9. Karyological Study of Three Intersexual Rainbow Trouts

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In fishes gonochorism and hermaphroditism are normal conditions. Although the Salmonid fishes are gonochoric, sex reversal can be produced by hormone manipulation (Johnstone *et al.* 1978, 1979; Nakamura 1981; Donaldson and Hunter 1982; Yamazaki 1983). In the gonochoric fishes, naturally occurring intersexual individuals have been observed rarely, but few reports of chromosomal studies on such individuals exist and the condition has not been reported in the Salmonidae. In the present report, the karyotypes of three intersexual rainbow trout (*Salmo gairdneri*) each with an ovotestis are described.

Materials and methods. Three intersexual rainbow trout (*S. gairdneri*; 3 years of age at maturity) from the Nikko Branch of the National Research Institute of Aquaculture, Fishery Agency, were provided as material for the study. Generally speaking, the external characteristics of both sexes appear at maturity; all three specimens examined here had a male head and a female trunk. Chromosome slides were made by culture (ovary, fin and kidney cells) and direct method (kidney cells), and by the routine air-drying method with conventional Giemsa stain. Ovary, fin, and kidney cells of individual No. 1, fin and kidney cells of individual No. 2, and ovary and fin cells of individual No. 3 were used. The cells were incubated in a medium (Eagle MEM + 10% fetal bovine serum + 100 IU/ml penicillin + 15 mM HEPES, and 20 μ l/ml PHA-M "Difco Co." was added in the kidney culture) at 18–20°C for 3–10 days; 4–6 hrs before they were harvested, colchicine was added to the medium at a final concentration of 0.15–0.2 μ g/ml. Direct chromosome preparations were made from the kidney cells with colchicine treatment (50 μ g/ml for 1.5 hrs at 18–20°C). Nomenclature of the chromosomes followed the Levan *et al.* system (1964).

Results. Intraindividual variations of the chromosome number are shown in Table I. Because no cells with just one subtelocentrics (ST) were observed, we considered that the number of ST was two and that each of the three individuals had a single karyotype. Fig. 1 is the karyotype of individual No. 1 ($2n$, 63); it consists of 41 meta- (M) and submetacentrics (SM), 2 ST, and 20 acrocentrics (A). This karyotype was characterized by 2 M or SM with satellites and 2 ST. Fig. 2 is the karyotype of individual No. 2 ($2n$, 60); it consists of 44 M and SM, 2 ST, and 14 A. This karyotype was characterized by 2 M or SM with satellites and 2 ST. The karyotype of individual No. 3, shown in Fig. 3, was the same as that of No. 2.

Discussion. Chromosomal polymorphisms, $2n=60$, 61, 62, 63 and 64 were recognized in the strain of rainbow trout of which these three intersexes are members (Ueda and Ojima 1984). These variations are induced by Robertsonian-

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Table I. Distribution of chromosome numbers in *in vitro* cells (Culture) and *in vivo* cells (Direct) of intersexual rainbow trouts

Fish	Tissue	Chromosome no.						2n=58-63	
		58	59	60	61	62	63	ST=2	ST=1 or 2
No. 1	Ovary (Culture)			2		1	18	25	7
	Fin (Culture)						5	5	
	Kidney (Direct)						9	18	3
	Kidney (Culture)					1	11	14	4
No. 2	Fin (Culture)			8				8	
	Kidney (Direct)			3				3	
	Kidney (Culture)			12				17	3
No. 3	Ovary (Culture)	1	5	22				30	8
	Fin (Culture)			10				10	

Cells with just one subtelocentrics (ST) were not observed.

Fig. 1. The karyotype of No. 1 individual. $2n=63$, M and SM=41, ST=2, A=20. The underline indicates 2 ST. The scale indicates 5 μ m.

Fig. 2. The karyotype of No. 2 individual. $2n=60$, M and SM=44, ST=2, A=14. The underline indicates 2 ST. The scale indicates 5 μ m.

type translocations in relation to interspecific differentiation. Because the male always has one less ST than the female, it was inferred that males have an X-Y, and females have an X-X sex chromosome complement. If that is the case, then all three individuals in this study had normal female karyotypes.

Intersex Salmonid fishes often result from artificial hormone treatment (Johnstone *et al.* 1979; Nakamura 1981). In the present study no artificial treatment for sex control had been carried out. As is often the case in human beings,

Fig. 3. The karyotype of No. 3 individual. $2n=60$, M and SM=44, ST=2, A=14. The underline indicates 2 ST. The scale indicates $5\mu\text{m}$.

intersexual individuals often appear as the result of mosaicism. We considered that each of the three individuals in this study had a single karyotype; there was no evidence of mosaicism. However, the cell counts leave something to be desired and there the slight possibility of mosaicism exists. In the future, we will have to do more examination to make clear the relation between intersex development and the chromosome constitution. Such an examination may elucidate sex development and sex chromosome differentiation.

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