Examensarbete
i ämnet biologi

The impact of founder events and introductions on genetic variation in the muskox *Ovibos moschatus*

Linda Englund

Photo: Stefan Mörtberg

Handledare: Göran Ericsson, Carl-Gustaf Thulin & Göran Spong

30 Poäng, D-nivå

Vilt, Fisk och Miljö
SLU
901 83 Umeå, Sweden

2008:12

Examensarbete
i ämnet biologi

The impact of founder events and introductions on genetic variation in the muskox *Ovibos moschatus*

Linda Englund

Photo: Stefan Mörtberg

Supervisors: Göran Ericsson, Carl-Gustaf Thulin & Göran Spong
30 Point, D-Level

Wildlife, Fish, and Environmental Studies
Swedish University of Agricultural Sciences
SE901 83 Umeå, Sweden

2008:12

ABSTRACT

The muskox is an ungulate that is well adapted to severe arctic conditions. Native populations are today found in northern Canada, the Canadian archipelago and on the northeastern coast of Greenland. Throughout its existence the muskox has been subject to many fluctuations in population size, both due to changing climate and intensive hunting during the hide trade in the 18- and 1900's. However, small refugia have persisted, allowing the muskox to increase in numbers again. In addition, introductions and translocations of the muskox around the Arctic have allowed the species to colonise new territories such as west Greenland and Norway. In 1971 five muskoxen left Norway and founded a population in Härjedalen, Sweden. Today (2008) the Swedish population consists of seven individuals. When trying to reinforce the genetic variation within the population one cow was mated with a captive Greenlandic bull, which resulted in a calf in 2006. To find out how muskox populations have been affected by sequential founder events muskox samples from the Canadian archipelago, east and west Greenland, Norway and Sweden were studied, using highly variable microsatellite markers. The result shows that the allelic variation follows the expectations of the founder events where Canada has the highest variation, followed by Greenland. However, the Swedish population has more genetic variation than Norway. This is explained by the contribution of two new alleles by the half-Greenlandic calf, indicating that one individual can make a large impact regarding the genetic variation. Also, the zoo population contains a higher degree of genetic variation than many of the introduced populations, revealing the importance of preserving the breeding programmes in zoos.

Key words: Canadian archipelago, genetic variation, Greenland, introductions, management, microsatellites, muskox, Norway, *Ovibos moschatus*, sequential founder events, Sweden.

CONTENTS

1 INTRODUCTION

1.1 Genetic variation

Genetic variation is an important factor in the long-term persistence of populations and is required for populations to adapt to, for example, environmental changes. The importance of genetic variation is particularly relevant in species with fragmented and isolated distribution, severe reductions in population size, and with continuously small numbers (Lacy 1997; Saccheri *et al.* 1998; Westemeier 1998; Frankham *et al.* 2002; Spielman *et al.* 2004). The population size of species may be affected by deterministic factors, such as habitat loss and overexploitation, which can cause reduced, fragmented and/or isolated populations. Consequently, when the population size is small, the influence of stochastic factors, such as demographic, and environmental, is much higher than for large population sizes, causing fluctuations in the population (Frankham *et al.* 2002; Reed *et al.* 2002). Fragmented and small populations are also more vulnerable to genetic stochasticity than a larger population, where in a small population genetic variation might be lost randomly through genetic drift (Hedrick and Kalinowski 2000; Spielman *et al.* 2004), causing a change in allele frequency and a loss of genetic variation (Frankham *et al.* 2002).

A decrease in population size and the following loss of genetic variation could be caused by bottlenecks or founder events. A bottleneck occurs when a large population experiences a prolonged decrease in the effective population size (N_e); and a founder event when a few individuals found a population (Nei *et al.* 1975; Frankham *et al.* 2002). According to Clegg *et al.* (2002) a single founder event is unlikely to affect the genetic variation within a population, while three or four sequential founder events are more likely to have an effect. Among populations of moose (*Alces alces*) in Canada, a single founder event resulted in an average reduction of 22% in observed heterozygosity, and a loss of nearly 46% for two successive founder events (Broders *et al.* 1999). The genetic variation that is retained after a bottleneck is dependent on the number of founders and the rate of the recovery. Unless the recovery is rapid, the genetic variation will decrease even further through genetic drift, resulting in lowered heterozygosity (DeYoung and Honeycutt 2005). In the long-term, this will increase the risks of extinction, especially if no immigration is possible due to isolation of the population. That a severe population bottleneck leads to a decrease in genetic variation has been shown in, among others, koalas, *Phascolarctos cinereus* (Houlden *et al.* 1996), pronghorn antelopes, *Antilocapra americana* (Stephen *et al.* 2005), northern elephant seal, *Mirounga angustirostris* (Weber *et al.* 2000) and in greater prairie chicken, *Tympanuchus cupido* (Bouzat *et al.* 1998).

In a small population directed matings between relatives might become unavoidable. This phenomenon, also known as inbreeding, may result in increased homozygosity and exposure of deleterious, recessive alleles. Further on this might lead to a reduced reproduction and survival, which will affect the growth rate and decrease the effective population size (Frankham *et al.* 2002). The smaller the population the more change there will be between the parental and offspring gene pools (Frankham *et al.* 2002). The negative impact of inbreeding is termed inbreeding depression. Inbreeding depression has been shown in both captive and wild mammalian populations (Laikre and Ryman 1991; Hedrick 1995; Laikre *et al.* 1996; Roldan *et al.* 1998; Slate *et al.* 2000; Cassinello 2005). Among 38 species, from 40 captive mammalian populations, the average mortality was 33% higher in inbred animals, than in offspring of unrelated parents (Ralls *et al.* 1988). Thus the possibility of inbreeding depression is important to consider preventing future fitness reduction, particularly among introduced and reared populations (Hedrick and Kalinowski 2000).

1.2 The muskox

The muskox, *Ovibos moschatus*, is an arctic living mammal, which can stand dry climate and low temperatures. The only weather condition that can cause them severe stress is the occurrence of very humid winters with heavy snowfall, rain and ice cover on the ground, which prevents the muskox from foraging (Vibe 1967; Barr 1991; Forchhammer and Boertmann 1993). They live in herds of a few up to about 20 individuals and their main predators are wolves and humans (Tener 1965; Vibe 1967; Borgen 1979; Nowak 1999). Muskox groups are not stable units, but the often mixed-sex groups change frequently; to some extent due to season (Tener 1965; Reynolds 1993). Native populations of muskoxen are today living in northern Canada, the Canadian archipelago, and along the northeastern coast of Greenland (McDonald and Davis 1989; Groves 1997; Bennike and Andreasen 2005) (Appendix I). The total population of muskoxen worldwide (both native and introduced) is today roughly estimated to be around 150 000 animals, but the data is incomplete (Table 1). There are two subspecies of muskox; the barren ground muskox (*Ovibos moschatus moschatus*), on the mainland of Canada, and the white faced muskox (*Ovibos moschatus wardi*), which populates the Canadian archipelago, and Greenland, and which also has been used for the many introductions around the Arctic (Borgen 1979; Rowell 1990; Lent 1999; Holst and Carlsen 2002).

Table 1 Estimated number of individuals of muskoxen worldwide, both native and introduced.

Country	Nr of individuals	Year	Reference
Canada	~130 000	2007	Van Coeverden De Groot (pers. comm.)
Alaska	~4000	2002	Patricia Reynolds (pers. comm.)
Greenland	~20 000	2007	Peter Aastrup (pers. comm.)
Norway	~200	2006	Rangbru and Andreassen 2006
Sweden	7	2008	Lars Rehnfeldt (pers. comm.)
Total:	**154 207**		

The earliest ancestors of the modern muskox evolved in southern central Asia during the late Miocene, more than ten million years ago. During the Pleistocene (1.8 million – 11 500 years ago) the muskox spread from Asia over the northern world (Lent 1999). It seems like the muskox spread to Alaska across the Bering Strait around 150 000-250 000 years ago (Tener 1965; Lent 1999). After our last ice age, around 10 000 years ago, the muskox was extinct in Europe, Asia and northwest North America (Borgen 1979). At the same time the diversity of the large mammal fauna of North America and Eurasia was reduced overall (Lent 1999). After the last glacial period, the muskox remained in Alaska and northern Canada and spread to northern and eastern Greenland, where they arrived at least 4000 years ago (Vibe 1967; Borgen 1979; Lent 1999; Bennike and Andreasen 2005). Even before the arrival of humans, it seems like the muskox was not an abundant species in the periglacial environments of Europe, Beringia and North America (Lent 1998; Lent 1999). According to fossil studies, by MacPhee *et al.* (2005), the *Ovibos* of late Quaternary (around 18 000 years ago) were supposedly more genetically diverse compared to modern muskoxen. In general, the muskox appear to have low genetic variation compared to other land mammals (Fleischman 1986; Groves 1997; Mikko *et al.* 1999), which makes the genetic definition of muskox populations difficult (Groves 1997; Van Coeverden De Groot unpublished results).

After the arrival of the Europeans to Canada and Alaska in late 17th to late 18th century, the muskox was threatened with extinction due to commercial exploitation. The already existing climate variation caused additional fluctuations among the populations of muskox, adding to the extinction risk (Tener 1965; Borgen 1979; Barr 1991; Reynolds 1998; Lent 1999). In the late 1800's the muskox had gone extinct in Alaska (Lent 1998; Lent 1999) and similar patterns were seen in Canada, where an estimated 400-500 muskoxen remained in 1917 (Barr 1991). This situation gave rise to the enactment of protective legislation of the muskox by the Canadian Government in 1917 (Tener 1965; Barr 1991; Lent 1999) and since then the populations have steadily grown (Barr 1991). During the beginning of the 1900's, several introductions of muskoxen to both former inhabited and uninhabited areas were done (Spencer and Lensink 1970; Jingfors and Klein 1982; Uspenski 1984; Le Hénaff and Crête 1989; Lundh 1996; Lent 1999) (Appendix I and II). In Greenland, the muskox populations have been fluctuating a lot, both due to overexploitation and to differing climate conditions (Jennov 1955; Lent 1999). To establish a new meat source and to guard the muskox from extinction, a total of 27 muskoxen were relocated, between 1962 and 1965, from northeast Greenland to the southwest side of Greenland, Angujaartorfiup Nunaa (Olesen 1993; Pedersen and Aastrup 2000; Raundrup 2002), which is not known to have been inhabited by muskoxen before (Vibe 1967). The population grew rapidly and in 1999 it stabilized at around 3000 animals (Pedersen and Aastrup 2000). The muskox has at least experienced two natural population fluctuations (Tener 1965) through the natural colonisation of the Canadian archipelago, followed by the colonisation of Greenland. Together with the numerous translocations and introductions of the species (Lent 1999), muskoxen might have experienced significant genetic bottlenecks in recent as well as in prehistoric times.

1.3 The Scandinavian muskoxen

The suggestion to introduce muskoxen from east Greenland to Norway was first postulated by G.E. Broms in 1900. The expedition failed along with the following six attempts by other expeditions, until 1947, when the first introduction of muskoxen to Norway succeeded (Lundh 1996). During the years of 1947-1953 an amount of 27 calves from east Greenland were released in Dovre, Norway. Several died during the first year, and the final founder population consisted of ten animals (Lundh 1996). Compared to introductions in Alaska and Greenland, the population development was low (Fig. 1), but the population slowly grew in size (Rangbru and Andreassen 2006), and today (2008) the estimated population size is 190 animals (Directorate for Nature Management, Norway 2008).

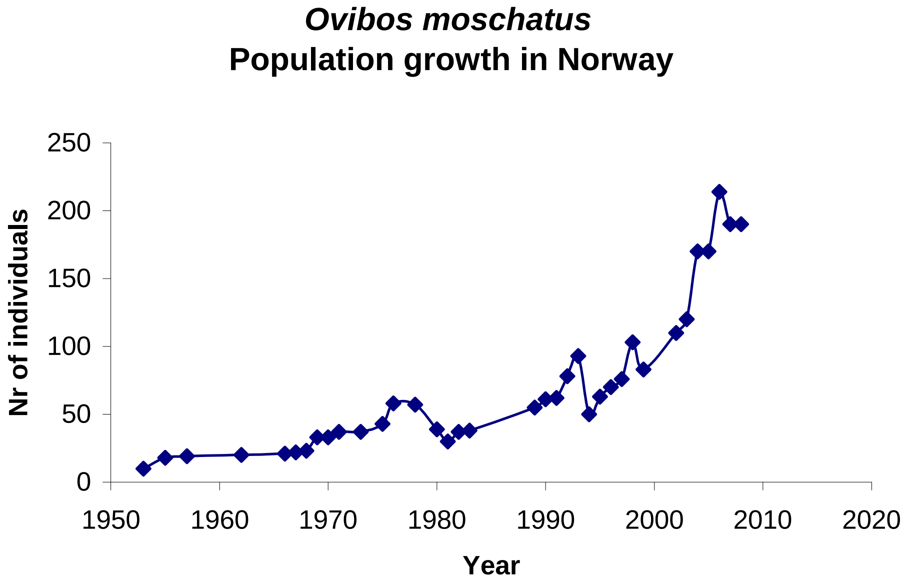


Figure 1 The population growth of the Norwegian population in Dovre, from year 1953 until 2008. (Directorate for Nature Management, Norway 2008; Williams Torg 2008).

In 1971 five animals from the Norwegian population left Norway, potentially a result of disturbance by photographers and tourists (Alendal 1974), and went into Härjedalen, Sweden. The small population had then walked around 200 km and they settled in an alpine area around Rogen nature reserve. The population consisted of two adult females with one calf each, and one adult male that was the father of the two calves (Alendal 1974). They slowly increased in numbers (Fig. 2) and in the mid 1980's the population peaked at 36 individuals (pers. comm. Lars Rehnfeldt). However, since then the population has steadily decreased in size and today (2008) there are only seven individuals left.

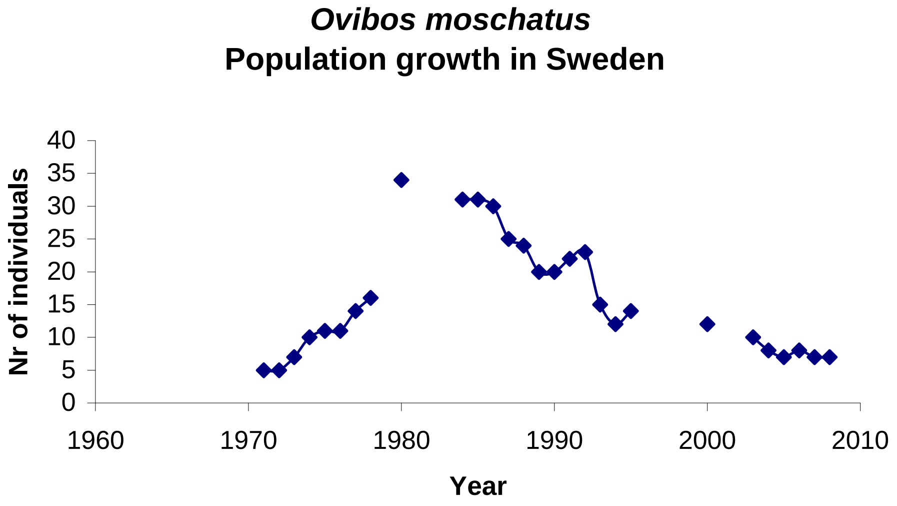


Figure 2 The population growth of the Swedish population from year 1971 until today, 2008. Data are missing for ten years. (Lundh 1996; Lent 1999; Myskoxarna).

Although there are several alternative explanations for the decline of the Swedish muskox population, the most widely accepted suggestion is that the population suffers from inbreeding (Laikre *et al.* 1997; Larsson 2003; Larsson and Larsson 2006). The five founders of the Swedish population have originated from a population that has passed at least two founder events. First, ten animals from east Greenland founded the Norwegian population in Dovre between 1947 and 1953, which had increased to 37 individuals when five of them went into Sweden in 1971 (Lundh 1996). The extent of inbreeding in the Swedish muskox population is not known in detail, but according to Laikre *et al.* (1997) it is probably high, where the five founders grew to 36 animals and then declined to seven individuals, suggesting that incestuous mating has occurred.

The decreasing numbers of animals in Härjedalen gave rise to a local committee (Project "Rädda myskoxen") that aims to secure the muskox population in Härjedalen. The male present in the population of eight individuals in 2004 was presumably related to some extent with the other individuals and was removed and replaced with a captive bred male, which unfortunately died before he managed to mate any of the cows. In another attempt to increase the gene pool of Swedish muskoxen, one of the wild cows was transferred to a zoo (Järvzoo) in central Sweden to mate with a captive bull from Greenland. This was successful and in 2006 the cow gave birth to a calf in the wild.

1.4 Genetic markers

To study long-term rates of population interchange and variation among populations affected by founder events and bottlenecks, highly variable genetic markers, such as microsatellites are commonly used (Cornuet and Luikart 1996; Haig 1998; Luikart *et al.* 1998a; Luikart *et al.* 1998b). Microsatellite loci are short tandem repeats (di, tri or tetra) of DNA sequences of 2-5 base pairs that are found in many regions in genomes of most species (Jarne and Lagoda 1996; Chambers and MacAvoy 2000; DeWoody 2005). They typically reveal high levels of polymorphism in populations and are considered as selectively neutral and co-dominant (Engel *et al.* 1996; Jarne and Lagoda 1996; Frankham *et al.* 2002; DeWoody 2005).

1.5 Objectives

The purpose of this study is to assess the degree of genetic variation and differences within and between introduced and native populations of muskoxen in Canada, Greenland, Norway and Sweden, using polymorphic microsatellite markers. The objective is also to see if the genetic variation gradually is reduced after each of the founder events the populations have experienced. Finally, I aim to assess the feasibility to use faecal samples to receive reliable genetic data, and to determine the degree of genetic variation in the Swedish muskox population in particular, in order to evaluate the need for genetic restoration.

2 MATERIALS AND METHODS

2.1 Samples

A total of 234 samples were collected, of which 25 were blood samples, 164 tissue samples (heart, kidney, liver and muscle tissue) and 45 faecal samples. All of the samples were stored in freezer after arrival, and 49 of the tissue samples were also stored in ethanol to secure the transportation of the samples. The samples were either collected in the field, or provided by researchers and colleagues worldwide (Table 2). The samples represent some of the wild muskox populations (Appendix I) and a large proportion of the current zoo population in Scandinavia. The objective was to collect DNA samples from 30 individuals per population, which would enable me to detect most of the variation present (Paetkau *et al.* 1999; Sjögren and Wyöni 1994). This was however not possible for the Swedish population, due to the small total population size (eight specimens in 2006). Thus, from the eight individuals in the Swedish population in Härjedalen, 27 fresh faecal samples were collected during August 2006. To avoid human DNA in the samples, and also to avoid contamination between the samples, each sample was handled separately with disposable plastic gloves and put into plastic tubes which were labelled with place, date, and, if possible, identity. As soon as possible after being collected, the samples were placed in dry ice. The death of one Swedish cow in late 2006 provided me with a reference tissue sample that was used to verify that cow's unique genotype among the faecal samples. Also, samples from deceased wild Swedish muskoxen were collected. From the Swedish Museum of Natural History five samples were received (A1984/5116, A1988/5095, A1989/5057, A1994 /5134, 2004/5253), found between 1984 and 2004 in Härjedalen. From the National Veterinary Institute in Uppsala two samples were received, found in 1994 and 1996. In total, samples from 15 individuals were collected from the wild Swedish population, including both the ones alive today, and seven deceased animals. From the Norwegian population in Dovre, samples from 28 specimens were sent from the Veterinarian Institute of Oslo, the Veterinarian Institute of Trondheim and one specimen from Kolmården zoo. Greenlandic (east and west) samples from 89 individuals were sent from Canada and Denmark. Another 22 samples from five islands (Appendix I) of the Canadian archipelago were received. To be able to analyse the genetic variation within the Scandinavian muskox population held at zoo's, samples of blood, faecal pellets and, if plausible, tissue and hair samples were collected from zoos in Scandinavia (Kolmården zoo, Lycksele zoo, Järvzoo, Copenhagen zoo). In total, samples were collected from 25 zoo individuals.

Table 2 Number of samples collected from each population studied, and the total number of individuals the samples derive from. The Swedish population is divided into the individuals from the present population, and those individuals that are deceased. The cow that died after collecting the samples is included in the present population. The samples from the present population of Sweden were collected from eight individuals but only five genotypes were received.

Population		**Number of samples**			**Number of animals from which samples were taken**	**Samples given by**
		Tissue	**Blood**	**Faeces**		
Canada		22			22	Peter J. Van Coeverden De Groot
Can/Gree		13			13	Peter J. Van Coeverden De Groot
Greenland	**East**	58			58	Peter J. Van Coeverden De Groot Mads Forchhammer and Niels Martin Schmidt
	West	29	1	2	31	Mads Forchhammer and Niels Martin Schmidt Katrine Raundrup and Gunnar Mylius Pedersen Jessica Åsbrink, SVA Carsten Grøndahl, Copenhagen Zoo
Norway		32	1		29	Nina Brekke Tvedt, vet.inst. Oslo Marthe Opland, vet. inst. Oslo Johan Schulze, vet. inst. Trondheim Bengt Röken and Anna Martinsson, Kolmården Zoo
Sweden - present pop		1	1	27	8 (5)	Lars Rehnfeldt Bengt Röken and Anna Martinsson, Kolmården Zoo Personal sampling
Sweden - deceased		8	1		7	Peter Mortensen and Göran Frisk, NRM Jessica Åsbrink, SVA Bengt Röken and Anna Martinsson, Kolmården Zoo
Zoo		1	21	16	25	Carsten Grøndahl, Copenhagen Zoo Olle Larsson, Järvzoo Bengt Röken and Anna Martinsson, Kolmården Zoo Lycksele Zoo
Total:		**164**	**25**	**45**	**185**	

2.2 DNA extractions

A small piece of tissue was placed in an eppendorf tube containing 500 μl chris lysis buffer (0.1 M Tris HCl pH 8.5, 0.05 M EDTA, 2% SDS, 0.2 M NaCl) and 5 μl proteinas K (20 μg/μl). The tube was placed in a heat cabinet for 2.5-3 hours, and was being shaken every half an hour. Thereafter it was centrifuged and the supernatant was recovered. It was mixed with 500 μl isopropanol to be able to recover the DNA, which in turn was washed with 1 ml ethanol. The DNA was redissolved in 10-100 μl of TE buffer, depending on how much DNA was visible, and kept in a freezer until used. When extracting DNA from the blood samples two different types of DNA kit were used. For those samples that contained quite a lot of blood the E.Z.N.A Blood DNA Miniprep Kit was used, following the manufactures protocol. For the samples that contained low volumes of blood (<10μl) the QIAGENE DNeasy Blood and Tissue Kit was used instead. For the faecal samples the E.Z.N.A Stool DNA Miniprep Kit was used, and the instructions in the protocol were followed. The DNA concentration was measured in most, but not in those samples showing visible DNA, using a spectrophotometer (Nanodrop), revealing concentrations between 0.2-4181.8 ng/μl.

2.3 Genotyping

To assess genetic variation 10 different microsatellite primers were used, all optimized from muskoxen and previously shown to amplify polymorphic loci among muskoxen (Appendix III). Primer OM54-23, OM58-06, OM53-38, OM50-08, OM51-19, OM56-30, OM51-16 were optimized by Van Coeverden De Groot and Boag (2004), and three other muskox primers, MoDIAS1, MoDIAS2, MoDIAS5, were designed by Holm *et al.* (1999). The PCR procedure was performed in a 10 μl solution, containing 1 μl 10X buffer (Biopool), 2.5-3.0 mM $MgCl_2$, 0.0625 mM dNTP each, 0.06 μl polymerase Taq, the interval of 0.2-1.0 μM of each forward and reverse Primer and 1-2 μl of the extracted DNA. The reason for the differing amounts of primer and extracted DNA was the varying quality of the DNA. For the PCR, two different touchdown cycles were used, TD65-55VL and MY68-63 (Appendix III). TD65-55VL have an initial denaturation step at 95°C for 3 min, 20 cycles with 20 s denaturation at 95°C, 30 s annealing at 65°C (lowered 0.5°C/cycle), and 30 s elongation at 72°C. This was followed by 25 cycles of 20 s in 95°C, 30 s in 55°C and 72°C in 30 s. The last cycle had a duration of 4 min in 72°C. The MY68-63 program started with an initial denaturation temperature of 94°C for 3 min and was followed by 10 cycles with denaturation temperature of 94°C for 30 s, 20 s annealing at 68°C (lowered 0.5°C/cycle), and 10 s elongation at 72°C. This was followed by 25 cycles of 30 s in 94°C, 20 s of 63°C, and 10 s of 72°C. The last cycle had a temperature of 72°C for 3 min. The amplified products were analysed on an automated DNA sequencer (Beckman Coulter CEQ 800). Sometimes, depending on the quality of the sample, multiplex was performed with OM58-06, OM53-38, MoDIAS5 and MoDIAS2. Multiplex was also done with the primers OM54-23 and MoDIAS2 and with OM51-19 and OM50-08.

2.4 Genetic analyses

The Excel add-in software Microsatellite Tool Kit (Park 2001), was used to calculate expected heterozygosity (H_e), observed heterozygosity (H_o), and allele frequency, number of alleles, number of polymorphic loci and to create both a genepop file and a text file. In the program Genepop on the Web (http://genepop.curtin.edu.au/), versions 3.1c-3.4 (Raymond and Rousset 1995), I conducted exact tests for deviations from Hardy-Weinberg equilibrium, together with linkage disequilibrium, and calculated inbreeding coefficient (F_{IS}). When calculating F_{IS}, a weighted ANOVA test was performed, following Weir and Cockerham (1984). To measure the degree of population differentiation, F_{ST} (Weir and Cockerham 1984) was calculated in Genetix 4.05 (Belkhir *et al.* 1996-2004). In addition to

F_{ST}, Nei's unbiased genetic distance (Nei's D) (Nei 1978; Takezaki and Nei 1996) was calculated, also in Genetix 4.02. Standard deviation of F_{ST} and Nei's D was also estimated in Genetix, using 10 000 permutations. To measure the genetic structuring among the muskox populations and calculate the probability of origin for each individual included in the study, an assignment test was performed using the software Geneclass2 (Piry *et al.* 2004). To calculate the probability of origin of individuals, standard criterion described by Rannala and Mountain (1997) was used, together with the simulation algorithm for population assignment described by Paetkau *et al.* (2004) simulating 10 000 genotypes. Probability tests of sampling efficiency and the probability of individuals with identical genotypes (PI) were performed in Excel. Calculations of the predicted genetic variation within the Swedish population, without immigration and assuming the initial population consists of one fertile male and five fertile females, was also done in Excel. When applicable, significance levels were corrected according to the Bonferroni procedure.

3 RESULTS

A total of 113 individuals were used in the final genetic analysis. The samples that produced less than 50% of the genotype were excluded from the analyses (Table 3). The number of genotyped specimens from each of the populations varied between 9 and 29 (Table 3). The six different populations are abbreviated MC (Canada), MGE (East Greenland), MGW (West Greenland), MN (Norway), MS (Sweden), and MZ (zoo).

Table 3 A schematic overview of all the samples prepared. The table shows the origin of the population and which region the samples came from. N_{TOT} is the total number of samples that were received from each population, D is the number of samples that were double, from the same individual, N_{NP} is samples that were not prepared, N_U is the number of samples that were used in the study, $G_{100\%}$ are those samples that were genotyped at 100%, and so on down to 50%. Those that were below 50% genotyped were excluded from the study.

Population	**Region**	N_{TOT}	D	N_{NP}	N_U	***G*100%**	***G*90%**	***G*80%**	***G*70%**	***G*60%**	***G*50%**	**G<50%**
Canada	North Islands	22			19	11	6		1	1		3
Can/Green	Unknown	13			0							13
Greenland	East	58		27	14	9	3		1		1	17
	West	32	1	1	29	22	6				1	1
Norway	Dovre	33	4		20	17	2	1				9
Sweden	Härjedalen	37	25		9	9						3
Zoo		38	13		22	6	6	4	2	3	1	3
Total		**233**	**43**	**28**	**113**	**74**	**23**	**5**	**4**	**4**	**3**	**49**

3.1 Genetic variation

The loci MoDIAS1 and OM58-06 were monomorphic and therefore excluded from the statistical analyses. No significant linkage between any of the loci was detected. Most polymorphism was detected in Canada, then west Greenland, followed by Sweden, east Greenland and Norway (Table 4). The 18 samples from the study by Van Coeverden De Groot and Boag (2004) represent the whole native distribution of muskoxen and, thus, detect more variation than in this study. For example, the loci MoDIAS1 and OM58-06 were monomorphic in this study, but in Van Coeverden De Groot and Boags (2004) they showed three and two alleles respectively. After Bonferroni corrections for multiple comparisons (Rice 1989), one locus (OM56-30) has a significant deviation from Hardy Weinberg equilibrium in the Canadian archipelago ($p<0.001$), east Greenland and the zoo population. Also a significant deviation was observed in OM54-23 in the east Greenland population ($p<0.01$). The highest number of identical genotypes was observed in the west Greenland population (2, 2, and 7 respectively). The samples were derived from separate individuals (pers. comm. Katrine Raundrup) but many of the samples were presumably collected from the same family group which potentially explains the high number of identical individuals. The Canadian archipelago has the highest observed heterozygosity, surprisingly followed by the Swedish population, and then by west Greenland and east Greenland (Table 4 and Fig. 3). The zoo population has comparably high genetic variation, which is not a surprise since the individuals originate both from Norway and Greenland. The inbreeding coefficient (F_{IS}) was highest in the native population of east Greenland and lowest in west Greenland, which is a little surprising (Table 4). The F_{IS} calculated for the Swedish population was negative, also a bit surprising.

Table 4 Genetic variation results in all the studied populations. The table shows the origin of the population and which region the samples came from. N is number of specimens used, N_A is total number of alleles, N_{PA} is number of private alleles for each population, P_{LOCI} is the number of polymorphic loci, $N_{A/LOCI}$ is the mean number of alleles per loci and population, H_E is the expected heterozygosity, H_O is the observed heterozygosity and F_{IS} is the inbreeding coefficient.

Population	**Region**	**N**	N_A	N_{PA}	P_{LOCI}	$N_{A/LOCI}$	H_E	H_O	F_{IS}
Canada	North Islands	19	21	8	8	2.63	0.35±0.08	0.26±0.04	0.28
Greenland	East	14	15	1	4	1.88	0.26±0.10	0.10±0.03	0.64
	West	29	14	1	5	1.75	0.13±0.04	0.13±0.02	0.02
Norway	Dovre	20	11	0	3	1.38	0.10±0.06	0.10±0.02	0.04
Sweden	Härjedalen	5	13	0	4	1.63	0.14±0.06	0.15±0.06	-0.07
Zoo	Copenhagen Järvzoo Kolmården Lycksele	22	15	0	3	1.88	0.15±0.08	0.13±0.03	0.10
Global		**109**	**26**	**10**	**8**	**3.25**	**0.33±0.10**	**0.14±0.01**	

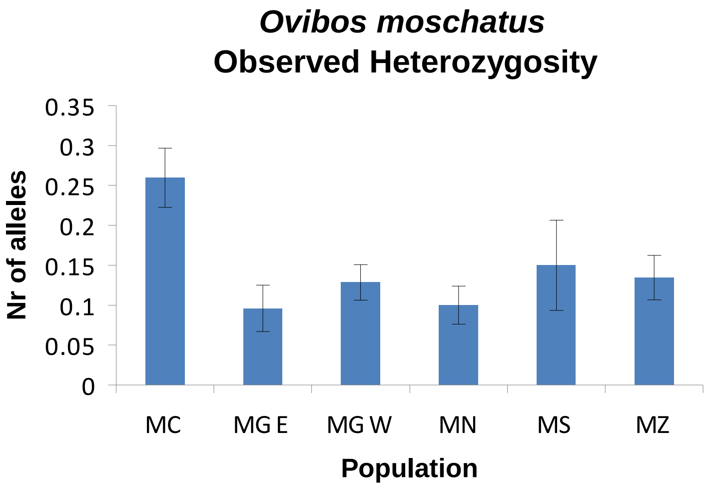


Figure 3 The observed heterozygosity, with standard deviation, among all investigated populations. MC is the Canadian archipelago; MGE is east Greenland, MGW is west Greenland; MN is Norway; MS is Sweden and MZ is the zoo population.

3.2 Differentiation

The allele frequencies differ quite a lot between loci and populations (Table 5). Interestingly, one allele (239) was detected in all other populations except Norway. In Sweden this allele occurred in the highest frequency. Two different alleles (160 and 178) were only discovered in two heterozygote individuals respectively; both found within the Canadian population.

Table 5 Observed allele frequency distributions by locus and population. MC is Canada; MGE is East Greenland; MGW is West Greenland; MN is Norway; MS is Sweden and MZ is the zoo population.

		Population					
Locus	**Allele**	**MC**	**MGE**	**MGW**	**MN**	**MS**	**MZ**
OM54-23	**104**	—	0.04	—	—	—	—
	106	0.74	0.25	0.84	—	0.10	—
	108	0.26	0.71	0.16	1.00	0.90	1.00
OM53-38	**159**	0.75	0.43	0.88	0.13	0.90	0.12
	161	0.25	0.57	0.13	0.88	0.10	0.88
MoDIAS5	**176**	0.97	1.00	1.00	1.00	1.00	1.00
	178	0.03	—	—	—	—	—
MoDIAS2	**204**	—	—	0.05	—	—	—
	206	0.83	1.00	0.95	1.00	1.00	1.00
	208	0.17	—	—	—	—	—
OM50-08	**158**	0.97	1.00	1.00	1.00	1.00	1.00
	160	0.03	—	—	—	—	—
OM51-19	**211**	0.50	—	—	—	—	—
	213	0.50	0.50	0.13	0.48	0.20	0.70
	215	—	0.20	0.88	0.53	0.80	0.30
OM56-30	**221**	0.21	—	—	—	—	—
	231	0.21	—	—	—	—	0.05
	235	0.34	0.31	0.88	—	—	0.05
	237	0.05	0.58	0.03	0.95	0.10	0.67
	239	0.16	0.08	0.09	—	0.80	0.17
	241	—	—	—	0.05	0.10	0.05
	243	—	0.04	—	—	—	0.02
	247	0.03	—	—	—	—	—
OM51-16	**263**	0.82	—	—	—	—	—
	267	0.11	1.00	1.00	1.00	1.00	1.00
	269	0.07	—	—	—	—	—

The pairwise F_{ST} estimate between populations was highest between west Greenland and Norway and lowest between Norway and the zoo animals (Table 6). According to the F_{ST} estimates, the Swedish population is more separated from the Norwegian population than from east Greenland. The population assignment test provides support for the differentiation revealed with the F_{ST} estimates, where the muskoxen in Norway and west Greenland almost doesn't assign to each other at all, while the Norwegian muskoxen assign to the zoo individuals (Table 7). Although some specimens may assign to several other populations when the threshold value of *p* has been set at 0.05, several of the populations are completely excluded as possible origin (Table 7). A surprising result, which is also seen in the F_{ST} estimates, is that the Swedish muskoxen does not assign to the Norwegian ones, but mostly to the individuals in Greenland (Table 7).

Table 6 Estimates of pairwise genetic differentiation between populations, computed using allele frequencies of the eight microsatellite markers. Estimates of F_{ST} (Weir and Cockerham 1984) in lower matrix, and Nei's unbiased genetic distance (Nei 1978) in the upper matrix. Estimates with * are significant at p<0.05, based on 10 000 permutations performed in Genetix 4.05 (Belkhir *et al.* 1996-2004), and with Bonferroni corrections (Rice 1989).

	MC	**MGE**	**MGW**	**MN**	**MS**	**MZ**
MC	—	0.30*	0.28*	0.49*	0.37*	0.42*
MGE	0.36*	—	0.15*	0.03	0.11	0.03
MGW	0.47*	0.40	—	0.37*	0.18*	0.37*
MN	0.58*	0.15	0.70*	—	0.21*	0.01
MS	0.42*	0.23	0.52	0.60*	—	0.19*
MZ	0.52*	0.11	0.66*	0.07	0.50*	—

Table 7 Results from population assignment test, performed in GENECLASS2 (Piry *et al.* 2004), using the standard criterion of Rannala and Mountain (1997), the simulation algorithm of Paetkau *et al.* (2004), and 10 000 simulated individuals. A threshold *p* value of 0.05 was applied for assignments. Thus, some specimens may assign to more than one population, and some not at all. The total number of assignments for each population are presented on the right hand side of the table.

	MC	**MGE**	**MGW**	**MN**	**MS**	**MZ**	**Total**
MC (19)	19	9	3	0	0	4	**35**
MGE (14)	9	14	7	9	4	14	**57**
MGW (29)	25	29	29	0	1	26	**110**
MN (20)	5	20	2	20	4	20	**71**
MS (5)	1	5	4	0	5	5	**20**
MZ (22)	11	22	6	15	4	22	**80**

According to the results in Table 4, the total number of alleles per population and the mean number of alleles per loci and population almost follows the assumption where the highest genetic variation should be found in the Canadian archipelago, followed by east Greenland, the introduced population in west Greenland together with the introduced Norwegian population and last the resulting population of two founder events, the Swedish population. This pattern of allele loss within the populations would follow the expectations for the sequential founder events (Fig. 4). However, the only population to deviate from this assumed pattern is the Swedish, which has more alleles than Norway.

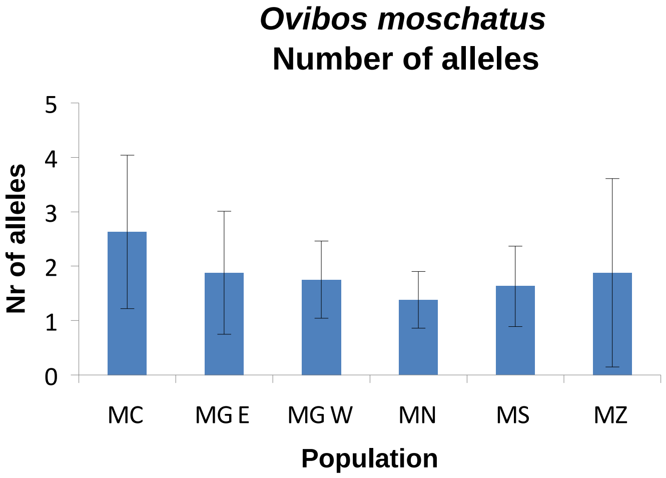


Figure 4 The mean number of alleles per locus and population with included standard deviation. MC is the Canadian archipelago; MGE is east Greenland, MGW is west Greenland; MN is Norway; MS is Sweden and MZ is the zoo population.

3.3 Faecal samples

The DNA extractions from the faecal samples produced high quality template. Out of the 27 faecal samples from the wild Swedish population of eight specimens, five separate genotypes were distinguished. The probability test showed that there is about an 80% possibility that all eight individuals within these 27 samples have been sampled. After calculating $P_{(ID)}$ (probability of identity) for the five genotypes discovered, there is around a 94% possibility that there are two or more identical genotypes within the samples (Fig. 5). This indicates that I have sampled all eight individuals in the wild Swedish population and that there are two or more specimens with identical genotypes.

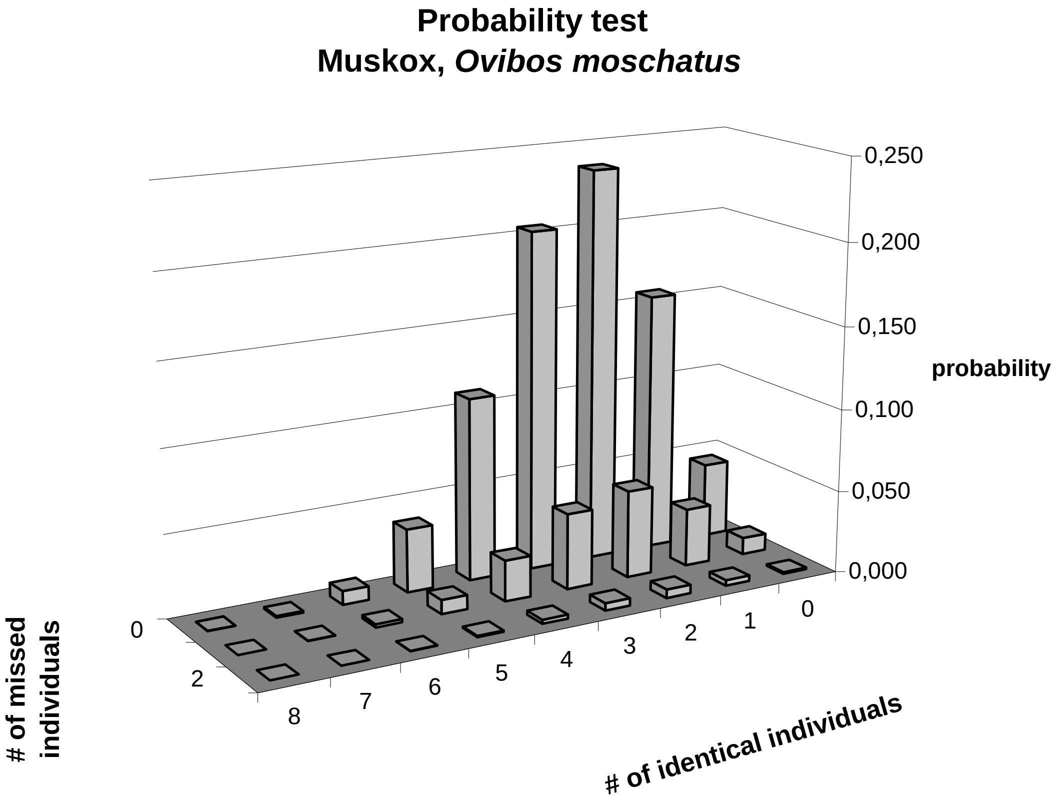


Figure 5 Probability test showing the possibility of identical individuals and how large the possibility is that individuals have been missed among the 27 samples, from eight individuals, of the wild Swedish population.

3.4 The Swedish population

The two alleles 106, locus OM54-23 and 161, locus OM53-38 found in the Swedish population are probably unique for the calf. The sire of this calf was a Greenlandic bull in Järvzoo. Although there is a possibility that another individual in the Swedish herd has exactly the same genotype, I regard it as highly unlikely, since allele 106 has not been observed in the Norwegian population. In Greenland, however, allele 106 is more abundant than allele 108, which is common in Sweden and Norway. If the half-Greenlandic calf hypothetically is removed from the population the Swedish population has exactly the same number of alleles as do Norway. When simulating the future appearance of the observed heterozygosity of the wild Swedish population, assuming one male and five females that all are sexually mature (Fig. 6), the result show that after only four generations the whole population of today would be homozygote if no new genetic material were to be introduced.

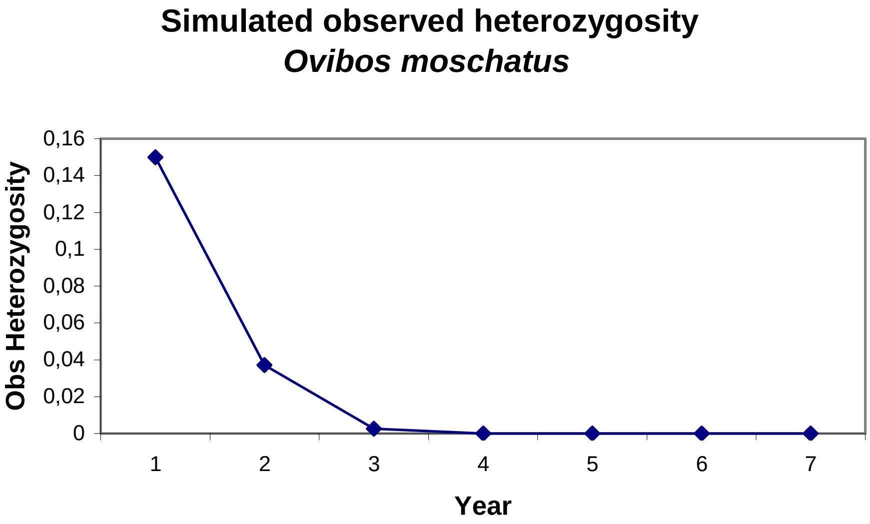


Figure 6 The simulated scenario for the wild Swedish population, starting with their observed heterozygosity of today, 0.1500, assuming no immigration or emigration, and one male and five females that all are sexually mature.

DISCUSSION

4.1 Genetic variation

Microsatellites reveal variation that is not expressed phenotypically. However, the loss of marker variation is indicative of a genome wide depletion of genetic variation, and is often associated with reduced viability or reproduction (Reed and Frankham 2003). The low genetic variation detected in previous studies of the muskox (Fleischman 1986; Groves 1997; Mikko *et al.* 1999; MacPhee *et al.* 2005) was confirmed by this study, where the native muskoxen of the Canadian archipelago has the highest heterozygosity (H_o = 0.26), which also has been concluded in previous studies (Van Coeverden De Groot unpublished results). Compared to other species, the heterozygosity in this study is much lower among muskoxen in the Canadian archipelago together with the global observed heterozygosity (0.14). For example a reintroduction of 17 pronghorn antelopes (*Antilocapra americ ana*) resulted in a population with lower variation and significant genotypic differentiation (Stephen *et al.* 2005). Another population with higher heterozygosity is a mouflon (*Ovis aries*) population founded by only two individuals in 1975, which had an average individual H_o of 0.48 in 2003 (Kauffer *et al.* 2007). Among introduced populations of moose (*Alces alces*) in Canada, two populations that derived from only six founders, had an observed heterozygosity of 0.22 and 0.31 respectively (Broders *et al.* 1999). The low genetic variation found in muskoxen seems to have persisted for a long time (MacPhee *et al.* 2005). Still, *Ovibos moschatus* is one of the few megafaunal mammals that have survived into recent times (MacPhee *et al.* 2005). High latitude species are experiencing both long-term and short-term fluctuations in numbers (Forchhammer and Boertmann 1993), which often results in a small long-term effective population size (Holm *et al.* 1999).

It was unexpected that the population in west Greenland, founded by 27 individuals, has a higher genetic variation (H_o = 0.13) than the original population in east Greenland (H_o = 0.10). An explanation to this might be that the muskox population of east Greenland, although native, seems to suffer from large fluctuations in population size (Vibe 1967) where genetic variation is lost through stochasticity. The muskoxen of east Greenland were apparently absent or at a very low number in the first half of the 1800 and not until 1869 the first live muskoxen was observed (Ferns 1974; Born *et al.* 1998). After that the population has increased, but severe winters have caused high mortality with years where no calves have been observed in some locations (Vibe 1967). Contrary to this population, the population of west Greenland has expanded rapidly after introduction, with no major fluctuations in population size.

The deviation from the Hardy Weinberg equilibrium detected in the Canadian archipelago, east Greenland, and the zoo population could be explained by inbreeding (i.e. non-random mating), where the higher number of homozygotes deviates from the equilibrium. Another explanation is the Wahlund effect where separated populations are pooled, which can result in fewer heterozygotes than anticipated from the number of alleles present. These three populations are sampled from several populations respectively. The Canadian samples derive from five locations from three islands, where supposedly the gene flow between the islands is quite low. The east Greenland population samples were collected from two populations approximately 500 kilometres apart. This might also explain the high estimate of the inbreeding coefficient found in Canada and east Greenland. That the Swedish population showed an excess of heterozygotes (negative F_{IS} estimate) is probably due to the genotype contributed by the half-Greenlandic calf.

4.2 Differentiation

The frequencies of alleles between the populations differ extensively in some of the loci, which also result in high F_{ST} estimates (Table 6). The reason for this could be an effect of random processes involved during the founder events, for example that few translocated specimens carried alleles that were rare in the ancestral population. The alleles that are in very low frequency and only detected in a few or only one of the populations, are examples of alleles with such a low frequency e.g. alleles 160 and 178 in locus OM50-08 and MoDIAS5 that was only observed in one individual respectively. The F_{ST} and Nei's D estimates show that Sweden is more separated from the Norwegian population than from east Greenland. Due to stochastic effects, the Swedish founders might have brought the genotypes that are more common in east Greenland than in Norway, from the Norwegian population. This might also explain the lack of the allele 239 in Norway, which was found with the highest frequency in Sweden. Also, considering that the five Swedish founders probably were a family group with high internal relatedness, they could have brought along a fraction of the gene pool that might have been reduced in Norway as a consequence. Another explanation for that Swedish muskoxen assign Greenlandic muskoxen more than Norwegian, is that the genotype of the half-Greenlandic calf has alleles that are more common in Greenland than in Norway. This show, that in such a small sample size as five individuals, one individual contributes substantially to the overall genetic variation. Both the change in allele frequencies and the loss of alleles is an example of how stochastic events affect small populations.

4.3 Sequential founder events

The mean number of alleles detected per locus and population (Fig. 4) almost follows the expectations from sequential founder events with subsequent bottlenecks. The reason why the observed heterozygosity (Fig. 3) part from these expectations could be explained by the assumption of Nei *et al.* (1975), that the amount of reduction of heterozygosity due to a bottleneck depends on both the size of the bottleneck and the rate of increase after the bottleneck, while loss of alleles mostly depends on the size of the bottleneck. Inevitably, when the population is very small the gene frequency will change through random genetic drift (Nei *et al.* 1975). However, if a population increases rapidly after a founder event, like the west Greenland population did, the reduction in average heterozygosity might be small indicating that populations in general loses rare alleles much faster than their heterozygosity (Nei *et al.* 1975). Also, as mentioned earlier, the result may differ in the east Greenland population due to the Wahlund effect. That the populations have been affected by the numerous founder events and prolonged bottlenecks are clearly seen in the allele variation (Fig. 4) and the overall low genetic variation.

4.4 Management suggestions

Among some studied species that have gone through large bottlenecks or founder events there are no apparent phenotypic deviances or negative fitness consequences (Paetkau *et al.* 1998; Broders *et al.* 1999). However, even if no visible effects are showing, species with decreased genetic variation might have harder to adapt to new changes in the environment. Inbred animals might be less resistant to parasites, which has been shown in soay sheep, *Ovis aries* (Coltman *et al.* 1999), experience decreased lifetime breeding success, which is seen among red deer, *Cervus elaphus* (Slate *et al.* 2000) and have lower birth weight and juvenile survival, shown in harbour seals, *Phoca vitulina* (Coltman *et al.* 1998). However, the effects of inbreeding on fitness vary and might be expressed differently between species (Mills and Smouse 1994; Hedrick and Kalinowski 2000). Also, some endangered species with successful breeding programs have had low founder numbers, for example four specimens of Speke's gazelle (*Gazella spekei*), 13 of Przewalski's horse (*Equus przewalskii*) and six black-footed ferret (*Mustela nigripes*) (Hedrick and Kalinowski 2000). These examples point out the difficulties in predicting the effect of inbreeding on fitness in an endangered population. Nevertheless, even if there is no evident inbreeding or inbreeding depression, the possibility is important to consider preventing future fitness reduction (Hedrick and Kalinowski 2000). Even though the muskox seems to have adapted to their low genetic variation, the Swedish population, with no visible inbreeding depression, might have difficulties adapting to a future change in their environment. Of course, with only seven individuals left, the population is extremely vulnerable to stochastic events such as diseases, unfavourable weather conditions or lightning. In 1978 a lightning strike killed 12 muskoxen in Norway, which reduced the Dovre population with 25% (Williams Torg 2008). The cause of the decline in the Swedish population is probably a combination of several factors, such as inbreeding, unsuitable climate and habitat, together with the additional disturbance from tourists.

Surprisingly the Norwegian population has a lower number of heterozygotes than the Swedish population. However, when removing the genotype of the half-Greenlandic calf from the Swedish population, the observed heterozygosity is lowered. This indicates, together with the two new alleles contributed by the calf, that the calf makes a large difference with respect to the genetic variation. This clearly shows the importance of releasing single individuals into small populations. It could, at least momentarily, increase the genetic variation considerably and potentially prevent inbreeding depression. According to the simulated heterozygosity of the Swedish population, assuming a generation time of 3-4 years in *Ovibos*, the whole population would be genetically monomorphic within approximately 15 years (Fig. 6). However, considering the fact that there is no mature male, and probably reduced fertility among the five females in the population today, the decline may be far more rapid. When homozygosity increases it also increases the chances of the expression of deleterious recessive alleles (Coltman *et al.* 1998), and thus the risk of extinction. To be able to reverse a population decline based on these factors, the main task would be to create a gene flow into the population. Many populations have been rescued back to pre-bottlenecked levels after immigration has occurred, which has been observed in the adder *Vipera berus* (Madsen *et al.* 1999) and in song sparrows *Melospiza melodia* (Keller *et al.* 2001). It has been suggested that genetic effective population size (N_e) should always be kept above 50 to retain evolutionary potential, and when considering long-term conservation, a N_e ranging between 500 and 5000 is said to be necessary to avoid loss of variation and evolutionary potential (Lande 1995; Allendorf and Ryman 2002). However, this differs between species and populations. For example Paetkau *et al.* (1998) reveals that Kodiak bears (*Ursus arctos*) have persisted and thrived in isolation although having a low N_e. Even small efforts can have a large impact, as seen with the half-Greenlandic calf. Mills and Allendorf (1996) suggest that one to ten migrants per generation into a population are sufficient to reduce the loss of genetic variation. However, sometimes an average of more than one migrant might be necessary, especially in a population where the N_e is much less than the total population size and where the receiving population has been isolated for many generations. First of all, the Swedish muskox population obviously needs at least one mature male and also several more fertile young females to survive. If the calf is a male it will, at the earliest, be sexually mature during 2009. The source population, for supplementary release, should be a more genetically diverse population than the Swedish, to increase the variation. It is important that the individuals are not taken from already introduced populations to avoid the risk of accumulated deleterious alleles. With these directions in mind, the Canadian archipelago and the east Greenland population seems to be the most suitable source populations. According to the assignment test the Canadian archipelago is the more divergent of those two populations. However, it should be done with caution, when local adaptations or diseases might create a negative impact on the receiving population (Swedish Environmental Protection Agency 2007). Due to the large genetic distance between the Canadian and the Swedish population, differences could have appeared which indicates that it might be better to use the east Greenland population as the source population. The Norwegian population should be avoided because it's been suffering from diseases lately, where several of the individuals have died.

Another possibility of managing the Swedish population is to transfer cows into zoo's to mate them with males that are genetically different, which was done with the cow in 2004. During the winter of 2007, five east Greenlandic muskoxen were moved from the small population in Tromsø, Norway, to Lycksele zoo, to reinforce the genetic variation among zoo individuals. This could, in turn, help the wild population in Sweden by providing possible specimens for breeding or supplementary release. When comparing my study with the one by Van Coeverden De Groot and Boag (2004), where they used samples from the native distribution of muskoxen, I found that for OM56-30 they detected six alleles while I detect eight, and for MoDIAS2 two compared to three. However, their result also revealed a higher variation than I have detected, in some of the loci. This might depend on that they collected samples from native populations, and also from the mainland of Canada (lacking in my study), whereas I collected from native, translocated, introduced and from zoo individuals. Also, as seen in Figure 4, the zoo animals have, together with east Greenland, the second highest allele variation observed. Thus, some genetic variation seems to be preserved among the introduced populations and among the zoo populations, which is an important finding. If this is the reason for the higher variation, it emphasizes the importance of captive breeding of endangered species with fragmented and/or limited distribution and limited gene flow. This could keep an overall population of a certain species that is more genetically variable, which in turn can help wild populations by introducing new individuals into the populations. If considering the Scandinavian zoo muskoxen (Appendix IV), the best male for future breeding would be the male in Copenhagen zoo (Jens). Otherwise, if only looking at the Swedish zoo individuals, one of the Tromsø males would probably be most useful, although the genotypes for them have not been established. The Greenlandic muskoxen, in general, have more variation than the Swedish muskoxen. The male in Kolmården zoo (Moses) might be appropriate to use as well, even though he is monomorph for all the loci, but he has the allele 237 and 161 that are at low frequencies within the Swedish population. Also, the male Aragorn and the male calf of Mura could be usable, when they both have the allele 237 and 161. If releasing captive held muskoxen into the wild, it is crucial to have enough knowledge about the animals before releasing them. The zoo populations of muskoxen in Sweden are today, to some extent, affected by intestinal worms, mites and overgrown hooves. It is treatable, but is still an important factor to keep in mind when an outbreak in the wild population could cause devastating results for the future survival of the already small population.

A few introductions or mating with bulls from zoo, as mentioned above, can make a large difference, but for the long-term management the population size needs to be expanded considerably. According to the management plan of 2002 the long-term goal is to maintain a population of 30 individuals in Härjedalen (Ericson 2002). First of all it might be difficult to maintain that many individuals in Rogen due to its low vegetation production (Nyqvist 2004) and secondly 30 individuals probably would be too few to secure the genetic variation needed to enable the future survival of the population. This is why I believe the best solution for enabling the survival of this small population would be to move the population to more suitable habitats. Areas known as preferable habitat by muskoxen are found not far from Rogen, located around the Skarsfjället or Lunndörrspasset, approximately 45 km north and 75 km northwest of Rogen (Nyqvist 2004). These areas show a higher productivity than Rogen and are also quite isolated from reindeer industry, infrastructure and tourism (Nyqvist 2004). If an increase in size would lead to separated populations at these locations, the chances for the population to survive would probably increase, and might lead to a natural gene flow between the populations. To increase the size of the population I suggest that several muskoxen from the east Greenland population are brought to Sweden. However, it is difficult to know exactly how many individuals would be enough to rescue the Swedish population. Maybe the best solution would be to start off with a couple of individuals and then continue releasing muskoxen into the population for the following years, until a steady and stable increase is seen. However, one thing is for sure, if we want to retain this high arctic mammal within the Swedish fauna, preservation actions needs to be done now.

Conclusion

The overall findings of this study is that the assumptions of the reduced genetic variation, following the sequential founder events and bottlenecks experienced, were confirmed in almost all the populations. This shows that the natural movement of muskoxen, colonising the Canadian archipelago and Greenland, have reduced the genetic variation, and also that the introduced populations of west Greenland and Norway have experienced some loss in genetic variation, compared to their source populations. This could depend on several factors including both founder events, the history of the source population and environmental fluctuations. That one individual makes a large difference regarding genetic variation has been observed in the Swedish population, where momentarily the genetic variation is higher than in Norway. However, stochastic factors might have a major impact on the now extremely small population, where the higher amount of variation will have no effect if the population size is not increased.

Acknowledgements

The study was financed by the Swedish University of Agricultural Sciences (SLU) in Umeå, and supported by the County Administration Board of Jämtland and the Muskox project in Härjedalen.

I wish to thank my supervisors Carl-Gustaf Thulin, Göran Ericsson and Göran Spong for their tremendous help and support. I also wish to thank Helena Königsson for her excellent supervision and help in the lab. I also would like to thank those involved in collecting samples and sending them to me: Daniel Ottosson, Lars Rehnfeldt, Stefan Mörtberg, Ronny Stålfjäll, Olle Larsson, Bengt Röken, Anna Martinsson, Mats Höggren, Staffan Åkeby, Bengt Holst, Carsten Grøndahl, Helle Flaga, Hans-Ove Larsson, Peter Mortensen, Torsten Mörner, Jessica Åsbrink, Göran Frisk, Arne Söderberg, Peter J. Van Coeverden De Groot, Patricia Reynolds, Mads Forchhammer, Niels Martin Schmidt, Peter Aastrup, Katrine Raundrup, Gunnar Mylius Pedersen, Jon Arnemo, Bjørn Rangbru, Bjørnar Ytrehus, Johan Schulze, Marthe Opland, Nina Brekke Tvedt and all the others that have helped me complete my work.

- Thank you!

5 REFERENCES

Aastrup, P., personal comment, 05/11/2007, e-mail, biologist, Aarhus University.

Alaska Department of Fish and Game. 2005. Muskox management report of survey-inventory activities 1 July 2002-30 June 2004. Brown, C., editor, Juneau, Alaska.

Alendal, E. 1974. Myskoxens historia i Sverige. – Fauna och Flora 2: 41-46.

Allendorf, F. W. and Ryman N. 2002. The role of genetics in population viability analysis. – In: Beissinger, S. R., McCullough, D. R. (eds.), Population viability analysis. University of Chicago Press, Chicago, pp. 50-85.

Barr, W. 1991. Back from the brink: The road to muskox conservation in the northwest territories. – The Arctic Institute of North America of the University of Calgary.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F. 1996-2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier, France.

Bennike, O., Andreasen, C. 2005. New dates of musk-ox (*Ovibos moschatus*) remains from northwest Greenland. – Polar Record 41: 125-129.

Boertmann, D., Forchhammer, M., Riis Olesen, C., Aastrup, P., Thing, H. 1992. The Greenland muskox population status 1990. – Rangifer 12: 5-12.

Borgen, U. 1979. Ett fynd av fossil myskoxe i Jämtland och något om myskoxarnas biologi och historia. – Fauna & Flora 1: 1-12.

Born, E., Jørgensen, M., Merkel, F., Cuyler, C., Neve, P., Rosing-Asvid, A. 1998. Grønlandske fugle, havpattedyr og landpattedyr - en status over vigtige ressourcer. – Pinngortitaleriffik, Grønlands Naturinstitut, Teknisk rapport, 16.

Bouzat, J. L., Cheng, H. H., Lewin, H. A., Westemeier, R. L., Brawn, J. D., Paige, K. N. 1998. Genetic evaluation of a demographic bottleneck in the greater prairie chicken. – Conservation Biology 12: 836-843.

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

Cassinello, J. 2005. Inbreeding depression on reproductiove performance and survival in captive gazelles of great conservation value. – Biological Conservation 122: 453-464.

Chambers, G. K., MacAvoy, E. S. 2000. Microsatellites: consensus and controversy. – Comparative Biochemistry and Physiology 126: 455-476.

Clegg, S. M., Degnan, S. M., Kikkawa, J., Moritz, C., Estoup, A., Owens, I. P. F. 2002. Genetic consequences of sequential founder events by an island-colonizing bird. – PNAS 99: 8127-8132.

Coltman, D. W., Bowen, W. D., Wright, J. M. 1998. Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. – Proceedings of the Royal Society of London, Series B 265: 803-809.

Coltman, D. W., Pilkington, J. G., Smith, J. A., Pemberton, J. M. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. – Evolution 53: 1259-1267.

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

Cuyler, C., Mølgaard, H. S. 2002. Overgrown hooves from muskoxen (*Ovibos moschatus*) of Kangaarsuk (Kap Atholl) Northwest Greenland. – Greenland Institute of Natural Resources. Technical report, 41.

DeWoody, J. A. 2005. Molecular approaches to the study of parentage, relatedness, and fitness: practical applications for wild animals. – Journal of Wildlife Management 69: 1400-1418.

DeYoung, R. W., Honeycutt, R. L. 2005. The molecular toolbox: genetic techniques in wildlife ecology and management. – Journal of Wildlife Management 69: 1362-1384.

Directorate for Nature Management, Norway (2008) Available at http://english.dirnat.no/.

Engel, S. R., Linn, R. A., Taylor, J. F., Davis, S. K. 1996. Conservation of microsatellite loci across species of artiodactyls: implications for population studies. – Journal of Mammalogy 77: 504-518.

Ericson, M. 2002. En lokal handlingsplan för att förbättra myskoxens möjligheter att leva vidare i vår svenska fjällvärld.

Ferguson, M. A. D., Gauthier, L. 1991. Status and trends of *Rangifer tarandus* and *Ovibos moschatus* populations in Canada. – Rangifer 12: 127-141.

Ferns, P. 1974. Muskox abundance in the southern part of the range in east Greenland. – Arctic 30: 52-60.

Fleischman, C. L. 1986. Genetic variation in muskoxen (*Ovibos moschatus*). MS thesis, University of Alaska, Fairbanks.

Forchhammer, M., Boertmann, D. 1993. The muskoxen *Ovibos moschatus* in north and northeast Greenland: population trends and the influence of abiotic parameters on population dynamics. – Ecography 16: 299-308.

Frankham, R., Ballou, J. D., Briscoe, D. A. 2002. Introduction to conservation genetics. Cambridge University Press.

Freeman, M. M. R. 1971. Population characteristics of musk-oxen in the Jones sound region of the northwest territories. – Journal of Wildlife Management 35: 103-108.

Fylkesmannen i Sør-Trøndelag. Available at http://www.fylkesmannen.no/enkel.aspx?m=4153&amid=1993273

Groves, P. 1997. Intraspecific variation in mitochondrial DNA of muskoxen, based on control-region sequences. – Canadian Journal of Zoology 75: 568-575.

Gundersen, L. H., Whist, A. C., Bretten, T., Røtvei, I., Klemetsen, C. E., Linnell, J. D. C., Asbjørnsen, E., Andersen, R., Lillehaug, A., Arnemo, J. M. 2005. Dødsårsaker hos moskus på Dovrefjell 1953-2005. – Norsk Veterinærtidsskrift 8: 613-619.

Gunn, A., Shank, C., McLean, B. 1991. The history, status and management of muskoxen on Banks Island. – Arctic 44: 188-195.

Haig, S. M. 1998. Molecular contributions to conservation. – Ecology 79: 413-425.

Hedrick, P. W. 1995. Gene flow and genetic restoration: the Florida panther as a case study. – Conservation Biology 9: 996-1007.

Hedrick, P. W., Kalinowski, S. T. 2000. Inbreeeding depression in conservation biology. – Annual Review of Ecology and Systematics 31: 139-162.

Holm, L. E., Forchhammer, M. C., Boomsma, J. J. 1999. Low genetic variation in muskoxen (*Ovibos moschatus*) from western Greenland using microsatellites. – Molecular Ecology 8: 675-679.

Holst, B., Carlsen, F. 2002. International studbook for the Muskox (*Ovibos moschatus*), Copenhagen Zoo, II.

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

Jarne, P., Lagoda, P. J. L. 1996. Microsatellites, from molecules to populations and back. – TREE 11: 424-429.

Jennov, J. G. 1955. Nordøstgrønlands moskusokser. – Tidsskriftet Grønland 2: 75-77.

Jingfors, K. T., Klein, D. R. 1982. Productivity in recently established muskox populations in Alaska. – Journal of Wildlife Management 46: 1092-1096.

Kauffer, R., Coltman, D. W., Chapuis, J-L., Pontier, D., Réale, D. 2007. Unexpected heterozygosity in an island mouflon population founded by a single pair of individuals. – Proceedings of the Royal Society of London, Series B 274: 527-533.

Keller, L. F., Jeffery, K. J., Arcese, P., Beaumont, M. A., Hochachka, W. M., Smith, J. N. M., Bruford, M. W. 2001. Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. – Proceedings of the Royal Society of London, Series B 268: 1387-1394.

Lacy, R. C. 1997. Importance of genetic variation to the viability of mammalian populations. – Journal of Mammalogy 78: 320-335.

Laikre, L., Ryman, N. 1991. Inbreeding depression in a captive wolf (*Canis lupus*) population. – Conservation Biology 5: 33-40.

Laikre, L., Andrén, R., Larsson, H., Ryman, N. 1996. Inbreeding depression in brown bear *Ursus arctos*. – Biological Conservation 76: 69-72.

Laikre, L., Ryman, N., Lundh, N. G. 1997. Estimated inbreeding in a small, wild muskox *Ovibos moschatus* population and its possible effects on population reproduction. – Biological Conservation 79: 197-204.

Lande, R. 1995. Mutation and conservation. – Conservation Biology 9: 782-791.

Larsson, H. 2003. En simulering av den framtida genetiska strukturen i den svenska populationen av myskoxar (*Ovibos moschatus*), vid introduktion av nya individer. Projektplan i populationsbiologi.

Larsson, H-O., Larsson, O. 2006. Förslag till fortsatt handlingsplan för Projekt Rädda Myskoxen. Stockholm 01/11/2006.

Le Hénaff, D., Crête, M. 1989. Introduction of muskoxen in northern Quebec: the demographic explosion of a colonizing herbivore. – Canadian Journal of Zoology 67: 1102-1105.

Lent, P. C. 1988. *Ovibos moschatus*. – Mammalian Species 302: 1-9.

Lent, P. C. 1998. Alaska's indigenous muskoxen: a history. – Rangifer 18: 133-144.

Lent, P. C. 1999 Muskoxen and their hunters: a history. – University of Oklahoma Press, Norman.

Luikart *et al.* a) Luikart, G., Allendorf, F. W., Cornuet, J. M., Sherwin, W. B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. – The American Genetic Association 89: 238-247.

Luikart *et al.* b) Luikart, G., Sherwin, B., Steele, B. M., Allendorf, F. W. 1998. Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. – Molecular Ecology 7: 963-974.

Lundh, N. G. 1979. Myskoxarna i Härjedalen. – Fauna och Flora 1: 13-26.

Lundh, N. G. 1996. Myskoxen i Sverige. – Naturvårdsverket Förlag, Rapport 4545.

MacPhee, R. D. E., Tikhonov, A. N., Mol, D., Greenwood, A. D. 2005. Late quaternary loss of genetic diversity in muskox (*Ovibos*). – BMC Evolutionary Biology 5.

Madsen, T., Shine, R., Olsson, M., Wittzell, H. 1999. Restoration of an inbred adder population. – Nature 402: 34-35.

McDonald, H. G., Davis, R. A. 1989. Fossil muskoxen of Ohio. – Canadian Journal of Zoology 67: 1159-1166.

Mikko, S., Røed, K., Schmutz, S., Andersson, L. 1999. Monomorphism and polymorphism at Mhc DRB loci in domestic and wild ruminants. – Immunological Reviews 167: 169-178.

Mills, L. S., Smouse, P.E. 1994. Demographic consequences of inbreeding in remnant populations. – The American Naturalist 144: 413-431.

Mills, L.S., Allendorf, F.W. 1996. The one-migrant-per-generation rule in conservation and management. – Conservation Biology 10: 1509-1518.

Myskoxarna. Available at www.myskoxe.se

Nei, M., Maruyama, T., Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. – Evolution 29: 1-10.

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

Nowak, R. M. 1999. Bovidae, genus *Ovibos*. – In: Walker's Mammals of the World, Vol II, 6th edn, The John Hopkins University Press, Baltimore/London, pp. 1215-1219.

Nyqvist, J. 2004. Ecological possibilities for musk oxen (*Ovibos moschatus*) in the Swedish mountain range. M. Sc. Degree Essay, Department of Forest animal ecology, University of Agricultural Sciences, Umeå, Sweden.

Olesen, C. R. 1993. Rapid population increase in an introduced muskox population, West Greenland. – Rangifer 13: 27-32.

Paetkau, D., Waits, L. P., Clarkson, P. L., Craighead, L., Vyse, E., Ward, R., Strobeck, C. 1998. Variation in genetic diversity across the range of north American brown bears. – Conservation Biology 12: 418-429.

Paetkau, D., Amstrup, S. C., Born, E. W., Calvert, W., Derocher, A. E., Garner, G. W., Messier, F., Stirling, I., Taylor, M. K., Wiig, Ø., Strobeck, C. 1999. Genetic structure of the world's polar bear populations. – Molecular Ecology 8: 1571-1584.

Paetkau, D., Slade, R., Burden, M., Estoup, A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: simulation-based exploration of accuracy and power. – Molecular Ecology 13: 55-65.

Park, S. D. E. 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection, Ph.D. thesis, University of Dublin.

Pedersen, C. B., Aastrup, P. 2000. Muskoxen in Angujaartorfiup Nunaa, west Greenland: monitoring, spatial distribution, population growth, and sustainable harvest. – Arctic 53: 18-26.

Piry, S., Alapetite, A., Cornuet,, J-M., Paetkau, D., Baudouin, L., Estoup, A. 2004. GeneClass2: A Software for Genetic Assignment and First-Generation Migrant Detection. – Journal of Heredity 95: 536-539.

Ralls, K., Ballou, J. D., Templeton, A. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. – Conservation Biology 2: 185-193.

Rangbru, B., Andreassen, S. 2006. Forvaltningsplan for moskusstammen på Dovrefjell. Rapport Nr 1, Fylkesmannen i Sør-Trøndelag, Miljøvernavdelingen, Trondheim.

Rannala, B., Mountain, J. L. 1997. Detecting immigration by using multilocus genotypes. – Proceedings of the National Academy of Science 94: 9197-9201.

Raundrup, K. 2002. Muskoxen and their gastrointestinal parasites – a field study in Kangerlussuaq, West Greenland. – In: International studbook for the muskox (*Ovibos moschatus*), II, Holst, B. and Carlsen, F., Copenhagen zoo.

Raundrup, K., personal comment, 08/10/2007, e-mail, biologist, Kangerlussuaq Tourism, Kangerlussuaq, Greenland.

Raymond, M., Rousset, F. 1995. GENEPOP (version 1.2), population genetics software for exact tests and ecumenicism. – Journal of Heredity 86: 248-249. Available from http://genepop.curtin.edu.au/

Reed, D. H., Briscoe, D. A., Frankham, R. 2002. Inbreeding and extinction: the effect of environmental stress and lineage. – Conservation Genetics 3: 301-307.

Reed, D. H., Frankham, R. 2003. Correlation between fitness and genetic diversity. – Conservation Biology 17: 230-237.

Rehnfeldt, L., personal comment, 2007, phone, naturbevakare, Länsstyrelsen Jämtlands län.

Reynolds, P. E. 1993. Dynamics of muskox groups in northeastern Alaska. – Rangifer 13: 83-89.

Reynolds, P. E. 1998. Dynamics and range expansion of a reestablished muskox population. – Journal of Wildlife Management 62: 734-744.

Reynolds, P. E., personal comment, 02/11/2007, e-mail, wildlife ecologist, USFWS Arctic NWR, Fairbanks.

Rice, W. R. 1989. Analyzing tables of statistical tests. – Evolution 43: 223-225.

Roldan, E. R. S., Cassinello, J., Abaigar, T., Gomendio, M. 1998. Inbreeding, fluctuating asymmetry, and ejaculate quality in an endangered ungulate. – Proceedings of the Royal Society of London, Series B 265: 243-248.

Rowell, J. 1990. The Muskox. – In: International studbook for the muskox (*Ovibos moschatus*), I, Holst, B., Copenhagen zoo.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., Hanski, I. 1998. Inbreeding and extinction in a butterfly metapopulation. – Nature 392: 491-494.

Sjögren, P., Wyöni, P. I. 1994. Conservation genetics and detection of rare alleles in finite populations. – Conservation Biology 8: 267-270.

Slate, J., Kruuk, L. E. B., Marshall, T. C., Pemberton, J. M., Clutton-Brock, T. H. 2000. Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). – Proceedings of the Royal Society of London, Series B 267: 1657-1662.

Spencer, D. L., Lensink, C. J. 1970. The muskox of Nunivak Island, Alaska. – Journal of Wildlife Management 34: 1-15.

Spielman, D., Brook, B. W., Frankham, R. 2004. Most species are not driven to extinction before genetic factors impact them. – PNAS 101: 15261-15264.

Stephen, C. L., Whittaker, D. G., Gillis, D., Cox, L. L., Rhodes, O. E. 2005. Genetic consequences of reintroductions: an example from Oregon pronghorn antelope (*Antilocapra americana*). – Journal of Wildlife Management 69: 1463-1474.

Swedish Environmental Protection Agency. 2007. Genetisk variation hos vilda växter och djur i Sverige. Rapport 5712.

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

Tener, J. S. 1965. Muskoxen in Canada: A biological and taxonomic review. – Monograph Series No. 2, Canadian Wildlife Service, Ottawa.

Uspenski, S. M. 1984. Muskoxen in the USSR: some results of and perspectives on their introduction. – Biological paper, University of Alaska, Special Report 4: 12-14.

Van Coeverden De Groot, P. J., Boag, P. 2004. Optimization of novel polymorphic microsatellites in muskox (*Ovibos moschatus*) leads to an increased estimate of muskox microsatellite diversity. – Molecular Ecology Notes 4: 713-715.

Van Coeverden De Groot P. J., personal comment, 01/10/2007, e-mail, Department of Biology, Queen's University, Canada.

Vibe, C. 1967. Arctic animals in relation to climatic fluctuations. – The Danish zoogeographical investigations in Greenland, C.A. Reitzels Forlag.

Vincent, D., Gunn, A. 1981. Population increase of muskoxen on Banks Island and implications for competition with Peary caribou. – Arctic 34: 175-179.

Weber, D. S., Stewart, B. S., Garza, J. C., Lehman, N. 2000. An empirical genetic assessment of the severity of the northern elephant seal population bottleneck. – Current Biology 10: 1287-1290.

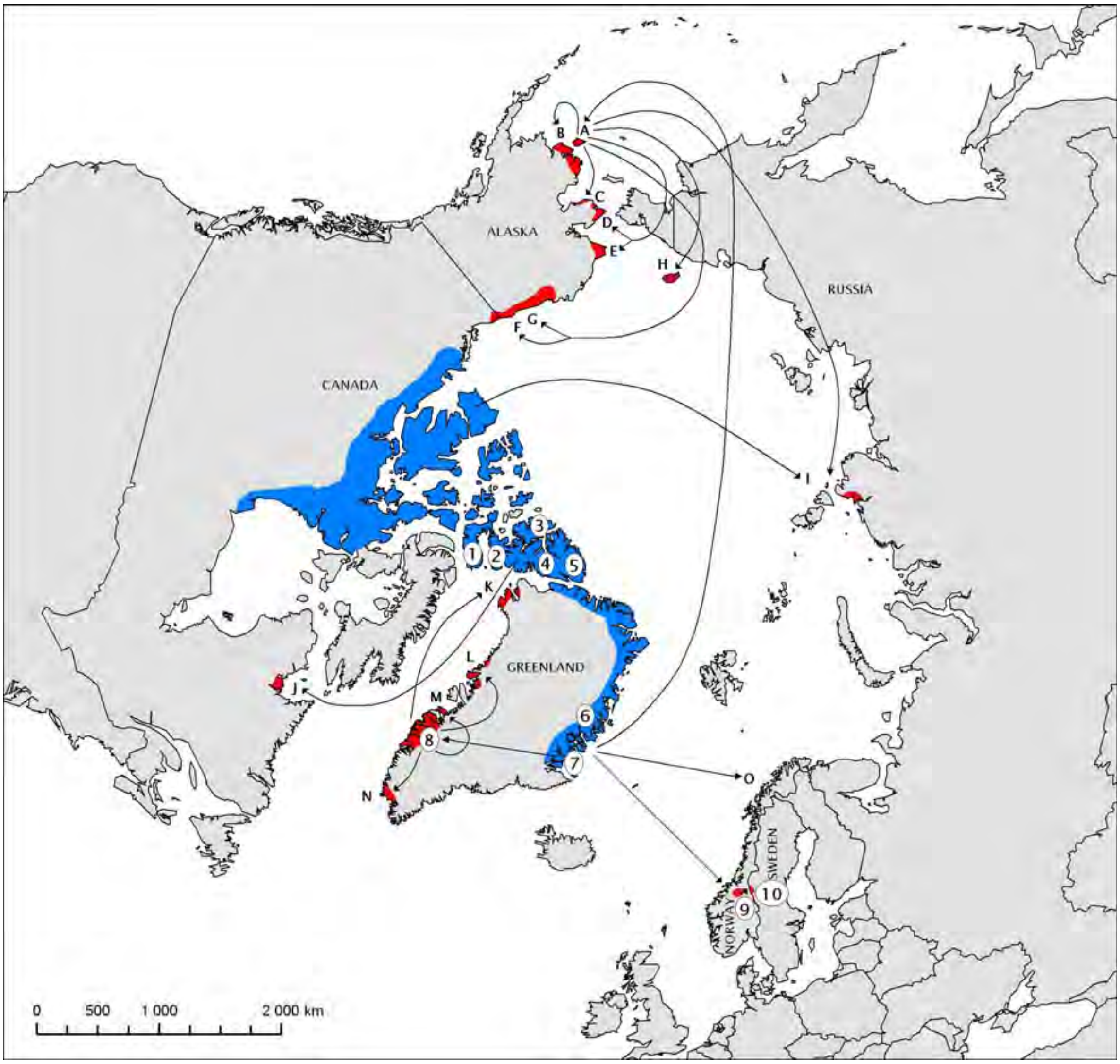
Weir, B. S., Cockerham, C. C. 1984. Esimating *F*-statistics for the analysis of population structure. – Evolution 38: 1358-1370.

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

Williams Torg. 2008. Avaliable at http://www.williamstorg.com/myskoxar.htm.

APPENDIX I

Map over the arctic showing native muskox populations in blue and most of the introduced or translocated populations in red. The samples for this study were sampled from the encirceled numbered locations; 1 – Devon Island, 2 – Grise Fjord, 3 – Axel Heiberg Island, 4 – Eureka, 5 – Northern Ellesmere Island, 6 – Zackenberg, 7 – Jameson Land, 8 - Angujaartorfiup Nunaa, 9 – Dovre and 10 – Härjedalen. The translocated or introduced populations are marked with letters, starting with A – Nunivak Island, B – Nelson Island, C – Seward Peninsula/Feather River, D – Seward Peninsula/Brevig mission, E – Ogotoruk Creek/Cape Thompson, F – Barter Island, G – Kavik River, H – Wrangel Island, I – Taimyr Peninsula, J – Ungava Peninsula, K – Thule area, L – Svartenhuk, M – Naternaq/Lersletten, N – Ivittuut area, and O – Tromsö. For more information regarding translocations see Appendix II.



APPENDIX II

The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

ALASKA

Map	Location	Year	Population size, translocations and/or introductions	Reference
		Illinoian time	Ovibos was first present in Alaska.	*Lent 1988*
		1858	The last known muskoxen was killed in the country.	*Lent 1998*
A	Nunivak Island	1935-1936	A number of 31 muskoxen from northeast Greenland were released on Nunivak Island to help ensure the survival of the species. During 1968 the number of animals had increased to around 750 animals. In 2004 the population was kept at 500-550 individuals.	*Lent 1999* *Spencer and Lensink 1970* *Alaska Dep. of Fish & Game 2005*
B	Nelson Island	1967	Eight yearlings (five males and three females) were moved from Nunivak Island to Nelson Island, 40 km from Nunivak Island.	*Lent 1999*
B	Nelson Island	1968	Another 15 animals were moved from Nunivak to Nelson. In 1969 at least 18 of the total 23 animals were alive. By 1973 the population had doubled and they started to spread to the mainland. By 1991 at least 125 muskoxen lived on the mainland of Alaska.	*Lent 1999* *Alaska Dep. of Fish & Game 2005*
F	Barter Island	1969	A total of 51 animals from Nunivak were moved to the northeastern of Alaska, Barter Island. Only 34 of the 51 remained alive after a few months.	*Reynolds 1998* *Lent 1999*
G	Kavik river	1970	A number of 13 muskoxen from Nunivak were released in the Kavik river area, about 130 km west of Barter Island.	*Lent 1999*

APPENDIX II continuing.

The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

ALASKA cont.

Map	Location	Year	Population size, translocations and/or introductions	Reference
	Barter Island and Kavik river		The Kavik river population and the Barter Island population grew slowly during the first few years, then they rapidly increased for almost a decade. The greatest rate of increase occurred between 1977 and 1981, when the annual rate of increase was 0.24. In 1982 these two introduced populations had formed three different populations. A number of 386 muskoxen were counted in 1986.	*Reynolds 1998*
C	Seward Peninsula, Feather river	1970	A release of 36 muskoxen from Nunivak, to the Feather river area, Seward Peninsula.	*Jingfors and Klein 1982* *Lent 1999*
D	Seward Peninsula, Brevig mission	1970	Another release of 36 animals from Nunivak were done in the Brevig mission.	*Alaska Dep. of Fish & Game 2005*
E	Ogotoruk Creek, Cape Thompson	1970	Again 36 animals from Nunivak were moved to the northwest coast of Alaska, in Ogotoruk Creek.	*Lent 1999* *Alaska Dep. of Fish & Game 2005*
E	Ogotoruk Creek, Cape Thompson	1977	An additional release of 35 animals from Nunivak to Ogotoruk Creek were done to supplement the population that had not been as successful as other introductions.	*Lent 1999*
		2002	There were around 4000 muskoxen to be found in five different populations in Alaska.	*Patricia Reynolds, pers. comment, 2007*

APPENDIX II continuing.
The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

CANADA

Map	Location	Year	Population size, translocations and/or introductions	Reference
		17th - 18th century	The first contact between muskoxen and Europeans happened. At this time muskoxen appeared in all the mainland tundra between the Anderson River basin in the northwest, Rae Isthmus in the northeast and south to the Churchill area.	*Barr 1991*
		1860-1915	The mainland population was heavily hunted mainly for their hides. The estimated number of hides traded between these years were around 21 000-22 000.	*Barr 1991*
		1917	The muskox population only consisted of around 400-500 animals. Therefore they became a protected species.	*Barr 1991*
		1967	The Canadian population had recovered so good that hunting was again allowed.	*Barr 1991*
J	Ungava Peninsula Quebec	1973-1983	Muskoxen from Ellesmere Island were introduced to the Ungava Peninsula in northern Quebec. 54 muskoxen were released along the south coast of Ungava Bay, in three locations. In 1986 the estimated population was 290 individuals.	*Le Hénaff and Crête 1989* *Ferguson and Gauthier 1991*
	Banks Island	1850-1853	Very few muskoxen were observed along the coasts of Banks Island, the Canadian archipelago. However, numerous remains of bones and skulls of muskoxen were found in old Inuit camps.	*Barr 1991*
	Banks Island	1913-1917	A Canadian expedition crossed the island several times all year round, until 1917, and no reports of live muskoxen were made. In total it seems like a small muskox population existed in 1850-53, which was followed by a modest increase and thereafter by a drastic decline that led to local extinctions by the end of the century.	*Barr 1991*

APPENDIX II continuing.
The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

CANADA cont.

Map	Location	Year	Population size, translocations and/or introductions	Reference
	Banks Island	1980	The increase of the population might be recolonisating animals from Melville or Victoria Island. In 1980 the population was estimated at around 19 000 animals.	*Vincent and Gunn 1981* *Barr 1991*
	Banks Island	1991	The Banks Island muskox population constituted almost a third of the world's muskoxen.	*Gunn et al. 1991*
1	Devon Island	1924	No muskoxen were found on the island, although searched for 11 days. However, findings of muskox skeletons were made.	*Barr 1991*
1	Devon Island	1966-1967	The populations along the north coast of Devon Island was estimated at 450 individuals.	*Freeman 1971*
2-5 **3**	Ellesmere- and Axel Heiberg Island	end of 1800	The muskox was more numerous here than in Banks Island during this time, although not abundant.	*Barr 1991*
2-5 **3**	Ellesmere- and Axel Heiberg Island	1875-1917	The population was drained by intense hunting, feeding the numerous dog teams of many expeditions.	*Barr 1991*
2-5 **3**	Ellesmere- and Axel Heiberg Island	1950	An estimation of the populations were made, where Ellesmere Island had around 2000 animals and Axel Heiberg Island had around 500.	*Barr 1991*
		2007	The estimated Canada mainland and archipelago population was around 130 000.	*Van Coeverden De Groot, pers. comment*

APPENDIX II continuing.

The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

RUSSIA

Map	Location	Year	Population size, translocations and/or introductions	Reference
I	Taimyr Peninsula	1974	Ten yearlings were captured on Banks Island, Canadian archipelago, and transported to the Taimyr Peninsula.	*Uspenski 1984* *Lent 1999*
I **H**	Taimyr Peninsula and Wrangel island	1975	40 muskoxen were captured on Nunivak and brought to Siberia. There they were split in two groups; 20 to the Taimyr Peninsula (together with the previously brought ten Canadian animals) and 20 were moved to Wrangel Island. The Taimyr population had a growth rate of 20% or more and reached around 500 individuals in 1991. The Wrangel population did not grow as fast as the other, but reached about a hundred individuals in 1995.	*Uspenski 1984* *Lent 1999*

APPENDIX II continuing.
The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

GREENLAND

Map	Location	Year	Population size, translocations and/or introductions	Reference
			There are no indications that muskoxen ever lived in west Greenland south of the Thule area. Its' southern migration might have been hindered by the Melville Bugt.	*Vibe 1967*
			There have been periods when muskoxen have had difficulties in reproducing and surviving on the east side of Greenland, mainly due to differing climate conditions, but also due to the presence of Inuit hunters.	*Jennov 1955* *Lent 1999*
7	Jameson Land, East Greenland	Prior to the 1700 century	Muskoxen were present in this region, indicated by bone remnants.	*Ferns 1974*
7	Jameson Land, East Greenland	The first half of the 1800	Muskoxen were absent or at a very low population size.	*Ferns 1974*
7	Jameson Land, East Greenland	1869	The first live muskoxen was reported.	*Ferns 1974*
		1900's	The sex ratio has sometimes been uneven in east Greenland, where in the 1950's there was an estimation of one cow for 26 males. In the beginning of the 1900 it was believed that the muskox was about to die out in northeast Greenland.	*Jennov 1955*
		1920-1940	Fortunate periods of favourable weather conditions made the population rise again, and during 1920-1940 the muskox population thrived in northeast Greenland.	*Jennov 1955* *Vibe 1967*
		1938/39 and 1953/54	Two catastrophic winters appeared, when the extreme weather diminished the muskox population severally. In several places no calves, either from the same year or the year before were observed.	*Vibe 1967*

APPENDIX II continuing.
The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

GREENLAND cont.

Map	Location	Year	Population size, translocations and/or introductions	Reference
8	Angujaartorfiup Nunaa	1962-1965	A number of 27 muskoxen were moved from northeast Greenland (Scoresby Sund south of Jameson Land) to west Greenland, Angujaartorfiup Nunaa. In 1999 the population had stabilized around 3000 animals, after the hunting had started in 1988. The mean annual rate of increase between 1977 and 1991 reached 32% and has declined since, but still remains high.	*Boertmann et al. 1992* *Olesen 1993* *Pedersen and Aastrup 2000*
K	Thule area	1986	27 muskoxen from Angujaartorfiup Nunaa divided into three small populations were introduced in Avanersuaq/Thule area. 14 of them went to Avannarliit (Inglefield Land), 6 to the Iterlassuaq area (Mac Cormick Fjord) and 7 to Kangaarsuk (Kap Atholl).	*Boertmann et al. 1992* *Born et al. 1998* *Cuyler and Mølgaard 1998*
N	Ivittuut area	1987	15 yearlings from Angujaartorfiup Nunaa were released near Kangilinnguit (Grönnedal) in the Ivittuut area. In 1990 this population was thriving and had a high rate of increase. In 1998 the population numbered around 200 animals.	*Boertmann et al. 1992* *Born et al. 1998*
L	Svartenhuk	1991	31 yearlings from Angujaartorfiup Nunaa were moved to Nunavik (Svartenhuk), Uummannaq and Upernavik. In 1998 the population consisted of less than 100.	*Boertmann et al. 1992* *Born et al. 1998*
M	Naternaq/Lersletten	1993	A bit north of Angujaartorfiup Nunaa 31 animals were released in Naternaq. In 1998 the population consisted of less than 100 individuals.	*Born et al. 1998*
		2007	The overall muskox population of Greenland was estimated at around 20 000 or more.	*Peter Aastrup, pers. comment 2007*

APPENDIX II continuing.
The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

NORWAY

Map	Location	Year	Population size, translocations and/or introductions	Reference
		1900	An expedition went to east Greenland to capture muskoxen, but failed, and only two calves were caught and brought to Norway, where they died.	*Lundh 1979*
	Svalbard	1929	A total of 17 muskoxen from east Greenland were moved to the Svalbard archipelago. They increased but had died out until the 1990's. As an explanation to the decrease could be that Svalbard lacks the willows that are present in almost all other winter ranges of muskoxen. The muskoxen were also disturbed a lot by tourists.	*Lent 1988* *Lundh 1996*
	Dovre	1932-1938	Ten animals from east Greenland were released in Dovre. In 1938 two more juveniles were released in the same area.They reproduced but at the end of the war in 1945 the population had died out by means of poaching and accidents.	*Lundh 1996* *Williams Torg 2008*
		1948	Eight calves from east Greenland were released near Bardufoss in Tromsö. The faith of the animals is unknown, but it is believed that they died during the 1960's.	*Lundh 1996*
9	Dovre	1947-1953	An amount of 27 calves from east Greenland were released in Dovre. Until August 1953 13 of them had been found dead, and there were suspicions that another four calves died within the same period. The remaining ten animals reproduced and created the foundation of the Norwegian population of today.	*Lundh 1979*

APPENDIX II continuing

The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

NORWAY cont.

Map	Location	Year	Population size, translocations and/or introductions	Reference
O	Tromsö	1969	Ten miles north of Narvik, in Bardu, Tromsö, a muskox farm was established. 25 muskoxen were caught on east Greenland and brought to Norway.	*Lundh 1996*
9	Dovre	1978	The population consisted of 51 animals in July 1978.	*Williams Torg 2008*
9	Dovre	1983	Due to several accidents the population was reduced to 36 animals.	*Williams Torg 2008*
9	Dovre	2004	There was an outbreak of the virus *Echtyma contagiosum,* where 14 calves and one cow died.	*Gundersen et al. 2005*
9	Dovre	2006	The Dovre population was decimated by the bacteria *Pasteurella,*which is naturally occurring in many species. The outbreak in Dovre was caused by very warm and humid weather.	*Fylkesmannen i Sør-Trøndelag 2008*
9	Dovre	Spring 2006	The population consisted of at least 214 animals.	*Directorate for Nature Management, Norway*
9	Dovre	2008	The population consisted of 190 individuals, where only 12 were calves. This might be a result from the bacteria outbreak in 2006.	*Fylkesmannen i Sør-Trøndelag 2008*

APPENDIX II continuing.
The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

SWEDEN

Map	Location	Year	Population size, translocations and/or introductions	Reference
	Jämtland	1900	Four calves from east Greenland were brought to Jämtland were they were placed in an enclosure. Three of them died and the fourth died in 1904.	*Alendal 1974* *Lundh 1996*
10	Härjedalen	1971	Five animals walked 200 km from the population in Norway to Härjedalen in Sweden.	*Alendal 1974* *Lundh 1996*
10	Härjedalen	1974	During this year a clear territory was seen among the muskoxen, with calving area, early summer area, winter area and the walking paths were the same as former years.	*Lundh 1979*
	Härjedalen	Middle of 1980's	The population consisted of 36 animals, but started to decline after that.	*Lars Rehnfeldt, pers. comment*
	Härjedalen	1990	The population consisted of 20 animals.	*Lundh 1996*
	Härjedalen	1995	The population had declined to 14 animals.	*Lundh 1996*
	Härjedalen	March-April 2004	The population were now down at nine individuals. Due to the expected inbreeding the adult bull was removed from the population and transported to Lycksele zoo.	*Personal observation*
		Aug-Sep 2004	A captive born bull from Kolmården zoo was released to the wild population. At the same time an adult cow from the wild was sedated and transported to Järvzoo, where the cow was placed with a Greenlandic bull. The thought was that they should mate and produce a calf.	*Personal observation*

APPENDIX II continuing.
The historic abundande of muskoxen worldwide and information about the many introductions and relocations of the muskox that has been made. In the column on the left, the map code (seen in Appendix I) for each location is found.

SWEDEN cont.

Map	Location	Year	Population size, translocations and/or introductions	Reference
		Nov 2004	The released bull left the cows and went in to Norway, where he was sedated and unfortunately died from the sedative.	*Lars Rehnfeldt, pers. comment*
		Spring 2006	The wild cow that was brought to Järvzoo earlier was pregnant and was therefore released back into the wild. In May/June she gave birth to a calf.	*Personal observation*
		2007	The population consisted of 8 individuals, 7 cows and one 1 year old calf.	*Lars Rehnfeldt, pers. comment*
		Spring 2007	One of the old adult cows separated from the others and went down to the village of Tänndalen. She lost one of her horns and seemed weak and unnourished. Therefore she was put to sleep by the authorities.	*Lars Rehnfeldt, pers. comment*
		2008	The herd now consists of 6 adult cows and 1 calf (sex still unknown) which will be 3 years old during spring 2009.	*Lars Rehnfeldt, pers. comment*

ZOO POPULATION - Scandinavia

Map	Location	Year	General information	Reference
		2002	The population of *Ovibos moschatus wardi* (which is the subspecies found in Scandinavian zoos) had 23 founders, of which seven were still alive.	*Holst and Carlsen 2002*

APPENDIX III

Details of the muskoxen microsatellites for all the populations treated as one population. Locus is the locus name, colour is the colour of the primer, repeat is the repeat array in the original clone, size is the size range of the alleles, left and right primer, which are listed 5’ → 3’, # is the number of alleles found per locus, H_E is the expected heterozygosity, H_O is the observed heterozygosity, and cycle is the touchdown PCR cycle optimized for the locus. The H_E and H_O are calculated when MoDIAS1 and OM58-06 have been removed, due to their monomorphism.

Locus	**Accession nr.**	**Colour**	**Repeat**	**Size**	**Left Primer**	**Right Primer**	**#**	**H_E**	**H_O**	**Cycle**
MoDIAS1	AF082895	green	$(TG)_{13}$	121	GCA CAG CTT AGA CAT TGT T	TTA TTG GTG GTA TCC TTT AG	1	—	—	TD65-55VL
MoDIAS2	AF082896	green	$(TG)_{21}$	204-208	ACT GGC AGG TGG ATT CTT AT	CCA AAC TTT CTG TCA TGA CC	3	0.0848	0.0680	TD65-55VL
MoDIAS5	AF082899	blue	$(CA)_{16}$	176-178	CAA ATT CAT GTC CAC AGA GG	ATG TCT CTC TGC CTC CTT CA	2	0.0098	0.0098	TD65-55VL
OM54-23	AY428656	blue	$(CA)_{20}$	104-108	TGG GAT TTA CAT AGG AAC AGA T	GTC AGT GGA TGA GTA GAC AAC A	3	0.4789	0.1748	TD65-55VL
OM58-06	AY428664	green	$(CA)_{19}$	155	GAG AAT CAC TTG GAC AGA GAA G	GTG GAC AGT GTT TGA TGT CTT A	1	—	—	TD65-55VL
OM53-38	AY428655	blue	$(CA)_{21}$	159-161	CCA TAG GGT GCA AAA TAA ATA A	GCA GTC ACA AAA GAA TCA GAT A	2	0.5023	0.2547	TD65-55VL
OM56-30	AY428662	green	$(CA)_{27}$	221-247	TCA TTG TAG AAC ATC TGG AGA A	GTT AGG TGG TTC CTT TCT ATG A	8	0.7008	0.1682	TD65-55VL
OM50-08	AY435177	blue	$(CA)_{18}$	158-160	CCT TTG TAG CCT CTT CAA TAA C	ACC TTT AGT GCA TAT GAG TTC C	2	0.0100	0.0100	MY68-63
OM51-19	AY428650	blue	$(CA)_{22}$	211-215	AAG AAA ATA GCA ACC TAC TCC A	AGC ATT AAC CAT CAT CAG TGT A	3	0.5723	0.4020	MY68-63
OM51-16	AY428649	green	$(CA)_{18}$	263-269	AGA ATA AAA TAA TGC CAT TTG C	TGT TAG TTT TGA GAT TCC ACA T	3	0.2411	0.0550	MY68-63

APPENDIX IV

The genotypes of the zoo individuals studied compared to the Swedish animals. MGW is animals from west Greenland, MN is animals from Norway, MZ are zoo individuals with mixed backgrounds and MS are the Swedish wild individuals.The individuals marked with an † are those known to me to be deceased. The alleles that are in low frequency within the Swedish population are bold among the zoo animals.

Zoo population

MGW15	**† Pitoraq ♂**	**106**	108	121	121	155	155	159	**161**	176	176	206	206	158	158	215	215	**235**	**235**	267	267
MGW200	**Laura**	**106**	108	0	0	0	0	0	0	0	0	0	0	158	158	215	215	**235**	**235**	267	267
MGW201	**Jens ♂**	**106**	**106**	0	0	155	155	159	159	176	176	206	206	0	0	215	215	**235**	**235**	267	267
MN1	**Moses ♂**	108	108	121	121	155	155	**161**	**161**	176	176	206	206	158	158	215	215	**237**	**237**	267	267
MZ1	**Olga**	108	108	121	121	0	0	**161**	**161**	0	0	0	0	158	158	0	0	**237**	**237**	267	267
MZ2	**Chewie ♂**	108	108	121	121	155	155	**161**	**161**	176	176	206	206	158	158	213	215	**237**	**237**	267	267
MZ3	**† Fenton ♂**	108	108	121	121	155	155	159	**161**	176	176	206	206	158	158	**213**	**213**	**237**	**237**	0	0
MZ4	**† Micke ♂**	108	108	0	0	155	155	**161**	**161**	176	176	206	206	158	158	**213**	**213**	**237**	**237**	0	0
MZ5	**† Willy ♂**	108	108	121	121	155	155	159	**161**	176	176	206	206	0	0	0	0	239	239	0	0
MZ6	**Pia**	108	108	121	121	155	155	159	**161**	176	176	206	206	158	158	**213**	**213**	**239**	**243**	267	267
MZ7	**Hedvig**	108	108	121	121	155	155	**161**	**161**	176	176	206	206	158	158	**213**	**213**	**237**	**237**	267	267
MZ8	**Aragorn ♂**	108	108	121	121	155	155	159	**161**	176	176	206	206	158	158	213	215	**237**	**237**	0	0
MZ9	**Arwen**	108	108	121	121	155	155	**161**	**161**	176	176	206	206	158	158	213	215	**235**	**237**	267	267
MZ10	**Sam ♂**	0	0	0	0	155	155	**161**	**161**	176	176	206	206	158	158	213	215	**231**	**231**	267	267
MZ11	**Frodo ♂**	108	108	0	0	155	155	**161**	**161**	176	176	206	206	158	158	213	215	**237**	**237**	267	267
MZ12	**Sulevi**	108	108	121	121	155	155	**161**	**161**	176	176	206	206	0	0	213	215	**237**	**237**	0	0
MZ13	**† Ymer ♂**	108	108	121	121	0	0	**161**	**161**	176	176	206	206	0	0	**213**	**213**	0	0	0	0
MZ14	**Saga**	108	108	0	0	0	0	**161**	**161**	0	0	0	0	0	0	213	215	**237**	**237**	267	267
MZ15	**Irja**	108	108	121	121	0	0	**161**	**161**	0	0	0	0	0	0	213	215	**237**	**237**	267	267
MZ16	**Undis**	108	108	0	0	155	155	**161**	**161**	176	176	206	206	158	158	213	215	**235**	**237**	0	0
MZ17	**Calf of Troja**	108	108	121	121	155	155	**161**	**161**	176	176	206	206	158	158	**213**	**213**	**237**	**237**	267	267
MZ18	**Troja**	108	108	121	121	155	155	**161**	**161**	176	176	206	206	158	158	**213**	**213**	**237**	**237**	0	0
MZ19	**Calf of Mura ♂**	108	108	121	121	155	155	**161**	**161**	176	176	206	206	158	158	213	215	**237**	**237**	267	267
MZ20	**Gronni**	108	108	121	121	155	155	**161**	**161**	176	176	206	206	0	0	**213**	**213**	**239**	**241**	267	267
MZ21	**MOS033 ♂**	0	0	0	0	155	155	0	0	176	176	206	206	158	158	213	215	239	239	267	267
MZ22	**MOS013**	108	108	0	0	155	155	159	**161**	176	176	206	206	158	158	213	215	**239**	**241**	267	267

Swedish population

MS9	**Sofie**	108	108	121	121	155	155	159	159	176	176	206	206	158	158	215	215	239	239	267	267
MS14		108	108	121	121	155	155	159	159	176	176	206	206	158	158	213	215	239	241	267	267
MS16		108	108	121	121	155	155	159	159	176	176	206	206	158	158	213	215	239	239	267	267
MS20	**Calf**	106	108	121	121	155	155	159	161	176	176	206	206	158	158	215	215	239	239	267	267
MS43	**† Moa**	108	108	121	121	155	155	159	159	176	176	206	206	158	158	215	215	237	239	267	267