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Historical and modern neutral genetic variability in Mednyi Arctic foxes passed through a severe bottleneck

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Abstract

Small isolated populations often show lower genetic variability. Demographic bottlenecks lead to loss of genetic variation too. Arctic foxes (*Vulpes lagopus*) have been isolated since the Pleistocene on Mednyi and Bering Islands (Commander Islands). In 1970–1980, the Mednyi population passed through a severe bottleneck due to a mange outbreak. Previous studies showed lack of genetic diversity in the contemporary Mednyi population that could be due to the long history of isolation and/or the recent bottleneck. To test both factors, we analyzed the mtDNA D-loop fragment and five microsatellites in pre-bottleneck Mednyi museum samples. Also, contemporary Mednyi, Bering and mainland Alaskan Arctic foxes were analyzed. Registered genetic variability in historical Mednyi was higher than in contemporary Mednyi Arctic foxes, but lower than in contemporary the Bering population. Our data confirms that the bottleneck reduced an already depleted polymorphism in Mednyi Arctic foxes. Lack of genetic variability could be a reason why the Mednyi population did not recover following the outbreak of mange.

Introduction

Genetic variation is an important component for both short- and long-term persistence of populations as lack of diversity has negative effects on fitness and may compromise the adaptive response to a changing environment (Hedrick & Kalinowski, 2000; Willi, Van Buskirk & Hoffmann, 2006). Small and/or isolated populations often show lower genetic variability compared with widely distributed ones in large continuous habitats (Frankham, 1998; Keller & Waller, 2002). Demographic bottlenecks result in a loss of genetic variation and increased inbreeding (Wright, 1969; Theodorou & Couvet, 2006).

The Arctic fox (*Vulpes lagopus*) is a canid with a circumpolar distribution. While most of the mainland populations show pronounced gene flow (Dalén *et al*., 2002, 2005; Geffen *et al*., 2007), Commander Islands (North Pacific, Russia) Arctic foxes are completely isolated in the south of the species' range (Fig. 1) and represent two endemic subspecies (Geptner & Naumov, 1967): *V. lagopus beringensis* Merriam, 1902 on Bering Island (S = 1667 m^2) and *V. l. seminovi* Ognev, 1921 on Mednyi Island (S = 186 m^2). Since their discovery in 1741, these foxes have been subject to intense hunting pressure (Il'ina, 1950). However, up to the middle of the 20th century, 2000 to 4000 foxes lived on Bering and up to 1000 inhabited Mednyi Island (Geptner & Naumov, 1967). In 1970–1980, the population on Mednyi Island crashed because of a mange epizootic (Goltsman, Kruchenkova & Macdonald, 1996; Goltsman *et al*., 2005). Currently, the Mednyi population numbers about 90 individuals (Goltsman *et al*., 2005).

An important factor determinant of the genetic differentiation of Arctic foxes on Commander Islands is the lack of sea ice. This prevents migration and corresponding gene flow (Dalén *et al*., 2005; Geffen *et al*., 2007). Previous genetic studies of the contemporary Mednyi population displayed low variability. Examination of the D-loop of the mitochondrial DNA revealed monomorphism in Mednyi foxes, while in the Bering population, seven haplotypes were observed (Dzhykiya *et al*., 2007). In other research, three mtDNA haplotypes were found in Mednyi and two haplotypes in the Bering population (Geffen *et al*., 2007). Analysis of 11 microsatellite loci in the latter study revealed low heterozygosity ($H_O \pm$ SD) in both contemporary Mednyi and Bering populations (0.19 ± 0.28 and 0.50 ± 0.17, respectively). Analysis of major histocompatibility complex class II genes showed low variability in historical Mednyi and monomorphism in contemporary Mednyi Arctic foxes (Ploshnitsa *et al*., 2011).

The lack of contemporary genetic diversity in the Mednyi population might result from the severe bottleneck and/or a founder effect and subsequent long history of isolation. The use of museum specimens has provided a unique opportunity to study genetic variability in pre-bottleneck populations and

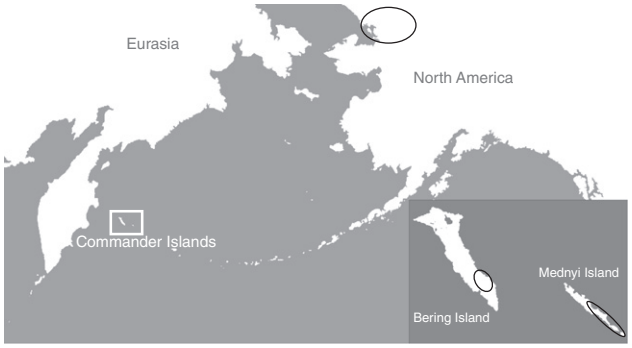

Figure 1 Map showing the geographical origin of the samples collected in northern continental Alaska, Mednyi and Bering Islands (encircled).

to test the suggested hypotheses. This approach has allowed the reconstruction of past levels of genetic variation, and estimation of changes in population genetic composition over time, in northern elephant seals (*Mirounga angustirostris*, Weber *et al*., 2000), sea otters (*Enhydra lutris*, Larson *et al*., 2002) and grey wolves (*Canis lupus*, Leonard, Vila & Wayne, 2005). The goals in this study were to document pre-bottleneck patterns of neutral genetic variability in Mednyi Arctic foxes and to evaluate impacts of the recent population decline and insular isolation on its genetic diversity.

Methods

Samples and DNA extraction

The sample comprised 49 Mednyi Arctic fox museum specimens and one Bering museum sample (collected in 1911–1946). Twenty-seven contemporary Mednyi animals (collected in 1997–2006), 12 contemporary Bering samples (collected during winter 2001) and 24 contemporary samples from Alaska were also analyzed. Previous surveys did not indicate population differentiation between Arctic foxes connected by land or pack ice (Dalén *et al*., 2005; Geffen *et al*., 2007), thus we choose Alaskan samples to represent the mainland outbred population. The historical material from the museum comprised of skulls. The contemporary material comprised of skin pieces stored in ethanol.

The DNA from historical samples was extracted from fine bone powder following a protocol suggested by Yang *et al*. (1998) using the 'QIAquick PCR purification kit' (Qiagen, Hilden, Germany). The detailed procedure is described in Ploshnitsa *et al*. (2011). Contemporary DNA was extracted using the phenol-chloroform method described by Sambrook, Fritsch & Maniatis (1989). Historical and modern DNA were stored separately at –20°C.

D-loop mtDNA

A fragment of the D-loop mtDNA including hypervariable region I (HVRI) was amplified using primers Pex1F and H3R (Dalén *et al*., 2002). For amplification of some museum samples, additional primers were used (Nyström, Angerbjörn & Dalén, 2006). Amplifications were performed in 20-μL volumes containing 5 μL museum DNA extract (2 μL for contemporary DNA); 0.3 mM deoxyribonucleotides (dNTPs); 1x polymerase chain reaction (PCR)-buffer without detergents; 5 U Hotstar *Taq* polymerase (Qiagen); 0.5 μM each primer; 1.8 mM $MgCl_2$ and sterile deionized water. PCR was set up with the following conditions: preheating 95°C 15 min; 40–45 cycles (30 cycles for contemporary DNA) of 95°C 30 s, 50°C (52°C for Pex2Fb and Pex2Rb primers) 30 s, 72°C 40 s; final elongation 72°C 5 min. Electrophoresis in 2% agarose gel was performed to confirm the absence of contamination. The PCR products (5 μL) were purified using 2U Exonuclease I and 10 U calf alkaline phosphotase by incubation at 37°C for 60 min with final heating at 85°C for 15 min. Sequences of each fragment were performed from both directions on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems Foster City, CA, USA) following the manufacturer's instructions.

Microsatellites

We genotyped five microsatellites (771, Mellersh *et al*., 1997; 377, Ostrander *et al*., 1995; CXX173, CXX250 and CXX140 Ostrander, Sprague & Rine, 1993), which had been successfully used for investigation of Scandinavian Arctic foxes (Nyström *et al*., 2006). Fragment analysis was performed using the universal fluorescent labelling method (Shimizu *et al*., 2002). PCR of each locus was performed separately and carried out in 10-μL volumes, containing 3 μL historical DNA (1 μL modern DNA), 0.3 mM dNTPs, 1x PCR-buffer, 2.25U Hotstar *Taq* polymerase (Qiagen), deionized water, 0.02 μM forward primer with 'tail' (complementary sequence to M13 primer), 0.5 μM each primers – reverse and M13, and 1.5 mM $MgCl_2$. Amplifications were performed using the 'touchdown' method with the following cycles: preheating 95°C for 10 min; 45 cycles for museum (35 cycles for contemporary DNA) with 95°C 30 s, primer annealing for 30 s with temperature dropping for the first 15 cycles from 65 to 58°C each cycle for 0.5°C, 72°C 1 min; final elongation 72°C for 5 min. Negative controls and concentration of PCR products were examined by agarose gel electrophoresis. PCR fragments were separated electrophoretically using an ABI PRISM 3100 Genetic Analyzer and allele sizes were scored against size standards ROX-400HD or ROX-500 (Applied Biosystems).

Contamination controls and authenticity

We followed the principal rules established for work with ancient DNA (Cooper & Poinar, 2001). Due to low copy number and the degraded nature of historical DNA, precautions to avoid contamination were taken during each procedure of extraction and amplification. Bone drilling, DNA extraction and PCR from the museum samples took place in a separate room in a laminar box dedicated to historical DNA research. Only one sample at a time was drilled. Before each drilling, work surfaces and equipment were sterilized with chlorine-containing solutions and/or ultraviolet irradiation.

Each amplification experiment contained at least two negative controls. To avoid allele dropout, two replications were performed for all homozygous samples. Second independent extracts from 10 museum samples were obtained. D-loop sequences and microsatellites from the second extractions were amplified and the analysis produced identical results.

Data analysis

Alignment of the obtained sequences was performed using the MEGA 4.0 software (Tamura *et al*., 2007). The software Arlequin version 3.1 (Schneider, Roessli & Excoffier, 2000) was used to calculate the genetic variability. Nucleotide diversity and gene (haplotype) diversity were calculated according to Nei (1987). NETWORK 4.6.1.0 (Bandelt, Forster & Röhl, 1999) was used to construct a median network. Microsatellite genetic variation was measured as the total number of observed alleles (N_A), allele richness (R_S) and average heterozygosity (H_A; Tajima, 1983). FSTAT ver. 2.9.3 (Goudet, 1995) was used to calculate allelic richness based on the minimum sample size and allele frequencies. The observed heterozygosity (H_O) and expected heterozygosity (H_E) from Hardy–Weinberg assumptions were also calculated and deviations from Hardy–Weinberg equilibrium were tested using an exact test based on a Markov chain algorithm with a forecast chain length of 100 000 steps and 1000 dememorization steps (Levene, 1949). A linkage disequilibrium test (10 000 permutations and 10 random initial conditions) was used to test for the presence of a significant association between all pairs of microsatellite loci (Slatkin, 1994), corrected for multiple testing using the Bonferroni correction (Rice, 1989). Evidence for a genetic bottleneck was evaluated using software BOTTLENECK (Piry, Luikart & Cornuet, 1999), which assumed that a signature of a severe reduction in the effective size was an excess of microsatellite heterozygosity relative to expected heterozygosity under Hardy–Weinberg equilibrium (Cornuet & Luikart, 1996). A Wilcoxon signed-rank test was used to test for such an excess (Cornuet & Luikart, 1996) and the two-phase model, consisting of the infinite allele model and the stepwise mutation model of microsatellite evolution, was used (Garza & Williamson, 2001). The distribution of allele frequencies in the studied populations of Arctic foxes was also evaluated to determine if a mode shift characteristic of a bottleneck had occurred (Luikart & Cornuet, 1998) as estimated by BOTTLENECK. The extent of population differentiation was examined by pairwise F_{ST} (10 000 permutations; Wright, 1969) using Arlequin Ver 3.1. Simulations using the software EASYPOP (Balloux, 2001) were carried out to investigate long-term isolation and bottleneck effect on genetic diversity in Commander Arctic foxes. For the first scenario, different total population size of Commander Arctic fixes (12 000, 6000, 2000 and 500 individuals) and Mednyi : Bering ratio (1 : 1, 1 : 4 and 1 : 24) were tested. From colonization of the Commander Islands until the moment of its split, 18 000 generations were set, and 6000 generations were set since the populations split. Equal sex ratio, random mating, five loci with maximal initial variability, free recombination between loci with 0.0001 mutation rate and single-step mutation model were set. For investigation of bottleneck impact, 20, 100, 500 and 1000 individuals were set for 20 generations as population passed through a bottleneck with initial allele diversity as it was observed in the historical Mednyi population. All simulations were replicated 10 times. Average heterozygosity, average allele number and F_{ST} were computed from the simulated data using the software ARLEQUIN as described above. Mann–Whitney *U*-test was used to test statistical significance when genetic diversity from different populations was compared.

Results

D-loop mtDNA

Consensus sequences of the HVRI of D-loop mtDNA (about 290 bp) were obtained from 31 Mednyi museum individuals, one Bering museum sample and 10 contemporary Alaskan Arctic foxes. The MEDN haplotype, which previously was observed in the contemporary Mednyi population (GenBank: DQ6307, Dzhykiya *et al*., 2007), was registered in all tested Mednyi museum samples. In addition, five museum Mednyi Arctic foxes had one polymorphic site (C/A in 37th position, Table 1). The sequence of the D-loop fragment from the historical Bering sample represents a new haplotype BA1 (GenBank: HM748647, Table 1). In Alaskan Arctic foxes, five haplotypes were identified. Seven polymorphic positions were found in Alaska haplotypes, while 16 variable sites were registered when compared to Commander haplotypes (Table 1). Nucleotide diversity was 0.009 (SD = 0.006) and haplotype diversity was 0.822 (SD = 0.097). All Alaskan haplotypes have previously been reported (Dalén *et al*., 2005). Median network (Fig. 2) showed that Commander Arctic foxes form a cluster, which is clearly distinct from continental populations.

Microsatellites

Thirty-six of the Mednyi museum individuals along with 27 contemporary Mednyi, 12 contemporary Bering and 24 Alaskan Arctic foxes were successfully genotyped for five microsatellites. Eleven alleles were found in museum, and eight alleles in contemporary, Mednyi Arctic foxes, 22 alleles in Bering and 43 alleles in Alaskan foxes (Table 2). Average allelic richness ($R_S \pm$ SD) for historical Mednyi was 2.1 ± 1.5; for contemporary Mednyi, it was 1.6 ± 1.1; for Bering and Alaska, it was 4.4 ± 2.7 and 7.5 ± 3.3, correspondingly. CXX140 was monomorphic in historical Mednyi, but after the epizootic outbreak, variability was lost in CXX250 too. All alleles identified in the contemporary Mednyi population were found in the museum samples. After the epizootic, the rarest alleles have not found in contemporary Mednyi Arctic foxes (Table 3). We did not identify linkage disequilibrium between any loci. Observed heterozygosity ($H_O \pm$ SD) was 0.36 ± 0.23, 0.29 ± 0.53, 0.65 ± 0.27 and 0.76 ± 0.03 in museum Mednyi, contemporary Mednyi, Bering and Alaskan samples, respec-

Table 1 Polymorphic positions in D-loop mtDNA sequences registered in historical Mednyi (haplotypes MEDN and MEDN with polymorphism), historical Bering (BA1) and contemporary Alaskan (S2, H2, C1, N1 and H5) Arctic foxes

														1	1	2	2	2
			2	2	2	3	3	3	3	3	3	3	9	2	5	2	3	8
		n	2	4	6	1	2	3	4	5	6	7	8	8	5	2	0	8
MEDN	DQ6307	26	C	C	C	–	–	C	C	C	C	A	T	T	A	T	T	T
MEDN	(polymorphic)	5	.	.	.	–	–	.	.	.	.	M	.	.	.	.	.	.
BA1	HM748647	1	.	.	.	–	–	.	.	.	.	C	.	C	.	.	.	.
S2	AY321133	4	T	A	A	C	T	T	A	A	A	.	.	C	G	.	.	.
H2	AY321125	2	T	A	A	C	T	T	A	A	A	.	.	C	.	.	C	.
C1	AY321143	1	T	G	A	C	T	T	A	A	A	.	C	C	.	.	.	.
N1	AY321136	2	T	A	A	–	T	T	A	A	A	.	.	C	.	C	.	C
H5	AY321127	1	T	A	A	–	T	T	A	A	A	.	.	C	.	.	.	C

n, number of observed animals with the same haplotype. Nucleotides: A, adenine; T, thymine; C, cytosine; G, guanine; M, polymorphic position with adenine and cytosine.

tively. No significant differences in average heterozygosity (H_A) were found between museum and contemporary Mednyi Arctic foxes. However, the average heterozygosity was significantly lower in both Mednyi samples compared with the Bering or Alaskan populations (Table 2). Departures from Hardy–Weinberg equilibrium were not significant for any of the loci in Mednyi Arctic foxes. One locus (CXX377) showed a significant deviation as deficit of observed heterozygosity in the Bering sample (P-value = 0.02; Table 2).

Using BOTTLENECK software, no significant excess of heterozygosity in either Commander population (museum Mednyi: $P = 0.09$; contemporary Mednyi: $P = 0.06$; Bering: $P = 0.50$) was observed. But there was significant excess of heterozygosity registered in Alaska samples ($P = 0.02$). However, evidence of a genetic bottleneck was detected using a test for mode shift of allele frequencies. In contemporary Mednyi Arctic foxes, allele frequencies had shifted mode, while museum Mednyi, Bering and Alaska populations showed normal L-shape distributions (Fig. 3).

Pairwise comparison between tested populations revealed significant difference between pre- and post-bottleneck Mednyi population ($F_{ST} = 0.111$, P-value = 0.000). The differentiation between Mednyi and Bering Arctic foxes was considerable and increased after the epizootic (Table 4). The greatest differentiation was observed between both Mednyi populations and either Bering or Alaskan Arctic foxes, while F_{ST} value between contemporary Bering and Alaska remained significantly low (0.084, P-value = 0.000).

The software EASYPOP revealed that the level of genetic variability in the simulation was the closest to the one observed in Commander Arctic foxes, when the total number of Commander Arctic foxes was set at 6000 individuals and Mednyi : Bering ratio was set at 1 : 4 (Fig. 4). Average heterozygosity ($H_A \pm SE$) for Mednyi was 0.26 ± 0.12, and for Bering, it was 0.61 ± 0.22. Average allele number ($N_A \pm SE$) was 1.9 ± 0.4 for Mednyi and 5.4 ± 0.6 for Bering. We also simulated a bottleneck event. The level of genetic diversity observed in contemporary Mednyi population was similar to the one registered in the simulation when population size was set at 100 individuals (Table 5).

Discussion

Founder effect and insular isolation of Commander Arctic foxes

In a study by Geffen *et al.* (2007), the high haplotype variability shown in D-loop mtDNA in the contemporary Mednyi population does not accord with both the very low allele variability found in 11 microsatellite loci published in the same study or with the mtDNA monomorphism reported by Dzhykiya *et al.* (2007). All tested museum Mednyi samples shared the same MEDN haplotype. The observed mtDNA variability reported by Geffen *et al.* (2007) is therefore puzzling and should be interpreted with caution.

The registered polymorphic site in five Mednyi samples located in the end of poly-C tract might be the result of degradation, analysis artefacts, nuclear mtDNA copy or heteroplasmy. Contamination is unlikely as MEDN is a unique haplotype characterized by several specific substitutions. Minimal errors were registered during research on historical mtDNA in Scandinavian Arctic foxes, where the same methods were applied (Nyström *et al.*, 2006). Still, to eliminate any possibility of analysis errors and degradation, we performed more than three iterations for each sample. The same results were obtained with different primer sets and with amplifying from different DNA extracts. We could not reject the possibility of heteroplasmy as mononucleotide stretch is reported as a region with a high probability of heteroplasmy (Bendall & Sykes, 1995). The Poly-C tract is specific for Commander Arctic foxes and causes high rates of *taq* polymerase mistakes, resulting in poor sequence following this region. This made it impossible to perform further investigation of the registered polymorphism.

Registered mtDNA monomorphism and reduced microsatellite variability in museum samples confirm that the Mednyi population already had depleted genetic variability before the bottleneck. A founder effect and subsequent insular isolation along with heavy hunting pressure are plausible explanations for the lack of genetic diversity in the Mednyi population.

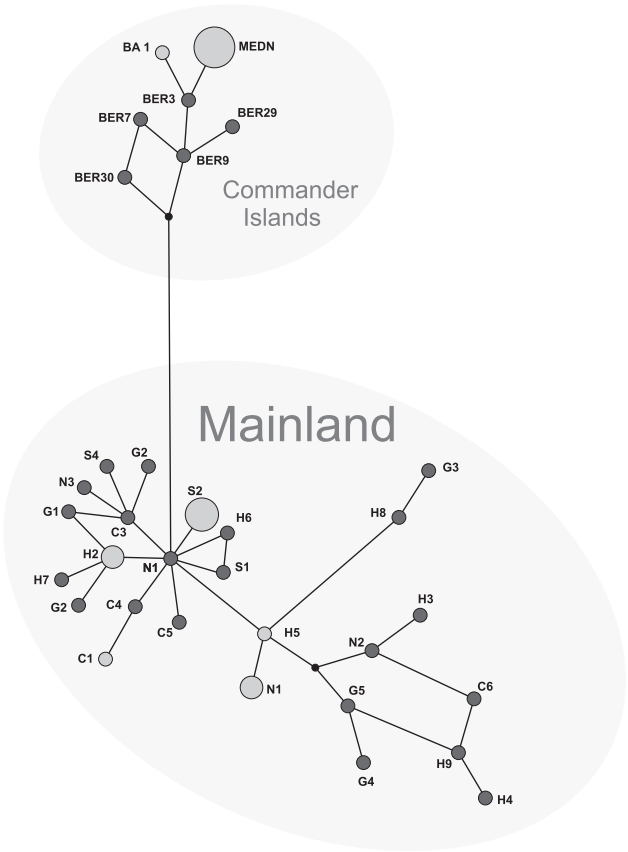

Figure 2 A median network of D-loop mtDNA haplotypes observed in historical Commander (BA1 and MEDN haplotypes) and modern Alaskan Arctic foxes in current study (marked with light-grey circles) and previously published haplotypes (marked with dark-grey circles; Dalén *et al.*, 2005; Dzhykiya *et al.*, 2007). Haplotypes H2, H5 were described before as Holarctic haplotypes distributed through the species natural habitat. Haplotypes N1 and C1 were previously described only for Nearctic and Canada, correspondingly. Distribution of the S2 was previously restricted to Siberia. Missing alleles (or latent vertices) are marked with small size dots. The distance between the alleles corresponds to the number of substitutions. The size of the light-grey circles corresponds to the number of samples with the same haplotype observed in this study.

High rates of hunting during earlier years might have reduced the genetic polymorphism on the Islands. In Scandinavia, comparatively few mtDNA haplotypes seems to have been lost as a consequence of the recolonization process due to climate change, whereas human over-exploitation caused a loss of about 60% of the haplotypes (Dalén *et al.*, 2007). Thus, the original (pre-1741) diversity of foxes on Mednyi Island might have been higher than we found in the museum samples. However, despite only 12 animals from the Bering population being genotyped, we registered higher genetic variability in Bering Arctic foxes in comparison with the historical Mednyi samples. Since the Bering population is larger, it might withstand hunting pressure better than the smaller Mednyi population. Therefore, we cannot reject a long-term difference in population size as a potential reason for lower genetic variability in the historical samples from Mednyi Island.

We observed strong divergence of Commander Arctic Foxes' mtDNA (Fig. 2) while observed diversity in Alaskan samples supports a previously published suggestion of high gene flow (Dalén *et al.*, 2005; Geffen *et al.*, 2007). Based on microsatellites, we obtained signs of high divergence of Mednyi Arctic foxes from either 'sister' Bering population or more distant Alaskan Arctic foxes. Also, the Bering population had lower F_{ST} value with Alaskan samples, rather than with those from Mednyi Arctic foxes (Table 4). Similar results were obtained by Geffen *et al.* (2007). The F_{ST} parameter might be affected by the very low allele diversity found in the Mednyi population, while Bering Arctic foxes remain more variable and subsequently share higher number of alleles with Alaskan foxes.

Using EASYPOP simulation of colonization and isolation of Commander Islands, we registered genetic diversity and differentiation between insular populations similar to the ones observed when founder Commander population was set at about 6000 individuals and split in ratio 1 : 4 (Fig. 4). The results from the simulation agreed with the number of Commander Arctic foxes observed at the moment of discovery (Il'ina, 1950; Geptner & Naumov, 1967).

Impact of the demographic bottleneck on genetic diversity

Our analysis revealed that foxes from the modern Mednyi population lost three alleles out of 11 registered in museum samples due to an epizootic and the subsequent drop in population size. Genetic drift affects smaller populations and mostly acts against rare alleles (Nei, Maruyama & Chakraborty, 1975). These alleles, which are lost in the contemporary Mednyi population, were at low frequency in museum samples (Table 3). Simulation by software EASYPOP showed that the loss of genetic diversity that we witnessed in contemporary Mednyi arctic foxes could be registered if the population effective size is set at about 100 individuals (Table 5).

At the same time, no significant difference in heterozygosity was observed for all tested Commander populations using BOTTLENECK software, while significant excess of heterozygosity was found in Alaskan Arctic foxes, which had not experienced any recent dramatic bottlenecks. The Alaskan Arctic foxes represent an outbred large population, which has big effective size and continuous distribution with pronounced influence of gene flow (Dalén *et al.*, 2005; Geffen *et al.*, 2007). Although a mode shift test showed a shift in allele frequencies indicating a recent genetic bottleneck in contemporary Mednyi Arctic foxes, other tested populations showed a normal L-shape distribution mode (Fig. 3).

A bottleneck of a translocated population of Roosevelt elk (*Cervus elaphus roosevelti*) with minimal effective size less than eight individuals did not show evidence of either heterozygosity excess or the mode shift tests (Hundertmark & Van Daele,

Table 2 Number of alleles, (N_A), observed (H_O) and expected (H_E) heterozygosity with corresponding *P*-value, allele richness (R_S) and average heterozygosity (H_A) with standard error (SE) calculated for five microsatellites loci

	Hist. Mednyi					Cont. Mednyi					Cont. Bering					Cont. Alaska				
	(*n* = 36)					(*n* = 27)					(*n* = 12)					(*n* = 24)				
Locus	N_A	H_O	H_E	*P*-value	R_S	N_A	H_O	H_E	*P*-value	R_S	N_A	H_O	H_E	*P*-value	R_S	N_A	H_O	H_E	*P*-value	R_S
CXX140	1	Monomorphic			1.00	1	Monomorphic			1.00	6	0.75	0.68	0.31	6.00	8	0.75	0.83	0.38	6.87
CXX173	2	0.47	0.42	0.68	2.00	2	0.44	0.50	0.69	2.00	3	0.75	0.70	1.00	3.00	5	0.75	0.76	0.88	4.94
CXX250	3	0.25	0.23	1.00	2.51	1	Monomorphic			1.00	3	0.67	0.67	1.00	3.00	10	0.79	0.88	0.06	8.86
377	2	0.53	0.46	0.47	2.00	2	0.33	0.48	0.12	2.00	5	0.42	0.49	0.02*	5.00	10	0.71	0.87	0.10	8.52
771	3	0.53	0.51	0.34	2.99	2	0.56	0.46	0.41	2.00	5	0.67	0.71	0.53	5.00	10	0.79	0.87	0.39	8.53
$H_A \pm$ SE	0.32 ± 0.10					0.29 ± 0.09					0.65 ± 0.17					0.84 ± 0.21				

n is the size of each sample. Significant *P*-value (<0.05) indicated by asterisk.

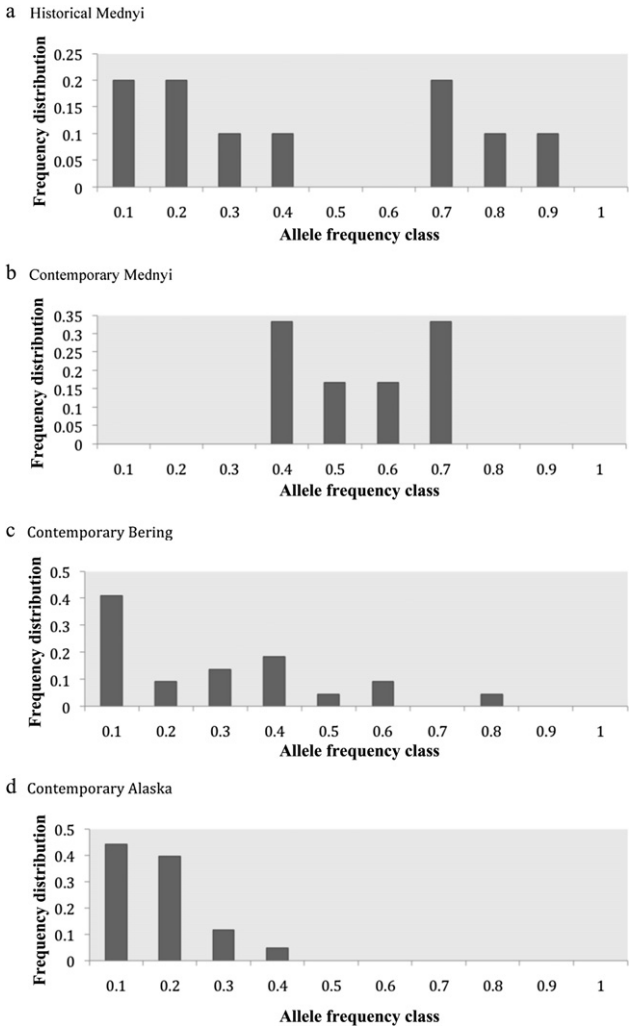

Figure 3 Mode shift test indicating a bottleneck event using software BOTTLENECK. (a) The normal L-shape distribution in historical Mednyi population. (b) Shifted mode in contemporary Mednyi population. (c) The normal L-shape distribution in contemporary Bering population. (d) The normal L-shape distribution in Alaskan Arctic foxes.

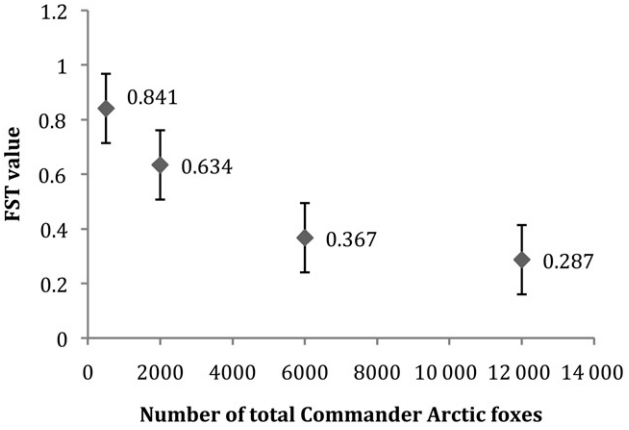

Figure 4 Results from simulation in EASYPOP, assuming different total population size for Commander Arctic foxes. At the moment of isolation, Mednyi and Bering were represented as 1 : 4 ratio. The expected pairwise F_{ST} values are averages from 10 replications.

2010). Bottleneck detection is critical for the interpretation of historical demography of populations and can be a valuable tool for endangered species management, but current tests for bottlenecks do not always detect them. Thus, the possibility of type I errors may be greater than sometimes anticipated (Williamson-Natesan, 2005; Hundertmark & Van Daele, 2010).

Implication to future evolution

Recently, much attention has been given to the ability of a population to adapt to changes in habitat availability (Teacher, Thomas & Barnes, 2011). Earlier research revealed no evidence that ensured that Arctic foxes have the capacity to track habit change (Dalén *et al.*, 2007). That makes this species, already a denizen of extreme latitudes (Hersteinsson & Macdonald, 1992), especially vulnerable to climate change. While the ability to track changing habitats may be important for mainland populations, Commander Arctic foxes are completely isolated and unable to migrate that makes their situation perilous. Absence of gene flow and

Table 3 Allele frequencies of five neutral microsatellite loci calculated for each population of Arctic foxes

	Hist. Mednyi $n = 36$	Cont. Mednyi $n = 27$	Cont. Bering $n = 12$	Cont. Alaska $n = 24$
Locus: CXX140				
136	–	–	–	0.08
140	–	–	0.04	0.02
142	–	–	0.04	0.02
144	1.00	1.00	0.29	0.29
146	–	–	–	0.10
148	–	–	0.50	0.19
150	–	–	0.08	0.08
152	–	–	0.04	0.21
Locus: CXX173				
120	–	–	–	0.13
122	0.29	0.44	0.33	0.35
124	0.71	0.56	0.33	0.31
126	–	–	0.33	0.10
128	–	–	–	0.10
Locus: CXX250				
115	0.03	–	–	–
119	–	–	–	0.13
121	–	–	0.42	0.23
123	–	–	–	0.06
125	–	–	–	0.19
127	0.88	1.00	0.38	0.04
129	0.10	–	0.21	0.13
131	–	–	–	0.10
133	–	–	–	0.04
137	–	–	–	0.04
139	–	–	–	0.04
Locus: 377				
173	–	–	–	0.02
175	–	–	–	0.08
177	–	–	–	0.19
179	–	–	0.08	0.06
181	0.35	0.39	0.71	0.25
183	0.65	0.61	0.04	0.10
185	–	–	0.13	0.10
187	–	–	–	0.13
189	–	–	0.04	0.04
191	–	–	–	0.02
Locus: 771				
90	–	–	–	0.10
92	–	–	–	0.04
94	–	–	–	0.02
96	–	–	0.13	–
98	–	–	–	0.06
100	–	–	0.21	0.27
102	–	–	0.08	0.13
104	0.17	–	0.50	0.08
106	–	–	0.08	0.13
108	0.67	0.35	–	0.15
112	0.17	0.65	–	–
118	–	–	–	0.02

The name of an allele is referred to its fragment size; *n* is the size of each sample.

Table 4 Pairwise F_{ST} values and *P*-value (upper triangle of the table) between Arctic fox populations computed on the basis of five neutral microsatellites loci

	Hist. Mednyi	Cont. Mednyi	Cont. Bering	Cont. Alaska
Hist. Mednyi	/	0.000	0.000	0.000
Cont. Mednyi	0.111	/	0.000	0.000
Cont. Bering	0.386	0.407	/	0.000
Cont. Alaska	0.307	0.322	0.084	/

Table 5 Average heterozygosity (H_A) and allele number (N_A) obtained from the simulation in EASYPOP when different population size was tested

	Tested population size for contemporary Mednyi Arctic foxes			
	20	100	500	1000
$H_A \pm SE$	0.11 ± 0.05	0.32 ± 0.11	0.41 ± 0.13	0.43 ± 0.13
$N_A \pm SE$	1.30 ± 0.22	2.00 ± 0.30	2.20 ± 0.37	2.20 ± 0.37

The values are averages from 10 replications. Significant correlations between simulated population with size 100 and observed in contemporary Mednyi Arctic foxes values were registered.

demographic fluctuation put Commander Arctic foxes at risk of depletion of genetic diversity and heightens the risk that they will lack the adaptability to adjust to environmental changes. Moreover, hunting exacerbates these risks (Dalén *et al.*, 2007).

No single factor explains the loss of genetic variability among Mednyi Island foxes, but rather a combination of the founder effect, long-term isolation, overexploitation, parasite invasion and a demographic crash. Nowadays, despite the fact that the population is protected and there are no any apparent environmental limitations, the Mednyi Arctic foxes have not recovered their former abundance (Goltsman *et al.*, 2005). We suggest that the genetic diversity of contemporary Mednyi foxes is so low that it constrains their reproduction and adaptability, and therefore has contributed to the failure of the population to recover from the outbreak of mange (Ploshnitsa *et al.*, 2011).

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