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Induced Gynogenesis in the Rainbow Trout: Sex and Survival of Progenies Production of All-Triploid Populations

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Summary. Gynogenesis could be an efficient way for producing inbred lines in commercial fish species. Gamma-irradiation of sperm gives haploid embryos that all die without hatching; in the present study, we optimized heat treatment of the eggs, in order to produce high rates of diploid gynogenetics. When the eggs are heated to 26 °C for 20 min after 25 min of development, 80% of the embryos hatch, and all the resulting fry are diploid; nevertheless, high mortalities are recorded until feeding start. The monosex female constitution of gynogenetic offspring confirms the female homogamety in the rainbow trout.

When the eggs are treated with the same heat shock 25 min after a fertilization with functional sperm, all-triploid populations are obtained; their survival until feeding start is not different from the control.

Key words: Gynogenesis – Triploidy – Sex determination – Rainbow trout

Introduction

In commercial fish species, heritability for growth performance is very low, and simple selection of faster growing individuals is generally considered inefficient (Ryman 1972; Chevassus 1976; Moav and Wohlfarth 1976; Refstie and Steine 1978). However, selection of superior families seems more promising (Kincaid et al. 1977).

In salmonids, cross-breeding of domesticated populations has not proved successful (Klupp 1979), a result generally attributed to the low genetic distances between strains as computed with biochemical techniques (Guyomard 1981).

About ten years ago, several teams undertook to cross inbred lines, a method new in fish genetics, but effective in plants. Kincaid (1978), obtaining high heterotic effects by crossing strains issuing from three successive fullsib mating, demonstrated the potential of this method in rainbow trout; but production of inbred lines would be a long term process by conventional means, because of the long interval of more than two years between generations.

Therefore, we developed new techniques for accelerating the rate of inbreeding. Viable gynogenesis, a type of parthenogenesis triggered by a genetically inert spermatozoa and recently induced in plaice (Purdom 1969), in grass carp (Stanley and Sneed 1974) and in common carp (Nagy et al. 1978), appears very promising.

In rainbow trout, gamma-irradiation of sperm allowed the production of haploid embryos which all died without hatching (Chourrout et al. 1980). Heat shocking eggs induced diploidization of embryos by retention of the second polar body, and resulted in viable fry production (Chourrout 1980).

In rainbow trout for which female homogamety has been demonstrated by mating sex-reversed males (Okada et al. 1979), gynogenesis is supposed to produce all-female progenies.

By applying similar heat shocks, Thoorgard et al. (1981) obtained triploid embryos (45% of the progeny and 15% of the treated eggs) after fertilizing eggs with functional sperm. Sterile triploids could be of a great interest in fish-farming, if they do not suffer the setback normally occurring at sexual maturation, such as mortalities, decreased growth rate and low meat quality.

In the present study, we confirm with appropriate caryology the inactivation of sperm by gamma-rays, and we optimize the heat shock technique by measuring the hatching rate following each treatment. Survivals at feeding start and sex of gynogenetics are determined and compared with controls. Finally, all-triploid populations are produced with optimal shocks and first results concerning their early survival are given here.

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Materials and Methods

Fertilization

We only used three-years-old breeding animals; sperm and eggs were removed by abdominal pressure. Fertilization consisted in mixing 100 eggs with 0.01 to 0.1 cc of a saline buffered diluent (Billard 1974); ten min later (zero development time), eggs were transferred into a 10 °C-thermoregulated recirculating system for incubation.

Sperm-irradiation

In order to induce gynogenesis, sperm was divided in three tubes receiving respectively 110, 150 and 190 krads from a Co^{60}-source, and finally mixed again before fertilization.

Heat Shocks

In order to induce retention of the second polar body, eggs were introduced 25 min after onset of incubation (optimum determined in previous studies) into a water-bath heated respectively to 24 °C, 26 °C and 28 °C, for 5 to 30 min.

Survival Rates

Counts of survivors were made in the different broods at day 20 (tail bud stage), at day 40 (hatched fry) and in some cases at day 60 (feeding start).

Caryology

Embryos: At day 17, eggs were placed for six hours into a 0.02% colchicine solution, and then dissected in 0.7% NaCl for removal of the embryo. Their tail was subjected to a hypotonic treatment in distilled water lasting 15 min, fixed in ethanol-acetic acid (3/1) for 3 min and rinsed in distilled water. The tail epithelium was gently dissociated on the slide in a drop of 50% acetic acid.

Hatched fry: at day 50, fry were kept swimming for 15 h in a 0.01% colchicine solution. After killing, gills were removed, put in distilled water for 45 min, fixed 3 min and then dissociated on the slide.

Three-months-old fish: we used the same method as for fry, except that colchicine was injected into the dorsal muscle (0.1 ml of 0.1% per 5 grams), five hours before killing.

In all cases, we squashed the dissociated cells under a coverslip that was immediatly removed into 50% acetic acid; after rinsing and air-drying, we stained the slides in 4% Giemsa for 10 min.

Results

Haploid Gynogenesis

The 50 caryological examinations performed on embryos from the batches fertilized with irradiated sperm without heat shock, revealed only haploid metaphases. Nevertheless, in some spreads, we observed near the chromosomes one or several chromosome fragments, and we think they are residues of paternal chromatin because we never saw them in diploid control metaphases (Fig. 1).

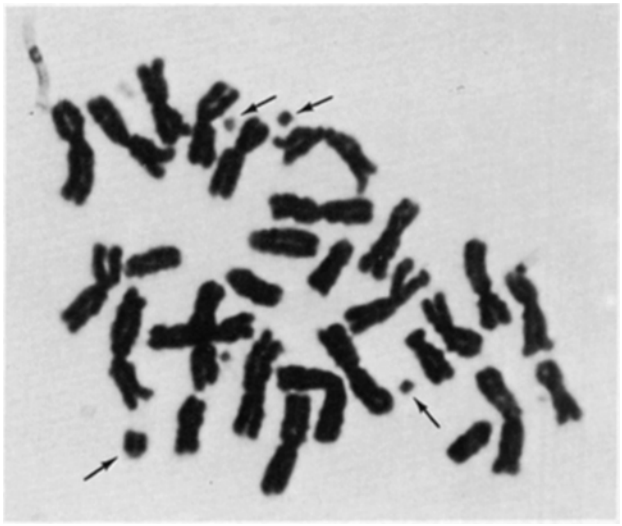

Fig. 1. Metaphase spread from haploid embryo, in which several supernumerary chromosome fragments were detected

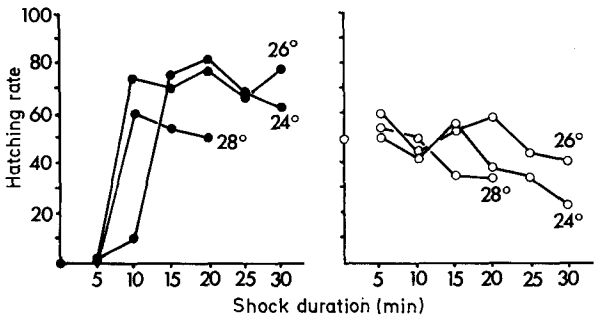

Fig. 2. Optimization of heat shocks beginning 25 min after fertilization with gamma-irradiated sperm (600 eggs per batch). ○ = survival at tail bud stage; ● = percentages of tail bud embryos that gave hatched fry

Diploid Gynogenesis; Optimization of Heat Shocks

We showed in a previous work (Chourrout 1980) that 10 min long treatments at temperatures between 24 °C and 30 °C applied to haploid eggs during the first hour of development resulted in high hatching rates. Repetitions of these experiments led us to conclude that eggs are especially sensitive to the diploidizing treatment between 20 min and 30 min of incubation.

Figure 2 shows that survival rate at tail bud stage was not very much decreased by shocks lasting up to 20 min. The percentage of tail bud embryos that hatched was appreciably improved by increasing durations of shock. The percentage of treated eggs giving viable fry reached its best values from shocks of 15 min at 24 °C, 10–15 min at 26 °C and 10 min at 28 °C.

Table 1. Survival rates of gynogenetics up to feeding stage (rainbow trout eggs) in percentages of initial number of treated eggs

Batch	Sperm origin	Survival rate at tail-bud stage	Survival rate at hatching	Survival rate at feeding stage
Diploid control	Rainbow trout	90%	85%	67%
Haploid control	Rainbow trout	41%	0%	
Haploid control	Coho salmon	28%	0%	
Diploid gynogenetic	Rainbow trout	60%	48%	26%
Diploid gynogenetic	Coho salmon	79%	63%	35%

Table 2. Sex of diploid gynogenetics

Batch	Sperm origin	Female	Male	Undetermined
Diploid control	Rainbow trout	26	24	0
Diploid gynogenetic	Rainbow trout	47	1	2[a]
Diploid gynogenetic	Coho salmon	50	0	0

[a] Ovary-like gonad, but without visible oocytes

In order to standardize further experiments, we chose a shock of 26 °C lasting 20 min, beginning 25 min after fertilization and producing 266 fry from 605 eggs.

Characteristics of Gynogenetics Obtained with Optimal Shock

The last experiment on gynogenesis used a mixture of four rainbow trout spawns, divided into five batches of 800 eggs each (Table 1):

– the diploid control (normal rainbow trout sperm; no shock) gave a high hatching rate, revealing the good quality of the spawns used.
– two haploid controls (respectively fertilized with irradiated sperm from rainbow trout and from coho salmon; no shock) did not produce any hatched fry and yield only haploid embryos. Nevertheless, we recognized supernumerary chromosome fragments in metaphases resulting from investigations of both batches.
– two gynogenetic batches (irradiated sperm from rainbow trout and from coho salmon; heat shock) gave satisfying hatching rates, but about half of the fry died before feeding as compared to 20% in the diploid control.

Most of the survivors at feeding start were normal in external appearance. These batches contained only diploid individuals (we analysed 20 embryos and 20 fry in each).

At last, we detected five months after hatching time a normal sex-ratio in the diploid control (1/1), while gynogenetics were all female (Table 2).

Triploid Rainbow Trout Production

Using ova collected from the same females, we applied the standard heat shock (26 °C lasting 20 min) 25 min after fertilization with normal rainbow trout sperm. The corresponding batch and the diploid control contained 800 eggs each.

In all cases (20/20 embryos; 59/60 fry in similar experiments), caryology revealed triploid chromosome complements (Fig. 3) after investigation of the treated batch.

No significant difference was detected between the hatching rates of the diploid control and of the triploid batch; a slightly lower survival rate was recorded at feeding start in the triploid batch (Table 3); all the corresponding fry were morphologically normal at this stage.

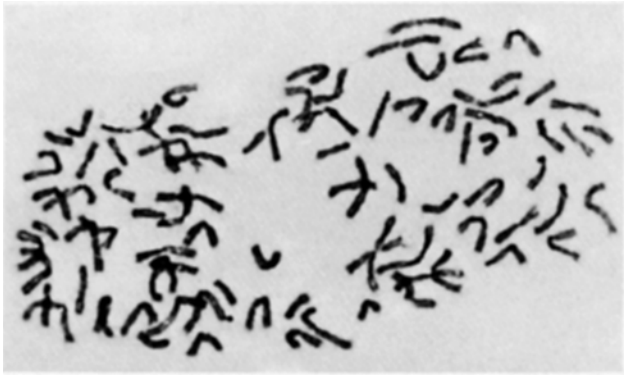

Fig. 3. Metaphase spread from triploid fry produced with heat shocks applied after fertilization with functional sperm

Table 3. Survival rates of rainbow trout up to feeding stage, in percentages of initial number of treated eggs

Batch	Hatching rate	Survival rate at feeding stage
Diploid control	85%	67%
Triploid	87%	63%

Discussion and Conclusions

(1) Our previous results allowed here a rapid optimization of the heat shock technique, confirmed by the all-triploid progenies obtained with the standard treatment applied after fertilization with normal sperm. By preventing the second polar body extrusion, we can expect through gynogenesis a substantial inbreeding increase, the value of which would depend on the mean post-reduction frequency (33 to 100% according to the locus position); estimations of this parameter have already been obtained in plaice (Purdom et al. 1976), in carp (Cherfas and Truweller 1978) and in trout (Guyomard in preparation).

(2) Even though our combined techniques (gamma-irradiation + heatshocks) succeeded in our initial purpose (they resulted in high rates of diploid gynogenetics), their future will depend on further survival of diploid gynogenetic fishes: the important mortalities occurring before the feeding stage raise some questions about the possibility of measuring quantitative parameters at later stages, and of applying selection intensities at the time of reproduction.

Optimistically, it might be possible to directly connect the low survival rates with a supposed high inbreeding level: in this case, we can hope that natural selection will improve survival in further generations.

A probably more realistic attitude consists in looking for eventual survival variations caused by modifying our techniques:

– Treatment intensity: using strong heat shocks in order to insure high diploidization rates could have in counterpart some depressive effects on fry survival (for instance by impairing the vitellus quality). On the other hand, using relatively low sperm irradiation doses that guarantee satisfying egg-activation rates, could favour an incomplete extrusion of male chromatin; in this respect, Jaylet (1972) showed in the newt *Pleurodeles waltlii* after UV sperm irradiation, that the most hypomorphic haploid embryos contained in their cells some additional chromosomes fragments analogous with those described here.

– Other treatments: optimizing other agents (ultraviolet sperm irradiation, hydrostatic compression of eggs), to compare them with our method and choose the combination producing the highest yields of feeding individuals.

(3) An interesting result is the all-female offspring obtained by gynogenesis. Although some authors observed it in two cyprinids (Stanley 1976; Nagy et al. 1978), other ones (Volpe and Dasgupta 1962; Richards and Nace 1978) reported various proportions of gynogenetic males in *Rana pipiens*, for which female homogamety has been demonstrated by mating sex-reversed males with standard females; nevertheless, crosses between gynogenetic males and wild females always produced all-female progenies.

Various hypothesis that were not supported by any experimental data, were proposed in order to explain the "XX" male occurrence in gynogenetic lines: effect of treatments used, special sensitiveness of inbred genomes to environmental effects, minor autosomal genes involved in sex determination.

In any case, gynogenetic progenies cannot be useful as monosex female populations in fish-farms, because of their weakness; gynogenesis must be considered as an interesting way of producing all-"XX" progenies, from which induced sex reversal will provide many neomales of female genotype; those ones, when mated with standard females will give monosex outbred populations directly fit for use in fish culture. As a matter of fact, and especially for sea-farming, females are of great interest because they sexually mature one year later than males: thus, all the problems connected with reproduction could be delayed if the males were eliminated.

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