Cukurova University
Journal of
Natural & Applied Sciences

Original Paper

Determination of Genetic Variations in Japanese Quails Improved for Meat and Egg Production Traits Using PCR-RAPD Method

Hacı Mansur Bozdoğan[1], Bahri Devrim Özcan[2]

[1]Çukurova University, Institute of Natural and Applied Sciences, Department of Animal Science, Adana, Türkiye
[2]Çukurova University, Faculty of Agriculture, Department of Animal Science, Adana, Turkey

ORCID IDs of the authors: H.M.B. 0009-0006-8656-9784; B.D.Ö. 0000-0002-9198-656X

Cite this article as: Bozdoğan, H. M., Özcan, B. D. (2023). Determination of Genetic Variations in Japanese Quails Improved for Meat and Egg Production Traits Using PCR-RAPD Method. Cukurova University Journal of Natural & Applied Sciences 2(3): 13-19.

Abstract

The study was conducted to assess the genetic characterization of Japanese quail (*Coturnix coturnix japonica*) lines bred according to their meat and egg production characteristics in the Quail Unit of Çukurova University Faculty of Agriculture Research and Application Farm was determined by Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD) method. Two RAPD primers, OPP-03 and OPC-11, were employed, determined through literature reviews to be monomorphic/polymorphic, and to generate a sufficient number of bands. In the study using the OPP-03 primer, the polymorphism was calculated as 83.33% and 100%, respectively, in layer and broiler individuals, while in the study using the OPC-11 primer, the polymorphism was calculated as 28.57% and 85.71%. These results indicate that the OPP-03 marker is more polymorphic in both meat and egg quail lines compared to the OPC-11 marker. On the other hand, it reveals that, in terms of both markers, meat individuals are more polymorphic than egg individuals.

Keywords: Japanese quail (*Coturnix coturnix japonica*), RAPD, Genetic characterization, Polymorphism

1. Introduction

Quails, which can be raised for many purposes today, have been the center of attention mostly as game animals and songbirds, especially after they were brought from China in 770-476 BC [1]. Quails, which have started to be raised as ornamental animals in terms of their sound and appearance over time, are now used as laboratory experiment materials in research fields such as genetics, physiology, endocrinology, immunology, and toxicology depending on the developing technologies, but they are also used as feeding material mainly due to their high-quality meat and eggs [2-5]. Housing more animals per unit area, low feed consumption, short intergenerational period, high egg yield per live weight, simple tools and equipment in production, and resistance to poultry diseases make quail breeding attractive [6].

Japanese quail (*Coturnix coturnix japonica*) are nowadays raised under intensive conditions mostly for their meat and eggs and have moved away from their ancestors in terms of morphology and yield ability [7]. In Japanese quails, females are 20-30 g heavier than males in terms of body weight, and females attract attention with more than 270 eggs in a 10-month laying period [6]. Quail eggs have an average mass of 10.70 g, yolk to water ratio of 45.25%, and whole egg-to-water ratio of 71.90% [8]. In proportion to the yolk ratio (50%), it contains more energy (9.5 kJ/gr) than other poultry eggs [9].

The RAPD method is widely and effectively employed as a molecular marker due to its numerous advantages. It finds extensive application in various scientific fields, including genome structure research, taxonomic classification, addressing evolutionary questions, establishing parentage, assessing genetic relatedness, exploring genetic variation in populations, genotype determination across organisms, ascertaining individual lineage and race, identifying specific genetic loci, and serving in forensic investigations, clinical diagnostics, genetic disease studies, ecological analyses, and epidemiological investigations involving diverse prokaryotic and eukaryotic organisms [10]. Numerous genetic characterization studies have been conducted on farm animals using the RAPD method, revealing polymorphisms based on the RAPD primers employed in these studies. This method has been instrumental in elucidating genetic variations within the farm animal populations.

Address for Correspondence:
Bahri Devrim Özcan, e-mail: bdozcan@gmail.com

Received: Nov 1, 2023
Accepted: Nov 22, 2023

The objective of this study is to partially determine variations in Japanese quail lines between developed based on meat and egg production characteristics at the Research and Application Farm of the Faculty of Agriculture, Çukurova University, using the RAPD primers. Two oligonucleotide primers of arbitrary sequences, having high GC content (60%) from the sets of OPP (OPP-03) and OPC (OPC-11) from Operon Technologies, Inc., (Almeda, California) were used.

2. Material and Method

2.1. Animal Materials Used in the Study

The animal materials used in this study were obtained by randomly selecting individuals from two Japanese quail lines that had been selectively bred for egg production (high egg yield, low body weight, and speckled feather color, 10 males and 10 females) and meat production (high body weight, low egg yield, and yellow feather color, 10 males and 5 females) at the Quail Experimental Unit of Çukurova University Faculty of Agriculture. Gender effect was not considered in the study, but male and female individuals were included in the study to ensure homogeneity of the samples. Blood samples from the animals were collected in accordance with ethical standards at the time of their slaughter in the experimental unit (Ethics Committee Decision Number: 9-2-2022). The blood was carefully collected directly into EDTA tubes to a volume of 2-3 ml. The tubes were labeled, transported to the laboratory on ice, and stored at -20°C until they were used for DNA isolation. DNA isolation from blood samples was performed according to Baqour [11].

2.2. RAPD Primers

Following a review of the literature, RAPD primers OPP-03 [12] and OPC-11 [13] (Table 1) were chosen for their demonstrated ability to produce a sufficient number of bands and their known polymorphic nature from Operon Technologies (Inc. Alameda, California). Reaction components were prepared in 0.2 ml PCR tubes with a total volume of 25 µl, comprising genomic DNA (150 ng), RAPD primers (20 pmol), MgCl2 (4 mM), PCR MasterMix (ThermoFisher Scientific, 6.5 µl), and distilled water (to reach a volume of 25 µl). This mixture was then placed in a thermal cycler (Blue-Ray Scientific, TurboCycler). The PCR process involved 45 cycles, beginning with an initial denaturation step at 95°C for 3 minutes, followed by denaturation at 94°C for 1 minute, primer annealing at 35°C for 1 minute, and extension at 72°C for 2 minutes. The PCR process concluded with a final extension step at 72°C for 7 minutes. The amplified DNA fragments, along with a marker DNA (Thermo Fisher Scientific, GeneRuler 1 kb DNA marker), were subjected to electrophoresis in a 1.5% agarose gel (Vivantis) at 100 volts. After electrophoresis, the gel was stained with ethidium bromide (0.5 µg/ml) for 30 minutes, and DNA bands were visualized and photographed using a UV transilluminator. Each study was repeated twice. The migration distances (in mm) of PCR products (amplicons) and DNA marker bands were measured using a ruler, and the obtained data were used to calculate log10 values in Microsoft Excel to create a linear regression curve. Subsequently, the molecular sizes of the amplicons were calculated using the regression coordinate values.

Table 1. The RAPD primers used in the study

Primer	Sequences (5'→3')	G+C content (%)	Tm (°C)
OPP-03	CTGATACGCC	60	35
OPC-11	AAAGCTGCGG	60	35

2.3. Evaluation of RAPD Loci in Meat and Egg Varieties

The assessment of RAPD loci for individuals in meat and egg varieties was carried out using the POPGENE32 (Version 1.31) package program. The genetic similarity between individuals was calculated using the equation Fxy=(2 Mxy)/(Mx+My), where Fxy represents genetic similarity, Mxy denotes the number of common RAPD bands between two individuals, and Mx and My represent the total RAPD band numbers for the first and second individuals, respectively.

3. Results and Discussion

Polymorphic bands were obtained following PCR reactions using both RAPD primers (Figures 1 and 2). In the study conducted using the OPC-11 primer, when all individuals are taken into account, a total of seven different PCR product bands (alleles) with sizes approximately around 1375, 1202, 923, 816, 598, 444, and 345 base pairs were obtained (Figure 1). However, upon examination of the PCR product DNA bands obtained using the OPP-03 primer, it was observed that, when considering all individuals, a total of six different bands with sizes approximately around 1223, 1083, 964, 785, 576, and 526 base pairs were obtained (Figure 2). Evaluation of the RAPD profiles for each sample was conducted with modifications from Koh et al [14].

Alleles found in each sample were scored as 1 (one), while absent alleles were scored as 0 (zero) (Tables 2 and 3). In the PCR study conducted using the OPC-11 primer, a band suitable for evaluation could not be obtained from sample 8 of the egg-laying individuals. Similarly, in the PCR study conducted using the OPP-03 primer, bands suitable for evaluation could not be obtained from sample 1 of the egg-laying individuals, as well as from samples 13 and 15 of the meat-type individuals.

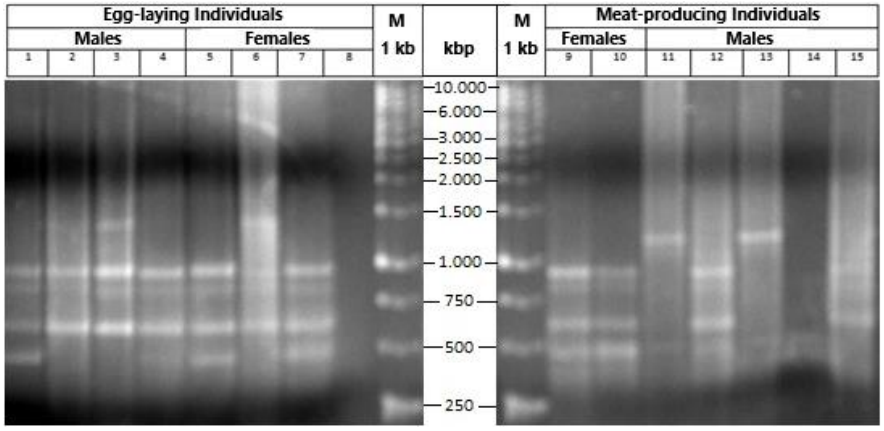

Figure 1. The agarose gel electrophoresis image of the PCR results obtained using the OPC-11 primer

Table 2. The presence-absence table of bands according to the agarose gel electrophoresis image of PCR results obtained using the OPC-11 primer (1: Band present, 0: Band absent).

PCR Product DNA Bands (bp)	Egg-laying individuals								Meat-producing individuals						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1375	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0
1202	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
923	1	1	1	1	1	1	1	0	1	1	0	1	0	0	1
816	1	1	1	1	1	1	1	0	1	1	0	1	0	0	0
598	1	1	1	1	1	1	1	0	1	1	0	1	0	0	1
444	1	0	0	1	1	0	1	0	1	1	1	1	1	1	0
345	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0

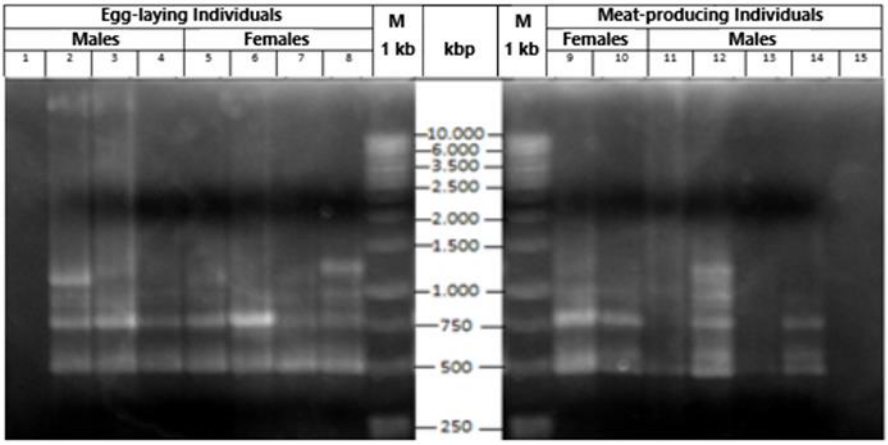

Figure 2. The agarose gel electrophoresis image of the PCR results obtained using the OPP-03 primer

Table 3. The presence-absence table of bands according to the agarose gel electrophoresis image of PCR results obtained using the OPP-03 primer (1: Band present, 0: Band absent).

PCR Product DNA Bands (bp)	Egg-laying individuals								Meat-producing individuals						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1223	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0
1083	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0
964	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0
785	0	1	1	1	1	1	1	1	1	1	0	1	0	1	0
576	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
526	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0
1223	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0

Analyses conducted with the PopGene32 program revealed that using the OPC-11 primer, the levels of polymorphism were calculated as 28.57% for egg-laying individuals (Table 4) and 85.71% for meat-producing individuals (Table 5), while for the study using the OPP-03 primer, polymorphism levels were calculated as 83.33% for egg-laying individuals (Table 6) and 100% for meat-producing individuals (Table 7). These results provide preliminary information on the use of existing markers for future breeding or selection studies in quails.

Table 4. Evaluation results of the bands obtained in egg-laying individuals with OPC-11 primer in PopGene32 program

Locus	Sample Size	na*	ne*	h*	I*
1375	7	2.0000	1.5848	0.3690	0.5557
1202	7	1.0000	1.0000	0.0000	0.0000
923	7	1.0000	1.0000	0.0000	0.0000
816	7	1.0000	1.0000	0.0000	0.0000
598	7	1.0000	1.0000	0.0000	0.0000
444	7	2.0000	1.8254	0.4522	0.6445
345	7	1.0000	1.0000	0.0000	0.0000
Mean	7	1.2857	1.2015	0.1173	0.1715
St. Dev.		0.4880	0.3510	0.2018	0.2939

*na: Observed number of alleles
*ne: Effective number of alleles
*h: Nei's gene diversity
*I: Shannon's Information index
The number of polymorphic loci is: 2
The percentage of polymorphic loci is: 28.57%

Table 5. Evaluation results of the bands obtained in meat producing individuals with OPC-11 primer in PopGene32 program

Locus	Sample Size	Ht	Hs	Gst	Nm*
1375	7	0.0000	0.0000	****	****
1202	7	0.2617	0.2617	0.0000	2000.0000
923	7	0.4522	0.4522	0.0000	2000.0000
816	7	0.3690	0.3690	0.0000	2000.0000
598	7	0.4522	0.4522	0.0000	2000.0000
444	7	0.4702	0.4702	0.0000	2000.0000
345	7	0.4976	0.4976	0.0000	2000.0000
Mean	7	0.3576	0.3576	0.0000	2000.0000
St. Dev.		0.0312	0.0312		

*Nm: Estimate of gene flow from Gst or Gcs. E.g., Nm=0.5(1-Gst)/Gst
The number of polymorphic loci is: 6
The percentage of polymorphic loci is: 85.71%

Table 6. Evaluation results of the bands obtained in egg-laying individuals with OPP-03 primer in PopGene32 program

Locus	Sample Size	na*	ne*	h*	I*
1223	8	2.0000	1.3022	0.2321	0.3939
1083	8	2.0000	1.4951	0.3311	0.5132
964	8	2.0000	1.8420	0.4571	0.6496
785	8	2.0000	1.8420	0.4571	0.6496
576	8	1.0000	1.0000	0.0000	0.0000
526	8	2.0000	1.8420	0.4571	0.6496
Mean	8	1.8333	1.5539	0.3224	0.4760
St. Dev.		0.4082	0.3529	0.1825	0.2550

*na: Observed number of alleles
*ne: Effective number of alleles
*h: Nei's gene diversity
*I: Shannon's Information index
The number of polymorphic loci is: 5
The percentage of polymorphic loci is: 83.33%

Table 7. Evaluation results of the bands obtained in meat producing individuals with OPP-03 primer in PopGene32 program

Locus	Sample Size	na*	ne*	h*	I*
1223	7	2.0000	1.3545	0.2617	0.4310
1083	7	2.0000	1.1592	0.1374	0.2643
964	7	2.0000	1.8254	0.4522	0.6445
785	7	2.0000	1.8254	0.4522	0.6445
576	7	2.0000	1.5848	0.3690	0.5557
526	7	2.0000	1.9905	0.4976	0.6908
Mean	7	2.0000	1.6233	0.3617	0.5385
St. Dev.		0.0000	0.3180	0.1380	0.1629

*na: Observed number of alleles
*ne: Effective number of alleles
*h: Nei's gene diversity
*I: Shannon's Information index
The number of polymorphic loci is: 6
The percentage of polymorphic loci is: 100.00%

Biomarkers have made substantial contributions to animal breeding from the discovery of these markers to the present day. These contributions have extended beyond breeding at the population level, as they have been extensively employed in accurately revealing genetic differences at lower taxonomic levels such as species, breeds, and strains within organisms, and this application continues.

A review of the literature indicates a limited number of studies conducted in our country using RAPD primers, particularly on quails, compared to studies conducted on chickens and other poultry species. In a comprehensive study on quails, Yeğenoğlu [15] utilized eight RAPD primers in Japanese quails that had not undergone any breeding work. A total of 69 RAPD loci were identified in this study, with 59 of them being polymorphic. The population's within-population genetic similarity rate was reported as 35%, and the total polymorphism rate was 65%.

Several polymorphism determination studies have been conducted in quails to determine genetic polymorphism, utilizing methods such as ISSR, allele frequency measurement [16], microchromosomes, and AFLP. Mansour et al. [17] compared four isolated quail lines using RAPD and ISSR methods and reported that individual and clustering results were dependent on the marker system used.

In this study, under the dominant diploid criteria of the POPGENE32 program, using the RAPD method with the OPP-11 primer, the presence-absence matrix of PCR product DNA bands revealed that there were 2 polymorphic loci (1375 and 444 bp-sized bands) in the egg-laying strain, and they exhibited a 28.57% polymorphism rate. In the meat-producing strain, 6 polymorphic loci (1202, 923, 816, 598, 444, and 345 bp-sized bands) were identified, and they demonstrated an 85.71% polymorphism rate. In the analysis of the presence-absence matrix of PCR product DNA bands obtained using the OPC-03 primer, the egg-laying strain had 5 polymorphic loci (1223, 1083, 964, 785, 576, and 526 bp-sized bands) and exhibited an 83.33% polymorphism rate. The meat-producing strain, on the other hand, had 6 loci (1223, 1083, 964, 785, 576, and 526 bp-sized bands), all of which were polymorphic, demonstrating a 100% polymorphism rate.

The results obtained from studies using RAPD primers may vary depending on the specific primers used [14]. The primary objective of polymorphism studies is to identify and reveal polymorphic regions among individuals. Some RAPD primers may not amplify polymorphic regions in the compared genomes, while others may not generate RAPD profiles due to a lack of compatibility with the genome of the species. Therefore, in polymorphism studies conducted using RAPD primers, it is advisable to employ as many RAPD primers as possible to obtain more reliable data. In this study, the use of only two RAPD primers may not be sufficient for a comprehensive evaluation of polymorphism among quail lines bred for meat and egg production. Continuing the study using additional RAPD primers will provide more accurate data for evaluation. The condition of RAPD profiles obtained is not solely dependent on the primer used but is also influenced by the concentrations of PCR components (e.g., MgCl2, target DNA) and the machine-specific parameters (e.g., primer annealing temperature), which have been minimized through optimization efforts.

When compared to other methods used for determining polymorphisms, the RAPD method was preferred in this study due to its low cost, ease of application, and quick results. However, due to the aforementioned disadvantages, the research was conducted with great care, repeated several times, and meticulous attention to obtain the most reliable results.

4. Conclusion

Using two RAPD primers, OPP-03 and OPC-11, comparisons were conducted between animals from two selectively bred Japanese quail lines, which were specifically bred for egg and meat production, within and between these lines, at the Quail Experimental Unit of Çukurova University Faculty of Agriculture. As the number of RAPD primers is increased in the continuation of the study, it is suggested that this will lead to a higher degree of polymorphism being revealed between the two quail lines. This recommendation forms the fundamental proposal of this study.

Acknowledgment

This study was supported by the Çukurova University Scientific Research Projects Unit under Project Number FYL-2019-11403. This article has been generated from Hacı Mansur Bozdoğan's master's thesis.

References

[1] Chang, B.G., Chang, H., Liu, X.P., Xu, W., Wang, H.Y., Zhao, M.W., Olowofeso, O. (2005). Developmental research on the origin and phylogeny of quail. World's Poultry Science Journal; 61(1): 105–112.

[2] Tığlı, R., Yaylak, E., Balcıoğlu, M.S. (1996). Japon bıldırcınlarının çeşitli verim özelliklerine ait fenotipik ve genetik parametreler. II. Canlı ağırlıklara ait fenotipik değerler. Ankara Üniversitesi Ziraat Fakültesi Dergisi; 9: 71-85.

[3] Kolar, R. (2006). Animal experimentation. Sci Eng Ethics; 12: 111-122.

[4] Ergün, Y. (2010). Hayvan deneylerinde etik. Arşiv 2010; 19: 220-235 https://dergipark.org.tr/tr/download/article-file/25384.

[5] Chain, J.R., Cawley, W.O. (1914). Japanese quail. The Texas Agricultural Extension Service; 3-12.

[6] Edwin, S.C. (2020). Commercial Japanese quail production and disease management. International Journal of Science, Environment and Technology; 9 (3): 416-422.

[7] Lukanov, H., Pavlova, I. (2020). Domestication changes in Japanese quail (Coturnix japonica): a Review. World's Poultry Science Journal; 76(4): 787-801.

[8] Balkan, M., Karakaş, R. (2007). Bıldırcın (Coturnix Coturnix Japonıca) yumurtalarına ilişkin bazı özellikler. Dicle Üniversitesi Ziya Gökalp Eğitim Fakültesi Dergisi; 8: 112-119.

[9] Sotherland, P.R., Rahn, H. (1987). On the composition of bird eggs. The Condor; 89: 48-65.

[10] Öz Aydın, S. (2004). RAPD (Rastgele Arttırılmış Polimofik DNA) belirleyicileri ve bitki sistematiği. Dumlupınar Üniversitesi Fen Bilimleri Enstitüsü Dergisi; 6: 113-130.

[11] Baqour, M. (2018). A minimum requirements method to isolate large quantities of highly purified DNA from one drop of poultry blood. Journal of Genetics; 29(1): 87-94.

[12] Sharma, D., Appa Rao, K.B.C., Singh, H.P., Totey, S.M. (1998). Randomly amplified polymorphic DNA (RAPD) for evaluating genetic relationships among varieties of guinea fowl. Genetic Analysis Biomolecular Engineering; 14: 125-128.

[13] Alatafi, A.K., Saleem, H.D., Singh, R.P., Herbert, S., Singh, V.K. (2013). DNA polymorphism among three native Indian chickens using RAPD markers. Res. Opin. Anim. Vet. Sci.; 3(4), 117-122.

[14] Koh, M.C., Lim, C.H., Chua, S.B., Chew, S.T., Phang, S.T.W. (1998). Random Amplified Polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. Meat Sci; 48: 275-285.

[15] Yeğenoğlu, E.D. (1999). Japon bıldırcınlarında (Coturnix coturnix japonica) DNA izolasyonu ve DNA parmakizlerinin çıkarılması. Yüksek Lisans Tezi. Ege Üniversitesi Fen Bilimleri Enstitüsü Zootekni Anabilim Dalı. İzmir.

[16] Amirinia, C., Emrani, H., Radjaee Arbbabe, M.A., Vaez Torshizi, R., Nejati, A. (2007). Evaluation of eight microsatellite loci polymorphism in four Japanese quail (Coturnix japonica) strain in Iran. Pakistan Journal of Biological Sciences; 10(8): 1195-1199.

[17] Mansour, A., Teixeira da Silva, J.A., El-Araby, E.E. (2010). Molecular markers associated with the development of new phenotypes of Japanese quail in Egypt. Global Science Books, Process Biotechnology and Molecular Biology; 4 (1): 79-84.