Genetic Diversity among Local Quail Using RAPD-DNA Marker

Diversidad Genética Entre Codornices Locales Usando Marcadores Dna-Rapd

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ABSTRACT

The randomly amplified polymorphic DNA (RAPD) markers were used to detect the genetic variations and polymorphism among three different colors of local quail (desert, brown and white). Out of twenty random primers used, thirteen were able to amplify and showed bands. The total fragment number arrived 310 with size range from 250 to 2800 bp. Polymorphic fragments and unique bands in all samples were (34.36 and 2), respectively. However, OPA-04, OPS-01, OPB-01 and 10- MER loci have generated high polymorphic bands. The Nei's gene diversity for overall lines averaged 0.1026.The higher distance found among brown female with both white and desert female (27.614), while the lowest distance (4.002) recorded between white and desert (male and female) quails. The overall dendrograms clustered the quail lines into two clusters. The 1st one consisted of white and desert, and the 2nd one included only brown. It was concluded that the white was closer to desert quail than to the brown quail.

Key words: RAPD; polymorphism; genetic diversity; local quail

RESUMEN

Los marcadores de ADN polimórfico amplificados al azar (RAPD) se utilizaron para detectar las variaciones genéticas y el polimorfismo entre tres colores diferentes de codornices locales (desierto, marrón y blanco). De los veinte cebadores aleatorios utilizados, trece pudieron amplificar y mostraron bandas. El número total de fragmentos llegó a 310 con un rango de tamaño de 250 a 2800 pb. Los fragmentos polimórficos y las bandas únicas en todas las muestras fueron (34,36 y 2), respectivamente. Sin embargo, los loci OPA-04, OPS-01, OPB-01 y 10-MER han generado altas bandas polimórficas. La distancia más alta se encontró entre las hembras marrones con hembras tanto, blancas como desérticas (27,614), mientras que la distancia más baja (4,002) se registró entre las codornices blancas y desérticas (machos y hembras). Los dendrogramas globales agruparon las líneas de codorniz en dos grupos. El primero consistía en blanco y desierto, y el segundo solo incluía marrón. Se concluyó que el blanco estaba más cerca de las codornices del desierto, que de las codornices marrones.

Palabras clave: RAPD; polimorfismo; diversidad genética; codornices locales

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INTRODUCTION

The Japanese quail (JQ) *Coturnix Coturnix Japonica*, at the start domesticated around the 11th century as a pet song fowl [5,11] is valued for its uniquely flavored egg and meat. It is additionally used broadly for laboratory researches due to the fact of its small physique size, speedy generation turnover, resistance to illnesses and high egg production [17]. It has been regarded as a appropriate model for poultry research [28]. From the phylogenetic factor of view, the JQ is intently related to the poultry [26]. Both (poultry and quail) have comparable karyotypes of 2n=78 chromosomes and a genome length of 1.2×109 base pair (bp), consisting of morphologically distinct macro chromosomes (1–8 and the ZW sex chromosomes) and cytologically indistinguishable micro chromosomes [22]. Conservation genetics for preservation of species has acquired increasing interest in the current years [7, 10]. Molecular markers are very beneficial to learn about of populace structure and gene drift on characteristics of economic importance, particularly characteristics that are challenging to select for on the field.

During the past decades, molecular genetics can be applied with classical breeding in various farm animals which include poultry. DNA markers are effective tools in characterization and estimation of relatedness between genotypes. The estimation of genetic variability of a species is an important for its conservation and genetic improvement [5] Mazandaran, Iran. Venous blood samples were col- lected from 100 birds of both sexes. The RAPD-PCR tech- nique was applied to generate a DNA fingerprint of individu- als. Initially, a total of 20 ten-nucleotide arbitrary primers were used but 14 of 20 primers revealed a pattern with scorable amplified bands. From a total number of 140 scored bands 63 (45%). Genetic variety research are undertaken to classify folks or populations; and has been accessed in farm animals via morphological, molecular or biochemical techniques. [9, 16, 20] showed that Random Amplified Polymorphic DNA (RAPD) techniques can be readily applied to different species. Therefore, the objective of current study was to determine the diversity and characterization among white, desert and brown local quail in Kurdistan Region of Iraq using RAPD-DNA marker technique.

MATERIALS AND METHODS

This study was carried out in Grdarasha researches unit, under the supervision and regulations of ethic committee at college of agriculture, University of Salahaddin-Erbil. A total of 144 blood samples were collected from both male and female local quails (desert, brown and white). From each bird, 0.5 mL blood sample was taken. Blood samples were collected in an anti-coagulant Ethylene Diamine Tetra acetic Acid (EDTA) tube, and stored at -20°C (Thermo Electron Corporation, ULT2540, USA) until DNA extraction. DNA was extracted the blood sample of each bird using the DNeasy® blood kit (GeNet Bio. Laboratory Korea) according to the manufactures instructions. The DNA extracts of the blood samples belong to quail birds of the same line. The quantity of DNA (purity and concentrations) has been measured by Thermo scientific NanoDropTM spectrophotometer (NanoDrop® ND-1000). The quality of DNA was determined on a 1% agarose gel electrophoresis. Twenty RAPD primers were initially applied (TABLE I). Genetic parameters of total fragment numbers, size range of fragments, polymorphic fragment numbers, Nei's gene diversity, Nei's genetic identity, genetic distance and phylogenetic tree construction were calculated using the genetics software Genepop version 3.3 [18]. Amplifications were performed using a thermal Cycler (aBioRad thermocycler), with the final reaction volume of 25 µL. Each reaction volume contained: 12.5 µL of Green Master Mix (0.5mM of each dNTP in 10 mM Tris-HCL, Ph 9.0, 4mM MgCl, enzyme stabilizer, loading dye and 1U Taq DNA polymerase), 2 µL of of each RAPD primer, 4 µL (40 ng) of DNA template and 6.5 µL of DNase free water. The Polymerase Chain Reaction program used for Primer (OPA-14): programmed for 35 cycles of denaturation at 95°C for 10 minutes (min), annealing at 34°C for 0.30 min and extension at 72°C for 0.30 min. An initial denaturation step of 1 min at 95°C and a final extension step of 5 min at 72°C were included in the first and last cycles, respectively. for the (OPG-05 and OPA-20) used the above program with annealing temperature replaced to 35°C and for Primers (OPM-06, OPM-20, OPN-16, OPP-14, OPQ-03, OPQ-7 and OPS-01) annealing temperature was set at 37°C and 4th protocol for primers (OPA-04, OPB-01 and 10-MER) annealing temperature was 42°C . Amplified DNA fragments were separated electrophoretically in 1.5% agarose gel and stained with ethidium-bromide in an electrophoresis tank 1x Tris-borate EDTA buffer. The gel was visualized by a Ultraviolet (UV) illumination (Proxima 2500 Isogene Life science, Netherland).

RESULTS AND DISCUSSION

Out of the twenty random primers, thirteen were amplified and showed bands. Twelve of the thirteen primers were polymorphisms and one primer were monomorphism in the local quails FIG. 1. The total fragment number (TFN) for the 13 primes was 310 fragments, ranged from 10 fragments in 10 MER to 30 fragments in OPG-05 and OPP-04 with fragments size ranged from 250 to 2800 bp (TABLE II). Similar results were reported [21] in six quail lines the number of bands ranged from 7 to 13 with size range of 250 to 4000 bp. While Eissa and Mahmoud [6] (2014) showed that the number of amplified DNA fragments ranging from 4-11. Karabag, and Balcioglub, [13] reported that the number of amplified bands ranged from 4 to 14 with band size ranged from150 to 2600 bp. in quail lines. The highest total numbers of bands were recorded in white (male and female), while the lowest were showed in brown female (TABLE II).

The overall polymorphic fragments number (PFN) was 34, were obtained out of 310 TFN from 13 primers (TABLE III). The highest PFN found at locus OPA-04 which had 4 bands, whereas the lowest PFN found at locus OPA-14, OPM-06, OPN-16, OPQ-07 and10-MER was 1band. These results were in range with that reported [21] found that the percentage of polymorphic was

TABLA I
NAME, SEQUENCES AND GC % OF ALL PRIMERS USED

No.	Primer Name	Sequence (5' - 3')	GC Content %	References
1	OPA-04	AATCGGGCTG	60	[24]
2	OPA-14	TCTGTGCTGG	60	
3	OPB-07	GGTGACGCAG	70	
4	OPB-19	ACCCCCGAAG	70	
5	OPG-03	GAGCCCTCCA	70	
6	OPG-05	CTGAGACGGA	60	
7	OPA-20	GTTGCGATCC	60	[23]
8	OPB-01	GTTTCGCTCC	60	
9	OPB-12	CCTTGACGCA	60	
10	OPC-02	GTGAGGCGTC	70	
11	OPC-13	AAGCCTCGTC	60	
12	OPM-05	GGGAACGTGT	60	[3]
13	OPM-06	CTGGGCAACT	60	
14	OPM-20	AGGTCTTGGG	60	
15	OPN-16	AAGCGACCTG	60	
16	OPP-04	GTGTCTCAGG	60	
17	OPQ-03	GGTCACCTCA	60	[12]
18	OPQ-07	CCCCGATGGT	70	[2]
19	OPS-01	CTACTGCGCT	60	[25]
20	10 MER	AACGCGCAAC	60	[4]

31.7% (19 bands) among the quail lines, and disagree with that detected 6, 13-15 Out of the 310 bands, 2 of them were unique bands (TABLE IV). The unique bands were obtained from OPQ-07 and OPB-01 locus in local brown breed with band size 1350 and 1500 bp, respectively.

The values of Nei's gene diversity (gene diversity / heterozygosity) overall quail lines averaged 0.1026. This result indicated the genetic diversity among local quails is moderately low. As in the (TABLE III) revealed that out of 13 amplified primers only one was monomorphism, the rest gives heterozygosity, the OPM-06, OPN-16 and OPQ-07 loci give highest heterozygosity were 0.444. Such results indicate possibility of using these loci more than others one in future studies. Similarly, [19] showed that the Nei's gene diversity were 0.3412, 0.2753, 0.2045, 0.3556 and 0.3200 for five populations of quails (Japanese, Fawn, Dhakaya, White and Rosetta), respectively. The genetic similarity among quail lines with a heterozygosis value was (0.125) [13]. The Shannon diversity index value in present study was 0.1469 for all over samples, (TABLE V).

Genetic distances among the studied three local quails were shown in TABLE VI. The genetic distances ranged from 0.262 between desert and brown. On the other hand the lowest distance 0.080 recorded between desert and white. The population pair white and desert showed higher genetic identity (0.9231) than other population pairs. Similar results were reported [19] in five population of quails namely Japanese, Fawn, Dhakya, White and Rosetta which the genetic distances value arrived 0.0675, 0.1138, 0.2088 and 0.0938 respectively among population.

The data obtained from the analysis of RAPD were used to draw precise relationships among the three local quail lines, and the resultant dendrograms are shown in FIG. 2 (A, B and C). Dendrograms of phylogenetic relationships were constructed based on the genetic similarity indices. Cluster analysis was conducted to generate a dendrograms illustrating possible relationships among the studied three quail lines based on molecular attributes. The overall dendrograms clustered the quail lines into two clusters (groups). The first group consisted of White males and females were delimited in separate group form one cluster from the rest of studied quail lines males and females. Desert line males and females, and brown line males and females were delimited in other separate group form another cluster. The overall genetic distance among local quails ranged from 0.040 to 0.107, the lowest genetic distance was recorded between the desert and white line, at 0.040. On the other hand, the highest genetic distance (0.107) was observed in brown line. The female and male dendrograms FIG. 2 (A and C) were exactly similar to the overall dendrograms FIG. (1 A) in the phylogenetic relationships, but different in the distances between breeds because in female dendrogram FIG. (2 B) the longest distance was (0.276) observed

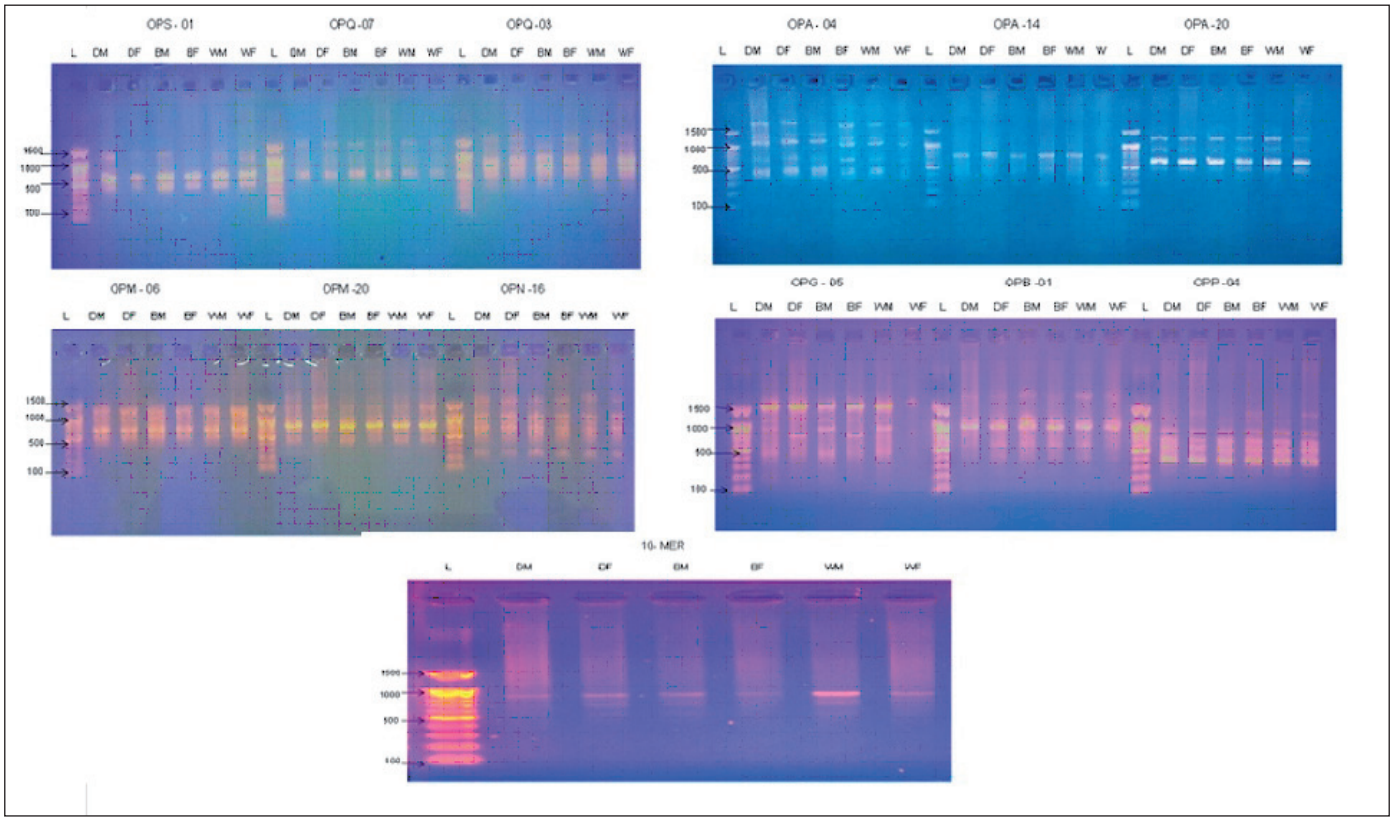

FIGURE 1. GEL ELECTROPHORESIS FOR THIRTEEN RAPD PRIMERS IN STUDIED LOCAL QUAIL BREEDS (DM= desert male, DF= desert female, BM= brown male, BF= brown female, WM= white male, WF = white female

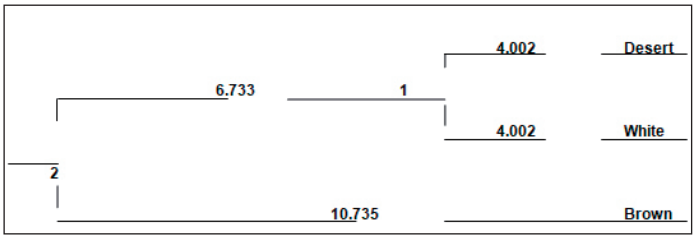

FIGURE (2A). UPGMA DENDOGRAMS SHOWING DIFFERENTIATION AMONG THE THREE LOCAL QUAILS

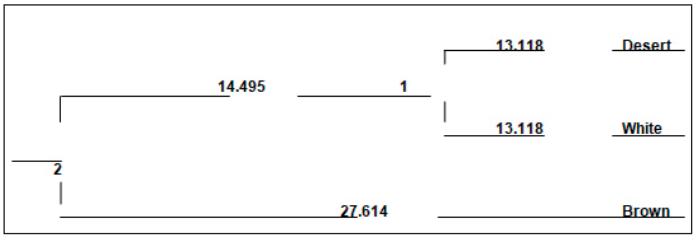

FIGURE (2B). UPGMA DENDOGRAMS SHOWING DIFFERENTIATION AMONG THE THREE LOCAL QUAILS (FEMALE)

in Brown female line, whereas the smallest distance was (0.131) between the desert and white, While in the male dendrogram FIG. (2 C) show the highest genetic distance in desert line was (0.187), thus, lowest genetic distance was (0.084) recorded between the brown and white line.

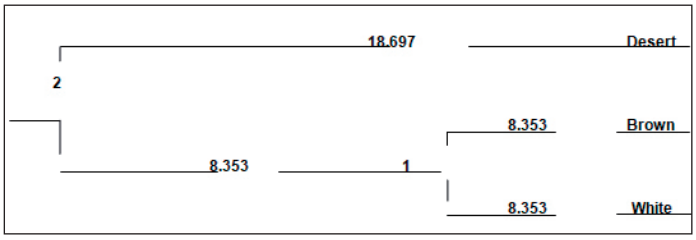

FIGURE (2C). UPGMA DENDOGRAMS SHOWING DIFFERENTIATION AMONG THE THREE LOCAL QUAILS (MALE)

Furthermore, these results were lower than that reported [19] in five quail strains. Moreover, the result showed that there were various genetic distances among studied quail breeds [1] indicating little genetic differentiation between the brown and the white quail was 0.0534, but [28] reported that the genetic identity (0.991) between white and brown quails are clustered into two clusters (groups), the 1st group included of desert lines, while, the 2nd cluster included the brown and white lines. Furthermore, these results were lower than that reported [19] in five quail strains. Moreover, the result showed that there were various genetic distances among studied quail breeds [1] indicating little genetic differentiation between the brown and the white quail was 0.0534 but [27] reported that the genetic identity (0.991) between white and brown quails.

TABLE II

BAND NUMBER AND FRAGMENTS SIXZE (*BP*) IN LOCAL QUAILS

No.	Primers name	Desert				Brown				White				Overall	
		Male		Female		Male		Female		Male		Female			
		No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	Total No. of band	Size range bp
1	OPA-04	5	450-2000	5	450-2000	4	450-1350	5	450-2000	5	450-2000	5	450-2000	29	450-1800
2	OPA-14	3	400-750	4	400-750	3	400-750	3	400-750	4	400-750	4	350-750	21	350-750
3	OPA-20	4	500-1250	4	500-1250	4	500-1250	4	500-1250	4	500-1250	4	500-1250	24	500-1250
4	OPM-06	5	400-1400	4	500-1400	5	400-1400	4	500-1400	5	400-1400	5	400-1400	28	400-1400
5	OPM-20	4	450-2000	3	600-2000	3	450-1500	2	600-1500	4	450-2000	4	450-2000	20	450-2000
6	OPN-16	5	250-2000	5	250-2000	5	250-2000	5	250-2000	5	250-2000	4	250-2000	29	250-2000
7	OPS-01	3	400-1400	3	400-1400	3	400-1400	2	400-700	3	400-1400	4	400-1400	18	400-1400
8	OPQ-07	3	700-2000	3	700-2000	3	700-2000	4	700-2000	3	700-2000	3	700-2000	19	700-2000
9	OPQ-03	5	600-1800	4	600-1800	4	600-1300	5	600-1800	5	600-1800	5	600-1800	28	600-1800
10	OPG-05	5	450-1800	5	450-1800	5	450-1800	5	450-1800	5	450-1800	5	450-1800	30	450-1800
11	OPB-01	4	400-2800	4	400-2800	5	400-2800	3	450-1300	4	400-2800	4	400-2800	24	400-2800
12	OPP-04	5	350-1300	5	350-1500	5	350-1300	6	350-1500	4	350-900	5	350-1500	30	350-1500
13	10 MER	1	900	2	600-900	2	600-900	2	600-900	2	600-900	1	900	10	600-900
	Total	52	250-2800	51	250-2800	51	250- 2800	50	250-2000	53	250-2800	53	250-2800	310	250-2800

TABLE III

UNIQUE BAND NUMBER AND FRAGMENTS SIZE (*BP*) IN LOCAL QUAILS

No.	Primers name	Desert				Brown				White				Overall	
		Male		Female		Male		Female		Male		Female			
		No. of Unique band	Fragment Size bp	No .of Unique band	Fragment Size bp	No. of Unique band	Fragment Size bp	No. of Unique band	Fragment Size bp	No .of Unique band	Fragment Size bp	No. of Unique band	Fragment Size bp	Total No. of Unique band	Size range bp
1	OPQ-07	0	–	0	–	0	–	1	1500	0	–	0	–	1	1500
2	OPB-01	0	–	0	–	1	1350	0	–	0	–	0	–	1	1350
	total	0		0		1		1		0		0		2	1350-1500

TABLE IV
TOTAL NUMBER OF FRAGMENTS, NUMBER AND PERCENTAGE POLYMORPHIC FRAGMENT FOR THIRTEEN PRIMERS USED

No.	Primer name	Total no. of fragment	No. of polymorphic fragment	% of polymorphic fragment
1	OPA-04	6	4	66.7
2	OPA-14	4	1	25
3	OPA-20	4	0	0
4	OPM-06	5	1	20
5	OPM-20	5	2	40
6	OPN-16	5	1	16.667
7	OPS-01	4	2	50
8	OPQ-07	4	1	25
9	OPQ-03	5	2	40
10	OPG-05	5	2	40
11	OPB-01	5	2	40
12	OPP-04	6	2	33.333
13	10 MER	2	1	50
Mean		**4.615**	**1.692**	**34.359**

TABLE V
OVERALL ESTIMATION OF NUMBER OF ALLELES, EFFECTIVE NUMBER OF ALLELES, NEI'S GENE DIVERSITRY AND SHANNON'S INFORMATION INDEX FOR LOCAL QUAIL BREEDS

No.	Primer name	na[1]	ne[2]	h[3]	I[4]
1	OPA-04	1.0000	1.0000	0.0000	0.0000
2	OPA-14	1.0000	1.0000	0.0000	0.0000
3	OPA-20	1.0000	1.0000	0.0000	0.0000
4	OPM-06	2.0000	1.8000	0.4444	0.6365
5	OPM-20	1.0000	1.0000	0.0000	0.0000
6	OPN-16	2.0000	1.8000	0.4444	0.6365
7	OPS-01	1.0000	1.0000	0.0000	0.0000
8	OPQ-07	2.0000	1.8000	0.4444	0.6365
9	OPQ-03	1.0000	1.0000	0.0000	0.0000
10	OPG-05	1.0000	1.0000	0.0000	0.0000
11	OPB-01	1.0000	1.0000	0.0000	0.0000
12	OPP-04	1.0000	1.0000	0.0000	0.0000
13	10 MER	1.0000	1.0000	0.0000	0.0000
Mean		**1.2308**	**1.1846**	**0.1026**	**0.1469**
	St. Dev	**0.4385**	**0.3508**	**0.1949**	**0.2791**

na[1] overall observed number of alleles.

ne[2] overall effective number of alleles.

h[3] overall Nei's gene diversity.

I[4] overall Shannon's information index

TABLE VI
NIES GENEYIC IDENTITY (ABOVE DIAGONAL) AND GENETIC DISTANCE (BELOW BIAGONAL) AMONG LOCAL QUAILS

Pop ID	Desert	Brown	White
Desert	***	0.7692	0.9231
Brown	0.2624	***	0.8462
White	0.08	0.1671	***

CONCLUSION

The data obtained from the lines included in this study are of great importance to the quail holders in Kurdistan Region – Iraq. The percentages of polymorphism (20 to 50%), the Nei's gene diversity (heterozygosity) and Shannon diversity index value averaged 0.1026, and 0.1469, respectively. The genetic distance (10.735 to 27.614) among three different quail colors found in this study indicates that these quail have the required amount of genetic variation to made genetic improvement in this type of bird in near further. This study helps us to clarify the image of the genetic diversity of the local Iraqi quail in and the breeders can used it for mating system when need to make the crossing among these quails

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