ORIGINAL ARTICLE

Genetic structure and population dynamics of autochthonous and modern porcine breeds. Analysis of the *IGF2* and *MC4R* genes that determine carcass characteristics

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ABSTRACT. To know the genetic situation of the Pampa Rocha, Celta, Bizaro Portuguese, Duroc, Iberian Extremeño and Iberian Andalusian porcine populations, their genetic structure and population dynamics were studied on the *IGF2* and *MC4R* genes, which determine meat characteristics and quality. The degree of genetic variability (He = 0.2511 in Pampa Rocha; 0.0278 in Celta; 0,1453 in Bizaro Portuguese; 0.3719 in Duroc; 0.0764 in Iberian Extremeño and 0.0384 in Iberian Andalusian), genetic distance, and the presence or absence of consanguinity were studied. The Fis values were positive for the Duroc population (0.00426) indicating a very low inbreeding, the rest of the populations did not present consanguinity. Significant deviations ($P \leq 0.05$) in the Hardy-Weinberg (HW) equilibrium were obtained for the *IGF2* locus in Celta, Iberian Extremeño, and Iberian Andalusian populations with the G allele fixed, while the Bizaro Portuguese, Pampa Rocha, and Duroc populations presented polymorphism, the G allelic frequency was much higher than A allele, except in the Duroc breed (0.15). These findings could help breeders to increase the presence of the A allele for the improvement of muscle mass and reduction in the back-fat thickness in this breed. All the studied populations presented polymorphism for the *MC4R* locus with different frequencies for each allele. Furthermore, these results could allow developing strategies against anthropogenic activities that hinder the conservation of the biodiversity of these porcine breeds.

Key words: pig populations, *IGF2, MC4R,* genes.

INTRODUCTION

The enormous scale at which porcine breeds are crossed to achieve greater production and better quality of meat has led to critical changes in the genetic structure of the various pig breeds farmed worldwide. Genetic variability has been drastically reduced and replaced by a genetic selection that seeks higher production. High rates of consanguinity, with the consequent loss of biodiversity, threaten these breeds. The knowledge of the genetics of swine breeds is important, especially for those genes that encode or regulate the traits desirable in the meat industry, as they are subject to high selection pressure (Andersson *et al* 1994, Azevedo *et al* 2015, De Campos *et al* 2015).

To identify the mechanisms that allow the maintenance of genetic variability, it is necessary to have suitable estimators of its magnitude, as well as to perform adequate characterisation of the patterns that are observed (Cockerham 1973). One method for the estimation of genetic variability is the use of DNA markers from each population (Avise 1994). These estimators of genetic populations allow us to determine whether genetic differences exist between two populations, identify if there is fragmentation in the population from a genetic perspective (population structure) and whether the identified variability can be explained by selection (Fisher 1930). However, in addition to selection, other effects can also affect the existing variability. For example, events that have occurred throughout history including drastic reductions in population size produced by a bottleneck such as an environmental disaster, the hunting of a species to the point of extinction, or habitat destruction that results in deaths (Fontdevila and Moya 1999). There is also the founder effect that occurs when a new population is established by a very small number of individuals from a larger population, the genetic drift that occurs when there is a change in the frequency of an existing gene variant (allele) in a population due to random sampling of organisms, and the mutations, among others. All of them can generate unexpected patterns in population parameters (Haldane 1932, Phillips *et al* 2019).

A population can quickly recover a high number of alleles if its structure is known and crosses are handled appropriately; however, it should be noted that if there are overlapping generations, as occurs in pig farms, the effect of the reduction of the population will be different. (Wellmann and Bennewitz 2019).

As indicated by González-Sarabia *et al* (2011), in some countries, such as Spain, Mexico, and Cuba, it has been observed that genetic variation in pig populations of autochthonous breeds is higher than that in commercial breeds. The autochthonous breeds are phylogenetically separated from the commercial breeds, which suggests that their genetic structures have been conserved owing to the

lack of systematized breeding programs (Lemus-Flores *et al* 2001). Although no productive selection programs have been applied to these breeds, they have survived for more than 500 years and have a genetic reservoir to produce better-adapted national varieties (Lemus-Flores *et al* 2001).

Candidate genes associated with meat quality, such as the *IGF2, MC4R* genes (Fassa *et al* 2015), are excellent markers to identify the presence or absence of genetic variability in porcine populations.

Insulin-like factor 2 (*IGF2*) plays a role in muscle growth and proliferation and differentiation of myeloblasts. Van Laere *et al* (2003) discovered a polymorphism in the *IGF2* locus, which was localised in the regulatory region of the gene. As indicated by Fassa *et al* (2015), the 3072 G>A substitution in the third intron of this gene alters the binding site of a nuclear repressor by tripling the expression of mRNA in skeletal muscle during postnatal growth, when the allele A is inherited from the male parent (genomic seal). This event leads to an increase in muscle mass and reduction in the back-fat thickness (BF), but does not affect the quality of the meat (Carrodeguas *et al* 2005, Estellé *et al* 2005, Den Maagdenberg *et al* 2008, Oczkowicz *et al* 2012, Fontanesi *et al* 2010 and 2012, Burgos *et al* 2012). The authors also established the association of this polymorphism with other characteristics of economic importance, such as body weight, growth rate, and conversion efficiency. One of the main objectives of selection in pigs during the past decades has been to obtain fast-growing and lean animals. It is assumed that the rapid evolution in this direction has accompanied the selection of the beneficial allele A of the *IGF2* gene in the different porcine breeds. Oczkowicz *et al* (2012) studied the genotype and genotypic frequencies of different breeds in Poland, reporting 100% and 91% for the AA genotype in the Duroc Jersey and Pietrain breeds, respectively, and a frequency of 27% in the local Pulawska breed.

The melanocortin 4 receptor gene (*MC4R*) codes for a G protein transmembrane receptor with an important role in the control of energy homeostasis and is a component of the central melanocortin pathway. In mammals, this receptor is expressed mainly in the central nervous system in regions that control food consumption, body weight, and energy homeostasis (Krashes *et al* 2016, Shen *et al* 2017). In pigs, Kim *et al* (2000) found a missense mutation, 1426 A> G, in the sequence encoding the seventh transmembrane region of the melanocortin receptor that results in the replacement Asp298Asn. The G allele was found to be associated with a lower BF, slower growth rate, and lower consumption (Burgos *et al* 2006, Bruun *et al* 2006, Meidtner *et al* 2006, Van den Maagdenberg *et al* 2008, Piorkowska *et al* 2010, Davoli *et al* 2012, Fontanesi *et al* 2013, Samoré and Fontanesi 2016).

Therefore, knowledge of the genetic structure of these breeds and their dynamics is crucial to plan appropriate strategies for the protection of native breeds and the conservation of their genetic diversity in the future.

The objective of this work was to study population dynamics and genetic structure in the Pampa Rocha, Celta, Bizaro Portuguese, Duroc, Iberian Extremeño and Iberian Andalusian porcine breeds, based on the *IGF2* and *MC4R* genes, which determine meat characteristics and quality. These analyses allowed us to determine the degree of genetic variability, the genetic distance between the breeds, and presence or absence of consanguinity to help develop strategies against anthropogenic activities that hinder the conservation of the biodiversity of these breeds.

To protect native breeds, it is important to consider the specific characteristics of each one of them, along with the genetic divergence between them, and acknowledge the effect of exploitation exercised by man to achieve and maintain the necessary balance between the desired characteristics and their genetic specificity in a breed.

MATERIAL AND METHODS

ANIMALS

We studied 233 animals of six different breeds of pigs (58 Pampa Rocha, 35 Celta, 45 Bizaro Portuguese, 40 Duroc, 24 Iberian Extremeño, Spain, and 25 Iberian Andalusian, Spain). Six wild boars were used as control animals and several hunters supplied samples of frozen muscles from wild boars. Frozen muscle samples from Iberian pigs were obtained from COVAP (Pozoblanco, Spain) and CENSYRA (Badajoz, Spain). Dr. Carballo García (University of Vigo, Spain) provided frozen muscle samples from Celta pigs and hair samples from Bizaro Portuguese pigs. Hair samples from the Pampa Rocha pigs were obtained from animals belonging to the reproductive group of the Pig Production Unit of the South Regional Center of the Experimental Station of the Faculty of Agronomy (University of the Republic) located in Progreso, Canelones (Uruguay). Semen samples of the Duroc pigs were obtained from Agropecuaria OBANOS S.A. Marcilla (Spain).

The Bioethical Committee Certification has been obtained and all researchers who have collaborated in this work have the Technical Competence in Animal Health, Husbandry and Handling.

DNA EXTRACTION

DNA was extracted from muscle, semen, or hair follicles by using the Realpure Genomic DNA Extraction Kit[1] and the GenomicPrep[TM] DNA isolation kit[2] following the manufacturer's instructions. The concentration of DNA in the extract was determined by using a NanoDrop[TM]

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[1]   Durviz (Valencia, Spain).

[2]   Amersham Biosciences (Little Chalfont, United Kingdom).

1000 spectrophotometer[3]. DNA was extracted from all the samples reaching a DNA concentration of about 4 x 10^2 ng/μL and diluted to a working concentration of 1 ng/μL.

GENOTYPING

Genotyping for *IGF2* and *MC4R* was performed by real-time PCR as described by Carrodeguas *et al* (2005), Burgos *et al* (2006 and 2012), Galve *et al* (2013) and Latorre *et al* (2016). The PCR reactions were carried out using an ABI-PRISM 7000 apparatus[4].

For the *IGF2* gene amplification, each assay contained 12.5 μL of TaqMan Universal PCR Master Mix, 0.6 μL of 40x Assay Mix, 5 μL of genomic DNA (1 ng/μL) and double distilled H_2O to 25 μL. PCR was carried out using an initial cycle of 10 min at 95 °C followed by 45 cycles of 15 s at 92 °C and 1 min at 61 °C. The primers used were IGF2F (AGCCAGGGACGAGCCT) and IGF2R (GAGGCCCGCGGACTC). The probes were IGF2V1 (CTAGGCTCGCAGCGC, labelled with the dye VIC) and IGF2M1 (CTAGGCTCACAGCGC, labelled with the dye FAM).

For the *MC4R* gene, the PCR assay sets contained 12.5 μL of TaqMan Universal PCR Master Mix, 0.6 μL of 40x Assay Mix, 5 μL of genomic DNA (1 ng/μL) and double distilled H_2O to 25 μL. PCR reaction was carried out using an initial cycle of 10 min at 95 °C followed by 50 cycles of 15 s at 92 °C and 1 min at 60 °C. The primers used were PIGBRAIN-MC4RF (TGCTTCATGTCTCACTTTAATTTGTATCTCA) and PIGBRAIN-MC4RR (GGCTCCGGAGTGCATAAATCA). The probes were PIGBRAIN-MC4RV1, attached to VIC fluorescent label, (CATCATCGATCCCC) and PIGBRAIN-MC4RM1, attached to FAM fluorescent label, (CATCATCAATCCCC).

STATISTICAL AND GENETIC ANALYSES

The analysis of gene and genotypic frequencies, as well as the heterozygosity for each gene in each breed, was performed according to Nei (1978) by using the BIOSYS-2 program (Swofford *et al* 1997). The number of alleles per locus (A), allele frequencies, observed heterozygosity (Ho) and expected heterozygosity (He) for genetic variation within local populations in the six porcine populations of this work have been estimated according to Hartl and Clark (1997) and Hedrick (2000).

We examined the total genetic diversity (HT), genetic diversity within populations (HS), genetic diversity among populations (DST), and genetic differentiation coefficient (GST) in genetic diversity within and among populations

(Weir and Cockerham 1984, Hedrick 2000, Culley *et al* 2002).

By using Wright's F-statistics (Wright 1978), the inbreeding coefficients between individuals within a population (Fis) and genetic differentiation between populations (Fst) were calculated.

The Fis coefficient is a measure of the deviation of the panmictic frequencies, in terms of excess or deficiency of heterozygotes, caused by the tendency for crosses to occur between related individuals; it is defined as the probability that two alleles in an individual are identical by descent with respect to the subpopulation. The value of this index varies between -1 and 1. The negative values indicate an excess of heterozygotes, with respect to the Hardy-Weinberg equilibrium while positive values indicate a deficiency of heterozygotes (inbreeding). To evaluate whether the Fis values were significantly different from zero, a test χ^2 = F2N (k-1) was performed with df = [k (k-1)] / 2, where F is the inbreeding coefficient, N is the sample size and k is the number of alleles (Li and Horvitz 1953).

Wright's Fst (Wright 1978) measures the effect of genetic subdivision or the reduction of heterozygosity in a population due to genetic drift; it is defined as the correlation of alleles of different individuals in the same population and varies between zero and one. When the estimate of Fst is 0.050 or less, it does not imply that genetic differentiation is negligible (Wright 1978). The zero value indicates panmixia or random matings, all subpopulations are in equilibrium and no genetic divergence occurs in the population. The value of one indicates complete isolation, an extreme subdivision of the population. To find out if the Fst values were significantly different from zero, we calculated χ^2 = 2NFst (k-1) with df = [(k-1) (s-1)], where N is the sample size, k is the number of alleles, and s is the number of subpopulations, in this case, the number of breeds (Workman and Niswander 1970).

Gene flow and genetic distances were also calculated. Gene flow is the transfer of genetic material from one population to another (Nm) (Slatkin 1987) and genetic distances (Nei 1978) reflect the number of changes that have occurred since the separation of two populations and is appropriate when populations diverge because of drift and mutations. This measure was based on the "normalised identity", which expresses the probability that when choosing one allele at random from each of the two populations considered, these are identical (Weir 1996, Ryman and Leimar 2008). The Nei distances used were corrected for small sample sizes (Nei 1978).

RESULTS

The allelic and genotypic frequencies are indicated in table 1. For *IGF2*, only Celta, Iberian Extremeño, and Iberian Andalusian populations showed significant deviations (*P*≤0.05) in the Hardy-Weinberg (HW) equilibrium, which could indicate a trend of directed matings or selection

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[3]   Thermo Fischer Scientific, (Wilmington, DE, USA).

[4]   Thermo Fischer Scientific, (Wilmington, DE, USA).

in these populations. The other populations were in HW equilibrium ($P \geq 0.05$). For *MC4R*, all populations were in HW equilibrium ($P \geq 0.05$).

In total, two alleles per locus in the two analysed loci were identified in all populations. The number of polymorphic loci was estimated, considering that a locus was polymorphic only if the frequency of the most common allele was less than an arbitrary value, which was typically 0.99. Similarly, only the Pampa Rocha, Bizaro Portuguese, and Duroc populations displayed polymorphism in the *IGF2* locus; this locus was considered non-polymorphic in the rest populations analysed. In contrast, all the analysed populations displayed polymorphism in the *MC4R* locus, except for wild boar samples that allele G was fixed.

The heterozygosity is indicated in table 2, in the *IGF2* and *MC4R* locus. The Celta, Iberian Extremeño and Iberian Andalusian populations had the lowest average genetic diversity within the populations; that is, the lowest He and Ho heterozygosities.

The genetic diversity (D) between populations is presented in table 4, being the greatest estimated distance between the populations of Celta and Duroc.

It was also calculated the coefficient of genetic differentiation for all loci, obtaining a value of GST = 0.5027, which indicated a genetic diversity of 50.27% among the populations and a genetic diversity of 49.73% within them.

WRIGHT'S F STATISTICS

The results obtained are shown in table 3. A positive Fis was obtained only in the Duroc breed, although a very low value; the rest of breeds did not indicate consanguinity,

Table 1. Allelic and genotype frequencies for the *IGF2* and *MC4R* genes analysed in the six pig breeds.

Breed	Allelic frequency		Genotype frequency		
	A	G	AA	AG	GG
IGF2					
Pampa Rocha	0.01	0.99	0.00	0.02	0.98
Celta	0.00	1.00[a]	0.00	0.00	1.00[a]
Bizaro Portuguese	0.02	0.98	0.00	0.04	0.96
Duroc	0.85	0.15	0.72	0.25	0.03
Iberian Extremeño	0.00	1.00[a]	0.00	0.00	1.00[a]
Iberian Andalusian	0.00	1.00[a]	0.00	0.00	1.00[a]
Wild boar	0.00	1.00[a]	0.00	0.00	1.00[a]
MC4R					
Pampa Rocha	0.59	0.41	0.31	0.55	0.14
Celta	0.03	0.97	0.00	0.06	0.94
Bizaro Portuguese	0.14	0.86	0.00	0.29	0.71
Duroc	0.57	0.43	0.33	0.50	0.17
Iberian Extremeño	0.08	0.92	0.00	0.17	0.83
Iberian Andalusian	0.04	0.96	0.00	0.08	0.92
Wild boar	0.00	1.00[a]	0.00	0.00	1.00[a]

[a]For the *IGF2* gene only the Celta, Iberian Extremeño, and Iberian Andalusian population groups showed significant deviations ($P \leq 0.05$) for the Hardy-Weinberg equilibrium analysis (HW). The other populations are in equilibrium of HW ($P \geq 0.05$).
For the *MC4R* gene all populations are in HW equilibrium ($P \geq 0.05$).

Table 2. Average expected and observed heterozigosity, on the loci analysed in the six populations studied.

	He	Ho	P (0.95)[a]	P (0.99)[a]
Pampa Rocha	0.2511	0.2845	0.5000	0.5000
Celta	0.0278	0.0286	0.0000	0.5000
Bizaro Portuguese	0.1453	0.1667	0.5000	1.0000
Duroc	0.3719	0.3750	1.0000	1.0000
Iberian Extremeño	0.0764	0.0833	0.5000	0.5000
Iberian Andalusian	0.0384	0.0400	0.0000	0.5000

[a]Threshold 95%, and 99% indicates that the locus is considered polymorphic if the most frequent allele does not exceed 95% or 99%, respectively.

with negative Fis values, Fis average values for each locus was negative for both loci.

The estimated values for Fst, according to Weir and Cockerham (1984), are indicated in table 5. The values obtained suggested that most of the genetic diversity was found within the populations.

A value of Nm > 1 indicates high gene flow between the populations (Wright 1969). The values of the genetic flow among the populations are shown in table 6. The genetic distances of Nei (1978) are presented in table 7.

Table 3. Fis values for each of the six porcine populations analysed.

Population	Fis	(IC 95 %)
Pampa Rocha	–0.12433	(–0.39877 - 0.10882)
Celta	–0.01493	(–0.06250 - –0.00000)
Bizaro Portuguese	–0.13597	(–0.22401 - –0.06344)
Duroc	0.00426	(–0.24877 - 0.25072)
Iberian Extremeño	–0.06977	(–0.17949 - 0.00000)
Iberian Andalusian	–0.02128	(–0.09091 - 0.00000)

Table 4. Genetic diversity (D) between each pair of populations analysed.

Population	Pampa Rocha	Celta	Bizaro Portuguese	Duroc	Iberian Extremeño	Iberian Andalusian
Pampa Rocha		0.64812	0.38121	0.76883	0.47787	0.57467
Celta			0.06029	1.21508	0.01429	0.01494
Bizaro Portuguese				0.99213	0.00315	0.03811
Duroc					1.03007	1.10591
Iberian Extremeño						0.00318
Iberian Andalusian						

Table 5. Fst values according to Weir and Cockerham (1984) for each pair of populations studied.

Population	Pampa Rocha	Celta	Bizaro Portuguese	Duroc	Iberian Extremeño	Iberian Andalusian
Pampa Rocha		0.47697	0.31696	0.53645	0.37990	0.43711
Celta			0.05851	0.70331	0.01419	0
Bizaro Portuguese				0.62921	0.00314	0.03740
Duroc					0.64302	0.66909
Iberian Extremeño						0

Table 6. Gene flow Nm (Wright, 1969) between each pair of populations analysed.

Population	Pampa Rocha	Celta	Bizaro Portuguese	Duroc	Iberian Extremeño	Iberian Andalusian
Pampa Rocha		0.27	0.54	0.22	0.41	0.32
Celta			4.02	0.11	17.37	a
Bizaro Portuguese				0.15	79.36	6.44
Duroc					0.14	0.12
Iberian Extremeño						a

[a]The gene flow between these populations has not been estimated, to reach values Fst ≤ 0, between these indicated populations.

Table 7. Genetic distances of Nei (1978) among the six porcine populations studied.

Population	Pampa Rocha	Celta	Bizaro Portuguese	Duroc	Iberian Extremeño	Iberian Andalusian
Pampa Rocha (58)	0.000	0.189	0.125	0.816	0.156	0.182
Celta (35)	0.189	0.000	0.004	1.123	0.000	0.001
Bizaro Portuguese (45)	0.125	0.004	0.000	0.989	0.000	0.003
Duroc (30)	0.816	1.123	0.989	0.000	1.080	1.114
Iberian Extremeño (24)	0.156	0.000	0.000	1.080	0.000	0.000
Iberian Andalusian (25)	0.182	0.001	0.003	1.114	0.000	0.000

DISCUSSION

During the process of selection, genetic variability can decrease compared with that of the original population. This decrease in genetic variability is a consequence of the selection process that involves the criteria and methods of selection with any degree of sophistication or use of current advanced techniques (Meffe 1986, Leberg 1992, Wellmann and Bennewitz 2019).

From the results obtained in this work, it was deduced that each analysed locus presented a different polymorphism in each porcine breed studied. In the *IGF2* locus (table 1), only the Bizaro Portuguese and Duroc populations presented interesting polymorphisms with a frequency of the G allele (0.98) much higher than the A allele (0.02) in the Bizaro Portuguese, unlike that the A allele (0.85) occurred at a higher frequency than the G allele (0.15) in Duroc; the A allele was observed in the Pampa Rocha population too but a very low frequency (0.01), similar to the frequency obtained by Burgos *et al* (2019) in this breed. In this locus, our results are very similar to those obtained by Fernandez *et al* (2017) for the Bizaro Portuguese breed (G allele with 0.99 and A allele with 0.01) and Burgos *et al* 2019 (G allele with 0.981 and A allele with 0.019). The rest of the populations do not have a frequency of A allele, as the G allele was fixed for the animals studied.

Fassa *et al* (2015) confirmed the greater effect of the A allele on BF and feed conversion, reporting that those pigs with the A allele had 1.8 mm less fat and better-feed conversion (they consumed 0.3 kg less of feed per kg of pork produced).

Oczkowicz *et al* (2009) reported that the frequency of the A allele was higher in the breeds or lines subjected to greater selection pressure for leanness and that the A allele increased the average daily weight gain and decreased consumption in Landrace populations. Fontanesi *et al* (2010, 2012) analysed a population of Large White Italian animals and, using as a registry of breeding values for BF, found that the animals with the highest expected breeding value for this character had a lower frequency of the G allele (0.38), whereas the animals with the lowest expected breeding value for BF had a higher frequency of the G allele (0.72).

In the *MC4R* locus, our results indicate that the populations of the Pampa Rocha and Duroc breeds have higher frequencies of the A allele (0.59 and 0.57, respectively), although they are values close to the G allele frequencies (0.41 and 0.43); however, their genetic structure is optimised for the objectives of higher performance, lower BF, and greater lean weight. Ovilo *et al* (2006), associated the identified missense mutation (Asp298Asn) located in a highly conserved region of this gene with backfat depth, feed intake and growth rate in different porcine lines. Our results differ slightly from those obtained by Klimenko *et al* (2014), who found a higher frequency of G allele (0.71)

compared with the A allele (0.29) in other pig breeds. It should be noted that Park *et al* (2002) and Fassa *et al* (2015) did not find significant differences between *MC4R* genotypes and production characteristics, except for the wild boar samples.

Population genetics is the discipline studying the genetic diversity and structuring of populations and the processes governing them, including changes in allele frequencies over time, and further embracing the aspects of quantitative genetics (Hartl and Clark 1997, Hedrick 2000). Our main objective was to determine the genetic variability of these pig populations because the use of genetic data in the establishment of conservation units is of great importance. According to Erwin (1991), it is necessary to recover and maintain processes within the species itself, instead of conserving only distinctive phenotypes, therefore, the utility of the genetic data is magnified. This argument is based on the fact that the functioning of microevolutionary processes requires intraspecific genetic variability that, although not an innovative perspective in conservation, was already identified in the 1970s (Frankel 1974) and has been strengthened since then by evolutionary biologists (Templeton 1986).

A good measure of genetic variation is the average frequency of heterozygous individuals per locus or, similarly, the heterozygosity of the population (Fontdevila and Moya 1999). In our work, the observed heterozygosities (Ho) were higher than those expected (He) (table 2), which was an indication that the heterozygosity of all the studied breeds was good and did not show consanguinity. Indeed, Fis values were mostly negative (Fis negative values represent the absence of inbreeding or consanguinity) (table 3) except for the Duroc population which, although a positive value, was a very low value. According to Hartl (2000) positive magnitudes of the Fis, Fis is considered low if Fis = 0 - 0.05, average Fis = 0.06 - 0.15, high Fis = 0.16 - 0.25 and very high Fis > 0.25.

The values obtained in our work for genetic diversity (D) among the populations range from the lowest estimated value, corresponding to the Bizaro Portuguese and Iberian Extremeño populations (table 4), to the highest value obtained, between the Duroc and Celta. As reported by Ryman and Leimar (2008), the effect of the mutation is small on the GST value in the early stages of divergence of populations, but it is unclear how long it has been since the separation of ancestral populations and whether the mutation may have affected heterozygosity. It is possible to think that the effect of the mutation is very small on that genetic diversity and that selection has been the most important effect on diversity. It is interesting to see how the genetic diversity obtained in our populations reaches a value of GST = 0.5027, which indicated a genetic diversity of 50.27% among the populations and a genetic diversity of 49.73% within them. These are expected results since that genetic diversity will be greater between different populations and breeds than within each population;

however, these results indicate that at one time there were genetic exchanges between all of them.

Similarly, for the estimated Fst (table 5), which measure the genetic differentiation between populations, the lowest value obtained is again between the Bizaro Portuguese and Iberian Extremeño (0.00314) and the highest value between the Celta and Duroc populations (0.70331). Regarding gene flow (Nm) or exchange of genes (table 6) that has occurred in the history in these populations, the highest value (79.36) was obtained between the Bizaro Portuguese and Iberian Extremeño, therefore, when having a greater gene flow, a lower genetic distance has been obtained (table 7) between both populations (0.00). In contrast, the lowest gene flow (Nm) corresponded to the Duroc and Celtic populations (0.11), along with the greatest genetic distance between these same populations (1,123).

Genomic selection is becoming a reality in livestock species. As indicated by Samoré and Fontanesi (2016), the applications of genomic selection are opening new opportunities in pig breeding. It is expected that the complete sequencing data will provide the best prediction accuracy since all causal mutations underlying a trait can be included (Zhang *et al* 2018). But sequencing is still too expensive for implementation in a large number of animals. Therefore, low-coverage DNA sequence fragment analysis strategies reduce costs and are well accepted.

In conclusion, it is very interesting to note that in our results the Pampa Rocha and Bizaro Portuguese autochthonous breeds present a certain frequency of the beneficial A allele at the IGF2 locus, although with a much lower frequency than that of the Duroc breed.

Moreover, in the Celta, Iberian Extremeño and Iberian Andalusian populations the imbalance observed for the alleles of the *IGF2* locus can be considered, in its broadest sense, to be due to selection, that is, that there had directed crosses at some point in their history. Finally, it is very pleasing to verify that the heterozygosity in the *MC4R* locus is high in all the analysed populations, which is indicating a good genetic "health" in all of them including the autochthonous breeds, knowing that they have started from a smaller number in their population components. These results could help breeders to manage their livestock and, at the same time, they highlight the need to continue controlling their populations through genetic analyses to avoid the loss of genetic variability and the presence of inbreeding at all times.

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