EVALUATION OF GENETIC SIMILARITY BETWEEN WHITE AND GREY VARIETIES OF GUINEA FOWL (*NUMIDA MELEAGRIS*)

OCENA PODOBIEŃSTWA GENETYCZNEGO MIĘDZY ODMIANĄ BIAŁĄ I SZARĄ PERLIC (*NUMIDA MELEAGRIS*)

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ABSTRACT

The aim of the study was to determine DNA polymorphism between and within white and grey varieties of guinea fowl. At the end of 12 weeks of age, blood was sampled from 13 white and 16 grey guinea fowl, and DNA was isolated. Genetic similarity between the birds was evaluated using RAPD-PCR technique. PCR with five primers was performed in birds studied. The largest number of bands appeared after electrophoresis with AB1-05 and AB1-09 primers, and the smallest number when AB1-08 primer was used. Genetic similarity between the white and grey varieties of guinea fowl, determined as the mean for the primers used, was 0.97. The coefficient of genetic similarity averaged 0.65 within white variety and 0.64 within grey variety.

Keywords: guinea fowl, genetic similarity, polymorphism, RAPD-PCR

STRESZCZENIE

Celem badań było określenie polimorfizmu DNA między perlicami odmiany białej i szarej oraz między osobnikami w obrębie danej odmiany. Z upływem 12 tygodnia życia perlic pobrano krew od 13 perlic białych i 16 szarych, a następnie wyizolowano DNA. Oceniono podobieństwo genetyczne między perlicami z użyciem techniki RAPD - PCR. U wszystkich badanych osobników przeprowadzono reakcję PCR z pięcioma wybranymi starterami. Najwięcej prążków uzyskano po elektroforezie dzięki starterom AB1-05 oraz AB1-09, natomiast najmniej przy użyciu AB1-08. Podobieństwo genetyczne między perlicami odmiany białej i szarej oznaczone jako średnia dla użytych starterów wynosiło 0,97. Średnia wartość współczynnika podobieństwa genetycznego w obrębie odmiany białej perlic wynosiła 0,65 a dla perlic odmiany szarej 0,64.

Słowa kluczowe: perlice, podobieństwo genetyczne, polimorfizm, RAPD-PCR

DETAILED ABSTRACT

Ocenie poddano 13 perlic białych i 16 szarych. Ptaki utrzymywano w budynku zamkniętym o regulowanych parametrach środowiska, w kojcach na słomie. Perlice żywiono *ad libitum* przemysłowymi mieszankami paszowymi zgodnie z polskimi normami żywienia perlic rzeźnych. Od każdego ptaka w wieku 12 tygodni pobrano krew z żyły skrzydłowej do probówek zawierających EDTA (antykoagulant) i przechowywano w temperaturze -20°C. Ocenę polimorfizmu DNA perlic przeprowadzono metodą RAPD – PCR. Średnie podobieństwo genetyczne między perlicami białymi i szarymi, oznaczone jako średnia dla użytych starterów było duże i wyniosło 0,97. Średnia wartość F dla perlic białych wynosiła 0,65 a dla perlic szarych 0,64. Większe podobieństwo genetyczne między badanymi odmianami perlic, niż w obrębie danej odmiany mogło wynikać z tego, że ptaki obu ocenianych odmian pochodziły z tego samego stada wyjściowego. Mniejsze podobieństwo genetyczne w obrębie odmiany związane było prawdopodobnie z większą zmiennością ocenianych osobników oraz uśrednianiem wyników otrzymanych przy użyciu zastosowanych starterów.

INTRODUCTION

Genetic variation has been investigated in different avian species around the world to determine the degree of relatedness or to identify genes responsible for traits of interest.

The development of molecular techniques has created new possibilities for genetic improvement of animals. Based mainly on DNA markers (RFLP, RAPD, VNTR, SSR, CR1-PCR), they enable the polymorphism of DNA fragments or whole genomes to be studied. RAPD (Randomly Amplified Polymorphic DNA) is a simple and rapid technique for determining relatedness and for identifying genes responsible for avian traits of interest. The RAPD (RAPD-PCR) method has been repeatedly used to study variation (or similarity) in populations of chickens (Ali, et al., 2003, Dehghanzadeh, et al., 2009, Smith, et al., 1996, Sharma, et al., 2001, Singh and Sharma, 2002, Semenova, et al., 2002), turkeys (Smith, et al., 1996), ducks (Dolmatova, et al., 2000a, b) and geese (Bednarczyk, et al., 2002, Lisowski, et al., 2008, Maciuszonek, et al., 2005, Zhang, et al., 2002, Xiao, et al., 2004). Few studies have dealt with genetic similarity (or polymorphism) of DNA markers in guinea fowl (Nahashon, et al., 2010, Sharma, et al., 1998, Sharma and Dhama, 2007).

Based on RAPD markers, Sharma, et al. (1998) found low levels of genetic variation within and between lavender, pearl (wild type) and white helmeted varieties of guinea fowl raised in India, which was probably due to the small size of the founder populations and the fact that they were bred without selective breeding for many years.

The improvement of guinea fowl meat production efficiency provided the basis for genetic improvement of this species in many countries. Due to the exquisite taste and nutritional value of their meat, guinea fowl are a valuable though undervalued

species of gallinaceous poultry. In Poland, guinea fowl farming was popular in the 1970s and 1980s, when guinea fowl breeding and multiplication farms were established. Live birds were purchased at buying stations and guinea fowl carcasses were exported. Today, however, there are no organized breeding and reproduction programmes for guinea fowl, which are mainly kept under backyard systems in Poland (Świerczewska, et al. 1999). A better understanding of different guinea fowl varieties may help to increase their popularity in Poland.
The aim of the study was to determine genetic similarity within and between white and grey varieties of guinea fowl.

MATERIAL AND METHODS

The analyses were performed at the Laboratory of Molecular Genetics of the Department of Poultry Breeding at the University of Technology and Life Sciences in Bydgoszcz. Blood from 13 white guinea fowl and 16 grey guinea fowl was investigated. Birds were kept in a confined facility (experimental farm of the Department of Poultry Breeding of the University of Technology and Life Sciences) under controlled environment conditions. Birds were penned on litter and fed commercial feed mixtures *ad libitum* in accordance with Polish nutrient requirements of broiler guinea fowl. At the end of 12 weeks of age, blood was collected from the wing vein in the arm near the elbow joint (Figure 1) into tubes with EDTA anticoagulant and stored at -20°C. DNA polymorphism in guinea fowl was analysed by RAPD-PCR.

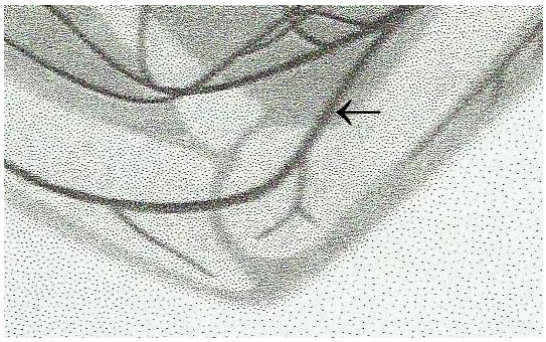

Figure 1 . Site at which blood was sampled from the wing vein
Rysunek 1. Miejsce pobrania prób krwi z żyły skrzydłowej

DNA for the analysis was isolated using a Master Pure™ DNA Purification Kit for Blood Contents (Epicentra) according to the manufacturer's instructions. DNA concentration and quality was then tested by agarose gel electrophoresis and using the spectrophotometric method (Perkin-Elmer, Lambda 25).
Twelve 10-nucleotide fragments (AB1 – 01, AB1 – 02, AB1 – 03, AB1 – 04, AB1 – 05, AB1 – 06, AB1 – 07, AB1 – 08, AB1 – 09, AB1 – 10, AB1 – 26, AB1 – 28) were tested to choose the optimum primers. Five 10-nucleotide primers (Sigma) were used for DNA amplification.

The analysed DNA was amplified in a T Gradient thermocycler (Biometra). Initial denaturation double-stranded DNA was carried out at 95°C for 5 min. This was followed by 45 cycles: 95°C for 1 min (short denaturation), 35°C for 1 min (primer annealing), 72°C for 2 min (DNA elongation). Complementary strands were synthesized at 72°C for 5 min.

PCR reaction mixture contained: DNA 25 ng, primer 100 pmol, 1x PCR buffer (75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 0.01% Tween20), $MgCl_2$ 2.5 mM, dNTP 0.2 mM, Taq polymerase (Fermentas) 1 unit. Total reaction volume was 25 μl.

PCR products were separated on 2% agarose gel with ethidium bromide, in 1x TBE buffer.

The Gene Ruler™ 100 bp DNA Ladder marker (100 – 3000 bp) (Fermentas) was added to each group of separated products. In all samples, 2 μl of Blue/Orange Loading Dye (Promega) was added to 10 μl of the product. Electrophoresis was performed at 100V power supply (Stabnap 200). Electrophoresis was about 90 min long. Gel was visualized on a Herolab UVT-20M transilluminator. Photographs were archived using the LightBIS system (Bioimaging Systems).

The fragments amplified in PCR reaction were used to calculate genetic similarity (F) between the white and grey guinea fowl, using the formula of Nei and Li (1979):

$$F = 2 \times N_{xy} / (N_x + N_y)$$

where:

N_{xy} is the number of PCR products shared by the groups of white (x) and grey (y) guinea fowl compared

N_x is the number of PCR products for the x group, and

N_y is the number of PCR products for the y group.

The numerical data were analysed statistically. Arithmetic means of the analysed traits were calculated using Excel software.

RESULTS AND DISCUSSION

PCR reaction with the five selected primers was performed in all guinea fowl of both colour varieties (Table 1). The number of bands common to both varieties was determined from the bands obtained as a result of electrophoresis (Figure 2).

Table 1. Sequences of the primers used.
Tabela 1. Sekwencje użytych starterów

Primer	sequence (5’ – 3’)
AB1 – 01	GTTTCGCTCC
AB1 – 03	CATCCCCCTG
AB1 – 05	TGCGCCCTTG
AB1 – 08	GTCCACACGG
AB1 – 09	TGGGGGACTC

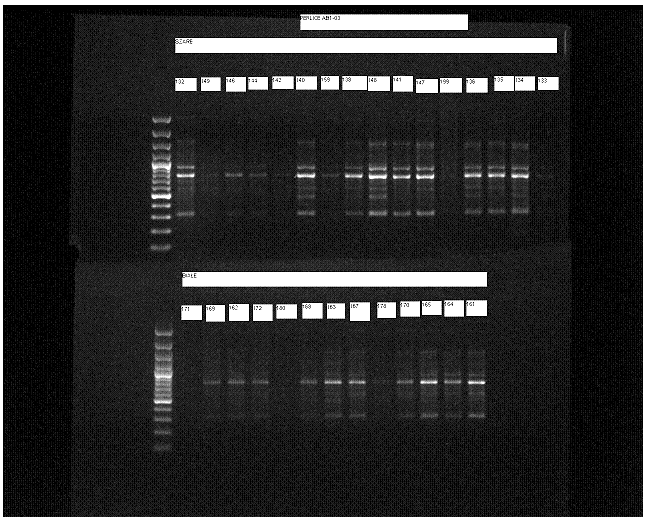

Figure 2. Electrophoretic pattern of genomic DNA amplification in white and grey guinea fowl using AB1 – 09 primer
Rysunek 2. Elektroforetyczny obraz amplifikacji genomowej DNA perlic białych i szarych ze starterem AB1 – 09

The number of PCR products for each primer and guinea fowl variety is given in Table 2. The largest number of bands was obtained with the AB1-05 and AB1-09 primers. The smallest number of electrophoresis products was obtained in both guinea fowl varieties with the AB1-08 primer. Within each guinea fowl variety there were birds for which no bands were generated using the AB1-03 and AB1-08 primers. No PCR products were obtained for the AB1-05 and AB1-09 primers in some birds of grey variety and for the AB1-01 primer in birds of the white variety

Table 2. Number of bands for different primers and groups
Tabela 2. Liczba prążków dla poszczególnych starterów i grup

Primer	AB1 – 01		AB1 – 03		AB1 – 05		AB1 – 08		AB1 – 09	
Guinea fowl variety	white	grey	white	grey	white	grey	white	grey	white	grey
No. of bands	0 - 4	1 - 4	0 - 7	0 - 6	2 -7	0 - 7	0 - 3	0 - 3	3 - 6	0 – 6
Total no. of bands	34	47	41	63	75	91	24	31	51	69
Mean no. of bands per bird	2.6	2.9	3.2	3.9	5.8	5.7	1.8	1.9	3.9	4.3
Mean no. of bands per primer	40.5		52		83		27.5		60	

Table 3 lists the coefficients of genetic similarity for the analysed guinea fowl varieties and for the primers used. F values for white and grey guinea fowl within each primer were the mean coefficients of genetic similarity for all birds of a given colour

Table 3. Coefficient of genetic similarity (F) for different groups and primers
Tabela 3. Wartość współczynnika podobieństwa genetycznego (F) dla poszczególnych grup i starterów

Primer	Within groups		
	white guinea fowl	grey guinea fowl	white guinea fowl/grey guinea fowl
AB1 – 01	0.75	0.74	1.0
AB1 – 03	0.43	0.55	0.93
AB1 – 05	0.82	0.78	1.0
AB1 – 08	0.41	0.44	1.0
AB1 – 09	0.82	0.69	0.92
Mean genetic similarity	0.65	0.64	0.97

variety. F value averaged 0.65 for white guinea fowl and 0.64 for grey guinea fowl. Within the AB1 – 01, AB1 – 05 and AB1 – 08 primers, the coefficient of genetic similarity between the colour varieties of guinea fowl was 1, which is indicative of complete similarity. Genetic similarity between white and grey guinea fowl, determined as the mean for the primers used, was high and averaged 0.97 (Table 3). When determining genetic similarity between hens and guinea fowl, Nahashon, et al. (2003) found that it ranged from 0.89 to 0.98 within the guinea fowl population, and from 0 to 0.46 between hens and guinea fowl, which is indicative of the low genetic similarity between these poultry species. Sharma, et al. (1998), who evaluated genetic variation between three varieties of guinea fowl (white, lavender and pearl) found genetic similarity to range from 0.946 to 0.971 within varieties and from 0.990 to 0.999 between varieties, which shows that genetic variation was low both within and between varieties.

The coefficient of similarity, calculated in the present study between white and grey guinea fowl (0.97) is slightly lower than that reported by Sharma, et al. (1998), but its mean value within primers ranged from 0.92 to 1.0. In the present study, genetic similarity within the colour varieties of guinea fowl (0.65 for white and 0.64 for grey guinea fowl) was markedly lower that that (0.95 – 0.97) reported by Sharma, et al. (1998).

It is known from research with different animal species that the same primers may produce different results in different laboratories depending on the reaction conditions (reagents and their concentration), which translates into the coefficients of genetic similarity. On the other hand, the use of different primers in the same species gives different amounts of PCR products, which translates into different values of the coefficient of genetic similarity. For this reason, the primers used in the experiments should be compared for a more complete comparison of the results obtained.

CONCLUSION

The higher genetic similarity between than within the analysed guinea fowl varieties could result from the fact that the birds of the analysed varieties originated from the same founder flock. The lower genetic similarity within variety was probably due to

the higher variation of the analysed animals and the fact that the results obtained using the primers were averaged.

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