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# CHROMOSOMAL MANIPULATION IN FISHES

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## Introduction

The research on chromosomal manipulation in fish has a little history compared to the vertebrate animals and crops. Since 1943, early attempts were initiated, and until recently various techniques have been developed to interfere with normal functioning of the metaphase spindle apparatus during nuclear cycles of cell division in fish eggs using several causal agents, both physical and chemical. As a result, individuals with differing genomic status, viz. polyploids (triploid and tetraploid), gynogenetics (both meiotic and mitotic gynogens) and androgenetics are being produced in fish population.

Fishes are different from other animals that are raised on farms because, for the most part fertilization occurs externally and embryological development takes place without the protection of an internal womb or a hard-shelled egg. This combination enables aqua-culturists to manipulate fertilized eggs or embryos (fry) to an extent not possible with mammals or birds.

Because fertilization occurs externally for most species of fish, it is relatively easy to manipulate chromosome number. A number of techniques have been developed to produce haploid, triploid and tetraploid fish and even to produce fish whose chromosomes come solely either from their mothers (gynogens) or from their father (androgens).

The techniques that are used to alter the chromosome number are similar and can be divided into three categories, temperature, pressure and chemical shocks. Most researchers use either pressure or temperature. These physical shocks are applied to eggs that are newly fertilized. The exact timing and duration of the shock, relative to both meiosis (extrusion of the second polar body) and mitosis (first cleavage-when the zygote divides to become a two-celled embryo), and the exact temperature or pressure determine not only the success rate but

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the type of chromosomal manipulation. Pressure chambers are used to apply pressure shocks. These devices range from cylinders attached to a mechanically operated screw-press, these controlled by computers.

These are several techniques that can be used to assess the success of chromosomal manipulation, which are as follow –

- One method is to count chromosomes.
- Second method is to examine the R.B.C. (erythrocyte) size.
- Third method is to examine erythrocyte nuclear volume.
- Fourth method is to determine DNA content i erythrocyte nuclei.
- Fifth method is to stain and count nucleoli.
- Sixth technique is to use electrophoresis.

Chromosomal manipulation may produce triploids, tetraploids, gynogenetic and androgenetic.

(1) **TRIPLOIDS:** The creation of triploid is the type of chromosomal manipulation. Triploids are created by shocking newly fertilized eggs to prevent extrusion of the second polar body. The fertilized egg thus contains an egg haploid nucleus a sperm haploid nucleus and a second polar body haploid nucleus which creates a triploid. Triploid have been created in many species, including *Heteropneustes fossilis* (Singhi), *Cyprinuscarpio* (common carp), *Ctenopharingo donidella* (grass carp), *Labeo rohita* (rohu), *Clarias gariepinus* (African catfish), *Oncorhynchus mykiss* (Rainbow trout), *Onchorhynchus gorbuscha* (Pink salmon), etc.

It is known that triploid fish have a great potential for commercial exploitation in aquaculture. Apart from that, they serve as an attractive model for basic research. As triploid fish are with abnormal physiological features, they provide a unique potential for discovery of new biochemical and molecular mechanisms in basic fish physiology. Future study needs data on various field performances of triploid fish before their commercial exploitation.

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(2) **TETRAPLOIDS:** Tetraploids are fish that have four sets of chromosomes instead of the normal two sets. Tetraploids can be created by shocking a diploid zygote when it is undergoing first cleavage. This prevents nuclear division so, the nucleus becomes 4n, and the zygote is a tetraploid. Tetraploid *Danio rerio* (Zebrafish), *Oreochromis niloticus* (Nile tilapia), *Oncorhynchus mykiss* (Rainbow trout), *Ictalurus punctatus* (Channel catfish), *Ctenopharyngo donidella* (Grass carp), *Labeorohita* (Rohu), *Catlacatla* (Catla/Bhakur) etc, have been produced.

The major interest in the creation of tetraploids is that they could be used to produce interploid triploid, which could eliminate the need to continually create triploids manually. Tetraploids are mates to diploids, and the ensuring progeny are interploidtriplids.

(3) **GYNOGENS:** These are fish that are engineered so, that both sets of chromosomes come from the mother. Gynogens are produced for several reasons. The first is to produce a monosex female population. If the species has the XY sex-determing system, female are XX, so, all gynogens will be females. Gynogens are produces in one of the two ways –

(i) Eggs are activated with sperms which have had their genetic material destroyed by X-rays, Gamma rays or UV irradiation. Shortly after activation, the egg is shocked to prevent the second leaving. The egg now contains two haploid (n) nuclei, the egg nucleus and the second polar body nucleus. These are sets of chromosomes originated in the mother.

(ii) The second technique being the same way. The first step is to activate eggs with sperm which have had their genetic material destroyed by irradiation. This will produce a gynogenetic haploid. When the haploid zygote undergoes first cleavage, the embryo is shocked, and this prevent cell division. The two haploid nuclei fuse to form a 2n zygote and both sets of chromosomes come from the mother.

Gynogenesis is a valuable tool for producing unisex populations, for locating and selecting recessive genes and for studying linkage and crossing over. Gynogenic *Labeorohita* (Rohu), *Catlacatla* (Catla/Bhakur), *Ctenopharyngo donidella*(Grass

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carp), *Cyprinus carpio* (Common carp), etc. have been produced by using this technique.

(4) **ANDROGENS:** Androgens are the opposite of gynogens. These are those fish whose chromosomes come solely from the father. Androgens can be produced by one of two methods. The most commonly used method is to fertilize eggs whose genetic material has been destroyed by gamma rays or x-rays with normal sperm. This produces a haploid androgenetic zygote undergoes first cleavage, it is shocked to prevent cell division and the two haploid nuclei fuse to form a diploid nucleus. All chromosomes in these fish come from the father. Androgenetic *Onchorhynchus mykiss* (Rainbow trout), *Salvelinus fontinalis* (Brook trout), *Ctenopharyngo donidella*(Grass carp) have been produced in this manner.

The second technique that can be used to produce androgens is to fertile eggs whose genetic material has been destroyed by irradiation with sperm from tetraploid males. Tetrploid males produce diploid sperm, consequently, the sperm pronucleus is diploid rather than haploid, which means the ensuring zygote will also be diploid.

(5) **HAPLOIDS:** Haploids are individuals that have only a single set of chromosomes. There are two ways to create haploids, and both involve the destruction of genetic material from one of the parents. One way is to "fertilize" eggs with sperm that have had their genetic material destroyed by x-ray, gamma rays, chemicals or UV irradiation. UV irradiation is probably the best method to destroy sperm DNA.

The second way to create haploids is to destroy the genetic material in eggs with x-rays or gamma rays and to fertilize these eggs with normal sperm. Because the sperm's haploid nucleus is the sole contributor to the zygote's genome, the embryo is a haploid. In this case, the haploid embryo is an androgenetic haploid, because all alleles come from the father.

Haploids are interesting because they enable breeders to study certain embryological genetic processes. On a practical level, it is important to know how to produce haploids, because that is the first step in the production of gynogenetic and androgenetic fish. Haploid *Heteropneustes fossilis* (Singhi) have been produced in this manner.

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UV light has been successfully used in irradiating eggs of *Axipensetrans montanus* (Sturgeon), *Cyprinus carpio* (Common carp), *Oreochromis niloticus* (Nile tilapia), *Misgurnusan guillicaudatus* (Loach), *Clarias gariepinus* (African catfish). UV irradiation is easy to use anywhere, inexpensive and safer to apply. Haploid androgenesis has been induced in the *Misgurnus anguillicaudatus* (Loach), *Oncorhyn chusmasou*, *Onchorhynchus mykiss* (Rainbow trout) and *Salvelinus fontinalis* (Brook trout).