Triploid Hybrids between Female Rainbow Trout and Male Brook Trout

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The hybrids are obtained by the routine artificial fertilization between a single female rainbow trout *Salmo gairdneri* and a male brook trout *Salvelinus fontinalis*. The chromosome spreads from the culture cells of eyed-embryos and fins of the parents and the hybrids. Two eyed-embryos have 72 chromosomes and eight eyed-embryos and fifteen fingerlings have 102 chromosomes, respectively. The individuals with 72 chromosomes (31 M and SM, 3 ST, 38 A) have an intermediate karyotype between the parents. The individuals with 102 chromosomes (53 M and SM, 4 ST, 45 A) are triploid having two genomes of rainbow trout and one genome of brook trout.

Though the karyotypes of many species in the Salmonidae were reported, there were a few reports about the chromosome studies of the hybrids. The chromosome study of the hybrids will surely give a major contribution to the taxonomy, genetics, or the study of breeding, etc. In the report of interspecific hybrids between chum salmon *Oncorhynchus keta*, and kokanee salmon *O. nerka*,¹⁾ the hybrids of both reciprocal crosses were found to have a karyotype exactly intermediate ($2n=66$) between chum salmon ($2n=74$) and kokanee salmon ($2n=58$). And, the karyotype of intergeneric hybrids between coho salmon *Oncorhynchus kisutch*, and brook trout *Salvelinus fontinalis* were reported by UYENO.²⁾ In his study, the hybrids between female brook trout and male coho salmon were found to have a karyotype intermediate ($2n=72$) between coho salmon ($2n=60$) and brook trout ($2n=84$). While the hybrids between female coho salmon and male brook trout were found to have the same karyotype of the coho salmon. In the present study of the intergeneric hybrids between female rainbow trout *Salmo gairdneri* and male brook trout *Salvelinus fontinalis*, a number of triploids were found to occur.

Materials and Methods

The hybrids were obtained by routine method of artificial fertilization between a female rainbow trout (3 years of age) and a male brook trout (3 years of age) at the Nikko Branch of the National Research Institute of Aquaculture, Fishery Agency (Tochigi Prefecture). The parents and the hybrids, ten eyed-embryos and fifteen fingerlings (7-9 months after hatching), were provided as materials for the present chromosome study. Eyed-embryos and fins (about 10×10 mm) of fingerlings were cultured in order to obtain dividing cells efficiently. The surface of the eggs and the fins were sterilized by immersion in sodium hypochloride (1%) for 30-60 s (the eyed-embryo) or 15-20 s (the fin). After washing two times, the embryos without chorion and the yolk as well as the fins were minced with scissors in a petri dish. After washing two times, the tissue fragments and cells were cultured in the medium Eagle MEM*⁴ +10% fetal bovine serum+100IU/ml penicillin +15 mM HEPES*⁵ in a plastic flask (Corning Co.) at 18-20°C. The medium was freshened at 4-5th day. At the 7-10th day, 4-6 h prior to harvest colchicine was added to culture media at a final concentration of 0.2 μ g/ml. Air-dried chromo-

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*⁴ Eagle minimum essential medium (Nissui Seiyaku Co.)

*⁵ N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

sone slides were made after hypotonic treatment (0.5% KCl for 30–40 min, at room temperature) and fixed with 3:1 ethanol-acetic acids, and then stained with the Giemsa solution. After the decolorization with 70% ethanol, the Giemsa C-banding technique³⁾ was applied for chromosome analyses.

Results

The chromosome numbers and the chromosome constitution are shown in Table 1.

Rainbow Trout

The metaphase figures and the karyotype of mother rainbow trout ($2n=60$), as shown in Figs. 1 and 2, consisted of 44 meta-(M) and submetacentrics (SM), 2 subtelocentrics (ST), and 14 acrocentrics (A). It is clear from Figs. 1 and 2 that C-bands appear in contact with the centromeres. One pair of ST with extraordinary dark C-bands close to the centromeres was also observed. One pair of M or SM with satellites had dark C-bands near the satellite regions.

Brook Trout

The metaphase figures and the karyotype of father brook trout ($2n=84$), as shown in Figs. 3 and 4, consisted of 18 M and SM, 4 ST, and 62 A. The karyotype was characterized by one pair of very small SM (near ST) with intense C-bands on

Table 1. The chromosomal numbers and the chromosome constitution

Specimen	Tissue	2n	M & SM	ST	A
Parents					
Rainbow trout	Fin	60	44	2	14
Brook trout	Fin	84	18	4	62
Hybrids					
No. 1	Embryo	72	31	3	38
No. 2	Embryo	72	31	3	38
No. 3	Embryo	102	53	4	45
No. 4	Embryo	102	53	4	45
No. 5	Embryo	102	53	4	45
No. 6	Embryo	102	53	4	45
No. 7	Embryo	102	53	4	45
No. 8	Embryo	102	53	4	45
No. 9	Embryo	102	53	4	45
No. 10	Embryo	102	53	4	45
No. 11	Fin	102	53	4	45
No. 12	Fin	102	53	4	45
No. 13	Fin	102	53	4	45
No. 14	Fin	102	53	4	45
No. 15	Fin	102	53	4	45
No. 16	Fin	102	53	4	45
No. 17	Fin	102	53	4	45
No. 18	Fin	102	53	4	45
No. 19	Fin	102	53	4	45
No. 20	Fin	102	53	4	45
No. 21	Fin	102	53	4	45
No. 22	Fin	102	53	4	45
No. 23	Fin	102	53	4	45
No. 24	Fin	102	53	4	45
No. 25	Fin	102	53	4	45

Fig. 1. The metaphase figures of rainbow trout. Conventional (a) and C-banding stain (b). The scales indicate 10 μ m.

Fig. 2. The karyotype of rainbow trout. Conventional (the upper row) and C-banding stain (the lower row). The underlines indicate one pair of ST with extraordinary dark C-bands close to the centromeres and one pair of M or SM with the satellite and intense C-bands near this satellite regions. The scale indicates 10 μ m.

Fig. 3. The metaphase figures of brook trout. Conventional (a) and C-banding stain (b). The scales indicate 10 μ m.

Fig. 4. The karyotype of brook trout. Conventional (the upper row) and C-banding stain (the lower row). The underlines indicate one pair of very small SM (near ST) with intense C-bands on the short arms, two pairs of big ST with intense C-bands on the short arms, and one pair of M or SM with intense C-bands on the terminal regions of the long arms. The scale indicates 10 μ m.

Fig. 5. The metaphase figures of the hybrid with 72 chromosomes. Conventional (a) and C-banding stain (b). The scales indicate 10 μ m.

Fig. 6. The karyotype of the hybrid with 72 chromosomes. Conventional (the upper row) and C-banding stain (the lower row). The underlines indicate one ST with extraordinary dark C-bands close to the centromere, and one M or SM with the satellite and intense C-bands near this satellite region, originated from rainbow trout (R), and one very small SM (near ST) with intense C-bands on the short arms, two big ST with intense C-bands on the short arms, and one M or SM with intense C-bands on the terminal regions of the long arms, originated from brook trout (B). The scale indicates 10 μ m.

the short arms, two pairs of big ST with intense C-bands on the short arms, and one pair of M or SM with intense C-bands on the terminal regions of the long arms.

Hybrids

Two eyed-embryos had 72 chromosomes, and eight eyed-embryos and all the fifteen fingerlings had 102 chromosomes, respectively. The metaphase figures and the karyotype of the individual with 72 chromosomes, as shown in Figs. 5 and 6,

consisted of 31 M and SM, 3 ST, and 38 A. This karyotype was characterized by one ST with extraordinary dark C-bands close to the centromere, and one M or SM with the satellite and intense C-bands near this satellite region, originated from rainbow trout, and one very small SM (near ST) with intense C-bands on the short arms, two big ST with intense C-bands on the short arms, and one M or SM with intense C-bands on the terminal regions of the long arms, originated from brook trout. This karyotype was intermediate between

Fig. 7. The metaphase figures of the hybrid with 102 chromosomes. Conventional (a) and C-banding stain (b). The scales indicate 10 μ m.

Fig. 8. The karyotype of the hybrid with 102 chromosomes. Conventional (the upper row) and C-banding stain (the lower row). The underlines indicate one pair of ST with extraordinary dark C-bands close to the centromeres, and one pair of M or SM with satellite and intense C-bands near this satellite regions, originated from rainbow trout (R), and one very small SM (near ST) with intense C-bands on the short arms, two big ST intense C-bands on the short arms, and one M or SM with intense C-bands on the terminal regions of the long arms, originated from brook trout (B). The scale indicates 10 μ m.

the parents.

The metaphase figures and the karyotype of the individual with 102 chromosomes, as shown in Figs. 7 and 8, consisted of 53 M and SM, 4 ST, and 45 A. This karyotype was characterized by

one pair of ST with extraordinary dark C-bands close to the centromeres, and one pair of M or SM with satellite and intense C-bands near this satellite regions, originated from rainbow trout, and one very small SM (near ST) with intense C-

bands on the short arms, two big ST intense C-bands on the short arms, and one M or SM with intense C-bands on the terminal regions of the long arms, originated from brook trout. This is evidently the triploid which have two genome of rainbow trout and one genome of brook trout.

Discussion

Identical karyotype of rainbow trout has been reported by FUKUOKA,⁴⁾ CUELLAR *et al.*,⁵⁾ MURAMOTO *et al.*,⁶⁾ and UEDA *et al.*⁷⁾ One pair of M or SM with satellites as shown in Figs. 1 and 2 had also been reported by CUELLAR *et al.*,⁵⁾ MURAMOTO *et al.*,⁶⁾ and UEDA *et al.*⁷⁾ The C-banding patterns were consistent with that reported by UEDA *et al.*⁷⁾

When we compared our karyotype of brook trout with that of MURAMOTO *et al.*,⁶⁾ ($2n=84$, M and SM=16, ST=6, A=62), minor differences has been shown. These differences may reflect the different nomenclatures used.

Spontaneous triploids in the family Salmonidae had been found by CUELLAR *et al.*,⁵⁾ ALLEN *et al.*,⁸⁾ THORGAARD *et al.*,⁹⁾ and UEDA *et al.*⁷⁾ There were no clear cause of producing those autotriploids, and it was not clear whether an additional genome originated from the maternal side or the paternal one. The allotriploids in the Salmonidae had not yet been reported. It is clear that triploids in the present study resulted from the digyny, judging from the chromosomal constitution. Studies on mammals have shown that digyny would be occurred by overripeness of eggs.¹⁰⁻¹²⁾ Triploids having probably two maternal genomes, would have been induced experimentally in fishes by the application of a thermal shock shortly after fertilization.¹³⁻¹⁷⁾ It was very unlikely that the drastic changes from the environmental factors (temperature, water pressure, or water quality, etc.) would have occurred during the development of embryos in the present study. Since the breeding trout are usually exposed to artificial conditions significantly different from the natural environmental factor,³⁾ the overripeness of eggs might be induced consequently. But, it is difficult to suspect that diploid eggs are to be produced through a unique maturation division such as *Ginbuna Carassius auratus langsdorfii*,¹⁸⁾ because the intermediate-type hybrids having 72 chromosomes, were found at the eyed-embryo-term.

Though the intermediate-type hybrids having 72 chromosomes, existed at 20% in the eyed-embryos, they were not found in the fingerlings.

The intermediate-type hybrids may have lower viability than triploid-type hybrids. In the previous report,¹⁹⁾ it was found that the hybrids obtained from the same combination as the present study had negligible survival rates. Their hybrids would probably be equal to our intermediate-types. The development in the hybrids reported here has been favorable so far (10 months of age). We can suggest triploid-types would have an enough viability and triploid-types have been produced at a high rate in our study.

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