Silver-banded karyotypes of the rainbow trout and the brook trout and their hybrids: Disappearance in allotriploids of Ag-NORs originated from the brook trout

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(Received December 1, 1987)

ABSTRACT

Silver-banded karyotypes of the rainbow trout *Salmo gairdneri*, the brook trout *Salvelinus fontinalis*, and their hybrids were described. Diploid-type and triploid-type hybrids were obtained. Triploid-type hybrids had two maternal genomes of the rainbow trout and one paternal genome of the brook trout. The rainbow trout had one pair of M or SM with Ag-NORs near the satellite. In the brook trout, Ag-NORs were observed in four pairs of chromosomes, but each cell had different number of chromosomes with Ag-NORs. In all observed cells of triploid-type hybrids, Ag-NORs originated from the brook trout were not recognized, whereas those from the rainbow trout were found.

1. INTRODUCTION

Silver staining methods to visualize the chromosomal nucleolar organizer regions (NORs) containing the genes coding for the ribosomal RNA (rDNA) have proved to be useful in karyotyping and chromosome identification (Henderson *et al.*, 1972, 1974a, b; Pardue and Hsu, 1975). In metaphase chromosomes NORs are generally located at the secondary constrictions, but both did not coincide in some cases (Hsu *et al.*, 1975). The investigations of NORs not only in mammals, but also in a wide variety of animals and plants have been reported (Goodpasture and Bloom, 1975; Howell and Black, 1980). The NORs have been studied by the silver staining in about 40 species of fishes since the report of Kligerman and Bloom (1977) using *Umbra limi*. In the salmonid fishes, the chromosomal polymorphisms in *Oncorhynchus masou* with the variation of Ag-NORs (silver stained nucleolar organizer regions) in ordinary air-dried chromosomes were observed by Ueda *et al.* (1985).

In the present study, we examined the pattern of Ag-NORs in the rainbow trout *Salmo gairdneri*, the brook trout *Salvelinus fontinalis*, and their intergeneric hybrids. Among these hybrids, the disappearance of Ag-NORs ori-

ginated from the brook trout was recognized in triploids.

2. MATERIAL AND METHODS

Two kinds of crossing between female rainbow trout *Salmo gairdneri* and male brook trout *Salvelinus fontinalis* were done at the Nikko Branch of the National Research Institute of Aquaculture, Fisheries Agency (Tochigi Prefecture). Specimens in the group I were obtained from only fertilization and those in the group II were obtained by an application of the pressure shock (650 kg/cm² for 6 min) 10 min after fertilization. Seven rainbow trouts, 7 brook trouts, and 12 hybrids were provided as materials for the present chromosome study (Table 1). The chromosome slides were made by short-term-culture methods of the fin and embryos (Ueda, 1986). The silver banding technique of Howell and Black (1980) was applied for staining NORs.

Table 1. *The number of specimens used for the chromosome analysis*

	Female	Male	Young* ¹	Embryo* ²	Total
Rainbow trout	2* ³	3		2	7
Brook trout	2	3* ⁴	1	1	7
Hybrid (group I)			2 (T)* ⁵	2 (D)* ⁶	4
Hybrid (group II)			3 (T)	5 (T)	8

*¹: 1 year old. *²: Eyed stage. *³ and *⁴: Including the mother and the father of the group I.

*⁵: Triploid. *⁶: Diploid.

3. RESULTS AND DISCUSSION

Silver-banded karyotype of the rainbow trout

The karyotype of the maternal rainbow trout of the group I ($2n=60$), as shown in Fig. 1, consisted of 44 meta- (M) or submetacentrics (SM) and 16 subtelo- (ST)

Fig. 1. Karyotype of rainbow trout. Routine Giemsa stain (the upper row) and Ag stain (the lower row). The arrows indicate Ag-NORs.

or acrocentrics (A). One pair of M or SM had Ag-NORs near the satellite (arrows in Fig. 1). In the remaining rainbow trout, $2n=60$; 44 M·SM+16 ST·A (2 males), $2n=61$; 43 M·SM+18 ST·A (1 female, 1 male and 1 eyed embryo), and $2n=62$; 42 M·SM+20 ST·A (1 eyed embryo) were observed. All specimens had the same fundamental number (NF=104). Robertsonian-type chromosomal variations have been reported within species (Ohno *et al.*, 1965; Fukuoka, 1972; Thorgaard, 1976; Ueda and Ojima, 1984). The chromosomal constitutions in the present results coincide with those of the previous study (Ueda and Ojima, 1984). One pair of M or SM with Ag-NORs were recognized in all of the fifteen metaphase cells observed in each individual.

Silver-banded karyotype of the brook trout

The brook trouts used here had the same chromosomal number ($2n=84$) and

Figs. 2 and 3. Karyotypes of brook trout. Routine Giemsa stain (the upper row) and Ag stain (the lower row). The arrows indicate Ag-NORs.

chromosomal constitution ($M \cdot SM = 18$, $ST \cdot A = 66$, $NF = 102$) (Figs. 2 and 3). The Ag-NORs were observed in four pairs of chromosomes — SM (a) and A (d) with Ag-NORs at the terminal regions of long arms, small SM (b) and large ST (c) with Ag-NORs on the short arms — but each cell had different number of chromosomes with Ag-NORs (Figs. 2 and 3, Table 2). The marker chromosomes (a, b, c and d) in Table 2 correspond with those in Figs. 2 and 3. For example, Fig. 2 is the (2a+2b+2d)-type. Whereas the pattern of Ag-NORs expression was fairly stable in the rainbow trout chromosomes, considerable inter- and intraindividual variations in the number of Ag-NOR-bearing chromosomes were apparent in the brook trout. In the Cyprinidae, variations in the Ag-NOR number were observed not only between individuals but also within individuals in several species (Takai and Ojima, 1986). And also, a predominant pattern of the chromosomes with the silver positive NORs in each individual was recognized in *Zacco platypus* (Takai and Ojima, 1986). In the present brook trout, chromosome "b" was predominant.

Table 2. *The chromosomes with Ag-NORs in each cell of 7 brook trouts*

Cell nos.	Individual numbers						
	1 (F)	2 (F)	3 (M)	4 (M)	5 (M)	6 (Y)	7 (E)
1	2b	1a+2b+1d	2b+1c	1a+2b	2b+2d	2a+2b+1c	2b+2d
2	2b	2b+1c+1d	2b+1c	1a+1b	2b+2d	2a+2b+2d	2b+2d
3	2b	2b+1c+1d	2b+1c	2b+2b	2b+2d	2a+2b+2d	2b+2d
4	2b	2b+1c	2b+1c	2b+2d	2b+1d	1a+2b+1d	2b+2d
5	2b	2b+1d	2b+1c	2b+1d	2b+1d	2b+2c+2d	2b+2d
6	2b	2b+1d	2b	2b+1d	2b+1d	2b+1c+2d	2b+2d
7	2b	2b+1d	2b	2b+1d	2b+1d	2b+1c+1d	2b+2d
8	2b	1b+2c	2b	2b	2b+1d	2b+2d	2b+2d
9	2b	1b+2d	2b	2b	1b+1d	2b+2d	2b+2d
10	2b	1b	2b	2b	1b+1d	2b+2d	2b+2d
11	2b		2b	1b+1c+2d	1b+1d	2b+2d	2b+2d
12	2b		1b+2c	1b+1c+2d		2b+2d	2b+2d
13	2b		1b+2c	1b+1c		2b+2d	2b+2d
14	2b		1b+2c	1b+1d		2b+1d	2b+2d
15	2b			2c+1d		2b+1d	
16				2d		2b+1d	
17				2d		2b+1d	
18						1b+2c+2d	
19						1b+2c+1d	
20						1b+2d	

a, b, c and d: these markers correspond with those in Figs. 2 and 3. No. 5 individual is the father of the group I.

Silver-banded karyotype of hybrids

Two eyed embryos in the group I were the diploid- and the others (2 specimens in the group I and 8 in the group II) were the triploid-type hybrids. The triploid-type hybrids produced by only fertilization were recognized in the group I, as the same result reported by Ueda *et al.* (1984). The diploid-type specimens were found only at the eyed stage, and in the previous reports it was considered that the survival rate of diploid-type hybrids in the same intergeneric cross was negligible (Suzuki and Fukuda, 1973; Ueda *et al.*, 1984).

1) Diploid-type hybrids

Two eyed embryos of diploid-type hybrids had 72 chromosomes, as shown in Fig. 4, consisted of 31 M or SM and 41 ST or A. Judging from the chromosomal constitution, this karyotype was intermediate between the parents. One M or SM with Ag-NORs near the satellite originated from the rainbow trout was found in all observed cells, but each cell had the different number of chromosomes with Ag-NORs originated from the brook trout (Table 3). The Ag-NORs originated from the brook trout were not observed in 3 cells of No. 1 specimen.

2) Triploid-type hybrids

All the 10 triploid-type hybrids had 102 chromosomes, consisted of 53 M or SM

Fig. 4. Metaphase figures of the diploid-type hybrid. Routine Giemsa stain (a) and Ag stain (b). The arrows indicate Ag-NORs originated from rainbow trout (R) and brook trout (B).

Table 3. *The chromosomes with Ag-NORs originated from brook trout in each cell of two diploid-type hybrids*

Cell nos.	Individual nos.	
	1	2
1	1b+1d	1b+1d
2	1b+1d	1b+1d
3	1b	1b+1d
4	1b	1b+1d
5	1b	1b+1d
6	1b	1b+1d
7	1b	1b+1d
8	1b	1b+1d
9	1b	1b+1d
10	1c	1b
11	1c	1b
12	1d	1b
13	1d	1b
14	1d	1b
15	1d	1d
16	—*	1d
17	—	1d
18	—	

b, c and d: these markers correspond with those in Figs. 2 and 3. The Ag-NORs originated from rainbow trout were found in all observed cells.

*: The Ag NORs originated from brook trout were not observed.

and 49 ST or A (NF=155), as shown in Fig. 5. On the basis of the chromosomal constitution, these triploid hybrids commonly had two genomes of the rainbow trout and one genome of the brook trout. Two M or SM with Ag-NORs near the satellite originated from the rainbow trout were observed in all cells. But Ag-NORs originated from the brook trout were not recognized at all.

In our allotriploid hybrids the chromosomal deletion was not observed, and further Ag-NORs originated from the brook trout were not recognized. Only actively transcribed NORs can be positively stained with silver (Miller *et al.*, 1976). The activation of rDNA originated from the brook trout in the allotriploid appeared to be repressed for some reason or other. The perfect disappearance of Ag-NORs on one parental species in fish hybrids has not been reported. The present phenomenon may be a single example of the so-called "nucleolar dominance". But, it is not clear whether the observed phenomenon is really the nucleolar dominance or simply the reflection of imbalance of genomic constitution

Fig. 5. Metaphase figures of the triploid-type hybrid. Routine Giemsa stain (a) and Ag stain (b). The arrows indicate Ag-NORs originated from rainbow trout. The Ag-NORs originated from brook trout were not observed.

in the triploid hybrids. The enough information about the mechanism of suppression of rDNA has not been obtained from our results. It is necessary to make more investigation for clarification of the mechanism about disappearance of Ag-NORs in the triploid.

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