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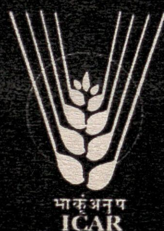
GENETICS AND BIOTECHNOLOGICAL TOOLS IN AQUACULTURE AND FISHERIES



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FISH CYTOGENETICS, GENOTOXICITY AND MUTAGENESIS

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FISH CYTOGENETICS

In recent years, fishes have been the subject of an increasing number of cytogenetic investigations in the area of Systematics, Pisciculture and Mutagenesis. All the same, cytogenetic studies on fishes were long plagued by technical problems. Though fishes represent forty three percent of the living vertebrates, even today many of the fish species still remain to be karyotyped.

The rapidly dividing blastomeres of the early embryo provided the suitable material in most of the pioneering studies on fish chromosomes. A major difficulty with regard to this method is the problem of obtaining embryos. Sectioning of testis and testis squashes have also been employed in several fish chromosome studies. However, this technique is useful only at times of active spermatogonial proliferation, which for most species is a month or two just prior to the spawning season.

Practical utilisation of fish karyotypes involving details of chromosome morphology, had to await the development of a readily applied technique, of gill epithelial squashes after treatment with colchicine and hypotonic treatment, by McPhail and Jones in 1966. But tissue squashes do often result in low numbers of poorly spread mitotic preparations and in addition require certain degree of manipulatory dexterity. Moreover, wet preparations have the disadvantage of a short useful life and require refrigerated storage.

Cell culture techniques using a variety of tissues, while providing a number of excellent spreads giving good resolution of chromosome details, require laboratory facilities not always available. The advent of the use of direct preparations by air dry method heralded in a new era in fish cytogenetic studies. Kligerman and Bloom in 1975 described a method for rapid chromosome preparations from solid tissues of fishes which is both inexpensive and simpler. The progress over the years has been rather slow compared to mammalian cytogenetics, and in many areas, Fish Cytogenetics still remains a virgin field.

This new source of data involving chromosome number and morphology, has been receiving increasing attention and shows high promise for interpreting the evolution of fishes. The systematic application of karyology is now assured, but it is still too early to know precisely what its value will be for different groups of fishes. Karyotypes are thus seen to be a useful tool for systematic appraisal of many intimately related compact groups of fishes, and it is predictable that they will be of value in other, recently differentiated groups as well. For example, many sibling species may have distinctly different karyotypes because of Robertsonian fusions, (Setzer,1970), where as many obviously distinct species in different families or even different orders show virtually identical karyotypes as in some flatfishes, sunfishes and anchovies. (Ohno, 1970). Fishes show a great diversity in karyotypes, the diploid no. ranging between as low as 16 and as high as 240. Pericentric inversions

played a major role in the evolution of karyotypes of many fishes, though certain less significant chromosome rearrangements often can be utilised to distinguish closely related species.

Perhaps in no group of vertebrates, other than fishes do remote crossings, both interspecific and intergeneric, occur so frequently in nature producing numerous hybrids, This is particularly true of fresh water fishes, where reproductive isolation is also violated more frequently than in marine fishes. Hybridisation, with a view to combine the beneficial qualities of two breeds, has already ensconced as an effective tool in fish culture. Information on the cytogenetic profile of parental species with a view to detect a possible marker chromosome in the hybrids can prove to be very useful in such studies. An effort in this direction has been made as early as 1975(Krishnaja and Rege). Cytogenetic, as well as biochemical genetic markers like haemoglobins can prove useful not only in pisciculture involving hybridisation but also in the identification of natural hybrids, especially since the gross morphological characters used for identification can at times be misleading. (Krishnaja, 1976; Krishnaja and Rege, 1977; Krishnaja and Rege,1979). The advantage of haemoglobin studies in this respect can hardly be over emphasised, since it is least affected by environmental factors unlike some other proteins. Chromosome studies employing epithelial cells from scales, fin, cornea etc. either directly or by short term culture would have wider scope in systematic hybridisation studies, since such a technique does not entail sacrificing the experimental animals. (Krishnaja and Rege,1980). A recent study stressed the importance of cytogenetic identification of breeders in the efficient genetic management of fish stocks (Porto-Foresti et. al.,1998). In this particular instance, an inversion involving the nucleolar organizer region , in homozygote condition was found to be lethal in stock rainbow trout and may cause economic losses in the culture of this species.

Polyploidy which has been very important in plant evolution, seems to have played almost no role in animal evolution. Polyploidy in animals usually upsets the sex determination mechanism and this has generally been assumed to block the successful establishment of ploidy. However in fishes, there are cases in which it appears that a single polyploid event has given rise to an entire taxonomic family in evolution. Salmonidae family of fishes comprising Salmon and Trout is a familiar example of a group, that appears to be originated through polyploidy. Salmonids have twice as much as DNA as related fish. Different Salmonid species have different chromosome numbers, but it has been discovered that the group has almost an invariant no. of chromosome arms (some fused in some species, that is twice the no. of arms in related groups). Hence the evidence points to the Salmonids, having evolved from a single event, that gave rise to a tetraploid. Sterility of triploids has been commercially exploited in animals as well as plants. Triploid oysters have been developed and have a commerciaal advantage over their diploid counterparts. The diploids go through a spawning season, when they are unplatable, but the triploids, because of their sterility do not spawn and are palatable the whole year around.

Today cytogentic studies in fishes have graduated from conventional karyotype analysis to high resolution techniques such as R-bands obtained after 5-BrdU incorporation, fluorochrome staining, restriction enzyme banding, in situ localisation of repetitive DNA probes and immunofluorescence. These studies have disclosed certain unique characteristics of fish species, like, evolution of polyploidy in certain genus, supernumerary B-chromosomes, natural triploids, a great level of polymorphism of NOR number and morphology, a rich variability of morphologically

differentiated sex chromosome systems, including simple and multiple systems, originated either by chromosome rearrangements involving the sex chromosomes, or else by heterochromatin addition/deletion. (Nanda et.al.,1992; Almeida-Toledo et.al.,1998; Denovan-wright et.al.,1998; Caputo et.al.,1998; Pisano and Ozouf-Costaz,1998; Nisi-Cerioni et. al.,1998; Fenocchio et.al.1998). Involvement of both A+T and G+C rich sequences has been observed. In contrast to other vertebrate classes, cytologically demonstrable sex chromosomes are rare among fishes. The demonstration of the chromosomal mode of sex determination via genetic experiments as well as the absence of heteromorphic sex chromosomes affirm Poeciliid fishes as a unique group among vertebrates that are endowed with the most primitive form of sex chromosomes. (Krishnaja and Rege,1983; Nanda et. al.,1993). In many different taxa the evolutionary process involved in the differentiation of advanced sex chromosomes is outlined through sex specifically organised repetitive sequences. Fishes represent the stem vertebrate condition and have maintained several gene rearrangements common to mammalian genomes throughout the 450Myr of divergence from a common ancestor. The cytogenetic and molecular genetic data obtained in fishes so far are a reminder of this fact.

GENOTOXICITY AND MUTAGENESIS

Current awareness of the potential hazards of pollutants in the aquatic environment has stimulated much interest in the use of fishes as indicators for the monitoring of environmental carcinogens., teratogens and mutagens. This is mainly because aquatic environments serve as convenient repositories for man's biological and technological wastes.(Krishnaja et.al.,1987). Epidemiological investigations have brought to light a higher incidence of tumours in fishes from polluted water systems compared with their counterparts from relatively non-polluted waters. Chromosomal aberrations (CA), sister chromatid exchanges and micronuclei are three major Cytogenetic end points that can be used as indicators of an in vitro/ in vivo mutagenic exposure. Many investigators in the past had attempted to use aquatic organisms to study the clastogenic effects of radiation and chemicals. Studies on the clastogenic effects of low level waterborne radionuclides were carried out by Tsytsugina(1973). In one of the field studies undertaken to examine chromosome abnormalities in aquatic organisms, Longwell(1976) analysed mackeral eggs collected from the New York Bight. An average of one third of all division figures scored from eggs collected in this highly polluted area were abnormal, showing misoriented spindles, chromosome stickiness, breakage and loss.

The major factor that has confounded the results of most clastogenic studies with aquatic organisms is the choice of species used in these experiments. Most fishes have large numbers of small chromosomes making chromosome breakage studies ardous if not impractical. These problems can be overcome by the utilisation of aquatic species with small numbers of large chromosomes and the adoption of modern cytogenetic methods of metaphase analysis. Through the selection of an in vivo model system approach using representative species with good karyotypes and the utilisation of metaphase chromosome and SCE analyses, sensitive short term test systems to detect waterborne genotoxic agents were developed. (Kligerman et.al.,1975; Prein et.al.,1978; Kligerman,1979; Alink et.al.,1980; Krishnaja,1980; Krishnaja and Rege,1982).

Krishnaja(1980) explored the possibilities of using fish species available in this part of the country as a cytogenetic model in vivo for the detection of potential

mutagens. Accordingly 20 species of fish were screened for the selection of a suitable karyotype. Other factors, such as easy maintenance in the laboratory, relatively small size, ability to withstand experimental conditions, availability in large numbers throughout the year and good yield of a number of metaphases from a wide variety of tissues, were also taken into consideration in the selection of the test species. A dose response effect was found with the reference mutagen mitomycin C. Clastogenic effects of metals such as Hg, Se and Cr in the form of phenyl mercury acetate, selenium dioxide and sodium dichromate following indirect (i.m. injections) and indirect(dissolved in the aquarium water) were studied. How ever, to bring this system to a standardised testing protocol, much needs to be done. Some of the aspects needing to be covered are (1) a more comprehensive screening involving a large number of fish species to detect an ideal karyotype, (2) cell cycle kinetics studies for the tissues concerned to make the chromosomal aberration analysis more meaningful, and (3) collection of more data to establish exposure time for indirect exposures. Rishi and Grewal(1995) carried out chromosome aberration analysis in Channa punctatus after indirect exposure (dissolved in aquarium water at a concentration level equal to that found in drainage from agricultural fields) to an organophosphorus insecticide, dichlorovos. One versatile end point, the frequency of anaphase aberrations has been used in several large marine assessments to evaluate genotoxicity. (Hose,1994).

By modifying the metaphase methodology to include incorporation of BrdU for two cell cycles prior to metaphase accumulation, SCE analysis can be performed. Many mutagenic carcinogens cause high levels of SCEs at doses that produce little or no chromosome breakage. Thus SCE analysis was used increasingly as a monitor for the presence of genotoxicants in both in vivo and in vitro studies. Most of the investigations in vivo are hampered by unreliable techniques. Kligerman ,1979 ,1980 , demonstrated the usefulness of Umbra limi as a model in vivo system for both CA and SCE analysis for the detection of mutagens. van de Kerkhoff and van der Gaag (1985) had discussed a few modifications, like BrdU incorporation from aqueous solutions, short interval between preparation and staining of slides and post treatment with HCl for SCD staining in Nothobranchius rachowi in vivo. These improvements resulted in a highly reliable SCD procedure in Nothobranchius rachowi, with a low level SCE frequency. Induction of SCEs and CA in haemopioetic tissue of a marine fish following in vivo exposure to genotoxicants was reported by Maddock et.al.(1986). It should be remembered that BrdU incorporation is always low in vivo, because of limited uptake and metabolism compared to in vitro conditions. Wrisberg and Van der Gaag(1992) investigated the genotoxicity of waste water from a wheat and rye straw pulp mill using SCEs and micronuclei.

Micronuclei (MN) assays in fish have been shown to be useful in vivo techniques for genotoxicity testing. The review by Al-Sabti and Metcalfe(1995) is an excellent one directed to assist laboratories in the development of fish genotoxicity assays for water quality monitoring. A summary of the various techniques that have been used for micronucleus analysis in fish is also included. Micronuclei in fish could be smaller in size, because most fish chromosomes are much smaller than mammalian chromosomes.(e.g. 1/10 to 1/30 of the main nucleus). So scoring criteria needs to be modified accordingly. Advantages over metaphase analysis are, (1)it is not dependent on a suitable karyotype, (2), it permits easier and faster scoring. Sanchez-Galan et.al.(1998) assessed the value of micronucleus test as an in situ indicator of environmental status of freshwater ecosystems by comparing its expression in kidney cells in wild brown trout populations from contrasting

ecosystems. The novel approach to genotoxicity testing using binucleated cytokinesis-blocked erythrocytes in fish could become an important part of the programs for detecting the genotoxic potential of waterborne pollutants in the laboratory and in natural aquatic ecosystems. However, considerable development is required before this technique can be used routinely in monitoring programmes.

Although it is clear that progress in any field depends primarily on the development of methodology, in general, the basic principles of fish CA, SCE and MN methods are the same as in mammalian cytogenetics. Intraspecific differences in xenobiotic metabolism, DNA repair, cell proliferation in the target organ and environmental conditions(temperature) are factors that may affect the sensitivity of fish species to genotoxicity. Considerable work is needed to standardise assay procedures. The fact that the studied species may not be readily available in areas where studies are desired may account for the apparent reluctance to pursue this promising approach to aquatic genotoxicology. It is surprising that very few attempts in India to develop other fish, crustacean or bivalve species as models have been reported. In the Indian context, there is an urgent need to develop few representative species of aquatic organisms as appropriate model systems to monitor the aquatic environment including the marine ecosystems.

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