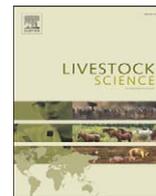




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Genetic diversity of Brazilian pig breeds evidenced by microsatellite markers

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ABSTRACT

The genetic diversity within and between five genetic groups of pigs in Brazil, ($n = 182$) represented by three naturalized breeds (Moura, Piau and Monteiro), one commercial breed (Landrace) and one commercial composite (MS60) was estimated using 28 microsatellite markers. The genetic structure within these groups was also tested. The results showed that 14% of the total variation ($p < 0.001$) observed was due to differences between breeds. Based on the UPGMA dendrogram obtained from Nei's D_A genetic distance it was possible to differentiate three groups. The first was formed by the commercial breed, Landrace, and the composite MS60, the second by two of the naturalized breeds (Piau and Monteiro) and the third by the Moura naturalized breed. Within breed variability indicated that the Piau breed had the highest value of expected heterozygosity among the naturalized breeds, whereas Landrace had the highest value between the commercial breeds. Using a Bayesian analysis, a population substructure was identified in Monteiro and Piau breeds. Furthermore, lower values for breed certification probability were observed for these two breeds as well as a significant genetic differentiation between the Moura and Landrace breeds and the composite MS60. The microsatellite marker panel possessed high precision (99.99%) when used in paternity exclusion in naturalized pig breeds and proved to be an effective tool for the management and conservation of naturalized pig breeds.

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1. Introduction

The Brazilian pig industry is based mainly on high technology and intensive production systems, using breeds with high genetic standards, specialized for meat production (Mariante et al., 2003). The pig breeds described as naturalized are the ones that guaranteed the sustainability of many Brazilian families for centuries. Since the beginning of the 20th century these populations have been gradually substituted by commercial breeds marked by a massive importation and use of exotic breeds, mainly from Europe (Cavalcanti, 2000). Nowadays, remaining animals of the naturalized pig breeds

are being raised only by smallholders and the demand of consumers has been focused on lean meat.

The naturalized pig breeds of Brazil are characterized by their toughness, resistance to diseases, low management requirements and feeding as well as a high adaptability. Between these naturalized pig breeds, the Piau is the most notorious. This breed is used in some programs of genetic improvement since 1939 (Vianna, 1956), aiming dual fitness like meat and fat, and it has some morphological characteristics similar to commercial pig breeds. The Monteiro is another naturalized pig breed found especially in Pantanal Matogrossense with morphological, physiological and behavioral adjustments very different from others naturalized and commercial pig breeds (Herrera et al., 1996). The Moura breed is the one that was spread out in southern region from Brazil

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Table 1
Sampling information and within population genetic diversity of five Brazilian naturalized and commercial pig breeds

Genetic group	Region	State*	N	Ho	He	PA	Ne	F _{IS}	HWE
Landrace	South	SC	24	0.6651	0.6316	8	2.70	-0.054	0.2493
	Center West	DF	7						
Monteiro	Center West	DF	19	0.5186	0.5735	7	2.34	0.097**	0.0001
		MT	16						
Moura	South	SC	37	0.6004	0.5698	3	2.32	-0.055	0.7186
MS60	South	SC	48	0.6145	0.6073	6	2.56	-0.012	0.0156
Piau	Center West	DF	15	0.5791	0.6612	13	2.94	0.126**	0.0001
	Southeast	MG	15						
	Northeast	PE	1						

*SC: Santa Catarina State; DF: Federal District; MT: Mato Grosso State; MG: Minas Gerais State; PE: Pernambuco State; ** $p < 0.01$.

N, number of animals analyzed; Ho and He, mean of the observed and unbiased expected heterozygosity; PA and Ne, private alleles and effective alleles; F_{IS}, inbreeding coefficient; HWE, p values for deviations to Hardy–Weinberg Equilibrium.

(Silva, 1987) and nowadays is being studied and conserved by Embrapa Suínos e Aves (Concórdia-SC).

Currently, there is no standardization in the phenotypic characterization of pig breeds as well as their denomination leading to a possible loss of genetic diversity. On the other hand these breeds can be also considered as a potential source of new allelic combinations of extreme importance for the future of the Brazilian pig industry. In Brazil just few works have been done with molecular markers emphasizing naturalized pig breeds (Tagliaro et al., 1995; Gonela, 2003; Guimaraes, 2003; Grossi et al., 2006).

Until now, among all the types of molecular markers, the microsatellite loci are the most widely used when studying the genetic diversity and population structure of livestock (Jordana et al., 2003; Yang et al., 2003; Parker et al., 2004; Martínez et al., 2006; Peter et al., 2007; Li et al., 2004; Kim et al., 2005; Behl et al., 2006). Although the Brazilian naturalized pig breeds have been little studied it is known that they present adaptation traits that could be useful in breeding programs aiming at future consumer demands. For this reason, the objective of this work was to evaluate the actual genetic diversity and the population structure of five natur-

Table 2
Details of 28 microsatellite loci typed on five Brazilian naturalized and commercial pig breeds

Locus	Cr	Na	Ho	He	PIC	EP1	EP2	F _{IT}	F _{ST}	F _{IS}
S0313	1	7	0.643	0.778	0.744	0.395	0.573	0.214	0.203	0.016
SW240	2	10	0.741	0.756	0.725	0.375	0.558	0.028	0.065	-0.040
SW72	3	5	0.636	0.638	0.569	0.221	0.371	0.025	0.088	0.070
SW445*	4	13	0.684	0.875	0.859	0.591	0.744	0.239	0.095	0.160
S0227*	4	10	0.419	0.569	0.546	0.194	0.377	0.305	0.229	0.112
S0226	4	10	0.623	0.720	0.684	0.327	0.509	0.192	0.223	-0.035
SW2425	5	7	0.559	0.756	0.713	0.351	0.527	0.295	0.192	0.121
SW995	5	9	0.626	0.779	0.745	0.397	0.575	0.235	0.173	0.067
SW2406*	6	10	0.395	0.647	0.611	0.250	0.431	0.440	0.279	0.223
S0025	7	9	0.634	0.784	0.757	0.415	0.597	0.225	0.173	0.059
SW632	7	12	0.697	0.791	0.771	0.445	0.625	0.143	0.136	0.009
OPN	8	10	0.778	0.796	0.769	0.435	0.612	0.032	0.059	-0.029
SW2410	8	7	0.713	0.56	0.515	0.170	0.331	-0.256	0.051	-0.324
S0225*	8	6	0.182	0.346	0.331	0.064	0.198	0.487	0.217	0.364
SW539	9	2	0.150	0.149	0.137	0.011	0.069	0.000	0.049	-0.053
SW951*	10	10	0.484	0.660	0.624	0.262	0.443	0.289	0.114	0.193
SW830	10	9	0.588	0.721	0.692	0.335	0.522	0.208	0.133	0.087
S0230	11	9	0.949	0.835	0.811	0.493	0.664	-0.105	0.135	-0.274
SW2008	11	9	0.571	0.757	0.723	0.370	0.551	0.286	0.188	0.120
SW957	12	13	0.646	0.686	0.661	0.305	0.494	0.076	0.085	-0.009
SW1962	12	6	0.744	0.755	0.712	0.346	0.524	0.060	0.163	-0.119
SWR1008	13	17	0.772	0.869	0.857	0.600	0.751	0.131	0.151	-0.023
SW769	13	6	0.299	0.311	0.295	0.051	0.170	0.041	0.070	-0.028
S0007	14	13	0.753	0.887	0.874	0.624	0.769	0.184	0.194	-0.013
SW857	15	8	0.75	0.819	0.793	0.464	0.639	0.112	0.145	-0.038
S0088	15	7	0.327	0.383	0.370	0.081	0.230	0.165	0.133	0.042
S0026	16	7	0.655	0.751	0.706	0.341	0.517	0.143	0.083	0.067
SW24*	17	10	0.645	0.821	0.798	0.478	0.652	0.237	0.149	0.104
Mean		8,96	0.5841	0.685	0.655	0.9999**	0.9999**	0.157	0.141	0.019

* $p < 0.001$ for deviations to Hardy–Weinberg Equilibrium.

** Combined power of exclusion.

Cr=*Sus scrofa* chromosome; Na=number of alleles; Ho and He=observed and expected heterozygosity PIC=Polymorphic Information Content; EP1 and EP2=Exclusion Probabilities for parentage analysis; F_{IT}=inbreeding coefficient related to the whole population; F_{ST}=genetic differentiation index; F_{IS}=inbreeding coefficient within populations.

alized and commercial pig breeds sampled in Brazil utilizing 28 microsatellite loci to infer about some primary tasks important in the area of conservations of pig genetic resources.

2. Material and methods

2.1. Sample collection and DNA extraction

Three naturalized pig breeds (Piau, Monteiro and Moura), one commercial breed (Landrace) and one composite (MS60) were studied. The composite MS60 was developed by Embrapa Swine and Poultry, located in Santa Catarina (SC), and is the result of the crossbreeding among the Duroc, Large White and Pietrain breeds. The samples, for both sex, were collected in five different states of Brazil: Federal District (DF), Santa Catarina (SC), Minas Gerais (MG), Pernambuco (PE) and Mato Grosso (MT), as can be seen in [Table 1](#). The animals were chosen for this analysis in order to assure that they were a representative sample of each breed, therefore, the chosen ones were non-related, or at least separated by three generations. Genomic DNA was extracted from blood or hair using the protocols of [Miller et al. \(1988\)](#) and [Boyce et al. \(1989\)](#), with minimum adaptations. All the samples were stored in the DNA Bank of the Laboratory of Animal Genetics located at Embrapa Genetic Resources and Biotechnology, Brasília (DF), where this experiment was developed.

2.2. Microsatellite data analyses

Thirty seven microsatellite loci were initially selected but only twenty-eight ([Table 2](#)) presented reliable amplification standards. Among these, nine (9) belong to the list of microsatellite markers recommended by [FAO/ISAG \(2004\)](#). The adopted strategy for the selection of the loci was to represent most of the autosomic pig chromosome.

The Polymerase Chain Reactions (PCR) was carried out in 20 μ L reactions consisting of 3 μ L of DNA (3ng/ μ L), 1.0 μ L of each forward and reverse starter (4 μ M); 1.6 μ L of dNTPs (2.5mM); 2.0 μ L buffer 10 \times (Tris HCl 1M, pH 8.4 100mM, KCl 500mM); 1.5 or 2.0 μ L of MgCl₂ (50mM); 0.2 μ L (1 unit) of Taq DNA Polymerase (5U/ μ L) and MilliQ sterilized water to complete the final volume. The concentrations of MgCl₂ varied depending on the optimization for each locus and negative and positive controls were used to identify contaminations. PCR was carried out using a Step Down amplification strategy for the majority of the loci. The amplified PCR products were initially visualized in 2% agarose gels stained with ethidium bromide to identify possible imperfections and to decrease the rate of failure in capillary electrophoresis. Multiplex systems were then assembled, to carry out capillary electrophoresis, according to criteria such as size of the observed alleles and the type of fluorescent labels used for each locus. The loci were analyzed using either an ABI Prism 3100 or an ABI Prism 3700 analyzer (Applied Biosystems). To guarantee the exemption of any bias from the electrophoresis of each analyzed plate, control samples were selected to be repeated in all runs. Therefore, any displacement of a determined allele, calculated in number of base pairs, could be identified between two independent injections.

Using a standard molecular size marker developed by [Brondani and Grattapaglia \(2001\)](#), the analysis of the sample

fragments was done using the GeneScan software (Applied Biosystems) while the allelic typing was done using Genotyper software (Applied Biosystems). The FlexiBinV.2.0 program ([Amos et al., 2006](#)) was used to standardize the binning of the established allele classes.

2.3. Statistical analyses

In the analysis of the within breed genetic variability of the 182 animals, the MS Tools software ([Park, 2001](#)) was used to calculate allelic frequencies and population genetic parameters such as: expected Heterozygosity (He), observed Heterozygosity (Ho) and allelic diversity for each locus. The Polymorphic Information Content (PIC) for each locus, was estimated using Cervus software ([Marshall et al., 1998](#)). The effective allele number (Ne) for each genetic group or locus was calculated using the following formula: $Ne = 1 / (1 - He)$, where He corresponds to the expected heterozygosity for each genetic group or locus, respectively.

Two exclusion probabilities for parentage analysis (EP) for each locus were estimated using Cervus software. The first exclusion probability (EP1) estimates the power of a randomly-selected unrelated candidate parent from parentage of an arbitrary offspring, given only the genotype of the offspring. While in the second exclusion probability (EP2) estimates the power to exclude a randomly-selected unrelated candidate parent from parentage of an arbitrary offspring, given the genotype of the offspring and of a known parent.

With the objective of assisting in the selection of the best loci to be used in a future panel for the genetic characterization of Brazilian pig breeds, for each marker, the allelic richness and the indices proposed by [Weir and Cockerham \(1984\)](#): F_{IT} , F_{ST} and F_{IS} were estimated, using the software FSTAT ([Goudet, 2002](#)). The model of infinite alleles was considered and bootstrap values ([Efron, 1985](#)) were obtained to estimate confidence intervals of 99% for each of the indices.

Within each genetic group, the inbreeding coefficient (F_{IS}) as well as its significance was estimated using the FSTAT software. The values of p were adjusted by the Sequential Test of Bonferroni. To evaluate the Hardy–Weinberg Equilibrium, within the markers and the analyzed pig populations, Arlequin software ([Excoffier et al., 2005](#)) was used. Global tests for Deficit and Excess of possible heterozygotes was estimated using Genepop software ([Raymond and Rousset, 1995](#)). Accurate values of p were estimated by the Markov Chains method using the following parameters: 10,000 dememorization, 150 batches and 5000iterations/batch.

The allelic frequencies of the populations were also compared using [Nei's standard genetic distances \(1972\)](#) and D_A ([Nei et al., 1983](#)) contained in the DISPAN software ([Ota, 1993](#)). Dendrograms were constructed using Neighbor Joining ([Saitou and Nei, 1987](#)) and the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean) ([Sneath and Sokal, 1973](#)) to be compared. Bootstrap analyses with 1000 replicates were used to evaluate the internal consistency of the suggested groupings, as well as the magnitude of the sampling errors.

To quantify the population structure within and between the analyzed genetic groups of pigs, the Analysis of Molecular Variance (AMOVA) was obtained using Arlequin software.

With the same program the genetic variability between the groups was also quantified by F_{ST} value, the Fixation Index proposed by Weir and Cockerham (1984), and tested using 100,000 iterations of the Markov Chains method and 10,000 permutations. Using STRUCTURE software (Pritchard et al., 2000), the individuals were probabilistically assigned using Bayesian inference, and inferring Admixture model, to determined populations or grouped to one or more populations. For these estimates the methods of Markov Chain Monte Carlo method was used, calculating, for each individual, the probability of one specific genotype X to be part of one given population K : $\ln Pr(X/K)$. The approximate posterior probabilities for values of K that varied from one to ten were estimated. To test the regularity of the results, each value of K was estimated four times independently from 500,000 iterations with a burn-in value of 100,000. Thus, it was possible to get an estimate of the number of existing subpopulations between the samples, assuming that these are in Hardy–Weinberg equilibrium.

3. Results

3.1. Within breed diversity

A total of 251 alleles from the 28 microsatellite loci were identified in the 182 evaluated samples of Brazilian pigs. Only the alleles observed only once were not included in the analyses. Thirty-seven private alleles were identified among the five analyzed genetic groups (Table 1) which were distributed between 19 of the 28 loci. Although possessing a lower observed number of alleles, the specific alleles of Moura were in higher frequencies (11%, 20% and 30%) when compared to the other breeds.

The number of alleles obtained individually inside the tested loci varied from two (SW539) to 17 (SWR1008) (Table 2). The effective number of alleles varied from 1.17 to 8.84 and was proportional to the values of expected heterozygosity found in loci SW539 (0.149) and S0007 (0.887), respectively. The estimates of allelic richness obtained in 22 animals of each studied genetic group, showed again that the locus SW539 presented the lowest value (1.981) and locus SWR1008 the highest one (13.547). It was observed that six of the 28 analyzed loci (SW445, SW24, S0227, SW951, S0225, and SW2406) deviated significantly from the Hardy–Weinberg Equilibrium (Table 2). F_{IS} , F_{IT} and F_{ST} indices estimated for each locus are identified also in Table 2.

The average values of observed heterozygosity in the studied genetic groups varied from 0.51 to 0.66 and they were lower than the average of the expected heterozygosity presented by the Piau and Monteiro breeds. For the Moura breed, the effective number of alleles (2.32) and the expected heterozygosity (0.569) were the lowest values amongst all analyzed genetic groups, with similar values compared with the Monteiro breed (2.34 and 0.573, respectively). On the other hand, although the Landrace breed presented the highest value of observed heterozygosity amongst all genetic groups, Piau was the breed that presented the highest estimated values of effective number of alleles and expected heterozygosity (2.94 and 0.66 respectively).

The Monteiro and Piau breeds presented the highest values of the F_{IS} Index (Table 1) and were not within the

expectations of the Hardy–Weinberg Equilibrium, revealing an amount of homozygous individuals beyond the expected. The other breeds (MS60, Moura and Landrace) did not present lower values for observed heterozygosities than for the expected ones, therefore, they remained in HWE for this test and presented negative values for F_{IS} .

The results of F_{IS} and HWE suggest the existence of a possible structure within the Piau and Monteiro breeds. The collected populations within each breed were analyzed separately to test this hypothesis. The value of F_{IS} and expected heterozygosity presented by the Piau population sampled in the Federal District were the highest amongst all analyzed populations (0.144 and 0.68, respectively). On the other hand, even though presenting a considerable degree of expected heterozygosity (0.57), the population of Minas Gerais did not present a high value of F_{IS} (0.007) and for the global test of deficit of heterozygosity in relation to the HWE. For the Monteiro breed, the two populations of the Federal District and Mato Grosso, also showed lower observed (H_o) than expected heterozygosities (H_e) and considerable values of F_{IS} (0.077 and 0.006, respectively). Only the population of the Federal District was significant ($p < 0.0001$) for the value of F_{IS} as well as for the HWE test.

3.2. Between breed divergence

In accordance with the analysis of existing genetic differentiation between the possible pairs of genetic groups, the F_{ST} values showed a higher differentiation between the Moura and Monteiro breeds (0.1995), being the highest, and between the Landrace breed and the MS60 composite (0.0823), the lowest. The AMOVA results showed that 14% of all observed diversity was due to differences between the breeds ($p > 0.0001$).

Comparing the results obtained using the matrix of D_A distance (Nei et al., 1983) and the matrix of Nei's standard distance (1972), it can be seen that the former made possible the inference of a more representative tree based on UPGMA method than the Neighbor joining. The highest genetic distance, based on the D_A distance, was the one found between the Moura and Landrace breeds (0.3185), similarly between Moura and Monteiro (0.3183), while the Piau and the Monteiro breeds were closer to each other (0.1676).

The selected dendrogram indicates a separation into three distinct groups (Fig. 1). The first cluster was formed by the commercial breed Landrace and the MS60 composite with a bootstrap value of 86%. Another cluster, with a bootstrap value of 83%, was formed by two naturalized breeds (Piau and Monteiro), while the third cluster that excluded the Moura breed from the others, was 99%.

The results obtained with the STRUCTURE software showed the distribution and the influence of each genetic group within

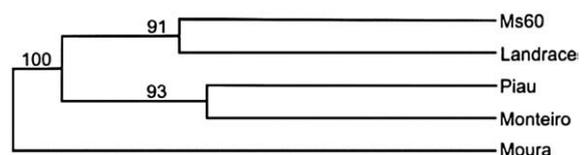


Fig. 1. UPGMA tree based on Nei's D_A genetic distance. Numbers indicate the proportion of bootstrap replicates sharing the labeled node.

the inferred populations. Among the tested possibilities, the value of K that proved to be the most adequate to explain the data set was seven (Fig. 2). As inferred by Pritchard et al. (2000), there are a couple of informal pointers which might be helpful in selecting K . The first one is the situation where the $Pr(K)$ value is very small for K less than the appropriate value (effectively zero). Also, where several values of K give similar estimates of $\log Pr(X/K)$, it seems that the smallest of these is often the one that better explains the data. The average probability of breed certification of the five sampled genetic groups throughout the seven inferred populations, was higher in the commercial composite MS60 (0.933), as well as in the Moura breed (0.929), followed by the Landrace breed (0.832). These genetic groups, represented respectively, by the orange, blue and white colors, presented a small number of individuals sharing alleles with other breeds. In the case of Landrace, the existence of some animals that share a high ratio of alleles with the sub-population of the Piau breed sampled in the Federal District was observed.

Corroborating the results of the intra-racial variability, the Piau and Monteiro breeds presented a sub-structure and, therefore, had the smaller probabilities of breed certification. Such sub-structure was basically among animals sampled in distinct regions for both breeds. In the Fig. 2, the purple and green colors represented, respectively, the Piau populations sampled in the Federal District and in Minas Gerais state, while the yellow and pink colors represented the Monteiro populations sampled in the Federal District and in Mato Grosso state.

4. Discussion

This is the first attempt to specifically quantify the genetic diversity of the naturalized Brazilian pig breeds with micro-

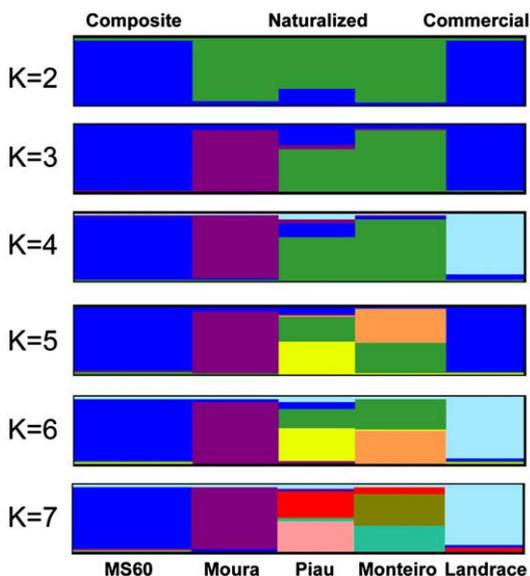


Fig. 2. Graphs of 182 individual Q-matrices obtained with STRUCTURE/Distruct softwares for $K=2$ to $K=7$ groups. Individuals were ordered by populations and separators between them were included. The length of each colored segment is equal to the estimated proportion of the individual's membership in the cluster of the correspondent color. The names of the breeds are presented below the figure, while their classification is presented above.

satellite markers. Amongst all the mammalian species used for animal production in Brazil, the naturalized pig breeds are the ones that, probably, present the smallest populations. An initial knowledge of the distribution of genetic variability will be a great help in management, conservation and improvement programs of this species.

In general, it was observed that the number of private alleles contributed to explaining the genetic diversity of the five genetic groups. Additionally, the high amount of rare alleles found, mainly in the naturalized breeds, shows their importance and the viability of their preservation. Caballero and Toro (2000) suggested that the ideal conservation management would be the one in which each descendant had the same ratio of genome as each one of its founders, as well as a maximum retention of their alleles. Thus, the mixture of the rare and common alleles will have to equal the genetic contributions.

The Moura and Monteiro breed, based on estimates of effective number of alleles and observed heterozygosity, can be considered the breeds with lowest genetic variability. The highest genetic diversity presented in the population sampled in the Federal District of the naturalized Piau breed can be the result of a high degree of outcrossing (Tapio et al., 2005). Based on analyses with Mexican pig breeds, Lemus-Flores et al. (2001) suggested that high heterozygosity could be explained by the occurrence of low selection pressure and by the lack of breeding programs, which also occurs with the Piau breed in Brazil, without considering the subpopulations.

Analyzing three local and one commercial pig breeds in India with 23 microsatellite markers, Behl et al. (2006) found higher values of expected heterozygosity (0.74 to 0.83) and an effective number of alleles varying between 4.78 and 5.34. On the other hand, Fang et al. (2005) obtained a lower mean value for this same parameter (0.50) and the effective number of alleles found was from 1.69 to 4.76, when analyzing 32 Chinese native breeds, three commercial breeds and two wild types with 34 microsatellite markers. This range is greater than the one found with the five Brazilian pig breeds studied (2.32 to 2.94).

An important pattern observed was the considerable genetic diversity presented by the commercial breeds in relation to the naturalized ones, suggesting a possible erosion of the genetic diversity of the latter. Comparing the estimates of expected heterozygosity and the effective number of alleles, Landrace showed a superior genetic variability compared to all other studied breeds, with the exception of the naturalized Piau breed (despite its low value when considered the observed heterozygosity). Kim et al., (2005) observed a value of expected heterozygosity of 0.702 for the Landrace breed, superior to all other commercial and native pig breeds from China, Korea and Europe studied.

The deviation to the HWE observed in six loci can be mainly attributed with the occurrence of a structure within some breeds, as proposed by Jordana et al., (2003), evaluating genetic diversity in cattle breeds. As observed in Table 1, two (Piau and Monteiro) of the five studied breeds had deviated from the HWE, which make evident the advantage of the Moura between he naturalized breed. The Monteiro and Piau breeds had both been collected in two regions. In such a way, the animals of these two breeds sampled in the Federal District belong to small farms where the matings are not

controlled and the population is reduced. The selection of non-related animals in populations with these characteristics generally is not easy, and can also influence the results in relation to the HWE (Fan et al., 2002). The other population of the Monteiro breed was collected in a conservation nucleus maintained in Mato Grosso state, where the population is also reduced. In contrast to the Monteiro breed, the Piau breed, sampled in Minas Gerais state did not show deviation to the HWE and presented low values of F_{IS} . These results may be due to the fact that these animals belong to a conservation nucleus with a controlled management aiming to maximize genetic variability.

On the other hand, the MS60, Landrace and Moura breeds remained in HWE, which was probably due to the fact that these animals had been sampled from larger herds with few migration events. Studying European native and commercial pig breeds, Laval et al., (2000) reported that the majority of them remained in HWE, and explained that deviation detected in some breeds was a consequence of the high values of F_{IS} presented by these breeds; what may be suggest in this present study.

The observed values of PIC in the loci revealed a panel that is capable of obtaining high paternity probability exclusion values in the analyzed genetic groups, including the naturalized breeds, even though only nine markers recommended by FAO/ISAG (2004) have been utilized. New studies to more specifically analyze the viability of these loci as panels for paternity exclusion tests are being developed. They should allow a reduction in execution time and cost of materials used in the analyses, after testing if a smaller number of loci can generate satisfactory results.

The value of the Fixation Index (F_{ST}) found in the Analysis of Molecular Variance (AMOVA) showed that approximately 14% of the genetic variability was distributed between the five breeds or Brazilian genetic groups of pigs. Lower values for such estimates (8%) have been found between three native pig breeds from India in an analysis of genetic diversity using 23 loci of microsatellite markers (Behl et al., 2006). In another study, San Cristobal et al., (2006) analyzed 70 populations of European pig breeds using 50 microsatellite markers and observed that 21% of all existing genetic variability was due to the differences between them.

The lower genetic distance estimates between the Piau and Monteiro breeds, suggests the occurrence of sharing of alleles mainly between the populations sampled in the Federal District. On the other hand, for being naturalized, the possibility of these breeds possessing the same genetic origin should not be discarded, but future studies with more appropriate markers, such as SNPs on mitochondrial and nuclear genes could clarify such assumption. The fact that the Moura breed did not cluster with the other two naturalized breeds can be due to the low actual gene flow. The Moura is a breed typical of the Southern region of Brazil, and probably the process of its formation was not influenced by breeds distinct from the Piau and Monteiro breeds, found in the Southeastern and Central-west regions, respectively. The genetic distancing of the Moura breed, also observed in the Landrace breed and in the composite MS60, suggests that their genetic origin, probably Iberian, differs from the commercial breeds. However, based on protein polymorphism, Tagliaro et al., (1995) showed a genetic similarity

between the Moura breed and the Landrace and Large White commercial breeds. The authors concluded that such similarity was due to recurrent crossbreedings, because they also assumed that their genetic origins were distinct. Considering that specific alleles in determined breeds can be a simple measure of differentiation of the populations, the high frequency of private alleles found in some loci can also have contributed to the distance of the Moura breed in relation to the other breeds. Although the true genetic processes that have lead to the current diversity still need to be known, some discussions have been brought around the use of genetic distances as a tool to be applied in conservations decisions (Olivier and Toro, 2008). The argument that genetic diversity could provide the best objective criterion for making conservation decisions, for example, breeds that are taxonomically distinct should be favored for conservation. In this way, the Moura breed can be considered as a naturalized breed that still possess high genetic distinction in comparison to the others. Such facts suggests the need for studies on their phenotypic, productive and reproductive characteristics, in order to consider their utilization in crossbreeding programs, as well as in other conservation programs.

Even though studies on the structure of populations in pig breeds can be generally based on methods of inference of distances, as considered by Laval et al., (2000) using an individual UPGMA tree, more recently, other refined methods have been proposed to test the existence of subdivisions in populations, breeds or varieties of domestic species such as those based on Bayesian assignment approach (Pritchard et al., 2000).

By means of the STRUCTURE software, that already has been used in analyses of naturalized pig breeds (Fabuel et al., 2004) the structure of populations was evidenced in the Piau and Monteiro breeds. It was also possible to observe that the animals of these two breeds sampled in the Federal District share alleles, probably due to the fact that the animals have been raised by smallholders and some unintentional crossbreeding may have occurred. This genetic structure observed can be considered either as a consequence of genetic introgression of individuals of other populations, or of a reproductive isolation that may have produced population bottlenecks, as suggested by Álvarez et al., (2004) when studying the genetic structure in sheep breeds. In general, the owners of naturalized pig breeds do not know much about the origin of the animals and the population size is reduced. The inclusion of new non-related and phenotypically characterized animals, can also contribute to a genetic differential for an increase in diversity of these herds, mainly those whose objective is conservation. For instance, the unique animal sampled from Pernambuco state presented a probability of breed certification of 94% with the Piau breed.

The high breed certification probabilities observed in the composite MS60, in the Moura naturalized breed and in the Landrace commercial breed, suggest the existence of well-defined allelic combinations among these breeds. The exclusion of animals that present alleles of other breeds can promote the improvement of the herds through a marker-assisted selection.

5. Conclusion

With the actual genetic diversity and the population structure of these five genetic groups evaluated, it was

possible to clarify the importance of the local or naturalized breeds as well as to propose some management strategies for these Brazilian genetic resources. If the molecular markers are used holistically together with other methodologies, like was done in this present study, they can become a very important tool in assisting the conservation and improvement of herds. Therefore, to preserve the genetic integrity of breeds is necessary, in order to prevent their disappearance, as well as to develop similar research aiming at their conservation. In this way, the genetic potential of these naturalized breeds can be used more effectively as a resource for unexpected future demands in the area of animal improvement.

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