

POSTER

PRELIMINARY STUDY OF INDIVIDUAL ASSIGNMENT OF IBERIAN PIGS USING DNA GENETIC MARKERS

ESTUDIO PRELIMINAR DE ASIGNACIÓN INDIVIDUAL DE CERDOS IBÉRICOS USANDO MARCADORES GENÉTICOS DE ADN

Vega-Pla¹, J.L., A.M. Martínez², A. Cabello³, P.P. Rodríguez-Gallardo¹ and J.V. Delgado²

¹Laboratorio de Genética Molecular. Servicio de Cría Caballar. Apartado Oficial Sucursal 2. 14071 Córdoba. España.

²Departamento de Genética. Campus de Rabanales. Ctra. Madrid-Cádiz, km 396. 14071 Córdoba. España.

³Delegación de Investigación y Desarrollo Agrícola y Ganadero. Diputación de Córdoba. Palacio de la Merced s/n. 14071 Córdoba. España.

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Genetic resources. Microsatellites. Genetic structure. PCR.

PALABRAS CLAVE ADICIONALES

Recursos genéticos. Microsatélites. Estructura genética. PCR.

SUMMARY

Assignment tests and related methods combined with the use of microsatellite markers enable the determination of cross-breeding among populations. The best classification of the Iberian Pig seems to be in three main varieties, and several local varieties, with different impacts on the genetic structure of the population. In this study 26 microsatellites were analysed in eight varieties of Iberian Pig and screened for between-breed and within-breed specificity. In Spain a *terminal cross* between Iberian females and Duroc boars is permitted for the production of typical Iberian meat products. Although the use of these hybrids are not recommended for further breeding, it is necessary to evaluate the possible influence of the Duroc pig in the Iberian pig, because the influence of this exotic breed (and others) began even before the establishment of the breed book. Molecular and statistical tools seem to be the best choice for that evaluation to complement detection based on morphological traits.

RESUMEN

Pruebas de asignación y métodos similares combinados con el uso de marcadores microsatélites permiten la determinación de cruces entre razas y la homogeneidad intraraza. La mejor clasificación del cerdo Ibérico parece ser en tres grandes variedades acompañadas por un gran número de ecotipos locales con influencia diferente en la estructura genética de la raza. En este estudio se han analizado 26 microsatélites en ocho variedades de cerdo Ibérico para describir la posible especificidad intra e inter-raza. Está permitido cruzar madres Ibéricas con sementales Duroc para obtener una primera generación de individuos destinados a la producción de los clásicos derivados cárnicos Ibéricos. Aunque el empleo de estos híbridos no se recomienda para la reproducción, es necesario evaluar la posible influencia de la raza Duroc en el cerdo Ibérico, ya que el uso de esta raza y de otras empezó incluso antes de que estuviera establecido el libro genealógico. Las herramientas moleculares y estadísticas parecen ser la mejor elección para esta

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evaluación como complemento de la detección basada en caracteres morfológicos.

INTRODUCTION

Recently, pig production, based on intensive exploitation of specialised meat and fast growing breeds, is entering in a new phase. The motivation for change are found in the emergence of new concepts such as the ecological impact of production, animal welfare and sustainable systems. Now, there is great interest in genetic conservation and the use of autochthonous breeds. The Iberian Pig is one example of economic success of traditional breeding. It originated from pigs with long-established adaptation to their environment. Now breeders and administrators have a great interest to define (or understand?) the genetic structure of Iberian Pig, and avoid its genetic erosion through crosses with other breeds. Duroc is frequently used to obtain hybrids with good production performance (Daza, 2001). The possibility to differentiate between pure and hybrids is necessary to protect and conserve the breed and the credibility of its products. The Iberian Pig has great diversity, incorporating several strains and ecological adaptations such as the Retinto, Lampiño, Torbiscal and Manchado de Jabugo (Delgado *et al.*, 2001). It is very important to differentiate each variety from the others to develop good conservation and improvement programs.

The availability of DNA genetic markers offers the opportunity to use individual genotypes to determine the population of origin of individuals

(Davies *et al.*, 1999). Based on differences in allele frequencies between populations and on observed marker genotypes of an individual, it is possible to calculate the individual probability of belonging to distinct populations. However, several factors will affect the possibility to identify both pure-breeds and hybrids, including the genetic differentiation between populations, class, number and variability of genetic marker scored, the number of animals analysed per breed and finally, the choice of assignment methods (Cornuet *et al.*, 1999; Hansen *et al.*, 2001).

Assignment methods can be applied in evolutionary, ecological and population genetic studies. DNA methods provide the possibility of analysing historical samples and a number of new statistical tools have been developed for such analysis (particularly those using microsatellite markers). Individual-based statistics methods for assigning individuals to populations have attracted particular interest (Bjornstad and Roed, 2002; Rannala and Mountain, 1997; Signer *et al.*, 2000; Waser and Strobeck, 1998). There are some examples of the assignment procedures used in studies of animal populations (Bjornstad and Roed, 2002; Estoup *et al.*, 1998; Gotz and Thaller, 1998; Hansen *et al.*, 2001; MacHugh *et al.*, 1998).

The main objective of this study was to develop a preliminary evaluation of the intra-breed and inter-breed assignment precision of individuals from some varieties of Iberian Pig breed and Duroc using microsatellites as genetic markers.

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MATERIALS AND METHODS

Blood or hair samples were collected from 276 Iberian and 38 Duroc pigs. The varieties sampled were according to the population structure obtained from previous studies (Delgado *et al.*,

2001; Martínez *et al.*, 2000) where Retinto is the most common group. The distribution of Iberian samples was as follows: Lampiño (48), Retinto (156), Torbiscal (101), Manchado de Jabugo (43). Also three small populations were sampled: Mamellado (7), Silvela (13)

Table 1. Individual self-assignments to variety using directly the Bayesian method. Next two rows after 10000 simulated individuals with a threshold of 1 and 5 percent to reject unlikely genotypes. (Autoasignaciones individuales a la variedad usando directamente el método Bayesiano. Las siguientes dos filas en cada caso son el resultado de autoasignación después de 10000 simulaciones de individuos con un umbral de rechazo de genotipos menos parecidos del 1 y 5 p.100 respectivamente).

Population	Nº	Iberian							Duroc	None
		Lamp.	Retin.	Torb.	Manch.	Mamel.	Silv.	Negro Pedroches		
Lampiño	48	47	1	-	-	-	-	-	-	-
1 p.100		45	12	5	-	-	-	-	-	1
5 p.100		45	7	2	-	-	-	-	-	1
Retinto	156	-	155	1	-	-	-	-	-	-
1 p.100		19	151	2	-	2	8	-	-	5
5 p.100		4	147	1	-	-	6	-	-	9
Torbiscal	101	-	1	99	-	-	1	-	-	-
1 p.100		5	15	88	-	-	5	-	-	2
5 p.100		3	7	95	-	-	1	-	-	5
Manchado de Jabugo	43	-	-	-	43	-	-	-	-	-
1 p.100		1	7	1	42	-	1	-	-	1
5 p.100		-	5	1	41	-	1	-	-	2
Mamellado	7	-	-	-	-	7	-	-	-	-
1p.100		-	-	-	-	7	1	-	-	-
5 p.100		-	-	-	-	7	1	-	-	-
Silvela	13	-	-	-	-	-	13	-	-	-
1 p.100		-	-	-	-	1	13	-	-	-
5 p.100		-	-	-	-	1	13	-	-	-
Negro de los Pedroches	22	-	-	-	-	-	-	22	-	-
1 p.100		-	-	-	-	1	8	21	-	-
5 p.100		-	-	-	-	-	5	21	-	1
Duroc	38	-	-	-	-	-	-	-	38	-
1 p.100		-	-	-	-	-	-	-	37	1
5 p.100		-	-	-	-	-	-	-	37	1

and Negro de Los Pedroches (22).

DNA was obtained following the usual protocol (Kawasaki, 1990). The 25 pig microsatellites studied were selected from the 27 markers recommended by FAO (FAO, 1998). PCR amplification was performed on a GeneAmp 9700 (Applied Biosystems, Foster City, CA, USA) Electrophoresis and analysis was performed using an ABI 377 XL automatic sequencer (Applied Biosystems, Foster City, CA, USA) and Genescan v. 3.1.2 software from the same manufacturer. Sizing of PCR products was accomplished both by the internal size standard and by the same two samples in each gel to correct for small variations in allele size assignment among runs. The GENECLASS version 1.0.02 computer program (Cornuet *et al.*, 1999) was used to obtain different assignment values. A measure of confidence that the individual truly belongs to a given population can be achieved by comparing the value of the criterion of the individual with values of the criterion for all individuals that belong to the population. To compute the distribution of the criterion for each population, a simulation of multilocus genotypes was made by randomly taking alleles according to their frequencies in the population.

RESULTS

Using Bayesian methods, 99.01 percent of individuals from the Iberian pig varieties were assigned correctly to their population. The assignment values for each individual were compared with those of the other members of the same population,

rejecting the 1 or 5 percent most unlikely genotypes; individuals with assignment values on the threshold for some populations appear several times (**table I**). Varieties had great homogeneity although some individuals showed a moderate degree of influence from other varieties with the simulation method. Also there have appeared some cases with no assignment to any population. Breed differentiation between the Iberian and the Duroc pigs based in the assignment test was clear despite the low number of samples of endangered populations. The Lampiño, the Retinto, the Manchado and the Torbiscal obtained very good results when 1 or 5 percent unlikely multilocus genotypes were rejected.

DISCUSSION

Differentiation between the Lampiño, the Retinto, and the Torbiscal varieties is similar to that shown by morphological and reproductive studies (Barba, 1999; Delgado *et al.*, 1998) but some individuals are not correctly allocated even though 25 microsatellites were used. It indicates that some individuals originated from crosses among several varieties, as a result of commercial exploitation of this pig without a controlled breeding or improvement plan. Bayesian assignment demonstrates a certain degree of similarity when all samples are included in the same Iberian population, and they are clearly differentiated from Duroc breed. Minor varieties showed different results. The Manchado de Jabugo is bred in only two farms and it is near the extinction, no other variety

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has been introduced for several decades and a high level of inbreeding is expected. The assignment test reveals a great uniformity of this variety. However, the Negro de Los Pedroches is a new Iberian pig variety submitted to a conservation program. Individuals of this population were rescued in the last two years based upon some morphological criteria. The assignment

test reveals influences from the Silvela population. Other minor varieties analysed do not permit conclusions because of the low number of samples. They were assigned correctly to the Iberian pig reaching poor values when simulation procedures were used. These varieties are contributing with private multilocus genotypes to the richness of the breed.

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