Chato Murciano pig breed: genetic and ethnozoological characterization

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Summary

The Chato Murciano is the only surviving breed of pig of those historically farmed in the region of Murcia for their quality meat. At present, it is on the verge of extinction, having a population of only 260 reproductive animals. This paper describes the genetic studies made in the conservation and recovery programme of this breed of pig. A study of the morphological characterization of these animals was carried out first, measuring thirteen quantitative and six qualitative variables in a sample of 24 adult animals, 8 males and 16 females.

Subsequently, investigation was made of the consanguinity of the individuals and of the population as well as the future influence of inbreeding in each generation. Finally, the accuracy and precision of the heterozygote-excess method was evaluated using two data sets from the Chato Murciano pig. One data set is an original population and the other is a F3+F4+F5 generation of a line created from mating a Chato Murciano female with a Large White boar as part of an absorption programme based on backcrosses with Chato Murciano boars.

Resumen

La raza Chato Murciano es a la única raza porcina superviviente de aquellas que históricamente se criaban en la región de Murcia por la calidad de su carne. Actualmente se encuentra en vía de extinción, ya que su población consta de tan solo 260 reproductores. Este artículo describe los estudios genéticos realizados para la conservación y programa de recuperación de esta raza. En primer lugar se llevó a cabo un estudio de la caracterización morfológica de estos animales, midiendo 13 variables cuantitativas y seis cualitativas en una muestra de 24 animales adultos, 8 machos y 16 hembras.

Como consecuencia, se realizó un estudio sobre la consanguinidad de los individuos y de la población, así como sobre la influencia futura de la consanguinidad en cada generación. Por fin, la exactitud y precisión del método heterozigote-excess fue evaluada utilizando dos series de datos de la raza Chato Murciano. Una de las series de datos proviene de una población y la segunda es una generación F3+F4+F5 de una línea creada por el cruce de hembra de Chato Murciano con macho Large White como parte de un programa de absorción basado en retrocruzamientos con machos de Chato Murciano.

Keywords: *Chato Murciano pig, Autochthonous breed, Morphological characterisation, Genetic studies.*

Introduction

The meat industry of south-eastern Spain required an improved pig from the primitive Murcian breed in their Gabana and Pintada variations and so they were crossed with the Yorkshire, Berkshire, Tamworth, Craonés and Alderney breeds. The Gabana type has a black coat and bristles while the Pintada type also has a black coat and bristles, but which were either tawny or had spots of red or white hair (Peinado *et al*., 2001; Poto *et al*., 2002).

The Murcian variety's abundance of fat was reduced and the yield and proportion of lean meat improved by crossing with the Yorkshire and Berkshire breeds. The primitive Murcian pigs also benefited from the precocity and prolificacy of these two breeds, without losing their adaptability characteristics or their capacity for agricultural farming and feeding on leftover food products from the traditional canning industry of the Murcian Region. At the beginning of the century 20th, this new breed was called the Chato Murciano (Figure 1) and spread all over the south-eastern coastal region of Spain, reaching the Catalan and Balearic coast, and was in great demand due to its precocity and lean meat (García *et al*., 1990).

However, the pressure imposed by commercial hybrids obtained from select breeds and submitted to highly technical intensive systems, has pushed this breed aside to the point of practically eliminating it (Martínez *et al*., 1998; Lobera, 1997). At present, the nucleus of pure breed specimens is very small, which has led the Ministry of Agriculture to declare the Chato Murciano a breed under special protection.

The Murcian Institute of Agricultural and Alimentary Research and Development (I.M.I.D.A.) and the Agricultural Training and Experiments Centre (CCEA), of the Murcian Regional Government started work some years ago on the conservation of the scarce specimens of this breed and on

Figure 1. Chato Murciano boar.

increasing the number of animals with the collaboration of a small number of stockbreeders.

As regards this first activity, semen from five male pigs of the Chato Murciano breed was frozen, and fresh semen is being used for the artificial insemination of females, not only in the CCEA, but also in private stock farms which request it, on the condition that they supply data on any animals obtained from this operation. Rotation of male pigs is taken into account when mating them with pure-breed or crossbred females, in order to keep inbreeding as low as possible. The aim of increasing the number of animals is to identify the characteristics of the Chato Murciano breed which are of most interest to the industry and which in the past provided the authentic products of the Region of Murcia.

The aims of this paper were to define the production situation of the Chato Murciano breed, defining its standard as it is today, and to investigate the genetic situation of the individuals and of the population in general, in order to undertake a recovery programme of the breed.

Materials and Methods

A total of 24 animals belonging to four different groups were used. The four groups were:

- Group 1: Three seven-month-old male pigs intended for slaughtering.
- Group 2: Four seven-month-old female pigs intended for reproduction.
- Group 3: Twelve reproductive female pigs in different reproductive conditions.
- Group 4: Five reproductive male pigs intended for the production of semen.

The variables measured for each of the animals were as follows.

Quantitative variables

- Head length: the distance between the occipital tip and the snout of the animal.
- Head width: the distance between both zygomatic arches.
- Length of the snout: the distance between the frontal nasal suture and the upper part of the snout.
- Distance between eyes: the shortest distance between the two eye sockets.
- Height at withers: the distance between the ground and the highest part of the withers.
- Breast height: the distance between the most sloping part of the withers and the most curved part of the sternum.
- Breast width: the distance between the ribs on each side using as a reference point the edges of the rib area with the front legs.
- Height of the rump: the distance between the ground and the highest point of the hip bones.
- Rump width: the distance between the external iliac tuberosities.
- Length of the rump: the distance between the beginning of the rump (external iliac tuberosity) and the end of the ischion.
- Longitudinal diameter: the distance between the ischiatic protuberance and the joint of the shoulder blade and the humorous.
- Thoracic circumference: the length of the circumference formed by the thorax around the back.
- Shank circumference: the length of the circumference of the metacarpus at the narrowest part.

Qualitative variables

Coat and hair, special features, hooves, mucous membrane, ears, number of mammary glands were observed. To study the breed's genetic situation the species/breed purity degree for all the population was determined by studying and analysing the available genealogical information and its proximity to the breed standard. Sex ratio and population age structure were determined.

Coefficient of consanguinity (Wright, 1922) for every animal in the population was calculated, and the average level of inbreeding in the population deduced, according to the average coefficient of the population consanguinity (Cavalli-Sforza and Bodmer, 1981). The expected increase in the rate of consanguinity of the population in following generations was estimated using the actual size of the population (Falconer, 1982).

Genetic molecular studies

The accuracy and precision of the heterozygote-excess method was evaluated using two data sets from the Chato Murciano pig. One of them is an original population and the other is a F3+F4+F5 generation of a line created from the mating of a Chato Murciano female with a Large White boar as part of an absorption programme based on backcrosses with Chato Murciano boars.

Eighty three animals were used for the molecular genetic identification as follows:

- 24 original Chato Murciano
- 7 Chato Murciano of unknown genealogy
- 7 Chato Murciano crossbred with other breeds (used as out-group in individual tree)
- Chato Murciano female x Large White boar for F1 (absorption of Large White influence in next generations with Chato Murciano boar):
- 1 F2 (F1 x Chato Murciano boar), 15 F3 (F2 x Chato Murciano boar), 17 F4 (F3 x Chato Murciano boar) and 12 F5 (F4 x Chato Murciano boar)

Twenty-five pig microsatellites were selected from the 27 markers published by Denis Milan and Martien Groenen (FAO, 1998). PCR amplification was performed on a GeneAmp 9600 (Perkin Elmer, Norwalk, CT, USA). Twenty-three microsatellites were amplified in seven multiplexed reactions (M1-7) and the remaining two markers in single PCR. PCR products were pooled in three tubes as follows: M1+M2+M3+S0355, M4+M5 and M6+M7+S0386.

A MICROSAT v.1.5b computer program (Minch, 1997) was used to calculate base pair distance values from inter-individual genetic distance, based on the proportion of alleles shared by 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 TREEVIEW application (Page, 1996).

The average heterozygosity and standard deviation for each *locus* and the probability of heterozygosity excess for the original Chato Murciano group and backcrossed group under IAM (infinite allele model) and SMM (step mutation model) were estimated using the computer program BOTTLENECK (Cornuet and Luikart, 1996).

This work was part of a conservation programme to recover the breed. When a population experiences a reduction followed by an expansion of its effective size, it is said that it has suffered a bottleneck. Bottlenecked populations generally develop a heterozygosity excess at selective neutral loci (Cornuet and Luikart, 1996), *i.e.* the heterozygosity computed from some genetic markers is larger than the heterozygosity expected at mutation drift equilibrium, given the number of alleles found in the population. 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 powerful statistical monitoring programs.

Results and Discussion

A zoometric study of 13 parameters was carried out on 24 animals of this breed from which the following results were obtained. The results of the quantitative variables are summarised in table 1 and 2.

Table 1. Average zoometric measurements in three groups of pigs of the Chato Murciano breed (cm).

Variable	Group 1 N=3 X±SD	Group 2 N=4 X±SD	Group 3 N=12 X±SD
Head length	25.16±0.28	24.5±1.0	25.65±1.95
Head width	13.0±2.17	13.17±1.26	16.32±1.17
Length of the snout	8.16±1.25	7.25±0.5	8.5±1.44
Distance between eyes	9.0±0.25	8.62±0.75	11.91±1.83
Height of the withers	58.33±4.16	56.76±1.25	65.25±3.01
Rump width	29.33±1.75	25.87±1.43	33.12±2.47
Longitudinal diameter	94.83±8.40	88.5±5.30	97.75±6.07
Breast height	31.16±2.02	30.25±0.95	37.87±2.98
Breast width	28.50±1.3	27.62±3.47	31.93±2.13
Height of the rump	64.33±1.15	65.25±2.21	71.12±2.47
Rump length	27.16±0.76	28.62±3.19	28.33±2.45
Thoracic circumference	105.66±5.03	96.25±2.98	112.87±87
Shank circumference	16.66±0.76	16±0	18±1.83

Group 1: Three seven-month-old male pigs intended for food and slaughtering.
Group 2: Four seven-month-old female pigs intended for reproduction.
Group 3: Twelve reproductive female pigs in different reproductive situations.

Table 2. Zoometric measurements found in the boar of this breed (cm).

Variable	Chato viejo	Chato 222	Chato 215	Chato 802	Chato 807
Age	12 years	4.5 years	3.5 years	1.5 years	1 year
Head length	28.5	29	29	28.5	27
Head width	20	18.5	18	17.5	16
Length of the snout	9	10.5	10.5	10	9.5
Distance between eyes	16	14	16	13	12.5
Height of the withers	82.5	87	85	73	71
Rump width	37	37	36.5	39	34
Longitudinal diameter	132	124	124	118	107.5
Breast height	43	42.5	42.5	42	36
Breast width	44	37.5	37	37	34
Height of the rump	85.5	86	86	78	81
Rump length	42	36	38	34	31
Thoracic circumference	140	134	126	120	109
Shank circumference	22	22	22	19	18

Qualitative variables

Coat and hair

The breed has a black coat, characteristic of the so-called "*Chato Murciano Negro*" variety, which was produced in the CCEA in Lorca (Murcia). The colour is uniform except for a few specimens which have white patches on their legs, snout and tail. The appearance of white parts is due to the characteristics inherited from the male pig of the Berkshire breed at the time of the breed formation.

The hair is a distinctive characteristic of the female pigs and is evenly distributed all

Table 3. The total population of the "Chato Murciano" according to the degree of purity, sex and age groups.

	Animals	Breed	Animals	Crossed
Born	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

over the body. The area of the mammary glands is free from hair in the female pigs.

Special features

When white colouring is found in the above mentioned areas, it not only appears on the skin, but also in the hair and on the hooves. The mucous membrane of the mouth is normally dark in colour, with the above mentioned exceptions, though it may have a whitish appearance with sections of white hair in the most closed areas.

Ears

They ears are large, triangular and point upwards and outwards.

Number of mammary glands

In the present specimens, the number of mammary glands varies between twelve and fourteen.

Genetic situation of the breed

The individual consanguinity coefficient ranged between 0% and 33.20% with an average of 10.95% calculated according to Cavalli-Sforza and Bodmer (1981). The average consanguinity of the population is found within a range of between 2.12% and 19.78%. The result of this consanguinity data can be considered alarming, as it includes some levels which exceed the limits for the appearance of inbreeding depression effects, according to Cardellino and Rovira (1987) and Legates and Warwick (1992) (Tables 3 and 4). The most immediate consequences of the inbreeding depression are the loss of adaptive values (productive and reproductive) as well as the appearance of the expression of deleterious genes in the population.

A cautionary note is that this figure only indicates the accumulated levels of inbreeding from the genetic information available, and as this breed has recovered from a small number of animals, it is possible that the rate of consanguinity 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, and the low viability of the piglets and the lack of hardiness in crossbreds (reduction of the body size) as consequences of the inbreeding depression.

Allelic frequencies for original and backcrossed groups are presented in table 5, only some exclusive alleles were found in each population with low frequency. Also the tree of genetic distances shows a mixture of individuals from two cited groups (Figure 2). Therefore the effect of the crossbreeding with a Large White individual seems to be absorbed after three backcross generations.

For both mutation models, IAM and SMM, the original population of Chato

Table 4. Average level of population consanguinity, according to Cavalli-Sforza and Bodmer (1981).

No. of animals	Relative frequency (P_i)	Consanguinity Coef. (F_i)	P_i x F_i
26 (11[a])	0.541	0.0000	0.000000
01 (01[a])	0.021	0.1250	0.002625
08 (08[a])	0.167	0.1568	0.026177
04 (2[a])	0.083	0.2500	0.020750
06	0.125	0.3125	0.039062
03 (03[a])	0.063	0.3320	0.020916
Total			0.109530

(*) Crossed animals in absorption process.

Table 5. Allele frequencies in pure and crossbred Chato Murciano pigs.

Marker	# alleles	Frequencies in decreasing order					
CGA	6	0.68056	0.13889	0.11806	0.03472	*0.00694*	*0.02083*
S0101	3	0.51852	0.27778	0.20370			
S0215	1	1					
S0355	2	0.78659	0.21341				
SW911	5	0.50000	0.27778	0.17284	0.04321	*0.00617*	
SW936	6	0.32927	0.26829	0.17073	0.14634	0.07927	0.00610
S0068	5	0.50625	0.26875	0.10000	0.09375	**0.03125**	
SW632	4	0.70139	0.18056	0.11111	0.00694		
SW24	4	0.90845	0.03521	0.02113	0.03521		
S0227	2	0.88889	0.11111				
50225	3	0.65000	0.23125	0.11875			
SW122	3	0.63971	0.33088	*0.02941*			
S0090	5	0.39103	0.30769	0.23718	*0.05769*	**0.00641**	
S0226	3	0.72222	0.17284	0.10494			
SW591	2	0.93210	0.06790				
S0228	2	0.59028	0.41171				
S0178	4	0.49242	0.33333	*0.12879*	0.04545		
S0005	5	0.26875	0.37500	0.33750	0.01250	*0.00625*	
S0386	4	0.48000	0.29333	0.15333	0.07333		
SW72	5	0.64557	0.25949	0.08861	0.00633		
S0002	3	0.69178	0.15068	0.15753			
SW857	4	0.65234	0.219.51	0.05488	0.07317		
S0026	4	0.60638	0.20213	0.14894	*0.04255*		
IGF1	4	0.51235	0.33333	0.14198	*0.01235*		
S0155	4	0.61585	0.31707	0.06098	*0.00610*		
SW240	2	0.56707	0.43293				

Notes: Exclusive alleles of the original population of Chatos are in bold.
Exclusive alleles of the F3+F4+F5 are in italic.

Murciano does not show heterozygosity excess, rejecting the hypothesis of a recent bottleneck (Table 6). Nevertheless the backcrossed group showed heterozygosity excess rejecting the drift equilibrium. This result was expected because all the individuals descend from one male and one female.

Table 6. Resuls of the aplication of both mutation models, IAM and SMM, on pure and cross-breed Chato Murciano animals.

Mutation model		Original	F3+F4+F5
IAM[1]	Expected markers with excess heterozygosity	12.8	12.62
	Markers with deficiency of heterozygosity	9	3
	Markers with excess heterozygosity	14	21
	Probability of mutation effect/bottleneck[3]	0.2905	0.0003
SMM[2]	Expected markers with excess heterozygosity	13.07	13.54
	Markers with deficiency of heterozygosity	11	6
	Markers with excess of heterozygosity	12	18
	Probability of mutation effect/bottleneck[3]	0.4013	0.0482

Note:
1. "Infinite Allele Model"
2. "Step Mutation Model"
3. $P<0.05$ reject the hypothesis of mutation drift equilibrium suggesting a recent reduction in population size

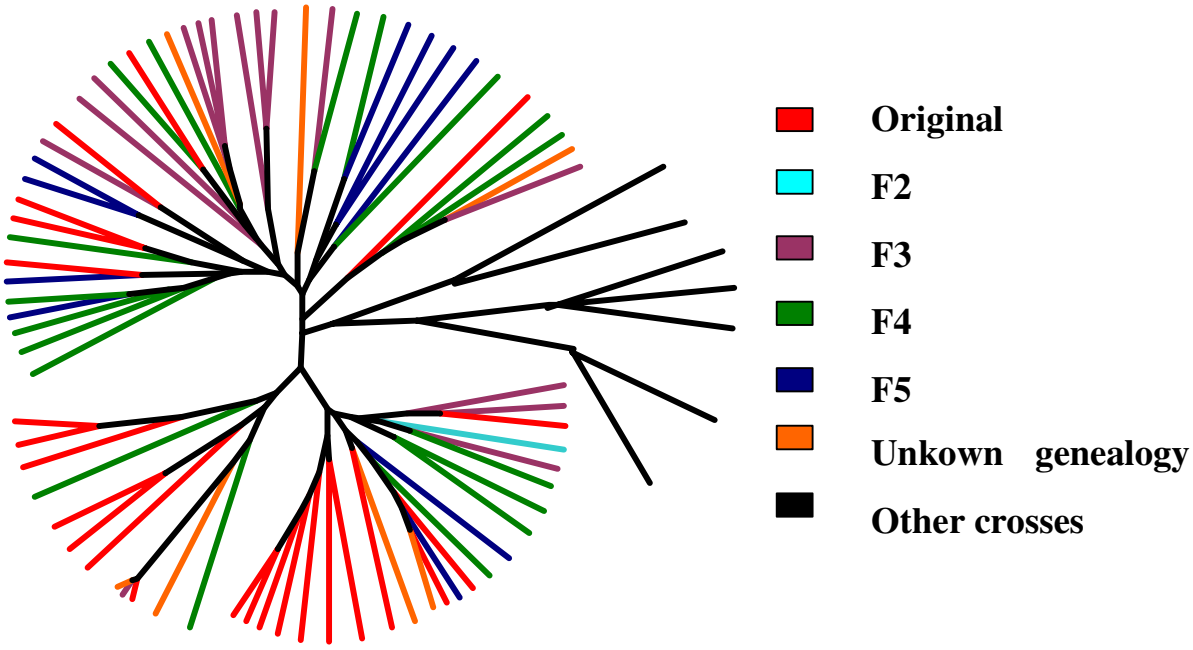

Figure 2. Individual tree UPGMA method.

Conclusions

For future measures a strict genealogical and reproductive control must be followed, by making a guided mating system compulsory. Use is being made of the tables of co-ancestry in order to determine the expected increase of consanguinity in future litters, and to minimize it. Furthermore, as a complementary measure, the Genetic Conservation Index (Alderson, 1992) can also be used, so that the variability in the breed is maximized, whilst the increase in consanguinity is minimized.

Allelic frequencies and genetic distances show minimum differences between original and backcrossed groups; therefore it is possible to establish a conservation plan based on absorption crosses to reduce inbreeding problems in enlarged populations.

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

The use of an external reproducer in the first steps is a very good tool to increase the size of a population in exceptional cases because its influence can be absorbed in a few generations.

List of References

Alderson, G.L. 1992. A system to maximize the maintenance of genetic variability in small populations. Genetic Conservation of Domestic Livestock. CAB International, 18–30.

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

Cardellino, R. & J. Rovira. 1987. Mejoramiento Genético Animal. Ed. Interamericana, 173-192.

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

Cornuet, J.M. & G. Luikart. 1996. Description and Power Analysis of Two Tests for Detecting Populations Bottlenecks From Allele Frequency Data. Genetics 144, 2001-2014.

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

FAO. 1998. Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans: Management of small populations at risk, FAO, Rome, pp 229.

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

García Dory, A., S. Martínez & F. Orozco. 1990. Guía de campo de las razas autóctonas de España. Madrid, Alianza (Ed), pp. 228.

Legates, J.E. & E.J. Warwick. 1992. Cría y Mejora del Ganado. Mc.Graw Hill (Ed), 229-239.

Lobera, J. 1997. El cerdo Chato Murciano. Antecedentes y creación. Primer Congreso Nacional de la Sociedad Española para los Recursos Genéticos Animales (S.E.R.G.A.). Córdoba, 14-17 December, pp. 61.

Martínez, M., B. Peinado, J. Martín, J. Lobera, C. Barba & A. Poto. 1998 El Chato Murciano, la raza autóctona de la Región. Situación actual desde el punto de vista genético. Adea-Asaja, no. 8, 24-26.

Minch, E. 1997. MICROSAT Version 1.5b (Macintosh). University of Stanford. Stanford.

Page, R.D.M. 1996. 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.

Peinado, B., A. Poto, M. Marín & J.B. Lobera. 2001. Raza porcina Chato Murciano. Porci: Conservación genética de razas autóctonas (II), no. 61, 39-55.

Poto, A., B. Peinado, M. Marín & J.B. Lobera. 2002. El cerdo Chato Murciano. Ediporc. no. 54, 7-17.

Sneath, P.H.A. & R.R. Sokal. 1973. Numerical taxonomy. Freeman Ed. San Francisco, pp. 549.

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