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Muñoz-Mejías M.E., González A., El Moutchou N., Rodero E.

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Genetic structure of two minor Spanish goat breeds: Blanca Andaluza and Blanca Celtibérica

M.E. Muñoz-Mejías[1], A. González[1], N. El Moutchou[2] and E. Rodero[1]

[1]Department of Animal Production, University of Córdoba, Campus de Rabanales, 14014, Córdoba (Spain)
[2]Faculty of Science and Technology, the University of Abelmaleck Essaädi, Tangier (Morocco)

Abstract. A total of 2149 individuals (Blanca Celtibérica (BC) = 1,204, Blanca Andaluza (BA) = 945) were sampled from 4 and 47 herds, respectively. The purpose of this work is to analyze the genetic structure of these specially endangered Spanish goat breeds. All of these sampled animals were registered in their Herdbook. Nineteen DNA microsatellite markers have been used following FAO and ISAG recommendations for genetic diversity studies. According to the correspondence analysis, both populations showed a high genetic similarity. In fact, values of gene flow (Nm = 20.550) and the Nei standard genetic distance (D_S = 0.034) suggested that the two breeds are closely related. Mean expected heterozygosity (BC = 0.731, BA = 0.722), mean polymorphic information content (PIC) (BC = 0.698, BA = 0.689) and F_{IS} value (BC = 0.011, BA = 0.027), within population, showed that BC has the largest genetic variability. Enough genetic variability within breed was observed in both Blanca Andaluza and Blanca Celtibérica breeds. In conclusion it is necessary to work on individual-population assignments.

Keywords. DNA – Conservation – Genetic characterization – Microsatellites – Risk of extinction.

Structure génétique de deux races caprines espagnoles : Blanca Andaluza et Blanca Celtibérica

Résumé. *Un total de 2149 individus (Blanca Celtibérica (BC) = 1204, Blanca Andaluza (BA) = 945) a été échantillonné à partir de 4 et 47 troupeaux respectivement. L'objectif de ce travail est d'analyser la structure génétique de ces deux races caprines espagnoles menacées d'extinction. Tous ces animaux échantillonnés ont été enregistrés dans leur livre généalogique. Dix-neuf marqueurs microsatellites ont été utilisés suivant les recommandations de la FAO et l'ISAG pour les études de diversité génétique. Selon l'analyse de la correspondance, les deux populations ont montré une grande similitude génétique. En effet, les valeurs du flux génétique (Nm = 20,550) et la distance génétique standard de Nei (D_s = 0,034) suggèrent que les deux races sont étroitement liées. L'hétérozygotie moyenne attendue (BC = 0,731, BA = 0,722), la moyenne du contenu de l'information polymorphe (PIC) (BC = 0,698, BA = 0,689) et la valeur de la F_{IS} (BC = 0,011, BA = 0,027), entre les populations, ont montré que BC possède la plus grande variabilité génétique. Une structure génétique racial assez variable a été observé pour les deux* races Blanca Andaluza et Blanca Celtibérica. En *conclusion, il est nécessaire de travailler en assignation des individus aux populations.*

Mots-clés. *ADN – Conservation – Caractérisation génétique – Microsatellites – Risque d'extinction.*

I – Introduction

Spanish goat population presents two different aptitudes: meat and milk. As it happens in most Mediterranean countries, the extensive production system for goats has been mainly devoted to the production of kids. This extensive production system plays an essential role in the use of natural resources in marginal areas. Previous research about European, Asian and African goat breeds (Luikart *et al*., 2001; Naderi *et al*., 2007) shows low levels of phylogeographic structure due to the high mobility of this specie. The large number of Spanish goat breeds originated from European and African populations. Nevertheless, the intensification of agriculture during the twentieth century endangered many Spanish breeds. The most endangered breeds were those reared exclusively for meat production, as they are linked to marginal areas. This applies to Blan-

ca Andaluza (BA) and Blanca Celtibérica (BC) breeds, two especially protected Spanish goats with great phenotypic similarity that shared area of influence. BA is an ancient breed with representative signs of Savana type and Nubiana breed (Herrera *et al.*, 2001). BC is an example of preservation of purity of Savana type due to isolation (Herrera and Luque, 2008), and it is even assumed that it formed part of the origin of BA (Herrera *et al.*, 2001).

The maintenance of animal genetic diversity depends on the definition and application of conservation programs, which must be based on the information of population structure (Notter, 1999). Microsatellite markers are recommended by the FAO (2011) because of their properties: high polymorphism, high repeatability through the genome and easy identification by Polymerase Chain Reaction (PCR). The purpose of this work is to analyze the genetic variability and relationship between two endangered meat production goat breeds, namely Blanca Andaluza (BA) and Blanca Celtibérica (BC), by microsatellite markers.

II – Material and methods

A total of 2,149 blood samples were obtained in natural habitats from the two studied breeds. The sample size for each breed was 945 of Blanca Andaluza goats (47 different herds in 24 counties) and 1,204 of Blanca Celtibérica goats (4 different flocks in 4 counties), covering its whole geographical distribution and principal locations. Sampling was done by the respective breeders associations and individuals were chosen at random from Herdbook (Table 1). Blood samples collected were placed into an EDTA tube for DNA isolation and they were analyzed by an independent molecular laboratory.

Table 1. Total population, sampled population, percentage of the population sampled, total herds in the population, number of sampled herds and percentage of total herds sampled of Blanca Andaluza (BA) and Blanca Celtibérica (BC) breeds

Breed	Total population	Sample size	Sampled animal (%)	Total herds in population	Sampled herds	Sampled herds (%)
BA	8,408	945	11.24	55	47	85.45
BC	5,271	1,204	22.84	58	4	6.90

A set of 19 microsatellite markers were selected following FAO and ISAG recommendations for genetic diversity studies (Hoffmann *et al.*, 2004). PCR amplification was performed following Saiki *et al*. (1988) methodology through a Multiplex PCR kit supplied by Qiagen®. Size calling was accomplished with Genemapper^{TM} software.

Fstat 2.9.3 software (Goudet, 1995) was used to calculate the total number of alleles (TNA), mean number of alleles per breed (MNA) and effective numbers of alleles (N_a). Genetix 4.05.2 software (Belkhir *et al.*, 2001) was employed to estimate the observed and expected heterozygozity (H_o and H_e), the F-statistics of Wright (1965) according to Weir and Cockerham (1984) for each locus and breed. Allelic richness and frequency were also obtained. The number of loci with exclusive alleles (LEA) was estimated by direct counting, considering an exclusive allele when it was found only in one breed. Polymorphic information content (PIC) and deviations from Hardy-Weinberg equilibrium (HW) were assessed using Cervus 3.0 (Kalinowski *et al.*, 2007). Deviations from Hardy-Weinberg expectations were tested per breed using the Markov chain Monte Carlo simulation as implemented in Genepop statistical package version 3.4 (Raymond and Rousset, 1995). Estimated genetic divergence (F_{ST}), Nei´s genetic distance (D_S) (Nei, 1978), Reynold´s genetic distance (D_R), gene flow or effective number of migrants (N_m) and the factorial correspondence analysis (Benzécri, 1973) were computed using Genetix.

III – Results and discussion

All the 19 microsatellite markers analyzed were found to be polymorphic (Table 2): TNA ranged between 5 (INRA005) and 25 (HSC), effective number of alleles (N_a) ranged from 2.101 (TGLA53) to 7.937 (HSC) and PIC value ranged from 0.483 (INRA063) to 0.874 (HSC) with a global mean of 0.699. Similar values were reported by Dixit *et al*. (2012) and Bruno-de-Sousa *et al*. (2011) for Indian and Portuguese goats, respectively.

Table 2. Microsatellite markers, total number of alleles (TNA), mean number of alleles per breed (MNA), effective numbers of alleles (Na), polymorphic information content (PIC), observed and expected heterozygozity (H_o and H_e), F-statistics (F_{IS}, F_{ST} and F_{IT}), and deviation from Hardy-Weinberg equilibrium (HW), for locus and total of loci

Microsatelites	TNA	MNA	N_a	PIC	H_o	H_e	F_{IS}	F_{ST}	F_{IT}	HW
BBM1258	15	13.000	6.369	0.827	0.828	0.843	0.018	0.003	0.021	NS
BM1329	11	10.500	5.102	0.778	0.784	0.804	0.025	0.001	0.026	NS
CSRD247	10	9.500	4.831	0.770	0.775	0.793	0.018	0.014	0.032	*
ETH10	6	5.500	2.653	0.554	0.609	0.623	0.023	0.012	0.034	NS
FCB20	9	8.000	2.525	0.571	0.596	0.604	0.012	0.033	0.044	NS
HSC	25	23.500	7.937	0.874	0.872	0.875	0.002	0.024	0.025	*
ILSTS11	8	8.000	3.115	0.647	0.665	0.679	0.019	0.010	0.029	*
ILSTS19	6	6.000	2.882	0.627	0.624	0.653	0.047	0.025	0.071	***
ILSTS30	19	17.000	7.246	0.852	0.833	0.862	0.032	0.008	0.040	**
ILSTS87	11	10.500	3.077	0.631	0.655	0.675	0.030	0.005	0.035	NS
INRA005	5	4.500	2.646	0.581	0.613	0.622	0.013	0.008	0.021	NS
INRA006	13	12.500	6.897	0.850	0.849	0.855	0.006	0.020	0.025	NS
INRA023	12	11.500	5.435	0.803	0.796	0.816	0.023	0.015	0.038	*
INRA063	8	7.500	2.242	0.483	0.551	0.554	0.006	0.006	0.012	NS
INRA172	9	9.000	5.102	0.785	0.791	0.804	0.013	0.014	0.026	NS
MAF65	19	17.000	6.849	0.847	0.841	0.854	0.015	0.013	0.028	***
SRCRSP5	10	9.500	3.125	0.656	0.657	0.680	0.030	0.004	0.034	NS
SRCRSP8	12	10.000	3.135	0.652	0.677	0.681	0.005	0.005	0.010	NS
TGLA53	11	10.500	2.101	0.500	0.521	0.524	0.003	0.006	0.009	NS
Overall	11.530	10.711	3.650	0.699	0.713	0.726	0.018	0.012	0.030	NS

NS: not significant; * P<0.05; ** P<0.01; P<0.005.

The H_o estimated was lower than H_e in all studied loci: both values were similar for each locus, suggesting a not significant departure from Hardy-Weinberg equilibrium. This assertion is strengthened by the remaining results: 7 of the 19 markers analyzed did not comply with Hardy-Weinberg equilibrium (P<0.05) and F-statistics estimated were close to zero for the 19 loci and the overall.

Mean results for the BA and BC breeds are displayed in Table 3. The mean number of alleles (BA = 11.21, BC = 10.21) and the estimated allelic richness (BA = 11.211, BC = 10.140) suggest a higher variability for BA breed. However estimations of F_{is} (BA = 0.027, BC = 0.011) and H_o (BA = 0.702, BC = 0.723) showed a low level of inbreeding and heterozygote deficiency for BC. Similar values were found by Martínez *et al.* (2004) for the BA breed and the results for other endangered native goats showed a significant homozygote excess (Oliveira *et al.*, 2010; Dixit *et al.*, 2012; Bruno-de-Sousa *et al.*, 2011). The genetic differentiation among breeds (F_{st}), Nei´s genetic distance (D_S) and Reynold´s genetic distance (D_R) were found to be 0.012, 0.034 and 0.012, respectively. This weak differentiation together with a considerable level of gene flow (20.550) shows that the two breeds are closely related. The factorial correspondence analysis supports this fact (Fig. 1).

Table 3. Mean number of alleles (MNA), effective numbers of alleles (Na), allelic richness (AR), polymorphic information content (PIC), number of loci with exclusive alleles (LEA), proportion of loci not in Hardy-Weinberg equilibrium (LHWD), observed and expected heterozygozity (H_o and H_e), within-breed deficit in heterozygosity (F_{IS}), estimated genetic divergence (F_{ST}), Nei´s genetic distance (D_S), Reynold´s genetic distance (D_R) and effective number of migrants (N_m) computed for Blanca Andaluza (BA) and Blanca Celtibérica (BC) breeds

	MNA	Na	AR	PIC	LEA	LHWD	Ho	He	F_{IS}	F_{ST}	D_S	D_R	N_m
BA	11.21	3.58	11.211	0.689	14	0.263	0.702	0.721	0.027	0.012	0.034	0.012	20.550
BC	10.21	3.72	10.140	0.698	6	0.368	0.723	0.731	0.011				

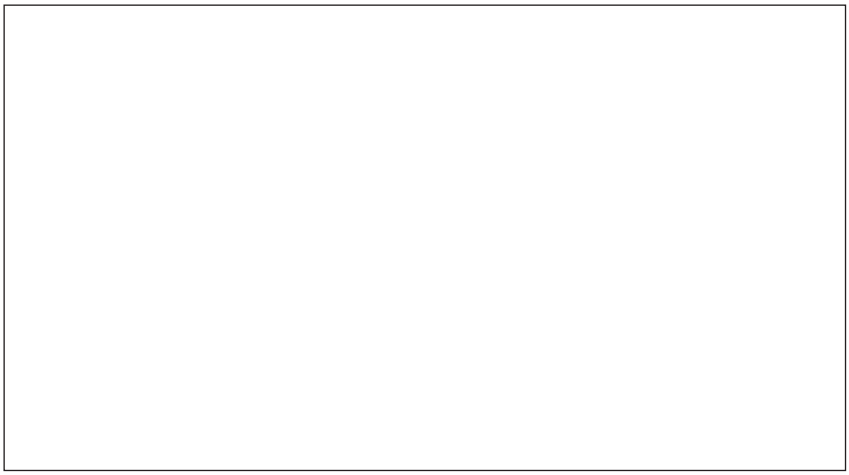

Fig. 1. Spatial representation of individuals from Blanca Andaluza (BA) and Blanca Celtibérica (BC) breeds resulting from a factorial correspondence analysis.

IV – Conclusions

Enough genetic variability within breed was observed in both Blanca Andaluza and Blanca Celtibérica breeds, without notable differences in the level of genetic diversity. This research corroborated the close genetic relationship between the two breeds, probably as a result of their common origin or past admixture. Population substructures and geographical groups should be studied further. It is also necessary to work on individual-population assignments in order to guarantee racial purity.

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