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Detection of farm fox and hybrid genotypes among wild arctic foxes in Scandinavia

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Abstract

In Scandinavia, farmed arctic foxes frequently escape from farms, raising concern about hybridization with the endangered wild population. This study was performed to find a genetic marker to distinguish escaped farm foxes from wild Scandinavian foxes. Microsatellite and mitochondrial control region variation were analyzed in 41 farm foxes. The results were compared with mitochondrial and microsatellite data from the wild population in Scandinavia. The farm foxes were genetically distinct from the wild foxes ($F_{ST} = 0.254$, $P < 0.00001$) and all farm foxes had a single control region haplotype different from those observed in the wild population. We developed a method based on Restriction Fragment Length Polymorphism (RFLP) on the mitochondrial control region to differentiate between farmed and wild arctic foxes. This test was subsequently successfully used on 25 samples from free-ranging foxes, of which four had a suspected farm origin. All four of the suspected foxes, and none of the others, carried the farm fox haplotype. Three of these were successfully genotyped for all eleven microsatellite loci. A population assignment test and a Bayesian Markov Chain Monte Carlo analysis indicated that two of these individuals were escaped farm foxes, and that the third possibly was a hybrid between a farmed and a wild arctic fox.

Introduction

Hybridization has led to extinction of many populations and species (Rhymer and Simberloff 1996; Allendorf et al. 2001). According to theoretical simulations, hybridization is one of the most significant threats to endangered species (Wolf et al. 2001). It has also been suggested that low population size greatly affects the extinction risk due to hybridization (Wolf et al. 2001). Evidence of hybridization between divergent species or populations in the wild has been described in a variety of carnivore species (e.g., Fergus 1991; Lehman et al. 1991; Rozhnov 1993; Reich et al. 1999).

Introgression of alien alleles can cause outbreeding depression and reduce fitness by two different mechanisms (Templeton 1986; Lynch 1991; Edmands and Timmerman 2003). Firstly, hybridization between genetically distinct populations, who are highly adapted to their local environment, can disrupt interactions between genes and the environment and thereby result in offspring with reduced fitness in both parental environments. Secondly, outbreeding depression can be caused by disruption of coadapted gene complexes. Outbreeding depression has been reported in a variety of species (e.g. Coyne and Orr 1989; Brown 1991; Garnier-Géré et al. 2002; McGinnity et al. 2003; Gilk et al. 2004),

including mammals like the Tatra mountain ibex (Greig 1979), the Arabian oryx (Marshall and Spalton 2000) and Goeldi's monkey (Vàsàrhelyi 2002).

Another aspect of hybridization concerns domesticated animals that escape from captivity and hybridize with their wild progenitors (Arnold 2004). This has previously been observed in carnivores like the gray wolf (Hope 1994; Vilá and Wayne 1999), the Scottish wildcat (Beaumont et al. 2001), the European wildcat (Hubbard et al. 1992) and the polecat (Davison et al. 1999). Domesticated populations are often subjected to intensive selection and thereby reduced genetic variation (Arnold 2004) and high rates of inbreeding (Ralls et al. 1988). These factors may, together with the altered natural and sexual selection in captivity, result in evolutionary divergence between wild and domesticated individuals (e.g., Swain et al. 1991; Petersson et al. 1996; Einum and Fleming 1997). Therefore, it is likely that hybridization between domesticated and wild animals could have detrimental effects on the wild population (e.g., Wiseman et al. 2000), especially when the wild population shows low numbers.

Farming of arctic foxes (*Alopex lagopus*), to produce fur for the clothing industry, has been performed for approximately 100 years (Konnerup-Madsen and Hansen 1980; Nes et al. 1987). The first arctic fox farm in Scandinavia was established in 1913 in Norway, using foxes imported from farms in Alaska (Nes et al. 1987; Frafjord 1993). Since the 1930s, breeding of arctic foxes have become more intensive and profitable (Konnerup-Madsen and Hansen 1980). Today, the farms in Scandinavia breed foxes originally imported from Alaska, Greenland, Jan Mayen and Svalbard (Nes et al. 1987; Nordrum 1994). Farm foxes are usually displayed to hard selective breeding due to breeding goals like fur quality, fecundity and litter size (Nordrum 1994). Also, poor reproductive performance and inbreeding effects has been documented in farm foxes (Braastad 1988; Nordrum 1994). Due to different origin, selective breeding and possible inbreeding, farm-bred arctic foxes are probably genetically distinct compared to wild arctic foxes in Fennoscandia.

The wild arctic fox population in Fennoscandia currently consists of approximately 150 adults and is classified as endangered in Sweden and Norway (Gärdenfors 2000; Linnell et al. 1999). The main threats to the population are interspecific competition with red foxes and food shortage (Tannerfeldt et al. 2002). Other possible threats are disease, inbreeding, low genetic variation and hybridization with farm foxes (Angerbjörn et al. 2004). Since there are frequent reports of farm-bred arctic foxes escaping from farms (Linnell et al. 1999), hybridization could lead to a loss of unique genetic variation and outbreeding depression or introduction of diseases or parasites (Linnell et al. 1999; Gharret et al. 1999; Edmands and Timmermann 2003). Although hybridization between farmed and wild arctic foxes in the wild has not yet been recorded in Scandinavia, such hybridization events have been documented in Iceland (Hersteinsson 1986, 2004). Given the low population size of the Fennoscandian population, it has been suggested that "a single reproduction event involving a farm-bred arctic fox could significantly alter the genetic structure of the wild population" (Linnell et al. 1999).

One approach to investigate the existence of escaped farm foxes and hybrids is to develop genetic markers specific for farm-bred foxes in Scandinavia. This could be accomplished by comparing mitochondrial control region sequences and microsatellite variation in farm foxes to wild arctic foxes in Scandinavia. Such a genetic marker could be used to identify escaped farm foxes and their possible hybrids in the wild, and thereby prevent genetic mixture with the wild population. In addition, wild arctic foxes often migrate long distances from their natal sites (Pulliainen 1965). Therefore, by finding a genetic marker specific for farm foxes, it would also be possible to identify wild individuals outside their regular distribution range and return them to the population.

This study was performed in three stages: (1) We used DNA samples from individuals with known origin, together with data from previous studies, to estimate the extent of genetic divergence between farmed and wild arctic foxes; (2) we used the data obtained in the first stage to develop a method to distinguish between the farmed and wild foxes; (3) we screened a number of unknown samples collected from the Scandinavian mountain tundra to investigate whether we can identify any escaped farm foxes or hybrids.

Methods

Samples

Samples from 41 farm foxes were collected from farms in Sweden, Finland and Norway. The tissues used for DNA extraction were muscle, blood, and skin biopsies. Samples were taken from four different farms with individuals belonging to five different breeding lines (Table 1). All 41 farm foxes were analyzed for microsatellite and mitochondrial control region variation. Samples from 22 wild arctic foxes were collected on the Scandinavian mountain tundra. The samples, consisting of muscle and skin biopsies, were taken from individuals that had been trapped, weighed, measured and had their behavior monitored. Since nothing unusual was observed during this procedure, we considered it safe to assume that these individuals were pure wild arctic foxes. These samples were used to analyze microsatellite variation. Known relatives from both farmed and wild foxes were excluded from the study. Data on mitochondrial DNA control region variation was collected from previous studies on Scandinavian arctic foxes (Strand et al. 1998; Dalén et al. 2002). To screen for escaped farm foxes in the wild, we used the Restriction Fragment Length Polymorphism (RFLP) method described below to analyze an additional 32 samples from wild foxes and four samples from individuals that were suspected to have farm origin due to rare fur color, unusual behavior or being found outside the normal distribution of wild arctic foxes. The samples consisted of muscle tissue (n = 18), skin biopsies (n = 3), blood (n = 2) and faeces (n = 13). All samples were coded so that the person performing the analyses did not know which samples were of suspected farm origin.

Table 1. Farmed arctic foxes from the five different breeding lines that were used in the study

Origin	n	Sample year	Tissue
Sweden	8	1994	Muscle tissue
Sweden	4	1994	Skin biopsy
Finland	4	2003	Blood
Norway	18	2003	Muscle tissue
Norway	7	2003	Muscle tissue

DNA extraction

Extraction of muscle tissue and ear tissue was performed using the DNeasy Tissue Kit (Qiagen) according to the manufacturers' protocol. Incubation with Buffer ATL and proteinase K in 55 °C was performed for 2 h for muscle tissue and overnight for ear tissue. The amount of tissue used was approximately 25 mg. DNA was extracted from blood according to the Chelex 100 method (Walsh et al. 1991). The amount of blood used was 20 μl . Thirteen out of the 36 samples used in the RFLP analysis originated from faecal samples. The faecal DNA was extracted using the Qiaamp DNA stool mini kit (Qiagen) according to the manufacturers' protocol. In all of the extraction methods mentioned above, one blank for every fifth sample was used to detect contamination.

mtDNA analysis

One mammalian specific primer (H3R) and one arctic fox specific primer (Pex1F) were used for amplification of an approximately 332 base pair (bp) long fragment of the mitochondrial control region (Dalén et al. 2002). Amplifications were performed in 25 μl reactions through the polymerase chain reaction method (PCR). Amplification reactions from tissue or blood samples contained 5 μl of DNA extract, 0.1 mM of each nucleotide, 2.5 mM $MgC1_2$, 0.2 μM of each primer, 1× PCR Buffer and 1 unit of Hot Star Taq (Qiagen). Amplification reactions (25 μl) on DNA extracted from faeces contained 2 μl DNA extract, 0.2 mM of each nucleotide, 2.5 mM $MgC1_2$, 0.1 mg/ml BSA, 0.5 μM of each primer, 1× PCR Gold Buffer, and 1 unit of AmpliTaq Gold (Perkin–Elmer). The PCR was performed on a Gene Amp PCR System 2400 (Perkin–Elmer) under the following conditions: 94 °C for 10 min, followed by 35 cycles of 94 °C for 20 s, 55 °C for 30 s, and 72 °C for 20 s, followed by a final extension at 72 °C for 7 min. Examination of the amplification success was made through electrophoresis, where 5 μl of the PCR-products were run on a 1.5% agarose gel in 1× TAE buffer. Successful amplifications were purified using the QlAquick Spin PCR Purification kit (Qiagen). Some samples showed too low concentration on the gel to be successfully sequenced and were therefore amplified in a new PCR reaction with 40 cycles.

Sequencing of both the heavy and light strand was performed by using a CEQ 8000 automated sequencer (Beckman Coulter) according to the manual.

Microsatellite analysis

Eleven different microsatellite loci, originally developed for the canine genome, were used in the study of microsatellite diversity (Ostrander et al. 1993; Ostrander et al. 1995; Fredholm and Winterø 1995), The different loci were arranged in five different multiplexes, using fluorescently labelled primers (Table 2).

The amplification reactions had a total volume of 15 μl and were composed of 3 μl DNA extract, 0.16 mM of each nucleotide, 1× PCR Buffer and 0.75 units of Hot Star Taq (Qiagen), The concentrations of primers and $MgCl_2$ varied in the different multiplexes (Table 2). The PCR thermal cycler used was a PTC100 Programmable Thermal Controller (MJ Reseach Inc.) and the following cycling conditions were used: 95 °C for 10 min, followed by three cycles of 94 °C for 30 s, 54 °C for 20 s, and 72 °C for 5 s, followed by 33 cycles of 94 °C for 15 s, 54 °C for 20 s, and 72 °C for 1 s, followed by a single cycle of 72 °C for 30 min. The PCR products were visualized on a CEQ 8000 automated sequencer (Beckman Coulter), using DNA Size Standard-400 (Beckman Coulter). Some samples displayed too low concentration to be successfully visualized, so a new PCR setup was made where the amount of Taq was increased to 1.5 units, and alternative cycling conditions were used: 95 °C for 10 min, followed by 40 cycles of 94 °C for 20 s, 54 °C for 30 s and 72 °C for 15 s, followed by 72 °C for 30 min.

Farm fox identification

The study of mtDNA variation showed that all farm foxes sampled shared haplotype H9, which does not exist in the wild Fennoscandian population (Strand et al. 1998; Dalén et al. 2005), Due to different base composition in the haplotypes, treatment with restriction enzymes Mnll and Ddel (New England Biolabs) gave restriction fragments of different sizes. Digestion with these enzymes gave a number of restriction fragments for each haplotype, where the size of the longest fragments varied between the different haplotypes (Table 3). The wild haplotypes H1, H3 and H7 gave after digestion fragments of 169, 187 and 171 bp, respectively. The farm fox specific haplotype, H9, gave a fragment of 141 bp. Since the longest fragment in H9 was shorter than in the wild haplotypes, the size difference made it possible to separate haplotype H9 from the three wild haplotypes by agarose gel electrophoresis, and thereby identifying foxes with haplotype, H9. However, it

Table 2. Primer concentrations (μM) and $MgCl_2$ concentrations (mM) in multiplex A–E

Multiplex	Locus	[Primer] (μM)	[$MgCl_2$] (mM)	Farm foxes		*P*	Wild foxes		*P*
				H_O	H_E		H_O	H_E	
A	CXX20[a]	0.16	2.5	0.707	0.807	0.05	0.727	0.802	0.62
A	CXX110[a]	0.24	2.5	0.512	0.591	0.26	0.727	0.832	0.13
B	CPH3[b]	0.16	2.5	0.854	0.783	0.91	0.818	0.827	0.39
B	CPH15[b]	0.08	2.5	0.390	0.463	0.62	0.409	0.450	0.07
B	758[c]	0.2	2.5	0.756	0.750	0.69	0.727	0.759	0.24
C	CXX140[a]	0.16	1.9	0.146	0.163	1.00	0.682	0.562	0.73
C	CXX250[a]	0.11	1.9	0.610	0.749	0.24	0.864	0.793	0.70
D	CPH9[b]	0.16	1.9	0.732	0.595	0.23	0.591	0.577	0.89
D	771[c]	0.16	1.9	0.024	0.048	1.00	0.818	0.871	0.09
E	CXX173[a]	0.16	1.9	0.341	0.419	0.28	0.591	0.559	0.77
E	377[c]	0.24	1.9	0.317	0.598	< 0.001	0.273	0.359	0.54

H_o and H_E for each microsatellite locus in farmed and wild arctic foxes.
[a]Ostrander et al. (1993).
[b]Fredholm and Winterø (1995).
[c]Ostrander et al. (1995).

Table 3. Restriction fragment sizes (bp) of each haplotype after digestion with Mnl1 and Dde1

H1	H3	H7	H9
169	187	171	141
124	144	126	123
121	121	121	121
< 100	< 100	< 100	< 100

was not possible to distinguish between haplotypes HI and H7 using agarose gel electrophoresis.

Digestion was performed in a total reaction volume of 10 μl which consisted of 5 μl PCR-product from amplification of the control region and 1 unit of restriction enzyme. 10× Reaction buffer and 10× BSA were added according to the manufacturers' protocol. Each sample was incubated separately with each restriction enzyme. The mixtures were incubated at 37 °C for 1 hour, followed by inactivation at 65 °C for 20 min. After inactivation, the Mnll- and Dde1-mixtures for each sample were pooled and a total volume of 20 μl was run on a 2% agarose gel in 1× TAE buffer at 100 V for 150 min. The method was optimized using eight samples of known farm foxes with haplotype H9 and eight samples of wild arctic foxes with the three known Scandinavian haplotypes. After optimization, 36 samples collected in the wild, of which four had suspected farm origin, were analyzed according to this method. Samples with haplotype H9, indicating farm origin, were subsequently analyzed for microsatellite variation as described below.

Statistical analysis

Population genetic analyses were performed using the software Arlequin (Schneider et al. 2000). Tests were made on non-random associations between alleles within loci (Hardy–Weinberg equilibrium) and between loci (linkage disequilibrium). Test of Hardy–Weinberg equilibrium was performed using a Markov chain for all loci, with a chain length of 100,000 and 3000 dememorization steps. The linkage disequilibrium test was performed with 1600 permutations and 100 initial conditions. The significance level for this analysis was corrected for multiple testing using the Bonferroni correction (Rice 1989). Genetic diversity was calculated as average expected heterozygosity (Schneider et al. 2000). Population differentiation was estimated using F_{ST}-statistics. Population pairwise F_{ST} was calculated using 10,100 permutations and a significance level of 0.05. A population assignment test, which assigns each individual to the population where its genotype is most likely to occur, was performed to quantify the degree of genetic differentiation between farmed and wild foxes (Paetkau et al. 1995). The test calculates the log-likelihood of each individual's genotype in each population, using the allele frequencies estimated in each sample. We used a Bayesian Markov Chain Monte Carlo (MCMC) approach to estimate the posterior probability (termed admixture coefficient, q) that each individual is from, or has a parent or grand parent that originates from each of K populations (Pritchard et al. 2000). The analyses were performed using the software STRUCTURE version 2 (Pritchard et al. 2000). We set $K=2$ in order to obtain pairwise comparisons of q between the farmed and wild samples. We used 100,000 burn in steps and 100,000 MCMC replicates in all simulations. The analysis allows for different settings of the probability that any random individual is either an immigrant or a hybrid (termed v). We used three different settings for v: 0.01, 0.05 and 0.10 (e.g. Pritchard et al. 2000).

Results

Genetic variation in farmed arctic foxes

Sequencing of the mitochondrial control region was successfully performed for all 41 farm foxes included in the study. One unique haplotype, H9, was found in all farm foxes that were sampled. Genotypes of the 11 microsatellite loci were determined for a total of 41 farm foxes and 22 wild foxes from Scandinavia. Allele frequencies of all loci in both farmed and wild foxes are listed in Appendix A. Several alleles were unique to either group (Appendix A). The average heterozygosity in the farm foxes was 0.53 (SE = 0.031), which was significantly lower than in the wild foxes where the average heterozygosity was 0.66 (SE = 0.053). The observed microsatellite heterozygosity per locus varied from 0.02 to 0.85 in farm foxes, while the wild samples varied from 0.27 to 0.86 (Table 2). Locus 377 and CXX20 deviated from

Hardy Weinberg equilibrium in the farm foxes ($P < 0.05$), showing excess of homozygotes. The other loci included did not show any deviation (Table 2). Linkage disequilibrium was observed in 22 out of 110 possible combinations when the significance level was set at 0.05 (data not shown). However, after applying the Bonferroni correction for multiple testing, no significant deviations were observed. The degree of genetic differentiation between farmed and wild foxes was calculated as F_{ST}. The F_{ST} value was 0.254 ($P < 0.00001$), showing a highly significant genetic differentiation between the two groups.

Identification of escaped farm foxes in Scandinavia

The eight known farm foxes used in optimization of the method showed the band characteristic for the farm fox specific haplotype H9, and the eight wild arctic foxes showed bands characteristic of the three wild haplotypes. The method was successfully performed in 25 of the 36 samples from free-ranging foxes. The remaining eleven samples did not contain sufficient amount of PCR-product after amplification, and therefore the method could not be successfully performed. The H9 band, characteristic for farm foxes, was identified in four of these samples, and verified through sequencing of one of the samples. These four individuals were the ones that were *a priori* suspected of having a farm origin. Microsatellite profiles were successfully recorded for three of the four "positive" samples. The fourth sample was a faecal sample, probably displaying too low concentration of DNA for microsatellite analysis. These samples deviated from both the farm and wild foxes in the population assignment test, but had a higher likelihood of belonging to the farm "population" (Figure 1). The MCMC analysis showed that all farm foxes had high posterior probabilities of belonging to the farm fox "population" (average = 99.2 %, SE = 0.5), indicating that all farm foxes had little or no recent ancestry from wild Scandinavian population. The 22 wild arctic foxes (excluding the three individuals with the farm fox haplotype, H9) had equally high posterior probabilities of belonging to the wild population (average = 98.8%, SE = 0.5). Different settings of the v parameter did not change the posterior probabilities significantly (data not shown). The three individuals carrying H9, and thus having a

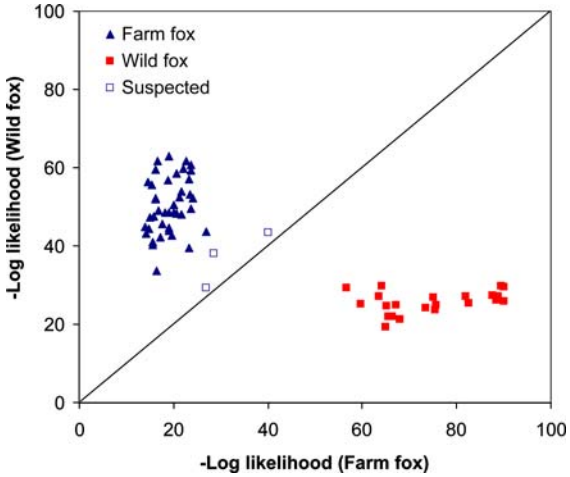

Figure 1. Population assignment test. Log likelihood of individual genotypes in all populations. A clear division between farmed and wild foxes is shown, except for the three individuals with suspected farm origin.

suspected farm origin, had less than 0.2% posterior probability of belonging to the wild population. Instead, two of the individuals had a posterior probability of *c.* 99% of being pure-bred farm foxes. The third individual had a 35.7% posterior probability of being of pure farm origin and 58.3% posterior probability of having a single parent from the farm fox "population". In an attempt to further resolve this, we used STRUCTURE (same setting as above, but without "prior population information") to approximate the 95% confidence intervals (CI) for the posterior probabilities of the individual being a hybrid or pure farm fox. The 95% CI of the posterior probabilities for the individual being pure farm and wild ranged from 58.9–100% and 0–41.1%, respectively, which suggests that it cannot be excluded that it is a pure farm fox.

Discussion

We found a strong genetic differentiation between the farmed and wild arctic foxes in Scandinavia, which is demonstrated by the high F_{ST} value between the groups. This differentiation is probably a result of different geographic origin and selective breeding. The results from the microsatellite study showed that a large number of alleles were unique either to the wild or farmed foxes,

while no fixed loci were detected (Appendix A). Therefore, although microsatellite analysis can be used to distinguish between farmed and wild foxes, it would require the use of several loci and would thus be time-consuming.

Deviation from Hardy–Weinberg equilibrium was observed in locus 377 and CXX20 in the farm foxes, while no deviation was seen in the wild population. The deviation was caused by a heterozygote deficiency and could possibly be explained by Wahlund's principle (Hartl 2000) or non-random mating, due to selective breeding in the farm population. However, since the heterozygote deficiency did not affect all loci, the most likely explanation is the existence of null alleles in the farm fox "population".

The deviation between the farmed and wild foxes in the mtDNA was clearer than for the Microsatellites, since the farm fox specific haplotype H9 does not exist among wild Scandinavian arctic foxes.. It was therefore possible to use it as a genetic marker for identification of escaped farm foxes in the wild. The actual analysis can be made either by sequencing of the control region or by performing the RFLP-based test described above. The RFLP-based test is quite fast and cheap, compared to sequencing. Both sequencing and the RFLP-based test identify escaped farm foxes of both sexes and hybrids with maternal farm origin. Unfortunately, hybrids with paternal farm origin are not identified. However, data from Iceland suggests a strong skew towards hybrids with maternal farm origin (13 out of 14 cases involved farmed females hybridizing with wild males; Hersteinsson 2004).

In this study four samples, collected in the wild, with the farm fox specific haplotype H9 have been identified. The three of these samples that were successfully analyzed for microsatellite variation (two from Norway and one from Sweden) had a higher likelihood of belonging to the farm fox samples than the wild samples in the population assignment test (Figure 1). The MCMC analysis showed that these three individuals had a negligible posterior probability of being pure wild arctic foxes. Instead, two of the individuals had a very high posterior probability of being pure farm-bred foxes, which suggests that they are escaped farm foxes. This is further supported by the observation of unusual behavior and rare fur color in these individuals. These two individuals (and the individual that we were unable to genotype) were found in the mountain area Hardangervidda/Nordfjella region in southwestern Norway, which traditionally is regarded as a high-quality area for wild arctic foxes (Østbye et al. 1978; Linnell et al. 1999). Since farmed and wild arctic foxes seem to coexist in the Hardangervidda region, there is a high risk that hybridization will occur. The origin of the third individual that was run over by a car in a Swedish boreal forest was more difficult to determine. It had a higher posterior probability of having a single parent from the farm fox "population" than being a pure farm fox, but since we were unable to exclude the possibility that it was of pure farm fox origin its status as a hybrid should be treated with caution.

Considering the low population size in Scandinavia, the geographic origin of farm foxes and previous documentations of such hybridization events (Hersteinsson 1986, 2004), the existence of escaped farm foxes on the Scandinavian mountain tundra must be regarded as a serious conservation problem. The arctic fox is well adapted for life in the arctic environment (Angerbjörn et al. 2004). Examples of local adaptations are the time for reproduction and pup rearing (Angerbjörn et al. 2004), as well as the insulating and camouflaging capabilities of the fur (Prestrud 1991). In addition, the litter size in Scandinavian arctic foxes seems to be adapted to the fluctuations in food availability caused by the lemming cycle (Tannerfeldt and Angerbjörn 1998). Since farm foxes have been bred for optimal fur appearance and reproductive output (Nordrum 1994), and since they partly originate from geographic regions that have a more predictable food availability (i.e., Greenland and Svalbard; Tannerfeldt and Angerbjörn 1998), hybridization may lead to loss of local adaptations in the wild population.

However, the high genetic differentiation (F_{ST} = 0.254) between the farmed and wild arctic foxes observed in this study suggests that the amount of gene flow from the farm fox "population" to the wild population so far has been limited. The occurrence of escaped farm foxes, and possibly hybrids, on the Scandinavian mountain tundra may thus be a recent phenomenon, but it is a problem that should be considered in the future management of the Fennoscandian arctic fox.

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Appendix A

Table A1. Observed allele frequency distribution in farmed and wild arctic foxes

Locus	Allele	Farm foxes	Wild foxes	Locus	Allele	Farm foxes	Wild foxes
CXX20	124	0.012	–	CXXX140	136	0.012	–
	126	0.329	0.295		140	–	0.091
	128	0.085	0.318		144	–	0.136
	130	–	0.114	–	146	0.024	–
	132	0.122	0.136		148	0.927	0.636
	136	0.049	0.091		150	–	0.136
	138	0.037	0.023		152	0.037	–
	140	0.122	–	CXX250	123	–	0.091
	142	0.232	0.023		125	0.305	0.318
	144	0.012	–		127	–	0.068
CXX110	84	0.098	0.341		129	0.354	–
	85	–	0.023		131	–	0.136
	107	0.085	0.182		133	0.146	0.318
	109	–	0.159	–	135	0.049	0.068
	111	0.622	–		137	0.134	–
	113	0.183	0.023		139	0.012	–
	115	–	0.068	CPH9	149	0.098	–
	117	–	0.091		151	0.500	0.068
	119	0.012	–		153	–	0.205
	123	–	0.114		155	0.402	0.614
CPH3	155	0.134	0.159		157	–	0.114
	157	0.012	0.295	771	98	–	0.114
	159	0.061	0.227		100	–	0.114
	163	0.354	0.114		102	0.988	0.114
	165	0.220	0.045		104	–	0.045
	167	0.049	–		106	0.012	–
	169	–	0.068		108	–	0.250
	173	–	0.091		112	–	0.136
	175	0.171	–		114	–	0.136
CPH15	153	0.305	–	**CXX173**	118	–	0.091
	155	0.012	–		122	0.049	0.136
	157	0.683	0.750		124	0.024	0.068
	159	–	0.023		126	0.171	0.636
	161	–	0.159	**377**	128	0.756	0.159
	163	–	0.068		177	0.098	–

Table A1. Continued

758	209	–	0.045	0.818
	228	–	0.341	–
	230	–	0.159	0.114
	232	0.049	–	0.023
	234	0.012	0.114	0.045
	235	–	0.023	
	237	0.280	–	
	239	0.317	–	
	241	–	0.318	
	247	0.073	–	
	249	0.268	–	

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