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Manipulation of chromosomes in fish : review of various techniques and their implications in aquaculture

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Abstract

Human ingenuity has made it possible to advent the chromosome manipulation techniques to produce individuals with differing genomic status in a number of fish using various causal agents such as physical shocks (temperature or hydrostatic pressure), chemical (endomitotics) and anaesthetic treatments either to suppress the second meiotic division shortly after fertilization of eggs or to prevent the first mitotic division shortly prior to mitotic cleavage formation. This results in the induction of polyploidy (triploidy and tetraploidy), gynogenesis (both meiotic and mitotic leading to clonal lines) and androgenesis in fish population. The rationale for the induction of such ploidy in fish has been its potential for generating sterile individuals, rapidly inbred lines and masculinized fish, which could be of benefit to fish farming and aquaculture. In this paper, these are critically reviewed and the implication of recently developed chromosome manipulation techniques to various fin fishes is discussed.

Key words : Cell divisions, Fish eggs, Polyploidy, Gynogenesis, Androgenesis

Introduction

Chromosome manipulation research has a short history in fish compared to that of crops and animals. 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.

Among polyploids, triploid individuals are expected to be functionally and endocrinologically sterile due to their meiotic inhibition of gametogenesis and lack of essential steroid hormone levels to support gonadal growth. Such sterility in triploid fish can be of advantage to aquaculture where control of reproduction and population is desirable. The production of putative tetraploids might have tremendous impact, because of promising future of large scale production of genetically sterile fish (triploids) population lies with the mating of normal diploid and tetraploid individuals.

Induction of two types of gynogenesis has its potential in generating nearly all female and all homozygous individuals in a single generation. Probable

application of meiotic gynogenetics in aquaculture relates to coupling with sex inversion using androgen hormones to produce all outbred monosex female population where growth rate of females are superior to males particularly in salmonids and cyprinids. Mitotic gynogenetics have limitations to use them directly for culture but they are potential and valuable as completely homozygous broodstock to produce second generation of clonal lines. Production of viable androgenetic diploids in fish has its great impact on commercial application of genetic masculinization technique to replace hormonal sex-reversal. Present review highlights mainly on the various chromosome manipulation techniques which could easily be used to suppress cell divisions in fish eggs and their probable implications to the cultured species to benefit aquaculture.

Suppression of meiotic events of cell division in fish eggs

Table 1 shows a brief review of suppression of second meiotic events of cell division in the fertilized eggs of various fishes.

Induction of triploidy

Triploidy is induced directly by blocking of second polar body extrusion during second meiotic division shortly after fertilization of fish eggs using various physical shocks and chemical treatments. The mating of normal diploid and tetraploid fish is an alternative method for producing hybrid triploids.

Many workers successfully used both temperature (cold and heat) and pressure stocks as effective agents in inducing triploidy in several fishes. In a thorough investigation in Nile tilapia, *Oreochromis niloticus*, conducted by Hussain *et al.* (1991) and Hussain *et al.* (1995), the cold shock survivals showed a lot of inter female difference despite the uniformly high control and pressure shocks survivals. Heat shock showed lower inter-individual variation than was found for cold, but on average were not as great as pressure in optimizing triploid yields. A similar situation following temperature shock for the induction of triploids, in the use of heat to produce triploid Atlantic salmon has been reported where, because of extreme variability in response to the same heat shock treatment of eggs from different females, pressure shock was the preferred method of triploidization (Benfey and Sutterlin 1984, Benfey *et al.* 1988, Johnstone 1989).

The use of chemical treatments such as cytochalasin B; colchicine (Refstie *et al.* 1977, Allen and Stanley 1979, Smith and Lemoine 1979) and anaesthetics such as nitrous oxide and Freon 22 (Sheldon *et al.* 1986, Johnstone *et al.* 1989, Santiago *et al.* 1992) to induce retention of the second polar body in several fishes have also been reported. Thorgaard (1983) suggested, in view of the success and ease of temperature and pressure shock treatments for inhibiting cell division in fish, chemical treatments may not be the method of choice. They are probably less adaptable to mass production than other methods.

Table 1. Suppression of second meiotic events of cell division in the fertilized eggs of various fishes

Fish species	Causal agents	Intensity level	Induction window	Ploidy status	Authors
Oreochromis niloticus	HS	41°C for 3.5 min	5 min a.f.	Triploid	Hussain *et al*. (1991)
Oreochromis niloticus	PS	8000 p.s.i. for 2 min	9 min a.f.	Triploidy	Hussain *et al*. (1991)
Oreochromis niloticus	CS	9°C for 30 min	7 min a.f.	Triploidy	Hussain *et al*. (1991)
Oreochromis niloticus	HS	41°C for 3.5 min	5 min a.f.	Meiotic gynogen	Hussain *et al*. (1993)
Oreochromis niloticus	PS	8000 p.s.i. for 2 min	9 min a.f.	Meiotic gynogen	Hussain *et al*. (1993)
Oreochromis niloticus	HS	41°C for 3.5 min	5 min a.f.	Meiotic gynogen	Mair (1988)
Oreochromis aureus	CS	11°C for 60 min	15 min a.f.	Triploidy	Valenti (1975)
Oreochromis mossambicus	HS	41.7°C for 3 min	32-54 min a.f.	Meiotic gynogen	Varadaraj & Pandian (1990)
Salmo salar	HS	32°C for 5 min	20 min a.f.	Triploidy	Benfey & Sutterlin(1984)
Salmo salar	PS	10150 p.s.i. for 6 min	20 min a.f.	Triploidy	Benfey & Sutterlin(1984)
Salmo salar	NO	11 amt for 30 min	0-30 min a.f.	Triploidy	Johnstone (1989)
Salmo gairdneri	HS	36 °C for 1 min	10 min a.f.	Triploidy	Thorgaard *et al.* (1981)
Salmo gairdneri	PS	7000 p.s.i. for 4 min	40 min a.f.	Triploidy	Chourrout (1984)
Salmo gairdneri	PS	7000 p.s.i. for 4 min	40 min a.f.	Meiotic gynogen	Chourrout (1984)
Clarias gareipinus	CS	5 °C for 40 min	2-4 min a.f.	Triploidy	Richter *et al*. (1987)

HS = Heat Shock, PS = Pressure Shock, CS = Cold Shock, NO = Nitrous Oxide.

Triploid individuals are expected to be functionally and endocrinologically sterile. Such sterility of triploid fish (both male and female) can be of benefit to aquaculture. The blocking of complete gametogenesis particularly in female triploids during early meiotic division results in complete inhibition of oocyte development and functional sterility (Thorgaard and Gall 1979, Richter *et al.* 1987, Hussain *et al.* 1994a, 1995). Despite gametic sterility triploid males due to meiotic inhibition of spermatogenesis, in fish species a proportion of such males are able to produce abnormal and aneuploid sperm. This ultimately leads to reproductive sterility of these males (Swarup 1957, Richter *et al.* 1987, Hussain *et al.* 1994a and 1995). Therefore, it was suggested that triploid males could be introduced into a wild population where suppression of natural reproduction of undesirable wild female fish (*viz. Oreochromis* spp.) is required to control their overpopulation, as any mating between the sterile male triploid and any females would result in inviable eggs and a reduction in recruitment. Such method can experimentally be applied in some of the Asian reservoirs

Induction of meiotic gynogenesis

In the process of meiotic gynogenesis, eggs are fertilized with UV irradiated sperm and then are exposed to a variety of physical shocks or chemical treatments which suppress the anaphase stages of second meiotic division by disruption of metaphase spindles, resulting artificial diploidization of maternal chromosome complement (retention of second polar body) of eggs (Purdom 1983).

The gynogenesis by the suppression of meiotic events has been induced since 1960 in numerous fish. At present there are few direct applications of gynogenesis in aquaculture because the fish are inbred and have a reduced variability compared to normal diploids. It has been commonly suggested that gynogenesis induction should be coupled with sex inversion such that functional XX all males could be produced (Nagy 1987, Thorgaard and Allen 1987). Such sex-reversed gynogenetic males are thought to be useful in crossbreeding experiments to produce all outbred monosex female population where the growth rate of females are superior to males particularly in salmonids and cyprinids. Presently sex-reversed gynogenetic males are being widely used in China for line breeding and genetic improvement of common carp and some other commercial strains (Prof. Wu Chingziag, personal communication). Roongrati *et al.* (1994) reported a model of producing all-female Thai silver barb, *Puntius gonionotus*, using the techniques of gynogenesis and sex reversal (Fig. 1). Attempts are being undertaken for mass scale production of such fish in Bangladesh and Thailand under the auspices DFID/Stirling University, Scotland for augmenting production of *P. gonionotus* in the rice fields and seasonal water bodies.

Suppression of mitotic events of cell division in fish eggs

Suppression of first cleavage of mitotic events of cell division has alraedy been carried out in a number fish species, a summary of these works is presented in Table 2.

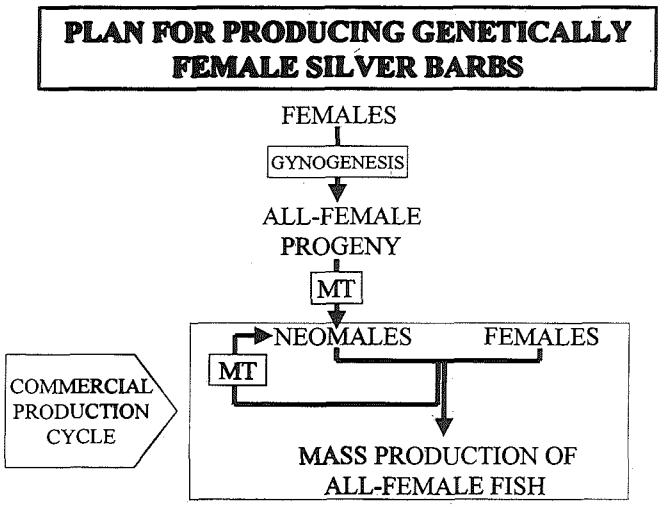

Fig. 1. A schematic model for commercial production cycle of producing all-female Thai silver barb, *Puntius gonionotus*.

Induction of tetraploidy

Several agents, both physical and chemical, that disrupt the first mitotic division shortly prior to formation of cleavage farrow, therefore, leading to the generation of tetraploid individuals having extra sets of chromosomes. Chourrout *et al.* (1986), who obtained fertile tetraploid male and female rainbow trout and successfully crossed tetraploid male and diploid females to produce viable triploids, although tetraploids have been produced in other fish species (Bidwell *et al.* 1985, Valenti 1975, Myers 1986, Pandian and Varadaraj 1987, Don and Avtalion 1988, Mair 1988). The aforementioned authors used various physical agents such as cold, heat and hydrostatic pressure shocks to induce tetraploidy in fish. Of the three shock treatments, pressure has been found most effective at blocking first mitotic cleavage in rainbow trout (Chourrout *et al.* 1986). Production of viable tetraploid individuals might have tremendous impact in aquaculture.

Induction of mitotic gynogenesis

In this process, putative gynogenetic progeny derive by the artificial diploidization of the maternal chromosome complement due to prevention of mitotic cleavage. The main rationale for gynogenesis induction in fish has been its potential for generating rapidly inbred lines. Han *et al.* (1991) suggested that homozygous inbred lines will never be produced by using meiotic gynogenetic diploids, even when reproduction is repeated for several generations. Therefore, induction of diploid gynogenesis by inhibition of first cleavage at mitotic division of a zygote might be more promising method for producing inbred lines which will be homozygous at every gene locus (Chourrout 1984, Streisinger *et al.* 1981, Hussain *et al.* 1994b).

The first example of the production of viable mitotic gynogenetics in a fish, *Brachyndanio rerio*, was that of Streisinger *et al.* (1981). The level of temperature or pressure shocks required to suppress first mitotic cleavage is the same as or

Table 2. Suppression of first mitotic events of cell division in the fertilized eggs of various fishes

Fish species	Causal agents	Intensity level	Induction window	Ploidy status	Authors
Oreochromis niloticus	Heat shock	41°C for 3.5 min	27.5-30 min a.f.	Mitotic gynogen	Hussain *et al.* (1993)
Oreochromis niloticus	Pressure shock	9000 p.s.i. for 2 min	40-50 min a.f.	Mitotic gynogen	Hussain *et al.* (1993)
Oreochromis niloticus	Heat shock	41°C for 3.5 min	25-35 min a.f.	Mitotic gynogen	Mair (1988)
Oreochromis niloticus	Pressure shock + Cold shock	7000 p.s.i. for 7 min + 7.5°C	57-60 min a.f.	Tetraploidy	Myers (1986)
Oreochromis aureus	Heat shock	41°C for 3.5 min	25-35 min a.f.	Mitotic gynogen	Mair (1988)
Oreochromis aureus	Cold shock	11°C for 60 min	92 min a.f.	Tetraploidy	Don & Avtalion (1988)
Cyprinus carpio	Heat shock	40°C for 2 min	28-30 min a.f.	Mitotic gynogen	Komen *et al.*(1991)
Cyprinus carpio	Heat shock	40°C for 2 min	24-28 min a.f.	Androgenesis	Bongers *et al.* (1993)
Salmo gairdneri	Pressure shock	7000 p.s.i. for 4 min	5 hr 50 min a.f.	Tetraploidy	Chorrout (1984)
Brachyndanio rerio	Heat shock	41°C for 2-3 min	13 min a.f.	Mitotic gynogen	Streisinger *et al.* (1981)
Brachyndanio rerio	Pressure shock	8000 p.s.i. for >1min	24-28 min a.f.	Mitotic gynogen	Streisinger *et al.* (1981)
Oryzias latipes	Heat shock	41°C for 2 min	85-95 min a.f.	Mitotic gynogen	Naruse *et al.* (1985)
Oryzias latipes	Pressure shock	10000 p.s.i. for 2 min	85-95 min a.f.	Mitotic gynogen	Naruse *et al.* (1985)
Plecoglossus altivelis	Pressure shock	9280 p.s.i. for 6 min	80 min a.f.	Mitotic gynogen	Taniguchi *et al.* (1988)

close to the level for inhibiting meiotic events. Recently, the technique has been applied successfully to common carp, *Cyprinus carpio* (Nagy 1987, Komen *et al.* 1991), medaka, *Oryzias latipes* (Iziri 1987), ayu, *Plecoglosus altivelis* (Taniguchi *et al.* 1988); Nile tilapia, *O. niloticus* (Mair *et al.* 1987, Hussain 1992, Hussain *et al.* 1993)); rainbow trout, *Onchorhynchus mykiss* (Quillet *et al.* 1991); channel catfish, *Ictalurus punctatus* (Goudie *et al.* 1991) and Asian carp, *Labeo rohita* (Hussain *et al.* 1997).

Despite the first generation of mitotic gynogenetics have limitations to use them directly for culture but they are potential and valuable as completely homozygous broodstock to produce second generation of clonal lines in fish.

Induction of androgenesis

Androgenesis is a genome manipulation technique which is the reverse of gynogenesis. It involves a genetically inactivated egg fertilized with normal sperm, resulting in the embryonic development with entirely parental chromosomal inheritance without any contribution from maternal genome.

The first androgenetic diploids in salmonids were produced by the suppression of first cleavage using pressure shock (Parsons and Thorgaard 1985, Scheerer *et al.* 1986, May *et al.* 1988) and later by heat shock (Thorgaard *et al.* 1990). The induction of androgenesis in fish could be useful in producing all male population in tilapia and some other fish to replace hormonal sex reversal (Myers, pers. communication). Another possible application of androgenetic diploids lies in recovering genotypes from cryopreserved sperm, which is important as egg and embryo cryopresrvation that has not yet been succeeded.

Production of clonal lines

Viable clonal or inbred lines in fish could be produced either by crossbreeding between a viable mitotic gynogenetic female and male (recessive mutation in a sex determining gene) or by gynogenetic reproduction (retention of 2nd polar body) of mitotic gynogenetic diploid(s) using optimal physical shocks (pressure, heat, cold etc.) or chemical treatments. Purdom and Lincoln (1973) pointed out that to produce inbred lines by conventional methods of sib-mating requires the maintenance of several lines with close inbreeding up 10 - 20 generations. But gynogenesis especially by inhibiting first mitotic division could dramatically shorten the time required to produce completely homozygous progeny in the first generation and an "inbred" or "clonal" lines with the unique gene combinations in the second (Komen *et al.* 1991, Hussain 1992, Hussain *et al.* 1994[b]).

Mitotic gynogenesis has already been induced in a number of fishes by several authors, but until recently, clones have only been produced in zebra fish (Streisinger *et al.* 1981); medaka (Naruse *et al.* 1985, Ijiri 1987); Common carp (Komen *et al.* 1991), ayu (Han *et al.* 1991), Nile tilapia (Hussain and McAndrew 1998) and Asian carps (Hussain *et al.* 1987).

Majority of these workers observed that clonal lines produced in the aforementioned fishes were mostly free of recessive, deleterious, low penetrance alleles and similar to the starting population. It was also appeared that the heterozygous clonal lines rather than the homozygous lines were preferred for the production of more viable and vigorous populations (Komen *et al.* 1991, Hussain and McAndrew 1998). It is expected that such vigorous clones will also be of great use as a pure "gene pool" for many genetic studies such as cell line and tissue culture, genetic fingerprinting, immunological, disease resistance, heritability and sex differentiation studies as well as developing breeding schemes based on the exploitation of heterosis.

Conclusions

In the recent past, the inland capture fisheries has registered a gradual decline in most of the countries of this region due to deterioration of aquatic environment, and partly or mostly, because of physical reduction of aquatic habitat. These have resulted in the threat of shrinking genetic base, causing the genetic vulnerability of fishes. Consequently, it gradually losses genetic diversity, which in turn, brings a havoc change in genetic stock structure of natural fish population. While the governments of most of the countries of this part of the world are seriously making all out efforts to rehabilitate the inland fisheries, they have focused their attention recently on aquaculture, which has tremendous opportunities in Asia. Therefore, apart from or in addition to the results of chromosome manipulation research of previous and present time, all the fishery biologists and geneticists should intense their attention to give more stress on further breeding plans and genetic studies to develop better breeds and improved stocks of commercially important cultured fish species to be useful for increasing sustainable aquaculture production.

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