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Genetic Diversity and Variation of ESR, RBP4 and FUT1 Genes in Mexican Creole and Yorkshire Pig Populations

¹C. Lemus-Flores, ¹K. Mejía-Martínez, ¹J.G. Rodríguez-Carpena,
²A. Barreras-Serrano, ³J.G. Herrera-Haro and ⁴R. Alonso-Morales

¹Postgrade of Biological and Veterinarian Sciences, Autonomous University of Nayarit,
City of Culture Amado Nervo. Tepic, Nayarit, CP 63190, México

²Instituto de Ciencias Veterinarias. Autonomous University of Baja California,

³Programa de Ganadería, Colegio de Posgraduados,

⁴Laboratory of Molecular Genetics, Faculty of Veterinary Medicine,
National Autonomous University of Mexico D.F., México

Abstract: The ESR, RBP4 and FUT1 candidate genes were studied in 103 pigs from the commercial breed Yorkshire and Mexican Creole, Pelón and Cuino animals, for establishing genic and genotypic differences, unbiased diversity and genetic distances by employing standard distances and phylogenetic tree build up by the Neighbor-Joining technique. Polymorphism was determined by PCR-RFLP methods using established procedures from other investigations. For ESR gene there were no homozygote BB animals in Pelón and Cuino; the allele B and A were more frequent in Yorkshire (0.38) and Cuino (0.84). Genetic distance was greater in Yorkshire and Cuino as compared to Pelón, with greatest heterozygosity for Yorkshire (0.479) followed by Pelón (0.390) and Cuino (0.275). The BB genotype for RBP4 gene was not detected in any breed examined; there was no dependence of genic and genotypic frequencies in any breed. Allele A frequency was similar in Yorkshire, Pelón and Cuino pigs (0.74, 0.74 and 0.75), in similitude of frequency of AA and AB genotypes in all breeds. Genetic distances and heterozygosity were similar in the three breeds (Yorkshire, 0.390; Pelón, 0.390 and Cuino, 0.382). For FUT1 gene a major frequency of allele G and genotype GG was found in Yorkshire (0.60 and 0.45) and Pelón (0.64 and 0.39) animals. The allele A was more frequent in Cuino pigs (0.55). There was a greater genetic distance between Yorkshire and Cuino animals. Cuino pigs showing a greater heterozygosity (0.503) as compared to Pelón (0.465) and Yorkshire (0.448) which in turn were no different between them.

Key words: Mexican creole pigs, genetic biodiversity, candidate genes

INTRODUCTION

Pig meat is the major protein source for human beings; this assumption is directly derived from World production (FAO, 2000). In this context, it is worth to mention that only a small number of pig breeds have been selected in an intensive manner as breeds of high commercial exploitation, with efficient levels of meat production. This factor has been decisive in the decrease of local breeds, which have been replaced by others through programs of assisted selection (Ramos *et al.*, 2003). According to a FAO (2007) report, during the last seven years a breed of domestic livestock has made extinct every month (<http://www.fao.org>). Besides, among pig breeds of high yield which are more utilized (Large White, Duroc and Landrace), genetic

diversity has been menaced by the use of reduced number of boars for improvement purposes.

Disappearance of creole breeds would be a non replaceable lost of the unique genetic heritability. This is the reason why of the importance of preservation of these local genotypes is essential, since they warrant the genetic variability contained in these breeds for future generations. In Mexico, in recent time an important impulse has been done for the study of animal genetic resources, since it has been understood the relevance of these generic variants which are so abundant, but at the same time so poorly characterized and even more when creole or local populations are concerned (Ramos *et al.*, 2003; Sierra *et al.*, 2005; Canul *et al.*, 2005). The Mexican creole pig, which has been reported by FAO as an animal in danger of extinction (FAO, 2000), is a poorly evaluated

species, which is thought it was introduced in the country in times of Spanish conquer and which has been survived in different ecological conditions, including infection factors and nutritional restraints in a natural way (Lemus *et al.*, 2003; Hurtado, 2006).

It has been found that genetic variation is greater in creole pig populations from Mexico, Cuba and Spain than those from commercial animals. In fact, a phylogenetic separation exists between Creole pigs and those of commercial origin. This state of the art strongly suggests that a conservation status has taken place in local breeds of this species, in spite of a lack of systematic programs of genetic improvement (Martínez *et al.*, 2000; Lemus *et al.*, 2001; Canul *et al.*, 2005). Indeed no programs of selection from the performance traits point of view have been applied in these types of creole pigs. However, those animals have survived for more than 500 years, thus representing a genetic reservoir for obtaining national varieties better adapted to the environment (Benítez and Sánchez, 2001; Lemus *et al.*, 2001; Sierra *et al.*, 2005; Lemus and Alonso, 2005).

Up to date technological advances in molecular biology and genetic engineering have led to more accurate methods for selection of more productive animals. These advances permit the identification of genes and regions of genome related to quantitative traits associated to characteristics of productive and economical importance (Dekkers, 2004; Hernández *et al.*, 2006).

Some investigations have shown that the estrogen receptor (ESR) gene, as well as retinol-binding protein 4 (RBP4) and alpha 1,2 fucosyl transferase (FUT1) genes, have alleles which participate in important traits from the point of view of economy. However, these alleles show its influence in different manners among breeds (Hernández *et al.*, 2006). The ESR gene has been studied as a candidate gene for litter size in pigs (Chen *et al.*, 2000). For its study, fragments of the ESR has been amplified by the PCR (polymerase chain reaction) methodology and it has been encountered diallelic polymorphism, with alleles called A and B. Several investigations shown that allele B is favorable to the increase of the number of piglets born alive (Drogemuller *et al.*, 2001; Isler *et al.*, 2002; Noguera *et al.*, 2003; Rothschild *et al.*, 1996; Short *et al.*, 1997). The RBP4 gene has been evaluated as candidate gene for litter size in pigs too, since the gene is involved in the development of embryos, transporting vitamin A *in utero* during the critical stage of gestation. In this connection, it has been observed that allele A is favorable for augmenting the number of either total born piglets or total piglets born alive (Rothschild *et al.*, 2000).

The FUT1 gene has been examined as candidate gene in resistance against infections caused by *Escherichia coli* in piglets from 4 to 12 week old, since it is associated

to synthesis of receptors for the k88 antigen (Vogeli *et al.*, 1996). For determining polymorphism of the FUT1 genotype, the amplification was carried out through the PCR-RFLP procedure. The results have shown an association between FUT1 genotype and the phenotype, where animals which are homozygote A are resistant to diarrhea originated by *Escherichia coli* and in contrast, animals being heterozygote and homozygote G are diarrhea susceptible in some breeds (Ciobanu *et al.*, 2001; Meijerink *et al.*, 1997; Yan *et al.*, 2003). Other studies have shown that polymorphism in this gene is related to litter size and that, as compared to sows exhibiting a genotype GG, sows from the genotype AA produce greater litter sizes from the point of view of total piglets born, total piglets born alive and total weaned piglets (Horák *et al.*, 2005).

The objective of the current study was to establish genic and genotypic differences, genetic diversity and distances, among pigs from three genotypes, commercial Yorkshire and local, Mexican Pelón and Cuino by its characterization through the ESR, RBP4 and FUT1 genes, related to sow reproductive performance.

MATERIALS AND METHODS

The current study was conducted in the Laboratory of Molecular Genetics, of the Academic Unit of Veterinary Medicine and Zootechnics, Autonomous University of Nayarit in México and is part of investigations directed to the genetic and productive characterization of creole pigs, topic: Biodiversity and genetic resources; was conducted from Jan. 2002 to Dec. 2008.

Animals: A total of 103 pigs were employed and its origin was from the Academic Unit of Veterinary Medicine and Zootechnics of the University. Three groups of animals were examined, 29 pigs corresponding to the commercial, improved Yorkshire breed, whereas other 74 were of the creole Mexican breeds and from those, 46 were Pelón animals and 28 were Cuino pigs.

Collection of the biological samples: Blood samples were collected from the external cava vein, in tubes containing 0.5 mg mL⁻¹ of EDTA. Blood was kept at -20°C until analysis. Extraction and purification of DNA was conducted by the phenol technique (Sambrook and Russell, 2001).

Polymorphism of ESR, RBP4 and FUT1 genes was determined by using the PCR-RFLP technique, following a procedure previously conducted by other investigators (Drogemuller *et al.*, 2001; Meijerink *et al.*, 1997; Rothschild *et al.*, 2000; Short *et al.*, 1997). Primers utilized

for amplification of ESR gene were F5' CCT GTT TTT ACA GAG 3' and R5' CAC TTC GAG GGT CAG TCC AAT TAG 3', for F RBP4 gene 5' GAG CAA GAT GGA GGT ATG T 3' and R 5' CTC GGT GTC TGT AAA GGT G 3' and for F FUT1 gene 5' CTG CCT GAA CGT CTA AGA TCA TC 3' and R 5' CTT CAG CCA GGG CTC CTT TAA G 3'.

Amplification by PCR (final volume, 25 µL), was by utilizing 100 ng DNA, 1 U of Taq polymerase, 0.2 mM dNTPs, 0.4 µM of every primer, 1xbuffer, 1.5 mM MgCl₂ and 15.55 µL H₂O, per reaction. The PCR conditions were 95°C for 8 min, followed by 30 cycles of 95°C 45 sec, 58°C 1 min, 73°C 1 min; finally 73°C by 10 min for both ESR and FUT1 genes. In the case of RBP4 gene, temperature of alienation was 62°C. The amplified fragments of ESR, FUT1 and RBP4 were 120, 421 and 550 pb. Five microliter from the PCR product were digested with 2.5 U of PvuII, MspI and HhaI enzymes, respectively. Fragment products from RFLPs were visualized by a agarose gel, previously stained with 4% ethidium bromide (0.5 µg mL⁻¹) in an ultraviolet transluminator. The pBR322/MspI, of known molecular weight, was used as reference standard for measuring the size of fragments originated in the RFLP process.

The ESR gene had only one site of restriction generating two fragments of 65 and 55 pb for allele B whereas allele A had no sites of restriction, therefore remaining a complete fragment of 120 pb. The RBP4 gene has two alleles and in this case, allele A generated three fragments of 190, 154 and 136 pb and allele B generated three fragments too, of 154, 136 and 125 pb. Allele A of FUT1 only had one site of restriction, generating two fragments of 328 and 93 pb, whereas allele G had two sites of restriction, with three fragments of 241, 93 and 87 pb.

Statistical analysis: Analysis of frequency and Hardy-Weinberg Equilibrium. An analysis of genic and genotype frequencies and unbiased heterozygosity per gen in every pig breed, using a χ^2 test, taking into account the methodology proposed by Nei (1978), by using the Biosys program (Swofford and Selander, 1981).

Genetic distances and phylogenetic trees: Genetic distances were calculated to build up phylogenetic trees existing among the breed groups, by employing the methods of standard distance and the Neighbor-Joining procedure (Nei, 1972; Nei *et al.*, 1983; Saitou and Nei, 1987). These calculations were carried out by the use of the Phylip 3.5 program (Felsenstein, 1997).

RESULTS

Genic and genotype frequencies: Table 1 shows genic and genotype frequencies for the three examined genes in the populations of creole and commercial pigs.

Genic and genotype frequencies of ESR gene were significantly ($p = 0.03$) different among breeds. On the other hand, allele B frequency, in similitude to genotype BB was higher in Yorkshire pigs. In previous conducted studies concerning the allele B, it has been considered that this entity is favorable for increasing litter size in pigs, if it is taken into account that the Yorkshire is one of the more prolific breed. Two allele were encountered for RBP4 gene and on the other hand were present in all breed evaluated. However, there were not significant ($p > 0.05$) differences among breeds. No homozygote BB animals were found. Genic and genotype frequencies were significantly ($p = 0.02$) different in the three breed populations for FUT1 gene. It was found a greater frequency of allele G in Yorkshire and Pelón pigs, as well as those frequencies of genotype GG.

Diversity, genetic distance and phylogenetic trees:

Genetic diversity and distances were obtained from the information concerning the genic frequencies for every gene. From the point of view or the ESR gene, it was observed that Yorkshire pigs were distant from Cuino pigs, whereas Pelón animals were nearer to Cuino than to Yorkshire pigs (Table 2). These findings explain that Pelón and Cuino breeds were nearest among them, due to crossing which could occur in these two creole breeds, since in previous years there were no programs for improvement and controlled selection. Heterozygosity for the ESR gen was higher in Yorkshire pigs (0.479), followed by Pelón (0.390) and Cuino animals (0.275). Genetic distances for RBP4 gene resulted to be similar among the studied breeds. In fact, heterozygosity was similar in the three populations examined, with values of 0.390, 0.390

Table 1: Genic and genotype frequencies of ESR, RBP4 and FUT1 genes in commercial and creole pigs

Gene	Breed	Genic frequency		Genotype frequency		
		A	B	AA	AB	BB
ESR	Yorkshire	0.62	0.38	0.35	0.55	0.10
	Pelón	0.74	0.26	0.48	0.52	0.00
	Cuino	0.84	0.16	0.68	0.32	0.00
RBP4	Yorkshire	0.74	0.26	0.48	0.52	0.00
	Pelón	0.74	0.26	0.48	0.52	0.00
	Cuino	0.75	0.25	0.50	0.50	0.00
Gene	Breed	Genic frequency		Genotype frequency		
		A	G	AA	AG	GG
FUT1	Yorkshire	0.33	0.67	0.10	0.45	0.45
	Pelón	0.36	0.64	0.11	0.50	0.39
	Cuino	0.55	0.45	0.39	0.32	0.29

ESR: Estrogen receptor; RBP4: Retinol binding protein 4; FUT1: Alpha 1, 2 fucosyltransferase; Pelón and Cuino are local Mexican pigs

Table 2: Matrix of standard distances of ESR, RBP4 and FUT1 genes in creole and commercial pigs

Gene	Breeds	Pelón	Cuino
ESR	Yorkshire	0.0226	0.0669
	Pelón		0.0112
RBP4	Breeds	Pelón	Cuino
	Yorkshire	0.0000	0.0001
	Pelón		0.0001
FUT1	Breeds	Pelón	Cuino
	Yorkshire	0.0015	0.0943
	Pelón		0.0711

ESR: Estrogen receptor; RBP4: Retinol binding protein 4; FUT1: Alpha 1, 2 fucosyltransferase; Pelón and Cuino are local Mexican pigs

and 0.382 for Yorkshire, Pelón and Cuino pigs, respectively. On the other hand, it could be observed that in the case of the FUT1 gene, there existed a major genetic distance between Yorkshire and Cuino pigs (0.0943) and that Pelón pigs were nearer to Yorkshire (0.0015) than to Cuino.

DISCUSSION

Genic and genotype frequencies: Results concerning the findings related to genic and genotype frequencies for ESR gene are very important since the population of this improved, commercial type, shows a major frequency of this favoring allele. Although, a genotype selection was not done, this appears to indicate that the gene is being fixed by indirect selection (Rothschild *et al.*, 1996). A contrary case was reflected in the creole Pelón and Cuino breeds, which accounted for a minor frequency of allele B, where besides, the genotype BB was not encountered in any case. The absence of genotype BB is in accordance to other results where homozygote B was not found in Duroc and Large White breeds (Drogemuller *et al.*, 2001) but in Iberian pig the homozygote B frequency was high (Muñoz *et al.*, 2009). Moreover, it has been reported lower frequencies for allele B in different pig breeds (Short *et al.*, 1997; Drogemuller *et al.*, 2001; Noguera *et al.*, 2003). Rothschild *et al.* (1996) have explained that allele B from ESR gene is only present in selected groups of breeds in pigs, which could in turn be an explanation for the absence of homozygote BB in the Pelón and Cuino breeds.

From the point of view of the RBP4 gene, the herein encountered values are in agreement to the report of Hernández *et al.* (2006) in commercial sows; genic and genotype frequencies were similar in the three examined pig breeds. High frequencies in allele A and genotype AA were expected to occur in Yorkshire animals due to the fact that it was the more prolific evaluated breed and because besides allele A, they have been considered that do determine an augmentation of litter size (Messer *et al.*, 1996; Rothschild *et al.*, 2000; Linville *et al.*, 2001). In this

connection, it has been observed that commercial sows from the genotype AA had 0.5 more piglets born in total and 0.6 more alive born piglets (Hernández *et al.*, 2006). It has been reported different allele frequencies for every breed (Drogemuller *et al.*, 2001), which indicate that these frequencies may be different among breeds, although it was not the case in the current investigation, where allele A frequencies were similar in Yorkshire, Pelón and Cuino pigs.

When genic and genotype frequencies are considered for FUT1 gene, it should be mentioned the study of Horák *et al.* (2005), who reported that sows of genotype AA had a minor number of total born piglets, piglets born alive and weaned piglets that those sows of genotype GG. On the other hand, it has been reported that allele A determines resistance to diarrhea caused by *Escherichia coli* in piglets (Meijerink *et al.*, 1997; Ciobanu *et al.*, 2001; Yan *et al.*, 2003). However, these researchers did not link this gene for productivity, to resistance or susceptibility to infections caused by this type of bacteria and then it follows that it is important to underline in present results that frequency of allele A was greater in Cuino pigs, which are considered as an important source of gene conferring resistance to diseases (Mejía-Martínez *et al.*, 2008).

Diversity, genetic distance and phylogenetic trees: When considering several reports in connection to the ESR gene and its relationship with prolific traits in pigs (Rothschild *et al.*, 1996; Short *et al.*, 1997; Horogh *et al.*, 2005; Chen *et al.*, 2000; Goliasova and Wolf, 2004), it should be logical to consider that in the Yorkshire, commercial population, it would exist more frequency of allele B, therefore influencing positively the litter size trait and being distant from the creole pig populations. On the other hand, it has been claimed that local Mexican breeds of pigs are less prolific types of pigs, having average litter size of 6.36 piglets for Pelón and 5.97 piglets for Cuino animals (Lemus *et al.*, 2003), if comparison is made to Yorkshire animals, accounting for 9.7 piglets, according to national reports in Mexico (PigChamp, 2007). These results suggest that in creole populations, the ESR gene, since there is a lack of increase of frequency of the desirable allele B, does not incline to a greater litter size, which is the contrary to what it could be happening in commercial pigs.

It could be suggested that the RBP4 gene is not a candidate gene which could be adequate to establish differences between creole and commercial herds of pigs according to data derived from this study.

The FUT1 gene has been studied as a candidate gene for resistance to diarrhea caused by *Escherichia coli*

(Meijerink *et al.*, 1997; Yan *et al.*, 2003) and on the other hand, the gene has been related to reproductive characteristics in pigs (Horák *et al.*, 2005; Hernández *et al.*, 2006). In terms of prolificity, it was observed that the breed with highest litter size (Yorkshire) was distant from those breeds with a little litter size (Pelón and Cuino). In terms of resistance to diseases, it is important to underline the distance obtained between the more resistant breed, the Cuino, having the major frequency in allele A and the less resistant breed, the Yorkshire (Mejía-Martínez *et al.*, 2008). On the other hand, heterozygosity was here greater in Cuino pigs (0.503) than in Pelón and Yorkshire animals (0.465 and 0.448, respectively), which in turn exhibited a major frequency in allele G.

Overall, in average values for the three evaluated genes, heterozygosity was higher in Yorkshire (0.439±0.026), followed by Pelón (0.415±0.025) and in minor extension, Cuino (0.387±0.066), which is contrary to what it has been reported by using markers of the microsatellite type, where Pelón animals showed a major diversity (Lemus *et al.*, 2001; Canul *et al.*, 2005), in similitude to Iberian pigs (Martínez *et al.*, 2000). As it was previously suggested, selection for improving prolificity has not been carried out in Mexican creole pigs and in consequence, from the point of view of those gene which it has been related to reproductive traits, genic frequencies have not been modified toward the allele which is favorable for improving pig production, as it has been done in commercial breeds.

In conclusion, there were not homozygote BB animals detected for ESR gene in the creole breeds of Pelón and Cuino; the allele B and A were more frequent in Yorkshire and Cuino pigs respectively. The genetic distance was greater between Yorkshire and Cuino pigs, whereas the heterozygosity was higher in Yorkshire (0.479), followed by Pelón (0.390) and Cuino in third place (0.275).

In gene RBP4, the genotype BB was not detected in any breed and there was no dependence on genic and genotype frequencies for Yorkshire, Pelón and Cuino breeds. The frequency of allele A was similar to that of allele B and the same held true for frequency of genotype AA and AB in any breed. Genetic distance and heterozygosity were similar in the three evaluated breeds. In the case of FUT1 gene, the greatest frequency of allele G and genotype GG was found in Yorkshire and Pelón animals. The allele A was more frequent in Cuino pigs and a major genetic distance existed between Yorkshire and Cuino animals, the heterozygosity being greater in Cuino (0.503) than in Pelón and Yorkshire pigs, which were similar (0.465 and 0.448).

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