GENETIC CHARACTERISATION OF BUFFALOES IN KERALA USING CYTOGENETIC TECHNIQUE

By

K. ANIL KUMAR

THESIS

Submitted in partial fulfilment of the requirement for the degree

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Animal Breeding and Genetics
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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DECLARATION

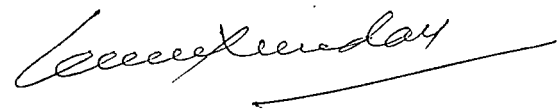
I hereby declare that this thesis entitled "Characterisation of buffaloes in Kerala using cytogenetic technique" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or society.

Mannuthy,

K. ANILKUMAR

CERTIFICATE

Certified that this thesis entitled **"Genetic Characterisation of buffaloes in Kerala using cytogenetic technique"** is a record of research work done independently by Dr.K.Anilkumar under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



Dr. G. Mukundan
(Chairman, Advisory Board)
Director,
Centre for Advanced Studies in Animal Genetics and Breeding
College of Veterinary and Animal Sciences, Mannuthy.

CERTIFICATE

We, the undersigned members of the Advisory Committee of Dr.K.ANILKUMAR, a candidate for the degree of Master of Veterinary Science in Animal Breeding and Genetics, agree that the thesis entitled **"Genetic characterisation of buffaloes in Kerala using cytogenetic technique"** may be submitted by Dr. K. ANILKUMAR in partial fulfilment of the requirement for the degree.

Dr.G.Mukundan,
Director,
Centre for Advanced Studies in Animal Genetics and Breeding,
College of Veterinary and Animal Sciences,
Mannuthy.

Dr. Sosamma Iype,
Professor,
Department of Animal Breeding and Genetics,
College of Veterinary and Animal Sciences, Mannuthy

Dr.K .V. Raghunandanan,
Associate Professor
Department of Animal Breeding and Genetics,
College of Veterinary and Animal Sciences, Mannuthy

Dr. C.P. Neelakanta Iyer,
Professor and Head,
Department of Animal Reproduction,
College of Veterinary and Animal Sciences, Mannuthy

EXTERNAL EXAMINER

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Introduction

INTRODUCTION

The word buffalo is considered to have originated from boubalos applied to cud chewing ox like ruminants. Buffaloes belong to the class Mammalia, order Ungulata, sub order Artiodactyla, family Bovidae, and tribe Bovini. Among the three groups under bovini, are included Bovina (cattle), Bubalina (Asian buffaloes) and Syncerina (African buffaloes). It is believed that the buffaloes were subjected to domestication around the period between 2500 and 2100 B.C, in the riverine civilisations of the Euphrates and Tigris, the Indus and Yangkzo. According to Cockrill (1974), the fossils recovered from the gravels of Narmada and topmost layers of the Sixalikis reflect the possible descent of Asian buffaloes from Bubalis palae indicus, of Pliocene period. The remnants of the progenitors of present day buffaloes have also been found in the valley of Godhavari and Narmada, along with stone implements which show that buffalo is a comtemporary of man. Representations of tame, and possible domesticated buffaloes appear on seals both in Mohenjo-Daro and in Mesopotamia from about the middle of the third millennium, B.C.

Domestic water buffalo are divided into two groups, the riverine and swamp types. Riverine groups are predominantly found on the banks of river and in India they are mainly concentrated in Indo-Gangetic plains. Swamp buffaloes are denizens of marshy land and prefer to wallow in mud.

At the material level, buffaloes contribute the biggest share of the total milk produced. It is estimated that 75 million buffaloes constituting more than half of the world population are present in India. This number is also showing an ascending trend. In Kerala state, as per the 14th quinquinal census (1987), there are only 3.3 lakh buffaloes and it can be seen that contribution of milk by the buffaloes in this state is only 8 per cent. These animals mostly serve as draft animals, mainly for ploughing. Hence male animals are more often retained than females. The reasons for the failure of buffaloes to be accepted as dairy animals vis-a-vis the cow by the people or the state need proper evaluation.

It is not known whether the buffaloes that existed in Kerala before the introduction of milk genes from Murrah and Surti through the various breeding programmes were riverine or swamp types. If the original types differed from the introduced breeds in chromosome profile, it is likely that crossbreeding might have resulted in chromosome polymorphism which would be reflected in the present day buffaloes.

Karyotyping can be employed to characterise the genetic nature of an animal. Karyotype is the complete chromosome complement of a cell which is the same for all diploid cells of the individual. This shows variation between species and between

the sexes. This variation may be in number and structure. By this technique the chromosome profile of an individual can be known in addition to finding out the degree of polymorphism, if present. It is worth mentioning that in man and some species of animals there are wide spectra of chromosome polymorphism.

It is seen that till date no investigation has been undertaken to evaluate the genetic profile of the buffaloes that exist in Kerala at present. Genetic characterisation is very much essential before embarking on scientific breeding programmes, whether for improving milk production or draft power or meat production capability. In addition many abnormalities have attributed to chromosomal aberrations. Reproductive disorders such as subfertility or infertility are not very uncommon in buffaloes and a proper evaluation is needed to know whether chromosome polymorphism has any role in the etiology of these conditions. Chromosome studies are significant in livestock breeding since chromosome abnormalities are transmitted from generation to generation. So this investigation has been planned for genetic characterisation of the buffaloes in Kerala by studying the chromosome profile using cytogenetic techniques. Such a study is very essential to initiate successful breeding programmes to improve the buffalo germ plasm in this state.

Review of Literature

REVIEW OF LITERATURE

As early as 1941, Macgregor realised the importance of buffaloes in agriculture and reviewed the details on its taxonomic position, variability in different types, husbandry practices and physiology. According to him the Bubalus genus was subdivided into B. africanus and B. asiaticus. He described the domesticated or degenerated species or type of arne or Indian buffaloe as domesticated water buffalo. These buffaloes were again classified into river type and swamp type. Karyotyping is one of the tools to identify the taxonomical pattern of the species.

Technique

Hsu and Pomerat (1953) evolved a method of spreading the chromosomes of mammalian cells in tissue culture. This was the first foundation of the present day karyotyping technique.

Peripheral blood leukocyte culture technique was first used by Hungerford et al (1956) to study human chromosomes of a phenotypic intersex. This technique was found more reliable and comparatively easy, and hence it became popular among cytogeneticists and became an effective tool for karyological

studies. Using this technique, Ulbrich and Weinfold (1963) determined the chromosome number in buffaloes.

Rathnasabapathy and Ganesh (1980) could obtain adequate number of metaphase plates with a technique of 72 hour culture of peripheral lymphocytes of buffaloes that reported at the Artificial breeding centre at Madras veterinary college. The diploid number of chromosomes was 50. Of the autosomes, four pairs were submetacentric, one pair metacentric and rest were telocentric. Both sex chromosomes were telocentric.

Rathnasabapathy and Venkatraj (1982) conducted a comparative study on the efficacy of the various techniques to obtain a consistantly large number of good metaphase plates from the buffalo lymphocytes and found that the method involving TC 199 dried medium and phytohemagglutinin was the best.

Thiagarajan et al. (1989) assessed the comparative efficacy of culture media TC 199, TC 199 with glutamine and RPMI 1640 and reported that RPMI 1640 was best suitable medium yielding higher mitotic index with consistant sucessful results. Similarly, the study on the comparative efficacy of two mitogens, phytohemagglutinin and pokeweed mitogen, revealed that pokeweed mitogen was superior for buffalo lymphocytes.

The reports on the effect of storage of blood samples intended for karyological studies of buffaloes are scanty. Thiagarajan et al. (1990) reported that blood sample could be stored upto 24 hours at refrigerated temperature and yielded an optimum mitotic index, declined rapidly beyond 24 hours of storage.

Chromosome Number and Structure.

Karyological studies of buffaloes were initiated by Makino (1944) using testicular material obtained from buffaloes found in swampy areas of southern Taiwan. He observed the chromosome number of 2n = 50 in his experimental material.

In India, studies on chromosomes of Murrah buffaloes was carried using testicular material by Dutt and Bhattacharya (1952) and revealed a diploid number of 48 chromosomes. They observed that four pairs of chromosomes were having median or submedian centromerses where as the rest were having terminal or nearly terminal centromere. The longest rod shaped chromosome was "X" where as "Y" was one among the smallest. The technique used by them was too elementary as they adopted paraffin sections under squash preparations which did not often lend themselves to accurate counting especially if chromosomes are too many.

DeGirolamo (1958) reported the diploid number of chromosomes in Italian buffaloes as 48. He also used testicuar material for the study.

Ulbrich and Fischer (1967), investigated the somatic chromosomes using blood culture methods. The diploid number of chromosomes Bubalus bubalis was 48. The autosome consisted of five metacentrics or submetacentrics and 18 pairs acrocentrics. The sex chromosomes were also acrocentrics. The diploid number of chromosomes of Syncerus caffer was 52. The autosomes consisted of four pairs of metacentric or submetacentric and 21 pairs of acrocentric chromosomes and two acrocentric sex chromosomes. The X chromosome was the largest and the Y chromosome the smallest of the acrocentrics.

The karyotypes of Turkish and south-east European buffaloes were identical with those of the Murrah buffalo. It consisted of five pairs of submetacentric and 19 pairs of acrocentric chromosomes. The sex chromosomes were also acrocentric (Ulbrich and Fischer, 1968).

Fischer and Ulbrich (1968) had described the karyotype of Asian swamp buffalo of Thailand and Malaysia. The diploid chromosomes number was 48 of which five pairs were metacentric or submetacentric and 19 pairs were acrocentric including two acrocentric sex chromosomes.

The karyotype of Australian swamp buffalo has been reported by Toll and Halnan (1976a) as similar to that of Asian swamp

buffalo found in Thailand and Malaysia. A hypothesis was advanced by them to account for movement of buffalo down the island chain from Malaya to Australia. Toll and Halnan (1976b) constructed a idiogram for the chromosomes of Australian swamp buffalo. Comparison with the G-banding patterns for goat, sheep and cattle chromosomes showed a remarkably close similarity between individual pairs, banding pattern homologies for the buffalo metacentric autosomes being identifiable among acrocentric autosomes of other species. The acrocentric X of buffalo was similar to goat 'X' in banding and beast like that of cattle. Buffalo 'Y' was unlike its counterpart of other species.

After examination of lymphocyte chromosomes of Thai and Murrah buffaloes using G-band staining, Scheurmann et al. (1976) reported differences in banding pattern between these two types in the autosomal pair of one and eight. Chromosome pair one in Murrah buffaloes was submetacentric and were no longer than the pair two and three. While chromosomes of pair one in Thai buffalo were considerably larger and more metacentric than those of other pairs near it. The acrocentric pair eight was identified in Murrah buffaloes.

Rommelt et al. (1978) studied metaphasic spreads of 11 swamp type and 11 river type buffaloes using C and G-banding methods. The difference in diploid chromosome number of 48 in swamp

buffaloes and 50 in river buffaloes was attributed to a balanced autosomal 2/9 tandem fusion in swamp buffaloes.

Khavary (1978) could not find any difference in chromosomes between Iranian and Ceylon buffaloes which showed a diploid number of 50. Similar observations were made by Cribiu and Obeidah (1978) in Egyptian water buffaloes with 50 chromosomes consisting of 10 submetacentric or metacentric and 15 pairs including 'X' and 'Y' acrocentric chromosomes. Pericentric or constitutive heterochromatin was absent from metacentric or submetacentric chromosomes excepting pair two, three and four. All the acrocentric chromosomes including 'X' and 'Y' had a pericentric constitutive heterochromatin.

Chandra et al. (1978) conducted karyological studies of desi water buffaloes found in southern Karnataka and reported that the metaphase chromosomes are of diploid number 50. Autosomes one, two, four and five were submetacentric and chromosome three was metacentric with a short arm which appeared pale with Giemsa and fluoresced weakly. The remaining 20 pairs including 'X' and 'Y' were acrocentric. The X chromosome was the longest acrocentric with a small faintly fluoresced and stainless proximal region. The Y chromosome was approximately intermediate in size among acrocentrics. Chromosome 10 was distinct with a large proximal region appearing very pale.

Using peripheral blood leukocyte culture technique, Bongso and Jainudeen (1979) reported 49 chromosomes in two Murrah-Malaysian crossbred buffaloes, as against 48 and 50 chromosomes in Malaysian swamp and Murrah buffaloes respectively. The chromosome pair one comprised of one metacentric and one submetacentric, similar to the corrsponding chromosomes of Malaysian and Murrah respectively. The metacentric was the larger of the two while one of the smaller acrocentrics lacked a partner.

In studies on six Murrah buffaloes and six local non-discript buffaloes maintained in Indian Veterinary Research Institute, and Nawab Gangh biological products division, Chakrabarti and Benjamin (1980), observed a diploid chromosome number of 50 in all the 24 animals. They could not find chromosomal polymorphism among these water buffaloes.

Mikaye et al.(1980), observed a diploid chromosome number of 48 in three swamp buffaloes consisting 10 meta or submetacentric chromosomes and 38 acrocentric chromosomes. The 'X' chromosome was the largest acrocentric and the 'Y' chromosome second smallest acrocentric. The 'X' chromosome had the broadest C band in centromere region while the 'Y' chromosomal arm was darkly stained in C-banding technique.

The Egyptian water buffalo (Bubalus bubalis) carried 24 pairs of autosomes comprising of three pairs submetacentrics, two pairs metacentrics and 19 pairs acrocentrics. Among the two acrocentric sex chromosomes, X chromsome was distinguishable from other acrocentrics by its greater length (Cribiu et al. 1980).

According to Goswami et al.(1980) analysis of chromosome preparations from three male and four female buffaloes revealed the eight chromosomes (three submetacentrics and three small and two large acrocentrics) as showing associations involving the telocentric ends of their short arms. Using amoniacal silver staining, these telomeric ends were shown to contain nucleolar organiser regions (NORs). There were differences between individuals in the number of NOR associations and type of chromosome involved.

Studies by DiBerardino et al. (1981) on the G,Q and R banding pattern compared the chromosomes between Murrah type of Bubalis bubalis (2n=50) and Holstein Friesian breed of Bos taurus (2n=60) and revealed that autosomes are similar in both. In the former, five pairs of submetacentrics corresponed to centric fusion of chromosomes 1-29, 2-22, 8-19, 5-28 and 16-25. In the later, silver staining on somatic cells of buffalo revealed telomeric Ag-NORs located on six pairs of autosomes, identified as 3p, 4p, 8,21,23 and 24. Only one pair of NOR chromosomes of buffaloes namely submetacentric chromosome 4p and acrocentric chromosomes

8,20,22 and 23 as containing NORs in silver staining. Murrah buffaloes had NORs located on six pairs of chromosomes, submetacentric 3p and 4p and acrocentric 8,21,23 and 24. They concluded that the chromosome number one of swamp buffalo resulted from tandem fusion between 4p and 9 of Murrah karyotype. The fusion had caused loss of telomeric NOR of chromosome four accounting for reduced number of NORs of chromosomes in swamp buffaloes. They further concluded that centromeric region of chromosome number nine was altered.

Balakrishnan and Yadav (1981) reported cases of x-trisomy, pericentric inversion of an autosome and C band reduction of an X chromosome in buffaloes with reproductive defeciencies. Balakrishnan et al. (1981) studied the sex chromosome chimerism in heterosexual murrah buffalo triplet. One male and one female of the triplet showed sex chromosome chimerism, the percentage of XY metaphases being 38.7 and 42.07 respectively. The other male of the triplet was normal.

Bongso and Hilmi (1982), studied the chromosomes of Murrah (river), swamp (Malaysian Kerbau), and F_1 hybrid (Murrah x Swamp) and first generation backcross (F_1 hybrid female x Murrah male) buffaloes (Bubalus bubalis) using Giemsa (G) and centromeric (C) banding technique. The diploid chromosome number of Murrah was 50, swamp 48, F_1 hybrid 49 and two backcrosses 49 and 50

respectively. The largest two metacentric chromosomes of the swamp resulted from a tandem fusion between the chromosomes 4p and 9 respectively of the Murrah karyotype. The F_1 hybrid and one of the back crosses (2=49), had karyotypes intermediate to Murrah and swamp parents. The C banding patterns were useful in identifying the X and Y chromosomes of the buffaloes and demonstrated that a major portion of the centromere region of chromosome nine was not incorporated into chromosome four during tandem fusion.

Proper identifying of sex chromosomes is important in cytogenetic studies of animals. Equivocal identification of the Y chromosomes of Indian river buffalo was not possible by conventional techniques as it could not be distinguished from small acrocentric autosomes based on C band characteristics and relative length measurements. It was proposed that Y chromosome was not the smallest acrocentric but is intermediary in length between 19th and 20th autosome pairs. This hypothesis was confirmed by G banding technique (Yadav and Balakrishnan, 1982).

Krasota et al. (1982) compared the cytogenetic profile of three buffalo populations of Caucasian type found in Azarbaijan and Georgia and the cross-breds of Murrah with Caucasian and found that the morphology of the first two pairs of submetacentrics did differ from that of Egyptian buffaloes.

Bongso and Hilmi (1983), observed numerical polymorphism in Murrah, swamp and Fl hybrids using Giemsa and Centromeric banding technique and attributed this anomaly to a tandem fusion between two chromosomes 4p and 9 of Murrah karyotype. During meiosis the 4/9T metacentric chromosome is likely to pair with either four or nine or both. Thus the possibilities include the formation of bivalent in which 4/9T of swamp buffaloe mother pairs with four as welll as nine of Murrah buffaloe father. A univalent and a bivalent in which 4/9T of swamp buffaloe pairs with either four or nine of Murrah leaving the alternative unpaired and three univalent, in which 4/9T of swamp buffaloe is not paired with genetic counter-parts of four or nine. All these possibilities were observed in diplotene and diakinesis stages of the hybrids examined in the studies. Semi-thin sections on the hybrid testis showed all stages of spermatogenesis and a variety of abnormal germ cells undergoing vacuole formation and degeneration. The Leydig cells and sertoli cells appeared normal although the lumen of the tubules were often filled with desquamated germ cells and para tubular space was more enlarged compared to similar sections from swamp buffaloes of corresponding age.

Yadav and Balakrishnan (1984) examined two sets of heterosexual triplets in the buffalo. All the animals comprising of one female and one male from one set and one female

from second set exibited sex chromosome chimerism. However they could not report the fertility of these females as they were too young.

Balakrishnan and Yadav (1984) observed a diploid number of 50 chromosomes in Murrah, Surthi and Jafferabadi buffaloes. On screening, chromosome abnormalities such as X triosomy, pericentric inversion and C band deletion could be detected in animals exhibiting reproductive disorders.

Shokry and Barakat (1984) screened the chromosome pattern of 50 Egyptian buffaloes aged between three and eight years. The incidence of chromosomal aberrations was found higher in buffaloes affected with upward fixation of patella. Out of the total abnormalities, 10.08 per cent were structural and 40 per cent were numerical as compared to normal.

Sethumadhavan et al. (1986) reported a complex chromosome pattern in a Murrah buffaloe with lymphoid leukaemia. The model number ranged from hypo-diploidy of thirty chromosomes to immeasurable hyper-diploidy leading to chromosomal fragmentation and pulverisation. Very few spreads of normal model numbers of 50 chromosomes were encountered. The preponderence of numerous proliferative cells in blast stage, the presence of lymphoblasts exibiting initital mitotic phases, the characteristic variation in model number, the effect of chromosomal pulverisation and structural abnormalities with chromatids gaps in one of the sex

chromosomes confirmed the characteristic karyological abnormalities described in lymphosarcoma/leukaemia complex.

Huang et al. (1987) conducted karyological studies on 35 buffaloes from Xilin, Fuzhong and Nanning of China and reported the diploid number as 48. C bands were absent in Y chromosome. G and C bands were identical in buffaloes from Xilin and Nanning.

Sethumadhavan et al. (1987) karyotyped Toda buffaloes using peripheral lymphocyte culture technique and reported diploid chromosome number as 50 comprising of ten submetacentric and forty acrocentric chromosomes conforming to river type buffaloes.

Batancourt (1988) investigated 120 Murrah buffaloes in Cuba and found out the chromosome number as 50. Of the autosomes, four pairs were submetacentric, one pair was metacentric and nineteen pairs were acrocentric.

The karyotype differences between Guangxi and Murrah buffaloes were sudied by Huang and Liu (1988). In G and C banded karyotypes, chromosome pair number one was submetacentric in Murrah, which was smaller than the metacentric. The Y chromosome was having a C band in Murrah whereas that was absent in Guangxi. The Y:X length ratios of the sex chromosomes were 0.410 and 0.366 respectively, in the two breeds.

Harisah et al.(1989) studied the chromosome seggregation patterns in crosses of swamp and river type buffaloes. The diploid chromosome number of swamp buffaloes which were studied from Malaysia and philippines was 48 and that of river type was 50. All the Fl Hybrids exibited 49 chromosomes. The F2 hybrids consisted of three different karyotypes categories (2n=48, 2n=50 and 2n=49) whereas back crosses included two different karyotype categories with 2n=48 and 2n=49 in the three quarter swamp types and 2n=49 and 2n=50 in the three quarter river types. Chi-square tests on pooled data from Malaysia and phillippines indicated that distribution of different karyotype categories of F2 animals did not deviate significantly from 1:2:1 ratio expected of, only balanced gametes with 24 and 25 chromosomes were produced by Fl hybrids. In the three quarter swamp and three quarter river types respective karyotype categories were in ratios approximately 1:1. The distribution of chromosomes categories among the Fl hybrids and back crosses suggested that only genetically balanced gametes of Fl hybrids were capable of producing viable F2 and backcross generations.

According to Sharar et al.(1989), metaphase chromosomes of the white blood cell culture of Murrah buffaloes obtained by innoculating buffalo plasma rich in leukocytes in autologous serum enriched within TC 199 medium exibited the diploid number

of 50. Relative length of five metacentric chromosomes, ranged from 6.40 to 8.75. Centromere index and arm ratio ranged from 35.97 to 48.72 and 0.54 to 0.96 respectively.

The frequency reports revealed that the chromosome number were not similar in swamp and river buffaloes and variation was exibited by buffaloes of different regions of the country. As a result of grading up local buffaloes with Murrah or Surti, the chromosome profile is liable to undergo change if the local buffaloes differ from the improved breed in chromosome number and structure.

Materials and Methods

MATERIAL AND METHODS

Blood collected from buffaloes from Pananchery, Manalur, Ollukkara, Varandrapally and Koorkanchery panchayaths of Trichur district, Attapady, Mundur, and Kakkayur of Palghat district, Aluva of Ernakulam district and Murikkassery of Idukky district formed the materials for the present study. Among the buffalo population are included working bullocks from Kakkayur and Mundur of Palghat and Ollukkara of Trichur district. Besides, the animals brought to the artificial insemination centre of the Veterinary college, Mannuthy, were also examined. In all, 54 animals have been karyotyped. The animals were designated as local, Murrah, Surti or their crosses. Since pedigree information was not available, it was difficult to classify them on the basis of their genotypes.

A great deal of variation was observed in the shape of horns of buffaloes. In the present study, the animals were classified into three groups based on horn shape viz., (1) sickle shaped horn type, (2) curled horn type and (3) non-discript horn type. The animals having the horn and forehead in the same plane and whose horns were curving inwards giving the appearence of sickle were grouped under the first group. The second group of animals was also having horns which were curling inwards and in the same plane of forehead. All the other animals were grouped as third

classification of non-descript. There were 16 sickle shaped horned, 15 curled type horned and 23 non-descript horned animals.

Collection and transportation of samples

Fifteen millilitres of blood was collected from either jugular vein or from the ear vein of buffaloes using 16 G needle. The blood was collected in centrifuge tubes containing heparin sodium (BIOLOGICALS) at a final concentration of 65 IU/ml of blood.

Blood samples collected from neighbourhood areas were transported to the laboratory within one hour. The temperature during transport ranged between 21-32°C. From distant places, collection tubes were kept inside thermoflask containing ice, without direct contact with the ice. The duration of transport never exceeded eight hours. At every step maximum precautions were taken to maintain aseptic conditions. After reaching the laboratory, culture was set within two hours.

Culture medium

The basal medium used was RPMI 1640 and the composition of the medium was as follows.

RPMI 1640 dried powder (SIGMA)	1000	mg
Sodium bi carbonate (MERCK)	75	mg
Mitogen [(Phytohaemagglutinin-M (PHA) or Pokeweed mitogen (PWM) - SIGMA)]	1	ml
Penicillin solution (5000 IU/ml)	0.5	ml
Double distilled autoclaved water	98.5	ml

Distilled water was taken in a sterile conical flask and dried medium and sodium bicarbonate were added to it. The solution was gently mixed to dissolve the powders.

Penicillin solution and mitogen were added to the medium. The pH was adjusted to 6.8 using 0.1 N hydrochloric acid. The whole medium was filtered through micro-filter of 0.22 micrometer pore size. The medium was then distributed to screwcapped culture vials, five millilitres each and stored at -5°C until use.

Culturing

From each sample one millilitre of blood was taken in a separate syringe and it was used to seed the culture. The rest of the blood was centrifuged to separate the plasma at 1500 rpm for ten minutes.

Into each culture vial containing five millilitre of medium, two millilitre of autologous plasma and nine drops of blood from a 21 G needle was added. The culture vial was then gently mixed by rotating between the palms of the hand. The culture vials were then incubated at 37°C in B.O.D. incubator for 71 hours. The cultures were gently agitated twice daily.

At the end of the incubation, colcemid (SIGMA) solution was added to the culture as mitotic arrester. The optimum concentration of colcemid was estimated by comparing the effects of different final concentrations viz., One microgram, two micrograms, three micrograms, four micrograms, eight micrograms and ten micrograms per millilitre. The duration of action of colcemid was also varied. The effect was studied at 30 minutes, 45 minutes, 60 minutes and 90 minutes.

Harvesting

At the end of incubation with colcemid solution, the contents of the culture vial was transferred to a centrifuge tube and the mixture was centifuged at 1500 rpm for 10 minutes. The supernatent was then removed leaving 0.5 ml above the sediment.

Hypotonic treatment was then done by adding 0.075 M potassium chloride solution, five times the volume of the sediment. The

hypotonic solution was prewarmed to 37°C and was added drop by drop to prevent the shock of the cells. After 20 minutes, the content was centrifuged at 1200 rpm for 10 minutes. The supernatent was discarded leaving 0.5 ml above the sediment.

Fixation of the cells was done using freshly prepared Carnoy's fixative, which contains three parts volume of methanol and one part of glacial acetic acid. After gently mixing the sediment with the left over supernatent solution, the fixative was added through the sides of the test tube little by little and mixed gently to break the cell button by drawing repeatedly into a pasteur pipette. The fixative was added to make up a total volume of eight millilitre, and the mixture was kept for ten minutes. Then it was centrifuged at 1500 rpm for eight minutes. The supernatent was pipetted out leaving about 0.5 ml of the solution above the cell button. The fixative treatment was continued with eight millilitres, six millilitres and four millilitres fixative till the clear supernatent solution was obtained. Finally the supernatent was removed leaving 0.5 ml and the cells were suspended in one millilitre fixative.

Slide preparation

Fresh slides were chilled in methanol and were used for smear preparation, The wet slides were kept in 60° slant and the

cell suspension was dropped into them at a height of two feet, using a pasteur pipette. Two to three drops of the the suspension was dropped into each slide, which was then waved from side to side as well as to and fro, to form a uniform spread of the material. These slides were then air-dried.

Staining

The slides were stained with Giemsa (SIGMA). The stock solution was diluted with Sorenson's phosphate buffer of pH 6.8 to four per cent. The slides were kept in a staining rack and were flooded with freshly prepared stain and kept as such for 40 minutes with occasional blowing of air, Then the slides were washed with distilled water and flooded with the distilled water and kept for another five minutes. The washing was then repeated three times. The slides were air-dried in slanting position.

The slides were screened in Karl Zeises Photomicroscope III. Those slides having good mitotic spreads were mounted in DPX mountant. The photographs of the spreads were taken.

Comparison on the efficacy of different mitogens and time of addition of colcemid to the culture

The effect of three mitogens viz., PHA, PWM and a mixture of these two on the buffalo lymphocytes was compared, together with the effect of addition of colcemid to the medium at intervals of 68 hours, 69 hours, 70 hours and 71 hours after the onset of the culturing. Mitotic drive and mitotic index were used as the criteria to assess the efficacy of these treatments. Mitotic drive is percentage of lymphoblasts and cells in metaphase whereas mitotic index is the percentage of cells in metaphase.

A randomised block design was adopted with the mitogens as treatments and time of addition of mitotic arrester as blocks. Five replications were done. Samples were allotted to any one of the treatments and blocks randomly.

Karyotype preparation

Good metaphase spreads were identified and photographs were taken. The chromosomes were identified and cut out from each plate. The homologous pair of chromosomes were identified and $karyotypes were prepared by pasting them on bond paper in descending order of their length. The chromosomes were grouped

on the basis of position of centromere and numbered in the karyotype. The moprhology of each chromosome was studied and comparative analysis was done.

Morphological measurements

The chromosomes were classified as metacentric, submetacentric or acrocentric according to the basis of the position of centromere. Morphological measurements were taken as per the recommendations of Denvar conference for describing human chromosomes.

The length of chromosomes in the karyotype was measured using a set of calipers with fine points. The morphology of individual chromosome was explained as relative length and centromere position. The relative length of chromosome is the ratio of length of the chromosome to the total length of the haploid set of chromosomes containing 'X' chromosome. The arm ratio of chromosome was taken as the ratio of long arm to that of the short arm. Centromere index was calculated as the ratio of length of short arm to that of total of the chromosome and expressed in percentage. This is an indication for nomenclature of the chromosome as submetacentric or metacentric.

Results

RESULTS

The comparative mitogenic efficacy of Pokeweed mitogen (PWM) (3.5 ug/ml of culture),Phytohaemagglutinin-M (PHA) (7 ug/ml culture) and a mixture of PHA and PWM (3.5 ug/ml and 1.75 ug/ml of culture) were assessed along with the effect of incubation time ie. the interval between the time of seeding the culture and the time of addition of colcemid as mitotic arrester on the basis of mitotic index and mitotic drive. The results of the assessment are presented in Tables 1 and 2.

During the incubation periods of 68, 69, 70 and 71 hours, the means of mitotic drive was 38.67±1.53, 37.38±3.25, 47.54±1.74 and 39.72±2.15 for PWM, 38.32±1.01, 34.74±2.73, 36.90±0.90 and 39.05±1.14 for PHA and 38.72±1.81, 44.24±1.84, 46.50±1.33 and 47.64±0.97 for the mixture of PWM and PHA, respectively. The average values of mitotic index of the mixture of the two mitogens were 2.8±0.8, 2.7±0.49, 2.8±1.25 and 4.4±0.91 for 68, 69, 70 and 71 hours incubation time, respectively. PHA induced the mitotic index of 1.6±0.4, 1.6±0.24, 1.8±0.37 and 2.0±0.45 and PWM had the mitotic index of 1.6±0.3, 2.4±0.93, 3.8±1.36 and 2.2±0.41 at incubation times of 68, 69, 70 and 71 hours, respectively.

Analysis of variance (Table.3) revealed that the difference due to the mitogens was significant. The mixture of PWM and PHA was significantly superior to PHA and PWM occupied the intermediary position.

In assessing the interaction of mitogen and the incubation time, (Table.4) it was found that the effect is significant for mitotic drive. The mixture of PWM and PHA and an incubation time of 71 hours was found to yield best results, as evidenced by the highest mitotic drive of 47.64 ± 0.97. The mixture of mitogens and incubation time of 70 hours was second with an average mitotic drive of 47.54 ± 1.74.

Among the final concentrations of one microgram, two micrograms, three micrograms, four micrograms eight micrograms and ten micrograms per millilitre of culture media, it was found that concentrations of two micrograms and three micrograms per millilitre of culture yielded better results. The higher concentrations caused condensation of the chromosomes affecting their morphology. Exposure to colcemid was found optimum between 45 minutes and 60 minutes when two micrograms per millilitre was the final concentration of colcemid.

Chromosome number and structure

Karyological studies of 54 buffaloes with regard to chromosome number revealed that there existed three classes of buffaloes. Five animals were included in the first class having the diploid chromosome number of 48 designated as swamp type. The number of buffaloes in the second class having diploid number of chromosomes of 50 were 45 and were called as river type. The remaining four animals possessed 49 chromosomes and were included in the third class and designated as 'Others'.

Among the 48 chromosomes in the first class 10 were submetacentric and others were acrocentric. Among the 50 chromosomes of the second class 10 chromosomes were submetacentric and 40 chromosomes were acrocentric. An interesting phenomenon was observed in the animals carrying 49 chromosomes. Although there were 10 submeticentric chromosomes in that class of animals, two were without homologues and one of them was largest among all the chromosomes. However an additional acrocentric chromosome over and above the 38 acrocentric chromosomes comparable to that in the second class could be detected.

The karyotypes of three buffaloes are presented in figures.1 to 5. It can be seen from the figures that sexual dimorphism was revealed in the first and second classes of buffaloes, as evidenced by the presence of XY chromosomes in males and XX chromosomes in females. The third class, 'Others', did not include any males.

The size of X and Y chromosomes varied to such an extent that they could be distinguished easily. The acrocentric X chromosome was one among the group of large chromosomes whereas Y chromosome was included in the group of smaller acrocentrics.

In the group having sickle shaped horns and non descript horns (miscellaneous), there were swamp type, river type and others, while in group having curled horns, there was only river type and others. The pooled population was having 45 river type, 5 swamp type and 4 others.

Morphological measurements

The relative length of buffalo chromosomes of the swamp type is presented in table 5. On the basis of the position of centromere, the autosomes of the swamp type were classified into two types viz. submetacentric and acrocentrics and both sex chromosomes were acrocentrics. Within each group, chromosomes

were arranged in descending order of their relative length. The relative length of submetacentric autosomes ranged between 4.911±0.118 and 6.921±0.152 in swamp buffaloes.

The largest acrocentric autosome was having a relative length of 4.953±0.136 and was larger than fifth submetacentric chromosome in swamp buffaloes. The smallest acrocentric autosome was with a relative length of 2.301±0.034.

The X chromosome of swamp buffaloes was the largest acrocentric chromosome of the complement with a relative length of 6.288±0.099 which was greater than the third metacentric chromosome. The Y chromosome had a relative length of 2.675±0.188 which occupied the position between chromosomes 20 and 21.

In river type buffaloes, the relative length of submetacentric chromosomes ranged between 5.050±0.107 and 7.278±0.094. Interestingly all the submetacentric chromosomes had relative length greater than the largest acrocentric chromosome in river type.

The relative length of the largest acrocentric autosome of the river type was 4.618±0.095, whereas the smallest autosome measured a relative length of 2.064±0.123. The X chromosome in

the river buffaloes was with a relative length of 6.220±0.136, which was larger than all the acrocentrics and three metacentric pairs of autosomes. In the case of Y chromosome, the average relative length was 2.550 ± 0.095 in between those of chromosomes 21 and 22.

Arm ratio

In both swamp type and river type buffaloes only five pairs of chromosomes were submetacentric. The measurement of arm ratio was therefore limited to these chromosomes and is presented in Table 6.

In swamp type buffaloes the arm ratio ranged between 1.479±0.055 and 2.183±1.169. The submetacentric chromosomes arranged on the basis of size showed arm ratios in chromosomes 1, 2,3, 4, and 5 as 2.183±0.169, 2.918±0.165, 1.961±0.122, 1.761±0.088 and 1.479±0.055 respectively, in swamp type buffaloes.

In river type buffaloes, the arm ratios were 2.289±0.221, 1.931±0.140, 1.712±0.200, 1.767±0.137 and 1.542±0.158 in chromosomes 1, 2, 3, 4 and 5, respectively.

Centromere index

From the Table 7, it can be seen that in swamp buffaloes the centromere index ranged between 33.2±2.1 per cent in first submetacentric chromosome to 40.4±0.9 per cnet in fifth submetacentric chromosome.

River buffaloes had the largest centromere index as 40.4±1.9 per cent in fifth chromosome and the smallest centromere index in first submetacentric autosome as 31.1±2.4 per cent.

Fig.1a.
KARYOTYPE OF A CLASS I (RIVER TYPE) MALE BUFFALO

Fig.1b.
MITOTIC METAPHASE SPREAD OF A CLASS I (RIVER TYPE) MALE BUFFALO

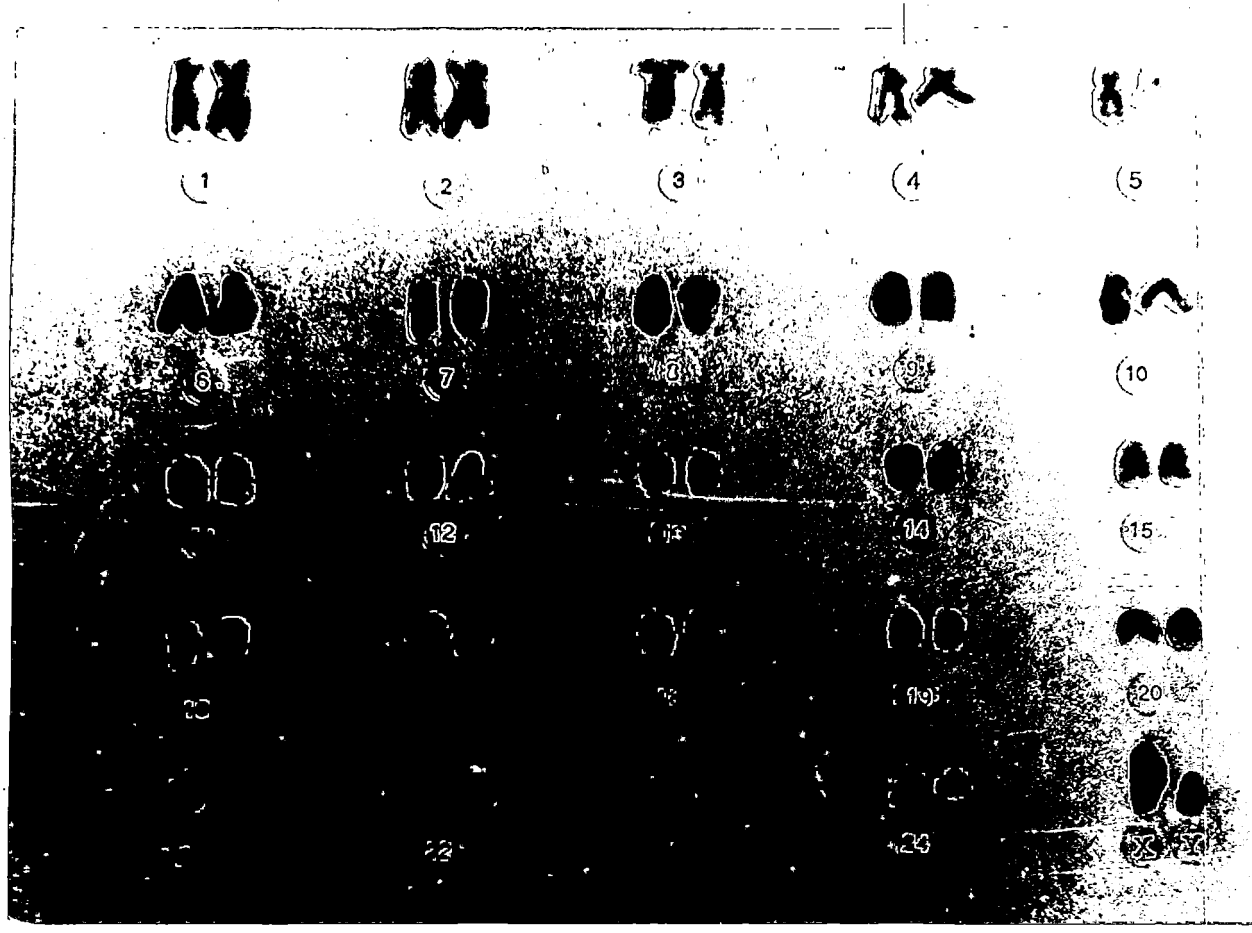
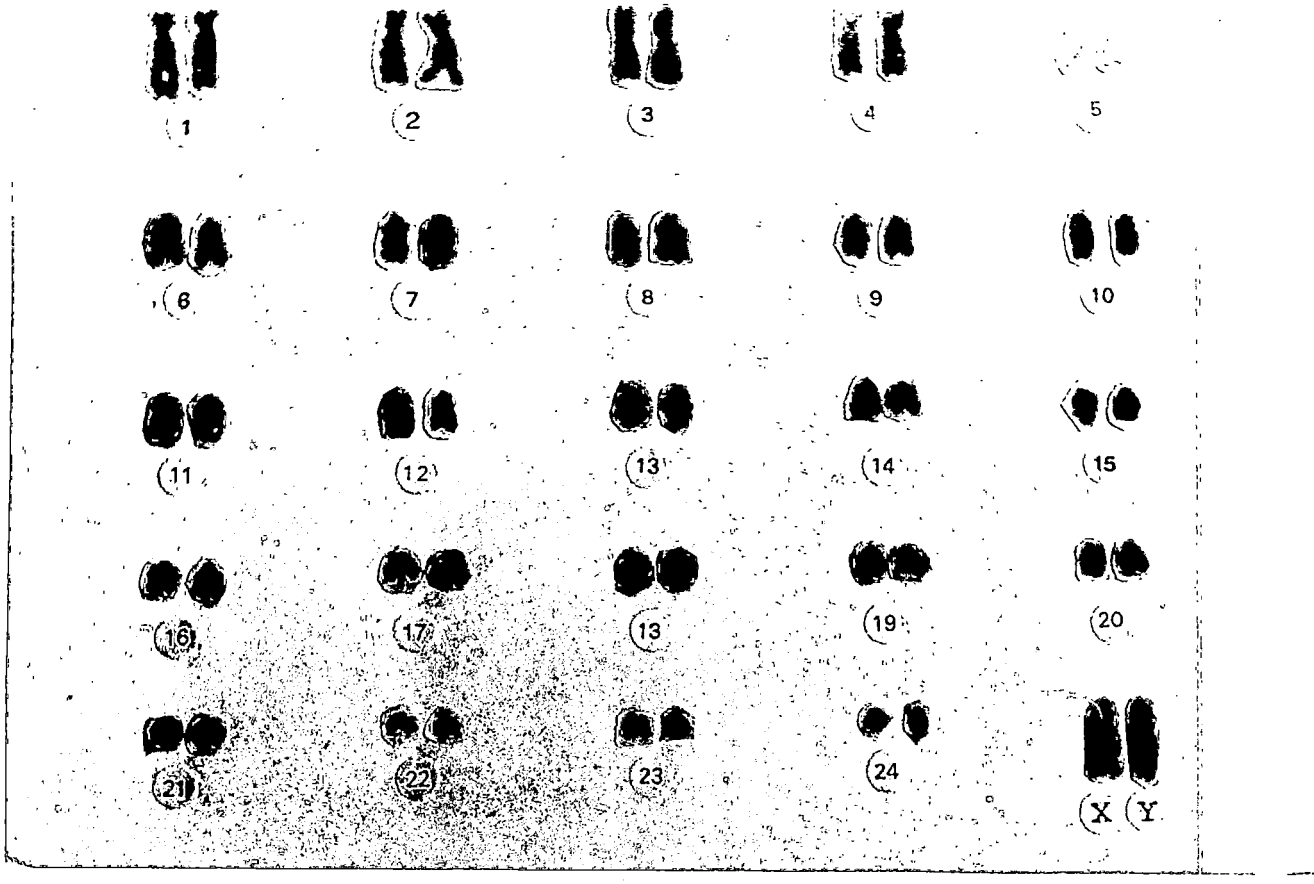


Fig.2a.
KARYOTYPE OF A CLASS I (RIVER TYPE) FEMALE BUFFALO

Fig.2b.
MITOTIC METAPHASE SPREAD OF A CLASS I (RIVER TYPE) FEMALE BUFFALO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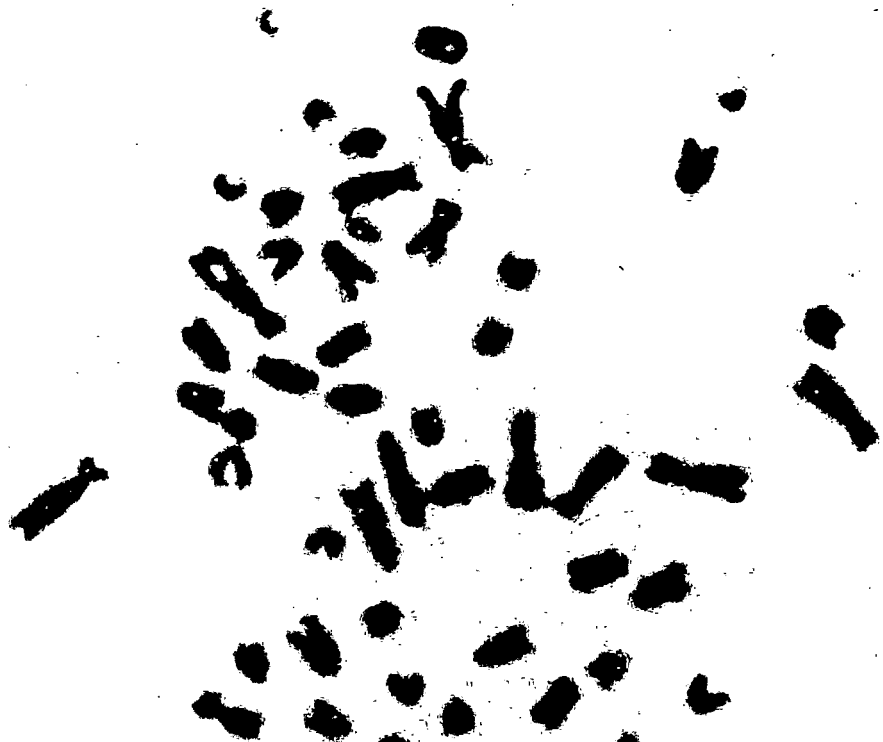
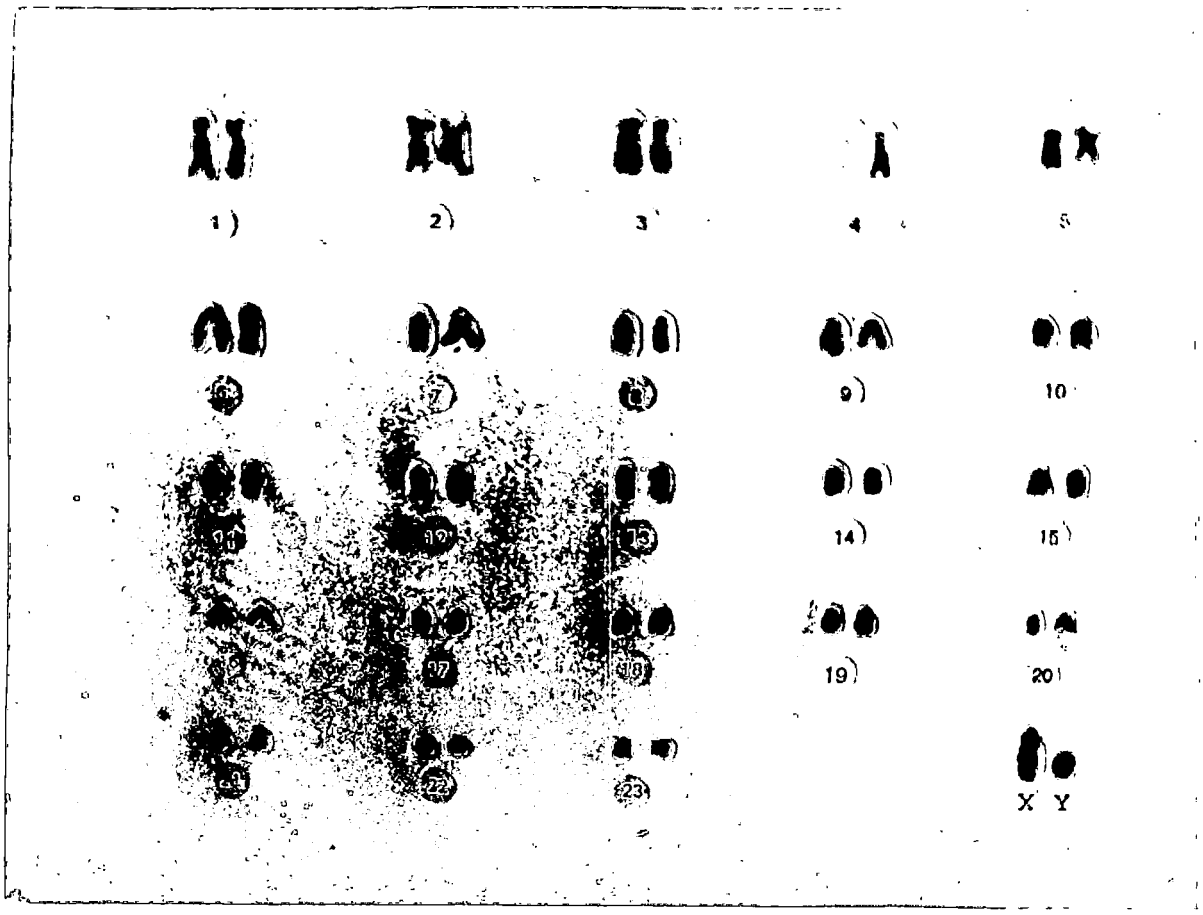


Fig.3a.
KARYOTYPE OF A CLASS II (SWAMP TYPE) MALE BUFFALO

Fig.3b.
MITOTIC METAPHASE SPREAD OF A CLASS II (SWAMP TYPE) MALE BUFFALO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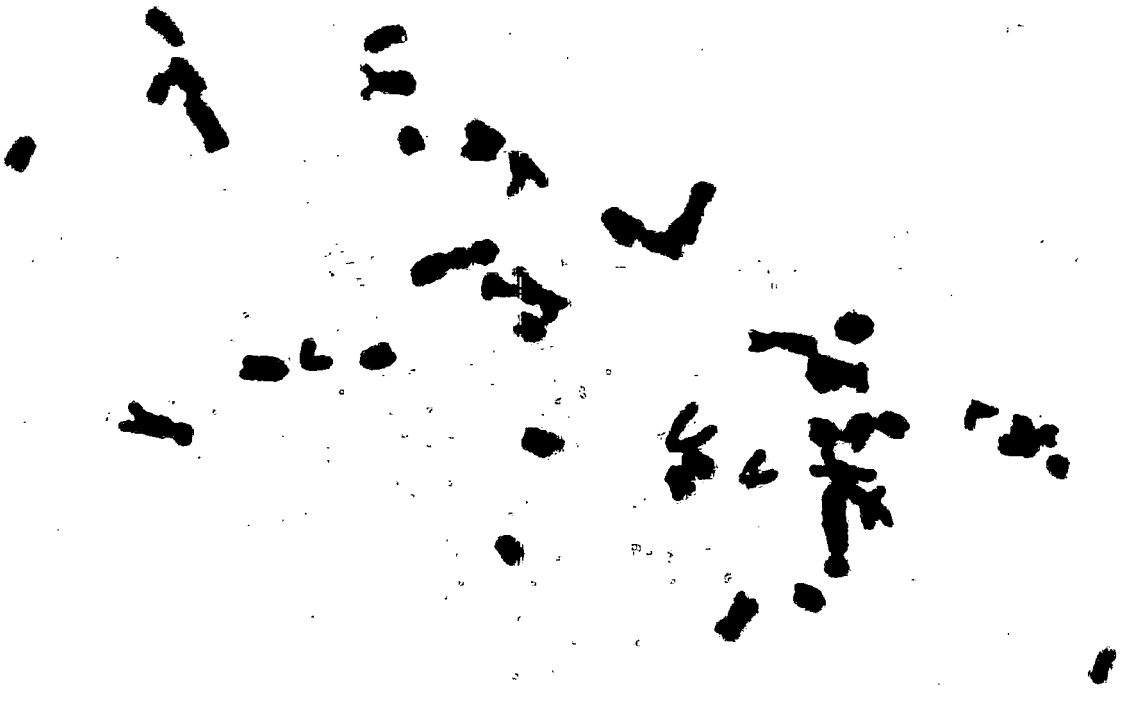

Fig.4a.
KARYOTYPE OF A CLASS II (SWAMP TYPE) FEMALE BUFFALO

Fig.4b.
MITOTIC METAPHASE SPREAD OF A CLASS II (SWAMP TYPE) FEMALE BUFFALO

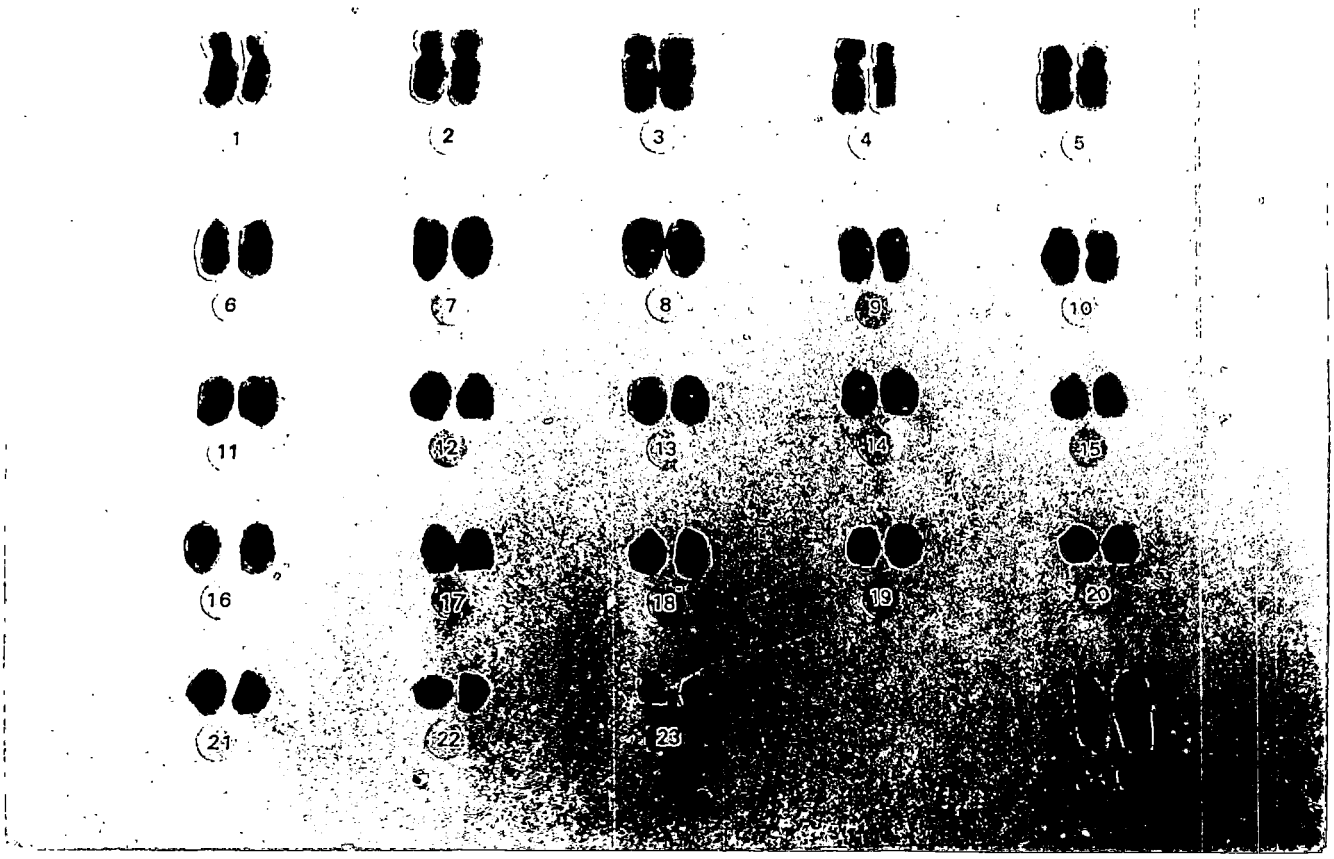
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Fig.5a.
KARYOTYPE OF A CLASS III (OTHERS) FEMALE BUFFALO

Fig.5b.
MITOTIC METAPHASE SPREAD OF A CLASS III (OTHERS) FEMALE BUFFALO

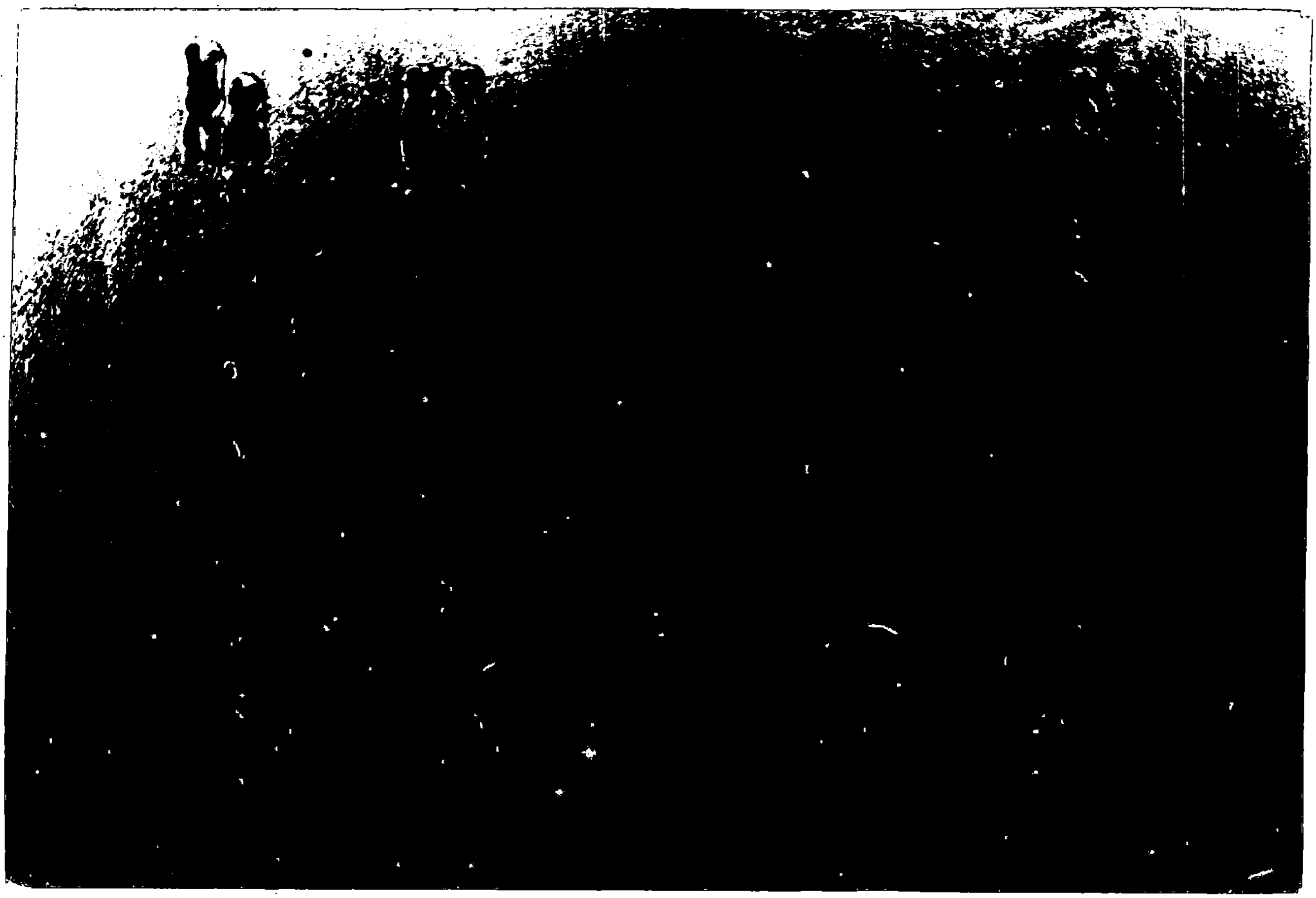


Table 1. Effect of mitogens and incubation time on mean mitotic index.

Mitogens	INCUBATION TIME (In hours)			
	68	69	70	71
Pokeweed mitogen (PWM)	1.6±0.3	2.4±0.93	3.8±1.36	2.2±0.41
Phytohemagglutinin (PHA)	1.6±0.4	1.6±0.24	1.8±0.37	2.0±0.45
Pokeweed mitogen and Phytohemagglutinin	2.8±0.8	2.7±0.49	2.8±1.25	4.4±0.91

Table 2. Effect of mitogens and incubation time on mean mitotic drive

Mitogens	INCUBATION TIME (In hours)			
	68	69	70	71
Pokeweed mitogen (PWM)	38.67±1.53	37.38±3.25	47.54±1.74	39.72±2.15
Phytohemagglutinin (PHA)	38.32±1.01	34.74±2.73	36.90±0.90	39.05±1.14
Pokeweed mitogen and Phytohemagglutinin	38.72±1.81	44.24±1.84	46.50±1.33	47.64±0.97

Table 3. Analysis of variance for the effect of mitogens and incubation time on mitotic index

Source	Degrees of freedom	Sum of squares	Mean sum squares	F value
Mitogens	2	61.880	30.941	4.667*
Incubation time	3	22.850	7.617	1.1489^{NS}
Interaction	6	34.041	5.674	0.8406^{NS}
Error	48	323.958	6.749	
Total	59			

* $P \leq 0.05$

NS - Not significant

Table 4. Analysis of variance for the effect of mitogens and incubation time on mitotic drive

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
Mitogens	2	491.125	245.563	4.6073*
Incubation time	3	283.031	94.344	1.7701^{NS}
Interaction	6	319.789	53.298	2.799*
Error	48	914.000	19.042	
Total	59			

* $P \leq 0.05$

NS - Non significant

Table 5. Relative length of chromosomes of swamp type and river type of buffaloes in Kerala

Chromosome number	Mean relative length		Chromosome number	Mean relative length	
	Swamp	River		Swamp	River
1.	6.921±0.152	7.278±0.094	14.	3.484±0.072	3.416±0.066
2.	6.546±0.090	6.724±0.127	15	3.365±0.045	3.260±0.073
3.	6.162±0.082	6.250±0.212	16.	3.236±0.033	3.130±0.058
4.	5.865±0.092	5.820±0.136	17.	3.141±0.027	2.940±0.049
5.	4.911±0.118	5.050±0.107	18.	3.056±0.144	2.865±0.056
6.	4.953±0.136	4.618±0.095	19.	2.910±0.116	2.778±0.073
7.	4.666±0.041	4.374±0.026	20.	2.847±0.060	2.706±0.072
8.	4.448±0.045	4.314±0.022	21.	2.633±0.068	2.616±0.058
9.	4.204±0.057	4.142±0.067	22.	2.486±0.046	2.530±0.062
10.	4.140±0.047	3.914±0.089	23.	2.301±0.034	2.310±0.062
11.	3.960±0.056	3.774±0.055	24.		2.064±0.123
12.	3.920±0.035	3.664±0.046	X	6.288±0.099	6.220±0.136
13.	3.712±0.045	3.490±0.050	Y	2.675±0.188	2.550±0.095

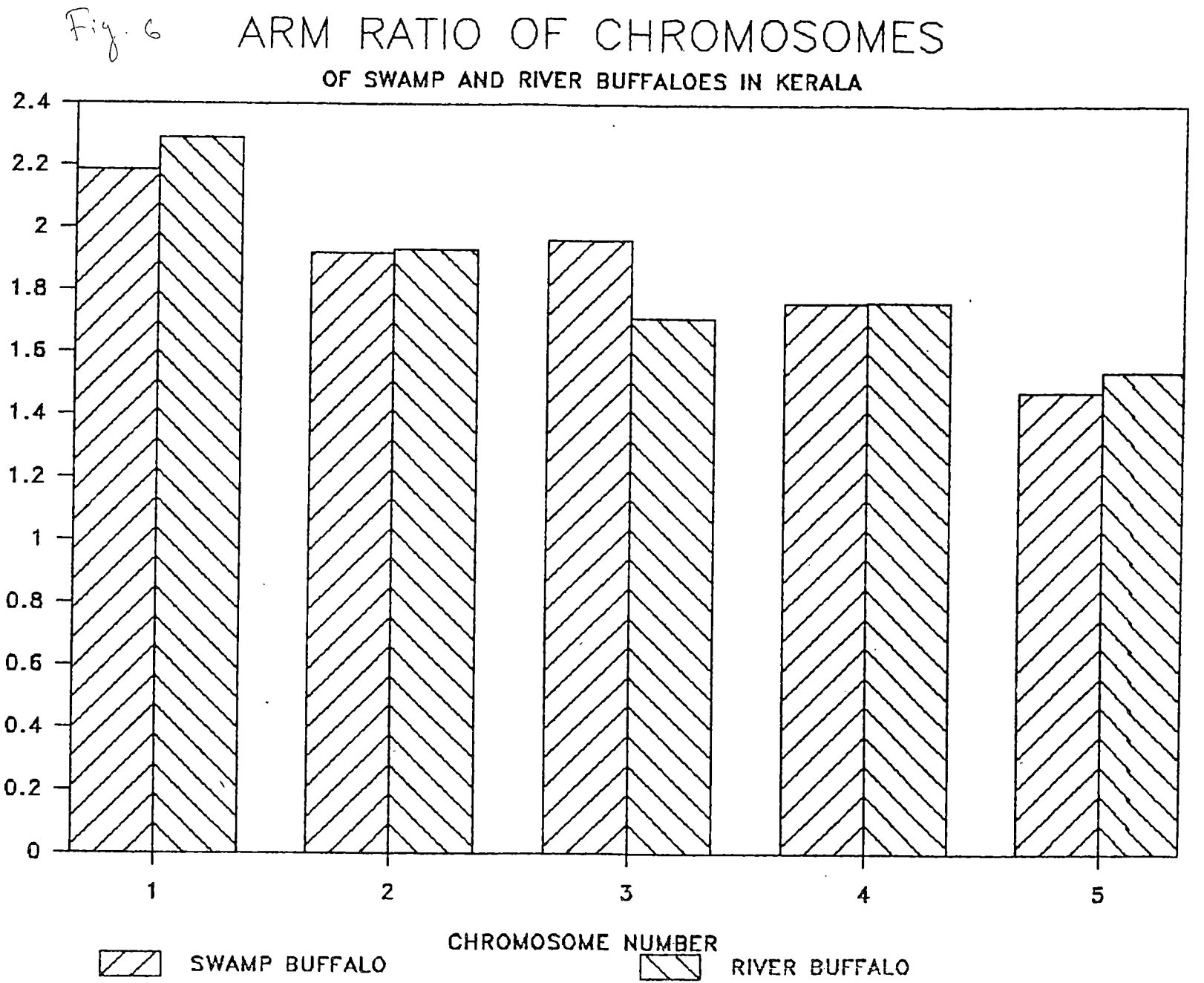
Table 6. Arm ratio of chromosomes of swamp type and river type of buffaloes in Kerala

Chromosome number	Mean arm ratio	
	Swamp	River
1	2.183±0.169	2.289±0.221
2	2.918±0.165	1.931±0.140
3	1.961±0.122	1.712±0.200
4	1.761±0.088	1.767±0.137
5	1.479±0.055	1.542±0.158

Table 7. Centromere index of chromosomes of swamp type and river type buffaloes in Kerala

Chromosome number	Mean centromere index	
	River	Swamp
1	31.1±2.4	33.2±2.1
2	34.8±1.7	35.1±1.7
3	38.5±2.5	34.3±1.4
4	37.0±1.9	36.5±1.1
5	40.4±1.9	40.4±0.9

Fig. 6


ARM RATIO OF CHROMOSOMES
OF SWAMP AND RIVER BUFFALOES IN KERALA
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3
4
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CHROMOSOME NUMBER
SWAMP BUFFALO
RIVER BUFFALO

Fig. 7

RELATIVE LENGTH OF CHROMOSOMES

OF SWAMP BUFFALOES IN KERALA

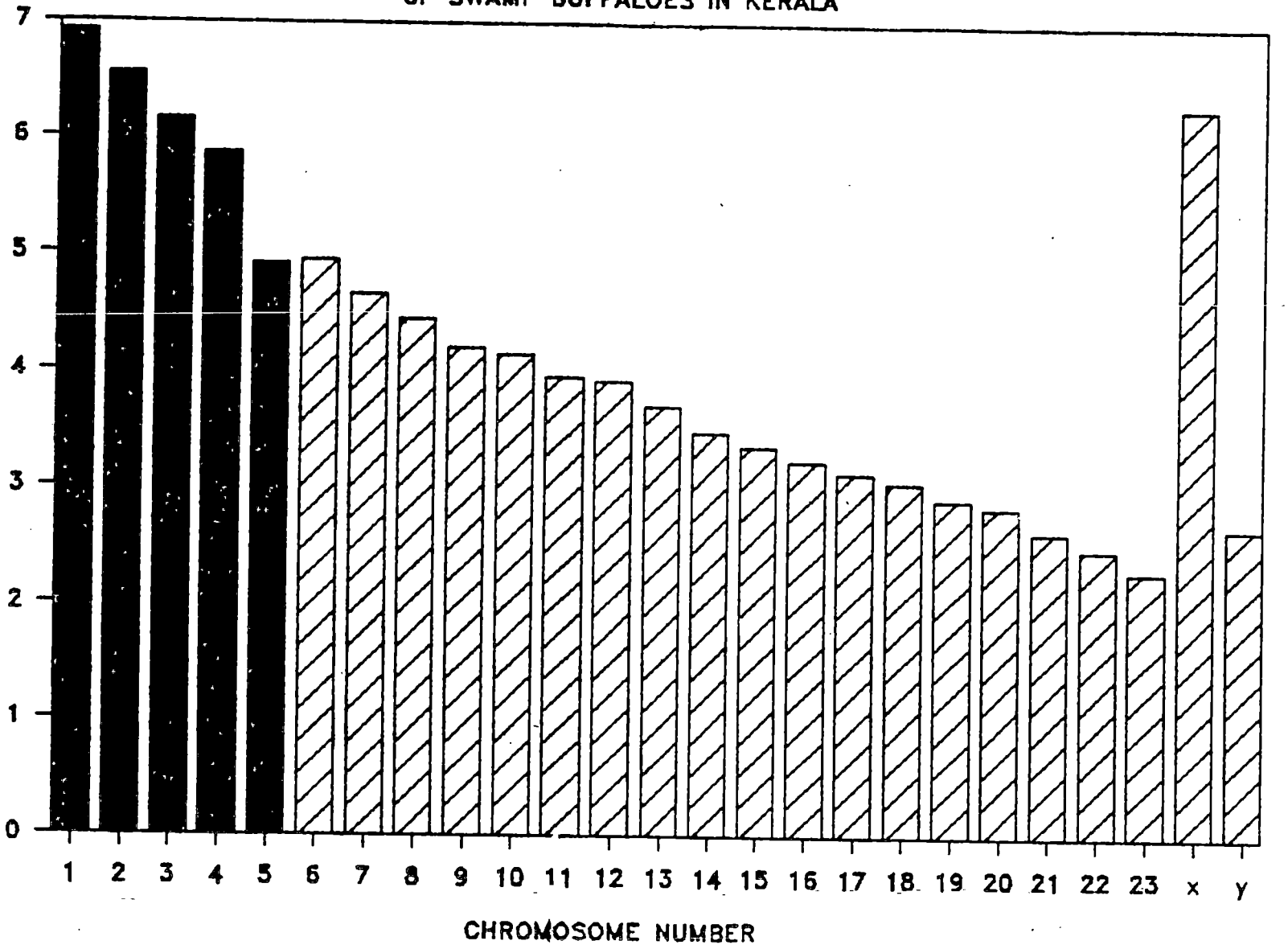
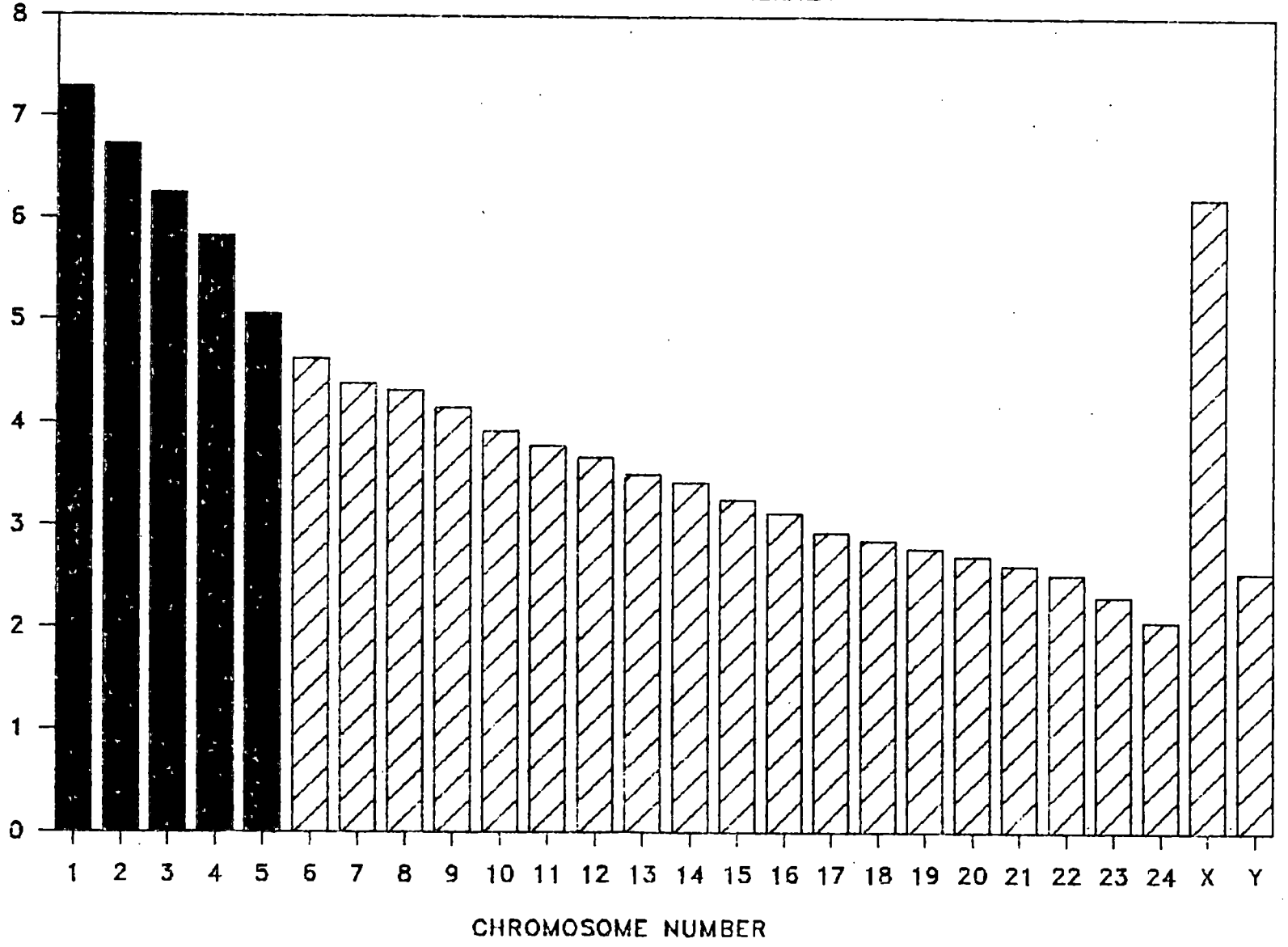


Fig. 8

RELATIVE LENGTH OF CHROMOSOMES

OF RIVER BUFFALOES IN KERALA



Discussion

DISCUSSION

In the present study, blood samples of 54 buffaloes distributed over five districts of Kerala, were collected and were used for karyological investigations. The lymphocytes of the blood samples were subjected to short term peripheral blood lymphocyte culture techique and the metaphase spreads obtained were analysed.

The medium in the present study was RPMI 1640 with extra sodium bicarbonate which was enriched with autologous plasma and mitogen at pH of 6.8. Comparison of the mitogenic effects of PWM, PHA and the mixture of PWM and PHA was assessed. It could be seen that mitotic drive and mitotic index were found to be greater when the mixture of PHA and PWM was used. This phenomenon may be due the differential stimulating property of the two mitogens. PHA stimulates mainly the T lymphocytes while PWM stimulates B lymphocytes. Combination of these two mitogens there fore stimulates both T and B lymphocytes resulting in large proportion of the lymphocytes, lymphoblasts and metaphase cells as reported by Halnan (1989).

Among the mitogens, PWM was superior to PHA in inducing mitosis in buffalo lymphocytes. Thiagarajan *et al*. (1989) reported that PWM yielded higher mitotic index with little or no hemagglutination and also reported that PWM is ideally suited for karyological work in buffaloes.

Incubation time i.e., the time from seeding to addition of mitotic arrester to the culture was also found to influence the mitotic index and mitotic drive. In the present study, the duration of culture ideally suited for buffalo lymphocytes is 71 hours when a combination of PWM and PHA was used and 70 hours when PWM alone was used.

According to Halnan (1989), records over twenty years on the cultures from large animals have not indicated that there is much difference whether the incubation time be 40-48 hours for two day culture or 60-74 hours for three day culture.

Exposure to two microgram or three micrograms per millilitre of culture medium colcemid, for 45-60 minutes yielded best condensation and highest mitotic index. Exposure to colcemid beyond 60 minutes gave more spreads but condensation was more.

Chromosome number and structure

Karyological studies of 54 buffaloes revealed that there existed three classes of buffaloes in Kerala. There were (a)

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swamp type with chromosome number of 2n=48, (b) river type with diploid chromosome number of 50 and (c) others with chromosome number 2n=49.

In swamp type, there were 10 submetacentric chromosomes, 36 acrocentric chromosomes and two acrocentric sex chromosomes. An extra pair of acrocentric chromosome was found in the river type, thus constituting a total of 50 chromosomes. In 'Other' type with 49 chromosomes, there were 10 submetacentics but two of them were without homologues. One of the non-homologues pair of metacentric was the largest among the chromosomes. Among the 37 acnocentrics, one of the autosomes was without a partner. Two acrocentric sex chromosomes were also present.

Crossbreeding between swamp and river type produces different karyotypes. In Kerala the Murrah and Surti belonging to river type are being used to breed the native local stock. The first pair in the serial order of chromosomes of swamp type was bigger which may be due to fusion between a pair of acrocentric chromosomes and one pair of submetacentric chromosomes of the river type. DiBerardino and Iannuzzi (1981) and Bongso and Hilmi (1982) reported that the tandem fusion of fourth and ninth chromosome pairs in river type became the first

pair in the swamp type. Since banding technique has not been used in the present study, a confirmative conclusion could not be made.

Whether the class of animals possessing karyotypes with imbalanced genetic material as in case of 49 chromosome results in reproductive inefficiency or affects the success of breeding programme in Kerala needs detailed investigation.

A diploid chromosome number of 48 in swamp buffaloes and 50 in river buffaloes have been reported by earlier workers (Basrur, 1989).

Chromosome number of 2n=49 in F1 crosses of swamp and Murrah buffaloes was reported by Fischer (1974), Bongso and Jainudeen (1979) and Bongso and Hilmi (1982). Bongso et al. (1988) could obtain a diploid chromosome number of 49 in crosses of Murrah and Malayasian swamp buffaloes. F2 crosses and back crosses of F1 with the parents yielded three types of diploid complements viz. 48, 49 and 50. Harisah et al. (1988) also reported similar polymorphism in chromosome numbers of F1 crosses. The present study indicated the existance of chromosome polymorphism in buffaloes found in Kerala State.

Morphological measurements

The autosomes were grouped into submetacentric and acrocentric groups. The sex chromosomes constituted the third group. The relative length of each group of chromosome was estimated and presented in Table 5. The chromosomes were arranged in pairs based on relative length in each group, since the identification of homologues could not be done by banding technique. Among the submetacentric group, the largest chromosome had a relative length of 6.921±0.152 in swamp type and 7.278±0.094 in river type. While the relative length of smallest metacentric was 4.911±0.118 in swamp type and 5.050±0.107 in river type, respectively. The relative length of acrocentric chromosome ranged from 2.301±0.034 to 4.953±0.130 in swamp type and 2.064±0.123 to 4.618±0.095 in river type.

Among the autosomes, first submetacentric was the largest in terms of relative length. The X chromosome occupies the third position in the swamp type and fourth position in river type.

In the case of sex chromosomes, the relative length of X was 6.288±0.099 in swamp type and 6.220±0.136 in river type, and Y chromosome had a relative length of 2.675±0.188 in swamp type and 2.550±0.095 in river type.

The X chromosome was the largest acrocentric, whereas Y chromosome occupied the 21st position in swamp type and 22nd position in river type in the relative length chart.

The findings of the present study was not in agreement with that of Ulbrich and Fisher (1968). Livescu (1981), Chuanchai and Lvesakul (1985) who reported Y chromosome as the smallest in Asiatic and African, Romanian and Thai buffaloes, respectively. However, the findings of Yadav and Balakrishnan (1982) were in partial agreement with those of the present study, that Y chromosome was not the smallest acrocentric but it was intermediat between 19th and 20th pairs.

Toll and Halnan (1976) reported that Y chromosome was a small acrocentric to submetacentric chromosome in which only the distal end of long arm appeared to stain darkly. Centromere did not stain. Mikaye et al. (1980) reported that the X chromosome was the largest acrocentric and the Y chromosome was the second smallest acrocentric in swamp buffaloes imported from Taiwan to Japan.

As regards to arm ratio, the swamp type and river type did not show any difference except in case of second, third and fourth chromosomes. In river type, the value for arm ratio in fourth chromosome was higher than that of third pair indicating that the centromere in third chromosome was comparatively nearer

to the mid point. This is in agreement with that of Sharer et al. (1986). In swamp type, the centromere in second pair is nearer to midpoint than in third chromosome. However, similarity is observed between the swamp and river type with regard to first and fourth chromosome.

Among the five pairs of submetacentric chromosomes, the centromere in the fifth pair is more proximal to the midpoint of chromosome than in other chromosome pairs. In first chromosome, the centromere is far away from the mid point compared to other chromosomes.

On going through the values of centromere indices, of five submetacentric chromosomes, it is seen that the largest submetacentric possessed centromere away from the midpoint while in the fifth submetacentric chromosome, the centromere is nearer to the midpoint in both types. In other words, the fifth chromosome is more metacentric than the other chromosomes. This finding confirms the estimate made on the basis of arm ratio. Sharer et al. (1989) reported the centromere index of first autosome as 31.17 per cent and that of autosome five as 48.72 per cent showing an agreement with the present study.

Summary

SUMMARY

The chromosome profile of the buffaloes in Kerala using Karyological technique has been investigated in this study.

Fifty four buffaloes selected from four districts of Kerala were karyotyped using peripheral blood lymphocyte culture. On the basis of horn shape, the animals were classified into three groups, viz. sickle shaped horn type, curled horn type and non-descript.

Blood was collected from external jugular / ear vein keeping 1.5 ml of the blood aside for seeding the culture, the rest of blood was centrifuged for separating the plasma. The medium used was RPMI 1640 supplemented with autologous plasma and sodium bicarbonate. The efficacy of different mitogens viz. phytohemagglutinin (PHA), pokeweed mitogen (PWM) and combination of these two was studied together with the effect of different incubation times of 68, 69, 70 and 71 hours. The concentration of colcemid and duration of its action were also standardised. The cells were arrested in metaphase stage of mitosis with colcemid, which were then treated with 0.075 M potassium chloride solution for 20 minutes. Fixative used was Carnoy's fixative. Slides were prepared and air dried. Four per cent Giemsa was used for staining.

Comparative assessment of the three mitogens based on the mitotic drive and mitotic index revealed that the combination of PWM and PHA was significantly superior to PHA in inducing mitosis of buffalo lymphocytes. PWM had an intermediary mitotic drive and mitotic index. Significant interaction between mitogen and incubation time was observed in the mitotic drive. The combination of PWM and PHA at an incubation time of 71 hours showed the best results followed by PWM at an incubation time of 70 hours.

Concentration of colcemid for arresting the mitosis was found to be best between 2 µg and 3 µg per ml of culture media. The time of action of colcemid at this concentration was one hour.

Three chromosome patterns such as 2n=48 (swamp type), 2n=49, and 2n=50 (river type), were observed in buffaloes of Kerala. Horned condition and diploid number of chromosome did not exhibit any correlation in the per cent study. In all the three chromosome types of animals 10 submetacentric autosomes were present. In swamp type animals 36 acrocentric autosomes were present, whereas in river type there were 38 acrocentric autosomes. In the animals having 49 diploid chromosomes the number of acrocentric autosomes was 37. Two of the submetacentric chromosomes in those animals were non-homologues.

The X chromosome was largest acrocentric chromosome of the complement. But the Y chromosome was acrocentric and took a position between 21st and 22nd in descending order based on relative length in the river buffaloes, whereas it occupied between 20th and 21st position in swamp type.

The longest submetacentric autosome of swamp type had a relative length of 6.921±0.152 whereas in river type it had a relative length of 7.278±0.094. The smallest submetacentric autosome was having an average relative length of 4.911±0.118 and 5.050±0.107 in swamp type and river type, respectively.

Relative length of the longest acrocentric autosome in swamp type and river type was 4.953±0.13 and 4.618±0.095, respectively. The shortest acrocentric in swamp type showed a relative length of 2.301±0.034, whereas in river type the shortest chromosome had a relative length of 2.064±0.123.

The X chromosome in river type had the relative length of 6.220±0.136 in river type as against the relative length of 6.288±0.188 in swamp type. The Y chromosome of swamp type and river type had relative lengths of 2.675±0.188 and 2.550±0.095 respectively. In terms of elative length X chromosome occupied fourth position in river type. Whereas it was in third position in swamp type.

In swamp buffalo, arm ratio ranged from 1.479±0.055 in fifth to 2.183±0.169 in first submetacentric autosome. In river type, the largest arm ratio was 2.289±0.221 in first submetacentric autosome and the smallest was 1.542±0.158 in fifth submetacentric autosome.

The highest centromere index of 40.4±0.9 was obtained for fifth submetacentric autosome and the least index observed for first chromosome was 33.2±2.1 in swamp type. In river buffaloes, fifth chromosome had a centromere index of 40.4±1.9, and first chromosome had the centromere index of 31.2±2.4.

Arm ratio and centromere index revealed that smallest submetacentric chromosome was more metacentric than other submetacentrics.

Out of the 54 buffaloes studied 45 animals had diploid chromosome number of 50, 5 had 48 numbers and 4 had 49. This indicated that there is chromosome polymorphism in the buffaloes found in Kerala. This observation is of practical significance and has to be borne in mind while drawing breeding programmes in buffaloes for improved production potential and reproductive efficiency.

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GENETIC CHARACTERISATION OF BUFFALOES IN KERALA USING CYTOGENETIC TECHNIQUE

By

K. ANIL KUMAR

ABSTRACT OF A THESIS

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Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Animal Breeding and Genetics
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Trichur

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ABSTRACT

Buffaloes from four districts of Kerala formed the materials for this study. Blood samples were collected from external jugular/ear vein, in heparinised tubes. The technique employed was peripheral leukocyte culture technique.

Out of the three different mitogens viz., pokeweed mitogen (PWM), phytohemaggultinin (PHA), and a mixture of these two, the mixture of PWM and PHA was found superior in inducing mitosis. A significant interaction between the mitogens and time of addition of mitotic arrester into the medium was observed. The mixture of PWM and PHA at an incubation time of 71 hours was found to produce best results in culture of buffalo lymphocytes, followed by PWM at 70 hours.

Ideal concentration of colcemid as mitotic arrester in this study was found to be 2 µg and 3 µg per ml of culture media, when the of colcemid was retained for one hour.

Out of the 54 animals studied 45 had 2n=50, 5 had 2n=48 and rest 4 had 2n=49. In all the three chromosome types of animals 10 submetacentric chromosomes were observed. The sex chromosomes were similar in all the three types. Number of acrocentric

autosomes was 38, 36 and 37 in the three classes respectively. The buffaloes having diploid chromosome number of 49 had two non homologues submetacentric chromosomes, one being the largest of the whole complement and one acrocentric was without a pair.

The longest submetacentric autosome of swamp type had a relative length of 6.925±0.152 whereas in river type it had a relative length of 7.228±0.094. The smallest submetacentric autosome was having an average relative length of 4.911±0.118 and 5.05±0.107 in swamp type and river type respectively.

Relative lengths of longest acrocentric autosome in swamp type and river type was 4.953±0.13 and 4.618±0.095 respectively. The shortest acrocentric in swamp type showed a relative length of 2.301±0.034 whereas in river type the shortest chromosome had a relative length of 2.064±0.275.

The X chromosome was largest acrocentric chromosome of the complement. The Y chromosome was acrocentric and took a position of 22 in descending order based on relative length in the river buffaloes, whereas it occupied 20th position in swamp type.

The X chromosome in swamp type had the relative length of 6.228±0.099 and that of river type was 6.220±0.136. The Y chromosome of swamp type and river type had relative length of 2.675±0.188 and 2.550±0.095 respectively. In terms of relative length X chromosome occupied fourth position in comparison to submetacentric autosomes of river type, whereas it was third position in swamp type.

In swamp buffaloes arm ratio ranged from 1.479±0.055 in fifth to 2.183±0.169 in first submetacentric autosome. In river type the largest arm ratio was 2.289±0.221 in first chromosome and the smallest was 1.542±0.158 in fifth chromosome.

The highest centromere index of 40.4±0.9 was obtained for fifth chromosome and the smallest centromere index of 33.2±2.1 was for first chromosome of swamp type. In river buffaloes fifth chromosome had a centromere index of 40.4±1.9 and first chromosome had the centromere index of 31.2±2.4.

The observation of chromosome polymorphism in buffaloes of Kerala State have to be borne in mind prior to drawing breeding strategies in buffaloes for improved production potential and reproductive efficiency.