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STATUS AND ORIGIN OF EGYPTIAN LOCAL RABBITS IN COMPARISON WITH SPANISH COMMON RABBITS USING MITOCHONDRIAL DNA SEQUENCE ANALYSIS

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Abstract: Mitochondrial DNA (mtDNA) and cytochrome b (cyt b) gene sequences were used to determine the status of genetic diversity and phylogeny for 132 individuals from local rabbit breeds in Egypt and Spain. The Egyptian local rabbit breeds were Egyptian Red Baladi (ERB), Egyptian Black Baladi (EBB) and Egyptian Gabali Sinai (EGS). However, the Spanish local rabbit breed was Spanish common rabbit (SCR). Previous breeds were compared with European Wild Rabbit taken from Albacete, Spain (EWR). A total of 353 mutations, 290 polymorphic sites, 14 haplotypes, 0.06126 haplotype diversity and –1.900 (P<0.05) for Tajima's D were defined in this study. Haplotype A mostly occurred in 83.3% of Egyptian rabbits and 11.7 % of EWR, while haplotype B occurred in 63.8% of Spanish rabbits and 36.2% of the EGS breed. A total of 47 domestic and wild *Oryctolagus cuniculus* published sequences were used to investigate the origin and relation among the rabbit breeds tested in this study. The most common haplotype (A) was combined with 44.7% of published sequences. However, haplotype B was combined with 8.5%. Haplotypes of Egyptian, SCR and EWR were scattered in cluster 1, while we found only one EGS haplotype with two haplotypes of EWR in cluster 2. Our results assumed that genetic diversity for ERB, EBB and SCR was very low. Egyptian breeds and SCR were introduced from European rabbits. We found that ERB and EBB belong to one breed.

Key Words: Egyptian rabbits, Spanish common rabbit, genetic diversity, mitochondrial DNA.

INTRODUCTION

The placing of farm animal genetic resources (AnGR) under the custody of national governments reflects the importance of identification of farm animal diversity. Knowing the origin and evaluating domestic animals arouses the curiosity of animal breeding and genetics researchers. Animal genetic resources are continually changed according to environmental conditions, markets and pathological cases, etc. The documentation and maintenance of AnGR are essential for the use and application of breeding strategies (Kim *et al.*, 2013). FAO (2007) stated that many indigenous domestic breeds are becoming extinct and are disappearing, being replaced or crossed with exotic breeds. Biodiversity studies can be used to enable relevant stakeholders to make properly informed decisions for breeding programme applications that contribute to food and livelihood security (Hall, 2004; FAO,2007; Owuor *et al.,* 2019).

Egypt and Spain are in the top six countries in rabbit production. They basically depend on exotic breeds and commercial strains, while totally neglecting their own local breeds in production. Egyptian rabbits have featured traits such as disease resistance, heat stress tolerance, prenatal abilities and postnatal maternal abilities (Galal

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and Khalil 1994; Khalil 2002; Emam *et al.,* 2017). However, Spanish common rabbits (SCR) are characterised by good fertility and maternal ability and are nowadays used only in backyard production in small population sizes (González-Redondo, 2007).

Mitochondrial DNA (mtDNA) is a popular molecular marker used for tracing wild animal forebear. It knows the center of the demonstration and an attractive marker for studying origin and diversity include high mutation rates and maternal inheritance (FAO, 2011; Gupta et al., 2015). It is also a method for finding out the control region (D-loop), haplotypes information and identifying relations among haplotypes by analysing software sequences (Achilli *et al.,* 2008). Over the last three decades, mtDNA has been widely used in rabbit diversity studies (Ennafaa *et al.,* 1987; Long *et al.,* 2003; Mougel *et al.,* 2003; Nguyen *et al.*, 2018; Owuor *et al.,* 2019). These genetic studies have focused on the European geographical expansion reported by Christensen and Peng (2012), and Valvo *et al.* (2017) mentioned that mtDNA is used to identify the primary origins for domestic rabbits.

In the present study, we sequenced 450 base pairs (bp) of mtDNA and Cyt b gene from three local Egyptian rabbits recorded in the FAO list, Egyptian Red Baladi (ERB), Egyptian Black Baladi (EBB) from delta (North of Egypt) and Egyptian Gabali Sinai (EGS) in the wild and under captivity. In addition, the undefined local breeds, Spanish common rabbit (SCR) gathered from south west Spain and the European wild rabbit (EWR) taken from Albacete (hybrid zone in south east Spain), were used. Moreover, this study investigates the origin of ERB and EBB breeds.

MATERIALS AND METHODS

Animal sampling

A Total of 70 blood samples from three local Egyptian breeds: ERB from research farms located in 30°11'27.8"N: 31°07'44.8"E and 30°48'06.4"N: 31°.07'56.8"E, EBB from research farms located in 31°05'15.8"N: 30°56'65.8"E and 30°49'57.0"N: 29°34'29.3"E and EGS from research farms and caught in Sinai located in 30°48'06.4"N: 31°.07'56.8", 31°05'15.8"N: 30°56'65.8"E and 31°22'67"N: 34°24'66.29"E were taken (7, 32 and 31 samples, respectively). Likewise, 32 hair samples of SCR were taken from backyard farms located in 37°35'64.2"N: 5°94'8.77"W, 37°.33'00.81"N: 5°88'19.74"W and 40°40'98.02"N: 3°67'01.21"W and 30 samples from EWR from a hunting preserve located in Albacete, Spain (38°37'20.6"N: 2°42'42.7"W). The geographical locations from each breed and EWR samples are shown in Figure 1. They were collected by phenotypic performance and body weight to make sure that they were pure breeds.

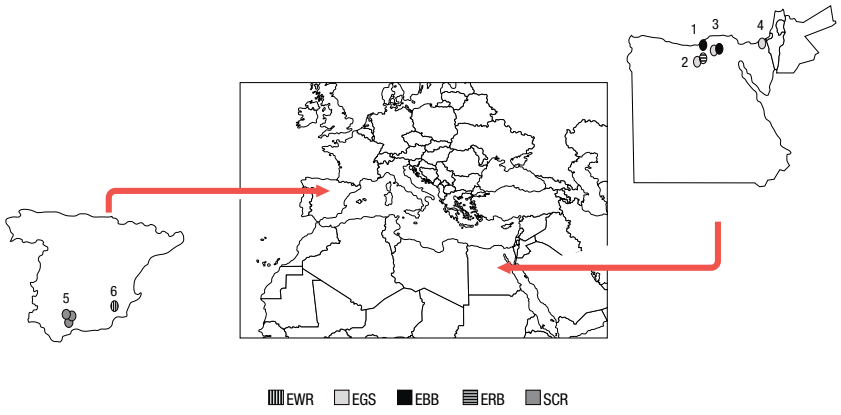

Figure 1: Collection sites of the three Egyptian breeds from north of Egypt 1. Porg El Arab, 4. El- Goura and Delta 2. El-Gimiza, 3. Sakha and. Spanish samples collected from 5. Seville and 6. Albacete.

DNA extraction and sequencing

DNA samples of Egyptian breeds were extracted by Qiamp Blood Mini Kit, Qiagen. However, the samples of SCR and EWR were hair samples, and DNA was extracted using the EasySpin Genomic DNA Tissue Kit from Citomed.

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'), with NC4 as a reverse primer (5'-GGTTCTTACCTCAGGGCCAT-3'). The nucleotide positions were numbered from complete published sequence of mtDNA (GenBank accession number AJ001588). The performance of polymerase chain reaction (PCR) used was 1.0 µL for genomics DNA in 9 µL mixture containing 5 µL Master Mix (Qiagen206145, Germany), 0.4 µL Prol, 0.4 µL NC4 (primers from Invitrogen, France) and 3.2 µL distilled water. The PCR products were cleaned using EXOSAP-IT, then purified by Sephadex™ (GE Healthcare). Sequencing was performed using 0.5 µL BigDye® Terminator v3. 1Kit with 0.6 µL forward primer, then carried out on a DNA sequencer (ABI PRISM 3130 XL–Genetic Analyzer).

Data analysis

Sequences were aligned with DNASTAR software (DNASTAR Inc., Madison, WI, USA). Purity of breeds was validated based on sequence alignment; all individuals with less than 90% of similarity were eliminated. Sequence data were submitted to GenBank (accession numbers: range KT029916 - KT030047). Numbers of nucleotide of polymorphic sites (S), haplotype (h), nucleotide diversity (π), haplotype diversity (Hd), total number of mutations (ETA) and Tajima's D (TD) were calculated in DNA sequence polymorphism Version 5.1 (Librado and Rozas 2009). Mega 6.0 (Tamura *et al.*, 2013) was used to estimate genetic distance among tested breeds by using UPGMA. Moreover, an unrooted neighbourhood joining (NJ) tree with the percentage of bootstrap values of 2000 replications was used to determine the relation between current study haplotypes and published haplotype sequences (Table 1). A median-joining network profile of the individuals was constructed by the phylogenetic Network Software, version 4.613 (*http://www.fluxus-engineering.com*).

RESULTS

Breeds status

The mtDNA diversity indices for the current study are shown in Table 2. In this study, a total of 14 haplotypes (H) and a total of 353 mutations (ETA) were observed. All rabbit breeds and EWR were polymorphic, with the number of haplotypes ranging from two (ERB, EBB and SCR) to eight (EWR). The highest haplotype Diversity (Hd) was observed in EWR (0.787±0.059). On the contrary, the lowest values were found in EBB (0.417±0.072). The differing nucleotide point site counted between sequences in the dataset, independently of the actual number of differences and hence independent of allele frequency, was estimated by number of polymorphic sites (S). It was recoded and 290 numbers of S ranged from 272 (EWR) to 1 (ERB, EBB and SCR). The average of nucleotide diversity (π) was used to measure genetic variation in breeds. Average value was 0.06126±0.01721 and ranged from 0.00093±0.00016 (EBB) to 0.1735±0.04998 (EWR). The frequency of allele distribution for nucleotide sequence data was measured by TD. The average of TD value was negatively significant (–1.90), EWR and EGS values were negatively significant (–1.63793 and –1.87766), while there was no significant difference among the other three breeds (ERB, EBB and SCR). Substantially, a higher level of genetic diversity was observed in EWR than in EGS (Table 2).

Genetic distance

The evolutionary relationship among tested breeds was inferred by the UPGMA method (Figure 2). The optimal tree with the sum of branch length was 0.183. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Codon positions were included in EWR, SCR and EGS. However, the noncoding positions were found in ERB and EBB. All positions containing gaps and missing data were eliminated.

Table 1: Sequence data list from Gene bank used in this study.

Type	Accession Number	Hap. Group	Abbreviation	Breed	Reference
Domestic	AJ293831	EU. DOM.	F. BOUR.	Fauve de Bourgogne	Bolet *et al.* (2000)
	AJ293834	EU. DOM.	ARG. C.	Argente de Champagne	Bolet *et al.* (2000)
	AJ293835	EU. DOM.	DOM. ENG.	Domestic English	Bolet *et al.* (2000)
	AJ293836	EU. DOM.	F. GIANT.	Flemish Giant	Bolet *et al.* (2000)
	AJ293838	EU. DOM.	H. GIANT.	Hungarian Giant	Bolet *et al.* (2000)
	AJ293840	EU. DOM.	FREN. L.	French Lop	Bolet *et al.* (2000)
	AJ293842	EU. DOM.	CHIN.	Chinchilla	Bolet *et al.* (2000)
	AF534080	ASIAN. DOM.	QIN.	Qixing	Long *et al.* (2003)
	AF534081	ASIAN. DOM.	HERB.W.	Haerbin White	Long *et al.* (2003)
	AF534082	ASIAN. DOM.	A.ANG.	Zhenhai Thick hair Angora	Long *et al.* (2003)
	AF534083	ASIAN. DOM.	B. E. B.	Big Ear Brown	Long *et al.* (2003)
	AF534084	ASIAN. DOM.	YUF. B.	Yufeng Brown	Long *et al.* (2003)
	AF534085	EU. DOM.	BELG.	Belgium	Long *et al.* (2003)
	AF534086	EU. DOM.	CALI.	California	Long *et al.* (2003)
	AF534087	EU. DOM.	DWAR.	Dwarf	Long *et al.* (2003)
	AF534088	ASIAN. DOM.	ELCO	Elco	Long *et al.* (2003)
	AF534089	EU. DOM.	NWZ.	New Zealand	Long *et al.* (2003)
	AF534090	EU. DOM.	R. U.S.A.	Rex USA	Long *et al.* (2003)
	AF534091	ASIAN. DOM.	ZIKA	Zika	Long *et al.* (2003)
	AF534092	ASIAN. DOM	SIC. W.	Sichuan White	Long *et al.* (2003)
	AF534093	ASIAN. DOM	JAP. W	Japanese White	Long *et al.* (2003)
	AF534094	EU. DOM.	Rex. G.	Rex Germany	Long *et al.* (2003)
	AF534095	EU. DOM.	ANG.	Angora	Long *et al.* (2003)
	AF534097	ASIAN. DOM.	FUJ.B	Fujian Brown	Long *et al.* (2003)
	AF534098	ASIAN. DOM.	TAI. M.	Taihang Mountain	Long *et al.* (2003)
	AF53100	ASIAN. DOM.	G. L.ZIKA	Germany line Zika	Long *et al.* (2003)
Wild	U62924	EWR-A.	EWR-A1	European Wild Rabbit- Australia	Fuller *et al.* (1997)
	U62925	EWR-A.	EWR-A2	European Wild Rabbit- Australia	Fuller *et al.* (1997)
	U62926	EWR-A.	EWR-A3	European Wild Rabbit- Australia	Fuller *et al.* (1997)
	U62927	EWR-A.	EWR-A4	European Wild Rabbit- Australia	Fuller *et al.* (1997)
	AJ535802	EWR-F.	EWR-F1	European Wild Rabbit- French	Mougel *et al.* (2002)
	AJ535805	EWR-F.	EWR-F2	European Wild Rabbit- French	Mougel *et al.* (2002)
	AJ535807	EWR-F.	EWR-F3	European Wild Rabbit- French	Mougel *et al.* (2002)
	AJ535811	EWR-F.	EWR-F4	European Wild Rabbit- French	Mougel *et al.* (2002)
	AJ535812	EWR-F.	EWR-F5	European Wild Rabbit- French	Mougel *et al.* (2002)
	Z83341	EWR-S.	EWR-S1	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83343	EWR-S.	EWR-S2	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83344	EWR-S.	EWR-S3	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83345	EWR-S.	EWR-S4	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83347	EWR-S.	EWR-S5	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83351	EWR-S.	EWR-S6	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83352	EWR-S.	EWR-S7	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83354	EWR-S.	EWR-S8	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83364	EWR-S.	EWR-S9	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83365	EWR-S.	EWR-S10	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83366	EWR-S.	EWR-S11	European Wild Rabbit- Spain	van der Loo *et al.* (1997)
	Z83367	EWR-S.	EWR-S12	European Wild Rabbit- Spain	van der Loo *et al.* (1997)

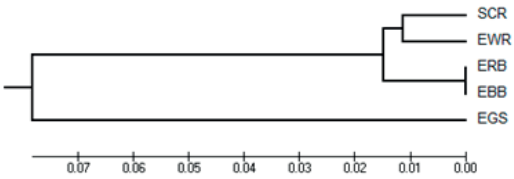

Figure 2: UPGMA tree to estimate genetic distance among tested breeds.

Sequence variation

Fourteen haplotypes (A: N) for 132 individuals were shown (Table 3). Most of the Egyptian rabbit breeds were in haplotype A and K. Haplotype A occurred in 36% of Egyptian breeds and 15% of EWR. On the other hand, haplotype K occurred in 42.9% of Egyptian breeds only. However, 52% of SCR and ERW-S and 39% of EGS occurred in haplotype B. Nevertheless, haplotype J occurred in 59% of SCR. Additionally, there were three haplotypes (L, M and N) for EGS breed and seven haplotypes (C, D, E, F, G, H and I).

Breeds origin

The unrooted NJ (with two lineages) of both wild and domestic rabbit breed mtDNA sequences is shown in Figure 3. In this study, we found 14 haplotypes (Table 3). Furthermore, a total of 47 sequences for domestic and wild published sequences breeds were used in this study to investigate tested breed origin (Table 1). Lineage A was composed of eight haplotypes of wild rabbit only (published Spanish and French sequences). However, in lineages B there were two clusters which included wild and domestic rabbits. We found 12 haplotypes (from our study) with 13 published sequence haplotypes (from 30 breeds) in cluster 1. However, in the second cluster only three haplotypes were observed (N, G and E).

Figure 4 shows the parsimony network for 28 haplotypes found in Lineage B. Both haplotypes A and B were in the centre with 26 haplotype sequences. Haplotype A was diverged from one site with haplotype K, EWR and four European domestic haplotype breeds (domestic English, Fauve de Bourgogne, Flemish Giant and Argente de Champagne). Haplotype B was differed with haplotypes J and I and with French Lop (European domestic breed).

Haplotype A was contained in Egyptian, Asian, European domestic rabbit breeds (California, New Zealand White, Rex, Angora, Dwarf, Belgium) and wild rabbits from Australia and Spain (from published and this study sequences). However, SCR, Germany Great Line Zika (Asian breed) and European wild rabbits from Spain and Australia were contained in haplotype B.

Table 2: The mtDNA diversity indices of tested breeds.

TD	ETA(η)	S	π ±SD	Hd ±SD	H	Breed
1.34164	1	1	0.00127 ± 0.00027	0.571 ±0.119	2	ERB
1.03928	1	1	0.00093 ±0.00016	0.417 ±0.072	2	EBB
-1.87766*	117	88	0.03305 ± 0.01099	0.684 ± 0.053	6	EGS
-1.63793*	286	272	0.17359 ± 0.04998	0.787 ± 0.059	8	EWR
1.5351	1	1	0.00111 ± 0.0009	0.498 ± 0.039	2	SCR
-1.900*	353	290	0.06126 ± 0.01721	0.801 ±0.015	14	Overall

H: Number of Haplotypes, Hd±SD: Haplotype Diversity±Standard Deviation, π±SD: Nucleotide diversity±Standard Deviation, S: Number of polymorphic sites, ETA: Total number of mutations (Fu and Li1993, K: Average number of nucleotide differences and TD: Tajima's D (*P<0.05).

ERB: Egyptian Red Baladi. EBB: Egyptian Black Baladi. EGS: Egyptian Gabali Sinai. EWR: European Wild Rabbit. SCR: Spanish Common Rabbit.

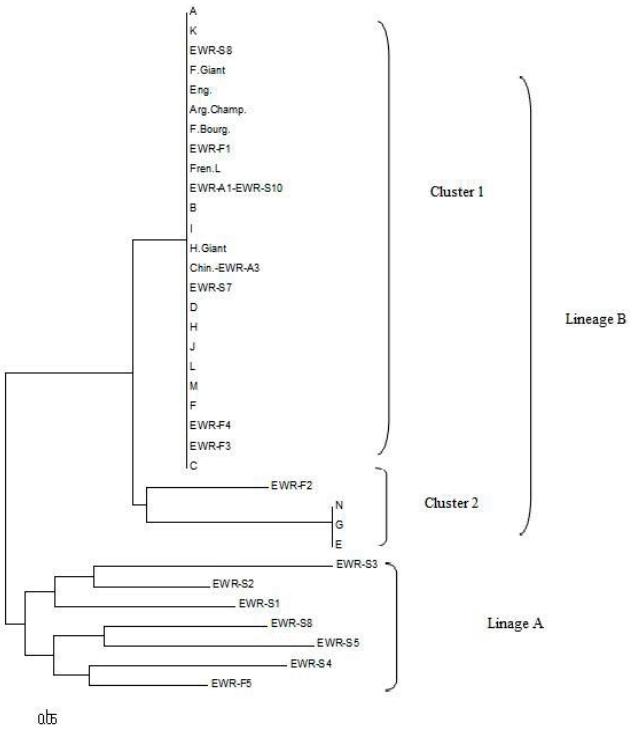

Figure 3: Unrooted Neighbor Joining (NJ) tree for sequences were studied in this study and gene bank investigated.

DISCUSSION

The present study represented the first comparison approach among local Egyptian rabbit breeds and both wild with SCR and EWR by mtDNA analysis. We selected EWR from Albacete because it was proved by Monnerot *et al.* (1994) that the origin of *Oryctolagus genus* is in southern Spain was 6-6.5 million years ago, as mentioned by Alves *et al.* (2015); Owuor *et al.* (2019).

High levels of genetic diversity were expressed in large population sizes (Carneiro *et al.,* 2012; Sakthivel *et al.,* 2019) which characterised EWR and EGS (Table 2). Conversely, endangered breeds such as local Egyptian breeds (Ministry of Agriculture and Land Reclamation and FAO, 2003) and SCR (González-Redondo, 2007) showed low genetic diversity and high levels of inbreeding (Brook, 2008; Kekkonen and Brommer, 2014; Emam, 2016). Generally, wild populations (EWR or EGS wild and under captivity) presented higher diversity than domestic breeds (ERB, EBB and SCR) which agreed with Carneiro *et al.* (2011) and Abrantas *et al.* (2013); Yu Yeh *et al.* (2019) for natural selection (Carneiro *et al.,* 2012). We observed that the diversity limitation in Egyptian domestic breeds (EBR and EBB) which agreed with Grimal *et al.* (2012). By the same token, in SCR which agreed with Zaragoza *et al.* (1987). They found high inbreeding levels in previous breeds by using microsatellites and blood protein electrophoresis, respectively.

The highest levels of π were shown in EWR (Geraldes *et al.,* 2006) and EGS breed (0.17359±0.04998 and 0.03305±0.01099, respectively). Conversely, local Egyptian and Spanish breeds showed the lowest π values (ERB 0.00127±0.00027, EBB 0.00093±0.00016 and SCR 0.00111±0.0009). This might be due to the natural selection effects of wild populations (Abrantes *et al.,* 2013; Schumer *et al.,* 2018). Low π values were found in domestic breeds that suffered from bottleneck and inbreeding (Gaggiotti 2003; Bortoluzzi *et al.,*2019).

Table 3: Fourteen mitochondrial DNA Haplotype Frequencies in this study.

Total	SCR	EWR	EGS	EBB	ERB	HAP code	HAP
3(0.0152)	-	3(0.0667)	-	-	-	C	1
30(0.2273)	-	5(0.1667)	13(0.4193)	9(0.2813)	3(0.4286)	A	2
3(0.0303)	-	3(0.1000)	-	-	-	D	3
4(0.0303)	-	4(0.1333)	-	-	-	E	4
36(0.2727)	13(0.4063)	11(0.3667)	12(0.3871)	-	-	B	5
2(0.0152)	-	2(0.0667)	-	-	-	F	6
1(0.0076)	-	1(0.0333)	-	-	-	G	7
1(0.0076)	-	1(0.0333)	-	-	-	H	8
1(0.0076)	-	1(0.0333)	-	-	-	I	9
19(0.1439)	19(0.5938)	-	-	-	-	J	10
30(0.2273)	-	-	3(0.0968)	23(0.7185)	4(0.5714)	K	11
1(0.0076)	-	-	1(0.0333)	-	-	L	12
1(0.0076)	-	-	1(0.0333)	-	-	M	13
1(0.0076)	-	-	1(0.0333)	-	-	N	14
132	32	30	31	32	7	Total	Total

ERB: Egyptian Red Baladi. EBB: Egyptian Black Baladi. EGS: Egyptian Gabali Sinai. EWR: European Wild Rabbit. SCR: Spanish Common Rabbit.

Genetic variation reflects genetic drift and mutation balance attributing a modest role in adaptation at the molecular level (Carneiro *et al.,* 2012). We noticed a limited number of mutations in domestic breeds (only one). The population size is an important factor for number of mutations (Jayaraman, 2011; Ghalayini *et al.,* 2018). Meanwhile, we recorded 286 mutations (ETA) in EWR. This was because EWR samples were collected from the hybrid zone in Iberian Peninsula in which Albacete is located (Campos *et al.,* 2012). Furthermore, the large population size increases the number of mutations (Bollback and Huelsenbeck, 2007; Peischl and Excoffier, 2015; Park *et al.,*2019) which was also observed in EGS (117). We observed the highest levels of Sin EWR; the high value was consistent with Geraldes *et al.* (2006) and Campos *et al.* (2012).

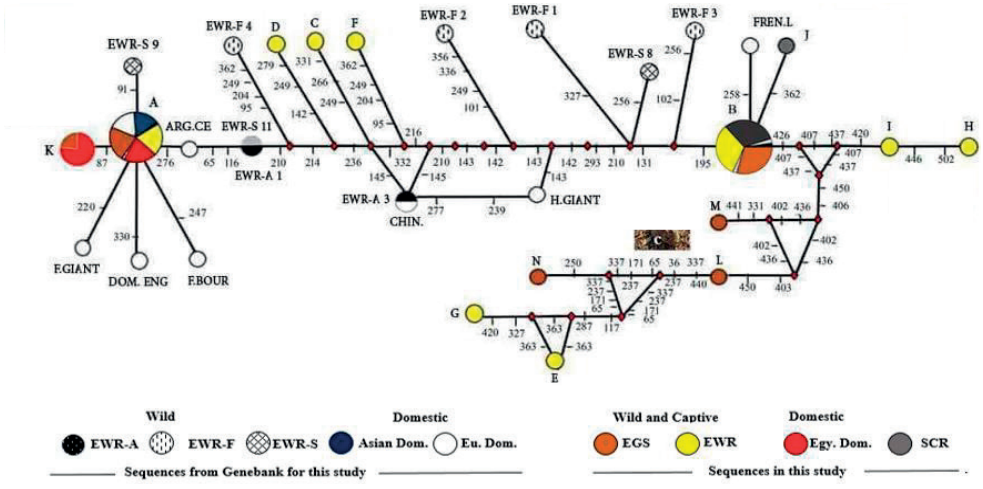

Figure 4: Maximum parsimony network analyses representing phylogenetic relationships among Linage B for this study and published sequences of rabbit breeds from gene bank mtDNA haplotypes. a. ERB b. EBB c. EGS d. SCR e. EWR.

Tajima's D values were found to be significantly negative in EWR and EGS. This is quite predictable, due to an excess of rare alleles (Christodoulakis *et al.,* 2007; Wares, 2010; Guo *et al.,* 2019) for EWR and EGS. However, positive values were recorded in domestic breeds as a result of the excess of intermediate frequency alleles (Schmidt and Pool 2002) for domestic breed.

In Egyptian breeds, it was summarised that EGS showed the highest values in all aforementioned parameters than the other two Egyptian domestic breeds (ERB and EBB). We suggested as probable cause that EGS is still raised in desert areas and only recently under captive conditions. EGS needs more study, especially among populations in desert areas (Emam *et al.,* 2016).

Genetic distance

The genetic distance among tested breeds is shown in Figure 2. In Spanish breeds, Genetic distance between EWR and SCR was 0.00-0.012. Our results are in total agreement with Martin-Burriel *et al.* (1996) who estimated the genetic distance among Spanish breeds (Contained of EWR and SCR) by using blood protein electrophoresis. In Egyptian local breeds, there was no genetic distance between ERB and EBB. This suggests that ERB and EBB belong to one breed. The ERB and EBB were a crossbreeding result between Egyptian native rabbit (1/4) and Flemish Giant (3/4), then selected according to red and black fur colour (Khalil, 2002). Correspondingly, the Ministry of Agriculture and Land Reclamation in Egypt & FAO (2003) considered ERB and EBB to be different breeds. Likewise, Grimal *et al.* (2012) claimed that ERB was an independent breed from EBB and EGS. With these results, we observed that EGS (captive and wild) showed the highest difference among tested breeds (>0.07), and it could be structurally separated from other Egyptian breeds. There is no literature for comparison study among all tested breeds.

Origin of Egyptian Rabbit breeds and Spanish Common Rabbits

We found that rabbit haplotypes were shown in two maternal lineages (Figure 3) based on mtDNA, which agreed with Watson and Davis (2019). Furthermore, all rabbits tested and most of the published sequences were located in second lineages, which is consistent with Long *et al.* (2003) and Owuor *et al.* (2019).

Sequence comparisons and phylogenetic analysis pointed out that haplotype A was represented in Egyptian breeds (Table 3). We noticed (Figures 3 and 4) that haplotype A contained 47% from all sequences used in this study. However, we noticed that the haplotype differed with some European haplotype breeds (Flemish Giant was one of them). This might be a strong indicator, as ERB and EBB were not subjected to any intensive selection programme (Khalil, 2002). Haplotype B contains 10.6% from all sequences used in this study. Logically, SCR and EWR were contained together in this haplotypes. The Origin of EGS rabbit was controversial before this study. This was shown (Figure 3) in second lineage (with other European rabbits). Moreover, we noticed that EGS haplotypes (L, M and N) were near to EWR haplotypes (G and E) in network analysis (Figure 4). In addition, EGS was diffused in both haplotypes A and B. Therefore, we concluded that EGS was originally introduced from European rabbits.

CONCLUSION

In this study, more light was shed on Egyptian rabbit origin by sequences analysis, showing that ERB and EBB belong to one breed. Phylogenetic trees confirmed that all the tested breeds originally came from European rabbits. The results presented in the current study can be used to help decision makers draw up necessary strategies to conserve important genetic resources, maintain biodiversity and help to design future genetic improvement programmes.

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