doi: 10.17221/2/2017-CJAS

Two Mitochondrial Genes Are Associated with Performance Traits in Farmed Raccoon Dogs (*Nyctereutes procyonoides*)

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ABSTRACT

Nisztuk-Pacek S., Slaska B., Zieba G., Rozempolska-Rucinska I. (2018): **Two mitochondrial genes are associated with performance traits in farmed raccoon dogs (*Nyctereutes procyonoides*)**. Czech J. Anim. Sci., 63, 110–118.

The relationships between chosen mitochondrial genes polymorphisms and performance traits in raccoon dogs were determined. The study involved 354 farmed raccoon dogs. Blood collected from the animals was the analysed biological material. Mitochondrial DNA genes, i.e. *MT-CO1* (mitochondrially encoded cytochrome c oxidase I), *MT-CO2* (mitochondrially encoded cytochrome c oxidase II), and *MT-CYB* (mitochondrially encoded cytochrome b) were amplified using the polymerase chain reaction method. The amplicons obtained were sequenced and subjected to bioinformatics analysis. Based on the nucleotide sequences, three haplotypes for the *MT-CO1* gene fragment and two haplotypes for the *MT-CO2* gene fragment were identified. The sequence of the *MT-CYB* gene was monomorphic. Based on the haplotypes, five previously undescribed mitochondrial haplogroups were determined. Statistical analysis revealed significant differences between the values of three of the five investigated performance traits of raccoon dogs and the identified haplotypes and mitochondrial haplogroups, taking into account predictors of direct additive effects, additive maternal effects, and fixed specific maternal environmental effects. The new mitochondrial haplogroups identified in the farmed raccoon dog population may imply constant emergence of adaptive mutations that are conserved in subsequent generations. The results of the association study indicate a statistically significant association between haplotypes and mitochondrial haplogroups of farmed raccoon dogs and their body weight, body size, and colour type, which allows considering *MT-CO1* and *MT-CO2* genes as candidate genes encoding these traits in raccoon dogs. The results of the molecular analyses can be applied to improve the performance traits in farmed raccoon dogs.

Keywords: haplotypes; polymorphism; fur-bearing animals; *MT-CO1*; *MT-CO2*; *MT-CYB*

The raccoon dog (*Nyctereutes procyonoides*, family Canidae), which is widely distributed in Europe, is a prominent example of a highly adaptable species (Norgaard et al. 2017). The raccoon dog breeding in Poland began with the introduction of 200 animals from Finland in 1979. In fur-bearing animal farms, breeding work is focused on the improvement of performance traits, primarily by selection of phenotypic traits; however, this leads to considerable fluctuations of the breeding value

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the paper.

doi: 10.17221/2/2017-CJAS

in successive generations, which is reflected in genetic trends. In turn, improvement of the hair coat and size traits is mainly associated with environmental progress (Wierzbicki and Filistowicz 2001; Gugolek et al. 2002; Slaska and Grzybowska-Szatkowska 2011).

The consequent increase in the breeding value requires analyses based on the genetic variability of animals. Modern research techniques facilitate the direct genome analysis and determination of the relationships between the animal genotype and phenotype. Mitochondria are one of the most important organelles present in the cytoplasm of almost all cells in the organism. Their primary function is to produce energy in the form of adenosine triphosphate (ATP) in the cell respiration process (Slaska and Grzybowska-Szatkowska 2011; Surdyka and Slaska 2016b). The mitochondrial genome includes, among others, 13 genes coding for respiratory chain proteins. A characteristic feature of mitochondrial DNA (mtDNA) is the absence of recombination; hence, changes in mtDNA emerge only through mutations. Consequently, the mitochondrial genome is a single haplotype inherited almost exclusively in the maternal line (Slaska and Grzybowska-Szatkowska 2011). Furthermore, it was found that the frequency of polymorphisms in the mitochondrial genome in the raccoon dog was twenty-fold higher than that in the nuclear genome (Nisztuk-Pacek 2016). With these features, mtDNA is often used in phylogenetic studies, molecular ecology, and veterinary medicine (Slaska and Grzybowska-Szatkowska 2011; Nisztuk-Pacek 2016; Surdyka and Slaska 2016a, b).

Different mitochondrial haplotypes affect the mitochondrial function to a certain extent, thus affecting the growth of cell, leading to diseases of individuals. Mutations in the genes of polypeptides encoded in mitochondrial DNA can cause defects in the mitochondrial oxidative phosphorylation (OXPHOS) system. An increased production of reactive oxygen species (ROS), accompanying the improper function of the respiratory chain and caused by mutations, seems to have a particularly significant effect (Slaska et al. 2013; Surdyka and Slaska 2016b; Zhuo et al. 2016). Simultaneously, the search for mutations that may be associated with economically important performance livestock traits is an interesting research trend based on mitochondrial DNA sequences. Mutations appearing in the mtDNA sequence may in fact lead to disturbances in the cell respiration process and malfunction of cells and tissues. Therefore, the aim of the study was to determine the relationship between chosen mitochondrial genes polymorphisms and the performance traits of raccoon dogs.

MATERIAL AND METHODS

Molecular analyses. The analyses involved 354 brown colour type raccoon dogs (*Nyctereutes procyonoides*) bred in one of fur-bearing animal farms in the south-east of Poland. Whole peripheral blood sampled intravitally into sterile vacuum tubes filled with anticoagulant K2 EDTA (dipotassium ethylenediaminetetraacetic acid) was the material for the molecular analyses. The biological material was used for isolating total DNA with the use of a commercial DNeasy Blood & Tissue Kit (Qiagen, Germany). The isolated DNA was subjected to qualitative and quantitative assessment. The genetic material was used for polymerase chain reaction (PCR) amplification of selected gene fragments performed with a Labcycler thermocycler (SensoQuest, Germany). The following mtDNA genes were amplified: *MT-CO1* (mitochondrially encoded cytochrome c oxidase I), *MT-CO2* (mitochondrially encoded cytochrome c oxidase II), and *MT-CYB* (mitochondrially encoded cytochrome b). Primer sequences for the *MT-CO1* gene fragment (m.5616-6272 – F: CCGGACATGGCATTCCCCCG, R: GGCGGACGTAAAGTACGCTCGTG) were designed on the basis of the nucleotide sequence (Accession No. NC_002008) with the program Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The complete mitochondrial sequence of *Nyctereutes procyonoides* has been available in GenBank since January 2010 (NC_013700), i.e. after accomplishing the analysis for this work. The primer sequences designed based on the dog's (NC_002008) and raccoon dog's (NC_013700) mitochondrial genome differ in single nucleotides in three (F-primer: CCaGACATaGCATTtCCCCG) and three (R-primer: aGCGGAtGTAAAGTAtGCTCGTG) positions. The differences in the primer sequences did not affect the amplification product yield. Primers available in literature, i.e. *MT-CYB* (m.15013–15312 – F: GCACGCAAATGGCGCTTCCA, R: GCATTGGCTAAGGGGCCGGA) and *MT-CO2* (m.7048–7713 – F: TACCCTTTCCAACTCG-

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GATT, R: GGCAGATCAGGTTTCGAAAT), were used for the other 2 gene fragments (Slaska and Grzybowska-Szatkowska 2011). The temporal temperature profile of the PCR reaction consisted of initial denaturation (95°C, 10 min), 35 denaturation cycles (95°C, 1 min), primer annealing (52°C (*MT-CO1*), 57°C (*MT-CO2*, *MT-CYB)*, 1 min) and extension (72°C, 1 min), and final primer extension (72°C, 20 min). The gene fragment amplicons were sequenced using a sequencing BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, USA). Purified products were sequenced on an ABI 377 automated sequencer (Applied Biosystems). The nucleotide sequences obtained were subjected to bioinformatics analysis carried out with the use of the DNA Baser Sequence Assembler v4 (2013). Haplotypes of the examined genes were established according to the nomenclature proposed by Slaska and Grzybowska-Szatkowska (2011).

Statistical methods. The analysed material comprised breeding documentation data from the fur-bearing animal farm. The documentation was compiled in accordance with the regulation for animal breeding and reproduction (1997) on reproduction and breeding farms. The observations involved 185 raccoon dog females and 169 males; the animals were weighed and the hair coat quality was assessed at maturity. The assessment of the conformation of young raccoon dogs was carried out in accordance with the relevant evaluation standard developed by the Central Animal Breeding Office (CSHZ 1997). A point-based trait evaluation was applied with a maximum score of 20 points for all traits. The following conformation traits were assessed: body size (0–6 points), colour type (0–3 points), colour purity (0–3 points), and hair coat quality (0–8 points). The phenotypic values of the raccoon dog conformation traits were shown as Least Squares Means (LSM), providing standard errors (SE) that determine the reliability of estimation, and were as follows: body weight (g) – LSM = 11 093 ± 94.8 (min. = 10 091, max. = 15 300); body size (points) LSM = 5.74 ± 0.04 (min. = 4, max. = 6); colour type (points) LSM = 2.98 ± 0.01 (min. = 2, max. = 3); colour purity (points) LSM = 2.50 ± 0.04 (min. = 1, max. = 3); hair coat quality (points) LSM = 6.10 ± 0.07 (min. = 3, max. = 8); total score (points) LSM = 17.30 ± 0.10 (min. = 11, max. = 20).

Pedigree data from a 4-generation population of raccoon dogs and their genotypes in mitochondrial gene fragments were used to determine the relationship between the genotypes and conformation traits (body weight, body size, colour type, colour purity, hair coat quality) and the total conformation value.

Estimation of variance components was performed with the REML method, and BLUP prediction of the breeding values was carried out in a mixed model:

$$y = \mathbf{X}b + \mathbf{Z}a + \mathbf{Z_m}m + \mathbf{W}pm + e$$

where:

y = observation vector
b = vector of fixed effects (sex, year of birth, sex × year of birth)
a = vector of random direct additive genetic effects
m = vector of random additive maternal effects
pm = vector of specific random maternal environmental effects
e = vector of random residual effects
$\mathbf{X}$, $\mathbf{Z}$, $\mathbf{Z_m}$, $\mathbf{W}$ = incidence matrices of experimental factors

The analyses were performed with the REML and BLUP methods using BLUPF90 family programs (BGF90) (Misztal et al. 2002).

The structure of the (co)variance matrix of random effects in the single-trait model was as follows:

$$\mathbf{V}\begin{bmatrix} \boldsymbol{a} \\ \boldsymbol{m} \\ \boldsymbol{pm} \\ \boldsymbol{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma_a^2 & \mathbf{A}\sigma_{am} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{am} & \mathbf{A}\sigma_m^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_{pm}^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix}$$

where:

$\mathbf{A}$ = pedigree linkage matrix (additive relatedness and inbreed)
σ_a^2 = direct additive variance
σ_m^2 = maternal additive genetic variance
σ_{pm}^2 = variance of fixed specific maternal environmental effect
σ_{am} = covariance between direct additive and additive maternal effects
σ_e^2 = error variance

The predictors of the traits of direct additive, maternal additive, and random specific maternal environmental effects were analyzed in a model comprising the genotype and residual effects. The analyses were performed with the use of the GLIMMIX procedure in the SAS software (Statistical Analysis System, Version 9.4, 2013). Multiple

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comparisons of the estimates of the differences in the haplotypes and mitochondrial haplogroups of the analyzed traits were subjected to the Bonferroni correction.

The study was approved by the II Local Ethical Commission for Animal Experiments in Lublin, Poland.

RESULTS

The encoding sequences of the raccoon dog *MT-CO1* (cytochrome c oxidase subunit I) and *MT-CO2* (cytochrome c oxidase subunit II) genes were deposited in the GenBank under Accession Nos. KX467623 and KX467624, respectively.

Analysis of the *MT-CO1* gene sequence revealed the presence of seven polymorphisms, which were grouped into three mitochondrial haplotypes: *A* (*TTCCTTC* – according to the reference sequence of *Nyctereutes procyonoides* in positions m.5713, m.5770, m.5929, m.6061, m.6088, m.6094, and m.6157, respectively), *B* (*TCCCTTC*), and *C* (*CTTTCCT*). The highest frequency was determined for haplotypes *A* (60.68%) and *C* (38.04%), whereas haplotype *B* (1.28%) exhibited lower frequency. Simultaneously, all the polymorphisms were found to be synonymous. Analysis of the *MT-CO2* gene sequence identified six polymorphisms in the group of the raccoon dogs, grouped into two haplotypes: *A* (*AAGAAC* – according to the reference sequence of *Nyctereutes procyonoides* in positions m.7133, m.7202, m.7229, m.7295, m.7586, and m.7673, respectively) and *B* (*GCAGGT*). Animals with identified haplotype *A* and haplotype *B* accounted for 38.89% and 61.11%, respectively. All the polymorphisms were found to have a synonymous character. Neither in *MT-CO1* nor in *MT-CO2* heteroplasmy was observed. The sequence analysis of the *MT-CYB* gene fragment showed no polymorphisms in the raccoon dogs.

Based on the haplotypes determined for the fragments of genes encoding cytochrome c oxidase subunits I and II, five mitochondrial haplogroups were distinguished for the raccoon dog population. The names of the haplogroups were assigned in accordance with the nomenclature proposed by Slaska and Grzybowska-Szatkowska (2011). Haplogroups Np9 and Np11 were characterized by the highest frequency (0.56 and 0.34, respectively) (Table 1).

Genes *MT-CO1* and *MT-CO2* are elements of complex IV of the respiratory chain and thus play a key role in cellular energy production. Given their important role in cells and the relatively high degree of intraspecific variability, they may be useful for e.g. investigations focused on identifying the relationship between polymorphisms and important performance traits of farmed raccoon dogs.

Association analyses

MT-CO1 gene haplotypes. Taking into account the predictors of direct additive effects, the analysis of the impact of the *MT-CO1* gene haplotypes on the performance traits of raccoon dogs revealed statistically significant differences in the body weight, body size, and colour type (Table 2). It was found that the animals representing mitochondrial haplotype *C* were characterized by significantly higher body weight than the raccoon dogs with haplotype *A*. Concurrently, it was shown that the raccoon dogs with haplotype *A* exhibited a significantly higher body size and higher scores for the colour type than the animals with haplotype *C* (Table 2).

Similarly, statistical analysis based on the predictors of additive maternal effects showed statistically significant differences in the case of the body weight, body size, and colour type. The raccoon dogs with haplotype *A* were characterized by significantly higher body weight and body size and had higher scores for the colour type than the animals with haplotype *C* (Table 2).

In the analysis of the association of *MT-CO1* haplotypes with colour purity, hair coat quality, and total score, no statistically significant differences were found when either direct additive effects or additive maternal effects were taken into account. In turn, there were no statistically significant differences in the values of any of the analyzed con-

Table 1. Mitochondrial haplogroups in the raccoon dog population identified on the basis of the *MT-CO1* and *MT-CO2* gene fragments

Mitochondrial haplogroup	Haplotype		Proportion (%)
	MT-CO1	*MT-CO2*	
Np8	*A*	*A*	4.70
Np9	*A*	*B*	55.98
Np10	*B*	*B*	1.28
Np11	*C*	*A*	34.19
Np12	*C*	*B*	3.85

doi: 10.17221/2/2017-CJAS

Table 2. Differences between selected performance traits in relation to the haplotypes of the *MT-CO1* gene, taking into account predictors of direct additive effects (*a*) and additive maternal effects (*m*)

Trait	Effect	Haplotypes	Difference	Confidence limits (95%)	
				lower	upper
Body weight	*a*	*A* v. *B* *TTCCTTC* v. *TCCCTTC*	–386	–1035	264
		A v. *C* *TTCCTTC* v. *CTTTCCT*	–176*	–327	–26
		B v. *C* *TCCCTTC* v. *CTTTCCT*	209	–444	862
	m	*A* v. *B* *TTCCTTC* v. *TCCCTTC*	362	–289	1012
		A v. *C* *TTCCTTC* v. *CTTTCCT*	171*	20	322
		B v. *C* *TCCCTTC* v. *CTTTCCT*	–190	–845	464
Body size	*a*	*A* v. *B* *TTCCTTC* v. *TCCCTTC*	6.82E-04	–0.002	0.003
		A v. *C* *TTCCTTC* v. *CTTTCCT*	7.51E-04**	1.61E-04	0.001
		B v. *C* *TCCCTTC* v. *CTTTCCT*	6.87E-05	–0.002	0.003
	m	*A* v. *B* *TTCCTTC* v. *TCCCTTC*	4.65E-05	–2.08E-04	3.01E-04
		A v. *C* *TTCCTTC* v. *CTTTCCT*	8.99E-05**	3.11E-05	1.49E-04
		B v. *C* *TCCCTTC* v. *CTTTCCT*	4.34E-05	–2.12E-04	2.99E-04
Colour type	*a*	*A* v. *B* *TTCCTTC* v. *TCCCTTC*	0.008	–0.002	0.019
		A v. *C* *TTCCTTC* v. *CTTTCCT*	0.005**	0.002	0.007
		B v. *C* *TCCCTTC* v. *CTTTCCT*	–0.004	–0.015	0.007
	m	*A* v. *B* *TTCCTTC* v. *TCCCTTC*	1.49E-04	–5.13E-04	0.001
		A v. *C* *TTCCTTC* v. *CTTTCCT*	2.38E-04**	8.50E-05	3.92E-04
		B v. *C* *TCCCTTC* v. *CTTTCCT*	8.93E-05	–5.76E-04	0.001

differences significant at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$

formation traits between the haplotypes when the fixed specific maternal environmental effect was included in the analysis (data not tabulated).

MT-CO2 gene haplotypes. The analysis based on the predictors of direct additive effects revealed statistically significant differences for the body weight, body size, and colour type between the haplotypes of the raccoon dogs (Table 3). Animals representing haplotype *A* were characterized by significantly higher body weight in comparison with the raccoon dogs with haplotype *B*. Different results were obtained for the other two charac-

Table 3. Differences between selected performance traits in relation to haplotypes of *MT-CO2* gene, taking into account predictors of direct additive effects (*a*), additive maternal effects (*m*), and fixed specific maternal environmental effects (*pm*)

Trait	Effect	Haplotypes	Difference	Confidence limits (95%)	
				lower	upper
Body weight	*a*	*A* v. *B* *AAGAAC* v. *GCAGGT*	211**	90	332
	m		–206**	–328	–85
	pm		26	–95	147
Body size	*a*		–0.001**	–0.002	–0.001
	m		1.15E-04**	1.62E-04	–6.86E-05
	pm		–0.417**	–0.689	–0.145
Colour type	*a*		–0.006**	–0.008	–0.004
	m		–3.05E-04**	–4.27E-04	–1.84E-04
	pm		–0.367**	–0.537	–0.198

differences significant at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$

doi: 10.17221/2/2017-CJAS

teristics. Animals with haplotype *B* exhibited significantly higher body size and colour type scores than the raccoon dogs representing haplotype *A* (Table 3). The analysis taking into account additive maternal variance showed statistically significant differences for the same performance traits as in the analysis based on the predictors of direct additive effects (Table 3). In this case, however, it was found that the raccoon dogs with haplotype *B* exhibited substantially higher body weight, body size, and colour type scores than animals with haplotype *A* (Table 3).

Statistical analysis of the association of *MT-CO2* haplotypes with the performance traits of raccoon dogs, based on predictors of the fixed specific maternal environmental effect, revealed statistically significant differences in the case of the body size and colour type. Raccoon dogs with haplotype *B* were characterized by significantly higher scores for the body size and colour type compared with the animals representing haplotype *A* (Table 3).

In the case of colour purity, hair coat quality, and total scores, there were no statistically significant differences in the values of the analyzed conformation traits between the *MT-CO2* haplotypes when the predictors of direct additive effects, additive maternal effects, and fixed specific maternal environmental effects were included in the analysis (data not tabulated).

Mitochondrial haplogroups. When the predictors of direct additive effects were included in the statistical analysis of the association of each mitochondrial haplogroup with the performance traits of the raccoon dogs, significant differences were shown for the body weight, body size, and colour type (Table 4). It was found that the raccoon dogs representing haplogroup Np9 were characterized by significantly higher body weight, body size, and colour type scores than the animals from haplogroup Np11. Simultaneously, it was demonstrated that the animals from haplogroup Np12 had significantly higher scores for the body size and colour type than the raccoon dogs from haplogroup Np11 (Table 4). In the case of the other performance traits, there were no statistically significant differences between the mitochondrial haplogroups (data not tabulated).

The analysis of the association of the mitochondrial haplogroups with the performance traits, based on the predictors of additive maternal effects, confirmed the results obtained in the analysis of the additive effects. Additionally, it was shown that the raccoon dogs from haplogroup Np11 were characterized by a significantly higher total

Table 4. Differences between selected performance traits in relation to the mitochondrial haplogroup, taking into account predictors of direct additive effects (*a*), additive maternal effects (*m*), and fixed specific maternal environmental effects (*pm*)

Trait	Effect	Mitochondrial haplogroups	Difference	Confidence limits (95%)	
				lower	upper
Body weight	*a*	Np9 v. Np11	–221**	–406	–37
	m	Np9 v. Np11	216**	32	401
Body size	*a*	Np9 v. Np11	0.001**	3.06E-04	0.002
		Np11 v. Np12	–0.002*	–0.004	–2.34E-04
	m	Np9 v. Np11	1.16E-04**	4.44E-05	1.87E-04
		Np11 v. Np12	–1.28E-04*	–4.62E-04	2.07E-04
Colour type	*a*	Np9 v. Np11	0.006**	0.003	0.009
		Np11 v. Np12	–0.009**	–0.016	–0.002
	m	Np9 v. Np11	3.06E-04**	1.21E-04	4.91E-04
		Np11 v. Np12	–4.90E-04*	–0.001	–3.07E-05
	pm	Np8 v. Np9	–0.859**	–1.425	–0.293
		Np8 v. Np12	–0.985**	–1.795	–0.175
		Np9 v. Np11	0.288*	0.032	0.543
Total score	*m*	Np11 v. Np12	0.691*	0.019	1.364

differences significant at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$

doi: 10.17221/2/2017-CJAS

score than that for the animals from haplogroup Np12 (Table 4).

The analysis of the association of the identified haplogroups with the performance traits, taking into account the predictors of fixed specific maternal environmental effects, revealed significant differences in the values of the colour type in the raccoon dogs. Animals representing mitochondrial haplogroup Np9 had significantly higher colour type scores in comparison with the animals from haplogroups Np8 and Np11. Furthermore, the animals from mitochondrial haplogroup Np12 exhibited significantly higher colour type scores than those in the animals from haplogroup Np8 (Table 4). No statistically significant differences were found between the mitochondrial haplogroups in the case of the other analyzed performance traits (data not tabulated).

DISCUSSION

To identify polymorphisms in mitochondrial DNA, which are associated with economically important performance traits, is of importance for breeders of fur-bearing animals. Currently, selection carried out on farms is based solely on the value of phenotypic traits. Therefore, application of the achievements of molecular genetics might contribute to improvement of ongoing breeding programs. The intensive development of molecular biology techniques facilitates rapid identification and selection of individuals with the most valuable genotype. Genomic selection, however, involves only nuclear markers.

Given the key role of mitochondrial DNA in the cell respiration process, there have been attempts in recent years at determining the association between mtDNA polymorphisms with economically important performance and functional traits in populations of various livestock animals (Sutarno et al. 2002; Mannen et al. 2003; Jeon et al. 2005; Yen et al. 2007; Fernandez et al. 2008; Reicher et al. 2012; Slaska et al. 2016).

The results obtained in the study by Slaska et al. (2016) indicate that *MT-CO2* gene can be involved in the development of hair coat. The results of the present study indicate an association between the haplotypes of *MT-CO2, MT-CO1*, mitochondrial haplogroups, and other performance traits: body weight, body size, and colour type of the farmed raccoon dogs, however not with hair coat quality, as described by Slaska et al. (2016).

Associations between reproduction traits and mtDNA haplotypes were investigated by Sutarno et al. (2002) and Reicher et al. (2012). They demonstrated statistically significant differences between the level of calving in beef cattle and mitochondrial haplotypes (Sutarno et al. 2002) and between haplogroups and Afec-Assaf sheep fecundity (Reicher et al. 2012). There are investigation results indicating a relationship between mitochondrial haplotypes and the surface area of *musculus longissimus* in Japanese Black cattle (Mannen et al. 2003), as well as meat quality in Iberian pigs (Fernandez et al. 2008).

The association between conformation traits and mtDNA haplotypes has been evidenced as well (Jeon et al. 2005; Yen et al. 2007; Zhang et al. 2008; Chen et al. 2009). Statistically significant differences between mtDNA haplotypes and piglet body weight (Yen et al. 2007) as well as the height and length of the body in Nanyang cattle have been proved (Zhang et al. 2008). In turn, Chen et al. (2009) demonstrated a statistically significant effect of *MT-CYB* gene haplotypes on rump width in Yunnan donkeys and body height in the Dezhou race. Due to its monomorphism, the *MT-CYB* sequence was not associated with the conformation traits in the raccoon dogs in the present study. Jeon et al. (2005) showed significant differences between genotypes identified in the *MT-CO2* gene and weight in Hanwoo cattle. Similarly, the results of this study indicate an association between haplotypes (*MT-CO1*, *MT-CO2*) and mitochondrial haplogroups as well as body weight and body size of the farmed raccoon dogs.

Cytochrome oxidase, which constitutes complex IV of the respiratory chain, is an important aerobic metabolism enzyme catalyzing the electron transport between cytochrome c and molecular oxygen and participating in production of energy in the form of ATP (Slaska and Grzybowska-Szatkowska 2011; Surdyka and Slaska 2016b), which may be related to growth and development. The association of mtDNA mutations with phenotypic features is confirmed. The symptoms of mitochondrial diseases are usually connected with tissues with high energy requirements such as skeletal muscle, which is manifested by myopathies (Zhuo et al. 2016). The results of the present study indicate a statistically significant association between

doi: 10.17221/2/2017-CJAS

the identified haplotypes and mitochondrial haplogroups and the growth (body weight and body size) of farmed raccoon dogs. It was found that the raccoon dogs representing haplotype *B* were characterized by a significantly higher value of the trait, compared with the animals with haplotype *A*. These results are important from the viewpoint of breeding fur-bearing animals, which is focused on production of the largest possible good quality pelts. Besides the hair coat quality, body weight, as well as body size evaluated as a conformation trait, is one of the traits improved on farms, as it directly contributes to breeders' profits.

CONCLUSION

In summary, the results of the present investigations allowed identify new mitochondrial haplogroups in the population of farmed raccoon dogs, which may imply constant emergence of adaptive mutations that are conserved in subsequent generations. Concurrently, the results of the association studies indicate a statistically significant relationship of the haplotypes and mitochondrial haplogroups in the farmed raccoon dogs with their body weight, body size, and colour type, which allows considering *MT-CO1* and *MT-CO2* as candidate genes for these traits. The results of the presented molecular analyses can therefore be applied in improving the performance traits of farmed raccoon dogs, which may contribute to increasing the body weight and size of these animals and improving the hair coat traits. Given the important role of mitochondria in biochemical processes associated with productivity, reproduction, and health, studies of associations between mtDNA polymorphisms and animal traits should be continued.

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Received: 2016–12–10
Accepted after corrections: 2018–01–03