Increased Viability of Allotriploid Pink Salmon $\times$ Japanese Char Hybrids^{*1}

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Triploidization of hybrids between female pink salmon *Oncorhynchus gorbuscha* and male Japanese char *Salvelinus leucomaenis* was performed by treating fertilized eggs with hydrostatic pressure. The embryos were confirmed to be allotriploids comprising two sets of maternally derived genome and one set of paternally derived genome by observations of chromosomes and measurement of erythrocytes. No diploid hybrid developed beyond the eyed stage, while 62.5% of triploid hybrids reached the eyed stage and 26.1% hatched out. This evidence showed an increased viability of hybrids by artificial allotriploidization. From the present and previous results on viabilities of allotriploids, it is estimated that an influence of paternally derived genome has an essential role for ruling survival potentials of triploid hybrids.

Allotriploids have been reported to show an increased developmental potential even in inviable crossings.¹⁻⁵⁾ Arai⁵⁾ reported that embryos from the experimental hybridization between chum salmon *Oncorhynchus keta* ♀ and brook trout *Salvelinus fontinalis* ♂ were inviable except for a small number of spontaneous allotriploids, but those from the artificial triploidization showed a high degree of developmental potential. These results are important from the viewpoint of breeding and developmental biology. Allotriploidization may provide a useful method to produce viable hybrids, even in the case when crossing ends in the production of inviable hybrids, and also provide an informative material toward understanding genome-genome and genome-cytoplasmic interactions.

In the present study, diploid and triploid hybrids between pink salmon *Oncorhynchus gorbuscha* ♀ and Japanese char *Salvelinus leucomaenis* ♂ were made. Resultant diploid hybrids were inviable, while triploid hybrids showed increased viability. An important role of paternally derived genome as a primary contributing factor in viabilities of allotriploids is discussed.

Materials and Methods

Pink salmon *Oncorhynchus gorbuscha* were provided by the Oshima Branch of Hokkaido Salmon Hatchery in Yakumo. Japanese char *Salvelinus*

leucomaenis was provided by the Nanae Fish Culture Experimental Station of Hokkaido University in Hakodate. Mature ova were collected from single female pink salmon and milt was collected from single male pink salmon and Japanese char.

Artificial crossings of pink salmon ♀ $\times$ pink salmon ♂ (P $\times$ P) and pink salmon ♀ $\times$ Japanese char ♂ (P $\times$ C) were carried out. Seven minutes after fertilization about a half of eggs from each crossing were treated to induce triploidy using 700 kg/cm² of hydrostatic pressure for seven minutes, according to the procedure by Onozato.⁶⁾ Each group was subdivided into two groups. One was used to check the daily survival rate, the other was used to examine the ploidy and the external appearance of developing embryos. Eggs were incubated in the hatching boxes at an average temperature of 12.2°C.

To identify the ploidy, chromosome preparations were made on 9 day-old embryos by the chopping method.⁷⁾ Thirty five to fifty day-old individuals were sectioned in thickness of 8 μ m and stained with hematoxylin-eosin. Nuclear diameter of 20 erythrocytes from each individual were measured. Statistical tests (*t*-test) for the difference in the average nuclear diameter of ten individuals of the P $\times$ P control group and the average nuclear diameter for each individual of both HP-shocked groups were carried out. The same tests were also carried out between the theoretical average

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Table 1. Ploidy check on the base of chromosome number on 9 day-old embryos

Crosses		Groups	Number of Embryos		Mode of Chromosome Number	
♀	♂		2n	3n	2n	3n
pink	× pink	Cont.	10	0	52	—
		HP	1	8	52	78
pink	× char	Cont.	7	0	68	—
		HP	0	7	—	94

HP: treated with hydrostatic pressure.

of triploids calculated from the average of the $P \times P$ control group ($3n = 2n \times (1.5)^{1/3}$) and the averages from each individual of both HP-shocked groups.

Several embryos were fixed with Bouin's fixative every 3–5 days and scanned for external appearances under a binocular microscope for morphological study.

Results

Ploidy Determination

Results from the chromosome observations of 9 day-old embryos are given in Table 1. All $P \times P$ controls had a diploid complement ($2n = 52$),⁸⁾ while eight of nine embryos from the HP-shocked $P \times P$ group showed a triploid karyotype ($3n = 78$) and the remaining one was diploid. These results indicate that HP treatment successfully induced a high percentage of triploidy (93.3%). In the $P \times C$ hybridization, all embryos from the control group had $2n = 68$ chromosomes which was equal to the sum of a maternally derived haploid set from the pink salmon ($n = 26$)⁸⁾ and a paternally derived set from the Japanese char ($n = 42$).⁹⁾ All embryos from the HP-shocked group showed an allotriploid karyotype with 94 chromosomes, which consisted of two sets of maternally derived haploid complements from the pink salmon and one set of a paternally derived chromosome complement from the Japanese char.

Results from the measurement on the nuclear diameter of erythrocytes from 35–50 day-old individuals are shown in Fig. 1. All but one individual from the HP-shocked $P \times P$ group (8/9) and all the individuals from the HP-shocked $P \times C$ hybrid group (4/4) were judged to be triploids. The remaining individual was judged to be diploid.

Viability

The survival curves of 4 groups were shown in Fig. 2. In the $P \times P$ control group, 100% of total eggs reached the eyed stage and 97.6% of those

Fig. 1. Nuclear diameter of erythrocytes from HP-shocked pink salmon (1–9) and HP-shocked hybrids (10–13). No. 1 was judged to be diploid. No. 2–13 were judged triploid. Vertical bars indicate 99% confidence limits. (A) shows the average nuclear diameter from the pink salmon control group. (B) shows the theoretical average calculated from the average of the pink salmon control group.

Fig. 2. Survival curves of four group. ■, pink salmon ♀ × pink salmon ♂ control; □, pink salmon ♀ × pink salmon ♂ treated with hydrostatic pressure; ●, pink salmon ♀ × Japanese char ♂ control; ○, pink salmon ♀ × Japanese char ♂ treated with hydrostatic pressure.

hatched out. The HP-shocked $P \times P$ group showed a slightly lower survival rate (95.5%) at the eyed stage than the $P \times P$ control, and 81.8% of these embryos reached hatching. There was a remarkable difference in survival rates between the $P \times C$ hybrid control and HP-shocked $P \times C$ hybrid groups.

No embryo from the $P \times C$ hybrid control group was able to develop beyond the eyed stage, while 62.5% of embryos from the HP-shocked $P \times C$ hybrid group reached the eyed stage and 26.1% of them hatched out. Some of these larvae survived more than 5 months.

Morphogenesis

At the eyed stage (25 days) no apparent difference was recognized in the appearance between the $P \times P$ control and HP-shocked $P \times P$ groups (Figs. 3a and b). Embryos from the $P \times C$ hybrid control group exhibited severe developmental arrest with malformations including small body size or disruption of embryos. Some embryos from

the HP-shocked $P \times C$ group developed normally, and some embryos showed abnormal body development with microphthalmia and microcephalia (Fig. 3c and d).

The $P \times P$ control, HP-shocked $P \times P$ and HP-shocked $P \times C$ hybrid groups began to hatch at 49, 50 and 36 days after fertilization, respectively. There was no morphological difference between larvae from the $P \times P$ control and HP-shocked groups. But external differences of larvae from the $P \times P$ control group versus those of the HP-shocked $P \times C$ hybrid group was evident. Larvae from the HP-shocked $P \times C$ hybrid group had more pigmented skin than the other two groups (Fig. 3e and f). Parr marks were not found in fingerlings from the $P \times P$ control and HP-shocked $P \times P$ groups but were found in those from the HP-shocked $P \times C$ group.

Discussion

From the ploidy determinations by chromosome observations and erythrocyte measurements, it was proved that triploidy was successfully induced by the HP treatment in both $P \times P$ and $P \times C$ crossing. Judging from the intermediate chromosome number ($2n=68$) being equal to the sum of each haploid genome from pink salmon ($n=26$) and Japanese char ($n=42$), diploid hybrid nature was confirmed in embryos from the $P \times C$ hybrid control. The triploid karyotype consisted of two sets of maternal pink salmon genome ($2n=52$) and one set of paternal Japanese char genome in embryos from the HP-shocked $P \times C$ group proved allotriploid nature. Better viability of triploid hybrids than of diploid hybrids was clearly demonstrated. This increased viability was assumed to be caused by doubling the female genome. The earlier initiation of hatching, the more heavily pigmented body and the presence of parr marks on the HP-shocked $P \times C$ group proved obvious expressions of paternally derived genes in allotriploids. Thorgaard⁹⁾ suggested in his review that a decrease of the ratio of the paternally derived genes might relate to the increased viability in allotriploids. In addition, Arai¹⁰⁾ pointed out that the function of maternal ooplasm must be considered because the hybrid incompatibility might also act between paternal genome and maternal cytoplasm. Previously reported result including this work regarding the viabilities of diploid and triploid hybrids are summarized in Fig. 4. Judging from these results, there is a possibility that survival

Fig. 3. External appearances of individuals from four groups. Twenty five day-old embryos from the control pink salmon (a), the HP-shocked pink salmon (b), the $P \times C$ hybrid control (c) and the HP-shocked $P \times C$ hybrid (d). Fifty days old larva from the control pink salmon (e) and forty five days old larva from the HP-shocked $P \times C$ hybrid (f). Scale bars indicate 5 mm.

♀ \ ♂	<i>S. leucomaenis</i>	<i>S. fontinalis</i>	<i>S. trutta</i>	<i>S. gairdneri</i>	<i>O. tshawytscha</i>	<i>O. keta</i>	<i>O. gorbuscha</i>	<i>O. kisutch</i>
<i>Salvelinus leucomaenis</i>						X		
<i>S. fontinalis</i>			2	X				
<i>Salmo trutta</i>		2		X				
<i>S. gairdneri</i>		1, 2	1, 2					1, 4
<i>Oncorhynchus tshawytscha</i>							10	
<i>O. keta</i>	3	5						
<i>O. gorbuscha</i>	P				10			
<i>O. kisutch</i>

 -DIPLOIDS-
 INVIABLE

 -TRIPLOIDS-
 INVIABLE

 VIABLE

 INVIABLE

 VIABLE

1-5, 10
 REFERENCE NUMBER

P
 PRESENT STUDY

Fig. 4. Viabilities of salmonid diploid and triploid hybrids from previous and present studies.

potential of triploid hybrids is caused by the paternal species. Because two sets of maternally derived genome in allotriploids are sufficient to support a normal embryonic development, survival potential of allotriploids may depend on the strength of the harmful influence by the paternally derived genome on maternally derived genome or ooplasm. For example, brook trout *Salvelinus fontinalis* has little harmful effect as a sperm donor, as three kinds of hybridization with male brook trout produced viable triploid hybrids. In contrast, rainbow trout *Salmo gairdneri* has a strong harmful effect, because two kinds of triploid hybrids using rainbow trout as paternal species resulted in failure. However, further studies about diploid hybrids and allotriploids consisting of two sets of the maternal genome and one set of the paternal genome ($\text{♀}2 \times \text{♂}1$) are needed to confirm this rule. Ueda *et al.*¹¹⁾ reported a method of sperm-fusion by polyethylenglycol. Allotriploids with $\text{♀}1 \times \text{♂}2$ genome made by this method may provide an informative experimental model to study the role of the surplus genome. Nucleo-cytoplasmic

hybrids with $\text{♀}0 \text{♂}2$ genome are especially useful to know interactions between the paternally derived genome and the maternally derived cytoplasm.

Allotriploidization has a possibility of producing useful hybrids for aquaculture. Parsons *et al.*⁴⁾ reported that diploid hybrids between rainbow trout $\text{♀} \times$ coho salmon ♂ were inviable but that triploid hybrids were viable and had inherited resistance to infectious hematopoietic necrosis (IHN) virus from the coho salmon. This kind of investigation on triploid hybrids also have to be carried out.

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