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Genetic characterisation of the Blanca Andaluza goat based on microsatellite markers

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Abstract

This is a genetic characterisation of the Blanca Serrana Andaluza goat breed based on microsatellite markers. Fifty animals from five herds were typed with a set of 27 microsatellites proposed by the FAO and ISAG for biodiversity studies. Our results showed that this is an extremely endangered breed, though it still possesses a high level of genetic variability, as demonstrated by the values for the expected and observed mean heterozygosity (0.71 and 0.66, respectively). All microsatellites were polymorphic, showing a mean number of alleles of 8.22. The present situation of the breed indicates that most of the microsatellites (18) show a H-W equilibrium, and the Fis shows a low value (0.07), suggesting a good strategy in the conservation plan of the breed.

Keywords: Molecular markers; characterisation, conservation, goat biodiversity
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Introduction

The Blanca Serrana Andaluza goat breed used to be widely distributed in the Andalusian region until the eighties of the 20th century. Its importance was that it was a zoogenetic resource, exploited under the extensive farming conditions of the Mediterranean region, contributing together with other farm species to maintain the ecological equilibrium. On the other hand, this breed contributed to the human fixation to the land based on the profitable use of the rusticity of the breed. After the eighties the milk goat farms displaced this breed from their traditional environment through two actions. The first was its substitution by specialised milk breeds such as Malagueña or Murciano-Granadina. The second was the crossbreeding with these breeds to produce its genetic substitution. Both actions produced genetic erosion leaving the breed close to extinction in present times. The first action to conserve the breed was its genetic characterisation in order to define the breed with respect to other genetic groups. This is the objective of the present paper.

Materials and Methods

We obtained DNA from blood samples of 50 animals most representative of the breed from five farms. The farms were located in four Andalusian provinces, Córdoba, Huelva, Jaén and Sevilla. DNA was extracted using the kit, BLOODCLEAN (BIOTOOLS - Biotechnological & Medical Laboratories, S.A. Madrid, Spain).

The following 27 microsatellites were studied: BM8125, BM1818, CSSM66, ILSTS011, INRA63, INRA23, SPS115, BM6506, ETH225, ETH10, INRA6, BM6526, HAUT27, CSRD247, MAF65, MAF209, OarFCB11, MM12, OarFCB304, BM1329, INRA5, TGLA122, HSC, MCM527, SRCRSP8, OarFCB48 and CSRM60. These microsatellites have been proposed by FAO and ISAG for biodiversity studies, because of having no linked location in the genome and their high level of variation.

These markers were amplified by means of the Polimerase Chain Reaction (PCR) technique according to the Martínez *et al.* (2000) method. To get the size separation of the obtained fragments we developed electrophoresis in polyacrylamide gel in an automatic sequencer ABI 377XL (Applied Biosystems, Foster

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City, CA, USA). The analyses of the fragments and the allelic typing were developed in the software Genescan Analysis® 3.1.2 and Genotyper® 2.5.2 respectively.

We calculated the allelic frequencies, the heterozygosity levels and the values of Fis by means of the software Genetix v. 4.02 (Belkhir, 2001). We have also calculated the content of polymorphic information (PIC) according to the algorithm proposed by Botstein *et al.* (1980). We also tested the Hardy-Weimberg (HW) equilibrium by means of the software Genepop v. 3.1c (Raymond & Rousset, 1995) which applies the chain method of Monte Carlo Markov (Guo & Thompson, 1992).

Results and Discussion

All 27 microsatellites were polymorphic, containing a minimum of three alleles for INRA5, ETH10 and MAF209 and a maximum of 17 for BM6526. This resulted in a mean number of 8.22 (Table 1). This value is higher than the 6.90 presented by Li *et al.* (2002) who studied 12 Chinese breeds of goats. This is also more than the values reported for other Asian (Barker *et al.* 2001) and French (Ouafi *et al.* 2002) breeds.

Table 1 Microsatellites analysed, number of alleles, expected (He) and observed heterozygosity (Ho), PIC (Polymorphic Information Content), Fis and probability value obtained for Hardy-Weinberg equilibrium

LOCUS	No of Alleles	He	Ho	PIC	Fis	P-Value
MM12	13	0.8926	0.8333	0.88	0.078	0.3476
CSSM66	16	0.8622	0.5476	0.85	0.375	0.0000
OarFCB48	9	0.8417	0.8478	0.82	0.004	0.4870
HSC	13	0.8274	0.7647	0.81	0.091	0.0386
MAF65	9	0.8282	0.9149	0.81	-0.094	0.0742
BM1329	9	0.8228	0.8864	0.80	-0.066	0.2052
OarFCB11	13	0.8163	0.7556	0.80	0.086	0.4180
CRSM60	7	0.8096	0.8936	0.78	-0.093	0.5407
TGLA122	7	0.7977	0.6087	0.77	0.247	0.0000
INRA23	7	0.7958	0.8824	0.77	-0.079	0.9018
BM1818	8	0.7789	0.6944	0.75	0.122	0.0684
BM6526	17	0.7769	0.7391	0.75	0.060	0.5875
CSRD247	9	0.7549	0.6591	0.72	0.138	0.7300
HAUT27	6	0.7597	0.6757	0.72	0.124	0.1582
BM6506	7	0.7453	0.7500	0.71	0.006	0.6435
OarFCB304	11	0.7476	0.6304	0.71	0.167	0.0252
INRA6	8	0.7378	0.6563	0.70	0.126	0.1908
SRCRSP8	9	0.7144	0.6818	0.68	0.057	0.1834
McM527	6	0.7102	0.5789	0.66	0.198	0.0078
ILSTS011	7	0.6933	0.5854	0.65	0.168	0.3474
BM8125	8	0.6652	0.6444	0.64	0.042	0.3142
ETH10	3	0.5797	0.6596	0.51	-0.127	0.5450
INRA63	5	0.5536	0.4118	0.48	0.270	0.1224
SPS115	5	0.4798	0.3415	0.43	0.300	0.0004
INRA5	3	0.5095	0.6897	0.40	-0.338	0.0896
ETH225	4	0.2942	0.3333	0.28	-0.114	1
MAF209	3	0.2407	0.2292	0.22	0.058	0.6084
Median	8.22	0.7050	0.6628		0.073	

The expected mean heterozygosity was 0.71 and the observed 0.66, rendering these values higher than those presented by Saitbekova *et al*, (1999) in nine Swiss breeds and those reported by Barker *et al.* (2001). But they are lower than those found by Yang *et al.* (1999) in five Chinese breeds and similar to those reported by Ouafi *et al.* (2002) and Li *et al.* (2002) in the papers mentioned before. These values indicate an important level of genetic variability for the breed considering its endangered state.

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In Table 1 the PIC values are presented, showing the level of informative capacity of the markers. All markers showed had acceptable informative capacities except ETH225 and MAF209.

Table 1 also shows the Fis values and the probability values for the H-W equilibrium. In both cases 18 microsatellites show H-W equilibrium, and that a high stability could be expected for the breed. It indicates that the population could be responding to the conservation activities developed. The mean value of the Fis for all loci was 0.07. It indicates a low level of inbreeding taking considering the present situation of the breed. The value is lower than those reported by Barker *et al.* (2001) for 11 Asiatic local breeds.

Conclusions

This breed possesses important levels of genetic variability and a low level of inbreeding, despite its extremely endangered situation. This set of microsatellites was a very good tool for the genetic characterization of the breed and the study of its genetic structure. The H-W equilibrium encountered in most of the microsatellites is showing a correct genetic management in the conservation plan of the population.

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