GENETIC VARIATION IN MUSKOXEN (*OVIBOS MOSCHATUS*)

A

THESIS

Presented to the Faculty of the University of Alaska

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

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Fairbanks, Alaska

May 1986

ABSTRACT

Populations of Alaskan muskoxen (*Ovibos moschatus*) are derived from 34 animals transplanted from East Greenland in the 1930s. The possibility of a founder effect following this transplant was investigated. Muscle, liver and plasma samples from 87 Alaskan animals and 39 Greenlandic animals were analyzed using polyacrylamide and starch gel electrophoresis. A total of 38 enzyme and non-enzymatic protein systems, coded by 58 presumptive loci, was tested for activity; 28 loci were considered usable. One locus (Esterase-2) was polymorphic; the proportion of polymorphic loci was 0.036 (95% criterion). The mean heterozygosity per individual was 0.006 in the Greenlandic population and 0.011 in the Alaskan population. The allele frequencies at the Est-2 locus were similar in both populations. No heterozygote deficiency and no evidence of a founder effect were seen in the Alaskan population. This may be a consequence of the low level of allozymic variation seen in muskoxen in general.

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ACKNOWLEDGEMENTS

Financial support for my research was provided primarily by Federal Aid in Wildlife Restoration funds from the Alaska Department of Fish and Game through the Alaska Cooperative Wildlife Research Unit, University of Alaska, Fairbanks (UAF). The experiments could not have occurred without the samples collected with the assistance of the Alaska Department of Fish and Game; the US Fish and Wildlife Service; the Department of Arctic Biology, University of Tromsø, Norway; the Vildtbiologisk Station, Rønde, Denmark; and the Institute of Arctic Biology (IAB), UAF. Many individuals at these agencies generously assisted me in the field or in the laboratory, but as one person has asked to remain anonymous, I prefer to mention no names and to thank everyone collectively. Also, I wish to thank the people of Mekoryuk, Alaska, for their assistance in sample collection and their hospitality. I am very grateful for the advice and assistance of my committee members: Dr. Stephen F. MacLean, Jr., Dr. Gerald F. Shields, Dr. Robert G. White, and especially my committee chairman, Dr. David R. Klein, who provided financial, logistic, and moral support.

I was introduced to polyacrylamide and starch gel electrophoresis techniques by Knut Røed at the Department of Zoology, Agricultural University of Norway (NLH), Aas, Norway, and Deanna Tolliver at Savannah River Ecology Laboratory (SREL), Aiken, South Carolina, respectively. I thank them and Drs. Hans Staaland, Chairman, Department of Zoology,

NLH, and Michael H. Smith, Director, SREL, for allowing me the use of their respective facilities and for their encouragement. A travel grant from the Vice-Chancellor for Research and Advanced Study, UAF, was awarded to help defray costs.

Of the many people at UAF who contributed to my project, I would especially like to thank Dr. Keith Miller for the loan of the circulating pump, Dr. George Stevens for editorial advice, Clay Cranor for statistical assistance, and Christopher A. Babcock for the good vibrations lab coat. For action exceeding the call of duty, may I single out Don Borchert, IAB, for incredible patience in matters of photography; my former roommates, Cristine Linesch and Connie O'Brien Zachel, for maintaining my blood sugar level; and the men in my life, my grandfather, Isaac Fleischman, my father, Milton W. Fleischman, and my husband, Richard S. Noll, for each taking his turn in contributing to the support of my academic development.

INTRODUCTION

The reintroduction of the muskox (Ovibos moschatus) to Alaska is an unquestioned success story in modern conservation biology. The species became extinct in Alaska before the turn of this century, but now thrives thanks to the rescue efforts of wildlife managers and conservationists. This intentional manipulation in the history of the species offers wildlife biologists the chance to assess the effects of such efforts and to attempt to predict the effects of further manipulation. It also offers geneticists the chance to investigate certain basic predictions about population genetics. These considerations are particularly important for a species which was once considered endangered throughout its range. Although much is now known of the ecology and behavior of the muskox, little is known of the genetics of muskox populations. This study addresses that gap in our knowledge of the muskox.

Genetic variation is the raw material upon which natural selection acts. However, studies demonstrating the selective advantage of specific alleles are few. That heterozygosity at a particular locus may be correlated with environmental variables has been demonstrated by Place and Powers (1979), who showed a correlation between LDH alleles in the killifish (Fundulus heteroclitus) and water temperature, and by Pipkin et al. (1975), who found a correlation between ADH alleles in fruit flies (Drosophila melanogaster) and extreme minimum ambient

temperature. Multi-locus heterozygosity was found to correlate with morphological characteristics in the killifish (Mitton, 1978) and mosquitofish (Gambusia affinis; Smith and Chesser, 1981). A correlation between higher heterozygosity and higher growth and reproduction characteristics has been documented for white-tailed deer (Smith et al., 1983) and for domestic livestock, laboratory animal, and domesticated plant species (Frankel and Soulé, 1981, and references therein). The converse has also been noted. Animal and plant breeders, who may reduce genetic variability by intentionally breeding for morphological or physiological homogeneity, frequently find that their genetically uniform stocks are more susceptible to pathogens, show reduced viability, or have low reproductive rates compared with outbred stocks. Managers of such stocks have learned to cope with inbreeding depression (reduced fitness) by crossing their inbred lines to produce hybrid lines (Falconer, 1981).

The importance of maintaining genetic variation has been demonstrated in wildlife species. Ballou and Ralls (1982) demonstrated a correlation between inbreeding and yearling mortality in 12 species of ungulates raised in zoos. O'Brien et al. (1985) showed the susceptibility to contagious disease of the cheetah (Acinonyx jubatus), which is monomorphic at antigen loci as well as at allozyme loci. In a remarkable demonstration of applied genetics, Templeton and Read (1983) were able to raise the survival rate and decrease the coefficient of inbreeding in a captive population of Speke's gazelle (Gazella spekei) descended from just five founders through a careful program of controlled breeding and pedigree analysis. The need to safeguard the

genetic resources contained in the gene pools of wild species (Harding, 1982; Vernhes, 1983) and the utility of zoos as repositories of these genetic resources, has been stressed (Mallinson, 1984;, Brisbin, 1980; Ralls, 1984). This is particularly critical for endangered species (Ryder et al., 1981; Foose, 1983).

Genetic variation within populations is commonly measured by examining the biochemical variation among enzymes and non-enzymatic proteins within and between individuals. The structurally different forms of enzymes are commonly called allozymes, and "genetic variation" is hereafter used synonymously with "allozymic variation". Allozymic variation is revealed using the technique of slab gel electrophoresis. Although electrophoresis using various media has been performed by physiologists for over 50 years, it was first applied to population biology in 1966 with the pioneering investigations of Harris (1966) on variation in humans, and Hubby and Lewontin (1966) on variation in fruit flies (Drosophila melanogaster). These early studies, in which unexpectedly large amounts of variation were discovered in natural populations, have stimulated hundreds of comparative electrophoretic surveys (cf. Smith et al., 1982). The latter studies revealed a wide range in level of genetic variation among species, the implications of which will be discussed later.

Electrophoretic data can be used for purposes other than describing the levels of genetic variation within species. Intensive study of selected species of interest to wildlife and range managers, including the white-footed mouse (Peromyscus spp.; Avise et al., 1979), vole (Clethrionomys spp.; Canham and Cameron, 1972), prairie dog (Cynomys

ludovicianus; Chesser, 1983), bighorn sheep (Ovis canadensis; Bunch and Valdez, 1976), white-tailed deer (Odocoileus virginianus; Ramsey et al., 1979), red deer (Cervus elaphus; Gyllensten et al.; 1980), reindeer (Rangifer tarandus tarandus and R. t. platyrhynchus; Soldal and Staaland, 1979; Røed, 1985b), and moose (Alces alces; Chesser et al., 1982), has revealed that intraspecific spatial variation in genetic parameters is a widespread phenomenon. Because the boundaries of genetic populations may not correspond with the geographic management units assigned by wildlife managers, the use of genetic data to define local populations has been urged (Smith et al., 1976). In game species subject to hunting pressure, different hunting regimes can have varying impacts on population parameters, and thereby on genetic variation within populations (Ryman et al., 1981).

The different alleles or allele frequencies revealed by electrophoretic analysis may be used as genetic markers to trace group movement and patterns of reproduction (Burns, 1975). This approach has been used to identify breeding stocks in migratory fish species from different parts of a single drainage (for a review see Ryman, 1983) and to show that even fish from the same lake may belong to sympatric (physically overlapping) but reproductively isolated populations (Ryman and Stahl, 1981).

Other applications of electrophoretic data that can be of use to wildlife managers include the identification of the species of origin of meat products (Kurth and Shaw, 1983) or verification of the species identity of carcasses and skins (Dilworth and McKenzie, 1970; Wolfe, 1983); the verification of paternity in wild (Foltz and Hoogland, 1981)

and zoo animals (O'Brien et al., 1984), and the identification as hybrids of individuals not positively identifiable as such on morphological grounds (Wishart, 1980; McClymont et al., 1982). Where the ranges of morphologically similar species overlap, electrophoresis can provide a quick and inexpensive method of identifying individuals without requiring sacrifice (Aquadro and Patton, 1980).

Finally, because electrophoretic data permit direct calculation of allele frequencies, they can be used in conjunction with studies of the breeding system to determine whether populations are breeding randomly or not. If skewed allele frequencies are seen, this may be an indication of inbreeding (Falconer, 1981) or selective mortality among genotypes (Scribner et al., 1983; Berry, 1978).

As mentioned previously, there is a wide range in the amount of genetic variation among species. Biologists do not agree on a paradigm that can predict or account for this. Factors that have been suggested to correlate with heterozygosity include mobility and body size (Selander and Kaufman, 1973), environmental heterogeneity (Levins, 1968), and predictability of trophic resources (Valentine, 1976). Nevo et al. (1984) surveyed electrophoretic analyses of 968 plant and animal species and concluded that ecological parameters show better correlations with genetic variation than do demographic or life history parameters. Mammals as a group show less variability than do other higher taxa (Nevo et al., 1984). Among the 184 species of mammals included in the survey, fossorial species showed lower variability than non-fossorial species; species with a restricted range had lower variability than more widespread species; arctic species showed lower

variability than species of other life zones, though the differences were not statistically significant; habitat specialists showed lower variability than habitat generalists; species of very arid or very mesic environments had lower variability than species from environments of intermediate aridity; solitary species had lower variability than social species; long-lived perennial species had lower variability than annual species; and large-bodied species had lower variability than small-bodied species, though the difference was not statistically significant (Nevo et al., 1984).

Based upon the trends seen in mammalian species (Nevo et al., 1984), muskoxen would be expected to show low genetic variability, as they live in the arctic life zone, have a restricted range, are habitat specialists, inhabit a very arid environment, are long-lived perennials, and have large bodies. Alaskan muskoxen in particular might be expected to have very low variability because of recent historical events which are summarized below.

Although arctic explorers of the 1800s reported numerous sightings and shootings of muskoxen in coastal Greenland, on many of the Canadian Arctic islands, and on the Canadian Arctic mainland (Hone, 1934, and citations therein), references to sightings of muskoxen in Alaska are confusing. Apparently, the presence of the species in Alaska at the time of European settlements was a subject of dispute among early naturalists (Allen, 1901; Hornaday and Brower, 1911). Nevertheless, the presence of muskox skulls and bones along the Alaskan arctic coast and the verbal testimony of native hunters (Allen, 1912) confirms their existence in Alaska at least in the previous century. Allen (1912)

suggested that the muskox had been eliminated in Alaska by the beginning of the present century by native hunters using high-powered rifles. In 1930, the United States Congress appropriated funds for the purpose of reintroducing the species to Alaska (Young, 1943). There were two objectives: (1) conservation of an endangered species, and (2) study of the possibility of domesticating the species to make "greater economic use of the vast forage resource of northern Alaska" (Palmer and Rouse, 1936). In the same year, 34 animals (17 calves, 16 yearlings, and one apparent 2-year old; Spencer and Lensink, 1970) were captured near Hold with Hope and on Clavering Island, East Greenland (Alendal, 1980). The animals were transported to the United States Biological Survey Experiment Station in College, Alaska, where they were held for feeding experiments and demographic observations. The animals thrived, although some were lost through black bear predation and accidental injury (Palmer and Rouse, 1936). In 1935, two males and two females were released on Nunivak Island (Fig. 1), off the Bering Sea coast (Rouse, 1936). Nunivak Island, though not part of the species' original range, was chosen for the release of the animals because of the quality and extent of suitable forage, the lack of predators, and the confined area (Rouse, 1936). The island had been made a National Wildlife Refuge in 1929 in anticipation of the transplant (Spencer and Lensink, 1970). The first four animals survived, and in 1936 the remaining 15 males and 12 females at the College research station were brought to the island. For several years, the population size fluctuated, reaching 49 in 1947, but thereafter it increased at an average annual rate of 16% (Spencer and Lensink, 1970). The peak size of approximately 750 animals was reached

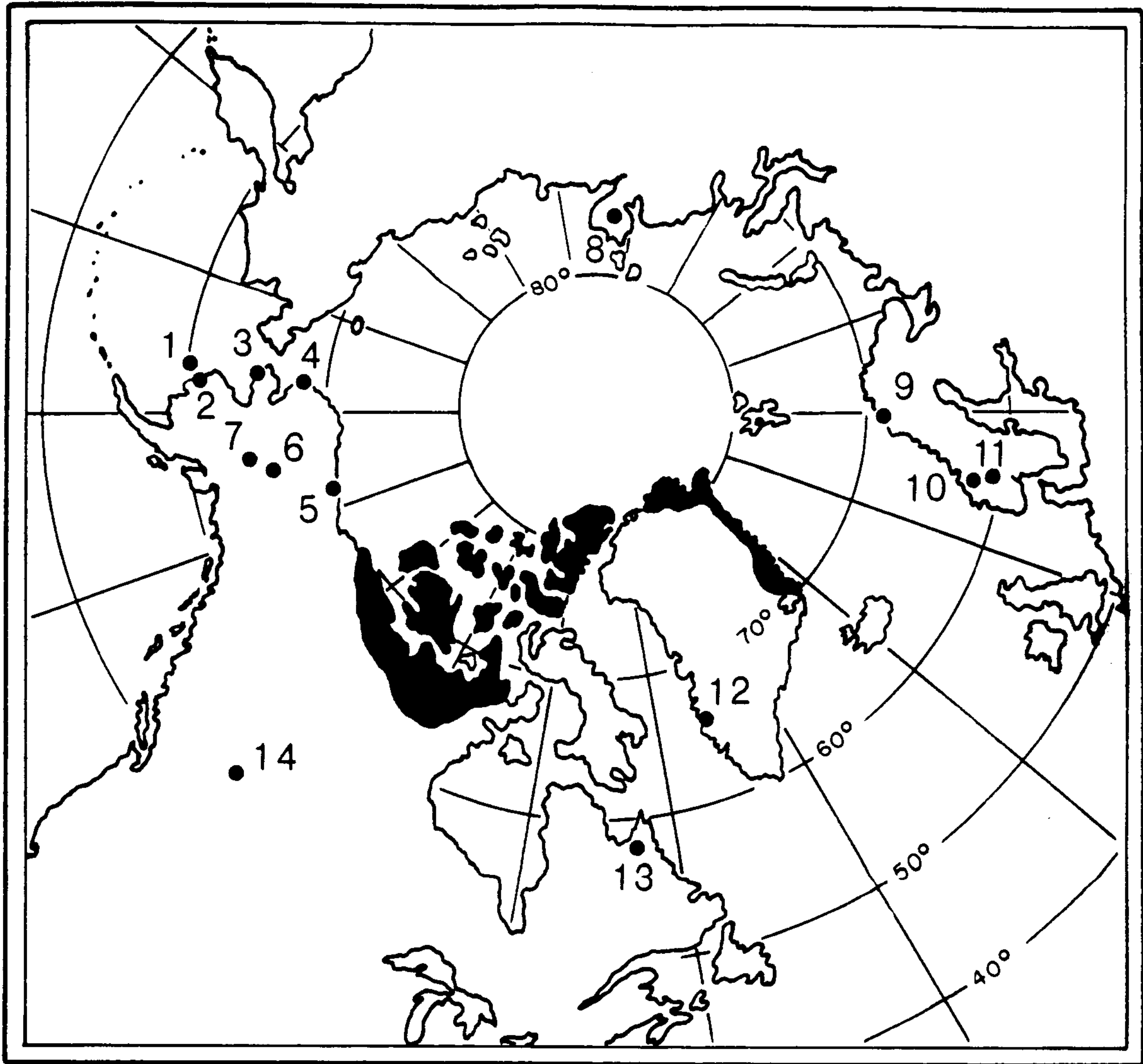

Fig. 1. Current distribution of muskoxen. Black area = approximate range of wild herds. Numbers refer to introduced herds: 1 = Nunivak Island, Alaska; 2 = Nelson Island, Alaska; 3 = Seward Peninsula, Alaska; 4 = Cape Thompson, Alaska; 5 = Arctic National Wildlife Refuge, Alaska; 6 = Large Animal Research Station, Fairbanks, Alaska (research herd); 7 = Muskox Domestication Project, Talkeetna, Alaska; 8 = Taimyr Peninsula, USSR; 9 = Tromsø, Norway (research herd); 10 = Dovre, Norway; 11 = Harjedalen, Sweden; 12 = Sondre Strømfjord, West Greenland; 13 = northern Quebec; 14 = University of Saskatchewan, Saskatoon (research herd).

in 1968 (Spencer and Lensink, 1970). Control of the population through transplants to other sites and to zoos was followed in 1975 by annual permit hunting (Smith, 1984). In addition to the Nunivak Island population, there are now two captive herds and four areas with wild populations in Alaska (Fig. 1). All mainland populations were started by transplants from the Nunivak Island herd, and surveys indicate that all the wild populations are successfully established and increasing in size (Smith, 1984). The number of muskoxen in Alaska in 1985 was estimated to be 1600-1700 (Klein, verb. comm.). It should be noted that Greenlandic muskoxen (and, therefore, the present Alaskan muskoxen) are of the subspecies _O. m. wardi_, which is also found on the Canadian High Arctic islands (Tener, 1965). The animals on the Canadian mainland are of the subspecies _O. m. moschatus_ (Tener, 1965).

Transplants of a small number of animals, as in the case of Alaskan muskoxen, provide excellent opportunities to investigate the genetics of founder populations and bottleneck situations. The founder effect refers to populations established from a subset drawn from a larger group and the bottleneck effect refers to populations which undergo a severe reduction in number lasting one or more generations (Hedrick, 1983). In both cases, genetic variation in the derived group may be reduced due to sampling error, and genetic drift (the random loss of genetic variation in small populations) may have an effect upon the subsequent level of variation in the resultant population (Hedrick, 1983). The level of genetic variation in a population that has passed through a bottleneck or is descended from a founder group can vary. The greater the number of breeders (i.e. the larger the founder group or

the wider the bottleneck), the greater is the probability that rare alleles which can contribute to the heterozygosity of the resultant population will be included. The greater the rate of reproductive increase of a founder or bottleneck population, the greater is the chance that each animal will have contributed its genotype to the gene pool before ceasing to reproduce, and the greater is the heterozygosity of the resultant population (Nei et al., 1975). Severe bottlenecks may not always cause a precipitous reduction in genetic variation. Even a single breeding pair may contain 75% of the genetic variance of the population from which it was drawn (Frankel and Soule, 1981). If that pair and the population descended from it reproduce at a growth rate (r) of less than 0.1, about 94% of the original heterozygosity will be lost, but if $r > 1.0$ is maintained for several generations, 65% or more of the original heterozygosity may be retained (Nei et al., 1975).

Electrophoretic analysis of populations begun by herd transplants, such as these in Alaska, allows investigation of the predictions of theoretical genetics. Of more immediate practical consideration, the unique history of the muskox in Alaska and its economic importance warrant the collection of genetic data as an aid to management of the species. Therefore, the present study was undertaken with the following objectives: 1) to determine whether the available field and laboratory methods were suitable for genetic analysis of muskox tissues; 2) (a) to collect baseline data on genetic variation of muskoxen in Alaska and Greenland, (b) to compare the genetic variation within these two populations and attempt to determine whether a founder effect had occurred in connection with the 1930 transplant, or inbreeding since

then; and (c) to compare the level of genetic variation in muskoxen with the levels of genetic variation reported in the literature for other species.

METHODS

Analysis of allozyme variation in muskox tissues has never been attempted before this study, hence, it was unknown whether the techniques used for analysis of other species would suffice. The polyacrylamide gel electrophoresis procedure used for analysis of reindeer tissues by Røed (1985a, 1985b) and the starch gel electrophoresis procedures used for analysis of white-tailed deer tissues by Manlove et al. (1976) were used as guides. Details and modifications are described below. Blood samples from white-tailed deer and caribou were run on gels with muskox blood samples to verify that differences in mobility were detectable with the systems used.

Two parameters of genetic variation within each population were computed. The proportion of loci that are polymorphic (p) is the percentage of loci analyzed in which the frequency of the most common allele is less than or equal to 95%. The mean heterozygosity per individual (H) is the frequency of heterozygous individuals at each locus, averaged for the number of loci analyzed; it was calculated by direct counting of heterozygotes. The distribution of genotypes at polymorphic loci was tested for goodness-of-fit to a Hardy-Weinberg equilibrium (expected distribution in an ideal, randomly-mating population) using log-likelihood ratios (Zar, 1974). The coefficient of inbreeding (f, heterozygote depression) was calculated using Wright's (1931) formula:

$$f = 1 - \frac{H}{2pq}$$

where H is the frequency of heterozygotes in the sample and p and q the allele frequencies at the locus under consideration.

Two parameters of genetic variation between populations were also computed. Genetic identity (I, proportion of identical genes between populations; Nei, 1972) was calculated using the formula:

$$I = \frac{\sum x_i y_i}{\sqrt{\sum x_i^2 \sum y_i^2}}$$

where x_i is the frequency of the ith allele at a locus in the first population and y_i is the frequency of the same allele at the same locus in the next population. Genetic distance (D, estimated number of allele substitutions per locus which could account for the diversity between the two populations; Nei, 1972) was calculated using the formula:

$$D = -\ln I.$$

Sample collection

Collecting localities are shown in Fig. 1. A list of all samples collected is provided in Appendix 1. Due to weather conditions in the field and the remoteness of the hunt sites, some samples deteriorated before they could be analysed. In Fairbanks, all samples were stored at -80 C. Procedural details relating to sample collection follow.

Muscle, liver, kidney, heart, and blood samples were collected from muskoxen on Nunivak Island, Nelson Island, and the Arctic National Wildlife Refuge, Alaska (Fig. 1), by hunters participating in scheduled

hunts in August 1983, February-March 1984, and August 1984. These hunts were overseen by the Alaska Department of Fish and Game (ADFG). Hunters were provided with kits containing an instruction sheet, labelled plastic sample bags, a syringe and needle, and one or two heparinized blood tubes. The tissues required were cut out during field-dressing of the freshly-shot animals. As ambient temperatures at the time of the hunts were below 0 C, the labelled samples were left on the snow surface to freeze. The blood tubes were kept from freezing by carrying them in an inside clothing pocket until the blood could be centrifuged or allowed to settle and the plasma and red cell fractions separated. Hunters brought the samples to ADFG officials in Mekoryuk, Bethel, or Fairbanks, Alaska, as part of their check-out procedure.

A small number of blood samples was obtained during radio-collaring operations by ADFG on Nelson Island. These samples were treated as described for those collected during hunts.

Muscle, liver, kidney, and heart samples from animals at the Large Animal Research Station (LARS), Fairbanks, Alaska, were obtained during necropsies of stillbirths and natural mortalities in spring 1983. In addition, blood samples were collected during routine examinations of research animals at LARS at various times in 1983 and 1984.

Blood samples were collected from research animals at the Department of Arctic Biology, University of Tromsø, Norway, in August 1983. These animals had been captured in Greenland, with the exception of one born in captivity in Norway. The samples were centrifuged in Tromsø, separated into red cell and plasma fractions, and then frozen for shipment to Alaska. In addition, sera which had been collected in

1982 and 1983 were provided by the Department.

Blood, muscle, heart, kidney and liver samples were collected during live capture and marking operations in Jameson Land, East Greenland, in summer 1983. Additional muscle and kidney samples were purchased from local hunters during the 1982-1984 hunting seasons. The samples were shipped under refrigeration from Greenland to Denmark and frozen there for shipment to Alaska. Muscle samples that had been collected in Jameson Land in 1975, when animals were shot for physiological studies, were also provided.

Polyacrylamide gel electrophoresis

Tissues were removed from the freezer and allowed to soften at room temperature until a 5-10 g piece could be cut off. This was placed in a 100-ml beaker (Nalgene) with an equal weight of chilled distilled deionized water and ground with a Tekmar Tissumizer sample grinder (Ultra-Turrax SDT-1810) until homogenized. After grinding each sample, the blade of the tissue grinder was spun in two changes of iced distilled water to rinse and cool it. The homogenate was poured into a chilled centrifuge tube and kept in the refrigerator until a full set of 8 samples had been ground. The homogenized samples were centrifuged for 20 min at 0 C and 17,000 g (Sorval SD-2 centrifuge). The supernatant was then withdrawn with a Pasteur pipette and transferred to chilled 400 1 micro-test tubes. The micro-test tubes containing subsamples from a single tissue sample were placed into 10-dram plastic vials and stored at -80 C for up to 12 months prior to electrophoresis. Each subsample was thawed no more than three times. When needed for electrophoresis,

the subsamples were allowed to thaw in a refrigerator. The appropriate volume of each subsample was withdrawn with a capillary tube (Corning disposable) and mixed with 200 1 of sample buffer in a spot plate well. Thirty 1 of the resultant mixture were transferred to a sample well in the prepared gel using a Hamilton microsyringe.

Blood samples were thawed over wet ice and then buffered and loaded as described above for tissue samples.

Electrophoresis was performed using a Protean Dual Slab Cell apparatus (Bio-Rad, Richmond, CA). Undiluted ethanol was used as coolant. A circulating pump (Lauda K-4/RD) was used to maintain the coolant at 2-4 C. Gels measured 14 x 16 cm. Gels of 1.5 mm and .75 mm thickness were used, but the 1.5 mm gels were found to give the best results as far as scorability of bands and ease of handling. Gel solutions were mixed to give a final acrylamide concentration of 7% in the running gel and 2.5% in the stacking gel. A 20-well comb (Bio-Rad) was used to form the stacking gel. The amount of catalyst used was that volume (Appendix 2) determined empirically to cause polymerization within 20 min. Bromphenol blue was used as the tracking dye.

The upper chamber buffer was prepared fresh from buffer stock solution before each run. The lower chamber buffer was used for 3 runs and then discarded. Sucrose, catalyst, and sample buffer solutions were made fresh every 5-7 runs and stored refrigerated. Acrylamide and gel buffer solutions were made up in 100-ml volumes and stored in the dark at room temperature. If not used routinely, solutions were discarded after 3 weeks. Electrode buffer stock solution was made up in 1-liter volumes and diluted 1:9 for use. It was stored refrigerated for up to 6

weeks, after which it was discarded.

Gels were run at 250 V through the stacking gel and 350 V through the running gel. A Bio-Rad Model 500/200 power supply was used. Electrophoresis was allowed to proceed until the tracking dye had migrated to within 2 cm of the bottom of the running gel (3-4 hr). Running gels were usually cast the night before the run. Occasionally, it was not possible to run them the following day, but gels used on the second day after casting showed no discernible reduction in resolution. Stacking gels were cast the morning of the run; sample dilution and loading was begun as soon as polymerization was evident. Gels were destained in 7% acetic acid.

Starch gel electrophoresis

Tissue samples for starch gel electrophoresis were prepared immediately prior to electrophoresis. Slivers (approximately 1 g) were shaved from the whole frozen tissues and placed into individual wells on a spot plate. A few drops of chilled grinding solution were added and the tissue was finely minced with a pair of scalpels. Wicks (Whatman filter paper # 2) were placed directly onto the minced tissue and allowed to soak. They were then blotted on fresh filter paper and loaded onto the gels. Minced samples were frozen and subsequently thawed for further electrophoresis only twice, and were then discarded.

Blood samples were thawed over wet ice. For plasma proteins, wicks were soaked in undiluted plasma and then blotted and loaded as described above. For red cell proteins, wicks were soaked in undiluted samples or in samples diluted 1:9 with grinding solution.

Horizontal starch gels were prepared with 9.5-10.5% starch concentrations (Electrostarch lot #392, Otto Hiller, Madison, WI, and Connaught starch, Connaught Laboratories, Toronto, Ontario), depending on the buffer (Appendix 3). The gel molds used were of plexiglass in two sizes, 10 x 180 x 210 mm and 15 x 180 x 210 mm. After pouring, gels were cooled, wrapped in plastic (Saran wrap), labelled and allowed to sit over night before being used for electrophoresis. Thick gels were loaded in the afternoon, run overnight, and sliced and stained the next morning. Thin gels were loaded in the morning and sliced and stained the same afternoon. Thick gels were sliced horizontally into eight 3-mm slices; thin gels were sliced into five 3-mm slices. Each slice was stained for a different enzyme or protein system. Amido black was used as the tracking dye. Tray buffer was discarded after each run. Gels were destained in a 1:5:5 (v:v:v) mixture of glacial acetic acid, methanol, and water.

Buffer systems

The buffer systems used in testing for enzymatic activity were:

Tris-citrate, pH 6.7 (Selander et al., 1971)

Tris-citrate, pH 8.0 (Selander et al., 1971)

Tris-citrate, pH 8.2 (Selander et al., 1971)

Tris-citrate-EDTA, pH 7.1 (Ayala et al., 1972)

Tris-maleate-EDTA, pH 7.4 (Selander et al., 1971)

Tris-borate-EDTA, pH 8.2-8.7 (Shaw and Prasad, 1970)

Tris-HCl, pH 6.7-8.9 (Davis, 1964)

Tris-HCl, pH 7.2-7.3 (Jolley and Allen, 1965)

Tris-HCl, pH 8.5 (Selander et al., 1971)

Amine citrate, pH 6.1 (Clayton and Tretiak, 1972)

Lithium hydroxide, pH 8.1 (Selander et al., 1971)

Whenever possible, all tissue types were tested on each buffer system. When this was not possible, at least muscle and liver were tested on each buffer. Complete recipes for gels, buffer and other solutions, and stains are given in Appendices 2 - 4, respectively.

RESULTS

Because of the small sample sizes for each tissue from each population (Table I), the samples from the Arctic National Wildlife Refuge, the Large Animal Research Station, Nelson Island and Nunivak Island were pooled for data analysis and are hereafter referred to as "the Alaska population". Likewise, the serum samples from Tromsø were pooled with those from Greenland, and are hereafter referred to as "the Greenland population".

A total of 38 enzyme and non-enzymatic protein systems, coded by 58 presumptive loci, was tested for electrophoretic activity. The choice of systems was made on the basis of availability of buffer and staining procedures, and not on prior knowledge of variability of the same systems in other species. Eighteen systems coded by 28 presumptive loci were considered reliably scorable. The gel type, buffer, and tissue used for scoring these systems are indicated in Table II.

The results of the electrophoretic analysis are shown in Table III. The locus designations therein follow the system of Allendorf and Utter (1979): when multiple forms of a protein are present, the acronym for each is given a hyphenated numeric suffix. The least anodal form is called '1', the next least is called '2', etc. Alleles at polymorphic loci are indicated by their relative electrophoretic mobility; the most common allele is designated '100' and other alleles are given a value depending upon their mobility relative to the most common allele.

Table I. Number of usable samples from two populations of muskoxen for analysis by electrophoresis.

Population[a]		Number of samples			Number of animals from which samples were taken
		Muscle	Liver	Plasma	
Alaska:	ANWR	4	5	3	5
	LARS	8	8	13	20
	NELS	4	3	2	6
	NU	50	50	30	56
Total		66	66	48	87
Greenland:	GR	8	6	23	31
	TR	-	-	8	8
Total		8	6	31	39

[a] ANWR = Alaska National Wildlife Refuge, LARS = Large Animal Research Station, NELS = Nelson Island, NU = Nunivak Island, GR = East Greenland, TR = Tromsø

Table II. Experimental procedures for best resolution of 28 enzyme and non-enzymatic protein systems in muskoxen.

System	Gel type	Buffer[a]	Tissue
AAT	Starch	TM	Liver
ADA	Starch	TM	Liver
ADH	Acrylamide	1	Liver
ALD	Starch	TM	Muscle
Est	Acrylamide	1	Liver
GDH	Acrylamide	2	Liver
GP	Acrylamide	1	Muscle
α-GPD	Acrylamide	2	Muscle
HK	Acrylamide	2	Liver
IDH	Starch	TM	Muscle
LAP	Starch	LiOH	Plasma
ME	Starch	TM	Muscle
MPI	Acrylamide	1	Muscle
6PGD	Starch	TM	Muscle
PGI	Acrylamide	2	Muscle
PGM	Acrylamide	2	Muscle
SOD	Acrylamide	1	Muscle
Tf	Acrylamide	1	Plasma

[a] TM = Tris-maleic acid, pH 7.4 (Selander et al., 1971), LiOH = Lithium hydroxide, pH 8.1 (Selander et al., 1971), 1 = Tris-HCl, pH 6.7 in stacking gel, pH 8.9 in running gel (Davis, 1964), 2 = Tris-HCl, pH 7.2 in stacking gel, pH 7.3 in running gel (Jolley and Allen, 1965)

Table III. Allele frequencies and sample sizes of 28 loci in two populations of muskoxen.

		Greenland		Alaska	
Locus	Allele	Frequency	N	Frequency	N
AAT-1		1.000	6	1.000	40
AAT-2		1.000	6	1.000	40
ADA		1.000	6	1.000	37
ADH		1.000	6	1.000	32
ALD		1.000	7	1.000	28
Est-1		1.000	6	1.000	61
Est-2	100	.750	6	.808	61
	98	.250		.192	
GDH		1.000	6	1.000	61
GP-2		1.000	8	1.000	59
GP-3		1.000	8	1.000	59
GP-4		1.000	8	1.000	59
α-GPD		1.000	8	1.000	59
HK		1.000	6	1.000	59
IDH-1		1.000	7	1.000	39
IDH-2		1.000	7	1.000	37
LAP		1.000	31	1.000	42
ME-1		1.000	7	1.000	28
ME-2		1.000	7	1.000	28
MPI-1		1.000	8	1.000	59
MPI-2		1.000	8	1.000	59
6PGD		1.000	7	1.000	28
PGI-1		1.000	8	1.000	59[a]
PGI-2		1.000	8	1.000	59[a]
PGM-1		1.000	8	1.000	59
PGM-2		1.000	8	1.000	59
SOD-1		1.000	8	1.000	59
SOD-2		1.000	8	1.000	59
Tf		1.000	31	1.000	41[b]
P[c]		0.036		0.036	
H		0.006		0.011	

[a] A single variant pattern with multiple bands was seen, but it could not be determined whether the polymorphism was at PGI-1 or PGI-2

[b] A single variant pattern was seen

[c] 95% criterion of polymorphism

A single polymorphic locus (Est-2) was detected in both the Greenland and the Alaska population; $p = 0.036$ in both populations. The same two alleles at this locus were seen in both populations. A single individual with a variant banding pattern was seen in the Alaska population at each of two other loci (PGI and Tf), but the frequency of the common allele at these loci was greater than 95%.

The number of heterozygotes was calculated by direct counting. The mean heterozygosity was low in both populations. For the Alaskan population, $H = 0.011$. In the Greenland population, $H = 0.006$. Because only one locus was variable, the values of H reflect the number of individuals scored for that locus (Alaska, $N = 60$, Greenland, $N = 6$). If the two populations are treated as subsamples of a single population and pooled, $H = 0.011$.

The distribution of genotypes at the Est-2 locus in the Alaska population was not significantly different from a Hardy-Weinberg equilibrium ($G = 2.3126$, $.10 < p < .25$); this calculation was not performed for the Greenland population because of the small sample sizes of the three genotypes. The coefficient of inbreeding in the Alaska population was $f = - 0.019$.

Genetic identity between the two populations was quite high, $I = 0.99987$. This value is higher than the average genetic identity between local populations of mammalian species (Ayala and Kiger, 1980), which is not surprising as the calculation is based upon a single polymorphic locus at which the same two alleles were present at similar frequencies. Genetic distance between the two populations is correspondingly low, $D = 0.00012$.

Detailed descriptions of the electrophoretic gels follow. These will be of interest to investigators wishing to compare banding patterns in muskoxen with banding patterns in other species and to those continuing the investigation of genetic variation in muskoxen. (E.C. = Enzyme Commission.)

Unusable loci

The following enzymes showed either no activity in any tissue tested or only faint activity in some liver samples, regardless of the buffer type, and were considered unusable in the present study: alkaline phosphatase (AKP, E.C. 3.1.3.1), aldehyde oxidase (AO), benzaldehyde dehydrogenase (BDH), diaphorase (DIA, E.C. 1.6.2.2), glucose-6-phosphate dehydrogenase (G6P, E.C. 1.1.1.49), β-hydroxybutyrate dehydrogenase (HBDH), octanol dehydrogenase (ODH, E.C. 1.1.1.73), xanthine dehydrogenase (XDH, E.C. 1.1.1.204).

The following systems showed fair to good activity in various tissues using selected buffers, but either the resolution was not sufficient for confident scoring, or activity was not seen in every sample.

Acid phosphatase (ACPH, E.C. 3.1.3.2) - One anodal zone of activity stained very well in liver, but the resolution was poor.

Aconitase (ACON, E.C. 4.2.1.3) - One weakly active zone in liver and kidney; migration was cathodal or anodal depending upon the buffer used, but was never far from the origin; resolution was poor.

Adenylate kinase (ADK, E.C. 2.7.4.3.) and creatine kinase (CK, E.C. 2.7.3.2) - Two anodal zones of high activity were seen in muscle and liver on gels stained for ADK. When additional slices of the same gels were stained for CK, patterns with identical mobilities appeared, as well as an additional cathodal area of heavy staining in both tissues. The resolution was poor, so that it could not be determined whether the bands in each zone were single or multiple.

Fumarase (FUM, E.C. 4.2.1.2) - activity was seen at one anodal zone in muscle, liver, and kidney, but the resolution was poor.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, E.C. 1.2.1.12) - One cathodal zone of activity was frequently seen in muscle and liver, and in kidney on one occasion. On starch gels to which NADP had been added, the bands were of good resolution but staining was inconsistent.

β-Glucuronidase (β-GUS) - One anodal zone of activity was seen in liver and kidney at the same mobility. Resolution was good, but this system was tested too late in the starch gel part of the analysis to allow scoring of the entire set of samples. Staining was poor on acrylamide gels.

Lactate dehydrogenase (LDH, E.C. 1.1.1.27) - Activity was seen at the same mobility in muscle, heart, liver, kidney, red cells, and plasma. The number of bands varied from 3 to 5 between tissues, but the patterns

could be interpreted as resulting from a tetrameric protein coded by two loci. Both loci could be scored in muscle, but only the faster one was consistently scorable in the other tissues. Sub-banding at the faster locus was seen in muscle after prolonged storage or repeated thawing. No variation was seen at either locus in any tissue, with the exception of red cells; however, this apparent considerable variation was not repeatable. Because of the question concerning the validity of the blood samples and in an effort to be conservative, LDH-1 and LDH-2 were not scored in the muscle samples despite good resolution.

Malate dehydrogenase (MDH, E.C. 1.1.1.37) - On starch gels, one anodal zone of activity was seen in muscle, liver, kidney, red cells, and plasma. A second cathodal zone was seen in muscle, liver, and kidney. Sub-banding and/or multiple banding was seen in some samples at the anodal zone, but the patterns were not repeatable and no basis for interpretation could be determined. Two anodal zones were seen on acrylamide gels.

Nucleoside phosphorylase (NSP, E.C. 2.4.2.1) - One anodal zone of activity was seen in muscle, liver, kidney, and red cells, but resolution was inconsistent.

Peptidases (E.C. 3.4.) - The substrates tested were leucylalanine, leucylglycine, and leucylglycylglycine. Anodal activity was seen in muscle, liver, kidney, heart, and plasma, but staining was inconsistent. When present, the resolution was usually good. In general, three zones

of activity could be seen in muscle and two in liver and kidney. The slowest band appeared at the same migration distance on gel slices stained with each of the three substrates. The other two zones of activity were quite close together and at least one always appeared on gels stained for leucylglycine or leucylglycylglycine. There were some patterns that indicated possible variation, but because of the inconsistency in staining they could not be interpreted.

Sorbitol dehydrogenase (SDH, E.C. 1.1.1.14) - Good activity was seen in muscle, liver, and kidney, but the enzyme either did not move from the origin or migrated only slightly and was poorly resolved. The mobility was the same on these tissues.

Monomorphic loci

The following systems showed good activity and good resolution but were invariant (95% criterion of polymorphism) in both populations. Unless otherwise noted, all zones of activity stained as a single band, which was assumed to reflect the expression of a single locus.

Aspartate aminotransferase (AAT, E.C. 2.6.1.1) - one anodal and one cathodal zone of activity. Same mobility in muscle, liver, kidney, and heart, though staining was inconsistent in the latter two tissues.

Adenosine deaminase (ADA, E.C. 3.5.4.4) - one anodal zone of activity in liver. Samples from calves migrated slower than samples from adults. Very weak activity in kidney at a different mobility.

Alcohol dehydrogenase (ADH, E.C. 1.1.1.1) - one anodal zone of activity in liver and kidney; no difference in mobility.

Aldolase (ALD, E.C. 4.1.2.13) - one cathodal zone of activity in muscle.

Glucose dehydrogenase (GDH, E.C. 1.1.1.47) - one anodal zone of activity in liver.

General Protein (GP) - four anodal zones of activity in muscle, as well as numerous other bands of poor resolution. The most anodal zone tended to overstain at sample concentrations optimal for scoring the other three, and was not used.

α-Glycerol-3-phosphate dehydrogenase (α-GPD, E.C. 1.1.1.8) - double bands at one anodal zone in muscle. Sub-banding seen in older samples. Active in liver but staining was inconsistent.

Hexokinase (HK, E.C. 2.7.1.1) - one anodal zone of activity in liver. With prolonged staining, a second, less anodal locus stained in some samples, at the same mobility as the bands on gels stained for GK. Only the former was scored.

Isocitrate dehydrogenase (IDH, E.C. 1.1.1.42) - one anodal and one cathodal zone of activity in muscle and liver, no difference in mobilty.

Poor quality samples exhibited anodal sub-banding at the anodal locus, which became a smear as staining progressed.

Leucine aminopeptidase (LAP, E.C. 3.4.1.1) - two anodal zones in plasma, only one of which (the more anodal) stained consistently and could be scored. Very weak activity in liver.

Malic enzyme (ME, E.C. 1.1.1.40) - two anodal zones. Same mobility in muscle, liver, kidney, and red cells. ME-2 was only seen when NADP was added to the gel solution prior to degassing.

Mannose phosphate isomerase (MPI, E.C. 5.3.1.8) - two anodal zones. Same mobility in muscle, liver, heart and kidney, but resolution much better in muscle. Sub-bands present in kidney and heart.

6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44) - one anodal zone. Same mobility but poorer resolution in liver and kidney than in muscle.

Phosphoglucose isomerase (PGI, E.C. 5.3.1.9) - two anodal zones of activity in muscle, heart, and kidney, with the typical three-banded pattern of a dimeric enzyme coded by two loci. Multiple sub-bands in all samples. A single variant individual with six primary bands of equal intensity was noted in the Alaskan population, but due to the many sub-bands, it could not be determined which of the loci was the variable one. An offspring of this individual showed the same pattern as all the

other animals.

Phosphoglucomutase (PGM, E.C. 2.7.5.1) - two anodal zones in muscle, liver, kidney, heart, and red cells; same mobility in all tissues. With HCl buffer, a third zone was seen in liver and kidney, but the resolution with this buffer was not as good. Activity was strong in adult muscle and heart, fair in kidney, and weak in fetal muscles.

Superoxide dismutase (SOD, E.C. 1.15.1.1) - two anodal zones of negative staining appeared on most gels stained for dehydrogenases and for aldolase, as well as for this enzyme specifically. No mobility differences between heart, kidney, and muscle, though the faster locus was not as active as the slower one.

Transferrin (Tf) - one anodal zone was observed. The presence of transferrin was confirmed by precipitation of other plasma proteins with 2% rivanol (Chen and Sutton, 1967). The common pattern was a single band with an anodal sub-band, though older samples showed multiple faster and slower sub-bands. A double-banded variant was seen in one Alaskan individual, in which the second band migrated less anodally than the common band. The bands were of equal intensity, as would be expected for codominant alleles for a monomeric protein. In the absence of other variant individuals, the genetic basis of this variation can not be determined.

Polymorphic locus

The following system showed good activity, good resolution, and variability (95% criterion of polymorphism) in both populations.

Esterase (Est, E.C. 3.1.) - Six zones of activity were seen in liver and were presumed to indicate the expression of six loci. However, staining was stopped after the first two zones had appeared, in order to prevent overstaining. The less anodal zone was invariant. The more anodal zone was variable, with single and double-banded phenotypes present in both populations. The patterns were consistent with allele segregation at a single locus coding for a monomeric enzyme. The less anodal (invariant) zone was present in kidney. The more anodal (variant) zone was not. No activity was observed in muscle or heart at either zone.

DISCUSSION

Hemolysate samples from wild Alaskan animals proved to be of limited usefulness after even short periods of storage. Red cell samples subjected to starch gel electrophoresis within three months of collection showed apparent variability for the loci LDH-2, PGM-2, and ME-1 with TM buffer. The patterns of "variability" were not repeatable on successive gels and there was no correlation between banding pattern and number of times a sample was thawed. Although there was good activity at these three loci and several others in red cells, they were considered unreliable for scoring purposes. Freezing conditions on Nunivak Island and in the Arctic National Wildlife Refuge at the time of muskox hunts made it very difficult to keep whole blood liquid until it could be brought back to a base camp and allowed to settle. Hunters often spent several days in the field before contacting wildlife authorities for check-out procedures. Perhaps the aid of guides and transporters who accompany hunters could be enlisted so that sample collection becomes a routine matter and the quality of samples is raised by familiarity with the technique. If field collection methods can be improved so that blood samples from remote parts of Alaska give consistent banding patterns on electrophoretic gels, data from animals immobilized during radio-collaring operations could be added to that from hunted animals, thereby widening the data base.

It is noteworthy that the oldest samples from Greenland, i.e. 17

muscle samples collected in 1975, exhibited considerable activity for several enzymes. Bands could be seen in more than 50% of the samples at the following loci with the buffers indicated: AAT-1 (1), peptidase-3 (1), MPI-1 (1), PGI-1 and PGI-2 (2), SOD-2 (1), and LDH-1 (TM). The mobility of the bands at these loci was not different from that in fresher samples. However, because of the irregularity of staining of these samples (not all samples stained for all systems), they are not included in Table I. Most reports of electrophoretic analyses use samples up to two years old, and the only systematic study of banding patterns in aging samples (Moore and Yates, 1983) continued for just 12 months. The results from these ten-year old samples suggest that stored tissue samples may be useful for a much longer period than previously assumed, although for a limited number of loci.

The genetic variation reported here for muskoxen is based upon electrophoretic analysis of 28 enzyme and non-enzymatic protein loci. These loci were easily visualized and consistently scorable using techniques reported for analysis of other ungulate species. Subsequent investigations into genetic variation in muskoxen should involve refinement of methods to improve the resolution of the 11 enzyme systems reported here in which activity was seen but could not be scored. Clearly, the greater the number of loci examined, the greater is the chance of finding polymorphisms. Nevertheless, the number of loci examined in this study is comparable to the number examined in other mammalian species for which genetic variation has been computed (Table IV). Inasmuch as there is no apparent pattern in the loci found to be polymorphic among the species examined, there is no basis for concluding

Table IV. Genetic variation in selected ungulate species and mean values for 184 mammalian species.

	n	H	p	number of loci	reference
Sika deer - *Cervus nippon*	25-40	0.0	0.0	19	Feldhamer et al, 1982
Muskoxen - *Ovibos moschatus*	39-87	0.011	0.036	28	this study
Pronghorn - *Antilocapra americana*	5	0.011	0.053	19	Baccus et al., 1983
Wapiti - *Cervus canadensis*	49	0.015	0.105	19	Baccus et al., 1983
Moose - *Alces alces*	165	0.017	0.158	19	Baccus et al., 1983
Bison - *Bos bison*	7	0.023	0.053	19	Baccus et al., 1983
Roe deer - *Capreolus capreolus*	24	0.024	0.105	19	Baccus et al., 1983
Cattle - *Bos taurus*	6	0.035	0.105	19	Baccus et al., 1983
Reindeer - *Rangifer tarandus*	198	0.043	0.086	34	Røed, 1985a
Red deer - *Cervus elaphus*	27	0.047	0.158	19	Baccus et al., 1983
Mule deer - *Odocoileus hemionus*	2	0.053	0.105	19	Baccus et al., 1983
White-tailed deer - *Odocoileus virginianus*	753	0.074	0.358	19	Baccus et al., 1983
184 species of mammals - mean value	199[a]	0.041	0.191	23[b]	Nevo et al., 1984

[a] minimum 10
[b] minimum 14

that examination of a different set of loci would have yielded significantly different results.

The distribution of genotypes at the one polymorphic locus (Est-2) in the Alaska population was not significantly different from a Hardy-Weinberg distribution. Although it is difficult to imagine that the genotype frequencies would be so similar to those of the Greenland population if inbreeding were occurring, this is not conclusive proof that random mating is occurring. In any polygynous species, some father-daughter mating may occur (mother-son mating is possible but less likely), but the coefficient of inbreeding may not be high enough to cause a significant deviation from a Hardy-Weinberg distribution. The coefficient of inbreeding calculated for the Alaska population was, indeed, negative ($f = -0.019$), indicating an excess of heterozygotes (i.e. random mating). However, if two fewer heterozygotes at the Est-2 locus had been observed in this sample, then the coefficient of inbreeding would become positive. The genotype frequencies in that case would still not be significantly different from a Hardy-Weinberg distribution, indicating that some inbreeding could be occurring but would not be detected. The present data would be of greatest utility with respect to inbreeding if compared with genotype frequencies in the Alaska population in the near future. The optimal situation would be regular sampling of Alaskan animals to monitor genotype frequencies and attempt to determine whether inbreeding equilibrium has been reached. A decline in heterozygote frequency from that seen here could indicate a deviation from random mating.

Because the reintroduction of muskoxen to Alaska involved a

founding event, it would be logical to expect to see evidence of this, i.e. lower genetic variation, in Alaskan animals. In fact, Alaskan muskoxen appear to have as much variation as Greenlandic animals. The same two alleles at the one polymorphic locus observed in Greenlandic animals were also seen at that locus in Alaskan individuals.

A significant decline in heterozygosity and the loss of rare alleles due to the founder effect may be avoided in two ways: by drawing a large random sample of animals for the founding group that, as a consequence of size, has a high probability of including all or most of the variation in the progenitor population; or by rapid reproduction of a small founder group following the founding event such that none of the variation included in the founders is lost due to lack of reproductive opportunity (Nei et al., 1975; Frankel and Soulé, 1981). Clearly, if a founding population consists of a monotypic group, then reproduction can not raise the heterozygosity of the next generation unless mutation introduces new variability into the gene pool. However, if a population consisting of few but genetically variable animals breeds rapidly, most, if not all, of the variation present in the parents may be passed on to the offspring before the parents become reproductively inactive.

Indeed, there are two lines of evidence to suggest that the transplantation of muskoxen from Greenland to Alaska did not involve detrimental genetic effects due to the founder effect. The first is the presence in the Alaska population of the single variant individuals for the PGI and Tf loci. It is possible that these variants are cases of new mutation. A more parsimonious explanation, considering the short

time since the transplant, is that they are rare alleles that were present in the Greenland population at the time of the transplant and were included in the founder group. They are now present in the Alaska gene pool at very low frequency ($q < .05$). This implies that the same variants should have been seen in the Greenland population. The Greenland sample for PGI was small ($N = 8$), so it is not unexpected that the rare allele ($q = .008$ in the Alaska population) was absent in it. The absence of the Tf variant in the Greenland sample ($N = 31$) is a little more surprising, yet consistent with its rarity ($q = .012$ in the Alaskan population).

The second line of evidence to suggest that detrimental genetic effects due to the founder effect did not occur is the size of the founding group and its subsequent reproductive history. Size will be considered first. The amount of diversity preserved in a founder population when the founders are not related and not inbred is given by the formula:

$$\text{Diversity} = 1 - \frac{1}{2N_e} \times 100\%$$

where N_e is the effect[illegible]33). It is known

$$\text{Diversity} = 1 - \frac{1}{2N_e} \times 100\%$$

that at least 12 of the original 19 founder cows produced calves during the holding period at the College research station, though there is no information about the number of breeding males (Palmer and Rouse, 1936). Even if only one bull bred all 12 cows, those 13 animals could have contained 86.5% of the genetic variation of the population from which they were drawn. Any bull that bred for the first time in subsequent years was a potential source of new genetic material, equivalent to an immigrant, for the presumed founder group of 13 animals. The maximum

genetic diversity preserved, if all 34 founders contributed to the gene pool of the present Alaska population, is 98.5%. The formula used here for proportion of diversity preserved assumes that the founding individuals were neither related nor inbred. This is speculative, as the founder animals were captured by a contracted hunter and information is not available concerning the number of herds from which they were taken. However, knowing that all but one were calves and yearlings (Palmer and Rouse, 1936) suggests that most of the animals were probably not related. As there are few if any reliable instances of live twinning in muskoxen (Dinneford and Anderson, 1984), though it is believed to occur in Greenland (Thing et al., 1986), it is unlikely that any of the calves were full sibs. If several calves were taken from the same herd, there is some chance that they were half-sibs sired by the same bull. Movements of males (see discussion below) assure that cows are usually bred by different bulls in subsequent years, but because yearlings may remain with their mothers, the possibility that some of the calves and yearlings captured in the same herd were half-sibs (by the same mother) must be considered. Few cows in Greenland successfully raise a calf every year, although this is reported from expanding populations in Canada and Alaska (Thing et al., 1986), so the number of calves and yearlings that were half-sibs in the original group was probably small. The two-year old in the original group may have been a half-sib of other animals.

The reproductive history of the Nunivak Island herd will be considered next. Although calves were produced, the total herd size did not increase significantly during the first decade after release, due to

adult mortality through accident, stranding on shore ice, and shooting (Spencer and Lensink, 1970). The average annual rate of increase thereafter, and until control was begun in 1968, was 16% (Spencer and Lensink, 1970). At the time this figure was reported, it was the fastest rate of increase then known for muskoxen (Spencer and Lensink, 1970). Extrapolating from the model of Nei et al. (1975), a growth rate of r = .16 would be sufficient to maintain a minimum of approximately 65% of the heterozygosity contained in a founder group of 10 individuals. As the founder group size in the muskox transplant was larger than 10, it may be assumed that a growth rate of 16% was rapid enough to maintain more than 65% of the founders' heterozygosity, as indicated by the heterozygosity observed. It is possible, of course, that not enough generations have passed since the founding event to reach the minimum level of heterozygosity. This could be tested by repeated electrophoretic examinations of animals from Nunivak Island.

If the genetic variation detected in this study is an accurate reflection of that in Greenlandic muskoxen, one must then consider whether Greenlandic muskoxen are inherently low in genetic variation or whether a higher level of diversity could have been depleted by genetic drift, inbreeding, or some other factor. The question of low level of genetic variation will be addressed first.

The genetic variation reported here for muskoxen is very low compared with that of other ungulate species (Table IV). However, low diversity is by no means a unique circumstance among mammals. In most mammals, most loci analyzed using electrophoresis are monomorphic. Several mammals have been found to be monomorphic at all loci examined,

including the northern elephant seal (*Mirounga angustirostris*; Bonnell and Selander, 1974), the cheetah (O'Brien et al., 1983), the polar bear (*Thalarctos maritimus*; Allendorf et al., 1979), the red fox (*Vulpes vulpes*), stoat (*Mustela erminea*), weasel (*Mustela nivalis*), polecat (*Mustela putorius*), pine marten (*Martes martes*), beech marten (*Martes foina*), and badger (*Meles meles*) (Simonsen, 1982), the sika deer (*Cervus nippon*; Feldhamer et al., 1982), four species of white-footed mouse (*Peromyscus eva carmeni*, *P. dickeyi*, *P. interparietalis*, *P. stephani*; Avise et al., 1974), and three species of mole (*Scapanus townsendi*, *S. orarius*, and *Condylura cristata* (of which only a single individual was available), Yates and Greenbaum, 1982). Explanations of the lack of genetic variation have been suggested for some of these species. The elephant seal is known to have passed through a severe population bottleneck in recent times (Bonnell and Selander, 1974) and it is suggested that the cheetah may have passed through a population bottleneck in historic times (O'Brien et al., 1983). The sika deer population examined in the study of Feldhamer et al. (1982) is derived from a small number of founders, which may account for the lack of variation; analysis of other sika deer populations is needed before this can be determined. The four monomorphic species of mouse are all island populations, which are particularly susceptible to reduction in variability through the founder effect and subsequent genetic drift (Avise et al., 1974). For the other species, no evidence of a bottleneck or of susceptibility to genetic drift is reported. Thus, it can not be determined with certainty whether the monomorphism seen is the result of such factors or a response to selective pressure. It

should be noted that the wapiti (Cervus canadensis), a species with heterozygosity similar to that of the muskox (Table IV), is not known to have passed through a population bottleneck (Cameron and Vyse, 1978).

The possibility of genetic drift within local muskox populations in Greenland will be considered next. Local coastal muskox populations in Greenland are subject to periodic catastrophic decline or failure of calf crop as a result of severe climatic fluctuations (Vibe, 1967). Mild winter weather, with alternate cycles of rain and sub-zero temperatures, causes a crust of ice to form on the snow, through which muskoxen are unable to break to reach the vegetation below (Vibe, 1958). More animals die in these years than in years of consistently cold dry winters (Vibe, 1958). Severe fluctuations in number are also known from the Canadian High Arctic (Gunn, 1984). However, geographic subdivision of the species is necessary if genetic drift or inbreeding are to have a significant effect in small local sub-populations. This would occur only if the sub-populations remain isolated for many generations.

Whether local groups of muskoxen in Greenland do remain isolated for extended periods is unknown. Regular censuses and marking of animals for movement studies have been conducted in Greenland only since 1981 and results are still preliminary (Lassen, 1984). Pedersen (1936) mentioned that the entire northeast coast of Greenland appeared to have been used by muskoxen at some time, based upon explorers' reports and contemporary sightings, tracks, and remains. It is known from recaptures or resightings of marked muskoxen elsewhere that they may travel extensively. Individual movements of 250 km and 300 km have been documented in the Soviet Union (Uspenski, 1984). A group movement of

170 km and an individual movement of 340 km are reported from Alaska (Grauvogel, 1984). A group movement of 200 km occurred with the migration of Norwegian animals to Sweden (Lundh, 1984). Although these examples all are cases of introduced animals in unfamiliar ranges, they indicate the ability of the animals to survive long-range movements.

The breeding behavior of muskoxen also involves movement of animals, possibly into unused parts of their range. Muskoxen are harem breeders, with dominance hierarchies within both sexes (Smith, 1976). Breeding bulls may displace one another as harems join and fracture during the rutting season (Smith, 1976; Gray, 1979). Subdominant bulls wander extensively during the rut in their search for available cows (Pedersen, 1936). Although more inclined to stay in herds, cows are also known to travel in pairs or small groups (Smith, 1976). Because of the extensive movements of bulls, the formation of new harems every year, a lack of fidelity to all but the best summer ranges, and limits to the number of cows a harem bull can successfully defend from single bulls, there is constant and thorough mixing of animals upon their range (Tener, 1965; Gray, 1979; Smith, 1976, Reynolds, 1984). This not only increases the opportunity for range expansion but also decreases the probability of inbreeding by ensuring that herds are not composed of the same animals from year to year. The concomitant difficulty in establishing the boundaries of muskox populations presents a problem for muskox managers (Gunn, 1984).

Based upon patterns of dental anomalies in skulls, Hendrichsen (1982) suggested that Greenland muskoxen are, indeed, subdivided into three geographic populations. According to his scheme, the progenitors

of the present Alaskan animals belong to one population and the animals sampled in this study to another. Electrophoretic studies of muskoxen from several points along the coast of Greenland might clarify this issue, for migration of even a single individual per generation is sufficient to maintain gene flow between populations (Kimura and Ohta, 1971). If alleles which are different from those seen here are found among Hendrichsen's (1982) putative populations, or if the same alleles are seen at significantly different frequencies, there may be reason to conclude that gene flow is not occurring along the extent of the Greenland coast.

There is a further possible explanation of the low heterozygosity in Greenland muskoxen. Early explorers suggested that muskoxen moved from the North American continent to the Canadian High Arctic islands and then over the frozen channel between Ellesmere Island and Greenland (Greely, 1888, cited in Hone, 1934; Feilden, 1895). It is possible that the original invasion of Greenland by muskoxen travelling over the sea ice from the Canadian High Arctic islands involved a severe bottleneck in the species' history, or that several invasions were necessary before a viable population was established. Colonization by a small group, followed by a slow increase in population size as the species' range increased, may have been accompanied by loss in genetic variation over time due to genetic drift. The date(s) of invasion of Greenland by muskoxen are unknown. Archeological evidence indicates that the earliest human inhabitants of north and northeast Greenland were muskox hunters who arrived from Canada approximately 4000 years ago, and that subsequent human cultures also subsisted largely on muskoxen (Knuth,

1967). Whether muskoxen have inhabited Greenland continuously for the last 4000 years is unknown. Hone's (1934) compilation of explorers' and naturalists' records of visits to Greenland between about 1820 and 1934 clearly indicates that muskoxen do not occupy the same portions of their range from year to year. It is not evident whether this is due to local extinction, movement of entire herds, or simply overlooking of animals that were further inland or at a higher elevation than the people looking for them.

It is now essential to obtain samples from Canadian High Arctic populations, as well as from other locations in Greenland, to see whether the genetic data contained in those groups can contribute to our knowledge of historic muskox movements. Analysis of mitochondrial DNA, in addition to allozyme electrophoresis, should be considered. Further analysis of Alaskan and Greenlandic samples will clarify the frequency of rare alleles and of inbreeding. If Canadian populations are found to have greater heterozygosity than the populations investigated here, introduction of Canadian animals into Alaskan herds and successful cross-breeding might raise the heterozygosity of Alaskan animals. Alaskan muskox managers would need to decide whether to maintain the integrity of the *O. m. wardi* subspecies in Alaska or compromise it by introduction of Canadian *O. m. moschatus*. This is complicated by the suggestion that the native Alaskan muskoxen were the *O. m. moschatus* subspecies (Allen, 1912). Such decisions would be helped by having electrophoretic data from several Canadian populations of both subspecies available.

It is important that Alaskan wildlife managers be aware of the low

level of genetic variation detected in the present study. Although many species that are completely monomorphic at electrophoretic loci appear to be stable, the small amount of variation that Alaskan and Greenlandic muskoxen possess should be safeguarded. Animal breeders and managers know how to reduce the genetic variation in a species, but there is no way to recover genetic resources that have been lost. Until more is known about the selective benefits of particular allozymes, it is prudent to consider that each genotype could confer a selective advantage should environmental conditions change, and that all genotypes should be maintained in the species' gene pool.

As with most preliminary studies, the original objectives of this project have been met to varying degrees. The utility of slab gel electrophoresis for analysis of muskox tissues has been demonstrated; the possibilty of obtaining a large increase in number of usable loci for some investment in methodological refinement was noted. No difference in genetic variation between the Alaska and Greenland populations, and no evidence of a founder effect resulting from the reintroduction of muskoxen into Alaska, was detected. Genotype frequencies in the Alaska population were not significantly different from those expected if random mating were occurring. Muskoxen were found to have less genetic variability than other ungulates which have been examined. These results should be of interest to muskox managers, and it is hoped that they will contribute to the maintenance of a species which thus far has been so successfully rescued from extinction.

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Appendix 1. List of samples collected.

Animal[a]	Date sample(s) taken	Age[b]	Sex[b]	M[c]	L[c]	K[c]	H[c]	P[c]	R[c]
NU-1	5 Sep. 1983	Adult	M		x	x	x		
NU-2	30 Aug. 1983	Adult	M	x	x	x	x		
NU-3	Feb./Mar. 1984	Adult	M	x	x	x	x	x	x
NU-4	Feb./Mar. 1984	Adult	M	x	x	x	x	x	
NU-5	15 Feb. 1984	Adult	M		x	x	x		x
NU-6	Feb./Mar. 1984	Adult	M	x	x	x	x	x	x
NU-7	Feb./Mar. 1984	Adult	M	x	x	x	x	x	x
NU-8	Feb./Mar. 1984	Adult	M	x	x		x		x
NU-9	Feb./Mar. 1984	Adult	M	x	x		x		x
NU-10	10 Mar. 1984	Adult	F	x	x	x	x	x	x
NU-11	5 Mar. 1984	3-4	F	x	x	x	x	x	x
NU-12	10 Mar. 1984	Adult	F		x	x	x	x	x
NU-13	10 Mar. 1984	Adult	F	x	x	x	x	x	x
NU-14	18 Feb. 1984	3	F	x	x	x	x	x	x
NU-15	3 Mar. 1984	Adult	F	x	x	x	x	x	x
NU-18	18 Feb. 1984	3	F	x	x		x	x	x
NU-19	18 Feb. 1984	3+	F	x	x	x	x	x	x
NU-20	13 Feb. 1984	Adult	F	x	x	x	x	x	x
NU-21	16 Feb. 1984	Adult	M	x	x	x	x	x	x
NU-22	19 Feb. 1984	3-4	F	x	x		x		x
NU-23	10 Mar. 1984	Adult	F	x	x	x	x	x	x
NU-24	28 Feb. 1984	3-4	F	x	x	x	x	x	x
NU-25	21 Feb. 1984	Adult	M	x					x
NU-26	26 Feb. 1984	Adult	M	x	x	x	x		
NU-27	21 Feb. 1984	Adult	M	x				x	x
NU-28	19 Feb. 1984	Adult	M	x	x	x	x	x	x
NU-29	17 Sep. 1984	Adult	M	x	x	x	x		
NU-30	26 Feb. 1984	Adult	M	x	x	x	x		
NU-32	28 Feb. 1984	Adult	F	x	x		x		x
NU-34	4 Sep. 1984	4	F	x	x	x	x		
NU-36	3 Sep. 1984	3	F	x	x	x	x		
NU-37	1 Sep. 1984	Adult	F	x	x	x	x		
NU-38	3 Sep. 1984	4	F	x	x	x	x		
NU-39	3 Sep. 1984	unk.	F	x	x	x	x		
NU-40	1 Sep. 1984	Adult	F	x	x	x	x		

Appendix 1. List of samples collected (continued).

Animal[a]	Date sample(s) taken	Age[b]	Sex[b]	M[c]	L[c]	K[c]	H[c]	P[c]	R[c]
NU-41	9 Sep. 1984	Adult	M	x	x	x	x		
NU-42	13 Sep. 1984	Adult	M	x	x	x	x		
NU-62	2 Mar. 1984	Adult	M		x	x	x		x
NU-63	2 Mar. 1984	Adult	F	x	x	x	x	x	x
NU-64	1 Mar. 1984	Adult	M	x	x	x	x	x	x
NU-65	28 Feb. 1984	Adult	F	x	x	x	x	x	x
NU-66	Feb./Mar. 1984	unk.	unk.	x	x	x	x		x
NU-67	3 Mar. 1984	Adult	M	x	x	x	x	x	x
NU-69	11 Mar. 1984	Adult	M	x	x	x	x	x	x
NU-70	10 Mar. 1984	Adult	F	x	x	x	x	x	x
NU-71	10 Mar. 1984	Adult	M	x	x	x	x	x	x
NU-72	11 Mar. 1984	3	F	x	x	x	x	x	x
NU-73	6 Sep. 1984	unk.	F	x	x	x	x		
NU-74	2 Mar. 1984	Adult	M	x	x	x	x		x
NU-75	3 Sep. 1984	Adult	F	x	x	x	x		
NU-76	8 Sep. 1984	4	F	x	x	x	x		
NU-RJH	11 Mar. 1984	3	F					x	x
NU-VS	Feb./Mar. 1984	unk.	F	x					
NU-LS	Feb. 1984	Adult	F	x					x
NU-JF	Feb./Mar. 1984	unk.	unk.	x	x				
NU-I	Feb./Mar. 1984	unk.	unk.						x
NU-II	Feb./Mar. 1984	unk.	unk.						x
NU-III	Feb./Mar. 1984	3	F					x	x
NELS-31	25 Mar. 1984	Adult	M	x	x	x	x		x
NELS-47	22 Mar. 1984	Adult	M					x	x
NELS-48	22 Mar. 1984	Adult	M					x	x
NELS-59	19 Mar. 1984	3+	M	x	x	x	x		x
NELS-60	1 Mar. 1984	unk.	M	x		x	x		x
NELS-X	23 Mar. 1984	Yearl.	M	x	x	x	x		
GR-50	Nov. 1982	unk.	unk.			x			
GR-51	Nov. 1982	unk.	unk.			x			
GR-52	Nov. 1982	unk.	unk.			x			
GR-53	Nov. 1982	unk.	unk.			x			
GR-54	Nov. 1982	unk.	unk.			x			
GR-55	Nov. 1982	unk.	unk.			x			

Appendix 1. List of samples collected (continued).

Animal[a]	Date sample(s) taken	Age[b]	Sex[b]	M[c]	L[c]	K[c]	H[c]	P[c]	R[c]
GR-56	Nov. 1982	unk.	unk.			x			
GR-57	Nov. 1982	unk.	unk.			x			
GR-58	Nov. 1982	unk.	unk.			x			
GR-45	Jul. 1983	unk.	unk.					x	x
GR-218	Jul. 1983	unk.	unk.					x	x
GR-364	Jul. 1983	unk.	unk.					x	x
GR-365	Jul. 1983	unk.	unk.					x	x
GR-366	Jul. 1983	unk.	unk.					x	x
GR-367	Jul. 1983	unk.	unk.					x	x
GR-368	Jul. 1983	unk.	unk.					x	x
GR-373	Jul. 1983	unk.	unk.					x	x
GR-375	Jul. 1983	unk.	unk.					x	x
GR-378	Jul. 1983	unk.	unk.					x	x
GR-379	Jul. 1983	unk.	unk.					x	x
GR-382	Jul. 1983	unk.	unk.					x	x
GR-384	Jul. 1983	unk.	unk.					x	x
GR-388	Jul. 1983	unk.	unk.					x	x
GR-389	Jul. 1983	unk.	unk.					x	x
GR-390	Jul. 1983	unk.	unk.					x	x
GR-392	Jul. 1983	unk.	unk.					x	x
GR-394	Jul. 1983	unk.	unk.					x	x
GR-395	Jul. 1983	unk.	unk.					x	x
GR-396	Jul. 1983	unk.	unk.					x	x
GR-400	Jul. 1983	unk.	unk.					x	x
GR-403	Jul. 1983	unk.	unk.					x	x
GR-409	Jul. 1983	unk.	unk.					x	x
GR-101	winter 1975	Adult	F	x					
GR-102	winter 1975	Adult	M	x					
GR-103	winter 1975	Young	unk.	x					
GR-104	winter 1975	unk.	unk.	x					
GR-105	winter 1975	Adult	M	x					
GR-106	winter 1975	Adult	F	x					
GR-107	winter 1975	Adult	M	x					
GR-108	winter 1975	Adult	M	x					
GR-109	winter 1975	Adult	F	x					

Appendix 1. List of samples collected (continued).

Animal[a]	Date sample(s) taken	Age[b]	Sex[b]	M[c]	L[c]	K[c]	H[c]	P[c]	R[c]
GR-110	winter 1975	Adult	M	x					
GR-111	winter 1975	Juv.	unk.	x					
GR-112	winter 1975	Juv.	unk.	x					
GR-113	winter 1975	Juv.	unk.	x					
GR-114	winter 1975	unk.	unk.	x					
GR-115	winter 1975	unk.	unk.	x					
GR-116	winter 1975	unk.	unk.	x					
GR-117	winter 1975	unk.	unk.	x					
GR-500	summer 1983	3	F	x	x	x	x		
GR-501	summer 1983	3	F	x	x	x	x		
GR-502	summer 1983	Adult	F			x			
GR-503	summer 1983	Adult	F			x			
GR-504	summer 1983	Adult	M	x		x			
GR-505	winter 1984	Adult	F	x	x		x		
GR-506	winter 1984	Young	M	x	x	x	x		
GR-507	winter 1984	Young	F	x		x	x		
GR-508	winter 1984	Adult	F	x	x	x	x		
GR-509	winter 1984	Adult	F	x	x	x	x		
TR-6	fall 1982	14	F					x	
TR-7	fall 1982	14	F					x	
TR-9	fall 1982	14	F					x	
TR-10	fall 1982	14	F					x	
TR-11	fall 1982	14	F					x	
TR-14	Aug. 1983	14	F					x	
TR-15	Aug. 1983	14	F					x	
TR-16	fall 1982	9	F					x	
ANWR-1	Mar. 1984	Adult	M	x	x		x		
ANWR-2	Mar. 1984	Adult	M		x		x		
ANWR-3	25 Mar. 1984	Adult	M	x	x	x	x	x	
ANWR-5	6 Mar. 1984	Adult	M	x	x	x	x	x	x
ANWR-6	6 Mar. 1984	Adult	M	x	x	x	x	x	x
LARS-63	spring 1983	Adult	M					x	x
LARS-66	28 Apr. 1983	5	F	x	x	x	x	x	x
LARS-66C	24 Apr. 1983	Fetus	F	x	x	x	x		
LARS-69	spring 1983	Adult	M					x	x

Appendix 1. List of samples collected (continued).

Animal[a]	Date sample(s) taken	Age[b]	Sex[b]	M[c]	L[c]	K[c]	H[c]	P[c]	R[c]
LARS-88	16 Apr. 1983	5	F	x	x	x			
LARS-88C	16 Apr. 1983	Fetus	F	x	x	x	x		
LARS-91	28 Apr. 1983	7	F	x	x	x	x		
LARS-91C	28 Apr. 1983	Fetus	M	x	x				x
LARS-99	spring 1983	Adult	M					x	x
LARS-BC	11 May 1983	Calf	M	x	x	x	x	x	
LARS-DC	9 May 1983	Calf	F	x	x	x	x		
LARS-Da	spring 1983	Adult	F					x	x
LARS-Un	spring 1984	Yearl.	F					x	x
LARS-Gr	spring 1984	Calf	M					x	x
LARS-Ta	spring 1984	Calf	F						x
LARS-Br	spring 1984	3	F					x	x
LARS-Si	spring 1984	2	F					x	
LARS-Nu	spring 1984	3	M					x	
LARS-Ik	spring 1984	3	M					x	
LARS-Re	spring 1984	3	F					x	

[a] NU - Nunivak Island; NELS - Nelson Island; GR - Jameson Land, East Greenland; TR - Tromsø; ANWR - Arctic National Wildlife Refuge; LARS - Large Animal Research Station, Fairbanks

[b] as provided by hunter or researcher who took the sample

[c] M = muscle, L = liver, H = heart, K = kidney, P = plasma or serum, R = red blood cells or whole blood

Appendix 2. Gel solutions.

I. Acrylamide gels (Davis, 1964)

A. running gel (7% acrylamide):
16 ml running gel acrylamide solution
8 ml running gel buffer solution
40 ml deionized water
7 ml catalyst solution

B. stacking gel (2.5% acrylamide):
3 ml stacking gel acrylamide solution
1.5 ml stacking gel buffer solution
6 ml sucrose solution
1.5 ml deionized water
1 ml catalyst solution

II. Starch gels

A. LiOH buffer (9.5 % starch)

thin gels:	thick gels:
9.5 g Electrostarch	14.25 g Electrostarch
29.0 g Connaught starch	43.0 g Connaught starch
400 ml gel buffer	600 ml gel buffer

B. TM buffer (10-10.5 % starch)

thin gels:	thick gels:
9.5 g Electrostarch	12.0 g Electrostarch
32.0 g Connaught starch	48.0 g Connaught starch
400 ml gel buffer	600 ml gel buffer

Appendix 3. Buffer and other stock solutions.

I. Acrylamide gels

A. Buffer stock solutions

1. medium pH discontinuous (2) (Jolley and Allen, 1965)

a. running gel buffer:
10.65 g Tris
0.23 ml TEMED
20 ml deionized water
adjust pH to 7.2 with 1N HCl (approx. 48 ml)
bring to 100 ml with deionized water

b. stacking gel buffer:
6.0 g Tris
0.46 ml TEMED
20 ml deionized water
adjust pH to 7.3 with 1N HCl (approx. 48 ml)
bring to 100 ml with deionized water

c. electrode buffer:
10.0 g Tris
28.8 g glycine
bring to 1 L with deionized water
adjust pH to 8.5 and dilute 1:9 before using

2. high pH discontinuous (1) (Davis, 1964)

a. running gel buffer:
36.6 g Tris
0.23 ml TEMED
20 ml deionized water
adjust pH to 8.9 with 1N HCl (approx. 48 ml)
bring to 100 ml with deionized water

b. stacking gel buffer:
5.98 g Tris
0.46 ml TEMED
20 ml deionized water
adjust pH to 6.7 with 1N HCl (approx. 48 ml)
bring to 100 ml with deionized water

c. electrode buffer:
6.0 g Tris
28.8 g glycine
bring to 1 L with deionized water
adjust pH to 8.3 and dilute 1:9 before using

Appendix 3. Buffer and stock solutions (continued).

B. Acrylamide stock solutions (Davis, 1964)

1. running gel:
   28.0 g polyacrylamide
   0.735 g bisacrylamide
   100 ml deionized water

2. stacking gel:
   10.0 g polyacrylamide
   2.5 g bisacrylamide
   100 ml deionized water

C. Other stock solutions

1. sucrose:
   10.0 g sucrose
   25.0 ml deionized water

2. catalyst:
   0.35 g ammonium persulfate
   25.0 ml deionized water

3. sample buffer:
   1 part stacking gel buffer solution
   3 parts deionized water
   4 parts sucrose solution
   5-10 drops 0.002% bromphenol blue solution

II. <u>Starch gels</u>

A. Tris-maleic acid (TM) (Selander et al., 1971)

1. tray (electrode) buffer:
   72.6 g Tris
   69.6 g maleic acid
   22.32 g $Na_2EDTA \cdot 2H_2O$
   12.18 g $MgCl_2 \cdot 6H_2O$
   6 L deionized water
   adjust pH to 7.4 with 2 M NaOH or 30 g NaOH pellets

2. gel buffer:
   1:9 dilution of tray buffer

Appendix 3. Buffer and other stock solutions (continued).

B. Lithium hydroxide (LiOH) (Selander et al., 1971)

1. tray (electrode) buffer:
   use solution A

2. gel buffer:
   1 part solution A : 9 parts solution B, pH 8.2 in final solution

   a. stock solution A, pH 8.1:
      7.2 g $LiOH \cdot H_2O$
      71.34 g H_3BO_3
      bring to 6 L with deionized water

   b. stock solution B, pH 8.4:
      9.6 g citric acid
      37.2 g Tris
      bring to 6 L with deionized water

C. Other solutions

1. grinding solution:
   1.21 g Tris
   0.37 g EDTA
   1 L deionized water
   adjust pH to 6.8 with HCl
   add 4 ml NADP stock solution

Appendix 4. Stain solutions.

I. Stock solutions

NAD:
1 g NAD
100 ml deionized water

NBT:
1 g NBT
100 ml deionized water

PMS:
1 g PMS
100 ml deionized water

NADP:
1 g NADP
100 ml deionized water

MTT:
1 g MTT
100 ml deionized water

α-NA esterase:
1 g α-naphthyl acetate
50 ml deionized water
50 ml acetone

1.0 M Na-arsenate:
31.2 g Na_2HAsO_4
100 ml deionized water

12% TCA:
120 g trichloroacetic acid
1 L deionized water

Coomassie blue:
250 mg Brilliant Blue R
100 ml deionized water

0.1 M $MgCl_2$:
20.3 $MgCl_2$
1 L deionized water

Appendix 4. Stain solutions (continued).

0.2 M Tris-HCl:
24.22 g Tris
1 L deionized water
adjust to desired pH with 1N HCl

0.5 M Tris-HCl:
60.55 g Tris
1 L deionized water
adjust to desired pH with 1N HCl

0.1 M Tris-maleic acid, pH 5.2:
12.1 g Tris
11.6 g maleic acid
900 ml deionized water
adjust pH with 2M NaOH
bring to 1 L with deionized water

2 M malic acid, pH 7.0:
268.2 g DL-malic acid
1 L deionized water
adjust pH with approx. 150 g NaOH added slowly with beaker placed in ice bath

0.5 M phosphate buffer, pH 7.0:
16.617 g monobasic potassium phosphate
21.75 g dibasic potassium phosphate
bring to 500 ml with deionized water
adjust pH with KOH pellets

II. Staining solutions

AAT (Manlove et al., 1976):
50 ml 0.2 M Tris-HCl, pH 8.0
0.5 mg pyrridoxal-5'-phosphate
200 mg L-aspartic acid
100 mg α-ketoglutaric acid
150 mg Fast Blue BB
Incubate at 37 C in dark

ADH (Harris and Hopkinson, 1976):
100 ml 0.05 M Tris-HCl, pH 8.0
1 ml ethanol
25 mg NAD
20 mg MTT
5 mg PMS
Incubate at 37 C in dark

Appendix 4. Stain solutions (continued).

ALD:
100 ml 0.1 M Tris-HCl, pH 8.0
1 ml 1 M Na-arsenate
40 u glyceraldehyde-3-phosphate dehydrogenase
60 mg D-fructose-1,6-diphosphate
0.8 ml NAD
0.5 ml NBT
0.2 ml PMS
Incubate at 37 C in dark

Esterase (Harris and Hopkinson, 1976):
0.05 M phosphate, pH 6.5
40 mg Fast Blue BB
4 ml α-NA
Incubate at room temperature in light

GDH:
100 ml 0.05 M phosphate, pH 7.5
5 g β-D-glucose
20 mg NAD
25 mg NBT
5 g PMS
Incubate at 37 C in dark

GP and Tf (Diezel et al., 1972):
Pour sufficient 12% TCA over gel to cover. Allow to stand 5 min. at room temperature. Add 5-10 ml Coomassie blue solution. Swirl. Cover, leave over night. Rinse in deionized water. Use several changes of destaining solution.

α-GPD (Manlove et al., 1976):
50 ml 0.2 M Tris-HCl, pH 8.0
1 ml 0.1 M $MgCl_2$
50 mg α-DL glycerophosphate
2 ml NAD
1.3 ml NBT
0.5 ml PMS
Incubate at 37 C in dark

HK (Ayala et al., 1972):
100 ml 0.05 M Tris-HCl, ph 7.1
900 mg glucose
20 mg $MgCl_2$
25 mg ATP
25 mg NADP
20 mg MTT
80 u glucose-6-phosphate dehydrogenase
3 mg PMS
Incubate at 37 C in dark

Appendix 4. Stain solutions (continued).

IDH:
45 ml deionized water
5 ml 0.5 M Tris-HCl, pH 7.0
0.2 ml 0.1 M $MgCl_2$
150 mg DL-isocitric acid
1.2 ml NADP
1.0 ml MTT
0.1 ml PMS
Incubate at 37 C in dark

LAP (Bush and Huettle, 1972):
30 ml 0.1 M Tris-maleic acid, pH 5.2
20 ml deionized water
12.5 mg Black K salt
10 mg L-leucyl-β-naphthylamide-HCl
Incubate at 37 C in dark

ME:
30 ml deionized water
10 ml 0.1 M Tris-HCl, pH 8.4
2.5 ml 2 M malic acid, pH 7.0
0.5 ml 0.1 M $MgCl_2$
1 ml NADP
1 ml NBT
0.5 ml PMS
Incubate at 37 C in dark

MPI (Ayala et al., 1976):
100 ml 0.05 M Tris-HCl, pH 8.0
30 mg mannose-6-phosphate
160 u phosphoglucose isomerase
80 u glucose-6-phosphate dehydrogenase
20 mg MTT
20 mg NADP
5 mg PMS
Incubate at 37 C in dark

6PGD (Manlove et al., 1976):
20 ml 0.02 M Tris-HCl, pH 8.0
14 ml 0.1 M $MgCl_2$
40 mg 6-phosphogluconic acid
0.2 ml NADP
0.8 ml MTT
0.2 ml PMS
Incubate at room temperature in dark

Appendix 4. Stain solutions (continued).

PGI (Ayala et al., 1972):
100 ml 0.1 M Tris-HCl, pH 7.1
20 mg fructose-6-phosphate
200 mg $MgCl_2$
25 mg EDTA
10 mg NADP
20 mg MTT
80 u glucose-6-phosphate dehydrogenase
5 mg PMS
Incubate at 37 C in dark

PGM (Ayala et al., 1972):
100 ml 0.1 M Tris-HCl, pH 7.1
600 mg glucose-1-phosphate
200 mg $MgCl_2$
10 mg NADP
20 mg MTT
80 u glucose-6-phosphate dehydrogenase
3 mg PMS
Incubate at 37 C in dark

SOD:
100 ml 0.1 M Tris-HCl, pH 8.0
10 mg $MgCl_2$
20 mg MTT
5 mg PMS
Incubate at room temperature in light