POSTER

GENETIC STUDY OF THE CHATO MURCIANO PIG BREED UNDER A RECOVERY PROGRAM

ESTUDIO GENÉTICO DEL CERDO CHATO MURCIANO SOMETIDO A UN PROGRAMA DE RECUPERACIÓN

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ADDITIONAL KEYWORDS

Genetic markers. Inbreeding. Conservation.

PALABRA CLAVE ADICIONALES

Marcadores genéticos. Consanguinidad. Conservación.

SUMMARY

The Chato Murciano is the only surviving local breed of pig historically produced in the region of Murcia (Spain). At the present time, it is at risk of extinction, with a census of no more of 40 reproductive animals.

In this paper, we are presenting a study of the inbreeding levels of the population, together with a genetic characterization of pure animals and individuals in several successive generations of absorption.

We observed high levels of inbreeding approaching 11 percent, which could explain the occurrence of malfunction and poor performance in the population.

The microsatellite study showed the genetic profile of the population and the genetic convergence of successive cross-bred generations towards the genetic profile of the purebred animals.

This study constitutes a follow up of the recovery program in development over the Chato Murciano.

RESUMEN

La raza Chato Murciano es la única raza local superviviente de los cerdos tradicionalmente producidos en la región de Murcia (España). En este momento está en peligro de extinción con un censo total de no más de 40 animales reproductores.

En este trabajo, presentamos un estudio sobre los niveles de endogamia de la población, junto con una caracterización genética de los animales puros así como de otros resultantes de sucesivas generaciones de cruces absorbentes.

Observamos altos niveles de consanguinidad, próximos al 11 p.100 lo que podría explicar la aparición de malformaciones y la pobre productividad de la población.

El estudio de microsatélites mostró el perfil genético de la población a la convergencia genética de las sucesivas generaciones de cruzamientos absorbentes hacia el perfil de los animales puros.

Este estudio constituye un paso adelante en

el programa de recuperación que desarrollamos sobre el Chato Murciano.

INTRODUCTION

The Chato Murciano Pig Breed has been characterised in the present paper by the study of genetic variability at 26 microsatellite loci, and was analysed in two populations of the breed. These populations were part of a selection programme to recover an old and rare pig variety on the verge of extinction. When the effective number of breeding animals in a population is small, the allele frequencies will be different in males and females, which causes an excess of heterozygotes in the progeny with respect to Hardy-Weinberg equilibrium expectations. It is important to detect population bottlenecks in threatened and managed species because bottlenecks can increase the risk of population extinction. Early detection is critical and can be facilitated by statistically powerful monitoring programs. We evaluate the accuracy and precision of the hetero-zygote-excess method using two data sets from the Chato Murciano pig. One is an original population and the other is a combination of F3+F4+F5 generations of a line created from the mating of one Chato Murciano boar with one Large White sow, followed by a absorption program. Analysis of highly polymorphic loci detects the experimental bottleneck and is a very good reference to estimate the magnitude of the bottleneck severity in the original population.

It is also the aim of this paper to show the genetic profile of individuals and of the population in general, in order to assist a recovery programme of the breed.

MATERIAL AND METHODS

COMPOSITION OF THE STUDIED POPULATION

The population was formed by 82 individuals distributed as follows: Chato Murciano pure (24); descendents of Chato Murciano X Large White F1 - F2 (1), F3 (15), F4 (17), F5 (12); Chato Murciano of unknown genealogy (7); Chato Murciano crossbreds (with other breeds) used as an outgroup in the individual tree (7).

STUDY OF THE GENETIC SITUATION

In order to study the genetic profile of this breed, the genealogical records of the complete population were examined to determine the degree of purity of all individuals, as well as an analysis of the population structure to find out the sex and age ratios.

Apart from this, we calculated the individual coefficient of inbreeding (Wright, 1922) in all the animals of the population using their genealogical information, as well as deducing the average level of inbreeding in the population, according to the average coefficient of the population inbreeding (Cavalli-Sforza and Bodmer, 1981).

In the same way, we calculated the likely increase of the rate of consanguinity of the population in following generations using the effective size of the population (Falconer, 1982).

MOLECULAR GENETIC CHARACTERIZATION

The 25 microsatellites studied were selected from the 27 markers published

Table I. *The total population of the Chato Murciano according to the degree of purity, sex and age groups.* (Población total de Chato Murciano de acuerdo con el grado de pureza, sexo y edad de los grupos).

Born	Animals Breed		Animals Crossed	
	Male	Female	Male	Female
Before 1995	2	2	0	0
1995	1	0	0	0
1996	0	8	0	6
1997	5	5	4	15
Total	8	15	4	21

by Denis Milan and Martien Groenen (FAO 1998): *CGA, IGF1, S0002, S0005, S0026, S0068, S0090, S0101, S0155, S0178, S0215, S0225, S0226, S0227, S0228, S0355, S0386, SW122, SW24, SW240, SW632, SW72, SW857, SW911, SW936* and *SW951*. The microsatellites were amplified using the polymerase chain reaction (PCR) by several multiplex reactions (Martí-nez *et al.*, 2000). Electrophoresis was carried out using the ABI 377 XL automatic sequencer (Applied Biosystems, Foster City, CA, USA).

A MICROSAT v.1.5b computer program (Minch 1997) was used to calculate pairwise distance values from inter-individual genetic distance, based on the proportion of alleles shared for two individuals averaged over loci (Bowcock *et al.,* 1994). These distance values were used to construct an UPGMA tree (Sneath and Sokal, 1973) using the NEIGHBOR module of the PHYLIP v.3.57c computer package (Felsenstein, 1995)and was drawn using the TREEWIEW application (Page, 1998).

RESULTS AND DISCUSSION

GENETIC PROFILE OF THE BREED

Values of the coefficient rate of inbreeding of individual animals ranged between nil and 32,30 percent. Regarding the average coefficient of

Table II. *The average level of inbreeding of the population, according to Cavalli-Sforza and Bodmer (1981).* (Media del nivel de consanguinidad de la población de acuerdo con Cavalli-Sforza y Bodmer (1981)).

Nº Animals	Crossed animals in the absorption process	Relative Frequency	F	$P_i * _{Fi}$
26	11	0.541	0.0000	0.000000
01	01	0.021	0.1250	0.002625
08	08	0.167	0.1568	0.026177
04	2	0.083	0.2500	0.020750
06		0.125	0.3125	0.039062
03	03	0.063	0.3320	0.020916
Total				0.109530

inbreeding, a value of 10,95 percent of the population, this offered us a margin between 2,12 and 19,78 percent, within which the average inbreeding of the population is found. This level of inbreeding can be considered dangerous, as it includes some relatively high levels which exceed the limits of the appearance of effects of inbreeding depression, according to Cardellino and Rovira (1987) and Legates and Warwick (1992) (see **tables I** and **II**). The most immediate consequences of the inbreeding depression are the loss of functional characteristics (productive and reproductive) in this breed, as well as the appearance of the expression of deleterious genes in the population.

Moreover, we must be very cau-

Table III. *Allelic frequencies of Chato Murciano pure and crosbred*. (Frecuencias alélicas de los Chatos Murcianos puros y cruzados).

Marker	# alleles	Frequencies					
CGA	6	0.681	0.139	0.118	0.035	*0.007*	*0.021*
S0101	3	0.518	0.278	0.204			
S0215	1	1					
S0355	2	0.787	0.213				
SW911	5	0.500	0.278	0.173	0.043	*0.006*	
SW936	6	0.329	0.268	0.170	0.146	0.079	0.006
S0068	5	0.506	0.269	0.100	0.094	**0.031**	
SW632	4	0.701	0.180	0.111	0.007		
SW24	4	0.908	0.035	0.021	0.035		
S0227	2	0.889	0.111				
S0225	3	0.650	0.231	0.119			
SW122	3	0.640	0.330	*0.029*			
S0090	5	0.391	0.308	0.237	*0.058*	**0.006**	
S0226	3	0.722	0.172	0.105			
SW591	2	0.932	0.068				
S0228	2	0.590	0.412				
S0178	4	0.492	0.333	*0.129*	0.045		
S0005	5	0.269	0.375	0.337	0.012	*0.006*	
S0386	4	0.480	0.293	0.153	0.073		
SW72	5	0.645	0.260	0.089	**0.006**		
S0002	3	0.692	0.150	0.158			
SW857	4	0.652	0.219	**0.055**	0.073		
S0026	4	0.606	0.202	0.149	*0.042*		
IGF1	4	0.512	0.333	0.142	*0.012*		
S0155	4	0.616	0.317	0.061	*0.006*		
SW240	2	0.567	0.433				

Note: Exclusive alleles of pure Chato population in bold. Exclusive alleles of F3+F4+F5 population in italic.
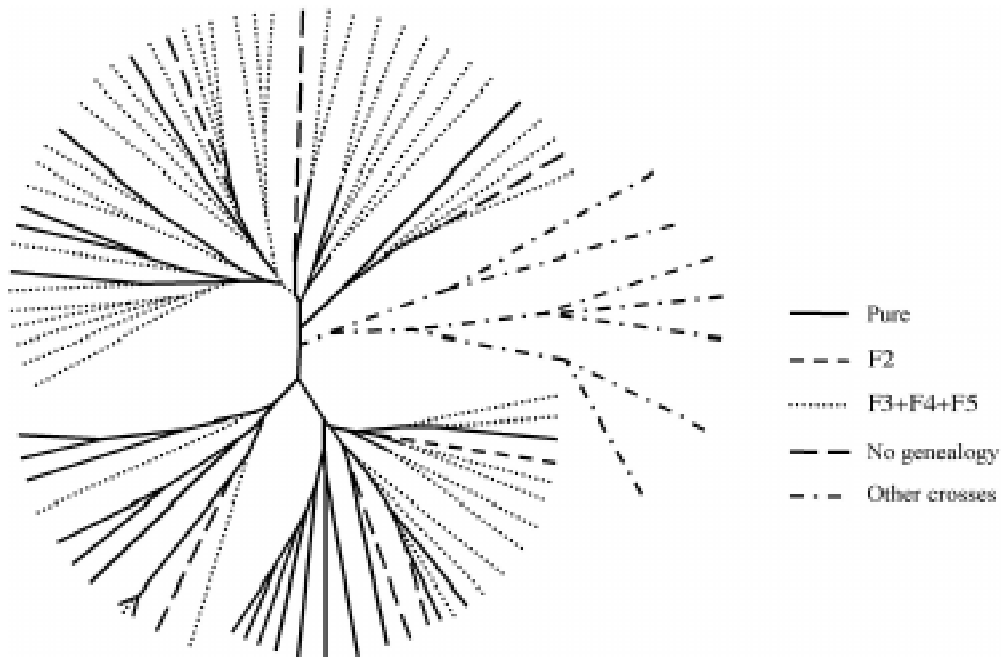
Figure 1. *UPGMA individual tree of Chato Murciano from average of shared alleles distances (Bowcock* et al., *1994).* (Árbol de distancia genética individual de cerdos Chatos Murcianos construido a partir de la matriz de pares de distancias basadas en los promedios de alelos compartidos (Bowcock *et al.,* 1994) mediante el método UPGMA).

tions, as this figure only indicates the accumulated levels of inbreeding from the genetic information available to us, and as this breed was recovered from a small number of animals, it is very possible that the rate of inbreeding prior to the genealogical check was high. For this reason, higher rates of underlying inbreeding are to be suspected, which would explain the fertility problems, the reduced number of piglets per litter, the low viability of the piglets, and the lack of hardiness in in the pure animals (reduction of the body size) as consequences of the inbreeding depression.

GENETIC CHARACTERIZATION

Table III shows the genetic profile of the studied population based on the genotypes of 25 microatellites. The population includes pure animals and individuals belonging to second, third, fourth and fifth generation of grading-up. The allelic frequencies of exclusive alleles are shown, of the pure population (in bold) and crossbred animals (in italic).

Allelic frequencies for original and F3+F4+F5 populations are close and the exclusive alleles found in each population are present in only a few cases with low frequency. Also

distance genetic tree shows three individual groups although all animals are related. The conclusion is the F3+F4+F5 population is similar to the original population of Chato Murciano. Therefore the effect of the crossbreeding with a LargeWhite individual seems to be absorbed.

For both mutation models the original population of Chato Murciano does not show a recent bottleneck. Nevertheless F3+F4+F5 population reject the mutation drift equilibrium as was expected because all individuals come from the same animal. Dinucleotide microsatelite markers seems to fit with IAM model because SMM model has more difficulties to show bottleneck effect.

There is an opportunity to reestablish the Chato Murciano breed because it shows some variability, does not show a strong allelic reduction and desequilibrium of genetic frequencies.

The use of an outcross to another breed as the first step is a very good tool to increase the size of population in exceptional cases because its influence can be absorbed in a few generations.

Microsatellite markers are very useful to understand the evolution of a conservation procedure of near extinction populations.

REFERENCES

Bowcock, A.M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J.R. Kidd and L.L. Cavalli-Sforza. 1994. High resolution of human evolution with polymorphic microsatellites. *Nature,* 368: 455-457.

Cardellino, R. y J. Rovira. 1987. Mejoramiento Genético Animal. Ed. Interamericana. pp: 173-192.

Cavalli-Sforza, L. y W. Bodmer. 1981. Genética de las poblaciones humanas. Ed. Omega. Barcelona. pp: 342-355.

Falconer, D. 1982. Introducción a la Genética Cuantitativa. Editorial Continental México. pp: 257-271.

FAO. 1998. Secondary Guidelines for Developmen of National Farm Animal Genetic Resources Management Plans: Management of small populations at risk, FAO. Rome.

Felsestein, J. 1995. PHYLIP (Phylogeny Inference Package) Version 3.5c. University of Washington.

Legates, J.E y E.J. Warwick. 1992. Cría y Mejora del Ganado. Mc.Graw Hill. pp: 229-239.

Martínez, A. M., B. Peinado, C. Barba, J.V. Delgado and J. L. Vega Pla. 2000. Genetic análisis of the Chato Murciano pig and its relationships with the Iberian pig using microsatellites. 27th International Conference on Animal Genetics. University of Minnesota.

Minch, E. 1998. MICROSAT Version 1.5b (Macintosh). University of Standford. Standford.

Page, R.D.M. 1998. Tree drawing software for Apple Macintosh and Microsoft Windows. In: http://taxonomy.zoology.gla.ac.uk/rod/rod.html. Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

Sneath, P.H.A. and H.H. Sokal. 1973. Numerical taxomomy. Freeman Ed. San Francisco.

Wright, S. 1922. Coefficients of inbreeding and relationship. *Amer. Nat.,* 56: 330-338.