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BIOTECHNOLOGICAL RESEARCHES AT THE PREFECTURAL FISHERIES EXPERIMENTAL STATION IN JAPAN

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ABSTRACT

Biotechnological studies have been conducted in 46 Prefectural Experimental Stations in Japan in 1991. In 41 of these, research focused on chromosome set manipulation including triploidy for sterilization and gynogenesis for sex control. Practical application of biotechnology for culture of each species is the main interest because each prefecture has its own project for promoting the local fisheries industry. Therefore, the commodities being studied are of commercial importance comprising of about 40 species.

The culture production of Japan in 1988 totaled 1,426,000 tons, 95% of which consisted of 10 species. Biotechnology is not widely used since most seeds are not from hatcheries, but from the wild. Recently, however, promising results on the study of sex determination mechanism in the Japanese flounder have been adopted for actual seed production. This has attracted attention as an approach to mass production of all-female seedlings.

INTRODUCTION

In Japan, biotechnological studies are carried out on various species of fish, shellfish, and algae. Most of them are not practical but basic research in aquaculture.

In the Prefectural Fisheries Experimental Stations, biotechnological studies focused on chromosome set manipulation, including triploidy for sterilization

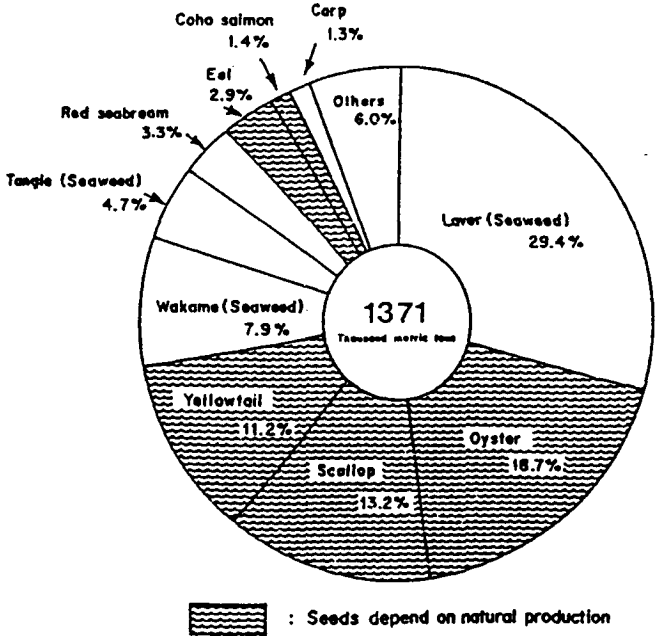

Fig. 1. Culture production of Japan in 1989

and gynogenesis for selective breeding and sex control. The stations' main concern is the practical application of biotechnology for aquaculture of each species because each prefecture has its own projects for promoting the local fisheries industry. As many as 40 species are being studied.

The culture production of Japan in 1989 totaled 1,370,600 tons, 94% consisted of 10 species (laver, oyster, scallop, yellowtail, wakame seaweed, tangle, red sea bream, eel, coho salmon, and carp in this order). Biotechnology has been rarely used in many of these commodities because most seeds are from the wild (Fig. 1). Recently, however, promising results were obtained on sex determination and sex differentiation of the Japanese flounder, *Paralichthys olivaceus* and these have been used for actual seed production. This has attracted attention because it is an approach to mass production of all-female seedlings (Fig. 2).

TRIPLOIDY

One of the most important requirements for aquaculture is constant production and supply of seedlings. This requires healthy and mature animals. However, culturists whose primary consideration is attaining marketable size in a short culture period may find maturation an undesirable process because

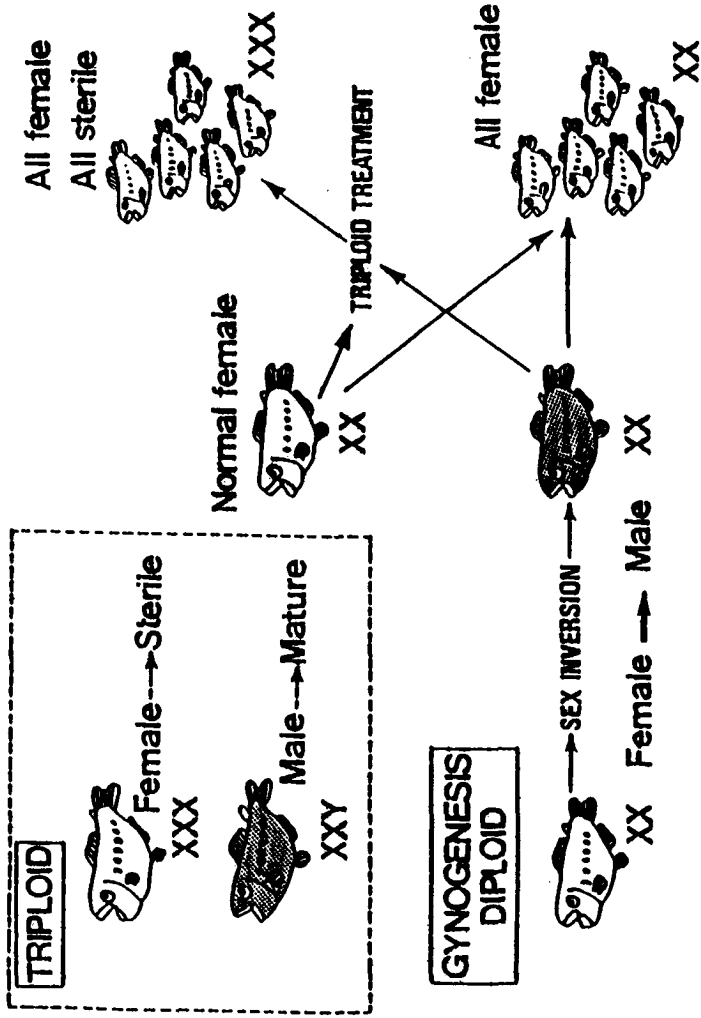

Fig. 2. All-female production systems by using chromosome set manipulation in rainbow trout

it retards growth and may cause onset of diseases. Studies on triploidy are being conducted to produce sterile seedlings. Triploidy has been studied in 41 Prefectural Fisheries Experimental Stations, using 20 species of fish *(Paralichthys olivaceus, Limanda yokohamae, Pagrus major, Acanthopagrus schlegeli, Oplenathus fasciatus, Fugu rubripes, Oncorhynchus kisutch, O. masou, O. nerka, O. rhodurus, O. mykiss, Salvelinus leucomaenis, Cyprinus carpio, Plecoglossus altivelis, Carassius buevgeri grandocutis, C. cuvieri, Misgurnus anguillicaudatus, Oreochromis mossambicus, Gnathopogon caerulescens,* and *Odontheste bonariensis)* and 4 species of shellfish *(Haliotis discus, Crassostrea gigas, Pinctada fucata martensii,* and *Chlamys nobilis).*

A triploid can be induced by inhibition of meiosis in eggs. Retention of the second polar body, which is normally extruded from the egg, may produce the embryo with three sets of genome consisting of female, male, and second polar nucleic chromosome. If eggs are stimulated after insemination by temperature or pressure, meiosis is inhibited, producing triploid rather easily.

Studies have been done to find out whether or not the triploid is sterile among many species. It is now clear that the extent of sterility in triploids varies among species, and, in addition, among sexes of the same species. In the triploids of rainbow trout and Japanese flounder the female triploid (XXX) is sterile but the male triploid (XXY) can mature (Tabata 1991a). Furthermore, the scallop, *C. nobilis,* is sterile in both sexes (Komaru and Wada 1989), while the Japanese pearl oyster, *P. fucata martensii,* matures in both female and male (Komaru and Wada 1990).

It is expected that growth rate of the triploid would be higher than that of diploid, but no studies have been done to confirm this.

Production of a triploid by mating a tetraploid with a diploid has not been well established in Japan because it is quite difficult to produce tetraploid.

GYNOGENESIS

In most fish species cultured in Japan, males mature earlier than females- Females are larger than males in general because maturity retards growth in males, while females continue to grow. Many Japanese are fond of eating fish eggs, thus the female fish with eggs commands a higher price than male. Although female fish are more profitable than the male, the sex of juveniles cannot be determined by external appearance and fish have been cultured in mixture of males and females.

Gynogenesis, which means development of embryos with only maternal nucleus, is an important technique for selective breeding and sex control. It will make all offsprings female in the type species with the male heterozygote (XY). Some 36 Prefectural Fisheries Experimental Stations have now studied gynogenesis using 17 fishes *(Paralichthys olivaceus, Pagrus major, Acanthopagrus schlegeli, Oncorhynchus kisutch, O. masou, O. nerka, O. rhodurus, O. mykiss, Salmo salar, Plecoglossus altivelis, Carassius buevgeri grandocutis, C. cuvieri, C. auratus, Cyprinus carpio, Gnathopogon caerulescens, Leptobotia curta,* and *Tribolodon hakonensis).*

The technique for gynogenesis involves two important steps. First is the inactivation of spermatozoa prior to insemination. Spermatozoa can be inactivated genetically by ultraviolet irradiation without damage to their mobility. Development of eggs is initiated by insemination of ultraviolet irradiated sperm. However, embryos will not survive during development because they have only half the desired number of chromosome (haploid) compared with the normal embryo (diploid). To make them survive, it is necessary to have the same number of chromosome as that of the diploid. Thus, the second important step is the inhibition of ejection of the second polar body to induce the gynogenetic diploid, which now contains 2 sets of chromosomes, from the egg nucleus and second polar body. With this method, the technique of mass production of gynogenetic diploid has been developed using 15 species of fish (*Paralichthys olivaceus, Pagrus major, Acanthopagrus schlegeli, Oncorhynchus kisutch, O. masou, O. rhodurus, O. mykiss, Plecoglossus altivelis, Carassius buevgeri grandocutis, C. auratus, Cyprinus carpio, Gnathopogon caerulescens, Leptobotia curia, Tribolodon hakonensis, and Misgurnus anguillicaudatus).*

Theoretically, chromosomes can also be diploidized by inhibition of the first cleavage. However, the survival and production rate of gynogenetic diploids through this procedure is rather low. Further improvements of this method are required.

SEX INVERSION

There is a sex inversion technique used in the mass production of all-female offsprings. Sex can be artificially reversed with sex hormones at the unstable stage of sex differentiation; thus, the female sex hormone is used for inversion to female, and the male sex hormone is used for inversion to male. When females are changed to males by sex inversion they mature like the primary male producing normal spermatozoa. In species where sex is determined by the XY system, sex in versed males will produce only X-chromosome sperms because they exhibit the XX type sex chromosomes. When they are crossed with normal females, all offspring will be of the XX type. These procedures can be used for mass production consisting of all-female seedling

In triploid male (XXY) of rainbow trout, mature triploid itself is not practical for producing sterile seedling. However, when spermatozoa of the sex-inversed male are used to induce triploidy offsprings are of all-female type and do not mature. Thus, we almost succeeded to produce all sterile seedlings (Fig. 2). This technique for producing all-female sterile fish has been developed using spermatozoa from the sex-inversed male, which is genetically female in 5 species offish *(Paralichthys olivaceus, Oncorhynchus kisutch, O. masou, O. rhodurus, and O. mykiss).*

TISSUE CULTURE

Tissue culture of laver *(Porphyra tenera* and *P. yezoensis)* has been studied in the Prefectural Fisheries Experimental Stations. Protoplasts from the leaf body of laver with excellent characteristics for seedling culture have been used.

The technique to culture protoplast has been developed to a fairly high degree but some problems persist such as the abnormal shape of the leaf body generated from the protoplast and difficulty in settling them on the laver net.

NEW TECHNOLOGY TO PRODUCE ALL-FEMALE SEEDLING OF JAPANESE FLOUNDER *(Paralichthys olivaceus)*

In 1989, production of flounder was 4,300 tons and ranked 16th among many species for aquaculture in Japan. Production has increased five times in recent years. In general, male flounder matures at 1 year of age, while it takes 2 years for the female. No difference in growth between male and female is observed up to the end of the first year, but the weight of the female is nearly twice that of the male at the end of the second year because maturation in the male retards growth. Consequently, it is more profitable to use females for commercial culture. Mass production of flounder seedling in the hatchery has been established since 1980s. However, a survey of the sex ratio of hatchery-produced flounders showed abnormally higher rate of males than in the natural population. This may be one of the reasons why many (13) Prefectural Fisheries Experimental Stations started studies on gynogenesis and triploidy in flounder to obtain all-female seedlings.

Recent studies revealed that the Japanese flounder is the male heterozygote type in genetic mechanism of sex differentiation (Tabata 1991a, b, Yamamoto et al. 1991). Consequently, all gynogenetic diploids are females with XX sex chromosome. However, most gynogenetic diploids produced by the ordinary hatchery method included many males. Sometimes over 50% of the offsprings were male, and even 100% male offsprings had been reported (Tabata 1991a). These results indicate that sex differentiation is not of the male heterogozygote type at some stage. After extensive studies, it has been shown that the mechanism of sex differentiation in the Japanese flounder may be genetically controlled by XX or XY type, and that the rearing environment may affect sex differentiation at the early unstable stage of development. Sex inversion from genotypic female to phenotypic male occurred at a high frequency in gynogenetic diploids (Tabata 1991a, b, Yamamoto et al. 1991). Accordingly, for production of all-female seedling it is necessary to do genetic as well as a physiological control to inhibit sex inversion at larval stage. A low dose of female sex hormone is needed to inhibit sex inversion from the genotypic female to phenotypic male. (Tabata 1991b, Yamamoto et al. 1991). The amount of hormone needed for inversion from the genotypic male to female is 10 or more times larger than that which inhibits inversion from female to male.

The inhibition of sex inversion from the genotypic female to male can also be done by controlling water temperatures during larval stage. Genotypic female larvae (16 millimeters in average total length) were raised for 2 months at 20°C which resulted in 93% phenotypic female. This sex ratio was similar to those treated with the female sex hormone. When the rearing temperature was increased to 25°C, the proportion of female was reduced to 66%, suggesting that water temperature may influence sex differentiation.

Production of phenotypic male which is inversed from genotypic female in gynogenetic diploids has been achieved by controlling water temperature at 25°C. Therefore, spermatozoa with only X chromosomes from these sex-inversed males can be used for all-female production. This method is simpler because it does not require any sex hormone treatment. By using the sex-inversed male, efficient mass production of all-female seedling of the flounder has been achieved.

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