Advances in Chromosome Engineering Research in Fish: Review of Methods, Achievements and Applications

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Abstract

Chromosome engineering research in fish has been conducted in a number of countries during the past and present decades. The techniques developed so far interfere with the normal function of the metaphase spindle during the nuclear cycles of cell division and alteration of gonial DNA structure in fertilized eggs, therefore, leading to the induction of polyploid (triploid and tetraploid), gynogenetic (haploid, meiotic and mitotic gynogens and clones) and androgegetic individuals. The rationale for the induction of such ploidy with differing genomic status in a number of fish is its potential to generate genetically sterile populations and rapidly inbred lines, which could ultimately benefit aquaculture. These approaches are critically reviewed and discussed.
Introduction

In recent years, there has been a rapid increase in the application of chromosome engineering techniques to various finfishes, either to improve cultured species or for research purposes. Many agents, such as physical shocks (temperature and hydrostatic pressure), chemicals (such as endomitotics) and anesthetics (such as nitrous oxide and freon 22) can interfere with the normal functioning of the spindle apparatus during cell division in fish eggs. This can lead to production of polyploid (triploid and tetraploid), gynogenetic and androgenetic individuals.

To be of benefit in aquaculture, triploid individuals are expected to be functionally and endocrinologically sterile, since maturation processes often have profound and, ultimately, limiting effects on growth. Triploids are commonly produced directly by the induction of second polar body retention during the second meiotic division of newly fertilized eggs, but may also be generated indirectly by crossing tetraploid and diploid individuals. The production of triploids from tetraploid x diploid matings could be invaluable in species such as tilapia and other fish where direct production of sterile triploid populations from diploid females and males is limited due to poor fecundity.
The production of gynogenetic individuals is of particular interest to fish breeders because a high level of inbreeding can be induced in a single generation. Meiotic gynogenesis may be used to develop partly or mostly inbred strains, to produce all-female populations, or to analyze traits such as sex-determination mechanisms in fish. Mitotic gynogenesis can produce completely homozygous individuals which in turn can be used to establish inbred or outbred clonal lines. Clones have great potential for fixing novel and superior genes in a line which could be used as a pure "gene pool" for genetic improvement of fish stocks.

Induction of androgenesis can produce all-male populations in fish which would have great advantages for commercial applications in tilapia and other fish to replace hormonal sex-reversal. Another direct application of androgenetic diploids may lie in recovery of genotypes from cryopreserved sperm. This is important as cryopreservation of eggs and embryos has not yet been successful in fish.

This review discusses advances in chromosome engineering research particularly those involving various procedures in inducing polyploidy, gynogenesis and androgenesis in fish; and explores the potential application of these techniques in breeding and farming a number of commercial fish species.

**Polyploidy**

Polyploidy is a genome manipulation technique which involves the production of individuals with extra set(s) of chromosomes, using physical or chemical treatments either to suppress the second meiotic division shortly after fertilization of eggs (triploidy induction), or to disrupt the first mitotic division prior to mitotic cleavage formation (tetraploidy induction). A schematic diagram of inducing polyploidy in fish using pressure, heat and cold shock treatments is shown in Fig. 1. Controlled use of these techniques has been applied to a variety of fish species in recent years (for review, see Purdom 1983; Chourrout 1987; Ihsen et al. 1990; Mair 1993).

Early attempts to induce triploidy in fish using cold shock were reported and observed in common carp *Cyprinus carpio* by Makino and Ozima (1943); and in Atlantic salmon *Salmo salar* by Svardson (1945). Swarup (1959a, 1959b) was the first to successfully induce triploidy in the stickleback *Gasterosteus aculeatus* by cold and heat shocks; and to rear triploid fish up to the mature stage. In the sturgeon *Acipenser gueldenstadtii*, both triploidy and tetraploidy were induced by heat shock by Vasetskii (1967). Following this, induction of triploidy in fish by retention of the second polar body was carried out by many workers. Cold shocks (Valenti 1975; Chourrout 1980; Don and Avtalion 1988a; Hussain et al. 1991), heat shocks (Chourrout 1980; Chourrout and Itskovich 1983; Johnstone 1985; Don and Avtalion 1986, 1988a; Varadaraj and Pandian 1990; Hussain et al. 1991), hydrostatic pressure shocks (Chourrout 1984; Allen and Myers 1985; Benfey et al. 1988; Lincoln 1989; Hussain et al. 1991), chemicals (Refsite et al. 1977; Allen and Stanley 1979; Allen et al. 1982) and anesthetics (Sheldon et al. 1986; Johnstone et al. 1989) have all been successful.
Fig. 1. A schematic diagram of inducing polyplody (triploidy and tetraploidy) in fish using pressure, heat and cold shocks. n = haploid chromosome set, 2n = diploid chromosome set, 3n = triploid chromosome set, 4n = tetraploid chromosome set.
Sublethal, long, cold or short heat shock treatments applied shortly after fertilization of eggs with normal sperm have been found to be the most convenient and easiest way of inducing triploidy in cold and warmwater fish. Cold shocks involving temperatures below 4°C effectively induced triploid individuals in channel catfish *Ictalurus punctatus* (Wolters et al. 1981, 1982). Moderate cold shocks (5-15°C) were equally effective at producing triploid tilapia (Valenti 1975; Don and Avtalion 1988a) and grass carp (Cassani and Caton 1985). In salmonids, cold shocks have been less effective in inducing triploidy (Chourrout 1980; Thorgaard et al. 1981; Purdom 1983). The reason might be either that the physical shock treatments had species-specific effectiveness or are less efficient in suppressing meiotic cell division in coldwater fish. Recently in Asia, Manickam (1991) and Na-Nakorn and Legrand (1991) successfully used cold shock to induce triploidy in Asian catfish *Clarias batrachus* and silver barb *Puntius gonionotus*.

According to Nagy (1987), heat shock is more effective than cold shock for coldwater fish. Chourrout and Quillet (1982) obtained high proportions of surviving triploid rainbow trout after heat shock treatments. Short heat shocks (3-4 minutes) at temperatures just below the lethal level (39.5-42.0°C) applied shortly (2.5-5 minutes) after fertilization of eggs efficiently suppressed the second meiotic division to produce nearly 100% triploid *Oreochromis* spp. (Chourrout and Itskovich 1983; Don and Avtalion 1986; Hussain et al. 1991).

Hydrostatic pressure shock was an effective agent in inducing triploidy in fish (Chourrout 1984; Hussain et al. 1991). Lincoln (1989) stated that pressure shock has some advantages over heat. Pressure shock affects all the eggs uniformly, giving rise to high triploid yields and lower embryo mortalities. This is not always achieved with heat because a large volume of eggs impair heat transfer, which may lead to reduced triploidy rates in rainbow trout.

The primary interest in induced triploid fish involves their sterility and increased chromosome complement which may affect phenotype and life expectancies, especially growth and survival in mature stages (Thorgaard 1986; Nagy 1987). A few authors reported an improved growth rate in triploid fish compared to diploids after maturation (Thorgaard and Gall 1979; Wolters et al. 1982); whereas others observed either no significant difference in juveniles (Gold and Avise 1976; Gervai et al. 1980) or even inferior growth rates at early stages (Penman et al. 1987). In adult triploids (*Pleurodeles platessa* × *Platichthys flesus* hybrids), higher fillet weights and dress-out weights were recorded by Lincoln (1981a). Some other differences, such as better food conversion efficiencies and lower condition factors of triploid fish compared to those of diploids, have been described (Wolters et al. 1982; Benfey and Sutterlin 1984).

Blocking complete gametogenesis, particularly in female triploids during early meiotic division, completely inhibits oocyte development and functional sterility (Thorgaard and Gall 1979; Lincoln 1981b; Wolters et al. 1982; Richter et al. 1987; Hussain et al., in press). Despite gametic sterility in triploid males due to meiotic inhibition of spermatogenesis, a proportion of such males in fish
species are able to produce abnormal and aneuploid sperm. This ultimately leads to reproductive sterility of these males (Swarup 1957; Lincoln 1981c; Wolters et al. 1982; Richter et al. 1987; Hussain et al., in press). There is evidence that although triploid males are sterile, they become sexually mature exhibiting endocrine profiles and secondary sexual characteristics similar to normal mature diploids (Lincoln and Bye 1987; Benfey et al. 1989; Hussain et al. 1995).

Such sterility of triploid fish (both male and female) can be of benefit in aquaculture. Thorgaard (1986) stated that sterility is advantageous when control of reproduction and population is desirable. Sterile grass carp have been stocked to control vegetation and to prevent natural reproduction in many water bodies (Wattendorf and Anderson 1986). The use of sterile salmon triploids has been suggested for cage culture as a way to minimize gene introgression and the threat to wild stocks (Anon. 1989a; 1989b; cited by IJssten et al. 1990). Therefore, it was suggested that as triploid males produce no sperm or aneuploid sperm, they could be introduced into a wild population to suppress the natural reproduction of undesirable wild, female fish (viz. Oreochromis spp.) in order to control their overpopulation, as any mating between sterile male triploids and any females would result in inviable eggs and a reduction in recruitment. This method may be applied experimentally in some Asian reservoirs, particularly the Kaptai Reservoir of Bangladesh where carp production has recently been reduced dramatically due to the accidental introduction and overpopulation of O. niloticus (M. M. Hussain, pers. comm.).

Mating normal diploid and tetraploid fishes is an alternative method for producing sterile hybrid triploids. Chourrout (1984) stated that direct production of triploids from diploid x tetraploid matings would be invaluable in species such as tilapia where direct production of triploids from diploid females and males is limited due to low fecundity and difficulty of artificial spawning. Therefore, a promising future for triploid fish production lies with viable tetraploid induction. It has been suggested that tetraploidy might be difficult to induce or it may be an inviable arrangement of chromosomes (Purdom 1983). However, it appears to have been at least partially successful in rainbow trout, Oncorhynchus mykiss, according to several authors (Thorgaard et al. 1981; Chourrout 1984; Chourrout et al. 1986; Diter et al. 1988). Chourrout et al. (1986) 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 species such as channel catfish I. punctatus (Bidwell et al. 1985) and Oreochromis spp. (Valenti 1975; Myers 1986; Pandian and Varadaraj 1987; Don and Avtalion 1988b; Mair 1988), none of these authors were able to produce viable tetraploids except Don and Avtalion (1988b). The aforementioned authors used various physical agents such as cold, heat and hydrostatic pressure shocks to induce tetraploidy. Of the three shock treatments, pressure (6,000-7,000 p.s.i.) has been most effective at blocking first mitotic cleavage in rainbow trout (Chourrout 1984; Chourrout et al. 1986).
Gynogenesis involves fertilizing eggs with inactivated sperm, and prevents any contribution of the male genome to the embryo. As a result, embryonic development proceeds with the inheritance of only maternal chromosome set(s). Artificial gynogenesis was first reported in the frog *Rana fusca* by Hertwig (1911), who demonstrated that if eggs were fertilized with sperm given increasingly higher dosages of gamma-radiation, the resulting embryos develop more normally than those fertilized with sperm irradiated with a lower dosage. This paradoxical phenomenon is known as the “Hertwig effect.”

The earliest observation of haploid gynogenesis in a fish, *Salmo trutta*, was first made by Oppermann (1913), who described a typical “Hertwig effect” of massive and delayed mortalities of embryos derived from eggs fertilized, respectively, with partially and completely inactivated sperm. Other workers also described an earlier study and development of parthenogenesis in Eurasian perch *Perca fluviatilis*, and roach *Rutilus rutilus* (Trifonowa 1931); shad *Paralosa lacustris* (Lestage 1934); northern pike *Esox lucius* (Kasansky 1934) and common carp *C. carpio* (Kasansky 1935).

Improved survival of viable gynogenetic diploids from eggs activated with X-irradiated sperm and exposed to cold shock treatments was first successfully demonstrated in common carp *C. carpio*, loach *Misgurnus fossilis* and sturgeon *A. ruthenus*, by Romashov et al. (1960) and Vassileva-Dryanovska and Belcheva (1965). Purdom (1969) and Tsoy (1972) were the first to induce gynogenetic diploids by suppressing the second meiotic division of salmonid eggs.

From the reports of most of the aforementioned authors, ionizing radiation (gamma or x-rays) effectively inactivates sperm, but because of residual paternal characteristics or chromosome fragments which may be present, it may not be the suitable technique for gynogenetic work (Ijiri 1980; Onozato 1984; Allen 1987).

Several authors also reported the use of chemical mutagens, such as diemethylsulphate and toluidine blue, to inactivate sperm in fish (Tsoy 1972; Chourrout 1986). Supernumary chromosome fragments were also detected in chemical mutagen treatment of rainbow trout *O. mykiss* sperm, but their frequency was much lower than gamma or x-rays (Chourrout 1986).

In this respect, ultraviolet (UV) light has been found to be suitable for sperm irradiation. Chourrout and Itskovich (1983), Mair et al. (1987) and Hussain et al. (1993) successfully induced gynogenesis in tilapia *O. niloticus* using UV irradiation of sperm, and did not find any residual chromosome fragments. The advantages of UV over other forms of irradiation are availability, transportability, ease of use, and safety (Allen 1987; Chourrout 1987).

According to Bohm (1891; cited by Ihssen et al. 1990), a mature fish egg completes first meiotic division before fertilization; therefore, only the remaining cell divisions can be manipulated. Viable gynogenetic progeny can thus be produced by artificial diploidization of the maternal chromosome complement (retention of the second polar body or inhibition of first cleavage) and several physical (such as long cold shocks, short heat shocks and short hydrostatic pressure shocks) and chemical (such as antimitotics) treatments are found
effective in fish (for review, see Purdom 1983; Chourrout 1987; Nagy 1987; Ihssen et al. 1990 and Mair 1993). A schematic diagram of inducing two types of gynogenesis (meiotic and mitotic) in fish is shown in Fig. 2. Long cold shocks were effective for inducing gynogenesis both in cold and warmwater fish. Gynogenetic diploids in loach Misgurnus anguillicaudatus, hirame Pralichthys olivaceus and common carp C. carpio, were successfully induced by cold shocks (0-1°C for 45-60 minutes) to eggs fertilized with irradiated sperm by Suzuki et al. (1985), Tabata et al. (1986) and Komen et al. (1988). Lincoln et al. (1974) reported that cold shocks below 0°C were not effective in salmonids. In contrast, cold shocks of < 0°C resulted in 33% gynogenetic diploid coho salmon O. kisutch (Refschie et al. 1982). Chourrout (1980) found this temperature to be partly effective in diploidizing gynogenetic rainbow trout.

Moderate cold shocks (4-15°C) were effective at inducing gynogenetic diploids in European catfish Silurus glanis (Krasznai and Marian 1987), Asian carp Labeo rohita (John et al. 1984) and silver barb P. gonionotus (Roongratri et al. 1984). Likewise, cold shocks, short heat and hydrostatic pressure shocks have been proven effective in restoring the viability of diploid gynogenetics in many fish species. Inhibition of second polar body formation by heat shock involves the dissolution of meiotic spindle fibers and suppression of cell membrane and cell proliferation (Allen and Stanley 1981). The high frequencies of diploid gynogenetic production in salmonids with heat shock (26-29°C for 10-20 minutes) were reported by Refschie (1983), Purdom et al. (1985) and Chourrout (1986).

Short heat shocks of sublethal level (35-42°C for 1.5-5 minutes) effectively diploidized the maternal genome in a number of warmwater fish (Streisinger et al. 1981; Chourrout and Itskovich 1983; Hollebecq et al. 1986; Mair et al. 1987; Don and Avtalion 1988b; Varadaraj 1990; Sugama et al. 1990a; Hussain et al. 1993).

Hydrostatic pressure shock (6,500-10,000 p.s.i. for 2-6 minutes) efficiently suppressed the anaphase stages of nuclear division by disrupting the metaphase spindle, resulting in diploidization of the chromosome set in eggs. The efficiency of hydrostatic pressure shock to induce gynogenesis was first demonstrated in zebra fish by Streisinger et al. (1981). After that, several authors reported gynogenesis using pressure shock (Benfey and Sutterlin 1984; Chourrout 1986; Ijiri 1987; Taniguchi et al. 1988; Hussain et al. 1993).

The use of chemical treatments such as cytochalasin B, colchicine (Refschie et al. 1977; Allen and Stanley 1979; Smith and Lemoine 1979) and anesthetics 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. In view of the success and ease of temperature and pressure shock treatments for inhibiting cell division in fish, Thorgaard (1983) suggested that chemical treatments may not be the method of choice. They are probably less adaptable to mass production than other methods.

The induction of gynogenetic diploids by artificial methods as discussed above is of interest for their practical use in inbreeding and producing all female population to develop modern breeding strategies for most commercially important fish species (Nagy et al. 1987). Gynogenesis induction could be
Fig. 2. A schematic diagram of inducing gynogenesis (meiotic and mitotic) in fish using pressure, heat and cold shocks. n = haploid chromosome set, 2n = diploid chromosome set.
coupled with sex inversion to produce XX all males which would be useful in crossbreeding experiments to generate all outbred monosex female population where growth rate is superior to males particularly in salmonids and cyprinids. Such technique is presently being applied in silver barb strains stock improvement programmes in Bangladesh and Thailand (D.S. Penman, pers. comm.). The suppression of second polar body extrusion would result in gynogenetic progeny heterozygous for many generations due to occurrence of recombination between chromatids during the first meiotic division of eggs (Purdom 1969; Nace et al. 1970).

The analysis of recombination data of several workers (see Table 1) reinforces the limitations of inducing meiotic gynogenesis in fish to produce completely homozygous inbred lines. Therefore, induction of diploid gynogenesis by suppression of first cleavage at meiotic division of a zygote might be a more promising method for producing inbred lines which will be homozygous at every gene locus (Streisinger et al. 1981; Chourrout 1984).

Table 1. Gene-centromere recombination rate estimated in gynogenetic diploids of different fishes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Loci (number)</th>
<th>Percent heterozygotes (average)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carp</td>
<td>Morphological and proteins</td>
<td>8-97 (35)</td>
<td>Cherfas (1977); Cherfas and Trueller (1978); Nagy et al. (1979); Nagy and Csanyi (1982); cited by Thorgaard (1983)</td>
</tr>
<tr>
<td>Carp</td>
<td>Morphological (12)</td>
<td>1-97 (45)</td>
<td>Reviewed by Komen (1990)</td>
</tr>
<tr>
<td>Plaice</td>
<td>Enzymes (5)</td>
<td>18-82 (44)</td>
<td>Thompson (1983; cited by Ihssen et al. 1990)</td>
</tr>
<tr>
<td>Zebra fish</td>
<td>Enzyme (1) Body color (6)</td>
<td>24-95 (66)</td>
<td>Streisinger et al. (1981, 1986)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Enzymes (10)</td>
<td>2-100 (55)</td>
<td>Thorgaard et al. (1983)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Enzymes (8)</td>
<td>11-100 (71)</td>
<td>Guyomard (1984)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Enzymes (4)</td>
<td>15.7-94.9 (62)</td>
<td>Thompson and Scott (1984)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Enzymes (25)</td>
<td>56</td>
<td>Allendorf et al. (1986)</td>
</tr>
<tr>
<td>Brown trout</td>
<td>Enzymes (12)</td>
<td>60-100 (88)</td>
<td>Guyomard (1986)</td>
</tr>
<tr>
<td>Brook trout</td>
<td>Enzymes (5)</td>
<td>6-50 (33)</td>
<td>Fujino et al. (1989)</td>
</tr>
<tr>
<td>Brook trout</td>
<td>Enzymes (17)</td>
<td>60-100 (81)</td>
<td>Ihssen (unpubl. data, cited by Ihssen et al. 1990)</td>
</tr>
<tr>
<td>Red sea bream</td>
<td>Enzymes (5)</td>
<td>19-87 (32)</td>
<td>Sugama et al. (1990b)</td>
</tr>
<tr>
<td>Nile tilapia</td>
<td>Enzymes (6)</td>
<td>0-100 (40.8)</td>
<td>Hussain et al. (1994a)</td>
</tr>
<tr>
<td>Nile tilapia</td>
<td>Body color (1)</td>
<td>20-100 (45)</td>
<td></td>
</tr>
<tr>
<td>Nitel tilapia</td>
<td>SDL-2 (1)</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Nile tilapia</td>
<td>Enzymes (2)</td>
<td>56.5-60 (58.3)</td>
<td>Mair et al. (1993)</td>
</tr>
</tbody>
</table>

Early attempts at inducing gynogenesis by restoring diplody at first mitosis of eggs in plaice *P. platessa* were unsuccessful (Purdom 1969). The first example of the production of viable mitotic gynogenetics in a fish, *Brachydanio rerio*, was that of Streisinger et al. (1981). After that, other workers also attempted to induce mitotic gynogenetics in fish with limited success, applying late cold shocks (Krasznai and Marian 1987); heat shocks (Purdom et al. 1985).
and pressure shocks (Chourrout 1984; Onozato 1984; Naruse et al. 1985). The level of temperature or pressure shock required to suppress first mitotic cleavage is the same as or close to the level for inhibiting meiotic events. Recently the technique has been applied successfully to common carp *C. carpio* (Nagy 1987; Komen et al. 1991), medaka *Oryzias latipes* (Ijiri 1987), ayu *Plecoglossus altivelis* (Taniguchi et al. 1988); Nile tilapia *O. niloticus* (Mair et al. 1987; Hussain et al. 1993); rainbow trout *O. mykiss* (Quillet et al. 1991); channel catfish *I. punctatus* (Goudie et al. 1991) and Asian carp *L. rohita* (Hussain et al. 1994b). Induction of mitotic gynogens in *L. rohita* is probably the first work in Asian carps which may lead to the development of inbred lines by gynogenetic reproduction and sex-reversal in the next generation.

Gynogenesis by blocking mitotic cleavage can dramatically shorten the time required to induce inbreeding, producing completely homozygous individuals in the first generation and clonal lines in the second. In contrast, homozygous inbred lines cannot be produced even after repeated meiotic gynogenetic reproduction or conventional methods of sib-mating for 10-20 generations. Clonal lines are supposed to be valuable products for improvement of fish stock (Han et al. 1991). Despite having made mitotic gynogens in the aforementioned different fish species, until now clones have been successfully produced only 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) and Nile tilapia (Hussain 1992). Heterozygous and homozygous clones have been successfully produced in common carp (by Komen et al. 1991) and Nile tilapia (by Hussain 1992). Majority of these workers observed that the clonal lines produced in the aforementioned fishes were mostly free of recessive, deleterious, low penetrance alleles; and were similar to the starting population. Clone individuals derived from common carp demonstrated a reduced variation for a variety of morphological traits and superior viability as a result of increased heterozygosity (Komen et al. 1991).

**Androgenesis**

Androgenesis is a genome manipulation technique which is the opposite of gynogenesis, involving a genetically inactivated egg fertilized with normal sperm. The resulting embryo develops with entirely paternal chromosomal inheritance, without any contribution from the maternal chromosomes. The eggs can be inactivated successfully by gamma or x-rays including UV irradiation. Meanwhile, androgenetic haploids have been induced using gamma radiation in loach *M. fossilis* (Romashov and Belyaeva 1964), founder *P. flesus* (Purdom 1969), salmon *O. masou* (Arai et al. 1979), rainbow trout *O. mykiss* (Parsons and Thorgaard 1985) and brook trout *Salvelinus fontinalis* (May et al. 1988). UV light has also been reported successful for inactivating eggs in common carp *C. carpio* (Bongers et al. 1993) and Nile tilapia, *O. niloticus* (Myers et al. 1993).

Stanley (1976) observed spontaneous androgenetic diploids among interspecific hybrids. A low incidence of androgenetic diploid grass carp *Ctenopharyngodon idella* was found among common carp female and grass
carp male hybrids, which might be due to incompatibility of the two genomes resulting in elimination of the female pronucleus, producing some progeny that had only paternal inheritance. The first androgenetic diploids in salmonids were produced by suppressing the first cleavage using pressure shock of 8,500-9,000 p.s.i. for 3 minutes (Parsons and Thorgaard 1985; Scheerer et al. 1986; May et al. 1988) and later by heat shock of 29°C for 10 minutes (Thorgaard et al. 1990). J. M. Myers (pers. comm.) reported the induction of androgenesis in *O. niloticus* using heat shock (42.5°C) applied 25-27.5 minutes post-fertilization of eggs for 4 minutes.

The overall rate of survival is low in the diploid androgenetics so far produced. This may be due to a number of reasons. According to Scheerer et al. (1986), there might be a number of associated factors contributing to such poor viability, such as homozygosity, genotype of the sperm, damage to the egg due to irradiation, and damaging effects of treatments blocking first cleavage division. However, induction of androgenesis in fish breeding could be extremely useful in producing inbred strains, and the most possible application of this technique is in recovery of genotypes from cryopreserved sperm (Stoss 1983; Thorgaard 1986). Recent work by J. M. Myers (pers. comm.) at Stirling University, Scotland, revealed androgenesis to be an effective method for Nile tilapia, where the male is heterogametic "XY", to produce homogametic "YY" males with potential as broodstock for the production of all-male progeny.

**Conclusions**

Over the last few decades, significant improvements in agriculture and livestock production have been achieved through the application of breeding plans and genetic manipulation research. The application of genetics has largely been neglected in fisheries and aquaculture. Therefore, fish and fish-like animals are less domesticated and farmed in many Asian and other developing countries. Efficient fish breeding and genetic stock improvement programs have wider implications in aquaculture. Development of precise and effective methodologies for their immediate application in fish farming has just begun. Apart from or in addition to the results of genetic engineering research, better team work between fish breeding biologists and geneticists is essential to develop improved commercial fish breeds and strains suitable for different farming conditions in Asia and other developing countries.

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