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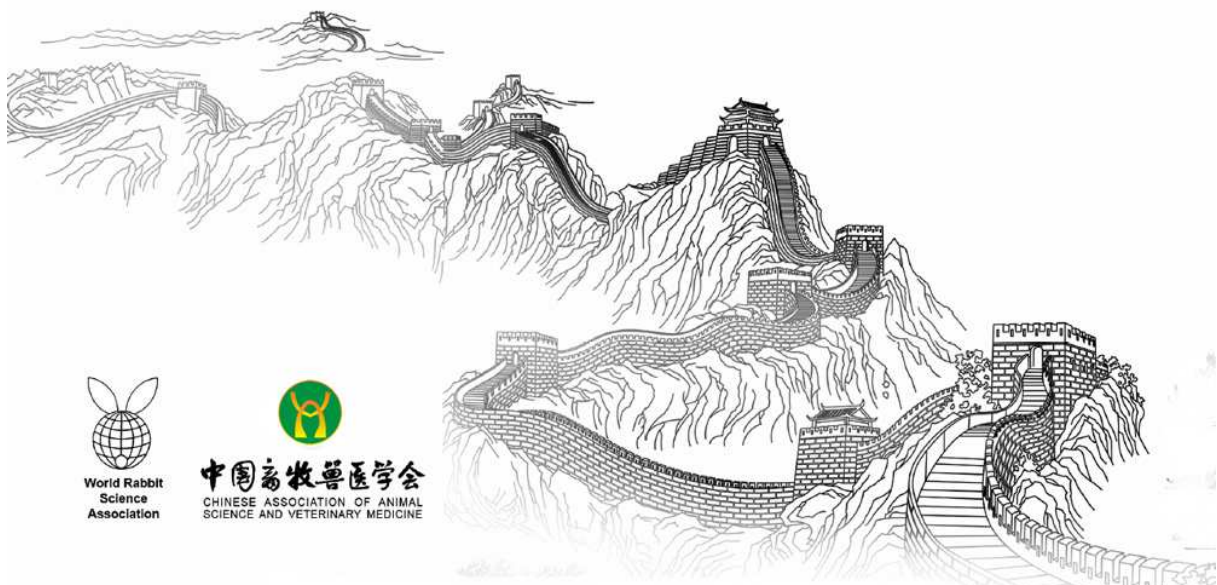
***Emam A.M., Afonso S., Azoz A.A.A., González-Redondo P.,
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MICROSATELLITE POLYMORPHISM IN SOME EGYPTIAN AND SPANISH COMMON RABBIT BREEDS

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ABSTRACT

Nineteen microsatellite loci were used to identify the genetic diversity for 3 Egyptian and one Spanish local rabbit breeds. Egyptian breeds were Egyptian Red Baladi (ERB), Egyptian Black Baladi (EBB) and Egyptian Gabali Sinai (EGS), while the Spanish breed belongs to a local domestic variety (Spanish Common rabbit, SCR) used in backyard raising. The previous breeds were compared with European Wild rabbits (EWR). This study recorded that lower genetic diversity exists in ERB, EBB and SCR than EGS and EWR. Results demonstrated that ERB and EBB belong to one breed. The phylogenetic analysis confirmed that there is separation between domestic rabbit breeds and wild rabbit in this study. The present study surely represented the first comparison approach among local Egyptian rabbit breeds and both SCR and EWR.

Key words: Egyptian rabbits, Spanish common rabbit, European wild rabbit, genetic diversity, microsatellites

INTRODUCTION

More than 17% of farm animals on the brink of extinction, with the highly endangered proportion for 45% of rabbit breeds (FAO, 2015). Diversity studies inform us about the necessity of the sustainable intensification of animal production (Traore *et al.*, 2009). Genetic resources documentation is one of the main areas of livestock conservation activities (Tian-Wen *et al.*, 2010). Microsatellites loci are widely used in genetic diversity analyses and population structure determination in rabbits (Grimal *et al.*, 2012; Ben Larbi *et al.*, 2014). Microsatellites are used to give information about the relationships between breeds and the domestication process (Erhardt & Weimann 2007). Molecular data have become more and more relevant for the characterization of genetic diversity (Groeneveld *et al.*, 2010).

In Egypt, the local rabbit breeds are suffering from endangered risk (Ministry of Agriculture and Land Reclamation in Egypt, FAO 2003). Those breeds are Egyptian Red Baladi (ERB), Egyptian Black Baladi (EBB) and Egyptian Gabali Sinai (EGS). In Spain, low population numbers of Spanish common rabbits (SCR), a local variety used in backyard raising are shown (González-Redondo, 2007).

In this context the aim of this study was to evaluate the genetic diversity and phylogenetical relationship among Egyptian breeds and Spanish local rabbit breed uses in backyards.

MATERIALS AND METHODS

Sampling and genotyping of microsatellites markers

A total of 145 rabbit samples belonging to Egyptian and Spanish local breeds were used to carry out this study. Egyptian breed samples (ERB, EBB and EGS) were selected according to Khalil (1997). The survey covered different farms in different regions in Egypt (Delta and, Sinai Peninsula). In Spain, 32 hair samples of SCR from backyard farms were collected from Seville (González-Redondo,

2007) and 32 European Wild Rabbit (EWR) samples were gathered from a hunting preserve located in Albacete (Table 1).

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).

For this study, 19 loci were obtained with Invitrogn (France). The PCR products were migrated on capillary sequencer (ABI PRISM 3130 XL) and scored by using GeneMapper version 4.0 (Applied Biosystems).

Analysis of microsatellite genotypes

POPGENE software (version 3.2, Yeh *et al.*, 1999) was used to estimate the mean number of alleles (MNA) per breed. The private number of alleles (PVT) was calculated by CONVERT software (Glaubitz, 2004). Genetic differentiation within the breeds was estimated based on F-statistics (FIS) according to Weir and Cockerham (1984). Also, the observed and expected heterozygosity within breeds was estimated by using the GENETIX software packages (Belkhir *et al.*, 1996-2004). Factorial correspondence analysis (FCA) based on alleles frequency was drawn by previous software. The population structure was evaluated based on a Bayesian clustering analysis by employing Structure 2.3 (Pritchard *et al.*, 2000). Phylogenetic trees were performed with 1000 bootstrap on locus. Neighbour-Joining Trees were edited by Mega 6 package (Tamura *et al.*, 2013).

RESULTS AND DISCUSSION

Within population diversity

A total of 277 alleles were detected across the 19 loci in the studied breeds. The mean number of observed alleles (MNA) and number of private alleles (PVT) was 93.2 and 5.28, respectively. The MNA values ranged from 3.526 in SCR to 9.421 in EWR. While, PVT values ranged from 1 in EBB to 92 in EWR (Table 1).

Table 1: Within-population summary statistics.

Country	Breed	N	Farms location	MNA ±SE	PVT	Ho±SE	He±SE	F _{IS}
Egypt	ERB	18	El-Gimiza and Gezeret El Shaer	3.684 ±0.287	11	0.325±0.048	0.524±0.042	0.073 ^c
	EBB	32	Porg El Arab and Sakha	3.684±0.351	1	0.458±0.031	0.506±0.039	0.046 ^{NS}
	EGS	31	Sakha, El-Gimiza and El- Goura	6.105±0.404	19	0.580±0.048	0.602±0.029	0.145 ^b
Spain	EWR	32	Albacete	9.421±0.933	92	0.648±0.041	0.795±0.019	0.192 ^a
	SCR	32	ETSIA and 2 backyard farms	3.526±0.268	13	0.426±0.053	0.458±0.043	0.088 ^c

Number of samples (N), (ETSIA) Escuela Técnica Superior de Ingeniería Agronómica (University of Seville), Mean number of observed alleles (MNA), Stander error (SE), number of private alleles (PVT), mean observed and expected heterozygosity (Ho and He), intra breed structure (F_{IS}).^{NS}: non-significant. (P<0.05)

We noticed that EWR and EGS expressed higher diversity than local domestic rabbit breeds (Table 1). The limitation of MNA was recorded by Alves *et al.* (2015) in domestic rabbit breeds; this may be due to inbred rabbit strains. Deeply, Grimal *et al.* (2012) found a low MNA values in Egyptian breeds (3.94 and 3.65 for ERB and EBB, respectively) vs. 3.684 in the current study. We expect convergence results ERB and EBB in low values of MNA for a categorization as previous endangered breeds according to Ministry of Agriculture and Land Reclamation in Egypt, FAO (2003). We noticed that ERB was higher in PVT than EBB which agreed with Grimal *et al.* (2012). The same conclusion was reached in SCR.

In this study, the large scale from EGS samples collection from different farms and Sinai Peninsula were reflected in high PVT and MNA (19 and 6.105, respectively) vs. 5 and 3.94 in Grimal *et al.* (2012). On the other hand, MNA and PVT for EWR were 9.421 and 92, respectively. These results are almost in agreement with Queney *et al.* (2001). They mentioned that Northeastern Iberian Peninsula (which Albacete is in the south) is a location for wild rabbit mutation and subsequent slipping occurs. Also, we found lower PVT in local domestic rabbits than wild rabbits. We noticed genetic bottleneck in local domestic breeds, while the bottleneck disappeared in wild rabbits. Queney *et al.* (2000)

declared that bottleneck is a result for low population size. High genetic variability with high number of PVT were recorded in EGS because it is still raised in desert areas and recently under captive conditions. The values of H_0 were low in Egyptian domestic breeds (0.325 for ERB and 0.458 for EBB) and SCR (0.426). In agreement with Grimal *et al.* (2012). The highest value of H_0 in Egyptian breeds was recorded in EGS, while the lowest value was recorded in ERB. In EGS and EWR, the H_0 was ranging from 0.58 to 0.65. These results are in general similarity to Queney *et al.* (2001) on wild rabbits.

Structure analysis

Figures 1A and 1B show the structure and K values of tested breeds, respectively. The highest ΔK value was obtained for K=4 (Figure 1B). When K=3 the out groups EWR and SCR formed one cluster and the ERB and EBB formed another cluster, whereas the EGS formed the third cluster. In the case of K=4 and K=5, EGS, EWR and SCR were in three separate clusters. Whereas ERB and EBB clustered together, although they were collected from different locations. These results suggest that ERB and EBB are belonging to one breed. Both of ERB and EBB were crossbred with the same originality Egyptian native rabbit (1/4) and Flemish Giant (3/4), then selected according to fur color red and black (Khalil, 2002). On the other hand, it implicates the uniqueness of ERB and EBB which are considered two different breeds by Ministry of Agriculture and Land Reclamation in Egypt, FAO (2003) and Galal (2007).

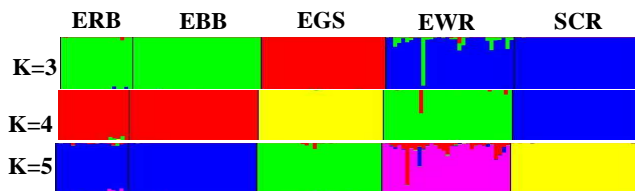


Figure 1A: STRUCTURE cluster analysis of the sample

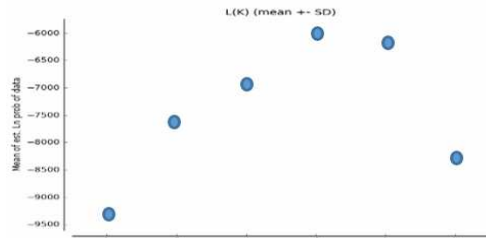


Figure 1B: ΔK calculated from K=1 to K=6
 Relation between breeds

Among populations diversity

Differentiation of the individuals within each breed was further assessed with the FCA by the construction of a three-dimensional plot in which the different animals took place (Figure 2A). Genetic relationships based on genetic distances among the populations were visualized by a neighbour-joining tree (Figure 2B). Results showed two main clusters: the domestic breeds (ERB, EBB and SCR) clustered together, and the second cluster included the wild and originally wild breed (EWR and EGS). These results are not similar to those obtained by Grimal *et al.* (2012), who found separation of the Egyptian populations from the Spanish line (NWZ).

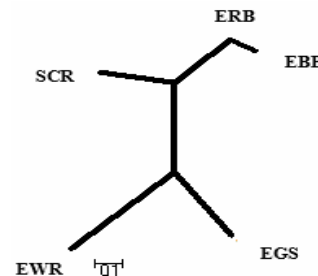
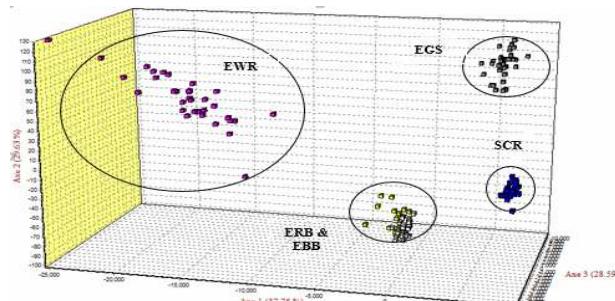


Figure 2A. FCA for tested breeds. **Figure 2B.** Neighbour-Joining tree between five rabbit breeds.

CONCLUSIONS

Microsatellite loci analysis revealed that all local domestic breeds are suffering from bottleneck in low population numbers affected badly in their diversity values. Finally, this study suggested according to structure analysis that ERB and EBB belonging to one breed.

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