

Study of Cyt B Gene Genetic Diversity for Japanese quail in Iraq (*Coturnix japonica*)

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Abstract

A 358-bp fragment of the cytochrome b gene was analyzed using primers 14816 and H15173 from twenty Japanese quail (*Coturnix japonica*) to assess genetic diversity and phylogenetic variation among individuals. Reference Cyt b gene sequences obtained from GenBank for quail from different countries were also included for comparison. Sequence alignment revealed two nucleotide substitutions at positions 81 (A>G) and 145 (G>A). The 81(A>G) mutation showed frequencies of 0.70 and 0.30 for the AA and GG variants, respectively, while the 145(G>A) mutation displayed frequencies of 0.75 and 0.25 for the corresponding variants. In contrast, 13 polymorphic sites were detected in the international sequences, producing six haplotypes, whereas the local quail exhibited three haplotypes. The nucleotide diversity (π) was 0.0022 in the local quail compared with 0.0093 in the global strains, and the haplotype diversity (Hd) was 0.484 and 0.641, respectively. Phylogenetic analysis showed that local samples clustered within a single branch comprising three sub-branches, indicating a shared maternal origin and a narrow genetic base. In contrast, the global quail populations were distributed across several distinct clades, reflecting broader genetic diversity. The G+C content ranged from 0.478 in local birds to 0.493 in global populations, indicating stable base composition. The Tajima's D value was positive (0.89508) for local quail and negative (-0.64464) for global strains, suggesting contrasting evolutionary dynamics. These findings highlight the influence of geographic isolation and small population size on reduced genetic diversity and emphasize the need for breeding programs that enhance genetic variation in local quail populations.

Keywords: Cyt B, Gene , Japanese quail, Genetic Diversity.

Introduction

The Japanese quail (*Coturnix japonica*) is one of the most popular small poultry species raised worldwide. It is recognized for its rapid growth, early sexual maturity, and high egg production, making it valuable for both meat and egg production [1]. In Iraq, interest in quail farming has increased in recent years because the species can easily adapt to hot climates and limited feed resources. Quails provide a good source of animal protein and are easier to manage compared with other poultry species. Despite these advantages, local quail populations in Iraq may face a loss of genetic variation. This reduction in

diversity can occur when breeding programs rely on a small number of individuals or apply intensive selection for specific traits. Decreased genetic diversity can lead to reduced adaptability and lower productivity over time [2]. Therefore, understanding the genetic structure of local quail populations and comparing them with those from other regions is essential for maintaining their long-term sustainability. One of the most useful genetic markers for studying genetic variation and population structure is the cytochrome b (Cyt b) gene. This gene, which is part of mitochondrial DNA

(mtDNA), plays a key role in cellular energy production. Because mtDNA is maternally inherited and evolves gradually over generations, it provides valuable insights into the genetic background and evolutionary history of species [3]. The Cyt b gene, in particular, has been widely used to detect genetic variation within and between bird species and to trace patterns of domestication and adaptation [2,4].

In Iraq, there is still limited information regarding the molecular diversity of Japanese quail. Studying the Cyt b gene can help clarify how local populations are genetically related to foreign breeds and whether they have developed unique genetic traits suited to Iraq's environmental conditions. The results of such studies can provide a scientific

Material and Methods

Twenty Japanese quail (*Coturnix japonica*) were obtained from a local breeder, approximately 10 µL of blood was collected from the wing vein of each bird. Genomic DNA was extracted using the (Geneaid) DNA extraction kit (Geneaid, South Korea) following the manufacturer's recommended protocol. The purity and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer. The DNA concentration ranged between 37 into 82 ng/µL, with an average purity ratio (A260/A280) is 1.76, indicating high-quality DNA suitable for downstream analysis. Polymerase Chain Reaction (PCR) was conducted using a Multigen thermal cycler (Germany) to amplify a 358 base-pair fragment of the cytochrome b (Cyt b) gene. The primer pairs 14816 (5'-

foundation for improving breeding programs, conserving local genetic resources, and supporting the sustainable development of quail production in the country. This study therefore aims to analyze the genetic diversity of the Japanese quail (*Coturnix japonica*) in Iraq by examining the cytochrome b (Cyt b) gene, which is part of the mitochondrial genome. The research seeks to determine the extent of genetic variation among local quail populations, compare them with international breeds, and assess the degree of genetic similarity or divergence. It also aims to explore evolutionary relationships among different quail groups and identify whether the local populations possess unique genetic signatures that reflect adaptation to Iraq's environmental conditions.

CCATCCAACATCTCAGCATGATGAA A-3') and H15173 (5'-CCCCTCAGAATGATATTTGTCCTCA-3') were adopted from [5]. The PCR mixture (25 µL total volume) consisted of 13 µL of MasterGreen 2× Mixture (Promega), 1 µL each of forward and reverse primers (10 pmol/µL), 4 µL of DNA template, and 6 µL of nuclease-free water.

The PCR amplification conditions were as follows: an initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 61°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 7 minutes. Amplified PCR products were electrophoresis on a 1.5% agarose gel at 85

V and 75 mA for 35 minutes. After confirming successful amplification, the products were sent to MacroGen Inc. (Seoul, South Korea) for sequencing. The obtained sequences were analyzed using MEGA version 11.0 [6]. To construct phylogenetic trees based on the Neighbor-Joining Tree (NJ) model. The relationships between Cyt b gene

sequences of local Japanese quail and reference sequences from the National Center for Biotechnology Information (NCBI) database were further examined using Network software version 10.2.0.0 [7]. Molecular diversity indices, including haplotype diversity (Hd) and nucleotide diversity (π), were estimated using DnaSP software version 5.10 [8].

Results and Discussion

The electrophoresis results of the amplified Cyt b gene fragment on a 1% agarose gel confirmed the successful amplification of

the target fragment, with an expected product size of 358 bp. This result was further verified by sequencing analysis of the PCR products conducted by MacroGen (Korea), as illustrated in fig. (1).

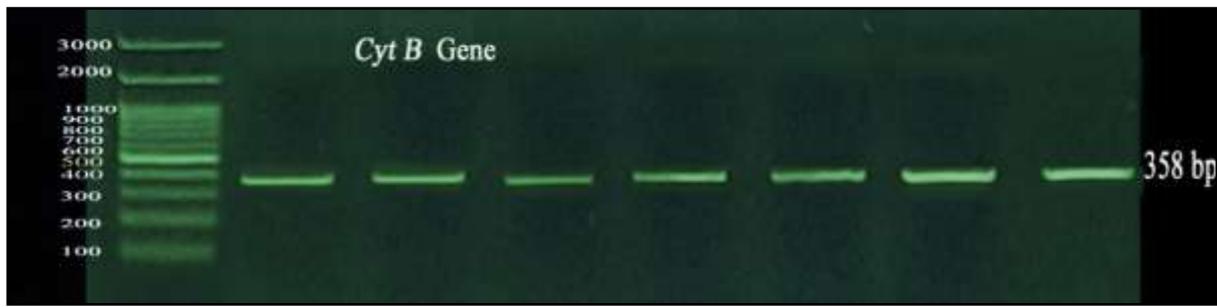


Figure 1: Electrophoresis analysis of PCR product amplified with *Cytb* Gene for Japanese quail in this study

The detection of two nucleotide substitutions (81.A>G and 145.G>A) in the Cyt b gene table (1) of Japanese quail indicates the presence of moderate genetic variation within the studied population. Such variation may reflect natural differences resulting from mutation and genetic drift rather than artificial selection. According to [2], the Cyt b gene is a sensitive marker for assessing genetic divergence among quail populations due to its relatively high rate of mitochondrial

mutation. Similarly, [4] noted that point mutations in mitochondrial genes can accumulate over time, providing insight into population differentiation and evolutionary adaptation. The observed polymorphism in this study suggest that the local Iraqi quail population retains a level of genetic variability comparable to that found in other quail breeds worldwide. This diversity may contribute to maintaining adaptability and resilience

under local environmental conditions, and conservation strategies. which is essential for sustainable breeding

Table. 1 Nucleotides Variants for Cyt B gene in local Japanese quail

The results of the multiple sequence the genotype GG. Regarding the second

Polymorphism Frequency	Polymorphism		Site
0.70	14	AA	81.A>G
0.30	6	GG	
0.75	15	GG	145.G>A
0.25	5	AA	

alignment analysis table (2) of the Cyt b gene sequences in the Japanese quail, performed using the CLUSTAL O. V. (1.2.4) tool (Figure 2), revealed two distinct mutations. The first mutation, located at position 81 (A>G), showed that 14 individuals possessed the homozygous genotype AA, while 6 individuals carried

mutation at position 145 (G>A), 15 birds exhibited the homozygous GG genotype, whereas 5 birds carried the AA genotype. No additional nucleotide variations were detected along the analyzed region of the Cyt b gene in the studied Japanese quail samples.

Table. 2 multiple sequence alignment between cyt B Gene for our sequences design by CLUSTAL O. V(1.2.4) tool

CytB3	CCATACACTACACCGCAGACGCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB8	CCATACACTACACCGCAGACGCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB9	CCATACACTACACCGCAGACGCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB10	CCATACACTACACCGCAGACGCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB13	CCATACACTACACCGCAGACGCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB14	CCATACACTACACCGCAGACGCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB1	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB2	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB4	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB5	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB6	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB7	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB11	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB12	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB15	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB16	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB17	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB18	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB19	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
CytB20	CCATACACTACACCGCAGACACCTCCCTAGCCTTCTCTCCGTAGCCCACACATGTCGAA	120
*****.*****		
CytB3	ACGTACAGTACGGCTGACTCATTCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB8	ACGTACAGTACGGCTGACTCATTCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB9	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB10	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB13	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB14	ACGTACAGTACGGCTGACTCATTCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB1	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB2	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB4	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB5	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB6	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB7	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB11	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB12	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB15	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB16	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB17	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180
CytB18	ACGTACAGTACGGCTGACTCATTGCAATCTCCATGCAAACGGCGCATCATTCTTCTTCA	180

Table (3) presents several molecular parameters of the *cyt b* gene region in

locally Japanese quail compared with reference sequences obtained from the NCBI GenBank database. The analysis included 20 sequences from local Japanese quail and 13 reference clones from China, Japan, India, and the USA. The results revealed that the local quail *cyt b* gene exhibited two nucleotide substitutions at positions 81 (A>G) and 145 (G>A), whereas the global reference sequences displayed 13 nucleotide variations. The nucleotide diversity (Pi) values were 0.0022 and 0.0093 for the local and global quail populations, respectively. These mutations resulted in the formation of three haplotypes in the local population and six haplotypes in the global one, with corresponding haplotype diversity (Hd) values of 0.484 and 0.641. The similarity in the G+C content ratios (0.478 for local and 0.493 for global quails) suggests a

relatively conserved base composition across populations.

The observed differences in nucleotide and haplotype diversity may be associated with geographical isolation, breeding practices, and population size, which influence gene flow and mutation rates [9]. The lower diversity observed in the local quail population indicates a possible limited genetic variation, likely due to inbreeding or a smaller effective population size compared with the global gene pool. Furthermore, the positive Tajima's D value (0.89508) in the local birds implies a population under balancing selection or a recent bottleneck, while the negative value (-0.64464) observed in the global birds suggests an excess of low-frequency polymorphism, possibly due to population expansion or purifying selection [10]. These findings collectively reflect the evolutionary and demographic history of local versus global Japanese quail populations.

Table (3) Molecular parameters of the cyt B gene fragment of locally bred Japanese quail compared to NCBI reference copeis

Japanese quail			parameter
All	Reference (NCBI)	This study	
33	13	20	Number of sequences
16	12	2	Number of variable sites
0.490	0.493	0.487	G+C content (%)
9	6	3	Number of Haplotypes
0.761	0.641	0.484	Haplotype diversity(Hd)
0.0831	0.0093	0.0022	Nucleotide diversity(Pi)
2.901	3.256	0.778	Average number of nucleotide differences(k)
-0.87996	-0.64464	0.89508	Tajima's D

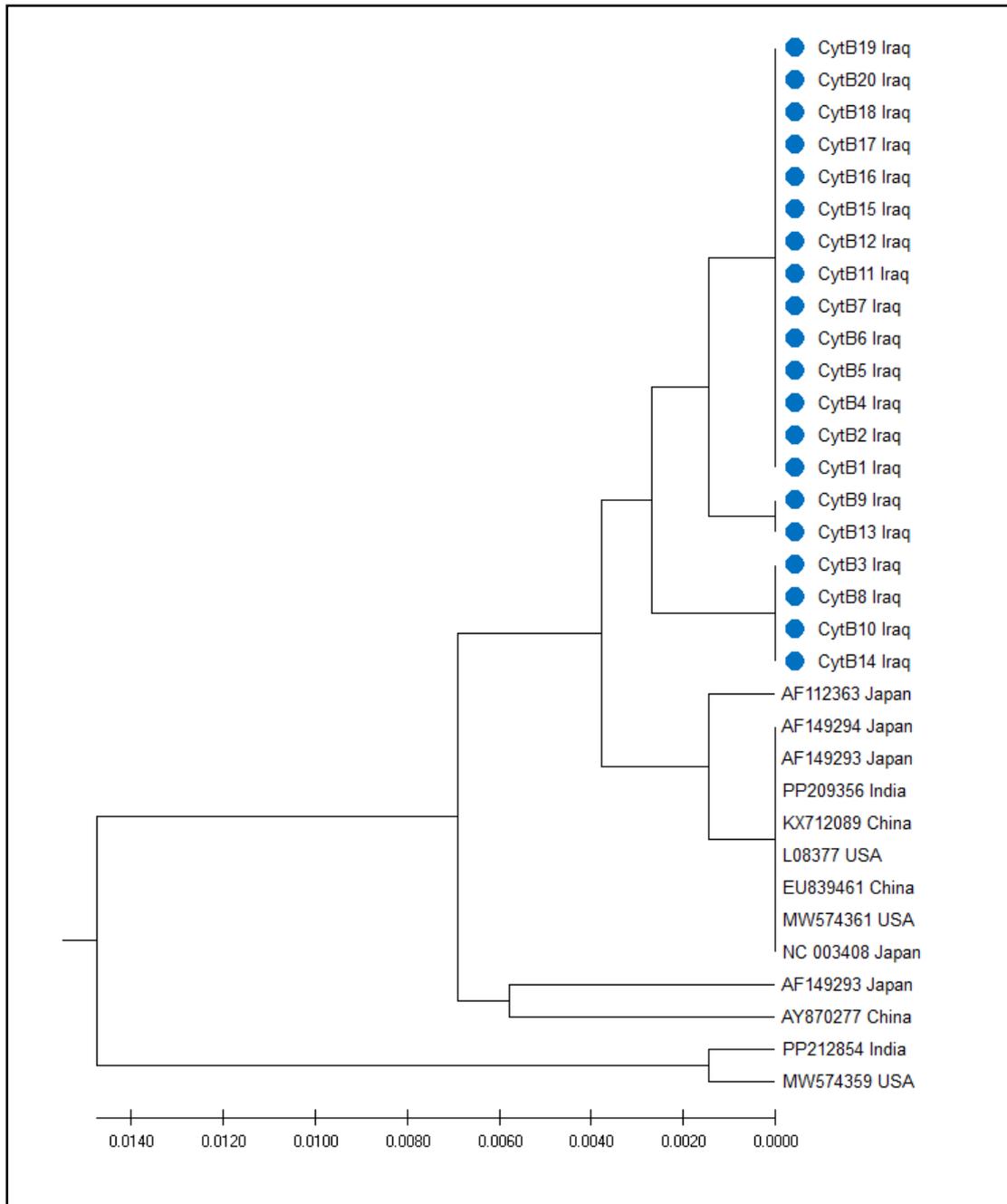


Figure. 2 phylogenetic tree of the nucleotide sequences for Japanese quail compared (Reference copies from NCBI)

Figure (2) shows the phylogenetic tree of the CytB gene in local Japanese quail compared with international strains registered in the NCBI GenBank. The tree reveals that all local strains (CytB1–CytB20) cluster within a single, distinct clade, branching into three subclades. This pattern indicates their close genetic proximity and shared maternal origin or a limited number of ancestral lineages. Such clustering may reflect the narrow genetic base of the local strain, its dependence on a restricted number of dams in breeding programs, or its geographical isolation, which limits the introduction of genetic variation from other lines or populations.

In contrast, the international Japanese quail strains (from Japan, China, India, and the United States) are distributed across several independent clades, reflecting greater genetic diversity compared with the local strains. This variation is likely attributable to differences in breeding and selection programs, regional environments,

and multiple domestication events that have contributed to the accumulation of distinct mutations in each geographic population [2]. The phylogenetic tree also demonstrates a clear separation between the Iraqi strain and the global strains, indicating limited gene flow between the two groups, likely due to geographic barriers, breeding practices, and long-term genetic isolation. This observation is consistent with findings in other avian species, where isolated populations often exhibit unique mitochondrial haplotypes [9]. Overall, the phylogenetic results align with previous molecular indicators such as nucleotide diversity (π) and haplotype diversity (H_d), both of which showed lower genetic variability in local birds compared with global strains. These findings emphasize the importance of implementing breeding programs that promote genetic diversity, or introducing new genotypes to preserve variability and enhance the adaptive and productive potential of local quail populations.

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