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RESEARCH ARTICLE

MtDNA Analysis of the Genetic Origin of Jordanian Indigenous Chickens in Comparison With Ancient, Commercial, and Fancy Breeds

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ABSTRACT

Jordan indigenous chickens (JIC) are threatened with extinction since they are crossed with exotic chickens to enhance their low levels of production. The study objective was to investigate the origin, biodiversity, and phylogeographic structure of JIC using mtDNA sequencing in reference to exotic chickens. We sampled 483 males and females by taking a blood spot on an FTA card. We also sampled ancient, commercial, and fancy chickens from Jordan as references. The sampling regions were the main cities and rural areas of the Middle, North and South governorates of Jordan. The extracted DNA from blood samples was sequenced for the mtDNA D-loop region. The results showed that most of the genetic diversity occurred within JIC rather than between populations. The highest expected heterozygosity was for JIC (0.666), whereas the lowest was for Cochin (0.503) chickens. In addition, the coefficients of differentiation (*F_{st}*) ranged from the lowest (0.271) between indigenous and Cochin (fancy) chickens to the highest (0.104) between Pharaonic chickens and Lamborghini. Particularly, a UPGMA-based evolutionary analysis showed high differentiation between JIC and the ancient chickens, indicating a lack of close phylogeographic relationship. This study provides maternal genetic evidence that the primary origin of JICs is linked to Indian chickens because they possess haplotypes of Asian and African origin, with additional groups indicating past and recent crossbreeding with exotic and commercial breeds. . . Finally, it is recommended to establish a genetic conservation program for JIC for their current-day diversity, to ensure better resilience to the current global warming hitting Jordan in recent years.

Keywords: Biodiversity, Food, Security, Conservation, Haplotype

Introduction

Jordanian indigenous chickens (JIC), like most domestic chicken populations, have accumulated substantial genetic diversity over their evolutionary and domestication history.¹ They are represented by several breeds and strains whose phenotypic characteristics vary across different regions of the Hashemite Kingdom of Jordan.² This diversity reflects the combined effects of natural and human-mediated selection for adaptation and production traits under diverse environmental conditions and management

systems.³ However, JICs' genetic resources are increasingly threatened by breed disappearance and the dilution of native gene pools through uncontrolled crossbreeding with exotic commercial chickens. The JIC's disappearance also involves the disappearance of treasured indigenous knowledge assembled by indigenous chicken owners.

JIC is considered a focal pool of genetic resources correlated with adaptation to harsh conditions and tolerance to stress and diseases, leading them for future breeding programs. A complete description of JIC needs a systematic characterization of

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phenotypes, breeding practices, and fundamental genetic variation. Both phenotype and genotype characterization are vital fundamentals for the efficient breeding, genetic improvement, and conservation programs.^{4,5} Consequently, the assessment of JIC as a genetic resource must involve the evaluation of genetic structure and diversity between populations. On the other hand, genetic DNA markers offer strong tools for examining genetic ancestry and variation both at specific sites and over the genome.⁶ Developments in technologies, mainly mitochondrial DNA (mtDNA) genotyping, have substantially boosted studies of detecting chickens' genetic origin and maternal lineage.⁷ MtDNA genotyping traces domestication pathways, identifies potential origin centers, and infers the chicken's ancestry.^{8–10} MtDNA control region (D-loop) sequencing has shown well-organized clarification of the genetic history and phylogeographic structure of chicken breeds through detecting haplogroups.^{11,12} For instance, many haplogroups were reported by Mwacharo et al.,¹² revealing high maternal genetic diversity in village chickens of East Africa. The results were linked to trading routes of ancient times that connected the Middle East with the Indian subcontinent. Similarly, Al-Jumaili et al.⁸ reported that Indian maternal chicken lineages were contributing to the genetic resources of chickens across West Asia and Africa.

Despite these advances, comprehensive studies on the genetic diversity and maternal origins of JIC remain limited. The primary objective of this study is to systematically assess the genetic diversity and population structure of JIC across distinct geographic and ecological regions using mitochondrial DNA (mtDNA) sequencing. The secondary objective is to elucidate the maternal genetic origins and phylogeographic relationships of these populations and to evaluate their implications for the conservation and sustainable management of JIC genetic resources. Collectively, these objectives could be utilized to generate scientific evidence to inform conservation strategies and support the long-term resilience of local poultry populations under changing climatic conditions and increasing pressures on food security.

Materials and methods

Chicken samples collection and study area

Jordanian indigenous chickens (JIC) were sampled from rural and urban locations across the three main geographic regions of Jordan: North, Middle, and South. Indigenous chickens were classified into ecotypes based on their geographic origin, with each ecotype named after the city from which it was sampled [Table 1](#). Chickens aged eight months or older

were randomly selected. Body weight was recorded, and plumage characteristics were documented for each individual following the methodology described by Al-Jumaili et al.⁸

A total of 585 chickens representing twelve ecotypes were included in the study. Of these, 483 were indigenous chickens, while 102 belonged to eight exotic breeds used as reference populations. The exotic breeds comprised commercial strains (Broiler, ISA-Brown, and Leghorn), ancient breeds (Pakistani, Pharaonic, and Lamborghini), and fancy breeds (Cochin and Fayoumi). Exotic chickens were sampled from private farms in Jordan where they were reared either independently or alongside indigenous chickens. Sample sizes for exotic breeds were as follows: Pakistani (n = 14), Broiler (n = 8), Cochin (n = 10), Fayoumi (n = 13), ISA-Brown (n = 13), Leghorn (n = 31), Pharaonic (n = 5), and Lamborghini (n = 8).

Blood sampling and ethical approval

Blood samples were collected from the wing vein of each bird and applied to FTA cards for DNA preservation. All animal handling and sampling procedures were performed according to Mwacharo et al.¹² and in accordance with the ethical guidelines approved by the Mutah University Ethics Committee (Approval ID: AGR-1-15-2018).

DNA extraction

Genomic DNA was extracted from dried blood spots on FTA cards (Whatman Biosciences; <https://www.sigmaaldrich.com/JO/en/product/sigma/whawb120206>). For each sample, 10–15 punches (1.2 mm diameter) were obtained using a QIAGEN UniCore Punch Kit. To prevent cross-contamination, the punch tip was decontaminated with sodium hypochlorite solution between samples. Punches were incubated overnight at 56°C in 200 µL of solid tissue buffer with 40 µL of proteinase K. DNA extraction was performed using the Quick-DNA Miniprep Plus Kit (Zymo Research, USA; <https://zymoresearch.eu/products/quick-dna-miniprep-plus-dna-extraction-kit>) following the manufacturer's protocol. DNA quality and concentration were measured using a Nabi spectrophotometer (MicroDigital, Korea; <https://www.bio3.my/nabi-korea>).

mtDNA amplification

A ~560 bp fragment of the mitochondrial DNA (mtDNA) control region (D-loop) was amplified by polymerase chain reaction (PCR). Each reaction contained 100 ng of genomic DNA,

10 μ L of 2 $\times$ i-MAX II PCR Master Mix (iNtRON Biotechnology, South Korea), 1 μ M of the forward primer AV1F2 (5'-AGGACTACGGCTTGAAAAGC-3'), 1 μ M of the reverse primer H547 (5'-ATGTGCCTGACCGAGGAACCAG-3'), and nuclease-free water to a final volume of 20 μ L. PCR was performed using a QuantGene 9600 thermal cycler (BIOER Technology) with the following conditions: initial denaturation at 94°C for 2 min; 40 cycles of denaturation at 94°C for 30 s, annealing at 63°C for 30 s, and extension at 72°C for 30 s; followed by a final extension at 72°C for 5 min. Gel Electrophoresis.

PCR products were resolved by agarose gel electrophoresis using 2% agarose E (Condalab, Spain) prepared in TE buffer and stained with RedSafe dye (iNtRON Biotechnology, South Korea). Electrophoresis was conducted at 70 V for 30 min. DNA fragments were visualized using a Vilber gel documentation system, and fragment sizes were estimated using the SiZer™-1000 DNA ladder (iNtRON Biotechnology, South Korea).

Sequencing read

PCR products of the expected size (approximately 500–600 bp) were purified and sequenced using Sanger sequencing by a commercial service provider (Genetics Company for Biotechnology, - www.genetics-jo.com/en-Jordan).

Genetic data analysis

Genetic diversity parameters, including the number of polymorphic sites, haplotype number and diversity, average number of nucleotide differences (K), and Tajima's D statistic, were calculated using Arlequin v3.5.2.¹³ Phylogenetic relationships among haplotypes were assessed by determining the best-fit nucleotide substitution model using jModelTest v2.1.7. Maximum likelihood (ML) phylogenetic trees with branch support were constructed using PhyML with 1,000 bootstrap replicates. Additionally, haplotype dendrograms were generated using MEGA v7.¹⁴ In addition, Analysis of Molecular Variance (AMOVA) was conducted in Arlequin with 1,000 permutations to partition genetic variation within and among breeds. For indigenous chickens, individuals from each city were grouped into ecotypes for intra- and inter-ecotype analyses.

Demographic analysis and neutrality tests

Demographic histories of each breed and ecotype were inferred using mismatch distribution analysis.

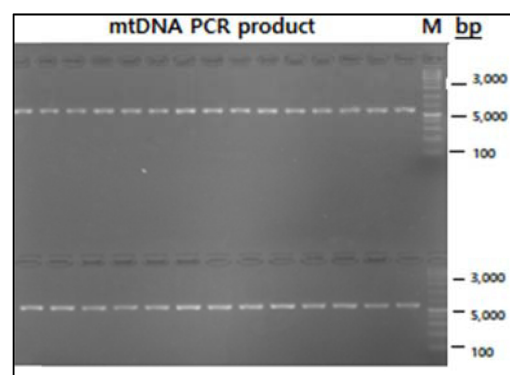


Fig. 1. Agarose gel electrophoresis of mtDNA PCR product (520 bp) DNA samples. Lane M contains a 1000 bp DNA ladder (vendor: SiZer™- DNA ladder) used for molecular weight estimation.

Neutrality tests, including Fu's Fs, Tajima's D, and Fu and Li's statistics, were calculated under the infinite-sites model implemented in Arlequin. In addition, MEGA v7¹⁴ was used to provide evolutionary analysis based on mtDNA haplogroups of the studied chicken populations.

Results

PCR products of mtDNA

JIC demonstrated big genetic variation, which caused difficulties in making inferences regarding genetic diversity and breed purity without mtDNA analyses. Firstly, PCR magnification effectively produced a 520 bp fragment for whole samples, proving successful DNA extraction and PCR and gel electrophoresis techniques Fig. 1. In brief, DNA fragment sizes were determined by comparison with the reference marker of 1 Kb. Prominent sample bands migrate in the ~3–5 kb range, consistent with the expected fragment size. Band alignment across lanes indicates reproducible electrophoretic separation and comparable DNA loading. Furthermore, sample bands align horizontally across lanes, allowing direct size comparison to the ladder bands. Reproducibility of bands appears in corresponding lanes at consistent heights, suggesting uniform electrophoresis conditions and minimal gel distortion.

Genetic variation and AMOVA

Analysis of molecular variance (AMOVA) indicated low genetic differentiation among the ten indigenous chicken ecotypes, with only 5.11% of total variation observed between ecotypes and 94.89% within ecotypes Table 1A. When both indigenous and exotic breeds were included, variation between breeds

Table 1. AMOVA analysis of chickens' ecotypes and breeds.

S.O.V	D.F	S.S	Components of Variance	Variation Percentage (%)
<i>A. AMOVA genetic variation for Indigenous chickens (Intra-Breed)</i>				
Among ecotypes	10	30247	10.283	5.11
Within ecotypes	3321	634671	191.11	94.89
Total	3331	664918	201.39	
<i>B. AMOVA genetic variation for all chicken breeds (Inter-Breeds)</i>				
Among breeds/ecotypes	8	34688	14.05	6.73
Within breeds/ecotypes	4755	926309.7	194.8	93.27
Total	4763	960998	208.86	

S.O.V: Source of Variation D.F: Degrees of freedom S.S : Sum of squares.

Table 2. Results from Tajima's neutrality test for indigenous, broiler, cochin, fayoumi, ISA brown, leghorns, pharaonic and lamborghini chicken breeds.

Breed	M	S	p_s	Θ	π	D	H_e
Indigenous (JIC)	483	162	1.000	0.148	0.662	10.626	0.666
Pakistani	14	599	1.000	0.314	0.711	5.71	0.666
Cochin	10	580	1.000	0.353	0.667	4.450	0.503
Fayoumi	13	559	1.000	0.322	0.683	5.171	0.632
ISA Brown	13	559	0.998	0.322	0.603	4.044	0.644
Leghorns	31	513	1.000	0.250	0.669	6.496	0.559
Broiler	8	542	1.000	0.386	0.704	4.533	0.601
Pharaonic	5	551	0.950	0.456	0.621	2.772	0.616
Lamborghini	8	544	0.977	0.377	0.595	3.181	0.518

m = number of sequences, S = Number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π = nucleotide diversity, and D is the Tajima test statistic, H_e is expected heterozygosity.

increased slightly to 6.73%, with a corresponding within-breed variation of 93.27% Table 1B.

Nucleotide diversity and haplotype analysis

The size of the sample (M), segregating sites (S), nucleotide diversity (π), Tajima's D statistic (D), and expected heterozygosity (H_e) for each population or breed were reported in Table 2. π was maximum in the Pakistani population, revealing significant genetic maternal variation in the populations. Among all populations, the H_e was 0.592 ± 0.06 , with the maximum estimate found in JIC ($H_e = 0.666$) and the lowest value in Cochins ($H_e = 0.503$). Haplogroups' diversity showed the dispersal and sharing of unique haplotypes among populations. The highest frequency haplotype (20%) was found in the Madaba region. Considering regions, for example, North Jarash chickens exposed the maximum haplotype diversity (0.944), revealing significant genetic variation within the population.

Phylogenetic analysis and neutrality tests

Indigenous chickens exhibited the highest values in Tajima's neutrality test, indicating significant departures from neutral evolution. The maximum likelihood (ML) estimate of the nucleotide substitution matrix for indigenous chickens was -8276.878 ,

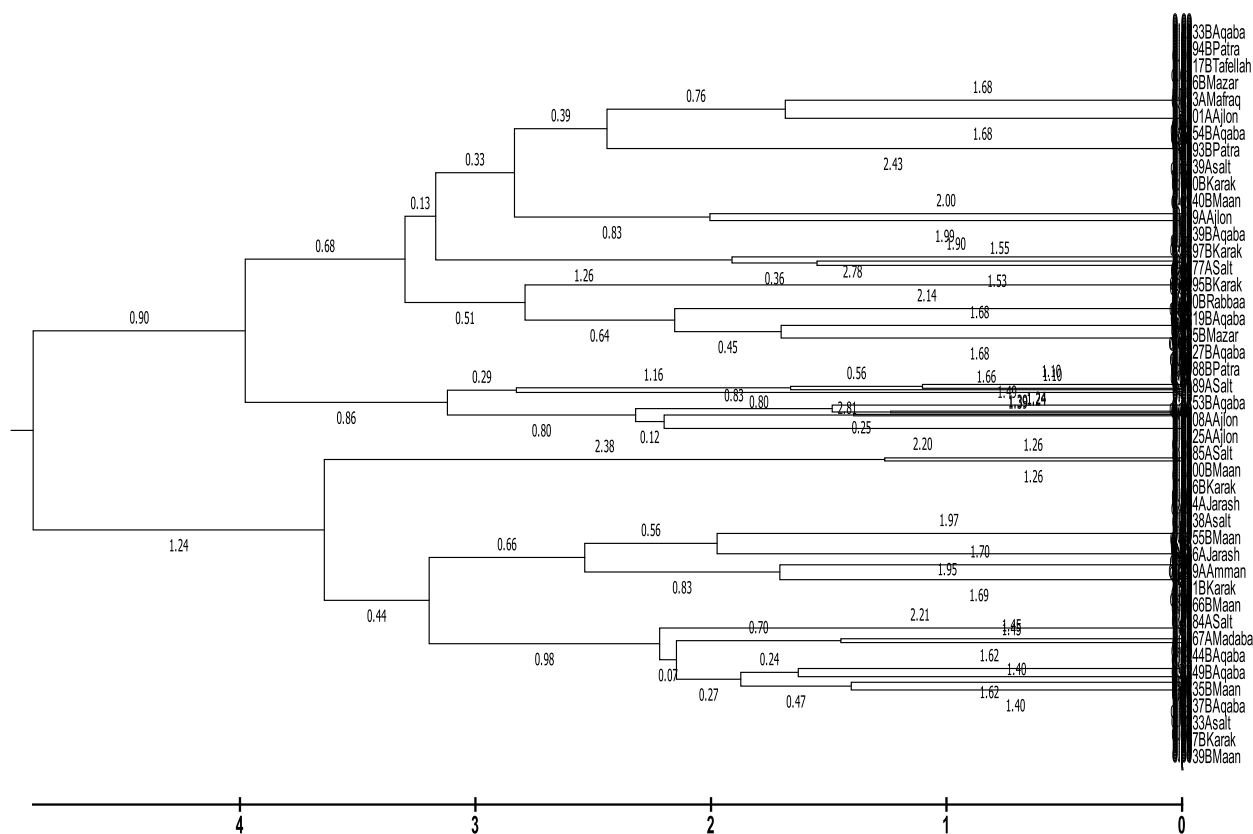
as shown in Table 3. Nucleotide substitution probabilities (r) were calculated under the Tamura-Nei model, with nucleotide frequencies revealing higher proportions of adenine (A) and thymine (T) (~31% each) and a lower frequency of guanine (G). Correspondingly, the lowest transversion substitution rate was observed for G, whereas A exhibited the highest transversion rate Table 3.

ML-based phylogenetic analysis produced a tree topology illustrating relationships among individuals within each indigenous chicken ecotype Fig. 2. In addition, a UPGMA dendrogram was constructed to depict evolutionary relationships among ecotypes and the overall evolutionary history Fig. 3. The optimal ML tree exhibited a total branch length of 82.23.

For enhanced visualization of genetic relationships among ecotypes, Fig. 4 presents a high-resolution plot of mtDNA-based genetic distances. Certain ecotypes, such as Madaba and Ajlon, are genetically distinct from other populations but closely related to each other. Conversely, several ecotypes clustered together, reflecting shared genetic backgrounds and similar D-loop haplotypes. Geographically proximate ecotypes also formed genetic clusters: chickens from Tafallah, Petra, and Maan (southern Jordan) grouped together, whereas Irbid and Ajlon (northern Jordan) formed a separate cluster, indicating regional patterns of genetic relatedness among indigenous chickens.

Table 3. Maximum likelihood estimate (–8276.878) * of substitution matrix in indigenous chickens. rates of different transitional substitutions are shown in bold and those of transversions substitutions are shown in italics.

	Frequency	A	T	C	G
A	29.22	-	8.32	6.78	7.17
T	30.35	8.11	-	10.00	3.32
C	27.25	8.11	12.27	-	3.32
G	13.18	17.50	8.32	6.78	-

**Fig. 2.** Maximum likelihood optimal tree with the sum of branch length = 82.23.

Genetic differentiation among breeds

Genetic differentiation between Jordanian indigenous chickens (JIC) and other domestic breeds was assessed using pairwise fixation indices (F_{ST}) Table 4. F_{ST} estimates based on the pairwise distance method were significant for all breed comparisons. Pairwise F_{ST} values ranged from 0.0271 between indigenous and Cochin (fancy) chickens, indicating low differentiation, to 0.104 between Pharaonic and Lamborghini chickens, representing the highest differentiation. Pharaonic chickens generally exhibited high levels of genetic differentiation relative to all other breeds. Overall, F_{ST} estimates indicated substantial genetic differentiation between indigenous chickens and ancient breeds, whereas differentiation between indigenous chickens and commercial breeds (Fayoumi, Leghorn, ISA-Brown) was comparatively lower. The relations are shown in Fig. 5, which con-

firms a slight distinction between JIC and Cochins. However, a marked distinction is obvious between JIC and Pharaonic chickens. The minimal variation was observed within commercial populations and between the strictly related populations.

Furthermore, genetic relationships, at the individual level, are pictured in Fig. 6. Cochins are closely grouped with JIC, revealing a common genetic origin, whereas commercial chickens are more genetically differentiated from JIC. The outcomes revealed various genetic differentiation and relationship patterns between JIC, commercial, ancient, and fancy chicken populations.

Discussion

The chickens of Jordan have long been assumed to be inherently derived from the earliest existing

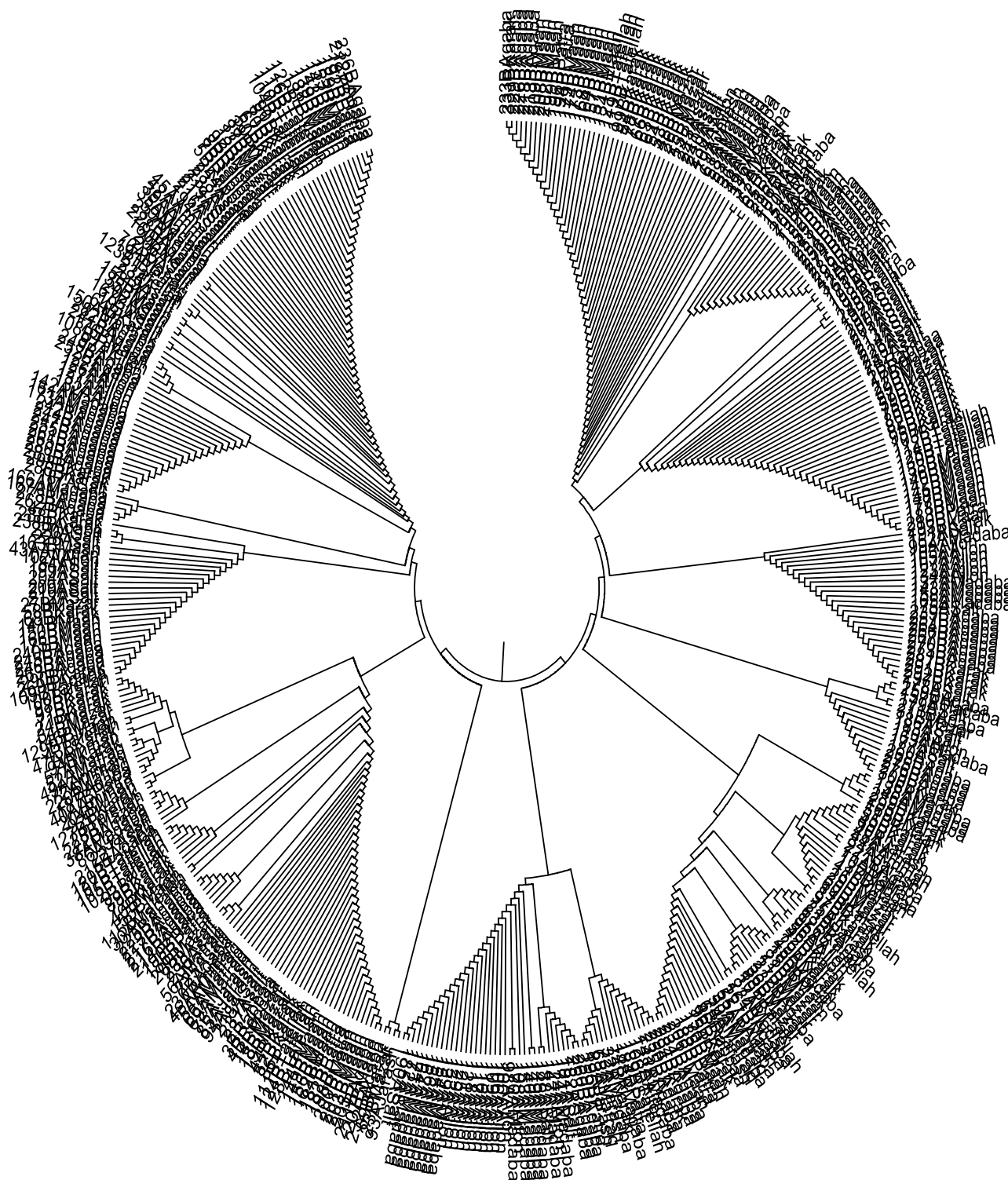


Fig. 3. Evolutionary relationships of Indigenous chickens using the UPGMA method for each individual of each ecotype. (The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site).

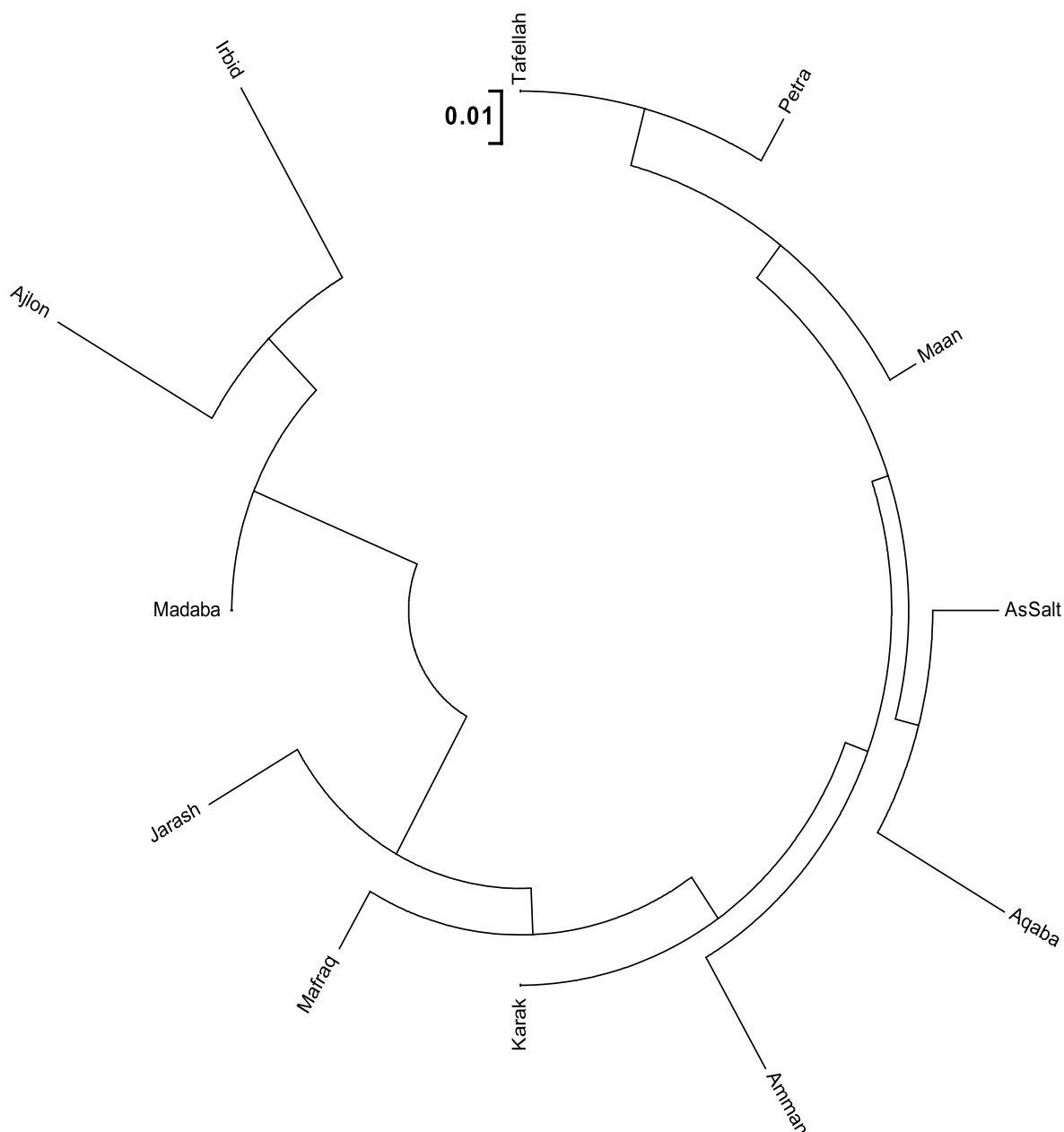


Fig. 4. Evolutionary relationships of Indigenous chickens' ecotypes.

Table 4. Pairwise differentiation coefficients (F_{st}) between the chicken populations.

Breed	Indigenous	Pakistani	Cochin	Pharaonic	Fayoumi	Leghorns	ISABrown	Broiler
Pakistani	0.047							
Cochin	0.034	0.085						
Pharaonic	0.120	0.164	0.166					
Fayoumi	0.037	0.089	0.060	0.171				
Leghorns	0.051	0.091	0.121	0.177	0.084			
ISABrown	0.051	0.129	0.069	0.208	0.093	0.138		
Broiler	0.052	0.099	0.107	0.171	0.083	0.098	0.136	
Lamborghini	0.108	0.149	0.130	0.271	0.126	0.184	0.097	0.158

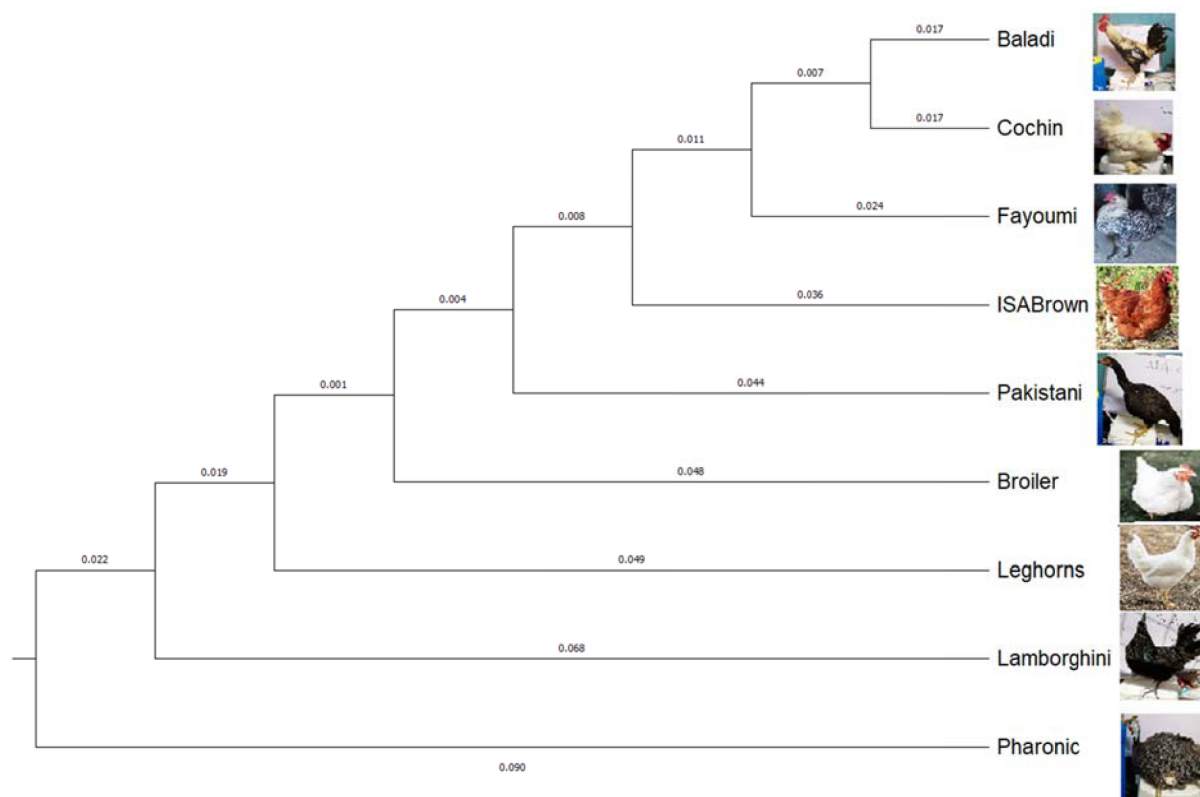


Fig. 5. Relationship of evolutionary distances and historical origin of chicken populations based on UPGMA methodology.

population, such as Pakistani, Malay, Chinese and Egyptian (Pharaonic) chickens. Some studies have indicated that modern JICs may represent crossbreds established throughout time by introducing many populations from distinctive regions.^{15–17} The current study offers suggestions focusing on JICs that are descendants of a sole ancestry. The evidence of mtDNA sequencing provided a precise judgement of maternal origins, indicating, as the first study, systematic research based on the maternal genetic material of JICs. These results provide further insights into the evolution, domestication and genetic development of JICs. Furthermore, the results showed differing levels of genetic diversity among JICs, which are grouped as ecotypes. For example, the ecotypes of the central region (As-Salt, Amman and Madaba) revealed greater nucleotide diversity in comparison with the Northern and Southern ecotypes. Particularly, the Southern ecotype showed the maximum haplotype diversity and nucleotide diversity (0.929 ± 0.021 and 0.0150 ± 0.0006 , respectively) [Fig. 4](#). Pairwise F_{ST} coefficients showed that JICs are genetically close to Pakistani and Cochin (fancy) chickens. Although they showed significant variation from ancient populations, such as Lamborghini and Pharaonic chickens. The findings are consistent with previous observa-

tions.¹⁶ JICs were also relatively closer to commercial breeds (Broiler, Leghorn, ISA-Brown and Fayoumi), indicating combined maternal ancestry and potential of historical and contemporary crossbreeding. The finding of genetic ancestry of maternal origin in JICs is consistent with findings in other indigenous populations of chicken in the world, including Africa, where several introductions of foreign populations and breeds have contributed to genetic variation and differentiation.¹²

JICs ecotypes of nearby geographical regions were usually genetically alike [Fig. 3](#), suggesting crossbreeding practices taking place amongst them. Past events of chicken movement, such as weekly chicken saleyards in Amman, the capital, live birds trading among farmers and from commercial farms in the central region, have likely facilitated gene flow between JICs and exotic breeds through crossbreeding. Many examples of crossbreds or individuals of gene flow from ancient, fancy, and commercial layer breeds into the indigenous gene pool were reported [Figs. 5 and 6](#). The findings are in accordance with previous studies.^{2,3,18}

Moreover, the maternal haplotypes identified in JICs manifested historical relationships of chicken dispersal and domestication in the region (Middle

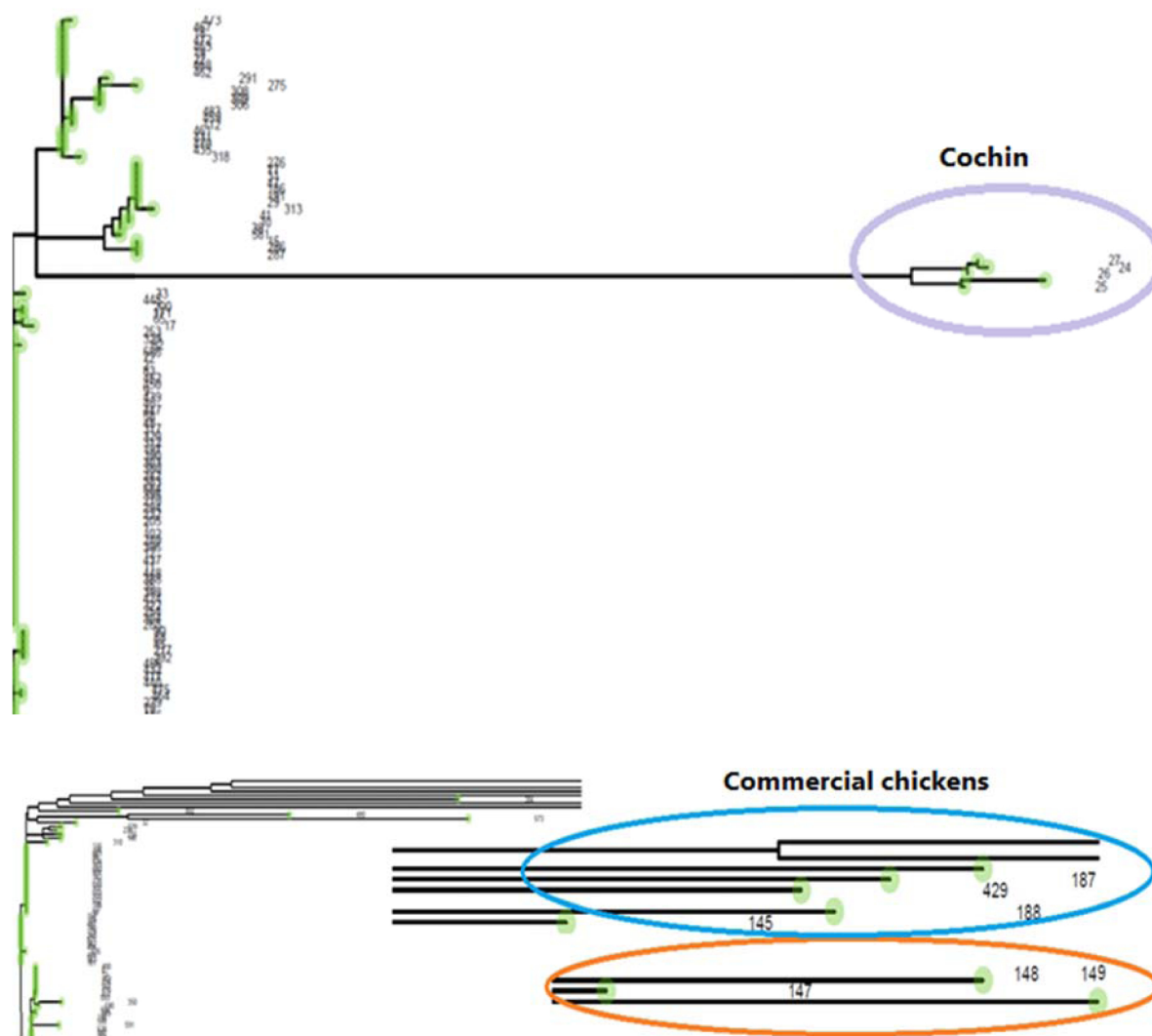


Fig. 6. Evolutionary relationships and history of chicken breeds using the UPGMA method.

East).^{8,19,20} Several haplotypes matched those of breeds from nearby regions, consistent with ancient trade routes between Europe and Asia during Antiquity. The trade routes, Silk and Spice Routes, were the main connection between ancient civilizations of the Old World, and they were a means of introducing Indian and Chinese chickens through terrestrial pathways along the Nile Basin or coastal routes via the Horn of Africa, passing over Asia.^{21–24} The Incense Route, a secondary route, may have been involved in introducing chickens to the Red Sea and Egypt from the Fertile Crescent and vice versa.^{8,25} Generally, JICs demonstrate great genetic variability and share haplotypes with global and regional chicken populations, underlining a distinctive ancient and evolutionary origin.

Conclusion

The study suggests maternal genetic evidence defending the theory that the origin of JICs is connected to Indian chickens. The chickens retain haplotypes of Asian and African origin, revealing historical and current crossbreeding practices with foreign and commercial breeds. The results support the extreme genetic differences and variation within JICs. Finding such knowledge of unique genetic resources and adaptation to local environmental conditions, *ex-situ* and *in situ* conservation of JICs is strongly recommended. Moreover, genome-wide investigations could enhance breeding strategies to identify economically important genes related to resilience to climate change, feed scarcity, and disease resistance.

Finally, the results might be employed for ensuring sustainable JIC production under Jordan's current and future environmental challenges.

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Authors' declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Any Figures and images, that are not mine, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No human studies are present in the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee at University of Mutah.

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Data availability

The data and needed information are available upon request

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تحليل الحمض النووي للميتوكوندريا لتحديد الأصل الجيني للدجاج المحلي الأردني مقارنةً بالسلالات القديمة والتجارية والزينة

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الخلاصة

يواجه الدجاج الأردني البلدي الأصل (المحلي) (JIC) خطر الانقراض نتيجة تهجينه مع سلالات دجاج أجنبية لتحسين إنتاجيته المنخفضة. وقد هدفت هذه الدراسة إلى التحقق من أصل الدجاج الأردني الأصل وتنوعه البيولوجي وبنيته الجغرافية الوراثية باستخدام تقنية تسلسل الحمض النووي للميتوكوندريا، وكذلك بالرجوع إلى سلالات الدجاج الأجنبية مرجعية. تم أخذ عينات دم من 483 دجاجة (ذكورًا وإناثًا) باستخدام بطاقة FTA. كما تم أخذ عينات من سلالات الدجاج المرجعية حيث مثلت دجاج قديم وتجاري وهجين، وهي سلالات موجودة في الأردن. شملت مناطق أخذ العينات المدن الرئيسية والمناطق الريفية في محافظات الوسط والشمال والجنوب الأردنية. خضع الحمض النووي المستخلص من عينات الدم لتحليل تسلسل منطقة حلقة D في الحمض النووي للميتوكوندريا. وقد أظهرت النتائج أن معظم التنوع الجيني يتركز داخل الدجاج الأردني الأصل وليس بين السلالات المختلفة. سُجلت أعلى نسبة تباين وراثي متوقعة في الدجاج الأردني الأصل (0.666)، بينما سُجلت أدنى نسبة في دجاج كوشين (0.503). بالإضافة إلى ذلك، تراوحت معاملات التمايز (Fst) من أدنى قيمة (0.271) بين الدجاج المحلي ودجاج كوشين إلى أعلى قيمة (0.104) بين الدجاج الفرعوني ودجاج لامبورغيني. وعلى وجه الخصوص، أظهر تحليل تطوري قائم على طريقة UPGMA تمايزًا عاليًا بين دجاج JIC والدجاج القديم، مما يشير إلى عدم وجود علاقة جغرافية وراثية وثيقة. تُقدم هذه الدراسة أدلة وراثية أمومية تُشير إلى أن الأصل الأساسي لدجاج JIC مرتبط بالدجاج الهندي نظرًا لامتلاكه أنماطًا وراثية من أصول آسيوية وأفريقية، مع وجود مجموعات إضافية تُشير إلى تهجين سابق وحديث مع سلالات أجنبية وتجارية. وأخيرًا، يُوصى بإنشاء برنامج للحفاظ على الجينات الخاصة بدجاج JIC نظرًا لتنوعه الحالي، بافتراض قدرته على التكيف بشكل أفضل مع ظاهرة الاحتباس الحراري التي تُؤثر على الأردن في السنوات الأخيرة.

الكلمات المفتاحية: النمط، تنوع، حفظ حيوي، أمن غذائي.