CHROMOSOME PROFILE OF INDIAN ELEPHANTS (*Elephas maximus indicus*)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Chromosome profile of Indian Elephants (*Elephas maximus indicus*)" 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.

A. Sakthi Kumar

Mannuthy,
19-1-1988

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CERTIFICATE

Certified that this thesis entitled "Chromosome profile of Indian Elephants (*Elephas maximus indicus*)" is a record of research work done independently by Shri. A. Saikthi Kumar 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.

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Dedicated to my

beloved parents

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A. SAKTHI KUMAR

CONTENTS

Introduction

INTRODUCTION

Cytogenetics is a hybrid of cytology and genetics which involves the study of correlation between cytologically observable chromosome changes and phenotype. Very recently, improvements in chromosome methodology have made a great impact on evolutionary studies and also in tracing the origin of particular herds.

Indian elephants (Elephas maximus indicus) - one of the most majestic and elegant of the living creatures on this planet occupy a highly important place in the religious, cultural and social life in our country. It is also considered as a symbol of wealth. In Hindu mythology, the white elephant 'Airavatha' has been depicted as the vehicle of Devendra, the king of Devas. In ancient days it was the number of well trained elephants that was a decisive factor in the wars. Most of the temples in India are particular to maintain a herd of elephants for temple festivals as well as to proclaim their status of wealth. In Kerala, any cultural activity, whatever be the religious tag attached to it, is considered incomplete if a caparisoned elephant and attendant ornate decorations are not included.

The use of elephants in gathering timber from deep forests inaccessible to vehicles and depositing at accessible places is indeed too valuable to be ignored. An outgrowth of the upper incisor teeth in the male Indian elephants, called "tusk", is a highly precious commodity. Expert artisans carve

out scintillating and enchanting items of art work including figurines, icons and intricately beautiful foligree articles from ivory. Elephant teeth have been found to possess medicinal properties in the Ayurvedic system of medicine.

Perusal of literature shows that information on cytogenetics of Indian elephants is scanty. Indian elephants exhibit sexual dimorphism, the male possessing tusks in addition to the differences in secondary sexual characteristics. It is also interesting to observe that the tusks in certain males called 'makhna' is rudimentary.

Cytogenetic reports already available on Indian elephants are based on one or two elephants using tissues not amenable to intense mitotic activity; and also were contradictory. Differences in chromosome morphology of male, including tuskers and makhnas, and females were not seen reported.

This study was therefore undertaken with the following objectives.

a) to evolve a technique for chromosome studies of elephants.

b) to find out the chromosome number in Indian elephants.

c) to suggest sex chromosome mechanism in sex determination.

Review of Literature

REVIEW OF LITERATURE

The intensity of mitosis of lymphocytes in artificial media is often dependent on the nature of media, temperature, mitogen, incubation period, etc. In order to get maximum mitosis in terms of mitotic drive and mitotic index, several attempts have been made to assess efficacy of the different media, mitogen and incubation period.

Moorehead et al. (1960) first demonstrated karyotype preparations using peripheral blood leukocytes. Ten ml. of venous blood was collected in heparinized syringe. Bacto-phytohaemagglutinin at a rate of 0.2 ml per 10 ml of blood was used to seperate the red blood cells by allowing the mixture to stand on ice bath for 30 to 60 minutes. This was then centrifuged at 300 to 350 revolutions per minute (RPM) for 5 to 10 minutes at 5°C to sediment the erythrocytes. The supernatant containing the leukocytes was separated out and a leukocyte count was made. About 1.0 to 1.2 x 10^6 cells per ml was planted in about 8 to 10 ml of a culture medium consisting of 30 to 40 per cent fresh autologous or homologous plasma and 60 to 70 per cent TC 199 medium. Penicillin and streptomycin were also added to the medium as antibiotics. The pH was adjusted to 7.0 to 7.2 using 0.1 N HCl or carbon-dioxide gas. Incubation was done at 37°C. After 65 to 70 hours of incubation, colchicine at a final concentration of 0.5 to 1.0 x 10^{-6}M was added and incubated again for another 6 hours. Afterwards the cells were loosened from the bottom

of the bottle using a pipette. The supernatant was removed and the cells resuspended in about 5 to 6 ml of warm Hank's or Earle's Balanced salt solution at pH 7.0. This was again centrifuged, supernatant removed and the cells resuspended in a small volume of balanced salt solution as before. Warm distilled water was added slowly two to three times the original volume and again incubated at 37°C for 3 to 5 minutes. The mixture was then centrifuged at 600 RPM for 5 minutes. The total time recommended for the treatment was 8 to 10 minutes. The supernatant was then removed and 3 to 4 ml of 3:1 methanol-acetic acid was added without disturbing the cell button. The mixture was allowed to stand for 30 minutes. The button was then broken up, the mixture centrifuged, supernatant removed and the cells resuspended in fixative. Slides were prepared by dropping 2 to 3 drops of the supernatant on chilled glass slides. Excess fluid was drained off by tilting the slide on to a blotter. Drying was accomplished in about 30 to 60 seconds by fanning or gently warming over a spirit flame. The quality of spreads was checked under phase contrast microscope; inadequate spreading was often corrected by addition of fixative even after long storage in refrigerator. Staining was done using acetorcein stain (1% natural orcein in 60% acetic acid). The slides were then mounted and observations of metaphase spreads were made.

In cattle, karyotyping using peripheral blood lymphocyte culture method was carried out by Nichols et al. (1962).

Ten ml of blood was collected from jugular vein. Coagulation was prevented using 0.25 to 0.5 ml of heparin containing 1000 units per ml. Sedimentation of erythrocytes was accomplished by the addition of 6 per cent bovine fibrinogen, at a rate of twice the volume of blood and allowing it to stand for 30 to 60 minutes at room temperature. Addition of PHA or dextran was not as successful as the addition of fibrinogen. It was imperative to filter the fibrinogen to prevent bacterial contamination. After sedimentation, the fibrinogen-plasma-white blood cell suspension was pipetted out and centrifuged at 1000 RPM for 5 minutes when the white blood cells formed a button at the bottom. Supernatant was carefully discarded. The cells were then suspended in one ml of culture medium which consisted of Parker's medium supplemented with 30 per cent calf serum. A cell count was performed. The final concentration of cells was adjusted to 10^6 cells per ml by the addition of more culture medium. Phytohaemagglutinin at a rate of 0.1 ml per 10 ml of culture medium was used to initiate mitosis. The mixture was incubated at 37°C in an atmosphere of 5 per cent carbondioxide. On day three and four of incubation, colchicine was added to a final concentration of 10^{-6} M/L for one hour. Hypotonic treatment was done using distilled water at a rate of three times the volume of the medium and allowing to stand for 15 minutes. The mixture was then centrifuged, supernatant removed, and one ml of freshly prepared fixative consisting of 60 per cent acetic acid and 0.1 N HCl was added without disturbing the cell button. The mixture was allowed to stand

for 10 minutes. Afterwards, the fixative was decanted as much as possible. The cells in the solution were stained using 2 per cent orcein acetic acid by adding a few drops of the stain to the cell suspension. Slides were prepared by dropping a few drops of the cell-stain mixture on a slide and squashing the cells using a cover slip.

Peripheral blood lymphocyte culture using whole blood in Asian elephants was reported by Jarofke and Noitzel (1983). They used Ham's F_{10} medium (4.0 ml) supplemented with 0.5 ml fetal calf serum, 0.12 ml PHA, 500 IU of penicillin and 500 mg of streptomycin sulphate as the medium for culturing the lymphocytes of a female Asian elephant. Heparinized whole blood at a rate of 0.4 ml per culture was used and the mixture incubated at 37°C for 4 to 5 days. Potassium chloride (0.075 M KCl) was used as a hypotonic solution and methanol-acetic acid (3:1) as fixative. Chromosomes were stained using 2 per cent Giemsa.

Separation of lymphocytes

Various methods and agents have been used for separating lymphocytes from whole blood for subsequent culturing of the separated cells. Phytohaemagglutinin was used as haemagglutinating and precipitating erythrocytes in the whole blood.

Moorehead et al. (1960) used phytohaemagglutinin at a rate of 0.2 ml per 10 ml of human blood. The mixture was allowed to stand on ice bath for about 30 to 60 minutes and complete sedimentation was facilitated by centrifugation at

300 to 350 RPM for 5 to 10 minutes at 5°C. Leucocytes present in the plasma was used to set up cultures.

Crossley and Clarke (1962) used PHA at a rate of 16 drops from a No.12 needle per 10 ml of cattle whole blood. Centrifugation was done immediately at 1000 RPM for 30 minutes to sediment the erythrocytes.

Nichols et al. (1962) found that 6 per cent bovine fibrinogen could be used successfully for sedimenting erythrocytes in cattle whole blood. Bovine fibrinogen at a rate of twice the volume of whole blood was added and erythrocyte sedimentation was accomplished by allowing the mixture to stand for 30 to 60 minutes at room temperature. It was imperative to filter the fibrinogen so as to avoid bacterial contamination. After sedimentation, the fibrinogen-plasma-white blood cell suspension was taken out and centrifuged at 1000 RPM for 5 minutes when the white blood cells formed a button at the bottom. According to the authors, PHA or dextran was not as successful as bovine fibrinogen in separating cattle erythrocytes.

Biggers and McFeely (1963) reported the use of Ficoll-Hypaque gradient technique for separating lymphocytes in domestic animals. The authors were of the opinion that PHA was not effective in domestic animals as in human beings for sedimenting erythrocytes.

Lin et al. (1976) followed the same method in domestic animals for separating lymphocytes. The opaque band obtained

which contained the lymphocytes was removed carefully and washed in Hank's Balanced salt solution and centrifuged at 800 to 1000 RPM for 20 minutes when a pellet of lymphocytes could be obtained at the bottom.

Whole blood collected from domestic animals was subjected to centrifugation at 800 RPM for 10 minutes to separate erythrocytes (Rumason and Sanders, 1963).

Schera and Louro (1963) accomplished the separation of erythrocytes by refrigerating the whole blood at 4 to 8°C for 2 to 3 hours in a vertical position, whereas Connolly et al. (1964) allowed the whole blood to stand at room temperature in the syringe itself when sedimentation of erythrocytes occurred.

Gustavsson (1964) used 30 ml dextran per 10 ml of heparinized whole blood. The mixture was refrigerated for about 3 hours to yield a cell deposit which was added to the culture medium.

The buffy coat containing lymphocytes obtained by centrifugation of whole blood was used by Hare et al. (1966) for setting up peripheral blood mononuclear leukocyte cultures.

Whole blood culture method

Arakaki and Sparkes (1963) reported the use of whole blood for lymphocyte cultures. About 0.2 ml of blood was drawn into one ml syringe wetted with heparin. The blood was directly inoculated into a sterile cylindrical vial containing 5 ml of Eagle's minimal medium and 0.1 ml of PHA supplemented with

15 per cent fetal calf serum. The blood and culture medium was mixed thoroughly by gently swirling the vial several times and incubated at 37°C. Colchicine at a final concentration of 0.02 µg per ml was added 4 to 5 hours prior to harvesting. Hypotonic treatment was accomplished by the addition of four volumes of warm distilled water to the cell suspension followed by incubation of the mixture at 37°C for 15 minutes. Cells were fixed using 1:3 acetic methanol. Air dried preparations were stained using chromosome stains.

Karyotype preparations using whole blood culture in domestic animals was reported by Basrur and Gilman (1964). They observed that addition of PHA or dextran was not efficient enough for the separation of leukocytes. One ml of whole blood was added to 9 ml of Connaught's H597 growth medium inclusive of 20 per cent inactivated calf serum and 0.5 ml PHA-M. The mixture was incubated at 38°C for 3 to 4 days. One ml of saline containing 100 µg of colchicine was used as mitotic inhibitor. Slides prepared by air-drying method were stained using 2 per cent natural orcein or carbol fuchsin. They also observed that storing heparinized blood at room temperature upto 6 hours did not significantly reduce the quality of chromosome preparations. Two volumes of distilled water was found to be sufficient enough to lyse the erythrocytes. Incubation upto 3 to 4 days was satisfactory. More than four day incubation tended to reduce the yield. The method was also found to be suitable for goats, sheep and mink.

This technique of whole blood culture was followed by Ponce De Leon (1975) and Berardino and Iannuzzi (1981) in sheep; Harvey (1976), Norberg et al. (1976); Halnan (1977), Miller (1977), Balakrishnan et al. (1979), Sharma et al. (1980) and Swarts and Vogt (1983) in cattle; Ma et al. (1980) in owl monkey; Winter et al. (1986) in Mithun, Siri and their crosses; and Jarofke and Neitzel (1985) in Asian elephants.

Peripheral blood lymphocyte culture technique

Several techniques have been employed to obtain better results in Karyotyping by incorporating modifications in the use of anticoagulant, media, supplementation of media, number of lymphocytes, quantity of whole blood, mitogen, incubation period, colchicine concentration, hypotonic treatment, fixative agent and staining. The modifications are furnished in table 1.

Transportation

Halnan (1977) suggested that heparinised whole blood may be transported by car upto 2 hours, by train upto 6 hours and by air upto 14 hours. Prolonged transport was found to reduce mitotic index. The temperature during transit was maintained between 21°C and 32°C. Best results could be obtained when blood was transported in the culture medium itself.

Ma et al. (1980) reported that successful chromosome preparations could be obtained from whole blood of owl monkeys transported to the laboratory by ship. Winter et al. (1986)

observed that whole blood collected from mithun, siri and mithun-siri crosses transported in ice contained in thermos-flasks yielded satisfactory results.

Storage

Basrur and Gilman (1964) observed that storage of heparinized whole blood at room temperature upto 6 hours did not significantly reduce the quality of chromosome preparations. Kanagawa and Basrur (1968) reported that successful cultures could be obtained from blood samples stored upto 72 hours at 5°C and 20°C. A comparison of results obtained from cultures prepared from blood samples stored for 72 hours at 5°C and those stored at room temperature showed that mitotic index dropped appreciably in the former.

Effect of antibiotics and Colchicine

In a human lymphocyte culture method a study was conducted on the effect of different concentrations of antibiotics in the culture medium by Neu et al. (1965). Mitotic indices varied according to different antibiotic concentrations. There was complete inhibition of mitosis at concentrations higher than 150 µg/ml of tetracycline. The chromosomes showed a tendency to clump together in high concentration of streptomycin, chlorum phenicol or tetracycline. In about 500 µg per ml, streptomycin apparently stimulated mitoses. There was only slight increase in mitotic activity with lower concentrations of penicillin and chloramphenicol.

Different concentrations of penicillin and streptomycin were used as antibiotics by several workers (Scherz and Louro, 1963; Hare et al. 1966; Lazary et al. 1974; Jarofke and Meitzel, 1985).

In a review of literature of chromosome cultures, Heath (1966) suggested that prolonged colchicine treatment or higher concentrations of colchicine increased mitotic index, but also produced highly contracted mitotic figures in older metaphases. The concentration of colchicine higher than 4 mg per ml reduced or even inhibited metaphase formation.

Chromosome number and morphology

Anatomically, elephants show many peculiarities (Mariappa, 1986). The trunk of the elephant is formed by an extension of the nose. The tusk is an outgrowth of the upper incisors, arising from pre-maxilla. Among the Indian elephants, only males possess tusks whereas females are tuskless.

In the males, testicles are placed inside the body cavity. The penis is very long with a pendulous part. The glans is inconspicuous. In females, the vulva is completely hidden from view being placed between the thighs below the pelvic symphysis; and it is placed in an oblique plane facing downward and backward. They possess pectoral mammary glands.

Elephants are highly social beings and lead a matriarchial system of life. Females staying together show alloparental care for the young ones. The gestation period range from 18 to 22 months. A new born may weigh about 110 to 120 kg.

On culturing the somatic cells from one male and one female Asian elephants, Hungerford et al. (1966) reported that the sex chromosomes were easily identifiable. The X was the only long chromosome with a nearly median centromere while Y was a short acrocentric with sharply defined centromere. The modal chromosome number in the case of African and Asian elephants was 2n=56. The first of the three pairs of autosomes with nearly median centromeres of the Asian elephant was found to possess a pronounced satellite, but was absent in the African elephant.

In a cytogenetic study using corneal and ovarian tissue of a single female Indian elephant which had to be sacrificed due to an incurable bilateral fracture of humerus, Norberg (1969) observed that the diploid chromosome number was 2n=56. It was possible to distinguish the largest acrocentric pair from the next two largest acrocentric pairs. The latter ones could not be separated and were therefore grouped together. There was no much difference between the remaining acrocentrics and hence were placed in a continuous series. The smallest pair of acrocentrics were different from other acrocentrics. The last group included three pairs of autosomes with nearly median centromeres. The authors could not identify any satellite on the first pair of these autosomes and hence were grouped together.

According to Jarofke and Neitzel (1985), the diploid chromosome number in both the Asian and African elephants

maintained in the Berlin zoo was 2n=56. In the African elephant, the karyotype consisted of 50 acrocentric and 4 metacentric autosomes. In the Asian elephants, the karyotype consisted of 48 acrocentric and 6 small metacentric autosomes. In both the species, X-chromosomes could be identified very easily because of their metacentric morphology and Y-chromosome was a small acrocentric.

Chromosomal abnormalities

On investigation, morphological as well as physiological abnormalities in animals are often found to be due to chromosome aberrations or gene mutations.

The first ever report of chromosome abnormality in domestic animals was published by Gustavson and Rockborn (1964). In three cases of overt lymphatic leukemia in cattle, all the mitoses were found to contain 59 chromosomes, with one chromosome diverging morphologically from the normal complement. The particular chromosome had a subterminally situated centromere and it was presumed to be a product of fusion of chromosomes 1 and 29.

An orange female kitten born to a Blue-and-white female and a tortoise-shell male cat was found to possess an abnormal karyotype 37, x 0 (Norby et al., 1974).

The case of an 18-month old heifer which failed to breed was investigated by Norberg et al. (1976). Clinical and post-mortem examination revealed poorly developed genitalia. On

cytogenetic analysis, the animal was found to possess a 61, XXX karyotype.

In a review, Sharma (1976) reported gross variation from normal karyotype leading to phenotypic effect in man. Alterations in the normal karyotype may involve change in a chromosome segment, in the number of chromosomes in a complement, that is, "Somy", or the entire genome, that is, "ploidy". The syndromes involving the number of chromosomes in man include Down's syndrome, Edward's syndrome and Patau's syndrome, while the syndromes described as Klinefelter's and Turner's pertain to those involving sex chromosomes.

Larsen et al. (1978) reported the case of a centric fusion of two acrocentric chromosomes in a Golden Retriever Cross and its offspring. Cytogenetic analysis of 16 of its pups and their sires revealed that 9 pups and the sires possessed normal karyotypes, while the remaining 7 pups were carriers for the translocation. The chromosomes involved were identified as 13 and 17 by G-banding.

Cytogenetic analysis of a tricolour male cat with normal sexual behaviour revealed a 39, XXY chromosome complement. A left-sided epididymal and deferential aplasia was detected after castration (Konig et al., 1984).

Johnson (1985) reported congenital abnormalities caused by chromosomal aberrations in (a) Maine x Anjou bull with thymic form of lymphosarcoma, (b) a Simmental male pseudohermaphrodite, (c) a Black and white x Simmental, (d) a German

yellow calf with muscular dystrophy, (e) a calf with bilateral anophthalmia and (f) a case of bovine hereditary paraketosis.

The reports on chromosome of Indian elephants do not, however, specify whether the male elephant is a tusker or makhna and whether there exists any chromosome variation between the two kinds of male elephants.

Table 1. Peripheral blood lymphocyte culture technique-methods and modifications

Author/Authors	Anticoagulant	Medium	Media supplementation	Number of lymphocytes/amount of whole blood per culture	Mitogen	Duration of culture	Colchicine	Hypotonic solution	Fixative	Stain
1	2	3	4	5	6	7	8	9	10	11
Moorhead et al.(1960)	Heparin	TC 199	30-40% fresh autologous or homologous plasma	1.0 to 1.2×10^6 cells/ml of plasma	PHA	65 to 70 hours	0.5 to 1.0×10^{-6}M for 6 hours	Warm distilled water for 3 to 5 minutes	3:1 methanol acetic acid	Acetorcein
Nichols et al. (1962)	0.25-0.5 ml of heparin/10 ml blood 1000 cu/ml	Parker's medium	30% calf serum	10^6 cells for ml of medium	PHA	3 to 4 days	10^{-6}M/L for one hour	Distilled water	Mixture of 60% acetic acid and 0.1 NHCl	Orcein acetic acid
Arakaki and Sparkes (1963)	Heparin	Eagle's minimal medium	15% fetal calf serum	0.2 ml of whole blood per 5 ml of culture	PHA	-	0.2 ug per ml	Warm distilled water at 37°C for 15 minutes	1:3 acetic methanol	chromosome stains
Biggers and McFeely (1963)	-	Eagle's minimal medium	10% calf serum	Upper layer of plasma	PHA	3 days	0.05 ug/ml for 3 hours	-	-	Giemsa
Humason and Snnders (1963)	0.2 ml heparin containing 1000 units per ml for 5-10 ml blood	F-10 Ham's	15% fetal bovine serum	3-4 drops of plasma per 5 ml culture	PHA	68 - 72 hours	0.1 ml of 10^{-6}M per 1 ml of culture for 4 to 5 hours	Warm water at 37°C for 10 minutes	3:1 methanol-acetic acid, 3:1 ethanol acetic acid	T. T. Puck's crosyl voilet acetate
Scherz and Louro (1963)	Heparin 1000 units per ml	TC 199 or NCTC 109	-	1.5 to 2.0 ml of plasma per 5 ml culture	PHA-M PHA-P	68 - 72 hours	Colchicine 0.25 ml of colchicine saline (40 ug/ml) per culture for 4 hours	1.12% sodium citrate	1 : 3 glacial acetic acid methanol	Acetic orcein
Ulbrich and Weinhold (1963)	2 to 4 ml heparin containing 200 mg of heparin per 10 ml blood	Medium 199	20% homologous plasma	1000 - 1200 cells/mm^3	PHA-M	72 hours	Colcemid 1 ml of 0.04 per cent for 3 hours	-	3 : 1 absolute alcohol glacial acetic acid	2% orcein
Basrur and Gilman (1964)	0.2 ml of 1% hepatin	Connaught's H 597	20% inactivated calf serum	1.0 ml of whole blood per 9ml culture	PHA-M	3 days	1 ml phosphate buffered saline containing 100 ug of colchicine/culture	Distilled water	Carnoyo solution	Natural orcein or carbol fuchsin

1	2	3	4	5	6	7	8	9	10	11
Gustavsson (1964)	-	Parker 199	30% calf serum	4 million cells per cc	PHA	65 - 70 hours	Colchicine 10^{-7} M for 30 minutes	Distilled water	9 parts of 60% acetic acid and 1 part of 0.1 NHCl	Acetic orcein
Connolly et al. (1964)	Heparin	Difco 199 culture medium	-	0.5 - 1.0 ml of plasma containing 5000 - 8000 cells per culture	PHA	68 - 72 hours	0.05 ml colchicine containing 10 ug of colchicine/ml for 3 to 4 hours	1% sodium citrate	3 : 1 absolute ethyl alcohol to glacial acetic acid	Acetic orcein Giemsa
Hare et al (1966)	Heparin - 100 USP units per 10 ml blood	Eagle's basal medium on TC 199, NCTC 109	15% calf serum 30% pig serum	1×10^7 mononuclear leucocytes per ml of autologous plasma	PHA-M	66 - 72 hours	Colcemid - 0.01 ug/ml, 0.08 ug/ml, 0.05 ug/ml, 0.40 ug/ml.	Warm mixture of one part fetal calf serum and 5 parts distilled water	3 : 1 methanol glacial acetic acid	Giemsa
Kanagawa and Basrur (1968)	-	-	-	-	-	72 hours	-	-	-	Acetic orcein
Ponce De Leon and Marcum (1975)	-	TC 199	-	1.5 ml whole blood per 7.5 ml culture medium	-	72 hours	0.06 ml colcemid (10 mg/ml) for 2 hours	0.075 MKCL	-	-
Harvey (1976)	Heparin	Waymouth's medium	-	-	PHA	48 hrs	Colchicine	-	-	-
Lin et al (1976)	-	Ham's F-10	20% fetal calf serum	Separated leucocyte pellets	PHA	70 hours	0.1 ug/ml of colchicine for 1 1/2 hours	0.075 M KCl	3 : 1 methanol acetic acid	-
Norberg et al (1976)	Heparin	TC 199	20% calf serum	18 - 19 drops whole blood/ 5 ml medium	PHA-M	72 hours	Colcemid 0.1 ug/ml of medium for 3 - 4 hours	Distilled water	1 : 3 acetic acid methanol	Aceto-orcein
Halnan (1977)	Heparin 10 IU/ml of blood	Medium 199	5% fetal calf serum	1.5 ml whole blood per 9 ml medium	PHA	48 - 72 hours	-	0.56% KCL	3 : 1 absolute methanol to acetic acid	Giemsa
Miller (1977)	Heparin	RPMI 1640	-	-	-	-	-	-	-	-
Balakrishnan et al (1979)	-	Ham's F-10	-	-	PHA	64 hours	-	0.075 M KCl	3 : 1 methanol acetic acid	Giemsa
Ma et al (1980)	Heparin	Ham's F-10	-	-	-	-	-	-	-	-
Sharma et al (1980)	-	TC 199	-	-	-	-	-	-	-	Carbol fuchsin
Berardino and Iannuzzi (1981)	-	McCoy's 5A medium	20% autologous plasma	-	PHA-M	-	Colcemid	0.075 M KCl	3 : 1 methanol acetic acid	-
Swartz and Vogt (1983)	Heparin	TC 199	15% fetal bovine serum	1 ml of whole blood per 10 ml medium	PHA-M	72 hours	Colcemid 0.1 ug per ml culture	0.075 M KCl	3 : 1 methanol acetic acid	Giemsa
Yadav and Balakrishnan (1983)	-	Ham's F-10	20% adult cattle serum	0.5 ml of whole blood per	Poke-weed mitogen	60-65 hours	Colchicine - one hour before harvest	0.075 M KCl	3 : 1 methanol acetic acid	Giemsa
Halnan (1985)	Heparin 20 IU/ml blood	Ham's F-10	-	-	-	44-66 hours	-	-	-	Giemsa
Jarofke and Neitzel (1985)	Heparin	Ham's F-10	Fetal calf serum	0.4 ml whole blood per culture	PHA	4 to 5 days	0.4 ug colcemid for 4 hours	0.075 M KCl	3 : 1 methanol acetic acid	Giemsa
Yadav and Balakrishnan (1985)	Sodium heparin	Ham's F-10	25% adult cattle serum	-	PWM	60-65 hours	1.3 ug of colchicine per 5 ml culture for one hour	0.075 M KCl	1 : 3 acetic alcohol	-

Materials and Methods

MATERIALS AND METHODS

Elephants belonging to some temples and private owners of Trichur District formed the materials for the present study. These elephants were captured from the western ghats and domesticated.

A male elephant with a highly developed outgrowth of the upper incisors called tusks is known as a 'tusker'. Tusks may be of different sizes and often grow downward and sideward and end in a pointed tip (Plate 1). The tusks of an Asian elephant may grow beyond 2.5 m and weigh about 70 kg.

'Makhna' is a male elephant which differ from the tusker by the absence of well developed tusks. Rudimentary tusks are seen in place of well developed tusks (Plate 5).

'Cow elephant' is a female elephant which does not have tusks and often possesses rudimentary outgrowth like that of the makhna (Plate 3).

In all, 15 healthy elephants comprising of 7 tuskers, 1 makhna and 7 cow elephants were subjected to cytogenetic study. Karyotype analysis was carried out using peripheral blood leukocyte culture technique.

Blood collection and transportation

Whole blood was collected using sterile 18 G needle from the ear vein of elephants. About 15 ml of blood was collected directly into sterile centrifuge tubes containing 0.5 ml of sodium heparin solution (5000 IU/ml). The samples were

transported to the laboratory in an ice box by bus/motor cycle. Precautions were taken to avoid undue agitation of the samples to prevent haemolysis. Samples were brought to the laboratory within one-and-a-half-hours after collection.

Culturing

In the laboratory, samples were kept outside for thawing. Cultures were set up within 2 hours after collection.

From each sample, 2 ml of whole blood was taken in separate syringes and the rest subjected to centrifugation at 1200 RPM for about 8 minutes to facilitate sedimentation of erythrocytes and extraction of autologous plasma.

Culture media TC 199 (Difco) and RPMI-1640 (GIBCO) were used to compare efficiency. In 5 ml of each medium containing 100 IU of penicillin, 2.5 ml of autologous plasma, 0.7 ml of whole blood (kept separately in the syringe) and 0.1 ml of mitogen were added one after the other and mixed gently by rotating the culture vial between the palms of the hands. Mitogens used were either phytohaemagglutinin-M (Difco) or Poke-weed mitogen (GIBCO). The cultures were then incubated at a temperature of 37 $\pm$0.5°C for 72 hours. The cultures were mixed gently twice daily.

At the end of 71 hours of incubation, colchicine solution (0.0001%) as a mitotic arrester was added to the cultures, mixed gently and incubated for a further one hour period. The optimum concentration and duration of colchicine treatment was

estimated observing the degree of condensation of metaphase chromosomes. The concentrations and duration used were 0.1 ml for one hour; 0.1 ml for two hours; 0.2 ml for one hour and 0.2 ml for two hours.

Harvesting

After 72 hours, the contents were transferred to individual centrifuge tubes and centrifuged at 1200 RPM for 10 minutes. The supernatant was discarded using pasteur pipette leaving about 0.5 ml of the supernatant above the sediment. About 2 ml of 0.075 M potassium chloride hypotonic solution was added to the tubes initially and the contents mixed gently. The volume was later made upto 6 ml by adding more hypotonic solution and allowed to stand for 10 minutes at room temperature. The contents were then centrifuged at 1200 RPM for 8 minutes and the supernatant discarded leaving about 0.5 ml of the solution above the sediment.

About 2 ml of 3:1 methanol acetic acid fixative was added to each tube and mixed gently to break the sediment by drawing repeatedly into a pasteur pipette. Fixative was added further to make up the volume to 6 ml and allowed to stand for 10 minutes. The mixture was then centrifuged at 1200 RPM for 8 minutes and the supernatant discarded leaving about 0.5 ml of the solution above the cell button. Fixative treatment was continued two to three times more until a clear supernatant was obtained. Finally, the supernatant was removed upto

about 0.5 ml above the cell button. A suspension of the cell button was prepared in the remaining solution and allowed to stand for a few minutes for the debris to settle down.

Slide preparation and staining

Fresh, chilled, wet slides were used for chromosome preparations. One or two drops of the cell suspension was dropped from a height of about 30 cm onto a glass slide kept in a horizontal plane on which 2 to 3 drops of 50% acetic acid was placed. The slides were allowed to air dry in the same position.

Staining was done on the same day using 4 per cent Giemsa prepared in phosphate buffer (pH 6.8) for 40 minutes. The slides were washed thrice in distilled water and allowed to dry in a slanting position. The chromosome spreads were viewed under the microscope.

The efficacy of two media viz., TC 199 (Difco) and RPMI-1640 (GIBCO) and two mitogens viz., phytohaemagglutinin-M (Difco) and Poke-weed mitogen (GIBCO) were assessed by observing 300 cells selected at random. The number of lymphocytes, lymphoblasts, cells in metaphase, mitotic drive and mitotic index were estimated (Table 2).

$$\text{Mitotic drive} = \frac{\text{Lymphoblasts + Number of cells in metaphase}}{\text{Total cells counted}} \times 100$$

$$\text{Mitotic index} = \frac{\text{Number of cells in metaphase}}{\text{Total cells counted}} \times 100$$

Whether the difference noticed between two media and the two mitogens was significantly different or not was determined using the method described by Snedecor and Cochran (1967).

Photography and karyotype preparation

Well scattered metaphase spreads without overlapping of chromosomes were identified and photographed on 2 to 3 plates using Carlzeiss photomicroscope III with a combination of research microscope and 35 mm camera with automatic exposure control. Individual chromosomes were cut out from one plate and others were kept for orientation and reference. Chromosomes were pasted on a bristol board and indicated by their number and group.

From the karyotypes thus prepared, the morphology of each chromosome was studied.

Morphological measurements

The chromosomes were classified as submetacentric, acrocentric or telocentric as described by Sharma and Talukder (1974).

The morphology of individual chromosomes was explained based on its total length and the position of the centromere. The size of the chromosomes was represented as the relative length that is in relation to the length of the haploid set containing the X-chromosome. The position of the centromere was indicated by

a) arm ratio = $\frac{\text{Length of long arm}}{\text{Length of short arm}} = \frac{q}{p}$

b) Centromeric index (C) = $\frac{\text{Length of short arm}}{\text{Total length}} = \frac{p}{p+q}$

RESULTS

In the present study, the elephants (*Elephas maximus indicus*) were categorized into three groups namely cow elephants (females), tuskers (males) and makhna (tuskless males). Whole blood collected from the ear vein of the animals was brought to the laboratory and subjected to peripheral blood lymphocyte culture.

Standardization of technique

The culture media TC 199 (Difco) and RPMI-1640 (GIBCO) and two mitogens viz., phytohaemagglutinin-M (Difco) and Poke-weed mitogen (GIBCO) were compared and the results obtained are presented in table 2. In the medium TC 199 having the mitogen phytohaemagglutinin-M, the mitotic drive and mitotic index were 18.66 and 0.33 per cent respectively; and with poke-weed mitogen the mitotic drive and mitotic index were 30.66 and 5.33 per cent respectively. In the medium RPMI-1640, the mitotic drive and mitotic index in the presence of phytohaemagglutinin-M were 23.0 and 0.66 per cent respectively; and with poke-weed mitogen, 31.33 per cent and 5.66 per cent respectively.

Statistical analysis revealed that the difference observed in the efficacy between the two media is not significant, whereas the difference due to mitogens in both the media was significant at five per cent level. Poke-weed mitogen was found to be more efficient than phytohaemagglutinin-M as far

as inducing mitosis in lymphocytes of elephants is concerned.

Among the four colchicine treatments of different concentrations and duration of treatment, it was found that the condensation of chromosomes using 0.1 ml colchicine (0.0001%) for a duration of one hour showed normal size and appearance of chromosomes. Sodium heparin was found to be satisfactory as an anticoagulant.

Transportation of blood samples in ice by bus/motorcycle did not interfere with the mitotic activity of elephant lymphocyte. Since the samples were transported always in ice, no comparison could be made on the effect of temperature during transit on the mitotic drive and mitotic index.

Hypotonic treatment using 0.075 M potassium chloride for 10 minutes was found to be satisfactory. Methanol acetic acid (3:1) fixative washings upto four times was found to be necessary to obtain a clear supernatant in which the final lymphocyte suspension was prepared.

Karyotype

The karyotype of tusker, cow elephant and makhna are presented in plates 2b, 4b and 6b. It can be seen from the plates that the diploid chromosome numbers in tusker, cow elephant and makhna is 2n=56 comprising of 54 autosomes and two sex chromosomes.

Morphometric measurements.

Morphological parameters viz., relative length, arm ratio and centromeric index are presented in table 3.

Based on the relative length of chromosomes, they were serially numbered in the karyotype prepared (Plates 2b, 4b, 6b; Fig.1). The largest chromosome with a relative length of 6.973 per cent was numbered as the first in the serial order of the whole complement. The first two pairs of autosomes in the acrocentric group (chromosome pairs 7 and 8) were the second and third largest with relative lengths of 5.900 and 5.700 per cent respectively. The X-chromosome with a relative length of 5.633 per cent was the fourth largest chromosome in the whole complement. The relative length of the remaining five pairs of autosomes in submetacentric group (pairs 2 to 6) ranged from 3.873 to 2.480 per cent. The relative lengths of the remaining acrocentric autosomes (pairs 9 to 27) ranged from 5.197 to 1.777 per cent. The Y-chromosome with a relative length measuring 1.710 per cent was the smallest of the complement. However, the sex chromosomes were classified separately.

Chromosome morphology.

Analysis of the arm ratio ($\frac{q}{p}$) revealed that all the biarmed chromosomes were submetacentric since no chromosome showed an arm ratio of 1.0.

The centromeric index (C) suggested that the position of the centromere was farthest from the centre of the chromosome in the second autosome pair and nearest to the centre in the X-chromosome. No metacentric or telocentric chromosomes were observed.

Based on the arm ratio and centromeric index, the autosomes were classified into two groups namely submetacentric and acrocentric. The group of submetacentric autosomes consisted of the first six pairs (pairs 1 to 6) and the group of acrocentric autosomes consisted of 21 pairs (pairs 7 to 27). Among the submetacentric autosomes, the first pair was the largest of the group as well as the whole complement. The remaining five pairs of the group were small submetacentrics. Among the acrocentric group of autosomes, the first pair of acrocentric (pair 7) was the largest of the group whereas it was the second largest chromosome in the whole complement. The submetacentric and acrocentric autosomes were arranged serially in the descending order of their size in their respective groups. The X-chromosome was a submetacentric in morphology whereas the Y-chromosome was an acrocentric and the smallest of the complement.

In all the three groups of elephants no satellite could be observed in the karyotype.

Sex chromosomes.

The Indian elephants (Elephas maximus indicus) exhibited XX/XY sex chromosome mechanism. Cow elephants possessed the XX sex chromosome complement and tuskers possessed the XY chromosome complement. The makhna also possessed the same sex chromosome complement (XY) as that of the tusker.

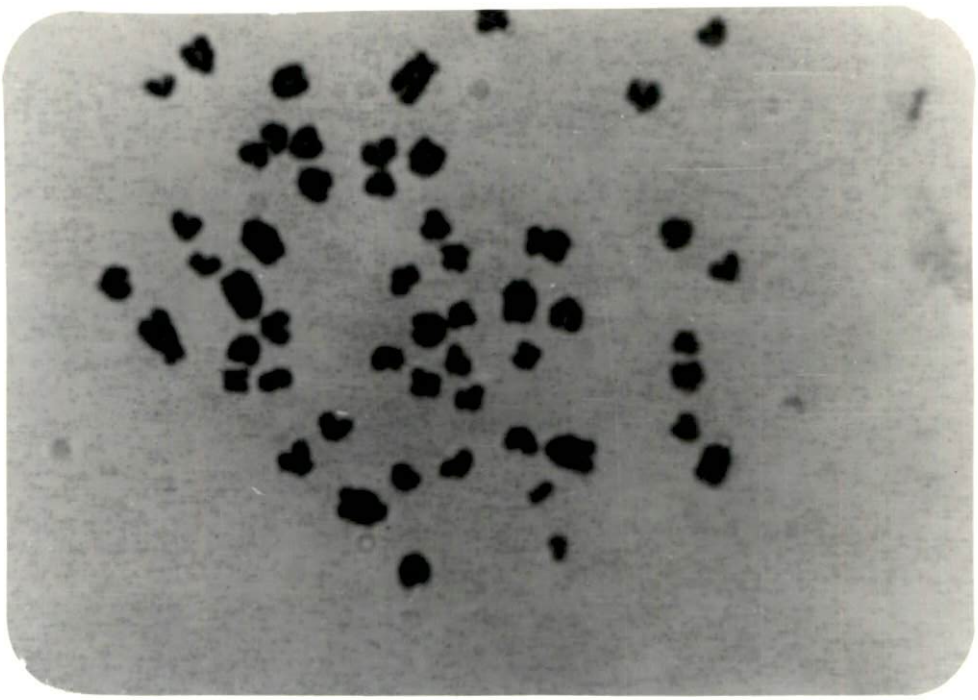
Morphologically all the autosomes were similar in both the tusker, makhna and cow elephant. The sex chromosomes were

XX and XY in the cow elephants and tuskers respectively. The karyotype of tusker and makhna showed no variation in morphology of autosomes and sex chromosomes.

Plate 1. TUSKER

Plate 2a. MITOTIC METAPHASE CHROMOSOME SPREAD OF TUSKER

Plate 2b. KARYOTYPE OF TUSKER



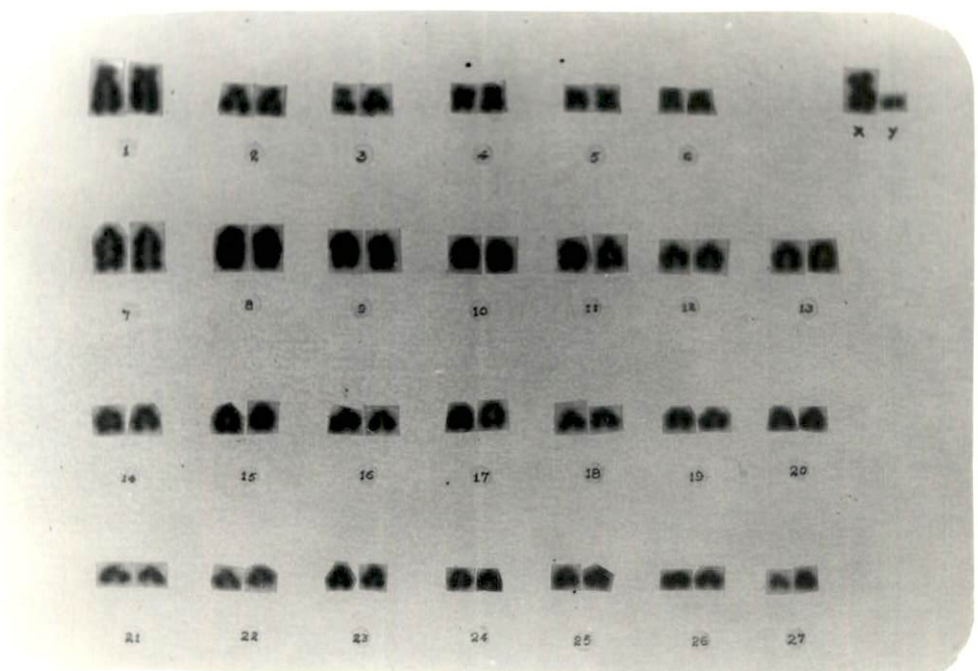

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Plate 3. COW ELEPHANT

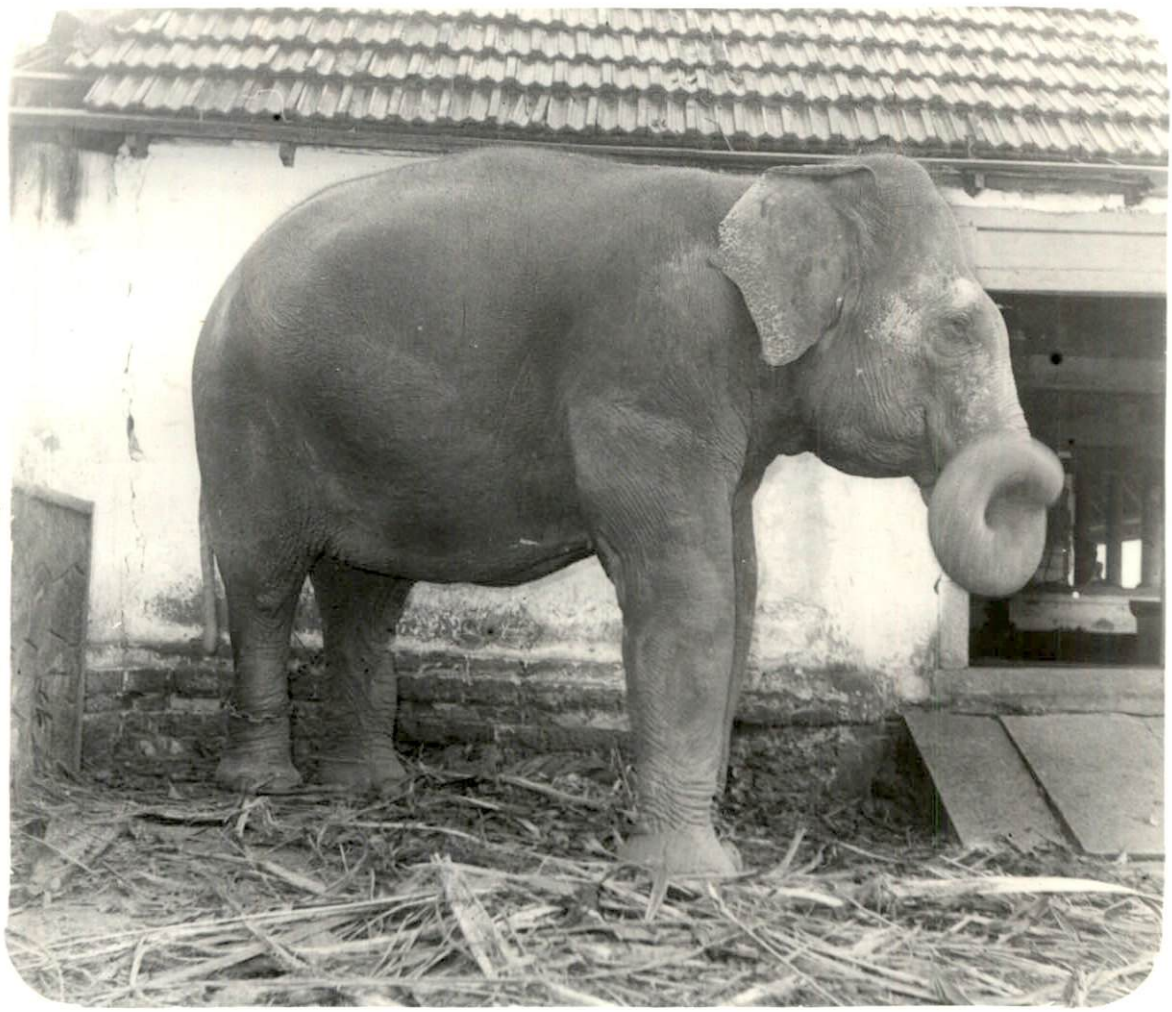
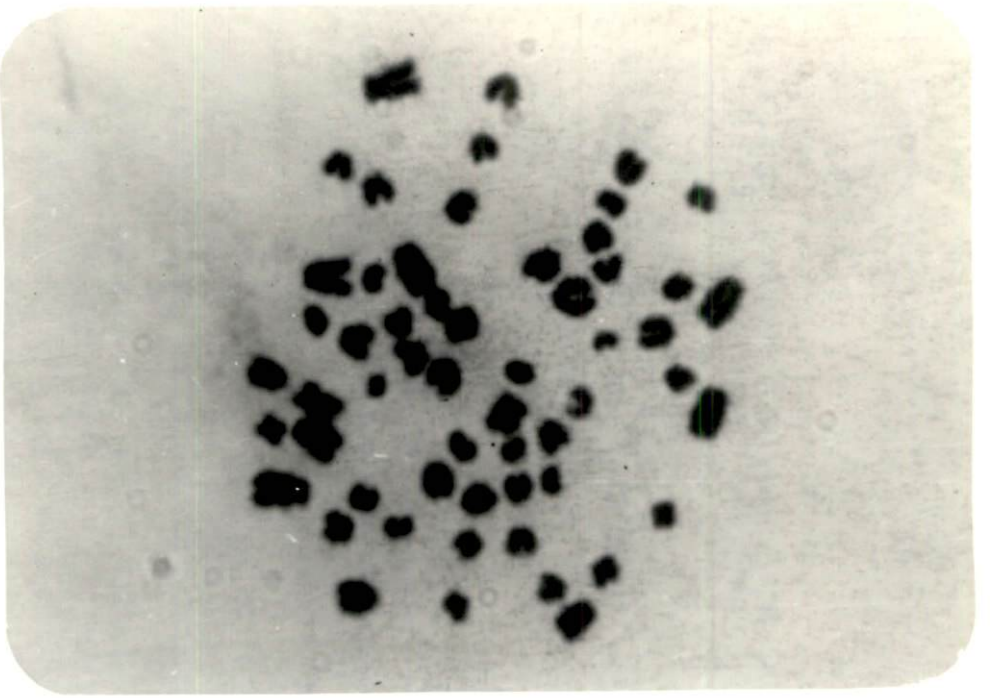


Plate 4a. MITOTIC METAPHASE CHROMOSOME SPREAD OF COW ELEPHANT

Plate 4b. KARYOTYPE OF COW ELEPHANT



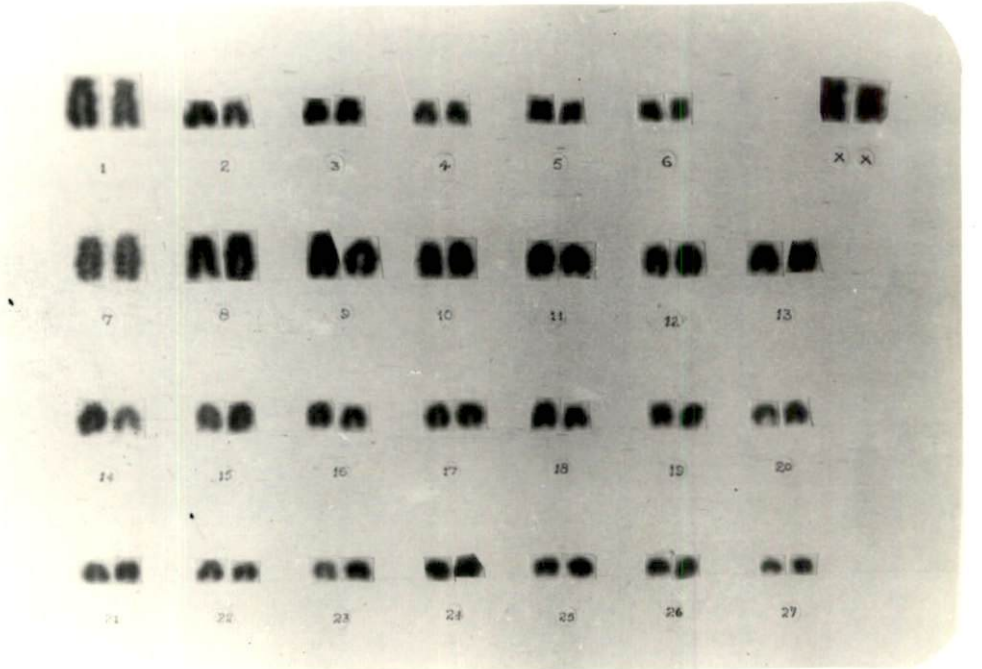

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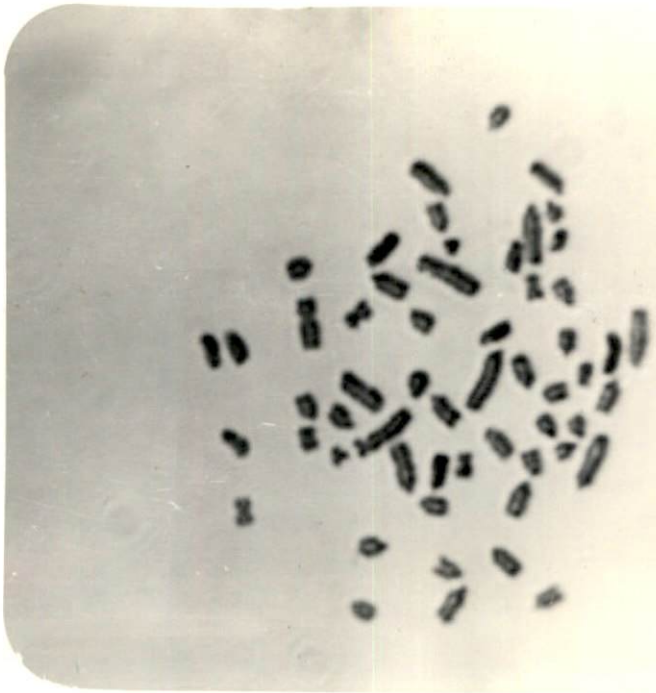
Plate 5. MAKHNA

Plate 6a. MITOTIC METAPHASE CHROMOSOME SPREAD OF MAKHNA

Plate 6b. KARYOTYPE OF MAKHNA



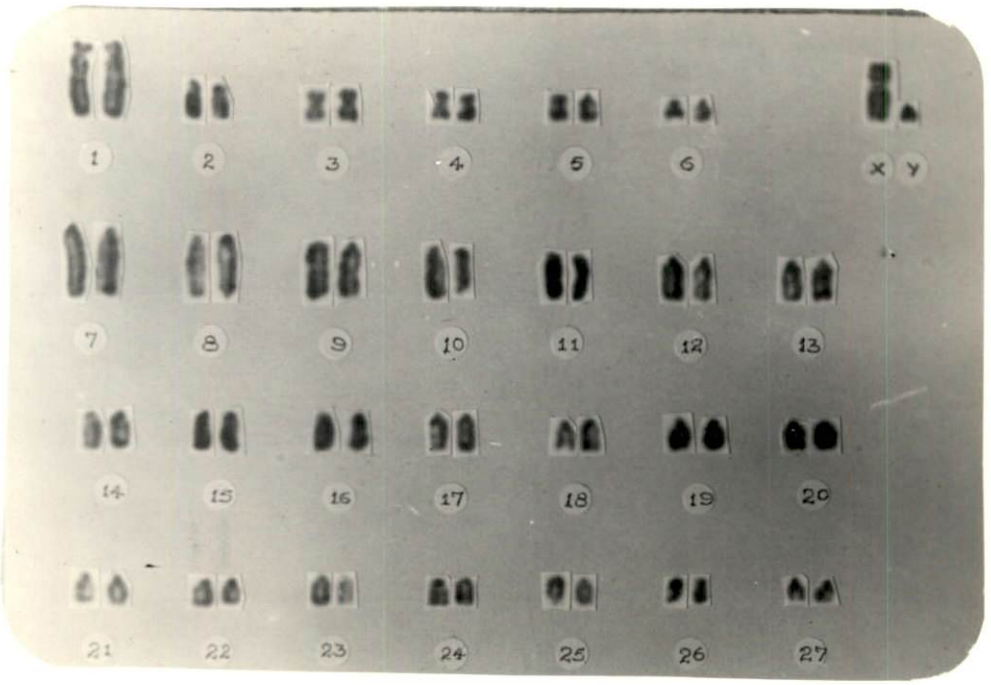

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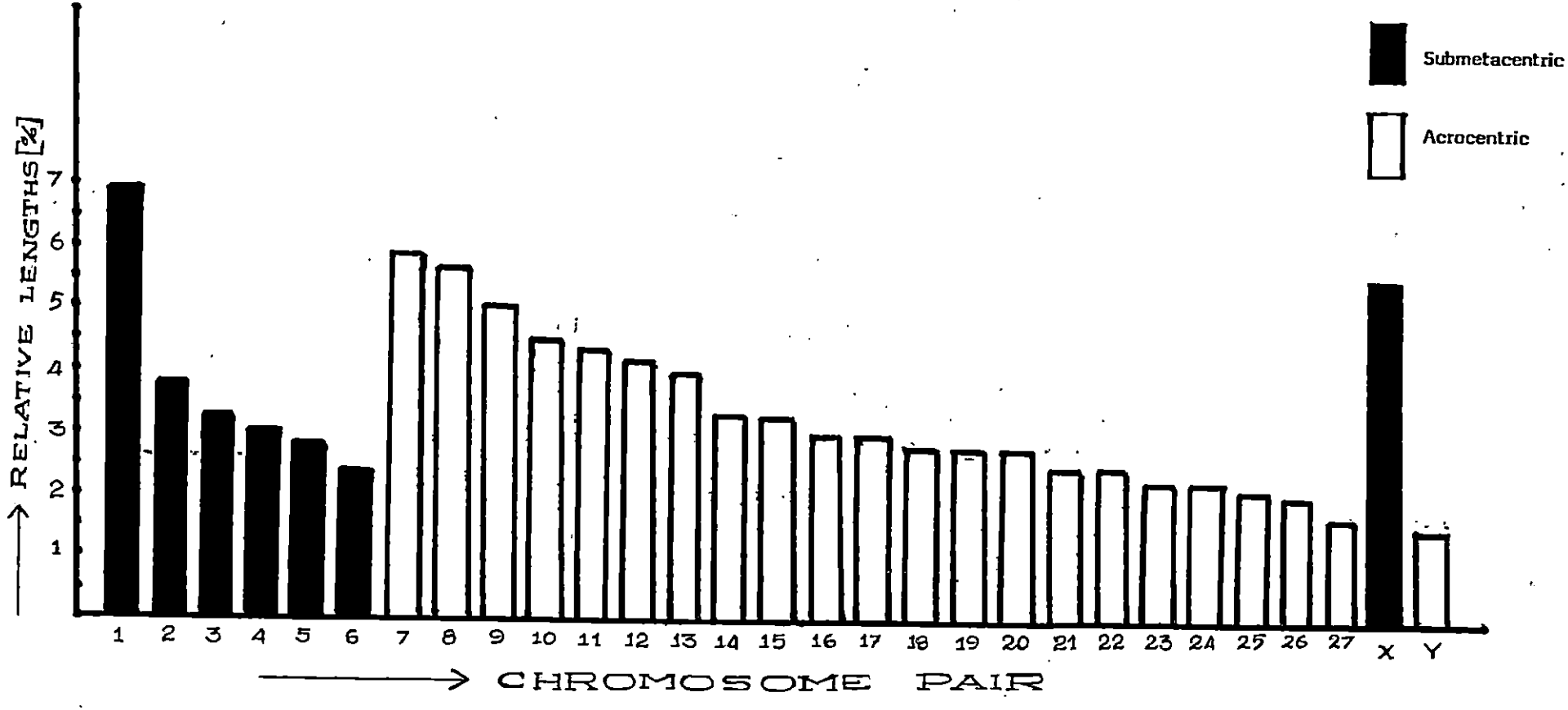
Table 2. Relative efficacy of mitogens in medium TC 199 and medium RPMI 1640 in Elephant lymphocyte cultures

Culture medium	Mitogen	Total cells counted	Lympho-cytes	Lympho-blasts	Mitotic spreads	Mitotic drive (%)	Mitotic index (%)
TC 199	Phytohaema-gglutinin-M	300	244	54	2	18.66	0.66
	Poke-weed mitogen	300	208	76	16	30.66	5.33
RPMI-1640	Phytohaema-gglutinin-M	300	231	68	1	23.0	0.33
	Poke-weed mitogen	300	196	87	17	31.33	5.66

Table 3. Morphometric characters of chromosomes of Indian elephants

Chromosome pair	Relative length (%)	Arm ratio (q/p)	Centromeric index (p/p+q)
1	6.973	2.49	0.304
2	3.873	4.33	0.189
3	3.317	2.0	0.333
4	3.083	1.97	0.356
5	2.813	1.72	0.387
6	2.480	1.94	0.359
7	5.900	-	-
8	5.700	-	-
9	5.197	-	-
10	4.590	-	-
11	4.457	-	-
12	4.223	-	-
13	4.090	-	-
14	3.383	-	-
15	3.383	-	-
16	3.114	-	-
17	3.114	-	-
18	2.881	-	-
19	2.881	-	-
20	2.881	-	-
21	2.513	-	-
22	2.513	-	-
23	2.380	-	-
24	2.380	-	-
25	2.280	-	-
26	2.143	-	-
27	1.777	-	-
X	5.633	1.38	0.421
Y	1.710	-	-

Fig. 1. IDIOGRAM REPRESENTING THE RELATIVE LENGTHS OF CHROMOSOMES OF INDIAN ELEPHANTS (Elephas maximus indicus)



Discussion

DISCUSSION

In the present study, elephant chromosome spreads were prepared following the technique of peripheral blood lymphocyte culture method using whole blood.

Comparison of two media namely TC 199 (Difco) and RPMI-1640 (GIBCO) revealed no significant difference in the mitotic activity of elephant lymphocytes as evidenced by the proportion of lymphocytes, lymphoblasts and cells in metaphase in both media. The mitotic drive and mitotic index revealed no significant difference between the two media and hence both can be used successfully for culturing elephant lymphocytes. Successful culturing using TC 199 medium was reported by Moorehead et al. (1960), Ulbrich and Weinhold (1963), Scherz and Louro (1963), Connolly et al. (1964), Hare et al. (1966), Ponce De Leon and Marcum (1975), Norberg et al.(1976), Halnan (1977), Sharma et al. (1980) and Swartz and Vogt (1983). Miller (1977) reported the suitability of medium RPMI-1640 for peripheral blood lymphocyte culture.

In the present study, phytohaemagglutinin-M (Difco) and poke-weed mitogen (GIBCO) were employed to assess the comparative efficacy of mitogens to initiate mitosis in elephant lymphocyte cultures in a 72 hour culture in medium TC 199 and medium RPMI-1640. Phytohaemagglutinin-M yielded a mitotic index of 0.33 per cent and 0.66 per cent in medium TC 199 and medium RPMI-1640 respectively, whereas poke-weed mitogen yielded a mitotic index of 5.33 per cent and 5.66 per cent in medium

TC 199 and medium RPMI-1640 respectively. The results indicated that in a 72 hour short-term peripheral blood lymphocyte culture method, the response of elephant lymphocytes was significantly higher to poke-weed mitogen than to phytohaemagglutinin-M irrespective of the culture medium used. Poke-weed mitogen as an efficient mitogen was reported by Yadav and Balakrishnan (1983, 1985).

Karyotype

a) Basic number.

The diploid chromosome number of 2n=56 as observed in the tusker and cow elephant is consistent with the reports of Hungerford et al. (1966) and Jarofke and Neitzel (1985). Norberg (1969) had reported the same chromosome number in a female Indian elephant. The chromosome number in makhna is however not reported by these authors.

The elephant chromosome complement consisted of 54 autosomes and 2 sex chromosomes (XX in males and XY in males and makhna). The XX/XY sex determining mechanism observed in this study is in agreement with the findings of Hungerford et al. (1966) and Jarofke and Neitzel (1985). Norberg (1969) had reported the existence of XX sex chromosome mechanism in an Indian female elephant. Between the tusker and makhna no variation could be observed in sex chromosome complement.

b) Relative length.

Based on the relative length of chromosomes, they are serially numbered in the karyotype prepared. The largest

chromosome with a relative length of 6.973 per cent was numbered as first in the serial order of the whole complement. The first two pairs of autosomes in the acrocentric group (chromosome pairs 7 and 8) were the second and third largest with a relative length of 5.900 and 5.700 per cent respectively. The X-chromosome with a relative length of 5.633 per cent was the fourth largest chromosome in the whole complement. The relative length of the remaining five pairs of autosomes in the submetacentric group (pairs 2 to 6) ranged from 3.873 to 2.480 per cent. The relative length of the remaining acrocentric autosomes (pairs 9 to 27) ranged from 5.197 to 1.777 per cent. The Y-chromosome with a relative length of 1.710 per cent was the smallest chromosome in the whole complement. However, the sex chromosomes were classified separately.

c) Position of centromere.

The present study revealed that out of the 54 autosomes in the karyotypes of three groups of elephants, 12 autosomes (6 pairs) were biarmed. Their arm ratio ranged from 1.72 to 4.33 and the centromeric index ranged from 0.387 to 0.189. The remaining 42 autosomes (21 pairs) are not biarmed and hence are termed acrocentrics. Among the sex chromosomes, the X-chromosome is submetacentric with arm ratio and centromeric index values as 1.38 and 0.421 respectively. Although 6 pairs of submetacentric and 21 pairs of acrocentric autosomes are observed in this study, Norberg (1969) reported the presence of only three pairs of autosomes with nearly median

centromeres and Jarofke and Neitzel (1985) reported that the autosomes of Indian elephants consisted of only 6 small metacentric and 48 acrocentric autosomes. However, Norberg (1969) observed autosomes with prominent short arms which were classified under acrocentric.

The arm ratio and centromeric index values suggested that none of the biarmed chromosomes were true metacentrics. The chromosome would be a true metacentric if the arm ratio ($\frac{q}{p}$) is unity or if the centromeric index (C) is 0.5. If $\frac{q}{p}$ is higher than unity and if C is less than 0.5, then it indicates that the centromere is away from the centre of the chromosome. Accordingly an analysis of the arm ratio and centromeric index obtained in this study revealed that among the biarmed chromosomes, including the X-chromosome, the position of centromere is nearest to the centre in the X-chromosome ($\frac{q}{p}$ = 1.38 and C = 0.421) and farthest from the centre in the second autosome pair ($\frac{q}{p}$ = 4.33 and C = 0.189). In the three groups of elephants, no variation could be observed as regards to the position of centromere in autosomes and sex chromosomes.

d) Presence of satellite.

No chromosome was found to possess any satellite in the present study. This finding is in agreement with the reports of Norberg (1969) and Jarofke and Neitzel (1985); whereas it is contradictory to the report of Hungerford et al. (1966) who reported the presence of satellites on one of the three submetacentric autosome pairs.

c) Chromosome variation.

In the three groups of elephants, viz., tusker, makhna and cow elephant studied, no difference was observed in the chromosome number. The chromosome number in all the three groups was 2n=56. The autosomes exhibited only two class of chromosomes namely submetacentric and acrocentric. The Y-chromosome was present only in tuskers and makhna and absent in cow elephants. The morphometric study did not show variations among the three types.

It can be inferred that the tusklessness in makhna is not associated with either euploidy or aneuploidy.

Summary

SUMMARY

A karyological study in Indian elephants (Elephas maximus indicus) using peripheral venous blood collected from 7 tuskers, 7 cow elephants and one makhna belonging to some temples and private owners of Trichur District was undertaken with objectives of

a) to evolve a technique for chromosome studies of elephants,
b) to find out the chromosome number in Indian elephants, and
c) to suggest sex chromosome mechanism in sex determination.

Standardization of technique

Comparison of efficacy of phytohaemagglutinin-M (Difco) and poke-weed mitogen (GIBCO), in two different culture media namely, TC 199 and RPMI-1640 as mitogens, showed that phytohaemagglutinin-M yielded a mitotic drive and mitotic index of 18.66 and 0.33 per cent respectively in medium TC 199, and 23.0 and 0.66 per cent respectively in medium RPMI-1640. Poke-weed mitogen yielded a mitotic drive and mitotic index of 30.66 and 5.33 per cent respectively in medium TC 199 and 31.33 and 5.66 per cent respectively in medium RPMI-1640. Statistical analysis revealed that the efficacy of the two different media used was not significantly different, whereas the efficacy of two mitogens differed significantly at 5 per cent level. Poke-weed mitogen was found to suit better than phytohaemagglutinin-M as far as inducing mitoses in elephant lymphocyte cultures over a 72 hour culture period was concerned. Colchicine treatment using 0.1 ml colchicine

solution (0.0001%) for a period of one hour yielded satisfactory chromosome preparations.

Karyotype

The karyotypes of three groups of elephants studied viz., tusker (male), makhna (tuskless male) and cow elephant (female) revealed a diploid chromosome number of 2n=56 comprising of 54 autosomes and 2 sex chromosomes. The autosomes were classified into two groups namely submetacentric group (chromosome pairs 1 to 6) and acrocentric group (chromosome pairs 7 to 27). The sex chromosomes were classified separately. The X-chromosome was a submetacentric in all the three groups studied, whereas the Y-chromosome which was observed only in the tusker and makhna was a small acrocentric chromosome.

Analysis of relative length of chromosomes showed that the largest chromosome pair measured a relative length of 6.973 per cent and the smallest chromosome was the Y-chromosome measuring a relative length of 1.710 per cent. The chromosome pairs 7 and 8 measuring a relative length of 5.900 and 5.700 per cent respectively were the second and third largest, whereas the X-chromosome with a relative length of 5.633 per cent was the fourth largest in the whole complement.

Position of the centromere on the basis of centromeric index suggested that the centromere was farthest from the centre of the chromosome in the second autosome pair and nearest to the centre in the X-chromosome among the submetacentric

chromosomes. No true metacentric chromosomes could be observed in the karyotype of the elephants studied.

Neither autosomes nor sex chromosomes possessed satellites on karyological examination.

The elephants studied exhibited XX/XY sex chromosome mechanism. The cow elephant possessed XX sex chromosome complement while both the tusker and makhna possessed XY sex chromosome complement.

A comparison between the karyotypes of tusker and makhna did not show any variation in (a) basic number, (b) relative length, (c) position of centromere, (d) absence of satellites on chromosomes and (e) sex chromosome complement.

It may be concluded that the tusklessness in makhna is not associated with either euploidy or aneuploidy.

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ABSTRACT

A karyological study in Indian elephants using peripheral venous blood collected from 7 tuskers, 7 cow elephants and one makhna belonging to some temples and private owners of Trichur District was undertaken with objectives of

a) to evolve a technique for chromosome studies of elephants,
b) to find out the chromosome number in Indian elephants, and
c) to suggest sex chromosome mechanism in sex determination.

Standardization of technique

Comparison of efficacy of phytohaemagglutinin-M (Difco) and Poke-weed mitogen (GIBCO), in two different culture media namely TC 199 and RPMI-1640, as mitogens showed that phytohaemagglutinin-M yielded a mitotic index of 0.33 and 0.66 per cent in medium TC 199 and RPMI-1640 respectively; poke-weed mitogen yielded a mitotic index of 5.33 and 5.66 per cent respectively in TC 199 and RPMI-1640. Statistical analysis revealed that the efficacy of the two different media used was not significantly different, whereas the efficacy of two mitogens differed significantly at five per cent level. Poke-weed mitogen was found to be better than phytohaemagglutinin-M as far as inducing mitoses in elephant lymphocyte cultures over a 72 hour culture period was concerned. Colchicine

treatment using 0.1 ml colchicine solution (0.0001%) for a period of one hour yielded satisfactory chromosome preparations.

Karyotype

The karyotype of the tusker, cow elephant and makhna revealed a diploid chromosome number of 2n=56, comprising of 54 autosomes and 2 sex chromosomes. The autosomes were classified into 6 submetacentric and 21 acrocentric chromosomes. The X-chromosome was a submetacentric in all the three groups of elephants whereas the Y-chromosome was a small acrocentric in the tusker as well as the makhna.

Analysis of relative length of chromosomes showed that the largest chromosome pair measured a relative length of 6.973 per cent and the smallest chromosome was the Y-chromosome measuring a relative length of 1.710 per cent.

Position of centromere on the basis of centromeric index suggested that the centromere was farthest from the centre of the chromosome in the second autosome pair and nearest to the centre in the X-chromosome among the submetacentric chromosomes.

Neither autosomes nor sex chromosomes possessed satellites on karyological examination.

The elephants studied exhibited XX/XY sex chromosome mechanism. The cow elephant possessed XX sex chromosome complement while both tusker and makhna possessed XY sex chromosome complement.

A comparison between the karyotypes of tusker and makhna did not show any variation in (a) basic number, (b) relative length (c) position of centromere, (d) absence of satellites on the chromosomes and (e) the sex chromosome complement.

It may be concluded that tusklessness in makhna is not associated with either euploidy or aneuploidy.