



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**THE PHYLOGENIC TREE OF WEST AZERBAIJAN NATIVE CHICKEN
COMPARE TO OTHER STRAINS BASED ON HVR-I REGION OF
MITOCHONDRIAL DNA**

ILKHANI M*, SEYEDABADI H, YEGANEH A AND ASGHARI GHELGHACHI A

Department of Animal science Shabestar Branch, Islamic Azad University- Shabestar- Iran

*Corresponding Author: E Mail: MeisamIlkhani@yahoo.com

ABSTRACT

Reserving genetic diversity in native chicken breeds of Iran because of little population size is necessary for breeding goals and increasing their production. The first step is determination of genetic diversity in existing populations. Studying mitochondrial HVR-I can give useful information about genetic diversity in those populations. This study was carried out for determination of the mitochondrial HVR-I sequence in west Azerbaijan native chicken in Iran. Blood samples were taken randomly from 20 birds. After extracting DNA, HVR-I region of mtDNA was amplified with specific primers using PCR and after purification was sequenced. Phylogenic tree, Composition index analysis of variance in haplotypes, Consensus sequence, and nucleotide composition percentage of Consensus determined using MEGA5 software. This is possible because of the very close genetic affinities with Asians is west Azerbaijan native chicken.

**Keywords: mtDNA, DNA Sequencing, HVR-I, West Azerbaijan Native Chicken,
Phylogeny**

INTRODUCTION

Diversity among farm animals within and among countries is of major interest to the scientific community as it is a significant resource for livestock development and for responding to changing needs and

production requirements. With increasing world population, there is concern that the growing demands for animal products are eroding these genetic resources especially in developing countries, where most of the

diversity is found. In recognition of this concern, many efforts have begun to characterize animals in developing countries to provide a foundation for developing sustainable genetic improvement approaches [1]. The control region (displacement loop: D-loop) is the most variable and non-coding portion of the mammalian mitochondrial genome [2, 3]. This region controls the mitochondrial DNA (mtDNA) replication by regulating the activities of various enzymes and proteins that are coded by the nuclear genes [4, 5]. In the last few years, there has been an accelerated accumulation of sequence data on animal mitochondrial genomes. Most of the complete mitochondrial DNA (mtDNA) sequences that have been published concern deuterostomes vertebrates and echinoderms: human [6], mouse [7], rat, cow [8]. The mtDNA gene content of coelomate metazoans is constant: it consists of 13 protein genes, two genes for the small and large ribosomal RNA subunits, and 22 tRNA genes, some of which are transcribed from one and some from the other mtDNA strand. Besides coding regions, there are also noncoding sequences in animal mtDNA and, more specifically, a major noncoding segment, high in deuterostomes (D-loop) contains a combination of sequence elements that are related with control of both replication and transcription. More

recently studies on DNA divergence are becoming more attractive in population genetic analysis. Mitochondrial DNA (mtDNA) is an available molecular tool for investigating evolutionary relationships and genetic variations within and between species because of its maternal inheritance and more rapid variability than nuclear DNA [9-12]. However, sequencing a specific fragment of mt DNA gives more accurate information on evolution and genetic diversity [13, 14]. Based on previous literatures, in this study, molecular analysis of west Azerbaijan native chicken population based on HVR-I region of mitochondrial DNA were investigated to develop molecular markers for breed identification.

MATERIAL AND METHODS

Animal and Blood Samples

West Azerbaijan native poultry lines which their reproductive characteristics studied for 12 generation were used in this study (Tala Tabeh, west Azerbaijan province, Iran). Blood samples (2ml in EDTA containing tubes) randomly collected from 20 birds via wing vein using disposable syringes in all birds and stored at -20 C° until used at hematology laboratory.

Establishment of a PCR-RFLP Assay

The PCR primers for the chicken were used in the forward between 16756-16776 bases and reverse between 845-865 bases

(forward: 5'-TTGTTCTCAACTACGGGAACA-3';
reverse: 5'-CAAAGTGCATCAGTGTCAAGAT-3')

using Primer premier software. DNA amplification of each individual bird was performed according to the following conditions: the PCR was performed in a total volume of 25 μ L, containing 2 μ L of genomic DNA, 10 pmol of each oligonucleotide primer, 2 μ L 25 mM MgCl₂, 2 μ L of 1 mM deoxynucleotide triphosphate mixture, and 1 U of Taq DNA polymerase; cycle parameters were 94 °C for 8 min then 35 cycles of 95 °C for 30 sec, 64 °C for 30 sec, and 72 °C for 5 min, with a final extension step for 2.07 min at 72 °C; the PCR products with length 776 bp were digested at 37 °C overnight with 10 U of Hinf I. Then the DNA primers electrophoresis on 1% agarose gel and NucleoSpin Extract II kit (Macherey-Nagel MN, Germany) used to purification DNA from agarose gel TAE/TBE.

Analysis of Primers

Obtained sequences analyzed using Chromas Lite 2.01 software. To investigate highest homology of west Azerbaijan native poultry lines, Blast procedure from NCBI was used. Consensus sequence of west Azerbaijan native poultry have done by SEQMAN software and recorded in NCBI using Sequin software. Phylogenic tree by

Neighbor-joining procedure, Composition index analysis of variance in haplotypes by Disparity Index Analysis procedure, Consensus sequence, nucleotide composition percentage of Consensus, were done using MEGA5 software.

RESULTS AND DISCUSSION

In this study for investigate of haplotypes, figure of the sequences performed using MEGA5 software using Neighbor- Joining procedure. As seen in **Figure 1**, 5 haplotype obtained which each of the samples of 16 in (B), 17 in (C), 2 and 9 in (D), 8, 15, 20 and 23 in (E) and the samples 1, 3, 4, 5, 7, 9, 10, 11, 12, 13, 14 and 22 was located in (A) haplotype, respectively.

In this study to investigate index analysis of variance in haplotypes, Disparity Index Analysis procedure was used by MEGA5 software. In this manner, variance in calculated using nucleotide differences in haplotypes. If the distance for 2 samples was 0, they were dependent to the same haplotypes. As seen in the **Figure 2**, samples 1, 3, 4, 5, 7, 9, 10, 11, 12, 13, 14, and 22 in haplotype (A), 16 (B), 17 (C), 2 and 19 in (D) and 8, 15, 20 and 23 in (E) groups, respectively. This result guarantees the Neighbor-Joining tree results for samples and haplotypes.

According to the data in **Figure 3**, to assume sequence index in west Azerbaijan native chicken, we used Consensus

sequence using BioEdit software in ~ 775 pair bases.

As presented in **Figure 4**, the Composition procedure of BioEdit software implied that 175 nucleotids was in group (A), 230 nucleotids in group (C), 140 nucleotids in group (G) and 240 nucleotids in group (T), respectively. Additionally, the G+C ratio was 46.45 and A+T was 53.55 percent. Furthermore, the molecular weight of this sequence was 236573 daltons and the molecular weight of pairs was 470691 daltons.

This result indicates that there is a common ancestor between the west Azerbaijan native chicken and other birds. It has been demonstrated that the mt DNARFLPs (restriction fragment length polymorphism) of jungle fowl have more extensive polymorphism than those of domestic fowl, which suggests the domestic fowl has a single and recent ancestor [15- 17]. However, sequencing a specific fragment of mtDNA gives more accurate information on evolution and genetic diversity [13, 14]. The D-loop region does not encode protein and evolves much faster than other regions of the mtDNA genome, so it is the most valuable and sensitive region in population genetic analysis, especially suited for genetic variation studies within species. Akishinomiya *et al.* [13, 14], in their study on the domestic fowl from the

Indonesian islands have large genetic differences compared with *G. g. bankiva* from the same place; in sharp contrast, all Thailand jungle fowls are very close to these Indonesian domestic fowls, which clearly excludes the involvement of *G. g. bankiva* in the domestication event, further indicating that the red junglefowl of Thailand may suffice as the matriarchic ancestor of all domestic fowl.

Chicken is the most widely distributed of all livestock and poultry species in African countries. It plays a very significant role as a source of income and high quality protein to the rural households. Previously, Bjørnstad *et al.*, [18] reported the sequences of the first 39 nucleotides were used for the analysis. Seventeen haplotypes were identified in the samples, 15 for Nigerian indigenous chicken population, 1 for Giriraja and 1 for Anak titan from 23 polymorphic sites. Phylogenetic analysis shows that Nigerian indigenous and Anak titan chicken were all grouped under clade IV, while the Indian Giriraja was under clade IIIc. Clade IV had 16 haplotypes, while clade IIIc had one haplotype. AMOVA analysis indicates that 97.32% of the total sequence variation between haplotypes was present within population and 2.68% between populations. Our results suggest single multiple maternal origins for the South Western Nigerian domestic

chicken. This study has proved that mtDNA and more specifically D-loop HV1 segment is a powerful molecular tool in resolving

phylogenetic relationships within a species and also understanding the genetic diversity.

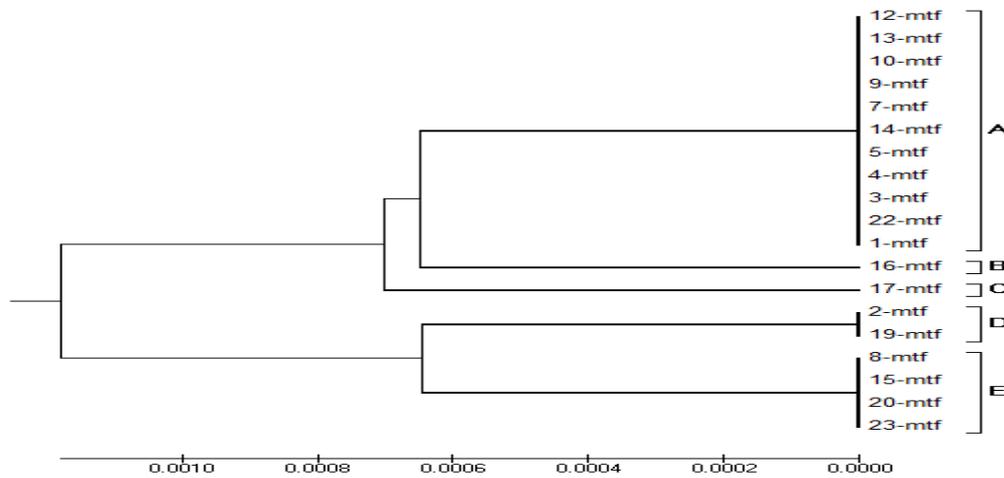


Figure 1: Phylogenetic Tree was Done Using Neighbor-Joining and the Haplotypes in West Azerbaijan Native Chicken

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1. 1-mtf																					
2. 2-mtf	0.001																				
3. 3-mtf	0.000	0.001																			
4. 4-mtf	0.000	0.001	0.000																		
5. 5-mtf	0.000	0.001	0.000	0.000																	
6. 7-mtf	0.000	0.001	0.000	0.000	0.000																
7. 8-mtf	0.003	0.001	0.003	0.003	0.003	0.003															
8. 9-mtf	0.000	0.001	0.000	0.000	0.000	0.000	0.003														
9. 10-mtf	0.000	0.001	0.000	0.000	0.000	0.000	0.003	0.000													
10. 11-mtf	0.000	0.001	0.000	0.000	0.000	0.000	0.003	0.000	0.000												
11. 12-mtf	0.000	0.001	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000											
12. 13-mtf	0.000	0.001	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000										
13. 14-mtf	0.000	0.001	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000									
14. 15-mtf	0.003	0.001	0.003	0.003	0.003	0.003	0.003	0.000	0.003	0.003	0.003	0.003	0.003								
15. 16-mtf	0.001	0.003	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.004							
16. 17-mtf	0.001	0.003	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003						
17. 19-mtf	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003					
18. 20-mtf	0.003	0.001	0.003	0.003	0.003	0.003	0.000	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.004	0.004	0.001				
19. 22-mtf	0.000	0.001	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.001	0.001	0.003			
20. 23-mtf	0.003	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.004	0.004	0.001	0.000	0.003		

Figure 2: Composition Index Analysis of Variance in Haplotypes in West Azerbaijan Native Chicken



Figure 3: Consensus Sequence in West Azerbaijan Native Chicken

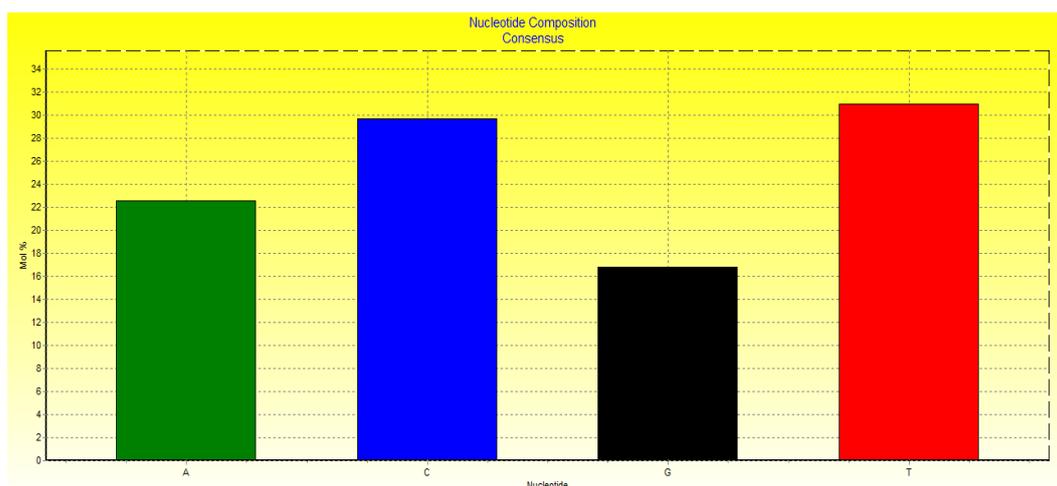


Figure 4: Nucleotide Composition Percentage of Consensus Sequence in West Azerbaijan Native Chicken

REFERENCES

- [1] Silva P, Guan X, Ho-Shing O, Jones J, Xu J, Hui D, Notter D and Smith E, Mitochondrial DNA-based Analysis of Genetic Variation and Relatedness among Sri Lankan Indigenous Chickens and the Ceylon Junglefowl (*Gallus lafayetti*), *Anim. Genet.*, 40 (1), 2009, 1-9.
- [2] Cann RL, Brown WM and Wilson AC, Polymorphic sites and the mechanism of evolution in human mitochondrial DNA, *Genetics*, 106, 1984, 479-499.
- [3] Wilson AC, Cann RL, Carr SM, George M, Gyllensten UB, Helm-Bychowski KM, Higuchi RJ, Palumbi SR, Prager EM, Sage RD and Stoneking M, Mitochondrial DNA and two perspectives on evolutionary genetics, *Biol. J. the Linnean Society*, 26, 1985, 375-400.
- [4] Ghivizzani SC, Madsen CS and Hauswirth WM, In organello footprinting: analysis of protein binding at regulatory regions in bovine mitochondrial DNA, *J. Biological Chem.* 268, 1993, 8675-8682.
- [5] Nasz MM, Precise sequence assignment of replication origin in the control region of chick mitochondrial DNA relative to 5' and 3' ends, secondary structure, DNA synthesis and protein binding. *Current Genetics* 28, 1995, 401-409.
- [6] Andersons AT, Bankier GB, De Bruijna MHL, Coulson R, *et al.*, Sequence and organization of the human mitochondrial genome, *Nature*, 290, 1981, 457-465.
- [7] Bibb MJ, Van etten RA, Wright T, Walberg MW, Clayton, D.A., Sequence and gene organization of

- mouse mitochondrial DNA. *Cell* 26, 1981, 167-180.
- [8] Andersons MH, De Bruijna L, Coulsoni R, Eperonf C, Sanger *et al.*, Complete sequence of bovine mitochondrial DNA: conserved features of the mammalian mitochondrial genome, *J. Mol. Biol.*, 156, 1982, 683-717.
- [9] Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA and Saunders NC, Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics, *Annu. Rev. Ecol. Syst.*, 18, 1987, 489.
- [10] Zhang YP and Shi LM, Mitochondrial DNA polymorphism in animals: A review, *Zool. Res.*, 13, 1992, 289.
- [11] Zhang YP and Shi LM, Phylogenetic relationships of macaques as inferred from restriction endonuclease analysis of mitochondrial DNA, *Folia Primatol.*, 60, 1993a, 7.
- [12] Zhang YP and Shi LM, Phylogeny of the slow lorises (genus *Nycticebus*): An approach using mitochondrial DNA restriction enzyme analysis, *Int. J. Primatol.*, 14, 1993b, 167.
- [13] Akishinonomiya F, Miyake T, Sumi S, Takada M, Ohno S, and Kondo N, One subspecies of the red junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds, *Proc. Natl. Acad. Sci., USA* 91, 1994, 12505.
- [14] Akishinonomiya F, Miyake T, Takada M, Shingu R, Endo T, Gojobori T, Kondo N, and Ohno S, Monophyletic origin and unique dispersal patterns of domestic fowl, *Proc. Natl. Acad. Sci., USA*, 93, 1996, 6792.
- [15] Glaus KR, Zassenhause HP, Fechheimer NS, Perlman PS, Avian mtDNA: Structure, organization and evolution, In Kroon AM and Saceon C, (eds.), *The Organization and Expression of the Mitochondrial Genome*, North-Holland Publishing, Amsterdam, 1980, 131-135.
- [16] Wakana S, Watanabe T, Hayashi Y, and Tomita T, A variant in the restriction endonuclease cleavage pattern of mitochondrial DNA in the domestic fowl, *Gallus gallus domesticus*, *Anim. Genet.*, 17, 1986, 159.
- [17] Wang W, Lan H, Liu AH and Shi LM, Variation of mitochondrial DNA among domestic fowl and red

junglefowl, Zool. Res., 15, 1994, 55.

- [18] Bjørnstada G, Bulimob W, Jianlina H, Kiersteina G, Mazhanid L, Podisid B, Hirboa J, Agyemangc K, Wollnye C, Gondwel T, Zeuhf V, Tadelleg D, Abebeg G, Abdoulayeh P, Pacoi S, Serunjogij L, Aberrahmanf M, Sowh R, Weigendm S, Sanfoi R, Gayec F, Ssewanyanaj E, Coulibalyk MD, Temek B and Hanottea O, Mitochondrial dna d-loop analysis of south western nigerian chicken analisis de d-loop adn mitochondrial de pollos de sw nigeria, Arch. Zootec., 58 (224), 2009, 637-643.