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Original article

Genetic variability among pig populations in Imo State, Nigeria using random amplified polymorphic DNA-PCR

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Abstract

Random amplified Polymorphic DNA polymerase chain reaction (RAPD DNA-PCR) was employed to access the genetic variability and phylogenetic relationship among pig populations in Imo State. Genomic DNA from 50 pigs comprising of at least 10 pigs per geopolitical zone of Imo state were extracted and two highly polymorphic primers utilized to estimate variability, phylogenetic relationship among the pigs and their genetic diversity. The genetic distance and genetic identity estimated showed that genetic distances (D) calculated ranged between 0.0300 (*Songhai* vs *FUTO*) and 0.1497 (*Mbaise* vs *Awo-Idemili*), while the genetic identity (I) calculated ranged between 0.8610 (*Mbaise* vs *Awo-Idemili*) and 0.9704 (*Songhai* vs *FUTO*). Similarity was observed between *Songhai, Mbaise* and *FUTO* populations, while *Mbano* and *Awo−idemili* exhibited dissimilarity. The phylogenetic tree showed that *Songhai, FUTO* and *Mbaise* populations are more closely related while *Mbano* and *Awo-idemili* are more genetically distant. The standard genetic diversity (h*) of total population ranged from 0.0001 to 0.4998 with an average of 0.3208, while Shannon's information index (I*) ranged from 0.0001 to 0.6929 with a mean of 0.4762. This study shows that genetic diversity of the RAPD DNA polymorphs amongst pig populations in Imo State is low while the genetic relationship between the different populations varies from population to population.

Keywords: Genomic; Diversity; Polymorphic; Shannon index; Genetic distance; Genetic identity; Phylogenetic; Genetic diversity

1. Introduction

Increased loss of genetic diversity has been observed for all domestic livestock species all over the world, with the exotic and developed breeds being more selected. It has been estimated that more than half of our once common livestock breeds are now endangered [6]. The population of Nigerian Indigenous Pigs (NIPs) is almost extinct, and these pigs are an important source of food and livelihood for some people especially in the rural South eastern and Middle Belt regions of Nigeria where they are reared mostly in extensive systems of production [16]. The NIPs have not been developed into breeds but can be differentiated from modern commercial pigs by their characteristic features of black, white, black and white or pied color with well-developed hair coat and erect ears, small, long and shallow bodies with a level back [1]. However, the existence of NIPs is fast eroding through large scale crossing with foreign commercial breeds such as Large White, Landrace, Hampshire and Duroc breeds to exploit heterosis [16]. There is evidence that suggests that most commercial pig farmers crossbreed their exotic stock with NIPs in order to attain to a great extent the hardiness and disease resistant characteristics of the NIPs in their flock [9].

Random amplified polymorphic DNA (RAPD) markers have proven to be a very useful tool for providing a convenient and rapid assessment of the genetic differences between genotypes [19]. Similarly, random amplified polymorphic DNA-

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polymerase chain reaction (RAPD-PCR) technique has been used for estimation of the genetic variability among the breeds/species, but has low reproducibility [19]. RAPD markers are the randomly amplified target regions of less functional part of the genome that do not strongly respond to selection of the phenotypic level. Such amplified regions might accumulate more mutations, thereby offering a wider potential in assessing the intra/interbred genetic differentiations. The RAPD-PCR has been used to estimate the genetic variabilities among livestock species [3,4,8,11]. However an application of the RAPD technique in the diversity and variability analysis of the crossbred commercial breeds of pigs is lacking. Hence, this study is aimed at identifying the level of genetic variability, similarity and diversity among the commercial pig population in Imo state using RAPD DNA-PCR markers by estimating the genetic distance (D) and identity (I) between populations, phylogenetic tree as well as genetic diversity index (h*) and Shannon Information Index (I*) respectively among 5 pig populations adapted and commercially used in pig production in the South-Eastern state of Imo State, Nigeria.

2. Materials and methods

2.1. Experimental animals

A random sample of 50 unrelated pigs comprising of at least 10 animals from each of the three geopolitical zone of Imo State were used for the experiment. Imo State is located in the tropical rainforest zone of South Eastern Nigeria, covered within Latitude 4^0 04′, $6^0$03′ N and Longitude $6^0$15′, $8^0$15′W of equator. The geopolitical zones include Owerri, Orlu and Okigwe, with the sources and sample sizes in each zone to include FUTO farm (10 pigs) and Mbaise farms (10 pigs) representing Owerri zone, Songhai farms (10 pigs) and Awo-idemili farms (10 pigs) representing Orlu zone and Mbano1 farms (5pigs) and Mbano 2 farms (5pigs) representing Okigwe zone.

2.2. Sample collection and processing

Approximately, 2 ml of whole blood samples was collected from each animal through the venepuncture of the marginal ear veins of pigs using 2 ml syringes, dispensed into clean plastic tubes containing EDTA (ethylene-di-amine-tetra-acetic acid), vigorously shaken and stored in ice packs and later transported to the National Institute for Medical Research (NIMR) Yaba Lagos State, Nigeria Biotechnology Laboratory where genomic DNA extraction, RAPD DNA PCR analysis and electrophoresis were carried out.

Each pig sampled was as a result of its phenotypic appearance which bears credence to possible crossbreeding with local animals.

2.3. DNA extraction

DNA was extracted using *Zymo-Spin*™ Genomic DNA Kit and purified following the manufacturer's standard i.e. with 100 μl whole blood, 400 μl of Genomic Lysis Buffer was added. Mixing was done by vortexing 4–6 s, and then let to stand for 5–10 min at room temperature. The mixture was later transferred to a *Zymo-Spin*™ Column in a Collection Tube and centrifuged at 10,000 × g for 1 min. The collection tube was discarded with the flow through. The *Zymo-Spin*™ Column was transferred to a new collection tube. 200 μl of DNA Pre-wash buffer was transferred to the spin column and centrifuged at 10,000 × g for 1 min. 500 μl of g-DNA Wash Buffer was added to the spin column and then centrifuged at 10,000 × g for 1 min. The spin column was transferred to a clean microcentrifuge tube, 50 μl of Elution Buffer was added to the spin column and incubated for 2–5 min at room temperature and then centrifuged at top speed for 30 s to elute the DNA. The eluted DNA was used immediately for molecular based applications and the remaining stored below −20 °C for future use.

2.4. PCR programme and conditions for RAPD marker

Two highly polymorphic RAPD primers (Operon Technologies Inc.) with 60–70% GC content (OPA-06 and OPA-19) was selected from literature and used based on their distinct polymorphism which produced more than 5 numbers of bands. The list of primers, their sequence and GC content are given in Table 1.

The PCR conditions with the final volume of 25 μl included; 2.5 μl of 12 × PCR Buffer, 1.5 μl of 12 mmol/L $MgCl_2$, 1 μl of dNTPs (12 mm dNTPs), 1.25 μl of each random primer, 0.5 μl *Taq* DNA polymerase, 13.25 μl distilled water and 5 μl of each template DNA from individual animal was used. PCR was performed in a thermal cycler machine which is programmable (i.e. thermal cycler of Biometra, Germany) with the following amplification conditions: initial denaturation at 94 °C for 2 min, followed by denaturation at 94 °C for 1 min, annealing at 40 °C for 1 min, extension at 72 °C for 2 min for 40 cycles and final extension at 72 °C for 5 min and cooling at 4 °C. The 20 μl PCR products was loaded in 1.5 agarose gel and run at 100 V for 4 h. Gel photograph was captured on gel documentation system.

Only distinct and prominent bands were scored for estimation of various parameters. The presence and absence of bands was recorded as "1" and "0", respectively. The binary coded characters (1,0) were used for genetic analysis.

2.5. Statistical analysis

From the gel results, band sizes were determined by visual appraisal with the aid of standard markers. Band presence and absence was designated 1 or 0, respectively. The genetic distance (D) and genetic identity (I) were estimated using [17]

Table 1
The primers with their decamer sequences and GC content.

Number	Oligo name	Sequence (5′->3′)	G + C content (%)
1.	OPA-06	GGTCCCTGAC	70
2.	OPA-19	CAAACGTCGG	60

according to [15] genetic distance and identity equations. While genetic similarity and dissimilarity among populations was analyzed by generating phylogenetic tree using Nei genetic distance with UPGMA (Un-weighed Pair Group Method of Arithmetic average) analysis through POPGENE software. The genetic diversity was also estimated using Nei's genetic diversity index (h*) and Shannon Information Index (I*) [17].

3. Results and discussion

The two primers of choice (OPA-06 and OPA-19) produced polymorphic band patterns, which were clear, scorable and interpretable in all the pig populations which ranged between 250 and 10,000 base pairs as shown in Fig. 1. Table 2 shows the [15] unbiased measures of genetic identity and genetic distance with the genetic distance (D) ranging between 0.0095 (*FUTO*vs*Mbaise*) and 0.1238 (*Mbaise* vs *Awo-Idemili*), while the genetic identity estimated ranging between 0.8836 (*Mbaise* vs *Awo-Idemili*) and 0.9905 (*FUTO*vs*Mbaise*), respectively.

Table 3 shows the summary of genetic variation for all the loci observed using Nei's genetic diversity index and Shannon Information Index. The genetic diversity index measured using [14] standard genetic diversity approach (h*) ranged from 0.0000 to 0.4998 with an average of 0.3208, while using Shannon's information index approach (I*), the genetic diversity estimated for both primers ranged from 0.0000 to 0.6929 with a mean of 0.4762.

It can be observed that the genetic distance is highest between *Mbaise* and *Awo-Idemili* (D = 0.1238) and lowest between FUTO and Mbaise (D = 0.0095) while the genetic identity was found to be high between FUTO and Mbaise (I = 0.9905) and lowest between Mbaise and Awo-idemili (I = 0.8836). Kumar et al. [13] reported a similar finding in sheep, where the genetic distance was found highest between Malpura and Garole (D = 0.1428) and the lowest (D = 0.0612) between Avikalin and Chokla. However the genetic identity was highest (I = 0.9406) between Avikalin and Chokla and the lowest (I = 0.8669) between Mapura and Garole. The highest genetic distance obtained in this study however was 0.1238 (Mbaise and Awo-Idemili) which is higher than all the genetic distances obtained between Taoyuan (T), Duroc (D) and Taiwan Black (TB) pigs which were 0.080, 0.064, and 0.096, respectively [20], using random RAPD markers in the three pig populations, which could be as a result of differences in breed types studied. This implies that the pig populations in FUTO and Mbaise are more genetically closer while those of Mbaise and Awo-Idemili were more distantly related. The inter population variability may be due to the difference in the breed/strain architecture and method of selection and breeding, as well as sources of samples for breeding and replacement stock. Most commercial farmers buy replacement boars for breeding from neighboring farms, and this could be shown in the close genetics of FUTO and Mbaise populations which are the closest neighbors in terms of distance. Fig. 2 shows the phylogenetic tree among the populations studied. This shows that pig populations from Mbano and Awo-Idemili farms are more genetically distant while FUTO, Mbaise and Songhai are more genetically close.

Table 2
Nei's [15] unbiased measures of genetic identity and genetic distance.

Pop. Id	Songhai	FUTO	Mbaise	Awo-Idemili	Mbano
Songhai	*****	0.9871	0.9468	0.9397	0.8932
FUTO	0.013	*****	0.9905	0.8956	0.8971
Mbaise	0.0547	0.0095	*****	0.8836	0.9425
Awo-Idemili	0.0622	0.1102	0.1238	*****	0.9040
Mbano	0.1129	0.1086	0.0593	0.1010	*****

* Values above the diagonal represent the genetic identity and below the diagonal represent the genetic distance.

Table 3
Summary of genetic variation for all loci using Nei's genetic diversity index (h*) and Shannon information index (I*).

Locus	Sample size	h*	I*
OPA06-1	50	0.3234	0.5043
OPA06-2	50	0.4994	0.6926
OPA06-3	50	0.0000	0.0000
OPA06-4	50	0.3198	0.5002
OPA06-5	50	0.0707	0.1573
OPA06-6	50	0.1106	0.2235
OPA19-1	50	0.4998	0.6929
OPA19-2	50	0.4683	0.6611
OPA19-3	50	0.3669	0.5534
OPA19-4	50	0.4594	0.6520
OPA19-5	50	0.4105	0.6008
Mean	50	0.3208	0.4762
S.E (0.05)		0.1802	0.2396

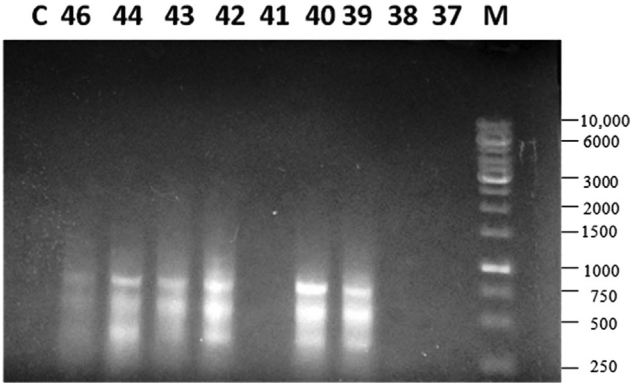

Fig. 1. RAPD PCR band patterns of some samples from *Awo-Idemili* and *Mbano* populations generated by using OPA-06 primer.

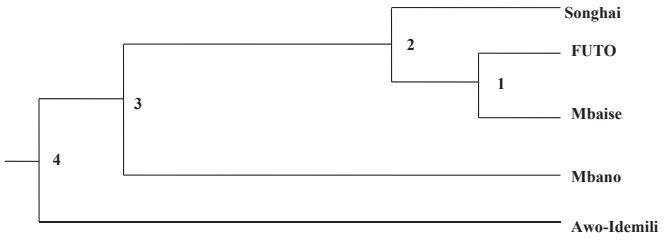

Fig. 2. Dendogram showing phylogenetic relationship between the pig populations.

4. Conclusion

Local pig farmers are advised to acquire their replacement stock from a more distant population than within their geopolitical zone, as this will reduce the chances of inbreeding and ensure exploitation of heterosis during crossbreeding.

The genetic diversity showed an average of 0.3208 and 0.4762 observed using the Nei's and Shannon's genetic index, respectively. Similar values of genetic polymorphism ($h = 0.298$ and $I = 0.455$) have been reported in see–see partridge [10] and other avian species such as Manchurian pheasant and Shiver ring-necked pheasant [7,12]. Agaviezor et al. [2] reported values ranging from 0.022 to 0.146 for H*s* and values of 0.033–0.211 for G*st* in the study of genetic diversity in Nigerian sheep breeds using microsatellite markers. Both H*s* and G*st* values were significantly different but revealed that gene diversity among the breeds of sheep were still low. Yale et al. (2001) also reported within-population genetic similarity (WGS) for T, D, and TB populations of 0.742, 0.747, and 0.745, respectively, and between-population genetic similarity (BGS) of 0.946 between T and TB; 0.953 between D and TB; and 0.934 between D and T were observed. However [5] reported an average genetic diversity of 0.53 in Balkan pig breeds in Europe which is low compared to that of conventional breeds such as Landrace, Duroc, and Large White. This shows that genetic diversity values reported in this study are still very low.

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