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INTRODUCTION OF TETRAPLOIDY IN *Rana tigrina* (INDIAN BULL FROG) BY THERMAL, HYDROSTATIC PRESSURE AND CHEMICAL SHOCKS

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AUTHORS' CONTRIBUTIONS

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Introduction of tertraploidy in R. *tigrina* was attempted, using thermal, hydrostatic pressure and chemical shocks. Heat and pressure shocks induced 100% tetraploidy. Chromosome count and RBC micro measurement were found to be reliable method for determination of ploidy. It can be achieved by using Anaesthetic- ether for giving Anaesthesia to frog. While Colchicine (0.02%) as a mitotic-inhibitor [1] and hypotonic -solution, (1952 Hugs, Makino, and Nishimpura), Methanol: Glacial acid (3:1)as freshly prepared cold-fixative, Giemsa stock-solution for staining chromosome,(Hsu and Hsu Pomerat [2] as well as Phosphate Buffer Salt (PBS) is used. Trypsin- solution is used for G-**banding** (**Plate-5a**). All the above solution were prepared using different types of chemical substances like, Sodium citrate, distilled water, Giemsa stock-solution by Giemsa powder, glycerol, sodium chloride, potassium – chloride, glucose, sodium bicarbonate etc [3]. (Tijio and Puck 1958). Karyotype is obtained in the animals like fishes, amphibians, mammals like rats can also be studied.

Then chromosomes are arranged on the basis of long arm and short arm (L/S) and classified as metaphase chromosome, as meta-centric, sub-metacentric, and acrocentric respectively. It can be obtained by centrifugation, air-dry method was followed for slide preparation. After drying slides were stained with 2% Giemsa for 15 minutes, and washed thoroughly in distilled water for clear-differentiation, under the microscope. Then after observing these plates, were selected and analyzed. Finally, good clear – differentiated slide were selected. Tetraploid slide was taken in which we got **52** chromosomes in plate number **5a-for** G-**banding** and **5b for tetraploidy** which is obtained by above mentioned method. Then Idiogram drawn on X- axis chromosome number and on Y-axis relative length. So in this work , which was carried out we got results similar to Stephenson (1977).

Keywords: Karyotype; tetraploidy*; Rana tigrina*; thermal; hydrostatic pressure and chemical shocks.

1. INTRODUCTION

For a long time, the frog has been a favorite object for the study of structure and function. Probably no species except man has been the subject of so many investigators. In early carboniferous period, some crossopterygian fishes developed in to the first land vertebrates. These were primitive stem Amphibian called Labyrihthodonia. Some of them gave rise to modern Amphibians such as frogs, toads, salamander. These are terrestrial like terapods. The name of the class Amphibia indicates that, these animals live at different times in their life – cycle in two environments water and air or land. Thus the class

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Amphibia represents a transitional group between the strictly aquatic, earlier vertebrates and strictly terrestrial later vertebrates. The animal widely studied as a representative of the class is the familiar frog.

Most frogs are similar in external appearance and internal morphology, differing only in colour, size and special features. Rana is found all over the world except Australia, New – Zealand, Southern South America. Five species occur in India. R. *tigrina*, R. *limnocharies*, R. *malabaricus*, R. *cyanophylyctis*, R. *hexadactyla*. Of these R. *tigrina* is the most common and largest Indian frog, commonly known as Bull frog due to its loud voice and large size.

A very large amount of recent work has brought to light many important features in the chromosome cytology of Amphibians (Shesachar 1941) , many of early studies were confined to chromosomes of common Anura and Uredela and it is only recently an extension of these studies has been made with respect to other Amphibians. Up till [4] the chromosome number up one species of Apoda,37of Urodela and 30 of Anura were known. The chromosome number of one another species of Apoda has since been added Shesachar [5,6-7]

From a study chromosome number in amphibian it becomes clear that the variation in the chromosome number within the group obeys fixed laws. In Apoda, the number in only 2 space is known n= 21 in *Ichthyophis glutinosus* and n = 18 in Urodela [6-7].

The variation is striking in Anura *Bufo* appears to have n=11 and in *Rana* n = 12 generally. Many other species this letter number observed in Anura is *Alytes obestricans*

2. CYTOGENETIC ANALYSIS OF FROG

2.1 Materials and Methods

The analysis of chromosome structure starts with the understanding of a clear delimitations between the chromosome of lower organisms on the one hand and those of higher organisms on the other. In the former the chromosome is a gonophore being a essentially a long chain of DNA molecule and in the later the chromosome is an extremely complex body which has other constituents in addition to the DNA gonophore and it may be analyses under a light microscope.

In 1938, Levan [1] introduced colchicine as a mitotic inhibitor of mammalian cells. This increasing the number of metaphase cells for chromosome analysis [8]

Hugs (1952), Makino and Nishimpura (1952) were used hypotonic solution for pre – treatment for swelling of the cells. Thus making possible the more facile spreading of the chromosomes within the swollen cytoplasm of the cells. This was the turning point in the vertebrate cytogenetic. This was confirmed by other Scientists, Hsu, 1952; Hsu and Pomerat [2].

The spreading of the chromosomes for analysis was further facilitated by the introduction of the air or flame drying technique [3]; Tijo and Puck, 1958) [9].

Karyotype is a phenotypic appearance of the Somatic chromosomes in constant to their genotype.

Levan and his associates in 1964, classified the chromosomes based on arm ratios. The long arm and short arm ratios (L/S) proposed by Levan be used as points of separation of different chromosomes [8].

Sexuality in frogs covers an almost complete range of those type found in vertebrate, [10]. In most cases sex – determination is considered as polygamic rather than chromosomal. [11].

1. Anaesthetic Ether.
2. Colchicine (0.02%) :-as a mitotic inhibitor
3. Hypotonic solution :-1gm of Sodium Citrate dissolved in 100ml distilled water. This is prewarmed to 37 degree Celsius before use.
4. Cold fixative :-Methanol : Glacial acetic acid (3:1) prepared freshly before use.
5. Giemsa stock solution :-1gm of Giemsa powder, crushed in mortar and dissolved in 54ml of glycerol kept as 60 degree Celsius for one hour by stirring intermittently. After one hour it was brought down to room temperature and added 8.5ml of CH – OH and stored at 4 degree Celsius which is used as stock solution.
6. Phosphate buffered salt (PBS) :-Nacl, Kcl, Glucose and Sodium Bicarbonate were dissolved in one liter of distilled water and PH is adjusted to 0.
7. Trypsin solution :-
   2 ml of stock Giemsa solution.
   2 ml of PBS.
   9 ml of distilled water.

2.2 Metaphase Chromosome Preparation Technique

Mitotic chromosome were prepared from bone marrow according to procedure described by Ojai et al. (1952) with modifications [12].

The mitotic spindle inhibitor colchicine (0.02%) was injected intraperitoneally at the abdomen. After two

hours the frog of Colchicine treatment, the frog was sacrificed by using anesthetic ether and dissected out the bones of femur [1] by using a strong scissors the ends of the bone were cut off.

The hypotonic solution (1% Sodium Citrate) which is pre – warmed at 37 degree Celsius, is taken in to hypodermic syringe and flushed the bone narrow in to a clean centrifuge tubes and the bone marrow was mixed with hypotonic solution thoroughly by agitating the solution, with the help of Pasteur pipette. This makes marrow clean from bone fragments. The tubes were kept at 37 degree Celsius in incubator for 20 – 30 min. This period is especially a critical for different species. After 30 min. the tubes were taken out from the incubator and centrifuged for 5 min. at 1000 rpm. The supernatant is discarded and added the 7 to 8 ml of fixative (Methanol : Glacial Acetic acid 3 : 1) gently to the cell pellet. The cells were to undergo fixation for 30 min at 4 degree Celsius. After 30 min. the fixatives was removed by centrifugation. This step has been repeated 3 -4 times for cleaning the cells from the cell – debris and for improving chromosomes spreading. The final cell pellet is resuspended in 1 – 2 ml of fixative depending on the quantity of cell pellet. The suspension is dropped on a clean and chilled slide from a Pasteur pipette 10 – 15 cm over the slide.

Air –dry method was followed for slide preparation. Preheating the slide was not preferred due to the change in gross morphology of the chromosomes. After drying the slides were stained with 2% Giemsa for 15min and washed thoroughly in distilled water for clear differentiation. Some of the slides were stored without staining for 2-3 days. (Rothfels and Siminovitch, 1959) [7].

3. OBSERVATIONS AND RESULTS

For chromosomal analysis we have screened nearly 25 metaphases of male and female. These were photographed by using automatic photometer with microscopic unit. After photography, the prints were prepared and each individual chromosome from photographs is arranged in the homologous pairs on the basis of meta-centric, sub-metacentric and acrocentric- [13-16,10,17].

The metaphase plates of male and female chromosomes are pasted. From these photographs, we can count the number of chromosomes.

The karyotypes were prepared by arranging homologous chromosomes and the males are having two types sex chromosomes. One is sub- metacentric and other is acrocentric. These were represented as X and Y chromosome. X-chromosome is longer than Y – Chromosome. The following photographs were included in this work [18,19,9,11,20,21,22,23,24,25,6].

There is a special type of chromosomes in a plate number **5a** for G-banding and **5b**- for **Tetraploidy.** Here the number of chromosomes is **52**. It shows polyploidy. But this polyploidy is different from the other polyploidies. Because here the identical homologous chromosomes from two cells, automatically arranged in to pairs. This might be an endomitosis.

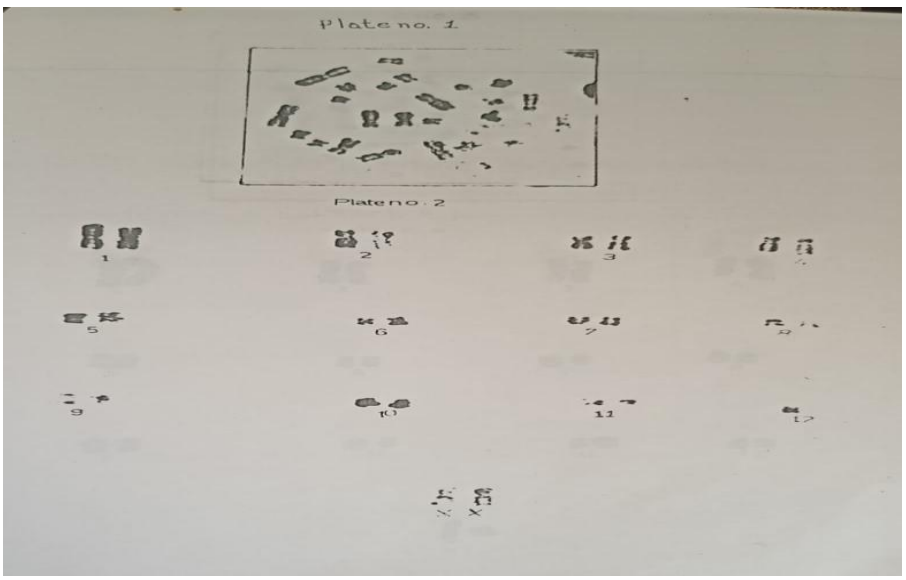

Plate. 1. Metaphase plate of normal female chromosomes (750X)

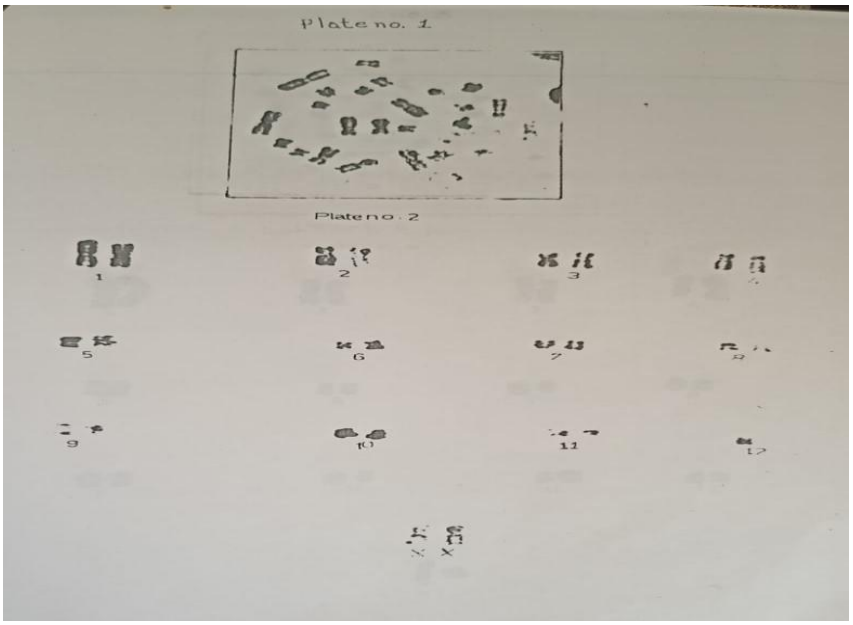

Plate. 2. Karyotype of normal female chromosome (750X)

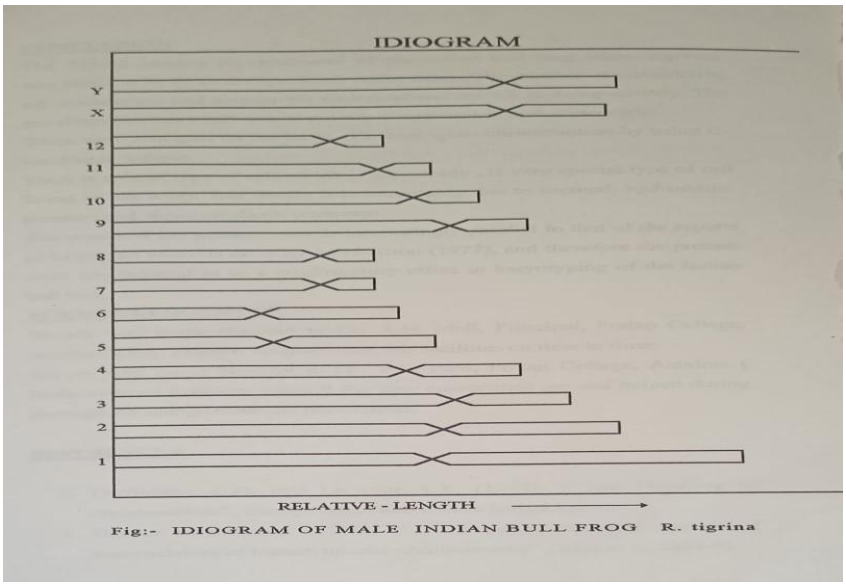

Plate. 3. Metaphase plate of normal male chromosomes (750X)

4. DISCUSSION

The present study encompassed karyotype preparation, trypsin Giemsa banding of chromosome complement of the mitotic chromosomes of *Rana tigrina*.

A format and style of presentation of human chromosomes has been established [26], which is now universally accepted. Such system of presentation can not be adopted for frogs as the chromosome complement composed of 26, which are divided in to large metacentric, large sub-metacentric, large acrocentric, small meta, sub meta and acrocentric. [1,2,5,27,28,29,18]

Homologous chromosomes were arranged in descending order of their size and length and it is hoped that, the proposed karyotype will be a useful tool in the hands of Cytotaxanomist, who desires something more than the morphological criteria for determining inter intra – species relationship [1].

The following photographs are included in this entire work.

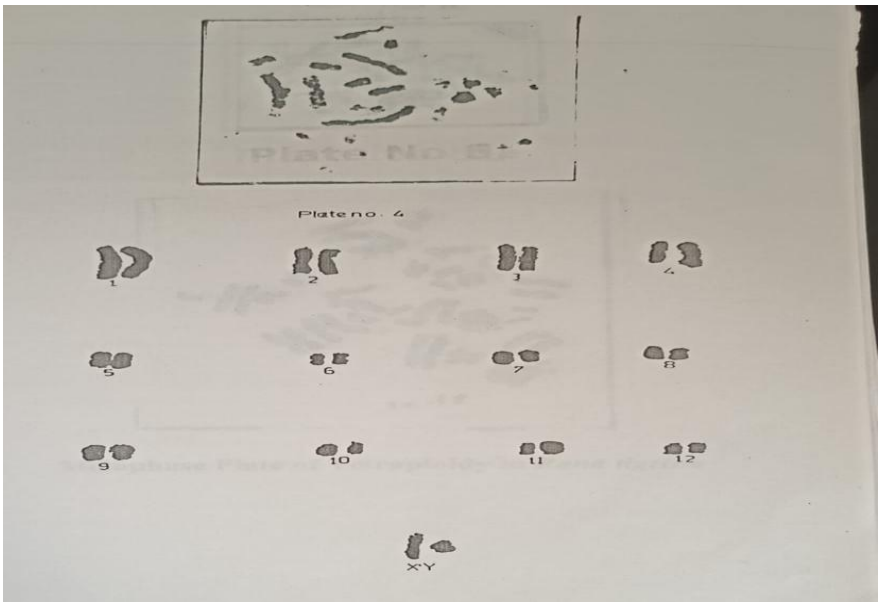

Plate. 4. Karyotype of normal male chromosomes (750X)

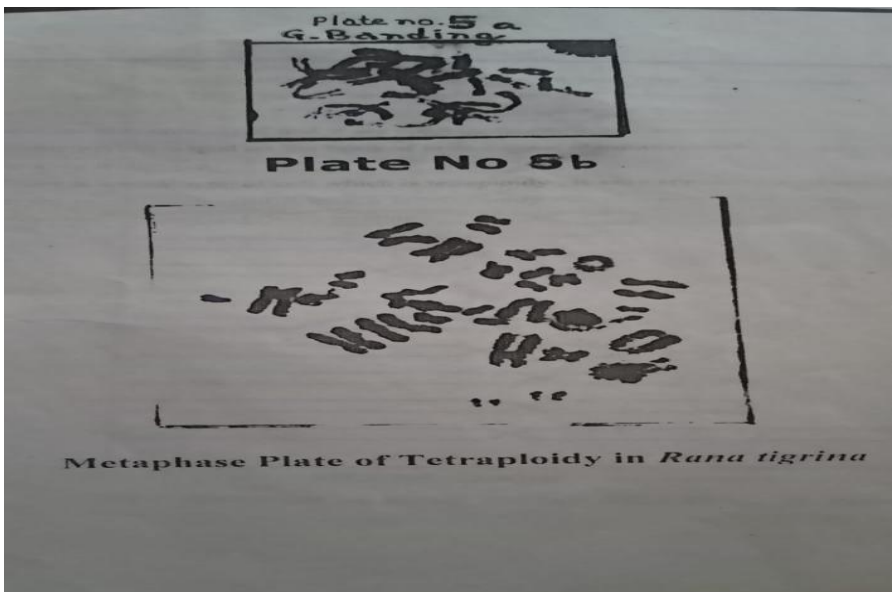

Plate. 5a and 5b. Metaphase plate of expected endomitosis (Tetraploidy) female chromosomes (600X)

For the foregoing account of the chromosome number in amphibia certain conclusions can be drawn . The legal number in amphibia appears to be n= 12 . Wherever there are variations in the majority of cases these variations may be traced to be a fragmentation of the chromosome resulting in a multiplication in the number. The work of the another has shown that the apparently diverse chromosome numbers. ln two species of Apoda where numbers are known as of double according to Robertson laws to the same basal number which in this case is n= 13. But the very large number of chromosome found in example of Cryptobranchidae and Hylobiidae can't apparently be explained by Robertson's laws and must have been brought about by a totally different kind of fragmentation from that which has resulted in the slight variations found in the some species of Urodela and and Apoda. The presence of very large number of V- shaped chromosomes with a telomatic attachment.

Whenever it occurs, it has not the same significance as in plants . Among Polyploidy generally in animals is a rare Amphibia tetraploid have been reported in *Rana esculenta* (Hartwig and Hartwig, 1920) and in Urodela *Triton palamatus* (Fankhauser,1937) *Triton vividescens* (Fankhauser and Kaylor,1933) partho

genetic triploid larvae have been reported as *Rana pipiens (*Parmenter, 1933)and in *Rana nigromaculatus* (Kawamura,1939) triploid and diploid larva have been found *in Euryces bisneata* (Frankauser,1939) [18,19,9,11,20,21,22,23,24,25,6].

5. CONCLUSION

The diploid number of the Indian bull frog (*Rana tigrina*), was found to be 26 in the specimen under study. The number of metacentric, sub – metacentric and acrocentric chromosomes are 3, 5 and 4 respectively. The sex chromosomes viz, X and Y are sub – metacentric and acrocentric.

These were arranged on the basis of homologous chromosomes by using G – banding technique.

There is special type of cell which might be an endomitosis, is very special type of cell found in our study. The reasons were not clear to get this type of cell how it was formed with tetraploidy *Rana tigrina* 52 chromosome numbers.

The experimental work was done in 1991, it was proved and well known fact that, low temperatures applied during meiosis in plants results in non-reduction of the chromosomes in the gametes which therefore retain diploid number. The fusion of dipliod gametes with normal haploid gametes produces triploid zygotes. Why low temperatures produce gametic duplication of the chromosomes, high temperatures produce somatic doubling?

The results of the present work is interestingly parallel to that of the reports of karyotype analysis done by Stephenson (1977), and therefore, the present work can referred to as a confirmatory effort in karyotyping of the Indian bull frog *Rana tigrina* with 52 chromosome number tetraploidy.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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