Full Length Research Paper

Cytogenetic characterization of Nigerian indigenous pig

Oluwole, O. O.* and Omitogun, O. G.

Department of Animal Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

Accepted 17 August, 2009

This work studied the chromosomal structure of the Nigerian indigenous pig (NIP). The karyotype was obtained and a detailed karyogram constructed. Using sixty-two metaphases of the NIP obtained from both mature male and female leucocytes culture, the chromosomal structure was studied. The karyotype obtained was based on the chromosome's relative length, centromere position and arm (p/q) ratio. The cells had the usual diploid complement of 38 chromosomes (2n = 38) and a *nombre fondamental* of 64 as in the exotic. The karyotype of NIP was characterized by submetacentric chromosome pairs 1 - 7; metacentric chromosome pairs 8 to 12 and telocentric chromosome pairs 13 to 18 with submetacentric XX for female and XY for male. A detailed karyogram was constructed from the obtained results.

Key words: Karyotype, Nombre fondamental, Nigerian indigenous pig.

INTRODUCTION

The black hairy NIP is known with special traits such as greater resistance to African swine fever and heat tolerance of high parasitic load, high percent of weaner weaned and low age at puberty (Adebambo,1982). NIP thrive better in extreme condition than exotic pig (Ilori, 1974). Due to extensive breeding with introduced exotic pig breed, there is a risk of loosing the gene pool of these traits (Adebambo, 2003). From genome analysis point of view (Galman et al., 1991), pig has the same genome compared to that of human (30 morgans or 3×10^9 bp in length). The karyotype of the domestic pig composed of 18 pairs of autosome and pairs of sex chromosomes (X or Y) are already well characterized of which 13 are metacentic and 6 are acrocentric (CSKSS, 1988;CTA, 1994; Fairbank 1999).

Much cytogentic work has been done on the exotic pig bread but none has been done on the NIP (Omitogun, 2004). This paper therefore aims at filling this gap by investigating the characteristics of the NIP at the chromosomal level and to compare its karyotype with the already published karyotype of the exotic pig.

MATERIALS AND METHODS

Animals were purchased from three remote areas of South Western

Nigeria; Otan Ile (Ilesa), Iwo and Ile-Ife and maintained at the Teaching and Research Farm of Obafemi Awolowo University. Blood was collected from the jugular veins using 18-gauge syringes and immediately transferred into heparinized bottles. The blood (0.7 ml) was cultured for 72 h in 10 ml of RPMI 1640 (SIGMA) supplemented with 0.1 ml of phytohaemaglutinin (SIGMA) and 10% fetal Bovine Serum (G1BCO). 1 h before harvesting, colchicine 0.8 µg/ml was added to arrest the cells at metaphase stage. After centrifugation at 1000 rpm for 10 min, the blood lymphocyte layer was collected and transferred into clean dry test-tubes.

5-10 ml of 0.075% of hypotonic solution was added, recentrifuged and fixed three times with cold 3:1 methanol and glacial acetic acid (v/v) fixative. The fixed cells were stored at 4^oC for 3 to 4 days. Chromosome suspensions were dropped on a pre-cleaned microscope slides, then stained. Stained and well-spread metaphase were analysed under the microscope, then photographed and karyotyped. Out of the 62 metaphasic chromosome photographs, 10 photographs with well-spread chromosome from 5 male and 5 female pigs were selected for karyotyping. From these 10 karyotypes of the NIP, means of each of 18 chromosomal arm lengths were obtained using a compass and a ruler to obtain the arm length ratios, centrometric indices and total chromosomal length which were required for classification of the chromosome (Abraham and Prasad, 1983). The karyogram showing size, variation and morphology of the chromosome were plotted using Microsoft Excel.

RESULTS AND DISCUSSION

The karyotypic data obtained from the analysis of each chromosome of the Nigerian Indigenous Pig (NIP) and Exotic pigs are shown in Table 1 and 2. These showed the cytogenetic analysis of the 18 pairs of chromosome of the NIP and exotic pigs. The short and long arm lengths

^{*}Corresponding author. E-mail: oluwafunmike@yahoo.co.uk. Tel: +234(0)8053570807.

Chromosome	P (± sd)	q (± sd)	p+q (±sd)	TCL	Arm ratio	C.I	C.T
pair No.	(µm)	(µm)	(µm)	(%)	p/q	100p/(p + q)	
1	0.72 (± 0.15)	1.67 (± 0.15)	2.39 (± 0.53)	9.32	0.43	30.13	nsm (-)
2	0.50 (± 0.19)	0.95 (± 0.18)	1.45 (± 0.37)	5.65	0.53	34.48	nsm (-)
3	0.45 (± 0.09)	0.96 (± 0.21)	1.41 (± 0.30)	5.50	0.47	31.91	nsm (-)
4	0.45 (± 0.13)	0.92 (± 0.19)	1.37 (± 0.32)	5.34	0.49	32.85	nsm (-)
5	0.34 (± 0.05)	0.80 (± 0.14)	1.14 (± 0.19)	4.45	0.43	29.82	nsm (-)
6	0.39 (± 0.24)	1.29 (± 0.38)	1.68 (± 0.62)	6.55	0.30	23.21	nsm (-)
7	0.36 (± 0.07)	0.75 (± 0.12)	1.11 (± 0.19)	4.33	0.48	32.43	nsm (-)
8	0.59 (± 0.11)	0.72 (± 0.11)	1.31 (± 0.22)	5.11	0.82	45.04	nm
9	0.45 (± 0.08)	0.56 (± 0.11)	1.01 (± 0.19)	3.94	0.80	44.94	nm
10	0.43 (± 0.18)	0.59 (± 0.10)	1.02 (± 0.28)	3.98	0.73	42.15	nm
11	0.37 (± 0.10)	0.46 (± 0.12)	0.83 (± 0.22)	3.24	0.80	44.58	nm
12	0.36 (± 0.07)	0.38 (± 0.12)	0.74 (± 0.19)	2.89	0.95	48.65	nm
13	0	2.1 (± 0.19)	2.1 (± 0.19)	8.19	0	0	Т
14	0	1.69 (± 0.28)	1.69 (± 0.28)	6.59	0	0	Т
15	0	1.47 (± 0.22)	1.47 (± 0.22)	5.73	0	0	Т
16	0	0.93 (± 0.25)	0.93 (± 0.25)	3.63	0	0	Т
17	0	0.79 (± 0.14)	0.79 (± 0.14)	3.08	0	0	Т
18	0	0.71 (± 0.14)	0.71 (± 0.14)	2.77	0	0	Т
Х	0.35 (± 0.15)	1.38 (± 0.26)	1.73 (± 0.41)	6.75	0.25	20.23	nsm (+)
Y	0.38 (± 0.13)	0.38 (± 0.16)	0.76 (± 0.29)	2.96	1.0	50	m

Table 1. The cytogenetic analysis of the 18 pairs of chromosome of the Nigerian Indigenous Pig (NIP).

CT = Chromosome type (Abraham and Prasad, 1983); TCL = total chromosome length percentage [(p + q)/ Σ (p + q)] x 100; Cl = centromere index = (p/p + q) x 100; p = short arm length; q = Long arm length; p + q = total chromosome length; nsm = nearly submetacentric; nm = nearly metacentric; T = telocentric.

are presented as well as the Centromere index, the total chromosome lengths in percentage and the chromosome type.

Figure 1 and 2 showed the solid stained metaphasic chromosomes of the male Nigerian indigenous pig and its karyotype. The Figure showed the unbanded karyotype of NIP having 38 chromosomes comprising of 19 pairs of autosomes, a large X and a very small Y sex chromosomes. The karyogram of NIP showing the morphology of the chromosomes is presented in Figure 2 while the karyogram with size variation among chromosomes is presented in Figure 3.

Chromosome number and nombre fondamental

The result obtained (Figures 1 and 2) showed that the NIP has a chromosome number of 2n = 38 and nombre fundamental (NF) of 64, which are the same with those of the exotic *Sus scrofa domestica* (CSKSS, 1988). Nombre Fondamental was coined by Mathey (1945) as an index for quick estimation of the nature of the chromosome in a karyotype in relation to the presence or absence of arms. This NF value of 64 showed that 6 diploid chromosomes are telocentric, that is, chromosomes 13 – 18. The agreement of the NF value of the NIP with that of the exotic pig indicates high degree of resemblance between the two. It

is however believed that diversity in NF indicated species at different evolutionary levels with the more advanced ones showing an increase in metacentric chromosome at the expense of the telocentric ones (Dallai and Telluri, 1969) but this study does not show that.

Chromosome morphology

The morphology of the NIP chromosomes observed could be divided into three main groups according to their structures and sizes:

Group I: The chromosomes under this group are chromosomes 1-7 including the X sex chromosomes. They are submetacentric and distinct by identifiable in all metaphase spreads.

Group II: This group consists of chromosomes 8-12 and Y sex chromosome. They are metacentric chromosomes. Group III: These consist of chromosomes 13-18 and are telocentric.

Each chromosome description is as follows:

Group I

Chromosome 1: This is the longest submetacentric

Table 2. The cytogenetic	analysis of th	e 18 pairs of chromo	some of the Exotic pigs.
--------------------------	----------------	----------------------	--------------------------

Chromosome	Р	q	p + q	TCL (%)	Arm	C.I	C.T
pair No.	(µm)	(µm)	(µm)		ratio p/q	100p/(p + q)	
1	0.72	1.40	2.10	10.40	0.50	33.30	nsm (-)
2	0.40	0.85	1.25	6.19	0.47	32.00	nsm (-)
3	0.40	0.60	1.00	4.95	0.67	40.00	nm (-)
4	0.30	0.60	0.90	4.46	0.50	33.30	nsm (-)
5	0.31	0.50	0.81	4.01	0.62	38.27	nm (-)
6	0.25	1.15	1.40	6.93	0.22	20.00	nsm (+)
7	0.25	0.70	0.95	4.71	0.36	26.30	nsm (-)
8	0.30	0.65	0.95	4.71	0.46	31.58	nsm(-)
9	0.50	0.60	1.10	5.45	0.803	45.45	nm
10	0.39	0.40	0.79	3.91	0.98	49.36	nm
11	0.30	0.35	0.65	3.22	0.86	46.45	nm
12	0.30	0.30	0.60	2.97	1.00	50.00	М
13	0	1.85	1.85	9.16	0	0	Т
14	0	1.45	1.45	7.18	0	0	Т
15	0	1.26	1.26	6.24	0	0	Т
16	0	0.80	0.80	3.96	0	0	Т
17	0	0.65	0.65	3.22	0	0	Т
18	0	0.60	0.60	2.97	0.66	0	Т
Х	0.43	0.65	1.08	5.35	1.00	39.81	nm
Y	0.25	0.25	0.50	2.48	1.00	50	М

CT = Chromosome type (Abraham and Prasad, 1983)

TCL = Total chromosome length percentage $[(p + q)/\Sigma(p + q)] \times 100$

 $CI = Centromere index = (p/p + q) \times 100$

p = Short arm length q = Long arm length

p + q = Total chromosome length

nsm =Nearly submetacentric

nm = Nearly metacentric

m = Metacentric

T = Telocentric

chromosome. It has p and q values of 0.72 and 1.67 μm respectively.

Chromosome 2: This chromosome is smaller than chromosome 1. It is nearly submetacentric. It has p and q values of 0.5 and 0.95 µm respectively.

Chromosome 3: This is also long but relatively shorter than chromosomes 1 and 2. It has p and q lengths of 0.45 and 0.96 μ m respectively.

Chromosome 4: A nearly submetacentric chromosome that has p and q lengths of 0.45 and 0.92 μ m respectively.

Chromosome 5: This also is a nearly submetacentric chromosome with p and q values of 0.34 and 0.80 μm respectively.

Chromosome 6: This is equally a nearly submetacentric chromosome with p and q values of 0.39 and 1.29 μm respectively.

Chromosome 7: This is a nearly submetacentric chromosome with p and q values of 0.36 and 0.75 μm respectively.

Chromosome X: This is nearly submetacentric. It has p

and q values of 0.35 and 1.38 µm respectively.

Group II

Chromosome 8: This is a long nearly metacentric chromosome with p and q values of 0.57 and 0.72 μm respectively.

Chromosome 9: This is a nearly metacentric chromosome. It has p and q values of 0.45 and 0.56 μ m respectively.

Chromosome 10: This also is a nearly metacentric chromosome with p and q values of 0.43 and 0.59 μ m respectively.

Chromosome 11: This is a short nearly median chromosome with p and q values of 0.37 and 0.46 μ m respectively.

Chromosome 12: This is also a short nearly median chromosome with p and q values of 0.36 and 0.38 μm respectively.

Chromosome Y: This is a very small metacentric chromo-



Figure 1. The unbanded (solid stain) chromosomes of the male NIP and its karyotype (38, XY).

some with p and q values of 0.38 and 0.38 μm respectively.

Group III

Chromosome 13: This is a long telocentric chromosome. It has a q value of 2.1 μ m.

Chromosome 14: This also is a long telocentric chromosome, but shorter than chromosome 13. It has a q value of 1.69 $\mu m.$

Chromosome 15: This telocentric chromosome has a q value of 1.47 $\mu m.$

Chromosome 16: This is a short telocentric chromosome with a q value of 0.93 μ m. Chromosome 17: This is also a short telocentric chromosome with a q value of 0.79 μ m. Chromosome 18: This is the shortest telocentric chromosome with q value of 0.71 μ m.

The karyotypic data

Below is the karyotypic data obtained from the measurement of chromosomal arms, the p/q ratio, the centromere indices and the chromosome type of the NIP are shown on Table 1. The chromosome arm length and



Figure 2 The karyogram of NIP showing the morphology of the chromosomes.



Figure 3. The karyogram of the NIP showing the size variation among the chromosomes.

p/q ratio of both were observed to be the same in chromosome 1, 2, 6, 9, 11, 12, 14 - 18 and Y sex chromosome within the S.E measurement while differences were obtained in chromosome 3, 4, 5, 7, 8, 10, 13 and X sex chromosomes but these differences do not cause distinction in chromosome type, except in the X sex chromosome which showed distinction in chromosome type (Oluwole, 2005).

The karyotypic data obtained was also used to construct the karyogram showing the morphology of each chromosomes and size variation amongs the chromosomes (Oluwole and Omitogun, 2007).

Conclusion

The NIP has a chromosome number of 2n = 38 and a nombre fondarmental of 64 which tally with that of the *Sus scrofa* domestica.

Similarities were found in p/q ratios NIP and exotic pig in chromosomes 1, 2, 6, 9, 11, 12, 14 - 18 and Y sex chromosome.

Differences were observed in the p/q ratios of NIP and exotic pig in chromosomes 3, 4, 5, 7, 8, 13 and X sex chromosome. It is assumed that these information will be of tremendous benefit in pig breeding programme aiming at enhancing diseases resistance and better adaptation.

ACKNOWLEDGEMENT

Our laboratory was funded by the Obafemi Awolowo University Research Council Research Grant (URC-11812 AVT). Acknowledgements are due to Ranjit Vandjopathay, Jackie Hughes and O. Mukangi of the International Institute of Tropical Agriculture (IITA), Ibadan for the assistance in digital photomicrographic aspect of this research.

REFERENCES

- Abraham Z, Prasad PN (1983). A system of chromosome classification and nomenclature cytology 48:95-101.
- Adebambo OA (1982). Evaluation of the genetic potential of Nigerian indigenous pigs. Proceeding 2nd Wld Cong. Genetic Appl. Livestock Prod. Madrid. pp. 543-553.
- Adebambo OA (2003). Animal breeds: A nation's heritage. Inaugural lecture, University of Agriculture, Abeokuta.
- Committee for the Standard Karyotype of *Sus scrofa* (1988). Standard Karyotype of the domestic pig *Heriditas* 109: 151 157.
- CTA (1994). Animal breeding. Mammalian Publishers. pp. 18 24.
- Dallai D, Talluri (1969). A Karyotypic study of three spiecies of *Scincidae* (Reptilia) chromosoma (Berl) 27:86-94
- Fairbanks DJ (1999). Genetics: The continuity of life. pp. 304 309, 322 326.
- Galman OG, Yerle M, Echard G (1991). The high resolution GTG banding karyotype of the *Sus scrofa domestica*. Genet. Set Evol. 23: 113s 116s.
- Ilori JA (1974). Assessing the productive potentials of local breeds of pigs. Ann. Conf. Nig. Soc. Anim. Prod. 1:100.
- Mattey R (1945). L'evolution de la formulae chrosominale chez les vertebras Experimentia (Basel) 1:78-86
- Omitogun OG (2004). Analysis of swine genome organization. Evolution of G-T-G bands in porcine chromosomes for physical mapping. In: (O.J. Oriyo C.O.N. Ikeobi, M.O. Ozoje, T. Omoniyi, and O.B. Kehinde, Eds.)..Proceedings of the 29th Annual Conference of the Genetics Society of Nigeria., NACGRAB, Moor Plantation, Ibadan pp. 54-57.
- Oluwole OO (2005). 'Cytogenetic characterization of the Nigerian Indigenous Pig' Unpublished MSc Thesis Obafemi Awolowo University, Ile-ife. ' Unpublished MSc Thesis Obafemi Awolowo University, Ile-ife, pp. 1-61
- Oluwole OO, Omitogun OG (2007). 'The karyomorphology of Nigerian indigenouspig chromosomes prepared from leucocyte culture' Trop. Anim. Sci. 10(2):37-40.