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Mitochondrial DNA Diversity in Five Egyptian Sheep Breeds

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Abstract: Threats to biodiversity are increasing, whether measured in terms of extinction rate, destruction of ecosystems and habitat or loss of genetic diversity within the species utilized in agriculture. In these conditions, it is more strategically important than ever to preserve as much the farm animal diversity as possible, to ensure a prompt and proper response to the needs of future generations. In this study, we used mtDNA to analyze genetic characterization of five sheep breeds reared in Egypt- Barki, Ossimi, Rahmani, Saidi and Sohagi- to clarify the genetic affinities of these breeds and their biodiversity. A 721-bp fragment from 15,541 to 16,261 bp (NC_001941.1) of the mtDNA control region was amplified using specific primers and the polymerase chain reaction was performed. The PCR products were purified and sequenced. The mitochondrial DNA analysis identified 58 haplotypes in the analyzed 90 animals with 127 polymorphic sites. The sequences of these haplotypes were submitted to NCBI/Bankit/GenBank with the accession numbers: KC291205 - KC291244 and KC461240 - KC461257. Within all tested breeds, the haplotype diversity and average number of pairwise differences were 0.98652 and 17.14782, respectively. The result showed that the highest nucleotide diversity in five tested breeds was in Sohagi breed (0.03654) and the lowest one in Rahmani breed where it was 0.01433 with the total nucleotide diversity in the five tested breeds 0.02378.The genetic distances (D) and the average number of pairwise differences (Dxy) between breeds were estimated. The lowest distance was observed between Barki and Rahmani breeds (D: 12.226 and Dxy: 0.01696) whereas the highest distance was observed between Saidi and Sohagi breeds (D: 26.068 and Dxy: 0.03616).The phylogeny result showed the presence of four haplogroups- HapA, HapB, HapC and HapE in the 90 examined samples with the absence of the fifth haplogroup HapD. The result showed that 76 out of 90 tested animals cluster with haplogroup B whereas nine tested animals cluster with haplogroup A, four animals cluster with haplogroup C and only one animal clusters with haplogroup E.

Key words: MtDNA · Diversity · Haplotypes · Phylogeny · Egyptian Sheep Breeds

INTRODUCTION

The history of domestication of human-related species (cow, sheep, goat, pig, dog, etc...) is clarified from the recent study of finer variations in the genomes. In sheep, search approaches for polymorphisms in broadband [1], polymorphisms insertions stabilized retroviruses [2] and the study of mitochondrial genome [3] have not yet determined the exact number of domestication events that occurred from wild populations to the present species. The first event of sheep domestication would have happened since 10 000 to 11 000 years in the Fertile Crescent [4]; the birthplace of agriculture, urbanization and trade. Its existence is supported by the presence of the oldest sheep archaeozoological evidence in present-day in Iran, Turkey and Cyprus.

Studies based mainly on sequencing of mitochondrial DNA showed that there are five maternal lineages in the world for domestic sheep breeds (*Ovis aries*) and these lineages called haplogroups A, B, C, D and E [5-8]. Haplogroup B includes breeds mainly distributed in Northern Europe and Eastern Mediterranean. Haplogroup A sheep breeds are particularly prevalent in Asia and Europe. Haplogroups C, D and E have been discovered in the Middle East and the Caucasus and they are

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characterized by fat tails with reserves that allow a tolerance to the lack of water for several days. Domestication of these breeds is not clearly established as independent from the haplogroups A and B.

The total population of domestic sheep in Egypt reaches to about 5.6 million animals. The so-called "majority" (Barki, Ossimi and Rahmani) breeds represent 65% of the sheep population while the so-called "minority" (Sohagi, Saidi, Awassi, Fallahi and Farafra) breeds represent 35% of the population [9].

Currently, more than 1300 different breeds of sheep are known for more than 1.1 billion animals [10]. However, 181 breeds are now extinct, more than 12% of breeds identified and many other breeds are threatened. They suffer such a loss of genetic variability due to an increase of inbreeding resulting from modern methods of selection exclusively oriented high productivity [11]. That is why it is urgent to maintain in countries with "old" breeds, not highly selected, a large genetic variability in the interest of genetic resources conservation.

Because of the eastern location of Egypt in the Mediterranean basin and the presence of fat tailed sheep breeds- character quite common in Turkey and Syria- where genotypes that seem quite primitive, the phylogenic studies of Egyptian sheep breeds is become particularly attractive. This work focused on the phylogeny study of five sheep breeds reared in Egypt to clarify the genetic affinities of these breeds and their biodiversity.

MATERIALS AND METHODS

Animals: Blood samples were collected from 90 animals belonging to five sheep breeds reared in Egypt, Barki (20 animals), Ossimi (22 animals) and Rahmani (25 animals), Saidi (11 animals) and Sohagi (12 animals). The selected animals were collected from different local farms; Barki (from Animal Breeding Research Station in Borg El-Arab, Alex), Ossimi (from Animal Breeding Research Station in Seds, Bani Sweif), Rahmani (from Animal Breeding Research Station in Sero, Damieta) and Saidi and Sohagi breed (From Faculty of Agriculture, Assiut University) and only 2-3 individuals were collected from one herd to minimize the likelihood of any close genetic relationships.

Genomic DNA Extraction: Blood was collected into EDTA tubes from unrelated animals (depending on the farm records) of each sheep breed. DNA was extracted from the blood samples according to established protocol [12] with minor modifications. Briefly, Blood samples were mixed with cold 2x sucrose-Triton and centrifuged at 5000 rpm for 15 min at 4°C. The nuclear pellet was suspended in lysis buffer, sodium dodecyl sulfate and proteinase K and incubated overnight in a shaking water bath at 37°C. Nucleic acids were extracted with saturated NaCl solution. The DNA was picked up and washed in 70% ethanol. The DNA was dissolved in 1x TE buffer. DNA concentration was determined, using Nano Drop1000 Thermo Scientific spectrophotometer and then diluted to the working concentration of 50ng/µl, which is suitable for polymerase chain reaction.

Polymerase Chain Reaction (PCR): Primers for the amplification of the partial D-loop region were designed using the software PolyPrimers [13], starting from the complete mtDNA sequence of *Ovis aries* in Genebank (NC_001941.1).

Primer OA_15346F: GGAGAACAACCAACCTCCCTA
Primer OA_157R: TGATTCGAAGGGCGTTACTC

A PCR cocktail consists of 4 µl of each oligonucleotide primer (10 pM/ µl), 5 µl of dNTPs (2 mM), 10 µl of 5X PCR buffer, 1 µl of Taq polymerase (5 units/µl) and 25 µl of sterile water. This PCR cocktail was added to 1 µl of genomic DNA (20 ng/ µl). The reaction mixture was preheated at 95°C for 5 min followed by 35 cycles; 30 sec. at 95°C, 30 sec. at 62°C and 2 min. at 72°C; followed by final extension at 72°C for 2 min.

Sequencing analysis of mtDNA: The forward primer (OA_15346F, GGAGAACAACCAACCTCCCTA) and an internal primer (OA_16032F, ATGCGTATCCTGT CCATTAG) were used for sequencing. The sequencing protocol was developed using the sequencer Ceq8800, after purification of the fragments with ExoSap-IT (USB Corporation) to remove residual primers and dNTPs. Sequencing was performed in Macrogen Incorporation (Seoul, Korea).

Data analysis:

- D-loop sequences were aligned using the BioEdit software [14] in order to identify and trace individual haplotype mutations.
- Haplotype structure, sequence variation, average number of nucleotide differences (D) and average number of nucleotide substitutions (Dxy) per site between breeds were calculated using DnaSP 5.00 software [15].

- Neighbour-joining (NJ) tree for all samples was constructed using Mega version 5.0 software [16].

RESULTS AND DISCUSSON

It is likely that a high number of breeds are being and will be lost in the near future, before their characteristics can be studied and their potential evaluated. This is particularly worrying because of uncertainties due to rapid climate change, increasing and differentiating market demand and human demographic expansion [17]. In these conditions it is more strategically important than ever to preserve as much the farm animal diversity as possible, to ensure a prompt and proper response to the needs of future generations.

Mitochondrial sequencing has been used to explain the origins of many modern domestic livestock species. Sheep data are beginning to match the pattern observed in other domestic species. Previous studies in goat [18-20], cattle [21, 22] and pig [23] have revealed additional maternal clades. Studies based mainly on sequencing of the control region in mitochondrial DNA showed that there are five maternal lineages in the world for domestic sheep breeds (*Ovis aries*) and these lineages called haplogroups A, B, C, D and E.

In this study, we used mtDNA to analyze genetic characterization of five sheep breeds reared in Egypt to clarify the genetic affinities of these breeds and their biodiversity.

A 721-bp fragment from 15,541 to 16,261 bp (NC_001941.1) of the mtDNA control region was amplified using specific primers and polymerase chain reaction. The PCR products were purified and sequenced. The analyzed samples in this study were 90 samples belonging to five sheep breeds reared in Egypt; Barki, Ossimi, Rahmani, Saidi and Sohagi. The alignment of all 90 analyzed samples was done using BioEdit software. DnaSP 5.00 software was used to identify the sequence variation and polymorphic sites in the aligned sequences.

In this study, we identified 58 haplotypes in the analyzed 90 animals with 127 polymorphic sites. The sequences of these haplotypes were submitted to NCBI/Bankit/GenBank with the accession numbers: KC291205 - KC291244 and KC461240 - KC461257. The highest frequency one of the 58 recorded haplotypes was recorded in 5 animals; 4 haplotypes were present in 4 animals; 4 haplotypes in 3 animals; 8 haplotypes in 2 animals and the other 41 haplotypes were occurred in one animal. Each tested breed has specific haplotypes; 16 in Barki, 12 in both Rahmani and Ossimi, 7 in Saidi and 11 in Sohagi breeds.

The statistical analysis of genetic diversity within 5 tested breeds (Table 1) showed that the highest number of haplotypes (16 haplotypes) was found in Barki breed where its 20 animals possessed 83 polymorphic sites, whereas the number of haplotypes was equal in Rahami and Ossimi where it was 12 in each breed (with 66 polymorphic sites). The lowest number of haplotypes (7 haplotypes) was found in Saidi breed where its 11 animals possessed 62 polymorphic sites followed by 11 haplotypes which was found in Sohagi breed (its 12 animals possessed 70 polymorphic sites).

The haplotype diversity in the 5 tested Egyptian breeds ranged from 0.98485 in Sohagi breed (with average number of pairwise differences K: 26.34848) to 0.90909 in Saidi breed (K: 24.10909). Within all tested breeds, the haplotype diversity and average number of pairwise differences were 0.98652 and 17.14782, respectively. The result showed that the highest nucleotide diversity in five tested breeds was in Sohagi breed (0.03654) and the lowest one in Rahmani breed where it was 0.01433 with the total nucleotide diversity in the five tested breeds 0.02378 (Table 1).

The genetic distances (D) and the average number of pairwise differences (Dxy) between breeds were estimated (Table 2). The lowest distance was observed between Barki and Rahmani breeds (D: 12.226 and Dxy: 0.01696) followed by distance between Rahmani and Ossimi breeds (D: 13.138 and Dxy: 0.01822 and then distance between Barki and Ossimi breeds (D: 13.936 and Dxy: 0.01933). On the other hand, the highest distance was observed between Saidi and Sohagi breeds (D: 26.068 and Dxy: 0.03616) followed by distance between Sohagi and Ossimi (D: 24.614 and Dxy: 0.03414).

Table 1: The genetic diversity data of the five tested Egyptian sheep breeds

Breed	Barki	Rahmani	Ossimi	Saidi	Sohagi	Total
No. of samples	20	25	22	11	12	90
No. of polymorphic sites (S)	83	66	66	62	70	127
No. of haplotypes (H)	16	12	12	7	11	58
Haplotype diversity (HD)	0.97368	0.92333	0.91342	0.90909	0.98485	0.98652
Average No. of pairwise differences (K)	13.32105	10.33333	14.20779	24.10909	26.34848	17.14782
Nucleotide diversity (π)	0.01848	0.01433	0.01971	0.03344	0.03654	0.02378

Table 2: Average pairwise differences between populations

	Barki	Rahmani	Ossimi	Saidi	Sohagi
Barki	------	0.01696	0.01933	0.02733	0.03261
Rahmani	12.226	------	0.01822	0.02644	0.03266
Ossimi	13.936	13.138	------	0.02868	0.03414
Saidi	19.705	19.065	20.678	-----	0.03616
Sohagi	23.508	23.550	24.614	26.068	------

Average number of nucleotide difference between populations, D (below)

Average number of nucleotide substitution per site between populations, Dxy (above)

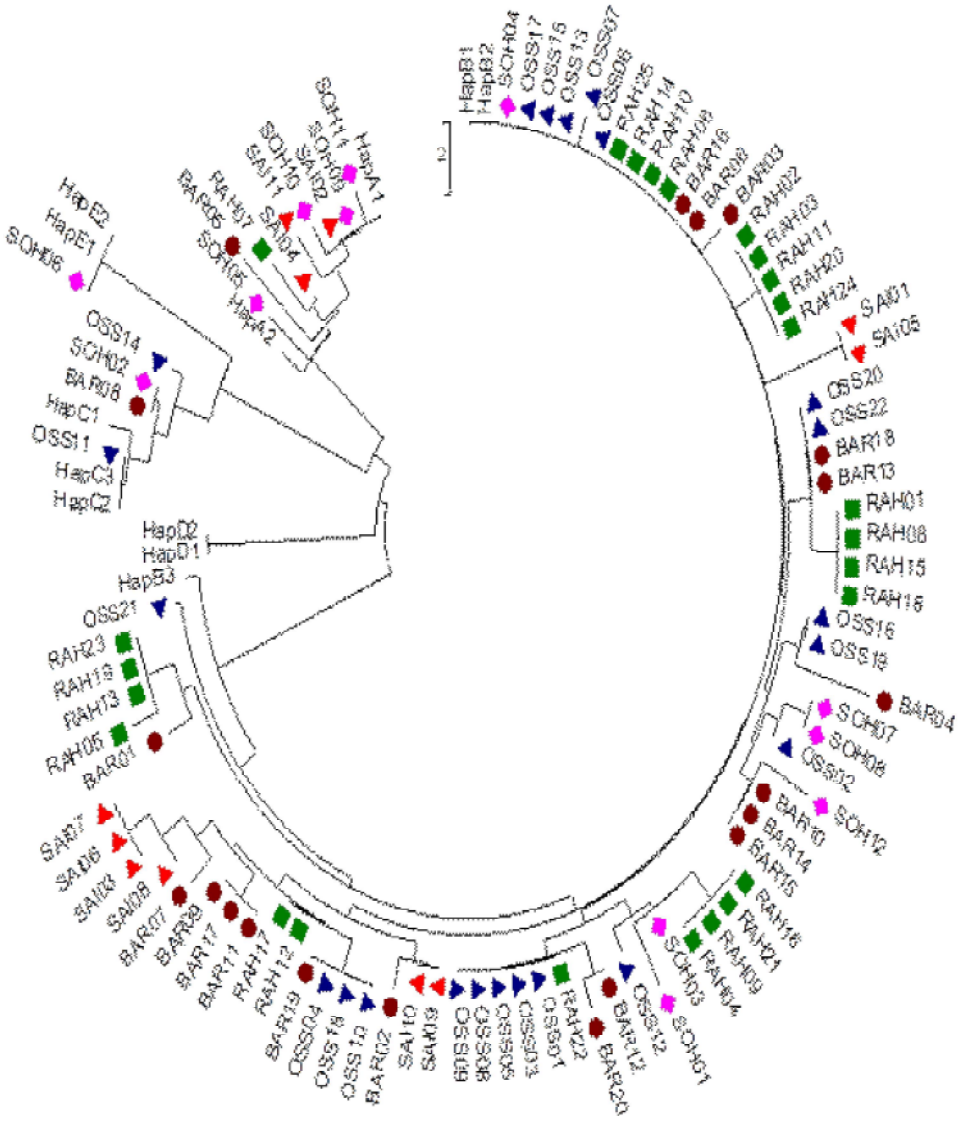

Fig. 1: Neighbour-joining (NJ) tree of the tested animals as circle
BAR: Barki, OSS: Ossimi, RAH: Rahmani, SAI: Saidi and SOH: Sohagi breeds

Neighbour-joining (Phylogeny) tree was constructed using Mega 5.0 software (Figs. 1 and 2). The sequences of the 90 analysed samples were aligned with references sequences of different haplogroups to define the haplogroups to which the analysed samples are belonged. References sequences used for defining haplogroups were: DQ852286 (A1) and DQ852287 (A2) for A haplogroup; DQ852285 (B1), DQ852282 (B2) and

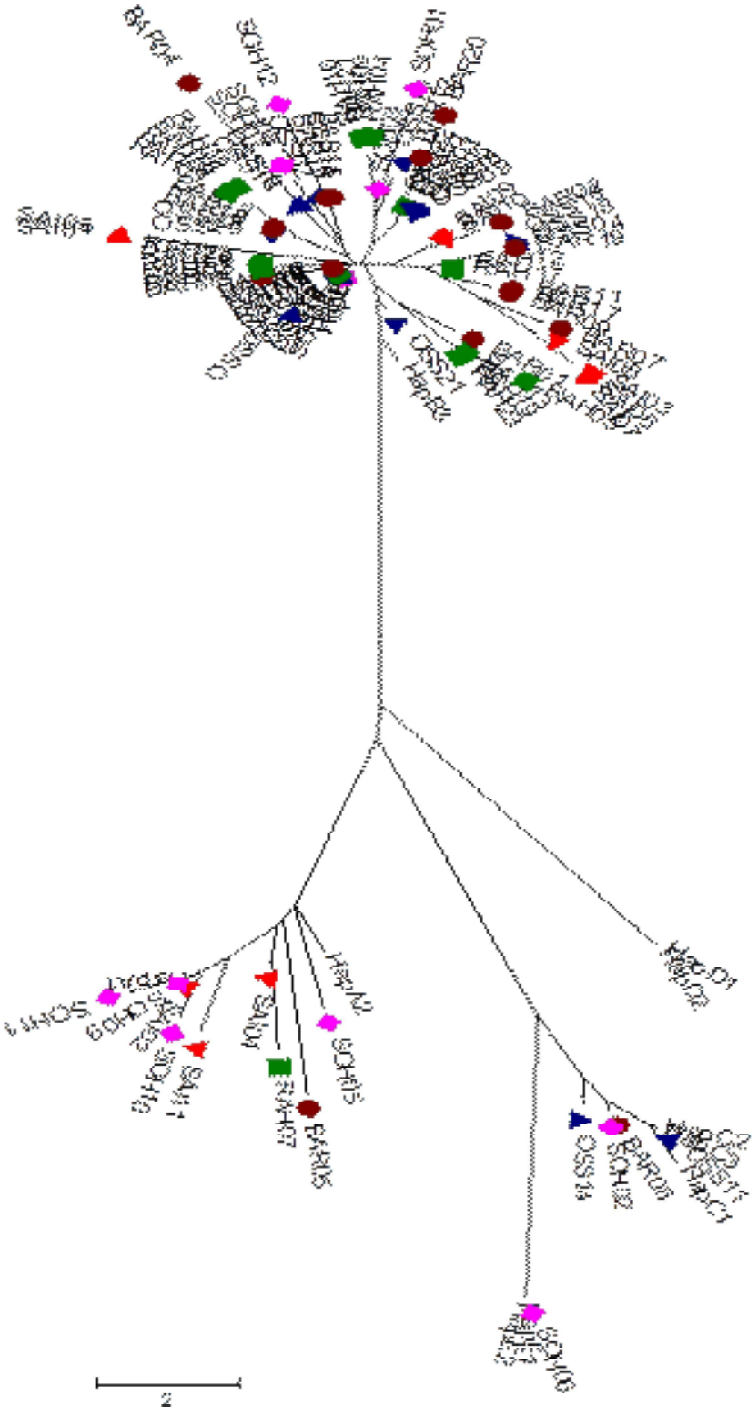

Fig. 2: Neighbour-joining (NJ) tree of the tested animals as radiation
BAR: Barki, OSS: Ossimi, RAH: Rahmani, SAI: Saidi and SOH: Sohagi breeds

AF039579 (B3) for B haplogroup; DQ097460 (C1), DQ097462 (C2) and DQ852283 (C3) for C haplogroup; DQ852288 (D1) and DQ852289 (D2) for D haplogroup and DQ852280 (E1) and DQ852281 (E2) for E haplogroup.

The phylogeny result showed the presence of four haplogroups (HapA, HapB, HapC and HapE) in the 90 examined samples with the absence of the fifth haplogroup described in literatures (HapD). The result

Table 3: The different haplogroups to which the tested animals are belonging

		Haplogroup A		Haplogroup B		Haplogroup C		Haplogroup E	
Breed	No. of samples	No.	%	No.	%	No.	%	No.	%
Barki	20	1	5%	18	90%	1	5%	0	0%
Rahmani	25	1	4%	24	96%	0	0%	0	0%
Ossimi	22	0	0%	20	90.9%	2	9.1%	0	0%
Saidi	11	3	27.3%	8	72.7%	0	0%	0	0%
Sohagi	12	4	33.4%	6	50%	1	8.3%	1	8.3%
Total	90	9	10%	76	84.5%	4	4.4%	1	1.1%

showed that 76 out of 90 tested animals cluster with haplogroup B (84.5%) whereas nine tested animals cluster with haplogroup A (10%), four animals cluster with haplogroup C (4.4%) and only one animal clusters with haplogroup E (1.1%) (Table 3).

The result declared that the most dominant haplogroup in Egyptian sheep breeds is haplogroup B which is present with the highest frequency in all tested breeds; Rahmani (96%), Barki and Ossimi (90%), Saidi (72.7%) and Sohagi (50%). This result agreed with literatures which reported that haplogroup B is dominant in Eastern Mediterranean countries like Syria and Turkey [7]. From this region; the birthplace of agriculture, urbanization and trade; the first event of domestication would have happened since 10 000 to 11 000 years [4] and enter Northern Africa via Sinai and the precursors of Egyptian sheep breeds came from Northern Syria and Southern Turkey [24].

Nine of the 90 analyzed sheep cluster with haplogroup A; seven of them belong to Saidi and Sohagi breeds which are present in Upper Egypt. This haplogroup is particularly dominant in Asia [25] and this is an indication for that the Egyptian breeds in Upper Egypt is genetically closer to Asian sheep breeds rather than breeds present in Eastern Mediterranean and European countries in which the haplogroup B is dominant.

Moreover, the presence of the haplotype C in the Egyptian sheep breeds is in agreement with literatures which reported the presence of haplogroup C in the steppe and semi-desert regions where the fat-tailed sheep are distributed [7] and exists mainly in Central Asia, India and China. On the other hand, the absence of haplogroup D and the presence of only one animal cluster with haplogroup E is a logical result because these haplogroups are present in Caucasus area which is far away from Egypt and there is no any migration for sheep domestication was done from this area.

CONCLUSION

This work declared that most Egyptian sheep animals are belonged, as expected, to haplogroup B which is dominant in Eastern Mediterranean countries. Also the appearance of haplogroups A and C is a logical result because these haplogroups are most frequently present in Asian countries with semi-desert regions.

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