Complete mitochondrial DNA and phylogenetic study of qionglai native black chicken

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Abstract. The complete mitochondrial genome sequence of the qionglai black chicken was measured by PCR-based methods，the molecular characterization and phyletic evolution analyzed in detail. Our result showed that the entire mitochondrial genome of the qionglai black chicken is a circular molecule consisting of 16,785 bp (GenBank accession number: KT958484). The contents of A, T, C, and G were 30.25%, 23.74%, 32.54% and 13.48% in the mitochondrial genome, respectively. The complete mitochondrial genome of the qionglai black chicken contains a typical structure, including 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes and 1 control region (D-loop region). The phyletic evolution analysis shown that this chicken was evolution between the red jungle fowl and the special egg chicken white loghorn chicken.This complete mitochondrial genome sequence provides essential information in understanding phylogenetic relationships among Gallus gallus domesticus mitochondrial genomes and the breeding of native chick

1 Introduction

In China, indigenous chickens making up 20% of the poultry market, and the indigenous chicken market is rapidly developing by a rate of 5 to 10% per year[1]. Native black chicken is one of the famous Chinese indigenous chickens in Sichuan province, and it has a large number of breeding in qionglai city which was called as qionglai black chicken. The qionglai black chicken, which has black feathers, black skin and red comb. The qionglai black chicken as the importance indigenous breed and genetic resource was a valuable resource for preserve genetic diversity.

Recently, the chicken market was occupied by specialied commercial populations, which originated in foreign countries. It was the enormous impact for the indigenous chicken market. Qionglai black chicken as the indigenous breeds was a large genetic resousrces as well as having well-adapted and stress toletant as its advantage. While it also have some disadvantage like low production performance. therefore, taking full advantage of this breeds was importance for the indgenous chicken breeding and save the gentic diversity .

Mitochondrial DNA(mtDNA) have the characteristic of quick evolution, matrilineal inherritance and simple molecular structure , which was the most being used as the molecular study marker. Recently, many studies have reported the results of using the fine-gained complete mtDNA to analyses and reconstruct the history of animal domestication, such as horses, pigs and cattle[2-7] .in this study, we research the mtDNA of qionglai black chicken to understanding it characterisric and phylogenetic.

2 Material and Methods

A total of 15 qionglai black chicken were sampled for this study, All birds were reared under the same condition: cage free (density of <35 chicken/100 m 2) and standard conditions of temperature (20° to 22°C), relative humidity (55 to 60%), and ventilation were maintained. The chickens were fed the same professional breeder diet and had free access to feed and water during the entire rearing period. Birds were managed with full consideration of bird welfare. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the National Institute of Agrobiological Sciences.

2.1 Sample collection

Approximately 2 mL blood was collected from the brachial vein of each individual according to the requirements of animal welfare of Sichuan Agricultural University, and stored at -20°C. The next day, genomic DNA was isolated by phenolic extraction. The quality and integrity of DNA was assessed using the A260/280 ratio and agarose gel electrophoresis.

2.2 Data Analysis

The complete mitochondrial genome was amplified by 22 pairs of primers designed according to the sequence of the Gallus gallus (GenBank accession number: AP003322). To determine whether the sequences obtained were correct, an NCBI nucleotide BLAST search was conducted (http://blast.ncbi.nlm.nih.gov/). The DNA sequence was analyzed using DNAStar 7.1 software (Madison, MI) and the base composition and distribution of the mitochondrial DNA sequence were analyzed using tRNA Scan-SE1.21 software (http://lowelab.ucsc.edu/tRNAScan-SE/)[8] and DOGMA software (http://dogma.ccbb.utexas.edu/) [9], the phylogenetic were analyzed by mega4.1, respectively.

3 Results and Discussion

3.1 The stucture of the mtDNA

We submission the sequence to the NCBI(http://www.ncbi.nlm.nih.gov/) and the genbank accession number was KT958484.The complete mitochondrial DNA of qionglai native chicken was 16785bp which contain 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes and 1 control region (D-loop region). The gene arrangement and composition were similar to those of other avian species[10, 11]. The overall nucleotide composition of A, T, C and G were found to be 30.28%, 23.74%, 32.50% and 13.48%, respectively, and in the order C>A>T>G. Most of the genes are encoded on the H-strand and they are similar in structure to the typical mitochondrial genome of vertebrates[12].

The molecular characterization of qionglai black chicken mitochondrial genome as followed (Table 1). Firstly, the initiation codon of the protein -coding genes is ATG, except for COX1, which show a GTG initiation codon. Secondly, have nine genes not encoded on the L-stand, including 1 protein-coding gene (ND6) and 8 tRNA genes (tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Pro and tRNA-Glu). Thirdly, these genes have four different types of termination codons: TAA AGG TAG and T--. Among all gene elements, there were overlaps and spaces. The D-loop contains regulatory elements that control mtDNA replication and transcription, which is a non-coding control region located between the tRNA-Glu and tRNA-Phe genes, and rich in A and T.

3.2 Phylogenetic analysis

We alignment the sequence of qionglai black chicken and other gallus (red jungle fowl, Ceylon jungle fowl, Green jungle fowl, grey jungle fowl, white leghorn), the outgroup were meleagris gallopavo, aix galericulata, alectoris chukar and tadorna ferruginea, the evolution tree shown that the qionglai black chicken phyletic evolution was between the red jungle fowl and white leghorn(Figure 1). As the results shwon, we speculate that the qionglai black chicken was suitable for developent in egg production.

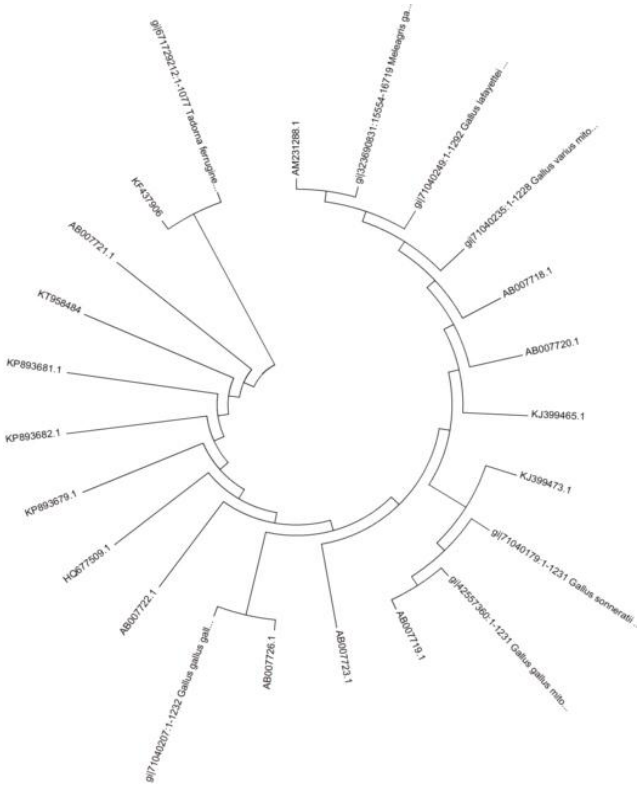

Figure 1. The Unrooted neighbor-joining (NJ) tree of qionglai black chicken and others.

Table 1. The molecular characterization of qionglai black chicken mtDNA

Gene	Position		size	codon			stand(H/L)	space(+)/ovrelap(-)
	start	end		start	stop	anti		
D-loop	1 16716	1162 16785	1232				H	0
tRNA-Phe	1163	1231	69			GAA	H	0
12S rRNA	1232	2207	976				H	0
tRNA-Val	2208	2280	73			GAC	H	0
16S rRNA	2281	3902	1622				H	0
tRNA-Leu	3903	3976	74			GAG	H	0
ND1	3986	4960	975	ATG	TAA		H	9

tRNA-Ile	4961	5032	72			GAT	H	0
tRNA-Gln	5038	5108	71			TTG	L	5
tRNA-Met	5108	5176	69			CAT	H	-1
ND2	5177	6217	1041	ATG	TAG		H	0
tRNA-Trp	6216	6291	76			CCA	H	-2
tRNA-Ala	6298	6366	69			AGC	L	6
tRNA-Asn	6370	6442	73			ATT	L	3
tRNA-Cys	6444	6509	66			GCA	L	1
tRNA-Tyr	6509	6579	71			GTA	L	-1
COI	6581	8131	1551	GTG	AGG		H	1
tRNA-Ser	8123	8197	75			TGA	L	-9
tRNA-Asp	8200	8268	69			GTC	H	2
COII	8270	8953	684	ATG	TAA		H	1
tRNA-Lys	8955	9022	68			CTT	H	1
ATPase 8	9024	9188	165	ATG	TAA		H	1
ATPase 6	9179	9862	684	ATG	TAA		H	-10
COIII	9862	10645	784	ATG	TAA		H	-1
tRNA-Gly	10647	10714	68			ACC	H	1
ND3	10716	11066	351	ATG	TAA		H	1
tRNA-Arg	11068	11135	68			TCT	H	1
ND4L	11136	11432	297	ATG	TAA		H	0
ND4	11426	12803	1378	ATG	TAT		H	-5
tRNA-His	12804	12872	69			ATG	H	0
tRNA-Ser	12874	12938	65			GCT	H	1
tRNA-Leu	12940	13010	71			AAG	H	1
ND5	13011	14828	1818	ATG	TAA		H	0
cytb	14833	15975	1143	ATG	TAA		H	4
tRNA-Thr	15979	16047	69			TGA	H	3
tRNA-Pro	16048	16117	70			TGG	L	0
ND6	16124	16645	522	ATG	TAA	CAT	L	6
tRNA-Glu	16648	16715	68			TTC	L	2

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