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Chromosomal Metaphase Spreads with G and NOR Bandings of Fresh Water Teleost, *Clarias batrachus*

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Background , The detail structure of chromosomes depicted by their banding pattern is very useful in drawing homology and analogy in the structure of chromosome within and between the species.

Results, In the present study, the diploid number of chromosomes was 50 in the fresh water teleost, *Clarias batrachus* in which 18 metacentric, 20 submetacentric and 12 telocentric chromosomes were observed. Structurally, the G bands revealed that transverse bands were visible after banding. These were due to the presence of heterochromatin and presence and absence of bases, these bands were observed at two pairs of submetacentric chromosomes. The NOR bands on the chromosomes were detected by silver staining in the two pairs of medium size telocentric chromosomes. It was believed that silver staining bound with the non-histone proteins of the transcriptionally active NOR region.

Conclusion, Our results showed that basic information of chromosome number and morphology with G and NOR banding can be used as an important tool for cytogenetic data of fresh water teleost *Clarias batrachus*.

Keywords: Chromosomal metaphase, G banding, NOR banding, *Clarias batrachus*.

Introduction

The study of fish chromosomes is based on histopathological sectioning of gonadal material particularly by the use of hypotonic treatment[1] and colchicine [2]. Chromosomal studies on fish started early when Post [3]investigated the chromosomes of the fish, *Salmo trutto fario*. Several cytogenetic studies have been carried out with fish, the progress has been reviewed by Blaxhall [4] and Kligerman and Bloom [5]. The model chromosome numbers in *Clarias batrachus* were reported to be 50 (2n number) by Pandey and Lakra [6]. Zhang and Tiersch [7] developed standardized karyotypes of *Ictalaris punctatus* (channel catfish). Studies on the fish chromosomes started from 1960's in the same way as in mammals. Karyological studies helped us to know basic information on the size, numbers and morphology of chromosomes. Many scientists including , Tan et al. [8] on crayfish, Kavaco et al. [9] on *Oligosarcus hepsetus*, Unal et al. [10] on *Psudophoxinus crassus* and *P. hittitoru*, Phimpham et al. [11] on five species of Lutjanid fish and Neeru et al. [12] on the Indian major carps were reported. The detailed structure of chromosomes is depicted by their banding pattern and is very useful in drawing homology and analogy in the structure of chromosomes within and between the species. The development

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of these differential staining techniques has allowed the demonstration of bandings characteristic of chromosomes. This has led to a better understanding of the fine characterization of individual chromosomes and to a better standardization of karyotypes. Karyotyping is the pairing of the chromosomes based on their size and morphology[13]. Researchers depended on ban.ding patterns for identification of chromosomes in a normal karyotype and in aneuploid situations as reported by O'Connor [14]. Howell and Black [15] described the detection of nuclear organizer region on the chromosomes by silver staining. It is relatively a simple method given and further modified by Gold et al. [16]. The mechanism of silver staining is not fully known but it is believed that silver staining binds with the non-histone proteins of the transcriptionally active NOR region. The banding techniques of fish chromosomes (G.Q. and R.) have been described for a few species of fishes [17,18]. The different arrangements of chromatin along the chromosomes of fishes make it difficult to obtain high resolution banding. The small size and large numbers of fish chromosomes also create additional difficulty in developing reliable banding technique. Since then, a multiple banding technique has been developed that enormously increases the power and sensitivity of chromosomal analysis [19]. The localization of C band heterochromatin has been studied in *Labeo rohita* and *Cirrhina mrigala* chromosomes *in vivo* by Khudabuksh and Chakrabarty [20]. Khudabuksh and Tiwary [21] obtained G. bands in *Anabas testudineus* and *Labeo rohita*. *In vivo,* the metaphase chromosomes in fish are extremely difficult to study and need a lot of pretreatment schedule. Lakra and Krishna [22] also suggested various techniques of chromosomal banding in fish. Khudabuksh and Dutta [23] successfully induced G and C bands in three species of larvivorous fishes (*Chanda nama, Esomus danrica* and *Puntius ticto*). Ag-NOR staining show the region containing the actively transcripted ribosomal RNA genes. As the chromosomal distribution patterns of NOR revealed by cytogenetic techniques are typically species specific in fish, they prove to be useful chromosome markers in fish studies[24,25] . It is to be mentioned that NOR staining can help in chromosome -polymorphism , studies of chromosomes with double satellites, and structural abnormalities involving satellite regions. Also,variations in NOR number and size could be caused by differential transcriptional activity necessary under changing ecophysiological conditions or due to other factors as age, disease,… [26, 27 and 10] . Phimphan et al. [11] proved through their studies the pair of nuclear organizer regions at sub-centromeric region of long arms of respective telocentric chromosomes pairs 1, 3, 4 and 9. The present study aimed to determine the chromosomal characteristics of *C. batrachus* collected from the local fish markets of Bareilly district ,India by using Giemsa staining as well as bandings like G and AgNOR.

Materials and Methods

The catfishes *Clarias batrachus* (30-40 gm.) (n=50) were used for chromosomal studies. The experimental fishes were collected from Rithora and Naryawal fish market, Bareilly district. The uninfected fishes were acclimatized to laboratory conditions. A total of 16 fishes of different sizes and body weight were selected. After 4 hours of colchicine (0.1%) treatment, the abdominal area was dissected out, kidney tissues were collected, immediately transferred to separate Petri dishes, cut into small pieces, kept in hypotonic solution for 30 minutes at room temperature, and subsequently, the hypotonic solution was aspirated out by a pipette. The fresh and chilled 5.0 ml of fixative (3 part Methanol and I part Glacial acetic acid) was added slowly along the sides of each tube and the contents were re-suspended, allowed to stand for 10 minutes and the fixative was pipetted out. The above step was repeated three times, until a supernatant along with a mass of white cells settled at the bottom of the tube. After fixation, the button was dried on filter paper, transferred to 40% glacial acetic acid solution and this suspension was dropped on pre-warmed slides from 12" inches height. Slides were air dried, matured for 24 hours and stained in 4% Giemsa stain [5]. The G banding method was carried out as described by Sumner [28].

Results

The diploid numbers of chromosomes in *Clarias batrachus* were observed to be 50. The total chromosomes recorded were 18 metacentric, 20 submetacentric and 12 telocentric (18M + 20SM + 12T) (Fig. A & A-1). Structurally, the G bands revealed that transverse bands on them were visible after banding. These were due to the presence of heterochromatin and presence and absence of bases. The stain appeared as a dark spot in the metaphase spreads (Fig. B). The NOR bands on the chromosomes were detected by silver staining. These bands were observed at 1- 4 places in the metaphase spreads (Fig. B). Structurally, the chromosomes had transverse bands that were visible after banding. The banding was due to the presence of heterochromatin and presence and absence of bases. The bands helped in pairing the homologous chromosomes (Fig. C).

The NOR regions were clearly observed at 2-3 places in the metaphase spread (Fig. A). Structurally, the chromosomes have transverse bands that are visible after banding. The banding is due to the presence of heterochromatin and presence and absence of bases. The bands help in pairing, the homologous chromosomes (Fig. B). After the silver staining, NOR binds to the non-histone proteins of the transcriptionally active NOR region as clearly observed at 2-3 places in the in the metaphase spreads (Fig. C).

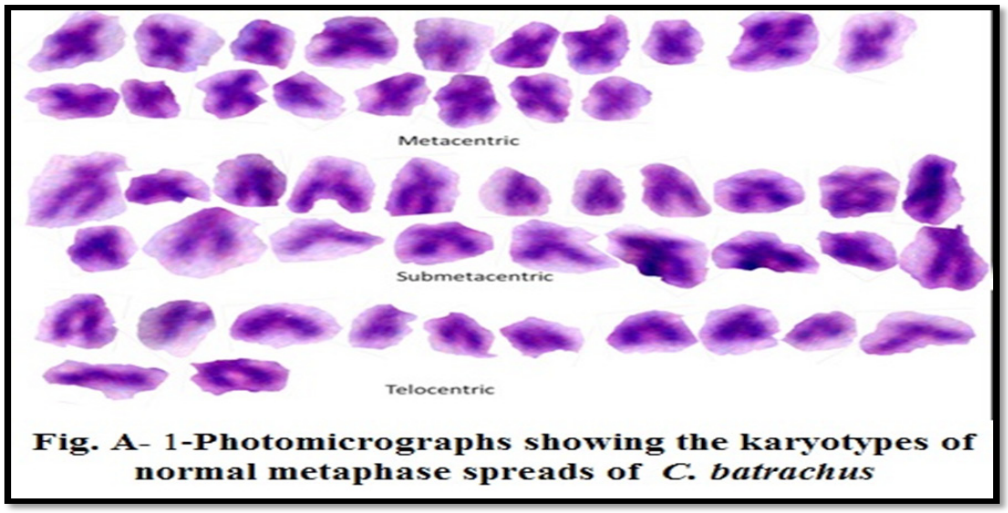

Fig. A- 1-Photomicrographs showing the karyotypes of normal metaphase spreads of *C. batrachus*

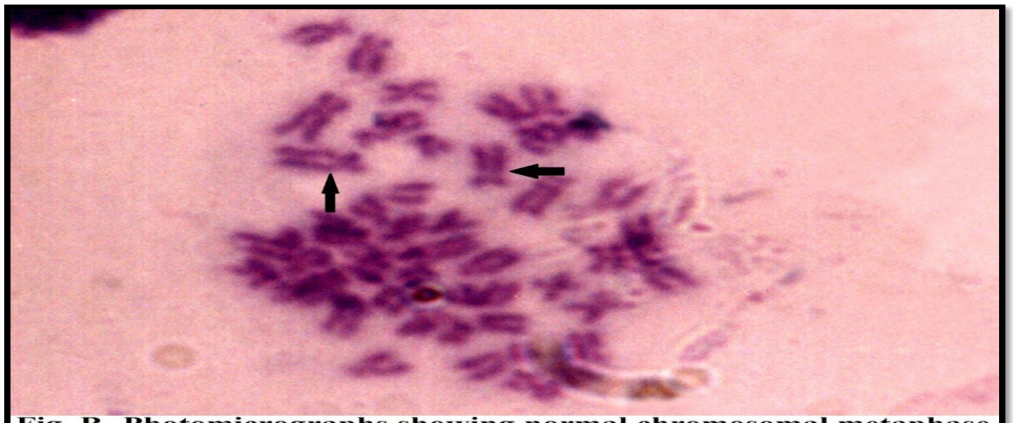

Fig. B- Photomicrographs showing normal chromosomal metaphase spread of *C. batrachus* with G banding

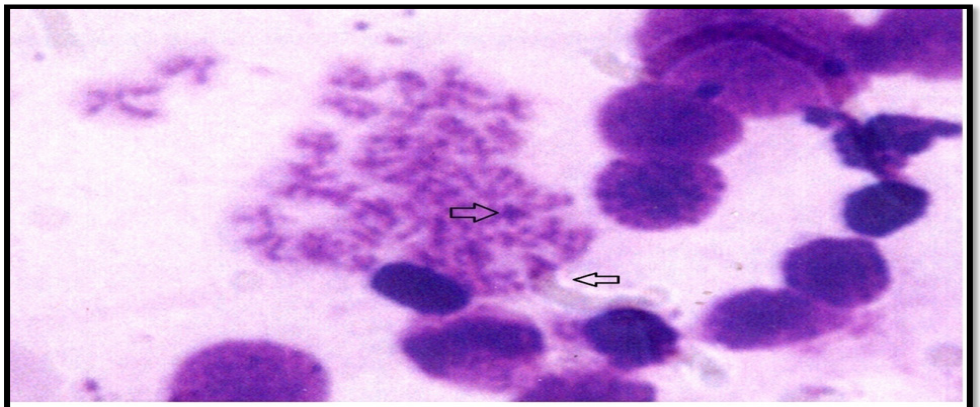

Fig. C- Photomicrographs showing normal chromosomal metaphase spread of *C. batrachus* with NOR banding

Discussion

Living in an aquatic system, fishes are particularly subjected to pollution hazards which directly or indirectly harm the fish. In the present study, the fingerlings of *C. batrachus* were used as the test material. The chromosomal preparation and banding can be considered as an art as well as science. Chromosomes are visualized individually only during mitosis and therefore techniques have been developed to trap the cell at metaphase stage by using cell inhibitors such as colchicine. Numerous methods are now available to identify chromosomes and prepare karyotypes for clinical and research purposes. The G banding technique is defined as a system of alternative dark and light spots throughout the length of euchromatic part of chromosomes [28]. G banding involves protease treated chromosomes with Giemsa dye and is thought to result from interaction of both DNA and proteins [29]. This technique has been routinely used in vertebrates and lower vertebrates like fish[30, 31 and 32]. Leitao et al. [33 and 34] described the G banding pattern in three species of oysters *C. gigas, Crassostrea angulate* and *C. virginica* and observed the dark and light band patterns on the two arms of 7 pairs of chromosomes. Karhan and Ergen [35] reported quite large heterochromatin region on metacentric chromosomal arms expressing the specific pattern of G banding which could be defined as genotypic markers for *Garra viariabilis*. Constitutive heterochromatin at telomeric and centromeric regions of chromosomes was found by Neeru et al. [12] in the chromosomes of *C. catla, L. rohita* and *C. mrigala* .

The present study confirmed the presence of heterochromatin in two pairs of sub-metacentric chromosomes in G-bands and successful silver staining in two pairs of medium sized telocentric chromosomes in NOR banding. It was believed that silver staining bound with the non histonic proteins of the transcriptionally active NOR located terminally on the short arms of small sub-metacentric chromosomes in European eel *Anguilla Anguilla* [36,37 and 38] observed silver marks terminally on the short arm of an acrocentric chromosome and these NORs were of the same size in all specimens studied in the Congo fish *Conger conger*. Vitturi et al. [39] reported the nuclear organizing regions as phenotype on the chromosomes of four species of the genus *Diplodus*. Wang et al. [40] observed that the numbers and location of the NORs were also often variable not allowing accurate location on the chromosomes of crosstree. Leitao and Chaves [41] reviewed 20 years history of karyotypes with NOR banding patterns in bivalves. Valic et al. [42] (2010) detected the NORs in the telomeres of the two pairs of medium sized sub-metacentric chromosomes of *Telestes ukliva*, while Nandini and Arokia[43] found NORs on two of sub-metacentric chromosomes in *Labeo rohita.* Homologous chromosome pair number 11 in *C. catla* and 15th chromosome pair in *L. rohita* were investigated for Ag-NOR bands [12].

The presence of darkly stained NORs on the terminal region of the long arms of one of the chromosome; was detected in *C. mrigala* ,by Ag- NOR staining . Chromosomal studies on commercially important fishes are important to achieve genetic improvement of fish production through chromosomally set manipulation and genetic selection necessitating individual identification of chromosome establishment of precise karyotypes.

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Conflict of interest :
The author has no conflict of interests to declare regarding the publication of this paper.

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الانتشار الطورى للكروموسومات ذو النطاقات جى و نور لأسماك المياه العذبة من نوع قراميط بتريكس مكتملة العظام

فى فيرما* ، **نيليما جوبتا**** ، **ديليب جوبتا***** و **سعيد شلبى******
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لقد أظهرت الدراسة أن عدد الكروموسومات الزوجية فى الأسماك كان ٥٠ وقد لوحظ وجود ١٨ كروموسوم متوسط التقسيم المركزى ، ٢٠ متوسطانى و ١٢ طرفى التقسيم المركزى.

تركيبيا، أظهرت نطاقات جى أن النطاقات العرضية كانت مرئية بعد عمل النطاقات وهذا يرجع الى وجود الهتروكرومين ووجود أو غياب القواعد .وقد ظهر هذا فى زوجين من الكروموسومات الموسطانية . لقد تم الكشف على نطاقات نور على الكروموسومات باستخدام صبغة الفضة فى نوعين من الكروموسومات موتوسطى الحجم من النوع طرفى التقسيم المركزى ، ويعتبر هذا وسيلة للدراسات الجينية الخلوية لأسماك المياه العذبة من نوع قراميط بتريكس مكتملة العظام.

الكلمات الدالة : طورية الكروموسومات - نطاقات جى و نور - قراميط بتريكس مكتملة العظام.