POPULATION GENETIC STRUCTURE AND THE EFFECT OF FOUNDER EVENTS ON THE GENETIC VARIABILITY OF MOOSE (Alces alces) IN CANADA

HUGH G. BRODERS

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

POPULATION GENETIC STRUCTURE AND THE EFFECT OF FOUNDER EVENTS ON THE GENETIC VARIABILITY OF MOOSE (*Alces alces*) IN CANADA

A thesis submitted to the School of Graduate Studies

in partial fulfillment of the requirements for the degree of

Master of Science

Biopsychology Programme

Memorial University of Newfoundland

St. John's, Newfoundland

1998

ABSTRACT

The evolutionary potential of any species is dependent upon its genetic variability. An understanding of the factors that influence loss or gain of genetic variability within a species can help us understand and prevent extinction. One such event that is expected to reduce genetic variation is the founding of a new population from a small number of individuals. Three such founder events have occurred through the founding of moose populations on the island of Cape Breton from Alberta, on the island of Newfoundland from New Brunswick and on the Avalon Peninsula from the island of Newfoundland. In order to determine the effects of these introductions on genetic variation in moose I have examined DNA microsatellite variation at five polymorphic loci in moose samples from throughout Canada, including all source and founder populations.

Canadian moose can be assigned to seven distinct populations: Avalon Peninsula-Newfoundland, Central Newfoundland-Northern Peninsula, Labrador, New Brunswick, Cape Breton, Ontario and Alberta. Cluster analysis shows two distinct groups of populations, one including Alberta and Cape Breton and the second including Avalon Peninsula-Newfoundland, Central Newfoundland-Northern Peninsula, Labrador, New Brunswick and Ontario. These two groups correspond to two recognized subspecies.

Four measures of genetic variability, observed and expected heterozygosity, the probability of identity and the mean number of alleles, show that genetic variability is

reduced in all founder populations relative to their source populations. However, genetic variability in the founder populations is in some cases comparable to that in populations that have not undergone founder events. Risks associated with any particular level of variability must be assessed relative to specific populations.

ACKNOWLEDGEMENTS

First and foremost I must thank my three supervisors, Willie Davidson, Shane Mahoney and Bill Montevecchi for attending all the meetings and providing constructive criticisms and encouragement throughout each phase of the project.

Secondly, I have to thank all the organizations who supported this research. Funding was provided by a Natural Sciences and Engineering Research Council scholarship to the author, the Newfoundland and Labrador Wildlife Division, and Bio-ID corporation. Samples were provided by 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. Also, 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.

This work would not have been possible without the support of the people who worked side by side with me in the lab, especially S. Bartlett, T. Benfey, C. McGowan, C. Walsh, M. Woods and T. Young. You were a continuous source of solutions to my many questions, especially in the early stages of this research when I was learning the techniques. I would also like to thank B. McLaren for providing assistance and access to

computers whenever they were required. M. Strapp for providing access to moose hunter returns. P. Wilson for very helpful discussion. P. Earle and Geographic Data Services for computer assistance and D. Butler for helping out whenever necessary.

Finally, I must thank my parents and Sarah who have been a continuous source of support and encouragement from the beginning.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

1.1 INTRODUCTION

1.1.1 BACKGROUND

The likelihood of persistence of a species in the face of environmental change or stress will depend on what the species has adapted to over evolutionary time (Noss 1992), as well as the demographic and genetic characteristics of the species at the time. For instance, the likelihood of persistence of a species at low numbers is reduced due to chance and inherent characteristics of the population, such as genetic variation. Small populations are expected to have reduced genetic variation relative to their pre-bottleneck (or source) population, the amount remaining will depend on the level and duration of population reduction (Nei *et al.*1975). Inbreeding (and therefore inbreeding depression) is also expected to be of increased importance in small populations (Allendorf and Leary 1986). These phenomena are important because the rate of extinction for birds and mammals is approximately 100 times the background level. At this pace, the natural rate of speciation cannot curb the net loss of species from the planet, and it seems, unless something is done, the species diversity and health of our planet will decline rapidly (Ehrlich 1986).

This thesis will determine the population genetic structure of moose (*Alces alces*) sampled from 11 Canadian regions and examine the effects of founder events on genetic variation. These founder events occurred when moose were introduced to the islands of

Newfoundland and Cape Breton from New Brunswick and Alberta, respectively. These introductions involved small numbers of animals and therefore offer ideal situations to test hypotheses on genetic variability and founder events.

1.1.2 Causes of and problems associated with population reductions

Phenomena other than founder events lead to small population sizes, and factors other than genetic variation may become important when a population is reduced. The acquisition of natural resources for an increasing human population has undoubtedly been the most important influence affecting natural populations (Schonewald-Cox 1983). Human exploitation has reduced the population size of many species such as the northern elephant seal, *Mirounga angustirostris* (Bonnell and Selander 1974); greater one-horned rhinoceros, *Rhinoceros unicornis* (Dinerstein and McCracken 1990) and has driven many others, such as the passenger pigeon, *Ectopistes migratorius* (Krebs 1988) and the great auk, *Pinguinus impennis* (Montevecchi and Kirk 1997), to extinction. Other phenomena that may reduce population size include disease and natural catastrophes (*e.g.* floods, earthquakes, *etc.*).

In certain cases, it is expected that individuals that have persisted through population reductions, or bottlenecks, had fitness advantages over individuals that did not, hence they were selected for. For example, in species that have been subjected to disease, only those individuals that were resistant to the disease would have survived. However, in the

event of natural catastrophes and human introductions (*i.e.* an artificial founder event) no selection is involved. Individuals persisting are expected to be a random sample of the original, or source, population.

One way to assess the risks associated with current population size is to determine the Minimum Viable Population (MVP) for the species. The MVP for "any given species in a given habitat is the smallest isolated population having a 99% chance of remaining extant for 1000 years, despite the foreseeable effects of demographic, environmental and genetic stochasticity, and natural catastrophes" (Shaffer 1981). It is important to note that the criteria for determining MVP size are arbitrary and specific for a species depending on which of the following goals are desired: (*i*) ensuring the short-term survival of the species, or extinction avoidance; (*ii*) ensuring that the population has the potential to adapt and persist in the face of a changing environment; (*iii*) the maintenance of the capability for species to speciate, which may offset declines in species diversity due to extinction (Soulé 1981). To determine the MVP for any species it is important to understand factors that affect fitness and also to know how much fitness a species can lose before it becomes imperiled (Lacy 1992).

The MVP size for a species is normally based on the effective population size rather than the census (or actual) population size. The effective population size is generally smaller than the census population and is dependent upon the number of reproductively active

females, the number of males that have a chance to mate with these females, the variance in fecundity of these females and the expected fluctuations in population size (Franklin 1980). It is "an "ideal" number of individuals whose decrease in genetic variation due to genetic drift equals that of the actual population being studied" (Shafer 1990).

Many species have successfully rebounded from small population sizes (Bonnell and Selander 1974; Montevecchi and Tuck 1987; Dinerstein and McCracken 1990; Ardern and Lambert 1997; see also Caughley and Gunn 1996). Nevertheless, chance phenomena increase the likelihood that small populations will go extinct, whereas these same phenomena may only cause a slight perturbation in numbers in large populations (Pimm *et al.* 1988). Three such chance phenomena are demographic stochasticity, environmental stochasticity (*i.e.* the effects of predators, disease, parasites, *etc.*) and natural catastrophes (Shaffer 1981; Gilpin 1987).

Another important source of uncertainty is genetic stochasticity, which encompasses all genetic changes that occur in a species due to genetic drift and inbreeding. Genetic drift is the unpredictable change in frequency of alleles from one generation to another due to sampling error (*i.e.* chance; Ayala and Kiger 1984). In large populations these changes are so small that they are negligible but in small populations even slight changes may cause large shifts in allele frequencies, especially if the population remains at low numbers for many generations. Genetic drift may also decrease the amount of variation

by eliminating alleles (generally rare ones) from the population (Ayala and Kiger 1984). In large populations, mutation will balance the rate of loss of alleles due to drift and an equilibrium will occur (Quammen 1996). However, in small populations the same mutation rate will produce fewer mutations which will not balance the loss of genetic variation due to drift, resulting in a net loss of genetic variation. This phenomenon may not be as important as the first three when the species is experiencing the reduction in numbers (Lande 1988) but if the species does rebound its effects are crucial for the evolutionary potential of the species.

Inbreeding, the mating of related individuals, is also important in small populations. The occurrence of inbreeding increases as population size decreases and may lead to inbreeding depression, characterized by a reduction in the viability, birth weight and fertility of offspring, and therefore the species' probability of survival (Templeton and Read 1983). There are two hypotheses regarding the causes of inbreeding depression. The first is that, as homozygosity increases due to inbreeding, so does the chance that recessive deleterious alleles will be expressed in the homozygous state. In an outbreeding population, the expression of the recessive deleterious allele will normally be prevented because it will be masked by a dominant and non-deleterious allele. A second hypothesis is that, at certain loci, heterozygotes are superior to homozygotes and that the additive benefits of each heterozygous locus will lead to increased fitness, termed a "heterozygote advantage" (see next section for examples: Lacy 1992; Avise 1994). In a study on the

effects of inbreeding on juvenile mortality in captive ungulates. Ralls *et al.* (1979) found that juvenile mortality was higher in inbred young than in non-inbred young in 15 of 16 species examined. Even though evidence for inbreeding depression in captive animals has been documented numerous times, it has yet to be shown as a factor causing the decline of a species in the wild (Caughley and Gunn 1996).

Invasion (or introduction) of foreign species also jeopardizes the persistence of the existing species in a community. Pimm (1987) reviews the literature on species introductions and describes three situations when communities will likely be adversely affected. The first occurs when species are introduced where there are no predators, the second is when the introduced species is polyphagous and the third is when the community is relatively simple. At least some of these conditions apply to the introduction of moose to the islands of Newfoundland and Cape Breton, depending on the definitions of 'polyphagus' and 'simple communities'.

1.1.3 Consequences of low genetic variability

The more genetic variability a species has the greater its potential to adapt to a changing environment. At any point in time, a particular allele (or variant) may be more suited to a particular environment than any other, but when or if environmental conditions change, the relative fitness of this allele may also change, and so will its frequency. If a species has only one allele (*i.e.* is monomorphic) at a locus it will be unable to adapt to new environmental conditions and its probability of survival will be lower than if there was

variation at this locus (Ayala and Kiger 1984). Since reductions in a species' population size are expected to cause a reduction in genetic variability, the evolutionary potential of the species should also be reduced.

Genetic variation has facilitated the survival of the English peppered moth, *Biston betularia*, through the industrial revolution in Britian. This moth has two morphs, one dark and one light. Prior to the industrial revolution the dark form was rare because it did not camouflage well and therefore had a higher incidence of predation. However, during the industrial revolution the colour of the landscape changed as lichens on rocks and trees were killed by pollution. This altered landscape now provided camouflage for the dark moths and the lighter morphs were more heavily preyed on. By the turn of this century, the frequency of the black and light coloured moths had completely reversed, due to a reversal of selection pressure (Campbell 1990). The important point is if the second morph had not been present the moth population may not have survived.

Genetic variation may influence physical attributes of an individual as well. Levels of heterozygosity have been shown to be positively correlated with weight gain (Singh and Zouros 1978; Koehn and Gaffney 1984; Garton *et al*. 1984; Manwell and Baker 1982; Shick *et al*. 1979) and four measures of fitness (survival, growth, fecundity and developmental stability; Quattro and Vrijenhoek 1989). It has been shown for the sulfur butterfly (*Colias philodice*) that the genotype present at the phosphoglucose isomerase

(PGI) locus affects the flight capability and survivorship of the individuals (Watt 1983; Watt *et al.* 1983). Individuals heterozygous at this locus had greater endurance and were capable of flying further and through a broader range of environmental conditions than any homozygote. This adaptation gave them access to more food and allowed them to escape predators and bad weather more effectively, thereby increasing their chances of survival.

Genetic variability may also effect a species' susceptibility to disease. Bacteria and viruses have a high rate of evolution and are therefore able to react to the defense systems of their host species rapidly (O'Brien and Evermann 1988). The more genetic variability present at loci coding for defense mechanisms, the greater the probability that the host species will persist. Low levels of genetic variation have been correlated with the susceptibility of cheetahs (*Acinonyx jubatus*) to the feline infectious peritonitis (FIP) coronavirus (O' Brien *et al.* 1985). It should be noted, however, that Caro and Laurenson (1994) believe that the high susceptibility is a result of husbandry practices in captivity rather than genetic characteristics.

1.1.4 Examples of species that have experienced low numbers and/or inbreeding

Some species have low levels of genetic variation, due to bottlenecks in their recent history, and appear to be thriving. For example, the northern elephant seal, which once numbered in the thousands, was nearly decimated due to hunting pressure. It is suspected

that in the early 1890s as few as 20 individuals remained, all in one isolated breeding location. With protection, these seals began a slow comeback (Bonnell and Selander 1974). By 1989 their numbers had increased to an estimated 125,000 individuals (Caughley and Gunn 1996). A study of 21 proteins encoded by 24 loci revealed that all loci were monomorphic at five breeding locations (Bonnell and Selander 1974). For comparison, the southern elephant seal (*Mirounga leonina*) had five polymorphic loci out of 18 examined (McDermid *et al.* 1972). Three of these five polymorphic loci were examined in the northern elephant seal and all were monomorphic.

Another species that has rebounded following an extreme population bottleneck is the black robin, *Petroica traversi*, of the Chatham Islands, near New Zealand. The present population of approximately 200 individuals is derived from a single breeding pair in the early 1980s. A genetic analysis of variation using minisatellites confirmed that the species manifests a severely impoverished genetic condition relative to related species. However, the species remains reproductively viable and there are no known threats to its survival at present (Ardern and Lambert 1997).

The greater one-horned rhinoceros population was reduced to an effective population size of 21-28 individuals in 1962 but rebounded to 400 individuals in 1988. Dinerstein and McCracken (1990) found nine polymorphic loci, out of 29 examined. The observed heterozygosity was among the highest reported for 140 mammal species examined using

similar techniques. The authors suggest that these high levels of variation exist because, historically, the population consisted of very large numbers (approximately 475,000) persisting over long periods of time (at least 100,000 rhinoceros' generations) which facilitated the accumulation of a large amount of genetic variation. Since recovery has been rapid, and only three generations have passed since the bottleneck, the decay of heterozygosity (as a measure of genetic variation) has not been severe.

As these examples illustrate, there is no clear pattern to the effects of population bottlenecks on genetic variation. Such effects are influenced by two principal factors: (*i*) the dynamics of the bottleneck or founder event (*e.g.* how low the numbers fell and for how many generations did these low numbers persist: Nei *et al.* 1975) and (*ii*) the mating strategy of the species. In polygynous species, like the northern elephant seal (Davies 1991), one dominant male may sire all offspring when population is severely reduced. However, in monogamous species many males will pass on their genes making it more likely that a particular allele will persist into the next generation. This is a major factor determining the effective population size for a species.

1.1.5 Importance of understanding population structure

Up to this point, discussion has dealt mainly with the viability of species. However, conservationists are now realizing that the viability of one or more distinct sub-populations may be crucial to the evolutionary potential of a species (Quammen 1996). If

a sub-population becomes extinct, a certain portion of the species' variability is lost, reducing the evolutionary potential of the species. For this reason, it is important to know the structure of, and interactions between, populations of a species in order to effectively manage wild populations to conserve biological diversity. For example, Atlantic cod (*Gadus morhua*) stocks in the Northwest Atlantic have been exploited to the point where, in 1992, it was necessary to impose a fishing moratorium. To implement effective management plans to ensure survival of the fish stocks it is necessary to understand the population structure. Using mtDNA, Carr *et al.* (1995) found that there were no genetically distinct populations in the Northwest Atlantic, meaning that all cod in the region could be managed as one population. However, Ruzzante *et al.* (1996), used more variable microsatellite loci and detected evidence of weak population structure. The presence of more than one population of cod in the Northwest Atlantic means that if genetic diversity, and therefore evolutionary potential, of the species is to be maintained, each population must be managed independently. This concept is becoming increasingly important as natural areas become fragmented and populations become more isolated from one another.

1.1.6 Moose biology and mating strategy

Moose, the largest member of the family Cervidae, inhabit the boreal coniferous forests of North America, Europe and Asia. They exhibit non-territorial behavior and are non-social, with some exceptions. One exception is that calves stay with their mothers for the

first year of life. Also, during some winters, 'yarding' behavior has been documented, when several animals congregate to obtain increased protection from predators and better foraging conditions (Peterson 1955). Moose also exhibit social behavior during the mating season, which peaks between September 20 and October 10 (Peterson 1955). During this period, bulls form temporary mating relationships with one cow at a time, but may mate with several females over the course of the breeding season (Peterson 1974).

1.1.7 Genetic variation in moose

There have been several studies of genetic variation in moose using different techniques. In an extensive allozyme study, Ryman *et al.* (1980) found comparable levels of genetic variation in moose relative to other mammalian species. Hundertmark *et al.* (1992) also found high levels of variation when examining 13 enzyme systems in a population of moose from the Kenai Peninsula, Alaska. These results contrast with previous studies suggesting that moose have extremely low levels of genetic variation (Ryman *et al.* 1977; Wilhelmson *et* al. 1978) suggesting that these studies have surveyed too few individuals or loci (Ryman *et al.* 1980).

Two unpublished studies have examined genetic variation of Newfoundland moose. In a study in the early 1980s, Payne and Fong examined allozyme variation at eight loci in two groups of moose from insular Newfoundland. They surveyed 12 individuals from the Grey River area and 15 from an introduced population on Brunette Island. Seven of

the eight loci were monomorphic. The eighth locus, malate dehydrogenase (MDH), had two alleles in both sampling locations. The seven momomorphic loci identified were not suprising, as all were monomorphic in Scandinavian moose (Ryman *et al.* 1977) and three of the seven that were examined by Hundertmark *et al.* (1992) were also monomorphic. In the second study of Newfoundland moose (P. Wilson, McMaster University, pers. comm.) levels of genetic diversity were examined in 29 individuals using minisatellites and a major histocompatability complex (MHC) locus. The band-sharing coefficient (a measure of genetic similarity) for minisatellite loci was high (approximately 70%) for moose from insular Newfoundland relative to moose in other regions of Canada (approximately 50%), suggesting low levels of variation in Newfoundland. Wilson also found low levels of variation at an MHC locus. In Canada, there were three alleles segregating at the exon coding for the peptide binding region. In Newfoundland only one of the three alleles was found. It is important to note that only one MHC locus was examined in this study and so this may not accurately represent the total variation at MHC loci.

1.1.8 Choosing a genetic marker

In order to study the genetic variation of a species, an appropriate marker is required. In this study the marker: (*i*) had to possess high levels of variation, so that any decrease in variability in the founded populations could be detected; (*ii*) could not be associated with any coding region, so that no selection pressure at the locus could be assumed (this will

allow any differences in genetic variation to be attributed to the founder effect and/or inbreeding); and (*iii*) had to be simple, such that a survey of a large number of individuals was possible.

In the late 1980s, a type of genetic marker was discovered with these features and it was used to address the questions posed in this study. This class of markers, known as microsatellites, consists of short sequences of tandemly repeated nucleotides (usually 2-5 base pairs long) that are flanked by unique sequences. Microsatellites are evenly distributed (every 100,000 base pairs in the human genome) on all chromosomes (Valdes *et al.* 1993). Other features of microsatellites that make them ideal for this study are: (*i*) they are codominant markers inherited in a Mendelian fashion (Ruzzante *et al.* 1996), (*ii*) they require only small amounts of DNA (Queller *et al.* 1993), (*iii*) they are short enough to allow amplification by the polymerase chain reaction (PCR), therefore Southern blot hybridization is not required (O'Reilly and Wright 1995) and (*iv*) they are relatively easy to use, interpret and analyze statistically.

The only disadvantage of microsatellites is that they must first be isolated and characterized so that sequences of regions flanking the microsatellite can be determined for the design of primers (Queller *et al.* 1993). Therefore, researchers are forced to determine sequences flanking microsatellites manually. Fortunately, once PCR primers have been designed in one species they may also be used in closely related species

(Moore *et al.* 1991). At the start of this study, there were no microsatellite primers published in the literature specifically for moose. There were, however, many primers designed for related cervid and bovid species. The following work has taken advantage of these published primers for related species and adapted some of them for moose.

1.2 RECENT HISTORY OF THE STUDY POPULATIONS

1.2.1 History of North American moose during and following the Wisconsin glaciation

During the Wisconsin age, glaciers reached a maximum in North America and moose became restricted to four isolated regions. As the glaciers retreated (approximately 10,000 years ago) moose expanded their range northward. Peterson (1955) recognizes four sub-species of moose in North America today that are believed to have originated from these isolated areas of glacial refugia. The natural ranges of these sub-species are as follows: *Alces alces gigas* (Miller) are found primarily in the Yukon and Alaska; *Alces alces shirasi* (Nelson) are found only along the southern part of the British Columbia - Alberta border and into the northwestern states of Montana, Wyoming and Idaho; *Alces alces andersoni* (Peterson) has the largest distribution that includes both territories and all provinces from British Columbia to Ontario. The fourth sub-species, *Alces alces americana* (Clinton), occur in all Atlantic provinces as well as Quebec and the eastern half of Ontario (Banfield 1974). In the past 100 years moose have been relocated to many areas where they did not occur naturally, substantially altering the distribution of the original sub-species.

1.2.2 Moose on insular Newfoundland

In 1878, two moose from Nova Scotia were released near Gander Bay, Newfoundland (Pimlott 1953) and, in 1904 an additional four animals were introduced to the Howley region from northeastern New Brunswick (Gale 1988; Figure 1). The success of the first introduction is uncertain. In 1912, a young bull moose was shot on the Gander River (Howley 1913), and over the next few years, there were a number of reports of moose in the area (Pimlott 1953). It is likely these individuals were descendants of the first pair introduced to that region. With the poor communications of the time and extremely low numbers of moose, it is possible that other sightings were not reported. The alternative explanation is that the first introduction failed and animals dispersed from the Howley region following the 1904 introduction, and that their descendants reached Gander Bay just eight years after their release. However, no sightings of moose were reported between Howley and Gander Bay prior to 1919, suggesting this explanation is unlikely (Pimlott 1953).

While studying a founding moose population in the Adirondack mountains Garner and Porter (1990) found that bulls moved much further in search of mates than cows. They also reported that individuals showed less fidelity to their home ranges in these circumstances, probably as a result of low intraspecific competition. These results could explain the presence of males but cannot explain how several animals could have moved from Howley to Gander Bay in only 10 years. Furthermore, Gasaway *et al.* (1980)

suggested that dispersal of moose does not occur in areas with low population densities. In addition, Howard (1960) suggested that dispersing individuals will only move far enough to reduce the stress of being associated with kin. These results provide additional support for the possible success of the first introduction.

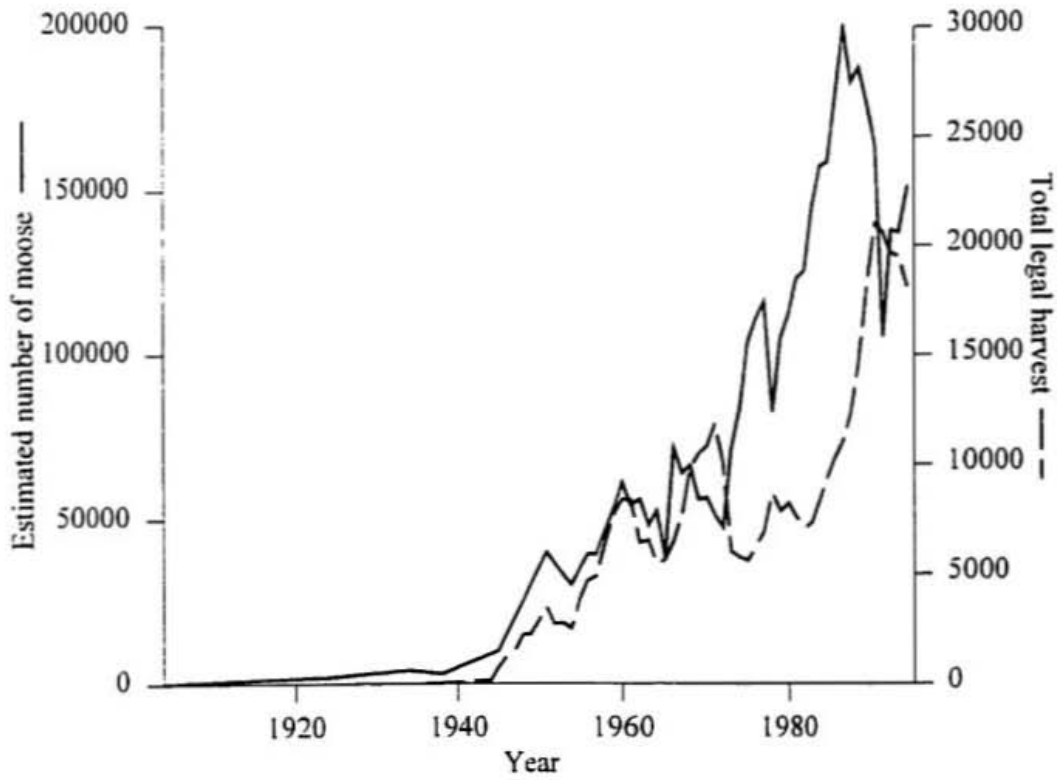
While we cannot determine with certainty whether one or both introductions succeeded, we do know that todays Newfoundland moose population was founded from a maximum of six adult animals (three males and three females). Furthermore, the moose introduced from New Brunswick were possibly related since they were captured from one social group (Gale 1988). If indeed they were related these animals would be less likely to have as much genetic variation as four unrelated individuals drawn at random from the population. Similar information on the animals introduced from Nova Scotia are not known.

Since introduction, the moose population on insular Newfoundland has grown dramatically. The first legal hunt occurred in 1936, when eight animals were taken from 33 issued licences. Since that time, more than 400,000 animals have been legally harvested and current population estimates are near 150,000 animals (Figure 2; Mercer G. Newfoundland and Labrador Wildlife Division unpublished report).

Figure 1: Moose introduction sites on the island of Newfoundland.

N
Two males, two females
1904
One male, one female
1878
0 50 100
Kilometres

Figure 2: Population size and legal harvest of moose on the island of Newfoundland since introduction (Newfoundland and Labrador Wildlife Division unpublished data).


200000
150000
100000
50000
0
Estimated number of moose
30000
25000
20000
15000
10000
5000
0
Total legal harvest
1920
1940
1960
1980
Year

1.2.3 Moose on insular Cape Breton

Moose were originally indigenous to the island of Cape Breton (Pimlott and Carberry 1958) but unrestricted harvests throughout much of the 1800s reduced their numbers. By the late 1800s regulations were in place to limit harvest, but by the turn of this century moose numbers had declined significantly (Pulsifer and Nette 1995). By 1924, the population had succumbed to hunting pressure and habitat destruction and was extirpated (Cameron 1958). In 1928 and 1929 an effort was made to re-establish moose in the Cape Breton Highlands when seven moose from mainland Nova Scotia were released in Inverness County (Peterson 1955). Dodds (1975) reported that the results of this introduction are scant and other authors do not even mention it. It seems from the lack of evidence available on this introduction that it was not successful.

In the late 1940s moose from Elk Island National Park, Alberta, were successfully introduced to Cape Breton island. Eight and 10 moose in 1947 and 1948, respectively, comprised of 11 females and seven males survived the road trip from Alberta and were released at Roper's Brook in Cape Breton Highlands National Park (Pimlott and Carberry 1958; Dodds 1975). By the early 1950s, moose were sighted in many areas of the park but did not significantly increase in numbers until the 1970s. By the winter of 1993/1994, the moose population in the park was estimated at 2000 animals, and it is anticipated to remain high in the foreseeable future (Corbett 1995).

1.3 HYPOTHESES

The main objective of this study is to determine the effects of founder events on genetic variability in moose. However, before addressing this question the population genetic structure of moose in Canada must be determined. The following hypotheses were developed at the start of the project and will be tested through the remainder of the thesis:

Hypothesis 1:

Part A

Hypothesis 1A(1)

H_0 : Moose from different Canadian regions have similar relative frequencies of alleles and therefore comprise a single homogeneous population.

H_1 : Moose from different Canadian regions have different relative frequencies of alleles and therefore consist of heterogeneous populations.

Part B

If the above 1A(1) H_0 is rejected the following hypotheses will be addressed:

Hypothesis 1B(1)

H_0 : Moose sampled from different regions on the island of Newfoundland (Avalon Peninsula, Central Newfoundland and the Northern Peninsula) have similar relative frequencies of alleles and therefore comprise a single homogeneous population.

H_1 : Moose sampled from different regions on the island of Newfoundland (Avalon Peninsula, Central Newfoundland and the Northern Peninsula) have different relative frequencies of alleles and therefore consist of heterogeneous populations.

Hypothesis 1B(2)

H_0 : Moose sampled from different regions within New Brunswick (Wildlife Management Zones 3, 7, 8 and 21) have similar relative frequencies of alleles and therefore comprise a single homogeneous population.

H_A: Moose sampled from different regions within New Brunswick (Wildlife Management zones 3, 7, 8 and 21) have different relative frequencies of alleles and therefore consist of heterogeneous populations.

Hypothesis 2:

H_0 The genetic variation (G.V.) of each recently founded moose population is comparable to the genetic variation of its source moose population.

H_0: G.V. (founded) = G.V. (source)

H_A The genetic variation of each recently founded moose population is less than the genetic variation of its source moose population.

H_A: G.V. (founded) < G.V. (source)

CHAPTER 2

2.1 MATERIALS AND METHODS

2.1.1 Sample type and quantity

Only muscle tissue samples were used in this study. Many samples were provided by hunters. Also, wildlife officials provided samples from road-killed animals and from any tissue remaining on jaw bones submitted by hunters to the Newfoundland and Labrador Wildlife Division for aging purposes. Most muscle tissue samples from jaw bones were highly decayed but it was still possible to isolate sufficient intact DNA for analysis. Wildlife officials from New Brunswick provided samples from moose processed at their hunter check stations.

2.1.2 Sample collection locations

Moose tissue samples were collected from 11 regions in five Canadian provinces (n=563: Figure 3). There were three core regions of sample collection on the island of Newfoundland, as well as 92 samples from outside these core regions. The three core regions were defined using the moose management zone (MMZ) designations of the Newfoundland and Labrador Wildlife Division. The Avalon Peninsula (n=64) core region consisted of MMZs 33, 35 and 36. The Central Newfoundland (n=77) core region consisted of MMZs 15, 15A, 16, 21, 22, 22A and 24 and the Northern Peninsula (n=44) core region consisted of Gros Morne National Park as well as MMZs 1, 2, 3, 3A, 39, 39a,

40 and 45 (Figure 4). Labrador samples were collected in the central region of Labrador near Happy Valley-Goose Bay.

There were also four core regions of sample collection in New Brunswick. Each core region consisted of one wildlife management zone (WMZ) as designated by the New Brunswick Department of Natural Resources and Energy (Fish and Game Division). A total of 148 individuals were sampled from WMZs 3 (n=46), 7 (n=23), 8 (n=51) and 21(n=28) (Figure 5).

There were 39 samples from the Cape Breton Highlands region, Nova Scotia, 10 from Algonquin Provincial Park, Ontario, and 50 from Alberta (Figure 3). Samples from Alberta originated mainly from the southwestern region of the province where the range of two sub-species (*Alces alces andersoni* and *Alces alces shirasi*) overlap (Peterson 1955). Therefore, these samples could be from one or both sub-species and/or hybrids between the two sub-species, if indeed these sub-species designations are warranted.

Figure 3: Map of Canada showing moose tissue collection areas.

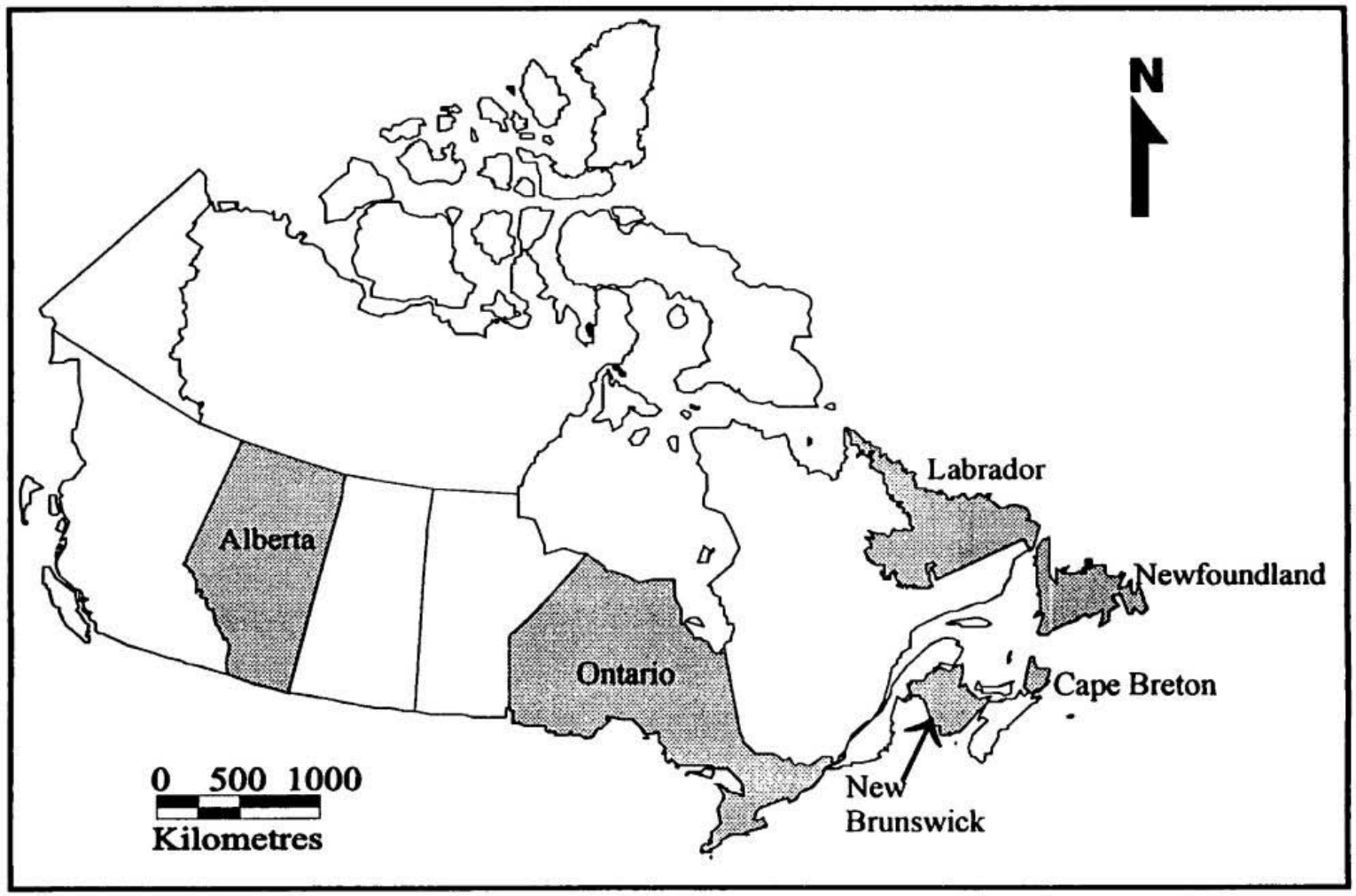

N
Labrador
Alberta
Newfoundland
Ontario
Cape Breton
New
Brunswick
0 500 1000
Kilometres

Figure 4: Map of Newfoundland showing moose tissue collection areas.

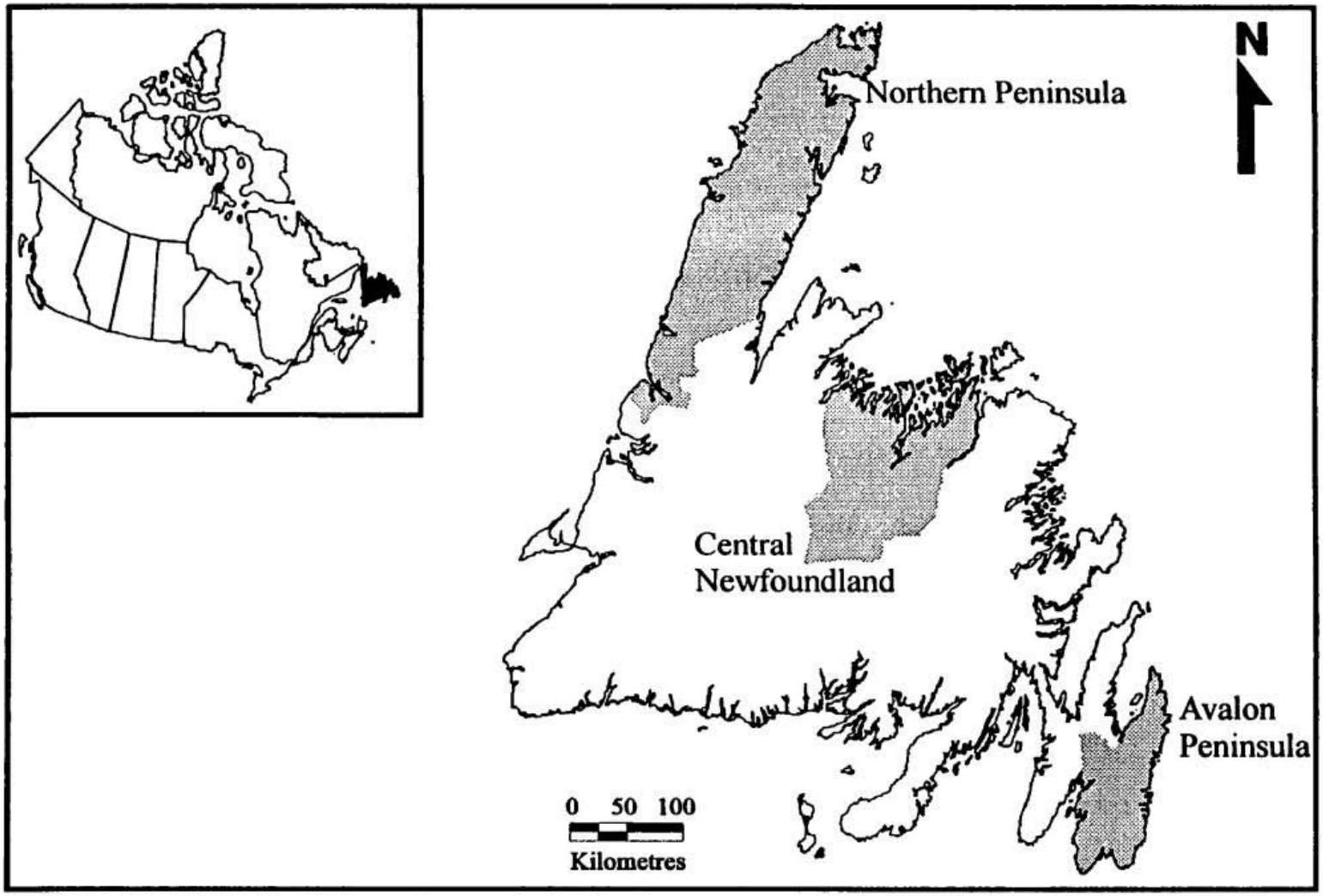
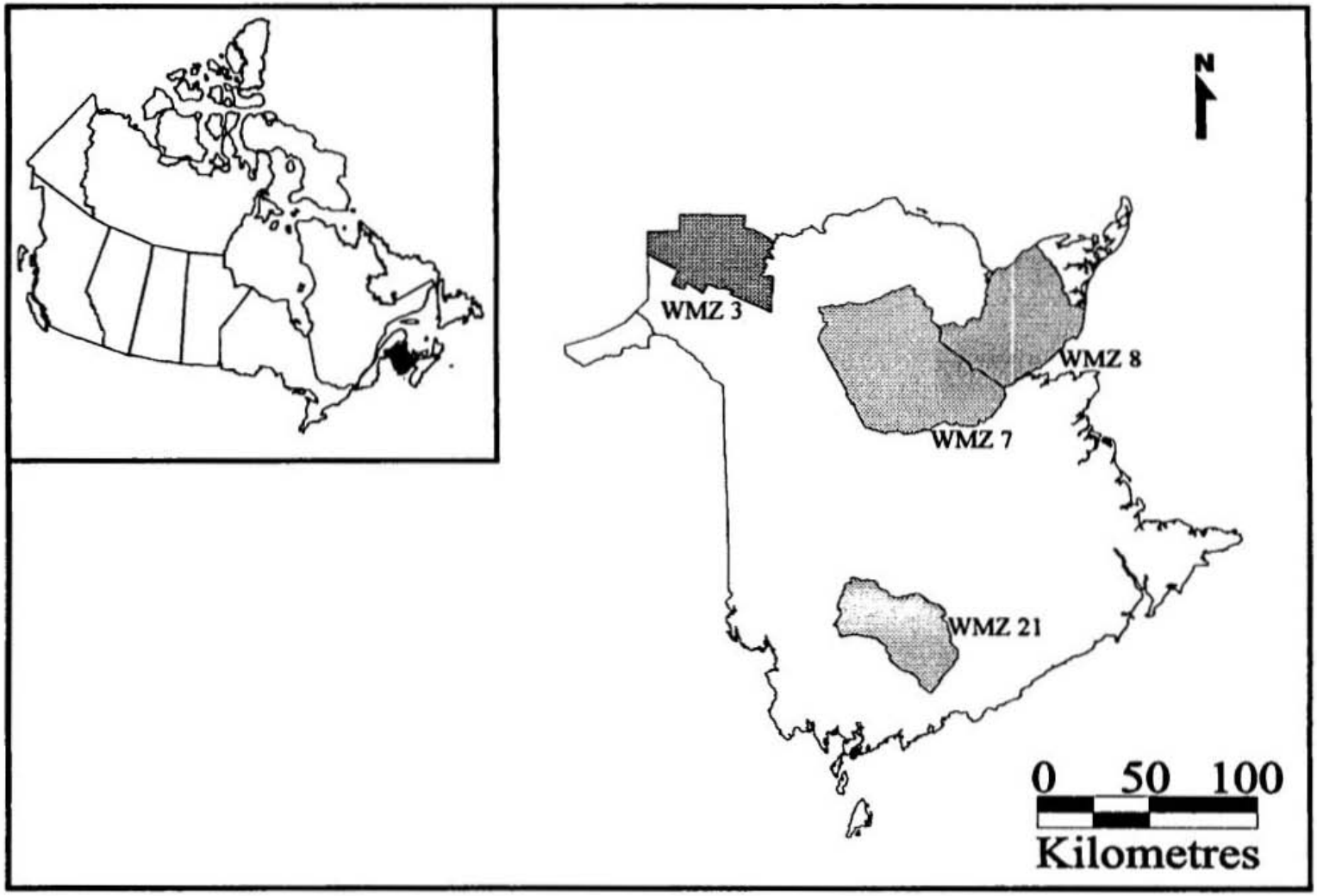

N
Northern Peninsula
Central
Newfoundland
Avalon
Peninsula
0 50 100
Kilometres

Figure 5: Map of New Brunswick showing core regions of moose tissue collection.


N
WMZ 3
WMZ 8
WMZ 7
WMZ 21
0 50 100
Kilometres

2.2 GENETIC ANALYSIS

2.2.1 DNA extraction

Samples were stored at -70°C until they were processed. Between 100 and 200 mg of muscle tissue from each sample was placed in 1.5 ml Eppendorf tubes along with 375 µl of 0.2 M EDTA and 0.5 % sarcosyl, and 25 µl of 20 mg/ml pronase. This mixture was mixed briefly and then incubated overnight at 37 °C. The following day 2 µl of 10 mg/ml DNAase-free RNAase was added to each tube, mixed briefly by hand and incubated at 37°C for 1 hour. After this incubation, 400 µl of phenol saturated with 0.1 M Tris, pH 8.0, was added, the mixture was then shaken vigorously by hand for 20 seconds, followed by 10 minutes of gentle mixing and then 10 minutes of centrifugation. After centrifugation, the top layer was decanted off and put into a new tube. This process was repeated twice more with the decanted material, first using a 1:1 mixture of phenol and chloroform (19:1 chloroform: isoamyl alcohol) and second with only chloroform, instead of 400 µl of phenol. Next, 2 volumes (approximately 800 µl) of ice-cold 95 % ethanol was added to each new tube and mixed by abrupt inversion of the tubes 5 or 6 times. The tubes were then placed at -20°C for 30 minutes. The samples were then centrifuged for 20 minutes and the ethanol decanted without disturbing the DNA pellet. The DNA was washed with 60 µl of 70 % ethanol and centrifuged for another 5 minutes. This ethanol was also decanted and the DNA was dried under reduced pressure and dissolved in 200 µl of sterile water or TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA).

2.2.2 Microsatellite analysis

There were no published primers for microsatellite loci in moose, so the literature was searched for primers in related species (*i.e.* other cervids and bovids). Once identified these primers were tested using moose DNA. Of the many 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 1X Tfl reaction buffer (Promega), 1.5 mM Mg SO_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 CORE DNA synthesis lab), 0.50 units of Tfl polymerase (Promega) and 0.07 µM primer labelled on the 5' end with [γ-^{32}P]ATP (Amersham) using polynucleotide kinase (Pharmacia) and ~100ng DNA. The general PCR conditions involved an initial denaturation at 94°C for 5 minutes followed by the appropriate number of cycles for each primer set (Table 2). Each cycle consisted of 94°C denaturation for 30 seconds followed by 30 seconds of annealing at the appropriate temperature for each primer set (Table 2) and final extension at 72°C for 30 seconds. When all cycles were complete the samples were stored at 4°C.

PCR products were separated on 6 % polyacrylamide gels containing 19:1 acrylamide:bis-acrylamide, 7 M urea and 1X TBE buffer. Gels were run for 1.5-4 hours (depending on product size) at constant power of 40 watts (~1600 volts, 25 mA), then

dried without fixing and autoradiographed either overnight at -70 °C using intensifying screens or at room temperature for ~48 hours. Each gel contained two reference samples from a previous gel to ensure correct scoring of alleles on all gels. Alleles were numbered arbitrarily with #1 being the smallest allele.

Table 1: Microsatellite primers tested on moose DNA. Primers producing reproducible and scorable products are in boldface.

Name	Source species	Reference
Cervid 1	**White-tailed deer**	**Dewoody *et al.* (1995)**
Cervid 2	White-tailed deer	Dewoody *et al.* (1995)
Cervid 3	White-tailed deer	Dewoody *et al.* (1995)
Cervid 4	White-tailed deer	Dewoody *et al.* (1995)
Cervid 14	White-tailed deer	Dewoody *et al.* (1995)
ORF 381	Red/Sika deer	Abernethy (1994)
OarFCB193	Red/Sika deer	Abernethy (1994)
BovirBP	**Bovine**	**D. MacHugh, Trinity College, Dublin**
CelJP15	**Red deer**	**Pemberton and Slate (1994)**
CelJP18	Red deer	Pemberton and Slate (1994)
CelJP27	Red deer	Pemberton and Slate (1994)
CelJP38	**Red deer**	**Pemberton and Slate (1994)**
BM-143	**Bovine**	**Bishop *et al.* (1994)**
BM-1225	**Bovine**	**Bishop *et al.* (1994)**
BM-2830	**Bovine**	**Bishop *et al.* (1994)**
BM4513	Bovine	Bishop *et al.* (1994)
MAF-70	Bovine	Bishop *et al.* (1994)
INRA003	**Bovine**	**Bishop *et al.* (1994)**
INRA023	Bovine	Bishop *et al.* (1994)

2.3 STATISTICAL ANALYSIS

2.3.1 Precautionary data check

The relative frequencies of different alleles and genotypes for each sampling region were calculated using two methods. First, relative frequency of alleles and genotypes were calculated by hand. Second, data sheets were built that could be used in GENEPOP (v3.1a; Raymond and Rousset 1995) to calculate relative frequencies as well as other statistics. To ensure data files and program algorithms were accurate, I compared relative frequencies calculated using both methods and corrected any errors in the data sheets before performing further analyses.

The data were examined using GENEPOP to see if they conformed to Hardy-Weinberg equilibrium and whether any of the loci were linked. Deviations of observed genotype frequencies from Hardy-Weinberg expectations could indicate more than one population within the sampling region (*i.e.* Wahlund effect) or such processes as inbreeding, assortative mating or selection may be occurring in the population (Nei 1987). Linkage was tested to determine if the occurrence of alleles at a locus is independent of alleles at other loci (*i.e.* in linkage equilibrium; Ayala and Kiger 1984). If linkage disequilibrium (*i.e.* non-random association) occurs loci are not independent of one another and only one of such associated loci should be used.

The critical value (α) for statistical significance in this study was set at 0.05.

I used three different tests of Hardy-Weinberg equilibrium in GENEPOP. The probability, or exact, test (Haldane 1954) examined the null hypothesis that union of gametes was random (H_A: non-random union of gametes) by computing the exact probability of obtaining the observed value and all other values with a greater deviation from what is expected. More powerful score (U) tests were also performed to assess the alternative hypotheses of heterozygote excess and deficiency (Guo and Thompson 1992; Rousset and Raymond 1995 and the GenepopV3.1a instruction manual).

Tests of genetic disequilibrium were also conducted using GENEPOP. The null hypothesis "genotypes at one locus are independent of genotypes at the other locus" was examined with Fisher's Exact Test, using a Markov chain permutation method (Guo and Thompson 1992) and a p-value computed across all populations using a global test (Fisher's method; GENEPOP instruction manual).

2.3.2 Analysis of hypothesis 1

G-tests were used to examine the similarity of allelic frequencies between a pair or more of populations. An advantage of G-statistics over other statistics (*i.e.* χ^2) is that they may be summed over all loci for each comparison and since the theoretical distribution of the G-statistic is approximately equal to the χ^2 distribution, p-values may also be computed (Sokal and Rohlf 1995). If the frequencies of alleles between populations were

statistically similar ($p>0.05$) the regions were considered part of one homogeneous population, rather than distinct sub-populations, and were combined for further analyses. Control files, executable in the statistical package Minitab (release 9), were built to calculate G-statistics (see Appendix 1). The control files were first tested for accuracy using published data and then employed to calculate G-statistics for this study. The degrees of freedom used to calculate the p-values were calculated as:

$$df = (n-1)*(m-1)$$

where n = the number of alleles, and

m = the number of sampling regions being compared

The NTSYS program (Rohlf 1992) was used to calculate Nei's genetic distance (Nei 1972) and Roger's genetic distance (Rogers 1972), between all population pairs. The genetic distance matrices of NTSYS were exported to the phylogenetic analysis using parsimony program (PAUP: test version v4.0: Swofford 1993) to perform an unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis.

2.3.3 Analysis of hypothesis 2

Four measures were used to examine the quantity of genetic variation in all moose populations: observed heterozygosity, expected heterozygosity, the probability of identity (POI) and the mean number of alleles in a population. The POI is the probability that two individuals drawn randomly from a population will have the same genotype at

all loci examined (Paetkau and Strobeck 1994). The observed and expected heterozygosity and the mean number of alleles increase as genetic variation increases, whereas POI decreases as genetic variation increases. Values were calculated using the following formulae:

$$H(\text{observed}) = \frac{\text{The number of heterozygotes}}{\text{Total number of individuals sampled}} \qquad \text{(Hedrick } et\ al.\text{ 1986)}$$

$$H(\text{expected}) = 1 - \sum_i p_i^2 \qquad \text{(Nei and Roychoudhury 1974)}$$

$$\text{POI} = \sum_i p_i^4 - \sum_i \sum_{j>i} (2p_i\, p_j)^2 \qquad \text{(Paetkau and Strobeck 1994)}$$

$$\text{Mean number of alleles} = \frac{\sum^{k} N_k}{k}$$

where p_i = relative frequency of the i-th allele,

p_j = relative frequency of the j-th allele,

N_k = number of alleles at the k-th locus,

k = number of loci

Control files (see Appendix 1) executable in the statistical package Minitab (release 9) were built for each statistic, tested using published data where possible and then employed to calculate the statistics for this study. The overall POI is the product of the value for each locus whereas the overall heterozygosity is the average heterozygosity of all loci.

The genetic variation in each founder and source population pair was compared to assess whether a reduction in genetic variation had occurred. ANOVAs were not used to do this because each source population had a different amount of variation which would influence the variation in the founded population. It is possible that a founded population could have more variation than other source populations. Therefore, these categorical tests could not accurately test the proposed hypothesis (*i.e.* hypothesis 2). In addition, it was not possible to use multiple regression to determine which factor (*i.e.* the number of founders, the amount of genetic variation in the source population or the time since colonization) contributed most to the loss of genetic variation because n was too low and the p-value calculated from such a test would not be reliable. Therefore, qualitative descriptions of the effects of each founder event will be presented.

CHAPTER 3

3.1 RESULTS

3.1.1 Data summary

Eight of the 19 primer pairs tested produced reproducible and scorable products (see appendix II). The number of alleles at these loci ranged from one to 10 in Canadian moose (Table 2). The size of alleles were determined by comparing the band mobility of the most intense band to that of a known sequence. The size of alleles and their relative frequencies are shown in Table 3.

Hardy-Weinberg tests where the alternative hypothesis was heterozygote deficiency suggested the presence of a null allele, at locus BM-143 ($p<0.05$ in six out of nine populations). Due to the difficulty and possible error associated with determining the allele frequencies at a locus with a null allele, this locus was removed from further statistical analysis. However, all three alleles at this locus (two amplifying and one null) are thought to be present in moose populations from all regions surveyed.

Observed genotype frequencies of moose from all regions conformed to Hardy-Weinberg proportions. Using the probability method there were two significant results ($p<0.05$) within a population at a locus. However, at $\alpha=0.05$ with 51 tests, approximately 3 significant results would be expected from chance alone. Also, when tests with alternative hypotheses of heterozygote excess and deficiency were employed only one

significant result in 51 tests for each method was recorded. Again, approximately 3 significant results would be expected by chance alone.

Only three tests for linkage disequilibrium out of 98 performed between pairs of loci were significant ($p<0.05$). Approximately five significant results were expected from chance alone. Furthermore, one of the three significant results occurred between two loci (INRA003 and BM-2830) known to be on different chromosomes in cattle (Bishop *et al.* 1994) which suggests that they are likely on different chromosomes in moose and therefore unlikely to be linked. When results for each locus pair were summed over all populations, no statistically significant results were obtained ($p>0.05$ in 10 tests). Therefore, there was no evidence of linkage disequilibrium in this study. Hence, independent assortment was assumed when other calculations were performed.

Table 2: The number of alleles and PCR amplification conditions for the eight microsatellite loci used to assess genetic variation in moose from Canada.

Locus	Number of alleles in moose from Canada	Annealing temperature (°C)	Number of cycles
Cervid1	1	55	30
CelJP38	1	52	30
BM-143	2 + null	61.5	32
BM-2830	4	58	35
BovirBP	3	46.5	27
CelJP15	4	45.5	35
BM-1225	7	61.5	32
INRA003	10	50	29

Table 3: Size (in bp) and relative frequency of alleles at seven independent microsatellite loci for seven moose populations in Canada. Private alleles (*i.e.* not present in any other population) in boldface*.

Locus	Allele (size)	Av.	Cen.-N.P.	N.B.	Lab.	C.B.	Ont.	Alta.
CelJP38	1 (?)	1.000	1.000	1.000	9.000	1.000	1.000	1.000
Cervid1	1 (?)	1.000	1.000	1.000	1.000	1.000	1.000	1.000
BM-2830	1 (83)	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	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)	0.000	0.000	0.000	0.000	0.000	0.000	0.020
BovirBP	1 (108)	0.204	0.205	0.358	0.055	0.750	0.050	0.690
	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	0.000	0.179
BM-1225	1 (227)	1.000	1.000	0.613	0.608	0.586	0.700	0.430
	2 (229)	0.000	0.000	0.000	0.000	0.000	0.000	**0.010**
	3 (235)	0.000	0.000	0.231	0.081	0.200	0.150	0.240
	4 (237)	0.000	0.000	0.000	0.000	0.086	0.000	0.070
	5 (247)	0.000	0.000	0.000	0.000	0.000	0.000	**0.070**
	6 (249)	0.000	0.000	0.000	0.000	0.000	0.000	**0.060**
	7 (251)	0.000	0.000	0.156	0.311	0.129	0.150	0.120
CelJP15	1 (78)	0.536	0.377	0.373	0.295	0.311	0.200	0.239
	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	0.000	0.013	0.050	0.033
INRA003	1 (178)	0.000	0.000	0.000	0.000	0.000	0.000	**0.038**
	2 (180)	0.000	0.000	0.237	0.263	0.700	0.500	0.238
	3 (182)	0.000	0.000	0.004	0.000	0.000	0.000	0.075
	4 (188)	0.000	0.000	0.011	0.013	0.057	0.000	0.188
	5 (190)	0.924	0.734	0.576	0.566	0.129	0.350	0.275
	6 (192)	0.000	0.000	0.031	0.000	0.114	0.000	0.088
	7 (194)	0.000	0.000	0.065	0.053	0.000	0.000	0.075
	8 (195)	0.065	0.260	0.076	0.105	0.000	0.150	0.013
	9 (197)	0.000	0.006	0.000	0.000	0.000	0.000	0.013
	10 (199)	**0.011**	0.000	0.000	0.000	0.000	0.000	0.000

* a second private allele was also recorded on the island of Newfoundland outside the core-regions of sample collection at locus BM-2830.

3.1.2 Population Genetic Structure of moose in Canada

Allele frequencies of moose from all 11 Canadian regions were dissimilar ($p<0.001$). Moose in Canada do not comprise one homogeneous. Furthermore, moose surveyed from the three core regions on the island of Newfoundland do not comprise one homogeneous population [$p=0.002$ $G=40(18)$]. However, allele frequencies from Central Newfoundland and the Northern Peninsula were similar [$p=0.556$, $G=7$ (8); Table 4], suggesting they comprise a genetically homogeneous population (Cen.-N.P.) that is distinct from the Avalon Peninsula population (Av.). Since moose on the Avalon Peninsula are distinct and partially geographically isolated from moose on the remainder of the island of Newfoundland, this population was considered a naturally founded population (Figure 4).

Moose from the four New Brunswick regions had similar allelic frequencies [$p=0.096$, $G=54(42)$ and only one of six pairwise comparisons produced a significant ($p<0.05$) result.

Results indicating homogeneous populations were also found when moose from Ontario and New Brunswick WMZ 8 [$p=0.056$, $G=23(14)$] and Ontario and Labrador [$p=0.065$, $G=20(12)$; see Table 4] were compared. However, these regions were not considered part of one populations because: (*i*) both p-values were very close to α . (*ii*) large geographic 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 the allele frequencies were similar by chance alone.

UPGMA cluster analysis using Nei's (1972) and Roger's (1972) measures of genetic distance (Table 5) showed the same clustering of populations (Figures 6 and 7, respectively). These results support the structure of the populations inferred from their geographic locations, sub-species designations and population histories. Both populations on the island of Newfoundland cluster together and this cluster is more similar to the source population in New Brunswick than any other. Likewise, the Cape Breton moose population (C.B.) cluster with the Alberta population (Alta.). Finally, the Labrador (Lab.) and Ontario (Ont.) populations cluster together and are more similar to the Newfoundland and New Brunswick cluster (which are part of the same sub-species) than the Cape Breton-Alberta cluster.

Table 4: G-statistics (df) for pairwise comparisons of moose from different Canadian regions above diagonal, and statistical significance below diagonal.

	Av.	Cen. NF.	N. Pen.	N.B. 7	N.B. 8	N.B. 3	N.B. 21	Lab.	C.B.	Ont.	Alta.
Av.		23 (8)	30 (9)	89 (13)	139 (15)	106 (14)	84 (14)	158 (13)	365 (14)	89 (11)	317 (22)
Cen. NF.	s		7 (8)	81 (12)	141 (14)	92 (13)	86 (13)	166 (12)	346 (13)	79 (10)	307 (20)
N. Pen.	s	0.5556		98 (13)	169 (15)	120 (14)	105 (14)	176 (13)	367 (14)	83 (11)	326 (21)
N.B. 7	s	s	s		23 (14)	10 (13)	13 (13)	51 (13)	144 (14)	23(12)	132(21)
N.B. 8	s	s	s	0.0637		28 (14)	19 (14)	69 (14)	185 (15)	23 (14	173 (21)
N.B. 3	s	s	s	0.6937	s		11 (13)	84 (13)	173 (14)	35 (13)	153 (21)
N.B. 21	s	s	s	0.4801	0.1788	0.6265		56 (14)	133 (14)	35 (13)	102 (21)
Lab.	s	s	s	s	s	s	s		219 (14)	20 (12)	222 (21)
C.B.	s	s	s	s	s	s	s	s		104 (13)	86 (21)
Ont.	s	s	s	s	0.0555	s	s	0.0646	s		104 (21)
Alta.	s	s	s	s	s	s	s	s	s	s	

s: Statistically significant at $\alpha = 0.05$

Table 5: Nei's (1972) genetic distance above diagonal and Rogers (1972) genetic distance below diagonal for pairwise comparisons of moose from seven Canadian populations.

	Av.	Cen.- N.P.	N.B.	Lab.	C.B.	Ont.	Alta.
Av.	0	0.013	0.050	0.110	0.298	0.121	0.213
Cen.- N.P.	0.044	0	0.047	0.117	0.284	0.097	0.201
N.B. (Tot)	0.084	0.073	0	0.055	0.145	0.053	0.099
Lab.	0.113	0.124	0.078	0	0.272	0.046	0.225
C.B.	0.202	0.201	0.147	0.144	0	0.217	0.043
Ont.	0.138	0.125	0.085	0.078	0.156	0	0.216
Alta.	0.165	0.162	0.115	0.130	0.072	0.144	0

Figure 6: UPGMA cluster analysis using Nei's (1972) genetic distance for seven moose populations in Canada. Branch lengths provided above each branch.

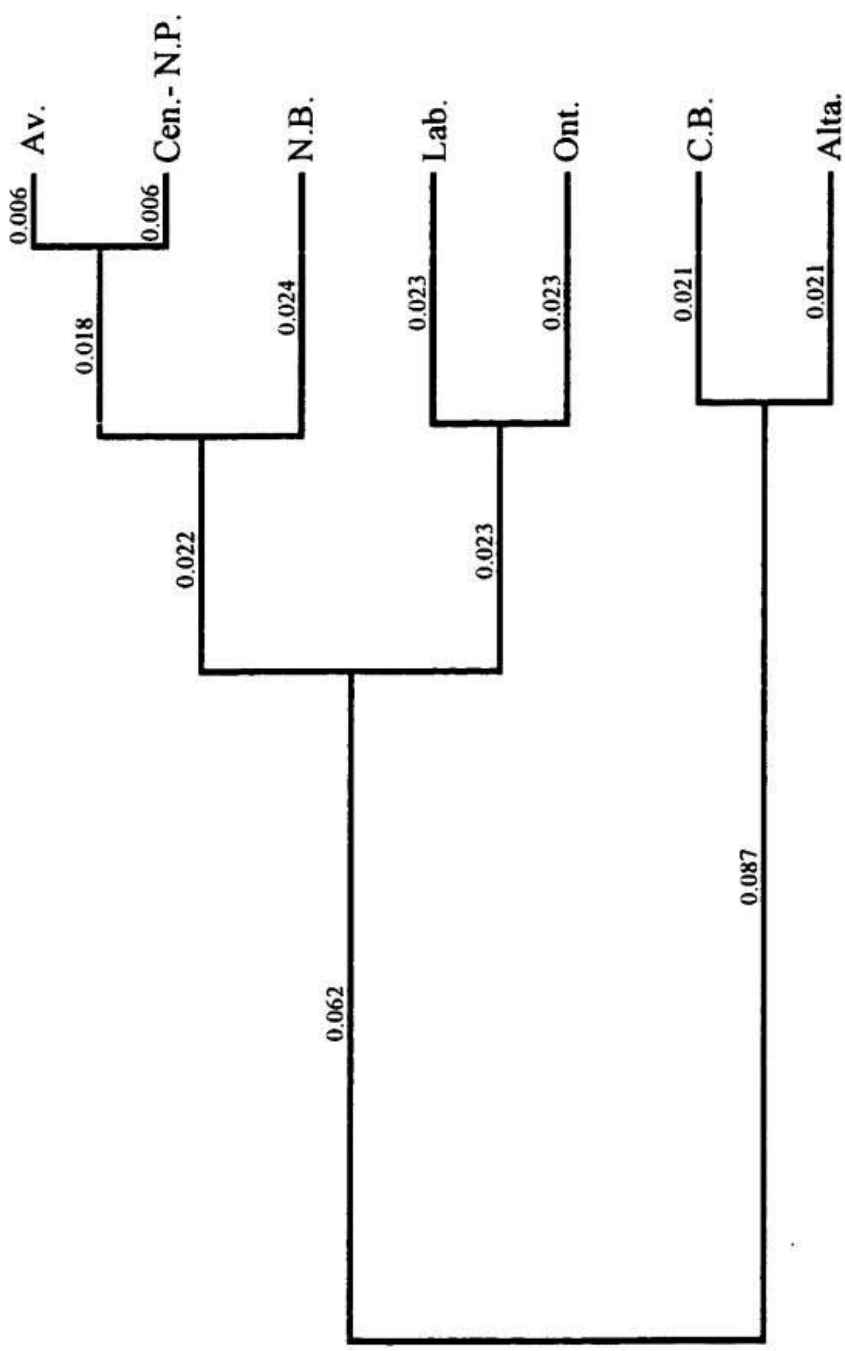
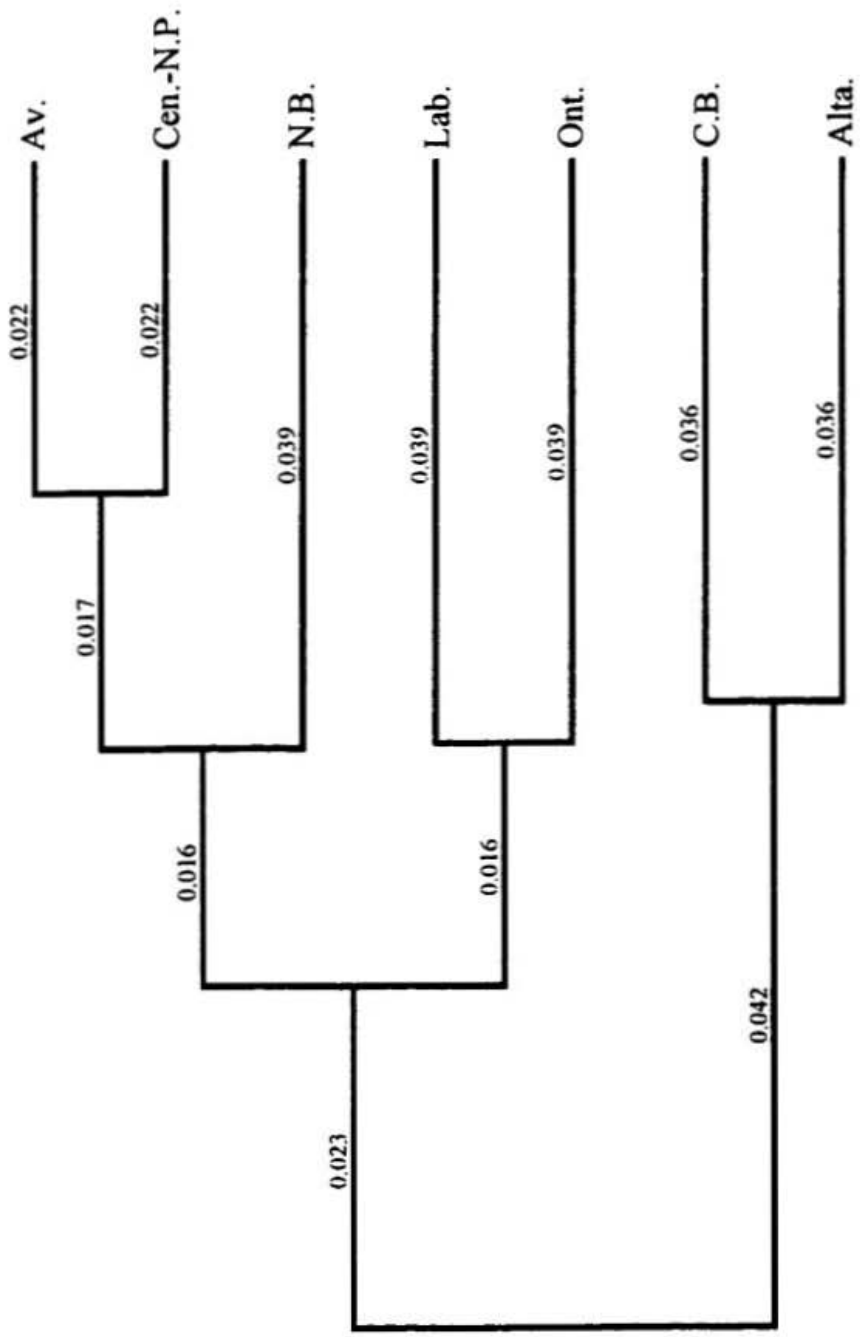

Av.
Cen.- N.P.
N.B.
Lab.
Ont.
C.B.
Alta.
0.006
0.006
0.018
0.024
0.023
0.023
0.021
0.021
0.022
0.023
0.062
0.087

Figure 7: UPGMA cluster analysis using Roger's (1972) genetic distance for seven moose populations in Canada. Branch lengths provided above each branch.


Av.
Cen.-N.P.
N.B.
Lab.
Ont.
C.B.
Alta.
0.022
0.022
0.039
0.039
0.039
0.036
0.036
0.017
0.016
0.016
0.042
0.023

3.1.3 Effects of founder events on genetic variability

Genetic variation is lower in all three founder populations (C.B., Cen.-N.P. and Av.) relative to their source populations (Alta., N.B. and Cen.-N.P., respectively). Observed and expected heterozygosities are provided in Table 6. The mean difference in expected heterozygosity for populations that have undergone a single founder event is 23.6%. For the Avalon sample, which has undergone two founder events, the reduction in expected heterozygosity is 43.3% (Table 7).

The POI suggests a similar pattern of genetic variation reduction from source to founder populations. The POI in the C.B. population is 1 250, whereas in the Alta. population it is 1 5,000. Similar reductions in genetic variation are evident in the founding events from N.B. to Cen.-N.P. and then to the Av. population where the POI falls from 1 1050, to 1 100, to 1 37 (Table 9), respectively.

The mean number of alleles was lower in founder populations relative to source populations in two out of three cases. There was a 32.5 % and 30 % reduction in the number of alleles in C.B. and Cen.-N.P. populations, respectively, relative to their source populations (Table 8). There was no change in the mean number of alleles between the Av. and Cen.-N.P. population. However, there was one private allele (*i.e.* an allele not present in any other population) in the Av. population (Table 4), therefore although the mean number of alleles is equal, those alleles are qualitatively different. A second private allele (locus BM-2830, allele 1) was found in Newfoundland outside the core regions.

The most probable source of these private alleles is new mutation. Their absence (or extremely low frequency) in the New Brunswick moose population and rarity in Newfoundland makes it unlikely that they were among the alleles of the original six introduced animals and have persisted at extremely low frequency since introduction. The Alberta population, which has the highest level of variation, has four private alleles (Table 4).

Table 6: Observed and expected heterozygosities for seven moose populations in Canada.

	CelJP38		Cervid1		BM-2830		BovirBP	
	observed	expected	observed	expected	observed	expected	observed	expected
Av.	0.000	0.000	0.000	0.000	0.438	0.469	0.426	0.432
Cen.-N.P.	0.000	0.000	0.000	0.000	0.562	0.498	0.545	0.511
N.B.	0.000	0.000	0.000	0.000	0.483	0.496	0.453	0.500
C.B.	0.000	0.000	0.000	0.000	0.459	0.354	0.361	0.400
Alta.	0.000	0.000	0.000	0.000	0.280	0.315	0.405	0.475
Lab.	0.000	0.000	0.000	0.000	0.359	0.444	0.139	0.130
Ont.	0.000	0.000	-	0.000	0.400	0.480	0.100	0.095

	CelJP15		BM-1225		INRA003		Total	
	observed	expected	observed	expected	observed	expected	observed	expected
Av.	0.518	0.585	0.000	0.000	0.152	0.142	0.219	0.232
Cen.-N.P.	0.670	0.638	0.000	0.000	0.403	0.394	0.311	0.292
N.B.	0.747	0.717	0.556	0.546	0.595	0.601	0.405	0.409
C.B.	0.541	0.513	0.543	0.593	0.514	0.477	0.345	0.334
Alta.	0.630	0.656	0.680	0.730	0.825	0.809	0.403	0.426
Lab.	0.513	0.586	0.595	0.527	0.474	0.596	0.297	0.326
Ont.	0.500	0.665	0.300	0.465	0.700	0.605	0.286	0.330

- none surveyed

Table 7: Percent reduction in the observed and expected heterozygosity following founder events.

	Alta.→C. B.	N. B. → Cen.-N.P.	Cen.-N.P. → Av.	N.B. → Cen.-N.P. →Av.
Observed heterozygosity	14.4	23.2	29.6	45.9
Expected heterozygosity	21.6	28.6	20.5	43.3

Table 8: Probability of identity (Paetkau *et al.* 1994) for seven moose populations in Canada.

Locus	Av.	Cen.-N.P.	N.B.	Lab.	C.B.	Ont.	Alta.	Canada
Cervid1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CelJP38	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
BM-2830	0.392	0.376	0.377	0.408	0.480	0.386	0.508	0.376
BovirBP	0.373	0.293	0.346	0.763	0.403	0.824	0.324	0.272
CelJP15	0.248	0.199	0.132	0.243	0.315	0.174	0.182	0.149
BM-1225	1.000	1.000	0.267	0.301	0.212	0.331	0.109	0.297
INRA003	0.744	0.441	0.205	0.211	0.306	0.234	0.062	0.195
Overall	0.027	0.010	0.0009	0.005	0.004	0.004	0.0002	0.0009

Table 9: Mean number of individuals sampled from each distinct population and the mean number and percent reduction of the mean number of alleles from source to founded population.

	Alta.	C.B.	N.B.	Cen.-N.P.	Av.
Mean number sampled	46	36	140	92	48
Mean number of alleles*	4.0	2.7	3.0	2.1	2.1
Percent reduction	32.5		30	0	

* Includes all alleles at any frequency.

CHAPTER 4

4.1 DISCUSSION

At least two of the four designated subspecies of moose in North America were sampled for this study. Comparisons of allelic frequencies at microsatellite loci have identified seven distinct populations (see Figures 6 and 7). Within the province of New Brunswick, all four core regions had similar allele frequencies ($p=0.096$) and therefore comprise a single population. This is not surprising since Peterson (1955) classified moose from this region as one subspecies (*A. a. americana*). Also, proximity of the regions to one another (Figure 5) and lack of geographic barriers allow moose to move freely throughout the landscape.

Unlike the moose population in New Brunswick, samples from the three core regions on the island of Newfoundland did not have similar allelic frequencies ($p=0.002$). However, pairwise comparisons of allelic frequencies indicate that Central Newfoundland and the Northern Peninsula moose were homogeneous ($p=0.556$) and both areas were distinct from moose from the Avalon Peninsula ($p<0.05$). Moose habitat between Central Newfoundland and the Northern Peninsula is continuous and, as in New Brunswick, moose can move unimpeded throughout the landscape which have allowed a thorough mixing of alleles and provided little opportunity for population sub-structuring to occur. The Avalon Peninsula, however, is separated from the remainder of the island of

Newfoundland by an isthmus that acts as a geographic barrier that limits migration and therefore the exchange of animals and facilitates differentiation of allelic frequencies.

The existence of significant spatial structure of moose on the island of Newfoundland should be incorporated into management decisions in order to conserve variability and therefore the evolutionary potential of the species. If, for example, the number of moose from a region in Central Newfoundland were to become reduced, animals from nearby areas that are part of the same population could move in and re-populate the area. However, if for some reason the moose population on the Avalon Peninsula were reduced, some unique genetic variants might be lost and the low migration rate to the area would not facilitate rapid re-growth of the population. Management decisions based on relatively small MMZs help to ensure that human exploitation will not cause significant declines in local populations, but the important point is that the same may not be true in other jurisdictions and/or especially for other species of less economic value.

The clustering of populations shown by UPGMA analysis of Nei's and Roger's genetic distance (Figures 6 and 7) are as expected given their geographic locations and origins of the founder populations. Therefore, this method should be useful for determining the population structure of species for which there is little extra information. The two Newfoundland populations are more similar to each other than they are to the source population in New Brunswick. Cape Breton moose are statistically different from

Alberta moose but they cluster together, and are quite distinct from all other eastern Canadian moose populations. The Ontario and Labrador moose populations cluster together and this cluster is significantly more similar to the Newfoundland/New Brunswick cluster than the Alberta Cape Breton cluster. These population relationships support Peterson's (1955) designations of at least two subspecies in Canada [one to the east of central Ontario (excluding Cape Breton) and one (or more) to the west]. An important question that still remains unanswered is: how much genetic differentiation is required for the designation of subspecies status (Avise 1994)?

The five polymorphic microsatellite loci used in this study have clearly and effectively documented the loss of genetic variation following founder events. All four measures of genetic variation were lower in founder populations relative to their source populations. These results support the prediction of Nei *et al.* (1975) that a population that has experienced more than one founder event (N.B.→Cen.-N.P.→Av.) will lose more variation than a population that has experienced only one founder event (Alta.→C.B., N.B.→Cen.-N.P., Cen.-N.P.→Av. ; see Table 7). Techniques such as allozyme electrophoresis would be less effective at documenting this reduction in genetic variation because of their low levels of variability. One strength of this study over other studies is that three founder events (*i.e.* replicates) have been examined and they all have similar results. Even though a reduction in genetic variation due to founder events has been documented the determination of which factor(s) (the amount of variation in the source

population, number of founders, inbreeding, genetic drift and/or the length of time the population remained at low numbers) most strongly influences the loss of genetic variation cannot be done with three replicates.

As mentioned earlier, when a population is at low numbers genetic factors are less important to the persistence of the species than are demographic stochasticity, environmental stochasticity and natural catastrophes (Lande 1988). However, on the islands of Newfoundland and Cape Breton at the time of the moose introductions these factors had little, if any, apparent effect on moose population growth, presumably because there were no competitors and predation was extremely low. Hunting was probably initially negligible, since people were generally unaware of the presence of moose while they were at low densities. These factors provided the introduced animals with unlimited food and cover, which facilitated rapid population growth and maintenance of significant portions of the genetic variation present in the founders. Also, because of the low moose densities following introduction, dominant males would have been less effective at preventing sub-ordinate males from mating. Therefore, each male should have had a relatively equal opportunity to mate and pass on his genes. This would have decreased the effects of drift and facilitated the maintenance of significant portions of the variability following the founder event.

Even though inbreeding would be inevitable in these small introduced populations there

is no evidence to suggest that any decrease in fitness occurred as a result. Growth of the populations has been phenomenal, and there are no apparent or reported phenotypic deviances in the populations. Genetic variation is reduced in each founded population relative to their source populations but there is no evidence that the viability of these founded populations is at greater risk than the 'typical' moose population in Canada. Levels of variation in the Cape Breton and Central Newfoundland-Northern Peninsula founder populations are comparable to other populations. Heterozygosities in the Labrador and Ontario moose populations are lower than that of the Cape Breton population and comparable to the Central Newfoundland-Northern Peninsula population (Table 6). The probability of identity in Cape Breton moose is similar to Ontario moose and less than those in Labrador (indicating more variability in Cape Breton). Therefore, risks to a population (or species) as a result of population reductions are greater relative to the source population but not necessarily to all other populations (or species).

Given the low number of founder events that have been examined, it is difficult to assess the relationship between genetic variability and species viability. Furthermore, it is not known how much variation a species (or population) needs in order to have a specific probability of survival. Also, is 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/if environmental conditions change) important for the viability of a species or population? Is the answer to this question the same in all cases or does it vary between

species and/or the particular circumstances of that species? Understanding these relationships are vital to understanding species and population viability. Only by increasing the number of studies on populations where the complete history is known and can be examined, like this study, can we begin to compile sound knowledge on the subject and attempt to make reasonable estimates of the potential of a species (or population) to persist under certain circumstances.

One problem with many studies that examine genetic variability after a reduction in population size is that the level of genetic variation in the original populations is not known (Ardern and Lambert 1997; Bonnell and Selander 1974). Researchers may only speculate that similar species that have not been reduced in size will have comparable levels of variation, and may serve as a comparison. This study has examined founder populations of a species whose source population still exists and therefore very few assumptions must be made.

Evolution cannot be anticipated and therefore, the genetic requirements of a species or population to avoid extinction at some point in the future cannot be predicted. Educated guesses based on indices of the genetic variability are the best available option. However, it is suspected that many island populations have been founded by one or a few individuals. Then, over evolutionary time scales, genetic variation has accumulated in the population providing more evolutionary potential. The major task for conservation

biologists today is not only to ensure populations at low numbers persist and maintain genetic variation but also to curb the loss of habitat, due to human exploitation. By ensuring that species have sufficient habitat and are not over-exploited we can ensure that their numbers will remain high, thereby limiting the effects of demographic stochasticity, environmental stochasticity and natural catastrophy and facilitating the accumulation of genetic variation, and therefore evolutionary potential.

LITERATURE CITED

Allendorf FW, Leary RF (1987) Heterozygosity and fitness in natural populations of animals. In: Soulé ME (ed.) *Conservation Biology: the science of scarcity and diversity*. pp 57-78. Sinauer Associates, Inc., Sunderland, MA.

Ardern SL, Lambert DM (1997) Is the black robin in genetic peril? *Molecular Ecology*. 6, 21-28.

Avise JC (1994) *Molecular markers, natural history and evolution*. Chapman and Hall. New York.

Ayala FJ, Kiger JA (1984) *Modern Genetics*. 2nd ed. Benjamin/Cummings Publishing Company, New York.

Banfield AWF (1974) *The Mammals of Canada*. University of Toronto Press, Toronto.

Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SLF, Hawkins GA, Toldo SS, Fries R, Grosz MD, Yoo J, Beattie CW (1994) A genetic linkage map for cattle. *Genetics*, 136, 619-639.

Bonnell ML, Selander RK (1974) Elephant seals: genetic variation and near extinction. *Science*, 184, 908-909.

Cameron AW (1958) *Mammals of the islands of the Gulf of St. Lawrence*. Bulletin of the National Museum of Canada. No. 154.

Campbell NA (1990) *Biology*. 2nd. ed. Benjamin/ Cummings Publishing Company, New York.

Caro TM, Laurenson MK (1994) Ecological and genetic factors in conservation: a cautionary tale. *Science*, 263, 485-486.

Carr SM, Snellen AJ, Howse KA, Wroblewski JS (1995) Mitochondrial DNA sequence variation and genetic stock structure of Atlantic cod (*Gadus morhua*) from bay and offshore locations on the Newfoundland continental shelf. *Molecular Ecology*, 4, 79-88.

Caughley C, Gunn A (1996) *Conservation biology in theory and practice*. Blackwell Science, Cambridge, MA.

Corbett GN (1995) Review of the history and present status of moose in the national parks of the Atlantic region: management implications? *Alces*, 31, 255-267.

Davies NB (1991) Mating systems. In: Krebs JR, Davies NB (eds.) *Behavioral ecology: an evolutionary approach*, 3rd ed., pp 263-294. Blackwell Scientific Publications, London.

Dewoody JA, Honeycutt RL, Skow LC (1995) Microsatellite markers in white-tailed deer. *Journal of Heredity*, 86, 317-319.

Dinerstein E, McCracken GF (1990) Endangered greater one-horned rhinoceros carry high levels of genetic variation. *Conservation Biology*, 4, 417-422.

Dodds DG (1975) Distribution, habitat and status of moose in the Atlantic provinces of Canada and northeastern United States. *Naturaliste Canadien*, 101, 51-65.

Erlich PR (1986) Extinction: what is happening now and what needs to be done. In: Elliot (ed.) *Dynamics of extinction*. John Wiley and Sons, New York.

Franklin IR (1980) Evolutionary changes in small populations. In: Soulé ME, Wilcox *BA* (eds.) *Conservation Biology: an evolutionary-ecological perspective*. pp 135-149. Sinauer Associates, Inc., Sunderland, MA.

Gale JM (1988) The moose let loose. *Newfoundland Quarterly*, 84(2), 19-20.

Garner DL, Porter WF (1990) Movements and seasonal home ranges of bull moose in a pioneering Adirondack population. *Alces*, 26, 80-85.

Garton DW, Koehn RK, Scott, TM (1984) Multiple locus heterozygosity and the physiological energetics of growth in the coot clam *Mutinia lateralis* from a natural population. *Genetics*, 108, 445-455.

Gasaway WC, DuBois SD, Brink KL (1980) Dispersal of subadult moose from a low density population in interior Alaska. *Proceeding of the North American Moose Conference and Workshop*, 16, 314-337.

Gilpin M (1987) Spatial structure and population vulnerability. In: Soulé ME (ed.) *Viable populations for conservation*. pp 125-139. Cambridge University Press.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics*, 48, 361-372.

Haldane JBS (1954) An exact test for randomness of mating. *Journal of Genetics*, 52, 631-635.

Hedrick PW, Brussard PF, Allendorf FW, Beardmore JA Orzack S (1986) Protein variation, fitness and captive propogation. *Zoo Biology*, 5, 91-99.

Howard WE (1960) Innate and environmental dispersal of individual vertebrates. *American Midland Naturalist*, 63, 152-161.

Howley JP (1913) Nature studies with observations on the natural history in Newfoundland.

Hundertmark KJ, Johns PE, Smith MH (1992) Genetic diversity of moose from the Kenai Peninsula, Alaska. *Alces*, 28, 15-20.

Koehn RK, Gaffney PM (1984) Genetic heterozygosity and the growth rate in *Mytilus edulis*. *Marine Biology*, 82, 1-7.

Krebs CJ (1988) *The message of ecology*. Harper and Row, New York.

Lacy RC (1992) The effects of inbreeding on isolated populations: are minimum viable population sizes predictable? In: Fiedler PL, Jain SK (eds.), *Conservation Biology*. Chapman and Hall, London.

Lande R (1988) Genetics and demography in biological conservation. *Science*, 241, 1455-1466.

Manwell C, Baker CMA (1982) Alcohol dehydrogenase in the marine polychaete, *Hyalinoecia tubicola*: heterozygote deficit and growth. *Comparative Biochemistry and Physiology*, 73B, 411-416.

McDermid EM. Ananthakrishnan R. Agar NS (1972) Electrophoretic investigation of plasma and red cell proteins and enzymes of Macquarie island elephant seals. *Animal Blood Groups and Biochemical Genetics*. 3. 85.

Mercer G (1995) Moose management plan for Newfoundland and Labrador. Newfoundland and Labrador Wildlife Division unpublished report.

Moore SS. Sargent LL. KingTJ. Mattick JS. Georges M. Hetzel DJS (1991). The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics*. 10. 654-660.

Montevecchi WA. Tuck LM (1987) *Newfoundland Birds*. Nuttall Ornithological Club. Cambridge. MA.

Montevecchi WA. Kirk DA (1996) Great Auk (*Pinguinus impennis*). In: Poole A. Gill F (eds.) The Birds of North America. No. 260. The Academy of Natural Sciences. Philadelphia. PA. and The American Ornithologists' Union. Washington. D.C.

Nei M (1972) Genetic distance between populations. *American Naturalist*. 106. 283-292.

Nei M. Roychoudhury AK (1974) Sampling variance of heterozygosity and genetic distance. *Genetics* 76. 379-390.

Nei M. Maruyama T. Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*. 29. 1-10.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press. New York.

Noss RF (1992) Issues of scale in conservation biology. In: Fiedler PL, Jain SK (eds.) *Conservation Biology*. pp 239-250. Chapman and Hall. London.

O' Brien SJ. Roeke ME. Marker L. Newman A. Winkler CA. Meltzer D. Colly L. Evermann JF. Bush M. Wildt DE (1985) Genetic basis for species vulnerability in the cheetah. *Science*. 227. 1428-1434.

O'Brien SJ. Evermann JF (1988) Interactive influence of infectious disease and genetic diversity in natural populations. *Trends in Ecology and Evolution*. 3. 254-259.

O'Reilly P. Wright JM (1995) The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. *Journal of Fish Biology*. 47. 29-55.

Paetkau D. Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*. 3.489-495.

Pemberton JM. Slate J (1994) Genetic studies of wild deer populations: a technical revolution. The third international congress on the biology of deer.

Peterson RL (1955) *North American Moose*. University of Toronto Press. Toronto.

Peterson RL (1974) A review of the general life history of moose. *Naturaliste Canadien*. 101. 9-21.

Pimlott DH (1953) Newfoundland Moose. *Transactions of the North American Wildlife Conference*. 18: 563-581.

Pimlott DH. Carberry WJ (1958) North American Moose transplantations and handling techniques. *Journal of Wildlife Management*. 22. 51-62.

Pimm SL (1987) Determining the effects of introduced species. *Trends in Ecology and Evolution*. 2. 293-295.

Pulsifer MD. Nette TL (1995) History. status and present distribution of moose in Nova Scotia. *Alces*. 31. 209-219.

Quammen D (1996) *The song of the dodo: Island biogeography in an age of extinctions*. Scribner. New York..

Quattro JM. Vrijenhoek RC (1989) Fitness differences among remnant populations of the endangered sonoran topminnow. *Science*. 245. 976-978.

Queller DC. Strassmann JE. Hughes CR (1993) Microsatellites and kinship. *Trends in Ecology and Evolution*. 8. 285-288.

Ralls K. Brugger K. Ballou J (1979) Inbreeding and juvenile mortality in small populations of ungulates. *Science*. 206. 1101-1103.

Raymond M. Rousset F (1995) An exact test for population differentiation. *Evolution*. 49. 1280-1283.

Rohlf FJ (1992) NTSYS-pc: Numerical taxonomy and multivariate analysis system. version 1.70. State University of New york. Stony Brook.

Rogers JS (1972) Measures of genetic similarity and genetic distance. University of Texas Publication No. 7213. pp 145-153.

Ruzzante DE. Taggart CT. Cook D. Goddard S (1996) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. *Canadian Journal of Fisheries and Aquatic Science*. 53. 634-645.

Ryman N. Beckman G. Bruun-Petersen. Reuterwall C (1977) Variability of red cell enzymes and genetic implications of management polocies in Scandinavian moose (*Alces alces*). *Hereditas*. 85. 157-162.

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

Schonewald-Cox C (1983) Conclusions: guidelines to management: a beginning attempt. In: Schonewald-Cox C. Chambers SM. MacBryde B. Thomas L (eds.) *Genetics and Conservation*. pp. 414-445. Benjamin Cummings Publishing Company. New York.

Shafer CL (1990) Nature reserves: island theory and conservation practice. Smithsonian Institute Press. Washington. D.C.

Shaffer ML (1981) Minimum population sizes for species conservation. *Bioscience*. 31. 131-134.

Shick JM. Hoffman RJ. Lamb AN (1979) Asexual reproduction, population structure, and genotype-environment interactions in sea anemones. *American Zoologist*. 19. 699-713.

Simonsen V, Born EW, Kistensen T (1982) Electrophoretic variation in large mammals. IV. The Atlantic walrus, Odobenus rosmarus rosmarus (L.). *Hereditas*, 97, 91-94.

Singh SM, Zouros E (1978) Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). *Evolution*, 32, 342-353.

Sokal RR, Rohlf FJ (1995) *Biometry*. W.H. Freeman and Company, New York.

Soulé ME (1981) Tresholds for survival: maintaining fitness and evolutionary potential. In: Soulé ME, Wilcox BA (eds.) *Conservation Biology: an evolutionary-ecological perspective*. pp 151-169. Sinauer Associates, Inc., Sunderland, MA.

Templeton AR, Read B (1983) The elimination of inbreeding depression in a captive herd of Speke's Gazelle. In: Schonewald-Cox C, Chambers SM, MacBryde B, Thomas L (eds.) *Genetics and Conservation*, pp. 241-261. Benjamin/Cummings Publishing Company, New York.

Valdes AM, Slatkin M, Freimer NB (1993) Allele frequencies at microsatellite loci: the stepwise mutation model revisited. *Genetics*, 133, 737-749.

Watt WB (1983) Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the *Colias* PGI polymorphism. *Genetics*, 103, 691-724.

Watt WB, Cassin RC, Swan MS (1983) Adaptation at specific loci. III. Field behavior and survivorship differences among *Colias* PGI genotypes are predictable from in vitro biochemistry. *Genetics*, 103, 725-739.

Wilhelmswon M. Juneja RK. Bengtsson S (1978) Lack of polymorphism in certain blood proteins and enzymes of European and Canadian moose (*Alces alces*). *Naturaliste Canadien*. 105. 445–449.

APPENDIX I

All control files provided below are executable in the statistical package MINITAB (release 9).

G-statistics

To compare actual (as opposed to relative) allele frequencies in two populations, *x* and *y* enter allele frequencies of population *x* in column 1 and allele frequencies of population *y* in column 2 and execute the following file. The G-statistic will be provided as 'Gtot'. If there are data for more than one locus enter the data for the second locus into columns 1 and 2 once analysis of locus one is complete. This control file will stack the G-statistics from each locus into column 26 and sum them over all loci to give the total G (*i.e.* 'Gtot'). It is important to note that to analyze a second pair of populations the values in column 26 from the previous analysis must be deleted because the program does not know when one analysis is done and the other begins.

Control file for a pair of populations: 'g2.etl'

```
let c3=c1-c2
let k1=sum(c1)+sum(c2)
let c4=c3-k1
let c5=c3*sum(c1)/k1
let c6=c3*sum(c2)/k1
stack c1 c2 c20
stack c5 c6 c21
let c22=c21*k1
let c23=c20-c21
name c20 'f' c21 'pN' c23 'res'
let c24= 'f' *log('f'/'pN')
let c25=2*c24
name c25 '2lnL'
let k2= sum (c25)
name k2 'sum_2lnL'
plot 'res' 'pN'
hist 'res'
hist '2lnL'
prin c20 c21 c23 c25
stack c26 k2 c26
let k3=sum (c26)
name k3 'Gtot'
```

```
prin k2 k3
end
```

Control file for four populations: 'g4.ctl'

```
let c5=c1-c2+c3+c4
let k1=sum(c1)+sum(c2)+sum(c3)+sum(c4)
let c6=c5/k1
let c7=c5*sum(c1)/k1
let c8=c5*sum(c2)/k1
let c9=c5*sum(c3)/k1
let c10=c5*sum(c4)/k1
stack c1 c2 c3 c4 c20
stack c7 c8 c9 c10 c21
let c22=c21*k1
let c23=c20-c21
name c20 'f' c21 'pN' c23 'res'
let c24= 'f' *log('f'/'pN')
let c25=2*c24
name c25 '2lnL'
let k2= sum (c25)
name k2 'sum_2lnL'
plot 'res' 'pN'
hist 'res'
hist '2lnL'
prin c20 c21 c23 c25
stack c26 k2 c26
let k3=sum (c26)
name k3 'Gtot'
prin k2 k3
end
```

Expected heterozygosity (and homozygosity): 'real hs.ctl'

To use this file the relative allele frequencies must be entered in to column 1. As a check to ensure data are entered correctly the control file will sum all of column, ensure the 'sum' value is 1 before trusting the heterozygosity value (*i.e.* 'hetero' value).

```
let c2=(c1)*(c1)
let k1=sum (c2)
let k2=1-sum(c2)
let k3=sum (c1)
name k1 'homo' k2 'hetero' k3 'sum'
prin k1 k2 k3
end
```

Probability of Identity (Paetkau *et al.* 1994)

Below are two examples of control files to calculate the probability of identity when there are four and six alleles at a locus, respectively. To use these files the relative frequency of *i*-th allele must be entered into the *i*-th column such that if the are *k* alleles there will be data in columns 1-*k*.

To ensure data are entered correctly into the columns the control file will sum the columns, and if they are entered correctly the value of 'sum' will be 1, and therefore the value of 'identity' will be the probability of identity.

Control file for calculating the probability of identity for a locus with four alleles: 'identity4.ctl'

```
let c5=(c1)*(c1)*(c1)*(c1)
let c6=(c2)*(c2)*(c2)*(c2)
let c7=(c3)*(c3)*(c3)*(c3)
let c8=(c4)*(c4)*(c4)*(c4)
let k1=(c5)+(c6)+(c7)+(c8)
let c9=(2*(c1)*(c2))*(2*(c1)*(c2))
let c10=(2*(c1)*(c3))*(2*(c1)*(c3))
let c11=(2*(c1)*(c4))*(2*(c1)*(c4))
let c12=(2*(c2)*(c3))*(2*(c2)*(c3))
let c13=(2*(c2)*(c4))*(2*(c2)*(c4))
```

```
let c14=(2*(c3)*(c4))*(2*(c3)*(c4))
let k2=(c9)+(c10)+(c11)+(c12)+(c13)+(c14)
let k8=k1+k2
let k9=(c1)+(c2)+(c3)+(c4)
name k8 'identity' k9 'sum'
prin k8 k9
end
```

Control file for calculating the probability of identity for a locus with six alleles:

'identity6.ctl'

```
let c10=(c1)*(c1)*(c1)*(c1)
let c11=(c2)*(c2)*(c2)*(c2)
let c12=(c3)*(c3)*(c3)*(c3)
let c13=(c4)*(c4)*(c4)*(c4)
let c14=(c5)*(c5)*(c5)*(c5)
let c15=(c6)*(c6)*(c6)*(c6)
let k1=(c10)+(c11)+(c12)+(c13)+(c14)+(c15)
let c20=(2*(c1)*(c2))*(2*(c1)*(c2))
let c21=(2*(c1)*(c3))*(2*(c1)*(c3))
let c22=(2*(c1)*(c4))*(2*(c1)*(c4))
let c23=(2*(c1)*(c5))*(2*(c1)*(c5))
let c24=(2*(c1)*(c6))*(2*(c1)*(c6))
let c25=(2*(c2)*(c3))*(2*(c2)*(c3))
let c26=(2*(c2)*(c4))*(2*(c2)*(c4))
let c27=(2*(c2)*(c5))*(2*(c2)*(c5))
let c28=(2*(c2)*(c6))*(2*(c2)*(c6))
let c29=(2*(c3)*(c4))*(2*(c3)*(c4))
let c30=(2*(c3)*(c5))*(2*(c3)*(c4))
let c31=(2*(c3)*(c6))*(2*(c3)*(c6))
let c32=(2*(c4)*(c5))*(2*(c4)*(c5))
let c33=(2*(c4)*(c6))*(2*(c4)*(c6))
let c34=(2*(c5)*(c6))*(2*(c5)*(c6))
let k2=(c20)+(c21)+(c22)+(c23)+(c24)+(c25)+(c26)+(c27)+(c28)+(c29)+(c30)
let k3=(c31)+(c32)+(c33)+(c34)
let k4=k2+k3
let k8=k1+k4
let k9=(c1)+(c2)+(c3)+(c4)+(c5)+(c6)
name k8 'identity' k9 'sum'
prin k8 k9
end
```

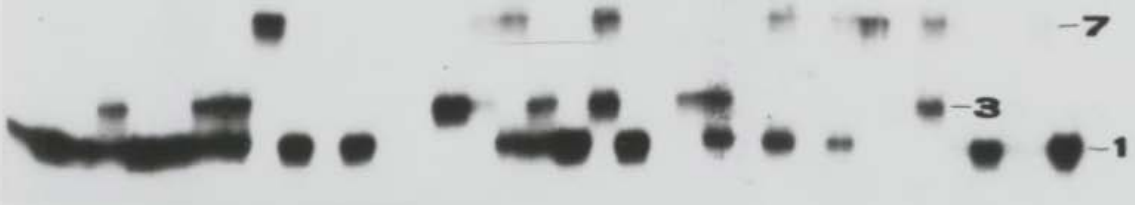
APPENDIX II

Polymerase chain reaction products for each polymorphic locus in several random individuals resolved using denaturing polyacrylamide gel electrophoresis. The smallest allele at each locus was designated 1, consecutively larger alleles were designated 2, 3, *etc*. Allele sizes (Table 3) were determined by comparing alleles to known sequences. The positive electrode was always toward the top of the photo. Allele numbers are shown at each locus and 'lm' indicates a lane marker.


LOCUS BM-2830
4
3
2
1


LOCUS BOVIRBP
3
2
1

LOCUS BM-1225



LOCUS CeLJP15


LOCUS INRA003
11
10
8
7
3
lm
lm