Evaluation of the effectiveness of introducing new alleles into the gene pool of a rare dog breed: Polish Hound as the example

I. Głażewska[1], B. Prusak[2]

[1]Department of Plant Taxonomy and Nature Conservation, University of Gdańsk, Gdynia, Poland
[2]Institute of Genetics and Animal Breeding PAS, Jastrzębiec, Poland

ABSTRACT: The objective of the analysis was to check the possibility of enriching a gene pool of a rare dog breed by breeding use of dogs of unknown origin that are phenotypically similar to a given breed. The evaluation was performed using pedigree and mtDNA analyses applied to Polish Hounds. The results indicated the very limited breeding success of such dogs in relation to their contributions to the gene pool and to the number of their descendants used in breeding. Dogs of unknown origin accounted for 80.9% of the total number of breed founders while the proportions of their descendants used in breeding were equal to just 14.3 and 4.7% of the total number of dams and sires, respectively. Breeders are unwilling to use such dogs and kennel judges are critical of their quality and appearance which are inconsistent with the breed standard. This may be connected with their distinct breed affiliation detected by the mtDNA analysis which showed the presence of three mtDNA haplotypes in Polish Hounds differing by a large number of substitutions. The study leads to the pessimistic conclusions that chances of enriching gene pools through breeding use of dogs of unknown origin are rather slim. The case of the Polish Hounds shows that the success of programmes for improving the genetic condition of endangered dog breeds can only be achieved in coordination between breeders and kennel authorities, and with financing from the state.

Keywords: dog; pedigree analysis; mtDNA

Many articles published in recent years have focused on pedigree analyses in rare dog breeds (Leroy et al., 2006, 2009; Głażewska, 2008; Oliehoek et al., 2009; Mäki, 2010). These analyses have indicated a number of unfavourable occurrences in dog breeding, such as a high level of inbreeding, high disproportion in the breeding use of sires, a low number of breed founders and strong imbalance in their contributions to a gene pool, all of which might result in the negative effects observed in the deteriorating health condition of a given breed and negatively influence its long-term perspectives. One way of enriching a limited gene pool is to introduce dogs of other breeds or of unknown origin that are phenotypically similar to a given breed (Leroy et al., 2006; Calboli et al., 2008; Mäki, 2010). However, the effectiveness of introducing new alleles into the gene pool of the breed depends mainly on breeders' will to use such dogs in breeding. This is of particular importance considering the specificity of dog breeding, with the high number of breeders and the low number of bitches in one kennel.

The objective of the present study was to evaluate the effects of using dogs of unknown origin in the breeding of a real population. The breeding success of dogs included in the breed and their breeding affiliation were evaluated with pedigree

Supported by the University of Gdańsk (Project No. BW/L 155-5-0345-9) and the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzębiec.

and mitochondrial DNA analyses, and the Polish Hound was used as an example of a rare dog breed.

The Polish Hound (ogar polski, PH) is a very small breed with a history that dates back to the 18th–19th centuries (Ściesiński, 2009). World War II resulted in the decline of this breed in Poland. The post-war restoration of PH began in the year 1959 using just two dogs and two bitches (first founders, FF) (Głażewska, 2008). The breed was recognized by the Fédération Cynologique Internationale (FCI) in 1966 (under FCI No. 52). Since then breeding has been conducted with dogs registered in the Polish Pedigree Book (Polska Księga Rodowodowa – PKR) and with PH-like dogs of unknown origin, which were registered together with two generations of their descendants, in the Introductory Book (Księga Wstępna – KW). The origin of the KW founders is unclear; they might be either purebred dogs bred without formal Polish Kennel Club documentation or dogs descended from other breeds or mixed-breed dogs. Polish Hounds are the hunting dogs, however, at present they are mostly kept as family dogs, and the only requirements to receive breeding qualifications in this breed are three positive evaluations from dog shows (see www.zkwp.pl). The limited size of the PH population resulted in the very high level of inbreeding. Considering this, worsening of basic anatomical measures and the declining health condition of the breed (Głażewska, 2008) can be recognized as signs of inbreeding depression. Since the 1970's, to improve this situation, dogs of unknown origin have been used in breeding.

MATERIAL AND METHODS

Pedigree analysis of dogs bred between the years 1960 and 2008 was conducted using the pedigree data available in the archives of the Polish Kennel Club (ZKwP). Three hundred and ten litters born in the period analysed were descendants of 6 male founders (2 FF and 4 KW dogs) and 15 female founders (2 FF and 13 KW bitches); the founders were defined as ancestors of unknown origin. The founders' contributions to the gene pool, i.e. the expected proportion of the population's gene pool that has descended from these founders (Lacy, 1989), were computed using GENES program v11.8 (Lacy, 1998).

Mitochondrial DNA was studied to examine the breed affiliation of dam line founders. Representatives of particular dam lines and branches that carried potentially different mitochondrial DNA sequences were chosen for laboratory analysis. Hair samples comprising 30–40 hairs each were taken from the backs and tails of the dogs. In total, eleven samples from dogs representing four existing dam lines and from last living representatives of two extinct lines (i.e. not continued after the year 2000), were collected. Samples from the remaining nine extinct lines were not available.

Total genomic DNA from hair bulbs was extracted according to the standard organic procedure (Wilson et al., 1995). DNA amplification was performed using primers designed in this study with the Primer (Rozen and Skaletsky, 2000): CRDOGF (15372 to 15392): 5'GTAACCGCCCTCCCTAAGAC3' and CRDOGR (16096–16117): 5'TGTCCTGAAACCATTGACTGA 3'. The PCR reaction was conducted in a GeneAmp PCR System 9600 Thermal Cycler (Applied Biosystems, Carlsbad, USA), according to the following parameters: 95°C for 10 min (denaturation) and next 94°C for 30 s, 55°C for 45 s, 72°C for 45 s – 35 cycles. The PCR reaction was conducted in a volume of 50 µl and the composition of the reaction mixture was as follows: 50–100 ng of genomic DNA, 200µM of each dNTP, 1 × PCR buffer (Applied Biosystems, Carlsbad, USA), 1.5mM $MgCl_2$, 1µM of each primer, 1.0 U AmpliTaq Gold® 360 DNA Polymerase (Applied Biosystems, Carlsbad, USA). The PCR products were purified through ultrafiltration using Microcon 100 microconcentrators (Amicon, Beverly, USA). The quantity and quality of PCR products were evaluated using Picodrop (Picodrop Limited, Saffron Walden, UK). Purified PCR products (660 base pair length) were sequenced with BigDye® Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, USA) according to the user's manual in a GeneAmp PCR System 9600 Thermal Cycler (Applied Biosystems, Carlsbad, USA). The sequencing products were purified with BigDye® XTerminator™ Purification Kit (Applied Biosystems, Carlsbad, USA) and separated in 3130*xl* Genetic Analyzer (Applied Biosystems, Carlsbad, USA). The electrophoretic data were collected by the ABI PRISM® Data Collection v.2.1. software and analysed by the DNA Sequencing Analysis Software v.3.0. (Applied Biosystems, Carlsbad, USA). The region of mitochondrial genome covering positions 15 426–16 085 (15 426–15 457 tRNA-Pro, 15 458–16 085 D-loop) was analysed according to the reference sequence of *Canis familiaris* mitochondrial genome, GenBank accession number U96639, published by Kim et al. (1998).

The sequences of the Polish Hounds were compared to 67 sequences found in 55 dog breeds representing different types and different FCI groups which were deposited in GenBank under accession numbers EU408245–408307 (Webb and Allard, 2009) and DQ4804489–480502 (Björnerfeldt et al., 2006). Phylogenetic analysis of the mtDNA haplotypes was performed using MEGA v4.0 software (Tamura et al., 2007). Bootstrap analysis (Felsenstein, 1985) with 1000 random permutations was performed to evaluate the reliability of the tree. The sequences determined in this study were deposited in GenBank under accession numbers HM007198–007200.

RESULTS

Pedigree analysis indicated the very limited breeding success of 17 KW founders and their descendants (designated later as KW dogs). Three hundred and ten litters of Polish Hounds were born during the 1960–2008 period. Nine litters were born of six male founders, including three of Burzan, the FF originator of the only active sire line. None of KW male founders did succeed in fixing a line. Regarding 15 dam lines established by the founder bitches, only four are still actively breeding, with the dominant FF line of Czita, with 259 litters (the FF line of Zorka had only two litters) (Figure 1). The number of litters born in 13 KW lines was significantly lower and ranged from one to seventeen (49 litters in total), and the length of the KW lines varied ranging from one to six generations (2.3 on average). The only KW founder bitch line that was more widely accepted by the breeders was Yuma Strapczyna (17 litters in 10 kennels).

A disproportionately small number of dogs and bitches born in KW dam lines were used in breeding. KW bitches were used in 32 (25.4%) kennels and only 23 mothers from 161 non-founder mothers represented these lines. Nor were KW males favoured by breeders and just five of 106 non-founder fathers were KW dogs, with a total of 15 litters. It should be also noted that the contribution of KW ancestors in the pedigrees of these five sires did not exceed 50% (range of 31–50%).

The limited breeding success of KW dogs was also reflected in the low contributions of the KW founders' genes to the gene pool of the population (Figure 2). The maximum contribution of the KW founders (18.2% in total) and the highest number of the KW founders in one period (10 from among 14 breed founders), were observed in the 1990–1994 period. The founder contributions to the gene pool of the litters born since the year 2000 are presented in details in Figure 3.

Three haplotypes were identified in the representatives of the six dam lines analysed. Haplotype PH1, which was present in the FF line of Czita,

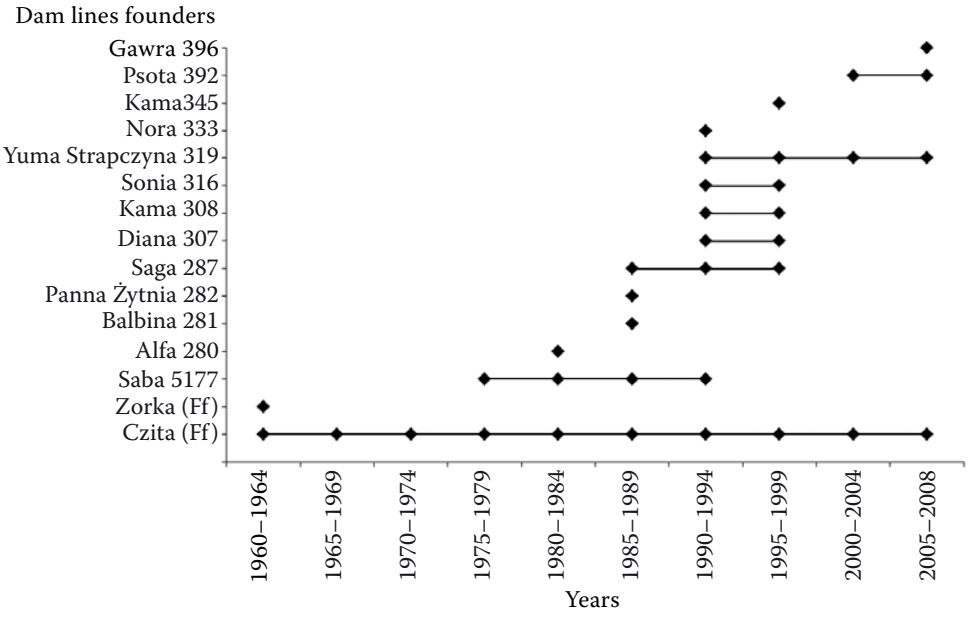

Figure 1. Breeding activity in dam lines of Polish Hounds (established by 2 FF and 13 KW female founders), observed in the four-year period within 1960–2008

Y-axis: names of the founder bitches followed by the registration number in the Introductory Book (KW). FF = first founder

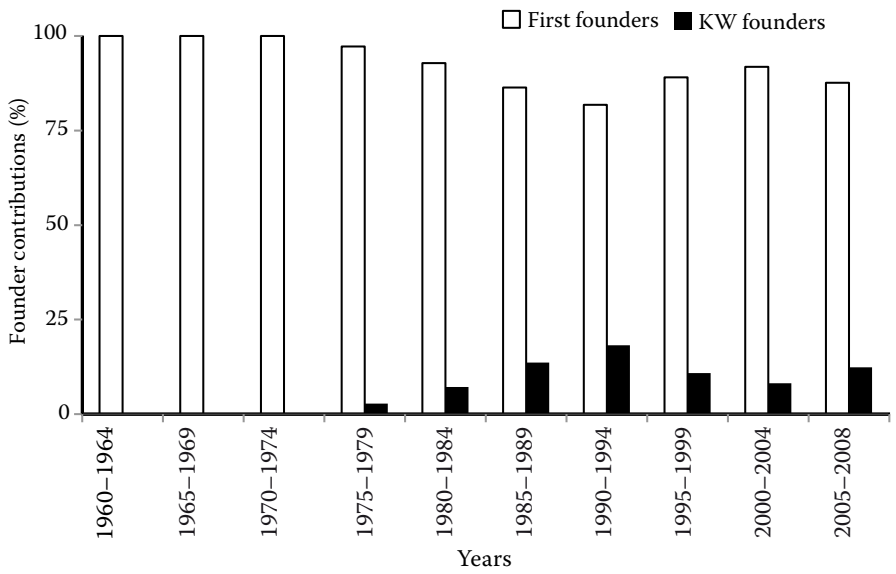

Figure 2. Contributions of 4 FF and 17 KW breed founders to the gene pool of 310 litters born within the years 1960–2008

was also found in the KW lines of Psota and Kama. Haplotype PH3 was in the KW lines of Saga and Gawra, while haplotype PH2, the most distinct, was found in the KW line of Yuma Strapczyna exclusively. Significant differences among particular haplotypes and the reference sequence were noted. The PH1 sequence differed from the reference sequence by five nucleotides (four transitions, one transversion), the PH2 sequence differed by 14 nucleotides (12 transitions, one transversion, one indel), and the PH3 sequence, which was the most similar, differed only by one transition. The sequences differed among themselves respectively by 14 (PH1 : PH2), 4 (PH1 : PH3), and 13 (PH2 : PH3) nucleotides. The comparison of the Polish Hound sequences with 55 sequences of other breeds indicated that the PH sequences belong to two clearly distinguishable haplogroups (Figure 4). Moreover, a set of sequences that were identical to PH1 and PH3 was found in other breeds. In the case of the PH1 haplotype, six identical sequences were found in mollosoid and companion dog breeds from FCI groups 2, 3, and 9. The PH3 sequence appeared to be identical to sequences of seven breeds, five of which were hunting dog breeds from different FCI groups (3, 4, 6, 7, 8). No sequences identical to PH2 were found among the sequences analysed and the most similar sequences were in the Walker Hound and Schipperke breeds which differ from PH2 haplotype by one nucleotide.

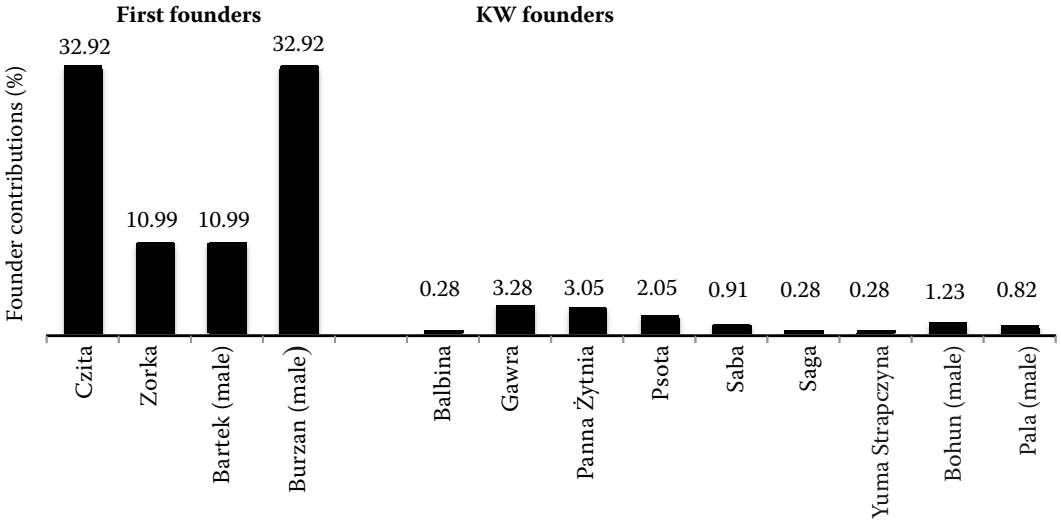

Figure 3. Founder contributions to the gene pool of 120 litters born within the years 2000–2008

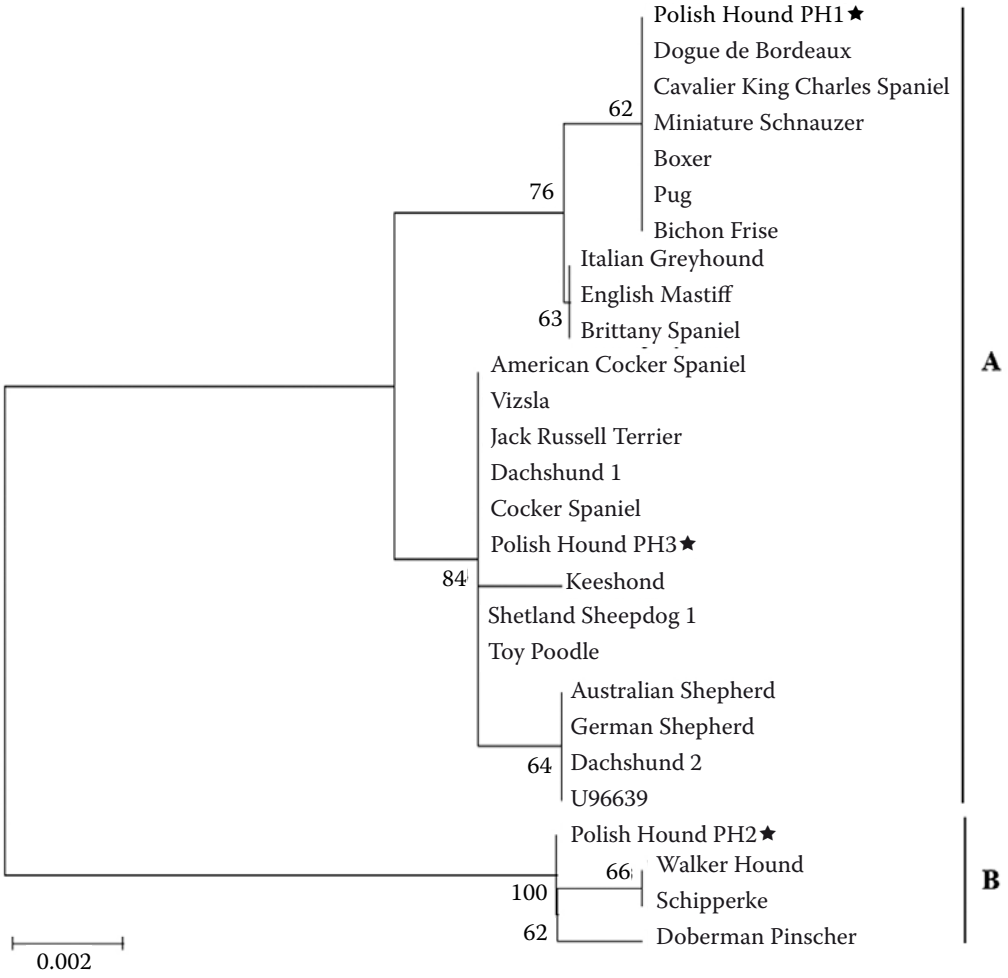

Figure 4. Relationship between Polish Hounds and the 22 most similar dog breeds from the 55 breeds analysed, shown by unrooted NJ tree of dog haplotypes based on 660 bp mtDNA control region sequences

PH1–PH3 = haplotypes identified in Polish Hounds; reference sequence GenBank accession No. U96639 included. Bootstrap support is indicated when found in at least 50% out of 1000 bootstrap replicates. Letters **A** and **B** correspond to the sequence clades determined by Savolainen et al. (2002)

DISCUSSION

The objective of the analysis was to evaluate the effectiveness of introducing new alleles from dogs of unknown origin into the gene pool of Polish Hounds. The results indicate the very limited success of new founders in relation to the number of individuals used in breeding as well as to their contributions to the gene pool. A disproportionately small number of KW bitches were used, and breeders also disapproved of using KW sires. Although the number of KW individuals was 80.9% of the total number of breed founders, their contribution to the gene pool was not even as much as 18.2% and the proportions of KW dams and sires were equal at just 14.3 and 4.7%, respectively, of the total number of breeding dogs.

The main cause of this appears to be the unsatisfactory quality of the KW dogs. According to Ściesiński (2009), the phenotype of KW dogs is frequently inconsistent with the breed standard, specifically with regard to the traits that are unacceptable in Polish Hounds (i.e. traits that suggest a relationship with the German Shepherd). Deviations from the breed standard in the KW dogs resulted in achieving worse show results and lower prices for their puppies (Głażewska, 2008). From a genetic point of view, the critical opinions of KW dog quality are not surprising. Qualifying KW founders for breeding based exclusively on their phenotypic similarity to the breed standard does not guarantee their purebred origin nor exclude the origin from other dog breeds. The results of mtDNA

analysis confirmed that the KW founders might be of distinct origin. Separate sequences in the lines of Czita, imported from Belarus in 1958, and those of Yuma Strapczyna, imported from Lithuania in 1990, were anticipated. In fact, the sequences in these lines were not only different, they also belonged to two clearly distinguishable haplogroups corresponding to clad A (PH1) and clad B (PH2) as determined by Savolainen et al. (2002). Regarding the remaining lines, two different results were possible: either the analysis would indicate sequences identical to PH1 or distinct sequences. The results of the mtDNA analysis showed that both variants were possible. The PH1 haplotype was found in the lines of Psota and Kama, meanwhile the third haplotype (PH3) was found in the lines of Saga and Gawra, which were bitches of unknown relationship and breed origin.

Taking into account the distinct origin of the KW founders, not only increased genetic diversity is expected in descendent generations, but also the appearance of phenotypes inconsistent with breed standards. While high genetic diversity is profitable for breeds, it simultaneously creates problems in dog breeding selection systems that focus on choosing individuals on the basis of their phenotypes. This highlights a paradox: increased phenotypic diversity in descendant generations is inevitable since new alleles are introduced into the gene pool by KW founders of distinct origin, but this increase is not acceptable to kennel judges or breeders. Introducing new dogs of unknown breed affiliation into PH breeding is also a financial risk that neither breeders nor buyers seeking pure-bred puppies are willing to take. Therefore, it appears there is little chance to overcome this impasse without external financial support. However, its obtaining from the state is impossible since local dog breeds are not included in any rare breeds conservation programmes.

Obviously, without breeders' acceptance, no improvement program can succeed, and changing the breeders' attitude is probably the most important issue facing PH breeding. Leroy et al. (2006), in their study of French dog breeds, also encountered problems convincing breeders to adopt certain behaviours. This is likely the main obstacle to the successful use of crossbreeding to improve the genetic condition of dog breeds. Mäki (2010), in her study on two breeds (Nova Scotia Retriever, Lancashire Heeler), proposed crosses with other breeds (NS) or with unregistered farm dogs (LS) to increase the genetic diversity of the breeds. Calboli et al. (2008) suggested relaxing breed rules to permit controlled outcrossing. Unfortunately, the case of the Polish Hounds indicates basic problems with such improvement programmes. Finally, the critical opinion of the quality of KW dogs resulted in the decision of the Polish Kennel Club to close the Introductory Book in November 2010.

The situation of the Polish Hound is a "trap of genetic impossibility": the breed has a very limited gene pool which cannot be enriched without dogs of distinct origin, but their breeding use is unacceptable for the reasons mentioned earlier. This case shows that the success of programmes for improving the genetic condition of dog breeds can only be achieved in coordination between breeders and kennel authorities, and with financing from the state.

Acknowledgement

We are grateful to the owners of Polish Hounds for their help in collecting hair samples.

REFERENCES

Björnerfeldt S., Webster M.T., Vila C. (2006): Relaxation of selective constraint on dog mitochondrial DNA following domestication. Genome Research, 16, 990–994.

Calboli F.C.F., Sampson J., Fretwell N., Balding D.J. (2008): Population structure and inbreeding from pedigree analysis of purebred dogs. Genetics, 179, 593–601.

Felsenstein J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39, 783–791.

Głażewska I. (2008): Genetic diversity in Polish Hounds estimated by pedigree analysis. Livestock Science, 2–3, 296–301.

Kim K.S., Lee S.E., Jeong H.W., Ha J.H. (1998): The complete nucleotide sequence of domestic dog (*Canis familiaris*) mitochondrial genome. Molecular Phylogenetics and Evolution, 10, 210–220.

Lacy R.C. (1989): Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. ZOO Biology, 8, 111–123.

Lacy R.C. (1998): GENES. Version 11.8. Software Package for Genetic Analysis of Studbook Data.

Leroy G., Rognon X., Varlet A., Joffrin C., Verrier E. (2006): Genetic variability in French dog breeds assessed by pedigree data. Journal of Animal Breeding and Genetics, 123, 1–9.

Leroy G., Verrier E., Meriaux J.C., Rognon X. (2009): Genetic diversity of dog breeding: within-breed diversity comparing genealogical and molecular data. Animal Genetics, 40, 323–332.

Mäki K. (2010): Population structure and genetic diversity of worldwide Nova Scotia Tolling Retriever and Lancashire Heeler dog populations. Journal of Animal Breeding and Genetics, 127, 318–326.

Oliehoek P.A., Bijma P., van der Meijden A. (2009): History and structure of the closed pedigreed population of Icelandic Sheepdogs. Genetics Selection Evolution, 41, 39, doi: 10.1186/1297-9686-41-39.

Rozen S., Skaletsky J.H. (2000): Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S. and Misener S. (eds): Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, USA, 365–386.

Savolainen P., Zhang Y.P., Luo J., Lundberg J., Leitner T. (2002): Genetic evidence for the East Asian origin of domestic dogs. Science, 298, 1610–1613.

Ściesiński K. (ed.) (2009): Polish Dog Breeds. Wydawnictwo SGGW, Warszawa, Poland. (in Polish)

Tamura K., Dudely J., Nei M., Kumar S. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24, 1596–1599.

Webb K.M., Allard M.W. (2009): Mitochondrial genome DNA analysis of the domestic dog: identifying informative SNPs outside of the control region. Journal of Forensic Science, 54, 275–288.

Wilson M.R., Polanskey D., Butler J., DiZinno J.A., Repogle J., Budowle B. (1995): Extraction, PCR amplification, and sequencing of mitochondrial DNA from human hair shafts. Biotechniques, 18, 662–669.

Received: 2011–09–21
Accepted after corrections: 2012–01–04

Corresponding Author

Dr. Iwona Głażewska, University of Gdańsk, Department of Plant Taxonomy and Nature Conservation,
Al. Piłsudskiego 46, 81-378 Gdynia, Poland
Tel. +48 585 236 306, e-mail: i.glazewska@ug.edu.pl