CHROMOSOME ARCHITECTURE OF DESI PIGS OF KERALA

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Chromosome architecture of desi pigs of Kerala"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"Chromosome architecture of desi pigs of Kerala"** is a record of research work done independently by **Dr.K.C.Jayan,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Dedicated to my grand mother

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Introduction

INTRODUCTION

The pig has associated itself with man from olden times as confirmed from its bones being found at the sites of Stone Age Settlements. Pigs were domesticated as early as 2000 BC even though some ancient cultural and religious taboos existed against it. The potential of pig industry as a source of human food is well established. This animal is well recognised for its efficiency to convert garbage and other wastes to valuable animal protein. The pig (*Sus scrofa domestica*), due to its high prolificacy, short generation interval, fast growth rate and other biological advantages, can play a very important role to overcome our animal protein deficit. In India, pig rearing is followed traditionally as a part of agriculture by people mostly coming under the lowest socio-economic strata.

Pig belong to the phylum Chordata, class Mammalia, order Artyodactila, sub order Suiformes and infraorder Suina which contain the superfamily Suoidae, all the members of which are loosely called pigs and superficially resemble the common pig. The superfamily Suoidae contain families, Tayassuidae which are natives of Central and South America, and Suidae, which are the true or old world pigs. The family Suidae contain five living genera of which the genus Sus contains several species including *Sus scrofa, Sus vittatus, Sus cristatus* and *Sus wadituaneus*. The European breeds of domestic swine were derived from *Sus scrofa*, whereas the breeds in the Far Eastern parts of the world were derived from *Sus vittatus*. The modern breeds of pig evolved from different crossings between these two types and

the present day domestic pig *Sus domesticus* is the result of thousands of years of evolution through gradual domestication. The pigs found in the Indian subcontinent were derived from *Sus cristatus*. Currently there are some 87 recognised breeds of domestic pigs in the world and other 225 and more varieties.

In early period, pig rearing and pig industry were in the hands of traditional pig farmers with no means to undertake intensive pig farming. The present local desi pigs were the type of animals kept by them. During the second and third five year plan periods, exotic pigs were introduced to upgrade the local stock. Three genetic groups of pigs now exist in India. They are the local desi pigs, the exotic pigs imported from Western countries and cross breeds of these two. According to the livestock census of 1996, the pig population in Kerala was 1.43 lakhs (Anon, 1996). The local desi pigs of Kerala are black in colour, small in size, highly adaptable to the local conditions and well known for their resistance to diseases. They need no special attention. This breed is on the verge of extinction now and urgent steps have to be taken to prevent this. A study to analyse and understand the genetic make up or chromosome constitution of desi pigs is an important step in the process of conservation. Karyotyping is an effective technique for genetic characterisation, and is less expensive and easily adaptable.

Karyotype include the complete chromosome complement present in every diploid cell. The karyotype varies between different species and within the species itself, it varies between different varieties and between sexes. The

domestic pig is having a chromosome number of 38. But some wild pigs like the European Wild boar have a chromosome number of 36.

Efforts are now on to take advantage of the local adaptability and disease resistance of the local desi pigs by cross breeding them with exotic pigs. Genetic characterisation is highly essential before starting such cross breeding programmes on a large scale.

So this study was taken up with the following objectives.

1. To investigate and establish the chromosome complement of black desi pigs of Kerala.
2. To analyse the chromosomal status of black desi pigs and compare it to that of Large White Yorkshire pigs.

Review of Literature

REVIEW OF LITERATURE

A method evolved by Hsu and Pomerat (1953) for spreading the chromosomes of mammalian cells in tissue culture was the preliminary step in karyotyping.

Moorhead *et al.* (1960) described peripheral blood leukocyte culture technique for studying chromosomes. The venous blood collected in heparinised vial was mixed with phytohaemagglutinin and held on ice for 60 minutes. The supernatant plasma obtained by centrifugation was cultured in medium 199 enriched with bovine plasma. After 70 hours of culturing at 37°C, colchicine was used to arrest the mitosis. The cells were fixed with methanol, glacial acetic acid mixture (3:1) and subsequently slides were prepared, air dried and stained. McConnell *et al.* (1963) used this technique in pigs.

Lin *et al.* (1976) isolated leukocytes from heparinised whole blood using a six per cent Ficoll - 10 per cent Hypaque gradient system. The leukocytes isolated were cultured and metaphase spreads were prepared.

Stanyon and Galleni (1991) used a fibroblast culture technique suitable for biopsy samples collected from small mammals for high resolution karyotyping.

Chromosome number

Melander (1951) found that the diploid chromosome number in testicular tissue of Large White Yorkshire pigs was 38 and that of Old Swedish pigs was 30.

Sachs (1954) studied the chromosome number of Old Swedish variety of pigs and concluded that the 2n number is 40. He ruled out the possibility of different chromosome numbers in different varieties.

Gimenez-Martin *et al.* (1962) determined the chromosome number of pigs from bone marrow cells of Large White pigs as 38.

McConnell *et al.* (1963) studied the chromosomes of the domestic pig using leukocyte culture technique. He studied the chromosome number of six breeds namely the Large White Yorkshire, Landrace, Poland China, Berkshire, Hampshire and Duroc. The diploid number of chromosomes was found to be 38.

Chromosome preparations were made using leukocytes from peripheral blood collected from Hampshire pigs by Stone(1963). The normal diploid complement of chromosomes in both sexes was found to be 38.

Di Antonio (1964) reported the diploid chromosome number in pigs as 38 using kidney cells and leukocyte cultures of pigs. He classified the chromosomes into three groups, 12 pairs of non-acrocentric autosomes, six pairs of acrocentric autosomes and one pair of heterosomes.

Muramoto *et al.* (1965) studied the chromosome complements of the Wild boar (*Sus vittatus* leucomystax Major) and the hybrids of male domestic pig and female wild boar using peripheral blood leukocyte culture technique and found the chromosome number to be 38 in both.

McFee *et al.* (1966) observed some variations in pig chromosome number among European wild pigs. Seventy three per cent of the animals had 36 chromosomes while 27 per cent had 37. According to them, the 36 chromosome individuals differed from the domestic pigs in that they had one pair of sub-metacentric chromosomes not found in the latter and lacked two pair of the domestic telocentrics. The 37 chromosome animals had chromosome of each of these three pairs, but lacked their homologous members.

Nizza (1966) studied the modal diploid number of chromosomes of the Corsican Swine and found it as 38. He found the X-chromosome as a medium sized one with a paramedian centromere and the Y-chromosome as the smallest metacentric chromosome with a median centromere.

The chromosomes of a Large White boar was studied by Krasavtsev and Yu (1968) using bone marrow biopsy. The diploid number was 38. The location of the centromere was reported to be submedian in four pairs of chromosomes and median in eight pairs. Six pairs were acrocentrics. The X-chromosome had an almost median centromere while the Y-chromosome was the smallest metacentric.

Rary *et al.* (1968) studied the cytogenetics of Swine in the Tellico Wildlife Management Area, Tennessee. Of the 108 pigs, 31.5 per cent had 36 chromosomes, 53.7 per cent had 37 chromosomes and 14.8 per cent had 38 chromosomes.

Srivastava and Lasley (1968) described a technique for chromosome preparation using blood collected under farm conditions. They estimated the chromosome number of Poland China, Duroc and their crossbred pigs as 38.

Gropp *et al.* (1969) found that the chromosome complement of wild pigs from Germany was 36 with 4 pairs of acrocentrics. They described that the change in chromosome number from domestic pigs (38) may be the result of intraspecific Robertsonian changes and a preferential selection of the 38 chromosome type in the course of domestication.

Mc Fee and Banner (1969) made all possible crosses between European Wild pig with either 36 or 37 chromosomes and domestic pigs with 38 chromosomes. They found that the cross between pigs with 36 chromosomes produced only pigs with 36 chromosomes. The 36 x 37 and 37 x 38 crosses yielded the parent numbers in about equal numbers of pigs. All pigs resulting from the 36 x 38 cross had 37, while the progenies had 36, 37 or 38 chromosomes in about a 1:2:1 ratio.

Rittmannsperger (1970) found the diploid number of chromosomes of Landrace, Minnesota miniature and Vietnamese pigs to be 38 with no breed differences.

Strelchenko *et al.* (1977) studied samples of bone marrow of European wild pigs and the domestic Russian Large White pigs and found them to have a karyotype of 38 autosomes and two sex chromosomes.

Krasavtsev and Yu (1978) found the diploid number of chromosomes to be 38 in metaphase plates of bone marrow cells from pigs.

Melander and Hansen-Melander (1980) conducted chromosome studies in African wild pigs. The chromosomes of the giant forest hog (*Hylochoerus meinertzhageni*),the warthog (*Phaeochoerus aethiopicus*), the bush pig (*Potamochoerus porcus*) and the domestic pig (*Sus scrofa*) were studied and were found to have 32, 34, 34 and 38 chromosomes, respectively.

Popescu *et al.* (1980) made chromosome analysis of French wild boar (*Sus scrofa scrofa*) and found that of the 36 wild boars from continental Europe one had 38 chromosomes and all the others had 36 chromosomes. The Corsican wild boars were found to have 38 chromosomes. Hybrids of domestic Corsican pig and Corsican wild boar had 38 chromosomes.

Guo-Jie (1981) prepared karyotypes of the Beijing Black Pig, a dual purpose breed developed by crossing the Northern native chinese pig, the Large White and the Berkshire breeds. The diploid chromosome number was found to be 38.

A diploid chromosome number of 38 was reported by Vijh *et al.* (1990) and Palegar *et al.* (1991) for the desi pigs in India.

Chromosome morphology

Haag and Nizza (1969) studied the karyotypes of pigs using peripheral blood leukocyte culture technique and found the diploid number as

38 (18 pairs of autosomes and a pair of sex chromosomes). They proposed that the variations in chromosome morphology permited the identification of 12 pairs of autosomes and the Y-chromosome. The remaining 6 pairs of autosomes and the X-chromosome could easily be distinguished because of their similarity in size and that of the position (median or submedian) of the centromere. They have divided the pig chromosomes into six groups (plus the Y-chromosome) according to size and morphology.

An analysis of the chromosomes of wild pigs in various parts of Yugoslavia was made by Zivkovic *et al.* (1971). They found the diploid number of chromosomes to be 38 of which six pairs were acrocentric and 12 pairs metacentric or submetacentric.

Bhatnagar and Srivastava (1972) showed that the diploid chromosome number of Indian domestic pig (*Sus cristatus* wagner) was 38. They described the X-chromosome as a medium sized almost metacentric chromosome and the Y as a submetacentric chromosome. They could observe some non-staining gaps in certain chromosomes.

Tikhnov and Troshina (1974) described the karyotypes of *Sus scrofa nigripes, Sus scrofa ussuricus* and *Sus scrofa attila*. The diploid chromosome number ranged from 36 to 38. They suggested the formation of new submetacentric chromosomes from acrocentric ones.

Nechiporenko (1974) subjected Russian Large White, Mirgorod, Landrace and Pietrain pigs to karyotyping. He found the percentage of

metaphase spreads having 2n = 38 chromosomes as 82.0, 77.1, 75.5 and 96.2 respectively for the above breeds. He measured the centromere indices and relative chromosome lengths and could not find any difference between the breeds.

Chromosome karyotype

Ruddle (1964) prepared detailed karyotypes of male and female Hampshire pigs. He suggested methods for comparison of arm lengths between different and independent cell populations, the recognition of homologue pairs within groups of very similar non-homologous chromosomes and the characterisation of the X-chromosome.

Goldman and Zhivalec (1970) made idiogram of the chromosomes of Russian Large White pigs and revealed 13 pairs of metacentric or sub-metacentric and six pairs of acrocentric chromosomes. The X-chromosome was in the 12^{th} pair according to them.

Karyotype of Kakhetian, Vietnamese, Mangalitsa, Landrace and Omsk Grey breeds of pig were studied by Tikhonov and Troshina (1971). None of the five breeds exhibited polymorphism in chromosome number or structure and had a diploid chromosome number of 38.

Berger (1972) studied the karyotype of the pig by a new technique which involved Giemsa staining after treatment with 8 M urea. The results obtained were similar to quinacrine mustard staining technique except for the

Y-chromosome. He identified the individual chromosomes by their banding patterns.

Rary and Murphree (1973) made quantitative analysis of chromosomes in pigs with a diploid chromosome number of 36, 37 or 38.

Gorin and Chudon (1978) made metaphase chromosome preparations from bone marrow cells of the Russian Large White pigs. They classified the autosomes into seven groups as follows. Group A had very large subacrocentric chromosomes of the first pair, Group B was of very large acrocentrics of the second pair, Group C of medium sized acrocentrics of three and four, Group D of pairs five to eleven with submetacentric morphology, Group E included medium sized acrocentrics of 12 and 13 pairs, Group F had small metacentrics of pairs 14 and 15 and Group G consisted of the very small acrocentrics of pairs 16 to 18. The X-chromosome was a submetacentric one and the Y-chromosome a very small metacentric.

Krasavtsev and Yu (1978) examined 3283 chromosome spreads of bone marrow cells of 31 Large White, Landrace, Mangalitsa and Berkshire pigs and tabulated the absolute and relative lengths of each of the chromosomes and their centromeric indices. The chromosomes were classified into eight groups and the sex chromosomes as a separate group.

Hansen (1980) studied chromosomes of Danish Landrace pig using Quinacrine and Giemsa staining technique. He tabulated the data on the relative lengths of the p and q arms and the whole chromosomes. The relative

length of the X-chromosome was five per cent of the haploid chromosome complement.

The karyotype of the village pig of Papua-New Guinea was prepared by Popescu *et al.* (1982). The diploid chromosome number was 38 with 12 pairs of metacentrics or submetacentrics, six pairs of acrocentrics, a submetacentric X- and a small submetacentric Y-chromosome. The karyotype was similar to that of the European domestic pig and they suggested a common origin for both the pigs.

Zengyang-Zhi *et al.* (1982) made karyotype studies of domestic pigs in Southern China. In the karyotype, chromosomes one to five were submetacentric, six and seven subtelocentric, eight to twelve and the X- and Y-chromosomes were metacentric and chromosomes thirteen to eighteen were telocentric.

Karyotype studies on cultured lymphocytes of Korean native pigs by Jung and Yoo (1990) showed that the pigs had 36 autosomes and two sex chromosomes. Among the autosomes there were five submetacentric, two acrocentric, five metacentric and six telocentric pairs. The X- and Y-chromosomes were metacentric.

Liu and Lu (1990) used cultured peripheral lymphocytes to study the G- and Q-banding patterns of Bamei, Guanzhong Black, Berkshire and Duroc pigs. The banded chromosomes were divided into 63 regions by them.

Vijh *et al.* (1990) evaluated the somatic metaphase chromosomes of Indian domestic pig and found that the complement consisted of metacentric, submetacentric, subtelocentric and telocentric chromosomes. They divided it into four groups (excluding sex chromosomes). Group A consisted of five pairs of submetacentrics, group B had five pairs of submetacentric/metacentric, group C of two pairs of subtelocentric and group D of six pairs of acrocentric or telocentric chromosomes. The X-chromosome was large submetacentric and the Y- was metacentric and smallest of the chromosomes. They observed a non-stainable area near the centromere of the tenth pair of chromosome.

Palegar *et al.* (1991) studied the karyotypes of Landrace, Yorkshire, Hampshire and desi pigs. All the breeds studied had a diploid chromosome number of 38, of these the first five pairs were submetacentric, two pairs subtelocentric, five pairs metacentric and the remaining six pairs telocentric. Both the sex chromosomes were metacentric in all breeds studied.

Chromosome morphology of Yorkshire, Landrace and Duroc pigs were studied by Kim *et al.* (1994) using peripheral blood leukocyte culture technique. The Y-chromosome was found to be the shortest with 1.9 per cent relative length. Chromosome 1 which was the longest chromosome had a relative length of 11 per cent. The chromosomes were classified into four groups. They were submetacentric and metacentric in group I, acrocentric and submetacentric in group II, metacentric in group III and telomeric and acrocentric in group IV. The centromeric index was 40 and 49.4 per cent in the

X- and Y-chromosomes respectively. They found that the centromeric index differed significantly among chromosomes 1, 6 and 9.

Chromosome banding

Hageltorn and Gustavsson (1973) demonstrated that a technique utilising trypsin pre-treatment provided the most consistent results in G-banding patterns in the pig karyotype. They found that chromosome nine could be distinguished from the X by a broad and strongly stained band in the distal region of the short arm, compared with the distinct narrow central band in the short arm of the X-chromosome and the two banded pattern in the long arm appeared more distinct in the X-chromosome than in chromosome nine.

Hansen-Melander and Melander (1974) studied the karyotype of the pig (*Sus scrofa domestica*) after applying G-band and C-band staining procedures.

Schnedl (1974) reviewed the banding techniques and banding patterns of various rodents, cattle, sheep, goats, pigs and birds in relation to human chromosomes.

Pace *et al.* (1975) could obtain G bands in pig chromosomes by either SSC or trypsin pretreatment methods.

Bosma (1976) studied chromosomal polymorphism and G-banding patterns of wild boar (*Sus scrofa* L.) in Netherlands and found that the extra

submetacentric chromosome of wild boar was homologous with chromosome 15 and 17 of the domestic pigs.

Michelmann *et al.* (1977) made a comparison of chromosomes in breeding and fattening pigs using the Giemsa staining and banding techniques and found that exchanges were more clearly recognisable with the G-banding technique.

Christensen and Smedegard (1978) suggested the use of C-band polymorphism as a marker in chromosome mapping and as an aid to check the parentage of pigs in Danish Landrace (DL), Danish Large White (DLW) and it crosses. They revealed that C-band patterns was inherited in a regular Mendelian manner.

Miyake and Ishikawa (1978) successfully identified 18 pairs of autosomes and both sex chromosomes in pigs by a modified trypsin-Giemsa chromosome banding technique.

The First International Conference for the standardisation of banded karyotype of domestic animals held at Reading, England described the main G-band patterns with sufficient detail to permit the unequivocal identification of individual chromosomes of pigs (Ford *et al.*, 1980).

Lin *et al.* (1980) identified all the individual autosomes and sex chromosomes in the pig using various banding techniques. The X-chromosome was shown to have a banding pattern similar to that of the

human X-chromosome. The porcine genome was found to contain at least three types of heterochromatin as a result of C-banding and olivomycin fluorescent banding techniques.

Giannoni *et al.* (1981) had schematically presented the C-banding patterns of wild and domestic pigs.

Hansen (1982) found that in pig the C-band patterns of the different pairs were very similar and that the C-band method could not be used for chromosome identification except for pair number 16 and for the Y-Chromosome.

Rønne *et al.* (1987) reported that the number of R-bands in the haploid set including the X- and Y-chromosomes were 541 in domestic pig (*Sus scrofa domestica*) using high resolution R-banded karyotypes.

Gustavsson (1988) made representative G- and R-banded karyotypes of the domestic pig.

Yu and Xin (1989) described the high resolution chromosome G-banding pattern of pigs. Five hundred and three bands per haploid set of chromosomes (including the X- and Y-chromosomes) were identified in the prometaphase stage and 300 bands in the metaphase stage.

Arroyo Nombela *et al.* (1990) made cytogenetic analysis of a wild boar population (*Sus scrofa scrofa*) with chromosomal polymorphism in the South East of Spain and observed three variants of diploid numbers (2n = 38,

2n = 37 and 2n = 36). The polymorphisms were shown to be due to a Robertsonian translocation between chromosome number 15 and 17 in karyotypes with 2n = 38. CBG band analysis of chromosomes by them revealed two types of heterochromatin; a dark staining type characteristic of the acrocentric chromosomes and a pale staining type characteristic of sub metacentric chromosomes. They found dark heterochromatin over the entire length of the long arm of the Y-chromosome.

Chen *et al.* (1991) found that banding patterns did not differ among breeds after making high resolution G-banding patterns of chromosomes of seven breeds of domestic pigs. They could detect 444 bands per haploid set including X- and Y-chromosomes.

Vijh *et al.* (1991) studied the G-banded chromosomal profile of Indian domestic pig. The chromosomes with G-banding patterns were classified into four morphological groups, viz. submetracentric (one to five), subtelocentric (six and seven), metacentric (eight to twelve) and telocentric (thirteen to eighteen). The results were comparable with the standard G-banded karyotypes of domestic pigs.

A study on high resolution banded karyotype of Erhualian pig was made by Wang *et al.* (1991) in which prometaphase chromosomes were studied by G-, R-, C- and DA/DAPI banding and silver staining of nucleolus organizer regions. They found that the G- and R-banding patterns were similar

to those of other domestic pig breeds. But the C-bands and Ag-NORs were polymorphic.

Li (1992) made chromosome comparison of Landrace, Yorkshire and Duroc pigs and found that they had similar conventional karyotypes and G-banding patterns, but had different C-banding patterns.

Switonski and Pietrzak (1992) conducted cytogenetic survey of AI boars in Poland using four staining methods viz. conventional Giemsa staining, G-banding, C-banding and Ag-NOR staining. They could observe polymorphism of block size of constitutive heterochromatin in 15 boars and polymorphism of nucleolar organiser region (NOR) size in the karyotype of four boars. The C-band polymorphism was observed in acrocentric chromosome pairs thirteen to seventeen while Ag-NOR polymorphism was found in NOR bearing pairs eight and ten.

Rao *et al.* (1993) found no differences in banding patterns of Indian domestic pig and Landrace using G-, C- and NOR banding patterns.

Xu *et al.* (1994) suggested that C-bands of pig chromosomes can be used as genetic markers as the C-band sizes of the same chromosomes in the cells with an individual were highly repeatable and C-band sizes at different growth stages of an individual were similar.

Representative RBG-banded chromosomes of *Sus scrofa domestica* and diagrammatic representation of the banding patterns at the

600-band resolution were presented by Rønne (1995). He compared the different classification systems of chromosomes and suggested the application of the details obtained to gene mapping, location of chromosome aberrations, comparative cytogenetics and translation between the different classification systems.

Xu *et al.* (1995) measured the band length, band area and quantity of heterochromatin of C-banded chromosomes of Erhualian and Large White pigs and their reciprocal crosses. According to them the frequency of C-band area polymorphism differed significantly for chromosome 13 among the breeds.

Ma Yong Xing *et al.* (1996) analysed the chromosomes of a new pig stain evolved by crossing the Chenhua and Landrace breeds using peripheral blood leukocyte culture. Various banding techniques (G-, C-, R- and Ag-NOR) were used for chromosome analysis. They could not found any noticeable difference in G- and R-band karyotypes of the new pig strain and its parents. They opined that C-banding can be used to differentiate strains of pigs.

Meo *et al.* (2000) studied the distribution of constitutive heterochromatin (C-bands) in chromosomes of two Italian pig breeds, Calabrian and Siena Belted and found that the use of high resolution banding techniques allowed a detailed description of heterochromatin distribution in pig chromosomes which appeared highly polymorphic within and among chromosome pairs.

Materials and Methods

MATERIALS AND METHODS

The Suidae family contain five living genera, of which the genus Sus contains several species including *Sus scrofa* the domestic pig. The five genera are Sus (the domestic pig), Potamochoerus (the bush pig), Phaeochoerus (the wart hog), Hylochoerus (the forest hog) and Baby rousa (the celebes hog). The attempts to analyse and characterise the chromosome architecture of the species was of interest for scientists during last few decades.

Desi pigs and Large White Yorkshire pigs stationed at AICRP on Pigs, Centre for Pig Production and Research, Mannuthy formed the material for the study. Fifty four Desi pigs and 45 Large White Yorkshire pigs were subjected to cytogenetic analysis.

1 Cytogenetic study

Cytogenetic screening of the animals included under the study was performed. Metaphase spreads of the pigs were obtained by peripheral blood leukocyte culture technique.

1.1 Collection and transportation of blood

Ten ml of venous blood was aseptically collected from the ear vein or anterior vena cava in sterile tubes with a heparin concentration of 15 IU/ml of blood.

Blood samples were transported to the cytogenetic laboratory within 30 minutes of collection and kept at 8°C. Culture of the samples were set within two hours of collection.

1.2 Preparation of culture medium

Culturing of blood was done in a composite tissue culture medium having the following composition.

RPMI 1640 lyophilised powder (Sigma) – 1 g

Benzyl penicillin solution (10,000 IU/ml) - 1.0 ml

Streptomycin Sulphate solution (10 mg/ml) – 1.0 ml

Pokeweed mitogen solution (1 mg/ml) – 1.0 ml

Phytohemagglutinin M solution (2 mg/ml) – 0.5 ml

Sodium bicarbonate solution (3.5 per cent) – 1.0 ml

Autoclaved, double distilled water – 95.5 ml

The pH of the medium was adjusted to 7.2. The medium was then filtered through membrane filter having a pore size of 0.22 µ. One hundred ml of culture medium was prepared at a time and was divided into five ml aliquots in the culture vials. The culture vials containing the medium were stored at – 5°C.

1.3 Leukocyte culturing technique

Leukocyte cultures were set up by placing 0.5 ml of whole blood into a culture vial containing the medium. Cultures were set up in duplicate for

each blood sample. Two ml of autologous plasma was also added to each culture.

The culture vials were incubated at 37°C for 70 hours. During the period of incubation, the cultures were gently mixed two times a day, to avoid lymphocytes from being trapped in agglutinated erythrocytes.

1.4 Culture harvesting

The following procedures were adopted to get the metaphase chromosomes on microscope slides from the cells in the culture.

(a) Colcemid (PAA lab) solution (10 µg/ml) was added at the rate of 0.05 ml per culture and was incubated for one hour at 37°C.

(b) The cultures were centrifuged at 1500 rpm for 15 minutes.

(c) The supernatant was discarded and the cell button was resuspended in ten ml of 0.075 M potassium chloride (0.56 per cent) prewarmed to 37°C.

(d) The suspension was incubated for 30 minutes at 37°C.

(e) The suspension was centrifuged at 1000 rpm for 10 minutes.

(f) The supernatant was discarded and the cell button was resuspended with five ml of chilled Carnoy's fixative (methyl alcohol : glacial acetic acid in 3:1 ratio) and left undisturbed for 30 minutes.

(g) The suspension was centrifuged at 1000 rpm for 15 minutes.

(h) The supernatant was removed and the cell button was resuspended with five ml of Carnoy's fixative.

(i) Procedures (g) and (h) were repeated till the supernatant was clear.

(j) A cell suspension of about one ml was made using fresh fixative.

During the harvesting, sterile siliconized pasteur pippettes were used to avoid loss of cells.

1.5 Slide preparation and staining

Clean grease free slides were chilled in cold methyl alcohol. Three to four drops of the cell suspension was dropped on to the slide from a height of about three feet. If the suspension was thick a gentle blow was given to spread the drops on the slide. The slides were dried on top of a flame.

Staining of the dried slides were done with 4 per cent Giemsa stain. Slides were stained for 30 minutes with occasional blowing of air, rinsed with distilled water and air dried.

1.6 Photomicrography

Three slides were prepared from each culture and screened under the microscope for metaphase spreads. Each slide was screened systematically under the low power (10x). About 30 metaphase spreads of each pig were identified and good ones were studied for assessing the morphology of the chromosomes.

The metaphase spreads with minimum chromosome overlapping and sufficient chromosome length were photographed, to give a final magnification of 945x using Carl-Zeiss photomicroscope III. The photographs were taken on 125 ASA black and white film and/or 100 ASA colour film.

1.7 Morphometric measurements

The size of various chromosomes of the pig were precisely measured using micrometer.

The size of the chromosomes were represented as relative lengths. Relative length of a chromosome was calculated by dividing the length of the chromosome with total length of the haploid set of chromosomes including X-chromosome and expressed as per cent. Centromeric indices and arm ratios of the chromosomes were calculated as follows

$$\text{Centromeric index} = \frac{\text{Length of short arm}}{\text{Total length}} \times 100$$

i.e, $\frac{p}{p+q} \times 100$

$$\text{Arm ratio} = \frac{\text{Length of long arm}}{\text{Length of short arm}}$$

i.e, $\frac{q}{p}$

Relative length of X- and Y-chromosomes and the arm ratio and centromeric index of X-chromosome of the two breeds were compared (Snedecor and Cochran, 1967).

1.8 Preparation of karyotype

Karyotypes were prepared from photographic prints of metaphase spreads. The photograph of good metaphase spread was developed and the chromosomes in the spread were cut and removed. The paired chromosomes were grouped based on their size and morphology. Karyotypes were arranged based on the length of chromosomes and centromeric position. The X- and Y-chromosomes formed the last pair. Idiogram was prepared.

1.9 G–banding

G-banding was performed using the method described by Ibrahim *et al.* (1983) with minor modifications. The method was a combination of the methods described by Sumner *et al.* (1971) and Seabright (1971).

Trypsin solution for banding was prepared by dissolving 0.025 g of trypsin (Gibco, 1:250), 6.0 g Na_2HPO_4, 1.0 g KH_2Po_4 and 1.0 g EDTA in 500 ml of deionised distilled water. The slides prepared were aged for three to seven days at room temperature. The aged slides were immersed in coupling jars with 50 ml 2 x SSC (0.3 M NaCl, and 0.03 M trisodium citrate) containing 1.0 ml of trypsin solution, and incubated at 60°C for 45 minutes in a water bath. The slides were treated with 70 per cent methyl alcohol to stop the action of trypsin. The slides were rinsed in distilled water and then in Sorenson's buffer (pH 6.8).

Slides were stained in 10 per cent Giemsa for five minutes, rinsed in distilled water and air dried.

1.10 Preparation of G–banded karyotype

Good metaphase spreads subjected to G-banding were identified and photographed using Carl–Zeiss photomicroscope III. The chromosomes were cut out and arranged in pairs. The banding pattern of each chromosome pair was studied as per the recommendations given by Ford *et al.* (1980).

Results

RESULTS

A programme to analyse and delineate chromosomes of local desi pigs of Kerala (Plate 1), Large White Yorkshire pigs (Plate 2) and their comparative evaluation was planned and carried out.

I. Materials and technique

Peripheral blood leukocyte culture using heparinised blood from 54 desi pigs and 45 Large White Yorkshire pigs was performed. Good metaphase spreads without any overlapping prepared from the cultures were used for the study (Plates 3 and 4). The karyotypes were prepared based on the chromosome length and position of the centromere and the chromosomes were numbered accordingly. Selected slides from the desi pigs were subjected to G-banding and the bands observed were analysed and described.

II. Chromosome number

The karyotype analysis revealed that in desi pigs of Kerala, the diploid chromosome number was 38 or 19 pairs which consisted of 18 pairs of autosomes and a pair of sex chromosomes which were XX in female and XY in male.

In Large White Yorkshire pigs also, the chromosome complement was 18 pairs of autosomes and a pair of sex chromosomes (XX in female and XY in male).

III. Chromosome morphology

The karyotypes of desi pigs (Plates 5 and 6) prepared were analysed for chromosome morphology. The autosomes consisted of 12 pairs of biarmed chromosomes which included eight pairs of submetacentric and four pairs of metacentric chromosomes. Six pairs of autosomes were single armed (acrocentric). The X-chromosome was submetacentric in morphology whereas the Y-chromosome was metacentric and the smallest of the group. Large White Yorkshire pigs were also found to have 12 pairs of biarmed and six pairs of single armed autosomes (Plates 7 and 8). Of the biarmed chromosomes, eight pairs were of the submetacentric morphology and four pairs of metacentric morphology. The sex chromosomes of Large White Yorkshire pigs were also morphologically similar to that of the desi pigs with the X-chromosome having a submetacentric morphology and the Y-chromosome with a metacentric morphology was the smallest.

Thus in both desi and Large White Yorkshire pigs, the autosomes can be described under three classes depending on their centromere positions - submetacentric, metacentric and the acrocentric.

IV. Chromosome morphometry

A better description of the karyotype and chromosomes was done using certain morphometric measurements. These include

1. Relative length

The mean relative length of chromosomes of the desi pigs studied varied from 11.69 ± 0.19 per cent for the largest to 1.95 ± 0.13 per cent for the

smallest. The largest chromosome was a submetacentric chromosome (chromosome 1) and the smallest was the Y-chromosome. The smallest autosome (chromosome 18) had a mean relative length of 2.21 ± 0.11 per cent and had an acrocentric morphology. The mean relative length of the X-chromosome of the desi pig was 4.63 ± 0.25 per cent and it was the eleventh largest chromosome in desi pigs. The mean relative length of the chromosomes of desi pigs of Kerala are tabulated in Table 1. An idiogram of desi pigs of Kerala is presented in Figure 1.

Mean relative length of chromosomes in Large White Yorkshire pigs are presented in Table 2. In Large White Yorkshire pigs, the mean relative length of chromosomes varied from 11.35 ± 0.37 per cent for the largest (chromosome 1) to 1.7 ± 0.07 per cent for the smallest (chromosome Y). A mean relative length of 2.03 ± 0.21 per cent was observed for the smallest autosome of the Large White Yorkshire pigs which was acrocentric. The X-chromosome of the Large White Yorkshire pig had a mean relative length of 5.01 ± 0.22 per cent. Idiogram of the Large White Yorkshire pig is presented as Figure 2.

2. Arm ratio

The mean arm ratio of the submetacentric chromosomes of the desi pigs varied from 1.35 ± 0.04 to 3.31 ± 0.13. The highest arm ratio was for chromosome 2 and the lowest for chromosome 8. The X-chromosome had an arm ratio of 1.97 ± 0.08. The metacentric autosomes (chromosomes 9-12) and

the Y-chromosome had an arm ratio of one. Table 3 shows the arm ratios of the chromosomes of the desi pigs.

In Large White Yorkshire pigs, the mean arm ratios ranged from 1.54 ± 0.07 to 2.86 ± 0.13. The highest arm ratio was for chromosome 2 and the smallest for chromosome 5. The X-chromosome had an arm ratio of 1.81 ± 0.15. Arm ratios of chromosomes of Large White Yorkshire pigs are tabulated in Table 4.

3. Centromeric Index

Centromeric index indicates the position of the centromere of the chromosomes. All the metacentric chromosomes in which the centromeres were situated in the centre had a centromeric index of 50. The centromeric index was lowest for chromosome 2 in desi pigs and had a mean value of 23.06 ± 0.84. Highest mean centromeric index of 42.68 ± 0.71 was observed for chromosome 8 in desi pigs. The X-chromosome of desi pigs had a mean centromeric index value of 32.09 ± 1.17. Table 5 shows the mean centromeric index values for the submetacentric chromosomes of desi pigs.

Chromosome 2 had the lowest mean centromeric index value of 26.2 ± 0.89 in Large White Yorkshire Pigs and chromosome 8 having the highest of 39.45 ± 1.51 among the submetacentric chromosomes. The X-chromosome had a mean centromeric index value of 36.33 ± 1.64. The metacentric chromosomes (chromosome Y and autosomes 9-12) had the

highest centromeric index of 50. The mean centromeric index values of Large White Yorkshire Pig chromosomes are shown in Table 6.

V. Karyotype

Karyotypes were prepared from very distinct metaphase spreads. The desi pigs and the Large White Yorkshire pigs had similar karyotypes. Karyotype of male and female desi pigs are given in Plates 5 and 6 and that of male and female Large White Yorkshire pigs in Plates 7 and 8, respectively. The autosomes were classified into three groups according to their morphology as sub metacentric (chromosome 1-8), metacentric (chromosome 9-12) and acrocentric. The sex chromosomes formed the fourth group. Within the group, the chromosomes were arranged in the descending order of size.

VI. G-banded karyotype

G-banded karyotypes of Large White Yorkshire pigs were described already by several workers and standard karyotype is available. G-banded karyotypes of desi pigs in Kerala were not available. The chromosomes of desi pigs were subjected to G-banding (Plate 9). A representative G-banded karyotype obtained is presented in Plate 10. Homologous chromosomes in the metaphase spread were paired by their characteristic banding patterns and arranged into a karyotype according to the international standard (Ford *et al.*, 1980). The chromosomes were arranged and described under three groups namely submetacentric, metacentric and acrocentric. The sex chromosomes were grouped separately. A diagrammatic representation of the G-banded karyotype is shown in Figure 3. The distinct visible bands observed in the

karyotype were only described. The banding pattern of chromosomes are as follows:

Sub-metacentric group - This group consist of chromosome pairs one to eight.

Chromosome No.1 – Largest of the submetacentric chromosomes. The short arm has a light central band. The long arm has two dark central bands.

Chromosome No.2 – A dark band on the short arm. On the long arm there was a broad dark area forming dark band distally and a narrow dark band near the centromere.

Chromosome No. 3 – The short arm has a distinct dark band centrally and a less distinct distal band. The long arm has three to five distal bands with a tendency to fuse and form single band.

Chromosome No. 4 – The short arm has a dark band near the centromere. A light band present on the long arm divides it into two, a conspicuous proximal dark band and a distal dark area.

Chromosome No. 5 – The short arm has a dark band near centromere and a light band at the distal end. The long arm has dark proximal band adjacent to centromere, light central band and two dark distal bands.

Chromosome No. 6 – A dark band is located centrally in the short arm. The proximal part of the long arm has two light bands and the distal part has a broad dark band.

Chromosome No. 7 – The short arm has one dark central band. The long arm has two dark bands proximally and a light band distally.

Chromosome No. 8 – Two dark bands in the short arm. The long arm has one broad dark proximal band and two dark distal bands.

Metacentric group – Chromosome nine to twelve were included in this group.

Chromosome No. 9 – There are two distinct dark bands in the p arm. The q arm has two dark bands proximally and a light band distally.

Chromosome No. 10 – A large faintly stained centromeric region is the most characteristic feature of this chromosome. There are two dark distal bands in the p arm and two dark bands in the q arm.

Chromosome No. 11 – A dark band present on the proximal part of the p arm and two dark bands on the q arm.

Chromosome No. 12 – There is a dark proximal band and a narrow dark distal band on the p arm and two dark bands in the q arm with tendency to fuse.

Acrocentric group – This group include chromosome number thirteen to eighteen.

Chromosome No.13 – It is the largest acrocentric chromosome. It has four distinct regions delineated by three light bands. The dark bands are sub divisible.

Chromosome No.14 – There were two dark proximal bands and three dark distal bands separated by a light central band.

Chromosome No.15 – A dark band near the centre divides the chromosomes into two regions. The proximal region has two dark bands and the distal region has three dark bands.

Chromosome No.16 – Two dark bands were present proximally and a light with dark band distally.

Chromosome No.17 – It has two distinct dark bands proximally and a narrow dark band distally.

Chromosome No.18 – A dark central band was present.

Sex chromosomes – The X-chromosome was submetacentric and the Y-chromosome metacentric.

Chromosome X – It has a dark band in the middle of the short arm. The long arm had a distinct dark band located in the proximal end and a less distinct dark band at the distal end.

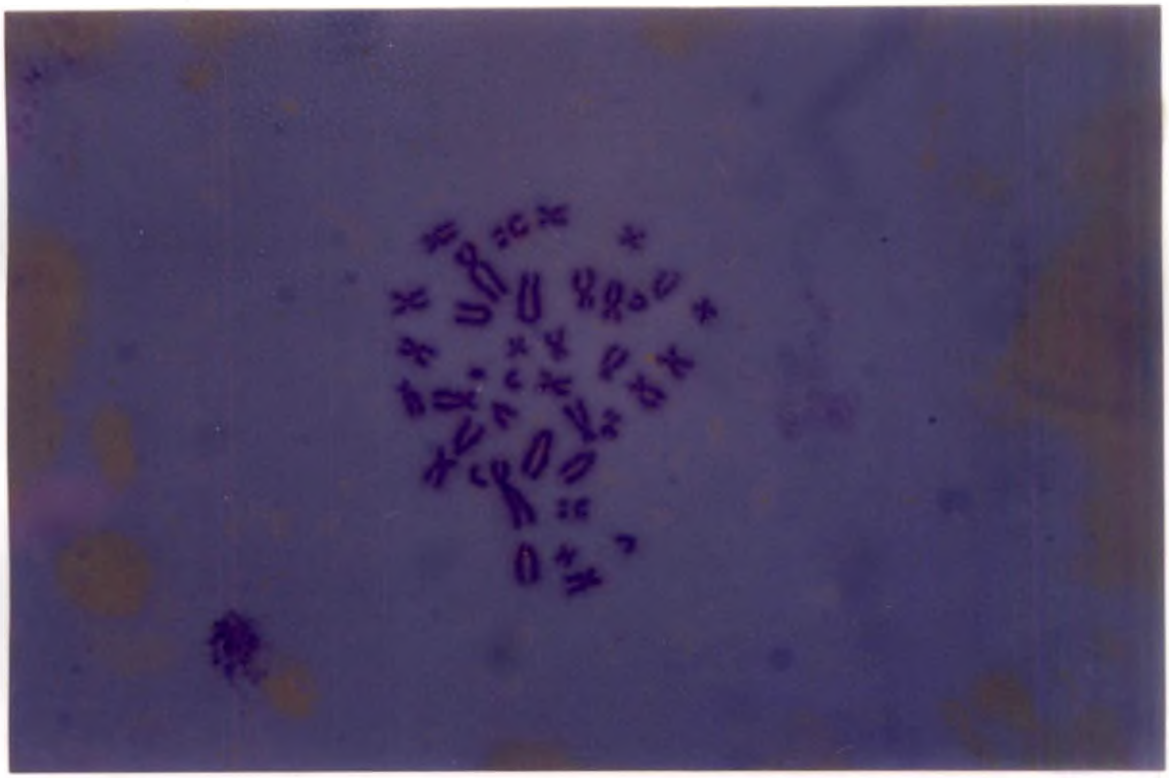
Chromosome Y – It was the smallest metacentric chromosome. The p arm was darkly stained and less a dark band in the middle. The q arm was faintly stained.

Plate 1. Local desi pig of Kerala

Plate 2. Large White Yorkshire pig

Plate 3. Representative metaphase spread - Desi pig

Plate 4. Representative metaphase spread - Large White Yorkshire pig



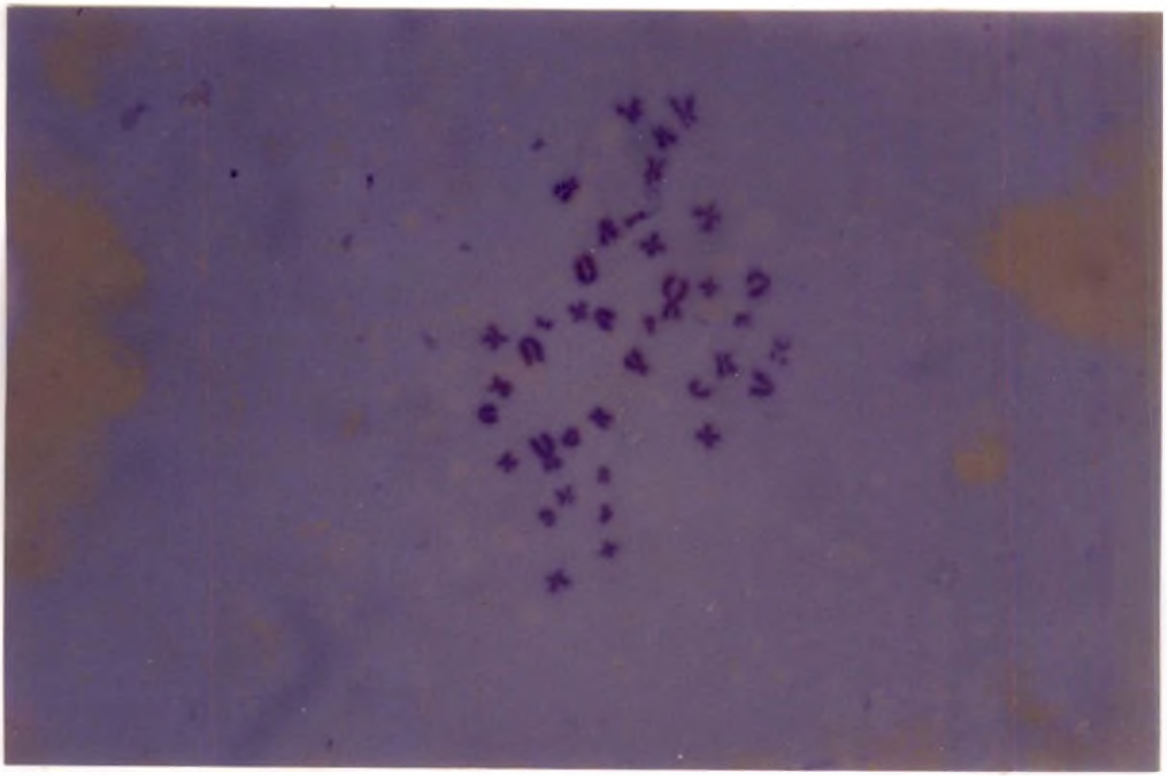
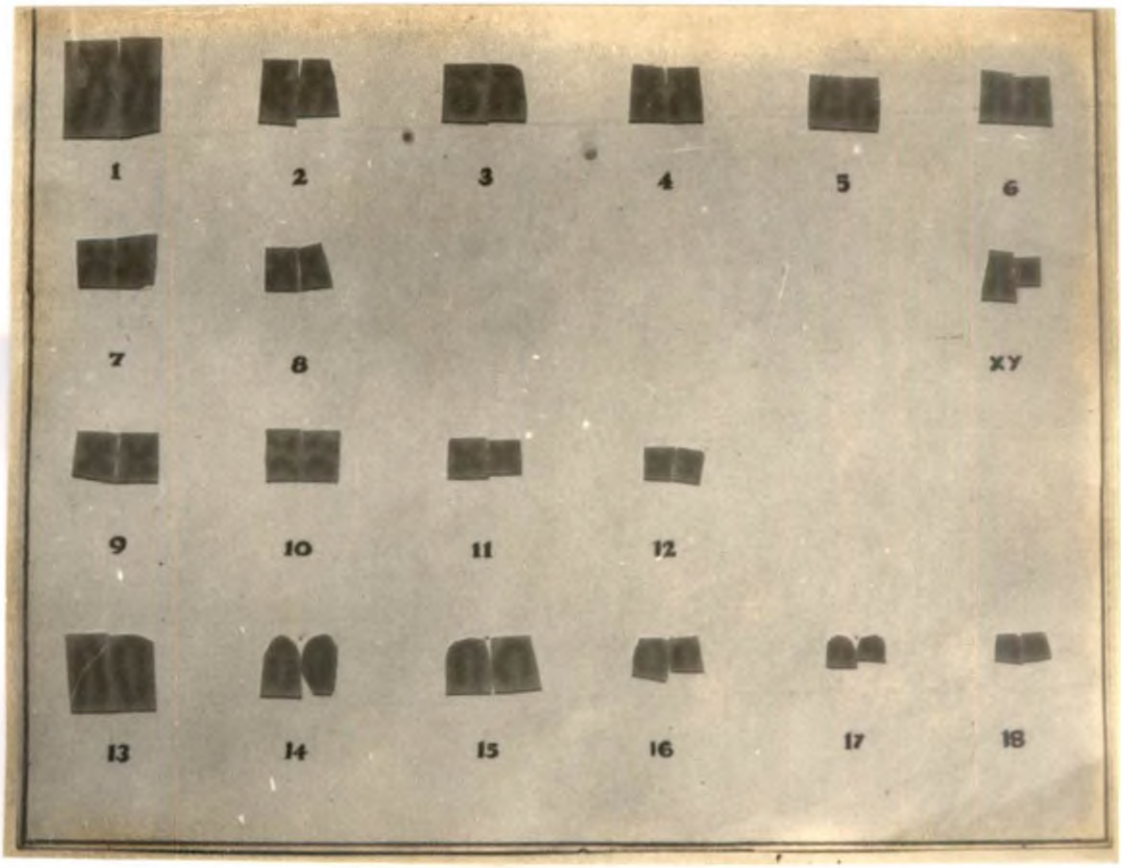


Plate 5. Karyotype of desi pig (male)

Plate 6. Karyotype of desi pig (female)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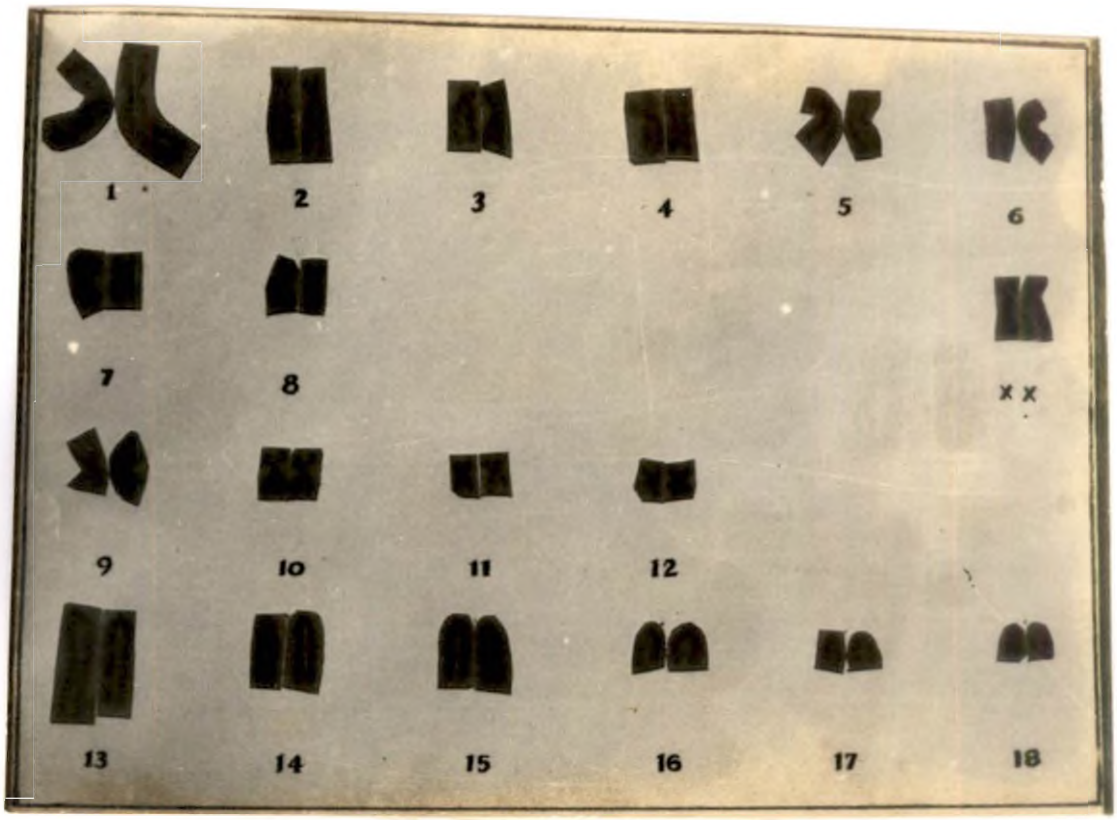
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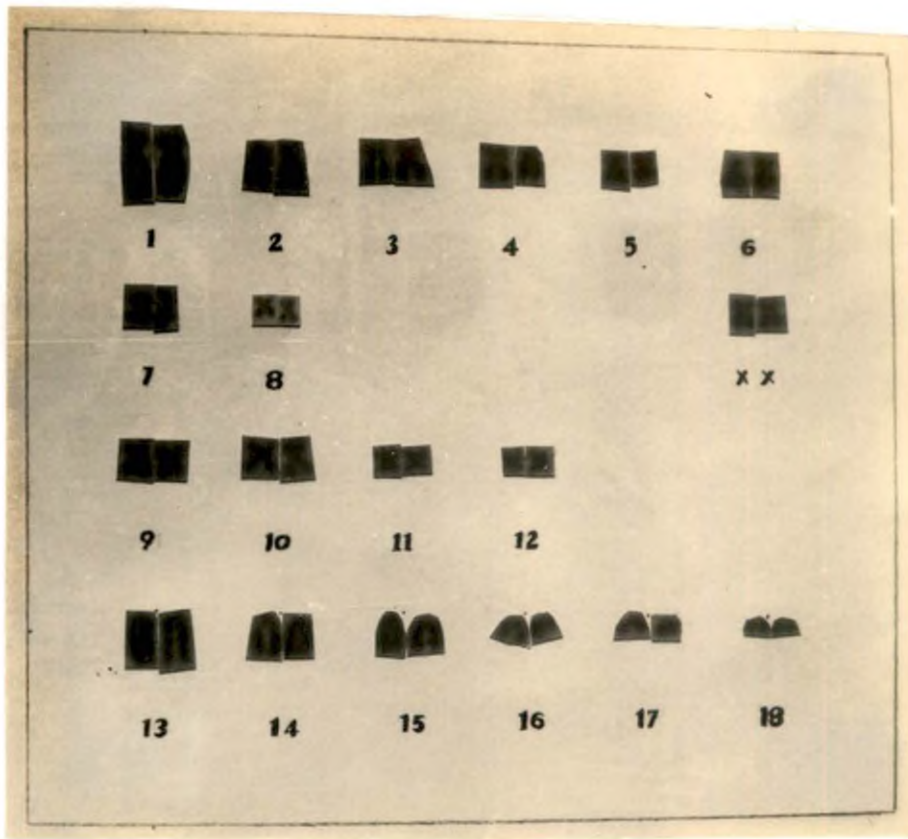
Plate 7. Karyotype of Large White Yorkshire pig (male)

Plate 8. Karyotype of Large White Yorkshire pig (female)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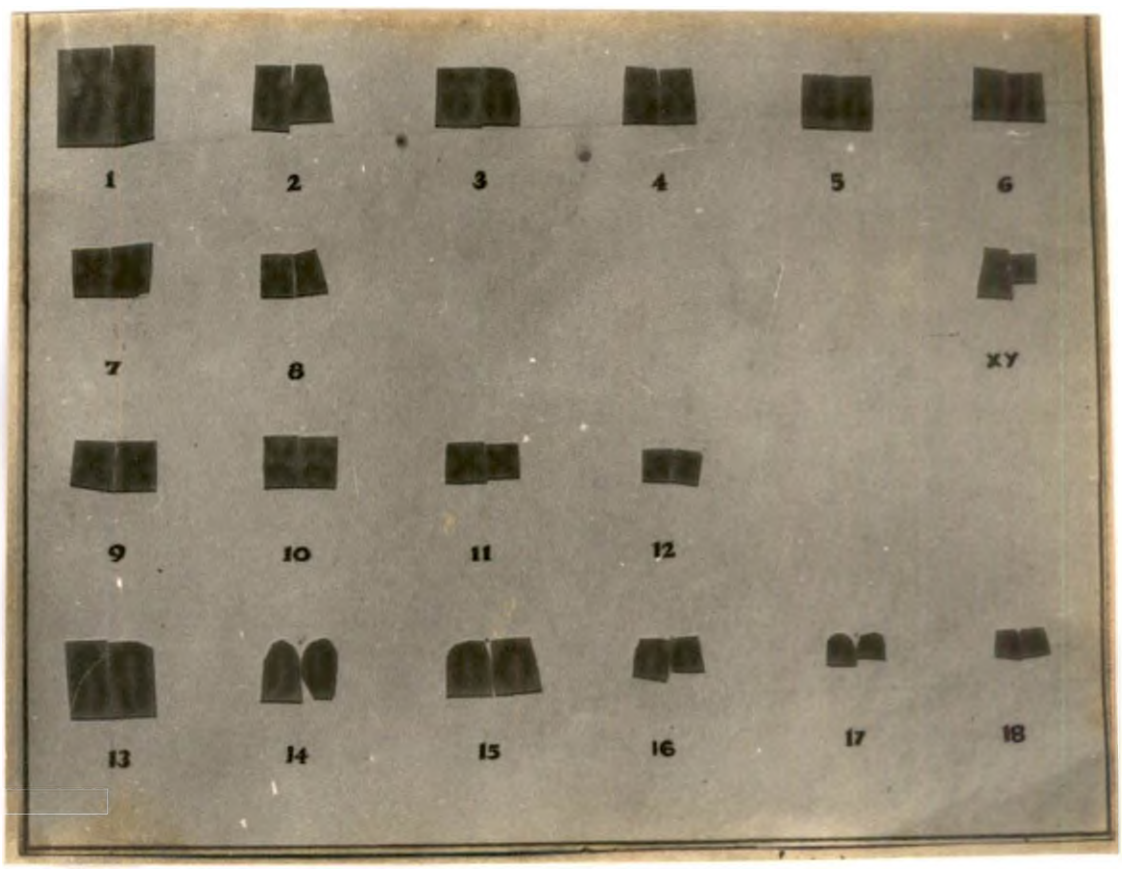
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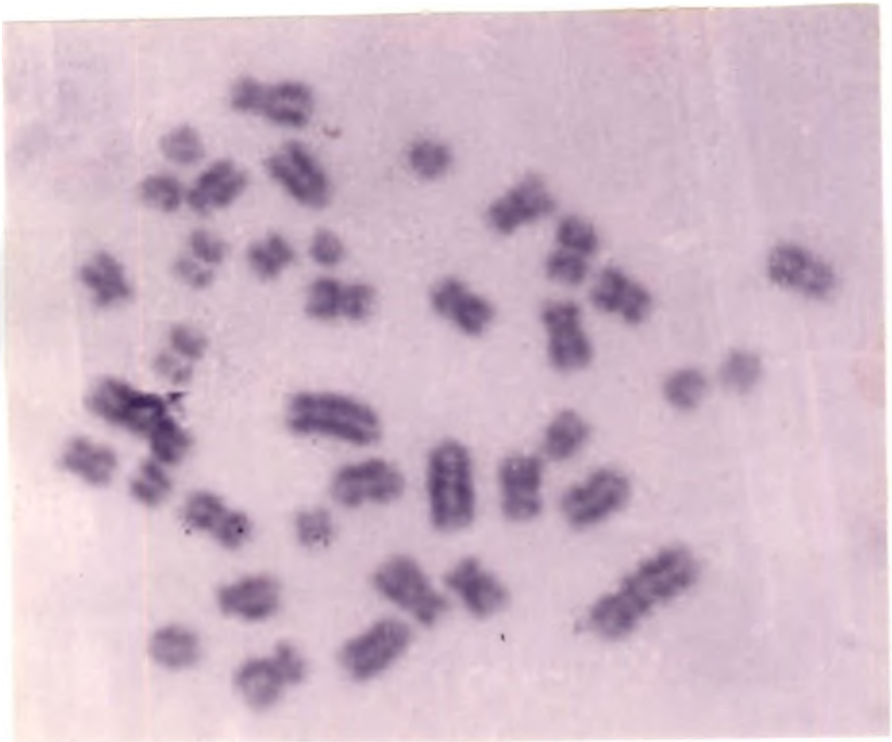
Plate 9. G-banded metaphase spread of desi pig

Plate 10. Representative G-banded karyotype of desi pig



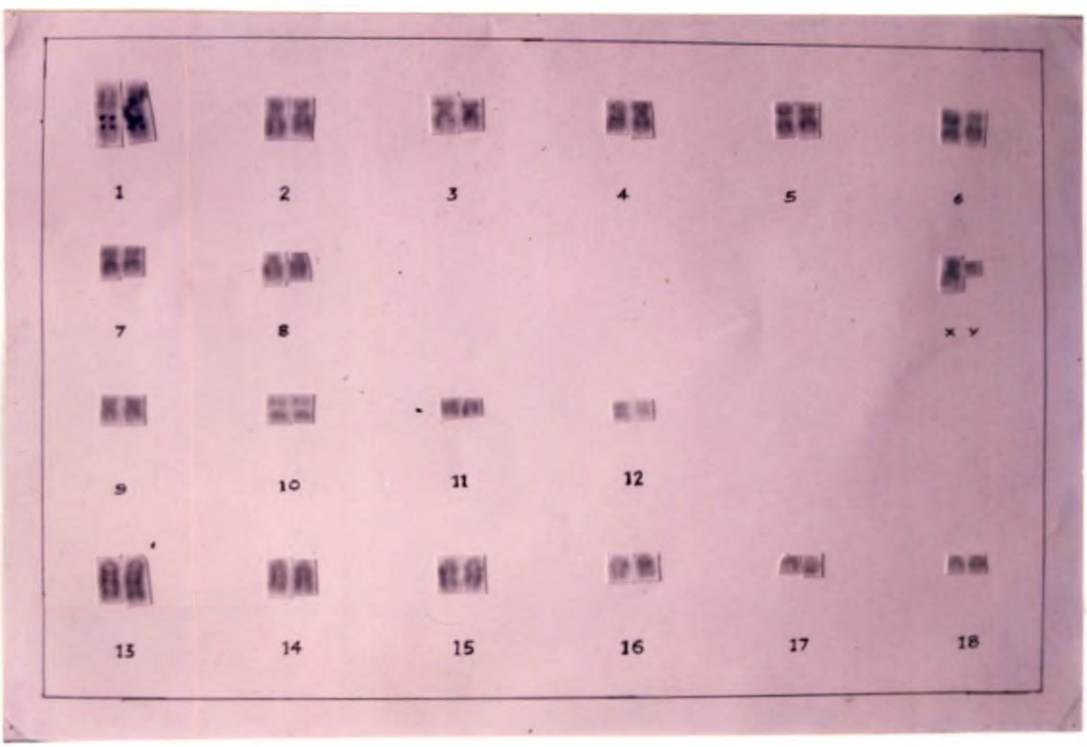

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TABLE 1

RELATIVE LENGTH OF CHROMOSOME – DESI PIG

Chromosome Number	Relative Length Mean ± SE	Range
1	11.69± 0.19	10.76 – 13.19
2	7.26 ± 0.19	6.55 – 8.58
3	6.34 ± 0.14	5.97 – 7.39
4	5.92 ± 0.08	5.58 – 6.37
5	5.68 ± 0.08	5.15 – 6.11
6	5.27 ± 0.08	4.73 – 5.68
7	4.54 ± 0.11	4.10 – 5.01
8	4.22 ± 0.11	3.63 – 4.52
9	5.29 ± 0.11	4.78 – 5.97
10	4.00 ± 0.08	3.60 – 4.37
11	3.38 ± 0.15	2.64 – 4.29
12	2.78 ± 0.16	2.11 – 3.49
13	8.62 ± 0.14	7.86 – 9.33
14	6.41 ± 0.15	5.68 – 7.46
15	5.21 ± 0.19	3.96 – 6.31
16	3.52 ± 0.19	2.64 – 4.29
17	2.71 ± 0.09	2.24 – 3.11
18	2.21 ± 0.11	1.58 – 2.70
X	4.63 ± 0.25	4.22 – 6.07
Y	1.95 ± 0.12	1.72 – 2.07

TABLE 2

RELATIVE LENGTH OF CHROMOSOME-LARGE WHITE YORKSHIRE PIG

Chromosome Number	Relative Length Mean ± SE	Range
1	11.35 ± 0.37	10.14 – 13.74
2	7.65 ± 0.13	7.11 – 8.25
3	6.58 ± 0.12	5.79 – 7.27
4	6.09 ± 0.13	5.26 – 6.67
5	5.69 ± 0.17	4.55 – 6.31
6	5.63 ± 0.13	5.26 – 6.67
7	4.29 ± 0.23	2.73 – 5.31
8	3.91 ± 0.24	2.73 – 4.85
9	5.37 ± 0.21	4.58 – 6.67
10	4.56 ± 0.26	3.81 – 6.32
11	3.33 ± 0.16	2.22 – 3.86
12	2.91 ± 0.11	2.22 – 3.64
13	8.53 ± 0.21	7.73 – 9.92
14	6.19 ± 0.11	5.78 – 6.67
15	5.36 ± 0.10	4.83 – 5.79
16	3.29 ± 0.09	2.86 – 3.78
17	2.33 ± 0.14	1.52 – 3.09
18	2.03 ± 0.21	1.53 – 2.41
X	5.01 ± 0.22	3.64 – 5.83
Y	1.70 ± 0.07	1.37 – 1.94

TABLE 3

ARM RATIO OF CHROMOSOMES – DESI PIG

Chromosome Number	Arm ratio Mean ± SE	Range
1	2.05 ± 0.08	1.71 – 2.38
2	3.31 ± 0.13	2.40 – 4.00
3	1.46 ± 0.06	1.28 – 2.00
4	1.86 ± 0.11	1.60 – 2.83
5	2.31 ± 0.14	1.71 – 2.80
6	1.92 ± 0.21	1.17 – 2.60
7	1.36 ± 0.04	1.17 – 1.67
8	1.35 ± 0.03	1.14 – 1.50
X	1.97 ± 0.08	1.60 – 2.33

TABLE 4

ARM RATIO OF CHROMOSOMES – LARGE WHITE YORKSHIRE PIG

Chromosome Number	Arm ratio Mean ± SE	Range
1	2.33 ± 0.15	1.73 – 3.50
2	2.86 ± 0.13	2.33 – 3.67
3	2.12 ± 0.09	1.67 – 2.50
4	1.94 ± 0.14	1.33 – 2.50
5	1.54 ± 0.07	1.60 – 2.00
6	1.89 ± 0.09	1.40 – 2.50
7	1.59 ± 0.07	1.10 – 2.00
8	1.57 ± 0.10	1.16 – 2.00
X	1.81 ± 0.15	1.40 – 3.00

TABLE 5

CENTROMERIC INDEX OF CHROMOSOMES – DESI PIG

Chromosome Number	Arm ratio Mean ± SE	Range
1	33.06 ± 0.95	29.63 – 36.84
2	23.06 ± 0.84	20.00 – 29.41
3	41.05 ± 0.98	33.33 – 43.75
4	36.21 ± 0.49	33.33 – 38.46
5	30.59 ± 1.29	25.00 – 36.84
6	36.91 ± 2.37	25.00 – 46.15
7	42.49 ± 0.80	37.50 – 46.15
8	42.68 ± 0.71	40.00 – 46.67
X	32.09 ± 1.17	26.09 – 37.50

TABLE 6

CENTROMERIC INDEX OF CHROMOSOMES - LARGE WHITE YORKSHIRE PIG

Chromosome Number	Arm ratio Mean ± SE	Range
1	30.50 ±1.26	28.57 – 36.67
2	26.20 ± 0.89	21.43 – 30.00
3	32.26 ± 0.98	28.57 – 36.36
4	34.80 ± 1.79	28.57 – 42.86
5	39.39 ± 0.80	33.33 – 42.86
6	34.85 ± 1.18	28.57 – 41.66
7	38.78 ± 1.03	33.33 – 45.45
8	39.45 ± 1.51	33.33 – 46.15
X	36.33 ± 1.64	25.00 – 41.67

Fig. 1. Idiogram of desi pig of Kerala.

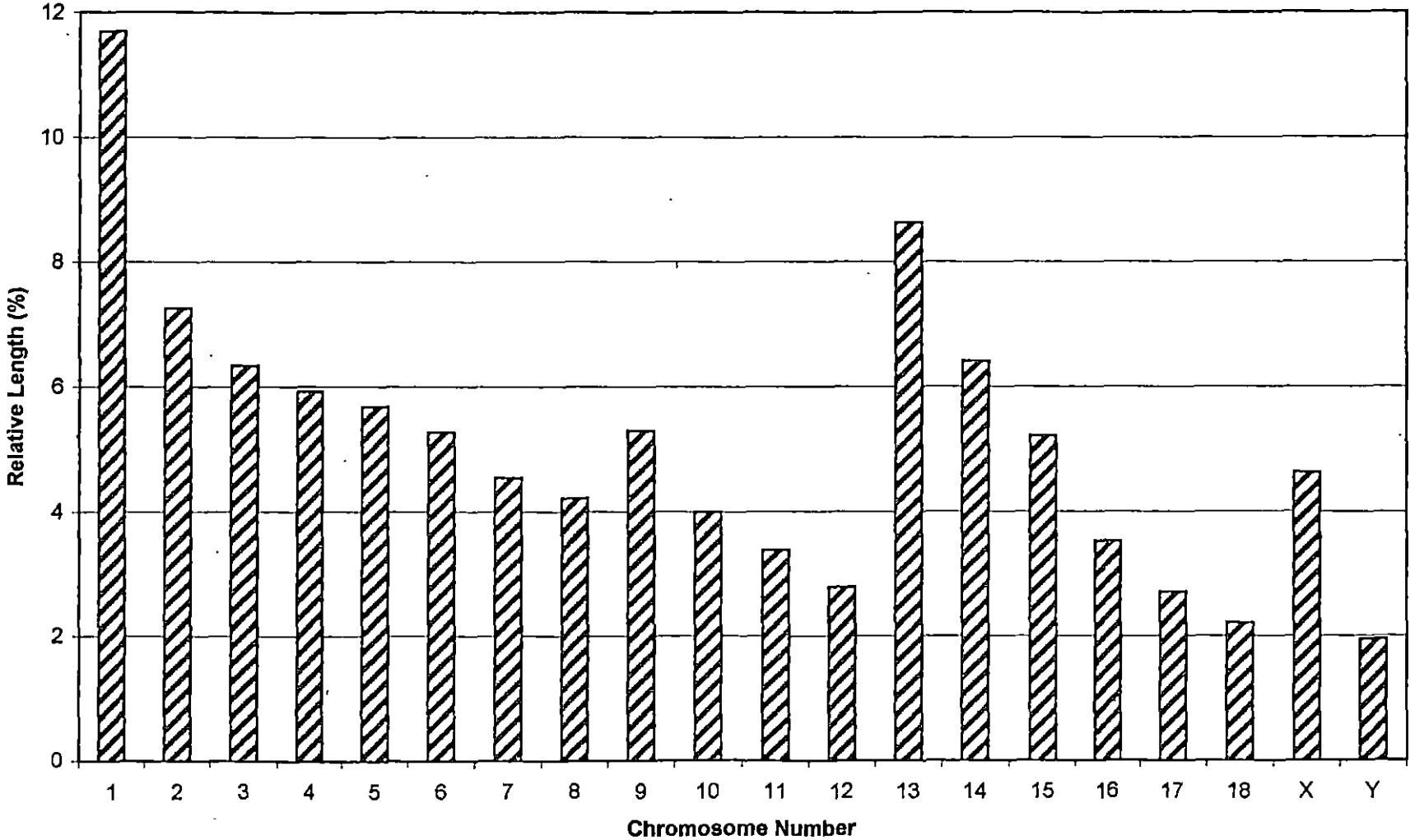
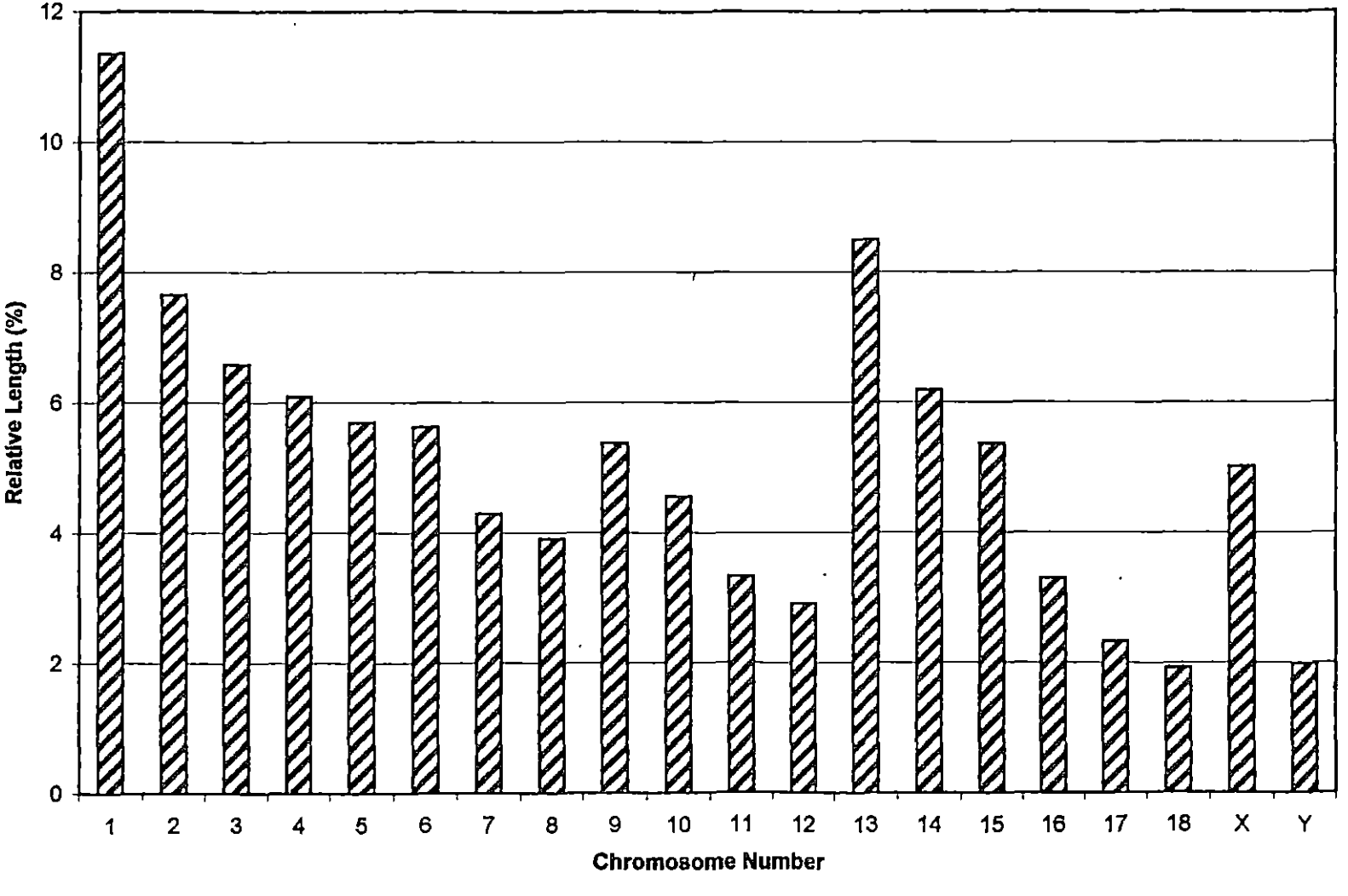


Fig. 2. Idiogram of Large White Yorkshire pig.



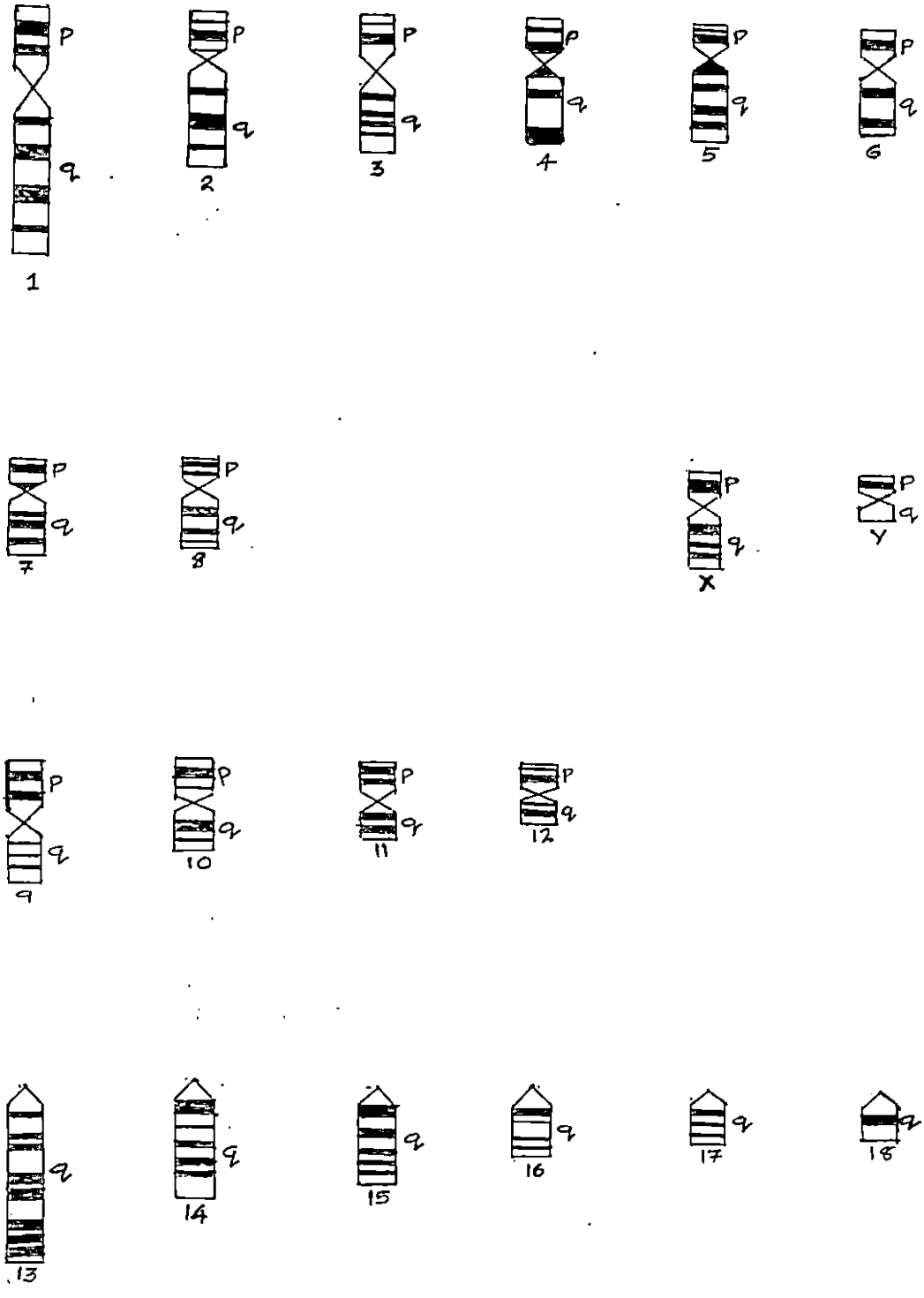


Fig.3. Diagrammatic representation of G-banded karyotype of desi pig

Discussion

DISCUSSION

Cytogenetic techniques such as karyotyping can play a very important role in the genetic characterization of animals. Chromosome of desi pigs of Kerala were subjected to cytogenetic investigation by kayotyping and a comparison was made with the karyotype of Large WhiteYorkshire pigs.

Chromosome identification becomes easy by subjecting them to banding techniques. Most commonly used banding is the G-banding which produce alternate dark stained and light stained areas. Each chromosome has its own characteristic banding pattern. The number, position, width and nature of bands are specific for each chromosome pair facilitating their easy identification. Chromosomes of desi pigs were subjected to G-banding in the present study.

Chromosome number

A diploid chromosome number of 38 was obtained for desi pigs of Kerala in the present study. A similar diploid number of chromosomes were obtained for the Indian domestic pig by Bhatnagar and Srivastava (1972), Vijh *et al.* (1990) and Palegar *et al.* (1991) in different parts of the country.

In Large White Yorkshire pigs also, a diploid chromosome number of 38 was obtained. A similar diploid chromosome number was obtained for Large White Yorkshire pigs in different parts of the world as reported by several workers (Melander, 1951; Gimenez-Martin *et al.*, 1962; Mc Connell *et al.*, 1963; Krasavtsev and Yu, 1978; Nechiporenko, 1974; Goldman and

Zhivalec, 1970; Gorin and Chudon, 1978; Kim *et al.*, 1994). A diploid chromosome number of 38 has been reported by Palegar *et al.* (1991) in Large White Yorkshire pigs of India.

The karyotypes with variations in their diploid chromosome numbers were reported by various workers (Mc Fee *et al.*, 1966; Rary *et al.*, 1968; Gropp *et al.*, 1969; Strelchenko, 1977; Melander and Hansen-Melander, 1980; Popescu, 1980). Gropp *et al.* (1969) suggest a preferential selection of the 38 chromosome type in the course of domestication as the domestic breeds of Asia and Europe contain 38 chromosomes. The occurrence of a diploid chromosome number of 38 in the desi pigs is indicative of a high level of karyological stability (Vijh *et al.*, 1990).

Chromosome morphology

The chromosomes of desi pigs and Large White Yorkshire pigs were morphologically similar. Both the breeds had 19 pairs of chromosomes which included 18 pairs of autosomes and a pair of sex chromosomes (XX or XY). The autosomes included 12 pairs of submetacentric or metacentric chromosomes and six pairs of acrocentric ones. This is in agreement with the observations were made by several workers (Di Antonio, 1964; Krasavtsev and Yu, 1968; Zivkovic *et al.*, 1971; Goldman and Zhivalec, 1970) in various breeds of pigs. Indian domestic pigs were also found to have similar chromosome morphologies as observed by Bhatnagar and Srivastava (1972), Vijh *et al.* (1990) and Palegar *et al.* (1991).

Grouping of the biarmed chromosomes was based on their centromeric positions. The desi pigs and Large White Yorkshire pigs were found to have eight pairs of submetacentric chromosomes (1-8) and four pairs of metacentrics (9-12). Variations were observed in the grouping of biarmed chromosomes by different workers. Vijh *et al.* (1990) made three groups as Group A (5 pairs of submetacentrics), Group B (5 pairs of submetacentric/metacentric) and Group C (2 pairs of sub telocentrics) for the biarmed chromosomes of Indian domestic pig. Palegar *et al.* (1991) also had similar observation in desi pigs.

The X-chromosome was found to be of sub metacentric morphology in both the breeds studied. Similar observations were made by Vijh *et al.* (1990) in Indian domestic pig. The X-chromosome in desi pigs was termed as a metacentric one by Bhatnagar and Srivastava (1972) and Palegar *et al.* (1991).

In the present study, the Y-chromosome was the smallest metacentric chromosome in both breeds. This is in accordance with the findings of Vijh *et al.* (1990) and Palegar *et al.* (1991) in Indian domestic pigs. But Bhatnagar and Srivastava (1972) has termed the Y-chromosome as submetacentric in desi pigs.

Chromosome morphometry

The morphometry of chromosomes is of considerable help in the comparative evaluation of individual chromosome pairs. Chromosomal

morphometric measurements include relative length, arm ratio and centromeric index.

Relative length

The relative length of chromosomes of desi pigs ranged from 1.94 to 11.69. This was in agreement with the values obtained by Kim *et al.* (1994). Vijh *et al.* (1990) obtained values from 2.18 to 10.62 for the chromosomes of Indian domestic pigs. In the present study the relative length of X-chromosome of desi pigs was 4.63 per cent. This value was comparable to the value obtained by Vijh *et al.* (1990) for Indian domestic pigs.

The Large White Yorkshire pigs studied had a range of 1.7 to 11.35 for the relative length of their chromosomes. The X-chromosome of Large White Yorkshire pigs had a relative length of 5 per cent which was in accordance with earlier findings.

Arm ratio

Arm ratio was highest for chromosome 2 in both desi pigs and Large White Yorkshire pigs. However, the highest arm ratio was observed for chromosome 7 by Vijh *et al.* (1990) in Indian domestic pig.

Centromeric Index

This value hints to the position of the centromere. As the value decreases, the position of the centromere is away from the centre. The centromeric index values of submetacentric chromosomes were highest for chromosome number 8 and lowest for chromosome number 2 in both the

breeds studied. The X-chromosome was having a centromeric index of 32.09 in desi pigs and 36.33 in Large White pigs. A centromeric index value of 40 was observed for the X-chromosomes by Kim *et al.* (1994).

G-banding patterns

An attempt was made for G-banding of the chromosomes of desi pigs. The clearly visible bands in the various chromosomes of Desi pigs were identified and compared with that of the standard established in the First International Conference for standardization of Banded Karyotypes of Domestic Animals (Ford *et al.*, 1980). But all the bands established and described were not observed in the present study. Similar bands were observed by Vijh *et al.* (1990) in Indian domestic pigs.

Summary

SUMMARY

Karyological investigation was carried out to reveal the chromosome architecture of the black desi pigs of Kerala. Black desi pigs available at the Centre for Pig Production and Research, Mannuthy and AICRP on pigs were utilized for the study. The metaphase chromosome spreads obtained were stained by conventional staining and by G-banding technique to study the morphology, morphometry and G-banding pattern of chromosomes. Comparison was made with those obtained for Large White Yorkshire pigs.

A chromosome number of 2n = 38 was obtained for all the pigs in their metaphase spreads. The sex chromosomes were XX in female and XY in male.

The metaphase spreads of both breeds revealed six pairs of submetacentric chromosomes, four pairs of metacentric chromosomes, six pairs of acrocentric chromosomes and a pair of sex chromosome, either XX or XY. The X-chromosome was having a submetacentric morphology where as the Y-chromosome was metacentric.

The largest chromosomes was a submetacentric chromosome and the smallest was the Y-chromosome in both breeds. The mean relative length ranged from 1.95 ± 0.12 to 11.69 ± 0.19 in desi pigs and 1.7 ± 0.07 to 11.35 ± 0.37 in Large White Yorkshire pigs. The X-chromosome had a relative length of 4.63 ± 0.25 in desi pigs and 5.01 ± 0.22 in Large White Yorkshire pigs.

The arm ratio of the submetacentric chromosomes ranged from 1.35 ± 0.03 to 3.31 ± 0.13 for the desi pigs and 1.54 ± 0.07 to 2.86 ± 0.13 for Large White Yorkshire pigs. The highest arm ratio was for chromosome 2 in both the breeds whereas the lowest arm ratio was for chromosome 8 in desi pigs and chromosome 5 in Large White Yorkshire pigs. The arm ratio of the X-chromosome was 1.97 ± 0.08 for desi pigs and 1.81 ± 0.15 for Large White Yorkshire pigs.

The centromeric index measurements of submetacentric chromosomes ranged from 23.06 ± 0.84 to 42.68 ± 0.71 for desi pigs and 26.2 ± 0.89 to 39.45 ± 1.51 for Large White Yorkshire pigs. The highest centromeric index value was for chromosome 8 and lowest for chromosome 2 in both breeds. The X-chromosome had a centromeric index of 32.09 ± 1.17 in desi pigs and 36.33 ± 1.64 in Large White Yorkshire pigs.

The G-banded karyotype prepared for the desi pigs was comparable to the standard karyotype of pigs.

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*Originals not seen

CHROMOSOME ARCHITECTURE OF DESI PIGS OF KERALA

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ABSTRACT OF A THESIS

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ABSTRACT

A cytogenetic analysis of the chromosomes of black desi pigs of Kerala was carried out. Fifty four black desi pigs housed at the AICRP on pigs, centre for Pig Production and Research, Mannuthy formed the material for study. Forty five Large White Yorkshire pigs were also studied for comparison of the chromosome architecture.

Metaphase spreads were obtained by peripheral blood leukocyte culture in RPMI 1640 medium. A combination of pokeweed nitrogen and phytohemagglutinin was used for initiating mitosis. The metaphase spreads were G-banded by incubating them in 2 x SSC containing trypsin solution for 45 minutes at 60°C.

The number, morphology and morphometric measurements of chromosomes were studied. The distinct visible bands observed in G-banding was compared to that of the standard G-banded karyotype of pigs.

The karyotype revealed in desi pigs a chromosome diploid number of 38 (2n = 19). This consist of six pairs of submetacentric chromosomes, four pairs of metacentric chromosomes, six pairs of acrocentric chromosomes and a pair of sex chromosomes, either XX or XY. The X-chromosome was submetacentric and Y-chromosome metacentric. In Large White Yorkshire pigs included in the present study also the diploid number of chromosomes is 38, with similar morphological characteristics for the chromosomes as that of

the desi pigs. Thus in morphology and number of chromosomes, the desi pigs maintained a similarity to that of Large White Yorkshire pigs.

The relative length of the largest chromosome which was a submetacentric one in both breeds was 11.69 ± 0.19 in desi pigs and 11.35 ± 0.37 in Large White Yorkshire pigs. The Y-chromosome was the smallest chromosome in desi and Large White Yorkshire pigs. The Y-chromosome had a relative length of 1.95 ± 0.12 in desi pigs and 1.7 ± 0.07 in Large White Yorkshire pigs. The relative length of X-chromosome of desi and Large White Yorkshire pigs were 4.63 ± 0.25 and 5.01 ± 0.22 respectively.

The arm ratio of the submetacentric chromosomes was highest for chromosome 2 in both the breeds. The arm ratio was lowest for chromosome 8 in desi pigs and chromosome 5 in Large White Yorkshire pigs. The arm ratio of the X-chromosome was 1.97 ± 0.08 for desi pigs and 1.81 ± 0.15 for the Large White Yorkshire pigs.

The centromeric index measurements varied from 23.06 ± 0.84 to 42.68 ± 0.71 for desi pigs and 26.2 ± 0.89 to 39.45 ± 1.51 for Large White Yorkshire pigs. The centromeric index value was highest for chromosome 8 and lowest for chromosome 2 in both breeds. The X-chromosome had a Centromeric Index of 32.09 ± 1.17 in desi pigs and 36.33 ± 1.64 in Large White Yorkshire pigs.

The bands obtained by G-banding of the chromosomes of desi pigs were comparable to the standard G-banded karyotype of pigs.