PHYLOGENETIC RELATIONSHIP AMONG FOUR EGYPTIAN AND ONE SPANISH RABBIT POPULATIONS BASED ON MICROSATELLITE MARKERS

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ABSTRACT

Seventeen microsatellite loci were used to identify the phylogenetic relationship among four Egyptian breeds and one Spanish line of rabbits. A total of 114 bucks rabbits belonging to four Egyptian breeds -Black Baladi (EBB), Gabali (EG), Red Baladi (ERB) and White Giza (EWG)- and Spanish White New Zealand line from Universidad Politécnica de Valencia (NZW) were studied. All microsatellite loci typed were polymorphic. The average number of alleles per locus was 5.41, ranging from 2 to 12. A total of 16 private alleles were found in 7 out of 17 microsatellite loci used. Mean observed heterozygosity was 0.527, ranging from 0.477 in the NZW breed to 0.581 in the EWG. Lower values for Ho were found for all populations. The inbreeding coefficient of individuals relative to the total population (F_{IT}) was 0.279. The overall within-population heterozygote deficit (F_{IS}) was 0.165, ranging from 0.045 in NZW breed to 0.266 in EBB breed. The overall variation between population (F_{ST}) was 0.137, where the NZW breed showed the most differentiated population (F_{ST} = 0.194). The Neighbour-Joining tree of the Reynolds genetic distances (D_R) among populations shows a clear separation of the Spanish population (NZW) from the Egyptians breeds and there is a population mixture in the Egyptian populations. Only the ERB may to cluster in one independent population.

Keywords: Rabbit populations, microsatellite markers, heterozygosity, heterozygote deficit, genetic distances.

INTRODUCTION

Rabbit is the only domesticated mammal originating from Western Europe. Both wild and domestic European rabbit belong to the single species *Oryctolagus cuniculus*, which history of its wild populations is well documented through both archeological and genetic studies (see Ferrand, 2008; Rogel-Gaillard *et al.,* 2009). A consequence of ancestral breeding and recent selection practices more than 60-70 breeds were described, where size, fur type and colour vary greatly (Rogel-Gaillard *et al.,* 2009). Genetic studies have focused on the European geographical expansion of this species (Branco *et al.,* 2000 and Queney *et al.,* 2001). So, a very strong phylogeographical pattern of two highly divergent mtDNA lineages in Iberian wild rabbits was observed by Branco *et al.* (2000), while Queney *et al.* (2001) defined the main routes of rabbit migration and observed a subset of genetic diversity from French wild rabbit populations. Geographical isolation of the populations could lead to substructuring through drift, mutation and different natural selection forces (Muchadeyi *et al.*, 2007). Others biology approaches of genetic markers has been, the studies of social and reproductive organization and the impact of viral epizootics on genetic diversity (Monoulou *et al.,* 2003). Genetic markers are also a powerful tool to assess genetic variation within and between domestic stocks in the conservation programs of genetic resources (Bolet *et al.,* 2000; Berthouly *et al.,* 2008).
The aim of this study was to evaluate the phylogenetical relationship among four Egyptian breeds and one Spanish line rabbit.

MATERIAL AND METHODS

Sampling animals

A total of 114 bucks belonging to four Egyptian breeds of rabbit described by Khalil (1997 and 2002) and New Zealand White breed from Spain (A Line, described by Estany *et al*., 1989) were used in this study. Blood samples were collected from 23 bucks per each Egyptian breeds named -Black Baladi (EBB), Gabali (EG), Red Baladi (ERB) and White Giza (EWG)- raised in the Agriculture Experimental Station of Faculty of Agriculture (Cairo University), and 22 bucks of A line raised in the Universidad Politécnica de Valencia (NZW).

Microsatellite genotyping analysis

Blood sample from the central artery of the ear of each individual was collected into tubes containing EDTAK3 as anticoagulant and was then preserved in a deep-freezer at -20°C. Genomic DNA was extracted using a commercial kit (GenElute Blood Genomic DNA Kit, Sigma-Aldrich, Madrid, Spain). Seventeen microsatellites were used in this study. Microsatellites were selected according to the number of alleles, the allele size and the chromosome localization. In this way, only those microsatellites with more than four alleles and well distributed along the whole genome were used. Based on prior information of PCR product size (Chantry-Darmon *et al.,* 2005), the 17 microsatellites were grouped into 4 multiplex reactions (Table 1).
The PCR amplification of DNA was performed on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA) in a final volume of 15 µL, using *Taq* PCR Master Mix (Quiagen) and variable concentration of forward and reverse primers. Every PCR was carried out following the same conditions, including an initial step of 94ºC (3 min), followed by 35 cycles (95ºC for 30s, 60ºC for 60s, 72ºC for 30s) and ending with a final extension step of 72ºC (10 min). The sequencing of the PCR products was performed with a sequencer CEQ 8000 Beckman Coultek by capillary electrophoresis with fluorescent labeling.

Population diversity analysis

In order to estimate genetic variation within populations, total number of alleles, number of alleles per locus (k), private alleles (PA, alleles found in only one breed), expected heterozygosity (He, estimated by Nei, 1987) and observed heterozygosity (Ho) calculated by Genetix 4.03 of Belkhir *et al.* (2000). Reduction in heterozygosity due to inbreeding (FIS) per population were determined using the Genepop 4.1 program (Raymond & Rousset 1995; Rousset, 2008). Values of pairwise genetic differentiation among breeds (Fst; proportion of genetic variability due to population substucturing) were computed for all pairs of the five populations using Genepop 4.1 software. The pattern of population differentiation was described by a factorial correspondence of the individual multilocus scores, computed using Genetix 4.03. The first three major components were plotted on a three-dimensional scatter diagram for the five rabbit populations. Two genetic distances were performed using Populations 1.2.31 package (Langella, 1999). Reynolds genetic distances (DR) were estimated among pairs of populations. Individual distances were calculated to asses if there is population mixture or not. In this sense, alleles shared distances (DAS) among individuals were also estimated. Phylogenetic trees were performed with 1000 bootstrap on locus. Neighbour-Joining Trees were edited by Mega 5 package (Tamura *et al*., 2007).

RESULTS AND DISCUSSION

Genetic markers polymorphism

All microsatellite loci typed were polymorphic. The number of alleles per locus, private alleles, and expected and observed heterozygosity across all the populations used are given in Table (1). A total of 92 alleles were found. The average number of alleles per locus was 5.41, ranging from 2 (INRACCDDV0105) to 12 (INRACCDDV0087). A total of 16 private alleles were found in 7 out of the 17 microsatellite loci used.

Table 1.- Genetic variability for each locus in all populations

Multiplex rections	Locus (Microsatellite name)	Accession number	Range	K	P_A	He	Ho
M1	INRACCDDV0241	AJ874574	137-150	5	0	0.623	0.602
	INRACCDDV0259	AJ874589	154-168	4	0	0.598	0.613
	INRACCDDV0235	AJ874568	191-195	3	0	0.339	0.335
	INRACCDDV0137	AJ874477	239-247	3	0	0.523	0.494
M2	INRACCDDV0105	AJ874447	121-123	2	0	0.121	0.118
	INRACCDDV0036	AJ874398	175-183	4	0	0.489	0.559
	INRACCDDV0016	AJ874380	191-207	7	0	0.712	0.716
	INRACCDDV0183	AJ874521	195-209	6	1	0.582	0.449
M3	INRACCDDV0157	AJ874497	118-124	4	0	0.548	0.408
	INRACCDDV0087	AJ874430	190-214	12	2	0.721	0.754
	INRACCDDV0337	AJ874654	280-286	4	0	0.501	0.455
	INRACCDDV0035	AJ874397	111-139	7	5	0.389	0.304
	INRACCDDV0071	AJ874415	170-194	8	1	0.682	0.215
M4	INRACCDDV0106	AJ74448	148-158	4	0	0.640	0.523
	INRACCDDV0213	AJ874547	195-211	9	3	0.608	0.202
	INRACCDDV0221	AJ874555	101-125	5	2	0.363	0.210
	INRACCDDV0248	AJ874579	264-272	5	2	0.519	0.459

K = number of alleles. P_A = private alleles. He = expected heterocygosity. Ho = observed heterocygosity.

Within-population diversity

The average number of alleles per locus, number of private alleles, values of expected and observed heterozygosity (He and Ho), and values of heterozygosis deficit (F_{IS}) per each of the 5 populations are given in Table (2). The average number of alleles per locus per populations was 3.6, ranging from 2.7 in the NZW breed to 3.9 in the ERB and EG breeds. Mean observed heterozygosity was 0.527, ranging from 0.477 in the NZW breed to 0.581 in the EWG. Lower values for Ho were found for all populations. The inbreeding coefficient of individuals relative to the total population (F_{IT}) was 0.279. The overall within-population heterozygote deficit (F_{IS}) was 0.165, ranging from 0.045 in NZW breed to 0.266 in EBB breed. As already reported in rabbit by Bolet *et al.* (2000) and Queney *et al.* (2001), a smaller number of alleles was observed but this may be due in part to the use of inbred rabbit strains for building the reference families.

Table 2.- Within population genetic variability for all 17 microsatellite loci used.

Population	K	P_A	He	Ho	F_{IS}	F_{ST}
Egyptian Black Baladi (EBB)	3.65	1	0.510	0.365	0.266	0.124
Egyptian Gabali (EG)	3.94	5	0.574	0.451	0.207	0.001
Egyptian Red Baladi (ERB)	3.94	8	0.492	0.456	0.057	0.150
Egyptian White Giza (EWG)	3.88	1	0.581	0.482	0.158	0.101
Spanish New Zealand White (NZW)	2.71	1	0.477	0.434	0.045	0.194

K = number of alleles. P_A = private alleles. He = expected heterocygosity. Ho = observed heterocygosity.

$\mathbf{F_{IS}}$ = heterozygosis deficit. F_{ST} = population variation.

Among populations diversity

The overall variation among populations (F_{ST}) was 0.137 where, the NZW breed was the most differentiated population (F_{ST} = 0.194). In the factorial correspondence analysis including all samples, the first three principal factors (PCs) explained 38.18%, 31.70% and 19.58% of the total variation, respectively (Figure 1A). Analysis of genetic distances and overlapped population structure indicated that three of the Egyptian breeds (EBB, EWG and EG) are structurally separated from the ERB and Spanish line (NZW). Also, Neighbour-Joining tree analyses of the Reynolds genetic distances (D_R) among populations shows a clear structurally separation of the Egyptian populations from the Spanish line (Figure 1B).

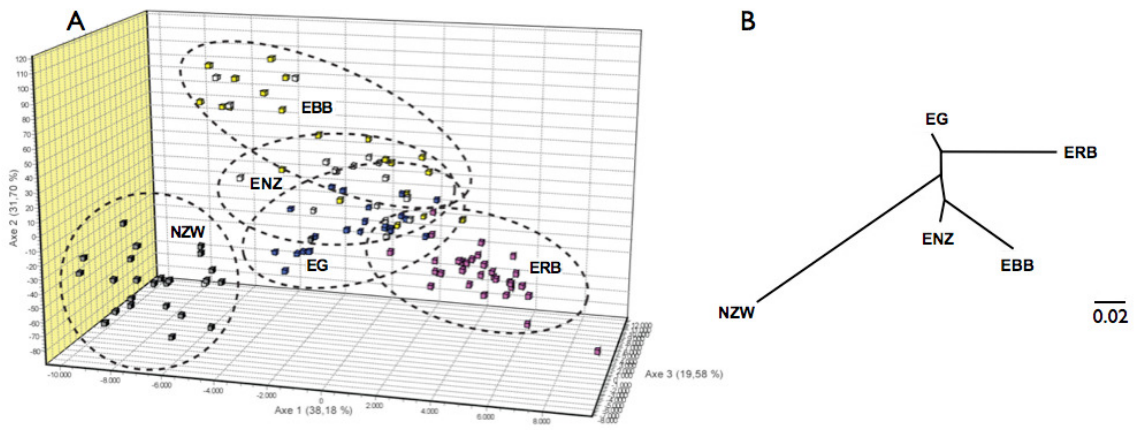

Figure 1A. Factorial correspondence analysis based on the allele frequencies from 17 mocrosatellite loci genotyped in five rabbit populations.

Figure 1B. Neigbour-Joining tree between five rabbit populations using Reynolds Distance. EBB: Egyptian Black Baladi, EG: Egyptian Gabali, ERB: Egyptian Red Baladi, EWG: Egyptian White Giza, NZW: Spanish New Zealand White line.

Using the individuals as taxonomic units and estimating the Allele Share Distance (D_{AS}), the Neighbour-Joining tree analysis reveals that there is a population mixture in the Egyptian populations, as seen with the AFC analysis (Figure 2). Only the ERB may to cluster in one independent population.

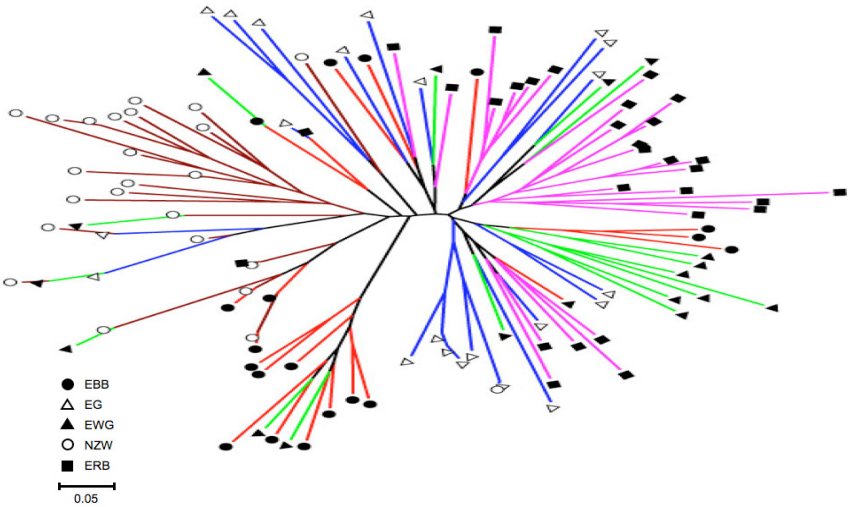

Figure 2. Neigbour-Joining tree among 144 individuals using Allele Shared Distance. EBB: Egyptian Black Baladi, EG: Egyptian Gabali, ERB: Egyptian Red Baladi, EWG: Egyptian White Giza, NZW: Spanish New Zealand line.

In conclusion, microsatellite loci analysis reveals that ERB breed could be structurally separated from the other Egyptians breeds, which are overlapped. It is necessary a more extend study to know if the others three Egyptian populations could be treated as a unique population at a genetic level with variations on their phenotypes.

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