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Genetic Variability among the Wild Boars (*Sus Scrofa Scrofa*), Crossbred Animals and Pigs Using Microsatellite Markers (STRs)

Paula Viana Correa da Silva[1], Jeffrey Frederico Lui[1*], Guilherme de Oliveira Band[1], Luciana Correia de Almeida Regitano[2], Selma de Fátima Grossi[3], Bruna Pena Sollero[4] and Cleujosí da Silva Nunes[5]

[1]Departamento de Zootecnia; Universidade Estadual Paulista; Via de Acesso Prof. Paulo Donato Castellane, s/n; 148844-900; Jaboticabal - SP - Brasil. [2]Embrapa Sudeste Pecuária; São Carlos - SP - Brasil. [3]Instituição Moura Lacerda; Ribeirão Preto - SP - Brasil. [4]Universidade Federal de Viçosa; Viçosa - MG - Brasil. [5]Universidade Federal de São Carlos - São Carlos - SP - Brasil

ABSTRACT

The aim of this work was to study the genetic variability among the wild boars, crossbred animals and pigs using microsatellite markers. Five genetic groups were studied. The fragments of three microsatellites developed for Sus scrofa domestica *- IGF1, ACTG2 and TNFB - were amplified through PCR technique to evaluate the expected intra populacion variability (He) and observed (Ho) heterozygosity, and endogamy coefficient (F_{IS}) within each population and inter population variability F_{ST}, testing relationship among five genetic groups to establish the genetic distance among them. The high level of observed heterozygosity values varied between 0.537 and 0.7871. Generally, F_{IS} was low, suggesting that the endogamy did not exist between the tested animals.*

Key words: molecular genetics, wild boars, microsatellites, fragments, amplification

INTRODUCTION

In Brazil, there has been an increase in breeding the wild animals at specific farms, aiming the reproduction for economic exploitation. Originating from Northern Africa and Southeast Asia, wild boars are mammals of *Artiodactyla* order, *Suidae* family, represented by five genres, including the *Sus* (Bosma et al., 1996). The need to increase the productivity at wild boar farms has led to crossings between the wild boars and pigs. These crosses originate the animals with new genotype, as a fusion between wild boar and pig genotypes (crossbred animals). Standard cariotype for European wild boars (*Sus scrofa scrofa*) is 2n=36 chromosomes (Darré et al., 1992) and hybrid animals could be 2n=37 and 2n=38 chromosomes, resulting from crossings between the wild boars and pigs. Despite these differences in chromosome number, these animals can mate and produce fertile hybrids (Grossi et al., 2006). Miranda et al. (2003), through cytogenetic characterization, reported a great deal of polymorphisms for the wild boars, with

*Author for correspondence: jeffrey@fcav.unesp.br

chromosome number varying between 36 and 38 that generated difficulties to obtain pure animals for farm development.

Currently, distinction between the pure and hybrid animals is made not only by phenotype observation, but also by the means of number of chromosomes analysis in diploid cells. In some cases, these methods are insufficient for safe determination of animal origin, since the hybrid phenotypes could be close to the pure animals and the chromosomal analysis does not determine the individual pureness but population pureness (Gimenez et al., 2003). Other molecular methods, as molecular markers, can collaborate with this distinction. Genetic markers as allozymes, microsatellites, mitochondrial and nuclear DNA sequences can be used to estimate many parameters of interest to study (Selkoe and Toonen, 2006).

The present work aimed to use the microsatellite markers (Single Tandem Repetition Polymorphisms - STRPs) developed to the domestic pigs for genetic characterization of pure wild boars (*Sus scrofa scrofa*) and its hybrids and determine the genetic variability and endogamy among the groups.

MATERIALS AND METHODS

Blood of 151 animals (wild boars, hybrids and pigs) of well defined genetic groups was used, which included 46 pure wild boars of origin, with 2n= 36 chromosomes, from two private wild boars farms in São Paulo, SP, Brazil; 46 hybrids, with 2n=36, 37 and 38 chromosomes, from a third wild boars private farm, also in São Paulo; and 59 three cross pigs (Landrace, Large White and Duroc). From each animal, 10 mL of cranial vein blood was collected with disposable syringe and placed in vacutainer tubes containing 0.05 ml EDTA (15.0%) solution.

The animals were grouped in five genetic groups, accordingly to pure and hybrid wild boars ploidy for lymphocytes cytogenetic analyses (Moorhead et al. 1960): group I, consisting of 59 domestic pigs with 2n = 38; ; group II, 46 pure wild boars of origin (PO) with 2n = 36; group III, 6 hybrids, with 2n=36, from the matings between hybrids and backcrossed animals; group IV, 30 hybrids with ploidy of 37 chromosomes; and group V, 10 hybrids, 2n=38, known popularly as *Javaporcos*, due to cariotype and phenotype similarity with the domestic pigs. Genomic DNA was extracted according to Zadworny and Kuhnlein (1990).

In this study three microsatellite *loci* (Single Tandem Repetitions Polymorphisms- STRPs) were analyzed, using Polymerase Chain Reaction technique (PCR). The markers were selected from Miranda (2005) as described in Table 1.

These genetics markers were chosen because they amplified well for this species according to Mendel segregation. PCR were carried out in 20 µl volumes. Initially standardized conditions for the genome of domestic swine were used; from these data, adjustments were carried through so that the starters developed for domestic swine were amplified in wild boar. The population parameters such as expected heterozigosity (He) and observed heterozigosity (Ho) also were calculated by means of MS_Tools program (Park, 2001). The endogamy coefficient (F_{IS}) within each population was calculated by FSTAT program (Goudet, 2002).

Table 1 – Primers to *Sus scrofa domestica* and *Sus scrofa scrofa.*

Primer	*SSC**	5'–3' sequence
ACTG2	3	CATCTTCCTCTTCCCTTCCCTGTGGACTCAAGGCTGTAAG
IGF1	5	GCTTGGATGGACCATGTTGCACTTGAGGGGCAAATGATT
TNFB	7	CTGGTCAGCCACCAAGATTTGGAAATGAGAAGTGTGGAGACC

**Sus scrofa* chromosome.

To analyze the existence of Hardy-Weinberg Equilibrium within the populations, global tests for deficit and excess of heterozygotes were taken using Genepop program (Raymond and Rousset, 1995). Accurate *p* values were obtained by Markov Chain Method through analysis using the following parameters: 10000 dememorizations, 40 batches and 2000 permutations.

To test the existing relations between the five analyzed genetic groups, the index of setting F_{ST} was estimated as in Weir and Cockerham (1984), calculated by Genepop program. The allelic

frequencies for the populations were also compared by the genetic distances, established by DISPAN program (Ota, 1993).

The pattern of genetic distance D_A (Nei et al., 1983) was tested for the construction of dendograms by the Neighbor-Joining method (Saitou and Nei, 1987). Bootstrap analysis with 1000 replications was used to evaluate the internal consistency of the suggested groupings, as well as the magnitude effect of sampling errors.

RESULTS AND DISCUSSION

Average observed heterozygosity values varied between 0.537 and 0.7871 and were inferior to the average values for expected heterozygosity (0.6749-0.8279). This value of observed heterozygosity (0.71) was similar to that found by Martinez et al. (2004) and indicated an important level of genetic variability. In hybrid genetic group 2n=38 (Group V), the effective number of alleles (3.0761) and observed heterozygosity (0.5357) were the lowest values among all the analyzed groups and the F_{IS} was the highest. In this group the endogamy existed. However, genetic hybrid groups 2n=37 (Group IV) and swines (Group I) presented the highest values for these estimates and for effective number of alleles (5.8 and 5.6 respectively). The values of F_{IS} (endogamy coefficient) for Groups II and III were negative (-0.005 and -0.037, respectively - Table 2) this genic diversity could be due to the fact that these populations were not under intense artificial selection. In the other groups (I, IV, V), the F_{IS} values were positive.

Amongst the five analyzed genetic groups, the swines (Group I) and hybrid 2n=37 (Group IV) did not present the equilibrium accordingly to data obtained by the global test of Hardy-Weinberg Equilibrium (EHW) for heterozygotes deficit (Table 2). Swines had been obtained from two small farms and endogamy could have occurred. The selection of inbred animals in the populations with these characteristics generally make it difficult and can also influence deviation results in relation to EHW.

Table 2 – Genetic diversity between populations for the five genetic groups obtained from three microsatellite markers

Genetic Groups	N	Na	Nm	He	Ho	Ne	F_{IS}	EHW1	EHW2
Group I	59	28	9.33	0.824	0.7871	5.6804	0.045	0.0510 (0.0037)*	0.9982 (0.0007)
Group II	46	19	6.33	0.7657	0.7685	4.2685	-0.005	0.5567 (0.0181)	0.4397 (0.0181)
Group III	6	10	3.33	0.7556	0.7778	4.0909	-0.037	0.6489 (0.0060)	0.6309 (0.0057)
Group IV	30	28	9.33	0.8279	0.7244	5.8109	0.128	0.0003 (0.0002)*	0.9927 (0.0024)
Group V	10	12	4.00	0.6749	0.5357	3.0761	0.220	0.0255 (0.0019)	0.9719 (0.0024)

Group I = swines, Group II = wild boars, Group III = crossed animals with 36 chromosomes ploidy, Group IV = crossed animals with 37 chromosomes ploidy, Group V = crossed animals with 38 chromosomes ploidy. sample size (N), number of identified alleles (Na), average allele number for each population (Nm), expected heterozygosity (He), observed heterozygosity (Ho), effective allele number (Ne), endogamy coefficient to the level of individual inside each population (F_{IS}), *p* values obtained by Hardy-Weinberg Equilibrium tests for heterozygotes deficit (EHW1), for heterozygotes excess (EHW2) and standard errors for each *p* value between parentheses.
*=P<0.01

Selkoe and Toonen (2006) found that a large fraction of "non-Medelian" ratios of alleles in offspring of defined crosses was apparently caused by null alleles. The potential causes of true non-Medelian behavior were sex linkage, physical association with the genes under strong selection, centers of recombination, transposable elements or processes during meioses such as non-disjunction or meiotic drive (segregation distortion). These processes may have severe effects, such as only one parental allele being passed on to all the offspring.

Beside inbreeding, other sources also could be involved or to be equally responsible for the disequilibrium of Hardy-Weinberg, as shown by the errors in genotyping individuals and presence

of null alleles (Silva, 2006), which originated due to a mutation in the hybridization site of at least one of the initiating oligonucleotides of the microsatellite to be amplified. This led to detection of an excess of apparent homozygotes, resulting in incorrect estimates of allelic frequencies, causing overestimation of inbreeding coefficients (Marshall et al., 1998).

Although hybrids were from the same farm, a difference occurred in EHW test results, where hybrid groups 2n=36 and 2n=38 presented equilibrium but hybrid 2n=37 did not. This could be explained by the fact that hybrids 2n=36 and 2n=38 possessed low sampling in relation to hybrid 2n=37.

In the present study, the number of average alleles of Group II presented reduction regarding to Group I, suggesting two explanations: the first one could be related to the animal husbandry in the farms, where individuals of a population did not cross with the ones of others, favoring endogamy. Another explanation for these reduction could be related to the presence of null alleles, which represented a problem when the starters of one species were used in another species.

Inter-population variability

According to the existing genetic differentiation analysis between the possible genetic groups pairs, F_{ST} values (Table 3) showed a higher differentiation (0.2007) between the wild boar (Group II) and hybrid 2n=38 (Group V) genetic groups, and the lowest (0.0222) between the hybrid 2n=36 (Group III) and 2n=37 (Group IV). Hampton et al. (2004) used microsatellites markers for feral pigs that belonged to the regions of Australia and all the populations had moderate heterozygosity (He=0.68) and moderate to high levels of differentiation between the populations (F_{ST} =0,118).

Table 4 showed that the highest genetic distance (0.6420) was between the wild boar (Group II) and hybrid 2n=38 (Group V) genetic groups, while the swines (Group I) appeared more related (0.233) to the hybrid group 2n=37 (Group IV). When all the crossed animals were considered as an only group, the highest distance (0.4084) was observed for the wild boars (Group II) and swines (Group I), followed by the hybrids and wild boars (0.4059), and hybrid groups (Groups III, IV and V) and swines were more related (0.2235).

Table 3 - Matrix of corresponding values to index F_{ST} obtained between the possible pairs of five analyzed genetic groups (all markers).

Genetic groups	Group I	Group II	Group III	Group IV
Group II	0.1085*	-	-	-
Group III	0.0467	0.0869	-	-
Group IV	0.0351	0.1021	0.0222	-
Group V	0.0803	0.2007	0.0513	0.0527

Group I = swines, Group II = wild boars, Group III = crossed animals with 36 chromosomes ploidy, Group IV = crossed animals with 37 chromosomes ploidy, Group V = crossed animals with 38 chromosomes ploidy.
*=P<0.01

Table 4 - Matrix of distance D_A with respective values for the possible pairs of five analyzed genetic groups.

Genetic groups	Group I	Group II	Group III	Group IV
Group II	0.4084	-	-	-
Group III	0.4122	0.3988	-	-
Group IV	0.2333	0.4359	0.2848	-
Group V	0.4093	0.6420	0.5172	0.3040

Group I = swines, Group II = wild boars, Group III = crossed animals with 36 chromosomes ploidy, Group IV = crossed animals with 37 chromosomes ploidy, Group V = crossed animals with 38 chromosomes ploidy.

The combined data sets obtained with the five groups were used to construct a dendogram (Fig. 1), which indicated three distinct groups. The first grouping formed by the group IV and group V was composed by crossed animals 2n=36 crossed animals 2n=38, respectively, with 74% similarity. The second grouping, formed by the group I, swines 2n=38, presented 82% of similarity with the first grouping. The third was represented by the groups II and III and revealed it self more differentiated and distant in relation to the others, with less than 50% of similarity (Rodrigues et al., 2008).

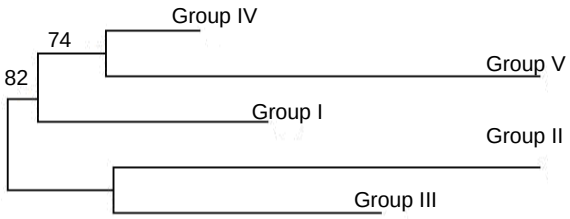

Figure 1 – UPGMA dendogram based on the distance D_A with the respective values of bootstrapping in each grouping.

CONCLUSIONS

The efficiency of heterologue amplification using microsatellite markers developed for the domestic swines (*Sus scrofa scrofa*) and applied to wild boars were proved. Pure wild boars were genetically different from the swines and hybrids and differences were in size and alleles frequencies for the three microsatellite *loci*. The estimates of variability pointed, in a general way, loss of heterozygosity.

These results could serve as a starting point for another studies aiming to clarify the phylogenetic relations between the genetic groups of wild boars, swines and hybrids, to obtain essential information for implementation and maintenance of conservation works.

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REFERENCES

Bosma, A. A.; De Haan, N. A.; Mellink, C. H. M.; Yerle, M.; Zijlstra, C. (1996), Chromosome homology between the domestic pig and the babirusa (family Suidae) elucidated with the use of porcine painting probes. *Cytogenet Cell Genet*, Basel, **75**, 32-35.

Darré, R.; Berland, H. M.; Goustat, P. (1992), Chromosomal status of free-ranging and farmed wild boar populations in France. *Rev Med Vet*, **3**, 225-232.

Gimenez D. L.; Mota L. S. L. S.; Curi R. A.; Rosa G. J. M.; Gimenes M. A.; Lopes C. R.; Lucca E. J. (2003), Análise cromossômica e molecular do javali europeu Sus scrofa scrofa e do suíno doméstico Sus scrofa domestica. *Braz J Vet Res Anim Sci*., **40**, 2, 146-154.

Goudet, J. (2002). FSTAT Version 2.9.3.2 for windows: a computer program to calculate F-statistics.
URL:http://www2.unil.ch/popgen/softwares/fstat.

Grossi, S. F.; Lui, J. F.; Garcia, J. E.; Meirelles, F. V. (2006), Genetic diversity in wild (*Sus scrofa scrofa*) and domestic (*Sus scrofa domestica*) pigs and their hybrids based on polymorphism of a fragment of D-loop region in the mitochondrial DNA. *Genet Mol Res*, **5** (4), 564-568.

Hampton, J. O.; Spencer, P. B. S.; Alpers, D. L. A.; Twigg, L. E.; Woolnough, A. P.; Doust, J. Higgs, T.; Pluske, J. (2004), Molecular techiniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. *J Applied Ecol*, **41**, 735-743.

Marshall, T. C.; Slate, J., Kruuk, L. E. B., Pemberton, J. M. (1998), Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol*, **7 (**5), 639-655.

Martinez, A. M.; Carrera, M. P.; Acosta, J. M.; Rodriguez-Gallardo, P. P.; Cabello, A.; Camacho, E.; Delgado, J. V. (2004), Genetic characterization of Blanca andaluza goat based on microsatellite markers. *S Afr J Anim Sci*, **34 (**1).

Miranda, L. L.; Lui, J. F. (2003), Citogenética do javali em criatórios comerciais das regiões Sul e Sudeste do Brasil. Pesqui Agropecu Bras, **38** (11), 1289-1295.

Miranda L. L. (2005). Caracterização genética de javalis (*Sus scrofa scrofa*) através de marcadores microssatélites – STRs. PhD Thesis, Universidade de São Paulo, Riberão Preto, Brazil.

Moorhead, P. S.; Howell, P. C.; Mellman, W. J.; Battips, D. M.; Hundgerford, D. A. (1960), Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp Cell Res*, New York, **20**, 613-616.

Nei, M..; Tajima, F.; Tateno, Y. (1983), Accuracy of estimated phylogenetic trees from molecular data. *J Mol Evol*, **19**, 153-170.

Ota T. (1993), Dispan: Genetic distance and phylogenetic analysis. Institute of Molecular Evolutionary Genetics, The Pennsylvania State University, University Park.

Park, S. D. E. (2001), Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection. Ph.D. Thesis, University of Dublin, Ireland.

Raymond, M. L. and Rousset, F (1995), An exact test for population differentiation. *Evolution*, **49**, 1280-1283.

Rodrigues, R.; Schneider, H.; Santos, S.; Vallinoto, M.; Sain-Paul, U.; Sampaio, I. (2008), Low levels of genetic diversity depicted from mitochondrial DNA sequences in a heavily exploited marine fish (*Cynoscion acoupa*, Sciaenidae) from Northern coast of Brazil. *Genet Mol Biol*, **31** (2), 487-491.

Saitou, N.; Nei, M. (1987), The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, **4**, 406-425.

Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. 2nd Edition. New York: Cold Spring Harbor Laboratory Press, p. 63.

Silva, R. W. (2006), Avaliação da variabilidade genética em Tayassu tajacu (cateto) e Tayassu pecari (Queixada) por meio da utilização de marcadores microssatélites. Master Thesis, Universidade Federal do Paraná, Curitiba, Brazil.

Selkoe, K. A.; Toonen, R. J. (2006), Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol Lett*, **9**, 615-629.

Weir, B. S.; Cockerham, C. C. (1984), Estimating F statistics for the analysis of the population structure. *Evolution*, **38**, 1358-1370.

Zadworny, D.; Kuhlein, U. (1990), The identification of the kappa-casein genotype in Holstein dairy cattle using the polymerase chain reaction. *Theo. Appl Genetic*, **80**, 631-63

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