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RESEARCH PAPER

**Journal of Biodiversity and Environmental Sciences (JBES)**

ISSN: 2220-6663 (Print) 2222-3045 (Online)

Vol. 7, No. 1, p. 537-542, 2015

<http://www.innspub.net>**OPEN ACCESS**

## Genetic diversity in the mitochondrial DNA of the Iranian commomnatvie and exotic chicken breeds

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Article published on July 30, 2015

**Key words:** Genetic diversity, Mitochondrial DNA, Chicken, Breeds.

### Abstract

Genetic diversity which exists among animals has been created as a result of evolution process during so many years and considering different problems such as economic conditions and the time duration of performance of this program, first, genetic potential and similarities existing among different races should be examined. According to sensible characteristics of the native breeds of the animals existing in Iran and in order to maintain and save original and native animals, researches should be carried out. One of the indicators in determining genetic diversity in animals is the area of controlling of the gene of the mitochondrion. And in this research, using this indicator, the similarities and differences between native common chickens of Iran and some foreign exotic breeds of broiler and layer breeds of chickens determine and the genetic diversity among them estimated. In this research a number of 20 native common chickens were used among which there were 17 different specific haplotype with haplotype 13<sup>th</sup> including four samples of mentioned chickens. Achieved haplotype 13<sup>th</sup> with three sub-category of Jungle red chickens, two types of White Leghorn chickens, one type of New Hampshire red chickens and three different types of Asian-breed chickens (Tibetan, Silky, Laos) totally showed difference in 80 gene place. The results of sequence analysis, the heliotype and nucleotide diversity were calculated 1 and 0.163 respectively. Considering the phylogenetic graph among the created haplotypes.

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## Introduction

With easy and convenient use of genetic markers it is possible to identify the difference between individuals. The genetic markers can be protein and or DNA (nuclear or mitochondrial). In addition, the sensitive methods and genetic markers are very importance to determine the genetic diversity using the difference of charges existing in DNA. In the recent years, the molecular methods have been mostly used among researchers. The researchers modify the primary principles of these methods regarding their testing materials. Rapid loss of test results, the low number of markers, availability of special devices as well as a long period of time to test are some characteristics of protein markers that compared with these characteristics, DNA markers have the great importance (Berkman, 2001). The detection degree of polymorphisms, the existence of bands, replication of results, statistical analysis, being economical as well as easy-to-use can be considered as the identifying characteristics of polymorphisms (Akin, 2002). In the world, 40 animal species and about 45000 races as the genetic resources are used for laboratory works and more than 30 percent of races are in the danger of extinction due to the small number of individuals and in the future this amount will affect the world. The tolerance to unfavorable climatic conditions, resistance to various diseases, and low production as well as having specific characteristics can be outstanding characteristics of indigenous breeds of animals and their being in danger of extinction can be mentioned as other characteristics of these animals. As we know it should be identified that which of mentioned characteristics are important and preserving their genetic resources should be considered regarding the available facilities (Ertugrul, 2000).

The country of Iran has a high degree in terms of biological diversity and diverse animal genetic resources and various reasons including the ability to propagate and to breed in unfavorable climatic conditions can be expressed in order to preserve the indigenous animals in Iran. The research on breeding

about indigenous animals and their proliferation with maintaining their genetic resources is possible. On the other words, maintaining the genetic resources is heterosis (genetic diversity). Considering changes of climatic conditions, position and hygiene of indigenous animals, it is critical to maintain the available resources and their proliferation. Considering diverse genetic combinations, using heterosis animals, selecting the excellent animals, cultural condition as well as having testing materials in order to carry out the genetic researches are necessities to maintain the various animal genetic resources. Nowadays, poultries include two main groups of pure breeds and hybrid breeds. The standard pure breeds have gave their place to hybrid breeds and they also have been protected in order to carry out eugenic research, to produce hybrids and chickens resistance to diseases, to produce biological productions as well as to be used in the same mentioned purposes. In the country of Iran, the chicken farming had been generally done by rural and or urban families in order to supply their daily needs. The modern chicken farming was flourished after the Islamic revolution and it has been rapidly developed during the last 20 years. The native breeds and strains were imported to Iran due to the low production of domestic chickens and due to this the breeds of domestic chickens are decreasing. Nowadays, apart from the domestic chicken farming by rural households, the Animal Research Center of Tabriz, Isfahan, Shiraz, Urmia and Tehran are also breeding and modifying the domestic chickens. Modifying and breeding domestic chickens has become as a culture in the country and it has found some applications in the genetic and phylogenetic researches and in the recent years, the conservation of genetic resources of domestic chickens has been considered by researchers.

One of the determining markers of genetic diversity in animals is mitochondrial genome. Having maternal inheritance and haplotype mode, showing the rapid evolution and the lack of recombination (Wenink and *et al.*, 1994) are characteristics of mitochondrial

genome and it is considered as an important marker in terms of phylogenetic analysis and it is one of the most applicable markers in the molecular genetics (Sorenson and *et al.*, 1999). The mitochondrial genome of poultries having about 17 kb weight is yet in circular-shaped DNA and has about 37 genes that 2 gens are related to rRNA, 22 gens are related to tRNA and 13 gens are related to coding polypeptides required in oxidation phases (Chinnery and schon, 2003, Guan and *et al.*, 2007, and Bao and *et al.*, 2008) and in vertebrates, the main function of these gens is supplying energy, metabolism, cellular balance and cell death. The mitochondrial genome has different regions in terms of evolutionary rate. One of these regions is control region that is the starting point of replication of the heavy strand of mitochondrial DNA as well as the starting point of the replication of the light strand. This point does not code any enzyme and or protein and has 2-5 times faster evolution compared to the remaining genome regions (Wenink and *et al.*, 1994). Nowadays, the mitochondrial sequence has been identified indomestic poultries and a number of vertebrates (Desjardins and Morais, 1990) and the research on this region of mitochondrial genome is in the first step.

The aim of this research is, has been tried to identify the genetic similarities and differences available among the Iranian common native chicken and broiler and laying breeds and to meet the genetic diversity among them.

## Material and methods

### Data

For this research, about 20 sequences of the control region of mitochondrial genome of Iranian common native chicken breed located in the Animal Science Research Center of the country were prepared and along with these sequences, 10 sequences of the native breed available in the gene bank were genetically compared including subgroups of the Red Jungle fowls (AP 00332, AP 3323.1 and AP 003323), Tibetan Jungle fowls (DQ 648776.1), two different

breeds of White Leghorn Fowls (X 52392 and AP 003317), Plymouth Rock fowls (AP 003318.1), Silky breed fowls (AB 086102.1), Laos breed fowls (AP 003319.1) as well as the fowls from the Red New Hampshire fowls.

### Determining DNA sequence

According to results obtained from computer programs with 17 haplotypes between domestic poultries of the Iranian common native chicken breed in this research it was found that they show 80 polymorphism gene positions so that haplotype 13 includes 4 samples of chickens and the remaining haplotypes contain 1 sample of chickens. So that, from results obtained from genetic analysis among haplotypes in the 11th position from 708 times repeated among haplotypes, 1,2,3,4,5,7,10 and 14 mutations of displacement ( $A \rightarrow G$ ) have been occurred and in the position 254, except haplotype 17, the mutation of displacement ( $T \rightarrow C$ ) has been occurred in all haplotypes. In addition, in the most of haplotypes, the following displacement have been respectively occurred in positions 318, 333, 343, 379 and 483: purine to purine displacement ( $G \rightarrow A$ ), pyrimidine to pyrimidine displacement ( $T \rightarrow C$ ), pyrimidine to pyrimidine displacement ( $C \rightarrow T$ ), purine to purine displacement ( $G \rightarrow A$ ) and pyrimidine to pyrimidine displacement ( $C \rightarrow T$ ).

## Result and discussion

Among the native breeds available in gene bank, the silky breed and one of the breeds of Leghorn fowls (X 52392) as well as haplotype 17 in the gene position 204, the above-mentioned poultries of Leghorn breed in the positions 247, 262, 280, 293, 298, 347 showed some differences compared to other breeds and haplotypes available in the Iranian common native chicken breed.

One of the subgroups of the Red Jungle Fowls (AP 003321) in the gene positions 249, 267, 280, 283, 293, 298, 347, 352, 379, and 507 shows polymorphism compared to other breeds. As well, Tibetan breed in the gene positions 271, 273, 283,

291, 352 and 354 shows polymorphism compared to other breeds and haplotypes available in Iranian common native chicken breed.

Finally, the New Hampshire breed in the gene positions 259 and 367 shows some differences compared to the position of other breeds of domestic

poulties. According to genetic analysis in the program Mega 5, the genetic distance existing among available haplotypes in Iranian common native chicken breed was variable between 0-0.072 that is shown in table (1) and the average genetic distance has been calculated as 0.016.

**Table 1.** The existing genetic distance between haplotypes of the Iranian common native chicken breed in terms of the control region of mitochondrial genome.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1. G.G.gallus2																											
2. G.G.gallus1	0.019																										
3. Tibetan	0.014	0.016																									
4. N.H.red	0.011	0.019	0.011																								
5. silky	0.016	0.011	0.019	0.016																							
6. laos	0.007	0.017	0.010	0.004	0.014																						
7. P.rock	0.009	0.016	0.009	0.003	0.013	0.001																					
8. W.lghorn1	0.009	0.016	0.009	0.003	0.013	0.001	0.000																				
9. W.lghorn2	0.016	0.011	0.019	0.016	0.003	0.014	0.013	0.013																			
10. G.G.gallus3	0.001	0.017	0.013	0.010	0.014	0.006	0.007	0.007	0.014																		
11. Hap1-121	0.010	0.017	0.010	0.004	0.014	0.003	0.001	0.001	0.014	0.009																	
12. Hap2-123	0.014	0.022	0.014	0.009	0.019	0.007	0.006	0.006	0.019	0.013	0.004																
13. Hap3-124	0.011	0.019	0.011	0.006	0.016	0.004	0.003	0.003	0.016	0.010	0.001	0.006															
14. Hap4-125	0.010	0.017	0.010	0.004	0.014	0.003	0.001	0.001	0.014	0.009	0.003	0.007	0.001														
15. Hap5-127	0.013	0.020	0.013	0.001	0.017	0.006	0.004	0.004	0.017	0.011	0.006	0.010	0.007	0.006													
16. Hap6-128	0.017	0.025	0.017	0.006	0.022	0.010	0.009	0.009	0.022	0.016	0.007	0.011	0.006	0.007	0.007												
17. Hap7-129	0.043	0.051	0.043	0.037	0.048	0.035	0.034	0.034	0.048	0.041	0.034	0.038	0.032	0.032	0.038	0.035											
18. Hap8-131	0.013	0.020	0.013	0.007	0.017	0.006	0.004	0.004	0.017	0.011	0.003	0.007	0.004	0.006	0.009	0.010	0.035										
19. Hap9-132	0.014	0.022	0.014	0.009	0.019	0.007	0.006	0.006	0.019	0.013	0.007	0.011	0.006	0.004	0.010	0.009	0.031	0.007									
20. Hap10-133	0.064	0.072	0.064	0.057	0.069	0.056	0.054	0.054	0.069	0.062	0.054	0.059	0.053	0.053	0.059	0.056	0.049	0.053	0.049								
21. Hap11-134	0.013	0.020	0.013	0.007	0.017	0.006	0.004	0.004	0.017	0.011	0.003	0.007	0.001	0.003	0.009	0.007	0.034	0.006	0.007	0.054							
22. Hap12-135	0.016	0.023	0.016	0.010	0.020	0.009	0.007	0.007	0.020	0.014	0.009	0.013	0.007	0.006	0.011	0.013	0.037	0.012	0.010	0.054	0.009						
23. Hap13-136	0.009	0.016	0.009	0.003	0.013	0.001	0.000	0.000	0.013	0.007	0.001	0.006	0.003	0.001	0.004	0.009	0.034	0.004	0.006	0.054	0.004	0.007					
24. Hap14-143	0.010	0.017	0.010	0.004	0.014	0.003	0.001	0.001	0.014	0.009	0.003	0.007	0.004	0.003	0.006	0.010	0.035	0.006	0.007	0.056	0.006	0.009	0.001				
25. Hap15-147	0.010	0.017	0.010	0.004	0.014	0.003	0.001	0.001	0.014	0.009	0.003	0.007	0.004	0.003	0.006	0.010	0.035	0.006	0.007	0.056	0.003	0.009	0.001	0.003			
26. Hap16-149	0.013	0.020	0.013	0.007	0.017	0.006	0.004	0.004	0.017	0.011	0.006	0.010	0.004	0.003	0.009	0.010	0.035	0.009	0.007	0.056	0.006	0.009	0.004	0.006	0.006		
27. Hap17-150	0.017	0.014	0.022	0.019	0.006	0.017	0.016	0.016	0.006	0.016	0.017	0.022	0.019	0.017	0.020	0.025	0.051	0.020	0.022	0.072	0.020	0.023	0.016	0.017	0.017	0.020	

According to the results of the determination of sequence, 17 haplotypes have been observed among the samples applied in this research and the reason of low genetic distance can be resulted from the following factors:

Sampling has not been carried out among different herds.

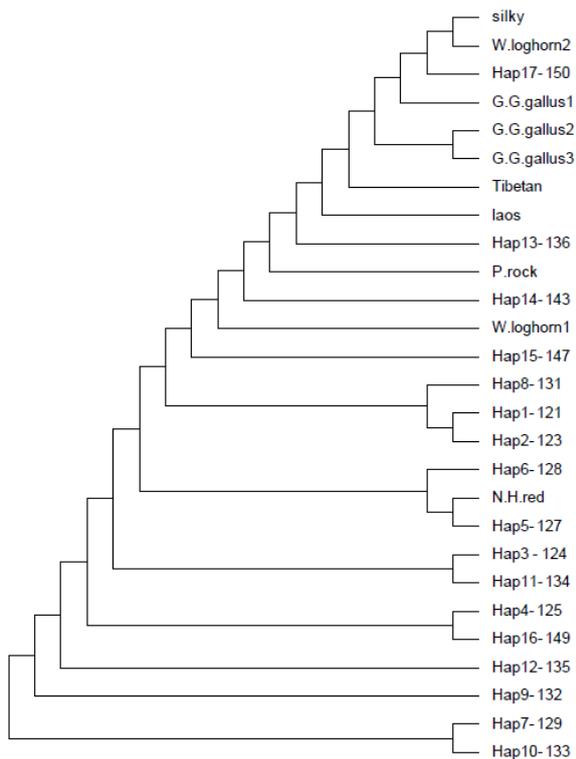
The population of herd is low.

The number of mothers in the herd is low.

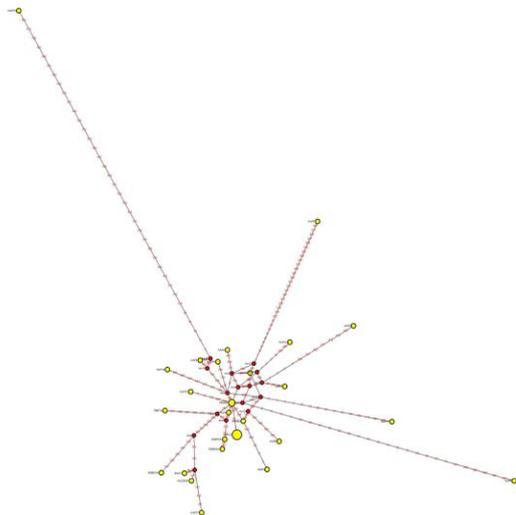
According to the results observed in the analysis of sequences obtained from the region of mitochondrial genome of the population of Iranian common native chicken breed, the haplotype diversity has been calculated as 1 and the nucleotide diversity as 0.163 according the nucleotide differences among the created haplotypes.

According to the results of sequences obtained from the control region of mitochondrial genome of the population of Iranian common native chicken breed with intragroup similarity percent resulted from 1000 times sampling, the phylogenetic diagram (the tree diagram with NJ and the network diagram MJ) was drawn that is shown in Fig. 1 and 2.

Considering the phylogenetic diagram it can be stated that among haplotypes created from the Iranian common native chicken breed, haplotype 5 is in a same cluster with New Hampshire fowls and among the silky breed and white Leghorn 2 (X52392) is in a same cluster and the chickens from the of Tibetan breed, laos, and Plymouth Rock are in different clusters. Finally, it can be concluded from phylogenetic diagram that sequences available in the gene bank have been collected in different times from different populations and in a few number.



**Fig. 1.** Phylogenetic diagram drawn with using software Mega 5, using sequences of 17 haplotypes related to the Iranian common native and exotic chicken breeds. haplotypes have been shown with letters Hapx.



**Fig. 2.** The network diagram MJ drawn with software Network from sequences of 17 haplotypes related to the domestic poultries of Iranian typical breed and native breeds.

One of the determining markers of genetic diversity in animals is mitochondrial genome. Having maternal inheritance and haplotype mode, showing the rapid evolution and the lack of recombination (Wenink and *et al.*, 1994) are characteristics of mitochondrial genome and it is considered as an important marker in terms of phylogenetic analysis and it is one of the most applicable markers in the molecular genetics (Sorenson and *et al.*, 1999). The mitochondrial genome of poultries having about 17 kb weight is yet in circular-shaped DNA and has about 37 genes that 2 gens are related to rRNA, 22 gens are related to tRNA and 13 gens are related to coding polypeptides required in oxidation phases (Chinnery and schon, 2003, Guan and *et al.*, 2007, and Bao and *et al.*, 2008) and in vertebrates, the main function of these gens is supplying energy, metabolism, cellular balance and cell death. The mitochondrial genome has different regions in terms of evolutionary rate. One of these regions is control region that is the starting point of replication of the heavy strand of mitochondrial DNA as well as the starting point of the replication of the light strand. This point does not code any enzyme and or protein and has 2-5 times faster evolution compared to the remaining genome regions (Wenink and *et al.*, 1994). Nowadays, the mitochondrial sequence has been identified indomestic poultries and a number of vertebrates (Desjardins and Morais, 1990) and the research on this region of mitochondrial genome is in the first step.

### Conclusion

In this research, the control region of mitochondrial genome of 20 samples of Iranian common native chicken breed was multiplied and their genetic changes have been specified using obtained haplotypes. Using practices carried out with 17 different haplotypes it was identified that haplotype 13 included 4 samples of the above- mentioned poultries. The haplotypes obtained along with three subgroups of Red Jungle fowls and two species of White Leghornfowls and one species of New Hampshire red chickens and three different species of

Asian chicken (Tibetan, silky and laos), totally showed differences in 80 gene sites. This research is one of the three researches carried out on the Iranian common native chicken breed that have been previously carried out by Pirani and *et al.* (2009) on the Mazandaran native chicken breed as well as the research by Mohammadipestebik and *et al.* (2011) on the Marandian native chicken breed. Pirani and *et al.* reported 6 haplotypes in Mazandaran native chicken breed that had polymorphism in 10 gene sites. And also, in the results obtained from the research by Mohammadi pestebik and *et al.* on the Marandian native chicken breed, 6 different haplotypes were identified. In this research, the Iranian common native chicken breed were genetically compared with other different native breeds using different computer genetic software that only haplotype 5 has been places in the same cluster with New Hampshire Red breed and it indicated the existence of traits of New Hampshire breed among Iranian common native chicken breed.

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