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Genetic diversity of pheasants from natural habitat and farm breeding in Eastern Poland

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The present model of wildlife management in relationship to pheasants is based on closed breeding and insertion of grown birds into the hunting grounds. The survival rate of pheasants from farm breeding does not exceed 10%. One can pose a hypothesis about the genetic determination of survivability of pheasants originating from farm breeding. A study of the DNA polymorphism of two groups of pheasants was performed. The first group comprised birds living in a natural habitat in Central-Eastern Poland, where no farm-bred pheasants had been inserted for seven years and where wildlife management was focused on the formation of good habitat conditions, an optimum nutritional base, and low-level shooting of cocks. The second group was of birds bred on a farm and from this farm, pheasants were inserted within the area of Central-Eastern Poland. Their DNA was isolated from their feathers. Amplification of fragments of DNA was with the random amplified polymorphic DNA polymrease chain reactrion (RAPD-PCR) method. Pheasants living in the natural environment were characterised by greater polymorphism. The original source of the pheasants living in the natural habitat is the farm, and the present genetic variation between the two groups of birds can be interpreted as an effect of natural selection.

Key words: Common pheasant (*Phasianus colchicus*), genetic distance, genetic polymorphism, genetic similarities, genetic variation, Random Amplified Polymorphic DNA.

INTRODUCTION

Pheasants have been present in Central Europe for about 600 years and for around 150 years have been the object of hunts. The contemporary model of wildlife management with relation to pheasants is based on closed breeding and insertion of grown birds in the hunting grounds. In Poland, the annual production of pheasants in closed breeding farms is over 300 thousand individuals. The survival rate of pheasants from farm breeding does not exceed 10%. During the pheasant hunting season, the highest percentage of cocks from insertions was recorded in November, while in February, the pheasants shot included cocks that had not originated from the last insertions (Czyżowski, 2000). The model of wildlife management with respect to pheasants in Poland accepts the insertion of birds from closed breeding, but also supports exploitation of populations in areas where no insertion from closed breeding is performed. The release of birds from closed breeding is followed by a high rate of mortality, but a certain small percentage of the birds survive. In Central Poland, one cock managed to survive seven years in the natural habitat after it had been released from breeding in a closed farm (Dziedzic,

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Figure 1. The map showing the research area of pheasant in Poland.

unpublished). One can pose a hypothesis about the genetic determination of survivability of pheasants originating from farm breeding. The low survival of farm-bred pheasants may be caused by difficulties in adapting to living in the wild, plus high predation level, hunting pressure and environmental conditions.

According to Weigend and Romanov, random amplified fragments of DNA (RAPD) are among the most frequently applied methods for the estimation of biodiversity (Weigend and Romanov, 2001). Described for the first time by Williams et al. (1990), it is a proven technique of genome analysis that, apart from the identification of genotypes, estimates the occurrence of genetic diversity and affinity among them. In this study, we utilised the random amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Nusser et al., 1996; Khaliq et al., 2010) in order to estimate the genetic diversity of the pheasant populations (*Phasianus colchicus*). The study of polymorphism with this method does not require preliminary information on the sequence of the analysed DNA and the exact primer sequence (Grosberg et al., 1996).

The possibility of survivable sampling of material for analyses, with no interference whatsoever, and isolation of genome DNA from the urine, faeces or feathers, the ease of performance of the analysis and rapid verification of results, make RAPD one of the most useful techniques for the genetic estimation of populations (Backeljau et al., 1995). The method permits the identification of genetic diversity without knowledge of preliminary information about the sequence of the analysed DNA. RAPD-PCR has found an extensive practical application in studies on closed-bred and wild-living populations of example crocodiles (Yau et al., 2002), insects (Moya et al., 2001) including bird species (Semenova et al., 1996; Giesel et al., 1997; Dolmatova et al., 2000; Muhammad et al., 2010; Riaz et al., 2011) and many other organisms.

The objective of the study presented here was to compare pheasants from a farm-bred environment and from wild-living, where no insertions had been performed over a long period of time, which appears to be an interesting problem in both the research and the practical aspects, using genetic molecular analyses as a comparison criterion with a high level of credibility.

MATERIALS AND METHODS

Study sites and sampling

The study was conducted based on pheasants living in the area of the Lublin Upland situated in Central-Eastern Poland (Figure 1). The research material was pheasants living in a natural habitat and birds bred on a farm. The number of pheasants on the Lublin region is about 40 thousand, and in Poland about 435 thousand. 45 cocks

were acquired from each of the two locations. The pheasants from the natural habitat were acquired through shooting, from a hunting district with a surface area of around 62,000 ha (62 km^2). That area is characterised by a ca. 9% share of forests, and a considerable percentage of horticultural and orchard cultures. Within this area, no pheasant insertions had been performed for seven years, while wildlife management relating to pheasants was focused on ensuring good nutritional and shielding conditions, and the annual shot of cocks was about 50. The comparative group of pheasants were cocks from a pheasant breeding farm situated in the Lublin Province. All grown birds from the farm were destined for hunting districts of the Lublin Province. All material for the study was acquired in December of the same year. The pheasants from farm-breeding were designated as (A) and those from the natural habitat as (B).

Laboratory procedures

From each population, 45 feathers were collected. Their DNA was isolated from their feathers using a commercial QIAamp® Mini Kit (from Nails and Hairs), with modifications consisting in the extension of incubation at 56°C to 24 h. Residues of connecting tissue of the papilla inside the pith of the feather quill were scraped off into one test tube. Amplification of the DNA fragments with the RAPD-PCR method was conducted on the basis of two pairs of Proligo starters with the following sequence: pair I: 1) ABI-01 5'GTTTCGCTCC3', 2. ABI-02 5'TGACCCTGG3'; pair II: 1.PRZ-01 5'ATGAGGTTA3', 2) PRZ-02 5'ATGACGTTGA3'. Total volume of each PCR reaction was 25 µl containing 50 ng/µl of DNA, 2.5 mM $Mgcl_2$, 10 x PCR buffer, 2.5 mM of each dNTP, 1 U.I. of Taq polymerase and 50 ng/µl of each primer in the first reaction gTTTCgCTCC and TgACCCTgg, and in the second reaction, ATgAggTTa and ATgACgTTgA. The reaction was conducted using the following thermal cycle: initial denaturation at 94°C for 5 min, followed by 46 cycles, each of which included three phases: denaturation at 94°C for 1 min, starter addition at 36°C for 2 min, elongation at 72°C for 1 min. The final stage of the programme was final elongation at 72°C for 10 min. The PCR-RAPD reaction was conducted in a MJ Research PTC-225 Peltier Thermal Cycler.

After the amplification, the product was separated with the electrophoretic method on 1.8% agarose gel with an admixture of ethidium bromide at 80 V for 210 min. Tagging with ethidium bromide as an intercalating agent consists in its excitation with light with a wavelength of 302 nm. The molecular weight standard was the marker GeneRuler™ 50bp DNA Ladder, from Fermentas, that contained 13 visible fragments with the following lengths: 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 and 1031 base pairs. Electrophoresis was conducted in Tris borate EDTA (TBE) buffer using the apparatus Sub-cell® GT, BIORAD. Data acquisition was performed using a CCD system composed of a CCD camera, a dark room, a UV transilluminator, Syngen BTX - 26.M. Data processing was made using the Scion Image software.

Data analysis

Documentation of the study was photographs of the gel in which the separation by electro-phoresis took place. The resulting matrix was imported into different programmes for data elaboration. Each locus was treated as a two-allele system. Each amplified band was treated as a dominant allele and scored as 1 and the absence of allele was scored as 0. Analysis of the results of electrophoresis was made using the PopGen (Yeh et al., 1999). A single allelic ladder was created for all birds studied and then every single individual was compared to it. The presence/absence of each band was scored by analysing the electro-phoretic profile obtained for each specimen. The Nei's average gene diversity (H) and the Shannon Index (S) were used for statistical analysis (Lewontin, 1972; Nei, 1973). The genetic identity and genetic distance (Nei, 1972, 1978) was calculated by PopGen software (V. 1.31). A genetic similarity dendrogram among the specimens were constructed by using the coefficient and the unweighted pair group method, with an arithmetic mean (UPGMA) cluster analysis algorithm in the computer program (MEGA4) (Tamura et al. 2007).

RESULTS

For the first pair of starters: (1. ABI-01 5'GTTTCGCTCC3', 2. ABI-02 5'TGACCCTGG3') results obtained indicated that polymorphism of DNA from farm-bred and wild-living pheasants. Nine phenotypes were recorded, out of which five different ones were obtained for the farm-bred birds (A) and seven among the wild-living birds (B) (Figure 2).

Types observed phenotypes (A-I) for farm-bred and wild-living pheasant are presented on Figure 1. One band was characteristic of individuals denoted as A, two bands of G, three bandsof H. Four bands were obtained for birds denoted as C and F, six bands for B and D, and the largest number of bands, eight bands was found for individuals denoted as I.

For the phenotypes from farm-bred pheasants (using the first pair of starters), five different (A, B, C, D and E) ones were obtained, and seven for wild-living pheasant (C, D, E, F, G, H and I) (Figure 3).

The level of genetic diversity in the two populations were H = 0.339 and S = 0.4963 (Table 1) in farm-bred pheasants, and H = 0.253 and S = 0.3643 (Table 1) in wild-living pheasants. High levels of results of genetic diversity were indicated in this species.

Table 2 presents the genetic similarities and genetic distances between farm-bred pheasants' and wild-living pheasants' genotypes using the first pair of starters. The genetic distance among the two populations was 0.6435 and genetic identity was 0.5219 (Table 2).

The dendrogram (Figure 4) illustrates the genetic distances between the phenotypes studied using the first pair of starter. Based on the dendrogram, it was determined that the greatest distances were noted between individuals from the farm-bred population and the wild-living population. The smallest distances were noted between individuals from the same population.

The second reaction (pair of starters: 1.PRZ-01 5'ATGAGGTTA3', 2. PRZ-02 5'ATGACGTTGA3') also revealed the occurrence of one to eight bands located at the height of around 250 to about 1050 bp (Figure 5). On the basis of analysis of the results obtained it was determined that there occurred a characteristic band at the height of about 250 bp. Within the numbers of bands observed, the numbers of phenotypes determined by the bands were studied. A single band was characteristic of individuals denoted as C, four bands as D and E, five bands as B, six bands as A, while the highest number of bands eight was recorded for individuals denoted as F. Using the second pair of starters, six different phenotypes were obtained, three of which were A, B and C among

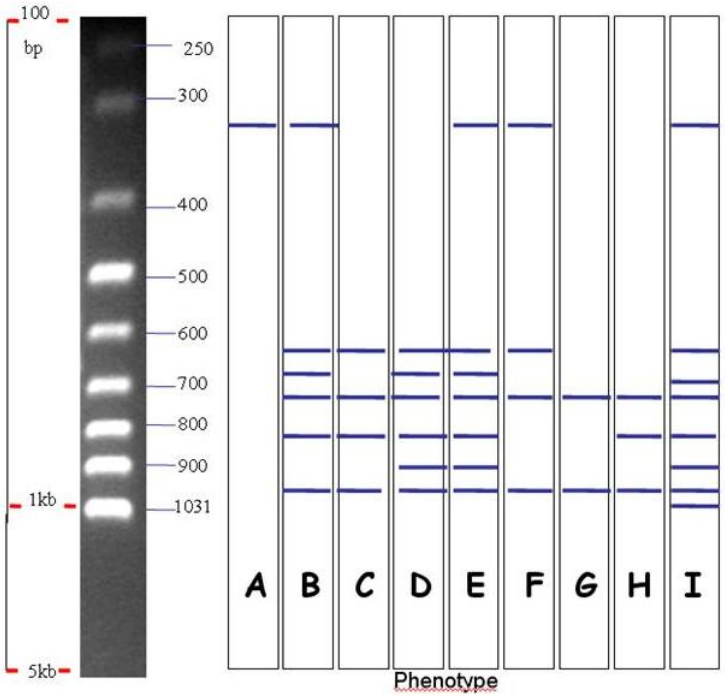

Figure 2. Kinds of pheasant phenotype identified using the first pair of starters with the RAPD-PDR method.

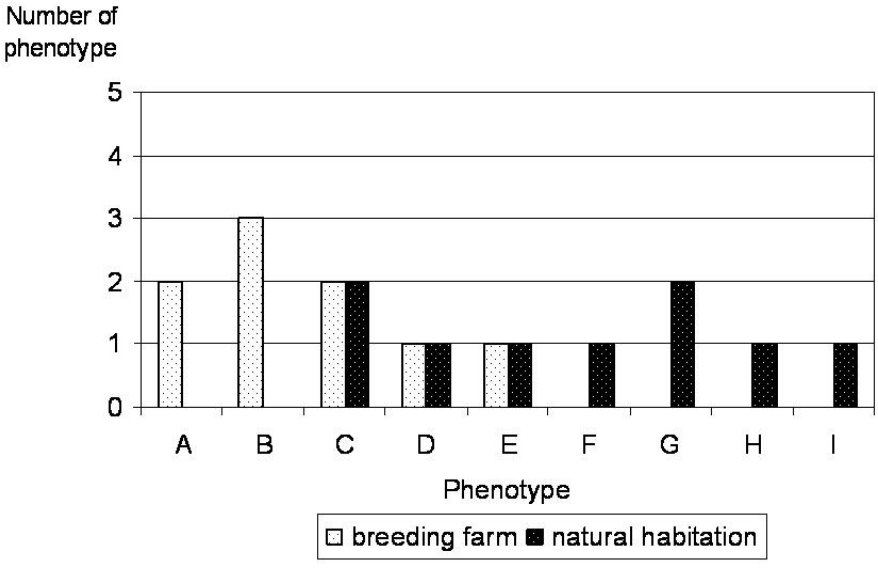

Figure 3. Numbers of particular phenotype obtained after electrophoresis with the use of the first pair of starters with the RAPD-PDR method.

Table 1. Genetic variation within population.

Population	Nt	Np	P	H	S
Farm-bred pheasants	8	8	100.00	0.339	0.4963
Wild-living pheasants	8	7	87.50	0.253	0.3643

Nt, Number of total loci; Np, number of polymorphic loci; P, percentage of polymorphic loci; H, Nei's (1973) gene diversity; S, Shannon's Information index.

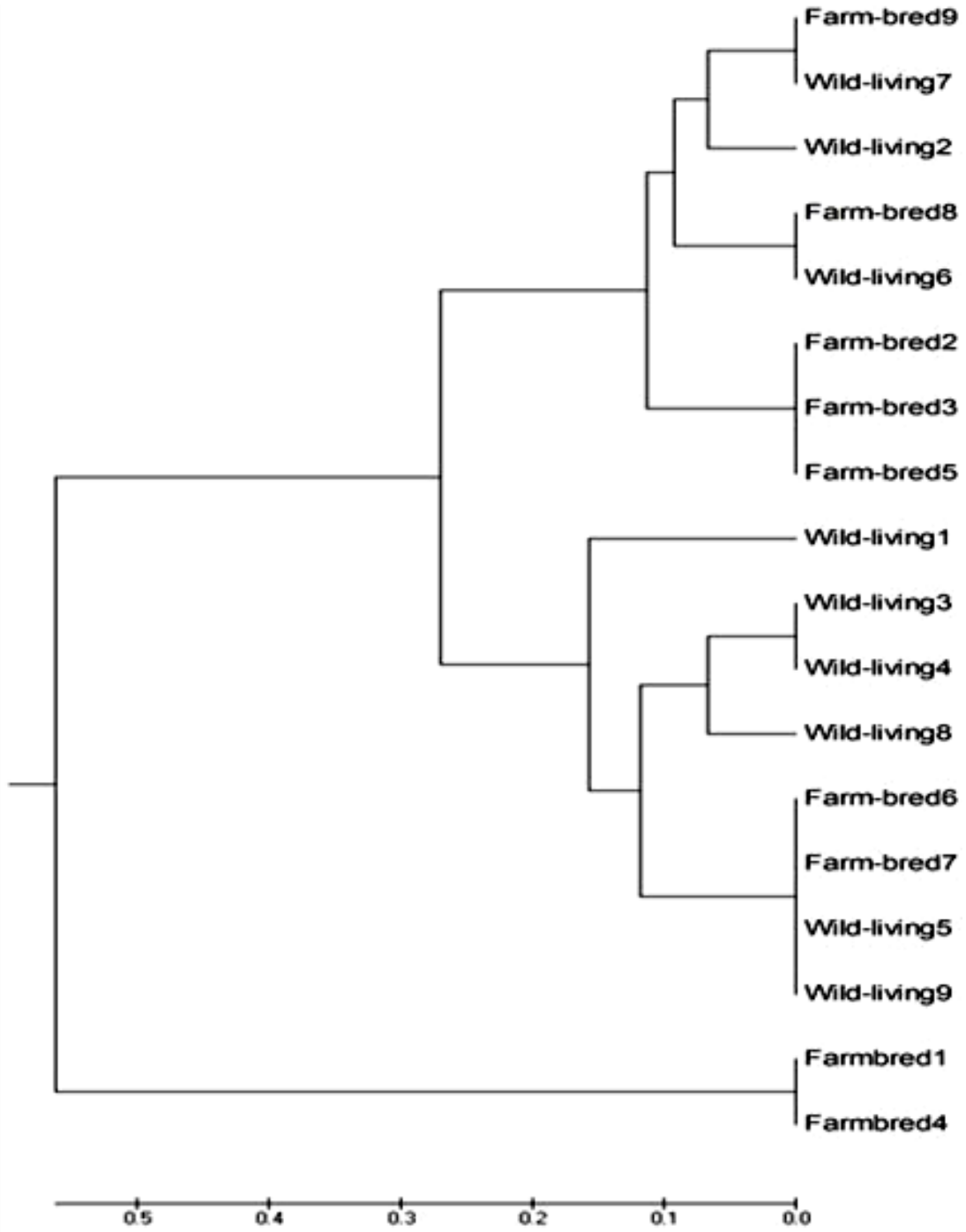

Figure 4. Dendrogram of the Nei genetic (Nei, 1978) distances between the studied pheasant genotype with the use the first pair of starters (UPGMA method).

pheasants from farm-breeding and five among the wild-living birds of B, C, D, E and F (Figure 6).

Table 3 presents the genetic similarities and genetic distances between farm-bred pheasants' and wild-living pheasants' genotypes using the second pair of starters. The genetic distance among the two populations was 0.7746 and genetic identity was 0.3554 (Table 3).

The dendrogram (Figure 7) illustrates the genetic distances between the phenotype studied using the second pair of starters. On the basis of the dendrogram, it was determined that the greatest distances were noted between individuals from the farm-bred population and those from the wild-living population. The least distances were noted between individuals from the same population.

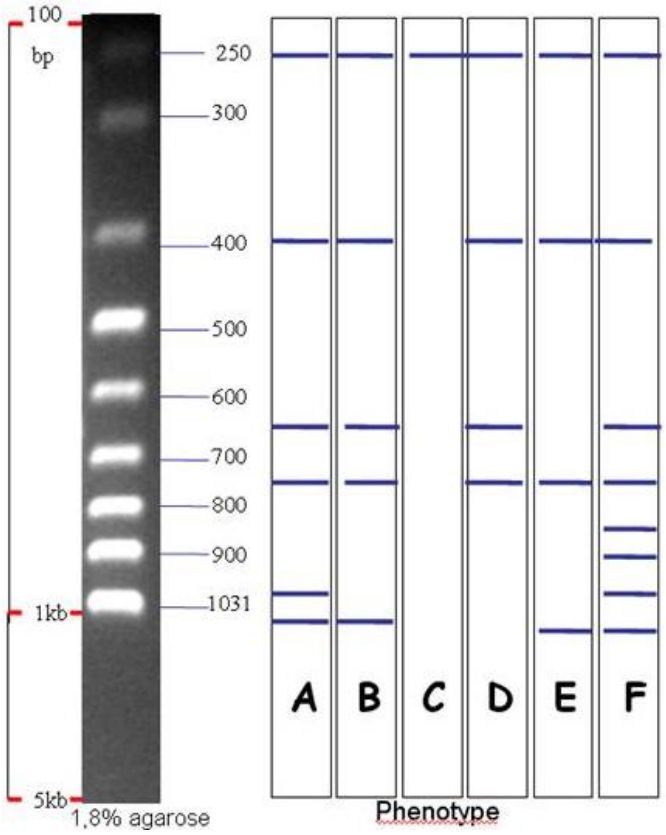

Figure 5. Kinds of pheasant phenotype determined with the use of the second pair of starters with the RAPD-PDR method

Table 2. Genetic similarities (above the diagonal) and Nei genetic distances (Nei, 1978) (below the diagonal) between farm-bred pheasants and wild-living pheasants genotypes using the first pair of starters.

Population	Farm-bred pheasant	Wild-living pheasant
Farm-bred pheasants	*****	0.6435
Wild-living pheasants	0.5219	*****

Table 3. Genetic similarities (above the diagonal) and Nei genetic distances (below the diagonal) between farm-bred pheasants and wild-living pheasants genotypes using the second pair of starters.

Population	Farm-bred pheasant	Wild-living pheasant
Farm-bred pheasants	*****	0.7746
Wild-living pheasants	0.3554	*****

DISCUSSION

The objective of this study was to analyse the genetic of variation between two populations of *P. colchicus*. The first came from a breeding farm and the second was from a natural habitat.

A team of American researchers applied the RAPD technique in a study on the genetic diversity of *P. colchicus* in Iowa (United States) and found a high level of applicability of the technique for the study of genetic diversity of *P. Colchicus* (Giesel et al., 1997). However, a Pakistani team rated differentiated genetic of diversity *Acridotheres tristis* species revealed by RAPD-PCR (Imtiaz et al., 2011).

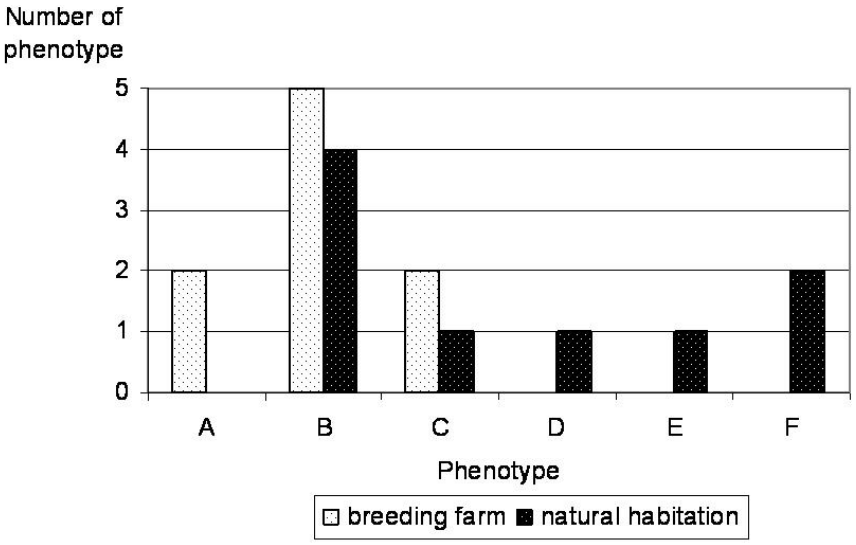

Figure 6. Numbers of particular phenotype obtained during electrophoresis with the use of the second pair of starters with the RAPD-PDR method.

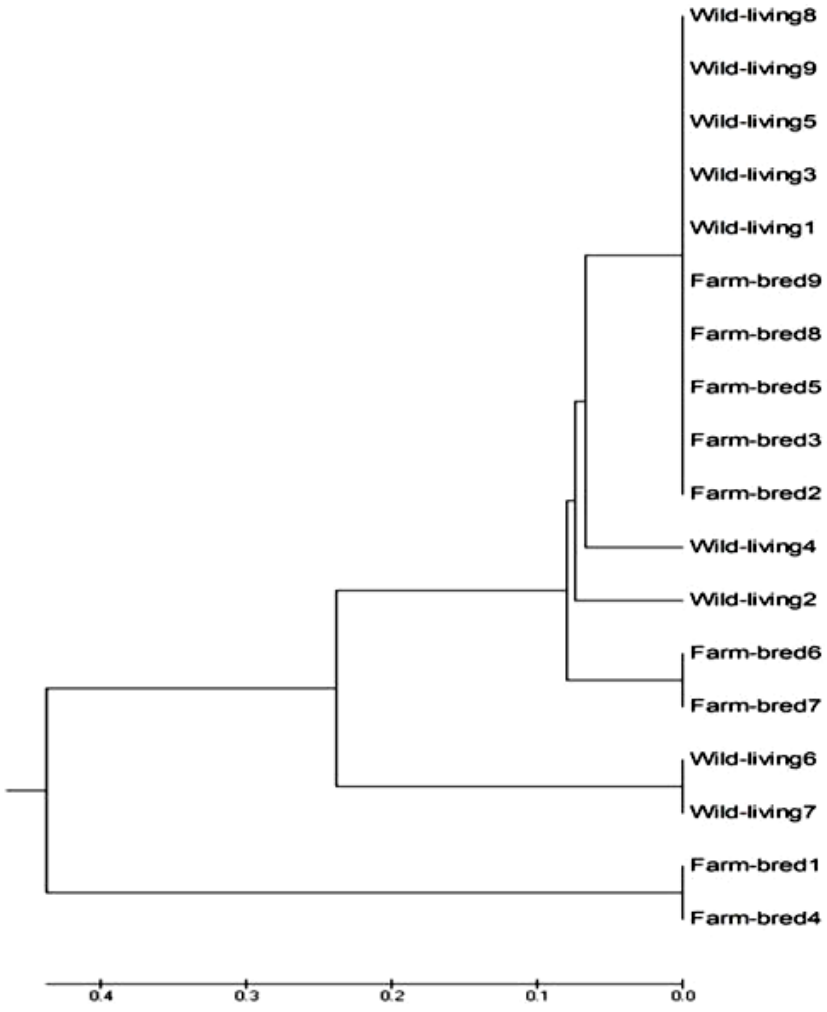

Figure 7. Dendrogram of the Nei genetic (Nei, 1978) between the studied pheasant genotype with the use the second pair of starters (UPGMA method).

In *P. colchicus*, the polymorphism was quite high for the first pair of starters (P=100.00%), and lower for the second pair of starters (P=87.50%) (Table 1); a result comparable with that obtained from the local population of the Manchurian pheasant (*P. colchicus*) while applying RAPD (P = 79.4%) (Kulikova et al., 2002); also wild Iran and captive Azerbaijan Pheasant populations (P = 86.70%) (Zarringhabaie et al., 2012). However, Muhammad et al. (2010) for Prinia (*Prinia burnesii*) obtained percentage of polymorphic loci from 48.11 to 93.07 (Muhammad et al., 2010). Different authors using RAPD markers reported either low (P = 25) for red-cockaded woodpecker *Picoides borealis* (Haig et al., 1994), light-footed clapper rail, (Nusser et al., 1996) *Acridotheres tristis* (Imtiaz et al., 2011), or high for carrion *Corvus corone*, jungle crows *Corvus macrorhynchos* (Spiridonova et al., 2003), poultry (Gryzińska, 2008) levels of genetic diversity in avian species.

Genetic distance is another parameter providing important insight into the differentiation among populations. The genetic distance between farm breeding and natural habitat was similar among the first and second pairs of primers (Figures 3 and 6). The greater value of the Nei's genetic distance (1978) was disclosed between pheasants from farm-breeding and wild-living using the second pair of starters - D = 0.7746 (Tables 2 and 3).

Pheasants living in the natural habitat, with no individuals inserted from the farm, were characterised by stronger polymorphism, which was manifest in a greater number of phenotypes. For the first pair of starters, the number of phenotypes specific only for pheasants from the natural habitat was twice as high as for those from farm breeding. For the second pair of starters, the difference was three-fold. The pheasants living in the natural habitat originally came from farm breeding.

High polymorphism and gene diversity of pheasants in natural habitat indicates good signs of rich genetic. The results concerning the genetic structure of the two populations of pheasants have implications from the conservation viewpoint. It is a well known fact that understanding the pattern of genetic variation is a critical step to assess species' fitness and to plan a conservation strategy (Reed et al., 2002). This study will help in the future conservation plans of this species in Poland.

The study reveals the occurrence of DNA polymorphism in the case of pheasants. Within the material studied, there occurred from between one to eight bands of different lengths for the two pairs of starters. On the basis of the material collected, we can state the presence of certain characteristic bands that occur in most of the farm-bred and wild-living individuals studied. In the two RAPD-PCR reactions performed with different pairs of starters, similar results were obtained.

Based on the analyses performed within this study, we can state that the method can be successfully applied for the determination of differences between various populations of animals belonging to a single species.

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