced and the alleles observed, using single-stranded conformational polymorphism (SSCP) according to Murray and White (1998).

Genetic analyses

Genetic variation was estimated at minisatellite loci using DNA fingerprints using the average percent difference (APD), which is one minus the mean band-sharing coefficient (BSC) (Guglich et al. 1993). Genetic variation at microsatellite loci and the *DRB* locus was assessed using allelic diversity (number of alleles) and expected heterozygosity (H_E). H_E is a less-biased measure of heterozygosity, because it is less influenced by sample size (Nei 1978).

Allele frequency distributions, exact tests of Hardy–Weinberg expectations (HWE), and estimates of the *F* statistics (F_{IS} and F_{ST}) and R_{ST} were assessed with GENEPOP version 3.1b (Raymond and Rousset 1995), using the method described by Weir and Cockerham (1984). Isolation-by-

Table 1. Sampling sites of moose (*Alces alces*).

Geographical region	Sites	*N*	Proposed history	Source(s)
Manitoba	Riding Mountain National Park	23	Island prairie population	Karns 1998
Northwestern Ontario	Red Lake, Sioux Lookout	27	Continuous Ontario population	Banfield 1974
Nipigon, Ontario	Lake Nipigon, Geraldton, Thunder Bay	39	Continuous Ontario population	Banfield 1974
Isle Royale, Michigan	Isle Royale National Park	17	Founded in the early 1900s, increasing in size to about 200 moose in 1915, population expansion in the late 1920s to 5000 animals, followed by a population crash in early 1940s. Levels remained at approximately 1000 moose upon introduction of wolves to the island in the late 1940s	Mech 1966
Pukaskwa, Ontario	Pukaskwa National Park	37	Continuous Ontario population	Banfield 1974
Central Ontario	North Bay, Sault Ste. Marie, Sudbury	32	Continuous Ontario population	Banfield 1974
Northeastern Ontario	Kirkland Lake, Cochrane, Moosonee	36	Continuous Ontario population	Banfield 1974
New Brunswick	North shore	19	Unknown	
Newfoundland	Province-wide	29	Moose introduced from Nova Scotia in 1870 and four moose introduced from New Brunswick in the early 1900s	Pimlott 1953; Broders et al. 1999

Note: *N* is the total sample size used in profiling multilocus DNA fingerprinting, microsatellite loci, and (or) the *DRB* locus.

Fig. 1. Map indicating the geographic regions in which moose (*Alces alces*) were sampled for this study.

distance among moose populations was also analysed in GENEPOP version 3.1b (Raymond and Rousset 1995). Nei's unbiased genetic distance (Nei 1978; Takezaki and Nei 1996) was calculated using the program GENETIX 3.3 (Belkhir et al. 1999), and a neighbor-joining tree was constructed in the computer program PHYLIP, using the program NEIGHBOR (Felsenstein 1993).

Results

Genetic variation and comparison of neutral DNA markers

Multilocus DNA fingerprints were generated and average percent differences (APDs) were calculated for the pairwise comparisons between individual moose of a particular geographic region (Table 1). Isle Royale and Newfoundland populations showed the lowest genetic variation, consistent with the proposed small founding events for each island. Moose from the Nipigon region, specifically Thunder Bay, showed variation comparable with those from Newfoundland. Riding Mountain and other regions in Ontario showed comparably higher levels of variation based on APD values. Unfortunately, multilocus DNA fingerprints were not generated for Pukaskwa National Park moose, because of the low quality of the DNA sample.

The microsatellite loci had 2–5 alleles per locus; the total number of alleles over the five loci ranged from 11 to 22 within the nine regions, with a mean observed number of al-

leles per locus of between 2.3 and 4.4 (Table 2). Moose in Manitoba and Ontario had a total of 20–22 alleles, while 14 were observed in the New Brunswick moose population. The island populations of Isle Royale and Newfoundland had 14 and 11 alleles, respectively. Expected heterozygosity (H_E) (Table 2) at the microsatellite loci was calculated for each region. Again, the populations of Isle Royale and Newfoundland showed the lowest variation (H_E), while the remaining regions showed relatively similar levels of heterozygosity (Table 2). Tests of heterozygosity at the microsatellite loci were consistent with HWE forn the majority of population–locus combinations. Three comparisons deviated significantly from HWE: Nipigon–BM4513 ($p < 0.050$), northeastern Ontario – BM4513 ($p < 0.001$), and Riding Mountain – IGF-1 ($p < 0.050$). F_{IS} values (Table 4) indicated a deficiency in the number of heterozygotes at the microsatellite loci for moose in both Riding Mountain and Isle Royale.

A comparison of genetic variation (APDs from multilocus DNA fingerprinting and H_E from microsatellite loci) showed that the two DNA markers demonstrated relative consistency among the majority of the regions. Discrepancies were found in two regions: Nipigon moose showed relatively low APD values compared with the highest observed H_E, and Isle Royale moose, while showing relatively low values for both APD values and H_E compared with the other populations, showed considerably lower APD values than H_E (Fig. 2).

A comparison of island populations with proposed small founding events (Isle Royale and Newfoundland) with their potential source populations (Nipigon and New Brunswick, respectively) using APD and microsatellite data (H_E and allelic diversity) identified a loss of genetic variation consistent with the proposed history of these two populations (Table 3). These reductions in genetic variation are consistent with previous genetic surveys of moose from source and founded island populations, including Newfoundland (Broders et al. 1999).

The loss of genetic variation detected using APD and microsatellite allelic diversity was almost identical for the Isle Royale comparison and the Newfoundland comparison. The loss of genetic variation observed with microsatellite locus H_E was smaller than that observed with APDs and microsatellite allelic diversity for the Isle Royale comparison and greater for the Newfoundland comparison (Table 3).

Genetic variation of the functional *DRB* locus

The *DRB* alleles found were identical to the alleles previously characterized by Mikko and Andersson (1995). The allele designations in our study and the corresponding alleles in the previously published study are, respectively, as follows: *DRB*-a is allele ala1-5; *DRB*-b is allele ala1-7; *DRB*-c is allele ala1-3; and *DRB*-d is allele ala1-10. The number of alleles within each population at the *DRB* locus varied from 1 to 4 (Table 2). The population of Riding Mountain National Park had all four of the alleles observed within North American moose (Mikko and Andersson 1995). Isle Royale, northwestern Ontario, Pukaskwa National Park, and New Brunswick moose had three of the four alleles. Nipigon, central Ontario, and northeastern Ontario moose had two alleles and the Newfoundland population was fixed for the *DRB*-a allele. Newfoundland moose were all homozygous for *DRB*-a, probably as a result of the founding event in the transfer of animals to the island. In contrast, little or no allelic diversity was lost by the founding event on Isle Royale, if mainland Ontario represents the founding population for the island, implying that allelic diversity may have been lost from Ontario mainland moose subsequent to the founding event. Three populations deviated from HWE at the *DRB* locus: Riding Mountain moose ($p < 0.050$), Isle Royale moose ($p < 0.050$), and New Brunswick moose ($p < 0.050$). A deficiency of heterozygotes at the *DRB* locus was further supported by F_{IS} values and was consistent with the microsatellite data from Riding Mountain and Isle Royale.

Comparison of the functional *DRB* locus with neutral DNA markers

Genetic variability of the neutral markers was inconsistent with variation at the functional *DRB* locus when comparing H_E and APDs versus H_E (*DRB*) (Fig. 2). Several inconsistencies in the levels of genetic variation were observed among the populations at the two types of loci (Table 2, Fig. 2). Isle Royale moose demonstrated low levels of genetic variation at the neutral microsatellite and minisatellite loci but demonstrated relatively high levels of variation at the *DRB* locus. Moose from regions within Ontario demonstrated relatively high genetic variation at both neutral markers but relatively low variation at the MHC locus by comparison. Moose from Riding Mountain and Pukaskwa national parks had relatively high levels of variation consistent among loci, while those from Newfoundland were consistently low at both the neutral and functional loci.

The allele frequency distributions compared among different regions at the neutral microsatellite loci and functional *DRB* locus indicated that the two types of loci were not in close agreement (Table A1). The populations with historic small founding events, that is, those of Isle Royale and Newfoundland, demonstrated close to bimodal distributions at three of the five microsatellite loci, with a fixed allele being found at one microsatellite locus in Newfoundland moose. The IGF-1 microsatellite locus was bimodal in all the populations examined. The criteria for a bimodal-like distribution of allele frequencies were the presence of 1–3 alleles, with at least one high frequency allele (>0.600). Similar criteria were used by Houlden et al. (1996). Bimodal distributions were identified in populations that had undergone severe population bottlenecks due to the rapid loss of rarer alleles (Houlden et al. 1996). The remaining populations had a wider range of allele frequency distributions, with the presence of rarer alleles. The *DRB* allele frequency distributions differed from the microsatellite loci in the majority of populations. The Ontario and Isle Royale populations demonstrated opposite patterns between the neutral and functional loci. Mainland Ontario populations (excluding Pukaskwa National Park) demonstrated bimodal allele frequencies at the *DRB* locus. In Isle Royale moose, while *DRB*-a was common, there was a more even distribution of two other alleles, contrary to the microsatellite data. In the two Canadian national park (Riding Mountain and Pukaskwa) populations allele frequency distributions between the neutral and functional loci were similar.

Population structure

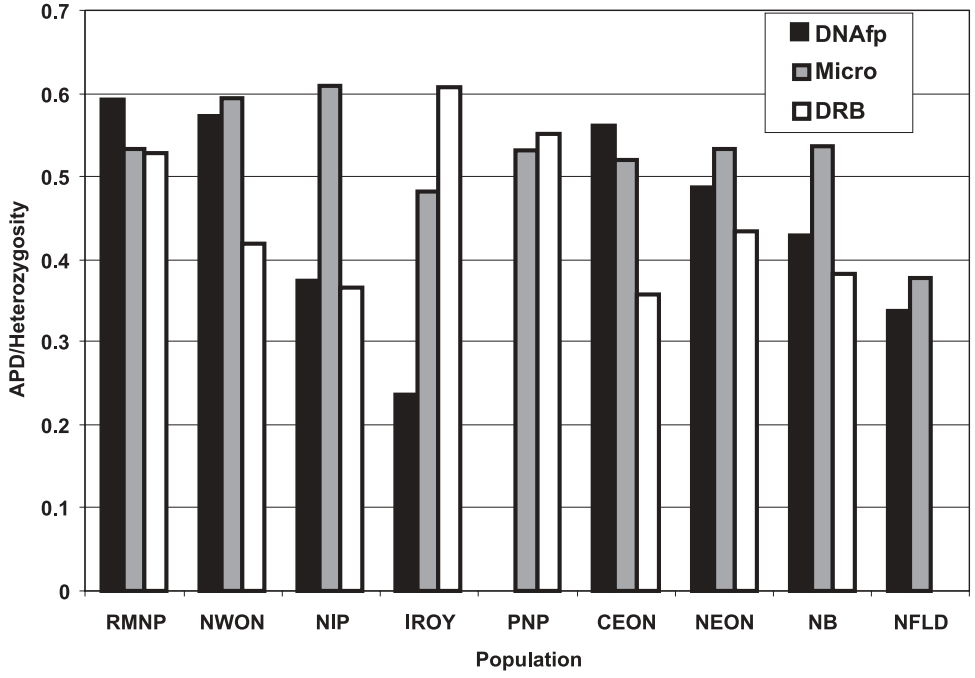
The population structure of the moose representing the

Table 2. Average percent difference (APD) from multilocus DNA fingerprints and number of alleles, expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}) for five microsatellite loci and the *DRB* locus for nine moose populations; *N* is sample size (actual or mean) analysed per type of locus.

	Multilocus DNA fingerprints		*Microsatellite loci*				DRB locus			
Population	N^a	APD	N^a	Alleles[b]	H_E	F_{IS}	N^c	Allele[d]	H_E	F_{IS}
Riding Mountain	10	0.593	23	4.4	0.534	0.120	23	4.0	0.529	0.171
Northwestern Ontario	18	0.573	24	4.4	0.595	0.039	27	3.0	0.419	0.047
Nipigon, Ontario	9	0.375	38	4.2	0.610	0.098	25	2.0	0.365	0.143
Isle Royale, Michigan	9	0.238	15	2.8	0.482	0.176	17	3.0	0.607	0.329
Pukaskwa	—	—	33	4.2	0.532	–0.037	37	3.0	0.551	0.082
Central Ontario	10	0.561	31	4.2	0.520	0.057	15	2.0	0.358	–0.273
Northeastern Ontario	19	0.487	36	4.0	0.534	0.075	30	2.0	0.433	0.016
New Brunswick	18	0.429	18	2.8	0.537	–0.132	19	3.0	0.381	0.060
Newfoundland	17	0.337	19	2.2	0.378	0.017	29	1.0	0.000	—

[a]Mean sample size.
[b]Mean number of alleles.
[c]Actual sample size.
[d]Total number of alleles.

Fig. 2. Graph showing average percent difference (APD) for multilocus DNA fingerprint (DNAfp) profiles and expected heterozygosity (H_E) for five microsatellite loci and the MHC *DRB* locus for moose from nine regions. RMNP, Riding Mountain National Park; NWON, northwestern Ontario; NIP, Nipigon; IROY, Isle Royale; PNP, Pukaskwa National Park; CEON, Central Ontario; NEON, northeastern Ontario; NB, New Brunswick; NFLD, Newfoundland.



nine geographic regions was assessed on the basis of microsatellite allele frequencies using F_{ST} and R_{ST} statistics (Table 4) (Table 1A). F_{ST} typically had higher values than R_{ST}. A comparison of these two estimates of population structuring for domestic and bighorn sheep indicated that F_{ST} is more sensitive to allopatry and semi-isolation and R_{ST} is more sensitive to longer historical separations (Forbes et al. 1995). In general, pairwise R_{ST} and F_{ST} estimates of less than 5% were interpreted to mean that some level of gene flow has been maintained, estimates of 5–10% to mean that populations are semi-isolated, and estimates of greater than 10% to mean that populations are isolated. Pairwise R_{ST} and F_{ST} comparisons were considered in the context of geography or history, that is, neighboring regions or the founding population, for direct comparisons.

Both measures differentiated Riding Mountain moose from moose in the nearest neighboring region, northwestern Ontario. F_{ST} and R_{ST} differentiated Isle Royale moose from those in the closest mainland regions of northwestern Ontario and Nipigon. Also, Newfoundland moose were differentiated from the mainland New Brunswick population, from which the island moose had originated approximately 100

Table 3. Loss of genetic variation between proposed source populations and the island populations of Isle Royale and Newfoundland.

Founding event	DNA fingerprinting: average percent difference	Microsatellite: Expected heterozygosity (H_E)	Microsatellite: Allelic diversity
Nipigon to Isle Royale	36.0% decrease	21.0% decrease	36.4% decrease
New Brunswick to Newfoundland	21.4% decrease	30.0% decrease	21.4% decrease

Table 4. R_{ST} (top diagonal) and F_{ST} (bottom diagonal) for each pairwise comparison of nine moose populations.

Population	RMNP	NWON	NIP	IROY	PNP	CEON	NEON	NB	NFLD
RMNP	—	0.1042	0.0579	0.0424	0.0601	0.0944	0.1072	0.1138	0.2011
NWON	0.1033	—	0.0011	0.0389	0.0343	0.0648	0.0579	0.0792	0.1300
NIP	0.0918	0.0239	—	0.0439	0.0045	0.0350	0.0294	0.0664	0.1652
IROY	0.1366	0.1036	0.1154	—	0.0673	0.1416	0.1150	0.0913	0.1095
PNP	0.0602	0.0237	0.0430	0.0927	—	0.0282	0.0124	0.1041	0.1553
CEON	0.0872	0.0688	0.0871	0.1674	0.0255	—	0.0266	0.1898	0.2523
NEON	0.0829	0.0645	0.0790	0.1647	0.0265	0.0343	—	0.1386	0.2110
NB	0.1228	0.1252	0.1132	0.1869	0.1083	0.1465	0.0737	—	0.1727
NFLD	0.3013	0.1614	0.1897	0.2371	0.1758	0.2028	0.1321	0.1370	—

Note: RMNP, Riding Mountain National Park; NWON, northwestern Ontario; NIP, Nipigon; IROY, Isle Royale; PNP, Pukaskwa National Park; CEON, Central Ontario; NEON, northeastern Ontario; NB, New Brunswick; NFLD, Newfoundland.

Table 5. Nei's unbiased genetic distance for the *DRB* locus (top diagonal) and five microsatellite loci (bottom diagonal).

Population	RMNP	NWON	NIP	IROY	PNP	CEON	NEON	NB	NFLD
RMNP	—	0.0212	0.0257	0.0458	0.0248	0.0370	0.0455	0.0142	0.0454
NWON	0.2085	—	0.0013	0.0552	0.0015	0.0256	0.0075	0.0070	0.0538
NIP	0.1980	0.0680	—	0.0736	0.0001	0.0265	0.0082	0.0046	0.0475
IROY	0.2043	0.1740	0.1947	—	0.0742	0.0935	0.0758	0.0815	0.1362
PNP	0.1068	0.0520	0.0764	0.1380	—	0.0280	0.0096	0.0040	0.0443
CEON	0.1528	0.1258	0.1560	0.2638	0.0491	—	0.0168	0.0283	0.1194
NEON	0.1401	0.1263	0.1586	0.2497	0.0534	0.0068	—	0.0218	0.0973
NB	0.2404	0.2610	0.2495	0.3291	0.1925	0.2661	0.1435	—	0.0305
NFLD	0.5449	0.4201	0.5574	0.4715	0.3998	0.4540	0.2620	0.2142	—

Note: RMNP, Riding Mountain National Park; NWON, northwestern Ontario; NIP, Nipigon; IROY, Isle Royale; PNP, Pukaskwa National Park; CEON, Central Ontario; NEON, northeastern Ontario; NB, New Brunswick; NFLD, Newfoundland.

years ago. The majority of neighboring populations in Ontario demonstrated high levels of gene flow, with the exception of moose from Nipigon and from the northeastern part of the province — these showed low levels of differentiation (Table 4). Animals appeared to be exchanged readily among the populations of Pukaskwa National Park and those of the northwest, northeast, and central regions of Ontario.

Genetic distances

Pairwise genetic distance measures, based on allele frequencies, were calculated for the microsatellite loci and the *DRB* locus, to determine the relationship among populations. Using Nei's unbiased genetic distance (Nei 1978), neighbor-joining trees (Table 3) were generated for the microsatellite loci (Fig. 3*a*) and for the *DRB* locus (Fig. 3*b*). Nei's unbiased genetic distance (Nei 1978) values were tenfold higher for the microsatellite loci than for the *DRB* locus, likely as a result of prominent alleles at the *DRB* locus within the majority of moose populations, that is, the *DRB*-a allele (Table 5). The topology of the microsatellite genetic distance tree (Fig. 3*a*) was consistent with the population structure estimated using F_{ST} and R_{ST} values, with neighboring regions clustering together and with the isolated and island populations more distant from the larger continuous population in Ontario and the population in New Brunswick.

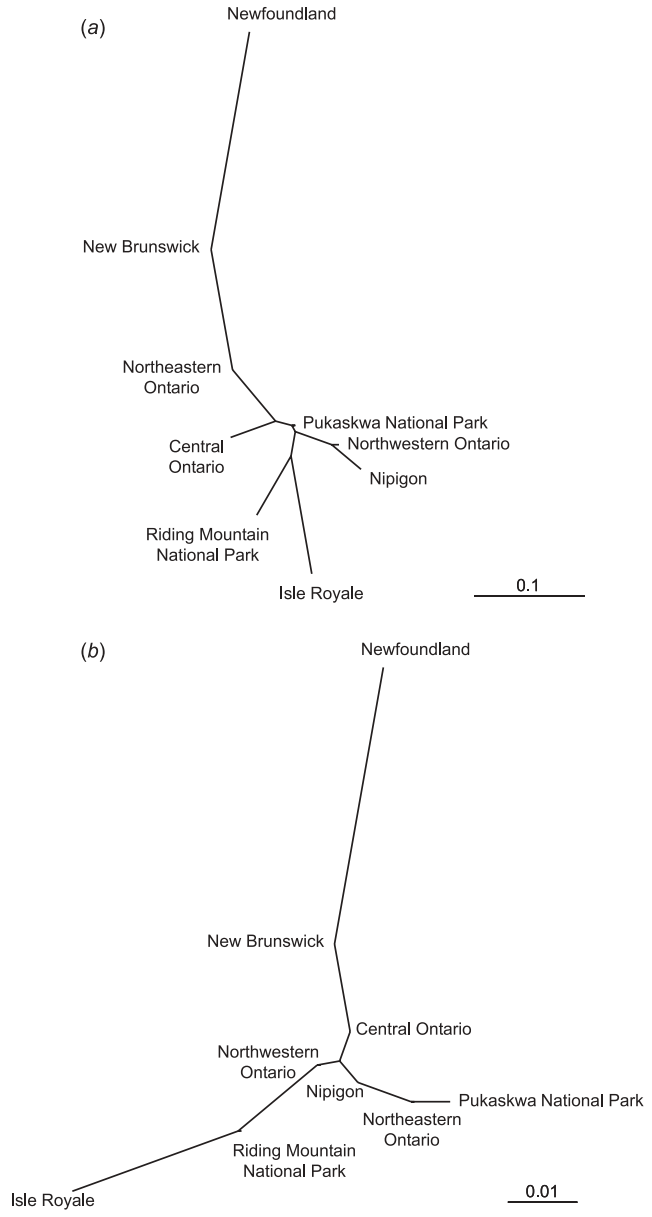
F_{ST} values for *DRB* showed no differentiation among populations (data not shown); this likely reflects the low allelic diversity and the use of a single locus. However, Nei's genetic distance provided some insight into the relationships among the different regions. The island populations and Pukaskwa National Park moose showed increased distance from the other regions (Fig. 3*b*). The distance of the Pukaskwa National Park population from other populations estimated using the functional locus compared with the distance estimated using the microsatellite loci reflects the differences in the higher genetic variability at *DRB* within the moose in the park compared with other Ontario moose populations, despite the gene flow detected by microsatellite analysis.

Discussion

Comparison of DNA marker systems

An assessment of the genetic variation of both neutral and functional genetic markers indicated overall low levels com-

Fig. 3. Neighbour-joining trees using Nei's unbiased genetic distance on allele frequencies from five microsatellite loci (*a*) and the exon-2 region of the MHC *DRB* gene (*b*).



pared with other wildlife species, specifically allelic diversity at the microsatellite loci (Paetkau and Strobeck 1994; Roy et al. 1994), the *DRB* locus (Mikko et al. 1999), and multilocus DNA fingerprinting (Guglich et al. 1993). This finding is consistent with other studies examining genetic variation in moose (Guglich et al. 1993; Mikko and Andersson 1995; Ellegren et al. 1996; Broders et al. 1999). The low genetic variability within moose has been attributed to population-size bottlenecks during the Pleistocene (Mikko and Andersson 1995; Ellegren et al. 1996).

The deficiency of heterozygotes based on deviations from HWE or F_{IS} values in the Riding Mountain and Isle Royale national park populations (Table 2) can result from three factors: nonassortative mating; selection; and pooling samples from local structured populations within the island populations, that is, a Wahlund effect. No evidence for nonassortative mating was observed when examining other ungulates using MHC loci (Paterson and Pemberton 1997), and selection at MHC loci is predicted to result in an excess of heterozygotes through balancing selection (Hedrick and Thomson 1983) (see below). The heterozygote deficiency in both the neutral microsatellite loci and the functional MHC gene supports a Wahlund effect. Riding Mountain and Isle Royale populations may maintain local subpopulations within these "island" populations. The higher densities in these populations, estimated at between 1.0 and 4.0 individuals/km^2 (Telfer 1984), may have resulted in the formation of local congregates of moose within the boundaries of the isolated populations; this is consistent with the apparent Wahlund effect. As a result, the presence of local structured populations may have actually caused expected heterozygosity to be underestimated at both the microsatellite and *DRB* loci. In African buffalo (*Synercus caffer*) from national parks, a consistent deficiency in heterozygotes was observed at the *DRB* locus (Wenink et al. 1998) and at microsatellite loci (Simonsen et al. 1999).

The Isle Royale and Newfoundland populations demonstrated the lowest genetic variability at the neutral DNA markers, consistent with the small founding events of approximately 100 years ago. Two discrepancies were observed when comparing the two neutral markers. Firstly, Nipigon moose demonstrated a relatively low APD value compared with microsatellite H_E; this is likely the result of using moose from a localized region around Thunder Bay, Ontario. DNA fingerprint profiles may detect higher genetic relatedness on smaller geographical scales than microsatellites, owing to the resolution of the two markers with higher mutation rates at minisatellite loci (Estoup and Angers 1998; Hansson et al. 2000). Unfortunately, technical limitations associated with DNA fingerprinting make large numbers of samples difficult to compare (intra-gel comparisons; Guglich et al. 1993), thereby limiting the scale at which this technique can effectively be used for monitoring. These limitations have resulted in a decline in the use of DNA fingerprinting in such studies in favor of using microsatellite loci (Estoup and Angers 1998).

Secondly, although consistently lower than for moose in other regions, the H_E of microsatellite loci for moose from Isle Royale was considerably higher than its comparable APD value. This discrepancy is likely the result of H_E being less sensitive to population bottlenecks – small founding events and of APD reflecting allelic loss and not heterozygosity. Levels of heterozygosity can be maintained following a population bottleneck, despite the loss of alleles (Spencer et al. 2000), or increase immediately following a bottleneck event (Cornuet and Luikart 1996). Bottlenecks can equalize allele frequencies as rare alleles are lost through population reduction causing an increase in heterozygosity (Leberg 1992; Spencer et al. 2000). Natural and experimental studies have shown that allelic diversity is significantly affected by population bottlenecks and more accurately reflects the loss of genetic variation (Leberg 1992; Luikart and Cornuet 1998; Spencer et al. 2000).

This relationship between allele loss and bottlenecks was supported by the observed loss of genetic variation at APD for minisatellite loci and H_E and allelic diversity for micro-

satellite loci between source and founded island populations (Table 3). The DNA fingerprint data (APD) showed a loss identical to microsatellite allelic diversity in both comparisons of source population to island founding events. Again, DNA fingerprinting analyses examine allele sharing (Guglich et al. 1993) and therefore reflect the loss of rarer alleles through a bottleneck.

At present, heterozygosity is one of the most common measures of assessing genetic variation. The recent study examining experimentally bottlenecked populations by Spencer et al. (2000) and other studies (Leberg 1992; Cornuet and Luikart 1996; Luikart and Cornuet 1998; Luikart et. al. 1999) have demonstrated the importance of allelic diversity in considering genetic diversity for conservation biology. Allelic diversity of MHC loci in particular has been recommended for consideration in captive-breeding programs (Hughes 1991) and natural populations (Yuhki and O'Brien 1990). Our data suggest that the assessment of neutral genetic variation may not accurately reflect adpative loci such as those of the MHC.

We observed several discordant results in this study when comparing neutral and functional loci. Moose in Isle Royale, Riding Mountain, and Pukaskwa national parks show relatively high levels of genetic variation at the *DRB* locus compared with MHC diversity in the other populations of Ontario that maintain gene flow and relatively high levels of genetic variation at the neutral DNA loci. The *DRB* variation observed in Isle Royale moose is contrary to the variation observed at the mini- and micro-satellite loci that is more consistent with the history of the island population, specifically a small founding event. The Newfoundland population has a history similar to that of Isle Royale with its small founding population and shows consistently low levels of genetic variation at the micro- and mini-satellite loci and monomorphism at the *DRB* locus.

Concordance between MHC and micro- or mini-satellite variation is seen in a number of studies: bighorn sheep (*Ovis canadensis*) (Boyce et al. 1997), pocket gophers (*Thomomys* spp.) (Sanjayan et al. 1996), and European beavers (*Caster fiber*) (Ellegren et al. 1993). However, a survey of three marine mammal species (Slade 1992) indicated lower MHC diversity compared with terrestrial mammals with no equivalent reduction at allozyme loci in the one seal and two whale species examined. This finding was attributed to reduced pathogen exposure in a marine environment. A similar explanation was proposed for the overall low MHC variation observed in Swedish moose (Ellegren et al. 1996), suggesting that the solitary lifestyle of moose may reduce the lateral transmission of pathogens. The marine-mammal and moose studies concluded that the reduced genetic variation at the MHC was not indicative of low genome-wide variation and supported reduced balancing selection pressures.

Balancing selection is predicted to result in high allelic diversity and an even distribution of allele frequencies at MHC loci that are maintained through heterozygote advantage (Hedrick and Thomson 1983). However, empirical evidence in nonhumans of balancing selection in natural populations is sparse. A study of island populations of the bush rat (*Rattus fuscipes greyii*) reported a loss of allelic diversity at neutral and MHC loci but an excess of heterozygosity at the MHC gene *RT1.Ba* in 3 of 14 island populations, suggestive of balancing selection through overdominance (Seddon and Baverstock 1999). Paterson and Pemberton (1997) showed direct evidence of selection acting on specific alleles rather than heterozygosity (predicted under balancing selection) in a natural ungulate population, as alleles at the *DRB* locus in Soay sheep (*Ovis aries*) were significantly associated with juvenile survival and resistance to intestinal nematodes. However, the heterozygosity at the *DRB* locus in Soay sheep of all age classes in the population revealed an even allele frequency distribution, supporting balancing selection (Paterson 1998). Both the bush rat and Soay sheep studies (Seddon and Baverstock 1999; Paterson and Pemberton 1997; Paterson 1998) observed greater nonsynonymous to synonymous substitutions at *DRB*, further supporting balancing selection through the favoring of novel MHC alleles.

Although the *DRB*-a allele was prevalent (Table A1), we observed higher *DRB* allelic diversity and more even allele frequency distributions within national parks. Although a deficiency in H_E was detected, discordant levels of genetic variation at the *DRB* locus compared with the neutral micro- and mini-satellite DNA markers within Isle Royale (high and low, respectively) and mainland Ontario (low and high, respectively) populations support differential selection pressures acting on the MHC locus. High allelic diversity and even distributions of allele frequencies at *DRB* were also observed in African buffalo national park populations, despite historic population bottlenecks caused by disease (Wenink et al. 1998). The analyses of genetic distances and tree topologies revealed different spatial patterns of variation between the microsatellite and DRB loci with respect to the Pukaskwa populations (Fig. 3). Similar results were interpreted as limited evidence for selection on the MHC locus (Boyce et al. 1997). Furthermore, the *DRB* sequences of this study and of Mikko and Andersson (1995) have greater nonsynonymous to synonymous substitutions in the ARS region, supporting long-term balancing selection. The differential patterns are suggestive of balancing selection within the park systems and pathogen-driven selection, that is, selection acting on specific alleles in nonpark regions. The levels of gene flow we observed do not support genetic drift as a factor resulting in a loss of alleles in the continuous Ontario region. We considered two potential factors that differ between the parks and neighboring regions we examined: the absence of white-tailed deer infected with the parasite *P. tenuis* within national parks and the absence of hunting within the park systems.

Firstly, the impact of white-tailed deer infected with the menengial worm *P. tenuis* may be influencing the MHC locus independently of the neutral loci, resulting in a shift to predominant-allele frequency within nonpark regions inhabited by deer. The populaton in Pukaskwa National Park has contact with moose from the northern and central regions of Ontario, yet moose in the other regions within the province show a more pronounced trend towards the *DRB*-a allele. White-tailed deer and *P. tenuis* are absent within Pukaskwa National Park (Whitlaw and Lankester 1994), the moose population of which maintains an additional allele not observed in moose exterior to the park. The dominant *DRB*-a distribution in moose in other regions of Ontario, despite the

gene flow detected with the microsatellite loci, is consistent with the presence of *P. tenuis*. Also supporting the potential impact of *P. tenuis* on *DRB* allele distributions are the alleles observed in British Columbian moose (Mikko and Andersson 1995). These moose have maintained the four North American *DRB* alleles and do not demonstrate the predominant *DRB*-a allele frequency observed in the more eastern range of moose we examined in this study, consistent with the absence of *P. tenuis* in western Canada.

In the absence of *P. tenuis*, balancing selection may be the primary factor influencing MHC variation and this may be further enhanced by high moose densities in two of the park systems: Isle Royale and Riding Mountain. The higher densities of moose in these populations may be atypical of the solitary life history of moose in other regions (Ellegren et al. 1996), resulting in increased balancing selection due to increased contact and pathogen exchange. High population densities in Isle Royale National Park and Algonquin Provincial Park, with the highest number of moose per square kilometre in Ontario (Whitlaw and Lankester 1994), have suffered high incidences of mortality from the winter tick (D. Strickland, personal communication). Pukaskwa National Park maintains a stable population of approximately 0.200 moose/km^2 (Whitlaw and Lankester 1994) compared with 1.0–4.0 moose/km^2 in Isle Royale and Riding Mountain national parks.

Secondly, although hunting has not been directly associated with a loss of diversity at MHC loci, there is the potential for such an association similar to pathogen-driven selection. An examination of the impacts of selective hunting, that is, a preference for large body size and high-quality antler racks, on red deer indicated a loss in the frequency of alleles associated with such traits (Hartl et al. 1991). MHC alleles have been shown to have an association with growth in cattle (Grignola et al. 1995), and this gene complex is linked to a large number of growth-related genes (Lewin et al. 1992). Hunting pressure on "larger" moose outside of protected areas may influence a loss of allelic diversity observed for the MHC genes that would not be readily detected at micro- or mini-satellite loci.

Our data is consistent with the suggestion that the presence or absence of white-tailed deer with *P. tenuis* and differential hunting pressure, singly or in combination, are potential factors influencing MHC allelic diversity in moose populations. At present, it would be premature to connect specific *DRB* alleles to any disease resistance against *P. tenuis* or fitness characteristics; however, MHC alleles are inherited in haplotypic combinations (Murray et al. 1999) and alleles at other genes in linkage may be influencing the observed shift in the *DRB* alleles. Furthermore, a complete assessment of the population structure in isolated parks would be required to fully assess heterozygote excess due to balancing selection versus a deficiency based on a Wahlund effect. More direct comparisons would be required to examine the role, if any, of hunting pressure on moose populations and its impact on levels of genetic variation at MHC or other functional loci.

Population structure and metapopulations

An assessment of connectivity and gene flow among different geographic areas identified both nonmigratory and migratory moose populations. Riding Mountain National Park was shown to be isolated from its nearest geographic neighbor, northwestern Ontario, a result consistent with a nonmigratory prairie "island" population (Karns 1998). Isle Royale was confirmed as being isolated from its corresponding mainland populations, that is, northwestern Ontario – Nipigon. Moose movement and gene flow were prevalent throughout Ontario, extending along the periphery of Lake Superior. Differentiation between northwestern and northeastern Ontario likely reflects isolation by distance (Table 4). Genetic distances between populations can reflect the effects of geographical separation (Simonsen et al. 1998), and the neighbor-joining tree of Nei's unbiased genetic distance (Nei 1978) supports isolation by distance within the continuous Ontario range (Table 4, Fig. 3*a*).

The monomorphic *DRB* locus in Newfoundland moose, in the absence of deer and *P. tenuis* on the island, indicates that historic population events such as founder events can significantly influence levels of genetic variation at functional DNA markers. Similar monomorphism at the *DRB* locus was observed in a number of the island populations of bush rat (Seddon and Baverstock 1999). The population turnover that may result from overhunting (Goddard 1970) may accelerate genetic drift in isolation, owing to decreased effective population size, thereby reducing genetic variation (Harrison and Hastings 1996). However, our findings suggest that the recovery of moose numbers in continuous geographic ranges such as Ontario is supplemented by immigration, contrary to the proposed limited impact of neighboring populations (Goddard 1970; Hundertmark 1998). The recovery of moose populations through local movements, immigration, or recolonization is consistent with a metapopulation of potential "source" and "sink" subpopulations (Harrison and Hastings 1996).

National and provincial parks represent important areas for maintaining genetic variability and, further, represent potential "source" populations within a connected metapopulation structure. The one notable example from this study is Pukaskwa National Park, which appears to represent an important reservoir of genetic material within the northern Ontario moose metapopulation. Different levels of genetic variability at the *DRB* locus in moose within the park (Fig. 3*b*) were observed despite connectivity to other regions in Ontario (Table 3, Fig. 3*a*). The maintenance of an additional allele at this one MHC locus within this region provides the potential transfer of allelic diversity to other regions of Ontario through the existing levels of gene flow, if local selection pressures are altered. Contrary to the situation in Pukaskwa National Park, Riding Mountain and Isle Royale national parks, while maintaining potentially important allelic diversity, are in apparent isolation from neighboring regions, limiting their contribution as natural "source" populations.

Recent studies have examined the genetic structure of populations within a metapopulation context by examining genetic variation combined with local extinction and recolonization (Saccheri et al. 1998; Westemeier et al. 1998). Also, local selection pressures affecting morphological traits despite gene flow have been observed in conifers (Karhu et al. 1996), and positive selection has been identified to be acting on the transferrin gene in salmon populations (Ford et al. 2000). Our data demonstrate the potential of combining

DNA profiles from neutral loci, such as microsatellites, to functional loci, such as moose MHC loci, to completely elucidate metapopulation structure. Although variable neutral loci such as microsatellites can be useful in assessing population structure, they may have limited use as a proxy for genome-wide variation, particularly in detecting local selection pressures influencing genetic variation within local "source" and "sink" populations.

Conclusions

There are obvious limits to the range of genetic variability that can exist in moose populations based on the number of alleles present in North American animals. As a result, large differences in allelic diversity and heterozygosity are constrained and any interpretation regarding the role of specific selection pressures, such as disease and hunting, influencing MHC allele frequencies in moose populations requires large sample sizes from a number of sites with different selection pressures. Certainly stochastic events such as bottlenecks could generate similar levels of allelic diversity. However, the low overall levels of genetic variation in moose in otherwise viable populations can provide a useful model for understanding genetic variation for comparison with threatened and endangered populations.

This study has identified differences between neutral genetic variation and variation at an adaptively significant locus. Used in combination, neutral and functional loci may provide a very different interpretation of genetic variation and population structure than either would provide used alone. A comprehensive comparison of neutral and functional genetic loci would provide a more accurate composite of population structure and genetic variation within a metapopulation framework. The management strategies for wildlife species should examine larger metapopulations as the primary conservation unit. Many studies have focused on isolated populations, considering isolation to be the primary criterion for identifying conservation or management units (Moritz 1994). Isolation may be a reasonable criterion for recommending protection, but identifying adaptive variation provides a sound basis for effective management and is a pro-active approach to preventing isolation among the differential selection pressures within a larger metapopulation. Priority should be placed on identifying locally adaptive genomes, using morphological, physiological, or genetic characters; high allelic diversity; and population structure, migration, and gene flow.

For moose specifically, increasing the number of park systems and surrounding areas, as well as increasing the number of microsatellite and MHC loci would allow a more accurate reconstruction of the metapopulation structure and the potential local selection pressures acting on moose from different regions. Based on our present findings, we recommend managing moose as a metapopulation, contained within protected park systems with no hunting and no white-tailed deer (or more importantly, no *P. tenuis*) that maintain connectivity to neighboring regions through corridors to support migration and gene flow.

Acknowledgements

We thank the Enforcement Branch of the Ontario Ministry of Natural Resources (OMNR) for providing samples used in this study. This research was funded by a grant from OMNR and a grant from the Natural Sciences and Engineering Research Council of Canada provided to B.N. White.

References

Bancroft, A.W.F. 1974. The mammals of Canada. University of Toronto Press, Toronto, Ontario.

Belkhir, K., Borsa, P., Goudet, J., Chikhi, L., and Bonhomme, F. 1999. GENETIX 3.3, logiciel sous WINDOWS TM pour la génétique des populations. Laboratoire Génome et Populations, Unité Propre de Recherche nº 9060 du Centre National de la Recherche Scientifiqe, Université de Montpellier II, Montpellier, France.

Boyce, W.M., Hedrick, P.W., Muggli-Cockett, N.E, Kalinowski, S., Penedo, M.C., and Ramey, R.R. 1997. Genetic variation of major histocompatibility complex and microsatellite loci: a comparison in bighorn sheep. Genetics, **145**: 421–433.

Broders, H.G., Mahoney, S.P., Montevecchi, W.A., and Davidson, W.S. 1999. Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces*, in Canada. Mol. Ecol. **8**: 1309–1315.

Carvalho, G.R. 1998. Molecular ecology: origins and approaches. *In* Advances in molecular ecology. *Edited by* G.R. Carvalho. NATO Science Series, Series A: Life Sciences, Amsterdam, the Netherlands. Vol. 306. pp. 1–16.

Cederlund, G., Sandegren, F., and Larsson, K. 1987. Summer movements of female moose and dispersal of their offspring. J. Wildl. Manage. **51**: 342–352.

Chesser, R.K., Reuterwall, C., and Ryman, C. 1982. Genetic differentiation of Scandinavian moose *Alces alces* populations over short geographic distances. Oikos, **39**: 125–130.

Cornuet, J., and Luikart, G. 1996. Description and power analysis of two tests for detecting recent demographic bottlenecks from allele frequency data. Genetics, **144**: 2001–2014.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M., and Wayne, R.K. 2000. Considering evolutionary processes in conservation biology. Trends Ecol. Evol. **15**: 290–295.

Cronin, M.A. 1992. Intraspecific variation in mitochondrial DNA of North American cervids. J. Mammal. **73**: 70–82.

Cumming, H.G. 1974. Moose management in Ontario from 1948–1973. Nat. Can. **101**: 673–687.

DeWoody, J.A., Honeycutt, R.L., and Skow, L.C. 1995. Microsatellite markers in white-tailed deer. J. Hered. **86**: 317–319.

Ellegren, H., Hartman, G., Johansson, M., and Andersson, L. 1993. Major histocompatibility complex monomorphism and low levels of DNA fingerprinting variability in a reintroduced and rapidly expanding population of beavers. Proc. Natl. Acad. Sci. U.S.A. **90**: 8150–8153.

Ellegren, H., Mikko, S., Wallin, K., and Andersson, L. 1996. Limited polymorphism at major histocompatibility complex (*Mhc*) loci in the Swedish moose *A. alces*. Mol. Ecol. **5**: 3–9.

Estoup, A., and Angers, B. 1998. Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations *In* Advances in molecular ecology. *Edited by* G.R. Carvalho. NATO Science Series, Series A: Life Sciences, Amsterdam, the Netherlands. Vol. 306. pp. 55–86.

Felsenstein, J. 1993. PHYLIP: phylogenetic inference package, version 3.5c. University of Washington, Seattle.

Ferris, J.D., and Palumbi, S.R. 1996. Molecular zoology: advances, strategies and protocols. Wiley–Liss, New York.

Forbes, S.H., Hogg, J.T., Buchanan, F.C., Crawford, A.M., and Allendorf, F.W. 1995. Microsatellite evolution in congeneric

mammals: domestic and bighorn sheep. Mol. Biol. Evol. **12**: 1106–1113.

Ford, M.J. 2000. Effects of natural selection on patterns of DNA sequence variation at the transferrin, somatolactin, and *p53* genes within and among chinook salmon (*Oncorhynchus tshawytscha*) populations. Mol. Ecol. **9**: 843–855.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genet. Res. **66**: 95–107.

Goddard, J. 1970. Movements of moose in a heavily hunted are in Ontario. J. Wildl. Manage. **34**: 439–445.

Grignola, F., Mao, I.L., Mejdell, C., Lie, O., and Solbu, H. 1995. Association of alleles for the class I bovine lymphocyte antigens with conformation, semen traits, and growth rate of young bulls. J. Dairy Sci. **78**: 908–913.

Guglich, E.A., Wilson, P.J., and White, B.N. 1993. Application of DNA fingerprinting to enforcement of hunting regulations in Ontario. J. For. Sci. **38**: 48–59.

Gurd, D.B., and Nudds, T.D. 1999. Insular biogeography of mammals in Canadian parks: a re-analysis. J. Biogeogr. **26**: 973–982.

Hansson, B., Bensch, S., Hasselquist, D., Lillandt, B.G., Wennerberg, L., and von Schantz, T. 2000. Increase in genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNA fingerprinting. Mol. Ecol. **9**: 1529–1538.

Harrison, S., and Hastings, A. 1996. Genetic and evolutionary consequences of metapopulation structure. Trends Ecol. Evol. **11**: 180–183.

Hartl, G.B., Lang, G., Klein, F., and Willing, R. 1991. Relationships between allozymes, heterozygosity and morphological characters in red deer (*Cervus elaphus*), and the influence of selective hunting on allele frequency distributions. Heredity, **66**: 343–350.

Hedrick, P.W. 1994. Evolutionary genetics of the major histocompatibility complex. Am. Nat. **143**: 945–964.

Hedrick, P.W. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution, **53**: 313–318.

Hedrick, P.W., and Thomson, G. 1983. Evidence for balancing selection at HLA. Genetics, **104**: 449–456.

Houlden, B.A., England, P.R., Taylor, A.C., Greville, W.D., and Sherwin, W.B. 1996. Low genetic variability of the koala *Phascolarctos cinerus* in south-eastern Australia following a severe population bottleneck. Mol. Ecol. **5**: 269–281.

Hughes, A.L. 1991. MHC polymorphism and the design of captive breeding programs. Conserv. Biol. **5**: 249–251.

Hughes, A.L., and Nei, M. 1988. Pattern of nucleotide substitution at major histocompatibility complex class-I loci reveals overdominant selection. Nature (Lond.), **335**: 167–170.

Hughes, A.L., and Yeager, M. 1998. Natural selection at major histocompatibility complex loci of vertebrates. Annu. Rev. Genet. **32**: 415–435.

Hundertmark, K.J. 1998. Home range, dispersal and migration *In* Ecology and management of the North American moose. *Edited by* A.W. Franzmann and C.C. Schwartz. Smithsonian Institution Press, Washington, D.C. pp. 303–336.

Karhu, A., Hurme, P., Karjalainen, M., Karvonen, P., Kärkkäinen, K., Neale, D., and Savolainen, O. 1996. Do molecular markers reflect patterns of differentiation in adaptive traits of conifers. Theor. Appl. Genet. **93**: 215–221.

Karns, P.D. 1998. Population distribution, density and trends. *In* Ecology and management of the North American moose. *Edited by* A.W. Franzmann and C.C. Schwartz. Smithsonian Institution Press, Washington, D.C. pp. 125–140.

King, T.L., and Burke, T. 1999. Special issue on gene conservation: identification and management of genetic diversity. Mol. Ecol. **8**: S1–S3.

Lacy, R.C. 1997. Importance of genetic variation to the viability of mammalian populations. J. Mammal. **78**: 320–335.

Leberg, P.L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution, **46**: 477–494.

LeResche, R.E. 1974. Moose migrations in North America. Nat. Can. **101**: 393–415.

Lewin, H.A., Schmitt, K., Hubert, R., van Eijk, M.J., and Arnheim, N. 1992. Close linkage between bovine prolactin and BoLA-DRB3 genes: genetic mapping in cattle by single sperm typing. Genomics, **13**: 44–48.

Luikart, G., and Cornuet, J. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conserv. Biol. **12**: 228–237.

Luikart, G., Allendorf, F.W., and Cornuet, J. 1999. Temporal changes in allele frequencies provide useful estimates of population size. Conserv. Biol. **13**: 523–530.

Mech, D. 1966. The wolves of Isle Royale. National Park Service Publication No. 7. National Park Service, Washington, D.C.

Mikko, S., and Andersson, L. 1995. Low major histocompatibility complex class II diversity in European and North American moose. Proc. Natl. Acad. Sci. U.S.A. **92**: 4259–4263.

Mikko, S., Roed, K., Schumtz, S., and Andersson, L. 1999. Monomorphism and polymorphism at the Mhc DRB loci in domestic and wild ruminants. Immunol. Rev. **167**: 169–178.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. **3**: 401–411.

Murray, B., Michaud, R., and White, B.N. 1999. Allelic and haplotype variation of major histocompatibility complex class II *DRB*1 and *DQB* loci in the St. Lawrence beluga (*Delphinapterus leucas*). Mol. Ecol. **8**: 1127–1139.

Murray, B.W., and White, B.N. 1998. Sequence variation at the major histocompatibility complex *DRB* loci in beluga (*Delphinapterus leucas*) and narwhale (*Monodon monoceros*). Immunogenetics, **48**: 242–252.

Murray, B.W., Malik, S., and White, B.N. 1995. Sequence variation at the major histocompatibility complex locus *DQB* in beluga whales (*Delphinapterus leucas*). Mol. Biol. Evol. **12**: 582–593.

Nei, M. 1978. Estimation of the average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**: 583–590.

O'Brien, S.J., and Evermann, J.F. 1988. Interactive influence of infectious disease and genetic diversity in natural populations. Trends Ecol. Evol. **3**: 254–259.

Paetkau, D., and Strobeck, C. 1994. Microsatellite analysis of genetic variation in black bear populations. Mol. Ecol. **3**: 489–495.

Paterson, S. 1998. Evidence for balancing selection at the major histocompatibility complex in a free-living ruminant. J. Hered. **89**: 289–294.

Paterson, S., and Pemberton, J.M. 1997. No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population. Proc. R. Soc. Lond. B Biol. Sci. **264**: 1813–1819.

Pimlott, D.H. 1953. Newfoundland moose. Trans. North Am. Wildl. Conf. **18**: 563–581.

Potts, W.K., and Wakeland, E.K. 1993. Evolution of MHC genetic diversity: a tale of incest, pestilence and mating preference. Trends Genet. **9**: 408–412.

Raymond, M., and Rousset, F. 1995. GENEPOP (ver. 1.2): a population genetics software for exact test and ecumenicism. J. Hered. **86**: 248–249.

Ritchey, R.W. 1974. Moose harvesting programs in Canada. Nat. Can. (Que.), **101**: 631–642.

Roy, M.S., Geffen, E., Smith, D., Ostrander, E.A., and Wayne, R.K. 1994. Pattern of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Mol. Biol. Evol. **11**: 553–570.

Ryman, N., Reuterwall, C., Nygren, K., and Nygren, T. 1980. Genetic variation and differentiation in Scandinavian moose (*Alces alces*): are large mammals monomorphic? Evolution, **34**: 1037–1049.

Ryman, N., Baccus, R., Reuterwall, C., and Smith, M.H. 1981. Effective population size, generation interval and potential loss of genetic variability in game species under different hunting regimes. Oikos, **36**: 257–266.

Saccheri, I., Kuusaaru, M., Kankare, M., Vikman, P., Fortelius, W., and Hanski, I. 1998. Inbreeding and extinction in a butterfly population. Nature (Lond.), **392**: 491–494.

Sanjayan, M.A., Crooks, K., Zegers, G., and Foran, D. 1996. Genetic variation and the immune response in natural populations of pocket gophers. Conserv. Biol. **10**: 1519–1527.

Schenk, A., Obbard, M.E., and Kovacs, K.M. 1998. Genetic relatedness and home-range overlap among female black bears (*Ursus americanus*) in northern Ontario, Canada. Can. J. Zool. **76**: 1511–1519.

Seddon, J.M., and Baverstock, P.R. 1999. Variation on islands: major histocompatibility complex (*Mhc*) polymorphism in populations of the Australian bush rat. Mol. Ecol. **8**: 2071–2079.

Simonsen, B.T., Siegismund, H.R., and Arctander, P. 1998. Population structure of African buffalo inferred from mtDNA sequences and microsatellite loci: high variation but low differentiation. Mol. Ecol. **7**: 225–237.

Slade, R.W. 1992. Limited MHC polymorphism in the southern elephant seal: implications for MHC evolution and marine mammal population biology. Proc. R. Soc. Lond. B Biol. Sci. **249**: 163–171.

Slatkin, M. 1995. A measure of population sub-division based on microsatellite allele frequencies. Genetics, **139**: 457–462.

Spencer, C.C., Neigel, J.E., and Leberg, P.L. 2000. Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. Mol. Ecol. **9**: 1517–1528.

Takezaki, N., and Nei, M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics, **144**: 389–399.

Telfer, E.S. 1984. Circumpolar distribution and habitat requirements of moose (*Alces alces*). *In* Northern ecology and resource management. *Edited by* R. Olson, R. Hastings, and F. Gedes. University of Alberta Press, Edmonton. pp. 145–182.

Timmerman, H.R., and Buss, M.E. 1998. Population and harvest management. *In* Ecology and management of the North American moose. *Edited by* A.W. Franzmann and C.C. Schwartz. Smithsonian Institution Press, Washington, D.C. pp. 559–615.

Weir, B.S., and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Wenink, P.W., Groen, A.F., Roelke-Parker, M.E., and Prins, H.H.T. 1998. African buffalo maintain high genetic diversity in the major histocompatibility complex in spite of historically known population bottlenecks. Mol. Ecol. **7**: 1315–1322.

Westemeier, R.L., Brawn, J.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W., Kershner, E.L., Bouzat, J.L., and Paige, K.N. 1998. Tracking the long-term decline and recovery of an isolated population. Science (Washington, D.C.), **282**: 1695–1698.

Whitlaw, H., and Lankester, M.W. 1994. The co-occurrence of moose, white-tailed deer, and *Parelaphostrongylus tenuis* in Ontario. Can. J. Zool. **72**: 819–825.

Wilson, P.J., Wood, C.M., Maina, J.N., and White, B.N. 2000. Genetic structure of Lake Magadi tilapia populations. J. Fish Biol. **56**: 590–603.

Yuhki, N., and O'Brien, S.J. 1990. DNA variation of the mammalian major histocompatibility complex reflects genomic diversity and population history. Proc. Natl. Acad. Sci. U.S.A. **87**: 836–840.

Appendix A

Table A1. Allele frequencies for five microsatellite loci (Map2C, Cervid14, IGF-1, BM1225, and BM4513) and the *DRB* locus in nine moose populations; for the microsatellite loci, sizes are in base pairs.

Locus	Allele	RMNP	NWON	NIP	IROY	PNP	CEON	NEON	NB	NFLD
MAP2C	113	0.000	0.042	0.083	0.000	0.015	0.000	0.015	0.000	0.000
	111	0.115	0.063	0.000	0.000	0.000	0.000	0.000	0.531	0.625
	109	0.577	0.604	0.514	0.867	0.676	0.545	0.424	0.250	0.000
	107	0.192	0.167	0.306	0.067	0.191	0.121	0.348	0.219	0.375
	105	0.115	0.125	0.097	0.067	0.118	0.333	0.212	0.000	0.000
Cervid 14	221	0.000	0.000	0.038	0.000	0.000	0.016	0.013	0.000	0.000
	219	0.694	0.660	0.700	0.367	0.809	0.891	0.795	0.650	0.694
	217	0.167	0.080	0.000	0.000	0.015	0.031	0.064	0.000	0.167
	215	0.028	0.120	0.200	0.633	0.147	0.047	0.115	0.350	0.028
	213	0.000	0.140	0.063	0.000	0.029	0.016	0.013	0.000	0.000
	207	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111
IGF-1	109	0.786	0.333	0.500	0.500	0.485	0.438	0.500	0.700	0.159
	107	0.214	0.667	0.500	0.500	0.515	0.563	0.500	0.300	0.841
BM1225	250	0.053	0.022	0.100	0.000	0.045	0.172	0.132	0.184	0.000
	248	0.132	0.174	0.000	0.000	0.015	0.063	0.000	0.000	0.000
	246	0.000	0.022	0.050	0.167	0.015	0.016	0.000	0.000	0.000
	238	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	236	0.105	0.413	0.412	0.233	0.348	0.172	0.118	0.237	0.000
	232	0.026	0.022	0.262	0.000	0.000	0.000	0.000	0.000	0.000
	230	0.658	0.348	0.175	0.600	0.576	0.578	0.750	0.579	1.000

Table A1 (*concluded*).

Locus	Allele	RMNP	NWON	NIP	IROY	PNP	CEON	NEON	NB	NFLD
BM4513	142	0.079	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	140	0.079	0.000	0.053	0.000	0.145	0.400	0.092	0.000	0.000
	138	0.079	0.083	0.053	0.000	0.129	0.033	0.197	0.263	0.452
	136	0.421	0.167	0.276	0.433	0.226	0.200	0.066	0.053	0.024
	134	0.289	0.354	0.329	0.367	0.210	0.100	0.197	0.158	0.214
	132	0.053	0.104	0.013	0.033	0.032	0.017	0.066	0.000	0.000
	130	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000
	122	0.000	0.292	0.263	0.167	0.258	0.250	0.382	0.526	0.310
DRB	*DRB*-a	0.656	0.722	0.760	0.529	0.595	0.767	0.683	0.763	1.000
	DRB-b	0.100	0.037	0.000	0.206	0.000	0.000	0.000	0.000	0.000
	DRB-c	0.144	0.241	0.240	0.265	0.283	0.233	0.317	0.184	0.000
	DRB-d	0.100	0.000	0.000	0.000	0.122	0.000	0.000	0.053	0.000

Note: RMNP, Riding Mountain National Park; NWON, northwestern Ontario; NIP, Nipigon; IROY, Isle Royale; PNP, Pukaskwa National Park; CEON, Central Ontario; NEON, northeastern Ontario; NB, New Brunswick; NFLD, Newfoundland.