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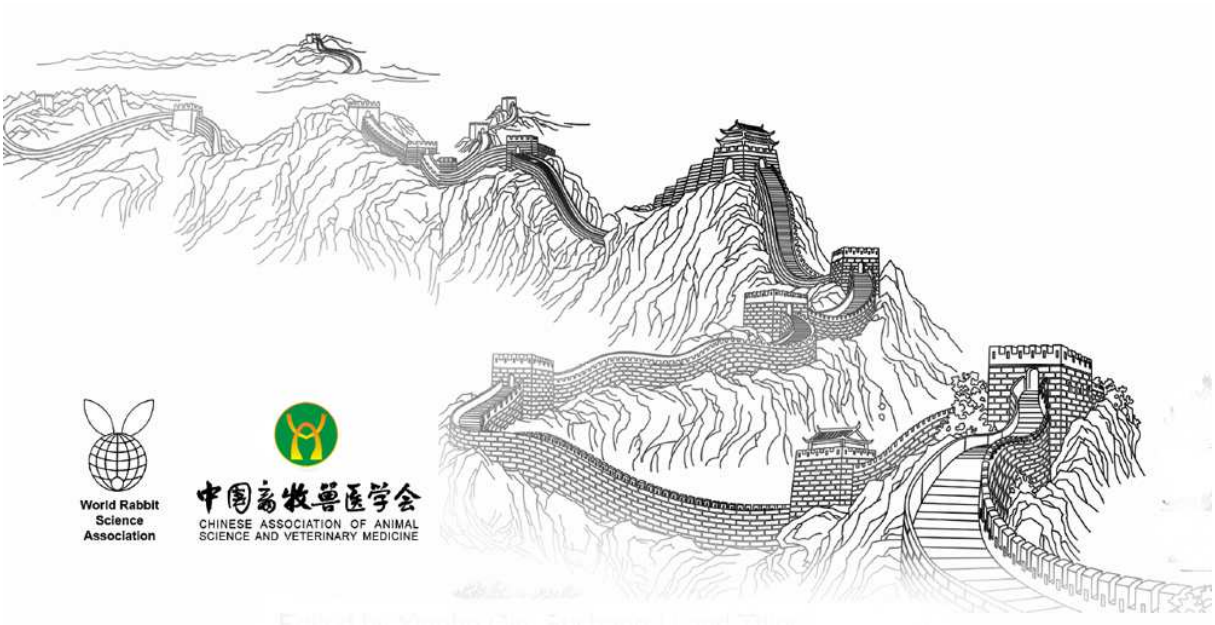

ORIGIN OF EGYPTIAN AND SPANISH COMMON RABBITS:EVIDENCE FROM MITOCHONDRIAL DNA CYTOCHROME B SEQUENCE ANALYSIS

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ABSTRACT

Mitochondrial DNA (mtDNA) cytochrome b (cyt b) gene sequences were used to determine the phylogeny and origin of local rabbit breeds in Egypt and Spain. The Egyptian local rabbit breeds included Egyptian Red Baladi (ERB), Egyptian Black Baladi (EBB) and Egyptian Gabali Sinai (EGS), while the Spanish local rabbit breed was Spanish common rabbit (SCR). Breeds were compared with European Wild Rabbit (EWR) taken from Albacete, Spain. The most common haplotype (A) was combined with 43.5% of published sequences, while haplotype B was combined with only 4.3%. This study provides evidence that Egyptian breeds and SCR were introduced from European rabbits. The most frequent haplotypes were recorded in EWR and EGS (50% and 21.4, respectively).

Key words: Egyptian rabbits, Spanish common rabbit, European wild rabbit, Origin, mtDNA.

INTRODUCTION

Mitochondrial DNA (mtDNA) is a genetic marker widely used to find out the control region, haplotypes information and identification of relations among haplotypes by software sequences analyzing (Achilli *et al*., 2008). Also, it is used for tracing of forebears wild animals and guide researchers to determine species demonstration by ability to generate some signal about population history over short time (FAO, 2011). The reasons for the adoption of mtDNA as marker of choice are well known. Experimentally, mtDNA is relatively easy to amplify because it appears in multiple copies in the cell. Mitochondrial gene content is strongly conserved across animals, with very few duplications, no intron, and very short intergenic regions (Gissi *et al*., 2008).

During the last three decades, mtDNA has been widely used in rabbits (Ennafaa *et al*., 1987; Seixas *et al*., 2014). The genetic studies have focused on the European geographical expansion of this species (Branco *et al*., 2000; Campos *et al*., 2012). Christensen and Peng (2012) mentioned that mtDNA is used to identify the primary origins for domestic rabbits.

In the current study, our aim was to sequence 450 base pairs of mtDNA cytochrome b (cyt b) gene to investigate genetic diversity among local Egyptian and Spanish local breeds. Egyptian breeds were Egyptian Red Baladi (ERB), Egyptian Black Baladi (EBB) and Egyptian Gabali Sinai (EGS), while the Spanish local rabbit breed was Spanish common rabbit (SCR). Previous breeds were compared with European Wild Rabbit (EWR) to perform those unspecified origin through phylogenetic analysis.

MATERIALS AND METHODS

Sampling and mtDNA sequencing

A total of 132 rabbit samples belonging to Egyptian and Spanish local breeds were used. Egyptian breed samples (ERB, EBB and EGS) were selected according to Khalil (1999; 2002). The survey covered different farms in different sectors in Egypt (Delta, Sinai Peninsula in Egypt). Seven blood samples of ERB collected from two research farms in Gemeza and Gezeret Al shaeer. Thirty two blood samples of EBB were collected from two research farms in Sakha and Bourg Al Arab. Thirty one blood and hair samples of EGS were collected from Sakha and Gmeza (research farms) and El Goura market. In Spain, 32 hair samples of SCR from two backyard farms in Seville and Higher Technical School of Agricultural Engineering rabbit farm were selected according to González-Redondo (2007), and 30 European Wild Rabbit (EWR) samples were also gathered from a hunting preserve located in Albacete.

Samples of DNA for Egyptian breeds were extracted by minikits blood (Qiaamp, Qiagen, GmbH, Hilden, Germany). While DNA extraction from hair samples of SCR and EWR was performed using the EasySpin Genomic DNA Tissue Kit (SP- TD-250, Citomed, Lisbon, Portugal).

MtDNA Cytb sequence (450 bp) fragmentation was used in this study. Total genomics of DNA were amplified using Pro1 as a forward primer (5'-CCACCATCAGCACCCAAAGCT-3'), and NC4 as a reverse primer (5'-GGTTCTTACCTCAGGGCCAT-3'). The positions of nucleotide were numbered from complete published sequence of mtDNA (GenBank accession number AJ001588). The PCR products were sequenced in the Centre for Molecular Analysis (CTM, Porto, Portugal) using automated DNA sequencer (ABI PRISM 3130 XL).

Data analysis

Sequences were aligned with DNASTAR software (DNASTAR Inc., Madison, WI, USA). Breeds purity was evaluated based on sequence alignment and each breed individuals of a certain group should be similar in 90% or less. The differentiation sequences higher than 90% percent were eliminated. Sequence data have been submitted to GenBank (accession numbers: range KT029916 - KT030047). Mega 6.0 (Tamura *et al,*. 2013) was used to estimate unrooted neighbour joining (NJ) tree with the percentage of bootstrap values 2000 replications was used to determine the relation between current study haplotypes and published haplotypes sequences in GenBank. A median-joining network profile of the individuals was constructed by the phylogenetic Network Software, version 4.613 (http://www.fluxus-engineering.com).

RESULTS AND DISCUSSION

In this study, we recorded 14 haplotypes (A-N) for 132 individual rabbits. Figure 1 shows the parsimony network for 14 haplotypes. Parsimony network analysis of mtDNA sequences was used to

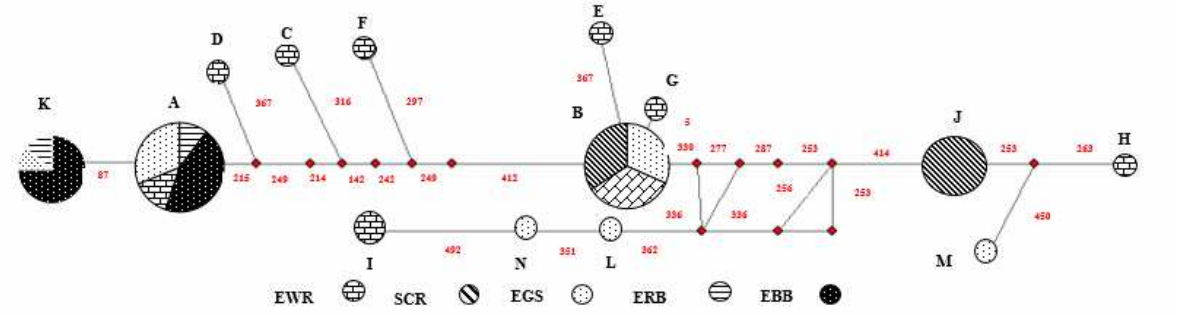

Figure 1: Maximum parsimony network representing the phylogenetic relationships between 14 mtDNA haplotypes.

delimit phylogenetic species of tested rabbits in an objective, formal manner based on cyt b gene. The highest number of different haplotypes (7 haplotypes) was recorded in EWR (C, D, E, F, G, H, I), while only 3 haplotypes were recorded in EGS (L, M, N). Haplotype A shared Egyptian rabbits and

EWR (83.3 and 16.6%, respectively), while haplotype B shared Spanish rabbits and EGS (66.6 and 33.3%, respectively). The percentage of Egyptian rabbit in haplotype A were 35.7, 37.1 and 10.5 for EGS, EBB and ERB, respectively. The Spanish rabbits percent in haplotype B were 33.6 and 33.1 for SCR and EWR, respectively. While, haplotypes K and J occurred as 42.9% and 59.4 for Egyptian rabbits and SCR, respectively.

The unrooted NJ tree for 14 haplotypes (Fig. 2) showed 2 main linages. The linage A contained 3 haplotypes for wild rabbits (G, E for EWR and N for EGS). The linage B contained 11 haplotypes. Linage B haplotypes for EWR (I, D, H, C and F), EGS (L and M), Egyptian rabbits (K) and SCR (J). Also, it contained haplotypes A and the NJ based on two main linages which is consistent with van der Loo *et al.* (1997), Branco *et al*. (2000) and Long *et al*. (2003).We found all domestic rabbits belong to the lineage B with wild rabbits which is agreed with Monnerot *et al.* (1996) and Long *et al*. (2003). The origin of EGS rabbits was controversial before this study. While as shown in this study, it is in the same lineages with other European rabbits. We can confirm that EGS haplotypes introduced from European rabbits (*Oryctolagus cuniculus*). Our results also confirmed that the domestic breeds (ERB, EBB and SCR) introduced from European wild rabbit as other domestic rabbit breeds (Alves *et al*., 2015).

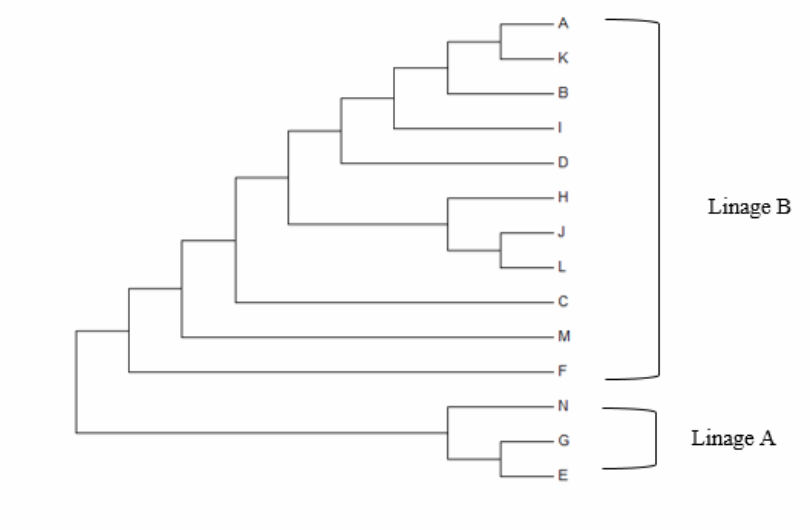

Figure 2: Unrooted Neighbour-joining (NJ) tree for haplotypes mtDNA sequences identified in current.

CONCLUSIONS

The current study is the first detailed analysis that investigated the direct phylogenetic relationships among local rabbit breeds in Egypt and Spain by using mtDNA. This study demystifies the relationship and origin of EGS rabbit in Egypt. Finally, all tested breeds were found to be originally introduced from European rabbits. Future work may help our understanding of the detailed history and evolutionary consequences process for EGS. We may need more studies among populations in Sinai Peninsula desert areas. In addition, tested breeds need huge comparison among European rabbit breeds belonging to *Oryctolagus cuniculus*.

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