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DEVELOPMENTAL GENETIC STUDIES ON SALMONIDS. MORPHOGENESIS, ISOZYME PHENOTYPES AND CHROMOSOMES IN HYBRID EMBRYOS.

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荒井克俊

1984

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INTRODUCTION

The management of embryos from fertilization to hatching is regarded as having an established technique with no room for improvement at present. Shortages in basic information, however, may leave some unsolved problems including frequent incidence of embryonic death and deformed larvae (Yamazaki and Arai, 1982). The presence of stages of vulnerability to environmental changes during early development is known in general, but its scientific interpretation remains to be presented from a standpoint of the genetics of the embryology of fishes with reference to its importance in the management of embryos. The mechanism underlying the embryogenetic process in fish remains unknown in spite of the great number of morphological, physiological, and biochemical approaches that have been taken (Yamamoto, 1943 ; Neyfakh and Abramova, 1974). Recent progress in developmental genetics, based on molecular biology, has pointed out that the developmental event can be understood as the process of differential control of gene activation (Davidson, 1976). Further studies from such a viewpoint will be indispensable in understanding embryological problems related to the artificial propagation of fishes, including salmonids.

Interspecific hybrids between different species play an informative role in the study of the onset of the genomic function in early embryogenesis, because it can be determined by first detecting the appearance of paternal characters, which are under the control of the embryonic genome itself in contrast to the maternal characters, on the appearance of which

there is a possibility of cytoplasmic influence. On the other hand, it is generally known that extreme mortality and abnormality are frequently found in hybrids between phylogenetically distant species. When such phenomena are specific to a certain hybrid combination, an examination of these hybrids will provide another useful tool for gaining insight into the mechanisms responsible for abnormal development in fishes. Newman (1914), at the beginning of the present century, extensively investigated the development of teleost hybrids of fourteen species and concluded, based on the morphology, developmental rate, and developmental capacity of viable and inviable teleostean hybrids, that the first step in embryonic differentiation started at the stage of gastrulation. Other similar studies on fishes, however, did not progress due to technical limitations that prevented the use of morphological characteristics and developmental parameters as markers of genomic function

Another approach to genetic control of fish embryogenesis relied on physical inactivation of embryonic nuclei, Neyfakh (1959, 1964) examined the survival potentials of loach embryos of which the nuclei had been inactivated by X-ray irradiation at progressive stages of development and concluded that the morphogenetic function was under the control of the embryonic nuclei from the stage of gastrulation. Although this approach provided valuable information for understanding the total function of the embryonic genome, the precise timing and the exact process of differential gene activation could not be shown because of the lack of direct genetic markers and the harmful

influence of X-ray irradiation on the total embryonic genome and cytoplasm.

The definition of the relationship between genes and proteins and the subsequent formulation of the concept of "isozyme" from 1950-1960's made available certain tools for developmental studies of fishes (Markert and Møller, 1959 ; Markert, 1963). As an isozyme is constructed by a subunit polypeptide assembly encoded in a specific gene (Markert and Møller, 1959 ; Markert, 1963), electrophoretic differences among isozymic bands must indicate differences among the genes controlling these isozymes. Therefore, it can be concluded that the isozyme, visually detected, is the most useful gene marker, and that the appearance of isozymic activity indicates the expression of the gene activity. In addition to this molecular basis, technical advances in the detection of isozymes using electrophoresis and histochemical staining have accelerated progress in this field of study. For example, Champion et al (1975) reported finding sixteen kinds of isozymes expressed during the early development of the chubsucker, Erymizon succer, and the green sunfish, Lepomis cyanellus, and pointed out the significance of the hatching stage. Shaklee et al (1974) stated that the appearance of tissue-specific isozymes was related to the morphological and functional differentiation of the concerned tissues in the ontogeny of the lake chubsucker. Similar work, including measurement of enzymatic activity, was carried out for other species of sunfish (Champion and Whitt, 1976 ; Shaklee and Whitt, 1977).

The use of isozymes in hybrid experiments provided a more

effective methodology for the study of gene activation during embryogenesis. The precise timing of the gene activation could be determined by utilizing as a genetic marker interspecific distinctions in electrophoretic mobilities of isozymes, since the appearance of isozymic components containing paternal gene products means activation of the embryonic genome itself. In this regard, embryonic lactate dehydrogenase (LDH) and phosphoglucomutase (PGM) were studies in the embryos of the hybrids of Salmo irideus x S. trutta, and it was suggested that the "augenpunkt" stage was significant in being the first stage in the genomic function of the embryos (Klose et al., 1968). Similar studies of ontogeny were carried out on an intraspecific hybrid of Rutilus rutilus with 6-phosphogluconate dehydrogenase (6PGDH) (Klose and Wolf, 1970), on interspecific Salvelinus hybrids with LDH (Goldberg et al., 1969), on Brachidanio hybrids with creatine kinase (CK) (Pontier and Hart, 1979), and on intergeneric Salmo trutta x Salvelinus fontinalis hybrids with glucosephosphate isomerase (GPI) (Engel et al., 1977).

Such experiments on hybrids using isozymic gene markers may also be of assistance in determining hybrid incompatibilities affecting gene expression, between the two parental genomes as well as between exotic genome and maternal cytoplasm. Hitzeroth et al. (1969) reported that activation of the maternal allele occurs earlier than that of the paternal allele at the loci of eye specific LDH and liver specific alcohol dehydrogenase (ADH) in hybrids between phylogenetically distant salmonid species. Similar asynchromisms of allelic expression

were reported in glucose-6-phosphate dehydrogenase (G6PDH) of Salvelinus fontinalis x S. namaycush hybrids (Yamauchi and Goldberg, 1974, 1975); however, synchronous expression was reported at the GPI locus in hybrids between closely related species of sunfish (Champion and Whitt, 1976b).

At present, however, the information obtained from isozymic ontogeny in fish hybrids is still fragmentary and inconclusive for the stage at which the onset of the genomic function occurs. Materials in most previous works were limited to normal embryos from viable hybrid combinations, and no experiments have been conducted to clarify the nature of the differential gene expressions in inviable and abnormal hybrid combinations, except for the studies by Whitt et al. (1977) of multilocus isozyme systems of inviable green sunfish x largemouth bass hybrids. Further developmental genetic work is required using as many isozyme markers as possible, on the development of various types of hybrids exhibiting various kinds of developmental capacities from viable to inviable.

Isozymic experiments provide valuable information for understanding the differential gene activation during fish embryogenesis, but the mitotic process of cellular division is another important factor, as it also encourages normal development of fish embryos. Mitosis means regular and cyclic replication and division of chromosomes. Any physical or chemical disturbance before or during this process results in a chromosomal aberration. This suggests that chromosomes can be used as visible markers for inspecting mitotic aber-

rations in the course of embryonic development.

Recently, Yamazaki (1981, 1983) reported that chromosome aberrations in abnormal embryos resulted from the aging of ovulated oocytes, and pointed out the morphological analogy between these anomolous embryos and hybrid embryos that resulted from inviable combinations. This strongly suggests the coexistence of cytological and cytogenetic causes common to abnormal development and indicates a need for chromosomal studies on abnormal hybrid embryos. Chromosomal investigations have been conducted on fish hybrids, including salmonids (Sasaki et al, 1968 ; Nygren et al, 1972 ; Uyeno, 1972 ; Gjedrem et al, 1977), but all of these have been restricted to viable hybrids capable of developing as far as the adult stage. At present, no work has been done on inviable hybrids, which die at early stages of embryogenesis, except for the classic studies using primitive cytological techniques done at the beginning of the present century (Pinney, 1918, 1922, 1928). Such a shortage of experimentally obtained cytogenetic information on fish development may result in the failure to examine the genomic constitution of hybrid embryos, such as those used in previous isozymic studies on gene activation. In the case of inviable hybrids, it is absolutely necessary to determine whether the embryonic abnormality is due to a disturbance in the genomic function or to a chromosomal aberration resulting from anomalous mitosis.

The hybrid experiment utilizing isozymes and chromosomes as visible genetic markers is one of the most effective methodologies in the developmental genetics of fishes. How-

ever, it can be used only situations such as the heterogenetic diploid state, where both the maternally and paternally derived genomes coexist in the maternal egg cytoplasm. Chromosome engineering has recently been progressing, and at present it is possible in several fish species including salmonids to induce gynogenetic and androgenetic development through insemination by gamma-ray, X-ray and Ultraviolet ray irradiated gamates, and also to produce polyploids by physical treatment after fertilization (Purdom, 1969 ; Onozato, 1981, 1982, 1983 ; Onozato and Yamaha, 1983). Such a procedure may be available not only for breeding techniques aimed at genetic improvement of commercial fish and shellfish, but also for developmental research on fishes (Onozato, 1983 ; Yamazaki, 1981).

When the release of the second polar body is fully blocked to produce triploid hybrids after the insemination with exotic milt from other species, the resultant hybrid embryos should consist of two sets of maternal genomes and one set of paternal genomes. Such a genomic constitution in triploid hybrids means a relative decrease in the proportion of exotic genomes to endemic genomes or egg cytoplasm. This relationship between paternal and maternal genomes and egg cytoplasm in triploid hybrids may also make it possible to diminish certain deleterious influences of exotic genomes on development, which have often been observed for inviable hybrids in the diploid state. This idea may be supported by Stanley (1976) who reported that no diploid hybrid survived and that only the triploid hybrids which spontaneously occurred could survive beyond hatching, in the artificial crossing of common carp and grass carp. Exper-

imental research on the embryonic differentiation and cellular division in triploid hybrids, utilizing isozymes and chromosomes, will be more valuable than research on diploid hybrids for understanding the developmental genetic events in normal and abnormal embryogenesis.

The purposes of the present study are to determine the timing and the process of differential genomic control for normal ontogenesis in salmonids, and to gain insights into the developmental genetic and cytogenetic mechanisms responsible for embryonic abnormality. The four major goals of the experiments carried out can be summarized as follows:

(1). Observations of morphogenesis and survival rates during early development for twelve kinds of interspecific or intergeneric hybrids from six species of salmonids, and the classification of each hybrid according to its developmental capacity.

(2). A determination of the stage at which embryonic gene activities are first expressed during early development of viable hybrids by examining the appearance of paternal gene products in lactate dehydrogenase (LDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGDH), and phosphoglucomutase (PGM) isozyme systems, and a further determination as to mechanisms responsible for abnormal development by inspecting aberrant gene expression in inviable hybrid embryos using the same methodology.

(3). A detailed report on chromosome numbers and karyotypes, in order to understand the cytological and cytogenetic events affecting the developmental abilities of the salmonid hybrids.

(4). An assessment of interactions between an exotic genome and a maternal genome, or between these two genomes and egg cytoplasm, by examining the influences of artificial allotriploidization on developmental capacity and isozymic gene expression in inviable hybrids.

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mature parental fish for the present study. The author is also greatly indebted to Dr. Toshiro Terao, Mr. Houji Okada and Mr. Haruyoshi Matsumoto of the Hokkaido Fish Hatchery for the collection of parental salmonid fishes. The author thanks Messrs. Yo Sugimoto, Motohiko Fukami, Kouichi Mano, Fumitaka Naito and Miss Yukari Kimura, School of Fisheries Sciences, Kitasato University, for their careful assistance in the present study. The present study was supported, in part, by Grant-in-Aids (No. 56760128 and No. 57760159) from the Japan Ministry of Education, Science and Culture.

MATERIALS AND METHODS

Origin of specimens

Mature parental fish of the following six species were used for crossing in this study: pink salmon, Oncorhynchus gorbuscha, masu salmon, O. masou, chinook salmon, O. tschawytscha, chum salmon, O. keta, Japanese char, Salvelinus leucomaenis and brook trout, S. fontinalis. Some of these fish were captured during their spawning season from rivers in Hokkaido and in the northern area of the main island of Honshu in Japan, and some were provided from the rearing stocks of the fish ponds of Nanae Experimental Fish Culture Station of Hokkaido University, Tadami Fish Culture Station of Kitasato University and the Chitose and Mori branches of Hokkaido Fish Hatchery (Text-Fig. 1). Locations, sampling years, sexes and fork-lengths are summarized in Table 1.

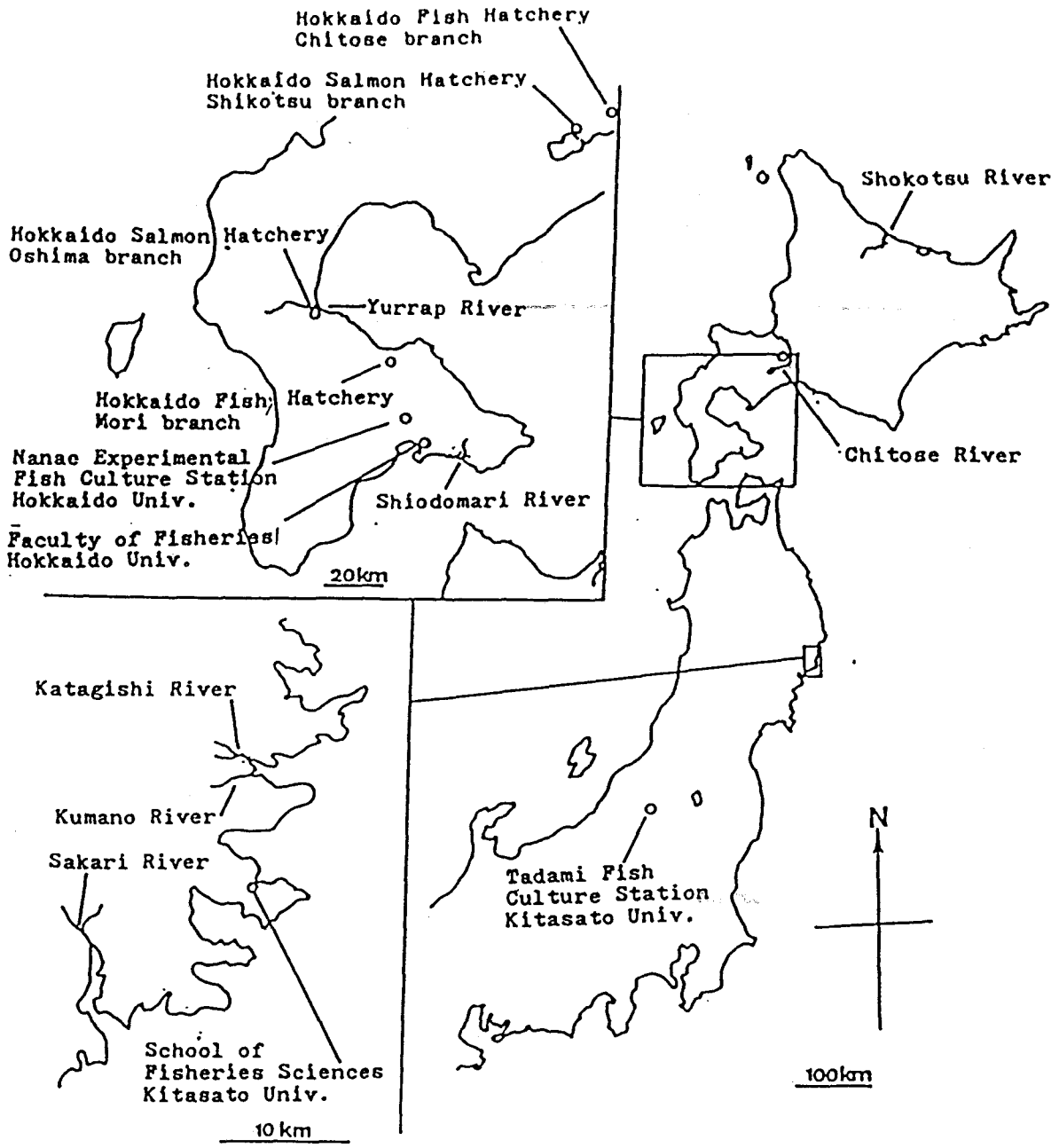
Table 1. Summary of specimens used as parental fishes for artificial crossing and materials for chromosome preparation.

species	fish No.	locality	sex	fork length(cm)	year of sampling
Pink salmon	101	Yurrap River	♀	54.0	1977
Oncorhynchus gorbuscha	102		♂	60.0	
	103		♀	51.5	
	104	Shokotsu River	♀	8.9	1979
	105		♂	8.2	
	106		♂	7.3	
	107		?	6.8	
Chum salmon	201	Yurrap River	♀	74.8	1977
Oncorhynchus keta	202		♂	72.3	
	203		♀	69.5	
	204		♂	67.0	
	205	Shiodomari River	♀	73.0	1979
	206		♂	83.0	
	207		♀	64.0	1980
	208		♂	76.0	
	209		♀	69.0	1981
	210		♂	68.0	
	211	Katagishi River	♀	81.5	1981
	212		♂	82.5	
	213	Sakari River	♀	61.0	1982
	214		♂	62.0	
	215	Sakari River	♀	68.0	1982
	216		♂	61.0	
Masu salmon	301	Hokkaido Fish Hatchery, Mori branch	♀	40.1	1977
Oncorhynchus masou	302		♀	32.5	
	303		♀	24.5	
	304		♀	33.5	
	305		♀	25.7	
	306		♀	26.5	
	307		♂	26.5	
	308	Hokkaido Fish Hatchery, Chitose branch	♀	29.0	1977
	309		♀	28.6	
	310		♀	28.7	
	311		♂	40.3	1977
	312		♀	52.8	
	313		♀	48.9	
	314	Shiodomari River	♀	53.0	1979
	315		♀	57.5	
	316	Nanae Experimental Fish Culture Station Hokkaido Univ.	♂	17.5	1979
	317		♂	18.0	
	318		♂	13.5	
	319		♂	11.9	
	320	Shiodomari River	♀	54.5	1980
	321	Nanae Experimental Fish Culture Station Hokkaido Univ.	♂	-	
	322		♂	12.8	
	323		♂	17.8	1981
	324	Kumano River	♀	11.5	1982
	325		♀	12.2	
	326		♂	11.7	
	327		♀	12.0	
	328		♀	11.8	
	329		♂	18.3	

Table 1. (continued)

species	fish No.	locality	sex	fork length(cm)	year of sampling
Chinook salmon	401	Hokkaido Fish Hatchery, Chitose branch	♂	50.9	1977
Oncorhynchus tschawytscha	402		♂	26.4	
Japanese Char	501	Nanae Experimental Fish Culture Station Hokkaido Univ.	♂	26.0	1979
Salvelinus leucomaenis	502		♂	25.5	
	503		♀	31.7	
	504		♀	40.6	
	505		♂	28.3	
	506		♀	36.2	
	507		♂	-	
	508		♂	39.0	1980
	509		♀	33.5	
	510		♀	35.1	1981
	511		♀	41.8	
	512		♂	41.3	
	513	Tadami Fish Culture Station Kitasato Univ.	♂	29.5	
	514		♀	42.0	
	515		♀	59.2	
	516		♂	36.5	
	517		♀	44.0	
	518		♀	46.0	
	519		♀	-	1982
	520		♀	-	
	521		♂	-	
	522		♂	-	
Brook trout	601	Tadami Fish Culture Station Kitasato Univ.	♂	17.9	1980
Salvelinus fontinalis	602		♀	15.7	
	603		♀	18.7	
	604		♂	26.5	1981
	605		♀	38.0	
	606		♂	26.8	
Masu salmon♀ x Pink salmon♂*	701	Faculty of Fisheries, Hokkaido Univ.	♀	9.4	1979
O. masou♀ x *O. gorbuscha*♂	702		♀	10.6	
	703		♀	9.4	

*: Originated from hybrid combination No. 7704.



Text-Fig. 1. Locations where specimens were sampled and artificial crossings were carried out for the present study.

Besides these parental fishes, several young dolly varden char, Salvelinus malma, coho salmon, Oncorhynchus kisutch, Japanese huchen, Hucho perryi and rainbow trout, Salmo gairdneri were provided by the Nanae Experimental Fish Culture Station of Hokkaido University, in order that interspecific differences in isozymes could be examined. Kokanee salmon, O. nerka were also provided by the Shikotsu branch of Hokkaido Salmon Hatchery.

Embryos and juveniles, which originated from parental and hybrid crossings of fishes shown in Table 1, were used for chromosome preparation. Besides these, pink salmon from the Shokotsu River in Hokkaido, masu salmon from the Kumano River and brook trout from Tadami Fish Culture Station were also used (Table 1).

Artificial hybridization and incubation of embryos

Artificial hybridization carried out among five species of salmonids resulted in the following twelve hybrid combinations: masu salmon♀ x pink salmon♂ (O. masou♀ x O. gorbuscha♂), pink salmon♀ x masu salmon♂ (O. gorbuscha♀ x O. masou♂), masu salmon♀ x chum salmon♂ (O. masou♀ x O. keta♂), chum salmon♀ x masu salmon♂ (O. keta♀ x O. masou♂), masu salmon♀ x chinook salmon♂ (O. masou♀ x O. tschawytscha♂), Japanese char♀ x brook trout♂ (S. leucomaenis♀ x S. fontinalis♂), masu salmon♀ x Japanese char♂ (O. masou♀ x S. leucomaenis♂), Japanese char♀ x masu salmon♂ (S. leucomaenis♀ x O. masou♂), chum salmon♀ x Japanese char♂ (O. keta♀ x S. leucomaenis♂), Japanese char♀ x chum salmon♂ (S. leucomaenis♀ x O. keta♂), chum salmon♀ x brook trout♂ (O. keta♀ x S. fontinalis♂) and

brook trout♀ x chum salmon♂ (S. fontinalis♀ x O. keta♂). Parental crossings were carried out in the same way as the hybrid crossings and the resultant combinations were utilized as controls. A summary of the results, including combinations of species, individual fish used as mothers and fathers, dates of crossing and locations of crossing, is shown in Table 2.

In all the crossings except for those carried out in 1977, mature ova were collected from a single female used as the mother by cutting the abdomen with scissors. After rinsing with isotonic physiological saline containing 9.04g of NaCl, 0.24g of KCl and 0.25g of $CaCl_2$ in 1l of distilled water, the eggs were inseminated with milt stripped from a single male using the dry method. In the case of crossings No. 7703, 77-08, 7710 in masu♀ x masu salmon♂, 7704 in masu♀ x pink salmon♂, 7705 in masu♀ x chum salmon♂ and 7709, 7711 in masu♀ x chinook salmon♂, eggs from two to six females were stocked because of insufficient numbers of ova from a single female. They were devided into several groups for mating as shown in Table 2. Even in these cases, only a single male fish was used as the father.

After the complete hardening of the chorion by absorption of water, fertilized eggs from each crossed group were packed in a jar with some ice cubes and transported by car from the locality of mating to the laboratory of either the Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido (in 1977) or the School of Fisheries Sciences, Kitasato University, Sanriku, Iwate (in 1980. 1981 and 1982). Fertilized eggs for each hybrid combination were placed in a small bascket (30 x

Table 2. Summary of Artificial Crossing

No.	combination	fish No. of maternal parent	fish No. of paternal parent	date of crossing	locality of crossing
7701	Pink x Pink	101	102	1977, Sept., 27	Hokkaido Salmon Hatchery, Oshima branch.
7702	Pink x Masu	103	307		
7703	Masu x Masu	301-306	307		
7704	Masu x Pink	301-306	102		
7705	Masu x Chum	301-306	204		
7706	Chum x Chum	201	202		
7707	Chum x Masu	203	307		
7708	Masu x Masu	308-310	311	1977, Oct., 12	Hokkaido Fish Hatchery, Chitose branch
7709	Masu x Chinook	308-310	401		
7710	Masu x Masu	312-313	311		
7711	Masu x Chinook	312-313	402		
7901	Masu x Masu	315	317	1979, Oct., 11	Shiodomari River
7902	Masu x Char	315	501		
7903	Masu x Char	315	502		
7904	Masu x Masu	314	316	1979, Oct., 12	Shiodomari R.
7905	Chum x Chum	205	206		
7906	Masu x Chum	314	206		
7907	Chum x Masu	205	316		
7908	Char x Masu	503	318	1979, Oct., 13	Nanae Experimental Fish Culture Station Hokkaido Univ.
7909	Char x Char	504	505	1979, Oct., 14	
7910	Char x Masu	504	319	1979, Oct., 14	
7911	Char x Char	506	507	1979, Oct., 16	
8001	Masu x Masu	320	321	1980, Oct., 9	Shiodomari River
8002	Masu x Char	320	508		
8003	Masu x Chum	320	208		
8004	Chum x Chum	207	208		
8005	Chum x Masu	207	322		
8006	Chum x Char	207	508		
8007	Char x Char	509	508		
8008	Char x Chum	509	208		
8101	Chum x Chum	209	210	1981, Oct., 8	Shiodomari R.
8102	Chum x Char	209	512		
8103	Char x Char	510	512		
8104	Char x Masu	511	323		
8105	Char x Chum	511	210		
8106	Char x Char	515	516	1981, Nov., 13	Tadami Fish Culture Station Kitasato Univ.
8107	Char x Brook	517	604		
8108	Char x Brook	518	604		
8109	Chum x Chum	211	212	1981, Nov., 14	Katagishi R.
8110	Chum x Char	211	513		
8111	Chum x Brook	211	606		
8112	Char x Chum	514	212		
8113	Brook x Brook	605	606	1981, Nov., 14	Katagishi River
8114	Brook x Chum	605	212		
8201	Chum x Chum	216	214	1982, Nov., 20	School of Fisheries Sciences, Kitasato Univ.
8202	Chum x Char	216	521		
8203	Char x Char	519	521		
8204	Char x Chum	519	214		
8205	Chum x Chum	215	216		
8206	Chum x Char	215	522		
8207	Char x Char	520	522		
8208	Char x Chum	520	216		

20 x 10cm) and incubated in a hatching box (270 x 32 x20cm) that was fully aerated by a motorized water recirculation system. The water temperature in the hatching box was maintained at 8.5-10.5°C using a refrigerator system.

Observations on viability and morphogenesis

Fertilized eggs from the hybrid and parental combinations of the crossings carried out from 1977 to 1981 were divided into two groups. One group was used as specimens for electrophoretic research, chromosomal preparation and morphological observation. As for the other group, the survival rate of its eggs was monitored daily during the experimental period by counting and removing the dead ones. In the experiment of 1982, each crossed group was further divided into two groups. Eggs in one group were exposed to hydrostatic pressure treatment after fertilization, in order to make them triploid; the other group was used as an intact control. Each divided group was further subdivided into two, and from one subdivided group samples of chromosomal, isozymic and morphological specimens were taken, while the other group was checked for viability.

During the course of embryonic development, five to ten fertilized eggs were collected and fixed with Bouin's solution every three or four days. The fixed embryos were observed under a binocular dissection microscope and sorted by external morphological appearance after the manual removal of their chorions with forceps. These embryos were photographed as the occasion demanded.

Electrophoresis and staining of isozymes

Eyes, heart, liver, kidneys and skeletal muscle were cut out from the parental fishes used for artificial mating, in order that adult tissue specificity and interspecific distinctions in isozymes may be electrophoretically examined. The parts used were kept frozen at -20°C until electrophoretic analysis. For the investigation of isozymic ontogeny, twenty to forty embryos of each crossed group were sampled at various stages during embryogenesis. Collected specimens were dissected on CO_2 ice in order to obtain embryonic parts with a little bit of yolk attached. Embryonic parts from various stages and blastodiscs from unfertilized eggs were also frozen and kept at -20°C. Cell-lysates or drip component directly extracted from adult tissues and developing embryos were absorbed by filter paper of 4 x 5 or 4 x 10mm in size as electrophoretic preparation of enzymatic samples.

The buffer systems used in the present study are summarized in Table 3. Gel plates were prepared by adding 12% hydrolyzed starch to the appropriate gel buffer. The starch-buffer mixture was then vigorously swirled and heated to boiling. After degassing using a water aspirator, the starch gel was poured into a plexiglass frame of 200 x 150 x 5 or 200 x 150 x 10 mm in size and allowed to cool to room temperature. A slit was cut at 5-6 cm from the cathodal side of the gel plate and 20-25 filter papers containing sufficient tissue or embryonic extract were placed vertically into the cut. Absorbant cloths such as paper towels or gauze were used to conduct electric current between the ends of the gel plate and the

electrode buffer in the anodal and cathodal chambers. After electrophoresis at a constant voltage of 50 V for 30 minutes, filter papers were removed from the cut and the surface of the gel was covered with a vinyl film to protect it from dryness. Then horizontal electrophoresis was carried out at a constant voltage of 150-200 V for 4-7 hours, until the dye indicator, amido black 10B, migrated to a line of 5-7 cm from the origin. Electrophoresis was done at $4^{\circ}C$ in a refrigerator.

After this, the gel was horizontally sliced into 5-10 sheets of one mm thickness each. Each sheet of gel was placed in a staining tray and incubated in an appropriate mixture according to the objective isozyme at $37^{\circ}C$ for 1-3 hours, untill the enzymatic activities appeared as stained bands or zones (Table 4). The stained gel was fixed with 5 % of acetic acid. After the infiltration of 10 % glyceric solution, the gel was tightly enclosed with cellophane film and photographed after drying.

Nomenclature of isozymes, genetic loci and alleles

Numerous researchers have studied many kinds of isozymes in various species of organisms and different systems of nomenclature have been used. At present, these nomenclature systems are not standardized and remain complicated. In the present study, the abbreviations for the names of enzymes and for the gene loci responsible for the isozymes are designated by Gothic type and Italics, respectively. For example, lactate dehydrogenase is abbreviated as LDH for the isozyme and *Ldh* for the gene locus involved. The distinctions between genetic loci are designated by using capitals, but

Table 3. Summary of buffer systems for the electrophoresis in the present study

type	bridge buffer	gel buffer	reference
I.	0.3M boric acid 0.085M sodium hydrate (pH. 8.5)	0.03M boric acid 0.005M sodium hydrate (pH. 8.5)	Schwaltz (1960)
II.	0.135M tris[1]. 0.045M citric acid 0.0012M EDTA-2Na[2]. (pH. 7.0)	0.009M tris 0.003M citric acid 0.0012M EDTA-2Na (pH. 7.0)	Ayala et al (1973)
III.	0.04M citric acid adjusted pH. with n-(3-aminopropyl)- diethanolamine (pH. 7.0)	0.002M citric acid adjusted pH. with n-(3-aminopropyl)- diethanolamine (pH. 7.0)	Clayton and Tretiak (1972)
IV.	0.04M citric acid adjusted pH. with n-(3-aminopropyl)- morpholine (pH. 6.1)	0.002M citric acid adjusted pH. with n-(3-aminopropyl)- morpholine (pH. 6.1)	Clayton and Tretiak (1972)

1. tris(hydroxymethyl)aminomethane
2. disodium ethylenediaminetetraacetate

Table 4. Comparison of staining solutions for four enzymes. All of the stains were mixed in 40 ml of 0.05M Tris-Hcl buffer (pH. 8.7)

enzymes	abbreviation	buffer system	tetrazolium salt (5 mg)	PMS[1] (2 mg)	co-enzyme (10 mg)	other components
Lactate Dehydrogenase (EC 1.1.1.27)	LDH	I, II	NBT[2]	+	NAD[3]	3ml of 0.5M sodium lactate.
Malate Dehydrogenase (EC 1.1.1.37)	MDH	III, IV	MTT[4]	+	NAD	3ml of 0.5M disodium malate
6-Phosphogluconate Dehydrogenase (EC 1.1.1.44)	6PGDH	III, IV	MTT	+	NADP[5]	10mg of trisodium 6-phosphogluconate
Phosphoglucomutase (EC 2.7.5.1)	PGM	III, IV	MTT	+	NADP	75mg of dipotassium glucose-1-phosphate 50 units of glucose-6-phosphate dehydrogenase

1. phenazine methosulfate
2. nitro blue tetrazolium
3. diphosphopyridine nucleotide
4. MTT tetrazolium
5. triphosphopyridine nucleotide

in the case of duplicated loci, superscript numbers are used. Allelic differences at those loci are then expressed by superscript small letters. By using subscript numbers, quarternary structures of enzymes can be distinguished.

As isozyme patterns and their genomic control in salmonids are quite complicated, it was difficult to indicate the names of all the isozymic bands, alleles and gene loci in photographs of the gel plates. Therefore, in most cases in this study, only the positions of representative monomeric bands, homodimers and homotetramers are indicated.

Chromosome preparations

For the preparation of embryonic chromosomes, the chopping method of Yamazaki et al (1981) was adopted for the present study with some modification in colchicine and hypotonic treatment. Embryos were manually removed from developing eggs by cutting the chorion with forceps under a stereoscopic microscope, and the resultant embryonic parts were rinsed with physiological saline to remove any adhesive yolk or oil droplets. These embryos were treated for 1 to 2 hours by soaking them in 0.02 % colchicine solution dissolved in physiological saline. They then underwent hypotonic treatment with 0.075M KCl for 50-80 minutes, and this was followed by two washes with chilled Carnoy's fixative (3:1=methanol:acetic acid). The fixation continued for at least 60 minutes in Carnoy's solution. A fixed embryo was placed on a clean slide and was chopped to a milky cell suspension in a drop of distilled water according to the method of Yamazaki et al.

(1981). In the present study, a whole embryo was placed on one slide. The cell suspension was expanded over the entire slide by dropping Carnoy's fixative on the slide, and this was followed by flame-drying or air-drying. Then the prepared slide was stained with Giemsa solution diluted to 30 times by a phosphate buffer (pH. 6.8).

Besides salmonid embryos, individual adults and juveniles of several species and hybrids (Table 1) were used for cytogenetic analysis. In these cases, the procedure was the same as for embryos. To obtain a large number of metaphase spreads, these adult or juvenile specimens were intramuscularly or intraperitoneally injected with a dose of 10μg/g body weight colchicine. After 12-20 hours, the specimens were killed and dissected to remove the head kidney. The tissue sample was treated for 50-60 minutes with hypotonic 0.075M KCl solution and then fixed with Carnoy's fixative. Then the fixed tissue was cut into pieces of a proper size of 1-2 mm^3 and chopped up for the slides. Giemsa staining was done in the same way as it was done for the embryo.

All procedures for chromosome preparation were carried out at room temperature.

Chromosome slides were scanned under 100X and the counts were made under 1000X with a Nikon microscope. Since chromosome numbers in salmonids are quite high, as pointed out by Yamazaki (1981), most metaphase spreads were microphotographed, and the chromosomes were counted on the printed photographs, in order to determine precise chromosome numbers. Fully expanded spreads were used for karyological analyses according

to the standard proposed by Levan et al (1964).

Artificial induction of triploid

In the 1982 experiment, artificial triploidization was attempted according to the method reported by Onozato (1983), who used hydrostatic pressure as a stimulant for blocking the release of a second polar body and the first cleavage. Half the embryos in the crossed groups Nos. 8201-8208 (Table 2) were treated for 7 minutes, 15 minutes after insemination, at 650 atm/cm^2 by a French Press. Immediately after the treatment, the embryos were placed in the hatching box and reared in the same manner as the intact groups. Observations and examinations of their survival rates , morphologies, isozyme patterns and chromosomes were made as previously described.

RESULTS

Part I. Survival potential and developmental capacity of hybrid salmonid embryo.

A number of hybrid experiments with salmonid fishes have produced viable F_1 hybrids suitable for commercial culture (Terao, 1970 ; Suzuki and Fukuda, 1971a, 1971b, 1973a, 1973b). However, no work has been done with salmonids from the viewpoint of developmental genetics. For the present study, artificial hybridization experiments were carried out among six species of salmonids. Their survival potentials and morphogenetic capacities were observed in order to determine

the biological influence of interspecific crossings on the development of hybrid embryos. In this regard, special consideration was paid to the drastic decrease in the survival rates of several inviable hybrids and also to the differential developmental capacities of reciprocal crossings.

1. Normal development of salmonids

Development of pink salmon, Oncorhynchus gorbuscha

In the parental species of pink salmon, Oncorhynchus gorbuscha (Table 2), eggs were successfully fertilized and most of the resultant embryos developed normally beyond hatching (Table 5, Text-Fig. 2). The hatching activity began at 49 days after fertilization under an average temperature of 10.5°C (Table 5).

Morphogenesis of pink salmon is demonstrated in Pl. I, Figs. 1-19. As shown in the figures, the cytoplasm became concentrated in the blastodisc after the fertilization of eggs. The blastodisc began to divide and progressed to the morula stage at 2 days after fertilization (Fig. 1). At 4 days, the eggs showed the typical morphology of the blastula stage and began to spread over the yolk mass (Fig. 2). At 6 days, the posterior region of the blastodisc became thicker than the anterior border and the formation of the embryonic shield became apparent (Fig. 3). This suggested the beginning of invagination and the differentiation of the embryonic axis, and therfore indicated that gastrulation was occurring. The embryonic body could be recognized at 8 days

Table 5. Summary of survival rates during the embryogenesis of hybrid salmonids

No.	combination	no. of embryos observed	survival rate (%) at 10 days after fertilization							date of hatching	average water temperature °C (range)
			10	15	20	25	30	40	50		
7701	pink x pink	224	99.6	99.6	99.6	99.6	98.7	95.1	83.9	49	10.5(10.0-11.0)
7702	pink x masu	410	99.8	99.3	97.8	96.1	91.7	87.6	77.1	47	10.5(10.0-11.0)
7703	masu x masu	389	98.5	95.1	91.5	78.9	59.9	41.9	36.8	38	10.5(10.0-11.0)
7704	masu x pink	325	98.2	96.6	91.4	75.4	48.3	22.2	17.5	47	10.5(10.0-11.0)
7705	masu x chum	333	94.3	64.6	13.2	7.2	3.9	0.6	0	-	10.5(10.0-11.0)
7706	chum x chum	421	99.5	99.3	99.3	99.1	99.1	97.6	96.2	49	10.5(10.0-11.0)
7707	chum x masu	293	99.0	98.3	32.4	15.0	6.5	1.0	0	-	10.5(10.0-11.0)
7708	masu x masu	139	100.0	100.0	92.1	85.6	78.4	73.4	61.9	43	10.1(10.0-11.0)
7709	masu x chinook	128	99.2	68.0	58.6	47.7	43.8	34.4	32.8	52	10.1(10.0-11.0)
7710	masu x masu	125	100.0	96.8	96.0	96.0	96.0	96.0	88.8	43	10.1(10.0-11.0)
7711	masu x chinook	129	99.2	92.2	88.4	86.8	85.3	81.4	75.2	52	10.1(10.0-11.0)
7901	masu x masu	317	100.0	100.0	99.7	99.7	99.7	99.7	99.1	39	10.5(9.8-11.5)
7902	masu x char	335	100.0	100.0	100.0	100.0	100.0	94.3	89.9	35	10.5(9.8-11.5)
7903	masu x char	313	100.0	100.0	99.7	99.7	99.7	99.0	99.0	35	10.5(9.8-11.5)
7904	masu x masu	332	100.0	100.0	99.1	99.1	99.1	99.1	98.5	36	10.5(9.8-11.5)
7905	chum x chum	525	99.8	99.8	99.8	99.8	99.8	99.8	99.6	45	10.5(9.8-11.5)
7906	masu x chum	548	99.8	99.6	99.3	98.7	98.2	76.0	8.7	-	10.5(9.8-11.5)
7907	chum x masu	170	100.0	97.7	94.7	94.7	94.7	94.7	60.2	36	10.5(9.8-11.5)
7908	char x masu	445	99.8	99.6	99.6	99.6	99.6	99.6	99.6	38	10.5(9.8-11.5)
7909	char x char	480	99.8	99.6	99.6	99.6	99.6	99.6	99.6	40	10.5(9.8-11.5)
7910	char x masu	270	99.6	98.1	98.1	98.1	98.1	98.1	98.1	38	10.5(9.8-11.5)
7911	char x char	468	100.0	99.8	99.6	98.9	97.9	95.7	95.7	40	10.5(9.8-11.5)
8001	masu x masu	111	100.0	77.5	65.8	62.2	59.5	-	-	-	9.2(8.5- 9.5)
8002	masu x char	237	99.2	87.8	75.1	70.7	68.4	60.8	-	-	9.2(8.5- 9.5)
8003	masu x chum	236	100.0	14.4	5.1	2.1	0	0	0	-	9.2(8.5- 9.5)
8004	chum x chum	217	99.5	98.6	82.5	80.2	77.4	-	-	-	9.2(8.5- 9.5)
8005	chum x masu	238	100.0	84.5	1.3	0.8	0	0	0	-	9.2(8.5- 9.5)
8006	chum x char	243	100.0	54.6	2.9	1.2	0	0	0	-	9.2(8.5- 9.5)
8007	char x char	102	100.0	100.0	100.0	100.0	-	-	-	-	9.2(8.5- 9.5)
8008	char x chum	72	100.0	98.6	61.1	22.2	2.8	1.4	0	-	9.2(8.5- 9.5)
8101	chum x chum	145	99.3	99.3	95.2	78.6	77.2	73.8	73.8	57	8.3(7.8- 9.0)
8102	chum x char	220	97.7	79.5	5.0	5.0	4.5	0	0	-	8.3(7.8- 9.0)
8103	char x char	380	99.7	99.5	98.7	98.7	97.9	96.2	91.9	48	8.3(7.8- 9.0)
8104	char x masu	408	100.0	100.0	100.0	99.5	99.5	99.0	94.1	48	8.3(7.8- 9.0)
8105	char x chum	294	96.9	96.6	91.8	81.3	58.0	11.1	1.7	-	8.3(7.8- 9.0)
8106	char x char	385	100.0	99.5	99.5	98.4	93.2	73.5	59.7	48	8.5(7.8- 9.0)
8107	char x brook	390	99.5	97.9	96.7	95.9	94.1	88.2	72.1	44	8.5(7.8- 9.0)
8108	char x brook	382	99.0	99.0	98.7	98.7	98.2	93.7	81.9	44	8.5(7.8- 9.0)
8109	chum x chum	172	100.0	100.0	97.1	93.6	89.0	87.2	72.7	58	8.5(7.8- 9.0)
8110	chum x char	315	100.0	94.9	1.6	0	0	0	0	-	8.3(7.5- 9.0)
8111	chum x brook	183	100.0	100.0	2.2	0	0	0	0	-	8.3(7.5- 9.0)
8112	char x chum	514	100.0	99.6	51.2	23.2	17.1	0	0	-	8.3(7.5- 9.0)
8113	brook x brook	92	100.0	100.0	91.3	84.8	65.2	65.2	65.2	44	8.3(7.5- 9.0)
8114	brook x chum	527	100.0	99.8	54.8	25.6	13.5	0	0	-	8.3(7.5- 9.0)
8201	chum x chum	380	97.4	85.5	71.3	65.8	61.8	58.9	29.7	60	8.1(6.5- 9.0)
8202	chum x char	429	94.4	81.8	21.4	0.7	0	0	0	-	8.1(6.5- 9.0)
8203	char x char	51	100.0	94.1	90.2	80.4	64.7	43.1	43.1	52	8.1(6.5- 9.0)
8204	char x chum	71	97.2	4.2	2.8	1.4	1.4	0	0	-	8.1(6.5- 9.0)
8205	chum x chum	274	93.8	93.4	93.1	92.0	92.0	88.3	88.3	58	8.1(6.5- 9.0)
8206	chum x char	320	96.3	94.4	27.8	19.7	17.5	14.4	12.6	-	8.1(6.5- 9.0)
8207	char x char	111	100.0	97.3	91.9	84.7	76.6	50.5	28.8	54	8.1(6.5- 9.0)
8208	char x chum	299	97.7	93.6	42.1	9.7	3.0	1.3	0	-	8.1(6.5- 9.0)

after fertilization, when epiboly was 1/3 completed (Fig. 4). At 10 days, the formation of the neural keel and differentiation of the optic vesicles were observed in the embryo and epiboly was 1/2 completed (Fig. 5). At 12 days after fertilization, the embryonic body continued to grow and the morphology of the yolk plug appeared (Fig. 6). After this, the blastodisc was closed at the caudal end of the embryo. The stage at 14 days after fertilization was characterized by prominence of the differentiation of the brain region, lens formation and tail bud formation (Fig. 7). At 16 days, the tail bud further developed and differentiation of the mesenchephalon, metenchephalon and myelenchephalon was recognized in the head region of the embryos (Fig. 8). Anlage of the pectoral fins became visible on the surface of the yolk sac and a primitive intestinal tract also appeared at this stage (Fig. 8). The heartbeat occurred at 18 days after fertilization when embryonic blood had already begun to circulate (Figs. 9, 10). The embryo reached a stage of continual growth and further differentiation of somites (Fig. 11). A primordial liver appeared at 24 days (Fig. 12) and the first muscle contraction occurred at 26 days after fertilization, when the eyes were only slightly pigmented (Fig. 13). The embryo reached the eyed stage at 28 days after fertilization (Fig. 14). Further embryonic development is shown in Figs. 15-18, and the hatching activity that occurred at 49 days after fertilization in Fig. 19.

Development of masu salmon, Oncorhynchus masou

Six parental fertilizations were performed as control experiments for the hybridization (Table 2). In these crosses, eggs were successfully fertilized with unfertilized eggs being scarcely found. Survival rates, dates of crossing and average temperatures for incubation are summarized in Table 5. Survival rates at the hatching varied from almost 100 % to about 40 % in different crossings. The hatching began at 36-39 days under an average temperature of 10.5°C, and at 43 days under a temperature of 10.1°C.

The morphogenesis of masu salmon is demonstrated in Pl. I, Figs. 20-31. After the cleavage, the egg reached the morula stage at 2 days, then progressed to the blastula stage at 4 days after fertilization (Fig. 20). Invagination proceeded and the embryonic body appeared at 6 days after fertilization (Fig. 21). Differentiation of the neural tube and optic vesicles was prominent in 8-day embryos, which showed 1/2 epiboly (Fig. 22). After the yolk plug stage of 10 days after fertilization (Fig. 23), the blastopore was completely closed. The tail bud differentiated at 12-14 days after fertilization (Figs. 24 and 25). The heart began to beat at 18 days after fertilization (Fig. 26) and muscle contraction was observed at 20 days (Fig. 27). Eye pigmentation prominently appeared at 22-24 days (Figs. 28 and 29) and most embryos began to hatch at 36-43 days (Fig. 31).

Development of chum salmon, Oncorhynchus keta

Intraspecific crossings of chum salmon, Oncorhynchus keta,

were performed as a control experiment (Table 2). As seen in Table 5, chum salmon embryos from the experiments of 1977 and 1979 (crossings No. 7706 and 7905) showed survival rates of more than 96 %, but other experiments, of 1981 and 1982, indicated relatively lower survival potentials. The hatching activities were observed at 45-49 days after fertilization under an average temperature of 10.5°C, and at 57-60 days under a temperature of 8.3°C.

A normal morphogenetic process in the chum salmon, from crossing No. 7905, is shown in Pl. II, Figs. 32-44. After fertilization, the cytoplasm became concentrated to form the blastodisc and the fertilized eggs displayed the blastula at 4 days after fertilization (Fig. 32). At 6 days, the embryonic shield was formed (Fig. 33). At 8 days, epiboly was 1/2 completed and the neural tube and optic vesicles were recognized (Fig. 34). At 10 days, epiboly was 2/3 completed and the euchephalon became distinct in the head region of the embryonic body (Fig. 35). At 12 days, the embryo was in the yolk plug stage, and three vesicles were recognized in the region of the euchephalon (Fig. 36). Differentiation of the tail bud was recognized at 14 days after fertilization (Fig. 37). After the further development of the tail bud and primordial pectoral fins (Fig. 38), blood circulation and heartbeat were detected and the primordial liver became distinct (Fig. 39). At 20 days, the embryo showed its first muscle contraction (Fig. 40). After active organogenesis and further development of the embryonic body (Figs. 41-43), the embryo reached the hatching stage at 49-60 days after fertilization (Table

5, Fig. 44).

Development of Japanese char, Salvelinus leucomaenis

Intraspecific crossings within the Japanese char, Salvelinus leucomaenis, were carried out as control experiments for the hybridizations between Japanese char and brook trout, masu salmon or chum salmon (Table 2). Survival rates at 50 days after fertilization varied widely, from 99.6 % to 28.8 %, in different crossings (Table 5). However, the morphogenetic processes of these control groups were normal and no abnormal embryo was seen. The hatching activity was detected at 40 days under an average temperature of 8.1-8.3°C (Table 5).

A normal morphogenetic process is shown in Pl. II, Figs. 45-59. The segmentation of the blastodisc continued and reached the morula stage at 2 days after fertilization (Fig. 45). At 4 days, the blastula began its movement over the yolk mass (Fig. 46). The germ ring became visible and the formation of the embryonic zone was recognized at 6 days (Fig. 47). The embryonic body was clearly visible at 8 days, when epiboly was 1/2 completed and the differentiation of the optic vesicles had become distinct (Fig. 48). The embryo reached the yolk plug stage at 10 days after fertilization (Fig. 49). The blastopore was enclosed at 12 days (Fig. 50) and the embryo reached the tail bud stage at 14 days after fertilization (Fig. 51). At 16 days, the tail bud differentiated further (Fig. 52). Blood began to circulate at 18 days (Fig. 53) and the heartbeat was recognized at 20 days after fertilization (Fig. 54). Muscle contraction was detected at 22

days (Fig. 55). After eye pigmentation (Figs. 56-58), the embryo reached the hatching stage at 40-52 days after fertilization (Table 5, Fig. 59).

Development of brook trout, Salvelinus fontinalis

In the present study, an interspecific crossing of the brook trout, Salvelinus fontinalis, was conducted as the control experiment for hybridizations between brook trout and other salmonid species (Table 2). The survival rate of this control crossing was about 65 % at the hatching stage, which began at 44 days after fertilization under an average temperature of 8.3°C (Table 5). The brook trout egg was smaller than that of other salmonids but the morphogenetic process for this species was almost the same.

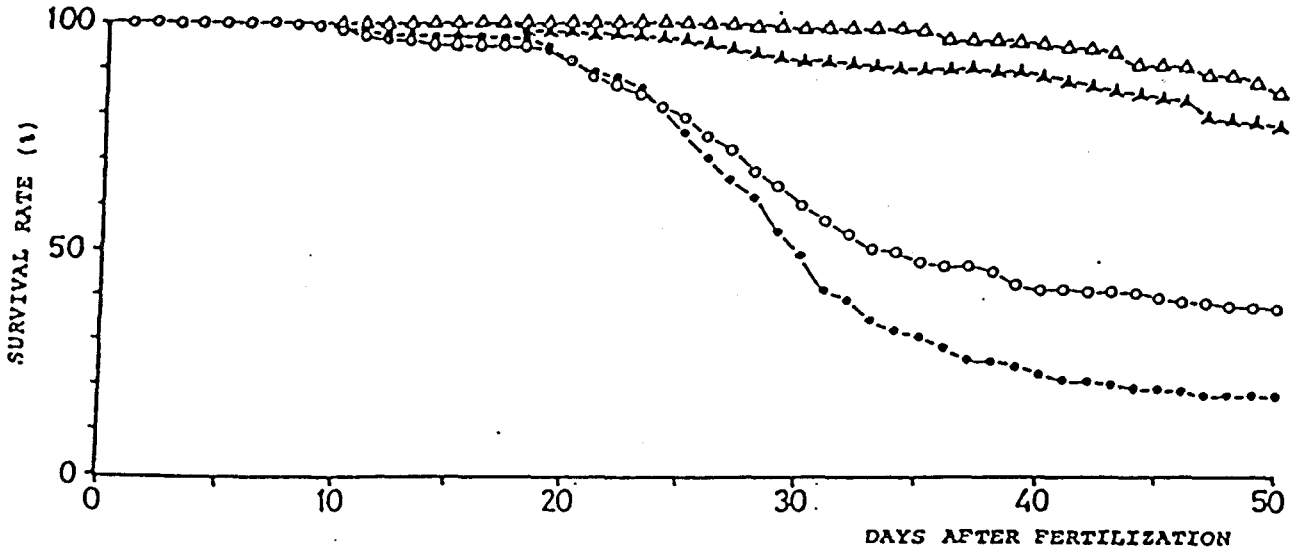
2. Developmental capacity and morphogenesis of hybrid salmonids.

Reciprocal hybrid between masu salmon and pink salmon

Interspecific hybridization between masu salmon and pink salmon was carried out in 1977 (Table 2). The survival rates, the dates of hatching and water temperatures of incubation are given in Table 5 and Text-Fig. 2. The resultant hybrid, pink salmon♀ x masu salmon♂, successfully developed and its survival rate was about 77 % at hatching (Table 5). On the contrary, the survival rate of the crossing of masu salmon♀ x pink salmon♂ was 17.5 % at hatching, which corelated with the low survival rate of the control, masu salmon. As shown

in Text-Fig. 2, no change in the survival rate was found until 10 days after fertilization. At this time, a drop in the survival rate was noticed. The hatching activities began at 47 days after fertilization, both in the masu salmon♀ x pink salmon♂ and pink salmon♀ x masu salmon♂ crossings (Table 5). Although the survival rate of masu salmon♀ x pink salmon♂ was not satisfactory, this hybrid demonstrated an almost normal process of morphogenesis. No deformed embryo was detected.

The morphogenetic process in the pink salmon♀ x masu salmon♂ crossing is given in Pl. III, Figs. 60-78. Following fertilization, the cytoplasm concentrated to form the blastodisc. As the segmentation of the blastodisc advanced, the egg entered the morula stage (Fig. 60). After the blastula stage (Fig. 61), invagination started, and the germ ring and embryonic zone were formed at 6 days after fertilization (Fig. 62). Embryonic body formation became visible at 8 days (Fig. 63) when epiboly was 1/2 completed (Fig. 64). At 12 days after fertilization, the euchephalon fully developed at the anterior region of the embryonic body, where the optic vesicles and auditory vesicles became clearly visible. Epiboly was now 4/5 completed and the morphology was in the yolk plug stage (Fig. 65). By 14 days after fertilization, the embryo had reached the tail bud stage (Fig. 66). At 16 days (Fig. 67) and 18 days (Fig. 68), the tail bud further developed and the anlage of the pectoral fins was visible on the surface of the yolk sac. The primordial intestinal tract was also observed at this stage. At 20 days after fertilization,



Text-Fig. 2. Survival curves in the hybrid experiment between pink salmon and masu salmon. △ ; pink salmon (crossing No. 7701), ○ ; masu salmon (No. 7703), ▲ ; pink salmon♀ x masu salmon♂ (No. 7702), ● ; masu salmon♀ x pink salmon♂ (No. 7704).

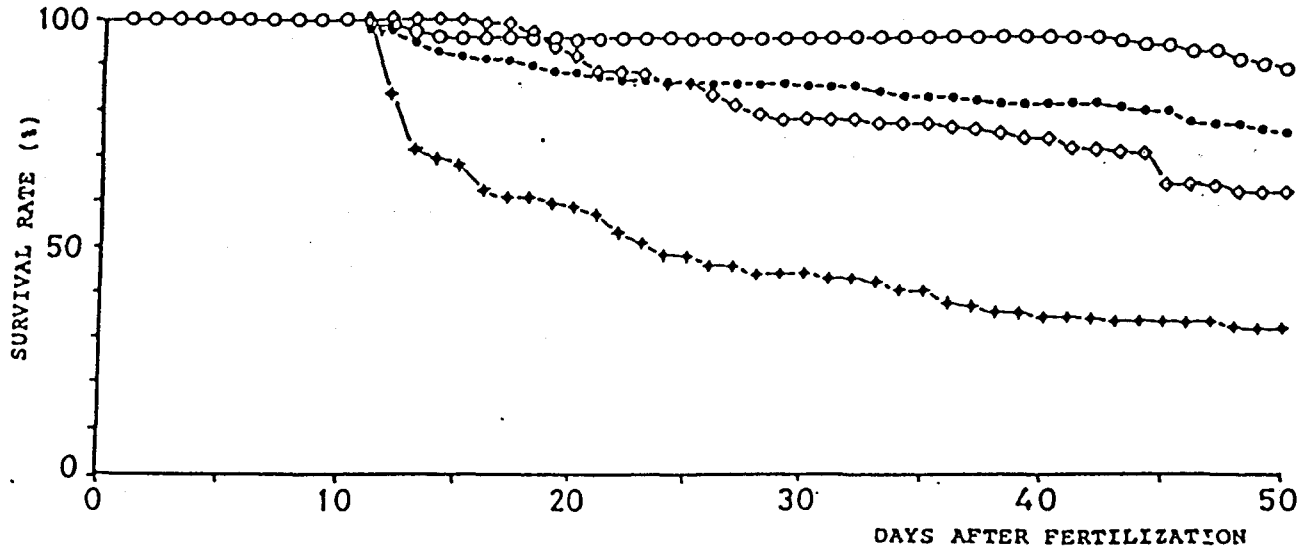
the heart began to beat and the blood circulation was apparent (Fig. 69). At 22 days, the anlage of the liver was recognized (Fig. 70), and the embryonal muscle began to contract at 24 days after fertilization (Fig. 71). After the occurence of eye pigmentation, the embryo continued to grow (Figs. 72-77) and hatched at 47 days after fertilization (Fig. 78).

Hybrid between masu salmon♀ and chinook salmon♂

Hybrid crossings between masu salmon♀ and chinook salmon♂ were performed in 1977 (Table 2), but intraspecific crossings within chinook and reciprocal crossings between chinook salmon♀ and masu salmon♂ could not be carried out due to differences in maturation times of the two species. The survival rates, dates of hatching and temperature conditions for incubation are shown in Table 5. As shown in Text-Fig. 3, the survival rate remained 100 % in both groups of masu salmon♀ x chinook salmon♂ until 12 days after fertilization, when embryonic death began to occur and the survival rate significantly decreased. The morphogenesis of masu salmon♀ x chinook salmon♂ was normal, and the developmental process was almost the same as that of masu salmon during the early developmental stages. The hybrid embryos began to hatch at 52 days after fertilization under an average temperature of 10.1°C.

Reciprocal hybrid between masu salmon and chum salmon

Interspecific hybridization experiments were carried out between masu salmon and chum salmon in 1977, 1979 and 1980

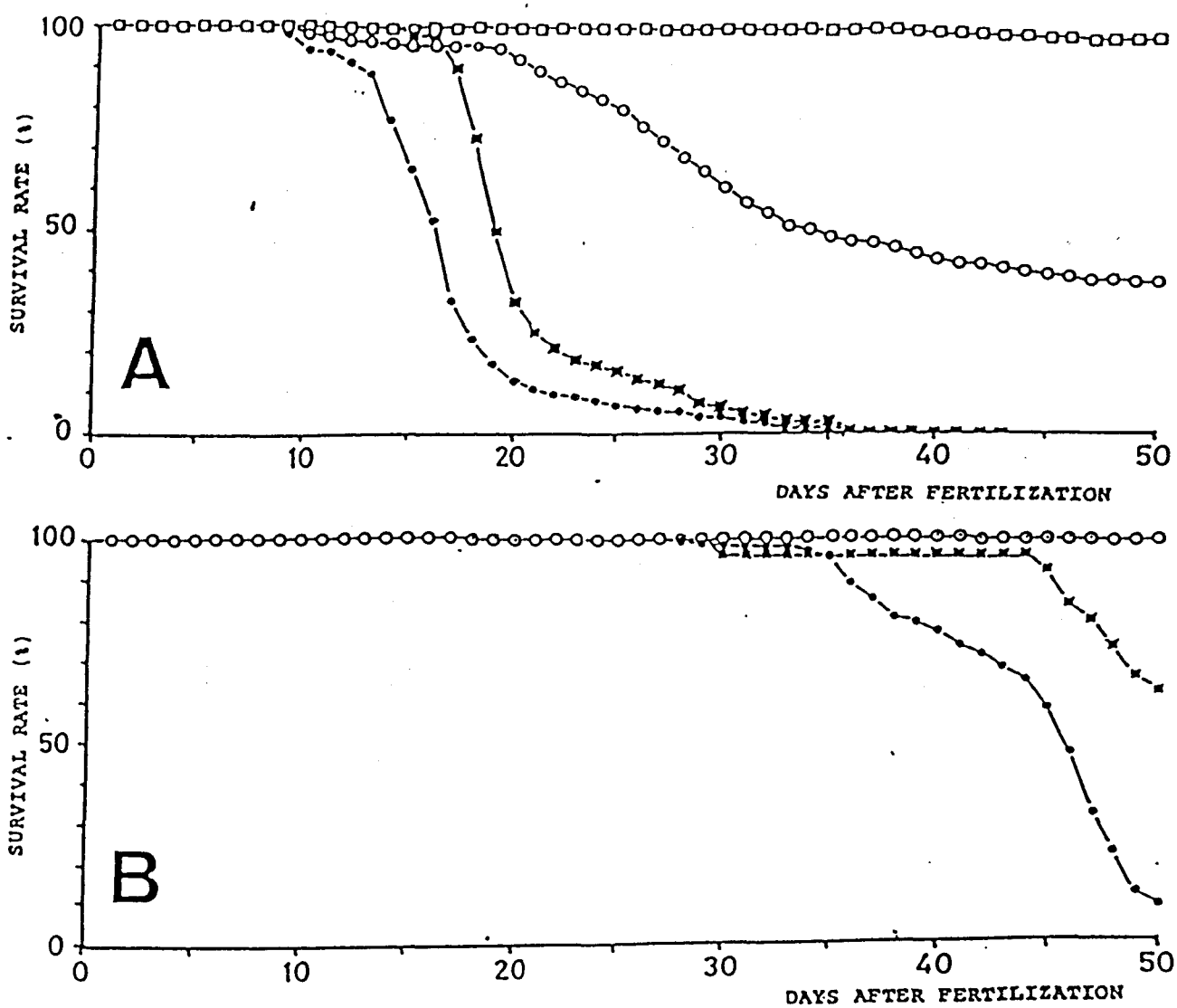


Text-Fig. 3. Survival curves in the hybrid experiment between masu salmon and chinook salmon. ◇; masu salmon (crossing No. 7708), ○; masu salmon (No. 7710), ◆; masu salmon♀ x chinook salmon♂ (No. 7709), ●; masu salmon♀ x chinook salmon♂ (No. 7711).

(Table 2). For the resultant six groups of crossings, Nos. 7705, 7707, 7906, 7907, 8003 and 8005 (Table 5), there were conspicuous differences between the results of the 1977 and 1978 hybrid experiments and those of 1979, in regard to survival potentials and developmental capacities.

As shown in Text-Fig. 4A, the survival rate of masu salmon♀ x chum salmon♂ in 1977 was constant until 10 days after fertilization, but it rapidly dropped during the period from 14 to 20 days. In these crossings, no hatched larvae were obtained. The survival rate of the reciprocal chum salmon♀ x masu salmon♂ was invariable during its early development, until 16 days, but then dropped down to 15 % by 25 days after fertilization. Survival curves of these hybrids between masu salmon and chum salmon were similar, but there was a difference in the dates at which most embryos began to die.

The morphogenetic process in these hybrids and control species is shown in Pl. III, Figs. 79-82. As seen in these figures, the embryos of both chum and masu salmon displayed normal morphology with regular differentiation of the head region, somites and tail bud. On the contrary, the hybrid embryos demonstrated abnormalities in their morphogeneses, but the degree of deformity differed between masu salmon♀ x chum salmon♂ and chum salmon♀ x masu salmon♂. As shown in Fig. 81, chum salmon♀ x masu salmon♂ showed an abnormal morphology characterized by microcephalia, microphthalmia and dwarf embryonic body, and defective differentiation of the brain region and tail bud. The masu salmon♀ x chum salmon♂ hybrid showed an even more deformed morphology (Fig. 82).



Text-Fig. 4. Survival curves in the hybrid experiments between chum salmon and masu salmon of 1977 (A) and 1979 (B). □ ; chum salmon (crossing No. 7706 in A), ○ ; masu salmon (No. 7703 in A and No. 7904 in B), ■ ; chum salmon♀ x masu salmon♂ (No. 7707 in A and No. 7907 in B), ● ; masu salmon♀ x chum salmon♂ (No. 7705 in A and No. 7906 in B).

In this hybrid, only the formation of the embryonic body was visible, with no indication of organogenesis.

The hybrid experiments of 1980 (crossings No. 8003, 8005) produced almost the same results as those of 1977, but differed considerably from those of 1979. In the 1979 hybrid experiments, chum salmon♀ x masu salmon♂ demonstrated viability. The reciprocal masu salmon♀ x chum salmon♂ was essentially inviable but survived beyond the deformed eyed stage until 55 days after fertilization. As seen in Table 5 and Text-Fig. 4B, the chum salmon♀ x masu salmon♂ from crossing No. 7907 showed a constant survival rate of more than 95 % until 9 days after hatching. Its morphogenesis is shown in Pl. IV, Figs. 83-95, and it is clear that no abnormality was recognized in this combination.

The morphogenetic process in this hybrid was almost the same as in the parental fishes, at least in its early development. After the concentration of cytoplasm to form the blastodisc, the cleavage continued and the egg reached the morula stage at 2 days after fertilization. As seen in Fig. 83, the morphology of the blastula became visible at 4 days, and then gastrulation occurred. The embryo had reached the embryonic shield stage by 6 days after fertilization (Fig. 84). At 8 days, epiboly was 1/3 completed and the formation of the embryonic body was distinct (Fig. 85). At 10 days, epiboly was 1/2 completed and differentiation of the brain and optic and auditory vesicles was visible (Fig. 86). After this, the embryo showed the morphology of the yolk plug stage (Fig. 87) and the tail bud stage was then reached at 14 days after fertili-

zation (Fig. 88). Following the formation of the primordial pectoral fins and intestinal tract at 16 days (Fig. 89), the heartbeat and blood circulation began at 20 days after fertilization (Fig. 90). Muscle contraction and eye pigmentation were noted at further developmental stages (Figs. 91-93) and the embryo hatched at 36 days after fertilization (Fig. 94). All embryos showed the same normal development and no deformed embryo was found in this hybrid experiment.

On the contrary, the reciprocal masu salmon♀ x chum salmon♂ hybrid remained nonviable , but its developmental capacity differed from that of the same combination from 1977 and 1980. As shown in Table 5 and Text-Fig. 4B, this hybrid, from crossing No. 7906, showed a constant high survival rate until 35 days after fertilization, which then drastically decreased because of complete deformation of all embryos. Consequently, the hybrid embryos died within 55 days, except for one embryo which hatched with an abnormal morphology.

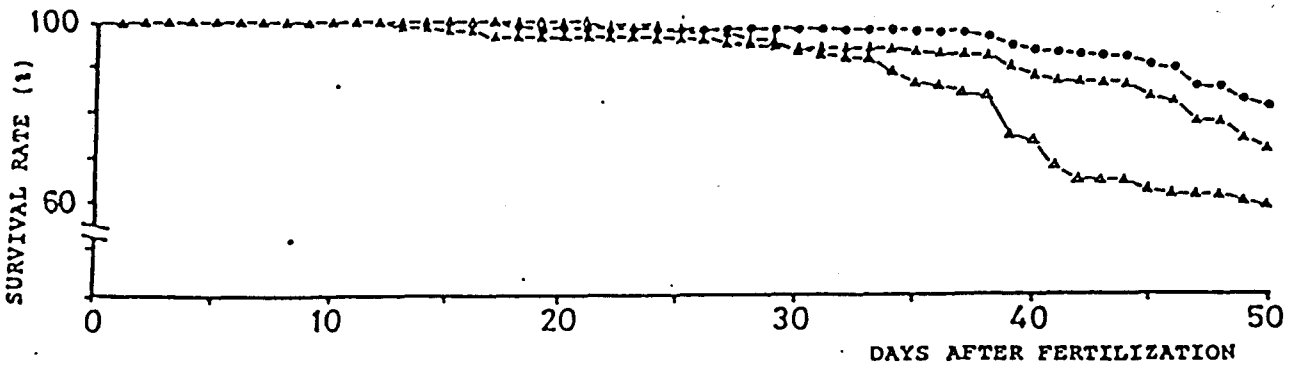
Morphogenetic changes in the masu salmon♀ x chum salmon♂ hybrid from the experiment of 1979 are shown in Pl. IV, Figs. 96-109. The morphology of this hybrid was grossly abnormal in spite of the constant rate of survival until 35 days after fertilization. At 4 days after fertilization, the egg reached the blastula stage (Fig. 96); after this, the posterior region of the blastodisc became thicker than the anterior border, so that the embryonic zone was formed by 6 days after fertilization (Fig. 97). The embryonic body was formed at 8 days after fertilization, but the extension of the body was significantly repressed as seen in Fig. 98. At 10 days after fertilization,

epiboly was 3/4 completed but differentiation of the brain region and the formation of the optic vesicles were behind normal when compared with viable hybrids and parental species (Fig. 99). Although the blastopore was not enclosed yet, the tail bud had already begun to differentiate in 12-day embryos. At this stage, abnormal formation of the brain and eyes was recognized with visible symptoms of microchephalia and microphthalmia (Fig. 100). Even at 14 days, most organogenesis was still undifferentiated and the extension of the embryonic body had defects (Fig. 101). Such a delay in embryonic development was continually observed during the period from 16 to 22 days (Figs. 102-105). An abnormal circulatory system was also recognized. Eye pigmentation was slightly detected in 26-28 day embryos, but the organogenesis of the eye was repressed so that the morphology was characterized by microphthalmia (Figs. 106-108). Only one individual hatched, at 50 days after fertilization, but it was quite deformed, as seen in Fig. 109.

Hybrid between Japanese char♀ and brook trout♂

Two crossings of Japanese char, Salvelinus leucomaenis♀ x brook trout, S. fontinalis♂ were conducted in 1981. As seen in Table 5 and Text-Fig. 5, the survival rates of these hybrids were more than 70 % at the hatching stage. Since the incubation temperature was lower than in the other experiments, these hybrids developed slowly. The morphogenesis of these hybrids was normal, as seen in Pl. V, Figs. 110-124.

Under an average water temperature of 8.5°C, fertilized eggs



Text-Fig. 5. Survival curves in the hybrid experiment between Japanese char and brook trout. △ ; Japanese char (crossing No. 8106), ▲ ; Japanese char♀ x brook trout♂ (No. 8107), ● ; Japanese char♀ x brook trout♂ (No. 8108).

reached the morula stage at 2 days after fertilization (Fig. 110). The blastodisc developed into the blastula at 4 days (Fig. 111). The blastula then began its movement over the yolk mass (Fig. 112) and reached the germ ring stage (Fig. 113). At 10 days after fertilization, embryos reached the embryonic shield; invagination had started by this time (Fig. 114). At 12 days after fertilization, epiboly was 1/2 completed and the embryonic body was visible (Fig. 115). At 14 days after fertilization, epiboly was 3/4 - 4/5 completed and the formation of vesicles and differentiation of the brain were prominent in the anterior region of the embryonic body (Fig. 116). At 16 days, the blastopore was enclosed and the formation of the optic vesicles and lens were observed (Fig. 117). The tail bud formation occurred at 18 days after fertilization (Fig. 118). At 20 days, primordial pectoral fins differentiated (Fig. 119). By 24 days after fertilization, the heart had been begun to beat (Figs, 120, 121), and at 28-30 days the embryo reached the eyed stage (Figs. 122, 123). At 44 days after fertilization, the first hatching activity was observed (Fig. 124).

Reciprocal hybrid between masu salmon and Japanese char

Intergeneric hybridizations between masu salmon and Japanese char were carried out in 1979, 1980 and 1981. As shown in Table 5, the survival rate of this combination was generally satisfactory, being over 90 % at 50 days after fertilization. Both the hybrids masu salmon♀ x Japanese char♂ and Japanese char♀ x masu salmon♂ showed almost the same mor-

phogenetic process as the parental species (Pl. V, Figs. 125-138). No differences were seen between survival potentials or developmental capacities in the reciprocal crossings.

The blastodisc of these hybrids segmented to form the morula at 2 days (Fig. 125). At 4 days after fertilization, the blastula began its movement over the yolk mass (Fig. 126) and the embryonic zone was formed at 6 days (Fig. 127). At 8 days, the embryonic body had become more distinct (Fig. 128). At 10 days, epiboly was 2/3 completed and the optic vesicles were recognized in the head region of the embryonic body (Fig. 129). At 12 days after fertilization, the embryo was in the yolk plug stage (Fig. 130). The blastopore was enclosed and the embryo reached the tail bud stage at 14 days after fertilization (Fig. 131). Primordial pectoral fins and the intestinal tract differentiated at 20 days after fertilization as the embryo developed (Fig. 132). The heart began to beat at 22 days after fertilization (Fig. 133). Eye pigmentation became visible around 24-26 days, and muscle contraction had been detected by this time (Figs. 134, 135). Embryos developed further to reach the eyed stage (Figs. 136, 137) and began to hatch at 35-40 days after fertilization (Fig. 138).

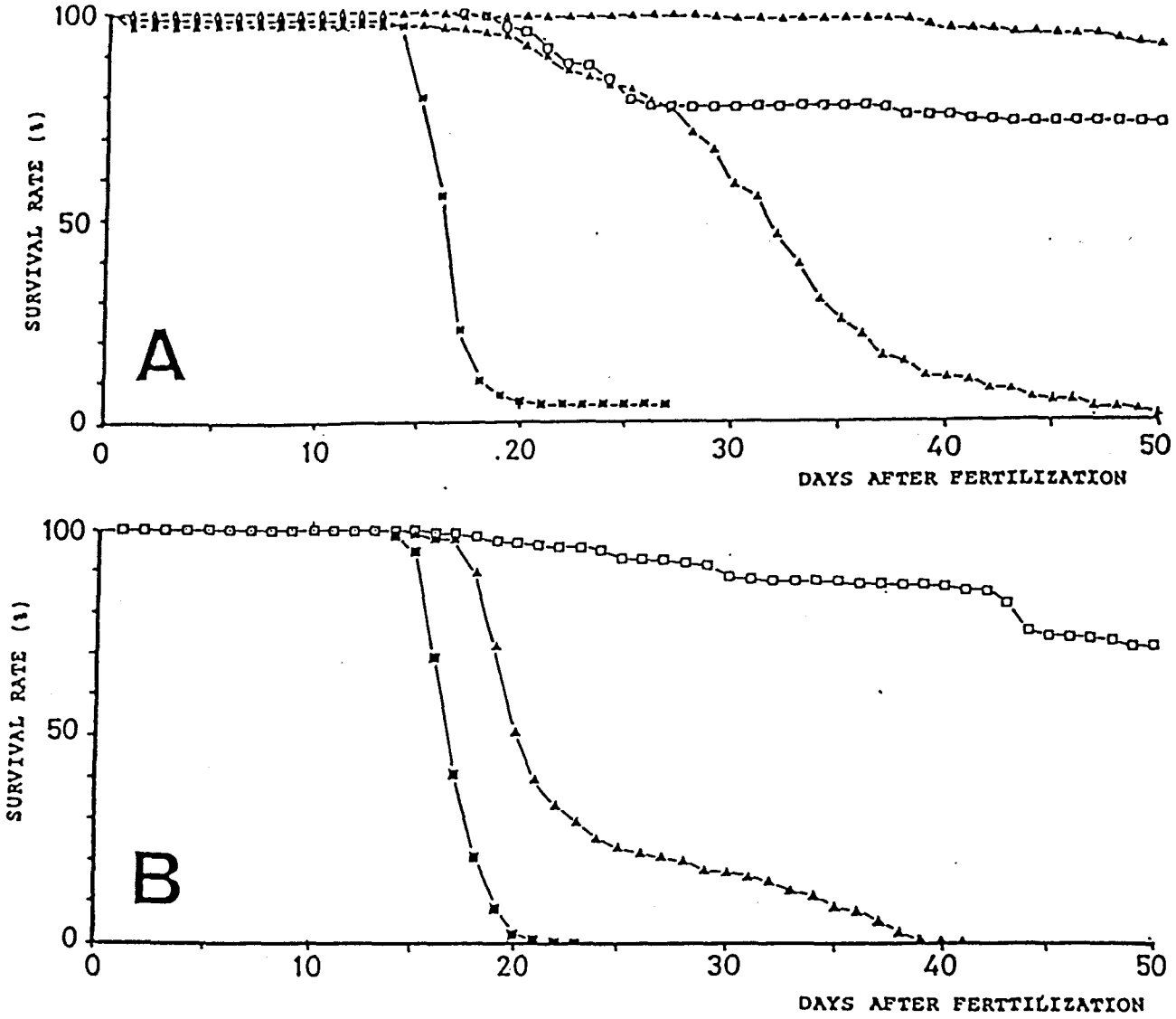
Reciprocal hybrid between chum salmon and Japanese char

Intergeneric crossings were carried out between chum salmon and Japanese char in 1980, 1981 and 1982 (Table 2). The hybrid from crossing No. 8006, chum salmon♀ x Japanese char♂, showed a constant survival rate until about 10 days after fertilization, but the rate drastically decreased from about 12

days, dropping down to 54.6 % at 15 days and to 2.9 % at 20 days (Table 5). Consequently, all the hybrid embryos died within 30 days (Table 5). For the reciprocal hybrid, Japanese char♀ x chum salmon♂, from crossing No. 8008, the survival rate began to drop after 15 days, but the curve was not as sharp as for chum salmon♀ x Japanese char♂. The hybrid Japanese char♀ x chum salmon♂ died within about 40 days (Table 5). No hatched larvae were obtained from either of these crossings (Table 5).

Similar results were obtained in hybrid experiments involving two reciprocal crossings (Nos. 8105 and 8112) in 1981 (Text-Fig. 6A). In the hybrid experiment carried out in October, 1981, chum salmon♀ x Japanese char♂ initially showed a constant survival rate, but it quickly dropped down after 15 days. On the contrary, the survival curve of Japanese char♀ x chum salmon♂ dropped fairly slowly, with most embryos dying within about 40 days. The same hybrid combination tested in November, 1981 showed almost the same results (Text-Fig. 6B). As seen in Text-Fig. 6B, the survival rate of the hybrid chum salmon♀ x Japanese char♂ was a constant 100 % until 15 days, but fell abruptly during the period from 16-20 days after fertilization. Therefore, the survival curve of Japanese char♀ x chum salmon♂ for crossing No. 8112 was sharpen than for No. 8105.

As described above, the survival potential of chum salmon♀ x Japanese char♂ was lower than that of Japanese char♀ x chum salmon♂ in all crossings performed in 1980 and 1981. However, the results were reversed in the two experimental crossings of



Text-Fig. 6. Survival curves in the hybrid experiments between chum salmon and Japanese char crossed in October, 1981 (A) and November, 1981 (B). □ ; chum salmon (crossing No. 8102 in A and No. 8109 in B), △ ; Japanese char (No. 8103 in A), ■ ; chum salmon♀ x Japanese char♂ (No. 8102 in A and No. 9110 in B), ▲ ; Japanese char♀ x chum salmon♂ (No. 8105 in A and No. 8112 in B).

1982, where the survival potential of chum salmon♀ x Japanese char♂ was higher than that of Japanese char♀ x chum salmon♂ (Table 5).

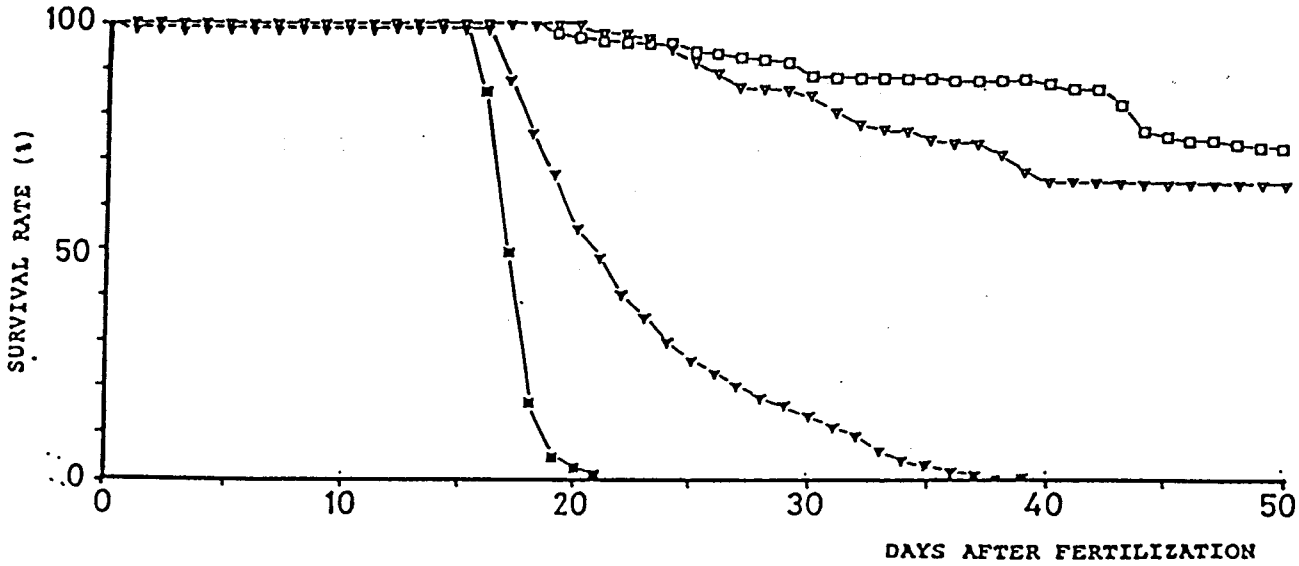
The morphogenetic process in the hybrid between chum salmon and Japanese char was examined and compared with the normal development in the control parental species (Pl. VI, Figs. 134 - 160). Hybrid embryos displayed almost the same morphology as the parental species, that of the embryonic shield stage, at 10 days after fertilization (Figs. 139-142). At 12 days, it was observed that epiboly was 1/2 completed in the Japanese char, and the embryonic body had become distinct (Fig. 143). The embryonic bodies of the parental chum salmon and chum salmon♀ x Japanese char♂ hybrid showed almost the same morphology at this time (Figs. 144, 146), but the development of Japanese char♀ x chum salmon♂ was slowed down (Fig. 145). At 14 days after fertilization, Japanese char embryos reached the yolk plug stage (Fig. 147), and for chum salmon epiboly was 1/3 completed (Fig. 148). At this same time, the Japanese char♀ x chum salmon♂ hybrid had reached 1/2 epiboly, which corresponded to the stage occurring at 12 days for the embryo of the control Japanese char (Fig. 149). On the other hand, embryonic body formation was inhibited in the hybrid between chum salmon♀ and Japanese char♂ (Fig. 150). At 18 days after fertilization, both the Japanese char and chum salmon reached the tail bud stage (Figs. 151, 152). However, the Japanese char♀ x chum salmon♂ hybrid was only at the yolk plug stage (Fig. 153) and the morphology of chum salmon♀ x Japanese char♂ showed an undifferentiated tail bud and symptoms of microcepha-

lia and microphthalmia (Fig. 154). Most hybrid embryos of chum salmon♀ x Japanese char♂ died after this period (Text-Fig. 6). At 24-26 days after fertilization, primordial pectoral fins and a primitive intestinal tract formed in the control embryos of both parental species (Figs. 158, 159). Defective eye pigmentation, weak blood circulation and undeveloped embryonic body were recognized in the hybrid, and the organogenesis was grossly repressed (Fig. 160).

Reciprocal hybrid between chum salmon and brook trout

Intergeneric hybridization was carried out between chum salmon and brook trout in 1981 (Table 2). As shown in Table 5 and Text-Fig. 7, the survival rate of chum salmon♀ x brook trout♂ remained at a high level until 15 days, with a rapid decrease in the rate occurring between 16 and 20 days. The hybrid brook trout♀ x chum salmon♂ showed a constant survival rate until 16 days, which then dropped down sharply (Text-Fig. 7).

The morphogenetic process in these hybrids is shown in Pl. VII, Figs. 161-183. As seen in the figures, morphogenesis was similar to that of the hybrids between chum salmon and Japanese char. At 12 days after fertilization, both control embryos were in the embryonic shield stage (Figs. 161, 162), and the hybrids brook trout♀ x chum salmon♂ and chum salmon♀ x brook trout♂ showed almost the same morphologies as the controls (Figs. 163, 164). Differences between embryonic morphology of the controls and the hybrids became recognizable at 14 days after fertilization. Both controls showed embryo-



Text-Fig. 7. Survival curves in the hybrid experiment between chum salmon and brook trout. □ ; chum salmon (crossing No. 8109), ▽ ; brook trout (No. 8113), ■ ; chum salmon♀ x brook trout♂ (No. 8111), ▼ ; brook trout♀ x chum salmon♂ (No. 8114).

nic body formation (Figs. 165, 166), but this process was repressed in both hybrid embryos (Figs. 167, 168). The difference in developmental rates between the controls and the hybrids was even more apparent at 16 days after fertilization (Figs. 169-172). At 18 days, the control embryos reached the tail bud stage and the brain region, primitive lens and tail bud were clearly differentiable (Figs. 173, 174). On the contrary, both hybrids showed an abnormal morphology characterized by undeveloped embryonic body and an absence of differentiation of the brain region and eyes (Figs. 175, 176). Most chum salmon♀ x brook trout♂ embryos died abruptly after this. At later stages in the development of the control embryos, the formation of the circulatory system and pigmentation of the eyes were apparent (Figs. 177, 178, 180, 181), but differentiation in the hybrid embryos was still repressed, such that the morphology was abnormal, characterized by microcephalia, microphthalmia and dwarf vented body (Figs. 179, 182, 183).

3. Discussion

Classification of salmonid hybrids

The following six hybrid combinations can be classified as viable, because they showed satisfactory survival rates similar to those of the parental species and in all cases demonstrated normal morphogenesis: pink salmon♀ x masu salmon♂, masu salmon♀ x pink salmon♂, masu salmon♀ x chinook salmon♂, Japanese char♀ x brook trout♂, Japanese char♀ x masu

salmon♂ and masu salmon♀ x Japanese char♂. Another five hybrids, as follows, can be classified as inviable, because they all showed grossly abnormal morphogenesis and died before hatching: masu salmon♀ x chum salmon♂, chum salmon♀ x Japanese char♂, Japanese char♀ x chum salmon♂, chum salmon♀ x brook trout♂ and brook trout♀ x chum salmon♂. In the hybrid experiments in which chum salmon♀ and masu salmon♂ were crossed, both inviable and viable cases were observed.

Interspecific hybrids within the same genus such as Japanese char, Salvelinus leucomaenis♀ x brook trout, S. fontinalis♂, masu salmon, Oncorhynchus masou♀ x chinook salmon, O. tschawytscha♂ and masu salmon, O. masou♀ x pink salmon, O. gorbuscha♂ were in general viable, but intergeneric hybrids between two different genera such as chum salmon, O. keta♀ x Japanese char, Salvelinus leucomaenis♂ demonstrated extreme mortality. However, this was not always the case, since the intergeneric hybrid between masu salmon and Japanese char demonstrated a satisfactory survival potential, similar to or higher than those of interspecific hybrids, and the intrageneric hybrid between masu salmon and chum salmon was usually inviable. Therefore , it is reasonable to conclude that a systematic analysis does not always corelate with the actual survival potentials.

The critical period in hybrid development

The results of hybridization indicate that hybrids can be derived into two categories, viable and inviable, as mentioned above. Although embryos from various inviable combinations

showed varying developmental capacities, it is worth noting that the stage at which most embryos began to die was almost the same for various crossed groups. In hybrid experiments of 1977, an abrupt fall in the survival rate occurred from 12 days in the masu salmon♀ x chum salmon♂ hybrid and from 17 days in chum salmon♀ x masu salmon♂ (Text-Fig. 4A). In the hybrid between female chum salmon and male Japanese char or brook trout, such a rapid drop in the survival rate was observed from 16 days after fertilization (Text-Figs. 6, 7). The same mode of embryonic mortality was also observed in Japanese char♀ x chum salmon♂ and brook trout♀ x chum salmon♂ from the 1981 hybridizations (Text-Figs. 6, 7). Morphologically, these periods may correspond to the stages between embryonic body formation and tail bud formation in the parental species and viable hybrids.

The presence of a stage at which most embryos begin to die has been generally known in most hybrid experiments. Moenkaus (1908) reported that most embryos from the hybridization between Fundulus heteroclitus and Menidia notata died before the stage of enclosure of the blastopore. Newman (1914) described the time of developmental obstruction as occurring at the onset of or during the process of gastrulation in many kinds of inviable teleost hybrids. Although there are slight differences in the developmental obstruction and the onset of mortality among hybrid experiments, Yamamoto (1943) reviewed developmental capacities of fish hybrids and concluded that most hybrids died at the stage of gastrulation, formation of tail and head or differentiation of the circula-

tory system.

It is interesting that the stage at which most inviable embryos died coincides with that which has been termed "critical" in many other physiological studies of fish embryogenesis. There has been a fairly large amount of work done on the resistance of developing embryos to environmental stimuli, such as abnormally high or low temperatures, unusual chemical components and mechanical shock. This is because of the recognized importance of testing the resistance of fish embryos with respect to optimum periods for transportation of fertilized eggs to fisheries fields. Yamamoto (1943) also reviewed this problem and found that the developing fish embryos were most vulnerable to mechanical shocks, such as shaking, pushing and dropping, at the stage of embryonic body formation. He also concluded that embryos which were exposed to abnormal temperatures or chemical stimulation showed relatively high mortality rates during gastrulation and during the process of embryonic body formation. Okada (1960) examined differential resistance to mechanical disturbance in each stage of development in the chum salmon and showed that it was most vulnerable around 10-15 days, which corresponds to the stage between optic and otic vesicle formation and the enclosure of the blastopore. Recently, Yamazaki (1981) and Yamazaki and Arai (1982) reported that the salmonid embryos originating from oocytes which had been irradiated by gamma-rays at the high dosage of 10^4 rad showed high mortality rates at 2 weeks after fertilization and died within 3 or 4 weeks. They found the same pattern of

embryonic mortality in salmonid embryos originating from artificially aged oocytes which were kept inside the body cavity for a few weeks after ovulation. Judging from the present observations on the embryonic development of salmonid hybrids, the most mortal stage for embryos from irradiated or artificially aged oocytes, as pointed out by Yamazaki ,1981), may correspond to the tail bud stage when inviable hybrids began to die abruptly. This is a noteworthy fact.

As described above, the stage at which mortality was high in inviable hybrids corresponds to the stage of "vulnerability" to physical stimuli. This strongly suggests that some important process may begin to take place at this stage in salmonid and teleost development. This ides is further supported by the fact that the survival rate was always constant up until this stage. Whatever happened at this time was the result of certain stimuli occuring before fertilization. Therfore, it is reasonable to conclude that such a developmental stage may be "critical" to embryonic morphogenesis, but further studies will be needed to analyze the developmental event occorring at this stage.

Differences in survival potentials and developmental capacities of reciprocal hybrids

Another noteworthy phenomenon in the present study was the difference in survival potentials and developmental capacities of reciprocal hybrids.

In the case of the viable hybrids between masu salmon and pink salmon, pink salmon♀ x masu salmon♂ demonstrated greater

viability than the masu salmon♀ x pink salmon♂ hybrid. In the case of the inviable hybrids between chum salmon and Japanese char from 1980 and 1981, Japanese char♀ x chum salmon♂ showed a slightly higher survival rate than the reciprocal chum salmon♀ x Japanese char♂. However, such a difference as was found in the case of the hybrids was also found for the two parental species. Therefore, it can be concluded that the differnce in survival potentials of the two hybrids is caused by differences in egg quality of the two species used as mother fish. Also, in the hybrid crossing between Japanese char and chum salmon, the results from 1980 and 1981 were the reverse of those obtained in 1982. In control experiments in 1980 and 1981, Japanese char showed a higher survival rate than chum salmon, but in 1982 this relation was reversed. Therefore, generalization concerning survival potential of this hybrid combination cannot be made, and differences in this potential may depend upon egg quality.

There was one case, however, where the difference between reciprocal hybrids could not due to differences in egg quality, but had to be due to certain events unique to the hybridization itself. In the experiment of 1977, chum salmon♀ x masu salmon♂ reached the eyed stage showing unusual morphology (Fig. 81), but the embryogenesis of masu salmon♀ x chum salmon♂ was repressed at or around the stage of embryonic body formation (Fig. 82). Such a difference between the two reciprocal hybrids was shown to an even greater extent in the hybrid experiment of 1979, in which the parental controls of both masu salmon and chum salmon showed survival rates of

nearly 100 % at hatching (Table 5, Text-Fig. 4B). In this experiment, chum salmon♀ x masu salmon♂ was viable and masu salmon♀ x chum salmon♂ inviable, but the latter survived beyond abnormal eye pigmentation until 55 days after fertilization (Text-Fig. 4B).

Similar differences between reciprocal hybrids have also been reported for other salmonids (Suzuki and Fukuda, 1971a) as well as for other teleostean fishes (Newman, 1914, 1915). Suzuki and Fukuda (1971a) observed that a hybrid rainbow trout♀ x masu salmon♂ reached the free swimming stage but that the reciprocal masu salmon♀ x rainbow trout♂ died before the eyed stage. They observed the same phenomenon in the hybrid combinations between brown trout and masu salmon and between brook trout and masu salmon (Suzuki and Fukuda, 1971a). Newman (1908) carried out reciprocal hybridizations between species with small sized eggs and species with large sized eggs in order to prove that differences in survival potentials between reciprocal hybrids depended upon differences in egg sizes, particularly on differences in the quantity of yolk mass. However, he obtained many other results which could not be explained by this theory and concluded that the differences were not a result of egg size alone (Newman, 1915). Suzuki (1968) performed artificial hybridizations among 23 species of cyprinid fishes and concluded that survival rates of reciprocal hybrids between species with small sized eggs and species with large sized eggs did not differ widely in many cases. Therefore, the differences in developmental capacities between reciprocal crosses may be due to other factors.

Hybridization is defined as the combining of heterogenic materials derived from two parental species in the maternal cytoplasm. An interaction between two parentally transmitted genomes can explain the differences in survival potentials among different kinds of hybrids, but not the different survival rates of reciprocal hybrids, because the sum of the two haploid genome sets from the parental species must be equal in the reciprocal hybrids. In this regard, the supposition made by Hubbs and Drewry (1959) may be worth considering. They believed that the differences found in reciprocal crosses might be due to some incompatibilities between maternal cytoplasm and paternal gene products.

Part II. Differential gene expression controlling isozyme phenotypes in the embryos of salmonids and their hybrids

The concept of "differential gene activation" is the most important concept in explaining embryonic development. In the present study, intra- and interspecific distinctions and tissue distributions of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGDH) and phosphoglucomutase (PGM) isozymes were electrophoretically analyzed in salmonid species to determine the genetic bases of these isozymes and to survey gene markers useful for developmental studies. Isozyme ontogenies in parental and hybrid salmonids were examined to map chronologically differential expression of embryonic gene activities controlling isozymes.

1. Lactate dehydrogenase (LDH)

LDH isozyme patterns

The tissue distribution and interspecific differences in the electrophoretic mobility of LDH isozymes were studied in the eyes, heart, liver and muscles from brook trout, Japanese char, chum salmon and masu salmon (Pl. VIII, Fig. 184). Genetic loci and alleles were then determined by observation of zymograms on the basis of their tetramic structures. As reported by many workers, LDH isozymes in salmonids were encoded at five different loci, which were differentially expressed within different tissues (Utter et al., 1973, Wright et al., 1975, Bailey et al., 1976). Ldh-A_1 and -A_2 activities were dominant in muscle tissue while the expression of Ldh-C was restricted to the eye (Fig. 184). Activities of Ldh-B_1 and -B_2 were detected in all tissues examined, but the expression of Ldh-B_1 predominated in the heart and Ldh-B_2 in the liver (Fig. 184).

Fig. 184 in Pl. VIII indicated that the LDH pattern of the brook trout eye consists of the five tissue ubiquitous isozymes B_4^{1c}, $B_3^{1c}B_1^{2a}$, $B_2^{1c}B_2^{2a}$, $B_1^{1c}B_3^{2a}$ and B_4^{2a} and an additional three eye specific isozymes containing LDH-C subunits. Tissue ubiquitous LDH-B^1 and -B^2 subunits were recognized in all tissues, but the predominance of LDH-B^1 and -B^2 subunits were observed respectively in the heart and liver of the brook trout. In muscle tissues, five isozyme bands controlled by the Ldh-A_1 and -A_2 loci occurred around the origin of the gel plate and two anodal isozymes showed electrophoretic mobility

identical to the $B_1^{1c}B_3^{2a}$ heterotetramer and B^{2a} homotetramer.

Similar isozymic expression within different tissues was observed in Japanese char but some interspecific distinctions were detected between char and brook trout (Fig. 184). As shown in Fig. 185, no distinction was observed between the mobilities of LDH-B_4^{1c} in these two species, but the isozyme homologous to LDH-B_4^{2a} migrated more anodally. In the liver of the F_1 hybrid between Japanese char and brook trout, five isozyme bands were made by the assembly of paternally derived LDH-B^{2a} subunits and maternally derived LDH-B^{2b} subunits (Fig. 186). This indicates an interspecific allelic difference between Japanese char and brook trout at the Ldh-B_2 locus.

Fig. 184 also showed that the electrophoretic mobilities of the LDH isozymes predominant in the liver and heart of chum salmon clearly differed from the homologous LDH isozymes observed in brook trout and Japanese char. In masu salmon, the isozyme which was common to all tissues was detected as a single band with a strong staining intensity under the present electrophoretic conditions (Table 3, 4, Fig. 184). Such an isozyme pattern indicates a similar electrophoretic mobility of gene products between Ldh-B_1 and -B_2.

In order to show interspecific differences among salmonid species, LDH isozymes were electrophoretically investigated in liver and heart of coho salmon, chinook salmon, chum salmon, masu salmon, kokanee salmon, pink salmon, Japanese char and dolly varden char (Fig. 187). In the liver, where the Ldh-B_2 locus was predominantly expressed, six Oncorhynchus species demonstrated the LDH-B_4^{2c} homotetramer, which differed in its

mobility from the homologous LDH-B_4^{2b} of Japanese char and dolly varden char (Fig. 187). In the heart, where the Ldh-B_1 locus was predominantly expressed, chum salmon, chinook salmon, coho salmon, kokanee salmon and pink salmon showed the same five-banded pattern with the predominance of cathodal LDH-B^{1a} subunits, though masu salmon displayed only one band corresponding to the mobility of the LDH-B_4^{1a} homotetramer in other Oncorhynchus species.

These results indicate allelic differences in Ldh-B_1 and -B_2 loci among salmonid species (Figs. 184-187). All Oncorhynchus species have an Ldh-B_{1a} allele with the same mobility, with the exception of the Ldh-B_{1b} allele of masu salmon. Salvelinus species, however, possess another allele, Ldh-B_{1c}. At the Ldh-B_2 locus, chum, pink, masu, coho, chinook and kokanee salmon have an Ldh-B_{2c} allele in common, but Japanese char and dolly varden char have a different allele, Ldh-B_{2b}. The Ldh-B_{2a} allele is unique to brook trout. Such interspecific distinctions may play an effective role as gene markers in developmental genetic examinations. As shown in Figs. 184-187, interspecific differences were found in muscle specific LDH isozyme systems among salmonid fishes, but such differentiation could be made due to the complicated isozyme patterns and the frequent presence of a number of variant forms between individuals.

LDH ontogeny

LDH ontogeny was examined in all parental species used as controls prior to electrophoretic analysis of hybrid embryos.

As shown in Pl. IX, Fig. 188, five isozyme bands corresponding to the LDH-B_4^{1c}, -$B_3^{1c}B_1^{2b}$, -$B_2^{1c}B_2^{2b}$, -$B_1^{1c}B_3^{2b}$ and -B_4^{2b} tetramers were observed, with LDH-B^{1b} subunit polypeptides predominating in the unfertilized egg of Japanese char from crossing No. 79-09. This pattern persisted until 12 days after fertilization. At 14 days, the embryo first showed a predominance of the LDH-B_4^{2b} homotetramer. Muscle specific LDH became visible at 26 days. No eye specific LDH was detected in this group during the experimental period.

Ontogenetic changes in LDH in chum salmon embryos from crossing No. 7905 are shown in Fig. 189. The five-banded pattern of LDH isozymes with a predominance of LDH-B^{1c} subunits was also observed in the fertilized egg of chum salmon. This pattern persisted until 10 days after fertilization, the pattern then changed, showing a significant increase in the staining intensity of the LDH-B_4^{2c} homotetramer at 12-14 days after fertilization. At 22 days, muscle specific LDH was first observed. Such a developmental progression of LDH isozymes was also observed in the ontogenesis of pink and chinook salmon.

The unfertilized egg of masu salmon, however, had only one isozyme corresponding to the LDH-B_4^{2c} and LDH-B_4^{1b} of the other salmonid species. This LDH band appeared during the developmental stages examined under the present electrophoretic conditions (Table 3, 4, Fig. 190).

The above observations indicate the presence of a common developmental pattern in the expression of LDH isozymes within salmonid species. In all experiments, isozymes containing

LDH-B^1 and -B^2 subunits were observed before fertilization and persisted during earlier stages of embryogenesis. The predominance of LDH-B^2 subunits occurred at 12-14 days and muscle specific LDH appeared at 20-24 days after fertilization.

Ontogenetic analysis was performed to evaluate the first expression of parental alleles in the course of embryogenesis in salmonid hybrids from crosses between parental species having LDH isozymes of different electrophoretic phenotypes. When the eggs of Japanese char possessing LDH-B^{2a} subunits were fertilized with the sperm of brook trout possessing LDH-B^{2b} subunits, the expression of the paternal Ldh-B^{2a} allele could be determined by the occurrence of hybrid LDH phenotypes. In the hybrid Japanese char♀ x brook trout♂ from crossing No. 8108, the LDH phenotype was completely maternal until 16 days after fertilization. LDH-$B_1^{2a}B_3^{2b}$ and -$B_2^{2a}B_2^{2b}$ heterotetramers containing paternal B^{2a} subunits of brook trout were detected at 18 days after fertilization (Fig. 191). At later developmental stages, the LDH-B_4^{2a} homotetramer appeared clearly and the embryo displayed the nine-banded type of LDH phenotype expected for this hybrid. Muscle specific LDH began to appear at 35 days after fertilization.

The existence of an exclusively maternal phenotype during the earlier stages and the later appearance of new isozymes containig paternally derived LDH-B^2 subunits were also observed in the embryogenesis of the other hybrid salmonids. As shown in Pl. X, Fig. 192, the masu salmon♀ x Japanese char♂ hybrid from crossing No. 7902 demonstrated only one

isozymic band, which was of the maternal masu salmon type, during the early stage of development until 12 days after fertilization. Appearance of hybrid isozymes containing paternal LDH-B^{2b} subunits occurred at 14 days after fertilization. Muscle specific LDH isozymes were expressed at 24 days, but no eye specific LDH was detected during the embryogenesis.

The onset time of the expression of the paternal Ldh-B_1 gene should be determinable by examining LDH ontogeny in other hybrids, between species showing a mutual allelic difference at the Ldh-B_1 locus. In this regard, an interspecific distinction of the Ldh-B_1 gene between masu salmon and other Oncorhynchus species such as pink salmon and chinook salmon can also be used in the developmental genetic study. When eggs of masu salmon with LDH-B_4^{1b} isozymes were inseminated with sperm of pink salmon with homologous LDH-B_4^{1a}, which clearly differed from masu salmon, in its electrophoretic mobility, the expression time of the paternal Ldh-B_a^1 allele could be estimated according to the first detection of hybrid heterotetramic isozymes containing paternal LDH-B^{1a} subunit polypeptides. As shown in Figs. 193 and 194, only the maternal LDH isozyme observed in the unfertilized egg of masu salmon was found up until 12 days in both masu♀ x chinook salmon♂ and in masu♀ x pink salmon♂. In these hybrids, the first appearance of a hybrid heterotetramer containing paternal LDH-B^{1a}subunits was detected at 14 days after fertilization.

In contrast to the LDH in the viable hybrids, the inviable

hybrid between masu salmon♀ and chum salmon♂, as described in Part I, showed an abnormal expression of LDH isozymes during its development. In the inviable hybrid masu♀ x chum salmon♂ from the 1979 experiment, the exclusive existence of the maternal phenotype continued for considerably longer than in the viable hybrids, and the appearance of a hybrid hetero-tetramer containing paternal subunits was only barely detectable at 35 days after fertilization (Fig. 195).

2. Malate dehydrogenase (MDH)

MDH isozyme patterns

Salmonid fishes have shown isozyme patterns of malate dehydrogenase (MDH) which are so complicated that their genetic bases cannot be interpreted. The involvement of tetrasomic genetic loci (Numachi et al., 1977) and of duplicated disomic loci (Allendorf et al., 1975) has been discussed. In the present study, genetic loci and allelic differences were established by electrophoretic analysis of the tissue distribution and interspecific comparison for salmonid species.

As shown in Pl. XI, Fig. 196, the MDH isozyme pattern of masu salmon consisted of four main isozymic bands. Although MDH-B_2^a and -C_2^b homodimers were widely present in most tissues examined, the MDH-B^a subunits were heavily expressed in the eye, liver and kidney tissues, and the MDH-C^b subunits in the heart and muscle tissues. The MDH-BC heterodimer had migrated to an intermediate position between these two homodimers. A strong staining intensity of the MDH-A_2 homodimer was recog-

nized in the muscle, heart and eye tissues of masu salmon, but MDH-A subunits did not assemble with other types of MDH subunits to form heterodimers. Although Japanese char displayed a similar distribution of MDH isozymes in different tissues, the electrophoretic mobility of MDH-B_2^b was faster than that of homologous MDH-B_2^a observed in masu salmon (Fig. 196). In the eye, heart and muscle tissues of Japanese char, three additional MDH isozymic bands were expressed. These may be homologous to MDH-A in other salmonid species, because the tissue distributions were the same. The three-banded pattern of MDH-A in Japanese char implies either a duplication of the Mdh-A locus or the presence of intraspecific polymorphic variants at this locus. The mobilities of the MDH-A_2 and -B_2^a isozymes in chum salmon were identical to the mobilities of the homologous isozymes in masu salmon. However, MDH-C_2^a in chum salmon clearly differed in its mobility from the homologous MDH-C_2^a in masu salmon and Japanese char.

MDH isozymes were also electrophoretically analyzed in the juvenile F_1 hybrids between Japanese char♀ and masu salmon♂ and between masu salmon♀ and pink salmon♂ (Fig. 197). The liver tissues of the interspecific hybrid Japanese char♀ x masu salmon♂ showed six MDH isozyme bands corresponding to the MDH-C_2^b, -B^bC^b, -B^aC^b, -B_2^b, -B^aB^b and -B_2^a isozymes, the expected isozyme phenotype for this hybrid. As shown in Fig. 197, pink salmon (o, p) possessed the same MDH phenotype as chum salmon (k, l) and the viable hybrid, masu salmon♀ x pink salmon♂, exhibited six MDH isozymes corresponding to

MDH-C_2^b, -C^aC^b, -C_2^a, -B^aC^b, -B^ac^a and -B_2^a isozymes (m, n).

These facts show that the MDH isozymes of salmonid fishes are encoded in at least three gene loci, Mdh-A, -B and -C, and also show the allelic distinctions that existed at the Mdh-B locus between chum salmon or pink salmon and masu salmon or Japanese char.

MDH ontogeny

As shown in Pl. XI, Fig. 198, four MDH bands, MDH-A_2, -B_2^a, -B^aC^b and -C_2^b, were recognized in the unfertilized eggs of masu salmon, and such an isozyme pattern existed throughout all developmental stages in this species without exhibiting any changes in the isozyme pattern. In the Japanese char from crossing No. 7911, MDH-C_2^b, -B^bC^b, -B_2^b and three additional bands that were probably homologous to the product of Mdh-A were detected in the unfertilized egg and these isozymes persisted throughout the embryogenesis of the species. In another Japanese char, from crossing No. 7909, a complicated eight-banded pattern of MDH isozymes was observed in the unfertilized egg, as seen in Fig. 199. The presence of such a pattern indicates intraspecific polymorphism of MDH-B and -C among individuals. However, the MDH pattern in the unfertilized egg of this specimen also persisted during the embryogenesis without any developmental changes.

The allelic difference between masu salmon and Japanese char at the Mdh-B locus can be used as an informative gene marker for determining the expression of parental alleles in the development of the hybrid between these two species. In

such a hybrid, the expression of the paternal Mdh-B gene in the embryo was expected to indicate the first appearance of the hybrid heterodimer containing paternal MDH-B subunits. As shown in Pl. XII, Fig. 200, only the maternal MDH phenotype was present in the unfertilized egg and in hybrid embryos in the early developmental stages upto 14 days after fertilization. The hybrid isozyme MDH-B^aB^b heterodimer containing paternal subunits of the Japanese char type was detected at 16 days after fertilization (Fig. 200). At 18 days, the hybrid embryos showed an MDH-B_2^b homodimer and an MDH-B^bC^b heterodimer which was formed by the combination of the paternal MDH-B^b subunit and the MDH-C^b subunit (Fig. 200).

MDH ontogeny was also examined in the reciprocal hybrid combination, Japanese char♀ x masu salmon♂. In the hybrid from crossing No. 7908, the MDH isozymes of maternal Japanese char in the unfertilized egg were continuously present until 14 days after fertilization. The appearance of the hybrid isozyme MDH-B^aB^b heterodimer occurred at 16 days (Fig. 201). Fig. 201 also showed that the MDH-B^aC^b heterodimer first appeared at 18 days after fertilization. A hybrid from another crossing, No. 7910, was also used in the examination of MDH ontogeny. In this hybrid, the maternal MDH phenotype was continually observed during the early stages of embryogenesis, until 12 days, and the hybrid phenotype was first detected at 14 days after fertilization as an MDH-B^aB^b heterodimer (Fig. 202).

The allelic difference between masu salmon and pink salmon or chum salmon at the Mdh-C locus can also be used in the

developmental research for estimating the time of the gene activation responsible for the synthesis of the MDH-C subunit. The ontogenetic change in the MDH isozyme pattern in the hybrid pink salmon♀ x masu salmon♂ from crossing No. 7702 is shown in Fig. 203. The maternal MDH phenotype of pink salmon was present in the unfertilized egg and in the following developmental stages until 16 days after fertilization (Fig. 203). Fig. 203 also showed that the hybrid isozymes MDH-C_2^b homodimer and -C^aC^b heterodimer, which contained paternally derived MDH-C^b subunits, were simultaneously detected at 19 days after fertilization.

Similar changes in MDH ontogeny were also observed in the viable hybrid chum salmon♀ x masu salmon♂ obtained from crossing No. 7907. As shown in Pl. XIII, Fig. 204, the maternal chum salmon pattern was observed in the unfertilized egg and this MDH phenotype persisted until 16 days after fertilization. The hybrid phenotype then clearly appeared at 18 days after fertilization (Fig. 204).

On the contrary the reciprocal hybrid, masu salmon♀ x chum salmon♂ demonstrated abnormal development, and its MDH ontogeny was quite different from that of the viable hybrids. As shown in Fig. 205, the inviable masu♀ x chum salmon♂ from crossing No. 7906 showed no sign of a hybrid MDH isozyme containing a paternally derived MDH-C^a subunit during the experimental period; only the maternal phenotype was present throughout the entire development

3. 6-Phosphogluconate dehydrogenase (6PGDH)

6PGDH isozyme pattern

In the eyes, heart, liver and muscles of all salmonid fishes examined, the activity of 6-phosphogluconate dehydrogenase (6PGDH) was electrophoretically detected as an isozymic band on a gel plate. There was no difference in the electrophoretic mobility of the 6PGDH isozyme among tissues examined, but the 6PGDH activity in the liver showed a stronger staining intensity than in the other tissues. Among salmonid species, however, there was an electrophoretic distinction in the mobility of the 6PGDH isozyme. As shown in Fig. 206, muscle tissues of chinook (a, h), chum (b), pink (c), coho (d) and masu (l-n) showed a 6PGDH-A_2^a homodimer that migrated more cathodally than the 6PGDH-A_2^b homodimer which was always observed in Japanese char (j) and brook trout (f). Japanese huchen possessed a A_2^b homodimer (Fig. 206, g) and rainbow trout a A_2^a homodimer (Fig. 206, i). In muscle tissues of the juvenile F_1 hybrids Japanese char♀ x masu salmon♂ and brook trout♀ x coho salmon♂, the 6PGDH isozyme system consisted of a cathodal A_2^a homodimer, anodal A_2^b homodimer and a hybrid band A^aA^b heterodimer which migrated to an intermediate position between the two parental isozyme bands (Fig. 206, e, k). Therefore 6PGDH, which was encoded at the single locus 6Pgdh-A, showed an interspecific allelic difference between Oncorhynchus species and Salvelinus species.

6PGDH ontogeny

In the developing masu salmon embryos, only the 6PGDH-A_2^a homodimer was observed and in the developing Japanese char, only the 6PGDH-A_2^b. As shown in Pl. XIII, Fig. 207, in the hybrid masu salmon♀ x Japanese char♂ from crossing No. 7907, only the maternal 6PGDH phenotype appeared in the unfertilized egg and in the developing embryos until 8 days after fertilization (Fig. 207). A similar ontogenetic pattern of 6PGDH isozymes was observed in the reciprocal hybrid, Japanese char♀ x masu salmon♂, from crossing No. 7908. As shown in Fig. 208, only the maternal 6PGDH-A_2^b homodimeric isozyme was present during the early developmental stages. The heterodimeric 6PGDH-A^aA^b isozyme containing paternal A^a subunits derived from paternal masu salmon was expressed at 10 days after fertilization.

4. Phosphoglucomutase (PGM)

PGM isozyme patterns

As seen in Pl. XIV, Fig. 209, three types of PGM isozymes were detected in the salmonid species examined. These zymograms indicate that three different loci, Pgm-A, -B and -C control the syntheses of PGM-A, -B and -C isozymes. As shown in the figure, there was an electrophoretic difference in PGM-B and PGM-C between Japanese char (a-c) and masu salmon (f-j) or chum salmon (d, e). The PGM-B^b of Japanese char differed in mobility from the homologous PGM-B^a of masu salmon and chum salmon (Fig. 209). The PGM-C^b of Japanese char also

differed in mobility from the homologous PGM-C^a of masu salmon and chum salmon (Fig. 209). There was an intraspecific polymorphism of PGM-A among masu salmon, and three phenotypes, PGM-A^a, -A^aA^b and -A^b, were observed as monomers as seen in Fig. 209. The PGM-A^b of masu salmon was identical to the homologous PGM of chum salmon and Japanese char, but the PGM-A^a was unique to some variant individuals of masu salmon. These facts prove that there is an allelic difference between Japanese char and masu salmon or chum salmon at the Pgm-B and -C loci, and also at the Pgm-A locus among individual masu salmon.

PGM ontogeny

PGM ontogeny was examined in the hybrid between female Japanese char with alleles Pgm-A^b, Pgm-B^b and Pgm-C^b (Fig. 210). The hybrid from crossing No. 7908 showed only the maternal PGM-A^b, -B^b and -C^b isozymes until 12 days after fertilization. Besides these three isozymes, an additional PGM isozyme, with a slightly different mobility from PGM-B^a, was observed in the unfertilized egg and in the early developmental stages. However, this stage-specific band disappeared at 14 days after fertilization. Paternal PGM-B^a was detected at 14 days and paternal PGM-A^a and -C^a were first recognized at 16 days after fertilization.

The first expression of the paternal PGM-A was also examined in the masu salmon embryo from intraspecific crossing No. 7901, between the female with PGM-A^b and the male with PGM-A^a. As shown in Fig. 211, the maternal PGM-A^b

persisted until 14 days after fertilization and the paternal PGM-A^a appeared at 16 days after fertilization. Similar analysis was performed for the viable hybrid chum salmon♀ x masu salmon♂ from crossing No. 7907. As seen in Fig. 212, the paternal PGM-A^a was detected at 18 days after fertilization in this hybrid.

5. Discussion

Pattern of the isozyme ontogeny

It was made clear in the present study that LDH-A^1, -A^2 and -C, which demonstrated strong tissue specific expression in adults, are expressed at relatively later stages in the early development of salmonids. On the contrary, isozymes such as LDH-B_4^1, -B_4^2, MDH-B_2, -C_2, 6PGDH-A_2, PGM-A, -B and -C, which were widely distributed within different tissues in adult salmonids and were expressed with little tissue specificity, were observed during all stages of development. These facts are of interest in that they prove certain corelations between the degree of tissue specificity and the ontogenetic pattern of isozymes in salmonids.

The present results concerning ontogenetic changes in isozyme patterns in salmonids agree with previous results reported for other teleost fishes (Shaklee *et al.*, 1974 ; Champion *et al.*, 1975 ; Champion and Whitt, 1976a ; Philipp and Whitt, 1977 ; Philipp *et al.*, 1979). Philipp and Whitt (1977) studies the tissue distribution and the developmental pattern of LDH isozymes in six species of teleosts —— medaka, *Oryzias*

latipes, mumihog, Fundulus heteroclitus, largemouth bass, Micropterus salmoides, green sunfish, Lepomis cyanellus, lake chubsucker, Erimyzon succer and rainbow trout, Salmo gairdneri —— and stated that the LDH isozymes which were present continuously throughout embryogenesis tended to be expressed in a variety of differentiated tissues of adult fish, and that those occurring later in the development showed a restricted distribution within the adult tissues. As for other isozymes, Champion and Whitt (1976a) reported that GPI (glucosephosphate isomerase)-A, MDH-A and CK (creatine kinase)-C in sunfish were expressed in all developmental stages as well as in most adult tissues, but that GPI-B, MDH-B and CK-A, which showed relatively high tissue specific expression, were detected only at later stages of embryogenesis. Similar results were obtained for largemouth bass and smallmouth bass (Philipp et al., 1979).

Another interesting phenomenon in the ontogeny of isozymes is the noticeable corelation between the first appearance of tissue specific isozymes and the differentiation of the tissues in embryos. In the present study, muscle specific LDH isozymes were observed at 26 days after fertilization in Japanese char, at 22 days in chum salmon and at 20 days in masu salmon (Figs. 188-190). The onset of muscle contraction in the embryos began at 24 days in Japanese char (Fig. 55), at 20 days in chum salmon (Fig. 40) and at 20 days in masu salmon (Fig. 28). It is clear from this evidence that the morphogenetic event coincides with the expression of muscle specific LDH in salmonids. Almost the same conclusion was drawn concerning medaka (Philipp and Whitt, 1977) and lake chubsucker (Champion et al.,

1975), in which muscle specific LDH was observed at the stage when the embryo displayed the first muscle contraction and the somites became fully differentiated.

The relationship between eye specific LDH and retinal development is a famous example in isozyme ontogeny. Although the appearance of eye specific LDH-C was not detected in the present study, Yamazaki and Arai (unpublished) observed in coho salmon and chum salmon that the expression of LDH-C occurred in accordance with the first retino-motor responses in the retinal tissues of posthatched larvae. Miller and Whitt (1975) also reported that the appearance of eye specific LDH was closely related to the ultrastructural differentiation of the retina in the developing green sun fish. Similar results have been obtained by Whitt (1970) and Whitt and Booth (1971).

It may be concluded that the isozyme which is expressed in most adult tissues tends to be present throughout all stages of development, and that the other type of isozyme, of high tissue specific distribution, appears at relatively later embryonic stages in accordance with the morphogenetic event of the tissue involved. Since tissue ubiquitous isozymes such as LDH-B^1, -B^2, MDH-B, -C and 6PGDH-A exist in most cell types in adult tissues of salmonids, these isozymes may play a "housekeeping" role, maintaining the essential metabolism in all salmonid adult and embryonic cells. On the other hand, the "tissue-specific" isozymes such as LDH-A^1, -A^2 and -C may corelate with specialized functions of the differentiated cells.

Exclusive existence of cytoplasmic isozymes during the early developmental stages

The time of the gene activation controlling tissue specific isozymes is easily determined by looking at the first appearance of these isozymes in the developing embryo. However, it is difficult to determine the onset of the gene activity responsible for the synthesis of "house-keeping" isozymes in experiments using parental pure bred species, because "house-keeping" isozymes exist throughout all stages of development without any changes. In the present study, the expression of parental gene products was investigated to determine the time of activation of the parentally derived genes in the development of hybrids between salmonid species with mutually distinguishable isozyme phenotypes.

The present results on the ontogeny of LDH (Figs. 191-195), MDH (Figs. 200-204), 6PGDH (Figs. 207, 208) and PGM (Figs. 210-212) in the viable hybrids show that only the maternal isozyme phenotypes exist during the early stages of development. This exclusiveness of maternal isozymes was also reported in other salmonid hybrids (Klose et al., 1968 ; Wright et al., 1975 ; Arai and Yamazaki, 1980) as well as in other teleost hybrids (Klose and Wolf, 1970 ; Neyfakh et al., 1973). Moreover, the same phenomenon has been found widely among vertebrates such as Amphibia (Wright and Moyer, 1966, 1968 ; Johnson, 1971; Johnson and Chapman, 1971a, b ; Wright, 1975) and Aves (Ohno et al., 1968 ; Leung and Haley, 1974 ; Meyerhof and Haley, 1975). Since the isozyme phenotype observed in the unfertilized egg continues after fertilization without

any changes, it is reasonable to assume that the maternal effect during the early stages is not due to a gene product of the embryonic genome but to a cytoplasmic substance stored in the eggs.

Wright and Subtelny (1971) first observed the exclusive existence of maternal dehydrogenases in the androgenetic nucleocytoplasmic hybrid embryos of the frog and concluded that the maternal effect on isozyme phenotype must be cytoplasmic in origin because the maternal genome was not present in the androgenetic hybrid (Wright and Subtelny, 1971). They also concluded that the maternal isozyme phenotype was not due to continued synthesis directed by messenger RNA, but to the isozyme itself stored in eggs, because no hybrid heteromeric isozyme could be detected and only paternal proteins appeared in the nucleo-cytoplasmic hybrid (Wright and Subtelny, 1971). A similar conclusion was obtained from the LDH and MDH ontogeny for a nucleo-cytoplasmic newt hybrid (Gallien et al., 1973). Recently, Ivanenkov (1980) performed a similar developmental study on esterase isozymes utilizing androgenetic loach embryos, and reported that the maternal esterase persisted until 110-120 hours after insemination and that the paternal esterase appeared at 40-50 hours . Although such experiments with artificial androgenesis have not been carried out with salmonids, it is reasonable to assume from the above evidence that the maternal LDH, MDH, 6PGDH and PGM isozyme phenotypes in the present hybrid salmonids are due to cytoplasmic isozymes stored in the eggs during oogenesis. Consequently, it can be concluded that

the early developmental stages of the salmonid depend entirely on the function of the maternally derived substances stored in the egg cytoplasm.

The first expression of embryonic gene activity during development

The first appearance of hybrid heteromeric isozymes or paternal isozymes containing paternal subunit polypeptides in the hybrid embryo indicates that isozyme properties have began to be controlled by the hybrid genome. This is indicative of the first expression of embryonic loci controlling isozymes in hybrid embryos.

The first appearance of hybrid isozymes containing paternal LDH-B^2 subunits at 18 days after fertilization in the Japanese char♀ x brook trout♂ hybrid indicates the expression of Ldh-B_2 gene activity (Fig. 191). This period might be referred to as the tail bud stage according to the morphogenetic observations made on this hybrid (Pl. V, Figs. 110-124). Isozymes containig paternal LDH-B^2 were first observed in a 14 day embryo of masu salmon♀ x Japanese char♂ (Fig. 192). This was also determined to be the stage of tail bud formation from observations on its morphogenesis (Fig. 131). This evidence clearly shows that the Ldh-B_2 locus is activated at the tail bud stage in salmonids. Similarly, the first expression of the hybrid heterotetramer containing paternal LDH-B^1 subunits in 14 day embryo of masu salmon♀ x pink salmon♂ and of masu salmon♀ x chinook salmon♂ (Figs. 193, 194) indicates that the gene activation at the Ldh-B_1 locus begins at the

tail bud stage (Fig. 66). These results on the activation of the Ldh-B_1 and -B_2 loci accord with those for the homologous loci in the brook trout♀ x lake trout♂ (Goldberg et al., 1969), masu salmon♀ x coho salmon♂ and masu salmon♀ x dolly varden char♂ hybrids (Arai and Yamazaki, 1980). In addition, the first isozymic change from the maternal phenotype to the predominant pattern of LDH-B^2 subunits observed in Japanese char and chum salmon embryo also occurred at the tail bud stage around 14 days after fertilization. This developmental event indicates that the genomic function also begins at this stage.

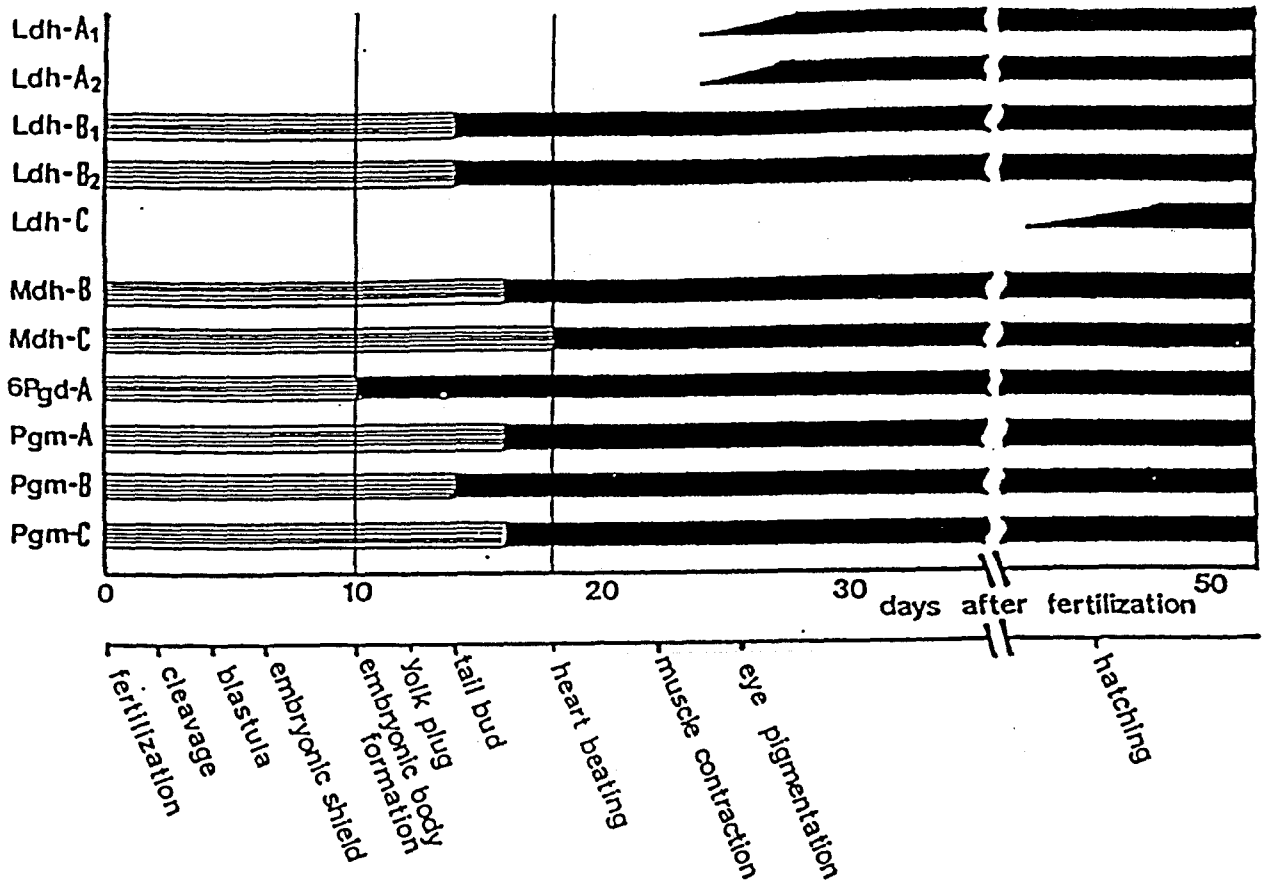
Gene activities of Mdh-B and -C loci may occur at slightly later stages than those of the Ldh-B_1 and -B_2 loci, and a few temporal differences are seen between the expression of the Mdh-B and -C loci. As shown in Figs. 200-202, in the hybrid between masu salmon and Japanese char, hybrid isozymes containing paternal MDH-B subunits appeared at 14-16 days after fertilization, when hybrid embryos reached the late tail bud stage. This suggests that the Mdh-B locus is activated at this stage. The appearance of the MDH-BC heterodimer in the hybrid between masu salmon and Japanese char (Figs. 200, 201) coincided with the appearance of heterodimeric MDH-C^aC^b in the hybrids between pink salmon♀ and masu salmon♂ and between chum salmon♀ and masu salmon♂ (Figs. 203, 204). Since both occurred at 18-19 days after fertilization, it is reasonable to conclude that the onset of gene activation at Mdh-C begins when hybrid embryos are in the late tail bud or heartbeat stage (Figs. 69, 90, 133).

As shown in Figs. 207 and 208, the hybrid heterodimer con-

taining paternal 6PGDH subunits appeared at 10 days after fertilization in both reciprocal crosses between masu salmon and Japanese char. This evidence clearly indicates that the 6Pgdh locus is activated at the stage at which the embryonic body is formed (Fig. 129).

The expression of Pgm genes can be determined as a direct result of the first appearance of the paternal type of PGM isozyme, because of its monomeric structure. As seen in Figs. 210-212, Pgm-A and -C are activated at 16-18 days after fertilization, which morphologically corresponds to the late tail bud stage, and Pgm-B is activated at 14 days after fertilization, when the tail bud begins to be formed in the hybrid embryos. Besides three types of PGM isozymes, additional PGM which was not distributed within adult tissues appeared during the period when only the maternal phenotype existed. This PGM isozyme disappeared just before the occurrence of a new paternal PGM-B isozyme (Fig. 210). These observations indicate that the additional PGM may be completely cytoplasmic in origin, and the significance of this isozyme remains unknown.

The activation times of all genetic loci controlling syntheses of various kinds of isozymes are summarized in Text-Fig. 8. In this chronological map of gene activations, it is notable that the activations of genetic loci responsible for the synthesis of "house-keeping" isozymes are concentrated in the relatively restricted period from 10 to 20 days after fertilization, morphologically corresponding to the stages from embryonic body formation to the first heartbeat. Because



Text-Fig. 8. Schematic representation of the onset of gene activation at 11 loci controlling isozymes during the normal development of salmonids. Lined area indicates the exclusive existence of maternal gene products stored in egg cytoplasm. Solid area indicates the expression of gene activity in embryonic genome. Note the change from cytoplasmic to genomic contribution on isozymic expression at and around the stages of embryonic body formation and tail bud.

the onsets of gene activation for several important isozymes are so concentrated, it might be assumed that all other genes controlling "house-keeping" proteins are activated during the same period. In fact, it has been reported that the first gene activation controlling the phosphoglucose isomerase (PGI) isozyme, which was not examined in the present study, occurs around the stage at which the embryonic body first appears in hybrids between brown and brook trout (Engel et al., 1977).

It is interesting that the gene activation coincided with the stage at which there was a high rate of embryonic death in most inviable hybrids. In addition, the early embryonic stage, where only the maternal isozyme phenotype was present, corresponded to the period when no embryonic death was detected. As suggested in Part I, the period showing the rapid drop in survival rates might be morphogenetically "critical", with a certain event indispensable to embryonic differentiation occurring during this time. Because of the coincidence of the onset of the genomic function and the beginning of embryonic death, it might be assumed that the certain important event of the "critical stage" is related to the synthesis of the "house-keeping" proteins, which are produced by gene activation of the embryonic genome.

Such a period in the development of salmonid embryos can therefore be a turning point in their biochemical differentiation. During this period, the early ontogenesis, directed only by the cytoplasmic function, may become the embryogenesis, which depends on gene products under the control of the genomic function of embryos.

Consequently, the "critical" stage in salmonid development is the stage of onset of "house-keeping" protein synthesis directed by the simultaneous activation of embryonic genes. Determination of the beginning of the nuclear function has been approached by other methodology. Neyfakh (1959, 1964) investigated the survival capacities of functionaly enucleated loach embryos of which the nuclei were inactivated at continual stages of development by X-ray irradiation. He concluded that the initial nuclear function at midblastula controlled the morphogenesis from late blastula to late gastrula, and that the next stage in the nuclear function at midgastrula directed the later organogenetic process. These results do not coincide with the present conclusion concerning biochemical differentiation. On the other hand, it is impossible to disregard the process of transcription of DNA, transportation of messenger RNA into the cytoplasm, translation and assembly of protein molecules into active enzymes. These problems remain to be solved by future experiments.

Aberrant gene expression in inviable hybrids

In most hybrid experiments using masu and chum salmon, all resultant embryos died within about three weeks after fertilization (Table 5). However, hybrid embryos from the 1979 crossing survived until 50 days after fertilization in spite of their extreme malformation (Text-Fig. 4, Pl. IV, Figs. 96-109). Ontogenetic analysis of LDH isozymes in this hybrid revealed that hybrid LDH phenotypes could not be detected at around 14 days after fertilization, when hybrid phenotypes usually ap-

peared in viable hybrids (Fig. 195). As shown in Fig. 195, the expression of paternal LDH began at 35 days after fertilization, about 20 days after *Ldh*-B_1 genes are normally activated. In the MDH ontogeny, no paternal gene products could be found throughout the entire development of the masu salmon♀ x chum salmon♂ hybrid (Fig. 205). Similar results were obtained by Yamazaki and Arai (1982), who reported that only the maternal LDH phenotype was present and that the paternal *Ldh* genes were not activated in another masu salmon♀ x chum salmon♂ hybrid that died within 3 weeks after fertilization. Johnson (1971) reported that no paternal gene function was recognized in the 6PGDH, MDH or PGM ontogeny of the inviable frog hybrid, *Rana pipiens*♀ x *R. sylvatica*♂. Wright (1975) found the same phenomenon in the inviable hybrid, *R. pipiens*♀ x *R. macroglossa*♂. Although these examples suggest aberrant gene expression in inviable embryos, there is also the possibility of gene deficiency due to chromosome aberration.

Although it has been observed that maternally and paternally derived alleles become activated simultaneously during embryogenesis in most vertebrate hybrids (Leung and Haley, 1974 ; Champion and Whitt, 1976 ; Pontier and Hart, 1979), asynchronous activation of parental alleles has been reported in some hybrids (Hitzeroth *et al.*, 1968 ; Castro-Siera and Ohno, 1968 ; Klose *et al.*, 1969 ; Yamauchi and Goldberg, 1974, 1975). This asynchronism in gene activation can be considered as a kind of aberrant gene expression in spite of its occurrence even in normally developing hybrid embryos. Ohno *et al.*(1969) believed that the degree of dissimilarity in the base sequence

of parental alleles might affect the time lag in gene activation. In this regard, Castro-Siera and Ohno (1968) suggested that the maternal cytoplasm might have more difficulty recognizing and activating exotic paternal allelic base sequences in hybrids between remotely related species. Therefore, in inviable hybrids with extremely high incompatibilities between maternal cytoplasm and paternal genes, asynchronous activation at the loci may occur to such a degree that the paternal genes are completely or almost completely repressed. Such a suppression of the exotic genome gives rise to "funtional" aneuploidy or haploidy in the hybrid genome and may result in the blocking of protein synthesis responsible for further embryonic development. In addition, it is possible that differences in survival potentials and developmental capacities of reciprocal hybrids and of different kinds of interspecific hybrids may be caused by the different degrees of incompatibility between the egg cytoplasm and the exotic genome.

These assumptions consistently account for the mechanisms involved in abnormal development. However, some contradictory phenomena have been reported. For example, Whitt et al. (1977) reported the precocious expression of paternal genes in a nonviable centrarchid hybrid between largemouth bass♀ and green sunfish♂, Schmidtke et al.(1976) found preferential activation of the paternally transmitted α-glycerophosphate dehydrogenase gene in hybrid trout embryos. Such findings strongly suggest that more complicated mechanisms might be involved in the process of developmental regulation of gene expression. In this regard, Engel et al. (1977) have tried

to explain it according to the theory of binding capacity of cytoplasmic regulatory substances with polymorphic receptor sites adjacent to structural genes. Whitt et al. (1977) also proposed a model to account for aberrant gene expression according to assumed threshold values of the affecting molecules in the cytoplasm to induce the transcription of genes. Such discussions generally came to the conclusion, however, that nucleo-cytoplasmic interaction was responsible after all. At present, no further discussion is possible on the regulatory systems of normal and aberrant gene activation owing to the shortage of experimental data.

Part III. Chromosomes of hybrid salmonids

There have been a number of reports on salmonid chromosomes concerning intra- and interspecific Robertsonian polymorphism (Ohno et al., 1965 ; Thorgaard, 1976), sex chromosomes (Thorgaard, 1977, 1978) and natural polyploidy (Cuellar and Uyeno, 1972 ; Thorgaard and Gall, 1979). Chromosome studies have been also carried out on hybrid salmonids. Sasaki et al. (1968) reported on chromosomes of salmonid hybrids between chum and kokanee salmon and showed that there was an exact sum of the haploid sets from the parent fish, numerically as well as morphologically. Similar results were obtained for the hybrid between Atlantic salmon and sea trout by Nygren et al. (1972) who observed that the hybrid had chromosome numbers of Atlantic salmon (N=29) and sea trout (N=40). On the other hand, Uyeno (1972) found that the karyotype of coho

salmon♀ x brook trout♂ was identical to that of the maternal species and that that of brook trout♀ x coho salmon♂ was intermediate between that of the parents. He concluded that there was a hybrid gynogenesis in the crossing between female coho salmon and male brook trout. However, all these results were obtained from normally developed hybrids and no chromosomal examinations have been done on salmonid hybrids showing high mortality rates or abnormal morphology. In the present, chromosomes of both viable and nonviable salmonid hybrids were cytogenetically examined following karyological observations of parental species. The results are discussed in terms of the presence or absence of unusual cellular division and abnormal cytological events in the embryonic genome of the hybrids.

1. Chromosomes of parental species.

Chromosomes of pink salmon, Oncorhynchus gorbuscha

Chromosome preparations were made from head-kidney tissues of four juvenile pink salmon collected from the Shokotsu River, Hokkaido (Table 1, Text-Fig. 1) and chromosomal counts were made for a total of 56 metaphase spreads. As shown in Table 6, the modal number of chromosomes in the pink salmon was 52 for all four specimens. This indicates that the diploid chromosome number in this species was 2N=52 (Text-Fig. 9). Karyotype analysis using the best plate proved that the diploid complement consisted of 24 pairs of meta- or submetacentric chromosomes and 2 pairs of subtelocentric chromosomes (Pl. XV, Figs. 213, 214).

Table 6. Distribution of chromosome numbers in Pink salmon, Oncorhynchus gorbuscha.

fish No.	chromosome counts											total
	45	46	47	48	49	50	51	52	53	54	55	
104					1		1	15	1			18
105					1	1	2	16	2	1		23
106			1		1	1	3	7				13
107								2				2
total			1		3	2	6	40	3	1		56

Chromosomes of masu salmon, Oncorhynchus masou

Chromosome preparations were made from head-kidney tissues of six juvenile masu salmon collected from the Kumano River, Iwate frefecture (Table 1, Text-Fig. 1) and from 10 developing embryos at 15 days after fertilization derived from the parental masu salmon crossing No. 8001 (Table 2). Chromosome counts were carried out on a total of 470 cells. As shown in Table 7 and Text-Fig. 9, the juveniles and embryos had a modal chromosome number of 66. Karyological analysis revealed that the diploid complements were comprised of 17 pairs of meta- or submetacentric chromosomes, 12 pairs of subtelocentric chromosomes and 4 pairs of acrocentric chromosomes (Pl. XVI, Figs. 215, 216). Among the second group, the largest pair of subtelocentric chromosomes is noteworthy in that it had satellites on the distal region of the short arm with a secondary constriction (Fig. 216).

Chromosomes of chum salmon, Oncorhynchus keta

Chromosomes of 49 developing chum salmon embryos from control fertilization of 1980, 1981 and 1982 were prepared and examined. As seen in Table 8, all 18 embryos from crossing No. 8004 had a modal chromosome number of 74. The same modal distribution was obtained for embryos from crossing No. 8109, 8201 and 8205. Consequently, 207 of 43 cells showed the modal chromosome number of 74 (Text-Fig. 10). Karyological study revealed that the diploid complements of chum salmon were comprised of 13 pairs of meta- or submetacentric chromosomes, 3 pairs of subtelocentric chromosomes and 21 pairs of

Table 7. Distribution of chromosome numbers in Masu salmon, Oncorhynchus masou.

No..of crossing	age of embryos (days)	fish or embryo No.	chromosome numbers																						total
			<50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70<		
		324.				1		1	1		1		1		1	2	1	2	13	1					25
		325.	1								1	1				2	2	2	8						17
		326.												1	3		4	1	6						15
		327.						1					1		1	3	4	7	14						31
		328.												1			3	3	13	1					21
		329.														4	4	4	12	2	1				27
		subtotal	1			1		2	1		2	1	2	2	5	11	18	19	66	4	1				136
8001	15	1.	1							1			2	3		3	6	6	20	2	1	1	2		48
		2.	3						2			2	1		2	2	1	1	9	2	1				26
		3.									1		1			4	3	4	22	2	1		2		40
		4.								1	1					1	5	4	19	1	1	2	1		36
		5.	1											1	2	1	2	1	11						19
		6.		1							1			1	2	2	5	3	24	1			1		41
		7.			1								2		3	2	1	1	10						20
		8.						1	1		1	1	4	1	2	3	4	3	22						43
		9.				1			1				1		1		3	1	11	1					20
		10.	1							1		2			1	4	3	8	17	3	1				41
		subtotal	6	1	1	1		1	4	3	4	5	11	6	13	22	33	32	165	12	5	3	6		334
		total	7	1	1	1		3	5	3	6	6	13	8	18	33	51	51	231	16	6	3	6		470

Table 8. Distribution of chromosome numbers in Chum salmon, Oncorhynchus keta

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																					total
			<59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79<	
8004	15	1.	1				1						1	2	1	1	2	4				1		14
		2.													2			4						6
		3.	1		1			1	1					1	1			2						8
		4.			1					1		1		1	2	1		4						11
		5.							1		1			1			1	4						8
		6.	1							1			1				1	2						6
		7.											1			2	2	5						10
		8.						1			1				1	2	1	3						9
		9.										1				1		2						4
		10.																1						1
		11.						1					1		1	2	1	3					1	10
		12.																1						1
		13.																2						2
		14.		1			1					1	2	2	1	2	2	17					1	30
		15.																2						2
		16.	1					1		1		1			2	1	2	8						17
		17.							1						1			2						4
		18.					1					1	1		1		2	5	1					12
	subtotal		4	1	2		2	5	3	3	2	5	7	7	13	12	14	71	1			1	2	155

Table 8. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																					total
			<59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79<	
8109	23	1.																1						1
		2.								1				1		1	1	4	1					9
		3.											1		1	1		1						4
		4.						1										2						3
		5.									1				1		1	7						10
		6.	1															2						3
		7.					1							1			1	3						6
		8.				1				1			1	1	1	3	1	3						12
		9.															1	3						4
		10.																1						1
	subtotal		1			1	1	1		2	1		2	3	3	5	5	27	1					53
8201	12	1.									1	1	1					4					2	9
		2.				1				1								3						5
		3.								1	1	1		1	2	1		7						14
		4.	1						1						1	1	1	4						9
		5.																2						2
		6.											1			1	1	1					1	5
	subtotal		1			1			1	2	2	2	2	1	3	3	2	21					3	44

Table 8. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																					total
			<59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79<	
8205	13	1.					1								1	1	1	1						5
		2.	1											1			1	5						8
		3.																2						2
		4.									1	1						3						5
		5.															1	3						4
		6.							1	1		1				1	1	5						10
		7.										1						1						2
		8.		1					1					1			1	2						6
		9.																1						1
	subtotal		1	1			1		2	1	1	3		2	1	2	5	23						43
8205	16	1.					1	1						2	1		1	14						20
		2.												1		2	2	9						15
		3.	1								1	2		2	1	1	3	13						24
		4.											1		1			10		1				13
		5.	1						1			1	2	1		3		9		1				19
		6.			1	1	1					1					2	10					1	17
	subtotal		2		1	1	2	1	1		1	5	3	6	3	6	8	65		2			1	108
	total		9	2	3	3	6	7	7	8	7	15	14	19	23	28	34	207	2	2		1	6	403

acrocentric chromosomes (Pl. XVII, Figs, 217, 218). Among the second group, the largest pair of subtelocentric chromosomes was noteworthy in that it had satellites on the distal region of the long arm with a secondary constriction (Fig. 218).

Chromosomes of Japanese char, Salvelinus leucomaenis

Chromosomes of Japanese char embryos derived from 1981 and 1982 experiments were examined at 15 or 16 days after fertilization. As shown in Table 9, all 21 embryos had a clear mode of 84 chromosomes. This indicates that the diploid chromosome number was 2N=84 in Japanese char (Text-Fig. 9). Karyotype analysis revealed that the diploid complements of Japanese char were comprised of 8 pairs of meta- or submetacentric chromosomes, 1 pair of subtelocentric chromosomes and 33 pairs of acrocentric chromosomes (Pl. XVIII, Figs, 219, 220).

Chromosomes of brook trout, Salvelinus fontinalis

Chromosomes of brook trout were examined in 3 individuals sampled from Tadami Fish Culture Station of Kitasato University, Tadami, Fukushima prefecture (Table 1, Text-Fig. 1). They were also examined in 2 embryos at 16 days after fertilization and in 7 embryos at 22 days, derived from parental crossings of 1981. As shown in Table 10, the modal chromosome number of the brook trout was 84 for all specimens examined. Thus, the diploid chromosome number was 2N=84 (Text-Fig. 9). Karyotype analysis revealed that the diploid complements consisted of 8 pairs of meta- or submetacentric

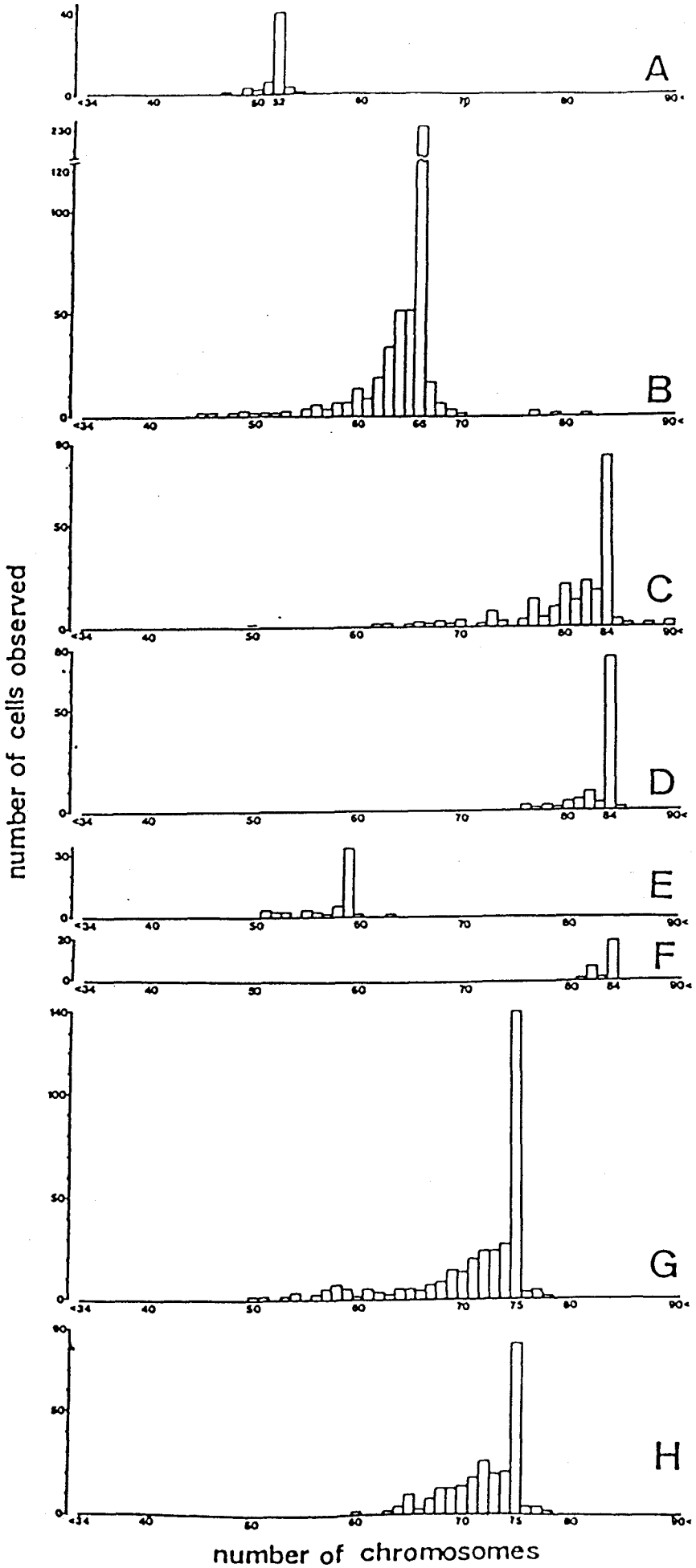
Table 9. Distribution of chromosome numbers in Japanese Char, Salvelinus leucomaenis

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																						total
			<68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88<		
8103	15	1.			2							1			3	2	3	2	5						18
		2.													1	1	2	2	6				1		13
		3									1				1	1	2	1	8	1					15
		4.										1		2	3	1	2	2	5						16
		5.										2	1	1	1	1	2	2	5				1		16
		6.						1						2	1	3	1	1	4		1				14
		7.						1	1						3		2	2	4				1		14
		8.			1						1	2			3	1		3	6						17
		9.					1	1				1	1	1	2		3		3	1					14
	subtotal				3		1	3	1		2	7	2	6	18	10	17	15	46	2	1		3		137
8203	10	1.	1				1					1	1			1	1		2						8
		2.	1					1											2						4
	12	1.	1																2						3
		2.	1																1						2
	subtotal		4				1	1				1	1			1	1		7						17
8207	16	1.	1									1	1	1					3						7
		2.																1	3						4
		3.													1	1	1		6	1					10
		4.	2	1				2											2						7
		5.	1					1											2						5
		6.	1									1		1		1	1		9						14
		7.							1		1	1		1					3						7
		8.										1			1		1	1	2						6
	subtotal		5	1				3	1		1	5	1	3	2	2	3	2	30	1					60
			9	1	3		2	7	2		3	13	4	9	20	13	21	17	83	3	1		3		214

Table 10. Distribution of chromosome numbers in Brook trout, Salvelinus fontinalis.

No. of crossing	age of embryos (days)	fish or embryo No.	chromosome numbers														total
			<64	74	75	76	77	78	79	80	81	82	83	84	85	86<	
		601.								1	2	4	2	18			27
		602.								2	1			17			20
		603.										2		20			22
		subtotal								3	3	6	2	55			69
8113	16	1.	1			1					1			7			10
		2.												2			2
		subtotal	1			1					1			9			12
8113	22	1.				1	1		1	1	1	1	1	3	1		11
		2.												3			3
		3.						1				1		1			3
		4.						1				1		1			3
		5.												1			1
		6.												1			1
		7.												1			1
		subtotal				1	1	2	1	1	1	3	1	11	1		35
		total	1			2	1	2	1	4	5	9	3	75	1		116

Text-Fig. 9. Schematic representation of total distribution of chromosome numbers in several viable hybrids. (A). pink salmon, (B). masu salmon, (C). Japanese char (D). brook trout, (E). masu salmon♀ x pink salmon♂, (F). Japanese char♀ x brook trout♂, (G). masu salmon♀ x Japanese char♂, (H). Japanese char♀ x masu salmon♂. The mode of each hybrid is the exact sum of the haploid numbers from the two parental species.


A
B
C
D
E
F
G
H
number of cells observed
number of chromosomes

chromosomes, 1 pair of subtelocentric chromosomes and 33 pairs of acrocentric chromosomes (Pl. XIX, Figs. 221, 222).

2. Chromosomes of viable hybrids

Chromosomes of the hybrid masu salmon♀ x pink salmon♂

Chromosomes of the viable hybrid masu salmon♀ x pink salmon♂ were examined in 3 juvenile F_1 individuals derived from the hybridization of 1977 (Table 1). As shown in Table 11 and Text-Fig. 9, all these hybrids had a prominent peak frequency of 59 chromosomes, and 33 of the 44 total had a modal chromosome number of 59. Thus, the diploid chromosome number was 2N=59 for this hybrid. Karyotype analysis showed that the hybrid had 42 meta- or submetacentric, 14 subtelocentric and 4 acrocentric elements (Pl. XX, Figs. 223, 224). As seen in Fig. 224, the number of diploid chromosome complements in the hybrid was the exact sum of two haploid sets from the parental species.

Chromosomes of the hybrid Japanese char♀ x brook trout♂

Chromosomes of the viable hybrid Japanese char♀ x brook trout♂ were examined in four F_1 individuals derived from the hybrid experiment of 1981. All these specimens had the modal chromosome number of 84 (Table 12, Text-Fig. 9). The karyotype of this hybrid consisted of 16 meta- or submetacentric, 2 subtelocentric and 66 acrocentric elements (Pl. XXI, Figs. 225, 226).

Table 11. Distribution of chromosome numbers in the hybrid Masu salmon♀ x Pink salmon♂.

fish No.	chromosome numbers															total
	<50	51	52	53	54	55	56	57	58	59	60	61	62	63	64<	
701										2						2
702			2	2		2	1	1	4	17				1		21
703		3				1	1		1	14	1					21
total		3	2	2		3	2	1	5	33	1			1		44

Table 12. Distribution of chromosome numbers in the hybrid Japanese char♀ x Brook trout♂.

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers							total
			<80	81	82	83	84	85	86<	
8107	27	1.		1	2	1	6			10
		2.			1		4			5
		3.					5			5
		4.			3		4			7
	total			1	6	1	19			27

Chromosomes of the hybrid masu salmon♀ x Japanese char♂

Chromosomes of the viable hybrid masu salmon♀ x Japanese char♂ derived from crossing No. 8002 were examined in 13 developing embryos at 15 days after fertilization. As shown in Table 13 and Text-Fig. 9, chromosomes in the observed cells showed a relatively wide distribution, but all embryos had a marked peak frquency of 75. A hundred thirty nine cells out of a total of 331 had the modal chromosome number of 75 (Table 13, Text-Fig. 9). The karyotype of this hybrid was comprised of 25 meta- or submetacentric, 13 subtelocentric and 37 acrocentric chromosomes (Pl. XXII, Figs. 226, 227). It is worth noting that one subtelocentric element had a satellite which was transmitted from the maternal masu salmon (Fig. 227). These observations showed that the chromosome number in masu salmon♀ x Japanese char♂ was numerically intermediate between the numbers found in the parental species (Text-Fig. 9). The karyotype showed that the number of chromosome elements was the sum of two haploid complements from the parental species (Table 20).

Chromosomes of the hybrid Japanese char♀ x masu salmon♂

Chromosomes of the viable hybrid Japanese char♀ x masu salmon♂ were examined in 12 embryos from the experiment of 1981. As shown in Table 14, the modal chromosome number was 75 for all specimens. Sixty cells out of a total of 195 had the modal chromosome number of 75 (Text-Fig. 9). Karyological analysis revealed that the karyotype of this hybrid was comprised of 25 meta- or submetacentric, 13 subtelocentric and 37 acrocentric chromosomes (Pl. XXIII, Figs. 229, 230).

Table 13. Distribution of chromosome numbers in the hybrid Masu salmon♀ x Japanese chard♂.

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																											total
			<53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	'85<	
8002	15	1.	2	1		1	1	2	3			1	1	1	2	2		1	3	1	2	2	3	3	15		1			48
		2.		1			1	1		1	1						2	1	2	2		3	1	2	8					26
		3.					1							1			2		2			1	1	2	4	1				15
		4.	1	1			1	1			1			2			1	2	3		3	3	3	4	26			1		53
		5.				1		1	1		1								1	1	2	2	3	3	9	1				26
		6.						1	1			1				1	1			2	2	1	2		2						14
		7.										1		1	2			1		1	3	4	3	2	24		1				43
		8.																			2	4	1	2	12	1			1		23
		9.					1	1								1		2	1	2	2	1	2	2	11						26
		10.															1	1	1	2	2			1	8						16
		11.																					1	1	7						9
		12.									1		1						1	2	1	2	3	4	9		2				26
		13.									1				1										4						6
		total	3	3		2	5	7	5	1	5	3	2	5	5	4	7	8	14	13	19	23	23	26	139	3	4	1	1	331

Table 14. Distribution of chromosome numbers in the hybrid Japanese char♀ x Masu salmon♂.

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																total
			<63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	
8104	16	1.						1	2		2	2	2	2	7		1		19
		2.									3	2	1		6	1	1	1	15
		3.	1		1			1		2		3	3	1	3				15
		4.			2			2	1	1		1	3	1	7				18
		5.			1		2	1	1	1	2	2	1	2	3				16
		6.			1	1		2		3		1	1	2	5				16
		7.							1					1	4				6
		8.						2		1	2	1	2	1	2				11
		9.			1		1		3	1	2	3		2	6				19
		10.	1		1		1			2	1	1	2		4	1			14
		11.		1	2		1	1	2		2	5	2	1	7		1		25
		12.		2			2		1	2	2	1	1	4	6				21
		total	2	3	9	1	7	10	11	13	16	22	18	17	60	2	3	1	195

A satellite chromosome with secondary constriction was also observed in this crossing (Fig. 230). The number of elements shown by the karyotype was the exact sum of the parental elements (Table 20).

3. Chromosomes of inviable hybrids.

Chromosomes of the hybrid chum salmon♀ x masu salmon♂

Hybrids between chum salmon♀ and masu salmon♂ were inviable in the 1977 and 1980 crossings, but they showed normal processes of morphogenesis and satisfactorily high survival potentials in the hybrid experiment of 1979. Chromosome distribution in this inviable hybrid was studied in a total of 40 embryos from crossing No. 8005. Fifteen embryos out of a total of 20 sampled at 13 days after fertilization had a marked modal frequency of 70 chromosomes and most of the other embryos also had cells with 70 chromosomes (Table 15). Thirteen embryos of a total of 20 showed a modal frequency of 70 chromosomes at 15 days after fertilization, and other embryos also had a cell with 70 chromosomes (Table 15). Consequently, 160 cells out of a total 488 observed had the modal chromosome number of 70 (Text-Fig. 10). This indicates that the diploid chromosome number in the inviable hybrid chum salmon♀ x masu salmon♂ is in general 2N 70. The result of the karyology is shown in Pl. XXIV, Figs. 231 and 232. The karyotype of this hybrid was comprised of 30 meta- or submetacentric, 15 subtelocentric and 25 acrocentric chromosomes (Fig. 232). In addition, it is noteworthy that the largest subtelocent-

Table 15. Distribution of chromosome numbers in the hybrid *Chum salmon♀ x Masu salmon♂*.

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																												total
			<48	'51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	'77<		
8005	13	1.				1	1						1						2				8	1							14
		2.							1			2		1				2			1		7								14
		3.													2		1		4	1	2		6								16
		4.					1		1					1			1			1	1	2	6	1							15
		5.	1									1	1	1			1	2	2		2	1	5					1			18
		6.															1					1	5		1						8
		7.	2					1				1			1	1	2		2			1	4			1					16
		8.									1							2	1	1	1		4								10
		9.											2			2			1				4						1		10
		10.							2			1			1			1	2				3						1		10
		11.													1			1		1	1		3								7
		12.							1		1		1					1	1	1	1		2								9
		13.								1		1					1			2			2								7
		14.													1								2						1		4
		15.																1					1								2
		16.											1				1	1	1	2			1						2		9
		17.	2		2					1		1			1			4	1				1								13
		18.	1							1		1	2	1	1		1	1		1			1								11
		19.						1					1		1			2			1										6
		20.		1						1					1					1											4
	subtotal		6	1	2	1	2	2	5	4	2	8	9	4	10	3	9	18	15	13	10	5	65	2	1	1		1	4	203

Table 15. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																											total
			<48	'51	52	53	54	55	56	57	58	59	60	61	62	63	64	'65	66	67	68	69	70	71	72	73	74	75	'77<	
8005	15	1.												1	1	1	2	3	3	5	2	2	13	1	1		2			37
		2.				1		1	2		1		1		1	2	2	2	2	5	2	2	13							37
		3.								1		1	2	1		1	1	6	4	2	3	2	12	2						38
		4.							1		1			1	1	2		2	2	2			9							21
		5.								1		1		3	1		3	3	3	1	3	2	8		1					30
		6.						1				3			2			2	3			6	8							25
		7.	1				1						1		1	2	2	2	3	2	1		5		1					22
		8.										1						1				1	5							8
		9.				1											1				2		4							8
		10.							1						1	1			1			1	4							9
		11.							1			1		1				1	1	1	2	3	3		1					15
		12.												1				2	1			1	3				1			9
		13.				1																	2							3
		14.												1				1					1							3
		15.									1								1				1		1					4
		16.				1						1						1					1							4
		17.							1												1		1							3
		18.																1			1		1					1		4
		19.																					1							1
		20.		11								1				1					1									4
		subtotal	1	1		4	2	1	6	2	3	9	4	9	8	10	11	27	24	18	18	20	95	3	5		3	1		285
		total	7	2	2	5	4	3	11	6	5	17	13	13	18	13	20	45	39	31	28	25	160	5	6	1	3	2	4	488

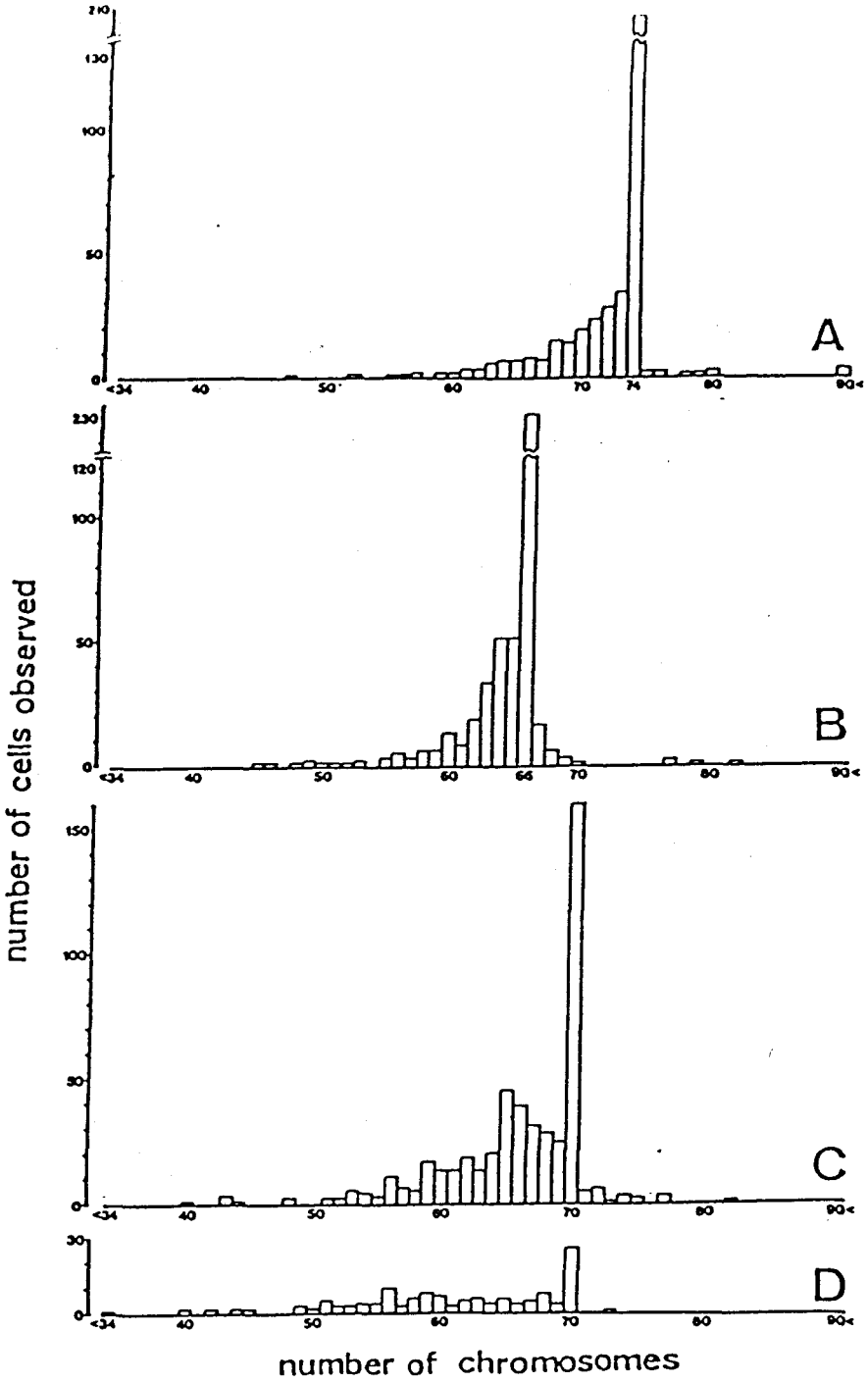
ric element had satellites at the distal portion of its short arm, indicating that it originated from the paternal masu salmon, and the second largest subtelocentric element had satellites at the distal portion of its long arm, suggesting that it originated from the maternal chum salmon (Fig. 232). A karyotype which was numerically and morphogenetically intermediate between the two parental species was also found in this inviable hybrid (Table 20).

Chromosomes of the hybrid masu salmon♀ x chum salmon♂

All hybridization between female masu salmon and male chum salmon resulted in nonviability, but whereas hybrids from crossing Nos. 7705 and 8004 were suppressed at the stage of formation of tha tail bud, the other hybrids, from No. 7904, developed beyond the tail bud stage to the eyed stage as described in Part I. Here, chromosome analysis was carried out on 19 embryos originating from the 1979 crossing. As shown in Table 16, chromosomes of this hybrid distributed widely, but 13 embryos out of a total of 19 had a modal chromosome number of 70, which was the same as the diploid number of the reciprocal chum salmon♀ x masu salmon♂ hybrid. In all, 25 cells out of 116 observed showed the modal chromosome number of 70 (Text-Fig. 10). Karyological analysis revealed the karyotype to be comprised of 30 meta- or submetacentric, 15 subtelocentric and 25 acrocentric elements (Pl. XXV, Figs. 233, 234). A satellite chromosome from the maternal masu salmon was observed in the subtelocentric group (Fig. 234).

Table 16. Distribution of chromosome numbers in the hybrid Masu salmon♀ x Chum salmon♂.

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																												total
			<45	'49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	'73	'92	'100<				
7906	37	1.						1		1	1	1	1	1		1	1			1		1	2	2	6		1	1				22
		2.	1									1		2	1		1				1	1	1		3							12
		3.				1					1			1			1				1				3	1						9
		4.												1	1	1			1	1	1		1		2							9
		5.	1							1			1	1	1			1					1		2							9
		6.				2			1						1								1		2			1				8
		7.	2	1							2		1					1							1							8
		8.			1						1							1					1	1	1							6
		9.					1				1						1					1			1							5
		10.									1		1												1							3
		11.													2										1							3
		12.				1	1																		1							3
		13.																		1		1			1							3
		14.								1			1					1	1	1												5
		15.																1	1	1												3
		16.							1		1			1																		3
		17.						1	1		1																					3
		18.		1																												1
		19.	1																													1
		total	5	2	1	4	2	2	3	3	9	2	5	7	6	2	4	5	3	5	3	4	7	3	25	1	1	2				116



Text-Fig. 10. Schematic representation of total distribution of chromosome numbers in inviable hybrids between chum salmon and masu salmon; (A). chum salmon, (B). masu salmon, (C). chum salmon♀ x masu salmon♂, (D). masu salmon♀ x chum salmon♂. The mode for reciprocal hybrids is an intermediate number (2N=70) between that of chum salmon (2N=74) and that of masu salmon (2N=66).

Chromosomes of the hybrid chum salmon♀ x Japanese char♂

All hybrids between chum salmon♀ and Japanese char♂ were inviable as described in Part I, but there were differences in the developmental capacities among hybrid groups. In the experiment here, chromosomes were examined in hybrid embryos from 1980, 1981 and 1982 crossings. As seen in Table 17, for hybrid embryos at 13, 14 and 15 days after fertilization derived from crossing No. 8006, 13 out of a total of 25 had a chromosome number of 79, which was expected as the theoretical intermediate number between those of the parental species. Among the hybrid groups from the 1981 crossing, 14 embryos out of a total of of 23 showed this theoretical mode of 79 chromosomes (Table 17). In the other hybrid groups, from the two crossings Nos. 8202 and 8206, most hybrid embryos had a marked peak frequency of 79 chromosomes (Table 17). In total, 150 cells out of 491 observed showed a peak frequency at the chromosome number of 79 (Text-Fig. 11). These facts suggest that most chum salmon♀ x Japanese char♂ have a diploid chromosome number of 79 (2N=79), but that some embryos may possess aneuploid cells in addition to normal cells. The results of karyological analysis on normal cells is given in Pl. XXVI, Figs. 235 and 236. As seen in the figure, the karyotype of this hybrid was comprised of 21 meta- or submetacentric, 4 subtelocentric and 54 acrocentric chromosomes and was just intermediate between that of the two parental species, numerically as well as morphologically (Table 20). In addition, it was remarked that only one of the subtelocentric elements had satellites in the distal portion of its long arm,

Table 17. Distribution of chromosome numbers in the hybrid Chum salmon♀ x Japanese chard♂

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																				total
			<50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81~	91~	101<				
8006	13	1.										1													1	1			2			2							7
		2.		1	1												1						1									1							5
		3.			1																								1			1			1				4
		4.								1		1	1			1		1	1			2				1	1		1		1	1							9
		5.								1		1	1			1					1		1					1					1						9
		6.															1			1											1								3
		7.										1					1								1	1	2			1							2		9
		8.	1												1												1	1											4
		9.																					1																1
		10.																				1																	1
	subtotal		1	1	2					1		3	1		1	1	3	1	1	1	1	3	3		3	3	4	2	4	1	2	5	1	1		2			52
	14	1.									1		2	1					1			2				1			1	1	1	4							15
		2.	2	1		1	1	1				1	1		2			1				1						1	1			3	1						18
		3.																		1								1	1		1	3							7
		4.														1							1				1				1	1							5
		5.																														1							1
		6.	1																					1	1			2											5
		7.	1			1								1				1	3		1	1												1					10
		8.																									1												1
		9.																				1																	1
	subtotal		4	1		2	1	1			1	1	3	2	2	1		2	4	1	1	5	1	1	1	1	2	4	3	1	3	12	1	1					63
	15	1.			1																1						1	1			1	2				1			8
		2.																								1	1	1	1			1							5
		3.																														1							1
		4.																														1							1
		5.	1						1						1	1		2		1			1		1		1	1											11
		6.				1																																	1
	subtotal		1		1	1			1						1	1		2		1	1		1		1	1	3	3	1		1	5				1			27

Table 17. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																	total
			<50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81~'91	~'101<	
8110	15	1.																1	1	1	2						1	1				3				10
		2.								1		1				2			2						1	1		1				3		1		13
		3.											1										1		1		2					3			1	9
		4.				1									1	1		1														2				6
		5.													1												1		1			2				5
		6.										1	1					1			1							1	2			1				8
		7.	1																1										1		1				4
		8.													1						1											1				3
		9.										1				1												1				1				4
		10.																														1				1
		11.																												1						1
		12.				1		1																					1							3
		13.																								11										1
		14.									1																									2
	subtotal		1			2		1		1	1	3	2		3	4		4	4	1	4		1		2	2	4	4	5	1		18		1	1	70
8110	16	1.					1													2	1	2	3		1	1	1			1	2	4				19
		2.					1								1						2	1		2	1	2						2				12
		3.	1															1	2					1								1				7
		4.										1		1	1			1			1	2	1		3	2	1	1				1				16
		5.														1						1								1						3
		6.	1							1				1					1		1				1	1	1	1							9
		7.	1					1									1	1							2		1							1		8
		8.																							1											1
		9.						1																												1
	subtotal		3				2	2		1		1		2	2	1	1	3	3	2	5	6	4	3	5	9	3	3	1	2	3	8			1	76

Table 17. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers <50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81~	91~	101<	total
8202	12	1.																						1	2	1				1		8		1			14
		2.																			1							1	1	1	1	3					8
		3.																														2					2
		4.																	1										1	1		1				1	5
		5.																									1					1					2
		6.																								1						1					2
		7.																				1	1									1					3
		8.																														1					1
		9.																						1			1										2
	20	1.																						2	1	1						2					7
	subtotal																		1		1	2	1	4	3	3	2	1	2	3	1	20		1		1	46
8206	13	1.																			1			1		1						4					7
		2.																										1	1	1	1	2					6
		3.																												1	1	1					3
		4.																			1											1					2
		5.																														1					1
		6.																								1											1
		7.																1								1											2
		8.																				1															1
	subtotal																	1			2	1		1		3		1	1	2	2	9					23
8206	16	1.																									1	3	2	2	5	23		2			38
		2.																	1				1	1		1	1	2	2	3	1	23		2			38
		3.																					1	1	1		1		2		2	7					15
		4.															1							1		1		1	1		1	7					13
		5.																								1	1	1				5		2			10
		6.																							1					1		3					5
		7.															1								1					1		3					6
		8.																							1		2		1	1	2	2					9
	subtotal																2		1				2	3	4	3	6	7	8	8	11	73		6			134
	total		9	3	2	5	4	4		4	2	8	6	4	8	8	7	11	16	5	15	18	13	12	19	25	24	25	25	18	23	150	2	10	2	4	491

indicating that it originated from the maternal chum salmon (Fig. 236).

Chromosomes of the hybrid Japanese char♀ x chum salmon♂

All hybrids between female Japanese char and male chum salmon were inviable, but some differences were recognized in developmental capacities among crossed groups as described in Part I. In the experiment here, chromosomes were analyzed in a total of 116 embryos obtained from the hybridizations of 1980, 1981 and 1982 using 637 cells. As seen in Table 18, no modal frequency was detected at 10 days in hybrid embryos from crossing No. 8008, and most embryonic cells showed a significantly lower chromosome number than the expected intermediate number between those of the parental species. A similar tendency was observed in other embryos from the same hybrid group at 14 or 15 days after fertilization. The chromosome numbers of most cells from these embryos were distributed within the range between 44 and nearly 60 and individual embryos were comprised of cells with various chromosome numbers as shown in Pl. XXVII, Figs. 237-240.

At 16 days after fertilization, most cells from embryos from crossing No. 8105 had chromosome numbers which were distributed within the range of 50 to 69 and no modal number was observed (Table 18). At 19 days, most cells from the same group of embryos showed chromosome numbers below 64 (Table 18). It is noteworthy that the range of the chromosome distribution for 19-day embryos had shifted downward to numbers lower than for 16-day embryos. In this case, however, the hybrid embryos did not have clear modes of chromosome

Table 18. Distribution of chromosome numbers in the hybrid Japanese char♀ x Chum salmon♂.

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																	total
			<39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70~'80~'100<		
8008	10	1.	1					1		1	1						1		1	1	1		1						1						1	11
		2.															1	1		1				1												4
		3.																1						1												2
		4.																						1												1
		5.																1																		1
		6.														2	1																			3
		7.						1																												1
	subtotal		1					2		1	1					2	3	3	1	2	1		1	1	2				1						1	23
	14	1.							1		1	1	1		1		1			2													1		9
		2.						1		1	1	1						1	1		1			1												8
		3.	1										1	2										1												5
		4.										1												1												2
		5.															1	1		2			1													5
		6.													1			1	1	1	1		1													6
		7.							1								1	2	2	1		1														8
		8.													1							1														2
		9.	1		1						1						1																			4
		10.								1																										1
	subtotal		2		1			1	2	2	3	3	2	2	3		4	5	4	6	2	2	2	3									1		50
	15	1.																															1		1
		2.													1	1		1		2			1													6
		3.																			1															1
		4.											2	1																						3
		5.	1										1																							2
		6.										1																								1
	subtotal		1									1	3	1	1	1		1		2	1		1										1		14

Table 18. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																	total
			<39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70~'80~'100<		
8105	16	1.															1	1			2	5	2		1		2	1					1			16
		2.																						2							1		1			4
		3.																					1	1			1			1						4
		4.												1										1	1	1			1							5
		5.																						1			2									3
		6.															1					1	2	1	1											6
		7.																							1											1
		8.																					1													1
		9.																			1		1													2
		10.																	1			1														2
	subtotal													1			2	1	1		3	7	7	6	4	1	5	1	1	1	1		2			44
	19	1.	2						2		2			1				1		1			1		1			1								13
		2.					1		1					1	1		1	1		2	1			1		2										12
		3.		1						1									1	1			1	1												6
		4.				1							1								1			1												4
		5.											1		1	3			1				1													7
		6.	2									1						1	1		1	1														7
		7.	2	1				1					2			1						1														8
		8.	1		1		1															1														4
		9.									1				1					2	2															6
		10.	2															1			1															4
		11.			1				1											1																3
		12.																	1																	1
		13.																1																		1
		14.	3																																	3
	subtotal		12	2	3	1	2	1	4	1	3	1	4	2	3	4	1	5	4	7	6	3	3	3	1	2		1								79

Table 18. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers: < 39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70~'80~'100<	total
8112	16	1.						1														1											1		3
		2.																					1				1								2
		3.																						1											1
		4.																						1											1
		5.																		1															1
		6.														1.																			1
		7.												1																					1
	subtotal							1						1		1				1		1	1	2			1						1		10
	17	1.															1													1			1		3
		2.	2																			1	1						1						5
		3.				1									1	1					1	1		1											6
		4.														1					1														2
		5.																			1														1
		6.																		1															1
		7.														1																			1
		8.					1		1	1																									3
	subtotal		2			1	1		1	1					1	3	1			1	3	2	1	1				1	1			1		22
	41	1.	1														1					1										1		4
		2.												1		1					1											1			4
		3.											1												2				1						4
		4.	1					1								1	1						1	1				1							7
		5.	1											1				2	1		1	1	2	1			1						1		12
		6.										1		1	1		2		1		1	1		1											9
		7.	1			2			1		1		1				3	1	1	1	2	2		1											17
		8.	1			1				1							2	2		1			1	1											10
		9.										1					2					1													4
		10.											1						1		1														3
		11.						1	1			1								1															4
		12.	1																1																2
		13.	1													1																			2
		14.				1																													1
	subtotal		7			4		2	2	1	1	3	3	3	1	3	11	5	5	3	6	6	4	5		2	1		1	1			1		1	1	83

Table 18. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																				total
			<39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70~	80~	100<			
8204	10	1.																																	1				1
		2.															1																	1					2
		3.		1													1	3		1		1		1	1		1			2	1	1	1	1	2	1			19
		4.									1		1	1		1	1	1		3	2	1	2		1	1		1					1							18
		5.										1					1	1	1			1											1			1				7
		6.							1							4	2	3	2	2	4				1				1	1		1								22
		7.															2	1		1												1								5
		8.																						1					1	1										3
		9.								1				1		1	1				1		1							1							1				8
		10.																										1												1
		11.																				1					1														2
		12.																			1						1														2
		13.																			1		1		1																3
	subtotal			1					1	1	1	1	1	2		6	9	9	3	7	9	4	4	2	4	1	3	2	2	5	1	3	3	2	3	3			93
	12	1.																																1					1
		2.														1																		1						2
		3.														1		1		3					1	1		2	1	1					1					12
		4.																		1					1					1										3
		5.													1						2																				3
		6.																				1																			1
	subtotal														1	2		1		4	2	1			2	1		2	1	2				2	1					22

Table 18. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																				total
			<39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70~'80~'100<					
8208	13	1.																				1		1	2			1			1								6
		2.								1								2		1		1	1											1						7
		3.											2	1		1				1	2	4	1											1						13
		4.											1					1		2	1	6			1															12
		5.																1		1			1																	3
	subtotal									1			3	1		1		4		5	3	12	3	1	3			1			1			2						41
	16	1.																1	1			1		1			1	1							1				7
		2.								1								3	2	2		2	2	2	3	1			1											19
		3.							1		1			3			1	1	1		1	1	1				1													12
		4.			1					1			1			1		2	3	1	5	4	5	3	3	2														32
		5.													2		1	1	1		1	4				1														11
		6.									1			2		1		1		1		3	1	3	1															14
		7.								1										1		3	1		1															7
		8.																		1	1	2	1	2																7
		9.											1										1	3																5
		10.														1				2			1	4																8
		11.			1															2	3			2																8
		12.																1		1		1		2																5
		13.																1			1	3		1																6
		14.	1																	1			1																	3
		15.					1					1								3		3																		8
		16.																	1	1		2																		4
	subtotal		1		2		1		1	3	2	1	2	5	2	3	2	11	9	16	12	29	14	23	8	4	2	1	1					1					156
	total		26	3	6	6	4	7	11	11	11	10	18	18	12	26	33	45	27	54	48	67	41	47	24	11	12	8	8	10	3	3	7	10	5	5			637

distribution (Table 18). Chromosomes from hybrid embryos from another hybridization were also analyzed. As shown in Table 18, a few cells showed chromosome numbers higher than 61, but more than 70 % of the embryonic cells from this hybridization were distributed within the range of 41 to 60.

Most cells obtained from the hybrid embryos at 10 or 12 days after fertilization showed wide distributions between 45 and 69, but some embryos showed chromosome numbers higher than 70. In these hybrid embryos, cells with chromosome numbers higher than 60 contrasted significantly with those of the other hybrid groups. In hybrid embryos from another crossed group, No. 8205, most cells showed distributions of chromosome numbers within the range of 50 to 65 at 13 and 16 days after fertilization. As seen in Pl. XXVIII, Figs. 241-244, embryos from the hybridization of 1982 was also comprised of cells with various chromosome numbers, with no modal chromosome number being recognized individually or as a whole. There was one hybrid embryo which had a nuclear plate characterized by aberrant chromosomes such as chromosome fragments, acentric chromosomes and dicentric chromosomes in addition to nuclear plates showing the aneuploid numbers of 54, 55 and 56 (Pl. XXIX, Figs. 245-248).

As described above, no modal distribution of chromosome numbers was detected in the cells of any of the hybrid embryos between female Japanese char and male chum salmon. Most cells of this hybrid had chromosome numbers ranging from 41 to 61 (Table 18, Text-Fig. 11). The hybrid showed a rather wide variation in chromosome numbers, and there was no embryo with

Table 19. Distribution of chromosome numbers in the hybrid Brook trout♀ x Chum salmon♂

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																						total
			<33	34	35	36'38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66'90	'117							
8114	16	1.	1																											1				1								3
		2.																														1										1
		3.	2					1				1		1			1	1		2	1			1		1						1										13
		4.	1	1						1																1					1											5
		5.											1					1		1		1		1		1	1		1														8
		6.																						1		1			1														3
		7.																											1													1
		8.																2			1							1														4
		9.																									2															2
		10.				1							1	1					1						1		1															6
		11.							1									1								2															4
		12.																1							1	1																3
		13.								1															1																	2
		14.																		2				1																		3
		15.			1		1								1			1	1					1																		6
	subtotal		4	1	1	1	1	1	1	2		1	2	2	1		1	7	2	5	2	1		5	3	7	4	1	2	2	1	2			1						64
		1.	1												1	1		1			1				1	2							1								9
	17	2.								1								1				1					1					1									5
		3.																1		1		1		3	3	2	1	1			1									14
		4.																							1		2				1										4
		5.																					1		1			1			1										4
		6.										2												1					1													4
		7.							1					1									1	1	1		1		1													7
		8.																					1						1													2
		9.														1				1								2													4
		10.																						2			1	1													4
		11.											1									1					1	1													4
		12.											1						1					1		1															4
		13.													1				1						1																	3
		14.												1						1																						2
	subtotal		1						1	1		2	2	2	2	2		3	2	3	1	3	3	8	8	5	6	7	3		3	1	1								70

Table 19. (continued)

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																						total
			<33	34	35	36,38		39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	90	117				
8114	32	1.	1					1	1			1									1	1	1		2	4	3	1						1							18
		2.																	1	1										1							1				4
		3.							1					1	1						2		2	1		1			1		1										11
		4.								1			2									1			2				1												7
		5.				1		1								2			1		3		2	1		2	1	1													15
		6.																										1													1
		7.												1				1			1				1	2																6
		8.								1														1																		2
		9.												1					1																							2
	subtotal		1			1		2	2	2		1	2	3	1	2		1	3	1	7	2	5	3	5	9	4	3	2		2			1			1			66
	total		6	1	1	1	1	3	3	4	3	1	5	7	5	5	2	2	13	5	15	5	9	6	18	20	16	13	10	5	4	4	3	2		1	1			200

Text-Fig. 11. Schematic representation of total distribution of chromosome numbers in inviable hybrids between chum salmon and Japanese char or brook trout; (A). chum salmon, (B). Japanese char, (C). brook trout, (D). chum salmon♀ x Japanese char♂, (E). Japanese char♀ x chum salmon♂, (F). brook trout♀ x chum salmon♂. The mode of the hybrid chum salmon♀ x Japanese char♂ shows an intermediate number (2N =79) between chum salmon (2N=74) and Japanese char (2N=84). Note the aberrant distribution of chromosome numbers in Japanese char♀ x chum salmon♂ and brook trout♀ x chum salmon♂.

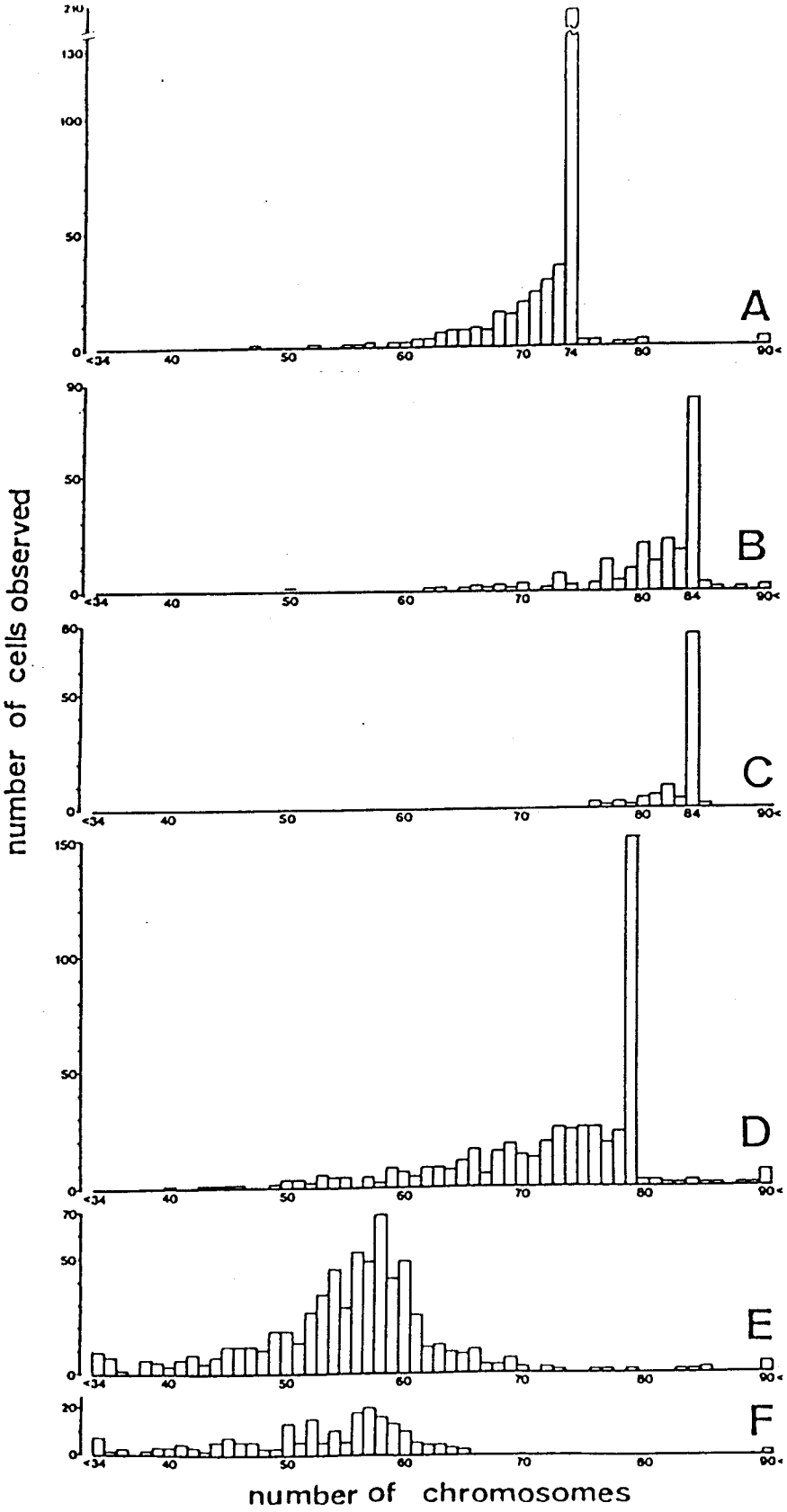

number of cells observed
number of chromosomes
A
B
C
D
E
F

Table 20. Summary of the karyotype of hybrid salmonids examined in the present study

	species or hybrids	diploid number (2N)	number of meta- and submeta-centric chromosome (M and SM)	number of subtelocentric chromosome (ST)	number of acrocentric chromosome (A)	fundamental number (NF)
Parental species	pink salmon	52	48	4	0	100
	masu salmon	66	34	24	8	100
	chum salmon	74	26	6	42	100
	Japanese char	84	16	2	66	100
	brook trout	84	16	2	66	100
Viable hybrids	masu♀ x pink♂	59	41	14	4	100
	char♀ x brook♂	84	16	2	66	100
	masu♀ x char♂	75	25	13	37	100
	char♀ x masu♂	75	25	13	37	100
Inviable hybrids	chum♀ x masu♂	70	30	15	25	100
	masu♀ x chum♂	70	30	15	25	100
	chum♀ x char♂	79	21	4	54	100
	char♀ x chum♂			aneuploidy		
	brook♀ x chum♂			aneuploidy		

the theoretical number of chromosomes ——— that is, the number intermediate between those of the two parental species. These facts indicate that the hybrid embryo Japanese char♀ x chum salmon♂ consisted of aneuploid cells.

Chromosomes of the hybrid brook trout♀ x chum salmon♂

The hybrid brook trout♀ x chum salmon♂ was also classified as inviable and its morphogenetic process closely resembled that of Japanese char♀ x chum salmon♂, as mentioned in Part I. Chromosome analysis was carried out on 38 embryos from crossing No. 8114. Distributions of chromosome numbers in this hybrid are shown in Table 19. The same tendency as was observed in Japanese char♀ x chum salmon♂ was found in the chromosome number distribution of brook trout♀ x chum salmon♂. Most cells of this hybrid had a chromosome number below 65, which is considerably lower than the theoretical number equivalent to the exact sum of the numbers of parental haploid sets (Text-Fig. 11). Among all embryos examined, there was no single embryo showing the modal frequency and each individual embryo was mosaically comprised of aneuploid cells as shown in Pl. XXX, Figs. 249-252.

4. Discussion

Chromosomes in the viable salmonid hybrids

Chromosome numbers and karyotypes of parental salmonid species and their hybrids examined in this study are shown in Table 20. The diploid numbers and karyotypes in pink salmon, masu salmon, chum salmon and Japanese char were in

agreement with the results of Muramoto et al.(1974). Those in brook trout were almost the same as in Japanese char, but the number of subtelocentric and acrocentric elements differed from those in the previous report (Muramoto et al. 1974 ; Uyeno, 1972).

The viable hybrid between masu salmon♀ and pink salmon♂ demonstrated a diploid chromosome number of 2N=59, equivalent to the exact sum of the haploid sets from masu salmon (N=33) and pink salmon (N=26). A similar result was obtained for the viable hybrid between masu salmon (N=33) and Japanese char (N=42), where the diploid chromosome number of 2N=75 was the exact sum of the haploid complements of the parental species. The karyological results concerning these viable hybrids also support the fact that the hybrid genome consists of the haploid sets from the parental species; the karyotype of the hybrids were just intermediate between those of the two parental species, numerically as well as morphologically.

Such intermediate chromosome numbers and karyotypes in hybrid salmonids have also been reported for viable hybrids between chum salmon and kokanee salmon (Sasaki et al.,1968), between Atlantic salmon and sea trout (Nygren et al., 1972) and between sea trout and Arctic char or Atlantic salmon (Gjedrem et al., 1977). These observations suggest that the cytological events occurring during the process of development may be normal in these hybrid salmonids.

Chromosomes in the inviable salmonid hybrids

It is worth noting that inviable salmonid hybrids can be cytogenetically categorized into two groups; one group has a complete hybrid genome equal to the exact sum of the haploid complements from the parental species, while the other group shows no definite modal distribution of chromosome numbers and shows aneuploid cells. Most offspring embryos from the reciprocal crossings between chum salmon and masu salmon in 1979 and 1980 not only showed a chromosomal mode at the theoretical chromosome number of 2N=70, calculated from the simple sum of the parental haploid complements, but also displayed karyotypes just intermediate between the two parental species, as shown in Text-Fig. 10 and Table 20. A similar cytogenetic result was obtained in the inviable hybrid between female chum salmon and male Japanese char. In this hybrid combination, embryos had a diploid chromosome number of 2N=79, which is equal to the exact sum of the haploid chromosomes from the two parental species, as seen in Text-Fig. 11 and Table 20. In the reciprocal hybrid, however, between female Japanese char and male chum salmon, no modal distribution of chromosome number was observed and distribution within most cells ranged from about 50 to 60. This was considerably lower than the theoretical diploid number for this hybrid, but higher than the haploid number for either parental species (Text-Fig. 11). Such an unusual chromosome distribution must therefore not due to hybrid gynogenesis, but to the aneuploidy originating from interspecific hybridization. Cells with aneuploid chromosomes were also observed in the inviable

hybrid between female brook trout and male chum salmon. The cells from this hybrid showed distributions within the range of about 50 to 60, and no modal number was found that was the theoretical sum of haploid sets from brook trout and chum salmon. These observations clearly suggest that chromosome aberration is caused by certain inviable hybridizations.

Although the number of aneuploid cells totally distributed within the range of 50 to 60 in both Japanese char♀ x chum salmon♂ and brook trout♀ x chum salmon♂, a notable phenomenon is discovered by revising the chromosome distributions of these inviable hybrids in the order of developmental age or stages. It is clear that the chromosome numbers are reduced during the embryogenesis of the inviable Japanese char♀ x chum salmon♂ hybrid, because 16 out of 44 cells observed in 16-day embryos had chromosome numbers which ranged over 60, but only 4 out of 79 cells in 19-day embryos showed distributions over 60 (Table 18). A similar phenomenon of differential reduction of chromosomes during development was observed in the hybrid experiment using Japanese char♀ x chum salmon♂ in 1982. In 10- and 12-day embryos of this hybrid, 37 of 115 cells possessed more than 60 chromosomes, but at 13 and 16 days only 9 of 197 cells showed over 60 (Table 18). These two cases clearly indicate that chromosome number may be reduced during the process of embryonic development in certain inviable hybrids such as Japanese char♀ x chum salmon♂. In addition, the facts that no distinct mode was recognized for any of the embryos and that each contained various aneuploid cells suggest that the reduction in chromosomes may

occur independently and continuously in various cells of hybrid embryos. All these cytogenetic results indicate that certain abnormalities in the division of embryonic cells may occur in certain inviable combinations. The presence of the chromosomal fragment and dicentric chromosomes might also serve as direct evidence of cytological abnormality in such hybrids. Hence, it can be concluded in the present study that certain hybrid combinations may be characterized by unusual cellular divisions causing the elimination of some chromosomes.

The present results, however, do not indicate whether or not the chromosomes are eliminated prferentially in these cells of inviable hybrids during the process of embryonic development. In this regard, the classic cytological studies of Pinney (1918, 1922, 1928) are informative. He reported lagging, non-disjunction and large-scale elimination of paternally transmitted chromosomes during the early cleavage period in inviable hybrids among various teleostean fishes such as Fundulus, Menidia, Prionotus, Ctenolabrus and Stenotomus. Further experimental studies may be needed not only to determine the developmental stage when aneuploid cells are formed, but also to find out what kinds of chromosomes are eliminated from the hybrid nucleus during embryogenesis.

The cytogenetics responsible for abnormal embryogenesis in the inviable hybrids.

The inviable hybrid chum salmon♀ x Japanese char♂ cytogenetically showed the theoretical number which can be calculated as the exact sum of the haploid sets from the two

parental species, but in the reciprocal cross, Japanese char♀ x chum salmon♂, there was clearly a broad distribution of aneuploid cells. It is possible to conclude from the present results that chromosome aberration is not due to the interaction of the two heterogenic genomes, but to the interaction of the maternal cytoplasm and foreign paternal chromosomes. The ooplasm of the Japanese char might have some molecular or subcellular mechanisms for recognizing exotic chromosomes from foreign species and eliminating them from the hybrid nucleus. However, it is doubtful that this mechanism can eliminate all exotic chromosomes and induce hybrid gynogenesis, because a large number of aneuploid cells were found to contain between just under 50 and 60 chromosomes, which are clearly more than the haploid number in the maternal species. Hence, the eliminating mechanisms may be related to the affinity between maternal cytoplasm and exotic chromosomes.

It is evident from the present data that the high mortality rate and abnormal embryogenesis observed in the hybrid embryos of Japanese char♀ x chum salmon♂ and brook trout♀ x chum salmon♂ are attributable to chromosome aberration. In these hybrids, aneuploidy may result in the large-scale deficiencies of embryonic genes, which inhibit the normal activities of the genes responsible for the synthesis of indispensable proteins The reason why the hybrid chum salmon♀ x Japanese char♂ displayed the same mortality rate and morphogenetic abnormalities as the reciprocal aneuploid hybrid, in spite of its complete genomic structure, may be related to repression of gene activities, as observed in the inviable hybrid, masu salmon♀ x

chum salmon♂. These hybrids showed abnormal gene expression for LDH and MDH isozymes, but have cytogenetically complete genome. The repression of certain gene activities in hybrid embryos may bring about a result similar to that of deficiency of genes by aneuploidization. Consequently, it can be concluded that abnormal development in certain hybrid salmonids is caused by two main mechanisms, the deficiency of genes by chromosome aberration and the repression of certain gene activities.

Part IV. Effects of artificial triploidization on the embryogenesis of hybrid salmonids

It was pointed out in the previous chapters that abnormal embryogenesis in hybrid salmonids probably results from the disturbance of gene activities and/or a deficiency of genes. It was supposed that the abnormalities might be connected with certain incompatibilities between maternal cytoplasm and the paternally derived exotic genome. Artificial reduction of the ratio of exotic genome to cytoplasm may be an interesting test for confirming the above supposition. When the release of the second polar body is completely blocked after insemination with milt from a different species, the resultant embryos will have two sets of maternal haploid complements and one set of paternal haploid complement in the maternally transmitted cytoplasm. This part of the paper describes the production of triploid hybrids between chum salmon and Japanese char, which was carried out by means of the hydrostatic pressure treatment of Onozato (1983). Developmental capacities,

morphogenesis, chromosomes and isozyme phenotypes in resultant embryos are also examined.

1. Survival potentials and developmental capacities of the triploid hybrid.

Survival rates during the development of triploid embryos

Two series of artificial crossings were made between chum salmon and Japanese char in 1982 (Nos. 8202, 8204, 8206, 8208). Some of the fertilized eggs from these crossings were subjected to artificial triploidization using the method described previously. Fertilized eggs from parental species of chum salmon (Nos. 8201, 8205) and Japanese char (Nos. 8203, 8207) were also used for triploidization. Non-treated eggs were used as control diploids. Survival rates, dates of hatching and water temperatures for incubation during development of the experimental and control embryos are given in Table 21.

As shown in Table 21, no significant difference was observed in the survival rates of control and experimental groups of chum salmon (No. 8205), and treated embryos showed more satisfactory survival rates than control embryos in crossing No. 8201. In the ontogenesis of Japanese char (Nos. 8203, 8207), the survival rate of the control group was lower than in other experiments of the present study (Table 5), and the control groups from Nos. 8201 and 8207, respectively, showed 43.1 % and 28.8 % survival rates at 50 days after fertilization. Experimental groups subjected to hydrostatic pressure showed almost the same survival rates as the control groups

Table 21. Summary of survival rates during the embryogenesis of embryos from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination

No.	combination		no. of embryos observed	survival rate (%) at 10 days after fertilization							date of hatching	average water temperature (range)
				10	15	20	25	30	40	50		
8201	chum x chum	cont.	380	97.4	85.5	71.3	65.8	61.8	58.9	29.7	60	8.1 (6.5-9.0) °C
		exp.	17	100.0	100.0	94.1	88.2	82.4	82.4	n.d.	60	8.1 (6.5-9.0)
8203	char x char	cont.	51	100.0	94.1	90.2	80.4	64.7	43.1	43.1	52	8.1 (6.5-9.0)
		exp.	34	96.4	67.8	67.8	64.3	53.6	46.4	39.3	55	8.1 (6.5-9.0)
8202	chum x char	cont.	429	94.4	81.8	21.2	0.7	0	0	0	-	8.1 (6.5-9.0)
		exp.	43	90.7	79.1	69.8	60.5	53.5	18.6	18.6	54	8.1 (6.5-9.0)
8204	char x chum	cont.	71	97.2	4.2	2.8	1.4	1.4	0	0	-	8.1 (6.5-9.0)
		exp.	50	96.0	8.0	6.0	0	0	0	0	-	8.1 (6.5-9.0)
8205	chum x chum	cont.	274	93.8	93.4	93.1	92.0	92.0	88.3	88.3	58	8.1 (6.5-9.0)
		exp.	66	89.1	89.1	89.1	89.1	89.1	89.1	89.1	58	8.1 (6.5-9.0)
8207	char x char	cont.	111	100.0	97.3	91.9	84.7	76.6	50.5	28.8	54	8.1 (6.5-9.0)
		exp.	116	96.6	88.8	69.8	64.7	53.4	25.9	25.9	57	8.1 (6.5-9.0)
8206	chum x char	cont.	320	96.3	94.4	27.8	19.7	17.5	14.4	12.6	-	8.1 (6.5-9.0)
		exp.	38	97.4	86.8	36.8	26.3	26.3	23.7	23.7	58	8.1 (6.5-9.0)
8208	char x chum	cont.	299	97.7	93.6	42.1	9.7	3.0	1.3	0	-	8.1 (6.5-9.0)
		exp.	117	99.1	82.1	44.4	23.9	11.1	3.6	0	-	8.1 (6.5-9.0)

On the contrary, a notable difference was observed between the survival potentials of control and experimental embryos from the crossing chum salmon♀ x Japanese char♂. The diploid hybrid chum salmon♀ x Japanese char♂ (No. 8202) showed a survival rate of close to 100 % until 10 days after fertilization, but the embryos began to die during the stage that followed, and most had died by 25 days after fertilization (Table 21). In the experimental group subjected to hydrostatic pressure, the drop in the survival rate was moderate, and about 19 % of the embryos reached the hatching stage, demonstrating their viability (Table 21). Although embryos from the experimental group showed a survival rate of about 26 % at 25 days, this rate remained constant from thereon until hatching.

As seen in Table 21, there was no significant difference between the survival rates of the control and experimental groups in the reciprocal Japanese char♀ x chum salmon♂ hybridization, since all embryos from both groups died during the process of embryonic development. Most embryos died during the period between 10 and 15 days after fertilization in both the control and experimental groups from crossing No. 8204. In another crossing, No 8208, all embryos died during the 40-50 day stage and no hatched larvae were observed.

Morphogenesis of triploid embryos

The morphogenetic process in embryos from control and experimental groups of chum salmon, Japanese char, chum salmon♀ x Japanese char♂ and Japanese char♀ x chum salmon♂ are represented in Pl. XXXI, Figs. 253-278. Each figure shows the

the morphology of the embryo at the tail bud stage (20 days), heartbeat stage (25 days), eyed stage (48 days) and hatching stage under an average water temperature of 8.1°C. As shown in Figs. 253-260, there were no morphological differences between diploid control and experimental groups of chum salmon during embryogenesis, and hatched larvae were obtained from both groups. A similar result was obtained for Japanese char. No difference was observed during the morphogenetic process between the diploid control group and the experimental group subjected to artificial triploidization (Figs. 261-268).

On the contrary, there was a conspicuous difference in morphogenesis between the diploid control and experimental groups of chum salmon♀ x Japanese char♂. The diploid hybrid showed an abnormal embryonic development characterized by microcephalia, microphthalmia, oedema and dwarf embryonic body, and no embryo survived until the hatching stage (Figs. 269-271). Embryos which originated from eggs subjected to hydrostatic pressure demonstrated normal development (Figs. 272-275). While embryos from the control group showed abnormal morphology at 20 days after insemination (Fig. 269), treated embryos showed the normal morphology of the tail bud stage (Fig. 272) and developed to more differentiated stages (Figs. 273, 274). In the chum salmon♀ x Japanese char♂ experimental group, about 19-24 % of the embryos reached the hatching stage and demonstrated their potential for further development and growth (Table 21, Fig. 275).

In the reciprocal crossing Japanese char♀ x chum salmon♂, there was no difference in embryonic development between the

control and experimental groups and both demonstrated similar process of abnormal embryogenesis, characterized by extreme mortality. As shown in Figs. 276 and 278, embryos from both these groups showed undifferentiated embryonic bodies at 20 and 25 days after fertilization, and no sign of recovery of morphogenetic capacity was observed in the treated embryo of this combination.

2. Chromosomes of the triploid hybrids

Chromosomes of the chum salmon embryo from eggs subjected to hydrostatic pressure

As shown in Table 8, the chromosome counts for the control groups from Nos. 8201 and 8205 showed the normal diploid number for chum salmon (2N=74). The karyotype was comprised of 26 meta- or submetacentric, 6 subtelocentric and 42 acrocentric elements (Pl. XVII, Figs. 217, 218). Chromosome distribution in embryos from chum salmon eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination with chum salmon milt is given in Table 22. Thirty-two of the 108 cells obtained from 20 embryos demonstrated a peak frequency at the chromosome number of 111, indicating that the embryos were triploid (Table 22, Text-Fig. 12). The result of a karyotype analysis of these triploid embryos are shown in Pl. XXXII, Figs. 279 and 280. As seen in the figures, the karyotype was comprised of 39 meta- or submetacentric, 9 subtelocentric and 63 acrocentric elements, apparently indicating the presence of three haploid sets. It is worth noting that a secondary constriction was observed in

Table 22. Distribution of chromosome numbers in the embryo of chum salmon, Oncorhynchus keta from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																						total
			<89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	<							
8201	12	1.																					1		3								1									5
		2.	1						1				1								2		1	1	2																	9
		3.	1								1											1			2																	5
		4.																1		1				1	2																	5
		5.													1							1			2																	4
		6.								1			1		1						1	1			1																	6
		7.	1		1													1							1								1									5
		8.												1		1									1																	3
		9.																1							1																	2
		10.																		1					1																	2
	subtotal		3		1				1	1	1		2	1	2	1	1	2	1	4		3	2	2	16								2									46
8205	13	1.	1		1										1	1	2	2				2		2	3		1		1												19
		2.																1					1		3																	5
		3.												1						1					2			1														5
		4.	1				1									1			1				1		2																8
		5.								1				1										1	2																	6
		6.			1											1							1		1																	5
		7.													1	1				1				1	1																	5
		8.																1		1	1				1																	4
		9.											1									1			1																	3
		10.						1												1												1										3
	subtotal		2		2		1	1		1			1	2	2	4	2	4	1	4	1	3	5	3	16		1	1	1			1	1	2							62
	total		5		3		1	1	1	2	1		3	3	4	5	3	6	2	8	1	6	7	5	32		1	1	1			1	3	2							108

the long arms of three telocentric chromosomes (Fig. 280).

Chromosomes of the Japanese char embryo from eggs subjected to hydrostatic pressure

The chromosome distribution in embryos of the control group (No. 8203) showed the modal number of 84, as seen in Table 9. As described in Part III, the diploid karyotype of the Japanese char was comprised of 16 meta- or submetacentric, 2 subtelocentric and 66 acrocentric elements (Pl. XVIII, Figs. 219, 220). The results of the chromosome counts on embryos from Japanese char eggs subjected to hydrostatic pressure are given in Table 23 and Text-Fig. 12. Sixteen of the 80 cells observed in 11 embryos from the experimental group had the modal chromosome number of 126 (Table 23, Text-Fig. 12). Karyological analysis revealed that the karyotype of the treated Japanese char embryo was comprised of 24 meta- or submetacentric, 3 subtelocentric and 99 acrocentric chromosomes (Pl. XXXIII, Figs. 281-282). These results indicate that the treated embryos had three sets of haploid complements from the Japanese char, numerically as well as morphologically.

Chromosomes of the allotriploid hybrid chum salmon♀ x Japanese char♂

Chromosome counts for control groups from crossing No. 8202 and No. 8206 are shown in Table 18. Embryos from diploid hybridization between chum salmon♀ and Japanese char♂ had a modal number of 79 chromosomes (Table 18) and their karyotype was comprised of 21 meta- or submetacentric, 4 subtelocentric and 54 acrocentric chromosomes (Pl.XXVI, Figs. 235, 236).

Table 23. Distribution of chromosome numbers in the embryo of Japanese char, Salvelinus leucomaenis from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																	total
			98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129		
8203	12	1.	2					1																		1					1					5
		2.																													1					1
		3.																													1					1
	subtotal		2					1																		1					3					7
8207	16	1.	1						1											1						1	1	2	1		5					13
		2.			1								1					1					1						1	1	3					9
		3.	1				1								1		1	1	2	1					1	1	1				2					13
		4.	1								1												1		1						2					6
		5.										1					1				1	1							1		1			1		7
		6.	1								1										1	1	1		1			1	1							8
		7.	2	1			1							1			2	1					1	1												10
		8.	2				1	1			1										1							1								7
	subtotal		8	1	1		3	1	1		3	1	1	1	1		4	3	2	2	3	2	4	1	3	2	2	4	4	1	13			1		73
	total		10	1	1		3	2	1		3	1	1	1	1		4	3	2	2	3	2	4	1	3	3	2	4	4	1	16			1		80

Therefore, the karyotype of the embryos consisted of the haploid sets from chum salmon and from Japanese char, numerically as well as morphologically. The chromosome distributions in the embryos from eggs subjected to hydrostatic pressure at 15 minutes after insemination are shown in Table 24. All 10 embryos from No. 8202 had the modal number of 116 chromosomes at 12 days of age (Table 24). The same result was obtained for treated embryos from crossing No. 8206 at 13 and 16 days after fertilization (Table 24). These results indicate that the treated embryos had a modal peak at 116 chromosomes (Text-Fig. 12). As shown in Pl. XXXIV, Figs. 283 and 284, the karyotype of a cell with 116 chromosomes was comprised of 34 meta- or submetacentric, 7 subtelocentric and 75 acrocentric elements, which was the exact sum of the diploid set from the maternal chum salmon and the haploid set from the paternal Japanese char. These facts show that the treated embryos were allotriploid hybrids: 2chum salmon♀ x Japanese char♂. It is noteworthy that two chromosomes in the subtelocentric group had satellites at the distal portions of their long arms, indicating their origin from the egg nucleus and second polar body of the maternal chum salmon. This observation also strongly supports the allotriploidy of the embryos from eggs subjected to hydrostatic pressure.

Chromosomes of the aneuploid hybrid Japanese char♀ x chum salmon♂

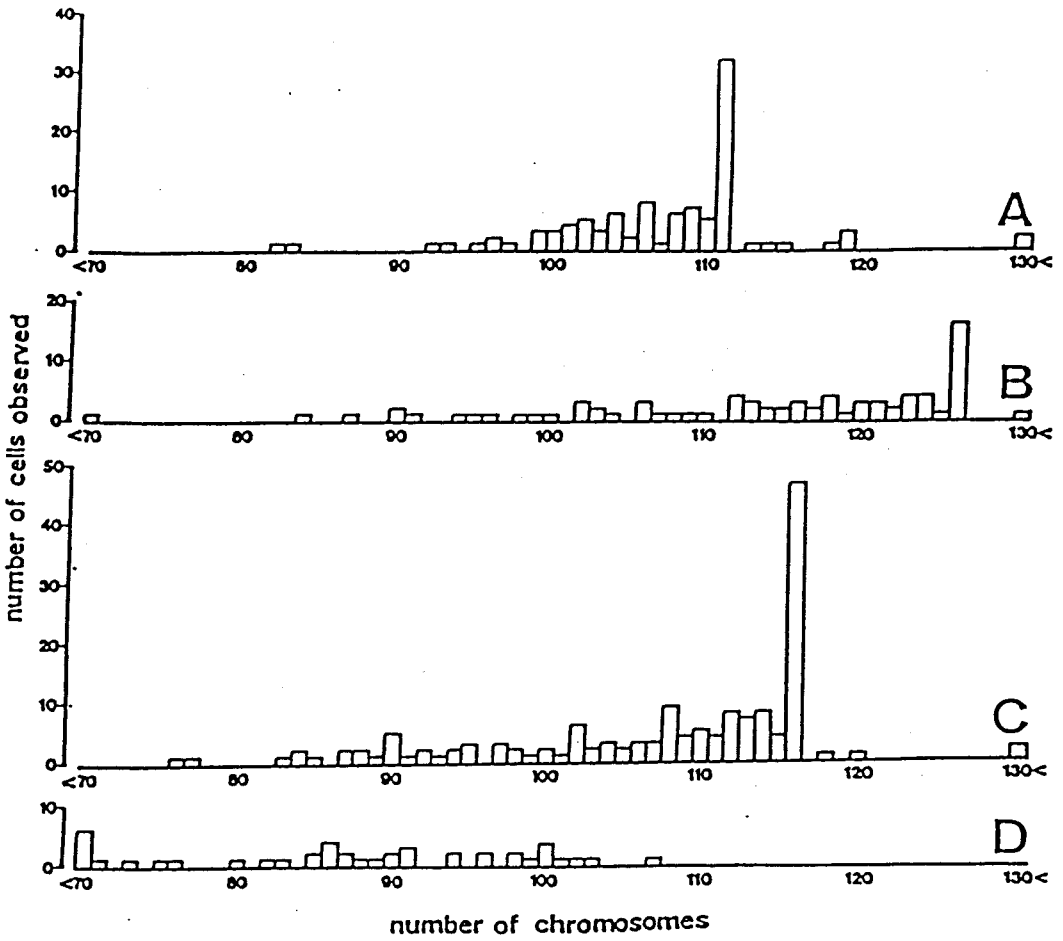
As mentioned in Part III, embryos from diploid hybridization between Japanese char♀ and chum salmon♂ did not show the modal peak individually and their chromosomes were distributed

Table 24. Distribution of chromosome numbers in the hybrid chum salmon♀ x Japanese chard♂ from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes at 15 minutes after insemination.

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																				total
			<89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120<					
8202	12	1.	1				1		1						1	2			1	1		2			1	1				8				1					21
		2.	1			1										1										1		2		5				1					12
		3.		1				1			1																2	1		4										10
		4.							1							2						1				1	1	3		3				1					12
		5.									1			1				1									1	1	1	3										9
		6.	3	1																				1			1			3									9
		7.																			1	2								3									6
		8.		1																1		1	1							2									6
		9.																						1						2									3
		10.																										1		1									2
	subtotal		5	3		1	1	1	2		2			1	1	5		1	1	2	1	6	1	2	1	3	5	7	1	34				3					90
8206	13	1.																		1		1			1					3									6
		2.			1			1													1									2									5
		3.		1																			1	1				1				1							5
		4.										1						1	1	1		1	1																	6
	subtotal			1	1			1				1						1	1	1	1	2	1	2	1	1			1		5		1						22
8206	16	1.	2			1					1														1	1	2	1		1	4								15
		2.										1				1	1					1				1	1			1	1									8
		3.	1						1				1					1					1			1			1	1									8
		4.		1																		1		1		1	1			1									6
		5.	3																																				3
	subtotal		6	1		1			1		1	1	1			1	1	1				2	1	2	2	5	2		3	7									40
	total		11	5	1	2	1	2	3		3	2	1	2	1	6	2	3	2	3	3	9	4	5	4	8	7	8	4	46		1		3					152

Table 25. Distribution of chromosome numbers in the hybrid Japanese char♀ x chum salmon♂ from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination

No. of crossing	age of embryos (days)	embryo No.	chromosome numbers																																	total
			<73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104<		
8204	12	1.																								2										2
		2.										1																								1
8208	16	1.																			1															1
		2.	1												1	1				1																4
		3.	1		1								1		1	1	1		1	1				1				1		1	1		1	1		14
		4.														1		1			1							1								4
		5.	3							1																										4
		6.	3			1										1					1			1					1	2						10
		7.															1															1				2
		total	8		1	1				1		1	1		2	4	2	1	1	2	3			2		2		2	1	3	1	1	1	1		49



Text-Fig. 12. Schematic representation of total distribution of chromosome numbers in embryos of chum salmon (A), Japanese char (B), chum salmon♀ x Japanese char♂ (C), and Japanese char♀ x chum salmon♂ (D) from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination. The mode of cells from treated embryos shows a triploid number in chum salmon (3N=111) and Japanese char (3N=126). The modal chromosome number of treated embryos from chum salmon♀ x Japanese char♂ is the exact sum (3N=116) of the diploid sets from maternal chum salmon (2N=74) and the haploid set from paternal Japanese char (N=42). Note the aberrant distribution of chromosome numbers in treated embryos from Japanese char♀ x chum salmon♂.

over the range of about 50 to 60, indicating aneuploidization during embryogenesis (Table 18, Pl. XXVII-XXIX, Figs. 237-248). Chromosome count distributions for embryos from experimental eggs subjected to hydrostatic pressure are given in Table 25 and Text-Fig. 12. As shown, the numbers of chromosomes ranged from 80 to 104. No cell with the expected triploid number of 121 chromosomes, equivalent to the exact sum of the diploid set from Japanese char and the haploid set from chum salmon, was observed. In addition, treated embryos did not show the modal peak individually. The embryos consisted of aneuploid cells with different hyperdiploid or hypotriploid chromosome numbers (Pl. XXXV, Figs. 285-287).

3. Isozyme phenotypes in the triploid hybrid.

Malate dehydrogenase (MDH) isozymes in the allotriploid hybrid

The expression of paternally transmitted genes from the Japanese char was examined in hatched larvae of the allotriploid hybrid, 2chum salmon♀ x Japanese char♂, utilizing electrophoretic differences in isozyme phenotypes as genetic markers. There were electrophoretic differences in mobilities of gene products between chum salmon and Japanese char at the Mdh-B and -C loci, as described in Part II. On the anodal side of the gel plate, the Japanese char had four isozymes, corresponding to MDH-C_2^b, MDH-B^bC^b, MDH-B_2^b and MDH-A_2 from anode to cathode (Pl. XXXVI, Fig. 288). The chum salmon, however, had three different isozymes, corresponding to MDH-C_2^a, MDH-B^aC^a and MDH-B_2^b on the anodal side of the gel plate

(Fig. 288). As seen in the figure, there were electrophoretic ifferences in mobilities between the homologous MDH-C_2^a and -C_2^b homodimers and between the homologous MDH-B_2^a and -B_2^b homodimers.

No distinction was observed in MDH isozyme patterns between diploid and triploid chum salmon (Fig. 288). The allotriploid hybrid 2chum salmon♀ x Japanese char♂ had a complicated isozyme pattern consisting of seven isozyme bands corresponding to MDH-C_2^b, MDH-C^aC^b, MDH-C_2^a (-B^bC^b), MDH-B^aC^b, MDH-B_2^b, MDH-B^aB^b and MDH-B_2^a (-A_2) (Fig. 288). Of these isozymes, MDH-C_2^b was the paternal char type and MDH-C^aC^b, -B^aC^b, -B^aC^a and -B^aB^b were hybrid heterodimers containing paternal gene products. The appearance of MDH isozymes containing paternal gene products suggested that the paternally derived Mdh-B_b and -C_b allelic activities were being expressed. In addition, the fact that the staining intensities of MDH-C_2^b, -C^aC^b and -C_2^a distributed according to a ratio of almost 4:4:1 proved the presence of two alleles of Mdh-C_a and one allele of Mdh-C_b at the Mdh-C locus, indicating the "gene-dosage effect" in the isozyme expression.

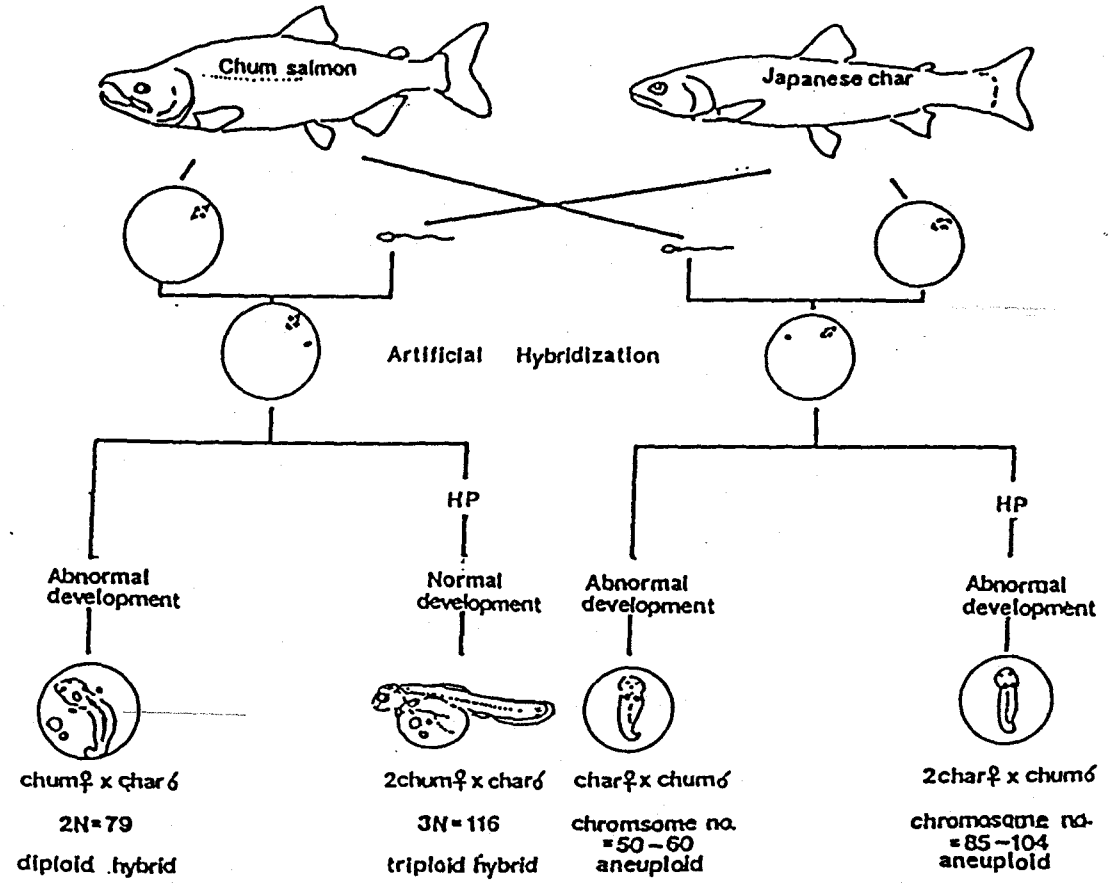
Phosphoglucomutase (PGM) isozymes in the allotriploid hybrid

As shown in Fig. 289, chum salmon and Japanese char had mutually distinguishable PGM-B^a and -B^b. There was no difference between the PGM isozyme patterns of the diploid and triploid chum salmon, but the allotriploid hybrid had two PGM isozymes, maternal PGM-B^a and paternal PGM-B^b (Fig. 289). In the allotriploid hybrid, maternal PGM-B^a had twice the staining capacity of paternal PGM-B^b, indicating two alleles of

maternal Pgm-B_a and one allele of paternal Pgm-B_b at the Pgm-B locus.

4. Discussion

It was proved from chromosomal observations that all embryos of chum salmon, Japanese char and chum salmon♀ x Japanese char♂ from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination had triploid karyotypes (Tables 22-24, Figs. 279-284). The event most worth noting is that the allotriploid hybrid, 2chum salmon♀ x Japanese char♂, demonstrated normal embryonic development in spite of the abnormal development observed in the diploid hybrid, chum salmon♀ x Japanese char♂ (Text-Fig. 13). However, in the case of Japanese char♀ x chum salmon♂, there was no difference in the survival potentials or developmental capacities between embryos from the control and those from the experimental groups (Text-Fig. 13). Those from both groups died during the embryonic stages by 40-50 days, and no hatched larvae were obtained. Although the triploid hybrid, 2chum salmon♀ x Japanese char♂, showed an allotriploid karyogram with 116 chromosomes, no embryo from treated eggs of Japanese char♀ x chum salmon♂ had a cell with 3N =121 chromosomes equal to the expected sum of the maternal diploid set (2N=84) and the paternal haploid set (N=37). Most cells from the treated embryos had chromosome numbers which ranged from 80 to 104 (Table 25, Text-Fig. 12). The fact that a number of cells had more chromosomes than the diploid number for the maternal species indicates that embryos from



Text-Fig. 13. Schematic representation of the developmental and the cytogenetic results from diploid and triploid hybridization between chum salmon and Japanese char. HP indicates the block of 2nd. meiosis for triploidization by means of hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after fertilization.

treated eggs may be aneuploidized during embryogenesis after triploidization by the blocking of the release of the second polar body. Similar mechanisms acted in the case of the diploid hybrid between Japanese char♀ and chum salmon♂. Hence, the aneuploidy in these treated embryos was probably hypotriploidy which may be characterized by the maternal diploid and some paternal chromosomes remaining in the hybrid nucleus after the process of chromosome elimination.

The morphogenetic evidence on the triploid hybrid 2chum salmon♀ x Japanese char♂ indicates that developmental capacity can be restored from the inviable state of the diploid hybrid by means of artificial allotriploidization with two sets of maternal chum salmon genome and one set of paternal char genome (Text-Fig. 13). The restoration of developmental capacity by artificial polyploidization has been reported for plaice♀ x halibut♂ and flounder♀ x halibut♂ (Purdom and Lincoln, 1974). However, the evidence used differed from the observations of the present study in that the restoration by polyploidization in flatfish hybrids was due to the diploidization of haploid gynogenetic plaice or flounder genome. The present result resembles that of Stanley (1981), who reported that most embryos from the crossing between carp and grass carp died before hatching; the few that survived were considered as androgenetic, gynogenetic diploid or triploid hybrids according to the measurement of erythrocytes, suggesting a recovery of developmental capacity by allotriploidization. This evidence suggests that the recovery of developmental capacity may be related to a reduction of the ratio of exotic genome in the hybrid nucleus to egg cytoplasm. Expression

of the paternal gene was confirmed by the isozyme experiment on the triploid hybrid which showed the isozyme phenotype containing paternal gene products in MDH and PGM (Figs. 288, 289). The triploid hybrid may differentiate according to the normal process of gene activation. On the contrary, the developmental capacity of the reciprocal hybrid, Japanese char♀ x chum salmon♂ was not restored by allotriploidization, and in treated hybrid embryos from this crossing there were conspicuous aneuploid number of chromosomes from hyperdiploid to hypotriploid. This evidence shows that the reduction in the ratio of paternally derived nucleus to maternal egg cytoplasm by allotriploidization is not effective in repressing the chromosome elimination mechanism in the hybrid genome, which suggests a strong nucleocytoplasmic incompatibility between the paternal genome from chum salmon and the maternal cytoplasm from Japanese char. The abnormality of the embryos Japanese char♀ x chum salmon♂ from eggs subjected to hydrostatic pressure must result from disturbances in genetic information due to aneuploidy of the hybrid genomes.

In conclusion, the developmental capacity of inviable hybrids can be restored, and the hybrids made viable, by artificial allotriploidization in cases such as chum salmon♀ x Japanese char♂ for which inviability is due not to aneuploidy but to aberrant gene expression. Therefore, survival potentials can be expected to be recovered in hybrids such as masu salmon♀ x chum salmon♂ and chum salmon♀ x masu salmon♂, which demonstrated abnormal embryogenesis in spite of their complete genomic structure. Similar experiments will be needed to

produce triploid viable hybrids of other combinations among salmonid species. Although the technique of "chromosome engineering" has been discussed from the viewpoint of breeding sciences in fish culture (Onozato, 1981, 1983), such a technique may also be effective for analyzing developmental genetic events in fishes, and hence as a tool for producing new experimental systems.

GENERAL DISCUSSION

Interspecific hybrids can be regarded as informative experimental systems for examining "differential gene activation" during embryogenesis, because it is easy to make the distinction between maternal and paternal gene products in hybrid species. Hybrids can also be used favorably as experimental systems for gaining insight into mechanisms which may be responsible for abnormal embryogenesis, because various abnormal hybrids morphogenetically resemble other abnormal embryos resulting from other biological factors such as overripening and irradiation of gamates (Yamazaki, 1981, 1983).

Interspecific hybrids among six species of salmonids can be categorized into two groups, "viable" and "inviable" based on their survival potentials and developmental capacities. Survival curves for the development of inviable hybrids show a persistence of the initial survival rate of near 100 % until around 10 days after fertilization, and a rapid drop in the rate between 10 and 20 days, corresponding to the morphological stages from the appearance of the embryonic body to the

first heartbeat (Text-Figs. 4, 6, 7). The stage when most embryo begin to die coincides with the stage when abnormal embryos from overripened or irradiated eggs begin to die (Yamazaki, 1981), and this stage is also characterized by "vulnerability" to physical or chemical stimulation (Yamamoto, 1943). This coincidence suggests that certain events important for embryonic development might occur during this stage. Furthermore, the constant survival rate without embryonic death prior to the stage at which the embryonic body appears suggests that the function of the embryonic genome has not been expressed during the cleavage stages.

Ontogenetic changes in several isozymes were analyzed in viable hybrids utilizing electrophoretic phenotypes as gene markers, in order to discover the first expression of embryonic genes, since the appearance of paternal gene products means that their synthesis is being controlled by the embryonic genome itself. Although the isozymes which appeared within most cell types of adult tissues may be related to the "house-keeping" function of embryonic cells, the appearance of "tissue-specific" isozymes may be related to the functional and morphological differentiation of the concerned tissues. It was proved from the isozyme ontogeny of LDH, MDH, 6PGDH and PGM in viable hybrid embryos that only the maternal phenotype existed during the earlier stages and that the hybrid phenotypes were first expressed during the period from the formation of the embryonic body to the beginning of the heartbeat (Text-Fig. 8). This evidence indicates that the early embryogenesis depends on cytoplasmic function, and that gene

function for the synthesis of "house-keeping" proteins may begin during the same period (text-Fig. 8). It is significant that the first expression of the genomic function as determined by isozyme experiments is in close accordance temporally with the stage when most inviable hybrids begin to die. This evidence strongly suggests that the stage between embryonic body formation and heartbeat is "critical" regarding differential gene activation in embryogenesis.

In one inviable hybrid, masu salmon♀ x chum salmon♂, the expression of the hybrid LDH phenotype began about 20 days later than normal (Pl. X, Fig. 195) and no paternal gene products could be observed for MDH ontogeny throughout all stages in which only the maternal phenotype appeared (Pl. XIII, Fig. 205). This observation may indicate that the partial and complete repressions of gene activation may both be related to abnormal embryogenesis in fishes.

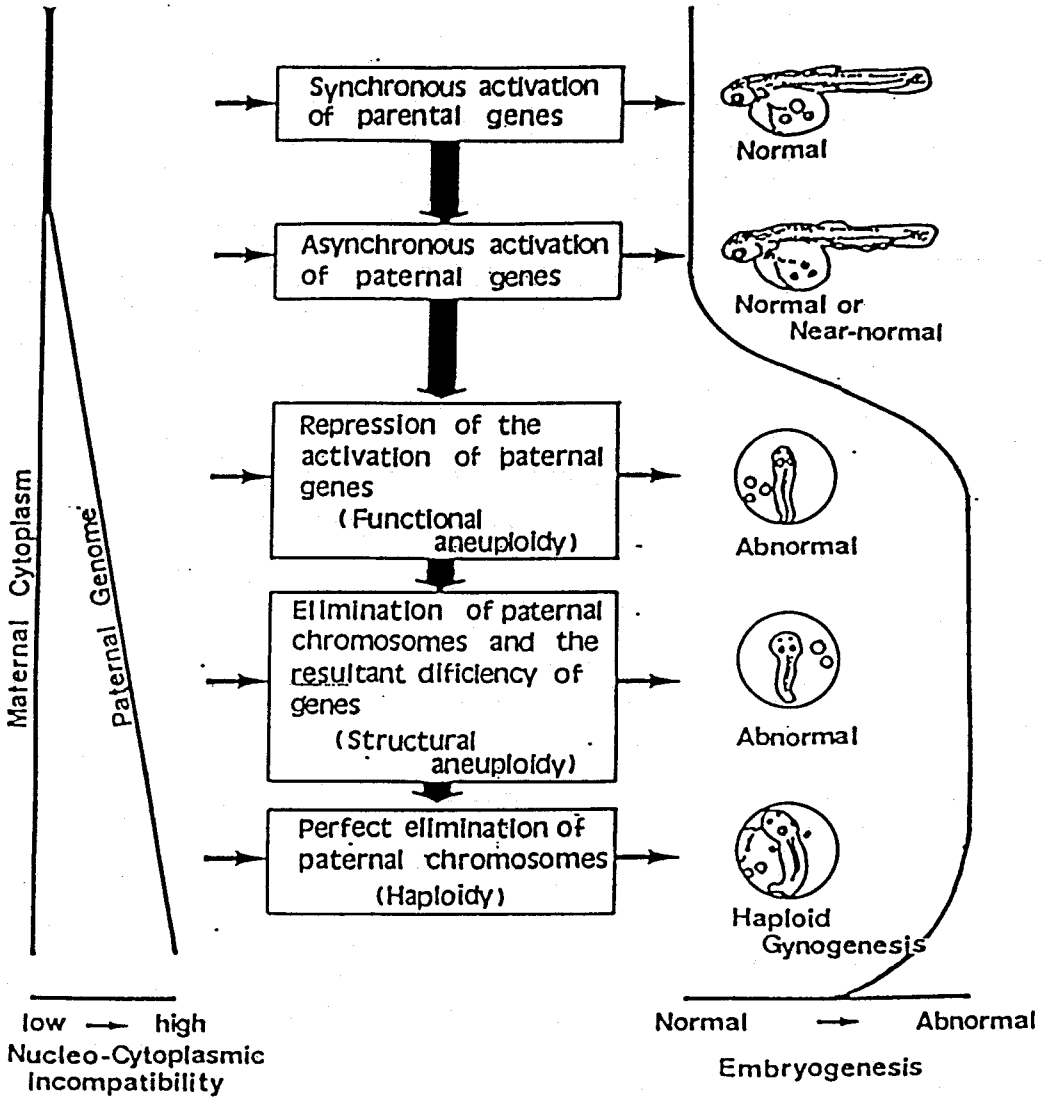
In the present study, embryos of viable and inviable hybrids were cytogenetically examined in order to check the chromosomal constitution of the hybrid genomes. It was concluded that the karyotypes of the viable hybrids are equal to the exact sum of the haploid sets from the parental species (Table 20, Text-Fig. 9). As for the inviable hybrids, some showed intermediate karyotypes between those of the two parental species, but others consisted of aneuploid cells with different hypodiploid chromosome numbers (Table 20, Text-Fig. 10, 11). Since the inviable hybrid masu salmon♀ x chum salmon♂, which showed aberrant gene expression such as excessive asynchronous activation of the paternal Ldh-B_1 gene and complete

repression of the paternal Mdh-C gene, had hybrid genomes comprised of the haploid sets from the two parental species, it is confirmed that morphological abnormality in such inviable hybrids is not due to structural deletion inducing gene deficiency but to a total lack of nuclear function due to the disturbance in the differential gene activation. Consequently, based on the isozymic and chromosomal studies on developing hybrids, abnormal development in hybrid embryos can be explained by the following two causes: (1). "Functional aneuploidy" owing to complete or partial repression of paternally transmitted gene activities in the diploid hybrid genome. (2). "Structural aneuploidy" inducing extensive deficiency of genes by massive chromosome elimination during the mitotic process of hybrid cells.

The aberrant gene expression and chromosome elimination induced in some interspecific hybrids suggest the presence of a common mechanism responsible for the two phenomena. In this regard, the different biological responses found in reciprocal hybrids are quite interesting. The hybrid chum salmon♀ x masu salmon♂ usually demonstrated a greater developmental capacity than the masu salmon♀ x chum salmon♂ hybrid. And although the inviable hybrid chum salmon♀ x Japanese char♂ exhibited the exact hybrid chromosome number, the reciprocal hybrid Japanese char♀ x chum salmon♂, consisted of mosaic aneuploid cells (Table 20, Text-Fig. 11, Figs. 236-248). These reciprocal difference cannot be interpreted from the interaction between maternal and paternal genomes. Therefore, the significance of the interaction between the maternal egg cyto-

plasm and the paternal genome from the exotic species must be considered in the future in analyzing the fundamental mechanisms causing abnormal embryogenesis. The restoration of developmental capacity of the diploid inviable hybrid chum salmon♀ x Japanese char♂ by means of artificial allotriploidization (Text-Fig. 13) shows the possible effect of reducing the ratio of exotic genome to a constant quantity of egg cytoplasm. This suggests the involvement of nucleocytoplasmic interaction in relation to the viability of the hybrids.

Various developmental genetic and cytogenetic abnormalities can be probably induced in hybrid salmonids according to the degree of nucleo-cytoplasmic incompatibility between the cytoplasm of the maternal species and the genome from the paternal species as schematically shown in Text-Fig. 14. Assuming the presence of a gene-regulatory system in egg cytoplasm, maternal cytoplasm may well be able to recognize and activate paternal genes in embryos from parental crossings within the same species and/or in hybrids between closely related species. Therefore, such a regulatory system is expected within the genome and the resultant gene products can maintain normal embryogenesis (Text-Fig. 14). However, when it becomes more difficult for the maternal cytoplasm to recognize and activate exotic alleles from foreign species, "asynchronous activation of the paternal gene" can be expected at many loci in the hybrid genome. Such a situation will not exert a major influence on synthesis of gene products, and hybrid embryos can be expected to be normal or near-normal (Text-Fig. 14). If nucleocytoplasmic relationships become more incompatible, so



Text-Fig. 14 Schematic representation of a possible mechanism responsible for the abnormal embryogenesis of teleosts.

as to induce a severe delay in or repression of the paternal genomic function, normal synthesis of gene products cannot proceed and such a lack of nuclear function may induce abnormal development. An even larger degree of incompatibility may have an effect on the mitotic process, because mitotic apparatus such as spindles and asters are apparently of cytoplasmic origin. Chromosome elimination and the resultant aneuploidy in hybrids must be the result of a mitotic abnormality due to incompatibility between mitotic apparatus and exotic chromosomes. "Structural aneuploidy" apparently gives rise to a large-scale deletion of genes, and hybrid embryos display abnormal development owing to a total lack of gene products.

Several authors have reported the occurrence of gynogenetic embryos from interspecific hybridization (Purdom and Lincoln, 1971 ; Uyeno, 1972 ; Stanley, 1981 ; Onozato, 1981), but no experimental evidence has been obtained regarding this phenomenon. It might be proposed from the above hypothesis that hybrid-gynogenesis is the result of a perfect elimination of paternal chromosomes during early embryogenesis after fertilization. The extremely high incompatibility between mitotic apparatus in the cytoplasm of eggs and exotic chromosomes from foreign species may give rise to excessive chromosome elimination, and the resultant embryos cannot help developing gynogenetically (Text-Fig. 14).

In the present study, the first expression of genomic function and the possible mechanisms responsible for abnormal embryogenesis were proposed from the experimental work on viable

and inviable hybrid embryos among salmonid species utilizing several isozymes and chromosomes as visible genetic markers. However, a number of important problems remain concerning the regulatory and triggering systems necessary to realize gene activation from transcription to protein synthesis and the complicated involvement of egg cytoplasm in relation to these systems. Knowledge of these matters is quite limited and fragmentary in regard to teleosts (Kafiani, 1970 ; Neyfakh, 1971). Further investigations will be called for to determine such steps of realization of genetic information in embryogenesis as well as cytoplasmic factors contributing to early development. There are also several things unknown about the process of and the differential steps involved in chromosome elimination, especially as regards the time when this phenomenon begins, the detailed mitotic function resulting in elimination and the origin of the chromosomes to be eliminated. These cytogenetic and cytological problems remain to be answered in future.

SUMMARY

1. Artificial hybridizations were carried out using six species of salmonids; pink salmon, masu salmon, chum salmon, chinook salmon, Japanese char and brook trout.
2. The resultant hybrids, masu salmon♀ x pink salmon♂, pink salmon♀ x masu salmon♂, masu salmon♀ x chinook salmon♂, masu salmon♀ x Japanese char♂, Japanese char♀ x masu salmon♂ and Japanese char♀ x brook trout♂ demonstrated satisfactory survival potentials as good as the parental controls and were classified as "viable" hybrids.

3. The resultant hybrids, masu salmon♀ x chum salmon♂, chum salmon♀ x masu salmon♂, chum salmon♀ x Japanese char♂, Japanese char♀ x chum salmon♂ and brook trout♀ x chum salmon♂ died during the embryonic stages before hatching and were classified as "inviable" hybrids.

4. For three hybrid experiments with chum salmon♀ x masu salmon♂, two cases resulted in inviability, while the other case resulted in normal embryogenesis.

5. Inviable hybrids exhibited a constant survival rates of almost 100 % before the stage of embryonic body formation, but after this most hybrid embryos died.

6. Differences in developmental capacities were always observed in the reciprocal hybrids masu salmon♀ x chum salmon♂ and chum salmon♀ x masu salmon♂.

7. The first activation of genes controlling isozyme phenotypes was studied utilizing inter- or intraspecific electrophoretic distinction of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGDH) and phosphoglucomutase (PGM) isozymes as biochemical gene markers.

8. The exclusive existence of maternal isozyme phenotypes was observed during the earlier stages in all hybrid embryos, suggesting that only maternal cytoplasmic substances contribute to the early development before the expression of the genomic function.

9. It was determined from the appearance of paternal gene products in viable hybrid embryos that embryonic genes controlling "house-keeping" isozymes such as 6PGDH-A, LDH-

B^1, $-B^2$, MDH-B, -C, PGM-A, -B and -C were expressed during the relatively restricted period between embryonic body formation and the first heartbeat. This period may be "critical" because the cytoplasmic contribution to embryogenesis was replaced by the nuclear function during this period.

10. Muscle-specific Ldh loci were activated in accordance with the functional differentiation of muscle tissues of embryos.

11. In the inviable hybrid masu salmon♀ x chum salmon♂, a noticeable delay in paternal Ldh-B_1 gene activation and complete repression of the paternal Mdh-C gene was recognized. These disturbances in differential gene activation may be related to the abnormal hybrid embryogenesis.

12. Chromosome analysis were conducted to examine cytogenetically both viable and inviable hybrid embryos.

13. The viable hybrid masu salmon♀ x pink salmon♂ showed a diploid karyotype of 2N=59, which was equal to the exact sum of the haploid sets from masu and pink salmon, numerically as well as morphologically.

14. The viable hybrid Japanese char♀ x brook trout♂ had a diploid karyotype of 2N=84, which was equal to the exact sum of the haploid sets from the parental species.

15. The viable hybrids masu salmon♀ x Japanese char♂ and Japanese char♀ x masu salmon♂ had diploid karyotypes of 2N=75, which was equal to the exact sum of the haploid sets from the parental masu salmon and Japanese char species, numerically as well as morphologically.

16. The inviable hybrids masu salmon♀ x chum salmon♂ and chum salmon♀ x masu salmon♂ had karyotypes of 2N=70, which was equal to the exact sum of the haploid sets from the parental masu salmon and chum salmon, numerically as well as morphologically.

17. Although the inviable hybrid chum salmon♀ x Japanese char♂ had a diploid karyotype of 2N=79, which was equal to the exact sum of the haploid sets from the parental species, the reciprocal hybrid, Japanese char♀ x chum salmon♂, was comprised of aneuploid cells with abnormal hypodiploid chromosome numbers.

18. The inviable hybrid brook trout♀ x chum salmon♂ also displayed chromosome aberration, showing aneuploid numbers.

19. Although all viable hybrids and some inviable hybrids showed karyotypes just intermediate between those of the parental species, some inviable hybrids consisted of aneuploid cells, indicating that chromosome aberration resulted in abnormal embryogenesis.

20. Triploid hybrids between chum salmon and Japanese char were produced by applying hydrostatic pressure just after insemination with milt. The resultant embryos were examined morphologically, cytogenetically and electrophoretically.

21. The triploid hybrid 2chum salmon♀ x Japanese char♂ displayed normal embryogenesis and a part of these embryos reached the hatching stage, in spite of the inviability of the diploid hybrid chum salmon♀ x Japanese char♂.

22. The triploid hybrid 2chum salmon♀ x Japanese char♂ had a karyotype of 3N=116, which was equal to the exact sum of

the diploid set from chum salmon and the haploid set from Japanese char, numerically as well as morphologically.

23. Embryos from Japanese char eggs subjected to hydrostatic pressure after insemination with milt from chum salmon demonstrated abnormal embryogenesis and were comprised of aneuploid cells with hyperdiploid and/or hypotriploid chromosome numbers.

24. The triploid hybrid 2chum salmon♀ x Japanese char♂ showed the expression of hybrid phenotypes in which paternal gene products were contained in the MDH and PGM isozymes, indicating the activation of paternally transmitted genes in the allotriploid genome.

25. The recovery of developmental potential through allotriploidization was assumed to be related to a reduction in the ratio of exotic genome within the hybrid genome to egg cytoplasm.

26. The significance of the first expression of genomic function was discussed from the standpoint of "critical" stages in fish development.

27. Disturbances in gene activation and chromosome aberration as the two main causes of abnormal development were discussed from the aspect of nucleo-cytoplasmic incompatibility between the maternal cytoplasm and the exotic paternal genome in hybrid embryos.

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EXPLANATION OF PLATES

PLATE I.

Surface views of the normal morphogenesis of the pink salmon, Oncorhynchus gorbuscha.

Fig. 1. Blastodisc at 2 days, morula stage.
Fig. 2. Blastodisc at 4 days, blastula stage.
Fig. 3. Blastodisc at 6 days, embryonic shield.
Fig. 4. Blastodisc at 8 days, appearance of embryonic body.
Fig. 5. Embryo at 10 days.
Fig. 6. Embryo at 12 days, yolk plug.
Fig. 7. Embryo at 14 days, tail bud stage.
Fig. 8. Embryo at 16 days.
Fig. 9. Embryo at 18 days, heart beating.
Fig. 10. Embryo at 20 days.
Fig. 11. Embryo at 22 days.
Fig. 12. Embryo at 24 days.
Fig. 13. Embryo at 26 days, muscle contraction.
Fig. 14. Embryo at 28 days, eye pigmentation.
Fig. 15. Embryo at 30 days.
Fig. 16. Embryo at 32 days.
Fig. 18. Embryo at 34 days.
Fig. 19. Hatched larva.
Scale in Figure indicates 5 mm.

Surface views of the normal morphogenesis of the masu salmon, Oncorhynchus masou.

Fig. 20. Blastodisc at 4 days, blastula stage.
Fig. 21. Blastodisc at 6 days, appearance of embryonic body.
Fig. 22. Embryo at 8 days.
Fig. 23. Embryo at 10 days, yolk plug.
Fig. 24. Embryo at 12 days, tail bud stage.
Fig. 25. Embryo at 14 days.
Fig. 26. Embryo at 18 days, heart beating.
Fig. 27. Embryo at 20 days, muscle contraction.
Fig. 28. Embryo at 22 days, eye pigmentation.
Fig. 29. Embryo at 24 days.
Fig. 30. Embryo at 30 days.
Fig. 31. Hatched larva.
Scale in Figure indicates 5 mm.

PLATE I.

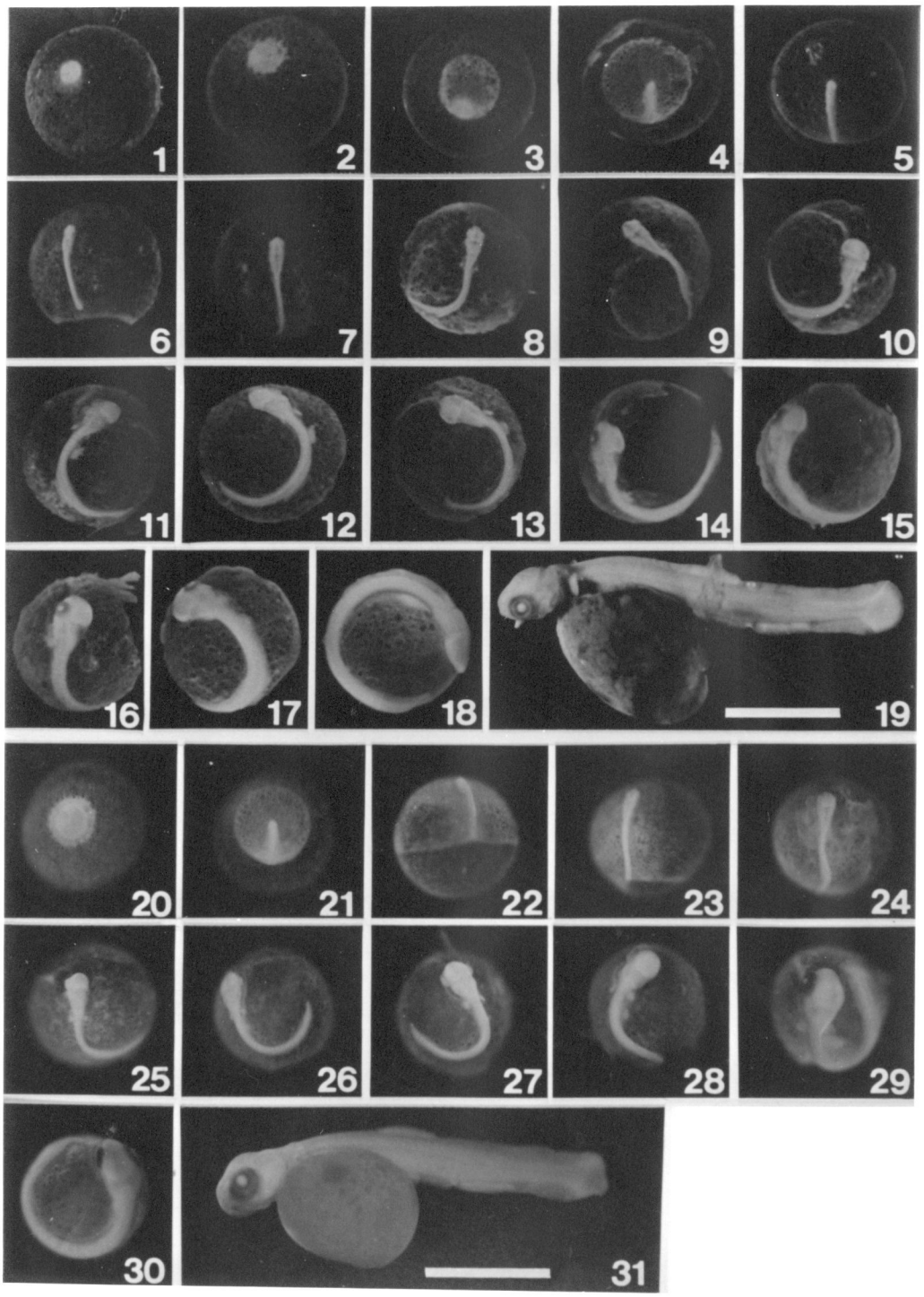

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PLATE II.

Surface views of the normal morphogenesis of the chum salmon, Oncorhynchus keta.

Fig. 32. Blastodisc at 4 days, blastula stage.
Fig. 33. Blastodisc at 6 days, embryonic shield.
Fig. 34. Blastodisc at 8 days, appearance of embryonic body.
Fig. 35. Embryo at 10 days.
Fig. 36. Embryo at 12 days, tail nod and yolk plug.
Fig. 37. Embryo at 14 days, tail bud stage.
Fig. 38. Embryo at 16 days.
Fig. 39. Embryo at 18 days, heart beating.
Fig. 40. Embryo at 20 days, muscle contraction.
Fig. 41. Embryo at 30 days.
Fig. 42. Embryo at 35 days.
Fig. 43. Embryo at 40 days.
Fig. 44. Hatched larva.
Scale in Figure indicates 5 mm.

Surface views of the normal morphogenesis of the Japanese char, Salvelinus leucomaenis.

Fig. 45. Blastodisc at 2 days, morula stage.
Fig. 46. Blastodisc at 4 days, blastula stage.
Fig. 47. Blastodisc at 6 days, germ ring.
Fig. 48. Blastodisc at 8 days, appearance of embryonic body.
Fig. 49. Embryo at 10 days, yolk plug.
Fig. 50. Embryo at 12 days, enclosure of blastopore.
Fig. 51. Embryo at 14 days, tail bud stage.
Fig. 52. Embryo at 16 days.
Fig. 53. Embryo at 18 days.
Fig. 54. Embryo at 20 days, heart beating.
Fig. 55. Embryo at 22 days, muscle contraction.
Fig. 56. Embryo at 26 days, eye pigmentation.
Fig. 57. Embryo at 30 days.
Fig. 58. Embryo at 36 days.
Fig. 59. Hatched larva.
Scale in Figure indicates 5 mm.

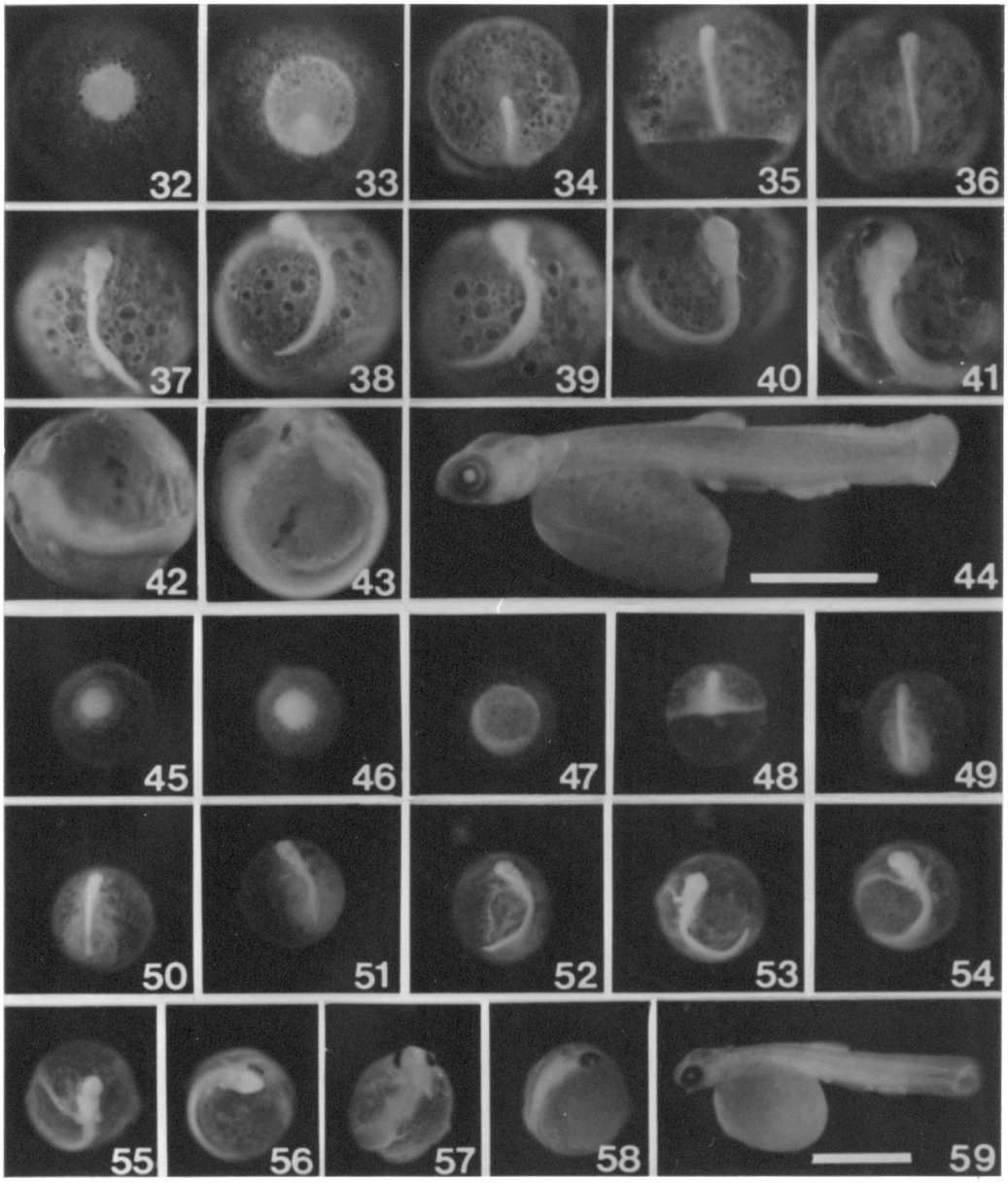

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PLATE III.

Surface views of the morphogenesis of the viable hybrid pink salmon♀ x masu salmon♂ from the 1977 experiment.

Fig. 60. Blastodisc at 2 days, morula stage.
Fig. 61. Blastodisc at 4 days, blastula stage.
Fig. 62. Blastodisc at 6 days, germ ring.
Fig. 63. Blastodisc at 8 days, appearance of embryonic body.
Fig. 64. Embryo at 10 days.
Fig. 65. Embryo at 12 days, yolk plug.
Fig. 66. Embryo at 14 days, tail bud stage.
Fig. 67. Embryo at 16 days.
Fig. 68. Embryo at 18 days.
Fig. 69. Embryo at 20 days, heart beating.
Fig. 70. Embryo at 22 days.
Fig. 71. Embryo at 24 days, muscle contraction.
Fig. 72. Embryo at 26 days, eye pigmentation.
Fig. 73. Embryo at 28 days.
Fig. 74. Embryo at 30 days.
Fig. 75. Embryo at 35 days.
Fig. 76. Embryo at 40 days.
Fig. 77. Embryo at 45 days.
Fig. 78. Hatched larva.

Scale in Figure indicates 5 mm.

Comparison of the morphologies of embryos of chum, masu salmon and their hybrids from the 1977 experiment.

Fig. 79. Embryos of the chum salmon at 18 days.
Fig. 80. Embryos of the masu salmon at 18 days.
Fig. 81. Embryos of the inviable hybrid chum salmon♀ x masu salmon♂ at 18 days.
Fig. 82. Embryos of the inviable hybrid masu salmon♀ x chum salmon♂ at 18 days. Note the repression of development at the stage of embryonic body formation.

Scales in Figures indicates 5 mm.

PLATE III.

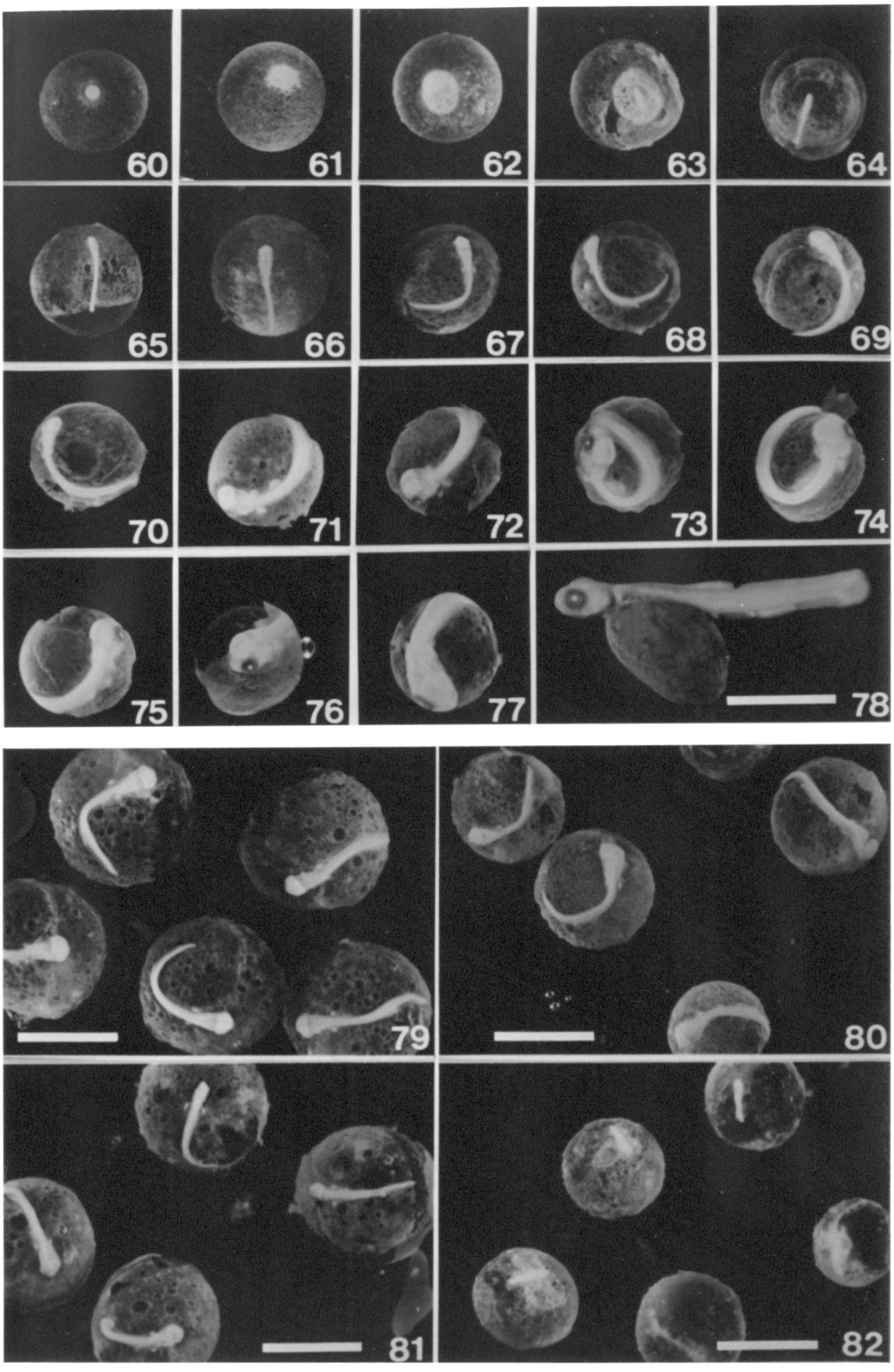


PLATE IV.

Surface views of the morphogenesis of the viable hybrid chum salmon♀ x masu salmon♂ from the 1979 experiment.

Fig. 83. Blastodisc at 4 days, blastula stage.
Fig. 84. Blastodisc at 6 days, embryonic shield.
Fig. 85. Blastodisc at 8 days, appearance of embryonic body.
Fig. 86. Embryo at 10 days, 1/2 epiboly.
Fig. 87. Embryo at 12 days, yolk plug.
Fig. 88. Embryo at 14 days, tail bud.
Fig. 89. Embryo at 16 days.
Fig. 90. Embryo at 18 days, heart beating.
Fig. 91. Embryo at 20 days.
Fig. 92. Embryo at 24 days, muscle contraction and eye pigmentation.
Fig. 93. Embryo at 30 days.
Fig. 94. Hatched larva at 36 days.
Fig. 95. Hatched larva at 40 days.
Scale in Figure indicates 5 mm.

Surface views of the morphogenesis of the lethal hybrid masu salmon♀ x chum salmon♂ from the 1979 experiment. Note the abnormal morphology of embryos with microcephalia and micropthalmia.

Fig. 96. Blastodisc at 4 days, blastula stage.
Fig. 97. Blastodisc at 6 days, embryonic shield.
Fig. 98. Blastodisc at 8 days, appearance of embryonic body.
Fig. 99. Embryo at 10 days.
Fig. 100. Embryo at 12 days.
Fig. 101. Embryo at 14 days.
Fig. 102. Embryo at 16 days.
Fig. 103. Embryo at 18 days.
Fig. 104. Embryo at 20 days.
Fig. 105. Embryo at 24 days.
Fig. 106. Embryo at 30 days.

PLATE IV.
(continued)

Fig. 107. Embryo at 36 days.
Fig. 108. Embryo at 40 days.
Fig. 109. Abnormal larva hatched at 50 days.
Scale in Figure indicates 5 mm.

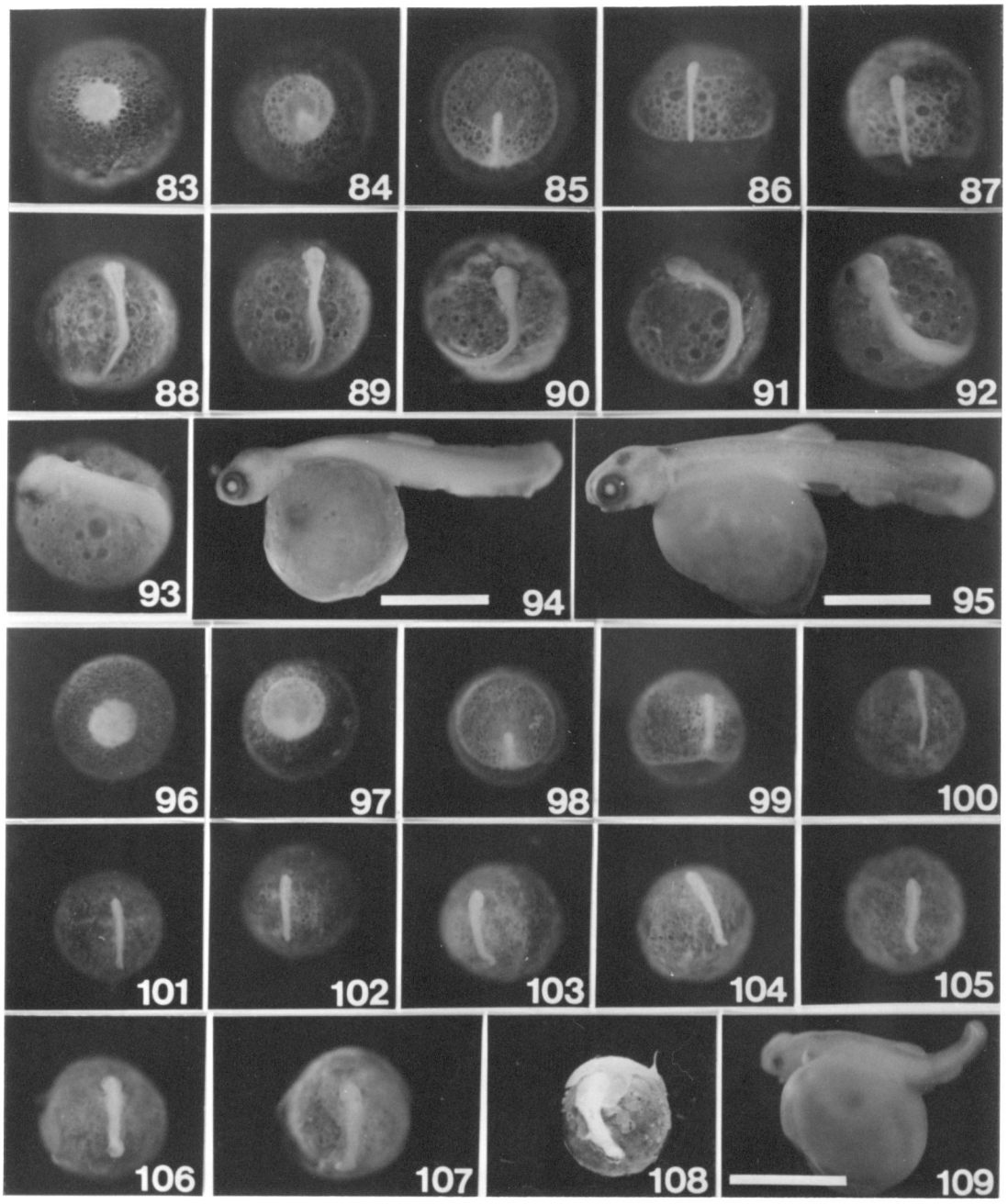

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PLATE V.

Surface views of the morphogenesis of the viable hybrid Japanese char♀ x brook trout♂ from the 1981 experiment.

Fig. 110. Blastodisc at 2 days, morula stage.
Fig. 111. Blastodisc at 4 days, blastula stage.
Fig. 112. Blastodisc at 6 days.
Fig. 113. Blastodisc at 8 days.
Fig. 114. Blastodisc at 10 days , embryonic shield.
Fig. 115. Embryo at 12 days, appearance of embryonic body.
Fig. 116. Embryo at 14 days, yolk plug.
Fig. 117. Embryo at 16 days, tail nod stage.
Fig. 118. Embryo at 18 days, tail bud stage.
Fig. 119. Embryo at 20 days.
Fig. 120. Embryo at 22 days.
Fig. 121. Embryo at 24 days, heart beating.
Fig. 122. Embryo at 28 days, eye pigmentation.
Fig. 123. Embryo at 30 days.
Fig. 124. Hatched larva.
Scale in Figure indicates 5 mm.

Surface views of the morphogenesis of the viable hybrid, masu salmon♀ x Japanese char♂ from the 1979 experiment.

Fig. 125. Blastodisc at 2 days, morula stage.
Fig. 126. Blastodisc at 4 days, blastula stage.
Fig. 127. Blastodisc at 6 days, germ ring.
Fig. 128. Blastodisc at 8 days, appearance of embryonic body.
Fig. 129. Embryo at 10 days.
Fig. 130. Embryo at 12 days, yolk plug.
Fig. 131. Embryo at 14 days, tail bud stage.
Fig. 132. Embryo at 16 days.
Fig. 133. Embryo at 20 days, heart beating.
Fig. 134. Embryo at 24 days, muscle contraction.
Fig. 135. Embryo at 26 days, eye pigmentation.
Fig. 136. Embryo at 28 days.
Fig. 137. Embryo at 30 days.
Fig. 138. Hatched larva.
Scale in Figure indicates 5 mm.

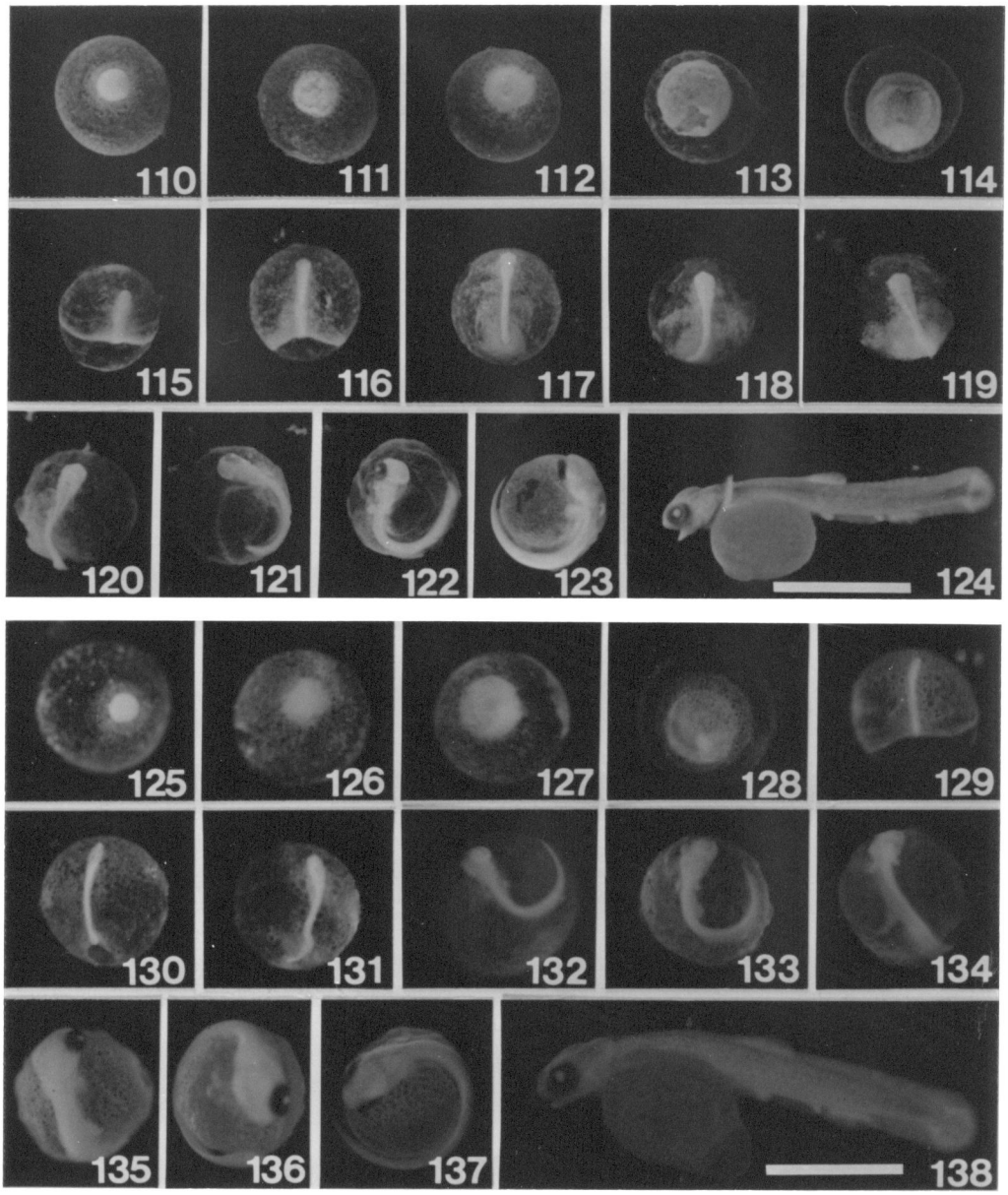

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PLATE VI.

Comparison of the morphologies of embryos of Japanese char, chum salmon and their hybrids from the 1981 experiments.

Fig. 139. Blastodiscs of the Japanese char at 10 days.
Fig. 140. Blastodiscs of the chum salmon at 10 days.
Fig. 141. Blastodiscs of the inviable hybrid Japanese char♀ x chum salmon♂ at 10 days.
Fig. 142. Blastodiscs of the inviable hybrid chum salmon♀ x Japanese char♂ at 10 days.

Fig. 143. Blastodiscs of the Japanese char at 12 days.
Fig. 144. Blastodiscs of the chum salmon at 12 days.
Fig. 145. Blastodiscs of the inviable hybrid Japanese char♀ x chum salmon♂ at 12 days.
Fig. 146. Blastodiscs of the inviable hybrid chum salmon♀ x Japanese char♂ at 12 days.

Fig. 147. Embryos of the Japanese char at 14 days.
Fig. 148. Embryos of the chum salmon at 14 days.
Fig. 149. Embryos of the inviable hybrid Japanese char♀ x chum salmon♂ at 14 days.
Fig. 150. Embryos of the inviable hybrid chum salmon♀ x Japanese char♂ at 14 days.

Fig. 151. Embryos of the Japanese char at 18 days.
Fig. 152. Embryos of the chum salmon at 18 days.
Fig. 153. Embryos of the inviable hybrid Japanese char♀ x chum salmon♂ at 18 days.
Fig. 154. Embryos of the inviable hybrid chum salmon♀ x Japanese char♂ at 18 days.

Fig. 155. Embryos of the Japanese char at 26 days.
Fig. 156. Embryos of the chum salmon at 24 days.
Fig. 157. Embryos of the inviable hybrid Japanese char♀ x chum salmon♂ at 24 days.

PLATE VI.
(continued)

Fig. 158. Embryos of the Japanese char at 30 days.
Fig. 159. Embryos of the chum salmon at 30 days.
Fig. 160. Embryos of the inviable hybrid Japanese char♀ x chum salmon♂ at 30 days.

Scales in Figures indicate 5 mm.

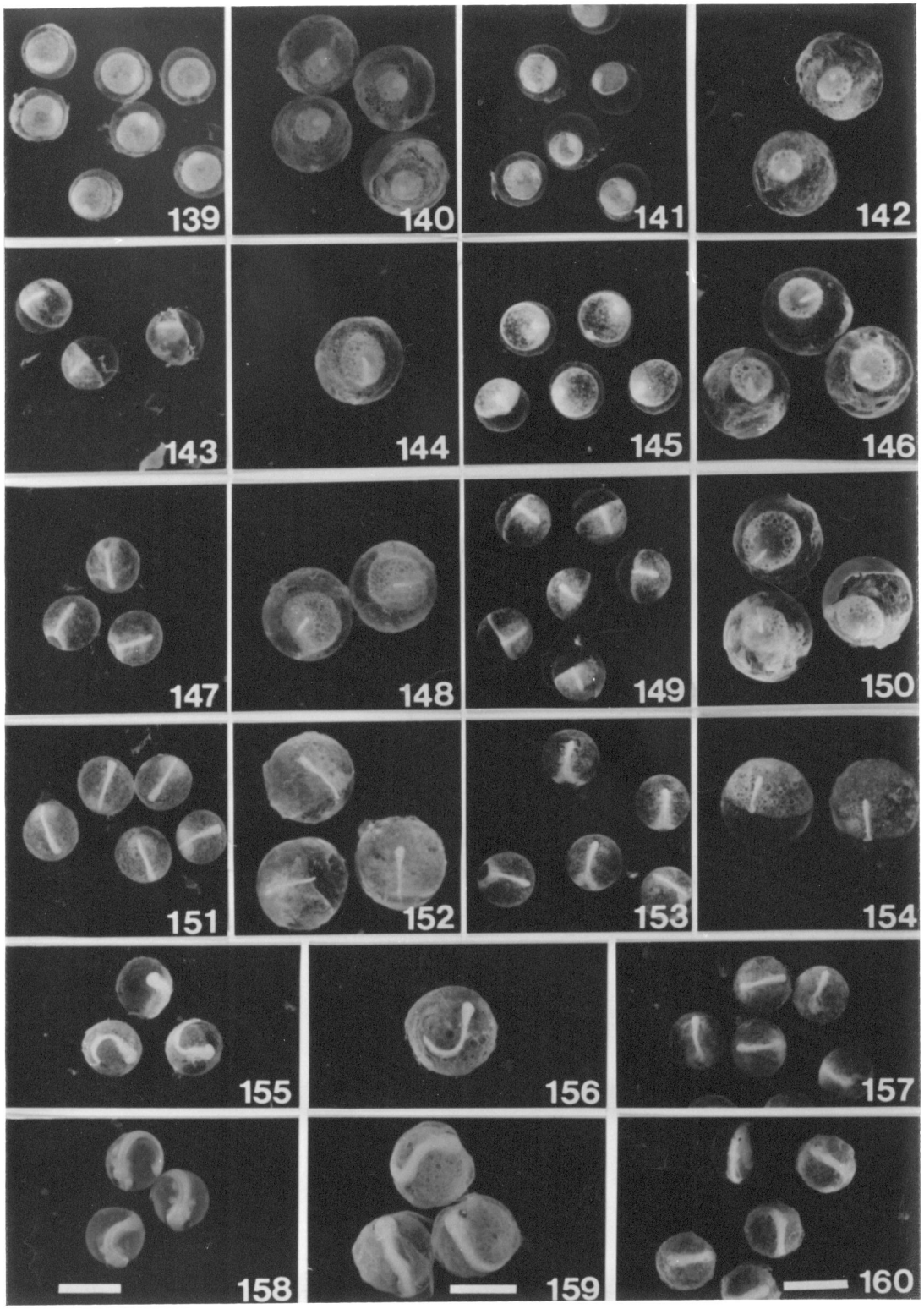

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PLATE VII.

Comparison of the morphologies of embryos of brook trout, chum salmon and their hybrids from the 1981 experiments.

Fig. 161. Blastodiscs of the brook trout at 10 days.
Fig. 162. Blastodiscs of the chum salmon at 10 days.
Fig. 163. Blastodiscs of the inviable hybrid brook trout♀ x chum salmon♂ at 10 days.
Fig. 164. Blastodiscs of the inviable hybrid chum salmon♀ x brook trout♂ at 10 days.

Fig. 165. Blastodiscs of the brook trout at 12 days.
Fig. 166. Blastodiscs of the chum salmon at 12 days.
Fig. 167. Blastodiscs of the inviable hybrid brook troue♀ x chum salmon♂ at 12 days.
Fig. 168. Blastodiscs of the inviable hybrid chum salmon♂ x brook trout♂ at 12 days.

Fig. 169. Embryos of the brook trout at 14 days.
Fig. 170. Embryos of the chum salmon at 14 days.
Fig. 171. Embryos of the inviable hybrid brook trout♀ x chum salmon♂.
Fig. 172. Embryos of the inviable hybrid chum salmon♀ x brook trout♂.

Fig. 173. Embryos of the brook trout at 18 days.
Fig. 174. Embryos of the chum salmon at 18 days.
Fig. 175. Embryos of the inviable hybrid brook trout♀ x chum salmon♂ at 18 days.
Fig. 176. Embryos of the inviable hybrid chum salmon♀ x brook trout♂ at 18 days.

Fig. 177. Embryos of the brook trout at 26 days.
Fig. 178. Embryos of the chum salmon at 24 days.
Fig. 179. Embryos of the inviable hybrid brook trout♀ x chum salmon♂ at 24 days.

PLATE VII.
(continued)

Fig. 180. Embryos of the brook trout at 30 days.

Fig. 181. Embryos of the chum salmon at 30 days.

Fig. 182. Embryos of the inviable hybrid brook trout♀ x chum salmon♂ at 30 days.

Fig. 183. Embryos of the inviable hybrid chum salmon♀ x brook trout♂ at 40 days.

Scales in Figures indicate 5 mm.

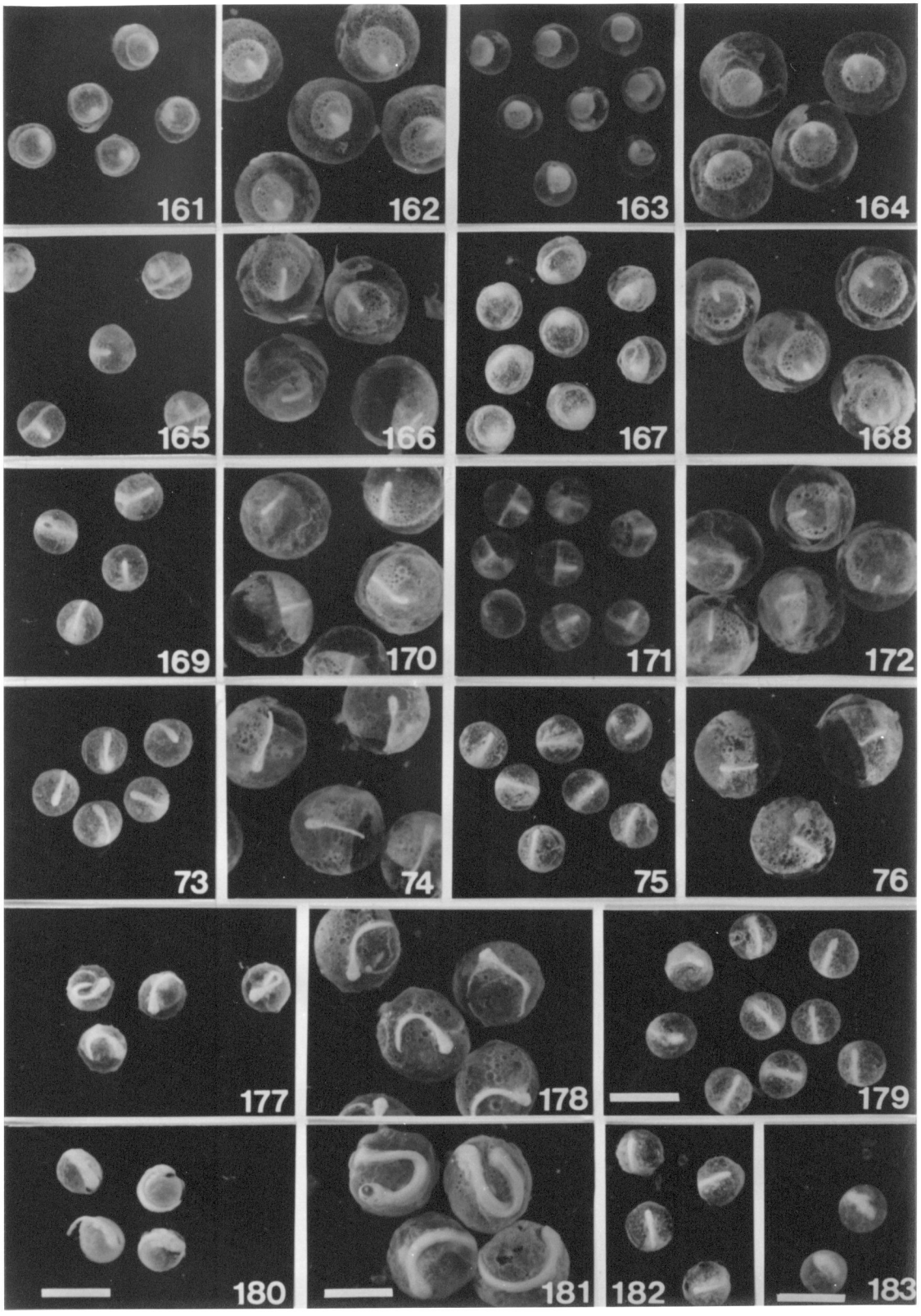

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PLATE VIII.

Fig. 184. Tissue distribution of lactate dehydrogenase (LDH) isozymes among eyes, heart, liver and muscles from adult brook trout, Japanese char, chum salmon and masu salmon.

Fig. 185. Comparison of LDH isozyme patterns in the muscle tissue of brook trout (a, c, d, and f) and Japanese char (b and e).

Fig. 186. Comparison of LDH isozyme patterns in the liver tissue of Japanese char (a and b), brook trout (c and d) and their hybrid, Japanese char♀ x brook trout♂ (e and f).

Fig. 187. Comparison of LDH isozyme patterns in the liver (a-h) and heart (i-p) of eight species of salmonids. (a). coho salmon, (b). chinook salmon, (c). chum salmon, (d). masu salmon, (e). kokanee salmon, (f). pink salmon, (g). Japanese char, (h). dolly varden char, (i). coho salmon, (j). chinook salmon, (k). chum salmon, (l). masu salmon, (m). kokanee salmon, (n). pink salmon, (o). Japanese char, (p). dolly varden char.

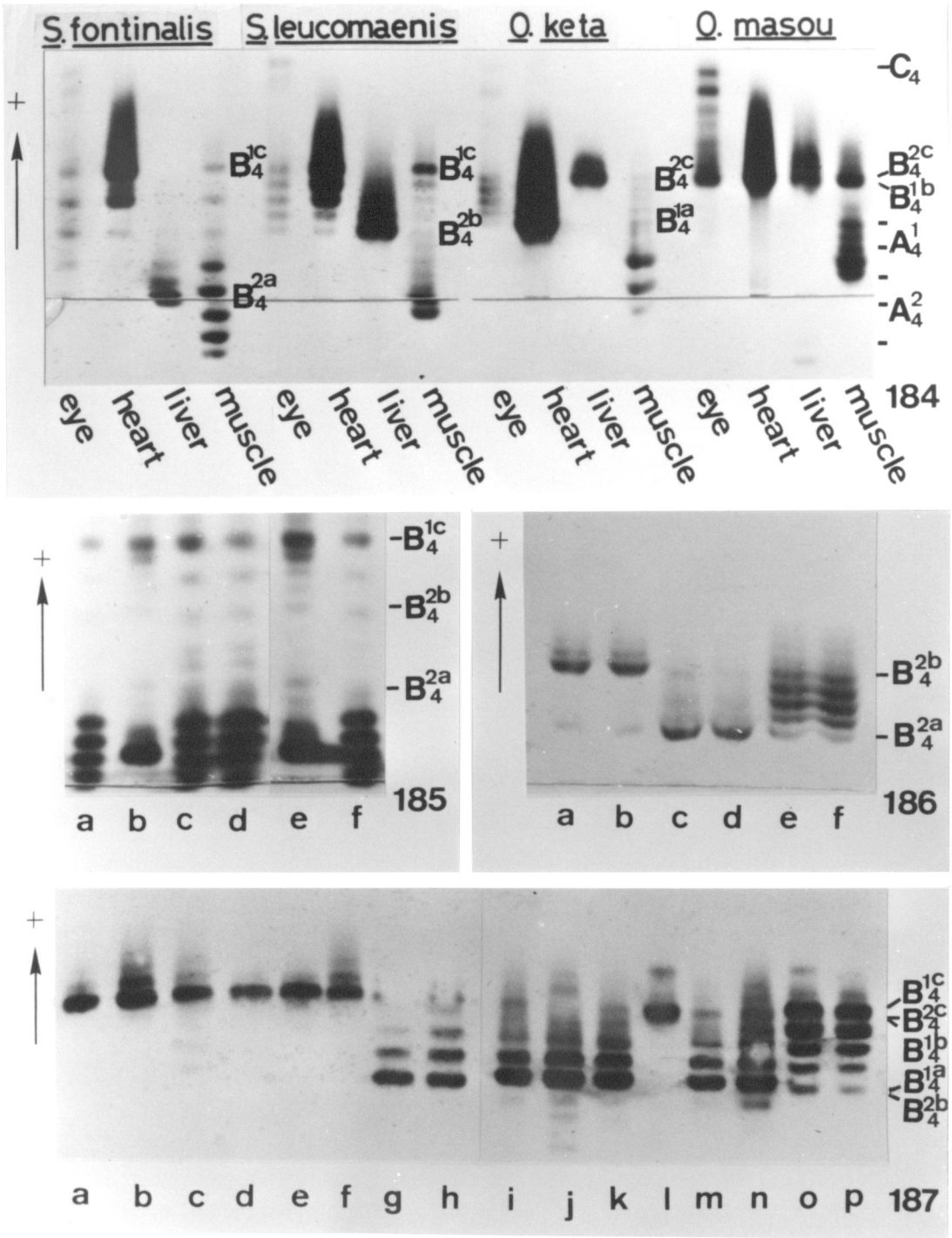

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B_4^{1a}
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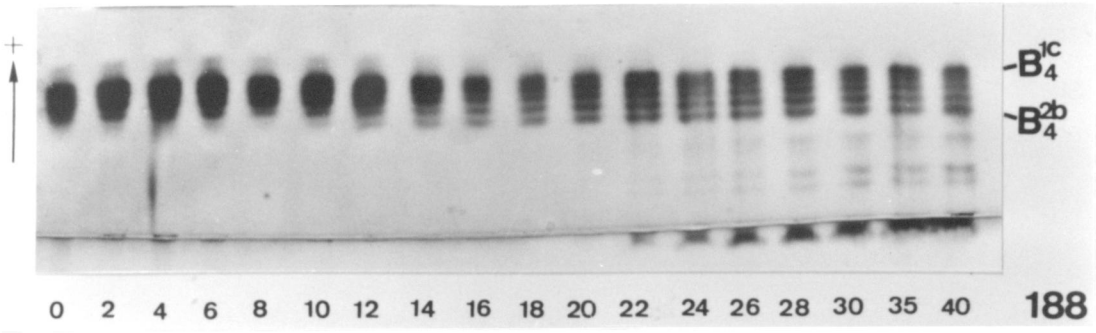
PLATE IX.

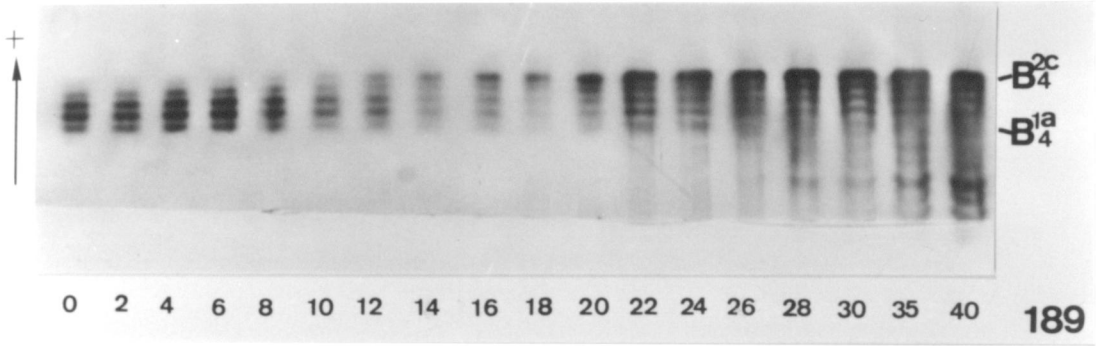
Fig. 188. LDH isozymes in the development of the Japanese char (No. 7909) from before fertilization of the egg until 40 days after fertilization. Numbers at bottom are days after fertilization.

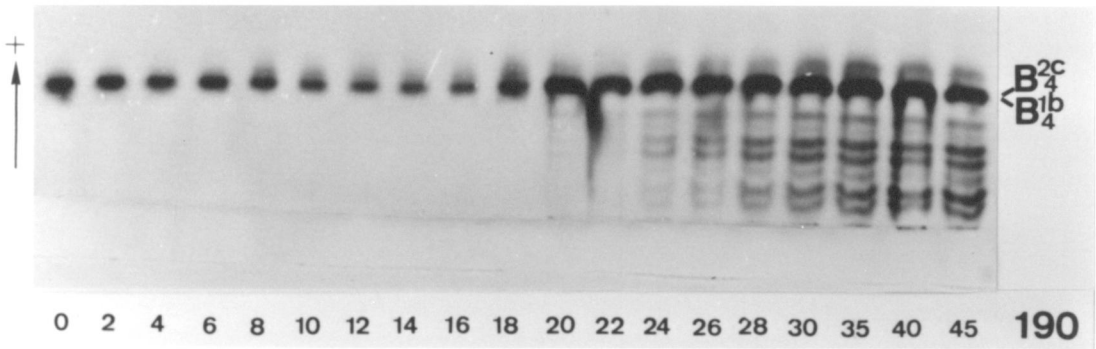
Fig. 189. LDH isozymes in the development of the chum salmon (No. 7905) from before fertilization of the egg until 40 days after fertilization. Numbers at bottom are days after fertilization.

Fig. 190. LDH isozymes in the development of the masu salmon (No. 7901) from before fertilization of the egg until 40 days after fertilization. Numbers at bottom are days after fertilization..

Fig. 191. LDH isozymes in the development of the viable hybrid Japanese char♀ x brook trout♂ (No. 8108) from before fertilization of the egg until 45 days after fertilization. Numbers at bottom are days after fertilization. Arrows in figure indicate the first appearance of hybrid heterotetramers containing paternal LDH-B^{2a} subunits.


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B_4^{2b}
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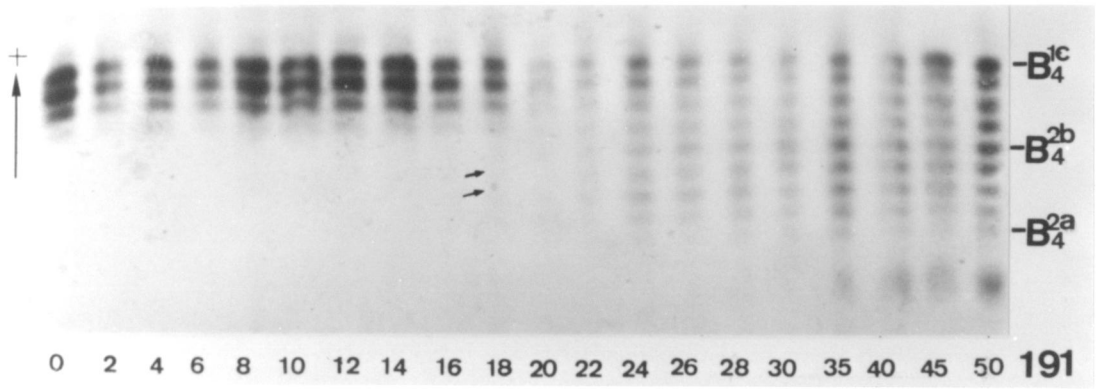

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PLATE X.

Fig. 192. LDH isozymes in the development of the viable hybrid masu salmon♀ x Japanese char♂ (No. 7902) from before fertilization of the egg until 45 days after fertilization. Numbers at bottom are days after fertilization. Arrow indicates the first appearance of hybrid heterotetramer containing paternal LDH-B^{2b} subunits.

Fig. 193. LDH isozymes in the development of the viable hybrid masu salmon♀ x chinook salmon♂ (No. 7711) from before fertilization of the egg until 35 days after fertilization. Numbers at bottom are days after fertilization. Arrow indicates the first appearance of hybrid heterotetramer containing paternal LDH-B^{1a} subunits.

Fig. 194. LDH isozymes in the development of the viable hybrid masu salmon♀ x pink salmon♂ (No. 7704) from before fertilization of the egg until 45 days after fertilization. Numbers at bottom are days after fertilization. Arrow indicates the first appearance of hybrid heterotetramer containing paternal LDH-B^{1a} subunits.

Fig. 195. LDH isozymes in the development of the inviable hybrid masu salmon♀ x chum salmon♂ (No. 7906) from before fertilization of the egg until 45 days after fertilization. Numbers at bottom are days after fertilization. Note the non-appearance of hybrid heterotetramer containing paternal LDH-B^{1a} subunits until 35 days after fertilization.

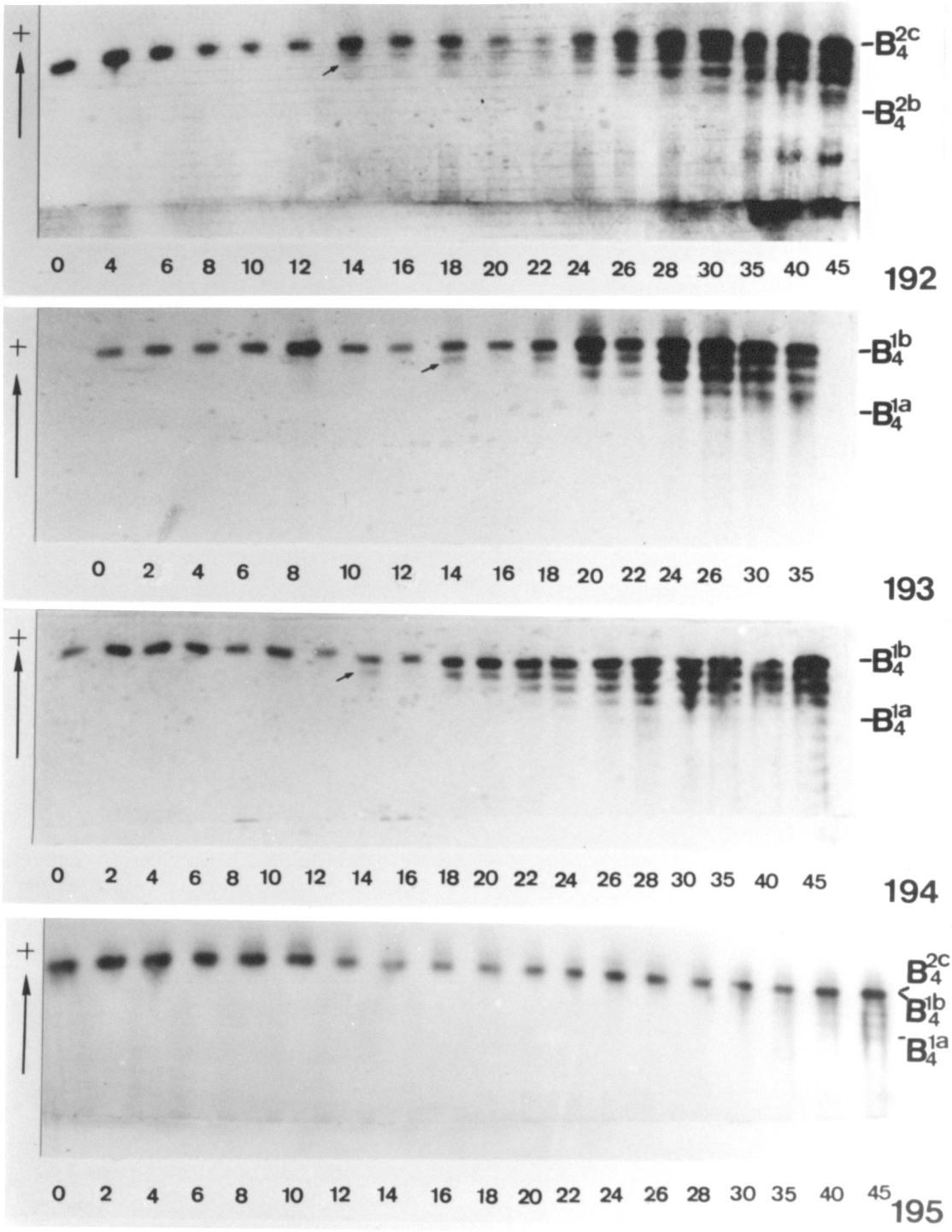

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0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 35 40 45
195

PLATE XI.

Fig. 196. Tissue distribution of malate dehydrogenase (MDH) isozymes among eyes, heart, liver, kidneys and muscles from adult masu salmon, Japanese char and chum salmon.

Fig. 197. Comparison of MDH isozyme patterns. (a). heart of Japanese char, (b). liver of Japanese char, (c). muscle of Japanese char, (d).(e). liver of a hybrid Japanese char♀ x masu salmon♂, (f). eye of masu salmon, (g). heart of masu salmon, (h). liver of masu salmon, (i). kidney of masu salmon, (j). muscle of masu salmon, (k). liver of chum salmon, (l). muscle of chum salmon, (m). liver of a hybrid pink salmon♀ x masu salmon♂, (n). muscle of a hybrid pink salmon♀ x masu salmon♂, (o). liver of pink salmon, (p). muscle of pink salmon.

Fig. 198. MDH isozymes in the development of the masu salmon (No. 7901) from before fertilization of the egg until 40 days after fertilization. Numbers at bottom are days after fertilization.

Fig. 199. MDH isozymes in the development of the Japanese char (No. 7909) from before fertilization of the egg until 40 days after fertilization. Numbers at bottom are days after fertilization.

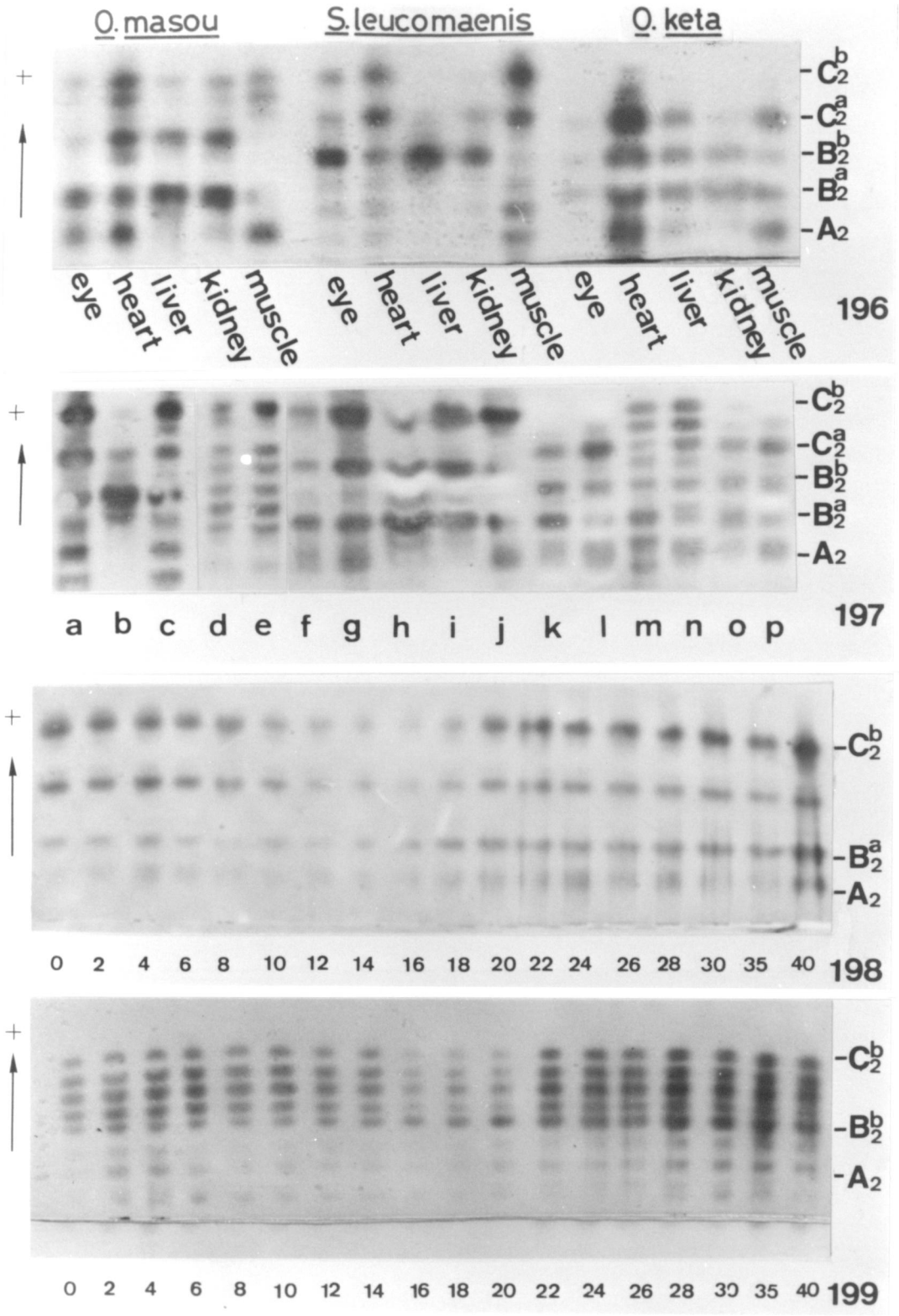

O. masou
S. leucomaenis
O. keta
+
C_2^b
C_2^a
B_2^b
B_2^a
A_2
eye
heart
liver
kidney
muscle
196
a b c d e f g h i j k l m n o p
197
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 35 40
198
199

PLATE XII.

Fig. 200. MDH isozymes in the development of the viable hybrid masu salmon♀ x Japanese char♂ (No. 7903) from before fertilization of the egg until 40 days after fertilization. Numbers at bottom are days after fertilization. Arrows (a) and (b) indicate the first appearance of hybrid heterodimers MDH-B^aB^b and MDH-B^bC^b, respectively.

Fig. 201. MDH isozymes in the development of the viable hybrid Japanese char♀ x masu salmon♂ (No. 7908) from before fertilization of the egg until 40 days after fertilization. Numbers at bottom are days after fertilization. Arrows (a) and (b) indicate the first appearance of hybrid heterodimers MDH-B^aB^b and MDH-B^aC^b, respectively.

Fig. 202. MDH isozymes in the development of the viable hybrid Japanese char♀ x masu salmon♂ (No. 7910) from before fertilization of the egg until 35 days after fertilization. Numbers at bottom are days after fertilization. Arrow indicates the first appearance of hybrid heterodimer MDH-B^aB^b.

Fig. 203. MDH isozymes in the development of the viable hybrid pink salmon♀ x masu salmon♂ (No. 7702) from before fertilization of the egg until 58 days after fertilization. Arrows (a) and (B) indicate the first appearance of hybrid heterodimers MDH-C^aC^b and MDH-B^aC^a, respectively.

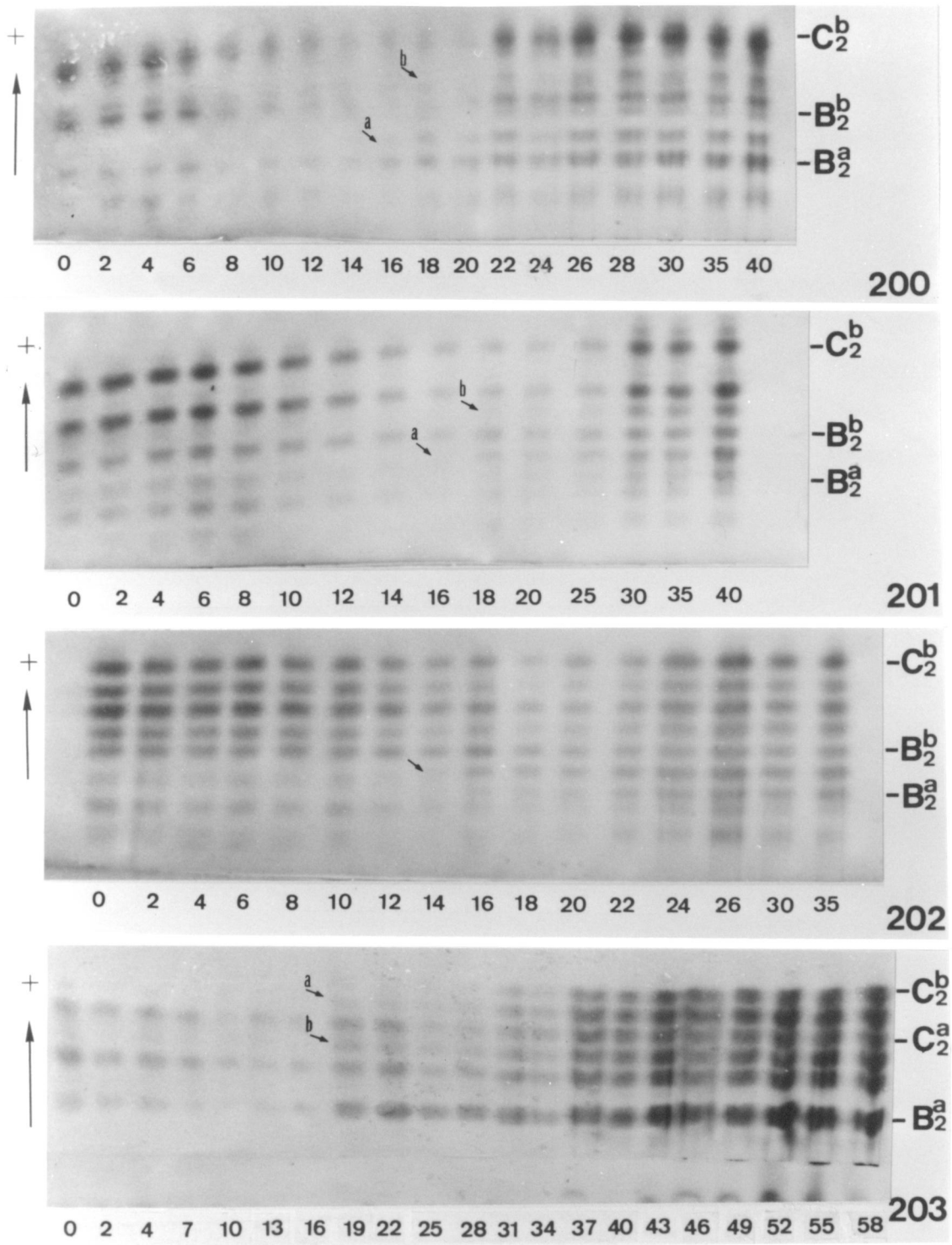

+
C_2^b
B_2^b
B_2^a
a
b
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 35 40
200
+
C_2^b
B_2^b
B_2^a
a
b
0 2 4 6 8 10 12 14 16 18 20 25 30 35 40
201
+
C_2^b
B_2^b
B_2^a
0 2 4 6 8 10 12 14 16 18 20 22 24 26 30 35
202
+
a
b
C_2^b
C_2^a
B_2^a
0 2 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
203

PLATE XIII.

Fig. 204. MDH isozymes in the development of the viable hybrid chum salmon♀ x masu salmon♂ (No. 7907) from before fertilization of the egg until 45 days after fertilization. Numbers at bottom are days after fertilization. Arrows (a) and (b) indicate the first appearance of hybrid heterodimes MDH-C^aC^b and MDH-B^aC^b, respectively.

Fig. 205. MDH isozymes in the development of the inviable hybrid masu salmon♀ x chum salmon♂ (No. 7906) from before fertilization of the egg until 45 days after fertilization. Numbers at bottom are days after fertilization. Note exclusive existence of maternal MDH phenotype and non-appearance of hybrid heterodimer.

Fig. 206. Interspecific comparison of 6-phosphogluconate dehydrogenase (6PGDH) isozyme patterns in muscle among nine species of salmonids. (a). chinook salmon, (b). chum salmon, (c). pink salmon, (d). coho salmon, (e). F_1 hybrid, brook trout♀ x coho salmon♂, (f). brook trout, (g). Japanese huchen, (h). chinook salmon, (i). rainbow trout, (j). Japanese char, (k). F_1 hybrid, Japanese char♀ x masu salmon♂, (l-n). masu salmon.

Fig. 207. 6PGDH isozymes in the development of the viable hybrid masu salmon♀ x Japanese char♂ (No. 7907) from before fertilization of the egg until 24 days after fertilization. Arrow indicates the first appearance of hybrid heterodimer containing paternal 6PGDH-A^b subunits.

Fig. 208. 6PGDH isozymes in the development of the viable hybrid Japanese char♀ x masu salmon♂ (No. 7908) from before fertilization of the egg until 24 days after fertilization. Arrow indicates the first appearance of hybrid heterodimer containing paternal 6PGDH-A^a subunits.

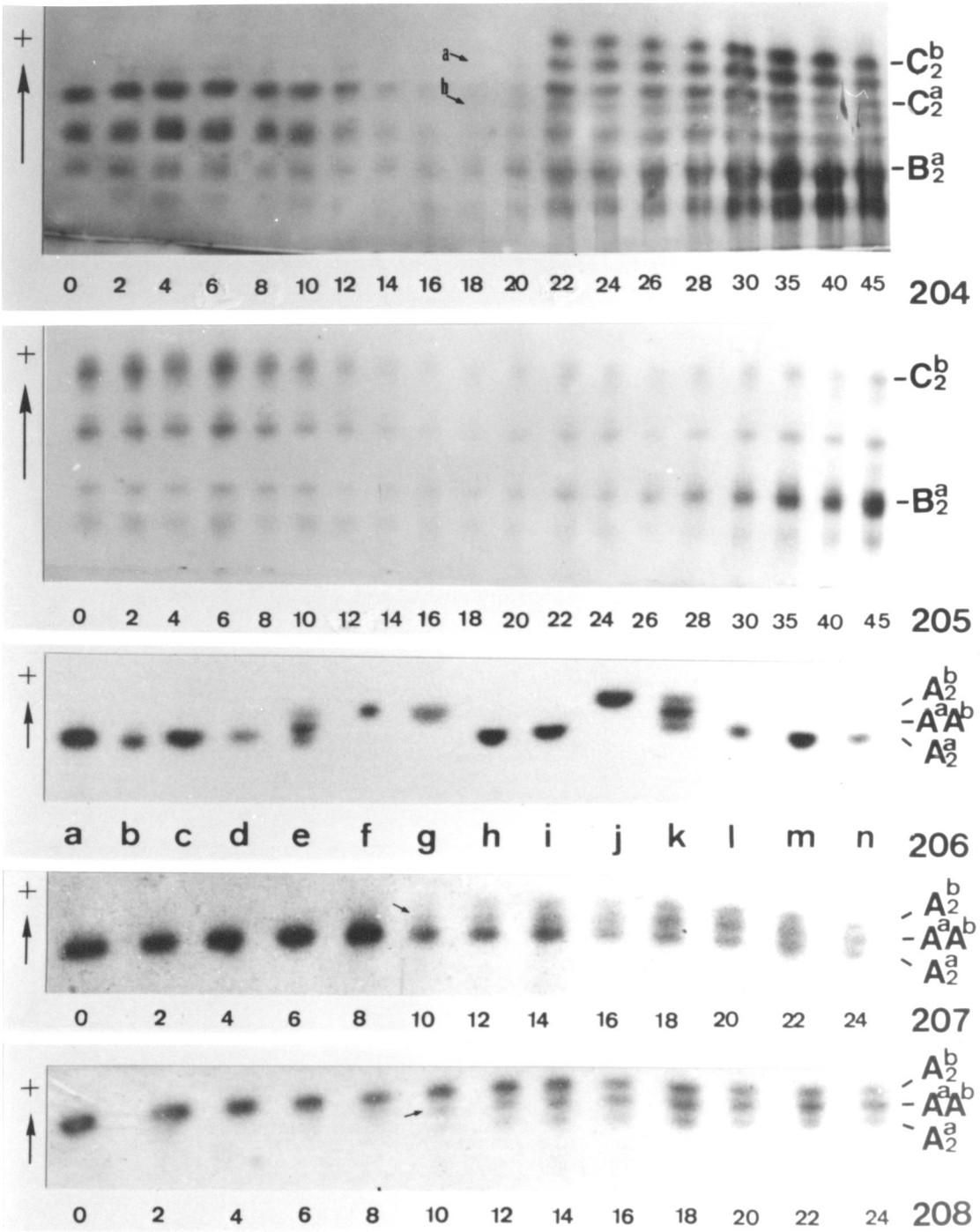

+
a
b
C_2^b
C_2^a
B_2^a
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 35 40 45
204
+
C_2^b
B_2^a
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 35 40 45
205
+
A_2^b
A^aA^b
A_2^a
a b c d e f g h i j k l m n
206
+
A_2^b
A^aA^b
A_2^a
0 2 4 6 8 10 12 14 16 18 20 22 24
207
+
A_2^b
A^aA^b
A_2^a
0 2 4 6 8 10 12 14 16 18 20 22 24
208

PLATE XIV.

Fig. 209. Comparison of phosphoglucomutase (PGM) isozyme patterns in muscles among Japanese char (a-c), chum salmon (d, e), masu salmon (f-j), and brook trout (k-r).

Fig. 210. PGM isozymes in the development of the viable hybrid Japanese char♀ x masu salmon♂ (No. 7908) from before fertilization of the egg until 35 days after fertilization. Arrows (a), (b) and (c) indicate the first appearance of paternal PGM isozymes PGM-B^a, PGM-A^a, and PGM-C^a, respectively. Open circle indicates the position of additional PGM band unique to unfertilized egg and early embryonic stages.

Fig. 211. PGM isozymes in the development of the masu salmon (No. 7901) from before fertilization of the egg until 40 days after fertilization. Numbers at bottom are days after fertilization. Arrow indicates the first appearance of paternal PGM-A^a isozyme.

Fig. 212. PGM isozymes in the development of the viable hybrid chum salmon♀ x masu salmon♂ (No. 7907) from before fertilization of the egg until 45 days after fertilization. Numbers at bottom are days after fertilization. Arrow indicates the first appearance of paternal PGM-A^a isozyme.

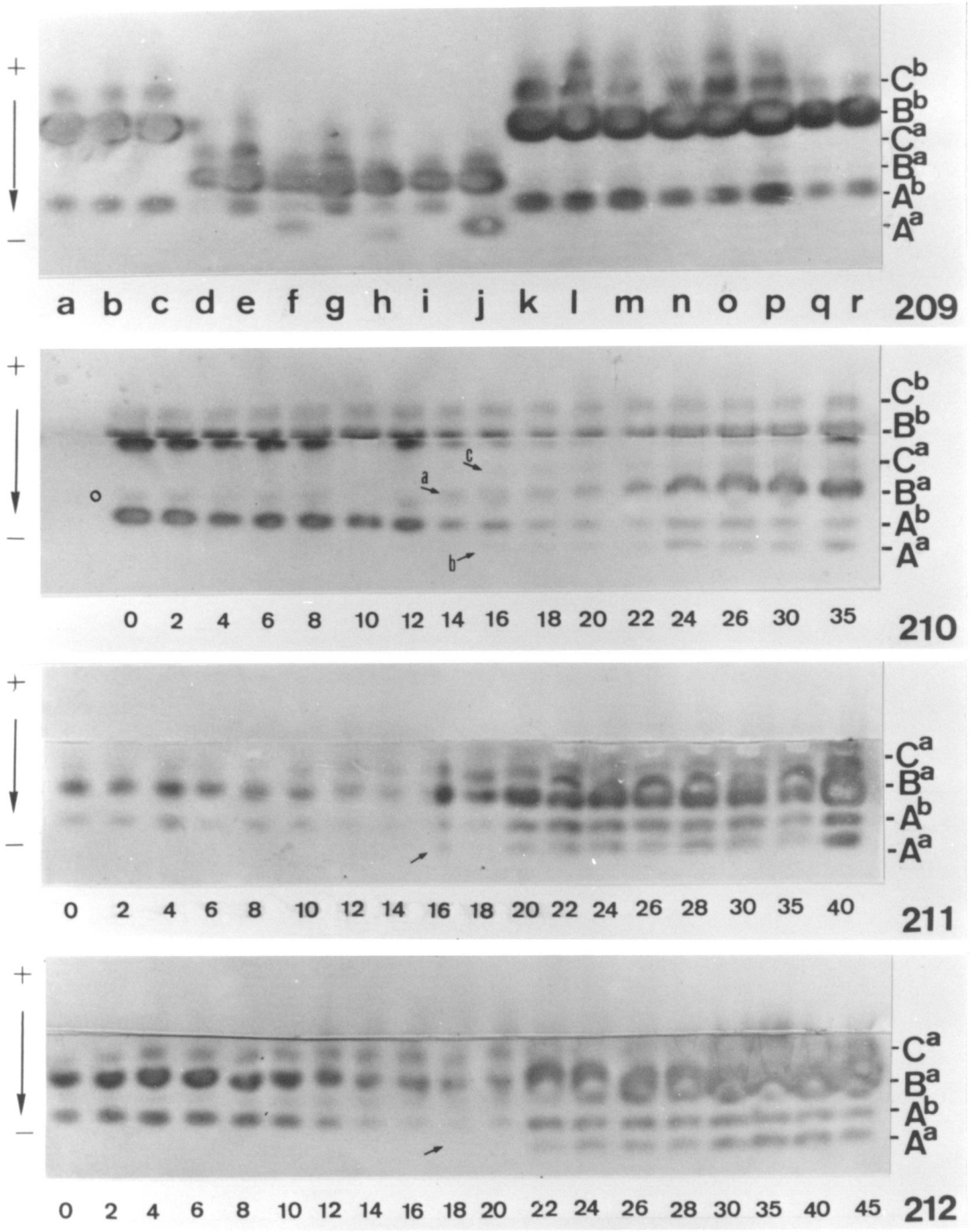

+
−
C^b
B^b
C^a
B^a
A^b
A^a
a b c d e f g h i j k l m n o p q r
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0 2 4 6 8 10 12 14 16 18 20 22 24 26 30 35
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0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 35 40 45
212

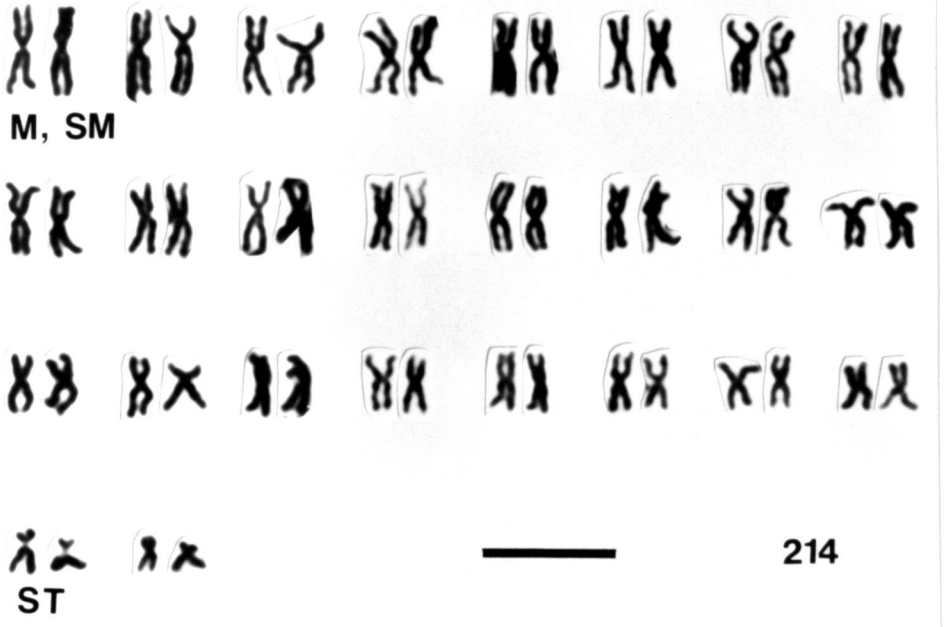
PLATE XV.

Fig. 213. A photomicrograph of metaphase chromosomes of the pink salmon, 2N=52. Scale indicates 10 μm.

Fig. 214. A karyogram of the pink salmon with 2N=52. The karyotype was comprised of 24 meta- or submetacentroc (M, SM) pairs and 2 subtelocentric (ST) pairs. Scale indicates 10 μm.



213

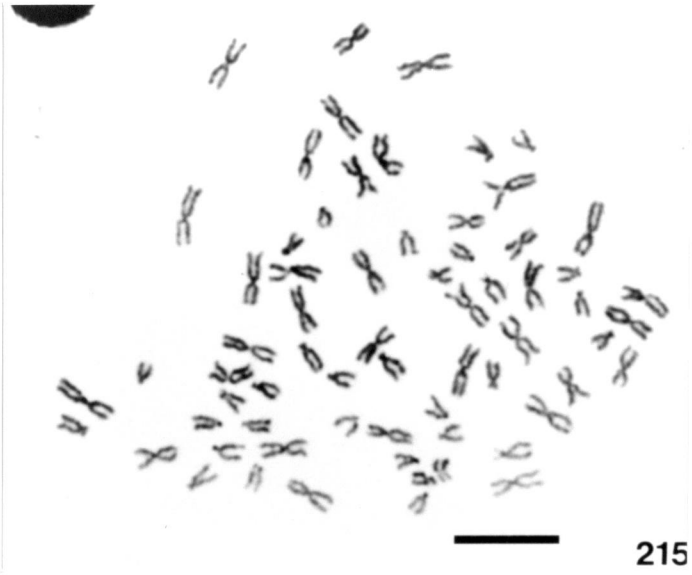


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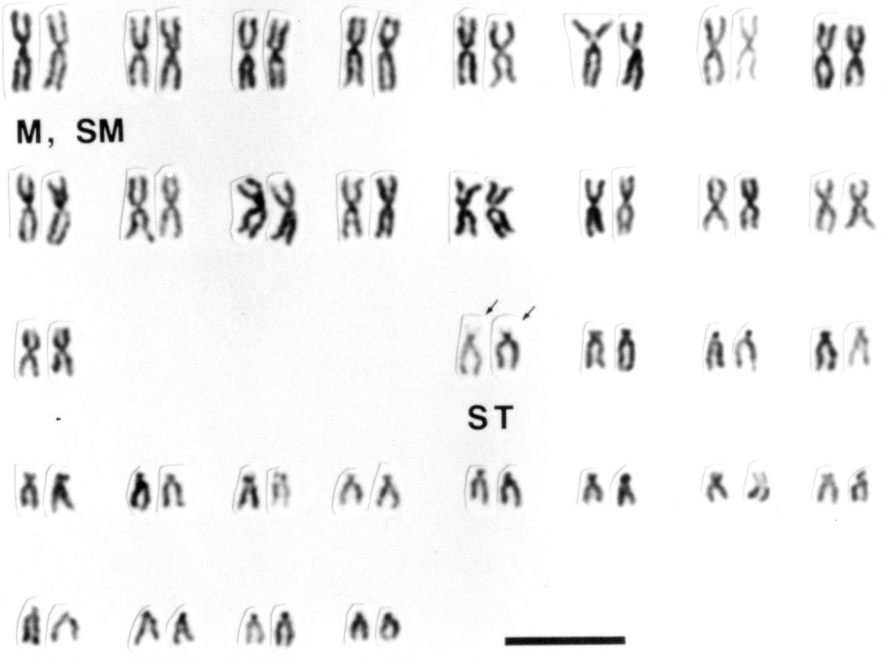
PLATE XVI.

Fig. 215. A photomicrograph of metaphase chromosomes of the masu salmon, 2N=66. Scale indicates 10 μm.

Fig. 216. A karyogram of the masu salmon with 2N=66. The karyotype was comprised of 17 meta- or submetacentric (M, SM), 12 subtelocentric (ST), and 4 acrocentric (A)pairs. Arrows indicate satellites at the distal portion of short arms of the largest subtelocentric pair. Scale indicates 10 μm.



215

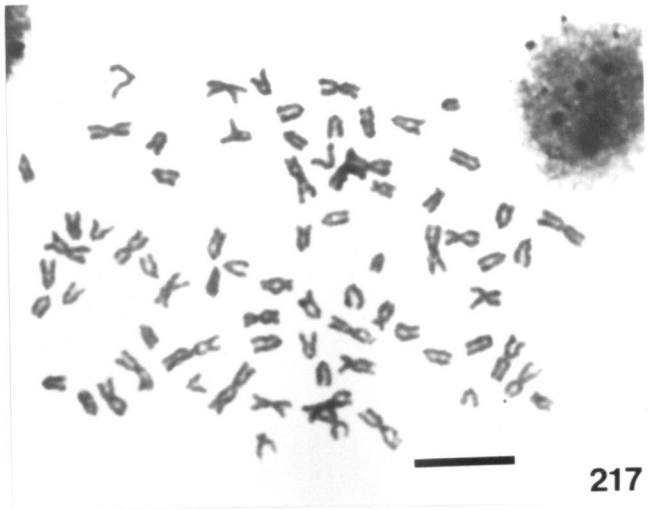


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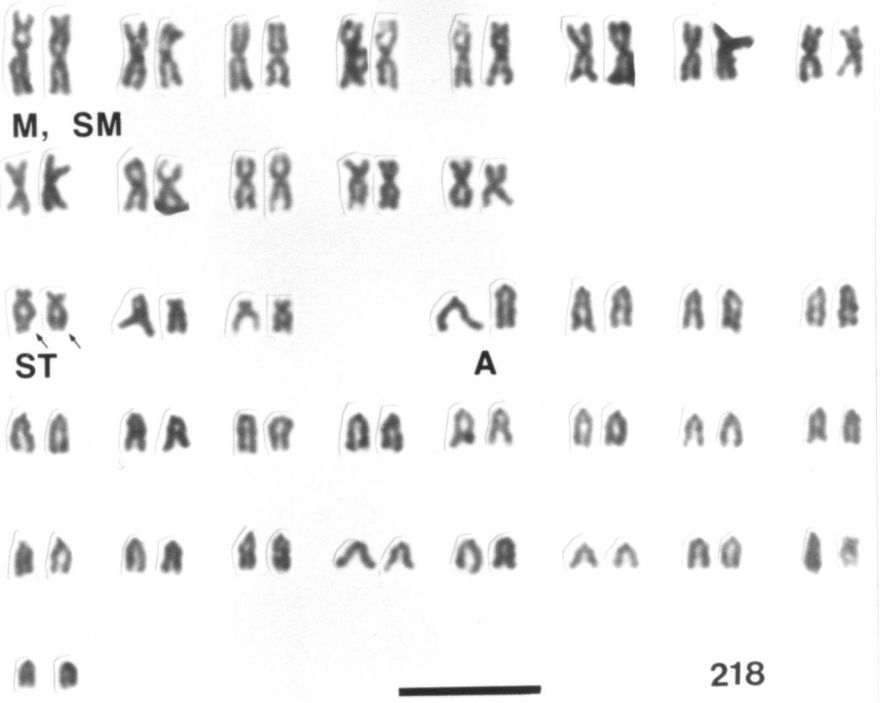
PLATE XVII.

Fig. 217. A photomicrograph of metaphase chromosomes of the chum salmon, 2N=74. Scale indicates 10 μm,

Fig. 218. A karyogram of the chum salmon with 2N=74. The karyotype was comprised of 13 meta- or submetacentric (M, SM), 3 subtelocentric (ST), and 21 acrocentric (A) pairs. Arrow indicate satellites at the distal portion of long arms of the largest subtelocentric pair. Scale indicates 10 μm.



217

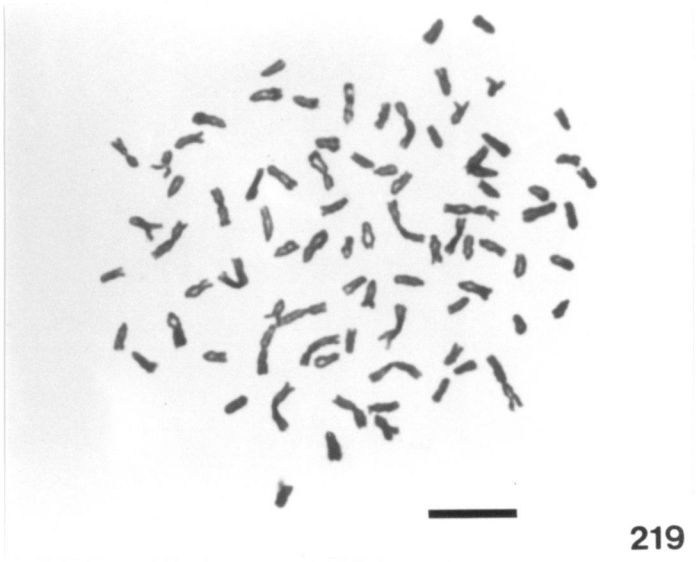


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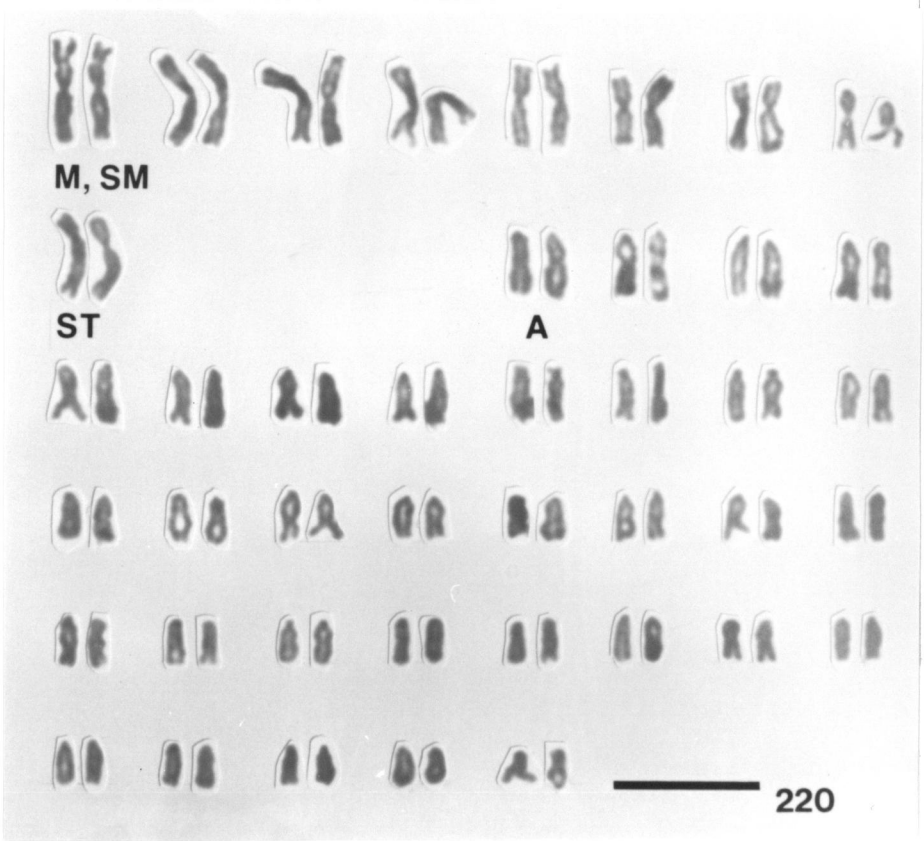
PLATE XVIII.

Fig. 219. A photomicrograph of metaphase chromosomes of the Japanese char, 2N=84. Scale indicates 10 μm.

Fig. 220. A karyogram of the Japanese char with 2N=84. The karyotype was comprised of 16 meta- or submeta-centric (M, SM), 1 subtelocentric (ST), and 33 acro-centric (A) pairs. Scale indicates 10 μm.



219

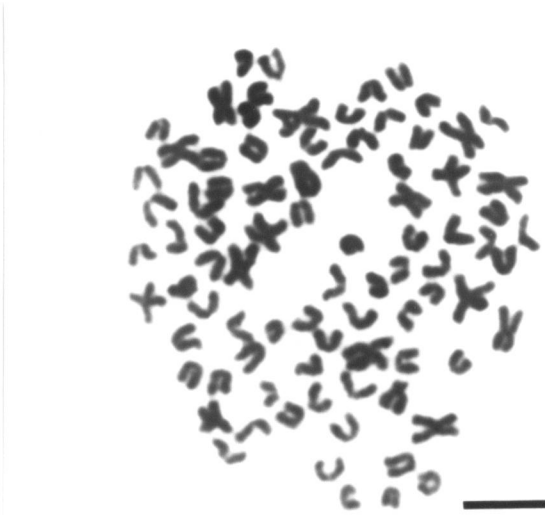


220

PLATE XIX.

Fig. 221. A photomicrograph of metaphase chromosomes of the brook trout, 2N=84. Scale indicates 10 μm.

Fig. 222. A karyogram of the brook trout with 2N=84. The karyotype was comprised of 16 meta- or submetacentric (M, SM), 1 subtelocentric (ST), and 33 acrocentric (A) pairs. Scale indicates 10 μm.



221

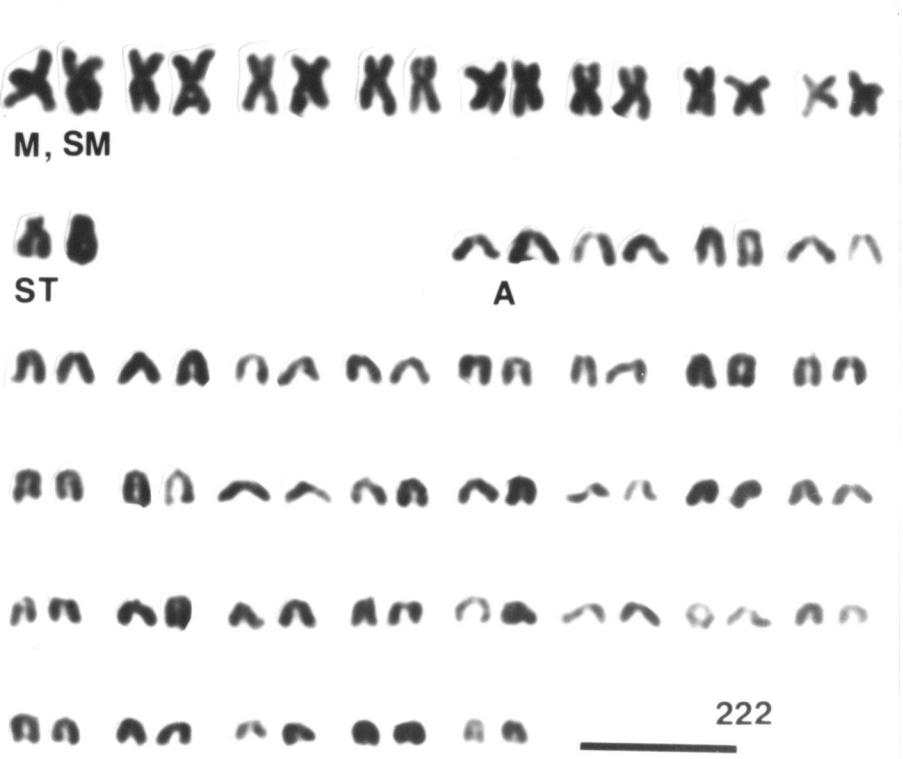
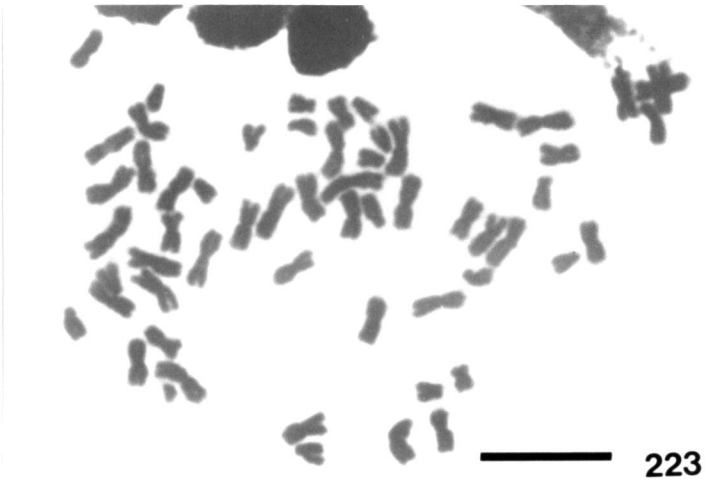


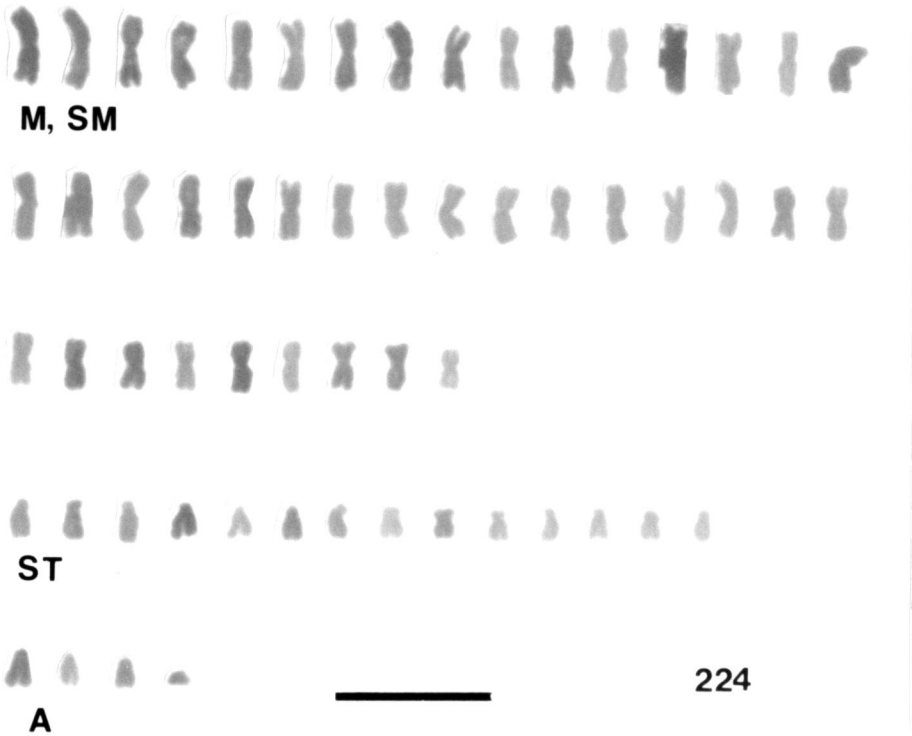
PLATE XX.

Fig. 223. A photomicrograph of metaphase chromosomes of the viable hybrid masu salmon♀ x pink salmon♂, 2N=59. Scale indicates 10 μm.

Fig. 224. A karyogram of the viable hybrid masu salmon♀ x pink salmon♂ with 2N=59. The karyotype was comprised of 41 meta- or submetacentric (M, SM), 14 subtelocentric (ST) and 4 acrocentric (A) chromosomes. Scale indicates 10 μm.



223



224

PLATE XXI.

Fig. 225. A photomicrograph of metaphase chromosomes of the viable hybrid Japanese char♀ x brook trout♂, 2N=84. Scale indicates 10 μm.

Fig. 226. A karyogram of the viable hybrid Japanese char♀ x brook trout♂ with 2N=84. The karyotype was comprised of 16 meta- or submetacentric (M, SM), 2 subtelocentric (ST) and 66 acrocentric (A) chromosomes. Scale indicates 10 μm.



225

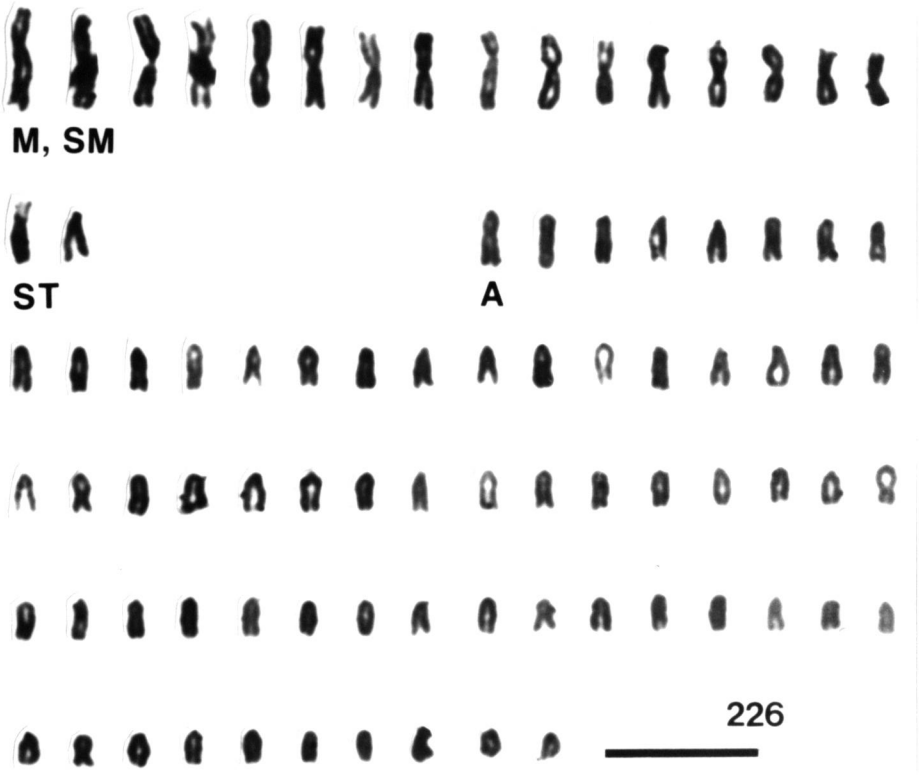
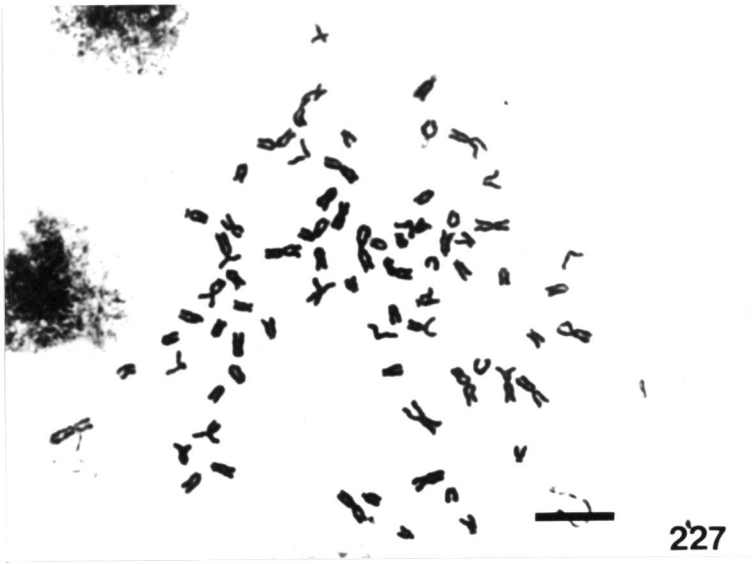


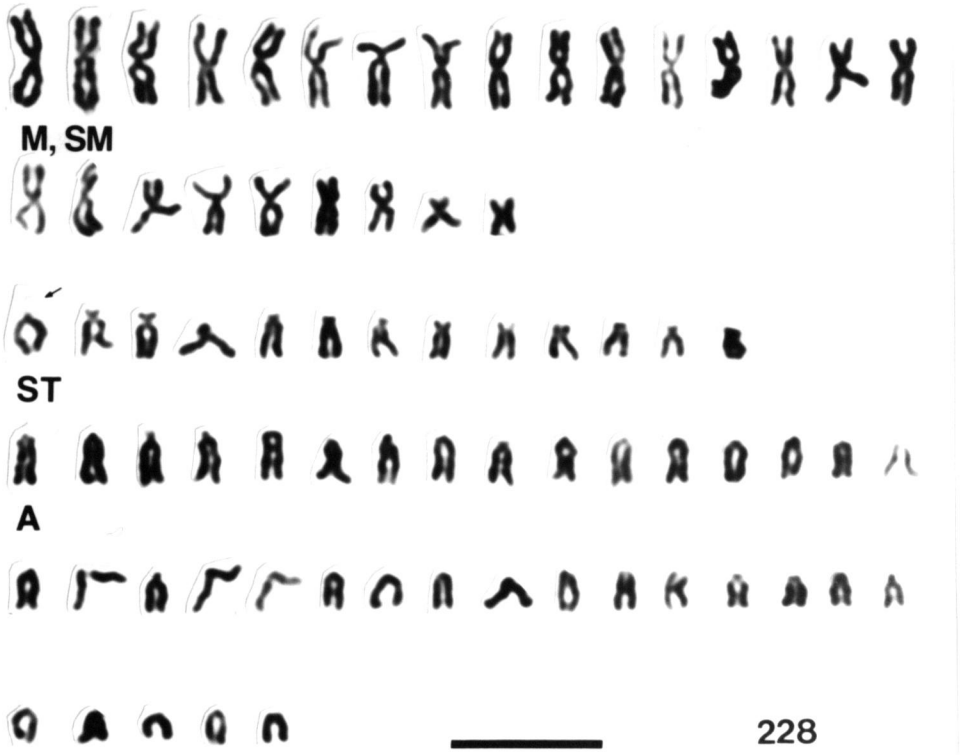
PLATE XXII.

Fig. 227. A photomicrograph of metaphase chromosomes of the viable hybrid masu salmon♀ x Japanese char♂, 2N=75. Scale indicates 10 μm.

Fig. 228. A karyogram of the viable hybrid masu salmon♀ x Japanese char♂ with 2N=75. The karyotype was comprised of 25 meta- or submetacentric (M, SM), 13 subtelocentric (ST) and 37 acrocentric (A) chromosomes. Arrow indicates the satellite at the distal portion of short arms of the largest subtelocentric chromosomes. Scale indicates 10 μm.



227

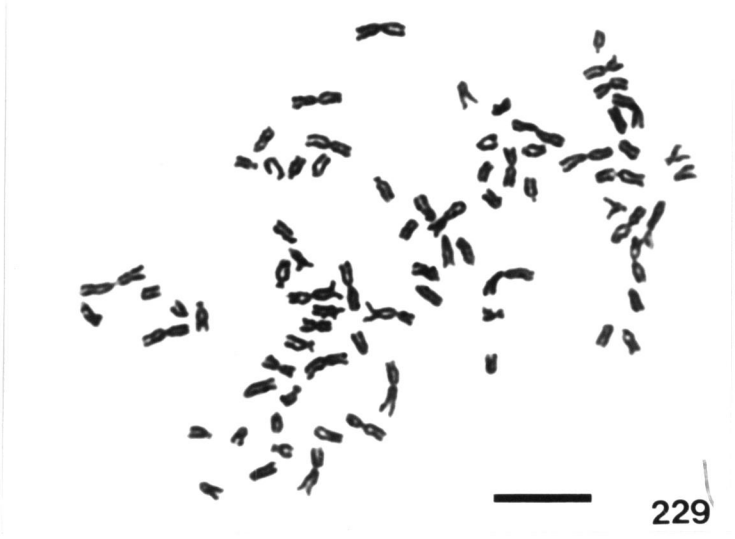


228

PLATE XXIII.

Fig. 229. A photomicrograph of metaphase chromosomes of the viable hybrid Japanese char♀ x masu salmon♂, 2N=75. Scale indicates 10 μm.

Fig. 230. A karyogram of the viable hybrid Japanese char♀ x masu salmon♂ with 2N=75. The karyotype was comprised of 25 meta- or submetacentric (M, SM), 13 subtelocentric (ST) and 37 acrocentric (A) chromosomes. Arrow indicates the satellite at the distal portion of short arms of the largest subtelocentric chromosomes. Scale indicates 10 μm.



229



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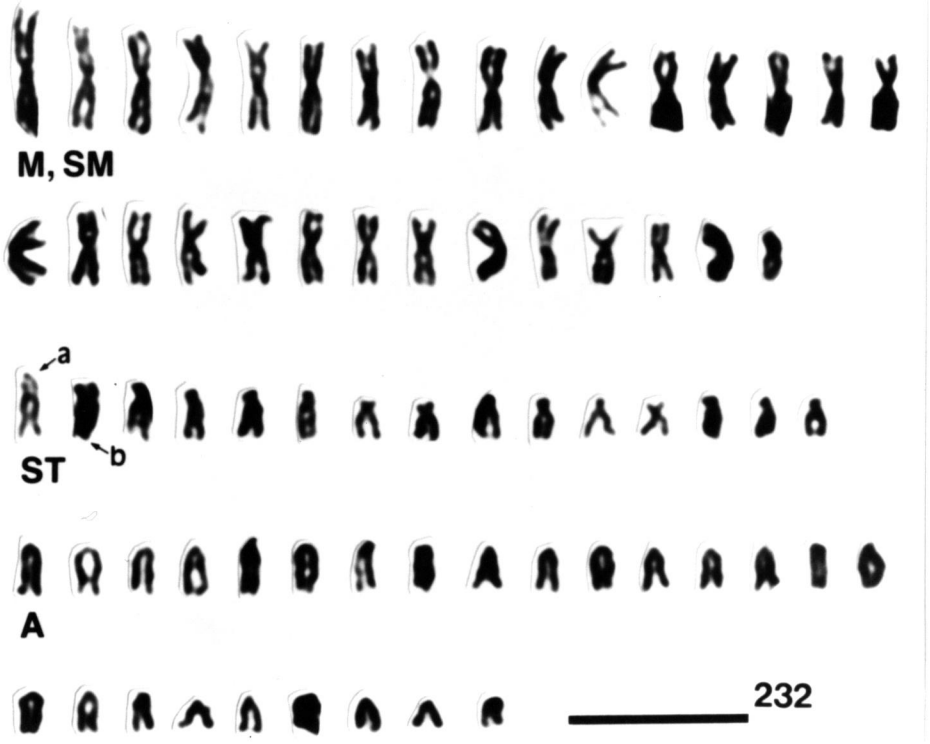
PLATE XXIV.

Fig. 231. A photomicrograph of metaphase chromosomes of the inviable hybrid chum salmon♀ x masu salmon♂, 2N=70. Scale indicates 10 μm.

Fig. 232. A karyotype of the inviable hybrid chum salmon♀ x masu salmon♂ with 2N=70. The karyotype was comprised of 30 meta- or submetacentric (M, SM), 15 subtelocentric (ST) and 25 acrocentric (A) chromosomes. Arrow (a) indicates the satellite at the distal portion of short arms of the largest subtelocentric chromosome, denoting its origin from paternal masu salmon. Arrow (b) indicates the satellite at the distal portion of long arms of the second largest subtelocentric chromosome, denoting its origin from maternal chum salmon. Scale indicates 10 μm.



231

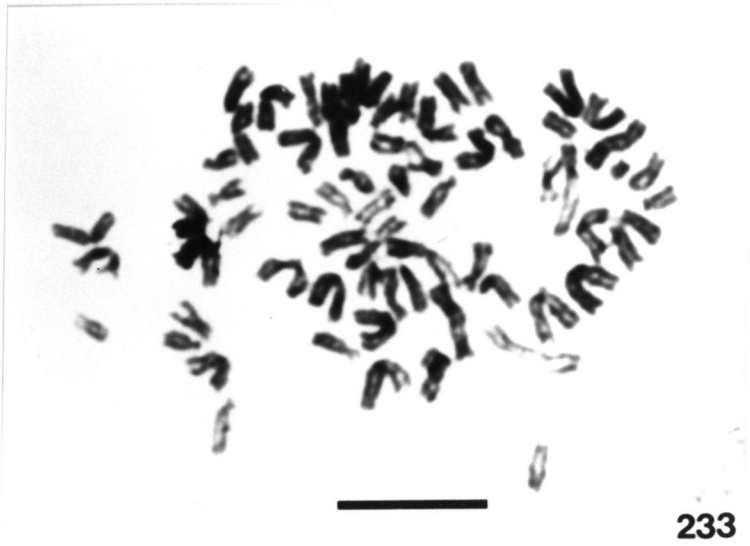


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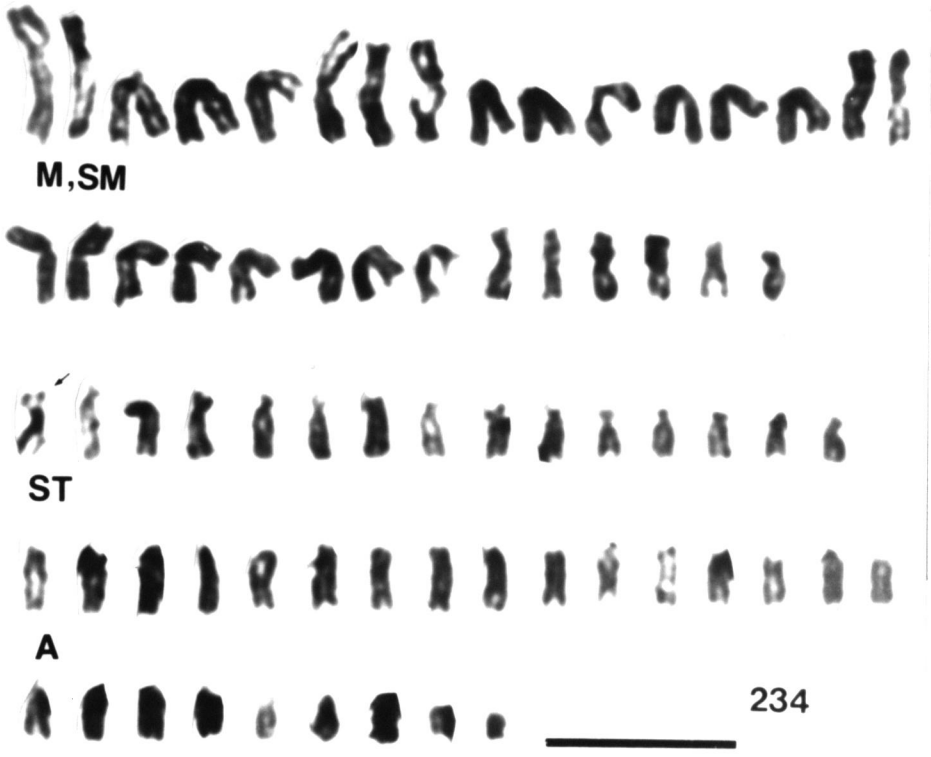
PLATE XXV.

Fig. 233. A photomicrograph of metaphase chromosomes of the inviable hybrid masu salmon♀ x chum salmon♂, 2N=70. Scale indicates 10 μm.

Fig. 234. A karyogram of the inviable hybrid masu salmon♀ x chum salmon♂ with 2N=70. The karyotype was comprised of 30 meta- or submetacentric (M, SM), 15 subtelocentric (ST) and 25 acrocentric (A) chromosomes. Arrow indicates the satellite at the distal portion of short arms of the largest subtelocentric chromosome. Scale indicates 10 μm.



233



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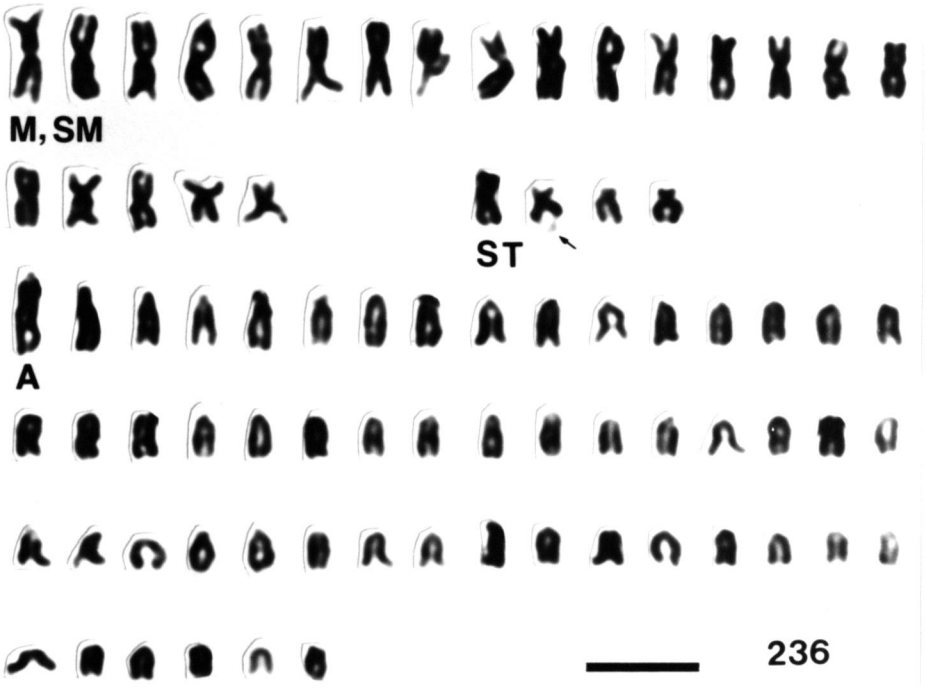
PLATE XXVI.

Fig. 235. A photomicrograph of metaphase chromosomes of the inviable hybrid chum salmon♀ x Japanese char♂, 2N 79. Scale indicates 10 μm.

Fig. 236. A karyogram of the inviable hybrid chum salmon♀ x Japanese char♂ with 2N 79. The karyotype was comprised of 21 meta- or submetacentric (M, SM), 4 subtelocentric (ST) and 54 acrocentric (A) chromosomes. Arrow indicates the satellite at the distal portion of long arms of one of the subtelocentric chromosomes, denoting its origin from maternal chum salmon. Scale indicates 10 μm.



235



236

PLATE XXVII.

Photomicrographs of metaphases of a 14-day embryo of the inviable hybrid Japanese char♀ x chum salmon♂ (No. 8008).

Fig. 237. An aneuploid cell with 45 chromosomes.

Fig. 238. An aneuploid cell with 56 chromosomes.

Fig. 239. An aneuploid cell with 56 chromosomes.

Fig. 240. An aneuploid cell with 72 chromosomes.

Scales indicate 10 μm.



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PLATE XXVIII.

Photomicrographs of metaphases of a 16-day embryo of the inviable hybrid Japanese char♀ x chum salmon♂ (No. 8105).

Fig. 241. An aneuploid cell with 55 chromosomes.

Fig. 242. An aneuploid cell with 58 chromosomes.

Fig. 243. An aneuploid cell with 58 chromosomes.

Fig. 244. An aneuploid cell with 61 chromosomes.

Scales indicate 10 μm.



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PLATE XXIX.

Photomicrographs of metaphases of a 16-day embryo of the inviable hybrid Japanese char♀ x chum salmon♂ (No. 8105).

Fig. 245. An aneuploid cell with 54 chromosomes.

Fig. 246. An aneuploid cell with 55 chromosomes.

Fig. 247. An aneuploid cell with 63 chromosomes.

Fig. 248. An aneuploid cell with a dicentric chromosome and a number of chromosome fragments. Arrow (f) indicates chromosome fragments and arrow (DC) indicates dicentric chromosomes.

Scales indicate 10 μm.



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PLATE XXX.

Photomicrographs of metaphases of a 32-day embryo of the inviable hybrid brook trout♀ x chum salmon♂ (No. 8114).

Fig. 249. An aneuploid cell with 47 chromosomes.

Fig. 250. An aneuploid cell with 54 chromosomes.

Fig. 251. An aneuploid cell with 54 chromosomes.

Fig. 252. An aneuploid cell with 58 chromosomes.

Scales indicate 10 μm.



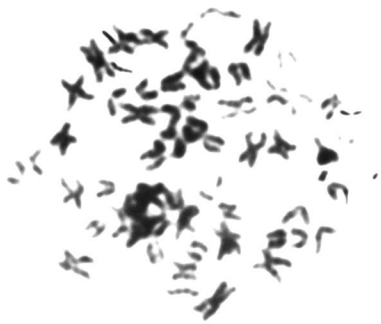
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PLATE XXXI.

Morphology of embryos of the chum salmon from the control group.

Fig. 253. Embryo at 20 days.
Fig. 254. Embryo at 25 days.
Fig. 255. Embryo at 48 days.
Fig. 256. Hatched larva.

Morphology of embryos of the triploid chum salmon from the experimental group subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination.

Fig. 257. Embryo at 20 days.
Fig. 258. Embryo at 25 days.
Fig. 259. Embryo at 48 days.
Fig. 260. Hatched larva.

Morphology of embryos of the Japanese char from the control group.

Fig. 261. Embryo at 20 days.
Fig. 262. Embryo at 25 days.
Fig. 263. Embryo at 48 days.
Fig. 264. Hatched larva.

Morphology of embryos of the triploid Japanese char from the experimental group subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination.

Fig. 265. Embryo at 20 days.
Fig. 266. Embryo at 25 days.
Fig. 267. Embryo at 48 days.
Fig. 268. Hatched larva.

Morphology of embryos of the inviable hybrid chum salmon♀ x Japanese char♂ from the control group.

Fig. 269. Embryo at 20 days.
Fig. 270. Embryo at 25 days.
Fig. 271. Embryo at 48 days.

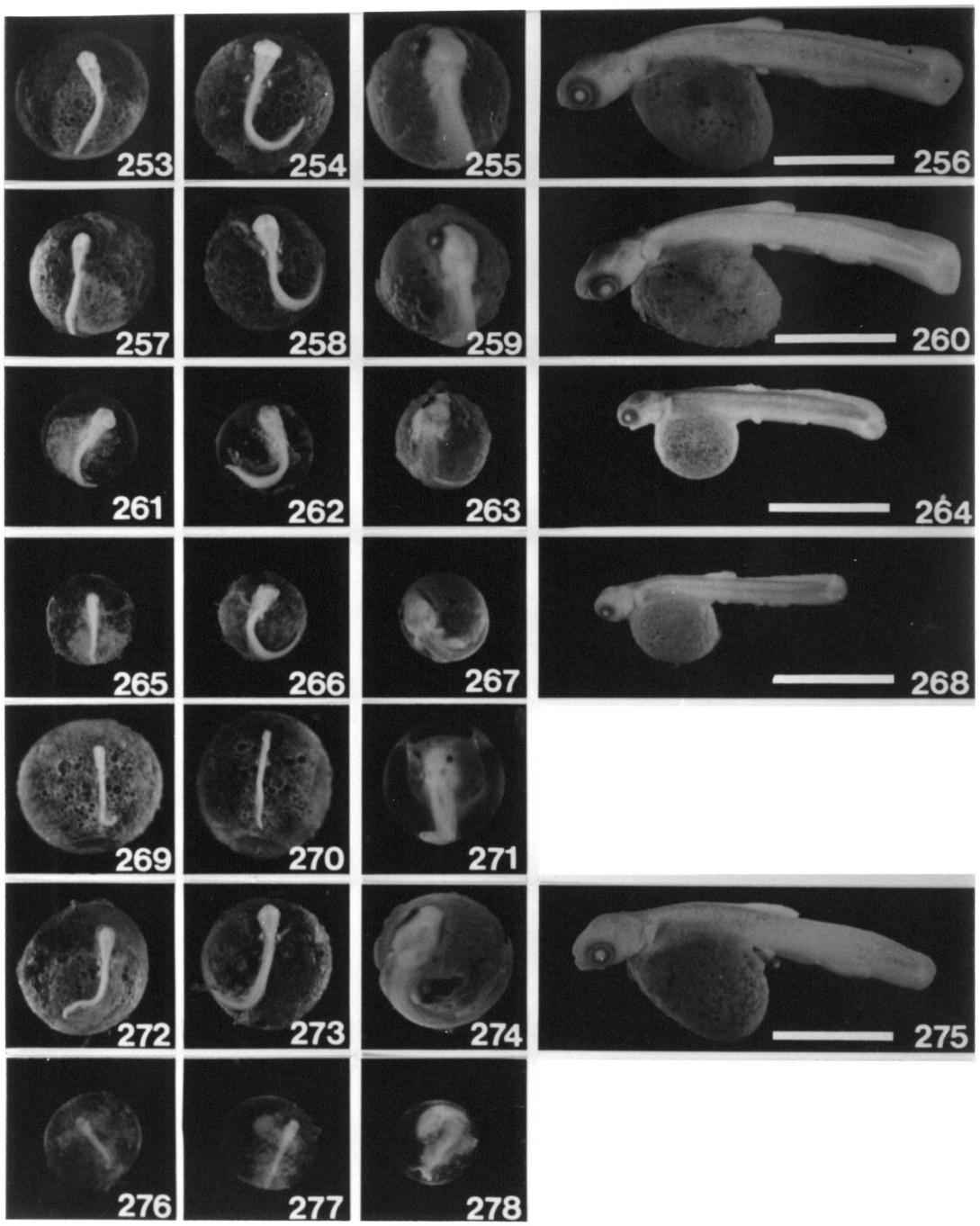

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PLATE XXXI.
(continued)

Morphology of embryos of the allotriploid hybrid 2chum salmon♀ x Japanese char♂ from the experimental group subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination.

Fig. 272. Embryo at 20 days.
Fig. 273. Embryo at 25 days.
Fig. 274. Embryo at 48 days.
Fig. 275. Hatched larva.

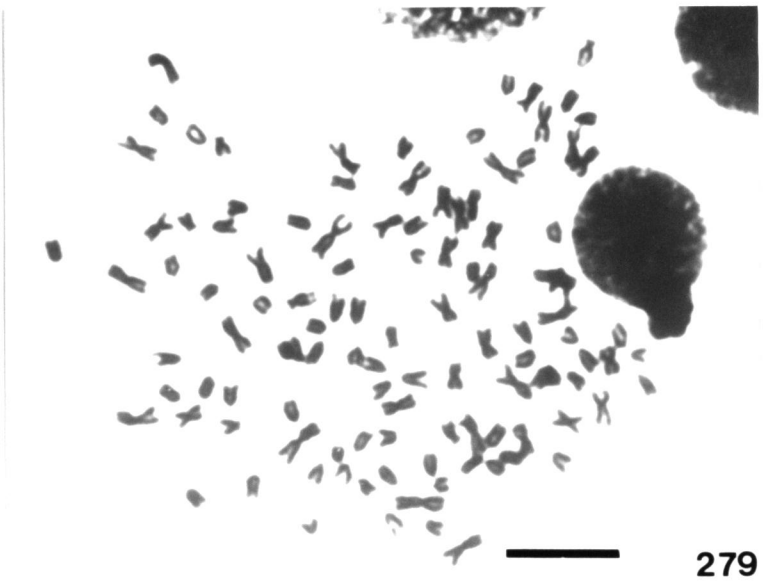
Morphology of embryos of the inviable hybrid Japanese char♀ x chum salmon♂ from the control group.

Fig. 276. Embryo at 20 days.

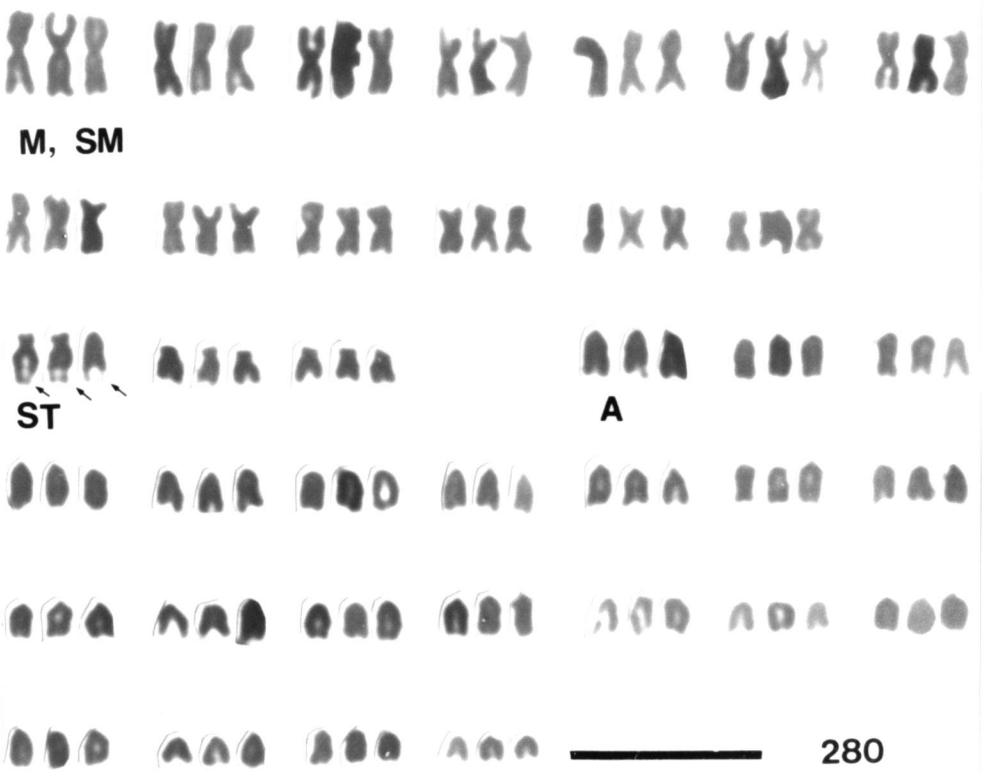
Morphology of embryos of the inviable hybrid Japanese char♀ x chum salmon♂ from the experimental group subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination.

Fig. 277. Embryo at 20 days.
Fig. 278. Embryo at 25 days.

Scales indicate 10 μm.



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PLATE XXXII.

Fig. 279. A photomicrograph of metaphase chromosomes of the triploid chum salmon embryo from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination, 3N=111. Scale indicates 10 μm.

Fig. 280. A karyogram of the triploid chum salmon with 3N=111. The karyotype was comprised of 39 meta- or submetacentric (M,,SM), 9 subtelocentric (ST) and 63 acrocentric (A) chromosomes, indicating triploidy. Arrows indicate satellites at the distal portion of long arms of three subtelocentric elements. Scale indicates 10 μm.

PLATE XXXIII.

Fig. 281. A photomicrograph of metaphase chromosomes of the triploid Japanese char embryo from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination, 3N=126. Scale indicates 10 μm.

Fig. 282. A karyogram of the triploid Japanese char with 3N=126. The karyotype was comprised of 24 meta- or submetacentric (M, SM), 3 subtelocentric (ST) and 99 acrocentric (A) chromosomes, indicating triploidy. Scale indicates 10 μm.



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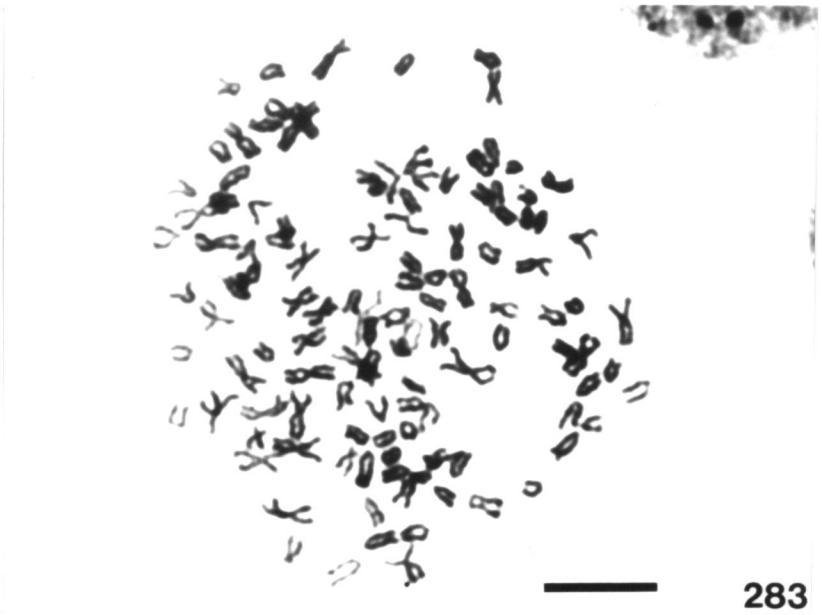


282

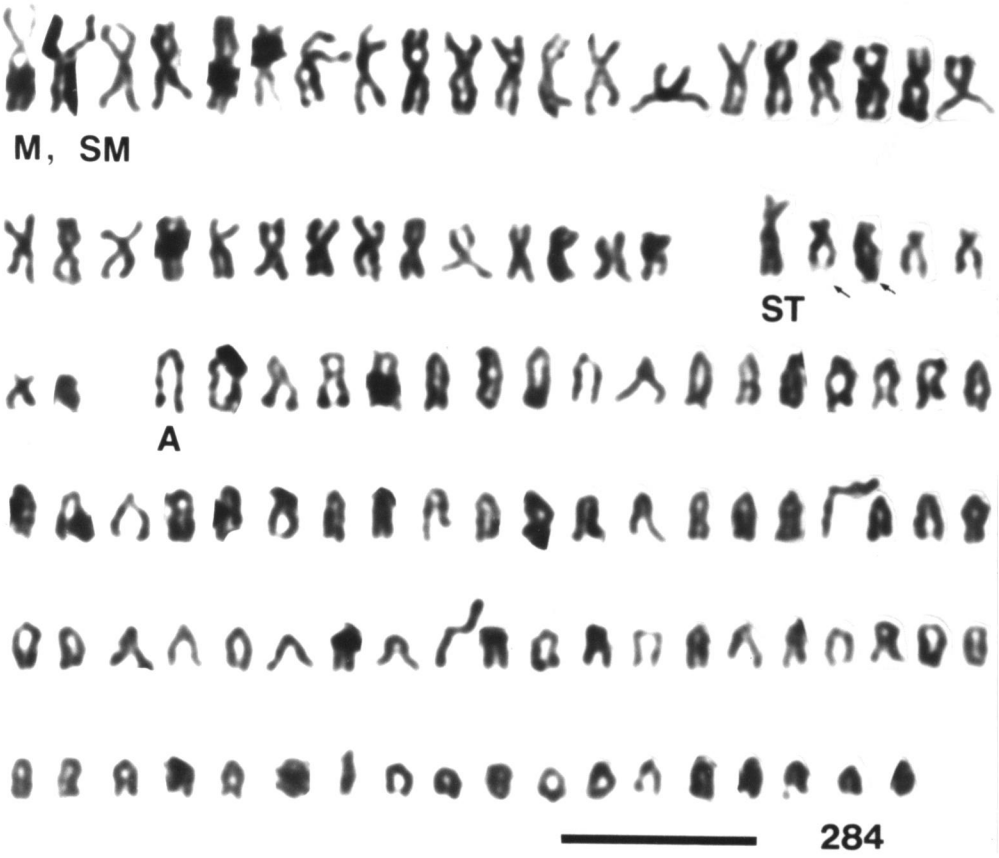
PLATE XXXIV.

Fig. 283. A photomicrograph of metaphase chromosomes of the allotriploid hybrid 2chum salmon♀ x Japanese char♂ embryo from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination, 3N=116. Scale indicates 10 μm.

Fig. 284. A karyogram of the allotriploid hybrid 2chum salmon♀ x Japanese char♂, with 3N=116. The karyotype was comprised of 34 meta- or submetacentric (M, SM), 7 subtelocentric (ST) and 75 acrocentric (A) chromosomes, which was the exact sum of the diploid set of maternal chum salmon and the haploid set of paternal Japanese char. Arrows indicate satellites at the distal portion of long arms of two subtelocentric chromosomes. Scale indicates 10 μm.



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PLATE XXXV.

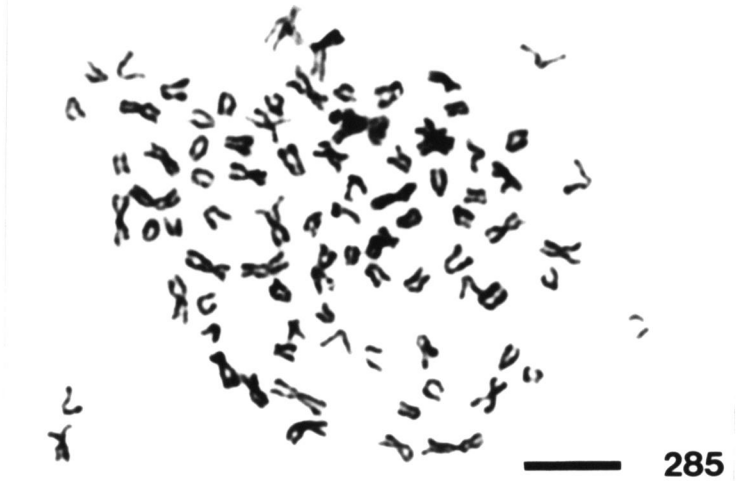
Photomicrographs of metaphases of a 16-day embryo of the aneuploid hybrid Japanese char♀ x chum salmon♂ from eggs subjected to hydrostatic pressure (650 Kg/cm^2 x 7 minutes) at 15 minutes after insemination.

Fig. 285. An aneuploid cell with 90 chromosomes.

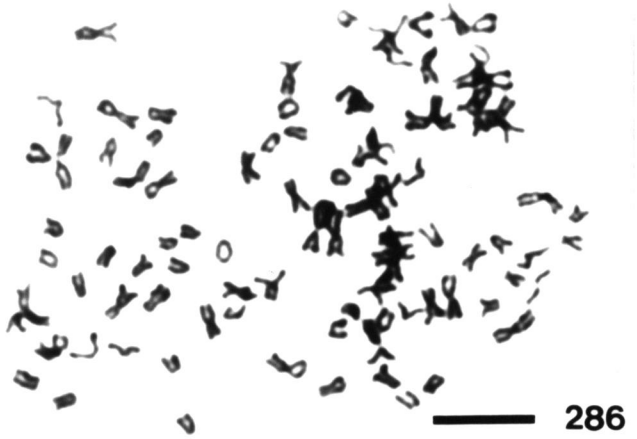
Fig. 286. An aneuploid cell with 98 chromosomes.

Fig. 287. An aneuploid cell with 103 chromosomes.

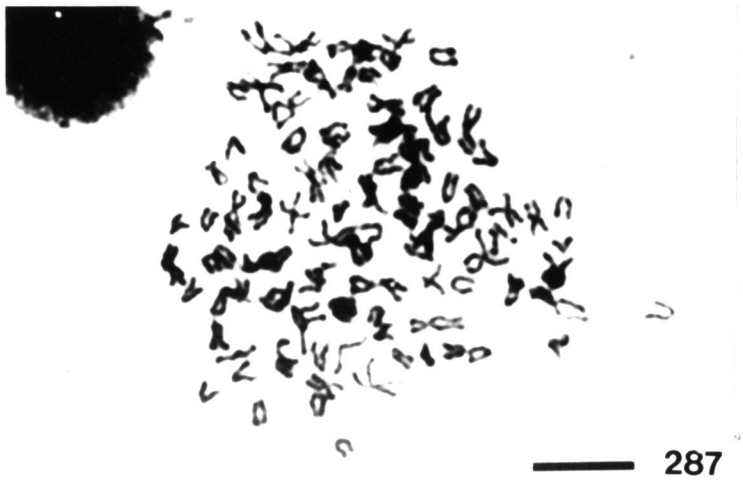
Scales indicate 10 μm.



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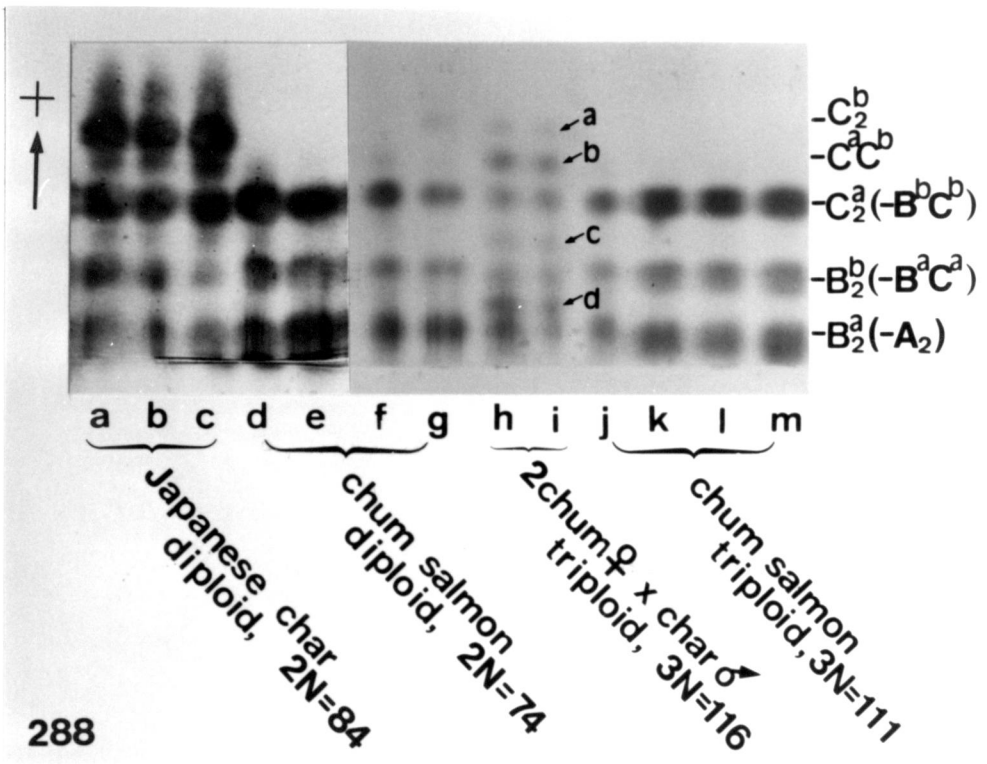


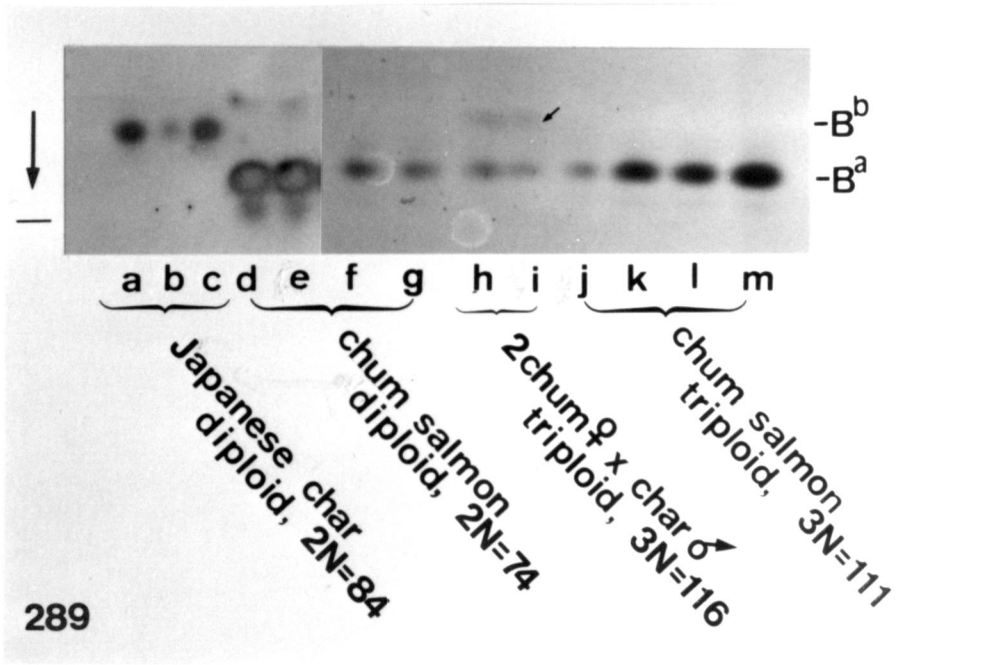
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PLATE XXXVI.

Fig. 288. Malate dehydrogenase (MDH) isozyme patterns in the triploid hybrid 2chum salmon♀ x Japanese char♂ with 116 chromosomes. Arrow (a) indicates paternal homodimer MDH-C_2^b, and arrows (b),(c) and (d) indicate heterodimers MDH-C^aC^b, MDH-B^aC^b, and MDH-B^aB^b, respectively.

Fig. 289. Phosphoglucomutase (PGM) isozyme patterns in the triploid hybrid 2chum salmon♀ x Japanese char♂ with 116 chromosomes. Arrow indicates paternal PGM-B^b isozyme.


+
a
b
c
d
$-C_2^b$
$-C^aC^b$
$-C_2^a(-B^bC^b)$
$-B_2^b(-B^aC^a)$
$-B_2^a(-A_2)$
a b c d e f g h i j k l m
Japanese char diploid, 2N=84
chum salmon diploid, 2N=74
2chum♀ x char♂ triploid, 3N=116
chum salmon triploid, 3N=111
288


−
$-B^b$
$-B^a$
a b c d e f g h i j k l m
Japanese char diploid, 2N=84
chum salmon diploid, 2N=74
2chum♀ x char♂ triploid, 3N=116
chum salmon triploid, 3N=111
289