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GENETIC RELATIONSHIP BETWEEN LOCAL GUINEA FOWL, QUAIL AND CHICKEN USING RAPD – PCR TECHNIQUE

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ABSTRACT

This study combines three of the most common bird species in both Iraq and the rest of the globe. This type of study demonstrates the degree of genetic convergence between these species. Blood samples from chicken, Guinea fowl, and quail were used in this study. The Four hundred and fifty birds are divided into 150 birds per species. Blood from a bird was sampled. The DNA samples' purities varied from 1.8 to 1.9. the (22) Gen Script USA primers are used. Overall, all of the primers produced (340) bands, 95 of which were polymorphic, with the maximum number of bands belonging to the OPA-20 and the lowest number of bands to the OPA-13. OPA-12 had the highest percentage (50.0), though. Primer OPA-20 has the widest molecular weight range (100- 1500 bp). Guinea fowl have a greatest number of bands overall (131), while quail reported the highest polymorphic bands overall (12) and polymorphism percentage (11.8). The maximum genetic similarity is found between chicken and quail (0.608), it is obvious that the genetic differences between the three groups are greatest between the Guinea fowl, quail and smallest between the quail and the chicken.

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INTRODUCTION

Domestic poultry is the most significant breed of poultry in Iraq. The process of domesticating poultry was initially begun by gathering eggs from wild species to hatch and raise young birds, but later kept the birds permanently in captivity. All domesticated bird species that generate products with a monetary value, such as meat, eggs, and excrement, in captivity are collectively referred to as poultry. The term "poultry" is used to describe a variety of bird species, including chicken, guinea, quail. Birds, together with eggs, are the second most popular meat source consumed worldwide because they contain high-quality protein and little fat, providing food that is nourishing (Fadhil *et al*., 2016). In various nations, guinea bird species have undergone genetic enhancement to increase the productivity of their meat output. Guinea fowl are an important but underappreciated species of poultry due to their high nutritional value and delicious flavour. There are currently no structured breeding programs or reproduction controllers for guinea birds, which are often kept in backyard systems in Iraq. Gaining a greater understanding of guinea bird lines could boost their appeal. Quail farming has recently gained popularity for the production of both meat and eggs. Quail eggs are the ideal source of wholesome, A

delicious food for human nutrition because it provides us with a large amount of high-quality nutrients, and it is also a great source of antioxidants, minerals, and vitamins and Quail are used in various studies for their ease of handling (Tunsaringkarn *et al*., 2013; Abdul-Majeed, and Al-Krad, 2023; Ameen *et al*., 2023).

The genetic variation in various avian species has been studied globally to determine the degree of relatedness or to pinpoint the genes in charge of intriguing features. The specific variations in the nucleotide sequences within genes are molecular in nature. Checking links between species and breeds is connected to genetic identity. The molecular advancement has opened up new opportunities due to the genetic enhancement of poultry. With the use of DNA markers (RAPD, RFLP, VNTR, CRI, SSR-PCR), entire genomes or polymorphic DNA segments can be analyzed. RAPD (Randomly Amplified Polymorphic DNA) is a quick and easy method for detecting relatedness and locating the genes responsible for an avian advantage characteristic. In the study of similarity or variation the RAPD - PCR has been used repeatedly in chickens populations (Abdulrazaq, *et al*., 2023, Dehghanzadeh, et, al., 2009, Smith, et, al., 1996, Sharma, et, al.,2001, Ali, et, al., 2003, Sharma and Singh, 2002, Semenova, *et al*., 2002), ducks (Dolmatova, *et al*., 2000a, b), turkey (Smith, et, al., 1996), animal (Abdulrazaq, et, al., 2019).guinea birds (Daham and Sharma, 2007; Nahashon *et al*., 2010; Sharma *et al*., 1998) Quail (Abdulrazaq, et, al., 2020) Phytoplasmas were detected by PCR and Nested-PCR (Alsawaf and Ali, 2022). The study is to know the genetic comparison of three widely distributed bird species and to knowing genetic affinity between them depends on the DNA.

MATERIALS AND METHODS

Ethics approval

The present study was approved by the Animal Research Ethics Committee (AREC) of College of Science (No. 4625 on 2023). In accordance with the ethical procedures and protocols of the College of Science, Salahuddin University - Erbil, Iraq.

Experimental procedure

Quail, chicken, and Guinea fowl blood samples were used in this study. 150 birds per species make up the 450 birds in total. A sample of 3 ml of bird blood was collected from the wing just above the elbow joint and put in tubes containing an Ethylenediaminetetraacetic acid, (EDTA) solution. The technique outlined in (Sharma *et al.,* 2016) then used to extract DNA. The DNA content and relative purity were measured using the Nano Drop® spectrometer; the purity of the DNA samples ranged from 1.8 to 1.9 (µg/ml). For RAPD-PCR use, the samples were diluted to 30 ng/µl. Using 22 primers from Gen Script USA for the RAPD produced results for the identification of genomic DNA complementary sequences Table (1). A thermal cycler with a T gradient was used to amplify the DNA analysis. Double-stranded DNA underwent first denaturation at 95 °C for five minutes. The next 40 cycles involved holding the temperature at 95°C for 1 minute, 42°C for 1 minute, and 72°C for 2 minutes. Five minutes at 75°C were used to form the complimentary strands. The PCR reaction includes the following elements: Each: (dATP, dCTP, dGTP, and

dTTP) (400 M), DNA (30 ng), primer (10 M), 1x GoTaq® Green Master Mix PCR buffer, and 1x, MgCl2 (3 mM). The total volume of the reaction was 25 µl. The Gene Ruler TM (100–1500 bp) DNA ladder marker at 100 bp. Each sample had 2 µl of Blue / Orange loading day mixed with 10 µl of the product. The functioning of the 100V power supply was finished. It took about 90 minutes to complete the electrophoresis. The PCR results were examined by electrophoresis on 2% agarose gel in 1X TBE buffer with ethidium bromide staining (Promega, USA). After the design had been enlarged by UV light, it was photographed. The enhanced pattern was seen and captured on a UV transluminator. The statistical analysis of Data recording RAPD patterns was recorded (0) due to (1) or absence. The polymorphism of each primer was determined using the formula polymorphism = (Np / Nt) 100, where NP = # polymorphic forms of random primer. The total number of sample primer domains is referred to as Nt (Bowditch, *et al.*, 1993). The numerical data were analyzed with statistics. The method of (Nei and Li.,1979) was used to determine similarity values in all available pair-wise comparisons of individuals between groups. The similarity matrix was submitted to cluster analysis using the unweighted pair group for arithmetic mean (UPGMA) cluster analysis technique, which resulted in the dendrogram. A dendrogram of genetic distance was produced using PAST version 1.34. (Rother, *et al.*, 2021) The closest neighbor technique was used in its creation.

RESULTS AND DISCUSSION

The current study evaluated the genetic relatedness of quail, chicken, and Guinea fowl using the RAPD-PCR method. 22 randomly selected genotypes from the samples were examined. The PCR reaction using the 12 chosen primers had an impact on all three species Table (1). The electrophoresis-acquired bands were used to calculate the number of bands shared by species birds groupings Figure (1). This study is that it combines three species of birds that are most widespread in Iraq and the world, which are used as an important food source for humans. In addition to using it as rich sources of oils used as a treatment or to increase the percentage of immunity in the body after eating a rich meal, for example, Omega 3-6-9. Where this type of studies shows the extent of the genetic convergence between these species, because it is a local species and studying it is considered an authentic work. Under the same reaction conditions, RAPD profiles using a set of 12 primers Table (1) showed consistent, strong, and well-defined bands. For various primers, the degree of polymorphism produced from the target genome in terms of polymorphic band pattern varied Figure (1). A successful use of molecular DNA markers for the discriminating of genetic resources that are economically significant, such as poultry and other farm animals, requires an investigation of genetic variation and relatedness across or within species, populations, and individuals. The RAPD PCR is used in the Deef *et al.* (2022) study to examine if the Egyptian Hoopoe, known as Upupa epops major, is a separate species from the European Hoopoe, known as Upupa epops epops, and to discover the relationships among them. In this study, we compared the use of RAPD-PCR genetic markers to PCR in order to ascertain the genetic relationship between species and the genetic similarity of chicken, quail, and guinea fowl.

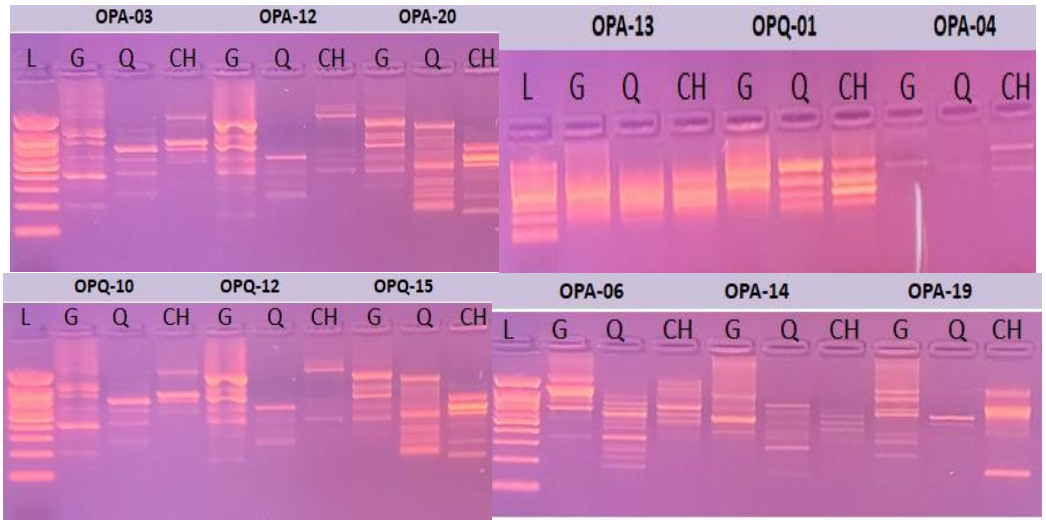

Figure (1): The electrophoretic pattern of genomic DNA amplification in (G) Guinea fowl, (Q) Quail and (CH) Chicken using OPA-03, OPA-12, OPA-20, OPA-13, OPQ-01, OPA-04, OPQ-10, OPQ-12, OPQ-15, OPA-06, OPA-14, OPA-19 primers

Table (1): The sequence of the primers and their % of GC content

Primer name	Sequence 5’ to 3	% GC content
OPA-03	AGTCAGCCAC	60%
OPA-12	TCGGCGATAG	60%
OPA-20	GTTGCGATCC	60%
OPA-13	CAGCACCCAC	70%
OPQ-01	GGGACGATGG	70%
OPA-04	AATCGGGCTG	60%
OPQ-10	GGCTAACCGA	60%
OPQ-12	TCTCCGCAAC	60%
OPQ-15	GGACGCTTCA	60%
OPA-06	GGTCCCTGAC	70%
OPA-14	TCTGTGCTGG	60%
OPA-19	CAAACGTCGG	60%

According to the findings in Table (2), the OPA-20 Primer produced the most bands (42 bands) out of all the species utilized, while the OPA-04 Primer produced the fewest bands (14 bands). Together, all of the Primers produced (340) bands in all, 95 of which were polymorphic, with the highest 13 and lowest 2 bands belonging to the OPA-20 and OPA-13, respectively. The primer OPA-13 showed the largest mono and monomorphic band when compared to the other primers utilized in this investigation. The primer OPA-13 had the lowest percentage of polymorphisms (4.88), while OPA-12 had the highest percentage (50.0). The molecular weight range of Primer OPA-20 is the greatest (100- 1500 bp), whereas the range of Primer OPA-04 is the shortest (400- 1500 bp).

Table (2): The name of primers (P N), Total number of band (T N B), Polymorphic band (P B), Mono band (M B), Monomorphic band (Mm B), % of polymorphism (% P) and size (bp)

P N	T N B	P B	M B	Mm B	% P	Size (bp)
OPA-03	27	5	7	22	18.52	200 - 1500
OPA-12	22	11	2	11	50.00	300 - 1500
OPA-20	42	13	7	29	30.95	100 -1500
OPA-13	41	2	13	39	4.88	200 -1500
OPQ-01	34	6	9	28	17.65	200 -1500
OPA-04	14	6	2	8	42.86	400 - 1500
OPQ-10	30	10	5	20	33.33	200 - 1500
OPQ-12	20	9	2	11	45.00	200 - 1500
OPQ-15	29	6	6	23	20.69	200 - 1500
OPA-06	21	8	3	13	38.10	150 - 1500
OPA-14	29	8	7	21	27.59	150 - 1500
OPA-19	31	11	5	20	35.48	150 - 1500
SUM	340	95	68	245	30.42	100-1500

For three local species, Guinea fowl, Quail, and chicken, there were no such publications on genetic diversity using molecular markers. In all, 340 (TNP) bands were produced, of which 95 were P B, 68 M B, and 245 Mm B. It exceeds because (Abdulrazaq, *et al*., 2020) investigated a total of 80 unique fragments (bands), 29 of which were polymorphic. The difference is bigger than if it were within the same species because of the comparison between species. Each Primer has between 14 and 42 bands that were amplified. The highest number of polymorphic bands (13), as well as the highest percentage of polymorphisms (50%), were found in each primer, which is more than what was indicated by Abdulrazaq (2022) referred to in order to evaluate the genetic relationship between groups of chickens and comprehend the magnitude of genetic differences between local chicken groups. The molecular weight ranges from 100 to 1500 bp at its maximum. According to Fadhil *et al*. (2016), the stated size disparity has a size range of 325 to 1325 bp.

In Table (3) displays the total number of bands, the percentage of polymorphism, and three different genotypes of local species birds (guinea fowl, quail, and chicken). Guinea fowl have the largest total number of bands (131), while quail reported the highest total number of polymorphic bands (12) and percentage of polymorphism (11.8). The genetic variability in a breeder flock of native fowl chickens was assessed using the RAPD approach (Rahimi *et al.*, 2005). In more closely related strains, fewer polymorphisms may be visible with the RAPD approach, but polymorphisms are likely to occur at moderate frequencies amongst distantly related lines (Levin *et al.*, 1994). Using RAPD markers, Guldehen (2002) found an average of 9.2 polymorphic bands per primer between chicken meat and layer pure lines. Five breeds of chicken chosen for early body weight and/or egg production were examined for polymorphism using RAPD markers (Sharma *et al.*, 2001). About 25% of the 96 amplified fragments showed

polymorphism. The presence of genetic variants, which are indicated by the number of alleles at a locus and their frequency of distribution in a population, is frequently the cause of polymorphism in a given population. According to Gupta *et al.* (2008), heterozygosity refers to the likelihood that two alleles chosen at random from a population may be differentiated using the relevant marker. In terms of the Nei's genetic diversity, Shannon's information index, coefficient of population differentiation, and total genotype diversity among and within populations, a useful quantitative assessment of marker utility and the polymorphism discovered may therefore be provided (Zhao *et al.*, 2006).

Table (3): Detail of Total number of band (TNB), Polymorphic band (PB), and % of polymorphism (%P) through three different local species birds genotypes: (Guinea fowl, Quail and Chicken)

Local species	T N B	P B	% P
Guinea fowl	131	10	7.6
Quail	102	12	11.8
chicken	107	11	10.3
sum	340	33	9.7

The genetic similarity between different species is shown in Table (4). The maximum genetic similarity is found between chicken and quail (0.608), while the lowest genetic similarity is found between quail and Guinea fowl (0.513). The dendrogram in Figure (2) shows three groups of birds: Guinea fowl, quail, and chicken. It is obvious that the genetic difference between the three groups is greatest between the Guinea fowl and quail and smallest between the quail and the chicken.

The level of polymorphism between three species with diverse phenotypes was found in the current investigation. In comparison to the Guinea fowl phenotype, the polymorphic bands in the Quail were more frequent and had a higher percentage of polymorphism (%11.8). The genotype with the highest genetic similarity across three different local species of birds is 0.608 in the present stude. The findings of Mollah *et al*. (2009), who indicated increased genetic similarity (82.45 to 90.03%) at genome level in indigenous chicken populations of Bangladesh, were supported by the genetic similarity index data. In the current study, the total loci acquired with the (12) RAPD markers were compared using the UPGMA method, and the dendrogram based on similarity coefficients was built as a result. The three species formed a single large group. The major group's quail and chicken species, which were initially grouped together and then with guinea fowl, had the closest genetic distance to one another.

Table (4): Genetic Similarity estimated among three different local species birds genotypes: (Guinea fowl, Quail and Chicken)

Local species	Guinea fowl	Quail	Chicken
Guinea fowl	1	0.513	0.556
Quail	0.513	1	0.608
Chicken	0.556	0.608	1

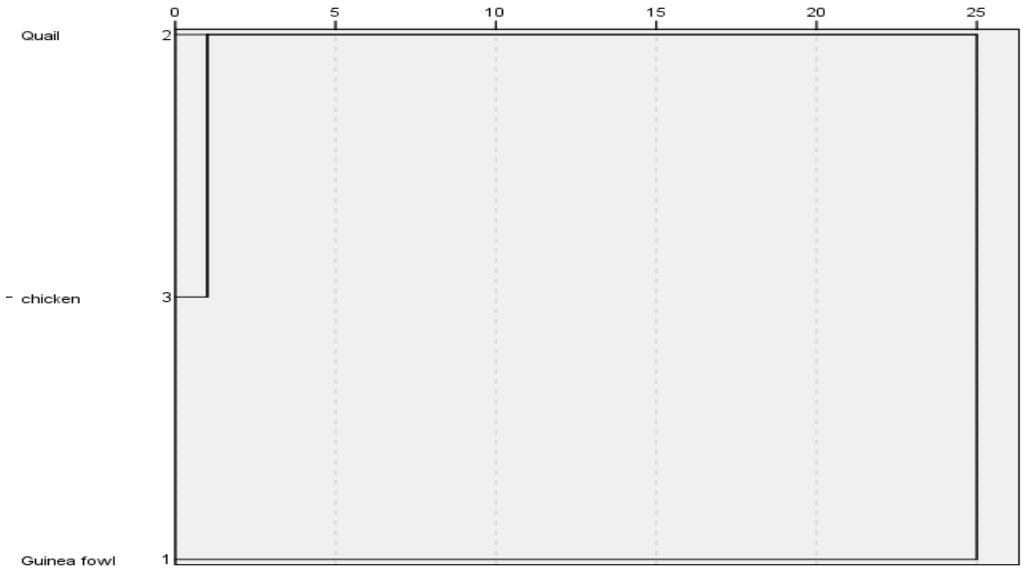

Figure (2): Dendrogram of genetic distance based on RAPD data of three different local species birds genotypes: (Guinea fowl, Quail and Chicken).

CONCLUSIONS

The results were showed, the use of RAPD-PCR to identify the genetic connections between species and the genetic similarity of chicken, quail, and guinea fowl to identify the genetic similarity. Based on the findings of this study, we can draw the conclusion that molecular RAPD markers are a helpful and effective tool for detecting polymorphism and have shown to be highly effective in differentiating between species. What's more, the study of the bands reveals several species-specific bands. Therefore, distinct bands could be cloned and sequenced in order to develop novel diagnostic primers more successful in genetic discriminating among examined species. The development of species-specific markers may also be required to look for quantitative trait loci within bird species that differ in their capacities for production. Therefore, additional research is needed to provide more information about the population structures.

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CONFLICT OF INTEREST

The authors state that there are no conflicts of interest with the publication of this work.

العلاقة الوراثية بين دجاج غينيا، سمان ودجاج المحلي باستخدام تقنية الحمض النووي متعدد الأشكال المضخم العشوائي– تفاعل البلمرة المتسلسل

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الخلاصة

تجمع هذه الدراسة بين ثلاثة من أكثر أنواع الطيور شيوعًا في كل من العراق وبقية العالم. نظرًا لكونه نوعًا محليًا. يوضح هذا النوع من الدراسة درجة التقارب الجيني بين هذه الأنواع. تم استخدام عينات دم من الدجاج وطيور غينيا والسمان. قسمت الطيور البالغ عددها 450 إلى 150 طائرًا لكل نوع. تم أخذ عينات دم من الطيور. تراوحت نقاء عينات الحمض النووي من 1.8 إلى 1.9. تم استخدام اثنان وعشرون بادئاً Gen Script USA. بشكل عام، أنتجت جميع البادئات (340) نطاقًا، 95 منها كانت متعددة الأشكال، مع أقصى عدد من النطاقات التي تنتمي إلى OPA-20 وأقل عدد من النطاقات لـ OPA-13. كان OPA-12 أعلى نسبة مئوية لمتعددة الأشكال (50.0). يحتوي Primer OPA-20 على أوسع نطاق للوزن الجزيئي (100- 1500 bp). تمتلك دجاج غينيا أكبر عدد من العصابات بشكل عام (131)، بينما سجل السمان أعلى عدد من العصابات متعددة الأشكال بشكل عام (12) والنسبة المئوية لتعدد الأشكال (11.8). تم العثور على أقصى تشابه وراثي بين الدجاج والسمان (0.608)، ومن الواضح أن الاختلاف الجيني بين المجموعات الثلاث أكبر بين دجاج غينيا والسمان وأصغر بين السمان والدجاج.

الكلمات المفتاحية: الطيور، الدم، البادئات، التشابه، الأنواع.

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