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Study of Karyotype Profiling of Fresh Water Fishes with G and NOR Banding Pattern

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Abstract

Cytogenetic studies of fishes are comparatively difficult, not much progress has been made in this field. In karyotyping 2n numbers of chromosomes were observed to be 56 total. The chromosomal formula was recorded as 18 metacentric, 22 submetacentric, and 16 telocentric with transverse bands on them that are visible after banding. Heterochromatin and the presence and lack of bases both contribute to the banding. In the metaphase spreads of the freshwater teleost Heteropneustes fossilis, the transcriptionally active nucleolar organiser region (NOR) was clearly seen at 2-3 locations after it bound to the non-histone proteins. Later, it was discovered that Clarias batrachus has 50 chromosomes, or 2n. 18 metacentric, 20 submetacentric, and 12 telocentric chromosomes in total were recorded; G bands showed that transverse bands remained evident on them after banding. By using silver staining, the NOR bands on the chromosomes were found.

Introduction

An increasing number of cytogenetic studies on fish have been conducted in the fields of systematics, mutagens, and aquaculture. The majority of fish karyotypes contain a high proportion of tiny chromosomes, and getting enough carefully prepared spreads of metaphase from different fish tissues has proven challenging. (KhudaBuksh 1984 ; KhudaBuksh *et al.* 1986) KhudaBuksh and Barat (1987) The histological sectioning of gonadal material, namely with the application of colchicine (Roberts, 1964) and hypotonic therapy (Sharma et al., 1960), has formed the foundation for the study of fish chromosomes. When Post (1965) examined the chromosomes of the fish Salmo trutto fario, chromosomal studies in fish had their start. Denton and Howell (1969) noted that the chromosomal metaphase was prepared using the flame drying procedure. In fact, the method used for human and mammalian chromosome preparation was applied to fish tissue such as gills, kidney and other organs. The development of several fish cytogenetic research has been summarised by Blaxhall (1975) and Hartley and Horne (1985). According to Tripathi and Sharma (1986), the chromosome numbers in *H. fossilis* are 56. The model chromosome). According to Pandey and Lakra's (1997) analysis of karyological investigations on the endangered American green sturgeon (Acipensor medirostris) and American white sturgeon (Acipensor transmontainus), Clarias batrachus has 50 (2n) chromosomes. Ictalaris punctatus (channel catfish) standardised karyotypes were created by Zhang and Tiersch in 1998 and had the number 249+8. (VanEenennaam *et al.* 1998, and VanEenennaam, 1999). It should be noted that chromosome polymorphism, investigations of chromosomes with double satellites, and structural problems involving satellite regions can all be aided by NOR staining. Additionally, fluctuations in NOR size and number may result from varied transcriptional activities required to adapt to shifting ecophysiological settings or from other factors like ageing or illness (Lakra *et al*., 2007; Neeru, 2014; Unal *etal*.,2014).The goal of the current study was to identify the chromosomal characteristics by using giemsa staining as well as bandings like G and AgNOR of *H. fossilis* and *C. batrachus* for further research purposes.

Material and Methods

1. **Karyotyping preparation**- The histological sectioning of gonadal material, namely with the application of colchicine (Roberts, 1964) and hypotonic therapy (Sharma et al., 1960), has formed the foundation for the study of fish chromosomes. When Post (1965) examined the chromosomes of the fish Salmo trutto fario, chromosomal studies in fish had their start. Denton and Howell (1969) noted that the chromosomal metaphase was prepared using the

flame drying procedure. In reality, fish tissue like gills, kidneys, and other organs were prepared using the same technique utilised to prepare human and animal chromosomes. Fish have been used in a number of cytogenetic studies, and the progress has been summarised by Blaxhall (1975), Kligerman and Bloom (1977), Gold (1979), and Hartley and Horne (1985).

2. **Bandings Patterns** - The banding pattern of chromosomes, which represents the intricate structure of chromosomes, is highly helpful in establishing homology and analogy in the chromosome structure within and between species. The demonstration of chromosome-specific bandings has been made possible by the advent of these differential staining techniques. This has improved karyotype standardisation as well as our comprehension of the subtle differences between individual chromosomes. Chromosomes are paired according to their size and form in a process called karyotyping. (Caspersson *et al*. 1968).

a. **NOR banding**- Silver staining can be used to identify the chromosomes' nuclear organiser region. Howell and Black (1980) first introduced the comparatively straightforward silver staining (NOR) technique, which Gold et al. (1986) later modified. Although the exact mechanism of silver staining is unknown, it is thought to bind to non-histone proteins in the transcriptionally active NOR region. For a few species of fish, the banding methods of the fish chromosomes (G.Q. and R.) have been described (Mayr et al., 1987; Medrano et al., 1988). It is challenging to get high resolution bands in fish because of the unusual chromatin organisation along their chromosomes. Creating a successful banding procedure is further made more challenging by the tiny size and vast number of fish chromosomes. Since then, the power and sensitivity of chromosomal analysis have been greatly increased by the multiple bands technique (Gold et al., 1990). Silver staining can be used to identify the chromosomes' nuclear organiser region. Howell and Black (1980) first introduced the comparatively straightforward silver staining (NOR) technique, which Gold et al. (1986) later modified. Although the exact mechanism of silver staining is unknown, it is thought to bind to non-histone proteins in the transcriptionally active NOR region. In cytohistopathology as it is used now, Trere (2000) explored the use of silver staining or Ag NOR staining to measure proteins. Karahan and Eregene (2010) discovered the NOR pattern in Garrra variables in many chromosomal pairs. Kushwaha et al. (2011) found variations in the NOR and C bands of the endangered marine species. Knytl et al. (2013) described the AgNOR in the chromosomes of the severely endangered crucian carp Carassius carassius. Neeru et al. (2018) used the NOR banding pattern on various karyotypes to observe the cytogenetic survey of the three species of Indian main carps, *C. catla, L. rohita, and C. mrigala.*

b. G banding - Khudabuksh and Chakrabarty (1994) examined the location of C band heterochromatin in vivo in the chromosomes of Labeo rohita and Cirrhina mrigala. Anabas testudineus and Labeo rohita were found to have G. bands by Khudabuksh and Tiwary in 1994. The majority of fish chromosomal banding methods that have been reported are limited to either C bands or NOR characterizations. The fish metaphase chromosomes are very challenging to investigate in vivo and require extensive preprocessing. Additionally, Lakra and Krishna (1996) proposed a number of methods for chromosomal banding in fish. Chanda nama, Esomus danrica, and Puntius ticto are three species of larvivorous fish that Khudabuksh and Dutta (1999) effectively induced G and C bands in.

Results

In the present study, the fingerlings of *H. fossilis* and *C. batrachus* were used for chromosomal studies. Healthy and small fishes *Heteropneustes fossili*s (10-20 gms.) and *Clarias batrachus* (30-40 gms.) were sacrificed for chromosome preparations. The chromosomal spreads were prepared from kidney tissues.

1. ***Heteropneustes fossilis***- A total of 56 chromosomes with 2n numbers were found. 18 metacentric, 22 submetacentric, and 16 telocentric chromosomes were identified (18M+22Sm+16T) (Fig. 1:A). Transverse bands on the chromosomes' structural makeup can

be seen following banding. Heterochromatin and the presence and lack of bases both contribute to the banding. The homologous chromosomes are paired with the aid of the bands (Fig. 1:B). According to Howell and Black (1980), the chromosomal spreads were stained for the nuclear organiser region. Following its binding to the non-histone proteins of the transcriptionally active NOR region, the silver staining NOR region. (1986; Banerjee and Banerjee). In the metaphase spreads, the NOR areas could be seen in two or three locations clearly (Fig. 1:C).

2. ***Clarias batrachus***- 50 chromosomes were found to be present in Clarias batrachus' 2n numbers. 18 metacentric, 20 submetacentric, and 12 telocentric chromosomes in total were found (Fig. 2:A). The G bands' structural analysis revealed that after banding, transverse bands remained on them. These were brought on by heterochromatin's presence as well as the presence and absence of bases. In the metaphase expands, the stain shows as a black patch (Fig. 2:B). By using silver staining, the NOR bands on the chromosomes were found. These bands were visible in the metaphase spreads between one and four locations (Fig. 2:C).

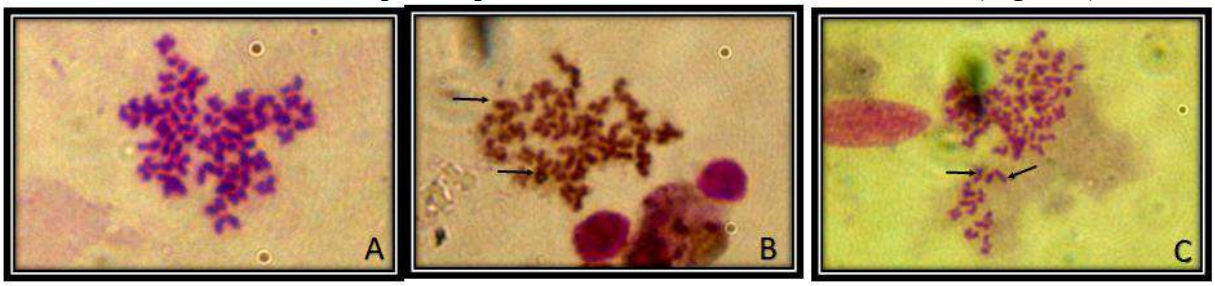

Fig. 1- A, B,C – A- Photomicrographs showing normal metaphase spread, B: NOR banding, C: G banding of *H. fossilis*.

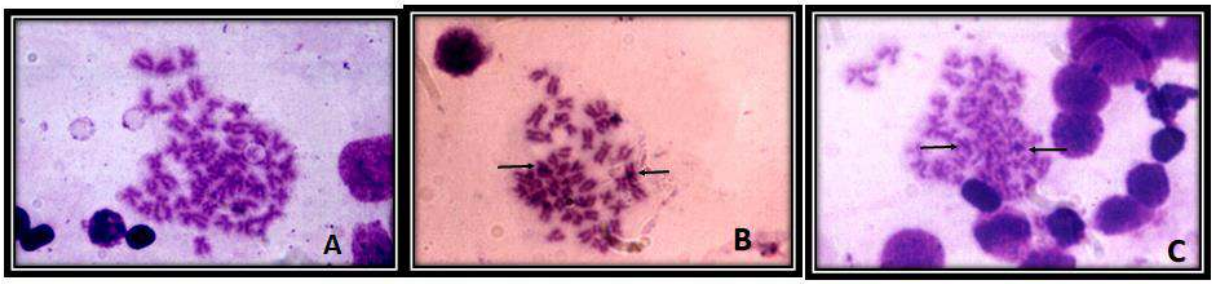

Fig. 2- A, B,C – A: Photomicrographs showing normal metaphase spread, B: NOR banding, C: G banding of *H. fossilis*.

3. Discussion

Fish play a crucial role in the study of animal evolution and the evolution of vertebrates in general. There hasn't been much advancement in this subject because fish cytogenetic investigations are relatively challenging. There are thought to be 20,000 different species of fish in the globe, 17,000 of which have been described in India. However, only 125 species have had their karyotypes satisfactorily established by fish cytologists. Fish cytogenetic research first started in the final decade of the 19th century. The data, technique, and benefits of fish chromosomal studies are all described in a number of publications that are currently available. The study of chromosomal number, size, and form at the metaphase stage is the foundation of cytogenetics. Karyotype analysis is important in taxonomy since it includes a heritable characteristic of each species. Cytotaxonomy is the study of how species differ from one another based on cytogenetic characteristics. Chromosomal rearrangement causes karyotypic variance in evolutionary lineages, which is important for disrupting the evolutionary interrelationship of particular taxa. Three different techniques can be used to find NOR on a chromosome: silver staining, chromomycin, and mithramyein.In the current study, the karyotypes of experimental fishes The NOR were dyed with AgNO3 in accordance with Howell and black (1980) and Gold and Elison (1983). Our findings concur with those of Salvadori *et al.* (1994), who found two silver cubes of the same size in every specimen they examined. In 48 total karyotypes, Vitturi *et al.* (1996) found that 5 pairs of

chromosomes were involved in nucleous organisation. In Diplodus annularis, the NOR were found terminally on the short arms of 1, 2, and 20, and on the long arms of the 4th and 5th pair. The majority of North American and European cyprinid species only have one pair of NOR (Amemiya *et al.* 1992). However, a few species have two pairs of NOR, including Phoxinus phoxinus (Boron 2001), Chondrostoma lusintanicum (Collares Pereira & Rab 1999), and Eupallasella perenurus (Boron *et al.* 1997). Morescalchi *et al.* (1998) who applied G banding patterns to the species' chromosomes. While Karahan and Ergene (2010) discovered the NOR region and observed numerous chromosomal pairs by silver staining, Valic *et al.* (2010) found two pairs of medium-sized submetacentric chromosomes in telomeres. By looking at 74 sparrow fish karyotypes, Kaewsri *et al.* (2014) were able to clearly see the NOR in 6 pairs of acrocentric chromosomes. By using diluted trypsin on the karyotype slides before staining with Giemsa, it is possible to discern dark and light striations over the chromosomes. While the lighter zone is made up of GC rich regions, the darker region comprises a region of facultative heterochromatin, also known as an AT rich region with G bands. After being treated with a digesting enzyme in the current work, the chromosomes display the heterochromatin-rich dark areas. Cuny *et al.* (1981) claimed that the presence of DNA segments known as isochers, which are enriched in GC base pairs and found in the genome of the majority of higher vertebrates, was related to the occurrence of G or R bands. As proof, Medrano *et al.* (1988) found that the G bands on the chromosomes of Aguilla anguilla were of higher quality. Anabas testudineus and Labeorohita were found to have G. bands by Khudabuksh and Tiwary in 1994. A significant majority of Indian fishes have G bands in their karyotypes, according to Lakra and Krishna (1996). Due to each chromosome's distinctive pattern of dark and light, G banding can be used to detect chromosomal aberrations including translocations (Uedo and Naoi 1999), and Karahan and Ergene (2010) examined cytogenetic studies using G.C.Q. and NOR staining of Garra variablis. As a result of treating mature chromosomal slides with AgNOR and tripsin enzyme, which causes silver binding with transcriptionally active non-histone protein, we have effectively done chromosomal spreads. However, transverse bands left over during digestion indicate the presence of heterochromatin and euchromatin regions in the chromosomes of the freshwater catfish Heteropneustes fossilis and *Clarias batrachus*, which can aid in the identification of genotypic aberration brought on by unfavourable environmental conditions.

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