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Further Evidence of Chromosome Abnormalities in Normal and Haploid Gynogenetic Progenies of Rainbow Trout, *Oncorhynchus mykiss*

NELSON COLIHUEQUE, PATRICIA ITURRA, NELSON F. DIAZ, AND ALBERTO VELOSO
Facultad de Ciencias, Departamento de Ciencias Ecologicas, Universidad de Chile, Casilla 653, Santiago, Chile

ABSTRACT Gynogenetic haploid progenies of rainbow trout, produced using UV light for sperm inactivation, were studied in comparison with normal progenies. In a total of 437 normal embryos, 3.5% of them showed chromosome abnormalities: 1.1% haploids, 1.8% triploids, and 0.4% tetraploids. In the haploid embryos, 90.6% showed NF=52 and 9.4% showed other NF (50, 51, 53, or 54); chromosome numbers for embryos with NF=52 were 28, 29, 30, and 31; two to three different chromosome numbers were observed in each progeny. Chromosome abnormalities in normal progenies seem to be a common phenomenon. Results from haploid progenies suggest that aneuploidy occurs during female meiosis. © 1996 Wiley-Liss, Inc.

The occurrence of spontaneous aneuploidy and polyploidy has been widely reported in Vertebrates, including many fish species (Benfey, '89; Solar et al., '92). In the rainbow trout both triploidy (Cuellar and Uyeno, '72; Thorgaard and Gall, '79; Ueda et al., '83; Flajshans and Rab, '87) and aneuploidy occur spontaneously in nature (Thorgaard, '83), and aneuploid individuals have been obtained in experimental gynogenetic haploid progenies (Nakayama and Chourrout, '93).

Cytogenetic studies focusing on natural chromosome abnormalities in rainbow trout are still scarce, requiring also a more systematic approach. In this work, experimental progenies were studied with the aim of contributing to a more detailed description and understanding of spontaneous karyotype aberrations.

Because chromosome abnormalities can reduce viability of individuals at advanced developmental stages, embryos of rainbow trout were used for the cytogenetic analysis. Basic characteristics of the karyotype, i.e., ploidy level, chromosome, and the chromosome arm number (NF), in embryos of normal and haploid gynogenetic progenies are described. Gynogenetic progenies were used because of the high quality information they provide on the occurrence of aneuploidy in the female germinal cell line, as well as on the inheritance pattern of the robertsonian polymorphism observed in the rainbow trout karyotype.

MATERIALS AND METHODS

The brood stock used in experimental crosses belong to the Kamloops strain (Veloso et al., '90) and breeders were obtained in '91 and '92 from a private hatchery (Sociedad Agrícola Macul Ltda.) in Santiago, Chile.

Thirty normal progenies and six gynogenetic haploid progenies were studied. The latter type of progenies were induced using UV light for sperm genetic inactivation (Colihueque et al., '92).

Mother's karyotype of each haploid progeny was established using the lymphocyte culture technique (Colihueque, '91). Chromosome slides were made from embryos (Iturra et al., '90). Slides were stained with 4% Giemsa in a phosphate buffer, pH 7.2.

Photographs (×1,000) were made from the best metaphase plates, i.e., without excessive chromosome condensation and with scarce overlapping of chromosomes. The karyotype for each individual was determined using from 2 to 20 metaphase plates. The chromosome arm number (NF) was checked in each metaphase plate. The nomenclature used to determinate chromosome types follows Levan et al. ('64).

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Address reprint requests to Dr. Nelson F. Diaz, Facultad de Ciencias, Departamento de Ciencias Ecologicas, Universidad de Chile, Casilla 653, Santiago, Chile.
Nelson Colihueque is now at Universidad de Los Lagos, Osorno-Chile.

RESULTS

Chromosome characteristics for normal progenies are shown in Table 1. From a total of 437 analyzed embryos, 3.5% of them show chromosome abnormalities with the following distribution: 5 haploids (1.1%), 8 triploids (1.8%), and 2 tetraploids (0.4%). The chromosome number of haploid, triploid, and tetraploid embryos, obtained from 9 progenies, were 30, 90, and 120, respectively (Fig. 1).

The chromosome numbers and the NF of the haploid gynogenetic progenies are shown in Table 2. All embryos in these progenies were haploids, which indicates that genetic inactivation of sperm using U.V. irradiation was effective. The diploid numbers observed in the mothers were 58, 60, and 61, all with NF = 104, which is characteristic of the rainbow trout karyotype. Among the haploid embryos, 29.3% of the offspring were not scorable for NF number; while among those where the NF could be determined, 90.6% of the offspring exhibited NF = 52, and 9.4% other chromosome arm numbers (50, 51, 53, or 54). The chromosome number observed for embryos with NF = 52 were 28, 29, 30, and 31. Furthermore, two or three different chromosome numbers were observed in different embryos from the same mother. For example, 28 and 29 chromosomes were found in different

TABLE 1. Chromosome analysis at ploidy level in normal progenies of rainbow trout

Cross	No. of analyzed embryos	Ploidy			
		n	2n	3n	4n
1. A	30		30		
2. B	21		21		
3. C	30		30		
4. D	32	1	31		
5. M	8		8		
6. Z	21		21		
7. 44	23		23		
8. 47	23		23		
9. 95	23		23		
10. 65	10		10		
11. 778	10		10		
12 902	10		10		
13. 988	10	1	9		
14. 989	10		10		
15. 1011	10		10		
16. 1012	10		10		
17. 1018	10		9	1	
18. 1060	11		11		
19. 1100	12		12		
20. 1101	10		10		
21. 1104	9		9		
22. 1105	10		9		1
23. 1140	10		10		
24. 1141	12		12		
25. 1172	13	1	9	3	
26. 1173	13	1	12		
27. 1174	12		8	3	1
28. 1175	12		12		
29. 1191	12		11	1	
30. 1192	10	1	9		
Total	437	5	422	8	2
%	100	1.1	96.5	1.8	0.45

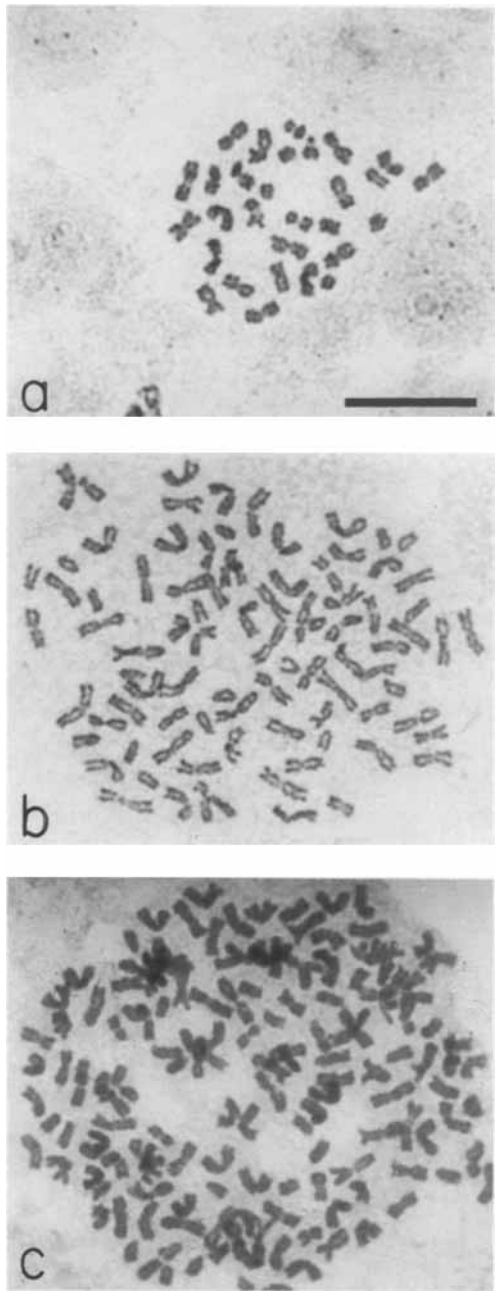

Fig. 1. Chromsome metaphase plates of haploid triploid and tetraploid rainbow trout embryos. **a**: haploid, **b**: triploid, and **c**: tetraploid. Bar represents 10 μm.

TABLE 2. Chromosome and chromosome arm numbers (NF) in haploid gynogenetic progenies of rainbow trout

	Haploid embryos				Chromosome number distribution of embryos with NF = 52			
Mothers (2n)	n[1]	NF (52)	NF (52 ± 1–2)	NF (unknown)	28	29	30	31
1012 (58)	9	6	0	3	2	4	0	0
65 (60)	13	11	0	2	0	2	9	0
95 (60)	12	7	1	4	0	1	4	2
98 (60)	23	12	1	10	0	8	4	0
902 (60)	11	6	2	3	0	1	2	3
778 (61)	7	6	1	0	0	0	2	4
Total	75	48	5	22				
% of embryos of known NF		90.6	9.4					

[1]Number of analyzed embryos.

embryos from the mother 1012 (2n = 58); embryos with 29 and 30 chromosomes in mothers 65 and 98 (2n = 60), while embryos having 29, 30, and 31 were observed in mothers 95 and 902 (2n = 60). Mother 778 (2n = 61) shows embryos with 30 or 31 chromosomes.

Five haploid karyotypes with 28, 29, 30, and 31 chromosomes (NF = 52) are shown in Figure 2a, b, c, and d, respectively, while that with 29 chromosome (NF = 53) is shown in Figure 2e. Karyotypes with 28, 29, 30, and 31 chromosomes had an NF = 52 because the combination of two-armed/one-armed chromosome were 24/4, 23/6, 22/8, and 21/10. The karyotype with 29 chromosomes and NF = 53 presented 24/5 two-armed/one-armed chromosomes. In all gynogenetic karyotypes three constant submetacentric chromosomes, the X chromosome and the secondary constriction carrier chromosome, were observed.

DISCUSSION

Our finding of natural triploid embryos in rainbow trout is an agreement with the reported spontaneous occurrence of adult triploids in the species (Cuellar and Uyeno, '72; Thorgaard and Gall, '79; Ueda et al., '83; Flajshans and Rab, '87).

Our study also verified the occurrence of spontaneous triploidy in the normal progenies of rainbow trout. The observations of a relatively high frequency of triploidy (1.8%, Table 1) suggest that this must be a more common phenomenon than previously thought. Our results also establish that haploid and tetraploid individuals are commonly seen. Furthermore, the results obtained from haploid progenies suggest that there are aneuploids among these embryos.

As far as we know, the natural haploidy and tetraploidy in rainbow trout have been not previously described, though the occurrence of spontaneous tetraploidy has been described in others fishes, like the common carp (Al-Sabti et al., '83) and the loach (Arai et al., '91). The lower viability of tetraploids and haploids at earliest developmental stages in the rainbow trout (Chourrout, '83; Colihueque et al., '92), together with the common use of adult specimens for cytogenetic studies may explain the lack of this class of individuals in previous reports.

Different mechanisms may explain the occurrence of haploid, triploid, and tetraploid individuals found in the present study. The spontaneous retention of the second polar body and the dispermy are often cited as the mechanisms that could explain the occurrence of natural triploidy. The fact that spontaneous diploidization can occur (Streisinger et al., '81; Kaastrup and Horlyck, '87) supports the idea that the migration of the polar body during the fertilization process may fail. The most likely hypothesis for the origin of tetraploid embryos seems to be the spontaneous inhibition of the first cleavage. In rainbow trout, as in other fishes, this process can be disrupted in vitro (Chourrout, '83). The mechanism of spontaneous suppression of first cleavage is unknown, though some factors associated to the aging process could be operating in the egg and may have an effect on the integrity of the spindle microtubules (Yamazaki et al., '89). The observation of spontaneous haploid embryos among normal progenies seems to be an uncommon phenomenon in rainbow trout, though Iturra et al. ('90) described this type of individual in manipulated progenies of rainbow trout and Swarup ('59) in *Gasterosteus aculeatus*. These authors suggest that either the elimination of maternal or paternal pronucleus by the thermal shock or the spontaneous gynogen-

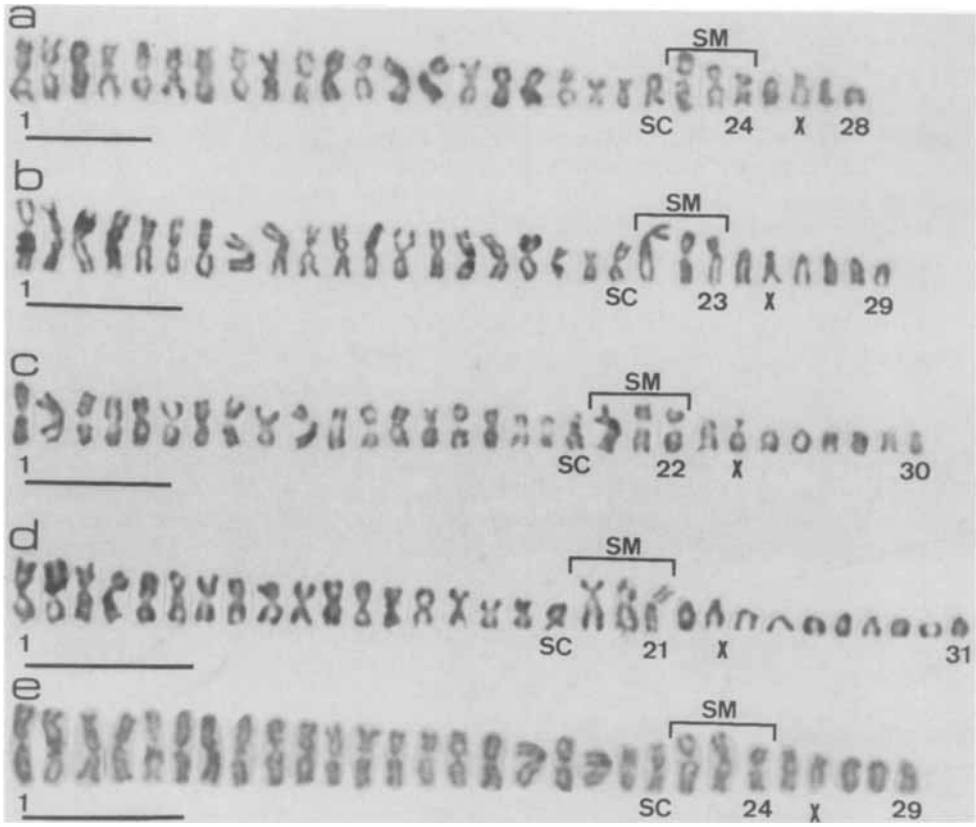

Fig. 2. Haploid karyotypes of rainbow trout with **(a)** 28, **(b)** 29, **(c)** 30, and **(d)** 31 chromosomes (NF = 52); and **(e)** with 29 chromosomes (NF = 53). The chromosomes are ordered from left to right according to their morphology (from metacentric to telocentric) and size. Number below each karyotype indicate number of two-armed and total number of chromosomes. The X chromosomes are indicated. SM: submetacentric chromosome; SC: secondary constriction carrier chromosome. Bar represents 10 μm.

esis may explain the occurrence of haploid individuals. In our study, the latter hypothesis is likely to explain the occurrence of haploid embryos, as these individuals appeared without any manipulation. However, with the present evidence, the occurrence of spontaneous haploid embryos through other mechanism, like androgenesis (Onozato, '93) cannot be ruled out. The use of genetic markers could help to know the exact origin of this kind of spontaneous variation.

Genetic and other factors contributing to the quality of eggs may explain the occurrence of chromosome abnormalities in rainbow trout. Arai et al. ('91) observed that spontaneous triploidy and tetraploidy in *Misgurnus anguillicaudatus* are often associated with cultured populations. Aging of the eggs appears to be also an important factor contributing to an increase in the rate of triploidy in rainbow trout (Díaz et al., '93), as well as to induce spontaneous haploidy in masu salmon (Yamasaki et al., '89). The variation in ploidy observed in this study could be associated to the type of cultured strain used (Kamloops) as well as to the egg quality at the moment of fertilization. For example, six out of nine mothers with chromosome abnormalities, belong to the first period of reproduction (April–May) at the hatchery, where eggs show low survival rates.

The study of the chromosome characteristics of the haploid gynogenetic progenies gives information about the chromosome segregation during female meiosis as well as on the inheritance pattern of the robertsonian polymorphism of the rainbow trout karyotype. In progenies analysis, the gynogenetic haploid can be considered as female gametes due to its uniparental origin. The relevancy of haploid gynogenetic individuals for this kind of studies have been previously pointed out

(Colihueque et al., '92). The finding of haploid embryos with NF different from the expected NF = 52 could be an indication of aneuploidy, though other type of rearrangement such as inversion cannot be ruled out. Our results are in agreement with those of Nakayama and Chourrout ('93), suggesting that non-disjunction events during female meiosis could occur. However, these segregation abnormalities seem to be limited, as a high percentage of female gametes (90.6%, Table 2) are balanced (NF = 52). The chromosome number distribution of the haploid progenies observed in the present analysis (Table 2) supports our previous report of chromosome number variability in haploid progenies as a result of arm rearrangements in the female germ line (Colihueque et al., '92), and the Nakayama and Chourrout ('93) results on the inheritance of robertsonian variation in rainbow trout. For example, mothers with 2n = 60 or 2n = 61 have embryos with two or three classes of chromosome number (29, 30, or 31) corresponding to a 1:2:1 segregation pattern (mothers 65, 95, and 902) as a result of a double heterozygous robertsonian rearrangement, or 1:1 segregation pattern product of a single heterozygous robertsonian rearrangement (mother 778) (Kolmogorov-Smirnov test, $P < 0.05$). However, one progeny (mother 98, Table 2) shows a statistically significant deviation from the chromosome classes expected ($P < 0.05$). Our results, and those of Nakayama and Chourrout ('93), show that the occurrence of this type of segregation pattern usually occur. The lack of a chromosome class (n = 31) in haploid embryos can be explained as due to meiotic drive or to a lower viability of this karyotype in the progeny of the mother, which could carry a double heterozygous robertsonian rearrangement. On the contrary, if the mother karyotypes does not carry this rearrangement, then a new type of centric fusion occurring in the female germ line cell could explain the unexpected chromosome class in this progeny.

Chromosome abnormalities as haploidy, tetraploidy, and mosaicism are usually related to morphological aberrations and decreasing viability in fishes (Chourrout, '83; Yamazaki et al., '89). In this work, it has been shown that spontaneous chromosome changes can be observed. It is recommended to closely examine the abnormal progenies looking for chromosome aberrations in other hatchery populations. The data here described (egg quality, strain, etc., vs. chromosome aberrations) may help to improve breeding programs and the chromosome manipulations of rainbow trout.

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