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A karyological study in indigenous and crossbred pigs

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Abstract
A karyotype analysis was carried out on 30 indigenous (15 of each gender) and 30 Large White Yorkshire crossbred (15 of each gender) pigs for the karyological study by adopting the short term peripheral blood leukocyte culture technique. There was significant difference between animals in relative length of all chromosomes in Large White Yorkshire crossbred females except for chromosome numbers 2nd, 3rd, 6th, 14th and 17th and in males except for chromosome numbers 2nd, 3rd, 7th, 12th, 17th and 18th respectively, whereas in indigenous pig females except for chromosome numbers 10th, 12th, 13th, 14th and 17th and in males except for chromosome numbers 3rd, 12th, 17th and 18th correspondingly. The significant difference between animals in relative length of indigenous pigs might be resulting from disassortative mating followed by the farmers, which is reflected in significant differences in relative length of some of the autosomes, whereas in crossbred pigs despite the selection was possibly due to differences in condensation rate of chromosomes and bending up or down of arms during chromosomal preparation which can only viewed into dimensions.

Keywords: Non-descript pig, large white Yorkshire crossbred pig, relative length, Ideogram.

Introduction
Today's domesticated pigs are having qualities that a farmer looks for in livestock, including high profitability as they have traits like high prolificacy, good feed conversion efficiency, rapid growth and short generation interval. Biological resemblance of pigs to humans, made them frequently used for human medical research. Pig population of India is 10.29 million ([1]) and it constitute about 2.01 per cent of the total livestock population. Andhra Pradesh contributes 3.83 per cent of pig population in India [1]. Now a days pig industry in India is developing step by step, but till now pigs raised are mostly of non-descript type [2] in the country.

Cytogenetic studies are useful to describe the chromosomal complements which are carriers of hereditary, which will determine all the characters of an individual or breed. The frequent chromosomal abnormalities occurs in pigs are reciprocal translocations, which causes a large negative effect on fertility of carrier animals, causes small litter size and increases the number of stillbirths and mummified foetus [3]. Karyotype analysis was aimed to identify chromosomal disorder related to genetic disorder [4]. For chromosomal classification, identification of bands on chromosomal arms, diagnosis of aberrations such as deletions, duplications, robertsonian translocations and mapping of genes on gene loci the relative length of chromosomes should be necessary [5]. Visible morphological differences in the chromosomes always may not be the reason for significant fertility problems, minor variations in the relative length and arm ratio of the chromosomes also disturb the reproduction process [6].

The aim of the present experiment was to study the relative length (%) of chromosomes in Indigenous and LWY crossbred pigs and finding the differences between animals in relative length of both the breeds.

Materials and Methods
The cytogenetic investigation was carried out on 30 indigenous (15 of each gender) and 30 Large White Yorkshire crossbred (15 of each gender) pigs maintained by the farmers in and around Tirupati and on Large White Yorkshire (LWY) crossbred pigs maintained at AICRP (All India Coordinated Research Project on pig), College of Veterinary Science, Tirupati, Chittoor District of Andhra Pradesh.

Blood samples (2 to 3ml per animal) were collected aseptically into a 10ml vaccutainer tube containing sodium heparin 68USP as an anticoagulant. The short term leukocyte culture technique, as described by [7] with minor modifications suitable to lab conditions was followed. The complete medium consisting of RPMI 1640 medium (8ml), Benzathine pencillin (16µl), Streptomycin sulphate (10µl), Autologous plasma (1.5ml), Phytohaemagglutin -M (0.1ml), Heparin (7.5IU/ml of culture media) with blood sample of 0.5 ml was incubated for 72 hrs at 37˚C and 8µg of colchicine was added 90 minutes prior to harvesting the culture to arresting the cell division at metaphase stage, then cell pellet was treated with hypotonic solution (0.075 M KCL) for 30 minutes at 37˚C and fixed in Methanol: Glacial acetic acid (3:1). Approximately 20 µl of the homogenous cell suspension was dropped on the chilled wet slides held at 45^0 angle from height of 2-3 feet with pressure. Slides were air dried and staining has done with 4% giemsa for 20 minutes. Slides were screened under 10**x** and 40**x** and 100**x** magnifications and the good metaphase spreads were identified and captured with the digital camera and karyotyped.

Statistical analysis

The relative length of chromosomes were calculated in percentage according to the formula given by [8]. Since the relative length of chromosomes were all below 30 percent, the data were transformed into angles using arc-sin transformation [9] for undertaking least squares analysis [10]. After the least squares analysis, the means were retransformed into percentages. The transformed data were subjected to least square analysis to find out the significant difference in relative length of animals within the breed.

Results and Discussion

A total of 900 karyotypes of indigenous and Large White Yorkshire crossbred pigs were prepared by pairing of chromosomes based on their length in descending order. Ideograms of indigenous and LWY crossbred pigs are shown in figures 1 and 2.

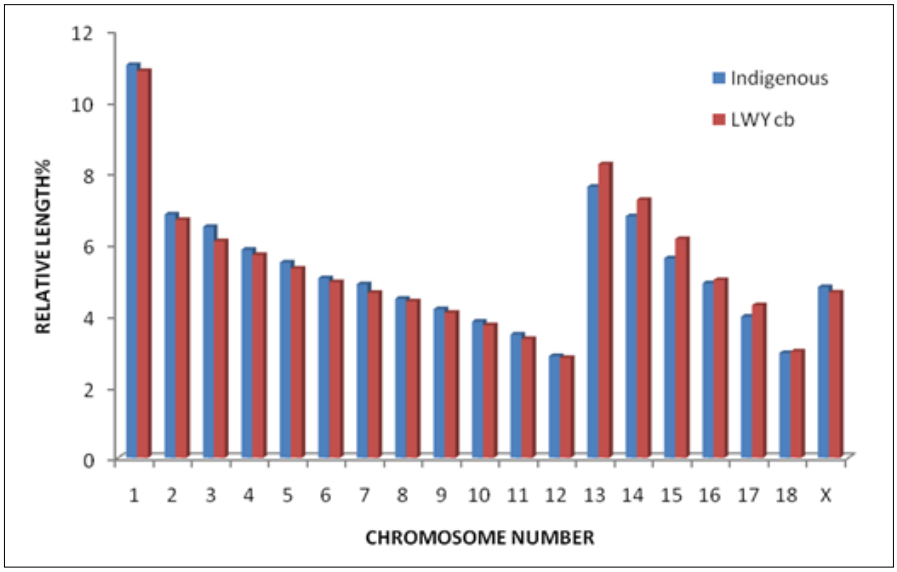

Fig 1: Comparative ideogram of indigenous and LWY crossbred female pig

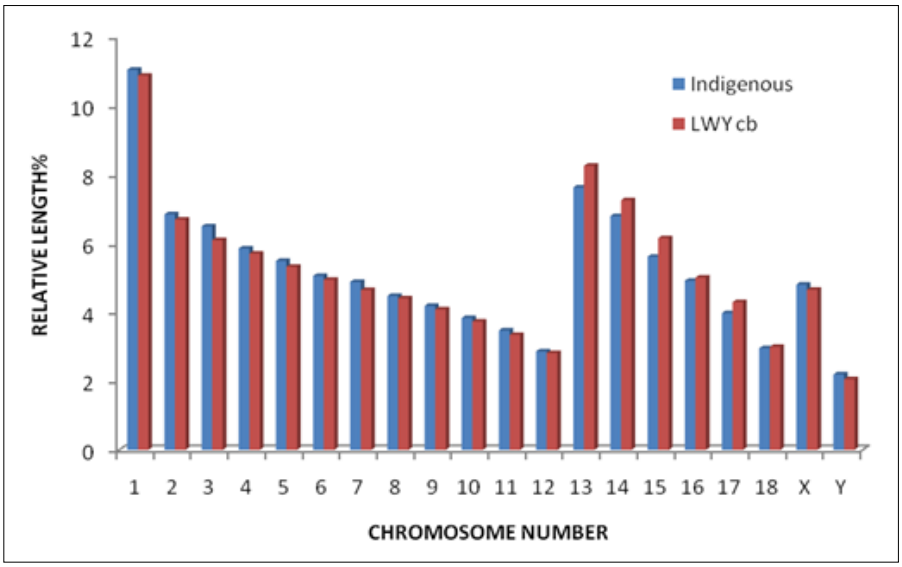

Fig 2: Comparative ideogram of indigenous and LWY crossbred male pig

The least squares analysis of variance for relative length (%) to study the variation between animals in indigenous female and male pigs were furnished in Table 1 and the overall least squares means are given in Table 2. There was a significant

difference between animals on relative length of autosomes and allosomes except for chromosome numbers 10th, 12th, 13th, 14th and 17th in females and in males except for chromosome numbers 3rd, 12th, 17th and 18th correspondingly which might be attributed to existence of wide phenotypic variations resulting from disassortative mating followed by the farmers. As the farmers do not practice any specific breeding strategy towards improving either the growth traits or the litter traits, uniformity among the indigenous pigs in the field would not be witnessed. This leads to the presence of wide genetic variations among the field stock. That is reflected in significant differences in relative length of some of the autosomes.

Table 1: Least squares analysis of variance of relative length (%) of autosomes and allosomes between animals in indigenous pigs.

Chromosome Number	Mean sum of squares			
	Females		Males	
	Between animals (d.f.=14)	Error (d.f.=135)	Between animals (d.f.=14)	Error (d.f.=135)
1	1.14*	0.63	1.11**	0.50
2	1.12*	0.64	0.63**	0.29
3	0.82*	0.45	0.5NS	0.34
4	1.33*	0.66	1.18*	0.60
5	1.14**	0.54	1.26*	0.69
6	0.90**	0.43	1.25*	0.73
7	0.53**	0.19	0.66**	0.22
8	0.89*	0.46	0.55**	0.20
9	0.84**	0.34	0.65**	0.29
10	1.21NS	0.76	1.27**	0.47
11	0.95*	0.49	1.02*	0.60
12	0.19NS	0.13	0.27NS	0.20
13	1.49NS	1.20	2.3**	1.14
14	1.32NS	1.09	2.01*	1.20
15	1.24**	0.55	1.54*	0.78
16	1.27**	0.60	1.60*	0.94
17	1.17NS	0.97	1.04NS	0.90
18	0.65*	0.39	0.96NS	0.86
X	2.33**	0.88	2.20**	0.98
Y	-	-	2.14*	1.26

* Significant at P<0.05; ** Significant at P<0.01; NS: Non - significant

Table 2: Least squares means of relative length (%) of chromosomes in indigenous female and male pigs

Chromosome number	Female		Male	
	Overall mean	S.E.	Overall mean	S.E.
1	11.01	0.09	11.04	0.08
2	6.88	0.09	6.84	0.06
3	6.47	0.08	6.49	0.06
4	5.90	0.09	5.85	0.09
5	5.53	0.09	5.49	0.10
6	5.08	0.08	5.05	0.10
7	4.87	0.05	4.88	0.05
8	4.46	0.08	4.47	0.05
9	4.16	0.07	4.18	0.06
10	3.82	0.10	3.83	0.08
11	3.45	0.08	3.47	0.09
12	2.84	0.04	2.86	0.05
13	7.60	0.13	7.62	0.13
14	6.78	0.12	6.79	0.13
15	5.58	0.09	5.61	0.10
16	4.89	0.09	4.91	0.11
17	3.95	0.19	3.97	0.21
18	2.94	0.07	2.95	0.11
X	4.82	0.11	4.80	0.12
Y	-	-	2.19	0.13

The least squares analysis of variance for relative length (%) of autosomes and allosomes to find out variation between animals of Large White Yorkshire crossbred pigs are given in Table 3. The least squares means of Large White Yorkshire crossbred pigs chromosome-wise for females and males are given in Table 4. There was significant difference between animals in relative length of all chromosomes in females except for chromosome numbers 2nd, 3rd, 6th, 14th and 17th, whereas in males except for chromosome numbers 2nd, 3rd, 7th, 12th, 17th and 18th respectively.

Table 3: Least squares analysis of variance of relative length (%) of autosomes and allosomes between animals in LWY crossbred pigs

Chromosome Number	Mean sum of squares			
	Females		Males	
	Between animals (d.f.=14)	Error (d.f.=135)	Between animals (d.f.=14)	Error (d.f.=135)
1	2.15*	1.23	1.34**	0.55
2	0.42NS	0.33	0.90NS	0.58
3	0.43NS	0.42	0.73NS	0.53
4	1.29**	0.53	1.36**	0.25
5	1.09**	0.41	1.35**	0.45
6	1.22 NS	0.92	0.76*	0.41
7	0.66*	0.33	0.46NS	0.45
8	1.22*	0.68	0.60*	0.33
9	0.95*	0.48	0.69*	0.38
10	1.16**	0.42	1.01*	0.59
11	0.90**	0.22	1.21**	0.51
12	0.43**	0.14	0.44NS	0.42
13	3.73**	1.29	1.69**	0.44
14	2.93NS	2.39	1.61**	0.46
15	2.82**	1.10	1.22**	0.42
16	2.73**	1.28	1.45**	0.57
17	1.72NS	1.56	1.00NS	0.76
18	1.25*	0.72	0.77NS	0.49
X	2.56**	1.05	2.51**	1.14
Y	-	-	1.97**	0.72

* Significant at P<0.05; ** Significant at P<0.01; NS; Non – significant

Table 4: Least squares means of relative length (%) of chromosomes in LWY crossbred female and male pigs

Chromosome number	Female		Male	
	Overall mean	S.E.	Overall mean	S.E.
1	10.83	0.09	10.87	0.09
2	6.68	0.07	6.69	0.09
3	6.07	0.07	6.09	0.08
4	5.68	0.09	5.70	0.06
5	5.30	0.07	5.32	0.08
6	4.92	0.12	4.94	0.07
7	4.69	0.07	4.64	0.08
8	4.38	0.10	4.40	0.07
9	4.07	0.08	4.08	0.07
10	3.76	0.08	3.73	0.09
11	3.36	0.05	3.34	0.08
12	2.79	0.04	2.81	0.07
13	8.23	0.13	8.25	0.08
14	7.26	0.18	7.25	0.08
15	6.13	0.12	6.15	0.08
16	5.03	0.13	5.00	0.09
17	4.31	0.61	4.29	0.19
18	2.98	0.10	2.99	0.08
X	4.67	0.12	4.65	0.13
Y	-	-	2.05	0.10

The significant differences among Large White Yorkshire crossbred pigs with respect to relative lengths of chromosomes, despite the selection was possibly due to differences in condensation rate of chromosomes and bending up or down of arms during chromosomal preparation which can only viewed into dimensions [11].

The mean relative length of autosomes in indigenous pig males contributed 2.86±0.05 to 11.04±0.08 per cent, while those of females contributed 2.84±0.04 to 11.01±0.09 per cent and the corresponding values of relative lengths in Large White Yorkshire crossbred pigs were 2.81±0.07 to 10.87±0.09 and 2.79±0.04 to 10.83±0.09 per cent, which agreed well with the reports of [12, 13, 14 and15].

The overall least squares mean relative length of X-chromosome in indigenous male pig was 4.80±0.12 and in females 4.82±0.11 per cent, whereas in Large White Yorkshire crossbred pigs the corresponding mean values were 4.65±0.13 and 4.67±0.12 per cent, which were in consonance with the reports of [12, 15, 16].

The mean relative length of Y-chromosome in indigenous and Large White Yorkshire crossbred pigs were 2.19±0.13 and 2.05±0.10 per cent respectively, which agreed well with the reports of [13, 14]. Results have indicated the existence of variation in phenotypic performance of animals studied over the generations of selection. The inclusion of more production and reproduction traits to find out significant correlations between karyotype characteristics and animal performance traits may be used as important consideration in selection of young piglets for next generation.

Conclusion

The modal chromosomal number in both the breeds was 38

with XX complement in females and XY complement in males and a fundamental number of 64 as in exotic. The significant differences between animals in relative lengths of indigenous pigs was due to disassortative mating followed by the farmers, whereas in Large White Yorkshire crossbred pigs due to selection and chromosomal condensation rates during cell preparation. The significant variance between animals in karyotype characteristics of Large White Yorkshire crossbred pig provide information that was usually associated with growth rates among animals over the generations of selection. The knowledge of chromosome characteristics and their effect on economic traits will also be helpful in animal breeding strategies.

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