Molecular Ecology (1999) 8, 1309–1315

Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces*, in Canada

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Abstract

Moose, *Alces alces*, occur naturally throughout most of Canada but successful introductions of known numbers of animals have been made to the islands of Newfoundland and Cape Breton. Five microsatellite loci were used to investigate the population genetic structure and any change in genetic variability due to founder events of moose in Canada. Comparisons of allele frequencies for moose from 11 regions of the country suggested that there are at least seven genetically distinct populations ($P < 0.05$) in North America, namely Alberta, eastern Ontario, New Brunswick, Cape Breton, Labrador, western Newfoundland, and the Avalon Peninsula of Newfoundland. The average population heterozygosity was approximately 33% (range from 22 to 41%). UPGMA analysis of Nei's genetic distances produced phenograms similar to what would be expected when geographical location and population history are considered. The loss of heterozygosity due to a single founder event ($n = 3$; two introductions and a natural colonization) ranged from 14 to 30%, and the cumulative loss of heterozygosity due to two successive founder events (an introduction followed by a natural colonization) was 46%. In these examples loss of genetic variability has not been associated with any known phenotypic deviances, suggesting that populations may be established from a small number of founders. However, the viability of these founded populations over evolutionary timescales cannot be determined and is highly dependent upon chance.

Keywords: *Alces alces*, founder effects, microsatellites, moose, population genetic structure

Received 17 August 1998; revision received 19 January 1999; accepted 12 March 1999

Introduction

Moose, *Alces alces*, the largest member of the family Cervidae, inhabit the boreal forests of North America, Europe and Asia. In North America, moose are classified into four subspecies, each believed to have originated from an isolated glacial refugium during the Wisconsin period (Peterson 1955). The natural ranges of these subspecies are as follows: *A.a. gigas* (Miller) is found primarily in the Yukon and Alaska; *A.a. shirasi* (Nelson) is found only along the southern part of the British Columbia–Alberta border and into Montana, Wyoming and Idaho; *A.a. andersoni* (Peterson) is distributed through the Yukon and Northwest Territories and all Canadian provinces from British Columbia to Ontario; and *A.a. americana* (Clinton) occurs in all Atlantic provinces as well as Québec and the eastern half of Ontario (Banfield 1974). In the past 100 years, moose have been relocated to several areas, including the island of Newfoundland, where moose had not previously existed (Pimlott 1953), and Cape Breton Island where the native population had been extirpated due to overhunting (Corbett 1995).

A total of six moose was introduced to the island of

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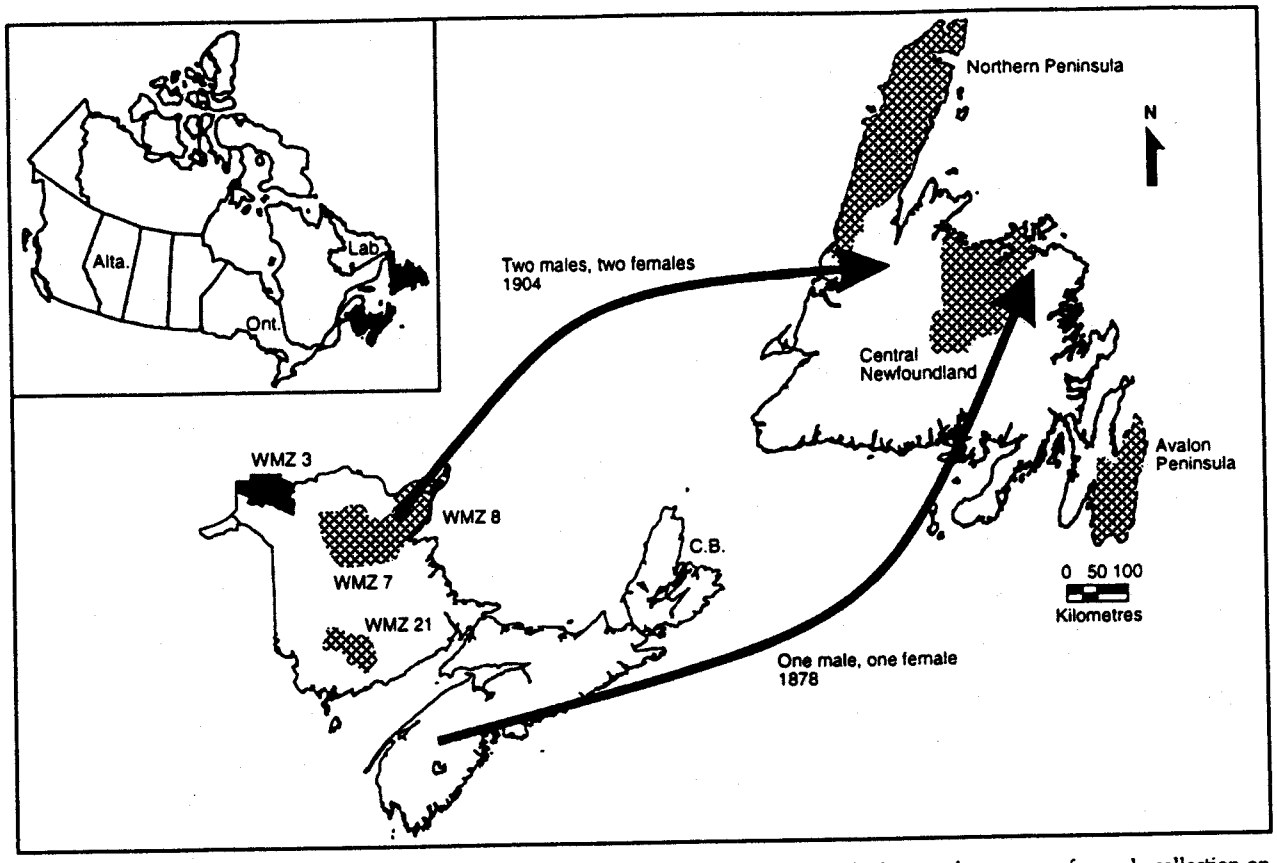

Fig. 1 Origins and release sites of moose introduced to the island of Newfoundland; hatched areas show areas of sample collection on Newfoundland and in New Brunswick.

Newfoundland from Nova Scotia (one male and one female) and New Brunswick (two males and two females) in 1878 and 1904, respectively (Pimlott 1953; Fig. 1). At present there are an estimated 150 000 animals on insular Newfoundland and since their introduction more than 400 000 have been legally harvested (Newfoundland and Labrador Wildlife Division, unpublished data). Eighteen moose were introduced to Cape Breton Island, Nova Scotia, in 1947–48 from Elk Island National Park, Alberta (Pimlott & Carberry 1958; Dodds 1975). By the winter of 1993–94, the moose population in Cape Breton Highlands National Park alone was estimated at 2000 animals (Corbett 1995), suggesting an entire population of 4000–6000 animals. We use these two introductions to assess the effect of founder events on genetic variability in moose.

Until recently, determining population structure has been limited by a lack of genetic markers sufficiently variable to detect differences between regional samples. For example, the levels of heterozygosity in moose detected using allozyme markers are low and range from 1.7 to 7.7% (Ryman *et al.* 1980; Baccus *et al.* 1983; Hundertmark *et al.* 1992). Low levels of polymorphism have also been reported in moose at the normally highly polymorphic major histocompatibility complex loci (Ellegren *et al.* 1996). However, levels of polymorphism at microsatellite loci were expected to be high (Bruford & Wayne 1993; Valdes *et al.* 1993). Therefore, we used microsatellite loci to investigate the population genetic structure of moose in Canada and to document any change in genetic variability due to introductions and founder events. Our results delineate population structure and clearly show the genetic effects of known demographic events in a natural setting.

Materials and methods

Sample collection

Muscle tissue samples of moose were collected from hunters and wildlife officials from 11 regions in Alberta, Ontario, New Brunswick, Nova Scotia, Newfoundland and Labrador ($n = 563$). There were three core collection regions on the island of Newfoundland: Avalon Peninsula ($n = 64$), Central Newfoundland ($n = 77$) and the Northern Peninsula ($n = 44$; Fig. 1). The Labrador samples ($n = 39$)

Table 1 Frequency of alleles at five independent microsatellite loci for five moose populations in Canada. PCR annealing temperature (°C) and number of cycles, respectively, are given in parentheses below each locus name

Locus	Allele (size)	Av.	Cen.-N.P.	N.B.	Lab.	C.B.	Ont.	Alta.
BM-2830[a]	1 (83)*	—	—	—	—	—	—	—
(58, 35)	2 (84)	0.625	0.529	0.542	0.667	0.770	0.400	0.810
	3 (86)	0.375	0.471	0.458	0.333	0.230	0.600	0.170
	4 (88)	—	—	—	—	—	—	0.020
BovirBP[b]	1 (108)	0.204	0.205	0.358	0.055	0.750	0.050	0.690
(46.5, 27)	2 (110)	0.722	0.653	0.609	0.931	0.069	0.950	0.131
	3 (112)	0.074	0.142	0.033	0.014	0.181	—	0.179
BM-1225[a]	1 (227)	1.000	1.000	0.613	0.608	0.586	0.700	0.430
(61.5, 32)	2 (229)	—	—	—	—	—	—	0.010
	3 (235)	—	—	0.231	0.081	0.200	0.150	0.240
	4 (237)	—	—	—	—	0.086	—	0.070
	5 (247)	—	—	—	—	—	—	0.070
	6 (249)	—	—	—	—	—	—	0.060
	7 (251)	—	—	0.156	0.311	0.129	0.150	0.120
CelJP15[c]	1 (78)	0.536	0.377	0.373	0.295	0.311	0.200	0.239
(45.5, 35)	2 (80)	0.348	0.453	0.253	0.154	0.054	0.300	0.261
	3 (82)	0.062	0.090	0.257	0.551	0.622	0.450	0.467
	4 (84)	0.054	0.080	0.116	—	0.013	0.050	0.033
INRA003[d]	1 (178)	—	—	—	—	—	—	0.038
(50, 29)	2 (180)	—	—	0.237	0.263	0.700	0.500	0.238
	3 (182)	—	—	0.004	—	—	—	0.075
	4 (188)	—	—	0.011	0.013	0.057	—	0.188
	5 (190)	0.924	0.734	0.576	0.566	0.129	0.350	0.275
	6 (192)	—	—	0.031	—	0.114	—	0.088
	7 (194)	—	—	0.065	0.053	—	—	0.075
	8 (195)	0.065	0.260	0.076	0.105	—	0.150	0.013
	9 (197)	—	0.006	—	—	—	—	0.013
	10 (199)	0.011	—	—	—	—	—	—

*A second private allele was also recorded on the island of Newfoundland outside the core-regions of sample collection at locus BM-2830.
a, Bishop *et al.* (1994); b, D. MacHugh, Trininty College, Dublin; c, Pemberton & Slate (1994); d, Vaiman *et al.* (1992).
Acronyms: Av. Avalon Peninsula; Cen.-N.P. Central Newfoundland-Northern Peninsula; N.B. New Brunswick; Lab: Labrador; C.B. Cape Breton; Ont: Algonquin Provincial Park, Ontario; Alta. Alberta.

were collected near Happy Valley–Goose Bay. There were four core collection regions in New Brunswick: wildlife management zones (WMZs) 3 (n = 46), 7 (n = 23), 8 (n = 51) and 21 (n = 28; Fig. 1). Thirty-nine samples were collected from the Cape Breton Highlands, Nova Scotia, 10 from Algonquin Provincial Park, Ontario, and 50 from Alberta. Samples from Alberta originated mainly from the southwestern region of the province where the range of two moose subspecies (*A.a. andersoni* and *A.a. shirasi*) overlap (Peterson 1955).

Microsatellite analysis

DNA was extracted from skeletal muscle tissue using a protocol for salmonid fishes (Taggart *et al.* 1992). In the absence of published primers for microsatellite loci in moose the literature was searched for primers in related species (i.e. other cervids and bovids) which were then tested using moose DNA. Of the 19 primers tested, polymerase chain reaction (PCR) conditions were optimized for eight (Table 1) and a clean microsatellite product was produced in each case. Microsatellites were amplified in a GeneAmp PCR system 9600 thermal-cycler (Perkin-Elmer) using 0.2 mL thin-walled microtubes (Gordon Technologies) in a final volume of 12 μL containing 1× Tfl reaction buffer (Promega), 1.5 mM $MgSO_4$ (Promega), 0.20 mM of each of dATP, dCTP, dGTP and dTTP (Pharmacia), 0.50 μM of each primer (Research Genetics or Queen's University CORE DNA Synthesis Lab), 0.50 units of Tfl polymerase (Promega) and 0.07 μM primer 5′-end-labelled with [γ-^{32}P]-ATP (Amersham) using polynucleotide kinase (Pharmacia) and approximately 100 ng of DNA. The general PCR conditions involved an initial denaturation at 94 °C for 5 min followed by the appropriate number of cycles for each primer set (Table 1). Each cycle consisted of 94 °C denaturation for 30 s followed by 30 s of annealing

We would also like to acknowledge the following groups that have contributed samples: Bio-ID Corporation, the Newfoundland and Labrador Wildlife Division, Gros Morne National Park, Cape Breton Highlands National Park, Nova Scotia Department of Natural Resources, New Brunswick Department of Natural Resources and Energy, Algonquin Provincial Park, Ontario, the Alberta Department of Natural Resources and many hunters. The Newfoundland and Labrador Wildlife Division, Nova Scotia Department of Natural Resources and New Brunswick Department of Natural Resources and Energy provided base maps and other support when required.

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This work was carried out by Hugh Broders as a Masters of Science research project in the Biopsychology Programme at Memorial University of Newfoundland in the Davidson Laboratory under the supervision of the other three authors. Shane Mahoney is the Chief of Wildlife Research and Inventory at the Newfoundland and Labrador Wildlife Division. Bill Montevecchi is the Chair of the Biopsychology Programme at Memorial University of Newfoundland and is involved with many conservation programmes dealing mainly with birds. Willie Davidson is Acting Dean of Science and a Professor of Biochemistry at Memorial University of Newfoundland, and studies molecular population genetics and the evolution of genomes.

	BM-2830	BovirBP	CelJP15	BM-1225	INRA003	Total
Av.	0.438	0.426	0.518	0.000	0.152	0.219
Cen.-N.P.	0.562	0.545	0.670	0.000	0.403	0.311
N.B.	0.483	0.453	0.747	0.556	0.595	0.405
C.B.	0.459	0.361	0.541	0.543	0.514	0.345
Alta.	0.280	0.405	0.630	0.680	0.825	0.403
Lab.	0.359	0.139	0.513	0.595	0.474	0.297
Ont.	0.400	0.100	0.500	0.300	0.700	0.286

Table 4 Observed heterozygosities for seven moose populations in Canada

Discussion

This study used microsatellites to address fundamental problems in conservation biology: (i) the population genetic structure of moose in Canada; and (ii) the effects that known demographic events have had on the genetic variation in introduced moose populations. Knowledge of the time since introduction and the initial number of founders, as well as the ability to sample from both source and founded populations, provided a unique opportunity to address these questions in a natural, yet highly controlled, setting. In most genetic studies of natural populations there are two problems that consistently hamper clear conclusions: (i) limited information on population history; and (ii) historical (or source) populations cannot be sampled (e.g. Bonnell & Selander 1974; Ardern & Lambert 1997). This study has quantified changes in genetic variation due to specific introduction events in natural settings. The three founder events (i.e. replicates) all showed a unidirectional reduction in genetic variation.

The concurrence of the cluster analysis (Fig. 2) and the population structure inferred from geographical locations, subspecies designations and population histories, indicates that this method should be useful for determining population structure in cases where general knowledge of the species being studied is lacking. The identification of a distinct moose population on the Avalon Peninsula, Newfoundland, strongly supports this argument. Approximately 50 years after the introduction of moose to insular Newfoundland the first moose were sighted on the Avalon Peninsula. However, because the Avalon Peninsula is connected to the remainder of insular Newfoundland by a narrow isthmus (Fig. 1) comprising maritime barrens (Damman 1983), a very suboptimal habitat for moose, it is likely that there were only a few initial founders of this population and that migration/dispersal between the Avalon Peninsula and the Central Newfoundland–Northern Peninsula population was, and has remained, low. These conditions continue to restrict gene flow and have lead to population differentiation.

A loss of genetic variation occurred in the founded populations with an average reduction of 22% in observed heterozygosity due to a single founder event and a loss of nearly 46% for two successive founder events. However, to date, there are no apparent or reported phenotypic deviances in these founded populations and we know of no negative fitness consequences that have affected or will affect the viability of these founded populations. In fact, populations derived from recent founder events have as much (or more) genetic variation as some long-established populations that have not experienced recent reductions in size (Table 4). How much variation a species (or population) needs in order to have a specific survival probability, and whether the actual amount of variation and/or the presence of certain alleles at particular loci (which may be selected for at some point in the future when and if environmental conditions change) is most important for the viability of a species or population are still important questions in conservation biology (Saccheri *et al.* 1998). Understanding these relationships is vital to predicting species and population viability. Only by examining populations or species where the complete demographic history is known can we begin to compile an empirical basis from which reasonable answers can be gleaned.

Factors other than genetic variation are also important to the persistence of the species that becomes reduced in numbers. These include demographic and environmental stochasticity and natural catastrophes (Sahffer 1981). It is expected, however, that these factors will be most important while the species' population size is reduced. With respect to the moose introductions investigated in the present study the conditions at the introduction sites were ideal (i.e. no predators or competitors and abundant food supplies), which permitted rapid population growth. Unless inbreeding depression is a factor (Saccheri *et al.* 1998), genetic variation will probably not affect the short-term population growth, but may, however, affect evolutionary potential by limiting selection flexibility (Ayala & Kiger 1984).

Acknowledgements

Financial support for this research was provided by an NSERC industrial postgraduate award to H.G.B., as well as the Newfoundland and Labrador Wildlife Division and Bio-ID Corporation.

Table 3 Nei's (1972) genetic distance for pairwise comparisons of moose from seven Canadian populations

	Cen.-N.P.	N.B.	Lab.	C.B.	Ont.	Alta.
Av.	0.013	0.050	0.110	0.298	0.121	0.213
Cen.-N.P.		0.047	0.117	0.284	0.097	0.201
N.B.			0.055	0.145	0.053	0.099
Lab.				0.272	0.046	0.225
C.B.					0.217	0.043
Ont.						0.216

on the Avalon Peninsula. Therefore, the Avalon Peninsula population was considered a naturally founded population whose source is the Central Newfoundland–Northern Peninsula population. No significant heterogeneity was detected for the moose sampled from the four New Brunswick regions [$P = 0.096$, $G = 54(42)$]. Furthermore, within New Brunswick only one of six pairwise comparisons showed significant heterogeneity at $\alpha = 0.05$, and none at $\alpha = 0.01$ (Table 2).

There was no significant heterogeneity detected between the Ontario and New Brunswick WMZ 8 [$P = 0.056$, $G = 23(14)$] and Ontario and Labrador [$P = 0.065$, $G = 20(12)$; Table 2] moose populations. However, these regions were not considered part of a single population because: (i) both P-values were very close to α; (ii) the large geographical distance between the regions makes it unlikely that the populations are homogeneous; and (iii) the low sample size ($n = 10$) from Ontario increases the probability that allele frequencies were similar by chance.

UPGMA cluster analysis of Nei's (1972) genetic distance (Table 3 and Fig. 2) showed that the two Newfoundland populations cluster together and are more similar to the New Brunswick moose population (i.e. their source) than any other group. Similarly, the moose population from Cape Breton is statistically different from the Alberta population, but they cluster together, and are quite distinct from all other eastern Canadian moose populations. Furthermore, the Ontario and Labrador moose populations cluster together and are significantly more similar to the Newfoundland/New Brunswick cluster than the Alberta/Cape Breton cluster. These results support Peterson's (1955) designation of at least two moose subspecies in Canada, one east of central Ontario (excluding Cape Breton) and one (or more) to the west.

Effects of founder events on genetic variability

The five polymorphic microsatellite loci used in this study clearly show a loss of genetic variation due to founder events. The observed heterozygosity was lower in the founded populations than in their source populations (Table 4). Reductions of heterozygosity due to single founder events were 14.4, 23.2 and 29.6% for the introductions of moose to the islands of Cape Breton and Newfoundland and the natural colonization of the Avalon Peninsula, respectively. Furthermore, a 45.9% reduction of heterozygosity in the Avalon Peninsula population relative to the New Brunswick population supports the prediction of Nei *et al.* (1975) that a population that has experienced more than one founder event will lose more variation than a population that has experienced only one founder event. However, all founded populations retained significant levels of genetic variation. In fact, the levels of genetic variation in the Cape Breton and Central Newfoundland–Northern Peninsula founder populations are comparable to, or greater than, other populations. Heterozygosity values in the Labrador and Ontario moose populations are less than that of the Cape Breton and Central Newfoundland–Northern Peninsula populations (Table 4).

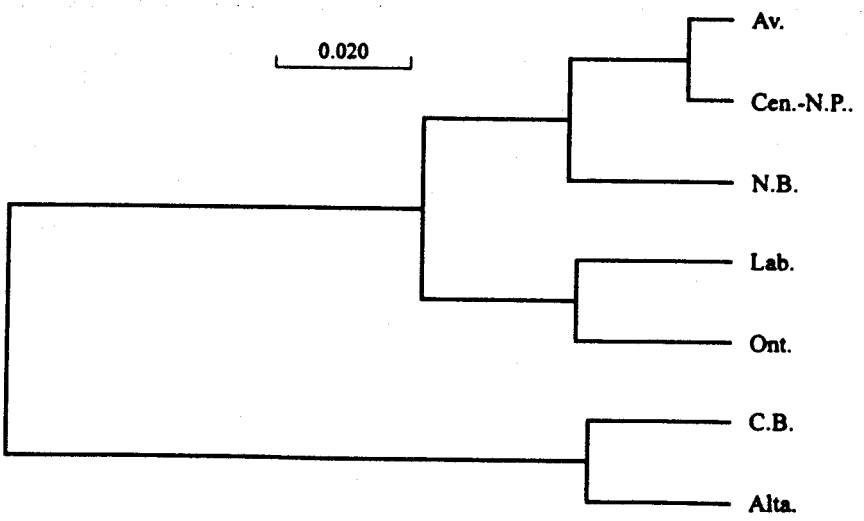

Fig. 2 UPGMA cluster analysis using Nei's (1972) genetic distance for seven moose populations in Canada.

Table 2 G-statistics (d.f.) for pairwise comparisons of moose from different Canadian regions above the diagonal, and statistical significance below the diagonal

	Av. Pen.	Cen. NF.	N. Pen.	N.B. 7	N.B. 8	N.B. 3	N.B. 21	Lab.	C.B.	Ont.	Alta.
Av. Pen.		23 (8)	30 (9)	89 (13)	139 (15)	106 (14)	84 (14)	158 (13)	365 (14)	89 (11)	317 (22)
Cen. NF.	**		7 (8)	81 (12)	141 (14)	92 (13)	86 (13)	166 (12)	346 (13)	79 (10)	307 (20)
N. Pen.	***	ns		98 (13)	169 (15)	120 (14)	105 (14)	176 (13)	367 (14)	83 (11)	326 (21)
N.B. 7	***	***	***		23 (14)	10 (13)	13 (13)	51 (13)	144 (14)	23 (12)	132 (21)
N.B. 8	***	***	***	ns		28 (14)	19 (14)	69 (14)	185 (15)	23 (14)	173 (21)
N.B. 3	***	***	***	ns	*		11 (13)	84 (13)	173 (14)	35 (13)	153 (21)
N.B. 21	***	***	***	ns	ns	ns		56 (14)	133 (14)	35 (13)	102 (21)
Lab.	***	***	***	***	***	***	***		219 (14)	20 (12)	222 (21)
C.B.	***	***	***	***	***	***	***	***		104 (13)	86 (21)
Ont.	***	***	***	*	ns	***	***	ns	***		104 (21)
Alta.	***	***	***	***	***	***	***	***	***	***	

ns: $P > 0.05$.
*, $0.05 > P > 0.01$.
**, $0.01 > P < 0.01$.
***, $P < 0.001$.

at the appropriate temperature for each primer set (Table 1) and final extension at 72 °C for 30 s. When all cycles were complete, samples were stored at 4 °C.

PCR products were separated on 6% polyacrylamide gels containing 19:1 acrylamide:bis-acrylamide, 7 M urea and 1× TBE buffer. Gels were run for 1.5–4 h (depending on product size) at a constant power of 40 W, then dried without fixing and autoradiographed either overnight at –70 °C using intensifying screens or at room temperature for ≈ 48 h. Each gel contained two reference samples to ensure consistent scoring of alleles on all gels. Alleles were numbered arbitrarily with no. 1 being the smallest allele. Before any of the data were analysed they were first checked using the program GENEPOP (version 3.1a; Raymond & Rousset 1995) to ensure conformation to Hardy–Weinberg expectations and that none of the loci were linked.

Population structure and the effects of founder events

Comparisons of allele frequencies between regions were performed using G-tests (Sokal & Rohlf 1995). If no significant heterogeneity was detected ($P > 0.05$) the regions were considered part of one homogeneous population and were combined for further analyses. The NTSYS program (Rohlf 1992) was used to calculate Nei's genetic distance (Nei 1972) between all population pairs. These genetic distance matrices were exported to the phylogenetic analysis using a parsimony program (Swofford & Begle 1993) to perform an unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis.

Observed heterozygosity (Hedrick *et al.* 1986) was used to quantify and compare the genetic variation in source and founder populations.

Results

Analysis of microsatellite data

The following primers were tested but clean amplification products were not obtained: Cervid 2, Cervid 3, Cervid 4, Cervid 14 (Dewoody *et al.* 1995), ORF 381, OarFCB 193 (Abernethy 1994), CelJP18, CelJP27 (Pemberton & Slate 1994), BM-4513, INRA023 (Bishop *et al.* 1994) and MAF70 (Buchanan & Crawford 1992). Genotypes were collected at eight microsatellite loci. There was no evidence of linkage between loci and therefore independent assortment was assumed. The only significant deviation from Hardy–Weinberg equilibrium occurred at locus BM-143. This locus showed an excess of homozygotes in six of nine regions, suggesting the presence of a null allele. Further evidence to support this came from the apparent inability to obtain amplification products from some samples that worked well with other primer pairs. Results from BM-143 as well as those from two monomorphic loci (Cervid 1 and CelJP38) are not considered further. Results from five polymorphic loci were used in this study (Table 1).

Population genetic structure of moose in Canada

Comparisons of allele frequencies from all 11 regions showed significant heterogeneity ($P < 0.001$) except in four cases. Moose from the three insular Newfoundland regions did not comprise a single homogeneous population [$P = 0.002$, $G = 40(18)$]. However, no significant heterogeneity was detected for allele frequencies of moose from Central Newfoundland and the Northern Peninsula [$P = 0.556$, $G = 7(8)$; Table 2], suggesting that they comprise a single population that is distinct from the population