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Chromosome studies of progenies of tetraploid female rainbow trout

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Summary. Nine induced tetraploid females were artificially inseminated by UV-irradiated sperm collected from diploid males, in order to induce the gynogenetic development of their ova. Most of the resulting embryos were diploid (or minor aneuploids). Several gynogenetic tetraploids, likely to issue from unreduced ova, were also detected in these progenies. The same females fertilized by normal sperm of diploid males gave a majority of triploids and several pentaploids, while the fertilization by normal sperm of tetraploid males gave rise to a majority of tetraploids and one hexaploid. The same crosses, after the eggs had been heat-shocked to double the maternal genetic contribution, yielded about three-quarters pentaploids and one quarter haploids (normal sperm of diploids), or three-quarters hexaploids and one quarter diploids (normal sperm of tetraploids). These haploids and diploids are likely to result from androgenesis.

Key words: Fish – Salmonids – Polyploidy – Gynogenesis

Introduction

Induced tetraploidy has been widely investigated in plants for two major reasons. The crosses between tetraploids and diploids result in triploids which give seedless fruits (Kihara 1951). On the other hand, tetraploids may be of direct interest when the size of their vegetative organs is greater than those of diploids. This superiority in yield may be partly due to the high level of heterozygosity which can result from successive inter-tetraploid mating (Bingham 1980). However, the fertility of tetraploids is usually lower than in diploids and this reduces their potential for plants harvested for their seeds or very dependent on their seeds for propagation (Dewey 1980). This inferiority is partly explained by meïotic multivalents which result in irregular chromosome segregation.

The potential of tetraploidy appears important in fish because it provides an alternative method of triploid production and tetraploid populations may respond differently to selective breeding than diploid ones. Few reports are available on tetraploid fish. Their production has been achieved in rainbow trout (Chourrout 1984), in channel catfish (Bidwell et al. 1985) and in tilapia (Myers 1985), and viable tetraploids were obtained in the former two. Fertile tetraploid males have been obtained and studied in rainbow trout (Chourrout et al. 1986): they provided triploids when mated with diploid females, and the production of second generation tetraploids has not required the utilization of tetraploid females. The maternal contribution could be doubled by heat shocks, so preventing the second meïotic division of the eggs.

We recently found the first mature females in our tetraploid broodstock. Their eggs were inseminated both by sperm collected from diploid and tetraploid males and by irradiated sperm from diploids for gynogenetic induction. Some have also been subjected to heat shocks to suppress the second meïotic division. Figure 1 shows the predictable results of such crosses and manipulations, i.e. various types of polyploids (3n, 4n, 5n, 6n) and new types of gynogenetics (2n and 4n). This model is true if the tetraploid females actually provide ova containing diploid pronuclei which undergo a normal karyogamy.

The present study is focused on ploidy determinations in the above progenies and examines the predictions in Fig. 1. It is a necessary preliminary to testing

Table 1. Symbols used to identify egg batches

		Diploid male	Tetraploid male	Irradiated sperm (diploid male)
Diploid female (control)	No heat shock	2n×2n	2n×4n	2n×2nirr
	Heat shock	2n×2nHS	2n×4nHS	–
Tetraploid female	No heat shock	4n×2n	4n×4n	4n×2nirr
	Heat shock	4n×2nHS	4n×4nHS	4n×2nirrHS

the potential of these novel genotypes for genetic improvement.

Materials and methods

The pressure shock technique for initial tetraploid induction has been described elsewhere (Chourrout 1984; Chourrout et al. 1986). Here, 12 3-year-old mature females which ovulated from October to December 1985 were taken from a broodstock of 100 animals; 9 were used in the present investigation. The procedures for artificial insemination, sperm irradiation by UV-rays and suppression of meïosis II by heat shock have been described (Chourrout 1986).

Six batches from the tetraploid females and five from diploid females were incubated for chromosome analysis, the latter being used as controls to check the efficiency of heat shocks and sperm irradiation, the haploid contribution of the six diploid males used and the diploid contribution of the two tetraploid males used (from the same broodstock). The symbols used for each batch are explained in Table 1.

The karyology was performed on 13- to 18-day-old embryos, according to a recent report (Chourrout and Happe 1986). To summarize, whole eggs were colchicine-treated and then dissected to remove the embryos. These were subjected to hypotonic treatment and fixation. Their epithelial cells were dissociated and spread on slides which were then Giemsa-stained. Particular care was taken in reading the chromosome preparations of batches 4n×2n irr., since their embryos result from the gynogenetic development of the ova, whose chromosome numbers they are likely to have in all cells.

The arm number (NF) was used for detecting eventual aneuploidies, as the rainbow trout shows robertsonian polymorphism (2n=58 to 64; NF=104). The counts were less accurate in polyploid embryos, but the ploidy levels were easily determined, although minor aneuploidies may not have been detected.

Results

The ploidy levels are all indicated in Table 2 for each female and each batch.

Controls: eggs of diploid females

We could not study all the possible groups for each mother either in the controls based on diploid females, or in the batches issuing from the tetraploid females. Three controls 2n×2n were performed on the fertilization dates of tetraploid females 1, 3 and 5 plus 6: they contained diploids only (32 embryos studied). The same eggs inseminated by the same sperm gave triploids when the heat shock had been applied (one batch 2n×2nHS made on the fertilization date of tetraploid females 5 and 6; 18 embryos studied). Triploids also resulted from eggs inseminated by sperm of tetraploid males (two batches 2n×4n made on the fertilization dates of females 3, 5 and 6, 18 embryos studied), while the heat shock gave 6 tetraploid and 1 triploid embryos after one such mating. Finally, one batch contemporaneous with the last experiment (involving tetraploid females 7, 8 and 9) and fertilized by UV-irradiated sperm yielded 17 haploids.

Eggs of tetraploid females

The eggs of six females were inseminated with UV-irradiated sperm collected from diploid males. Column "4n×2n irr" of Table 2 shows that most embryos thus produced were diploid (81 out of 86); the other 5 were tetraploid and originated from females 2 and 8. Attempts to classily the 81 diploid embryos by their NF gave the following results: 43 had NF of 104, i.e. were exactly diploid; 5 were aneuploids (2 hypodiploids NF=102; 3 hyperdiploids NF=105, 106); 33 could not be precisely NF determined.

Some of the eggs of females 3 and 6 inseminated with UV-irradiated sperm were subjected to heat shock. Among the 13 embryos analysed, 12 were tetraploid and 1 was diplo-hexaploid mosaic.

Fertilization by diploid and tetraploid males

92 of the 103 embryos from the fertilization of 7 females by diploid males were triploid, confirming the tetraploidy of the mothers used. Of 6 others which were pentaploid, 4 came from female 8, 1 from female 4 and 1 from female 9. The five other embryos were haploid, diploid, haplo-triploid and haplo-pentaploid mosaics. Similar data, but at one more level of ploidy, were recorded for the 91 embryos belonging to the six

Table 2. Detailed results of ploidy levels. Females 5 and 6 were fertilized on the same day, as were females 7, 8 and 9

Female	4n × 2nirr	4n × 2n	4n × 4n	4n × 2nHS	4n × 4nHS	4n × 2nirrHS
♀ 1		3n:19				
♀ 2	2n:14 4n:1	3n:12	4n:7 2n/6n:1	n:6	2n:9 2n/6n:1	
♀ 3	2n:14		4n:13 2n:2 hyper 3n:1	n:8 5n:7	2n:11 6n:1	4n:4
♀ 4	2n:9	3n:7 5n:1 n/3n:1				
♀ 5		3n:15 n:1	4n:19 2n:1		6n:7 4n:1	
♀ 6				5n:9	6n:9 2n:2	4n:8 2n/6n:1
♀ 7	2n:16	3n:15 2n:1	4n:15 hyper 3n:1	5n:11 3n:2 n:1	6n:14 4n:1	
♀ 8	2n:11 4n:4	3n:11 5n:4 2n:1	4n:13 6n:1 hyper 3n:1	5n:15	6n:8 4n:2 2n:1	
♀ 9	2n:17	3n:13 5n:1 n/5n:1	4n:15 2n/hypo 3n:1	5n:11 n:6	6n:12 2n:1 4n:1	
Total	2n:81 4n:5	3n:92 5n:6 2n:2 n:1	4n:82 2n:3 6n:1	5n:53 n:21 3n:2	6n:51 2n:24 4n:5	4n:12
Others		2	5		1	1

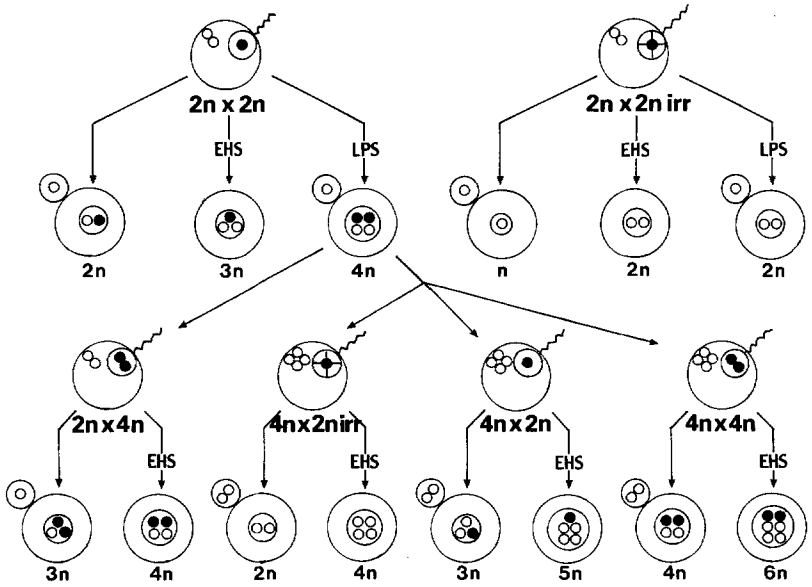

Fig. 1. The insemination of diploid females by irradiated or normal sperm of diploid males (*upper part*) and suppression of meiosis II by early heat shocks (EHS) allow the production of gynogenetics and triploids in fish. Normal insemination followed by late pressure treatment (LPS) inhibiting the fish mitosis results in viable and fertile tetraploids. Second generations of triploids and tetraploids were obtained from tetraploid males and diploid females (*lower left*) in previous study. When tetraploid females' eggs are fertilized by irradiated, normal sperm of diploid or tetraploid males and subjected or not to heat shocks they may provide new categories of polyploids (3n, 4n, 5n, 6n) and gynogenetics (2n, 4n)

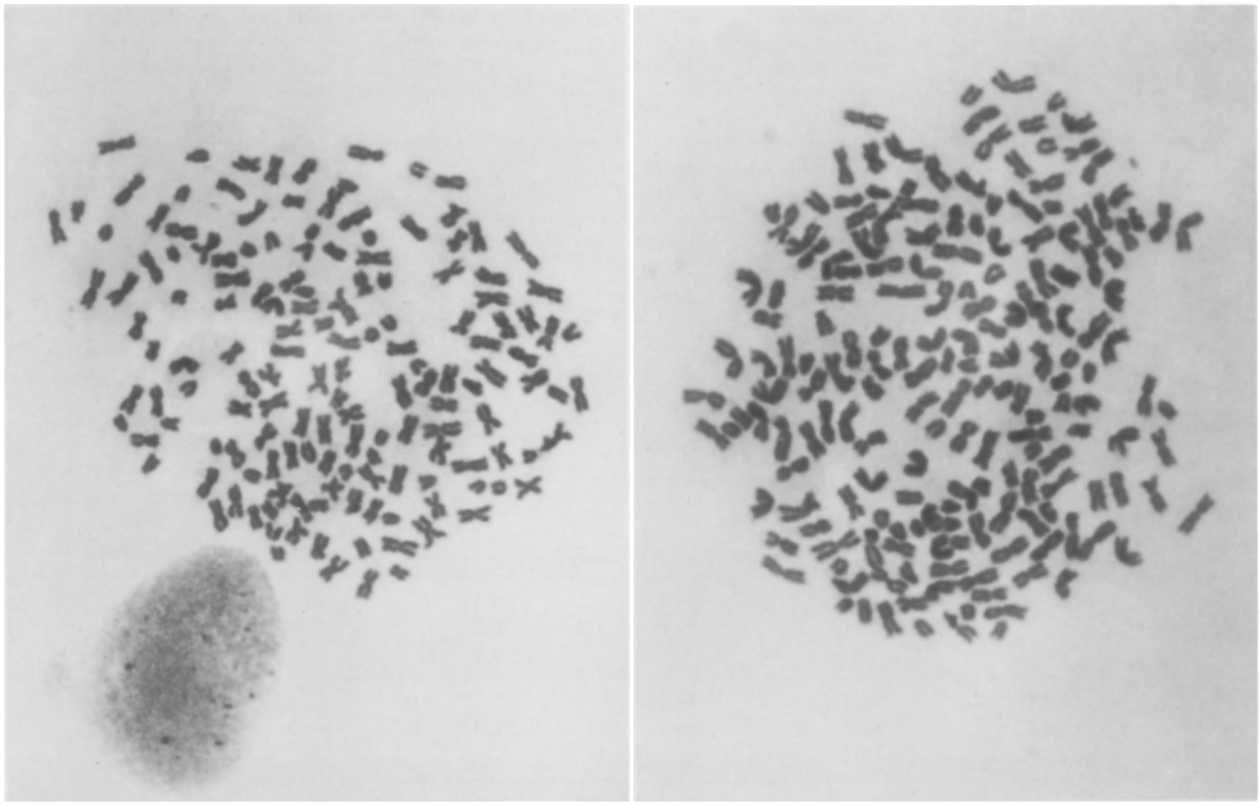

Fig. 2. Metaphase spreads of pentaploid (*left*) and hexaploid (*right*) embryos belonging to batches 4n × 2nHS and 4n × 4nHS, respectively

batches "4n × 4n": 82 were tetraploid and the other 9 were diploid, hexaploid, hypertriploid or mosaics.

Fertilization by diploid and tetraploid males and with heat shock

When a heat shock had been applied to the eggs after the same inseminations, the majority of embryos were high polyploids (pentaploids in batches "4n × 2nHS"; hexaploids in batches "4n × 4nHS"; Fig. 2). However, it is noteworthy that 27% of haploids were obtained in mixture with the pentaploids and 29% of diploids in mixture with the hexaploids. These "contaminants" were essentially provided by three females: the extreme cases are females 2 and 3 for which the haploids and diploids were more numerous than the polyploids.

Discussion

All the females tested here were tetraploid. The majority of the embryos analysed in the batches inseminated by irradiated sperm (4n × 2n irr), normal sperm of diploid (4n × 2n) and tetraploid males (4n × 4n) were diploid, triploid and tetraploid, as predicted by a model of normal meïotic reduction and karyogamy. This offers new possibilities for triploid and tetraploid production and will be discussed later. We will first concentrate on the deviations from the model illustrated by Fig. 1, which are revealed by the cytogenetic examinations.

Tetraploid females provide a minority of unbalanced gametes

The careful observation of the batches (4n × 2n irr) composed of a majority of diploid gynogenetics indicates slight aneuploidy in several embryos. This may be the consequence of meïotic irregularities in the tetraploid mothers and has been observed in many plants and several amphibians (Dawson 1962; Sakharov and Kuvarin 1970; Ellerström and Sjödin 1974; Humphrey and Fankhauser 1949; Fankhauser and Humphrey 1950, 1959; Beetschen 1962). In a recent study, we did not detect significant rates of aneuploids in triploids issued from tetraploid males and diploid rainbow trout females (Chourrout et al. 1986). We had not discarded this contingency, owing to the relatively

low accuracy of chromosome counts in triploid embryos. It might be interesting to reconsider this question for the tetraploid males by looking at the chromosome sets of diploid androgenetics produced by their sperm and irradiated ova (Parsons and Thorgaard 1985). Among the explanations for the difference between tetraploid males and females in the production of aneuploid offspring, could be selection against the unbalanced sperm occurring at fertilization, possibly by differential motility. Other situations, like differences in the events of meïotic pairing or disjunction between testes and ovaries could also be possible. Anyhow, it seems that both sexes of tetraploid rainbow trout do not produce embryos affected by major aneuploidies.

Tetraploid females provide a minority of unreduced ova

A minority of tetraploid embryos were found in the batches (4n × 2n irr). These individuals are most probably also gynogenetic. As a matter of fact, the irradiated sperm used to trigger the egg development originated from diploid males, and a partly inefficient irradiation would have given triploids and not tetraploids. Therefore, these tetraploids resulted either from unreduced ova or from a non-disjunction of the chromosomes in their first mitosis. This latter hypothesis is quite unlikely because it would have led to several hexaploid embryos in batches 4n × 2n and possibly to octoploids in batches 4n × 4n. The minority of pentaploids and hexaploids observed respectively in these groups suggests tetraploid ova. Summing up the information obtained from batches 4n × 2n irr, 4n × 2n and 4n × 4n, it appears that 12 of the 280 embryos analysed possibly issued from tetraploid ova. But 9 of them originated from one female, and it is therefore difficult to know whether the production of unreduced ova is linked directly to the tetraploidy of the mothers. Against this is the observation by Thorgaard and Gall (1979) of high frequencies of triploidy in a rainbow trout family with two diploid parents. In its favour, is the detection by Nishioka and Ueda (1983) of numerous hexaploids in progenies of tetraploid frogs.

Tetraploid ova can be produced in different ways (suppression of one or two meïotic divisions, normal meïosis started from an octoploid oogonium) and so it is interesting to examine the results obtained from batches 4n × 2nHS and 4n × 4nHS subjected to treatment inhibiting meïosis II. If the mechanism of non-reduction was not the suppression of meïosis II, the heat shock should have doubled the maternal contribution and led to several nonaploids and decaploids in the batches. Such embryos were not observed, but we cannot discard either mechanism for tetraploid ova as information on their viability up to 18 days old is not available.

Heat shocking the eggs of tetraploid females induces androgenetic developments

More striking is the significant proportion of haploids and diploids in batches 4n × 2nHS and 4n × 4nHS, respectively. These embryos, which are most probably androgenetic, were observed in three females out of six, in each of which two batches were studied. Occasional embryos, possibly arising by spontaneous androgenesis, are also found without heat shock (batches 4n × 2n and 4n × 4n). It appears therefore that heat shock greatly reduces karyogamy. This phenomenon is not observed with diploid parents (Chourrout 1986). It is noteworthy that we had also interpreted (Chourrout et al. 1986) several percent of diploids in the progenies of diploid females and tetraploid males as being the result of spontaneous androgenesis. The haploids "contaminating" groups 4n × 2nHS will die at hatching, but the diploids of groups 4n × 4nHS will certainly survive much longer. This underlines the necessity of cytogenetic analysis before undertaking studies of polyploid progenies.

Potential of tetraploid females

The fertility of tetraploid females opens new possibilities for sterile triploid production, in addition to the heat shock technique after crossing diploid parents, and to the mating of diploid females and tetraploid males. The 4n × 2n or 2n × 4n crosses seem of particular interest in species for which in vitro fertilization is not practicable on a large scale; indeed, the heat shock technique relies on handling and controlling the development time of many eggs. For tilapia, it is easier to build a small tetraploid broodstock in the laboratory and then to use it in mass natural spawning with diploid partners. However, the cross 4n × 2n will require a tetraploid broodstock larger than the reciprocal because the males are more fertile than the females. This cross would become of particular interest in species where the only tetraploid female is fertile, as in amphibians urodeles.

In salmonids, heat shock is easily practised on hundreds of thousands of eggs at once by the fish-culturists, and which of the three methods of triploid production is used will depend at the end on the relative performances of the triploid groups. The theoretical benefit of using a tetraploid parent is the higher level of heterozygosity of the triploid offspring. We showed recently (Diter et al. 1987) that tetraploid males and females provide on average 87% of heterozygous gametes; in contrast, the suppression of meïosis II by thermal shock leads to 60% of heterozygous ova (Thorgaard et al. 1983; Guyomard 1984; Thompson and Scott 1984; Allendorf et al. 1986).

Above all, the fertility of tetraploid rainbow trout females raises the alternative of tetraploid production. The present study shows that tetraploid strains may be reproduced by ordinary mating. In animals, this possibility was up to now restricted to frogs (Nishioka and Ueda 1983). Our method of producing second generation tetraploids is to cross tetraploid males and diploid females with meïosis II suppressed by heat shock. The mating between two tetraploid parents should lead to a higher level of heterozygosity, according to the above reasoning. The performances of both types of second generation tetraploids will be compared in the near future.

The other genotypes obtained in the present study (diploid and tetraploid gynogenetics, penta-and hexaploids) by using tetraploid females are, in our opinion, of little immediate interest. The major goal of gynogenetic production in fish is the rapid establishment of inbred lines, and those obtained here from tetraploid females are not expected to be as inbred as those issued from diploid females. The usefulness of the pentaploids and hexaploids is not yet evident, and studies of their performances will not be performed. The testing first requires a laborious culling in batches 4n×4nHS, owing to the contamination of the hexaploids by large numbers of diploids.

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