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High genetic distinctiveness of wild and farm fox (*Vulpes vulpes* L.) populations in Poland: evidence from mitochondrial DNA analysis

Beata HORECKA*, Kornel KASPEREK, Grażyna JEŻEWSKA-WITKOWSKA, Brygida ŚLASKA, Iwona ROZEMPOLSKA-RUCIŃSKA, Magdalena GRYZIŃSKA, Andrzej JAKUBCZAK
Department of Biological Basis of Animal Production, Faculty of Biology, Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Lublin, Poland

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Abstract: In Poland, the number of wild red foxes (*Vulpes vulpes* L.) and the size of the fur-farming industry are growing. There is concern that the gene pool of the wild foxes is being infiltrated by that of the farm animals. We analyzed three groups—Polish farm foxes and wild-living animals from Poland and North America—to investigate the gene flow or introgression between farm and wild red foxes. We took into account the breeding history of the species and the evolutionary relationships between fox populations on different continents. We compared the haplotypes based on the concatenated nucleotide sequences of *MT-CO1* (mitochondrially encoded cytochrome c oxidase I) and *MT-ATP6* (mitochondrially encoded ATP synthase 6) genes. It was confirmed that investigated fur-farm animals originated from wild individuals living in North America. We found a haplotype common to wild foxes from Europe (Poland) and wild North American individuals. The common haplotype shared by both investigated wild-living groups could indicate some degree of introgression between Polish farm and wild-living populations. Haplotypes characteristic of North American foxes were transferred to the Polish wild population and have been established. However, the pairwise Φ_{ST} values make it clear that North American wild and Polish wild foxes are genetically distinct evolutionary groups.

Key words: Genetic structure, phylogeography, farm fox, population conservation, *Vulpes vulpes*

1. Introduction

The red fox (*Vulpes vulpes*) originates from Eurasia, where it evolved from smaller ancestors in a transitional climatic period called the middle Villafranchian. It is probably a descendant of *Vulpes alopecoides* or Chinese *Vulpes chikushanensis*, because both of these species came from that period. During the penultimate glaciation, the species reached North America (Péwé and Hopkins, 1967). As indicated by the results of mitochondrial genetic analyses, maternal lines of foxes living in North America are derived from two clearly distinct clades, the Holarctic and the Nearctic. The Nearctic clade became isolated from the Holarctic clade nearly half a million years ago as a result of the progressive movement of glaciers, causing long-term isolation of the populations. In addition, within the Nearctic clade, a further three groups of red foxes are distinguished: western subclade, eastern subclade, and widespread subclade (Aubry et al., 2009). The eastern subclade includes insular populations of the Newfoundland red fox (*Vulpes vulpes deletrix*), which derived from disparate refugia isolated during the Wisconsinan glaciation (Aubry et al., 2009; Langille et al., 2014; Lounsberry et al., 2016). In the case of the populations of foxes living in Europe, some authors indicate no significant structural differences due to geographical or temporal distance (Teacher et al., 2011). At the same time, other studies show a high degree of phylogeographic structuring of the red fox across Europe and, consistent with paleontological and ancient DNA evidence, confirm via phylogenetic indicators that red foxes were persistent in areas outside peninsular refugia during the last Ice Age (Edwards et al., 2012; Statham et al., 2014). Other studies, discussing mitochondrial DNA control region haplotypes identified in red fox from four regions in Croatia, revealed haplotype diversity of Croatian red foxes to be among the highest of all European red fox populations studied to date, although genetic differentiation among regions was quite low (Galov et al., 2014). As evidenced by analysis of the nuclear and mitochondrial markers, Eurasian red foxes also form a separate group in comparison to the representatives of *Vulpes vulpes* living in North Africa (Leite et al., 2015). While taking into consideration Asia Minor, the majority of Turkish haplotypes grouped with those of Eurasia. Despite the great distance between the localities,

* Correspondence: beata.horecka@up.lublin.pl

two haplotypes from SW Anatolia in Turkey grouped with previously reported haplotypes from Hokkaido, Japan. The study showed that the Turkish red fox is nested within two main phylogroups and exhibits high genetic diversity (İbiş et al., 2014). *Vulpes vulpes* representatives commonly occur in various geographical areas, including on the islands of Japan, where individuals of this species also form genetically distinct populations classified by some researchers as a separate subspecies (Inoue et al., 2007). Analysis of South Korean individuals showed that they were separated into two lineages of Eurasian and North Pacific groups showing unclear phylogeographic structuring (Yu et al., 2012). Despite its wide distribution in the world, the red fox has been drastically reduced in South Korea due to habitat loss and poaching. Preserved red fox museum specimens were used to determine the genetic status of red foxes that had previously inhabited South Korea against red foxes from neighboring countries. This kind of biological material is now a valuable source of information for the population study and conservation genetics (Wandeler et al., 2007).

The red fox is also a native species commonly found in Poland. It has an incredible ability to adapt to environmental conditions; therefore, it creates numerous populations throughout the country. In recent years, a further significant increase in the number of foxes has been observed, driven by a large-scale vaccination program implemented for these animals against rabies. In recent years, there have been suspicions that the currently functioning population of red foxes in Poland consists not only of the animals that are the descendants of natives of the species. Because of its valuable fur, the red fox is maintained in breeding farms and is considered livestock. The wild ancestor of fox adapted by American trappers to farm-breeding was a subspecies inhabiting Canada, *Vulpes vulpes fulva* Desmarest (Petersen, 1914). In the population of these wild foxes, individuals with particularly valuable coat coloration—black or silver fur—were encountered. In the second half of the 19th century, specimens with such fur color were first farmed on Prince Edward Island, Canada. Much of the breeding stock came also from Newfoundland and later from Alaska (Balcom, 1916; Laut, 1921), with some share of individuals from the Cascade Range in Washington (Statham et al. 2012; Sacks et al., 2016).

In Poland, the breeding of fur animals, including the silver fox (a mutational color variation of the red fox), reaches back to the interwar years (1918–1939). Over the past 15 years, however, the farm fox population has declined in favor of the American mink (*Neovison vison*), which is more profitable in farm-breeding (Piórkowska, 2013). Almost simultaneously with the advent of fur farming, reports appeared of escaped or intentionally freed farm individuals that acclimate in the wild, contributing to free-living populations. It is therefore worthwhile to answer the question of whether the Polish fox is a descendant of the native red fox, or if the wild population on Polish territory is characterized by a significant share of genes of its American relative, from which the breeding fox originates. This is important in terms of the disquieting increase in the number of foxes, and the search for those possibly responsible for such a situation among breeders of these animals. On the other hand, in the case of a significant proportion of breeding individuals in the wild-living population, conservation activities designed to preserve the population of the representatives of indigenous species and natural biodiversity of fauna are important.

MtDNA studies provide wide opportunities for the identification and differentiation of groups of animals within a species, such as in the field of conservation activities aimed at protecting endangered populations (Perrine et al., 2007). In research on red foxes from North America, the use of mitochondrial markers also enabled the reconstruction of the evolutionary history of the studied *Vulpes vulpes* populations, and enabled the determination of the relationships between native and introduced representatives of the taxonomic group (Sacks et al., 2010, 2011). Red fox expansion over the Canadian Arctic was also considered a threat to the arctic fox (*Vulpes lagopus*). There were suspicions that the cause might be European red foxes introduced to eastern North America (Kamler and Ballard, 2002). Recent studies showed that mitochondrial DNA haplotypes in red foxes from Arctic locations were phylogenetically divergent from those in Eurasia, but were shared with neighboring indigenous North American populations (Berteaux et al., 2015).

The aim of this study was to compare the haplotypes based on concatenated nucleotide sequences of mitochondrial genes (*MT-CO1*: mitochondrially encoded cytochrome c oxidase I; *MT- ATP6*: mitochondrially encoded ATP synthase 6) for farm and free-living animals of the species *Vulpes vulpes*. The study included three groups of animals; along with Polish fur-farm and free-living foxes, wild foxes from North America were analyzed. This enabled adding new data to the genetic characterization of the species, as well as determination of the source of the current red fox population living in Poland.

2. Materials and methods

The research was carried out with the approval of the II Local Ethical Committee for experiments using animals, in Lublin (Resolution No. 83/2009). Analyzed samples included 60 farm foxes and 110 wild-living animals from each area (Poland and North America). Farm fox samples were sourced from commercial fur farms located in

southeastern Poland. The total number of fox farms in this area is greater than 30. Most of the analyzed samples (>40) came from a large commodity farm that did not allow kin mating. The rest of the fur-farm samples came from adjacent small operations. Wild-living animals from Poland were obtained by hunting associations in southeastern Poland from the area adjacent to the studied farms (n = 31), and from northern Poland (n = 79) (Figure 1). North American fox samples were taken from purchased skins delivered by trappers at auctions in Canada. The whole peripheral blood was collected from farmed animals into vacuum tubes containing EDTA anticoagulant, while pieces of skins with hair served as material for investigating wild animals from Poland and North America. Before and after DNA isolation, the test material was stored at –20 °C. DNA from tissues was isolated with a QIAamp DNA Mini Kit (QIAGEN). The isolation from blood was performed with a QIAamp DNA Blood Mini Kit, using the QIAcube system (QIAGEN). In both cases the extraction of nucleic acids was performed according to the manufacturer's procedure. The purity and concentration of the isolated DNA was determined spectrophometrically (260/280 nm) by BioPhotometer (Eppendorf). Evaluation of quality was assessed by electrophoretic separation in 1% agarose gel containing ethidium bromide in 1X TBE buffer at 70 V for 40 min. The samples were visualized under UV light and saved using Scion Image software.

The PCR for the investigated fragment (385 bp) of the *MT-ATP6* gene was performed using primers (F-5'GCCTCCCAATCGCTGTGTTAG3', R-5'GCTAAGGCCATAGGCTGGA3') designed from the sequence of the fox mitochondrial genome from the NCBI database (GenBank accession number AM181037), using the Primer-BLAST tool on the NCBI website. To amplify a fragment (646 bp) of the *MT-CO1* gene, the primer sequences (F-5'CCTGCAGGAGGAGGAGATCC3', R-5'AGTATAAGCGTCTGGGTAGTC3') were used, as in previous studies on canids by Wayne et al. (1997).

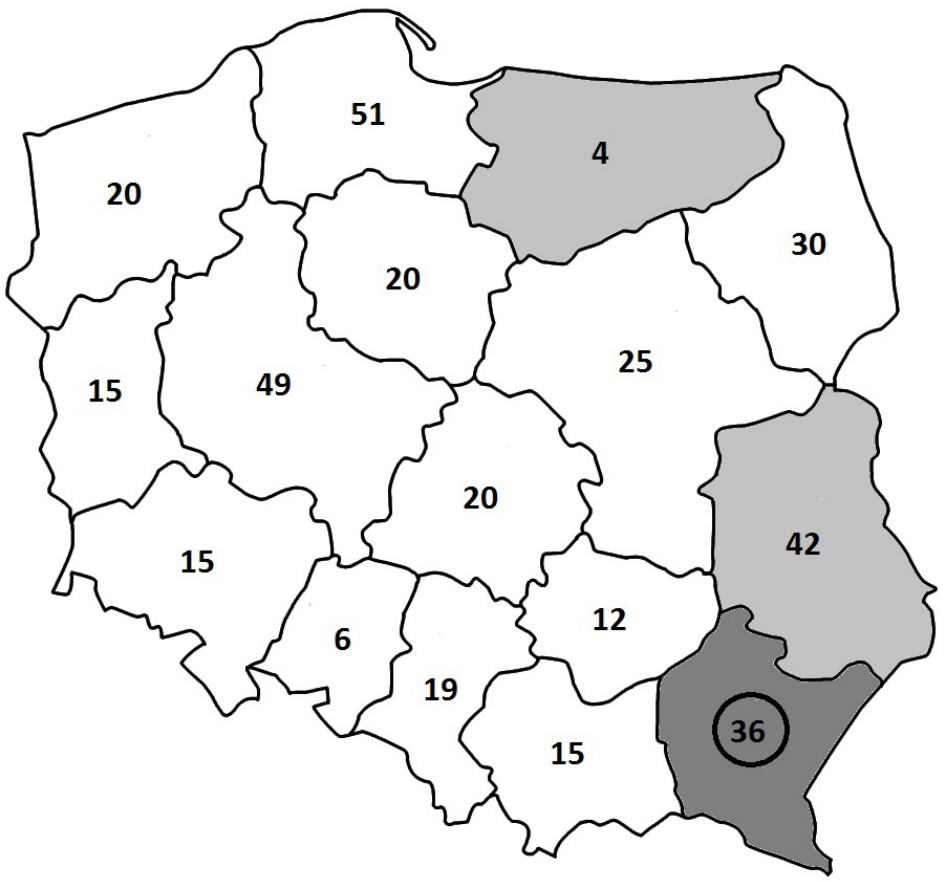

Figure 1. Distribution of sampling sites of wild and fur-farm red foxes in Poland: light gray areas represent the provinces from which samples of wild foxes were taken; the darker gray area indicated with a black circle shows the location of investigated fox farms; the numbers represent fox fur-farms in particular voivodeships.

Amplification of gene fragments was carried out using AmpliTaq Gold DNA Polymerase 360 (Life Technologies). PCR reactions were performed in a volume of 30 µL. A single sample contained 1X AmpliTaq Gold 360 buffer, 2.5 mM $MgCl_2$, 1 U of AmpliTaq Gold 360 DNA polymerase, 0.4 mM of each primer, 0.2 mM of each dNTP (Fermentas), and 5 µL of genomic DNA (130–170 ng/µL). PCR was performed on a Labcycler (SensoQuest) using the following thermal profile: 10 min at 95 °C prior to 35 cycles of 30 s at 95 °C, 60 s at 56 °C, and 60 s at 72 °C; this was followed by an extension step of 10 min at 72 °C. Spectrophotometric evaluation of the PCR product was then performed on BioPhotometr (Eppendorf AG). Electrophoresis of samples to check the length of the obtained DNA fragments was performed on a 2% concentration agarose gel with the presence of 1X TBE buffer and ethidium bromide, using Gene Ruler 100-bp DNA Ladder from Fermentas. The reaction was performed at a constant voltage of 70 V for 180 min. The gels were obtained using ScionImage. Sequencing PCR for both genes was performed using the BigDye Terminator v.3.1 Cycle Sequencing Kit from Life Technologies, with forward primers previously used in preparative PCR. Quantitative composition of the mixture and the temperature–time profile recommended by the manufacturer (final sample volume of 20 µL) were used. Sequencing PCR products were then purified from the reactants by using a DyeEx 2.0 Spin Kit (QIAGEN) using QIAcube and separated by electrophoresis in the 50-cm capillary in 6% polyacrylamide gel (POP-6). Sequencing was performed on a molecular analyzer (3100-Avant Genetic Analyzer).

Preliminary elaboration of sequencing results was performed with DNA Baser (DNA Baser Sequence Assembler v.3) and MEGA 4 (Tamura et al., 2007) software. It consisted of the location of SNP mutations for all animals in the test gene fragments. Mutation position was determined in relation to a reference sequence of the red fox mitochondrial genome from the NCBI database (accession number AM181037). On the basis of SNP changes identified in the concatenated sequences of *MT-CO1* and *MT-ATP6*, the occurring haplotypes were defined. The frequencies of haplotypes for each group individually were calculated using SAS v.9.1.3. The information concerning the type and frequency of haplotypes occurring in different populations was used to calculate the haplotype diversity (h), the nucleotide diversity (π), the average number of nucleotide differences between haplotypes (k), and the pairwise fixation index (Φ_{ST}) with the program Arlequin v.3.5 (Excofier and Lischer, 2010). The relationships among haplotypes were represented as a neighbor-joining haplotype network obtained by SplitsTree software (Huson and Bryant, 2006).

3. Results

In both fragments, almost all identified SNP changes were transitions. Only one transversion (m.6687A > T) occurred in the *MT-CO1* sequence. Greater polymorphism was characterized in a fragment of *MT-CO1* (29 SNPs; fewer in *MT-ATP6*: 17). Based on the concatenated sequences of *MT-CO1* and *MT-ATP6* in the three studied groups, a total of 22 different haplotypes were identified. The farm-bred population turned out to be the least differentiated, characterized by the presence of only three haplotypes, wherein the predominant parts were HP1 (40.0%) and HP2 (50.0%). In the Polish group of wild-living foxes, 11 haplotypes were noted, from which the most common was HP4 (35%), which was not identified in farm foxes. However, it appeared with a frequency of 6% in the population of individuals living in the wild in North America. Other haplotypes described for Polish wild foxes were unique to this group of animals. In the population of wild foxes from North America, the most frequently noted one was HP1, occurring in 34% of individuals, as it was in the farm animals. The second most common for Polish wild animals was HP5 (21%), which was not identified in the other analyzed groups. Other haplotypes described in this group of animals were characterized by a smaller share, but were specific only for this population of foxes. In the case of wild foxes from America, apart from HP1, HP2, and HP3, which also occurred in farm foxes, and HP4, which was common to both wild populations, eight specific haplotypes were found characteristic for this population. Characteristics of identified SNP changes and haplotype frequencies for each group of animals can be found in the Supplementary Table.

Due to the greatest number of unique haplotypes found for wild foxes from Poland, this group was characterized by the highest values of haplotype diversity (*h*), reaching 0.84. At the same time, the highest value of nucleotide diversity (π) was noted for the wild-living population from North America: 0.097 (Table 1).

Calculated values of pairwise Φ_{ST} showed that both wild populations proved to be highly genetically distinct, with pairwise Φ_{ST} of 0.66, in relation to the fixed differences arising from the separate evolutionary paths of *Vulpes vulpes* on separate continents. The highest value of this parameter was identified between the wild-living population from Poland and Polish farm foxes. The pairwise Φ_{ST} for these two groups reached the value of 0.82; such significant values show clear genetic distinctiveness of these two groups of foxes coexisting in Polish territory. They reflect differences between the wild-living populations, thereby demonstrating partial genetic distinction. This finding is supported by the lowest pairwise Φ_{ST} value of 0.13 between farm individuals and wild-living animals from North America, resulting from their common origin (Table 2).

Table. 1. Total number of haplotypes (*H*), number of private haplotypes (*U*), haplotype (*h*), and nucleotide diversity (π) in three investigated populations of foxes based on concatenated *MT-CO1* and *MT-ATP6* sequences.

	H	U	h	π
Farm fox	3	-	0.60 ± 0.06	0.015 ± 0.013
Wild fox Poland	11	10	0.84 ± 0.04	0.080 ±0.040
Wild fox North America	12	8	0.80 ± 0.05	0.097 ± 0.054

Table 2. Pairwise Φ_{ST} values between three investigated populations of foxes.

	Farm fox	Wild fox Poland	Wild fox North America
Farm fox	*		
Wild fox Poland	0.82	*	
Wild fox North America	0.13	0.66	*

The haplotype network is a graphic illustration of the relationship between the haplotypes identified in the studied populations (Figure 2). The areas of the circles are proportional to the number of foxes sharing each haplotype. Haplotypes observed in the groups are marked in accordance with the legend. As can be seen, haplotypes characteristic for wild individuals from North America are grouped together with haplotypes characteristic for farm animals, as well as haplotypes common to both mentioned populations. The intermediate part of the network involves a haplotype common to the two analyzed wild populations of foxes. On the other hand, a separate section of the haplotype network consists of haplotypes occurring only in wild individuals from Poland.

4. Discussion

The number of samples obtained in a particular location and the different number of determined haplotypes possibly resulting from it are of great importance for phylogeographic analysis using mtDNA (McDevitt et al., 2012). Based on analysis of D-loop and *CYTB* sequence, Aubry et al. (2009) identified two main clades of red foxes: the Holarctic clade (which includes foxes from Eurasia, Alaska, and western Canada), and the Nearctic clade

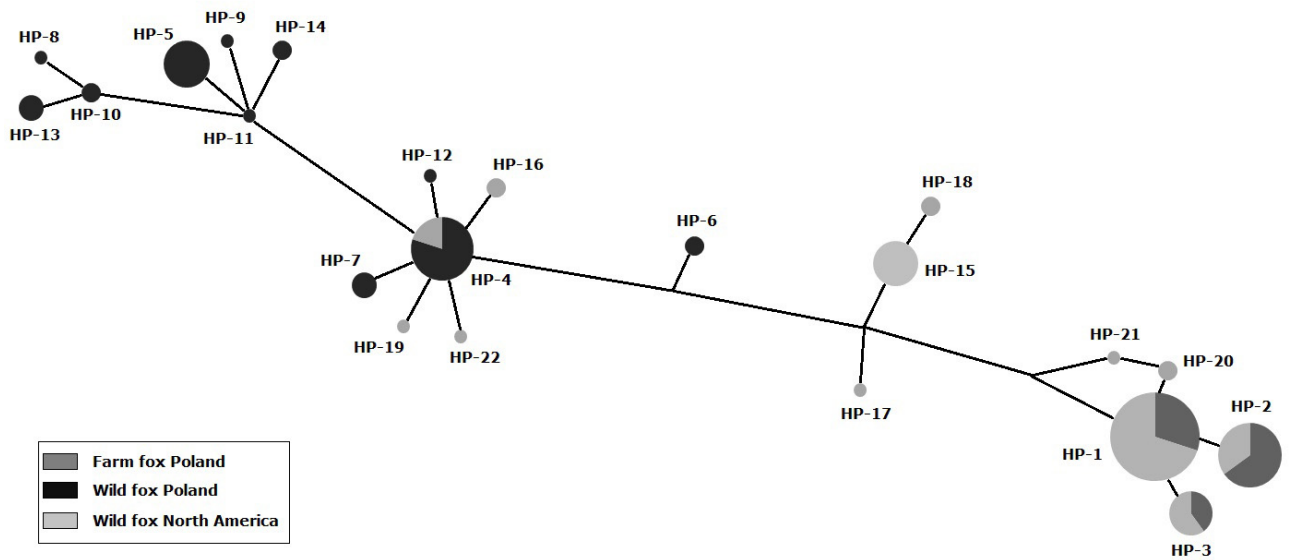

Figure 2. Neighbor-joining haplotype network based on frequencies showing relationships between concatenated *MT-CO1* and *MT-ATP6* sequences of fur farm and wild red foxes.

(further divided into three subgroups of foxes from North America, including populations in danger of extinction). The authors found greater genetic diversity within the North American populations compared with the Eurasian ones. It included haplotype diversity as well as single nucleotide changes. In our study, similar parameters of diversity of Polish and North American wild animals were found, with the smallest variability within the fur-farm individuals. Farm fox samples were taken mainly from one farm, with the addition of some individuals from smaller operations, but this group is quite representative for the Polish farm animals due to the intensive exchange of animals between farms.

Based on the sequence polymorphism analysis of mitochondrial genes *MT-ATP6* and *MT-CO1* in the three fox groups, it can be concluded that a significant share of common haplotypes in farm individuals and wild animals from North America confirms the common origin of both groups. Therefore, the data obtained from the mtDNA sequence are in accord with the results obtained by polymorphism analysis of microsatellite sequences by Jeżewska-Witkowska et al. (2012). A significant specificity of haplotypes identified in the Polish wild-living group probably results from the separate evolutionary paths of foxes from Poland and North America. Statham et al. (2011) drew similar conclusions from their research based on examining the origin of the captive breeding foxes on farms in Russia. The authors analyzed the sequence of the mtDNA gene encoding cytochrome b and the D-loop; based on the polymorphism, the study population was described using the seven haplotypes. No haplotypes indicating a Eurasian origin of these animals were found in the Russian fox breeding population. However, they unequivocally determined that the initial population for the compared populations of Russia had individuals living in the eastern part of North America, specifically eastern Canada. This is also reflected in the historical reports describing Prince Edward Island in Canada as a primary source of breeding material, from where foxes were imported at the advent of breeding programs in Europe.

In turn, Statham et al. (2012) studied the origin of recently established and currently coexisting fox populations in the southeastern and western parts of the United States. The authors tested the hypothesis of translocation of the European red fox ancestry to this part of North America. Based on the analysis of *CYTB* gene and D-loop sequences, they found a lack of Eurasian haplotypes in the newly established populations, noting at the same time the occurrence of haplotypes characteristic for native populations from eastern Canada and the northeastern United States. In turn, populations living in southern California were characterized by the presence of many different nonnative haplotypes, which is most likely the result of intracontinental translocation of animals from fur farms (ultimately eastern and Alaskan North America). Several common haplotypes frequently occurring in these populations originated from the areas where fox fur-farm stock originated.

Additionally, Aubry et al. (2009) found no haplotypes common for foxes from Europe and North America, while in our study one haplotype common to wild foxes from Poland and North America was identified. This is also in contradiction with the research by Kutschera et al. (2013). The authors, conducting phylogeographic research on wild common foxes from various continents, included individuals living in Polish territory. On the basis of the analysis of the mtDNA control region, they identified 13 haplotypes in Polish foxes, two of which were also observed in the German population, one in Swedish foxes, and one in individuals from Serbia. This indicates a similar variation in the control region of mtDNA in Polish foxes in comparison with the concatenated *MT-ATP6* and *MT-CO1* sequences, based upon which the 11 haplotypes were identified in our own study. However, Kutschera et al. (2013) did not identify haplotypes shared with North American populations within newly described Polish samples.

As in the studies by Lounsberry et al. (2016), our own results indicate that the populations of farm foxes and animals coexisting in natural conditions in Poland clearly differ genetically. This may prove a good organization of fur farming in Poland nowadays and appropriate isolation of farm individuals from free-living animals. It has also been confirmed in the studies of other species of fur animals reared in Poland—e.g., raccoon dog, where wild and farmed populations differed from each other with a number of haplotypes identified within mtDNA (Ślaska and Grzybowska-Szatkowska, 2011).

The presence of a number of specific haplotypes in Polish animals living in the wild that are absent among wild foxes from North America shows a relatively ancient divergence and different evolutionary paths of these groups of animals. However, the presence of a common haplotype for wild foxes from Poland and North America is interesting as a phenomenon that had not been observed by other researchers. Again, this could indicate the penetration of Polish fur-farm and wild living populations in the past. In this way, haplotypes characteristic of North American foxes, from which the farm-bred population originates, were transferred to the wild population living in Poland and have been established as a consequence of 'founder effect'. In contrast, the absence of these haplotypes in the currently existing farm population can be explained by their progressive elimination through selection of individuals on farms. Some previous studies indicate that mitochondrial DNA haplotypes are associated with

performance traits in fur animals (Ślaska et al., 2016). If these haplotypes, transferred in the past by farm animals to the wild populations, were not associated with better quality of skins, they might be lost on farms during many years of breeding work. However, there is another more probable explanation for this situation. The common haplotype shared by Polish wild foxes and North American red foxes suggests possible escapes from fur farms that were not sampled. The results indicate that investigated wild foxes in Poland do not have high maternal gene flow with the sampled fox farms. It should be emphasized in conclusion that farmed red foxes in Poland are a separate genetic group compared to the wild Polish representatives of the species. On the basis of these studies, there is no reason to think that farmed animals, by penetration into the environment, threaten native populations of this species or cause significant damage to natural ecosystems.

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Supplementary Table. Characteristics of the haplotypes based on concatenated *MT-ATP6* and *MT-CO1* sequences in three populations of foxes.

Gene	SNP*	Haplotype																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
MT-CO1	6069 C	T	T	T												T		T	T		T	T	
	6084 T																	C					
	6102 T			C																			
	6123 T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
	6126 T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
	6147 G																				A	A	
	6153 C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
	6231 T																						C
	6276 T	C	C	C												C		C	C		C	C	
	6312 C													T									
	6327 A	G	G	G												G		G	G		G	G	
	6357 A	G	G	G	G		G	G					G			G	G	G	G	G	G	G	G
	6441 T						C																
	6444 C														T								
	6465 C						T																
	6516 T	C	C	C												C		C	C		C	C	
	6525 A	G	G	G			G									G		G	G		G	G	
	6534 C																T						
	6555 C	T	T	T			T									T		T	T		T	T	
	6558 C	T	T	T	T		T	T					T			T	T	T	T	T	T	T	T
	6579 A	G	G	G												G		G	G		G	G	
	6597 T																		C				
	6600 A						G																
	6612 G								A														
	6645 G	A	A	A												A		A	A		A	A	
	6684 T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
	6687 A	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
	6690 C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
	6693 C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
MT-ATP6	8063 A						G																
	8069 T									T													
	8090 A							G															
	8105 G	A		A	A		A	A					A			A	A	A	A	A	A	A	A
	8120 T	C	C	C			C									C		C	C		C	C	
	8132 G	A	A	A																	A	A	
	8181 A														G								
	8207 G																			A			
	8213 T															C			C				
	8225 G												A										
	8231 C	T	T	T												T		T	T		T	T	
	8378 T					C																	
	8391 A								G		G			G									
	8402 A						G																
	8409 C	T	T	T																	T		
	8412 T																		C				
	8420 A	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
		Haplotype																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Frequency of haplotype (%)**	F	40	50	10																			
	WP				35	21	6	9	3	3	6	3	3	9	6								
	WNA	34	12	6	6											12	6	3	6	3	6	3	3

*position in *Vulpes vulpes* mitogenome (AM181037).** haplotype frequency in population: F-fur farm, WP-wild from Poland, WNA-wild from North America.