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ANALYSIS OF GENETIC VARIABILITY IN FARMED AND WILD POPULATIONS OF RACCOON DOG (*NYCTEREUTES PROCYONOIDES*) USING MICROSATELLITE SEQUENCES*

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Abstract

The aim of this study was to detect possible differences between farmed and wild-living raccoon dogs. Analysis of polymorphism in 15 microsatellite sequences led to the conclusion that raccoon dogs raised on Polish farms and wild raccoon dogs living in Poland are two genetically distinct groups of animals. Wild Polish raccoon dogs are genetically more similar to the population of wild animals from the Kaliningrad Region than to farmed animals. The analysis of microsatellite loci showed clear genetic differences between farmed and wild-living populations of raccoon dog, despite only 50 years of isolation of the two groups of animals. The farmed population was characterized by higher genetic variation than the wild-living population. On the basis of the analyses three microsatellite loci (INU014, Ren13J22 and Ren41D20) were proposed for determination of the origin of animals that have escaped from farms.

Key words: *Nyctereutes procyonoides*, wild and farmed animals, microsatellites, population assignment

One of the six subspecies of raccoon dog (Sheldon, 1992; Ward et al., 1987), kept as a fur animal, is the Chinese raccoon dog, *Nyctereutes procyonoides procyonoides* (Gray, 1834), which due to the geographical location of individuals introduced in Europe is often distinguished as the separate subspecies *Nyctereutes procyonoides ussuriensis* (Matschie, 1907). In the literature dealing with both farmed and wild raccoon dogs living in Europe, there is disagreement regarding their assignment to a specific taxonomic population (Kauhala and Kowalczyk, 2011; Korablev et al.,

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2011; Ślaska and Grzybowska-Szatkowska, 2011; Pitra et al., 2010; Ślaska et al., 2010 b, 2008; Rogalska-Niznik et al., 2003). The history of both wild and farmed raccoon dog populations in Europe began with the introduction of approx. 9,000 individuals in the years 1929–1955 from their original habitat in the Far East (the Amur River basin, Ussuria and Sungaria) to new locations such as the European part of the former Soviet Union (Lavrov, 1971). According to Lavrov (1971), the area of introduction included western Russia, Ukraine, Siberia, Kazakhstan, Kyrgyzstan and the Caucasus. Then the species began to spread rapidly to the west along the river valleys and colonize other countries of Europe (Kauhala and Kowalczyk, 2011). Wild raccoon dog was first recorded in Poland in 1955 (Dehnel, 1956). In the 1970s the species was commonly found in the wild in Poland (Kauhala and Kowalczyk, 2011), and in the following decades populated much of Europe (Kauhala and Saeki, 2004). In the late 1970s farm breeding of raccoon dogs began in Poland. Farm animals were imported from Finland, where they had been caught in the wild in the early 1970s. As a result, both the European wild population and the farmed population have their origin in individuals which had been introduced to the European part of Russia.

Keeping fur animals on farms is often controversial, due to the possibility of their escape and mating with wild animals, or the formation of new populations in areas where they did not previously exist. There have been similar concerns regarding the raccoon dog. Barrat et al. (2010) reported a second wave of introduction of raccoon dogs into the environment in Europe via accidental release of farm animals. Mulder (2013) suggests that farm animals have appeared in the wild in the Netherlands, while Heltai et al. (2000) argue that in certain areas of Hungary raccoon dogs from farms have formed new wild populations.

Long-term, intensive breeding of fur animal populations has led to the emergence of differences in production and behavioural traits between farmed and wild-living animals. In the case of raccoon dogs, differences in body weight and coat quality between wild and farmed individuals have been confirmed (Ślaska 2010 a, b). Differences have been found between farmed and wild individuals in plasma ghrelin and growth hormone levels, which according to the authors (Asikainen et al., 2004) is due to differing adaptation to long periods of food shortage in winter. These differences are also reflected in the genotypes of farmed and free-living animals. This is a consequence of selective pressure, which takes into account economically valuable traits which are often not favoured by natural selection in wild populations. Cytogenetic analyses of farmed and wild raccoon dog populations in Poland suggest that the two populations are distinct in terms of the number of B chromosomes and nucleolus organizer regions (NOR) (Bugno-Poniewierska et al., 2013). Sequencing studies based on three mitochondrial gene fragments, presented in a study by Ślaska and Grzybowska-Szatkowska (2011), confirm the genetic distinctiveness between farm animals and individuals living in the wild in Poland. This creates the opportunity to determine molecular differences between populations of farmed and free-living fur animals. Determination of population-specific alleles may be useful in identifying animals escaped from farms and their adaptation to the environment, as well as the possibility of interbreeding with wild representatives of the species. A promising tool

for identification based on genetic material in this context may be analysis of microsatellite loci, which usually have a high degree of polymorphism.

Hence the aim of this study was to detect possible differences between farmed and wild-living raccoon dogs on the basis of analysis of microsatellite sequences.

Material and methods

The material was collected from a total of 173 raccoon dogs. The study included 130 farmed individuals, unrelated for four generations, from two breeding farms in south-eastern Poland. In addition, animals from two wild populations were tested: 28 individuals from a Polish population and 15 from a Russian population. The animals from the Polish population came from the Lubelskie and Podkarpackie Provinces. The Russian raccoon dogs were acquired in the Kaliningrad Region. All procedures used during the research were approved by the Second Local Ethics Committee for Animal Testing at the University of Life Sciences in Lublin, Poland (Approval No. 83/2009 of 8 December 2009).

The material from the farm animals consisted of peripheral blood from the small saphenous vein collected into vacuum tubes containing EDTA as an anticoagulant. Soft tissues and skin were collected from the wild animals from Poland and Russia. The research material before and after DNA isolation was stored at –20ºC. Isolation of DNA from the blood was performed using the QIAamp DNA Blood Mini Kit, and isolation from the tissue with the QIAamp DNA Mini Kit (QIAGEN). On the basis of the literature, 15 microsatellite markers were selected from the genome of the domestic dog for genetic characterization of the studied populations: INU005, INU013 and INU014 (Ichikawa et al., 2002) and Ren01E05, Ren67C18, Ren02C20, Ren02P03, Ren04M22, Ren02K21, Ren39L15, Ren13J22, Ren44K10, Ren06C11, Ren41D20 and Ren01O23 (Jouquand et al., 2000) (Table 1). All analyzed microsatellites in raccoon dog were not mapped yet. PCR was performed using AmpliTaq Gold 360 DNA Polymerase in an MJResearch PTC 225 Tetrad thermal cycler. Optimization of PCR made it possible to form 4 multiplexes of 12 microsatellite sequences. Three microsatellite sequences were amplified individually. The characteristics of the PCR primers and cycling profiles are shown in Table 1. The volume of each sample was 10 µl: 9 µl of the reaction mixture and 1 µl of the DNA template. Electrophoresis of microsatellite fragments was performed in a capillary analyser – 3100 Avant Genetic Analyzer (Applied Biosystems). The length of the alleles obtained was analysed with reference to the GeneScan 500 Rox Size Standard marker using Gene Mapper Software v 3.5. Despite optimization of the reaction and repeated analyses on selected microsatellite loci, in a few individuals from each population no product was obtained. The number and length of alleles, as well as alleles specific to the farmed population and the two wild populations (Polish and Russian), were determined in the microsatellite loci. Alleles specific for both wild populations together were presented as well. The frequencies of specific alleles within each population were given. Intra- and inter-population variability was estimated using PopGene

software version 1.31 and Cervus version 3.0.3, based on polymorphic information content (PIC), expected heterozygosity (He) and observed heterozygosity (Ho). Genetic similarity and genetic distance were determined according to Nei (1978). Furthermore, the genetic structure of the analysed populations was determined using Structure software, version 2.3.2.1 (X.2009) (Pritchard et al., 2000), which on the basis of allele frequency enables selection of the most genetically similar individuals, which can then be joined in clusters. The method employs Bayesian clustering analysis of individuals using the algorithm of the Monte Carlo method and Markov chains (Markov Chain Monte Carlo, MCMC). A mixed model correlated with the frequency of alleles was used. The number of groups (K) was tested from 1 to 10. For each K, 20 iterations were performed to verify the reproducibility of the results of the grouping of individuals. The similarity index between repetitions for each K, ΔK and the probability of K (Evanno et al., 2005) were used to determine the optimal number of groups (K) for each analysis. The results are shown in a bar plot for individual clusters and the three populations established in the methodology.

Results

The lengths and frequencies of alleles specific for each population are given in Table 2. With the exception of locus Ren 06C11, which proved to be monomorphic, the total number of alleles per polymorphic locus ranged from 2 for the loci Ren67C18 and Ren02P03 to 9 for INU014, Ren02K21, Ren39L15 and Ren 13J22. In all three populations specific alleles were found (Table 2): 4 for wild animals from the Kaliningrad region, 5 in the farmed population and 6 in the wild population from Poland. Most of them were found in single individuals. However, alleles of 392 and 394 bp in locus Ren13J22 were noted in over 20% and over 50%, respectively, of animals from the farmed population. Comparison of the farmed population and the combined wild populations in terms of specific alleles reveals more such interpopulation differences (Table 2). A set of selected alleles for loci INU014, Ren13J22 and Ren41D20 was characteristic only for wild animals, and their total frequency was 55%, 59% and 23%, respectively. In consequence the shortest alleles, i.e. 164 and 168 bp for locus INU014 and 380, 382, 384 and 386 bp for locus Ren13J22, and the longest alleles, i.e. 205 and 209 bp for locus Ren41D20, can be used for analysis.

Five loci (Ren02P03, Ren02C20, Ren67C18, Ren04M22 and INU005) were characterized by a low degree of informativeness, due to the low level of polymorphic information content, which in at least one population fell below 0.5 in these loci (Table 3). The highest polymorphism was observed in loci Ren41D20, Ren39L15 and INU013, where the PIC value ranged from 0.612 to 0.734, depending on the population and the locus. In most cases, the observed heterozygosity was lower than expected, but in each population the reverse situation occurred in at least three loci.

Table 1. Primer characterization and temperature-time profile for PCR of microsatellite sequences

Multiplex	Primer	Primer sequence F – Forward; R – Reverse	Initial denaturation	Number of cycles	Denaturation	Annealing	Elongation	Final elongation	Dye
M1	INU005	F:5'CTTTCTACCAGCAAGGTTAC3' R:5'TTCCCATTTAATTGCCTCT3'	95ºC /10 min	31	95ºC /30 sec.	60ºC /1 min	72ºC /1 min	72ºC /20 min	VIC
	INU013	F:5'AGAGAAAAGCATCCAGTAAG3' R:5'AAATGGTCTTCCTGTATCCT3'							6-FAM
	INU014	F:5'ACATTTATCATAGTAAGTACCGAG3' R:5'AAAACCACAAAACCTAACCT3'							NED
M2	Ren01E05	F:5'TCATCACTTCCTGCTCCATT3' R:5'TCTCATGCCACACGGAACCT3'	95ºC /10 min	31	95ºC /30 sec.	52ºC /1 min	72ºC /1 min	72ºC /20 min	6-FAM
	Ren67C18	F:5'TCTGTGCGTTTCCGTTTATG3' R:5'TTAGTACCTGTTTGTTATCC3'							VIC
	Ren02C20	F:5'AGAAATTGCATCACTCACAT3' R:5'GCTGCTCCGAAAACTAACTT3'							VIC
	Ren02P03	F:5'CATTCTTATCCTTCAGTGCTGA3' R:5'GGCTCAGTCAGTAGTTGTGCGA3'							6-FAM
	Ren04M22	F:5'AGAGAAAAGCATCCAGTAAG3' R:5'AAATGGTCTTCCTGTATCCT3'							NED
M3	Ren02K21	F:5'CTTAGTTTTCAGGCTTTCAG3' R:5'TGATAGGAAGTAAAGATGTT3'	95ºC /10 min	36	95ºC /30 sec.	50ºC /1 min	72ºC /1 min	72ºC /10 min	NED
	Ren39L15	F:5'CTTGTTTTCTTTTGGATAGC3' R:5'CTGCCTTGAAGAATGATAAA3'							6-FAM
M4	Ren13J22	F:5'TATTGCAACTGTCTTATGTA3' R:5'TGTCTTAGTGATGGCTCCTG3'	95ºC /10 min	36	95ºC /30 sec.	50ºC /1 min	72ºC /1 min	72ºC /10 min	VIC
	Ren44K10	F:5'CATATTGGACCTTCACAT3' R:5'TTAACGCACAACTTCATC3'							NED
	Ren06C11	F:5'GGGGGTGTCGGTGGAGTTCT3' R:5'TGCAGGGCAGAGGCTGGAGG3'	95ºC /10 min	31	94ºC /50 sec.	58ºC /50 sec.	72ºC /50 sec.	72ºC /10 min	NED
	Ren41D20	F:5'TGTCTATGTAATATCACAGG3' R:5'TTCTGGGTATTTATCTGAAG3'	95ºC /10 min	36	95ºC /30 sec.	50ºC /1 min	72ºC /1 min	72ºC /10 min	6-FAM
	Ren01O23	F:5'TTCCCTGCAGCCCTTCCTCA3' R:5'TGTGCCTCATTCCTTTTTAT3'	95ºC /10 min	31	95ºC /50 sec.	55ºC /55 sec.	72ºC /50 sec.	72ºC /10 min	6-FAM

Table 2. The number (Na) and length of alleles and specific alleles noted in the loci analysed in the raccoon dog populations

Locus	Na	Length of alleles (bp)				
			specific alleles (frequency of alleles)			
		All populations	wild Polish raccoon dogs	wild Russian raccoon dogs	wild raccoon dogs (Polish + Russian)	farmed raccoon dogs
INU005	3	108; 110; 112	-	-	-	-
INU013	6	172; 174; 176; 178; 180; 182	176(0.11)	-	176(0.07); 178(0.12)	-
INU014	9	164; 168; 172; 174; 176; 178; 180; 182; 184	164(0.02); 184(10.02)	-	164(0.02);168(0.53); 184 (0.01)	174(0.08); 176(0.02)
Ren01E05	4	400; 402; 404; 406	-	-	-	-
Ren67C18	2	158; 160	-	-	160(0.12)	-
Ren02C20	3	297; 300; 303	-	-	-	-
Ren02P03	2	161; 170	-	-	161(0.06)	-
Ren04M22	5	191; 193; 197; 201; 203	-	203(0.03)	203(0.01)	-
Ren02K21	9	284; 286; 288; 292; 294; 296; 298; 300; 302	-	286(0.02)	286(0.01); 288(0.06)	302(0.03)
Ren39L15	9	200; 202; 204; 206; 208; 210; 216; 218; 220	206(0.02)	-	200(0.23); 206(0.01); 210(0.05)	204(>0.01)
Ren13J22	9	380; 382; 384; 386; 388; 390; 392; 394; 396	382(0.07)	380(0.04)	380(0.01); 382(0.07); 384(0.13); 386(0.38)	392(0.21); 394(0.53)
Ren44K10	5	196; 200; 204; 206; 208	-	-	-	-
Ren06C11	1	86	-	-	-	-
Ren41D20	7	185; 197; 199; 201; 203; 205; 209	-	209(0.03)	205(0.19); 209 (0.03)	-
Ren01O23	4	182; 185; 188; 191	-	-	191(0.12)	-

Table 3. The number of alleles (Na), polymorphic information content (PIC), observed heterozygosity (Ho) and expected heterozygosity (He) for each raccoon dog population in the analysed microsatellite loci

Locus	Wild Polish population (n=28)				Wild Russian population (n=15)				Farmed population (n=130)			
	N_a	H_o	H_e	PIC	N_a	H_o	H_e	PIC	N_a	H_o	H_e	PIC
INU005	3	0.538	0.595	0.501	2	***0.077***	***0.471***	0.350	3	0.403	0.450	0.364
INU013	6	0.773	0.788	0.734	5	0.833	0.779	0.707	5	***0.674***	***0.721***	0.668
INU014	6	***0.318***	***0.598***	0.537	5	0.583	0.714	0.627	6	***0.897***	***0.671***	0.613
Ren01E05	4	***0.462***	***0.696***	0.623	4	***0.333***	***0.701***	0.619	4	0.611	0.643	0.587
Ren67C18	2	0.077	0.075	0.071	2	***0.000***	***0.405***	0.315	1	0.000	0.000	0.000
Ren02C20	3	***0.174***	***0.672***	0.583	3	0.077	0.557	0.428	3	***0.460***	***0.548***	0.486
Ren02P03	2	0.038	0.038	0.037	2	0.267	0.239	0.204	1	0.000	0.000	0.000
Ren04M22	4	0.522	0.519	0.417	5	0.600	0.651	0.566	4	0.611	0.627	0.574
Ren02K21	7	***0.571***	***0.656***	0.613	6	***0.533***	***0.685***	0.630	7	0.528	0.541	0.512
Ren39L15	8	0.786	0.790	0.744	5	0.600	0.703	0.623	7	***0.432***	***0.703***	0.662
Ren13J22	6	0.704	0.728	0.672	6	***0.929***	***0.733***	0.661	5	0.631	0.632	0.573
Ren44K10	5	0.667	0.706	0.637	5	0.643	0.722	0.654	5	0.715	0.678	0.610
Ren06C11	1	0.000	0.000	0.000	1	0.000	0.000	0.000	1	0.000	0.000	0.000
Ren41D20	6	0.600	0.826	0.775	7	0.533	0.740	0.684	5	0.677	0.656	0.612
Ren01O23	4	***0.520***	***0.652***	0.580	4	0.667	0.660	0.590	3	0.600	0.668	0.591

Deviations from Hardy-Weinberg equilibrium (HWE) were calculated using PopGene version 1.31. Values in italics and bold are significantly different from Hardy-Weinberg equilibrium (HWE) at $P<0.05$.

The greatest genetic distance calculated on the basis of microsatellite sequences was found between farmed animals raised in Poland and wild animals from Russia (0.2958). The smallest distance separated individuals from the two groups of wild animals (0.04) (Table 4). On the basis of Bayesian analysis, carried out for all individuals from the farmed population and two wild populations, optimal division into groups, determined by the value of ΔK and the probability of K, was found in the case of seven clusters (Figure 1) K = 7. All animals living in the wild, whether from the Polish or the Russian population, represented one genetic group (Figure 1 and 2), whereas the farmed population was found to be described by 6 clusters (Figure 1 and 2).

Table 4. Genetic distance (below diagonal) and genetic similarity (above diagonal) according to Nei between the three raccoon dog populations on the basis of microsatellite sequences

		Genetic similarities		
		wild Polish population	wild Russian population	farmed population
Genetic distance	wild Polish population	***	0.9608	0.8120
	wild Russian population	0.0400	***	0.7439
	farmed population	0.2082	0.2958	***

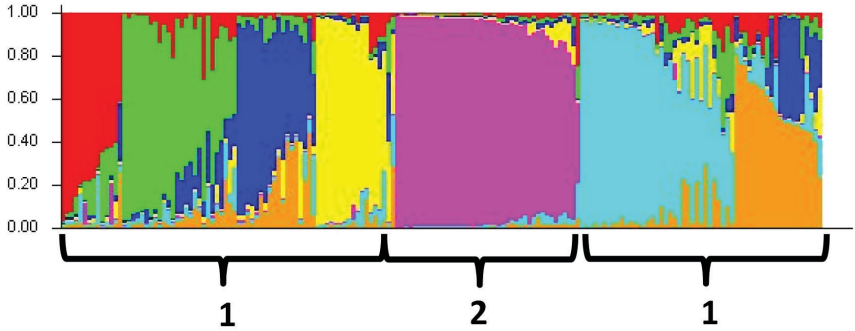

Figure 1. Bayesian analysis of raccoon dog individuals divided into seven genetic groups (clusters) based on allele frequency in 15 microsatellite loci. 1 – farmed population. 2 – wild population of Polish and Russian origin. Colours correspond to genetic pools (clusters)

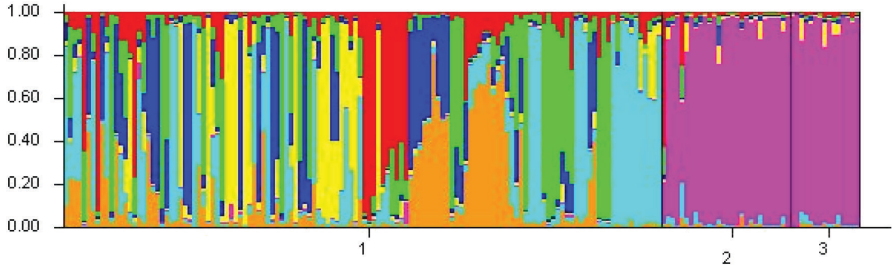

Figure 2. Bayesian analysis of raccoon dog individuals from the farmed population (1), the wild Polish population (2) and the wild population from the Kaliningrad Region (3), divided on the basis of allele frequency in 15 microsatellite loci. Colours correspond to genetic pools (clusters)

Discussion

The suitability of 15 microsatellite loci for analysis of membership in a given raccoon dog population was investigated. Irrespective of the animals' origin in one of three groups established in the methodology, 10 loci (60%) may be classified as highly informative markers (PIC>0.5). Consequently, the microsatellite loci used in these studies, i.e. INU013, INU014, Ren01E05, Ren02K21, Ren39L15, Ren13J22, Ren44K10, Ren06C11, Ren41D20 and Ren01O23, can be used in studying membership in a given population. The primer set used for the microsatellite loci, together with the Bayesian method of clustering genetically similar individuals, is sufficient to distinguish farmed and wild animals. The same microsatellite loci were successfully used to distinguish a Polish farmed fox population from wild animals living in Poland and Canada (Jeżewska-Witkowska et al., 2012). Based on the PIC (polymorphic information content) value and specific alleles occurring with high frequency, the loci INU014, Ren13J22 and Ren41D20 are sufficient to determine membership in a given raccoon dog population. For a stronger conclusion, microsatellite markers other than those used in this study should be tested in order to increase the number of loci. Nevertheless, our study has shown a genetic distinction between wild and farmed animals, which has also been indicated by other analyses. The data presented in the work of Ślaska and Grzybowska-Szatkowska (2011) confirm the genetic distinctiveness of farmed animals and individuals living in the wild in Poland. On the basis of the sequence of 3 fragments of mitochondrial genes (MT-CYTB, MT-COI and MT-COII) in 44 farmed individuals and 7 wild individuals from Poland, 7 haplogroups were established. Genetic differences between farmed and free-living individuals, with the first group represented by 4 and the second by 3 independent haplogroups (Ślaska and Grzybowska-Szatkowska, 2011), are quite distinct. The genetic distinctness of farmed and wild animals is also indicated by Bugno-Poniewierska et al. (2013), on the basis of analysis of the number of B chromosomes. These examples, as well as our own research, indicate a lack of gene flow to wild populations, which can be explained by a negligible number or complete lack of escapes, or by the inability of farmed animals to survive in the wild.

Bayesian analysis assigning individuals to a given group showed greater genetic diversity in the farmed animals than in the wild population. As a result of this analysis, the farmed population was described by 6 genetic groups, and the two wild populations by only one.

Our own research indicates a genetically homogeneous group of wild animals, in contrast to research by Pitra et al. (2010) and Korablev et al. (2011), who suggest the existence of two distinct clades of the European population. Sequencing of the control region of mtDNA from 78 wild Finnish and German raccoon dog populations made it possible to describe genetic variation using 9 haplotypes (Pitra et al., 2010). Five haplotypes proved to be common to both geographic regions, suggesting settlement in Germany from the east via Poland by the genetic pool of individuals from Scandinavia. On the other hand, the authors suggest (Pitra et al., 2010) settlement in Germany from the south by individuals reintroduced in Ukraine. In addition, Pitra et al. (2010) indicate the presence of two distinct clades, common

to German and Finnish populations that diverged probably approx. 500,000 years ago, which according to the authors is explained by the introduction of Asian raccoon dogs to Eastern Europe from various geographically isolated regions. Similar conclusions were drawn by Korablev et al. (2011), who indicate 2 haplogroups, significantly different in terms of mtDNA control region sequences, in a wild raccoon dog population in the lower Volga region. The authors (Korablev et al., 2011) point out that the division into haplogroups has no link to the geographical distribution of the animals. In their study, Korablev et al. (2011) found a surprising degree of genetic variability in the animals, with 18 different haplotypes noted in a group of 30 animals. Our own analyses, based on microsatellite sequences, do not allow for such clear distinctions between wild animals from either the Polish or the Russian population, which created a genetically homogeneous group, clearly distinguishable from the farmed population. This can be explained by two different types of genetic information. Toews and Brelsford (2012) emphasize the limitations of phylogenetic studies based purely on mitochondrial DNA sequencing, suggesting the use of a combined method using nuclear (nuDNA) and mitochondrial DNA (mtDNA). They justify their argument by the divergent phylogenetic inferences drawn from these two types of genetic information, providing 126 examples of such cases from the last 10 years (Toews and Brelsford, 2012). The reasons for such differences are to be found in adaptive introgression of mtDNA, demographic differences, and different rates of sex dispersion. Furthermore, microsatellite loci are subject to rapid evolutionary mutations and variations in comparison with conserved sequences of mitochondrial DNA. As a consequence, the level of genetic differentiation between populations as determined by microsatellite sequence is often lower than the record in the mitochondrial DNA suggests (Ngamprasertwong et al., 2008; Crochet et al., 2003). In cases where male and female individuals exhibit different patterns of migration, reconstruction of the history of the population based only on the mitochondrial genome inherited from the maternal line may not be comprehensive. This problem may be relevant in the case of the raccoon dog invasion of the European continent. As reported by Kauhala et al. (2006), the average migration distance of males is much greater than that of females, 14 and 19 km, respectively, with maximum values of 48 and 71 km. The problem can be avoided by using a skilful combination of information derived from analysis of mtDNA variation with information obtained from analysis of nuclear loci, including microsatellite loci. The situation is further complicated by the inclusion of phenotypic information for phylogenetic analysis. In contrast to Pitra et al. (2010), who reported two common clades of German and Finnish populations, Ansorge et al. (2009), on the basis of examination of the skull, concluded that there were two independent clusters for German and Finnish/Polish populations. Hence reliable phylogenetic inferences should be based on classification systems that require a large base of empirical knowledge, not only of the DNA nucleotide sequence of the species, but also phenotypic data and information on its ecology, biology and geographical distribution. The differences in the genetic diversity of wild animals between our study and the research by Pitra et al. (2010) may also result from the probability of having obtained individuals constituting a less numerous genetic clade for analysis. In a study of Finnish and German populations, the

probability was 1:10, while our own analyses included only 43 individuals living in the wild.

While wild animals formed one genetic group, farmed animals were classified into six different clusters. One reason for the increased genetic variability in farmed raccoon dogs may be the polyandrous mating system. Ślaska and Jeżewska (2008) demonstrated that in one litter there are two fathers in about 47% of cases and three in about 55% of cases. Another reason for the high variability of farmed animals may be the exchange of breeding material between farms in Poland and Finland. Research by Bugno-Poniewierska et al. (2013) confirms the higher genetic variability of farmed animals in comparison with wild ones. Analysis of metaphases in somatic cells of farmed and wild raccoon dogs in Poland showed from 0 to 4 B chromosomes in farmed animals and from 0 to 3 in wild populations (Bugno-Poniewierska et al., 2013). The change in the organization of the genome (Bugno-Poniewierska et al., 2013) and the presence of new mutations in microsatellite loci or in the mitochondrial DNA (Ślaska and Grzybowska-Szatkowska, 2011) in farmed animals compared to wild ones indicates the possibility of adaptive mutations that may have emerged as a result of changes in environment and the impact of directional selection.

In conclusion, the analysis of microsatellite loci showed clear genetic differences between farmed and wild populations of raccoon dog, despite only 50 years of isolation of the two groups of animals. The farmed population was characterized by higher genetic variation than the wild population. On the basis of the analyses, three microsatellite loci (INU014, Ren13J22 and Ren41D20) were proposed for use in identifying the origin of animals that have escaped from farms.

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