



## Investigation of Microsatellite Markers in Genetic Identification of Breeds in Untagged White Skinned Pig Populations

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### Article Info

#### Article history:

Received: May 24, 2023

Revised: January 25, 2024

Accepted: March 5, 2024

#### Keywords:

Exotic pigs,

Microsatellite markers,

Genetic diversity,

Phylogenetic tree

### Abstract

This study identifies genetic differences that distinguish one breed from another within white skinned pig populations. The Number of alleles ( $N_a$ ) total means was  $3.48 \pm 0.26$ . The Landrace had the highest number of allele ( $N_a$ )  $4.00 \pm 0.54$  among the 3 populations while the Large White had the least  $3.14 \pm 0.51$ . The mean of Observed heterozygosities ( $H_o$ ) of Crosses was highest ( $0.69 \pm 0.11$ ) among the three while they all had the same expected heterozygosities ( $H_e$ ) of 0.55. The means of  $F_{IT}$  (measures of the deviation from Hardy-Weinberg in the total population) and  $F_{ST}$  (measures of the genetic differentiation over subpopulations) among the markers were  $0.06 \pm 0.11$  and  $0.04 \pm 0.01$ , respectively.  $F_{IT}$  value was the highest for SW632 (0.41), while it was the lowest for SW226 (-0.05). The phylogenetic tree shows that the three pig populations originated from the same ancestor and branched into two where Landrace and Crosses clustered together while the Large White clustered alone. This study helps to distinguish between the pig breeds and revealed their genetic makeup and identity.

### Introduction

Livestock contribute significantly to the global agricultural output, supporting the livelihoods and food security of nearly a billion people. The world's animal production relies heavily on a small number of domesticated species, with 14 species accounting for 90% of the total production (Drago and Sumarstvo, 2011). In Nigeria, the increase in population rate has influenced the demand for protein intake where the 3.5g/caput/day is less than 35 g/caput/day recommended by the FAO (2011). Ironkwe and Amefule (2008) described pigs as one of the most prolific and fast-growing livestock that can convert food waste to valuable products. They excel in converting feed to flesh than other red meat animals such as cattle, sheep and goat.

The first set of exotic pigs, Mollington and Bradbuns strains now called the Large White or Yorkshire, were imported into the country at the

Institute of Agricultural Research and Training in Ibadan, in 1944 from Britain to improve Nigerian indigenous pig (NIP). While Duroc and Hampshire breeds were imported into Oyo and Lagos States in 1956, the Landrace breeds were imported from Republic of Togo (Adebambo, 2003). These pigs thrive well in Nigeria at this period. Currently, the most common ones in Nigeria are Large White, Landrace and Duroc. These breeds thrive well in Nigeria and made the farmers and pork consumers to neglect our indigenous because of its small body size.

Heritability is a measure of the genetic control of a trait. The KIT gene on chromosome 8 is responsible for the dominant white color in pigs due to a gene duplication and a splice mutation (Johanson et al., 1992; Marklund et al., 1998; Xu et al. 2020). White colour is a dominant gene in pig and its heterozygous or carrier always have white colour phenotype (Xu et al., 2020).

Microsatellites have been reported to be used intensely for population studies in commercial as well as rare pig breeds in the last 25 years (Giuffra et al., 2000; SanCristobal et al.,

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2006; Gama *et al.*, 2013). Due to its abundance, distribution in the genome, high level of polymorphism and ease of genotyping, microsatellite has been proposed as convenient markers for evaluating genetic diversity of domestic animals (Joshi *et al.*, 2012). It has been used for traceability of different pig breeds meat in meat products (Blasi *et al.*, 2003; Oh *et al.*, 2014; Zhao *et al.*, 2017; Xing *et al.*, 2018). The expected heterozygosity (He), observed heterozygosity (Ho) and polymorphism information contents (PIC) are important parameters for the selection of markers for pig breed identification (Zhao *et al.*, 2017; Joshi *et al.*, 2012).

Andersson and Georges (2004) reported that comparative genomic analysis of different domestic animal breeds can explain the genetic basis of phenotypic variation of several domestic animal breeds. The complete pig genome sequencing project provided information molecular mechanisms controlling phenotype and plays significant role in the pork meat production. Chen *et al.* (2019) reported an extensive information deriving from the *Sus scrofa* genome sequencing that the number of DNA molecular markers available for Quantitative Trait Loci (QTL) characterization and genotyping has risen dramatically since 2003. Preserving local breeds is essential to prevent the loss of genetic resources, especially for species crucial to food production, rural development, and environmental protection (Ceccobelli *et al.*, 2020). Toro *et al.* (2009) report that in recent years, estimation of genetic distances between different populations of livestock animals has been based on information on microsatellite polymorphism. Indiscriminate crossing of the commercial pigs with existing breed or unknown pedigree, in order to upgrade them, has caused the impurity of these breeds. This also caused some pig vendors selling mixed or non-identifiable breeds to farmers due to the white colors of these commercial exotic breeds. Fraudulent labeling of pigs to farmers as a pure breed are the common atrocities going on presently in Nigeria. This study aims to identify markers with sufficient genetic information to discriminate between the three main white coloured pig populations in Southwestern Nigeria and to estimate the level of their genetic diversity.

## Materials and Methods

### Animal materials and DNA isolation

Genomic DNA of thirty-three pigs obtained from Southwestern Nigeria, consisting of fourteen Landrace, eleven Large White and eight crosses between these breeds were extracted from FTA (Flinders Technology Associates) paper and quantify by method of Oluwole *et al.* (2016). The DNA concentration and quality were checked using Nanodrop™ spectrophotometer and 1% agarose gel electrophoresis, respectively. Genotyping was done using optimized primers recommended by ISAG (2011) for both breed and individual identification in pigs.

### Polymerase chain reaction Amplification

Polymerase chain reaction (PCR) reaction of 15µl volume each containing of 2 µl of genomic DNA, 8.25 µl of primermix, 1.5 µl of 10XPCR buffer (10mM Tris-HCl, 50mM The KCl, 1.5mM MgCl<sub>2</sub>, pH 8.3), 1.2µl dNTPs (2.5 mM), 0.4µl Taq polymerase (10 unit/µl). Multiplex PCR reaction of initial denaturation at 95°C for 15 min; 5 cycles at 94°C, 1min; 55°C, 1min; 72°C, 1min, 10 cycles at 94°C, 1min; 54°C, 1:15min; 72°C, 1min and 24cycles at 94°C, 1min; 53°C, 1:15min; 72°C, 1min and a final elongation at 65°C, 30min. The amplified products were checked on 1 % agarose gel and stained with green die (0.5µl)..

### Genotyping

This was achieved by labeling DNA fragments that were labeled with fluorescent dyes to make them visible under the machine's detection system. The labeled DNA fragments were loaded onto the GeneMapper® Software Version 4.0 (Applied Biosystems) (by following users guide), which uses capillary electrophoresis to separate the fragments based on their sizes and charges. The machine detected the labeled fragments and recorded their sizes and intensities of fluorescence as the DNA fragments move through the capillary tube. Electropherogram generated, which was a graphical representation of the DNA fragments' sizes and intensities, were analyzed by genemapper software with connection to the computer screen, where kit, panel and bins were created and bins set were generated by using auto bin feature. The electogram were matched to the reference database of genetic markers in order to map the location of specific genes within the

DNA sample. The final results were obtained and interpreted to determine the presence or absence of specific genes or genetic variations within the sample (band scoring).

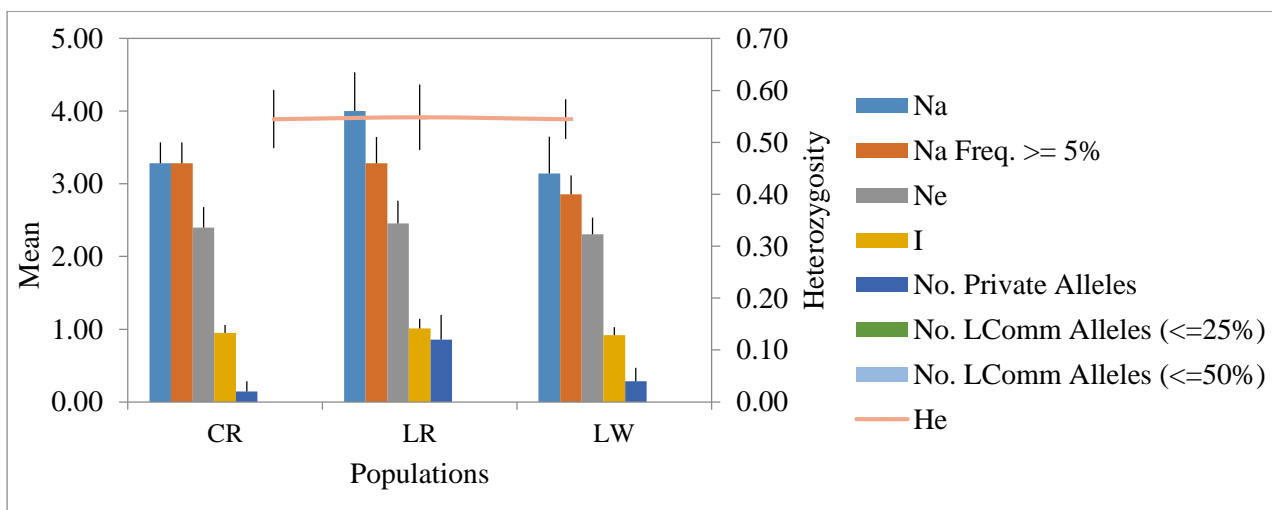
**Data Analysis**

The GenAIEX® software version 6.41 was used to estimate allele frequencies, mean number of alleles per locus (NA), observed heterozygosity (Ho), heterozygosity expected (He) from Hardy-Weinberg (HW) assumptions. The GenAIEX® manual was fully followed. The genetic variation among population and individual of the total genetic variance were calculated with Analysis of Molecular Variance (AMOVA).

The number of alleles, observed heterozygosity (HOb), expected total heterozygosity (HEx), Pairwise FST and inbreeding coefficients (FIS and FIT) and Expected probability of identity (PI) value among genotypes of random individuals were calculated using GenAIEX® software version 6.41. The polymorphic information contents (PIC) were calculated using Genecalculators (<https://www.genecalculators.net/pq-chwe-polypicker.html>). The phylogenetic tree was constructed by Poptree2® software (<http://www.med.kagawau.ac.jp/~genomelb/takezaki/poptree2/download.html#download>). A phylogenetic tree was constructed from allele frequency data using neighbor-joining method (Saitou and Nei 1987) with bootstrap tests of 1000.

**Results and Discussions**

The number of alleles (Na), effective number of alleles (Ne) and heterozygosity were presented graphically in Fig. 1. The genetic parameters of the 7 microsatellite markers were shown in Table 1. The Number of alleles Na total means was 3.48±0.26. The Landrace had the 4.00±0.54 among the 3 populations while the Large White had the least 3.14±0.51. The mean of Observed heterozygosities (Ho) of CR was highest (0.69±0.11) among the 3 while they all had the same expected heterozygosities (He) of 0.55. These results were higher than those reported by Scali *et al.* (2012) on genetic differentiation of Cinta Senese and commercial pigs on white colors (0.46 Ho and 0.53 He) and Jeju and Berkshire that ranged from 0.54 to 0.71(He) and 0.54 to 0.68 Ho on meat products (Oh *et al.*, 2017). Among the 7 microsatellites, Na ranged from 2.00 (CR-S009; LW-SW26 and SW226) to 7.00 (LR, SW122). The heterozygosities- Ho were ranged from 0.25 (LR-SW227 and 632) to 1.0 (CR-SW122 and 226) and He ranged from 0.23 (LR-SW127) to 0.74 (LR-SW122). From the previous studies, heterozygosities (He and Ho) values must be average between 0.3 and 0.8 before considering useful for identification and diversities of breeds (Takezaki *et al.*, 1996) and Oh *et al.*, 2014). From this study, He and Ho total mean were 0.55±0.03 and 0.61±0.05 (Table 2).



**Fig. 1:** Allelic Patterns across Populations

LW-Large White; LR- Land race; CR- Crosses; Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1 \* Sum F = Fixation Index = (He - Ho) / He = 1 - (Ho / He)(pi \* Ln (pi)). Ho = Observed Heterozygosity = No. of Hets / N; He = Expected Heterozygosity = 1 - Sum pi^2; F = Fixation Index = (He - Ho) / He = 1 - (Ho / He)

**Table 1:** Genetic Diversity Parameters of 7 Microsatellite Markers among the populations

Pop	Locus	Na	Ho	He
CR	SW26	3.00	0.83	0.54
	S009	2.00	0.50	0.38
	SW24	4.00	0.80	0.66
	SW122	4.00	1.00	0.63
	S0226	4.00	1.00	0.72
	S0227	3.00	0.38	0.32
	SW632	3.00	0.33	0.57
	Mean	3.29±0.29	0.69±0.11	0.55±0.06
LR	SW26	3.00	0.73	0.53
	S009	3.00	0.60	0.54
	SW24	4.00	0.57	0.66
	SW122	7.00	0.86	0.74
	S0226	4.00	0.83	0.65
	S0227	3.00	0.25	0.23
	SW632	4.00	0.25	0.484
	Mean	4.00±0.54	0.59±0.10	0.55±0.06
LW	SW26	2.00	0.67	0.44
	S009	3.00	0.50	0.57
	SW24	3.00	0.33	0.63
	SW122	6.00	0.91	0.71
	S0226	2.00	0.60	0.42
	S0227	3.00	0.46	0.53
	SW632	3.000	0.40	0.52
	Mean	3.14±0.51	0.55±0.07	0.55±0.04

LW-Large White; LR- Land race; CR- Crosses; Na = No. of Different Alleles, Ne = No. of Effective Alleles =  $1 / (\sum \pi^2)$ , I = Shannon's Information Index =  $-1 * \sum F$  = Fixation Index =  $(He - Ho) / He = 1 - (Ho / He)(\pi * \ln(\pi))$ . Ho = Observed Heterozygosity = No. of Hets / N; He = Expected Heterozygosity =  $1 - \sum \pi^2$

**Table 2:** Mean of the Genetic parameters for the 7 Microsatellite markers

Locus	Na	Ho	He
SW26	2.67±0.33	0.74±0.05	0.51±0.03
S009	2.67±0.33	0.53±0.03	0.50±0.06
SW24	3.67±0.33	0.57±0.14	0.65±0.01
SW122	5.67±0.88	0.92±0.04	0.70±0.03
S0226	3.33±0.67	0.81±0.12	0.60±0.09
S0227	3.00±0.00	0.36±0.06	0.36±0.09
SW632	3.33±0.33	0.33±0.04	0.52±0.03
Total Mean	3.48±0.26	0.61±0.05	0.55±0.03

Table 3 shows results of analyses of Wright’s  $F$ -statistics that were carried out using Genealex software. The mean  $F_{IT}$  and  $F_{ST}$  among the markers were  $0.06 \pm 0.11$  and  $0.04 \pm 0.01$ , respectively.  $F_{IT}$  value was the highest for SW632 (0.41), while it was the lowest for SW226 (-0.05).  $F_{ST}$  value of SW226 was the highest (0.08) and that of SW26 was the lowest (0.01). The negative values obtained in this study for  $F_{IS}$  and  $F_{IT}$  at loci SW26, SWS009, SW222, SW226 and SW 227 show that there was higher proportion of heterozygous genotypes than the homozygous genotypes at all those loci. Table 4 shows the pairwise  $F_{st}$  values among the 3 populations ranged from 0.03 to 0.04. The low pairwise  $F_{st}$  values among breeds studied indicated that 97.0% to 96.0% of the total genetic variation was explained by the individual variability. These values also corroborated with mean value of  $F_{st}$  (0.04) results obtained in the present study.

Table 5 show the Polymorphic information contents (PIC) of the three populations and their mean. The landrace had the largest mean and was also significant ( $P < 0.01$ ) at loci SW632 while the Large White was significant ( $P < 0.05$ ) at SW227. It has been reported that the PIC should be from 0.5 and above before considering useful for identification and diversities. Approximately all the mean will be equal to 0.50 that made all the markers good generally but 3 individual markers were lower than 0.45 (SW24, S009 and SW227) while others were greater than 0.45 approximately to 0.50. Therefore only 4 of the 7 markers can be used for traceability of coat color. The same results were reported by for SW26, S009 and SW228 that was close to SW227. PIC is a parameter for indicating genetic variation and for measurement of marker’s informativeness. PIC values can vary from 0 to 1, indicating a greater level of polymorphism or variation when the value is closer to 1 (Ricardo *et al.*, 2008).

**Table 3:** Analysis of Wright’s  $F$ -statistics

Pop	loci SW26	loci S009	Loci SW24	Loci SW122	Loci S0226	Loci S0227	Loci SW632	Mean
Fis	-0.47	-0.08	0.13	-0.33	-0.36	-0.00	0.37	-0.11±0.11
Fit	-0.45	-0.05	0.17	-0.35	-0.25	0.04	0.41	-0.06±0.11
Fst	0.01	0.03	0.05	0.03	0.08	0.04	0.06	0.04±0.01

Mean number of alleles ( $N_a$ ), number of effective alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), number of private alleles ( $P_a$ ), expected heterozygosity ( $H_e$ ) and Shanon Index (I) of all 7loci per population

**Table 4:** Nei Pairwise Population  $F_{st}$  Values for the 3 population

CR	LR	LW
0.00		CR
0.03	0.00	LR
0.04	0.03	0.000

LW- Large White; LR- Land race; CR- Crosses

**Table 5:** Polymorphic Information Contents (PIC) for the Three Populations

Parameters	Loci SW26	Loci S009	Loci SW24	Loci SW122	Loci S0226	Loci S0227	Loci SW632	PIC Mean
Crosses	0.46	0.30	0.61	0.56	0.67	0.29	0.48	0.48
Landrace	0.43	0.47	0.60	0.70	0.60	0.22	0.44**	0.49
Large White	0.35	0.51	0.38	0.67	0.33	0.47*	0.42	0.45
Mean	0.41	0.43	0.53	0.64	0.53	0.33	0.45	0.48

Key: ns=not significant, \* P<0.05, \*\* P<0.01. PIC - Polymorphic Information Contents

Table 6 show the Pairwise Population Matrix of Nei Genetic Distance (below diag-onal) and Pairwise Population Matrix of Nei Genetic Identity (above diagonal) among the 3 pig populations. The genetic similar coefficients varied from 0.90 to 0.94 with an average of 0.92. The Genetic distances between the three pigs were 0.08 (crosses and Landrace), 0.11 (Crosses and Large White) and 0.067 (Large White and Landrace) respectively. The land race and LW were closer compared with the crosses.

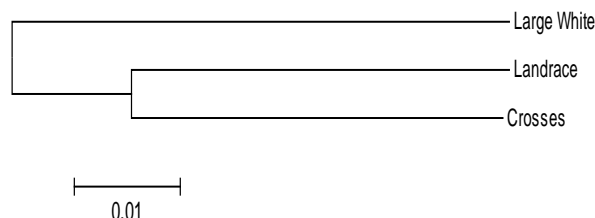
**Table 6:** Nei’s Matrix of standard genetic distances and Identity

CR	LR	LW	
0.00	0.92	0.90	CR
0.08	0.00	0.94	LR
0.11	0.07	0.00	LW

Note: Upper diagonal - Genetic Identity, Lower diagonal - Genetic Distance, LW-Large White; LR-Land race; CR- Crosses

A phylogenetic tree was estimated based on the equation of Nei *et al.* (1983), by the distribution of allele sharing by genetic distance (D) using POPTREE2 software. A phylogenetic tree constructed from allele frequency data, using neighbor-joining method (Saitou and Nei 1987) with bootstrap tests of 1000 as shown in Figure 2. The three pig populations originated from the same ancestor and branched into two where Landrace and crosses clustered together while the Large White clustered alone. This indicated that the crosses contain mostly the genes of Landrace, hence they are hybrid of Landrace.

They share a coat colour but the markers and the dendrogram were able to distinct them from each other and revealed their genetic makeup or identity.



**Fig. 2:** Phylogenetic tree of the 3 populations

**Conclusion**

This study concluded that there was genetic variation among the 2 exotic breeds (Large White and Landrace) and enough differences between them and the crosses used in this study, this was revealed by the genetic parameters using the molecular markers and the phylogenetic tree. There was enough polymorphism among the seven microsatellite markers but only four of them can be used for white coat traceability because of their low values in Polymorphic information contents and expected heterozygosities. This is just a preliminary work on the genetic variations of coat colours of pigs. It is recommended that more microsatellite markers can be used to investigate further.

**Acknowledgement**

The laboratory aspects of this project was partly/fully supported by the BecA-ILRI Hub through the Africa Biosciences Challenge Fund (ABCF) program and Africa women in Agriculture and development (AWARD). The ABCF Program is funded by the Australian

Department for Foreign Affairs and Trade (DFAT) through the BecA-CSIRO partnership; the Syngenta Foundation for Sustainable Agriculture (SFSA); the Bill & Melinda Gates Foundation (BMGF); the UK Department for International Development (DFID) and; the Swedish International Development Cooperation Agency (Sida).”

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