

Comparison of genetic (neutral DNA markers) and phenotypic (fresh meat quality traits) distances between Iberian pig strains

O. Polvillo, M. Juárez, A. Molina, A. Membrillo & I. Clemente

MERAGEM Research Group. University of Cordoba (Spain). Edif. Gregor Mendel. Campus Rabanales. 14071. Cordoba. (Spain).

Email: oppolo@us.es

Abstract

In 2007, a new National Quality Standard has been published in Spain to regulate the products derived from the Iberian pig carcass, including for first time fresh meat, and four different strains were recognized as official lines (Lampião, Entrepelado, Retinto and Torbiscal). A study of the neutral DNA markers (including the 27 microsatellites recommended by FAO for genotyping of pig populations) and the main meat quality traits of tenderloin from pigs from each strain and from the most common crossbreeding (Iberian x Duroc) was developed. The lowest genetic distances were those between Lampião and Torbiscal, and between Entrepelado and Retinto strains respectively. However Retinto and Lampião strains showed the lowest phenotypic distances, followed by Entrepelado strain. Torbiscal meat traits were the most different within the Iberian strains. Crossbreed was highly different to the pure Iberian pigs, as well genetic as phenotypically. Since the recommended DNA markers seem not to be highly related to meat quality, new studies including specific markers should be developed.

Introduction

The Iberian (IB) pig breed is the most important Mediterranean swine type, both in population size and economic importance. Most of IB pig meat is consumed as cured products (ham, loin and shoulder), which are highly priced. However, the importance of the consumption of fresh meat has recently increased. Due to this increasing consumption of fresh meat, in November 2007 a National Quality Standard was published to regulate the production, processing and marketing of products derived from the IB pig carcass (Real Decreto 1469/2007). That Quality Standard included for first time fresh meat, instead of cured products only.

On the other hand, several authors have reported important differences in productive, reproductive and morphologic parameters between different strains (Benito *et al.*, 1998; Petron, 2002; Tejada *et al.*, 2002). The traditional classification and selection of the strains have been based on morphologic parameters. In fact, in some studies the strains are named “morphological types” (Fernández *et al.*, 2003). In January 2007 four IB strains (Lampião, LA; Entrepelado, EN; Retinto, RE; Torbiscal, TO) were recognized as official varieties by the Spanish Ministry of Agriculture, Fisheries and Food, under the Official List of Livestock Breeds of Spain (Orden APA/53/2007). However in general no genetic characterization has been done to classify the IB pig populations within each official strain.

Therefore the aim of this study was to analyze if the genetic differences among IB subpopulations are reflected in meat quality traits.

Material and Methods

Genetic distances

The study was carried out on 140 animals (35 assigned to each official strain) from the Spanish Association of IB Pig Breeders (AECERIBER). A batch of 35 IB (no assigned to any official strain) × Duroc crossbreed pigs (Ix_D) was included into the study to compare their genetic and productive traits to those from IB purebred pigs.

Blood samples from the animals were collected and genomic DNA was isolated and purified using Dominion mbl Kit[®] (Cordoba, Spain). 34 microsatellites molecular markers, including the 27 recommended by FAO for genotyping of pig populations (FAO, 2004), were

amplified by Polymerase chain reaction (PCR), and the products were subjected to electrophoresis in an automatic sequencer ABI 3130 (Applied Biosystems, Foster City, CA, USA). The results were analyzed with GeneScan v.3.7 software. Genetix 4.05 software (Belkhir *et al.*, 2001) was used to compute the allelic frequencies, and a tree of genetic distances (Reynolds' distance); with the UPGMA algorithm was generated using the program Phylip 3.65 (Felsenstein, 2005).

Phenotypic distances

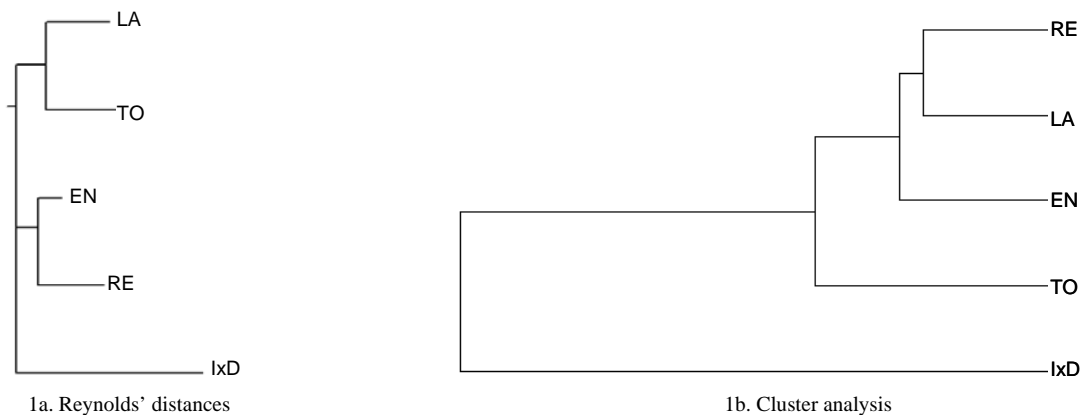
From the groups selected in the genetic study, 50 castrated male pigs were assigned to the meat quality study, 10 from each IB pig strain and 10 from IB × Duroc crossbreeding. All pigs were reared under regular semi-extensive management at the same experimental barn. The pigs were slaughtered when reached the commercial live weight (160-180 kg) according to the specifications outlined in the Spanish legislation (B.O.E., 147/1993). Tenderloins were collected 24 h post-mortem from the left side of the carcass and aged for 72 h at 4°C. Meat quality traits (moisture, ash, fat, protein, water holding capacity, shear force, colour indices and heme pigment contents) were determined according to AOAC (1990) methods. The total fatty acids were extracted, methylated and analysed by an adaptation of the method described by Aldai *et al.* (2006), which has been reported to be highly effective for PUFA analysis (Juárez *et al.*, 2008).

Data from meat quality analysis were analyzed with the Statistica 7.0 for Windows (StatSoft, 2006) statistical package. A tree diagram (Cluster analysis) was developed following Ward's method to show the distribution of the five groups of animals according to their meat quality traits.

Results and Discussions

Figure 1a shows the distribution of the IB strains according to their neutral DNA molecular markers. IB purebred strains dendrogram grouped the batches into two subgroups. One of them included the black hairless herds LA and the red strain TO. This similarity can be explained because the black hairless strains have contributed to the foundation of TO. (Rodrigáñez *et al.*, 1998) The chestnut coated populations (RE and EN) were grouped in the other main branch. This is explained because RE contributed to the formation of EN strain. IxD batch appears, as expected, clearly discriminated as another subgroup due to the contribution of another breed (Duroc). Therefore for all the IB subpopulations, the genetic distance to the Duroc breed is greater than that to any of the other subpopulations of the breed. A similar distribution of IB types has been reported by Martínez *et al.* (2000).

Figures 1a-1b. Dendrograms of genetic (Reynolds' distances) and phenotypic (Cluster analysis) distances among Iberian pig subpopulations.



The dendrogram from Cluster analysis of meat quality traits (Figure 1b) shows two main and well differentiated branches. One of them includes the group from crossbreeding (IxD) and the other one includes the four IB strains. Therefore a high difference between IxD and IB purebred meat quality is observed. Among the IB purebred branch, TO strain appears isolated.

And, finally, the figure shows a higher proximity between RE and LA than between those groups and EN strain. The distribution is different to that observed when genetic distances are studied, showing differences between TO meat quality and that from the other three strains. This difference is probably due to the selection for muscular conformation of the animals of the TO line. In fact TO variety was produced 50 years ago by crossing several IB herds (from Spain and Portugal) (Ovilo *et al.*, 2000) and it has been selected from this moment on for meat production.

Conclusions

Meat from pure IB pig strains has very different characteristics from that of the crossed pigs used in this experiment and commonly found in the meat market. Among the four official IB strains, the neutral DNA markers showed a distribution in accordance with the reported gene-flow between those strains. However meat quality characteristics showed an effect of TO genetic selection on those traits, and a higher proximity between RE and LA, even if they are genetically closer to other strains. Therefore neutral DNA markers are useful to discriminate closely related populations. Nevertheless their correlation with meat quality characteristics is limited to well differentiated populations.

References

- Aldai, N., Osoro, K., Barron, L. J. R. and Nájera, A. I. (2006). Gas-liquid chromatographic method for analysing complex mixtures of fatty acids including conjugated linoleic acids (cis9trans11 and trans10cis12 isomers) and long-chain (n-3 or n-6) polyunsaturated fatty acids - Application to the intramuscular fat of beef meat. *J. Chromatogr. A* 1110:133-139.
- AOAC. (1990). Official methods of analysis 15th ed. Assoc. Off. Anal. Chem., Arlington, VA. , 931-932.
- Benito, J., Vázquez, C., Menaya, C., Ferrera, J. L., Garcia-Casco, J.M., Silió, L., J. Rodríguez, J. and Rodríguez, M.C. 1998. Evaluación de los parámetros productivos en distintas líneas de cerdo Ibérico. In: Proceedings IV International Symposium of Mediterranean Pig. Evora, Portugal.
- Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. and Bonhomme, F. (2001). GENETIX 4.02, Logiciel sous Windows TM pour la Génétique des Populations. Laboratoire Génome, Populations, Interactions: CNRS UMR 5000. Université de Montpellier II, Montpellier (France)
- FAO. (2004). Secondary guidelines for development of national farm animal genetic resources management plans. Measurement of domestic animal diversity (MoDAD): recommended microsatellite markers. Rome, Italy
- Felsenstein, R. (2005). PHYLIP (Phylogeny Inference Package) Version 3.65. University of Washington, Seattle, Washington.
- Fernández, A., De Pedro, E., Núñez, N., Silió, L., Garcia-Casco, J. and Rodríguez, C. (2003). Genetic parameters for meta and fat quality and carcass composition traits in Iberian pigs. *Meat Sci.* 64:405-410
- Juárez, M., Polvillo, O., Contò, M., Ficco, A., Ballico, S. and Failla, S. (2008). Comparison of four extraction/methylation analytical methods to measure fatty acid composition by gas chromatography in meat. *J. Chromatogr. A.* 1190: 327-332.
- Martinez, A.M.; Delgado, J.V., Rodero, A. and Vega-Pla, J.L. (200) Genetic structure of the Iberian pig breed using microsatellites. *Animal Genetic* 31: 295-301.
- Ovilo, C., Barragán, M.C., Castellanos, C., Rodríguez, M.C., Silió, L. and Toro, M.A. (2000). Application of molecular markers (RAPD, AFLP and Microsatellites) to Iberian pig genotype characterization. *CIHEAM – Options Méditerranéennes*. Vol. 41: 79-84. 79-84.
- Petron, M.J. (2002). Estudio de la fracción lipídica intramuscular en diferentes tipos de jamón Ibérico. Ph. Thesis, Universidad de Extremadura.
- Rodríguez, J., Toro, M., Rodríguez, C. and Silió, L. (1998). Supervivencia de alelos de estirpes portuguesas y españolas en una población de cerdos ibéricos. In: IV Simposio Internacional do Porco Mediterrânico, Evora, Portugal.

- StatSoft, Inc. (2006). STATISTICA (data analysis software system), version 7.
www.statsoft.com.
- Tejeda, J.F., Garcia, C., Muriel, E. and Antequera, T. (2002). Muscle lipid composition of Iberian pig meat as related to genetic line. In: 48th International Congress of Meat Science and Technology. Vol. II, August 2002, Rome, Italy, pp. 734.