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Fertility of Hybrids between Female Masu Salmon, *Oncorhynchus masou* and Male Pink Salmon, *O. gorbuscha*

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Abstract

Gonads of the hybrids between female masu salmon and male pink salmon were investigated anatomically and light microscopically. One (of 22) was fertile and ovulated 125 eggs. All the rest were sterile with abnormal intersexual gonads which obviously could not produce any eggs or sperm. One allotetraploid hybrid of the same combination showed normal ovaries. Sex maturity in hybrid seems more to relate with the genetic distance of their parental species than the difference of chromosome numbers. The appearance of intersex is considered to have something to do with plasm factors. Tetraploid male fish may be difficult to use as paternal fish because they are deemed to produce sperm too large to fertilize eggs.

Development of gonads in hybrids made from different combinations of parental species varies significantly at different stages (Chevassus, 1983). Some of the combinations present normal maturation of both the sexes, for instance, *Oncorhynchus keta* × *Oncorhynchus nerka* hybrids (Terao et al., 1965, in salmonids), some just mature in one of the sexes such as carp × funa hybrids (Chiba et al., 1979, in cyprinids) and others can mature in neither of the sexes, for example *Salmo trutta* × *Salvelinus fontinalis* hybrids (Suzuki and Fukuda, 1973). Besides these, skewing of sex ratio in some of hybrid fish was also noticed by several authors (Hickling, 1969; Suzuki, 1961; Suzuki, 1973).

It is of great importance to aquaculture to know whether hybrids can mature or not. The hybrids, sterile of both the sexes, would be good materials for stocking in an open water system with assured control of the fish population without too many difficulties. In sterile female salmonid hybrids, the value of body weight without viscera against the total body weight are greater than those in the controls, and sterile salmonids were noticed to have assumed silvery lustre which is smoltification and their muscles retained reddish orange colour even when they reached their third autumn (Suzuki and Fukuda, 1973). That makes the fish of higher market value. With the use of hybrids fertile in both the sexes, the next generation may be available by intracross, backcross or 3-way-cross. Repeated backcrosses may give us a way to transport some characteristics from one species to another. With hybrids which mature in only one of the sexes is possible to make backcrosses with paternal or maternal species or 3-way crosses with other species. In *Tilapia*, the F_1 generation is all-male and the male individuals usually grow faster and have larger body size than females. Moreover, production of an all-male population can also avoid

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producing individuals too small body size due to over reproduction and crowding.

Masu salmon is an important economic fish in Japan and there have been many studies on this fish. Some experiments have proven that hybridization between masu salmon and pink salmon produce viable offspring (Arai, 1984). Several reports on such hybrids have described some genetic and physiological characteristics (Arai, 1984 ; Ma and Yamazaki, 1986). Those reports have confirmed that such combination gives birth to a new real hybrid instead of parthenogenesis. Consequently an investigation of the gonads of the hybrids between female masu salmon and male pink salmon was made both anatomically and light microscopically by the authors.

It is generally believed that sterile diploid hybrid can be made fertile by tetraploidization. Therefore we tried to make tetraploid hybrids with the same combination by the hydrostatic pressure method, as described by Onozato (1984), to see whether or not allotetraploid F_1 shows any difference from the diploid in sex maturation.

In the present paper, we are going to describe the features of the gonads in the diploid and tetraploid hybrids and discuss the phenomena in comparison to what has been reported by other authors.

Materials and methods

Twenty-two diploid hybrids and one 8-month old tetraploid hybrid were dissected to observe the gonads. Diploid hybrids were aritificially produced on October 15, 1983 as mentioned in a previous paper (Ma and Yamazaki, 1986). Tetraploid hybrids were made by treatment of the fertilized eggs with a hydrostatic pressure of 700 kg/cm^2 for 7 minutes 6.5 hours after incubation at 10°C on October 3, 1985. The parental fish were obtained from the same places as in 1983. All these experiments were carried out at the Faculty of Fisheries, Hokkaido University. Before used in this study, the chromosome number of the fish treated with hydrostatic pressure was investigated by cell culture and the result showed that the fish used was tetraploidy. The gonads of diploid hybrids were weighed and some were photographed before being fixed by Bouin solution. Sections of the gonads were cut in thicknesses of 3 and 7 μm and stained with hematoxylin-eosin. The preparations were scanned under a microscope and microphotographed.

Results

In the diploid hybrid fish investigated, one matured and ovulated 125 eggs at 2 years of age and all of the others were sterile. Fig. 1 and 2 show the appearances of both the fertile and sterile hybrids with their eggs or gonads. Silvery lustre of the matured female hybrid was concealed by the nuptial colouration but that of the sterile hybrids did not even though some were older than the female individual. We failed to check the capacity of fertilization of these eggs because the fish had died when we found it and sperm was not available. The numerical characteristics of the matured individual were counted and they confirmed it to be a true hybrid. The sterile fish had very small and abnormal gonads. The gonad weight, fork length and body weight of the fish older than 1.5 years are listed in Table 1.

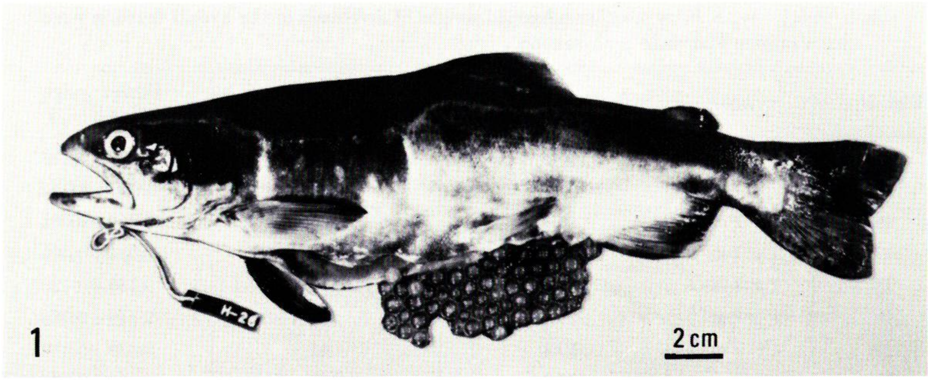


Fig. 1. External appearance of the maturing female hybrid at two years old with its ovulated eggs. The fork length is 29.9 cm.

Fig. 2. External appearance of a sterile specimen of the hybrid two years old with its gonads. The fork length is 28.5 cm.

Gonads of the fish numbered 14, 15 and 17 looked very much like ovaries, testes and hermaphroditism respectively from their external appearances. The gonads of fish No. 14 were full of small eggs about 1 mm in diameter and its tissue colour was reddish. The gonads of No. 15 were white in colour and no eggs could be externally observed. Number 17 had white gonads containing a few visible eggs in the front of the gonads. The three fish were selected as typical specimens and sections of their gonads were made. Figs. 3, 4, 5 show the light microscopic structure of these gonads. All the three were of abnormal microscopic structure. Apart from evident eggs, a testicular lobule-like structure containing cells in different stages was also found. Then sections of gonads of all the hybrids were cut and all fish showed the same phenomenon except for No. 26. The only difference between them was just the proportionate volume of eggs to testicular lobule-like tissue, but they were not essentially different from one another. The eggs seemed to abort in development. The 'testicular lobules' were very different from the normal and no sperm was observed. The cells in these 'lobules' did not seem to take synchronous division to shape up cysts. The developing cells got moved into the center by division and the nuclei began to shrink before they died perhaps due to lack of oxygen. Successive

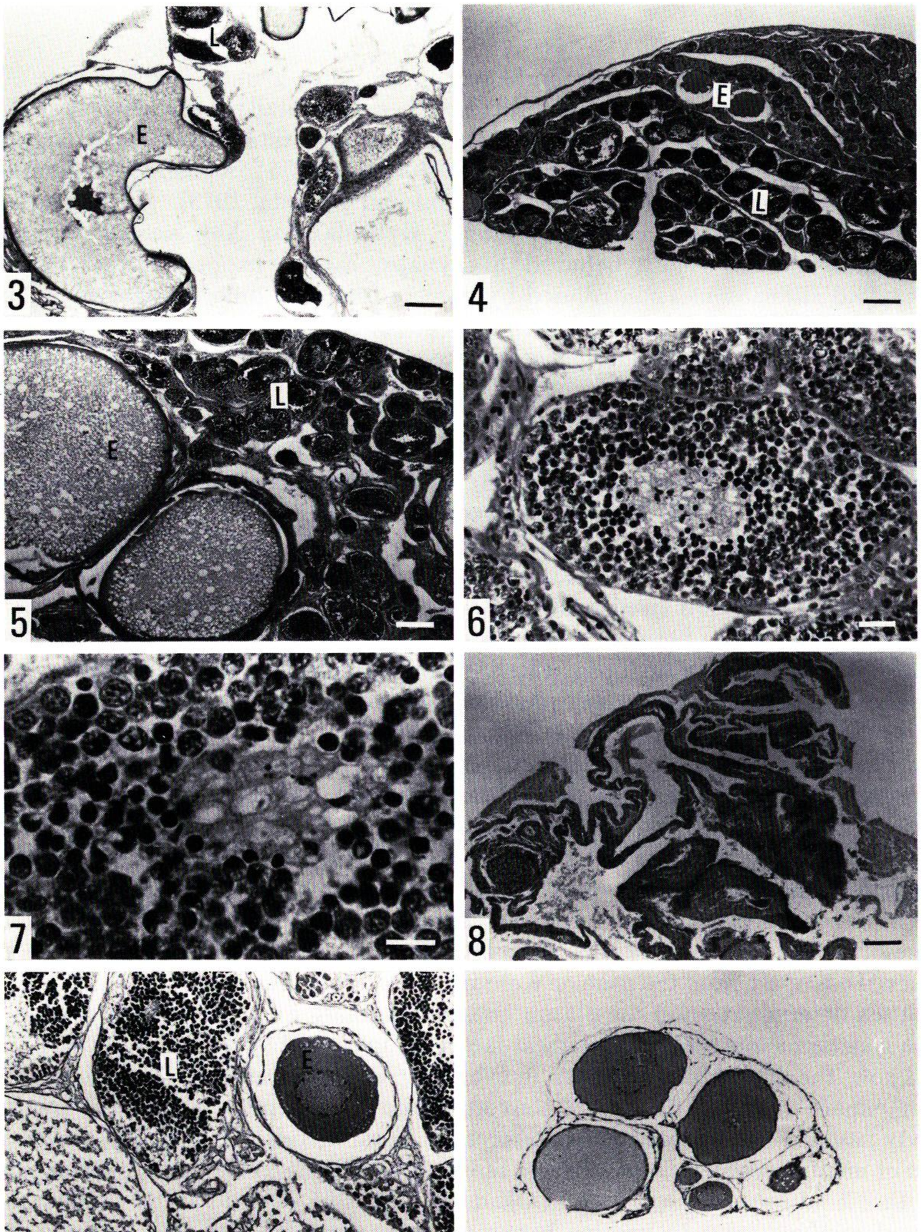
Table 1. Fork length, body weight and gonad weight of seventeen of the hybrid between female masu salmon and male pink salmon.

Fish No.	Age	Fork length (cm)	Body weight (g)	Gonad weight (L+R) (g)
14	1 y 7 m	28.8	263	0.360+0.253
15	1 y 7 m	27.0	210	0.050+0.0.82
16	1 y 7 m	26.4	205	0.046+0.061
17	1 y 7 m	26.1	208	0.084+0.095
18	1 y 7 m	28.5	277	0.133+0.120
19	1 y 7 m	27.8	251	0.110+0.028
20	1 y 8 m	19.4	41	0.020+0.010
21	1 y 9 m	26.8	218	0.194+0.234
22	1 y 9 m	23.7	148	0.087+0.050
23	1 y 9 m	24.1	156	0.035+0.023
24	1 y 10 m	26.5	156	0.092+0.076
25	1 y 10 m	26.2	190	0.030+0.034
26	2 y 0 m	29.9	281	*
27	2 y 0 m	25.2	144	0.142+0.000
28	2 y 2 m	28.5	244	0.050+0.204
29	2 y 4 m	33.0	431	0.519+0.679
30	2 y 4 m	20.4	78	0.103+0.078

* One hundred and twenty-five eggs were ovulated.
L-left; R-right; y-year; m-month.

absorption of the dead cells resulted in the formation of a vacant cavity in center of the 'lobules' (Fig. 6). We are positive that neither sperm nor mature eggs could be produced by such gonads. Fig. 7 shows the cells in different stages in a 'testicular lobule'. We cut sections of the gonads of fish No. 26 and found no testicular lobule-like tissues (Fig. 8). After finding these interesting phenomena, we dissected another 5 hybrids at 10-months of age to see the condition of younger gonads. What we saw was no different from the sterile hybrids described above (Fig. 9). The observation of the allotetraploid individual revealed the gonads of normal ovarian structure (Fig. 10).

Fig. 3. Microscopic structure of the gonad of hybrid numbered 14. Bar indicates 0.2 mm. (E : egg; L : lobule-like part).
Fig. 4. Microscopic structure of the gonad of hybrid numbered 15. Bar indicates 0.2 mm.
Fig. 5. Microscopic structure of the gonad of hybrid numbered 17. Bar indicates 0.2 mm.
Fig. 6. A lobule-like part in the gonads of sterile hybrids. Bar indicates 0.02 mm.
Fig. 7. An enlarged picture showing the cells in a 'lobule'. Bar indicates 0.01 mm.
Fig. 8. A part of the ovary of hybrid numbered 26 after ovulating. Bar indicates 0.2 mm.
Fig. 9. Microscopic structure of gonad of the hybrid at 10 months old. Bar indicates 0.1 mm.
Fig. 10. Microscopic structure of the gonad of the allotetraploid hybrid. Bar indicates 0.1 mm.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Discussion

Masu salmon and pink salmon belong to the same genus: *Oncorhynchus*. In salmonids, a number of different hybrids have been artificially made between species belonging to same or different genera. Of the intrageneric hybrids, sexual maturation has been reported by many researchers (Foeyster, 1935; Soguri, 1936; Stenton, 1950; Alm, 1955; Terao et al., 1965; Suzuki and Fukuda, 1973). In the present study, we too obtained a fertile individual. Hybrids often show sterility in higher vertebrates and it is usually believed that synapsis can not happen between two sets of haploid chromosomes contributed by parents from two different species because there are no homologous chromosomes between different species. Chromosomes can not pair with their homologous hence gametogenesis stops in primary spermatocytes. But, this illustration can not explain this question: Why are some hybrids in fish able to mature? In some interspecific crosses, fertility seems to have no relation to the difference of chromosome numbers between two parental species. For example, *O. keta* ($n=37$) $\times$ *O. gorbuscha* ($n=26$) hybrids are fertile, but although the difference of chromosome numbers between *O. rhodurus* ($n=33$) and *S. trutta* ($n=40$) is much less than that between *O. keta* and *O. gorbuscha* their hybrids can not mature sexually (Suzuki and Fukuda, 1973). Based on these results, it seems that genetic distance of two species has more impact on fertility than the difference in chromosome numbers between two parental species. If we want some sterile salmonid fish to stock in an open water system, intergeneric hybrids may be good materials. On the contrary, hybridization between two species in a same genus has the possibility of producing fertile salmonid hybrids.

The other phenomena in the present study is the sterility combined with intersexual gonads in most of the hybrids observed. Suzuki and Fukuda (1973) studied many hybrids between salmonids and obtained both fertile and sterile hybrids, however they did not cut sections of the gonads to observe the internal structure. Intersex is a phenomenon widely existing in many kinds of hybrids. No one however has given a satisfying explanation of these phenomena. It is difficult to resolve this problem by the theory of X-Y chromosome determination. We believe that apart from the gene on a chromosome, there must be some factors related with sex determination in the plasm. Sex determination in fish seems to depend on both genetic factors and physiological factors as indicated by Yamazaki (1983). As we know, the determination of sex in fish, especially in salmonids, is not so strict as in mammals. It is not difficult to convert female masu salmon into male by treating newly hatched fish with methyltestosterone. In salmonids, the optimum concentration of methyltestosterone required to induce masculinization is quite low compared to other kind fishes. According to Okada et al. (1983), over dosage of sex hormone caused rainbow trout to become hermaphroditic when females were induced into males. Therefore, any of the factors resulting in sexual physiological derangement may be possible to cause the phenomenon of intersex, sterility. Hybridization often induces the appearance of these factors. There are however too few studies on factors such as endocrine gland and salmon reproductive systems to make any conclusion. As to the sex ratio skewing in one direction in this study, it is perhaps because sterile fish were of stronger heterosis for survival than fertile fish, for the majority of the hybrids died before this experiment was concluded. It is not contradictory to Holdane's rule although only one female was obtained because it matured.

The allotetraploid hybrid showed normal gonad structure. Tetraploidy is very difficult to make in salmonids. Those we produced showed rather low survival and growth rates compared to the diploid hybrids. They were so weak and fragile that the longest lived individual died at 8 months after fertilization at a fork length of 4.8 cm though we took more care of it than the others. Because of the small number of the tetraploid hybrid, it is difficult to discuss the maturation and sex determination. The frequency of appearance of female individuals in the diploid hybrid seemed very low whereas the longest lived tetraploid hybrid was female. We could not get a male to check its gonads to determine whether male individuals can become grownup or if they will have normal testes. Whether a fertile tetraploid male fish, if any, can be used to mate normal females is still open to question. We suspect that the diploid sperm produced by fertile tetraploid male will not be able to fertilize normal eggs because they might be too large to enter eggs through the micropyles.

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