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Characteristics of the Hybrid F_1 Juveniles between Female Masu Salmon, *Oncorhynchus masou*, and Male Pink Salmon, *Oncorhynchus gorbuscha*

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Abstract

Large variations were noticed in the F_1 hybrid between female masu salmon and male pink salmon. The hybrids could be clearly divided into 4 types named W. S. B and N. These types were not only different in their appearance but also varied in survival rate and growth rate. W and S type hybrids lived only during alevin stage. B-type hybrids could not grow up to more than seven centimeters. N-type hybrids showed heterosis and salinity tolerance since early stage. The karyotype analysis of the hybrid embryos revealed also wide variations in chromosome number. One out of 14 embryos showed theoretically expected chromosome number of 59. There are however two modes noticed in total distribution of chromosome number having peaks at 52 and 59. Six out of seven numerical characters of N-type hybrids were invariably between those of masu salmon and pink salmon. The N-type hybrids having nature of parental species would be useful materials to analyze genetic mechanisms of life phase division or seaward migration of salmonids.

Introduction

In northern Japan, chum salmon, pink salmon and masu salmon are very important fish in salmon fisheries industry. These three fish species have different characteristics. The chum salmon and pink salmon go down to the sea after absorbing yolk and they migrate up to the north Pacific. When mature, they return to their mother rivers. This characteristic is greatly beneficial for the efficient utilization of space and production of the open sea.

On the other hand, masu salmon has a very complicated life cycle. They have two different life phases: fluvial life and sea life. They live in fresh water until about 10 cm then most females become smoltified and go down to live in the coastal waters. But most males remain in the rivers and mature in a residual form.

Hybridization between these species will help to understand the genetic bases of these characteristics. There have been many reports on interspecific hybridization between salmonids (Suzuki, 1966; Chevassus, 1979). Suzuki and Fukuda (1973) have reported the appearance and numerical characters of 25 kinds of hybrids between salmonids at one or two years of age. However, these reports did not include hybridization between masu salmon and chum or pink salmon. The interspecific hybrids between masu salmon and chum salmon are almost inviable (Arai, 1984).

In a previous study (Yamazaki and Ma, 1985), the endocrine organs of thyroids

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and internal tissues associated with the characteristics of smoltification, salinity tolerance and down stream migration were reported in one type of hybrids between masu salmon and pink salmon.

The purpose of this study is to assess the variability of the hybrids between masu salmon and pink salmon especially in the appearance, growth, silvering and numerical characteristics. To understand the genetic bases of the variability in appearance we have examined the chromosome number and morphology during embryonic stage of the hybrids. The salinity tolerance of the hybrids has also been examined to study the genetic aspect of down stream migration.

Material and Methods

Both parental fish, masu salmon (*Oncorhynchus masou*) and pink salmon (*O. gorbuscha*) were obtained from three places in Hokkaido. They were fertilized artificially on October 13 (lot 1) and October 15 (lot 2) 1983 and September 29 (lot 3) and October 8 (lot 4), 1984 respectively. Each of the lots included two groups: hybrids of female masu salmon and male pink salmon, and masu salmon control. The parental fish used for lot 1 and 2 were a male and a female masu salmon captured from the Shiyodomari River near Hakodate and a male pink salmon from the Abashiri River. Only sperm in this case was transported by air from Abashiri to Hakodate. It took 9 hours. The parental fish for lot 3 were a male and a female masu salmon captured from the Nakoma River and a male pink salmon from the Yakumo River southern Hokkaido. For lot 4 a male and six female masu salmon from the Mori Branch of Hokkaido Fish Hatchery and a male pink salmon from the Yakumo River in southern Hokkaido. All inseminations were carried out in our laboratory. All the inseminated eggs were incubated at about 10°C and reared at 10-14°C water temperature. The distribution of body weight of the hybrids were compared with those of the parental species when they were at about 6 months old. The pink salmon control were obtained from the Oshima Branch of Hokkaido Salmon Hatchery located in Yakumo before they started to feed. They were reared at the same condition as the hybrid and masu salmon control. The materials used in the observation of chromosomes were 13 day-old embryos of the hybrid from lot 4. The embryos were manually removed from the yolks by cutting the chorion with forceps under a stereoscopic microscope and rinsed with physiological saline to remove the remained yolk and oil droplets. The chromosome preparations were made according to the chopping method (Yamazaki *et al.*, 1981). The metaphase spreads were microphotographed to count the number of the chromosomes. Salinity tolerance of the N-type hybrids and their parental species controls at about 6 months of age were tested with use of 32‰ of artificial sea water.

Results

Appearances of the hybrids

In rearing the hybrids we recognized four types of variation in their appearance until 30 days after hatching. The first one was W-type with little pigmentation scattering in their bodies, wider head with larger distance between the eyes. They always lay on the bottom of the hatching basket in the aquarium and swam in screw

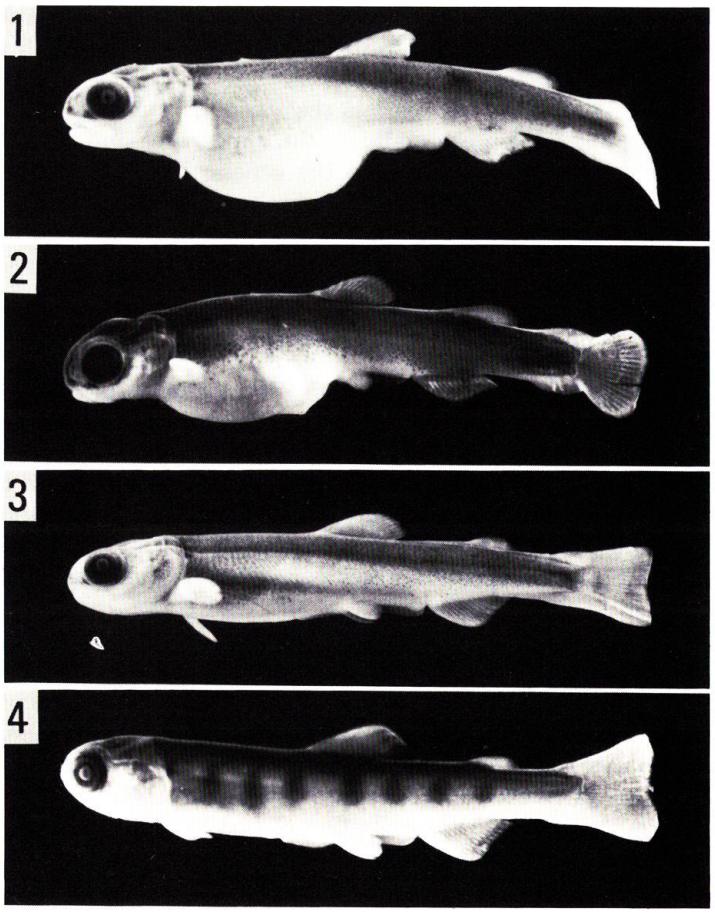

Fig. 1. The four types appeared in the hybrid fry of lot 3.
1 : W-type, BL=27.5 mm : 2 : S-type, BL=27.9 mm ;
3 : B-type, BL=31.1 mm ; 4 : N-type, BL=34.4 mm ;

Table 1. Numbers of fish and ratios of four types found in the F_1 hybrids between female masu salmon and male pink salmon in lot 3.

	DAYS AFTER FERTILIZATION	TYPES				TOTAL NUMBER
		W	S	B	N	
NUMBER	75	13	24	46	66	149
RATIO		1	1.8	3.5	5.1	

pattern for a short time, before sinking down to the bottom again. They died before to start feeding without absorbing yolk. The second type, S-type, was bluish in colour at first and they became darker and darker to blackish at death. Their heads were normal in shape. They lay on the bottom of the hatching basket and seldom swam up except when startled. They all died before complete absorption of the

yolk. The third one was the B-type with bluish colour on their back and sides, without any parr marks. The body form of this type was very thin and slender, quite different from that of masu salmon. The fourth type, N-type had brownish colouration on their backs with striking parr marks numbering 8 to 10 on each side of the body. The body shape was a little thinner than that of masu salmon. The parts of belly and where between parr marks were more silvery than those of masu salmon (Fig. 1). Table 1 shows the numbers and ratios of each type found in lot 3.

Characteristics in growth of the hybrids

The two viable types, N-type and B-type were investigated on the distribution of their weights at 3 and 6 months after hatching. These two types showed different distribution of the body weight (Fig. 2). The body weights of B-type ranged from 0.09 g to 0.16 g and those of N-type ranged from 0.14 g to 0.83 g at 3 months of age (Fig. 2). Fig. 3 shows the results of the weight of hybrid and their parental controls at 6 months old. The hybrids showed two distinct distribution of their body weight, the smaller one was B-type and the bigger one was N-type. The distribution of the weight of two parental species existed between those of B-type and N-type (Fig. 3).

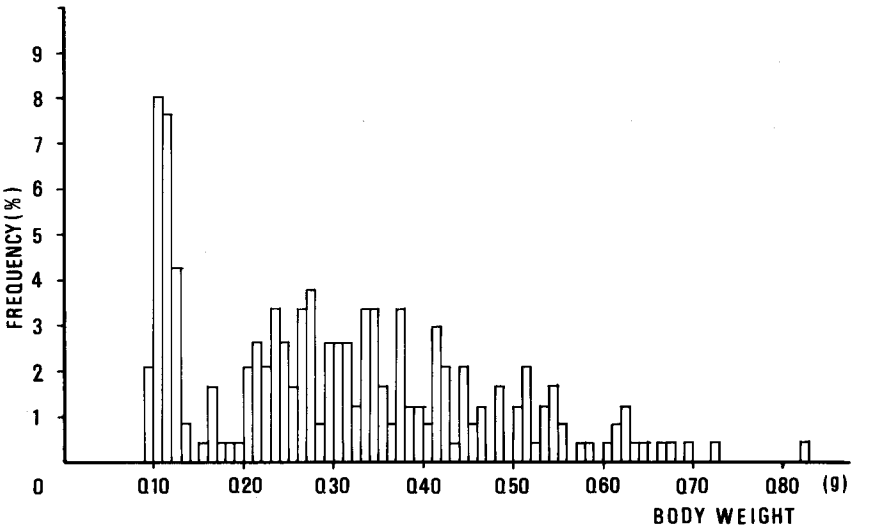

Fig. 2. The distribution of body weight in the hybrid at three months old.

Chromosomes of the hybrid embryos

After having found the four types in the F_1 hybrids, chromosome preparations were made to examine the chromosomal aberration in the embryonic stage because we failed to get clear metaphase spreads from larvae at the alevin stage or just after the yolk absorption. Fourteen embryos were used to count chromosome numbers. No. 14 out of these embryos gave rather good numbers of the nuclear plates. The results suggest the chromosome number of the hybrids is 59. The chromosome number of 59 is just the sum of two haploid sets, one from masu salmon (33) and the

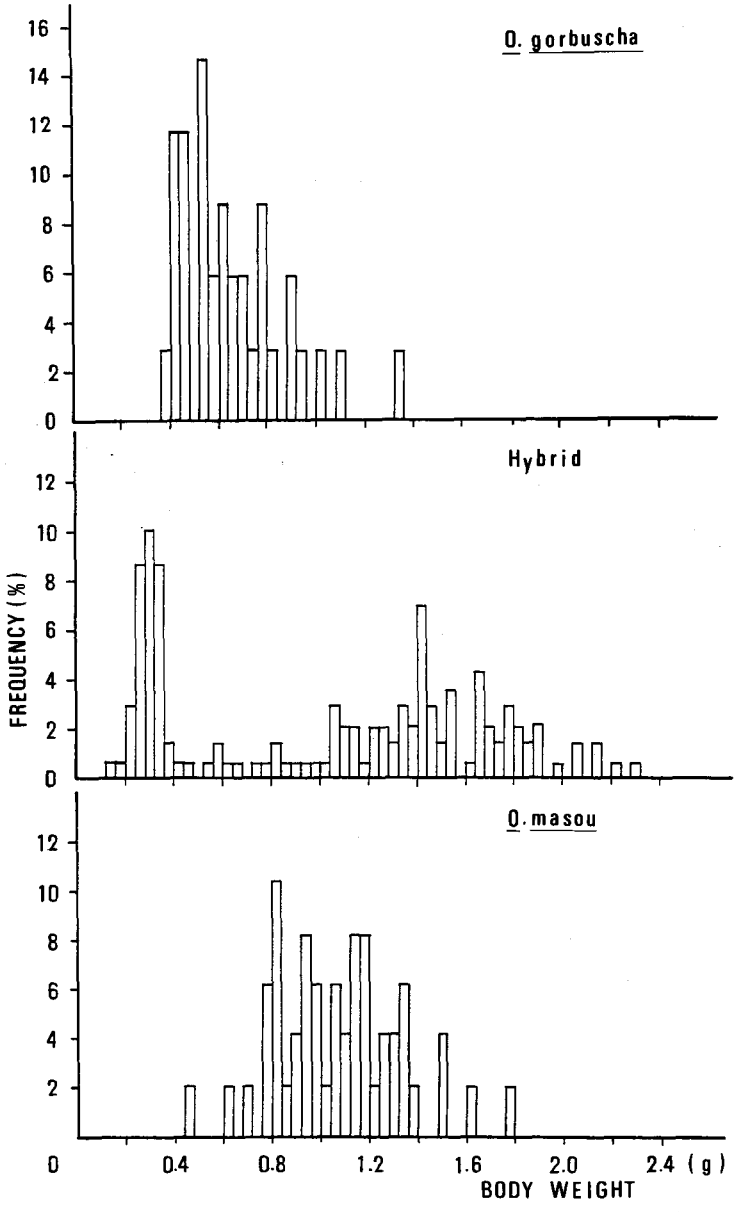

Fig. 3. The distribution of body weight in the hybrids and their parental species controls at six months old.

other from pink salmon (26).

There are however, two modes noticed in total distribution of chromosome numbers having peaks at 52 and 59 (Table 2). We also noticed that the chromosome number distributed very widely and that the cells having chromosome number of 52–59 took about 73% of the total cells examined.

Table 2. Distribution of chromosome numbers in embryos of the hybrid between female masu salmon and male pink salmon.

EMBRYO No.	NUMBER OF CHROMOSOME																					TOTAL
	<28	36	38	39	42	43	50	51	52	53	54	55	56	57	58	59	61	62	63	64	95	
1			1						1													2
2		1																				1
3									2	1		1	1									5
4																1						1
5	1												1			2					1	5
6										1				1								2
7									1		1											2
8									1	2												3
9	1								2					1		1						5
10							1							1	1							3
11									1				2			2		1				6
12				1					3				1	1								6
13										1	1					1		1	2	1		7
14	2				3	1		1	2	1	4	4	4	3	3	12	4	3				47
TOTAL	4	1	1	1	3	1	1	1	13	6	6	5	9	7	4	19	4	5	2	1	1	95

A fully expanded spread of 59 chromosomes was used for the analysis of karyotype according to the standard proposed by Laven *et al.* (1964). The karyotype analysis showed that there were 41 meta- or submetacentric, 14 subtelocentric and 4 acrocentric chromosomes (Fig. 4). In 52 chromosomes, there were several acrocentric chromosomes which do not exist in pink salmon.

Salinity tolerance

The fish of lot 4 were used in the salinity tolerance test. All control masu salmon died during this test period whereas the hybrids had strong salinity tolerance without mortality. (Table 3, 4, 5). The dehydration rates in hybrids were higher than those of the pink salmon at the beginning of the test. But they went down near to those of the pink salmon. The hybrids did not show clear silver colour. They had yet parr marks on their body sides but the distal sides of the dorsal and caudal fins were pigmented.

Numerical characters

Fig. 5 shows the appearance of masu salmon, pink salmon and their hybrids of N and B types. We counted the numbers of 7 numerical characters of the N-type hybrids and their parental species controls. Table 6 shows the results of the countings. The most different characteristics were found in the number of gill rakers, scales in the lateral lines and pyloric appendages among these species and the hybrids. The numbers of these numerical characters except dorsal rays in hybrids were invariably between those of masu salmon and pink salmon.

Fig. 4. A karyogram of a hybrid embryo with 59 chromosomes. Bars indicate 10 μm.

Table 3. Variances of body weights of masu salmon, pink salmon and their hybrid during the salinity tolerance test (mean±SD).

SPECIES	BODY WEIGHT (g)						
	START	DAY-1	DAY-2	DAY-3	DAY-4	DAY-5	DAY-8
MASU	4.8±1.3	3.8±1.2	2.65*				
HYBRID	5.3±1.4	4.9±1.5	4.9±1.4	4.8±1.4	4.6±1.4	4.5±1.3	4.4±1.3
PINK	3.3±0.87	3.2±0.86	3.2±0.88	3.0±0.86	3.0±0.85	2.9±0.82	2.8±0.84

The mark, * is the last one survived day-1

Table 4. Dehydration rates of masu salmon, pink salmon and their hybrid during the salinity tolerance test.

SPECIES	DEHYDRATION RATE (%)						
	START	DAY-1	DAY-2	DAY-3	DAY-4	DAY-5	DAY-8
MASU	0	20.83					
HYBRID	0	7.55	7.55	9.43	13.21	15.09	16.98
PINK	0	3.03	3.03	9.09	9.09	12.12	15.15

Table 5. Survival rates of masu salmon, pink salmon and their hybrid during the salinity tolerance test.

SPECIES	SURVIVAL RATE (%)						
	START	DAY-1	DAY-2	DAY-3	DAY-4	DAY-5	DAY-8
MASU	100	10	0	0	0	0	0
HYBRID	100	100	100	100	100	100	100
PINK	100	100	100	100	100	100	100

Table 6 Means and standard errors of seven numerical characters in masu salmon, pink salmon and their hybrid.

CHARACTER	MASU SALMON	HYBRID	PINK SALMON
NUMBER OF INDIVIDUALS	15	19	10
DORSAL RAYS	14.40±0.17	13.42±0.16	13.50±0.18
PECTORAL RAYS	13.47±0.20	15.16±0.14	15.60±0.23
PELVIC RAYS	9.20±0.11	10.05+0.05	10.50±0.24
ANAL RAYS	13.73±0.16	14.47±0.16	15.70±0.22
GILL RAKERS	17.33±0.13	25.21±0.29	28.70±0.32
SCALES IN LATERAL LINE	124.27±1.25	142.63±0.97	154.10±2.39
PYLORIC APPENDAGE	44.00±1.08	80.11±1.62	121.33±7.79

Fig. 5. Appearances in the juveniles of masu salmon (1 : FL=75 mm), pink salmon (4 : FL= 79 mm) and their hybrids of N-type (2 : FL=91 mm) and B-type (3 : FL=55 mm).

Discussion

It is worthwhile to note here that there were great variations in hybrids between female masu salmon and male pink salmon. Suzuki and Fukuda (1971b) reported on the growth and survival of many kinds of hybrids among salmonids and noticed also variation in the distribution of body weight in one and two-year old hybrids especially in intergeneric hybrids. These variations found in the hybrids between masu salmon and pink salmon may be caused by cytogenetic factors as suggested by Suzuki and Fukuda (1971b) in other hybrids of salmonids. These cytogenetic factors may act sometimes harmoniously to produce hybrid vigor and occasionally to produce abnormal embryogenesis when unbalanced. In the present study, four

types of the hybrids were conveniently identified. There are still many differences between the viable types of B and N. These types were quite different each other. W and S type hybrid died without complete absorption of yolk. B-type hybrid was quite slender and bluish in colour without any parr marks. The viability of this type was weak and the growth was very slow. But N-type hybrid having clear parr marks showed strong heterosis in growth. These appearances of the types were invariable because all lots from 1 to 3 which had been produced by one female masu salmon and one pink salmon showed the same characteristics. These results therefore clearly indicate that the hybrid are too varied to discuss only average nature having a modal characteristics.

Arai (1984) has reported that the hybrid juveniles have 59 chromosomes. This hybrid may be commensurated to N-type of the present study because none but N-type of hybrid could reach to 9 cm in fork length. In the present study, there have been two modes in total chromosome counts having peaks at 52 and 59 in chromosome numbers. These chromosome counts were made from 13 day-old embryos. In this embryonic stage the type classification were difficult but the variation of the chromosome numbers in this stage may associate with the abnormal embryogenesis or weak newly hatched larvae. The karyotypes of 59 chromosomes found in this stage were almost the same as that reported by Arai (1984). The modal chromosome number of 52 is the same chromosome number of pink salmon. But in the present study, the karyotype analysis of the chromosomes showed several acrocentric chromosomes which are never found in pink salmon. Therefore the embryos having 52 chromosomes are not androgenetic but may occasionally lose 7 chromosomes. The phenomenon of chromosome loss in the hybrids was first reported in the hybrids of masu salmon $\times$ chum salmon (Yamazaki, 1981). The same phenomenon was also observed in other inviable hybrids in salmonids (Arai, 1984). The losing of chromosomes during embryogenesis in inviable hybrids may be one of important reasons to induce gross anomalies of the embryos.

The results of salinity tolerance test did not vary from the previous results of Yamazaki and Ma (1985). The N-type hybrids expressed salinity tolerance at earlier stages before appearance of silvering in body colour. This salinity tolerance at early stages of N-type hybrids has therefore no direct relation to silvering colour of the body and would be inherited from the pink salmon. These results indicate that appearance of sea water tolerance at earlier stages could be controlled by genetic factors. The N-type hybrids having natures of parental species would be useful materials to analyze the genetic mechanisms of life phase division found in masu salmon.

Nearly all of the numerical characters in the N-type hybrids were between those of masu salmon and pink salmon. It seems however that the influence of pink salmon was larger than that of masu salmon such as those found in dorsal rays or scales in lateral line. The N-type hybrids grew faster than parental species and earlier salinity tolerance than masu salmon. They sometimes showed downstream migration behaviour in the experimental conditions (Yamazaki and Ma, 1985). These characteristics should be studied more in detail in future to understand seaward migration mechanisms of salmonids.

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