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RAPD-PCR Analysis of Various Goose Populations

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The aim of this study was to genetically analyse by the RAPD-PCR method four indigenous Polish goose breeds, Kartuska (Ka), Lubelska (Lu), Kielecka (Ki) and Podkarpacka (Pd), in order to determine the band-sharing frequency as well as bands characteristic of the evaluated breeds. The birds were maintained as conservative flocks, accounting for a reserve of genetic resources. A total of 102 scorable bands were obtained, their number ranging from 0 to 8, depending on one of seven primers used and the group of birds analyzed, within a mean of 3.64. For each genetic group specific bands with given primers were obtained, suggesting their potential for use as population-specific markers, especially in ex-situ conservation methods. The results also suggest that keeping endangered geese as separate flocks is relevant for their preservation.

Key words: Goose, RAPD, DNA polymorphism, PCR, conservative flocks.

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Domestic geese originating from Greylag geese (*Anser anser*) and wild Swan geese (*Anser cygnoides*) have been developed into a number of recognized breeds. Due to broad adaptability, geese can be kept in every climate as a multi-purpose animal, for meat, fine feathers and down as well as for fatty livers (Foie Gras). However, geese are not of worldwide importance as chicken and ducks are.

In Poland, one of the main geese producers in Eastern Europe, among many breeds, only the White Italian geese is being kept on a commercial scale, accounting for 50-52 per cent of total poultry products exported from Poland. The remaining Polish native breeds, despite commercial insignificance, are considered endangered and therefore they are maintained as conservative flocks, as they may be a valuable source of certain genes in further breeding programmes (ROSIŃSKI 2002). The conservative flocks were established in 1972 and now are supported through the Biological Development Fund (Ministry of Agriculture and Food Economy) (SMALEC 1991). Information including population size, performance results, special characteristics and status of most of the breeds, varieties and lines of farm animals are included in the Global Database, which is a part of the FAO Domestic Animal Diversity Information System (DAD-IS), one of the key elements of the Global Strategy for Management of Farm Animal Genetic Resources.

Goose in-situ conservation programmes, financially supported by government, maintain two reserve flocks, fifteen conservative flocks and a couple of cross-breeds (MAZANOWSKI 2001). An alternative method to the costly in-situ conservation is the ex-situ preservation of genetic resources. Cryopreservation of germ cells, like semen, ova, eggs, blastodermal cells and embryos as well as DNA from native breeds may be applicable. Reconstruction of the breeds is performed through transferring embryos or semen to surrogate mothers or, in poultry through mating of germline chimeric birds (BEDNARCZYK *et al.* 2002). In order to implement ex-situ conservation methods, genetic characterization of the breeds is indispensable as a means of molecular identification of the preserved germ cells.

The aim of this study was to genetically analyse by the RAPD-PCR method (WILLIAMS *et al.* 1990) four indigenous Polish goose breeds, maintained in conservative flocks, in order to determine band-sharing frequency as well as the bands characteristic for each breed evaluated.

Material and Methods

The breeds used in this study were four native Polish goose lines, named after the region of the country they derived from: Lubelska (Lu), Kielecka (Ki), Podkarpacka (Pd) and Kartuska (Ka). They were maintained in the Waterfowl Breeding Station at Dworzyska nr. Poznań as conservative flocks (SMALEC 1991). The blood samples (40 in all) were collected from ten males of each line. Two ml of venous blood was taken from the wing vein of each individual into tubes with EDTA as an anticoagulant. DNA was obtained using the phenol-chlorophorm-isoamyl alcohol extraction method and ethanol precipitation. The quantity and quality of DNA was determined using a spectrophotometer based on absorbency at 260 and 280 mm, respectively.

For PCRs, a set of seven 10-bp RAPD primers (Polish Academy of Sciences, Institute of Bioorganic Chemistry in Poznań, Poland) was used (Table 1). The amplified PCR products were obtained in a volume of 24.2 μl. Each PCR tube contained 1.0 μl of DNA template, 0.2μl of primer (0.25 μM), 0.5 μl of each dNTP, 0.2 μl of Taq Polymerase (Sigma-Aldrich). The reaction was buffered by the addition of 2,5 μl 10x buffer solution consisting of: 100mM TRIS-HCl pH=8,8, 500 mM KCl, 20 mM $MgCl_2$ and 1% Trixon X-100. The mixture was covered with 20 μl of mineral oil. PCR was carried out using MJ Research PTC 100 thermal cycler programmed for initial denaturation of double-stranded DNA at 95ºC (5 min) followed by 45 cycles of denaturation at 95ºC (1 min), primer annealing at 35ºC (1 min), extension at 72ºC (2 min) and final extension at 72ºC (5 min).

Table 1

Sequence of primers used for determination of genetic similarity between goose lines

Primer	Sequence (5'-3')
AB1-02	TGATCCCTGG
AB1-03	CATCCCCCTG
AB1-04	GGACTGGAGT
AB1-07	GGTGACGCAG
AB1-10	CTGCTGGGAC
AB1-26	TTTGCCCGGA
AB1-28	AGGGAACGAG

After sample amplification, the product was electrophoretically separated in horizontal, 2% agarose gel supplemented with ethidium bromide. Markers, pUC 18/Msp I (Sigma-Aldrich) and pUC 19/ Msp I (DNA Gdańsk II), were used as molecular weight standards. 10 μl of DNA samples suspended in 1 μl of bromophenol blue were placed on gels. Electrophoresis was carried out in 10x buffer at 100 V for about 80 min (APELEX ST 606 T). After developing the gels, they were observed under a transilluminator (Spectroline TC-312 A) in UV light. Gels were documented on photographs, which were archived using the Grab-it software, and analyzed using GelScan software (Kucharczyk T. E. Company).

To assess the genetic similarity between lines, RAPD patterns from mixed DNA samples were used to calculate band sharing (BS) by the formula of JEFFREYS *et al.* (1986):

$$BS = 2N_{ab}/(N_a + N_b)$$

Where N_{ab} is the number of bands common to lines a and b, and N_a and N_b are the total number of fragments scored in lines a and b, respectively.

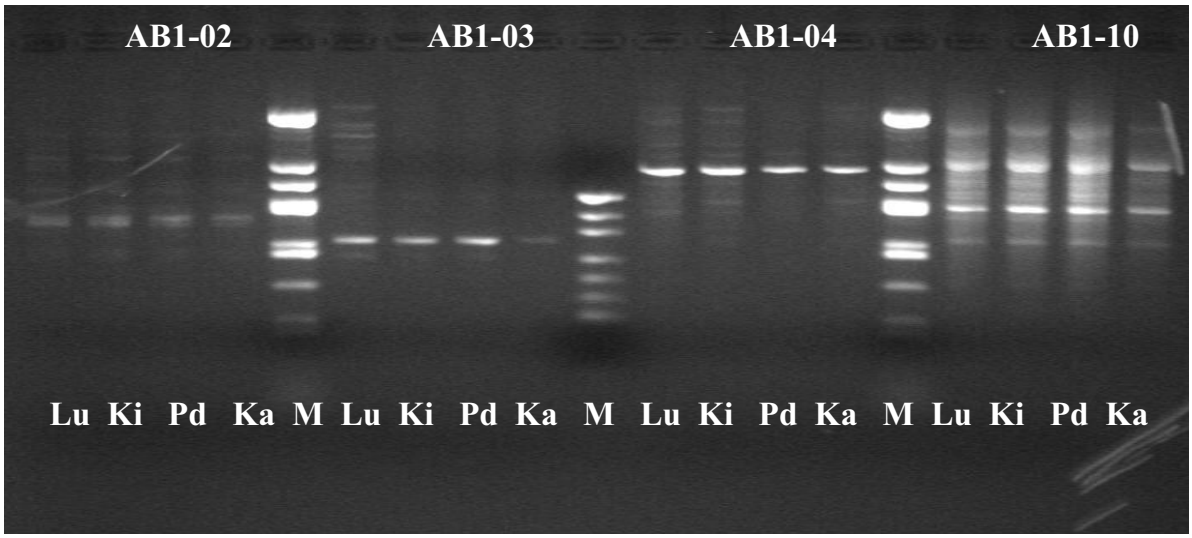

Fig. 1. Gel patterns of randomly amplified polymorphic DNA products of primers AB1-02, AB1-03 and AB1-04. The fragment patterns are identified as follows: Lane Lu-Lubelska Geese; Lane Ki-Kielecka Geese; Lane Pd-Podkarpacka Geese; Lane Ka-Kartuska Geese, Lane M-Molecular Standard.

Results and Discussion

In the present study the RAPD technique was used to assess genetic relatedness among goose lines. For this purpose samples containing ten randomly chosen genotypes were tested. Using mixed DNA samples was proven to be an effective approach when comparing among population patterns, as it helps to overcome limitation of the numbers of individuals (HILLEL *et al.* 1991). In total, the seven RAPD primers produced 102 scorable bands. The numbers of bands amplified with each primer ranged from 0 to 8, within a mean of 3.64. According to results of parallel studies, where the same RAPD primers were tested, higher values were received than reported in the goose (BEDNARCZYK *et al.* 2002) but lower than in the hen (BEDNARCZYK & SIWEK 1999). The results obtained were satisfactory but showed potential for designing primers more specific for geese in order to reveal higher DNA polymorphism, as did HORN *et al.* (1996) in the Magpie Goose, where he yielded 6.2 polymorphic bands per primer.

The size of scorable amplified fragment ranged from 200 to 1500 bp. A typical gel is shown in Figure 1. Some of the primers produced no amplification products and for some lines a monomorphic product was obtained. However, each of the primers used was effective in amplifying specific bands for a given line. According to reports comparing chickens and turkeys, where only 10 out of 60 primers produced specific bands (SMITH *et al.* 1996), the results of the present study were adequate. From 1 or 2 up to 13 specific bands per goose line were obtained. The specific bands described may serve as molecular markers and be the basis for designing SCAR markers (sequence characterized amplified region).

Analysis of scorable bands resulted in estimates of genetic similarity between populations, which was based on BS (Band Sharing) frequency. Among all the lines, the BS coefficient had a value of 0.638, from 0.37 to 0.89 (Table 2). The average BS value across 7 primers for pairwise comparisons of lines ranged from 0,37 for Ka vs. Lu to 0,89 for Ki vs. Lu. For homogenous populations a value of 1 is expected. In the case of relatedness between Pd and Ki lines, the BS was also very high, as it amounted to a value of 0,75. The results obtained are consistent to a previous study of blood protein polymorphism data, where higher polymorphism was observed between northern (Ka) and southern (Ki, Lu, Pd) groups of geese (SMALEC 1991). Also, an evaluation of Polish native geese by DNA fingerprinting showed a high diversity of Ka group when compared to others (ZAWADZKA 1999).

Table 2

Genetic similarity (Band Sharing coefficient) between goose lines

Lines	Lu	Ki	Pd	Ka
Lu		0.89	0.56	0.37
Ki			0.75	0.62
Pd				0.74
Mean	0.59	0.68	0.68	0.60
				0.638

The study revealed that the level of genetic relatedness among the groups tested has justified further keeping as separate flocks and preserving valuable genetic resources. Besides, several specific DNA fragments described may facilitate identification of genotypes, and as follows, may be used in germplasm conservation.

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