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Research Article

Estimation of Genetic Distance in Meat and Layer Pure Lines Using Randomly Amplified Polymorphic DNA*

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Abstract: The purpose of this study was to evaluate genetic similarities and distances among pure lines of poultry using the RAPD technique. In this study four layer and three meat type pure lines were used. Venous blood samples from five males and five females of each line were collected from the *venae cutenea ulnaris*. The RAPD technique was used to generate a DNA fingerprint of lines. Initially, a total of 35 primers having arbitrary sequences were used for RAPD analysis. The amplicons were resolved on 1.5% agarose gel and stained with ethidium bromide. To evaluate the bands, polymorphic and monomorphic bands were described. Genetic similarities and genetic distances were calculated in meat and layer pure lines. The RAPD analysis data from 23 primers were utilized in estimating genetic similarities, which ranged from 0.644 to 0.853 between the lines. The maximum genetic distance was observed between the L2 meat dam line and L4 layer sire line (0.440).

Key Words: RAPD-PCR, polymorphism, genetic similarity, genetic distance

Etçi ve Yumurtacı Saf Hatlarda RAPD (Randomly Amplified Polymorphic DNA) Yöntemiyle Genetik Uzaklığın Tahmini

Özet: Bu çalışmanın amacı, RAPD tekniğini kullanarak kanatlı saf hatları arasındaki genetik benzerlikleri ve genetik uzaklıkları saptamaktır. Çalışmada bir ticari işletmeden sağlanan pedigrili elit sürüden 4 yumurtacı ve 3 etçi saf hat kullanılmıştır. Her bir hattan 5 erkek ve 5 dişi olacak şekilde toplam 70 hayvanın kanat altı damarından (*venae cutenea ulnaris*) kan alınmıştır. RAPD yöntemi, hatlara ilişkin DNA parmak izlerinin çıkartılmasında kullanılmıştır. Polimeraz Zincir Reaksiyonu ürünleri %1,5' luk agaroz jel elektroforezi yoluyla ayrımlanmıştır. Jeller ethidium bromid ile boyanarak U.V. altında fotoğrafları çekilmiştir. Başlangıçta toplam 35 primer RAPD analizinde kullanılmış, 23 primerden elde edilen bantlar etçi ve yumurtacı saf hatlarda genetik benzerlik ve uzaklıkların hesaplanmasında kullanılmıştır. Tüm hatlar arasında genetik benzerlik değeri 0.644 ile 0.853 arasında değişmiştir. En büyük genetik uzaklık L2 etçi ana ve L4 yumurtacı baba hatları arasında (0.440) saptanmıştır.

Anahtar Sözcükler: RAPD-PCR, polimorfizim, genetik benzerlik, genetik uzaklık

Introduction

DNA polymerase techniques and molecular markers can be used in the poultry industry. One of the primary needs of breeders is to identify individuals with the appropriate combination of desired characteristics. Many methods have been developed over the past two decades that allow the detection of polymorphism in DNA. New techniques are emerging every day with advantages over previous ones. The invention of PCR has added a new dimension to the field of molecular biology. It is a simple technique by which small amounts of DNA can be amplified to thousands of folds quickly. The assay is non-radioactive, easy to execute and requires a few simple reagents.

There are many ways of using PCR technology and one powerful technique is Randomly Amplified Polymorphic DNA (RAPD) product analysis (1,2). The RAPD technique, on the basis of the polymerase chain reaction (PCR), is used to identify polymorphism of genome DNA through PCR products amplified on DNA studied using a single random oligonucleotide chain with a series of random base sequences. RAPD analyses have

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been used for genome mapping (3-5), parentage analysis, genetic analysis of relatedness and diversity in chickens and turkeys (6), genetic characterization of chicken lines (7), identification of marker for QTL (8) and estimation of genetic variation (1,9).

The objective of this study was to evaluate genetic similarities and distances among seven pure lines of poultry using pooled DNA samples by the RAPD technique.

Materials and Methods

Animal Material

This study included three meat and four layer type pure lines as shown below. A total of 70 individuals (five males and five females) from each line were utilized.

L1- Dam line
L2- Dam line
L3- Sire line
} Meat pure lines

L4- Sire line
L5- Sire line
L6- Dam line
L7- Dam line
} Layer pure lines

DNA Extraction and Pooling

Blood samples were collected from the *venae cutenea ulnaris* into 5 cc tubes including EDTA. Genomic DNAs were isolated from blood samples as described by Dunnington et al. (10). The purity and concentration of DNA samples were checked by DNA fluorometer. Equal quantities of DNA from the individuals of each line were mixed together to prepare the representative DNA pool.

Amplification Condition and Electrophoresis

A total of 35 primers of arbitrary sequences having high G+C content (60%) from OPA, OPQ, and OPU (OPA-1, OPA-2, OPA-3, OPA-4, OPA-5, OPA-6, OPA-7, OPA-8, OPA-9, OPA-10, OPA-11, OPA-12, OPA-13, OPA-14, OPA-15, OPA-16, OPA-17, OPA-18, OPA-19, OPA-20, OPQ-1, OPQ-3, OPQ-4, OPQ-5, OPQ-6, OPQ-8, OPQ-9, OPQ-10, OPQ-11, OPQ-12, OPQ-13, OPQ-14, OPQ-15, OPQ-16, and OPU-1) were tested using pooled DNA samples. RAPD reactions were carried out on a final volume of 15 µl containing 25 ng DNA, 100 µM each of dATP, dTTP, dCTP, and dGTP (Boehringer-Mannheim), 15 ng primer (Operon, CA, USA), 1x Super Therm polymerase buffer (20 mM Tris-HCl pH: 8, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol) and 1 unit of Super Therm DNA polymerase enzyme (SR Product, UK). The amplification profile was carried out in Thermal Cycler (Appligene) programmed for 30 s initial denaturation at 94 ºC and 35 cycles of 25 s at 94 ºC, 45 s at 35 ºC and 1 min at 72 ºC. After the cycles were completed, a 5 min elongation at 72 ºC was performed. Amplification products were analysed by electrophoresis in 1.5% agarose gel, visualized in a U.V. transilluminator after staining with ethidium bromide and photographed. Lambda DNA-EcoR I/Hind III double digest was used as a molecular size marker and run parallel to the amplified products.

Statistical Analysis

To evaluate the bands, polymorphic and monomorphic bands were described. A matrix for the presence or absence of markers was used to estimate the genetic similarity between lines in terms of average band sharing frequencies (11). Band sharing (genetic similarity) was calculated as

$$Mab = 2 (Nab)/(Na + Nb),$$

where Mab is the band sharing level between individuals a and b, Nab is the number of bands present in both individuals, Na is the total number of bands in individual a and Nb is the total number of bands in individual b.

Genetic variation (P = polymorphism) was found as

$$P = 1 - M$$

Genetic distance 'D' was calculated as -ln(M) (9).

Genetic distances were used to construct a dendrogram, using average linkage determined by the CLUSTER procedure of JMP (12).

Results and Discussion

Initially, a total of 35 random primers were tested for amplification using pooled DNA samples from seven lines, but 23 of the 35 primers (OPA-1, OPA-2, OPA-4, OPA-5, OPA-6, OPA-7, OPA-8, OPA-9, OPA-10, OPA-11, OPA-12, OPA-15, OPA-16, OPA-17, OPA-18, OPA-19, OPA-

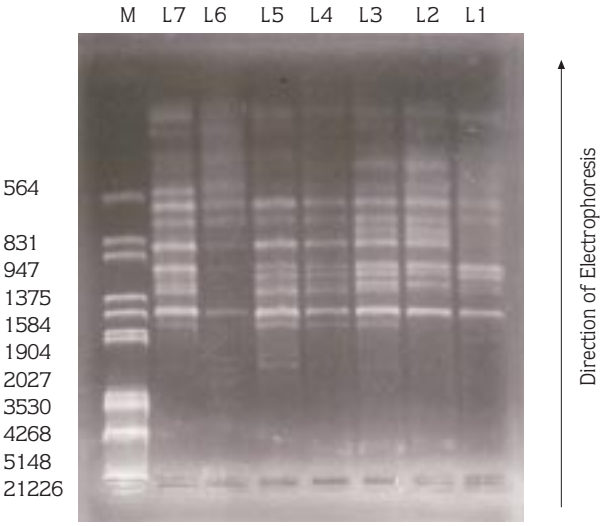

Figure 1. RAPD pattern of seven pure lines with OPQ-8 primer

M: Lambda DNA EcoR I/Hind III, L1-L3: meat pure lines, L4-L7: layer pure lines

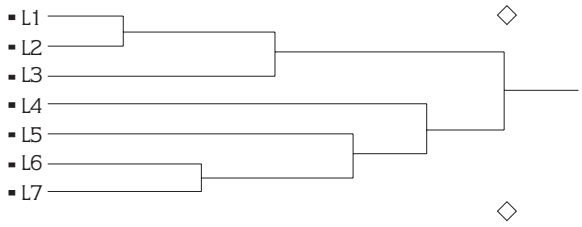

Figure 2. A dendrogram of meat and layer pure lines on average linkage cluster analysis using RAPD markers

20, OPQ-5, OPQ-8, OPQ-11, OPQ-12, OPQ-15, and OPU-1) revealed patterns with scorable amplified primers. A total of 274 bands revealed 213 (77.7%) of them to be polymorphic. The average number of polymorphic bands per primer was 9.2. The RAPD pattern of OPQ-8 is given as an example in Figure 1.

The average of genetic similarity in all lines was 0.736 while genetic variation was 0.264.

The maximum genetic similarity in meat pure lines was 0.853 between the L1 and L2 dam lines. On the other hand, the lowest genetic similarity occurred between the L3 sire and L1 dam line and was 0.755 (Table 1). In layer pure lines, the maximum genetic similarity was found between the L6 and L7 dam lines and was 0.845, while the lowest genetic similarity was obtained between the L4 sire and L7 dam lines (0.724). The maximum genetic variation among the meat and layer lines was found between the L2 meat dam line and L4 layer sire line (0.356). These results are supported by Lee et al. (13), who used Korean native chicken populations and three pure lines, and found that the highest genetic similarity occurred in pure lines.

The use of dam and sire lines having the lowest genetic similarity within both lines is suggested for crossbreeding in respect of potential genetic benefits.

The highest genetic distance was determined between the L1 and L3 lines within meat lines (0.281) (Table 2). L4 showed the maximum distance with L7 within layer lines (0.322). Between meat and layer lines, the

Table 1. Genetic similarity (M) and genetic variation (P) values between pure lines.

	L1	L2	L3	L4	L5	L6
L2	M = 0.853					
	P = 0.147					
L3	M = 0.755	M = 0.757				
	P = 0.245	P = 0.243				
L4	M = 0.681	M = 0.644	M = 0.752			
	P = 0.319	P = 0.356	P = 0.248			
L5	M = 0.674	M = 0.703	M = 0.753	M = 0.793		
	P = 0.326	P = 0.297	P = 0.247	P = 0.207		
L6	M = 0.672	M = 0.702	M = 0.692	M = 0.737	M = 0.821	
	P = 0.328	P = 0.298	P = 0.308	P = 0.263	P = 0.179	
L7	M = 0.684	M = 0.680	M = 0.720	M = 0.724	M = 0.827	M = 0.845
	P = 0.316	P = 0.320	P = 0.280	P = 0.276	P = 0.173	P = 0.155

Table 2. Genetic distance values between pure lines.

	L1	L2	L3	L4	L5	L6
L2	0.158					
L3	0.281	0.278				
L4	0.384	0.440	0.285			
L5	0.394	0.352	0.283	0.231		
L6	0.397	0.353	0.368	0.305	0.197	
L7	0.379	0.385	0.328	0.322	0.189	0.168

maximum genetic distance was observed between the L2 and L4 (0.440) followed by L1 and L6 (0.397) lines, as seen in Table 2.

A dendrogram based on the genetic distances among lines is shown in Figure 2. Two clusters were observed. The first cluster included L1, L2, and L3 meat pure lines while the second one included L4, L5, L6, and L7 layer pure lines.

In general, this study revealed that the genetic distance values within meat and layer lines were lower than those between meat and layer lines. Sharma et al. (9) used the RAPD technique to evaluate the genetic diversities among six breeds of poultry using pooled DNA samples and found that the genetic distances between meat and layer breeds were similar to those in this study. Siegel et al. (14) observed similar findings using DNA fingerprinting in chicken breeds. Dunnington et al. (15) studied genetic variation from a commercial chicken population using the DNA fingerprint method. They also observed a more distant relationship between meat and layer types.

The present study supports the applicability of the RAPD-PCR technique in DNA polymorphism and genetic distance studies in chickens.

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