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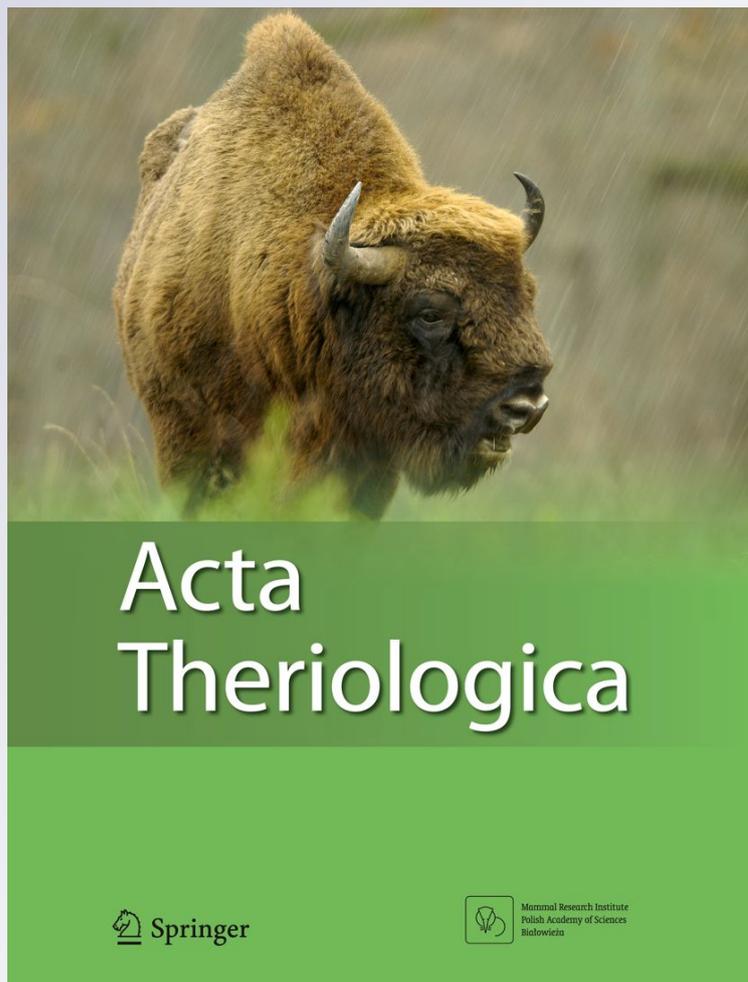
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The impact of founder events and introductions on genetic variation in the muskox *Ovibos moschatus* in Sweden

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Abstract The muskox *Ovibos moschatus* (Zimmerman 1780) is a specialised arctic mammal with a highly fragmented circumpolar distribution, with native populations in Canada and east Greenland and introduced populations in west Greenland, Alaska, Siberia and Eurasia. In 1971, five *O. moschatus* individuals from an introduced population in Norway migrated to Sweden. After a peak population of 36 individuals in the mid-1980s, the Swedish population now numbers seven individuals, making it vulnerable to both demographic and genetic stochasticity (i.e. inbreeding). Here, we analyse genetic variation among native and introduced populations of *O. moschatus* to evaluate the genetic effect of sequential founder events in this species. Our results show that genetic variation among native and introduced *O. moschatus* populations do not conform entirely to the expectations from sequential founder events, most likely because of random processes associated with introduction. In the Swedish population, a calf resulting from the mating of a wild cow and a captive Greenlandic bull contributes significantly to the current genetic variation. Thus, even a single outbreeding event may, at least momentarily,

increase the genetic variation and potentially prevent inbreeding depression. Our results should aid the long-term preservation of *O. moschatus* in Sweden and Europe.

Keywords Supplemental release · Conservation · Restoration · Microsatellites · Bottleneck · Sequential

Introduction

Genetic variation can be an important factor in the long-term persistence of populations, as it may affect a population's ability to adapt to ecological change. The importance of genetic variation is particularly relevant for species with fragmented and isolated distribution, severe reductions in population size and continuously small numbers (Lacy 1997; Saccheri et al. 1998; Westemeier et al. 1998; Frankham et al. 2002; Spielman et al. 2004). Fragmented and small populations are more vulnerable to genetic stochasticity because genetic variation is more easily lost randomly through genetic drift (Hedrick and Kalinowski 2000; Spielman et al. 2004), causing changes in allele frequencies and a loss of genetic variation (Frankham et al. 2002). Sequential founder events are likely to affect the genetic variation within a population more than a single event (i.e. stepping-stone model, Le Corre and Kremer 1998). Induced differentiation following sequential founder events in a stepping-stone manner has also been confirmed among island bird populations (Clegg et al. 2002; Pruett and Winker 2005). Among populations of moose *Alces alces* (Linnaeus 1758) 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, the rate of the

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subsequent recovery and random processes like drift (Nei et al. 1975; DeYoung and Honeycutt 2005). In small populations, inbreeding may become unavoidable and result in 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). In 38 of 40 mammalian populations held in captivity, 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 for preventing future fitness reduction, particularly among introduced and captive populations (Hedrick and Kalinowski 2000).

The muskox *Ovibos moschatus* (Zimmerman 1780) is an arctic mammal adapted to extreme environmental conditions. Today, native populations of *O. moschatus* are found in northern Canada, the Canadian archipelago and along the northeastern coast of Greenland (McDonald and Davis 1989; Groves 1997; Bennike and Andreassen 2005) (Fig. 1). The total number of *O. moschatus* worldwide (both native and introduced) is estimated at around 150,000 animals (Table 1). Although *O. moschatus* is considered to be of least concern (LC) according to the IUCN Red List (Gunn and Forchhammer 2008), there is still great uncertainty about how the populations will react to long-term climate changes. In general, *O. moschatus* appear to have low genetic variation compared to other land mammals (Fleischman 1986; Groves 1997; Mikko et al. 1999), possibly due to periodically low abundances because of climate fluctuations during the glacial periods in the northern hemisphere (Lent 1998, 1999; Campos et al. 2010). In addition, a more recent history of intensified hunting may have added to fluctuations in population size (Barr 1991; Lent 1999).

In the early twentieth century, several introductions of *O. moschatus* to both formerly inhabited and uninhabited areas were conducted (Spencer and Lensink 1970; Jingfors and Klein 1982; Uspenski 1984; Le Hénaff and Crête 1989; Lundh 1996; Lent 1999). Between 1962 and 1965, a total of 27 individuals were relocated from northeast Greenland to the previously uninhabited southwest side of Greenland, Angujaartorfiup Nunaa (Olesen 1993; Pedersen and Aastrup 2000). The population grew rapidly, and in 1999, it stabilised at around 3,000 animals (Pedersen and Aastrup 2000). Furthermore, during the years 1947–1953, 27 calves from east Greenland were released in Dovre, Norway. The final founder population was comprised of ten individuals (Lundh 1996). Compared to introductions in Alaska and Greenland, the Norwegian population size increased slowly, with 200 individuals in 2006 (Rangbru and Andreassen 2006).

In 1971, five animals from the Norwegian population migrated into Härjedalen, Sweden (Alendal 1974) and

established the second *O. moschatus* population in Europe. The small group dispersed approximately 200 km and settled in an alpine area around Rogen Nature Reserve. The group consisted of one bull, two cows and their two calves (Alendal 1974). The population grew until the mid-1980s and peaked at 36 individuals (Lars Rehnfeldt, personal communication). Thereafter, the population size has steadily declined to seven individuals in 2008 (Fig. 2). The most widely accepted explanation for the decline is that the population suffers from inbreeding depression (Laikre et al. 1997). In a recent attempt to increase the genetic variation in the Swedish *O. moschatus* population, one of the wild cows was allowed to mate with a captive bull, originally from Greenland, which resulted in a wild calf born in 2006.

In this study, we analyse genetic variation among native and introduced populations of *O. moschatus* to evaluate the impact of natural and anthropogenic factors on genetic diversity. We specifically focus on how subsequent founder events affect genetic variation and the impact of the effort to increase genetic variation among Swedish *O. moschatus*. The implications of the results for future conservation and management of the Swedish population are discussed specifically, along with the relevance of subsequent introductions in general.

Materials and methods

Samples

Muscle tissue samples were collected from native *O. moschatus* populations in the Canadian archipelago (Devon Island, Grise Fjord, Axel Heiberg Island, Eureka and Northern Ellesmere Island) ($n=22$) and east Greenland ($n=58$), as well as from introduced populations on southwest Greenland ($n=31$), Norway ($n=29$) and Sweden ($n=8$). In addition, blood, biopsies, faecal pellets and/or hair were collected from captive *O. moschatus* in Scandinavia ($n=25$). Faecal pellets from a total of 27 droppings from the Swedish wild population were collected in 2006 (eight individuals at that point) to facilitate successful genotyping of all individuals. The natural death of one Swedish cow in 2006 provided us with a reference tissue sample, used to verify one unique genotype among the faecal samples.

DNA extractions

DNA was extracted from tissue using a standard salt (NaCl) extraction protocol and ethanol precipitation (Miller et al. 1988). To extract DNA from blood samples, we used E.Z.N. A Blood DNA Miniprep Kit (Omega Bio-Tek, USA) for large amounts of blood and the Qiagen DNeasy Blood and Tissue Kit (Qiagen Sciences, USA) for small amounts

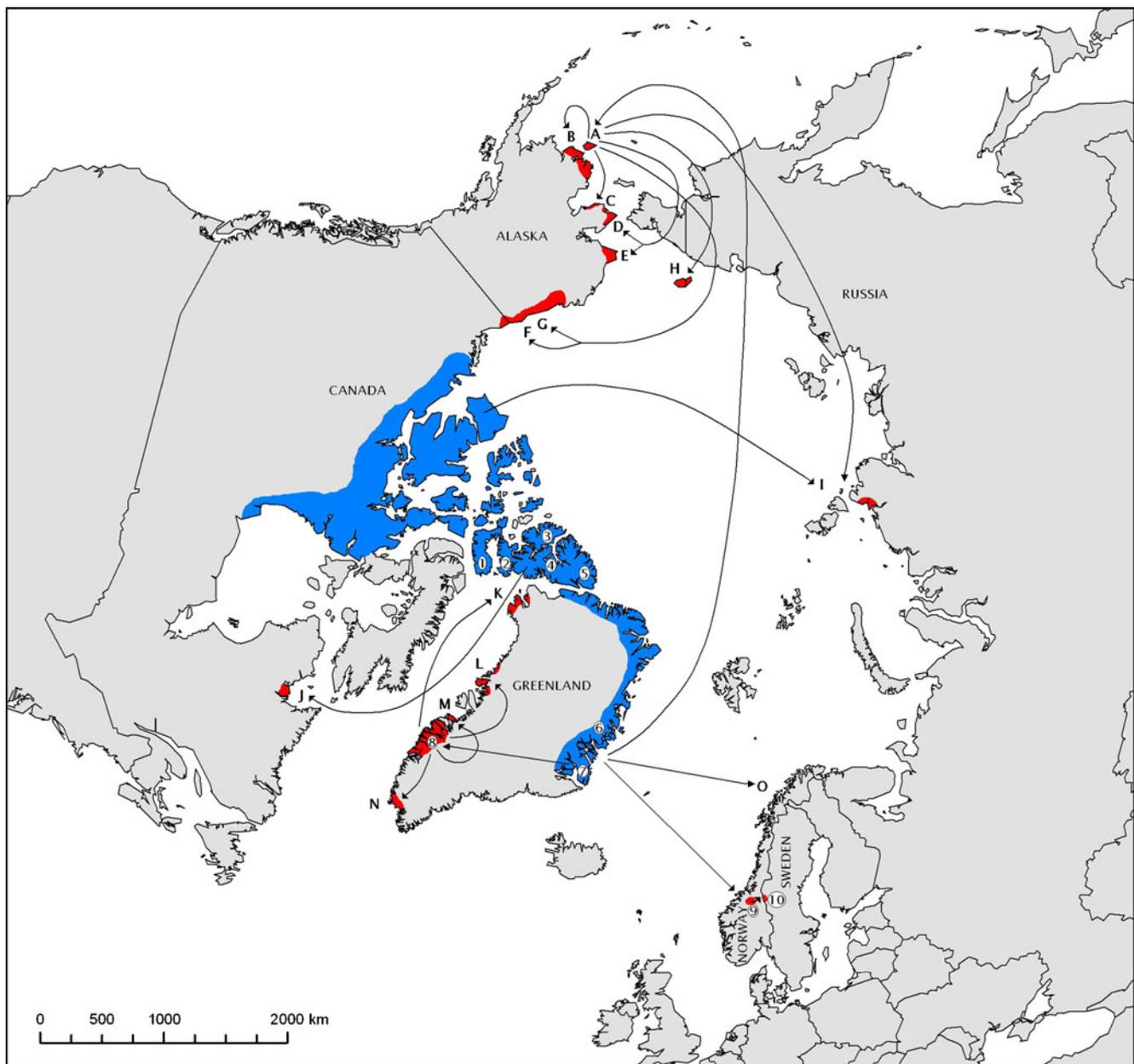


Fig. 1 Map showing native *O. moschatus* populations in blue and introduced or translocated populations in red. The samples for this study were collected from locations denominated with encircled numbers (numbers of individuals sampled): 1 Devon Island, 2 Grise Fjord, 3 Axel Heiberg Island, 4 Eureka, 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 Svartehuk, M Naternaq/Lersletten, N Ivittuut area, and O Tromsø

(<10 μ L). For faecal samples, we used the E.Z.N.A Stool DNA Miniprep Kit (Omega Bio-Tek, USA) following the manufacturer's instructions.

Genotyping

To assess genetic variation, we used ten different microsatellite loci previously shown polymorphic in *O. moscha-*

tus, OM54-23, OM58-06, OM53-38, OM50-08, OM51-19, OM56-30 and OM51-16 (Van Coeverden De Groot and Boag 2004), and MoDIAS1, MoDIAS2 and MoDIAS5 (Holm et al. 1999). The PCR procedure was performed in a 10- μ L solution, containing 1 μ L 10 \times buffer (Biopool), 2.5–3.0 mM MgCl₂, 0.0625 mM dNTP each, 0.06 μ L polymerase Taq, the interval of 0.2–1.0 μ M of each forward and reverse primer and 1–2 μ L of template DNA depending on

Table 1 Estimated number (N) of free-ranging *O. moschatus* individuals worldwide

Country	N	Year	References
Canada	~130,000	2007	P. J. Van Coeverden De Groot (personal communication)
Alaska	~4,000	2002	P. Reynolds (personal communication)
Greenland	~20,000	2007	P. Aastrup (personal communication)
Siberia	~4,000	-	Anonymous referee
Norway	~200	2006	Rangbru and Andreassen (2006)
Sweden	7	2010	S. Mörtberg (personal communication), also http://www.myskoxe.se
Total	150–160 000		

quality and concentration. For the PCR, two different touchdown cycles were used, TD65-55VL and MY68-63. 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 by 0.5°C/cycle) and 30 s extension at 72°C. This was followed by 25 cycles of 20 s at 95°C, 30 s at 55°C and 72°C at 30 s. The last cycle had a duration of 4 min at 72°C. The MY68-63 program started with an initial denaturation temperature of 94°C for 3 min and was followed by ten cycles with denaturation temperature of 94°C for 30 s, 20 s annealing at 68°C (lowered 0.5°C/cycle) and 10 s extension at 72°C. This was followed by 25 cycles of 30 s at 94°C, 20 s at 63°C and 10 s at 72°C. The last cycle had a temperature of 72°C for 3 min. The amplified products were visualised using an automated DNA sequencer (Beckman Coulter CEQ 800). Multiplex was performed with OM58-06, OM53-38, MoDIAS5 and MoDIAS2 (multiplex I), OM54-23 and MoDIAS2 (multiplex II) and OM51-19 and OM50-08 (multiplex III).

Genetic analyses

We used a microsatellite tool kit (Park 2001) to calculate expected and observed heterozygosity (H_e and H_o), allele frequency, number of alleles and number of polymorphic

loci, and to create input files for other software. In an effort to correct the estimates of allelic diversity for different sample sizes, we standardised our estimates of number of alleles per loci to the minimum number of five genotypes obtained in the Swedish population using the rarefaction procedure in the software HP-Rare 1.1 (Kalinowski 2005). Genepop on the Web (<http://genepop.curtin.edu.au/>), versions 3.1c–3.4 (Raymond and Rousset 1995) was used to test for Hardy–Weinberg equilibrium and linkage disequilibrium and to calculate inbreeding coefficients (F_{IS}). When calculating F_{IS} , a weighted ANOVA test was performed (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. 2004). In addition to F_{ST} , Nei's unbiased genetic distance (Nei's D) was calculated (Nei 1978; Takezaki and Nei 1996), also in Genetix 4.05 along with standard deviations for F_{ST} and Nei's D using 10,000 permutations. To measure the genetic structuring among *O. moschatus* 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, the standard criterion described by Rannala and Mountain (1997) was used, together with the simulation algorithm for population assignment described by

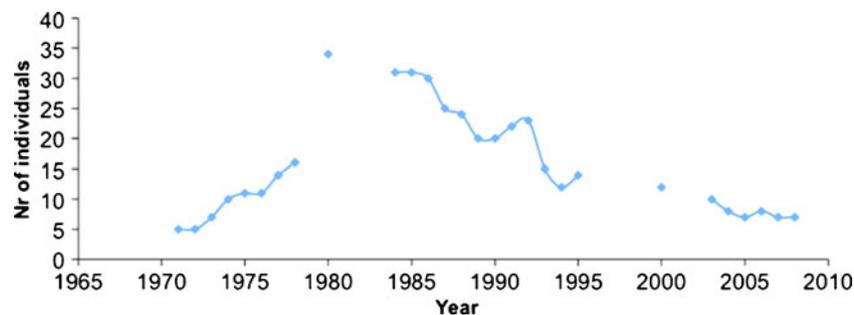


Fig. 2 The population size of the Swedish *O. moschatus* populations 1971–2008. Data are missing for 10 years (Lundh 1996; Lent 1999). After the birth of a calf in summer 2010 and the recent disappearance

of one older cow (probably diseased), the current population size is still seven (personal communication, S. Mörtberg, see also <http://www.myskoxe.se>)

Paetkau et al. (2004) simulating 10,000 genotypes. Probability tests of sampling efficiency and the probability of receiving individuals with identical genotypes (PI) were performed in Microsoft Excel. Finally, a deterministic projection of future genetic variation within the Swedish population was also performed in Microsoft Excel, assuming no immigration and an initial population of one fertile bull and five cows. Subsequent generations assumed an equal sex ratio of three males and three cows. When appropriate, significance levels were corrected according to the Bonferroni procedure (Rice 1989).

Results

Complete genotypes from all ten microsatellite loci were obtained from 74 individuals, genotypes consisting of nine loci were obtained from 23 individuals and genotypes consisting of at least five loci were obtained from an additional 12 individuals, adding up to 109 individuals used for the population genetic analysis (Table 2). The investigated microsatellite loci produced between one and eight alleles each per locus (Table 3). The loci MoDIAS1 and OM58-06 were monomorphic and excluded from further analyses.

No significant linkage between any of the loci was detected. Most allelic variation was observed in the samples from Canada, then east Greenland followed by west Greenland, Sweden and Norway (Table 2). After Bonferroni corrections for multiple comparisons (Rice 1989), one locus (OM56-30) still deviated significantly from Hardy Weinberg equilibrium in the Canadian archipelago east Greenland and the zoo population ($p <$

0.05). In addition, a significant deviation was observed in OM54-23 in the east Greenland population ($p < 0.05$). The Canadian archipelago had the highest observed heterozygosity, followed by the introduced population in Sweden, west Greenland, east Greenland and Norway (Table 2). The inbreeding coefficient (F_{IS}) was highest in the native population of east Greenland and lowest in west Greenland (Table 2). In comparison with wild muskoxen, there is a relatively high amount of genetic variation within the pooled sample from different zoological parks in Sweden and Denmark.

The pairwise F_{ST} estimate between populations was highest between west Greenland and Norway ($F_{ST}=0.70$, $p < 0.05$) and lowest between Norway and the individuals from zoological parks ($F_{ST}=0.07$, Table 4). The Swedish population is more separated from the Norwegian population ($F_{ST}=0.60$, $p < 0.05$) than from east Greenland ($F_{ST}=0.23$, NS). The estimates of F_{ST} are congruent with Nei's unbiased genetic distance and the population assignment tests in GeneClass2 (Tables 4 and 5). The *O. moschatus* in Norway rarely assign to the population from west Greenland and reverse, while the Norwegian *O. moschatus* assign to the group of individuals from zoological parks (Table 5). Although some individuals assigned to several other populations when the threshold value of p was set at 0.05, several of the populations were completely excluded as possible sources (Table 5). Interestingly, the Swedish *O. moschatus* did not assign to the Norwegian population, from which they recently emigrated. Mostly, the wild Swedish population assign to the population in east Greenland (Table 5).

Of the 27 faecal samples from the wild Swedish population of eight individuals, five unique genotypes were

Table 2 The origin and number of muskoxen sampled for the study ($N_{sampled}$) and those used for genetic analysis ($N_{genotyped}$)

Population	$N_{sampled}$	$N_{genotyped}$	N_A	N_{PA}	P_{LOCI}	$N_{A/LOCI}$	A_5	H_E	H_O	F_{IS}
Canada	22	19	21	8	8	2.63	1.82	0.35±0.08	0.26±0.04	0.28
E. Greenland	58	14	15	1	4	1.88	1.54	0.26±0.10	0.10±0.03	0.64
W. Greenland	31	29	14	1	5	1.75	1.30	0.13±0.04	0.13±0.02	0.02
Norway	29	20	11	0	3	1.38	1.21	0.10±0.06	0.10±0.02	0.04
Sweden	8	5	13	0	4	1.63	1.35	0.14±0.06	0.15±0.06	-0.07
Zool. Parks	25	22	15	0	3	1.88	1.34	0.15±0.08	0.13±0.03	0.10
Global	173	109	26	10	8	3.25		0.33±0.10	0.14±0.01	

The zoological park (Zool. Parks) samples are collected from the zoological gardens in Copenhagen (Denmark), Järvsö, Kolmården and Lycksele (Sweden). Data include total number of alleles for all ten loci investigated (N_A) and number of alleles private alleles for each population (N_{PA}). P_{LOCI} is the number of polymorphic loci, and $N_{A/LOCI}$ is the mean number of alleles per locus and population. A_5 is the allelic richness standardised to a sample size of five (as in the Swedish population), using the rarefaction procedure in HP-Rare 1.1 (Kalinowski 2005). The expected heterozygosity (H_E) and the observed heterozygosity (H_O) are provided, including standard deviations, calculated in Microsatellite Tool Kit (Park 2001). F_{IS} is the inbreeding coefficient, calculated in Genepop on the Web (<http://genepop.curtin.edu.au/>), versions 3.1c-3.4 (Raymond and Rousset 1995)

Table 3 Name, accession code, repeat composition and fragment size of the microsatellite loci used in the current study (data from Van Coeverden De Groot and Boag 2004; Holm et al. 1999)

The number of alleles (N_A) found for each locus along with expected heterozygosity (H_E) and the observed heterozygosity (H_O) for the whole sample combined is also shown. Calculations were done in a microsatellite tool kit (Park 2001)

Locus	Accession code	Repeat	Size	N_A	H_E	H_O
MoDIAS1	AF082895	(TG) ₁₃	121	1	–	–
MoDIAS2	AF082896	(TG) ₂₁	204–208	3	0.085	0.068
MoDIAS5	AF082899	(CA) ₁₆	176–178	2	0.010	0.010
OM54-23	AY428656	(CA) ₂₀	104–108	3	0.479	0.175
OM58-06	AY428664	(CA) ₁₉	155	1	–	–
OM53-38	AY428655	(CA) ₂₁	159–161	2	0.502	0.255
OM56-30	AY428662	(CA) ₂₇	221–247	8	0.701	0.168
OM50-08	AY435177	(CA) ₁₈	158–160	2	0.010	0.010
OM51-19	AY428650	(CA) ₂₂	211–215	3	0.572	0.402
OM51-16	AY428649	(CA) ₁₈	263–269	3	0.241	0.055

found. The probability test showed that there was an 80% possibility that all individuals within these 27 samples had been sampled. After calculating probability of identity (P_{ID}) for the five genotypes discovered, there was a 94% possibility that there were two or more identical genotypes within the samples (Fig. 3). This indicates that all eight individuals from the wild Swedish population (as of 2006) likely were represented in the samples and that there were two or more individuals with identical genotypes.

The allele 106 from locus OM54-23 occurs in the Swedish population, but has not been observed in the ancestral Norwegian population. This allele is however abundant among *O. moschatus* from Greenland (frequency of 0.25 in east Greenland and 0.84 in west Greenland). Thus, this allele likely originates from the half-Greenlandic calf in the Swedish population. Finally, the deterministic projection of future genetic variation within the Swedish population, assuming no immigration, an initial population of one fertile bull and five cows and thereafter an equal sex ratio, show that after four generations the whole population will be monomorphic for all of the investigated microsatellite loci (Fig. 4).

Discussion

The low genetic variation detected in previous studies of *O. moschatus* (Fleischman 1986; Groves 1997; Mikko et al. 1999; MacPhee et al. 2005; Campos et al. 2010) is confirmed here. The highest heterozygosity ($H_o=0.26$) and the largest genetic variation were observed in native *O. moschatus* from the Canadian archipelago (Table 2). High-latitude species commonly experience both long- 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). Paleontological data also provide support for a dramatic population decline among *Ovibos* throughout the Pleistocene, and the low genetic variation seems to have persisted for a long time (MacPhee et al. 2005; Campos et al. 2010). Still, *O. moschatus* is one of the few representatives of the Pleistocene mammalian megafauna that have survived into recent times (MacPhee et al. 2005), probably a result of a combination of long generation time, purging and favourable random processes along with the fact that this species inhabit areas less frequented by human hunters.

Table 4 Pairwise estimates of F_{ST} (Weir and Cockerham 1984) in lower matrix and Nei's unbiased genetic distance (Nei 1978) in the upper matrix (sample localities abbreviated), using eight polymorphic microsatellite loci

Country	C	EG	WG	N	S	ZP
Canada	–	0.30*	0.28*	0.49*	0.37*	0.42*
East Greenland	0.36*	–	0.15*	0.03	0.11	0.03
West Greenland	0.47*	0.40	–	0.37*	0.18*	0.37*
Norway	0.58*	0.15	0.70*	–	0.21*	0.01
Sweden	0.42*	0.23	0.52	0.60*	–	0.19*
Zoological Parks	0.52*	0.11	0.66*	0.07	0.50*	–

* $p < 0.05$, estimates significant at this level based on 10,000 permutations performed in Genetix 4.05 (Belkhir et al. 2004) and after Bonferroni corrections (Rice 1989)

Table 5 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

Country (<i>N</i>)	C	GE	GW	N	S	ZP	Total
Canada (19)	19	9	3	0	0	4	35
East Greenland (14)	9	14	7	9	4	14	57
West Greenland (29)	25	29	29	0	1	26	110
Norway (20)	5	20	2	20	4	20	71
Sweden (5)	1	5	4	0	5	5	20
Zoological Parks (22)	11	22	6	15	4	22	80

A threshold *p* value of 0.05 was applied for assignments. Thus, some individuals may be assigned to more than one population and some not at all. The total number of assignments (right column) may thus exceed the actual sample genotyped (*N*, in parenthesis). Source population in left column, and populations assigned to abbreviated in horizontal line

The highest number of alleles per population and the largest mean number of alleles per locus and population are also found in the Canadian archipelago, followed by east Greenland, west Greenland and Norway. Thus, the pattern of allelic variation within the populations almost follows the expectations for sequential founder events (e.g. Le Corre and Kremer 1998), also confirmed among for example island populations of birds (Clegg et al. 2002; Pruett and Winker 2005) and translocated *A. alces* (Broders et al. 1999). The observed heterozygosity does, however, give a slightly different impression: In the introduced *O. moschatus* population in west Greenland, we observed higher heterozygosity ($H_o=0.13$) than the native population in east Greenland ($H_o=0.10$). The reason may relate to the assumption of Nei et al. (1975) that the amount of reduction

Fig. 3 Combined probability distribution of the occurrence of identical genotypes and missing individuals in the 27 faecal samples analysed from the Swedish population

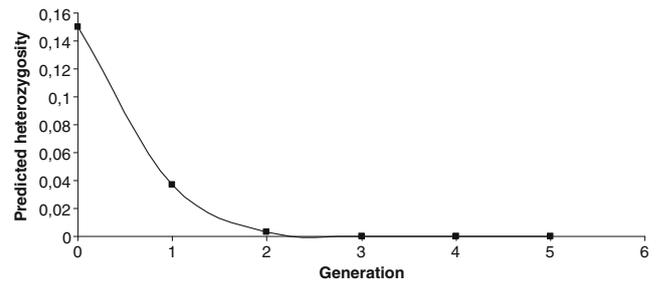
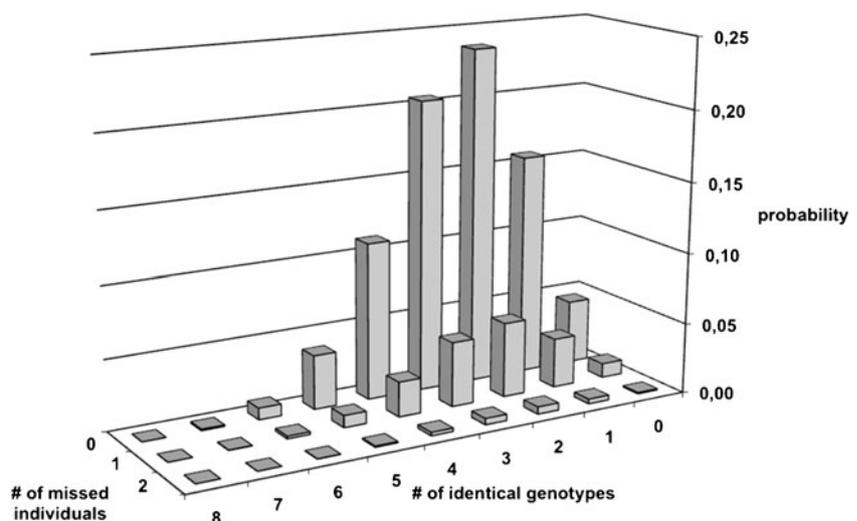


Fig. 4 Deterministic projection of future genetic variation within the Swedish *O. moschatus* population, starting with the current observed heterozygosity of 0.15, assuming no immigration, an initial population of one fertile bull and five cows and thereafter an equal sex ratio of three bulls and three cows

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). The *O. moschatus* population of east Greenland, although native, suffer from large numerical fluctuations because of high mortality caused by severe winters (Vibe 1967). The introduced population on west Greenland, however, has expanded rapidly after introduction, with a subsequent preservation of genetic diversity as a potential consequence. Thus, we believe that a temporary loss of alleles but retention of heterozygosity may explain the relatively high heterozygosity observed in the west Greenland muskox population.

The Swedish population also deviates from the sequential pattern because it contains more allelic variation and higher observed heterozygosity than the ancestral Norwegian population. When the allelic richness is standardised

for a sample size of five (as in the Swedish sample), the estimates from the Swedish sample even exceed that of the sample from introduced population of west Greenland as well as the admixed sample from zoological parks (Table 2). In addition, the estimates of genetic differentiation (F_{ST} and Nei's D) in the Swedish population reveal it to be more similar to the east Greenland populations than the ancestral population in Norway ($p < 0.05$). Thus, random processes during founder events may induce differentiation between populations, as seen for example among introduced populations of *Herpestes auropunctatus* (Thulin et al. 2006), *A. alces* (Broders et al. 1999; Hundertmark 2009) and *Cervus elaphus* (Hundertmark and Van Daele 2010).

The most important explanation for the Swedish population being more similar to the East Greenland population than the Norwegian is, however, the offspring sired by the Greenland bull. For example, allele 106 from locus OM54-23 is most likely unique for the calf that was sired by a captive bull with Greenlandic origin. That this allele has not been observed in the ancestral Norwegian population, but is abundant among *O. moschatus* from Greenland (frequency of 0.25 in east Greenland and 0.84 in the west), provides support for this conclusion. Thus, the half Greenlandic calf makes a significant difference with respect to the genetic variation, and our results clearly show the important contribution of even a single sire in a small population. Since inbreeding at the individual level is completely removed by a single outbreeding event, effects on population health by single individuals can be dramatic. It could, at least momentarily, increase the genetic variation considerably and potentially prevent inbreeding depression. Inbred animals might be less resistant to parasites (Coltman et al. 1999), experience decreased lifetime breeding success (Slate et al. 2000) or have lower birth weight and juvenile survival (Coltman et al. 1998). Even if there is no evident inbreeding or inbreeding depression, the possibility is important to consider in preventing future fitness reduction (Hedrick and Kalinowski 2000).

Conservation implications

The simulations of future heterozygosity within the Swedish population show that the population would be genetically monomorphic within approximately 15 years (Fig. 4). Since the now adult calf has proven to be a bull, and sired a recent offspring in the herd (first documented July 9, 2010, personal communication S. Mörtberg, see also <http://www.myskoxe.se>), there is still some hope for the striving population. There is nevertheless a large risk for a more rapid decline of heterozygosity than indicated in our simulation if genetic variation is lost because of sterility

due to old age among the five cows in the current population. Gradual exposure to deleterious recessive alleles due to increased homozygosity is an additional threat (Coltman et al. 1998). To reverse this negative trend, new introductions are necessary. The suggestion of Mills and Allendorf (1996) that one to ten migrants per generation into a population are sufficient to reduce the loss of genetic variation may be a suitable threshold [although Vucetich and Waite (2000) show that even more migrants are needed in small populations]. In addition, a relocation of the population to more suitable habitats is desirable (Nyqvist 2004). According to a local management plan outlined in 2002, the long-term goal is to maintain a population of 30 *O. moschatus* individuals in Härjedalen (Ericson 2002). We believe that this is a definite minimum to preserve *O. moschatus* in Sweden and suggest that two or more additional, viable family groups should be established with geographical connectivity to the current Swedish population.

To increase the genetic variation within the Swedish *O. moschatus* population, individuals used for supplemental introductions should preferably be drawn from more genetically diverse populations. With respect to these general recommendations and the estimates of genetic variation presented in this study, the populations in the Canadian archipelago and east Greenland population seem to be the most suitable sources. Although we only achieved samples from the Canadian archipelago (a minor proportion of the Canadian distribution, Fig. 1), the estimates of genetic differentiation and the population assignments show that the Canadian population is the most divergent, which may urge for some caution because there might be a risk that local adaptations or diseases might create a negative impact on the receiving population (Andersson et al. 2007). Thus, we believe that the population in east Greenland is the better choice for restocking. The Norwegian population should definitely be avoided because of a recent increase in mortality due to outbreaks of a bacterial skin disease (ecthyma) in the herd (Vikøren et al. 2008).

An alternative strategy is to continue using captive *O. moschatus* individuals for supplemental releases or reproduction as with the recent half-Greenlandic calf. The captive population in Scandinavia has the second highest allelic variation observed in this study (Table 2). Thus, genetic variation seems to be conserved within the captive population, most likely a consequence of the multiple origins of the captive individuals combined with careful breeding strategies that aim to preserve genetic variation (Mallinson 1995). This emphasises the importance of captive breeding of endangered species with fragmented and/or limited distribution and limited gene flow.

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Ethical standards Sample collections comply with the current law in Sweden.

Conflict of interest The authors declare that they have no conflict of interest.

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