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# Use of grayling sperm (*Thymallus thymallus*) as a marker for the production of gynogenetic rainbow trout (*Salmo gairdneri*)

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**Summary.** Experiments were conducted to estimate the viability of diploid and induced triploid hybrids between the rainbow trout female and the grayling male. Both are unable to hatch, even so the triploid dies later than the diploid. Insemination of rainbow trout eggs by UV irradiated sperm of grayling results in gynogenetic rainbow trouts when viability is restored by heat shocks inhibiting the second division of egg meiosis.

**Key words:** Gynogenesis – Hybridization – Salmonids – Triploidy – UV irradiation – Heat shocks

## Introduction

Gynogenesis, a type of parthenogenesis triggered by genetically irradiated sperm, has been induced in several fish in order to accelerate the production of inbred lines and monosex female populations (reviews by Chourrout 1982a; Purdom 1983; Thorgaard 1983). In rainbow trout, the prevention of the male contribution to development is achieved by sperm pretreatment with gamma rays (Purdom 1969; Chourrout et al. 1980), ultraviolet rays (Chourrout 1982b; Onozato and Yamaha 1983; Thorgaard et al. 1983) or chemical mutagens (Tsoi 1969; Chourrout 1986).

Ultraviolet rays seem preferrable to both gamma rays and dimethylsulphate which do not eliminate the passage of residual chromosome fragments by the sperm. However, UV rays are not very penetrating and some spermatozoas may accidentally escape from their effects, leading to the contamination of gynogenetic lines. Sperm donors in the same species homozygous and eventually heterozygous for dominant genetic markers are often used to control those paternal genes not transmitted (Nagy et al. 1978 in carp; Chourrout 1980 in trout; Ijiri and Egami in medaka; Streisinger et al. 1981 in zebrafish; Chourrout and Itskovich 1983 in tilapia).

A promising alternative is to use irradiated sperm of a foreign species which gives precociously inviable hybrids with the species subjected to gynogenesis. In this case, individuals arising from accidentally intact sperm will be eliminated by selective mortality. Such a procedure has been tested in grass carp with common carp sperm (Stanley and Jones 1976), in rainbow trout with coho salmon sperm (Chourrout and Quillet 1982) and in *Sarotherodon niloticus* with *Tilapia rendalli* sperm (Chourrout and Itskovich 1983).

However, haploid gynogenetics thus produced are diploidized shortly after insemination by suppression of the second meiotic division. Intact sperm after inefficient irradiation will therefore provide triploid hybrids which will contaminate the lines if they are viable in contrast to diploid hybrids. Recent studies have actually shown that induced triploid hybrids may survive when diploid hybrids do not (Chevassus et al. 1983; Chourrout and Itskovich 1983; Scheerer and Thorgaard 1983); viable triploid hybrids have also been found in grass carp×common carp crosses (Vasil'ev et al. 1975). As far as we know, none of the interspecific combinations used to control gynogenesis is valid because triploid hybrids are viable in each of them.

The species of salmonids which are commonly available (brown trout, coho salmon, brook trout, atlantic salmon ...) give inviable diploid hybrids but viable triploid hybrids with the rainbow trout female. We tested, therefore, the viability of the diploid and triploid hybrids between the rainbow trout and the grayling male (*Thymallus thymallus*), which is found in Europe and in North America, in order to know if the sperm of this species may be used as a marker in the production of rainbow trout gynogenetics.

## Materials and methods

### *Breeding animals, fertilization and incubation*

Rainbow trout sperm and ova were collected from animals grown up in the CSP-INRA fish farm of Gournay sur Aronde; grayling sperm was shipped from the CSP farm of Augerolles and used 15 h after collection. Dry fertilization involved batches of 400 ova mixed with 0.5 cc of milt and 30 cc of dilutent 532 (Billard 1974) and was followed 10 min later by incubation at 10 °C in freshwater. Early examination of eggs (day 10) permitted the gastrulated embryos, the abortions and the unfertilized eggs to be counted. Later counts at the eyed stage (day 20) did not require the sacrifice of the embryos which were studied for their ability to hatch (day 40).

### *Chromosome preparations*

They were made from 14 to 22 day-old embryos submitted to colchicine treatment (0.02% overnight), hypotonic treatment (0.8% trisodium citrate for 20 min) after dissection in 0.8% NaCl, and successive fixations in ethanol 3 : acetic acid 1. Preparations of slides were performed according to Chourrout (1984).

### *Heat shocks and UV irradiation*

In order to suppress the second meiotic division of the egg, we used 26 °C shocks starting after a 25 min incubation and lasting 20 min; UV irradiation of trout sperm lasted 4 min and was performed according to Chourrout (1982b); UV irradiation of grayling sperm lasted 1, 2 and 4 min.

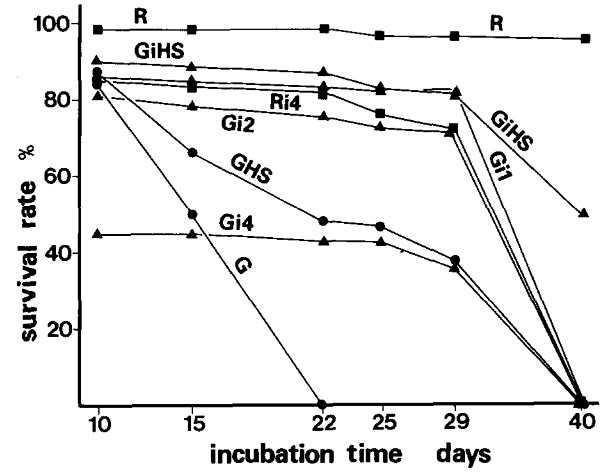

**Fig. 1.** Survival up to hatching stage in 8 batches, among which 6 have been inseminated by non-irradiated (G, GHS) or UV-irradiated grayling sperm (Gi1, Gi2, Gi4, GiHS); (R) and (Ri4) are controls fertilized by normal and irradiated rainbow trout sperm, respectively

## Results

### *Preliminary*

Grayling sperm has an aspect similar to that of rainbow trout. Its motility in the dilutent 532 established for trouts is satisfactory. Reversible immobility is observed in the same dilutent with the addition of 2 g/l of KCl, and these are the conditions under which irradiation is performed.

### *Experimental protocol*

Eggs provided by three rainbow trout females were kept separately and divided each into eight batches: two controls inseminated by rainbow trout sperm, irradiated for 4 min (Ri4) and not irradiated (R); one inseminated by non-irradiated grayling sperm (G); three inseminated by grayling sperm, irradiated for 1, 2, and 4 min (Gi1, Gi2, Gi4); two subjected to heat shock, and inseminated by grayling sperm, irradiated for 3 min (GiHS) and not irradiated (GHS).

### *Survival*

Results from female 1 only are given in Fig. 1. Patterns of survival from females 2 and 3 were similar.

The survival in the two controls fertilized by trout sperm was stable from the gastrula stage to the late eyed stage. Nearly all the embryos hatched in the diploid control (R) while no viable larvae emerged from the batch (Ri4) fertilized by trout irradiated sperm.

The batch (G) fertilized by normal grayling sperm contained many embryos, all of which were abnormal even at the gastrula stage (short and twisted bodies). They died within three weeks.

The three batches Gi had a stable survival from the gastrula stage to hatching. The embryos had a morphology similar to those of batch (Ri4) and died at the same time. The lower fertilization rates after 4 min of irradiation (batch Gi4) is probably due to the low sperm motility observed after irradiation. This low resistance of sperm to irradiation may be the result of bad conditions of shipment, in particular a lack of ice resulting in a too high temperature.

The heat shock after fertilization by normal grayling sperm (batch GHS) improved the survival, providing apparently normal gastrulae which became abnormal eyed embryos (all hypocephalic and microphtalmic) unable to give hatched larvae.

Finally, the heat shock after fertilization by grayling sperm irradiated for 3 min (batch GiHS) resulted in 50% normal larvae.

### *Karyology*

The results of examinations performed on embryos issuing from females 1 and 2 were as follows.

All 29 embryos belonging to controls (R) were diploid. The slides prepared from 15 very hypomorphic embryos of the batch (G) were not amenable to cytological examinations because of a very low mitotic index and the very poor quality of the preparations. However, the several, very approximate counts revealed chromosome numbers higher than 30, indicating, therefore, a likely karyogamy between rainbow trout female and grayling male pronuclei. This was confirmed by the examination of 67 embryos of the batch (GHS) which

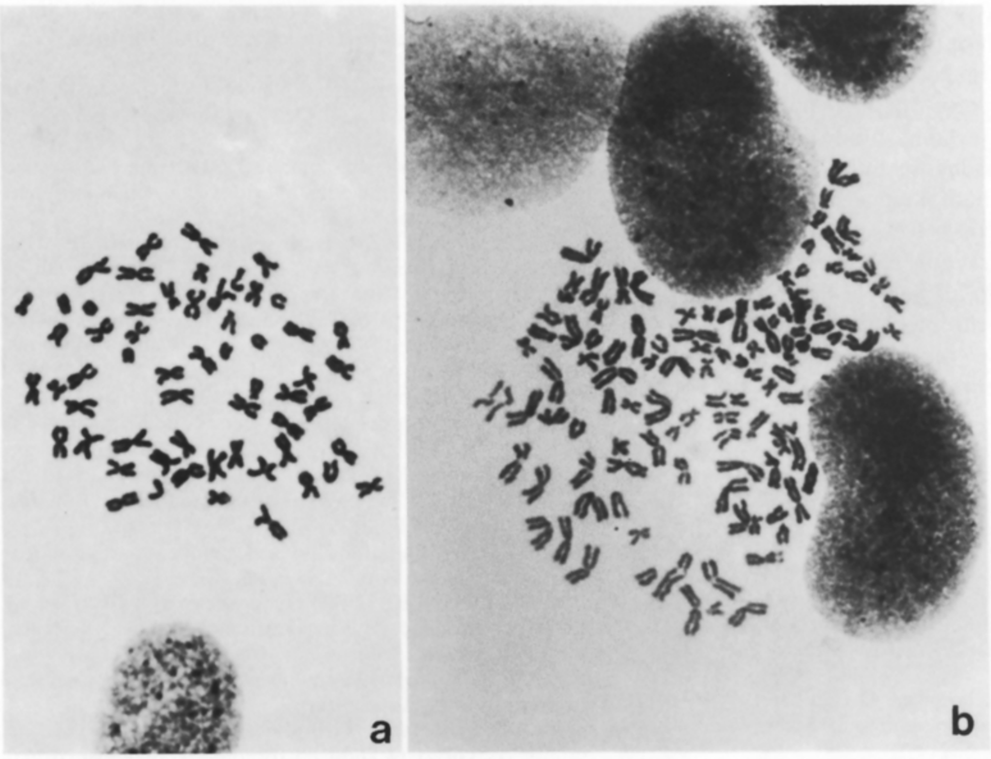

**Fig. 2.** Diploid metaphase of a gynogenetic rainbow trout embryo, produced by irradiation of grayling sperm (**a**). Triploid hybrid "rainbow trout × grayling" metaphase (**b**)

were all triploid hybrids: all counts were between 108 and 115 chromosomes. The presence of chromosomes from the grayling was confirmed by the observation of numerous small chromosomes and, particularly, of subtelocentrics of which there are never more than two in the rainbow trout diploid complement (Fig. 2).

The 41 embryos belonging to the batches (Gi) were all haploid without any supernumerary chromosome fragments (130 metaphases examined). This is remarkable in the case of the 15 embryos resulting from one minute of irradiation (35 metaphases).

Finally, a sample of 30 normal and 5 abnormal embryos was collected from the batch (GiHS). All 15 normal individuals analyzed were diploid rainbow trout, as well as one of the 5 abnormal ones. The four other abnormal embryos were haploid.

## Discussion and conclusions

Fertilization of rainbow trout eggs by grayling sperm provided true hybrids, as revealed by the observation of morphology and karyology on the resulting embryos. Some hybridizations in amphibians (Ting 1951; Volpe and Dasgupta 1962) and in flatfishes (Purdom and Lincoln 1974) result systematically in male pronucleus extrusion and are exploited for the gynogenesis induction. Similar events (spontaneous gynogenesis or androgenesis) may occasionally happen in other hybridizations in amphibians (Ferrier 1966) and in other fish (review by Chevassus 1983). Spontaneous gynogenesis would have resulted in haploids in the batch (G) and in diploids in the batch (GHS). The observation of these two batches ascertained that the embryos were neither haploid nor diploid rainbow trout. In the case of spontaneous androgenesis, they would have been androgenetic haploids in both cases but a look at the embryos of both batches showed that they differed markedly in external morphology. The karyology confirmed the presence of the grayling genome and therefore that karyogamy of the two pronuclei occurred systematically.

The fact that triploid hybrids were more viable than diploid hybrids extends recent information claiming that triploidization may restore the viability of inviable hybrids. What is interesting in our present purpose is that both diploid and triploid hybrids with the grayling male are unable to hatch, a fact making possible the use of its sperm as a marker for the induction of gynogenesis in the rainbow trout. One cannot exclude the hypothesis that some other rainbow trout females may provide viable hybrids with particular grayling males, and the addition of two small batches (G) and (GHS) in addition to a large batch of gynogenetics produced with grayling sperm is advised therefore.

The use of foreign sperm for the induction of gynogenesis has been also justified in newts by the fact that inactivated foreign chromatin may be eliminated more quickly than with homologous sperm (Ferrier 1967; Jaylet and Ferrier 1978). This might be the case here if we consider that grayling sperm irradiated for only one minute resulted in all haploid embryos without any supernumerary chromosome fragments, in contrast to what happens with rainbow trout sperm irradiated for the same duration (Chourrout 1982b; Chourrout 1986).

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