Genetic Diversity Analysis of Egyptian Native Chickens Using mtDNA D-loop Region

Ibrahim Elkhayat^{1,4}, Kotaro Kawabe², Kamal Saleh¹, Hassan Younis¹, Reiad Nofal¹,
Shinnya Masuda³, Takeshi Shimogiri⁴ and Shin Okamoto⁴

¹ Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt

² Natural Science Centre for Research and Education, Kagoshima University, Kagoshima 890-0065, Japan

³ Liberal Arts Education Center, Aso Campus, Tokai University, Minami Aso-mura, Kumamoto 869-1404, Japan

⁴ Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, Japan

This study was aimed to evaluate the genetic diversity of Egyptian native chickens by using mtDNA sequence polymorphism. A 546-bp fragment of the mtDNA D-loop region was sequenced for a total of 36 Egyptian native chickens with 14 reference sequences from DNA databank. Of the Egyptian chickens 5 haplotypes were identified. Haplotype diversity and nucleotide diversity of the Egyptian native chickens were 0.5635 ± 0.0845 and 0.00123 ± 0.00108 , respectively. The Egyptian native chickens were distributed within one clade, which were closed to the haplotypes from Indian subcontinent and Southeast Asia. Most of Egyptian native chickens were classified into the haplotype E1, which contains 63.9% of individuals followed by E4 (16.6%), E5 (11.1%), E2 (5.5%) and E3 (2.7%), respectively. These findings indicate that the maternal lineages was involved in the origin of domestic chicken in Egypt may have roots in Indian subcontinent and other Southeast Asia. The genetic information from this study will probably pave the way to further studies for evaluation, preservation and improvement of Egyptian native chickens as genetic resources in the future.

Key words: Egyptian chickens, genetic diversity, mtDNA

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Introduction

Egypt located in the north of Africa, bordering the Mediterranean Sea, between Libya and the Gaza Strip, and the Red Sea north of Sudan, and includes the Asian Sinai Peninsula. Egypt has four main native chicken breeds, Fayoumi, Dandarawy, Balady and the Sinai strain, moreover, the native chickens are well adapted to adverse environmental conditions and show resistance to some local diseases such as Rous sarcoma virus and avian leucosis (Hosny, 2006). Therefore, the evaluation and preservation of Egyptian native chickens as genetic resources, is essential for its improvement in the future.

Recently, mitochondrial DNA (mtDNA) is one of the useful tools to know the genetic structures or their diversities (Muchadeyi *et al.*, 2008). A number of studies have investigated the origin and dispersal of domestic chickens

using mtDNA sequence data. Akishinonmiya *et al.* (1996) reported that existing domestic chickens originated from *Gallus gallus gallus* in Thailand and adjacent regions. Kanginakudru *et al.* (2008) found the evidence for domestication of Indian chickens, which were originated from *Gallus gallus spadiceus*, *Gallus gallus gallus* and *Gallus gallus murghi*. Islam and Nishibori (2012) also reported that native fowls in Bangladesh were strongly influenced from *Gallus gallus murghi*. The domestication of chickens was occurred in Southeast Asia, South China and Indian subcontinent (Liu *et al.*, 2006; Oka *et al.*, 2007). Some studies using mtDNA D-loop sequences have addressed the origin of African native chickens. Muchadeyi *et al.* (2008) observed two distinct haplogroups in Zimbabwe native chickens, which may be originated from Southeast Asia and the Indian subcontinent. Accordingly, Razafindraibe *et al.* (2008) observed two haplotypes in Madagascar native chicken originated from Indonesia and African continent or an introgression from commercial lines. On the other hand, a single haplogroup seems to be of Indian origin was observed in Nigeria native chickens (Adebambo *et al.*, 2010). In addition, Mwacharo *et al.* (2011) analyzed partial mtDNA D-loop sequences in East Africa (Kenya,

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Correspondence: Dr. S. Okamoto, Laboratory of Animal Breeding and Genetics, Faculty of Agriculture, Kagoshima University, Korimoto 1-21-24, Kagoshima 890-0065, Japan.

(E-mail: chacha@agri.kagoshima-u.ac.jp)

Ethiopia, Sudan and Uganda) native chickens, and revealed the existence of at least five genetically distinct mtDNA D-loop haplogroups, which were originated from south and southwest China and/or surrounding regions as well as Southeast Asia such as Myanmar and Thailand. An investigation using microsatellite markers reported the three groups of Egyptian chickens (Eltanany *et al.*, 2011). Ramadan *et al.* (2011) compared the phylogenetic differentiation between two Egyptian chicken breeds with mtDNA D-loop region. However, there are few works to evaluate the genetic diversity on Egyptian native chickens using mtDNA sequences.

Therefore, the present study was undertaken to estimate the genetic diversity and origin of Egyptian native chickens as genetic resources in the future.

Materials and Methods

Sampling

A total of 36 Egyptian native chicken blood samples were collected from the preserving stations belong to Alazab Poultry Integrated Project, the Fayoum governorate, Egypt. To verify the genetic relationships of the study populations to Asiatic and other African chickens, 14 haplotypes from the GenBank were included in the analysis.

PCR Amplification and Sequencing

DNA extraction from whole blood samples was performed using standard phenol-chloroform extraction (Sambrook, 1989), which were stored at -20°C until use. A 546-bp in the D-loop hypervariable region in mtDNA was amplified with the following primer forward: L16750 (5'-AGGAC-TACGGCTTGAAAAGC-3'; Akishinonmiya *et al.*, 1994) and reverse: H522 (5'-ATGTGCCTGACCGAGGAACCAG-3'; Liu *et al.*, 2006). The PCR reaction included of 100 ng of template DNA, 4 pmol of each primers, 1x EX Taq buffer, 400 μmol of dNTPs mixture and 1 unit of EX Taq HS polymerase (Takara, Otsu, Japan). The PCR was conducted

at 94°C for 2 min, followed by 30 cycles consisting of 1 min denaturation at 94°C , 1 min annealing at 60°C and 1 min extension at 72°C , with final extension at 72°C for 7 min using a Gene Amp PCR System 9700 (Applied Biosystems Inc, Foster City, CA, USA). The PCR products were electrophoresed (100 V, 40 min) on 2% agarose gels, and purified with Viogene Gel/PCR DNA Isolation System GP1001 (Viogene BioTek Corp, Taipei, Taiwan). The DNA sequence analysis was performed with Big Dye Terminator cycle sequencing kit v.3.1 and ABI Prism 3500 genetic analyzer sequencer (Applied Biosystems Inc).

Data Analysis

Multiple alignment analysis for the mtDNA D-loop nucleotide sequence data was carried out using the Clustal X version 2.1 computer software (Larkin *et al.*, 2007). The position and number of polymorphic sites and corresponding haplotypes were calculated using MEGA v5.2.1 (Molecular Evolutionary Genetic Analysis Version 5.2.1 (Tamura *et al.*, 2011). Nucleotide diversity (Nei and Li, 1979) and haplotype diversity (Nei, 1987) were estimated using ARLEQUIN v.3.5.1.3 (Excoffier *et al.*, 2010). A median joining network (Bandelt *et al.*, 1999) was constructed using NETWORK 4.611 software (Fluxus technology Ltd.) to classify the haplotypes under the setting described by Cuc *et al.* (2011) into nine clades, following Liu *et al.* (2006), three clades, following Oka *et al.* (2007), and two clades, following Muchadeyi *et al.* (2008). The list of haplotypes used and the corresponding GenBank accession number are shown in Table 1.

Results and Discussion

Genetic Diversity and Haplotype Distribution

Sequences spanning the 546-bp in the D-loop hypervariable region in mtDNA were used for analysis. Out of 36 individuals, a total of 4 variable sites were identified (Fig. 1). No insertions or deletions were detected in the present

Table 1. Haplotype names and accession numbers of reference chicken mtDNA sequences from DNA databank were used in the present study

Haplotype	GenBank accession No.	Reference
E1 - E5		This study
Liu_A1	AB114069	Liu <i>et al.</i> (2006) haplotype A1
Liu_B1	AB007744	Liu <i>et al.</i> (2006) haplotype B1
Liu_C1	AB114070	Liu <i>et al.</i> (2006) haplotype C1
Liu_D1	AY588636	Liu <i>et al.</i> (2006) haplotype D1
Liu_E1	AB114076	Liu <i>et al.</i> (2006) haplotype E1
Liu_F1	AF512285	Liu <i>et al.</i> (2006) haplotype F1
Liu_G1	AF512288	Liu <i>et al.</i> (2006) haplotype G1
Liu_H1	D82904	Liu <i>et al.</i> (2006) haplotype H1
Liu_I1	AB009434	Liu <i>et al.</i> (2006) haplotype I1
Oka_D6	AB268535	Oka <i>et al.</i> (2007) haplotype D6
Oka_G1	AB268545	Oka <i>et al.</i> (2007) haplotype G1
Oka_F1	AB268543	Oka <i>et al.</i> (2007) haplotype F1
Z_A1	AM746024	Muchadeyi <i>et al.</i> (2008) haplotype A1
Z_C1	AM746040	Muchadeyi <i>et al.</i> (2008) haplotype C1

	1	1	2	2
	4	5	0	9
	5	1	8	4
E1	C	T	C	A
E2	.	.	.	G
E3	.	.	A	.
E4	A	.	.	.
E5	.	C	.	.

Fig. 1. **Variable sites for 5 haplotypes observed in the Egyptian native chicken populations.** Dots indicate nucleotide positions identical to those of Haplotype E1. Numbers at the top refer to variable sites and correspond to the nucleotide positions of Haplotype E1.

sequences. In the present study, the haplotypic diversity within the Egyptian chickens was 0.5635 ± 0.0845 which was higher than those of African populations (Ethiopian 0.374, Sudanese 0.413 and Ugandan 0.322: Mwacharo *et al.*, 2011) or (Nigerian 0.421: Adebambo *et al.*, 2010). However, the value is lower than those of other African fowls (Kenyan 0.857: Mwacharo *et al.*, 2011 or Zimbabwean 0.730: Muchadeyi *et al.*, 2008) and also lower than some Asian fowls (Vietnamese 0.615 to 0.942: Cuc *et al.*, 2011) or (Laotian 0.854: Kawabe *et al.*, 2014). The nucleotide diversity within the Egyptian chicken was 0.00123 ± 0.00108 , which was lower than other African and Asian fowls except Ugandan fowls (0.00096: Mwacharo *et al.*, 2011). Therefore, these results indicate that Egyptian native chicken have moderate genetic diversity in African chicken populations.

Network Analysis of Haplotypes

The five haplotypes were clustered into one clade, observed in the Egyptian native chickens (Fig. 2). Most of Egyptian native chickens were classified in the haplotype E1, which contains 23 individuals (63.9%) followed by E4 (6 individuals, 16.6%), E5 (4, 11.1%), E2 (2, 5.5%) and E3 (1, 2.7%), respectively. All haplotypes in this study clustered mainly with one haplogroup (clade C obtained from Muchadeyi *et al.*, 2008) was similar to Zimbabwean, Sudanese, Northwest European chickens and six purebred lines. The haplotype E1 in the present study is identical to the partial sequence of haplotype C3 (clade C from Muchadeyi *et al.*, 2008), and haplotype E1 (clade E from Liu *et al.*, 2006), which sequenced from the chickens of Europe, the Middle East (Iran, Azerbaijan and Turkmenistan), India, China (provinces adjacent to Yunnan, South East) and Japan. Whereas, the other quoted haplotypes were not detected with Egyptian chicken in the present study. Liu *et al.* (2006) stated that the maternal lineages associated with this clade could have originated from the Indian subcontinent. Oka *et*

al. (2007) suggested that this clade originated in Southeast Asia and was first introduced to the Indian subcontinent before spreading to other regions. Previous reports (MacDonald and Edwards, 1993; Fuller *et al.*, 2011; Mwacharo *et al.*, 2013) suggested that, chickens may have first entered into Africa overland from the North area such as Egypt, and then dispersed into the South along the Nile valley to Nubia and after that in West Africa along the Sudano-Sahelian corridor. MacDonald and Edwards (1993) reported that chickens distributed into West Africa from the East African coast through the Sudano-Sahelian belt, occurred much later, those seems to be related to Indian Ocean trade networks. Liu *et al.* (2006) suggests that the introduction of the haplogroup E into the African continent was almost the consequence of a maritime introduction. Its arrival might have followed the Chinese maritime trading expeditions across the Indian Ocean (Duyvendak, 1939; Beaujard, 2005; Mwacharo *et al.*, 2011). These findings indicate that the maternal lineages was involved in the origin of domestic chicken in Egypt may have roots in Indian subcontinent and other Southeast Asia. The genetic information from this study will probably pave the way to further studies for evaluation, preservation and improvement of Egyptian native chickens as genetic resources in the future.

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Fig. 2. **Median network profile of the mtDNA D-loop haplotypes observed in the present study.** Data merged with sequences of major haplotypes described from Liu *et al.* (2006), Oka *et al.* (2007) and Muchadeyi *et al.* (2008). The circle size corresponds to haplotype frequency. Empty circles are median vectors used in connecting haplotypes indirectly.

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