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

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. Akishinonomiya 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,
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′-AGGACTACGGCTTGAAAAGC-3′; Akishinonomiya et al., 1994) and reverse: H522 (5′-ATGTGCTGACCGAGGAACCAG-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 Clastal 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>
<thead>
<tr>
<th>Haplotype</th>
<th>GenBank accession No.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 - E5</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>Liu_A1</td>
<td>AB114069</td>
<td>Liu et al. (2006) haplotype A1</td>
</tr>
<tr>
<td>Liu_B1</td>
<td>AB007744</td>
<td>Liu et al. (2006) haplotype B1</td>
</tr>
<tr>
<td>Liu_C1</td>
<td>AB114070</td>
<td>Liu et al. (2006) haplotype C1</td>
</tr>
<tr>
<td>Liu_D1</td>
<td>AY588636</td>
<td>Liu et al. (2006) haplotype D1</td>
</tr>
<tr>
<td>Liu_E1</td>
<td>AB114076</td>
<td>Liu et al. (2006) haplotype E1</td>
</tr>
<tr>
<td>Liu_F1</td>
<td>AF512285</td>
<td>Liu et al. (2006) haplotype F1</td>
</tr>
<tr>
<td>Liu_G1</td>
<td>AF512288</td>
<td>Liu et al. (2006) haplotype G1</td>
</tr>
<tr>
<td>Liu_H1</td>
<td>D82904</td>
<td>Liu et al. (2006) haplotype H1</td>
</tr>
<tr>
<td>Liu_I1</td>
<td>AB009434</td>
<td>Liu et al. (2006) haplotype I1</td>
</tr>
<tr>
<td>Oka_D6</td>
<td>AB268535</td>
<td>Oka et al. (2007) haplotype D6</td>
</tr>
<tr>
<td>Oka_G1</td>
<td>AB268545</td>
<td>Oka et al. (2007) haplotype G1</td>
</tr>
<tr>
<td>Oka_F1</td>
<td>AB268543</td>
<td>Oka et al. (2007) haplotype F1</td>
</tr>
<tr>
<td>Z_A1</td>
<td>AM746024</td>
<td>Muchadeyi et al. (2008) haplotype A1</td>
</tr>
<tr>
<td>Z_C1</td>
<td>AM746040</td>
<td>Muchadeyi et al. (2008) haplotype C1</td>
</tr>
</tbody>
</table>
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.

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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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