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Research Article

Genetic Diversity among four Pig Breeds in Nigeria using Microsatellite Markers

Joseph A. Adepegba[1], Noah E.T. Tor[1,2*], Olaniyi A. Oni[1,3], Babatunde M. Ilori[1], Thomas I. Wamagi[4] and Mamfe E. Ate[2]

[1]Department of Animal Breeding and Genetics, College of Animal Science and Livestock Production, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Ogun State, Nigeria
[2]Department of Animal Breeding and Physiology, College of Animal Science, Federal University of Agriculture, Makurdi, Benue State, Nigeria
[3]Department of Agriculture and Natural Resource, Ondo State Local Government Service Commission, Alagbaka, Akure, Ondo State, Nigeria
[4]School of Agricultural Technology, Nuhu Bamali Polytechnic, Zaria, Kaduna State, Nigeria
***Corresponding author:** tor.noah2k@gmail.com

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ABSTRACT

A study on genetic diversity and genetic distances among pigs was carried out using a total of 51 genomic DNA randomly sampled from four pig breeds in Ogun and Ondo States of Nigeria. The gDNAs were amplified with six microsatellite markers, generating amplicons which were separated by Polyacrylamide solution. Bands on the gels were scored considering the six microsatellite markers used. Mean number of alleles (MNA), observed (Ho) and expected (He) heterozygosities, Polymorphism information content (PIC) and Genetic distances were calculated using GenAlex software.Exclusion probability (PEI) and combined exclusion probabilities (CPE) were also computed. The number of alleles observed ranged from 3 to 17, with the highest produced by SW71 and lowest by S0036 markers. MNA per breeds ranged from 5.333 in Landrace to 8.833 in Large White. The mean PIC across loci per breed ranged from 0.6863±0.047 in Landrace to 0.7924±0.040 in Large White. Mean observed and expected heterozygosities per breed ranged from 0.7305±0.019 in Duroc to 0.7916±0.020 in Large White, and from 0.7868±0.034 in Landrace to 0.8698±0.021 in Large White respectively. Genetic distances among the pig breeds ranged from 0.2570 (Large White Vs Landrace) to 0.6663 (DurocVs Large White). Mean value of inbreeding coefficient (F_{IS}) was -0.0698. Exclusion probability of each microsatellite marker when genotype of both parents were known (PE1) ranged from 0.9591 in Landrace to 0.9925 in Large White, when genotype of only one parent known (PE2) ranged from 0.9657 in Landrace to 0.9953 in Large White pigs, and when the genotype of the two parents unknown (PE3) ranged from 0.9568 in Landrace to 0.9999 in Large White. The Combined exclusion probability (CPE) for all the markers used when genotype of both parent were known (PE1) was 0.9999. The study concluded that there was high genetic diversity among the pig breeds in Ogun and Ondo States, Nigeria, indicative of their potentials for genetic improvement via selection or crossbreeding. Furthermore, the six microsatellite markers used in this study may be recommended based on their PIC and CP_E values for further analysis of genetic background and routine parentage verification of the Nigerian pig breeds.

Key words: Pigs, breeds, genetic diversity, microsatellite marker.

INTRODUCTION

Detailed knowledge of population structure among and within breeds of livestock is essential for establishing conservation priorities and strategies (Caballero and Toro, 2002). Many breeds of pigs in the world are on the edge of extinction, the intensification of agriculture that took place caused major changes to pig breeding, with traditional systems being replaced by intensive production based on an increased number of exotic breeds, while native breeds were progressively abandoned and became virtually extinct (Gama *et al.*, 2006). Therefore, a conservation of these pig breeds is highly important. The history of the domestic pig in Africa is highly controversial, its ancestor, the wild pig (*Susscrofa*) is native to North Africa and its range extends along the Atlantic coast (Ollivier, 2000). According to

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Bourn *et al*. (1994), pig population in Nigeria was estimated to be about 3.5 million and this is because swine farming is popular in many parts of the country.Earlier reports by Adebambo, (1982) shows Nigeria has the highest pig population in Africa. Pig breeds in South-West Nigeria are characterized by their hardiness, resistance to diseases and ability to survive on a varied diet (Adebambo, 1982; RIM, 1992).The assessment of genetic diversity within and among populations is important for the conservation of genetic resources. Genetic diversity assessment can be based on morphological, biochemical and molecular types of data. Molecular markers such as amplified fragment length polymorphism, random amplified polymorphic DNA, mitochondrial DNA, restriction fragment length polymorphism, single nucleotide polymorphism and Y chromosome markers are superior to both morphological and biochemical markers, because they are relatively simple to detect, abundant throughout the genome, completely independent of environmental conditions and can easily be used (Naqvi, 2007; Teneva *et al*., 2014). Detailed knowledge of genetic variation within and among different pig breeds is very important for understanding and improving economically important traits. Genetic diversity serves as a way for populations to adapt to changing environmental conditions (NBII, 2011). The indicators of genetic variation within a breed (that is, heterozygosity and effective number of alleles) at a locus are usually employed to describe a population. These indicators demonstrate the degree of diversity based upon analysed polymorphic loci within a breed. High heterozygosity and high effective number of alleles inform about a greater genetic diversity of a breed. Microsatellites have proved extremely useful for the analysis of population structure, relationships and have been used for genetic characterization of several species and animal populations (Chen *et al.,* 2004; San Cristobal *et al.,* 2006; Kamara *et al.,* 2007; Vicente *et al.,* 2008). In searched literature, few genetic diversity studies have been documented on Nigerian pig populations using microsatellite markers. Therefore, there is a need to carry out a research on genetic relationships among pig populations in Nigeria, where there is abundance of pigs and to examine the power (or the combined exclusion probabilities) of microsatellite markers with DNA obtained from these populations in order to ascertain their informativeness before employing them for routine parentage analysis.

MATERIALS AND METHODS

The laboratory analyses were carried out at the Biotechnology Centre, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Blood samples were obtained from nine commercial farms and one research farm scattered across Ogun and Ondo states of South Western Nigeria. Blood samples were obtained from a total of 51 animals consisting of Large White (n = 5); Duroc (n = 10); Landrace (n = 6) Pietran (5) from Ogun state and Large White (n = 13), Duroc (n = 8), Pietran (4) from Ondo State. Samples of each population were taken from different locations within each state in order to avoid closely-related individuals. Exactly, 1 ml of blood was collected from individual pig from the marginal ear vein aseptically using a 16 mm needle and 2 ml syringe then transferred into 1.5 ml EDTA tube, which was properly labeled and preserved at the collection site before been transported to the laboratory. Blood genomic DNA isolation was carried out with NORGEN GENOMIC DNA KIT following the manufacturer's protocol. Six microsatellite markers were selected from the database of the Food and Agriculture Organization of the United Nations (Table 1).

PCR was performed in a thermal cycler-Hybaid Express System 9600 (Perkin Elmer) programmable incubator with the following setting:94 ^{0}C, 300 seconds of initial denaturation, followed by 35 cycles of denaturation at 94 ^{0}C, 60 seconds, annealing temperature of the primers used (markers) was determined to be between the range of 48 °C to 55 °C, 45 seconds and extension temperature of 72 °C, 30 seconds, after preliminary optimization protocols. The microsatellite markers, purified DNA, double distilled water and other PCR reaction mixture was amplified in a total volume of 25 µl, This PCR mixtures contain 1 µl purified DNA, 2 µl primer (forward and reverse primer), 16.10 µl double distilled water and 5.90 µl PCR Master Mix which consists of (*Taq* DNA polymerase, Deoxyribonucloside5-triphosphates (dNTP) 10 x PCR buffer and cation (Mg^{2+}). PCR-products were heat-denatured for another 60 seconds in the PCR system and transferred to an ice box chilled at 0 ^{0}C.They were thereafter loaded into the gel containing 12% polyacrylamide solution made up of 210 g of 6 ml urea, acrylamide and N'N methylene bisacrylamide (19:1).25 µL of 30% TEMED ($C_6H_{12}N_2$) and 450 ml of 10% ammonium persulphate was added to each 60 ml of polyacrylamide gel solution to serve as crosslink. All constituents were mixed and poured into the gel cassette made up of 20 x 20 cm double-glass plates clamped together by iron clips. Exactly 1 ml bromophenol blue loading dye was placed on a tray and 10 ml of the amplified product was added, mixed and loaded to each lane of the glass trough with PCR Sizer 100 bp DNA Ladder used as internal marker for sizing. The electrophoresis process was allowed to run for two hours at 100 V, 10 Ma. A drop of ethidium bromide (EB) was used as staining agent before visualization and photography of the products under ultra-violet (UV) light. Fragment size data for each pig population were prepared into Excel Worksheet and analyzed using GenAlEx software (Peakall and Smouse,2012) so as to generate the allele frequencies, observed heterozygosity (H_o), expected heterozygosity (H_e),number of alleles per marker (N_A)and Mean number of allele (MNA). Genetic differentiation (i.eF_{ST}) and global fixation indices (i.eF_{IS} and F_{IT}) were calculated using programme FSTAT v.2.9.3 developed by Goudet (2001). Polymorphism information content (PIC) of each microsatellite marker was calculated using Botstein *et al.* (1980) formula. Nei's standard genetic distances (D_s) among pig populations were generated using Microsatellite Analyzer version 4.05 developed by Dieringer and Schlotterer (2003). Exclusion probability (P_{EI}) and combined exclusion probabilities (C_{PE}) otherwise known as power of microsatellite, across all markers used with pig

Table 1: Microsatellite markers used in this study

Marker	Sequence	Chromosom allocation	Size(bp)	Annealing Temperature (°C)
S0036	F-ATGCAGCTGTGGCGGCGCAG R-TTAGGCAGCCACATGCACAAG	2	114 -132	55
SW902	F-ATCAGTTGGAAATGATGGCC R-CTTGCCTCAAAGAGTTGTAAGG	3	130- 162	48
SW2	F-TGCCAATGGTGTGGCTATAA R-CCCTGAAGGCTCAGATGGT	5	83-119	50
SW71	F-GATCACCCTTATCCCCATTC R-TAGAAACACCATCATCCATTCA	6	81-121	48
SW205	F-CACAGGTCCATCACCTCATG R-GGGTATCTAATGTACATCACGG	8	144-160	50
SW445	F-CCTCCCTGGCACTCATTG R-CACACACACAAGCAGGTGC	4	184-208	55

Table 2: Pooled number of alleles at each microsatellite locus within the four pig breeds in Ogun and Ondo States, Nigeria

Locus	Total	Large White	Duroc	Pietran	Landrace
S0036	10	4	6	6	3
SW902	16	7	7	8	5
SW2	14	8	5	4	5
SW71	21	17	6	10	6
SW205	9	5	5	5	4
SW445	13	12	12	10	9
MNA	13.833	8.833	6.833	7.000	5.333
SEM	1.622	1.816	0.983	0.881	0.769

MNA: Mean number of alleles; SEM: Standard error of mean

populations were computed using the relationship developed by Jamieson and Taylor (1997) and employed by Olowofeso *et al.* (2016). These relations were depicted as:

Scenario I:

$$CP_E = 1 - (1 - P_1)(1 - P_2)(1 - P_3) \ldots (1 - P_n) \ldots (4)$$

Where

$$P_1 = 1 - 2\sum P_i^2 + \sum P_i^3 + 2\sum P_i^4 - 3\sum P_i^5 - 2(\sum P_i^2)^2 + 3\sum P_i^2 \sum P_i^3 (5)$$

CP_E is combined exclusion probability for n number of markers used and P_i is the allele frequencies produced by each marker per pig population.

Scenario 2:

$$P_2 = 1 - 4\sum P_i^2 + 2(\sum P_i^2)^2 + 4\sum P_i^3 - 3\sum P_i^4 \ldots (6)$$

and

Scenario 3:

$$P_{31} + 4\sum P_i^4 - 4\sum P_i^5 - 3\sum P_i^6 - 8(\sum P_i^2)^2 + 8(\sum P_i^2)(\sum P_i^3) + 2(\sum P_i^3)^2 \ldots (7) =$$

RESULTS

The number of alleles observed across the six (6) microsatellite markers varied between 3 (S0036) for Landrace and 17 (SW71) for the Large White. The total number of alleles and mean number of alleles identified in the entire pig population were 83 and 13.833±1.622 respectively, while for the four sub- populations, the values were 8.833±1.816 for the Large White, 6.833±0.983 for Duroc, 7.000±0.881 for Pietran and 5.333± 0.769 for Landrace (Table 2). Allele frequencies and numbers of rare alleles observed in the pig breeds are represented in Table 3.

Though very rare alleles (new mutation) are not very informative for assessing genetic diversity within a population or genetic structure among population (Hale *et al.,* 2012), previous study by (Piry *et al.,* 1999) reported that rare alleles contribute little heterozygosity as rare alleles tend to be lostwhile average number of alleles per locus and allelic diversity is reduced, heterozygosity however is not reduced proportionally. Mean PIC values across the loci per breeds ranged from 0.6863 in Landrace to 0.7924 in Large White. PIC values for all loci ranged from 0.4923 for S0036 in Landrace to 0.8921 for SW445 in Large White (Table 4).

Mean expected values across loci per population ranged from 0.7868 in Landrace breed to 0.8698 in the Large White breed. The highest value of expected heterozygosity (0.8891) was found in SW2 and SW71 for Large white and Land race breeds respectively. The lowest 0.6444 was produced by S0036 in the Landrace breeds. The Highest unbiased gene diversity however was revealed in the Large White pig breed with 87%, as compared to Pietran, 84%, Duroc, 82% and Landrace, 72% (Table 5).

The observed heterozygosity values (Table 5) shows mean values across loci ranged from 0.7305 in Duroc breed to 0.7916 in the Large White breeds. The highest value of observed heterozygosity (0.8827) was recorded for Landrace for the SW71, while the lowest value 0.6435 was observed in the Duroc for SW445. The mean value of inbreeding coefficient (F_{IS}) was -0.0698, while that of F_{IT}, F_{ST} and N_M were 0.0594, 0.1043and 7.128 respectively. The highest value of F_{IS}, 0.1145 was observed in SW445 while the lowest value of -0.1833 was estimated for the SW2. SW205 had the highest F_{ST} value 0.1831 and the lowest value, 0.0079 for F_{ST} was recorded in locus SW445. N_M values ranged from 1.265 in S0036 to 31.395 in SW445. Results on these are presented in Table 6.

Nei's genetic distance among the pig populations in Ogun and Ondo States of Nigeria is presented on Table 7. The Nei's genetic distance between the population pairs ranged from 0.2570 to 0.6663. The lowest genetic distance 0.2570 was observed between the Large White and Landrace, while the highest genetic distance value 0.6663 was observed between the Duroc and the Large White breeds.

The probability of exclusion of each microsatellite marker when genotype of both parents are known (PE1) ranged from 0.9591 in Landraceto 0.9925 in the Large White breed, while the probability of exclusion of each microsatellite marker and for increasing combinations of the 6 loci when genotype of only one parent is known (PE2)

Table 3: Allele frequencies and unique (rare) alleles of four pig populations in Ogun and Ondo States, Nigeria as revealed by microsatellite markers

Fragment (bp)	Breed			
S0036	Large White	Duroc	Pietran	Landrace
114	0.3125	0.0000	0.1428	0.5000
116	0.1250	0.0000	0.2857	0.0000
118	0.4375	0.0000	0.2142	0.4000
120	0.1250	0.0000	0.1429	0.1000
122	0.0000	0.7692	0.2314	0.0000
124	0.0000	0.1154	0.0714	0.0000
126	0.0000	0.2308	0.0000	0.0000
128	0.0000	0.0385	0.0000	0.0000
130	0.0000	0.0269	0.0000	0.0000
132	0.0000	0.2526	0.0000	0.0000
SW902	Large White	Duroc	Pietran	Landrace
130	0.0833	0.0000	0.0000	0.1667
132	0.1667	0.0000	0.0000	0.3333
134	0.0556	0.0000	0.0000	0.1667
136	0.1944	0.0000	0.0000	0.2500
138	0.2500	0.0000	0.0000	0.0833
140	0.1111	0.0000	0.0000	0.0000
144	0.1389	0.0000	0.0000	0.0000
146	0.0000	0.0000	0.2143	0.0000
148	0.0000	0.0000	0.7143	0.0000
150	0.0000	0.0333	0.3571	0.0000
152	0.0000	0.0667	0.0714	0.0000
154	0.0000	0.1333	0.3123	0.0000
156	0.0000	0.3333	0.0132	0.0000
158	0.0000	0.3000	0.2432	0.0000
160	0.0000	0.0667	0.0000	0.0000
162	0.0000	0.0667	0.7143	0.0000
SW2	Large White	Duroc	Pietran	Landrace
83	0.0000	0.1563	0.0000	0.0000
85	0.0000	0.2500	0.0000	0.0000
87	0.0000	0.1875	0.0000	0.0000
89	0.0000	0.2520	0.0000	0.0000
101	0.8333	0.1563	0.0000	0.2500
103	0.1667	0.0000	0.0000	0.4167
105	0.1389	0.0000	0.0000	0.1667
107	0.1389	0.0000	0.0000	0.0833
109	0.1111	0.0000	0.0000	0.0833
111	0.1389	0.0000	0.0000	0.0000
113	0.1667	0.0000	0.2134	0.0000
115	0.0556	0.0000	0.2564	0.0000
117	0.0000	0.0000	0.1667	0.0000
119	0.0000	0.0000	0.2778	0.0000
SW71	Large White	Duroc	Pietran	Landrace
81	0.0556	0.0000	0.0000	0.0000
83	0.0345	0.0000	0.0000	0.0000
85	0.3243	0.0000	0.0000	0.0000
87	0.0833	0.0000	0.0000	0.0000
89	0.1389	0.0000	0.0000	0.0000
91	0.0556	0.0000	0.0000	0.0000
93	0.0556	0.0000	0.1429	0.0000
95	0.0278	0.0000	0.0714	0.0000
97	0.3255	0.0000	0.3235	0.1000
99	0.2322	0.0000	0.1429	0.2000
101	0.2778	0.0000	0.3221	0.1000
103	0.2322	0.0000	0.0000	0.0000
105	0.0556	0.0000	0.0000	0.0000
107	0.0278	0.0000	0.0000	0.0000
109	0.0278	0.0000	0.0000	0.0000
111	0.0278	0.0588	0.0000	0.0000
113	0.0278	0.1176	0.0714	0.0000
115	0.0000	0.2731	0.1594	0.1000
117	0.0000	0.1471	0.2143	0.3000
119	0.0000	0.2353	0.1429	0.2000
121	0.0000	0.3235	0.0714	0.0000
SW205	Large White	Duroc	Pietran	Landrace
144	0.0000	0.1071	0.0000	0.0000
146	0.0000	0.3212	0.0000	0.0000
148	0.0000	0.2500	0.0714	0.0000
150	0.0000	0.4285	0.1429	0.0000
152	0.0357	0.0357	0.2857	0.0000
154	0.1071	0.0000	0.2857	0.0833
156	0.2857	0.0000	0.2143	0.1667
158	0.2354	0.0000	0.0000	0.2500
160	0.3243	0.0000	0.0000	0.5000
SW445	Large White	Duroc	Pietran	Landrace
184	0.0357	0.0000	0.0714	0.0833
186	0.1429	0.0352	0.0000	0.0833
188	0.1429	0.0714	0.0223	0.1667
190	0.0714	0.0714	0.0213	0.0237
192	0.0357	0.1429	0.0232	0.0433
194	0.7143	0.1221	0.0000	0.2500
196	0.1071	0.2322	0.0000	0.0756
198	0.0714	0.3571	0.2143	0.0000
200	0.0000	0.0714	0.0232	0.0000
202	0.2322	0.1429	0.1071	0.0000
204	0.1212	0.2143	0.2354	0.0000
206	0.3223	0.1071	0.0544	0.0833
208	0.2322	0.0351	0.3243	0.0833

Bold values represent frequencies of rare alleles

ranged from 0.9657 in Landrace to 0.9953 in Large White breed, while the probability of exclusion of each microsatellite marker and increasing combinations of the 6 loci, when the genotype of both parent are excluded (PE3) ranged from 0.9568 in Landrace to the 0.9999 in the Large White breed. The combined exclusion probability for one and both parents excluded was 0.9999 (Table 8).

DISCUSSION

The number of allele range observed in this study was wider compared to those reported in earlier studies suggesting higher genetic diversity among the pig breeds. The markers used for this study were appropriate since their polymorphisms were higher than the minimum of four alleles required for microsatellite markers to be used in the estimation of genetic diversity. The mean number of alleles (MNA) observed over the six microsatellite loci for the four studied pig breeds are considered to be good indicators in defining the genetic variability within the population. The high MNA value (13.833) recorded among the pig breeds is more than mean number of alleles when compared to the Czech (7.86), Vrtková *et al.* (2012), local Ghanaian pig (7.65) Ayizanga *et al.* (2016), Brazilian pig (6.18) Silva *et al.* (2011), Chinese pig (5.54) Wang *et al.*, (2004) and black Slovanian pig (2.50) Bradic *et al*. (2007). Thus indicating more genetic diversity in the Nigeria pigs which could have been influenced by selection of different alleles among the breeds over time. However, across the six microsatellite loci used the highest number of allele 21 was observed in SW71, which indicates that it is highly polymorphic while 9 was recorded for SW205, showing that it had less variability. This is in support of the report by SanCristobal *et al*. (2003) that differences in the reported number of alleles and other genetic diversity parameters might be caused by differences in the types of microsatellites used. All microsatellite markers used with the exception of SW445 were observed with at least two rare alleles across the pig breeds. Since rare/private alleles are alleles unique to a particular breed and/or population it is only fitting that

Table 4: The polymorphism information content (PIC) of the loci within the four pig breeds in Ogun and Ondo States, Nigeria

Locus	Large White	Duroc	Pietran	Landrace
S0036	0.6240	0.7471	0.7796	0.4923
SW902	0.8080	0.7332	0.7734	0.7262
SW2	0.8491	0.7582	0.6921	0.6814
SW71	0.8850	0.7576	0.8657	0.7721
SW205	0.6962	0.6632	0.7261	0.5991
SW445	0.8921	0.8752	0.8662	0.8472
Mean PIC across Loci per population ± SEM	0.7924±0.040	0.7557±0.025	0.7839±0.026	0.6863±0.047

Table 5: Observed and expected heterozygosities among four pig breeds in Ogun and Ondo States of Nigeria.

Locus	Large White		Duroc		Pietran		Landrace	
	H_{OBS}	H_{EXP}	H_{OBS}	H_{EXP}	H_{OBS}	H_{EXP}	H_{OBS}	H_{EXP}
S0036	0.7022	0.8751	0.7692	0.8121	0.8572	0.8682	0.8000	0.6444
SW902	0.8332	0.8542	0.6972	0.7932	0.7264	0.8572	0.6721	0.8332
SW2	0.8471	0.8891	0.7556	0.8173	0.7217	0.7842	0.8512	0.7884
SW71	0.7592	0.8542	0.7821	0.8112	0.7796	0.8455	0.8827	0.8891
SW205	0.7745	0.8574	0.7352	0.8661	0.7242	0.8241	0.6674	0.7125
SW445	0.8344	0.8890	0.6435	0.8182	0.8455	0.8498	0.8392	0.8533
Mean ±SEM	0.7916±0.020	0.8698±0.021	0.7305±0.019	0.8196±0.009	0.7757±0.023	0.8382±0.011	0.7854±0.034	0.7868±0.034

Table 6: Fixation indices and migrant rate or gene flow as revealed by six pig microsatellite markers

Locus	F_{IS}	F_{IT}	F_{ST}	N_M
S0036	-0.1335	0.0535	0.1650	1.265
SW902	-0.1028	0.0565	0.0582	4.045
SW2	-0.1833	-0.0128	0.1441	1.484
SW71	-0.0498	0.0209	0.0673	3.465
SW205	-0.0641	0.1307	0.1831	1.115
SW445	0.1145	0.1075	0.0079	31.395
Mean	-0.0698	0.0594	0.1043	7.128

F_{IS} =Inbreeding Coefficient of individual relative to the Sub-population, F_{IT} = Inbreeding coefficient of individual relative to the total population, F_{ST} = Inbreeding coefficient of the sub-population relative to the total population.

Table 7: Nei's genetic distances (below diagonal) and the pairwise F_{ST} (above diagonal) among four pig breeds in Ogun and Ondo States, Nigeria

Population	Large White	Duroc	Pietran	Landrace
Large White	******	0.1552	0.0673	0.0102
Duroc	0.6663	******	0.1062	0.1630
Pietran	0.4192	0.4342	******	0.1025
Landrace	0.2570	0.6213	0.5124	******

Table 8: Power of exclusion of the microsatellite markers used with four pig breeds in Ogun and Ondo States, Nigeria.

Breeds	PE1	PE2	PE3
Large White	0.9925	0.9953	0.9999
Duroc	0.9846	0.9879	0.9969
Pietran	0.9672	0.9893	0.9879
Landrace	0.9591	0.9657	0.9568
C_{PE}	0.9999	0.9999	0.9999

PE1= Probability of exclusion when genotype of both parent are known, PE2= Probability of exclusion when genotype of only one parent is known, PE3 = Probability of exclusion which exclude parents and CP_{E1}, CP_{E2} and CP_{E3} = Combined exclusion probabilities for genotypes of both parents known, genotype of one and both parents excluded respectively

such alleles are used in the genetic identification of such population. Toro *et al*. (2008) emphasized the importance of high frequency rare/private alleles in the genotyping and line identification of populations.

The PIC range observed in this study is similar to that reported in the Thai pig breeds (Yang *et al*., 2012) but wider compared to values reported in the Chinese (Wang *et al.*, 2004) and Czech pig breeds (Vrtková *et al.*, 2012). The values of the PIC recorded for this study showed that all microsatellite markers used for the analysis are highly polymorphic and informative for genetic diversity studies, since all loci PIC value in this study was greater than the threshold value of 0.5 suggested by Botstein *et al. (*1980). The observed heterozygosity and the expected heterozygosity, otherwise known as gene diversity in this study was wider compared to those reported for Ghanaian pig breeds (Ayizanga *et al.,* 2016), Czech pig breeds (Vrtková *et al.,* 2012), and also similar in range values to the reported studies of the Thai pig breeds (Yang *et al*., 2012). The observed heterozygosity obtained in this study was less than all the values for the expected heterozygosity in the pig populations, this however could be attributed to segregation of non-amplifying (null) alleles, and/or scoring bias (heterozygotes scored wrongly) (Zhu *et al.,* 2004). The high level of heterozygosity recorded in this study is similar to reports of Luetkemeier *et al*. (2010) who also reported higher observed heterozygosity in their studies of some Asian pig breeds. This could be attributable to the mixed nature of the breeds from historic mixing of strains of different populations.

Population differentiation was examined by the fixation indices, F_{IS}, F_{IT} and the F_{ST}, for each locus and across all loci. The average genetic differentiation among breeds (F_{ST}) was 0.1043which implies 89.6 % of the total genetic variation was explained by individual variability, this is low when compared to 0.2858 in previous study by Scali *et al*. (2012) on Cinta Senese and commercial pig breed of Tuscan territory. The mean inbreeding coefficient of the individual relative to the sub-population (F_{IS}) -0.0698 indicated the existence of outbreeding within the pig population which reflects a population not in panmixia. This is not in agreement with the inbreeding coefficient of the individual relative to sub-population (F_{IS}) in a previous study by Scali *et al*. (2012) with positive F_{IS} value which is believed to be as a result of inbreeding and a significant deviation from Hardy-Weinberg equilibrium. The low level of genetic differentiation 0.1043 was supported by the high-level gene flow rate N_M (Nm, number of migrants per generation) which suggests lack of isolation among the breeding pig population. The Nei's genetic distance was used to ascertain the degree of relationship among the four pig populations in Nigeria. The Large White and the Landrace pig breeds revealed the closest relationship, (0.2570) while the farthest relationship (0.6663) was recorded between the Duroc and Large White breeds of pig. This genetic distance relationship between Duroc and Large White breeds (0.6663) is similar to the value of genetic distance on previous study reported by (Kim *et al*., 2005) between the Korean native pig and south china pig breeds (that is Xiang pig 0.594 and Wuzhishan pig 0.672). The closely relatedness between the Large White and the Landrace pig in Ogun and Ondo States, Nigeria could be due to few samples of the Landrace population used in the study.

It has been reported that high exclusion power of microsatellite markers as such reported in this study makes them more valuable for parentage analysis (Rehout *et al.*, 2006; Davila *et al.*, 2009). The value of combine probability of exclusion when the genotype of one parent is known (PE2) and when both parents are excluded (PE3) in this study (0.9999) compares favourably with previous study by Fan *et al. (*2005) on New Zealand pig populations. The Combined exclusion probabilities for the six microsatellite markers among the four breeds of pigs (CP_{E2}, combined exclusion probability of the genotype of a single parent and CP_{E3}, combined probability of exclusion of genotype of both parents) were informative and suitable for parentage analysis since both CP_{E2} and CP_{E3} were above the 0.9995 threshold suggested by Rehout *et al*. (2006), Davila *et al*. (2009) and Souza *et al*. (2012).

Conclusion

High values of mean number of alleles across loci and the expected heterozygosities recorded across loci in the pig populations indicated high genetic diversity in the pig populations in Ogun and Ondo States of Nigeria. Microsatellite markers used in this study were highly polymorphic and informative in the characterization of the studied four pig breeds. The genetic distance values indicated the closest genetic relationship between the Large White and the Landrace pig breeds while the farthest genetic distance was recorded between the Large White and Duroc pig breeds. The low values of genetic differentiation with regard to the F-statistics indicated that the four pig breeds have more within breed variation than between breed variation. Results of the probability of exclusion and combined probability of exclusion of the markers indicate microsatellite markers used were not only informative, but suitable for routine parentage verifications of the pig populations in the states considered in the South-West, Nigeria. The microsatellite markers used in this study were polymorphic and informative for studying genetic diversity of pig populations. The microsatellite markers used are thus recommended for further studies with pig populations irrespective of origins and breeds and also recommended for routine parentage verifications of pig population.

REFERENCES

Adebambo OA, 1982. Evaluation of the genetic potential of Nigerian indigenous pigs, Proceedings of the Second World Conference on Genetics applied to Livestock Production, 543-53. Madrid, Spain.

Ayizanga RA, BB Kayang, K Adomako, C Adenyo, M Inoue-Murayama and L Asamoah 2016. Genetic diversity of some Ghanaian pigs based on micro-satellite markers. J Live Research Rural Dev 28: 150-152.

Botstein D, RL White, M Skolnick and RW Davis 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. J Human Gen, 3:385-387.

Bourn D, W Wint, R Blench, E Wooley, 1994. Nigeria livestock resources survey. World Anim Rev,78:49-58.

Bradic M, M Uremovic, B Mioc, M Konjacic, Z Lukovic and T Safner 2007. Microsatellite analysis of genetic diversity in the black Slavonian pig. ActaVeterinaria (Beograd), 57, (No. 2-3): 209-215.

Caballero A and MA Toro, 2002. Interrelations between effective population size and other pedigree tools for management of conserved population. Gen Res, 33:289-299.

Chen GH, XS Wu, DQ Wang, J Qin, SL Wu, QL Zhou, F Xie, R Cheng, Q Xu, B Liu, XY Zhang and O Olowofeso, 2004. Cluster analysis of 12 Chinese native chicken populations using microsatellite marker. Asian-Aust J AnimSc, 17(8): 1047-1052.

Davila SG, MG Gil, P Resino-Talavan and JL Campo 2009. Evalution of diversity between different Spanish chicken breeds. A tester line, and White Leghorn population based on microsatellite markers. Poultry Sci, 88: 2518-2525.

Dieringer DJ and C Schlottterer 2003. Nonneutral Admixture of Immigrant Genotypes in African Drosophila Melanogaster Population from Zimbabwe. Mol Bio and Evol, 20(8): 1329-13337.

Fan Y, R Kalaba and J Moore 2005. Arriving on time. J Optimization Theory and App, 127: 497-513.

Gama LT, MF Micarolino and JA Santos-Silva 2006. Prion protein in Portuguese breeds of sheep. Livestock J:175-184.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices. Version 2.9.3. Available from http://www2.unil.ch/popgen/sotwares/fstat.htm.

Hale ML, TM Burg and TE Steeves, 2012. Sampling for microsatellite Based Population Genetic Studies: 25 to 30 individuals per population is Enough to Accurately Estimate Allele Frequencies. PLOS ONE 7: e45 170.

Jamieson A and STCS Taylor, 1997. Comparison of three probability formulae for parentage exclusion. Anim Gen, 28: 397-400.

Kamara D, KB Gyenai, T Geng, H Hammade and EJ Smith, 2007. Microsatellite marker-based genetic analysis of relatedness among commercial and heritage turkeys. Poult Sc, 86: 46-49.

Kim TH, KS Kim, BH Choi, DH Yoon, GW Jang, KT Lee, HY Chung, HY Lee, HS Park and JW Lee, 2005. Genetic structure of pig breeds from Korea and China using microsatellite loci analysis. J AnimSci, 83: 2255-2263.

LuetkemeierES, M Sodhi, LB Schook and RS Malhi 2010. Multiple Asian pig origins revealed through genomic analyses. MolPhylogen and Evol 54: 680–686.

NaqviAN, 2007. Application of molecular genetics Technologies in Livestock Production: Potentials for Developing Countries. Advances in Biological Research 1: 72-84.

NBII, 2011. National biological information infrastructure. Introduction to Genetic Diversity. Us. Geological survey Archived from the original of the canary goat populations using microsatellites. Livestock. Sci 102, 140–145.

OllivierL, 2000. Genetic diversity of eleven European pig breeds. Genet SelEvol, 32, 187.

Olowofeso O, M Wheto, SO Durosaro, KO Bankole, DA Adepoju and OV Folarin, 2016. Combined exclusion probabilities of ten microsatellite markers used with Nigerian chicken populations. Euro Int J Sc and Tech, 5(4): 21-32.

Peakall AR and Smouse PE, 2012. GenAlex6.5: Genetic analysis in Excel: population genetic software for teaching and research on update. Bioinformatics 28: 2537-2539.

Piry S, G Luikart and JM Cornuet, 1999. Bottle neck: A computer program for detecting recent reductions in the effective population size using allele frequency data. J Heredity90: 502-503.

Rehout V, E Hradecka and J Citek, 2006. Evaluation of parentage testing in the Czech population of Holstein cattle. Czech. J AnimSci, 51: 503-509.

RIM, 1992. Nigeria Livestock Resource Survey. Volume 4. Report by Inventory and Management Limited (RIM) to FDL and PCS, Abuja, Nigeria.

SanCristobal M, C Chevalet, CS Haley, R Joosten, AP Rattink, B Harlizius, MAM Groenen, Y MY Amigues, G Boscher, A Russel, R Law, V Davoli, C Russo, L Desautes, L Alderson, E Fimland, M Bagga, JV Delgado, JL Vega-Pla, AM Martiniz, P Glodek, JN Meyer, GC Gandini, D Matassino, GS Plastow, KW Siggins, G Laval, AL Archibald, D Milan, K Hammond and R Cardellino, 2003. Genetic diversity within and between European pig breeds using microsatellite markers. Anim Genet, 37(3): 189-198.

Scali M, V Rita, B Jacopo, P Elisa, B Andrea, S Ottavia, N Neri and C Mauro, 2012. Genetic differentiation between Cintas senese and breeds using Microsatellite. JBiotech, 15: 1-11.

Silva EC, WM Dutra Jnr, WM jnrIanella, P Gomes Filho, MA Oliveiral, C Moura-Ferreira, DN Caetan, AR and SRPaiva, 2011.Patterns of genetic diversity of local pig populations in the State of Pernambuco, Brazil.Research of Brasília Zoo Technology, 40: 1691-1699.

Souza CA, SR Paiva, CM Mcmanus, HC Azevedo, AS Mariante and D Grattapaglia, 2012. Genetic diversity and assessment of 23 microsatellite markers for parentage testing of Santa Ines hair sheep in Brazil. Genet Mol Res, 11 (2): 1217-1229.

Teneva A, N Tomlekova and G Goujgoulova, 2014. Major features mutation mechanism and development of microsatellite as genetic markers. Bulg. J Agric Sci,20: 949-956.

Toro MA, J Rodriganez, C Barragan, E Alves, C Gortazar and L Silio, 2008. Genetic diversity and allelic richness in Spanish wild and domestic pig population estimated from microsatellite markers. Spanish J Agric Res6 (special issue) 107-115.

Vicente AA, MI Carolino, MCO Sousa, C Ginja, FS Silva, AM Martinez, JL Vega-Pla, N Carolino and LT Gama, 2008. Genetic diversity in native and commercial breeds of pigs in Portugal assessed by microsatellites. J Animl Sci. 86(5): 2496-2507.

Vrtková I, L Stehlík, L Putnová, L Kratochvílová and L Falková, 2012. Genetic Structure in Three Breeds of Pigs Populations Using Microsatellite Markers in the Czech Republic. Res In Pig Breeding 6 (2): 83-85.

Wang X, HH Cao1, SM Geng and HB Li, 2004. Genetic Diversity of 10 Indigenous Pig Breeds in China by Using Microsatellite Markers. Asian-Aust J AnimSci,17: 1219-1222.

Yang SL, B Surinton, NL Pongchan, NN Uthairat and ZH Shi, 2012. Genetic Variation and Population Structure of Thai Indigenous Pig populations based on mitochondrial and Microsatellite DNA Markers. JAnimlVet Adv11: 509-516.

Zhu CS, M Gore, ES Buckler and JM Yu 2004. Status and prospects of association mapping in plants. Plant Genome1: 5-20.