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Inbred strains of the medaka, *Oryzias latipes**

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The medaka, a small teleost fish, has been used extensively in the National Institute of Radiological Sciences (NIRS) to analyze the mechanisms of various radiation effects on living organisms since 1962 (Hyodo-Taguchi and Egami, 1969, 1976; Hyodo-Taguchi *et al.*, 1973). This fish has also been used to evaluate the carcinogenic properties of chemical agents (Ishikawa *et al.*, 1976; Aoki and Matsudaira, 1984). During the process of these studies, efforts to create a standard inbred strain of the medaka for laboratory use were started in 1974 by the author; several inbred strains of the medaka have since been successfully established. In 1980, the first paper on the inbred strains of medaka was published by Hyodo-Taguchi (1980); two pedigrees of the orange-red variety and three pedigrees of wild-type fish, were established. In our second paper on inbred strains of medaka, we reported two newly established inbred strains of medaka (Hyodo-Taguchi and Sakaizumi, 1993). Since then, we have been maintaining these inbred strains by full sister × brother mating for 60-71 generations. Moreover, a continuing effort has been made to establish other inbred strains from the medaka with different allozymic characteristics. Recently, two more inbred strains of medaka were established from Korean medaka and from the d-rr strain, respectively.

In this paper, I shall briefly review the process of the establishment of the inbred strains of the medaka and describe laboratory stocks of the inbred strains of the medaka maintained in National Institute of Radiological Sciences; then, as an example of the use of the strain, I shall review the use of the inbred strains of the medaka for studies of genetics and embryology.

General information about maintenance of inbred strains of the medaka is available only from NIRS, but many mutants of body color and morphology of this species have been bred in some laboratories, mainly as mass-cultured colonies, and in some places by the inbreeding method.

Overall survey of efforts to inbreed medaka

To establish inbred strains of the medaka, we carried out sister-brother mating of pairs of fish for successive generations since 1974 in the first group. In this system, one male and one female are selected from the fish born from one pair of parents and are mated in each generation. During inbreeding of many pedigrees, the reproductive potential was reduced, or there was high mortality. Two pedigrees of the orange-red variety of *Oryzias latipes* and three pedigrees of the wild-type fish, however, were successfully inbred by full sister-brother mating for more than 20 generations until 1979 (Hyodo-Taguchi, 1980) and now these experienced 63-73 generations of inbreeding as described in next section. Two families from different populations, a stock (d-rR) at Nagoya University and a stock (NI) at the University of Tokyo, moreover, have been inbred by sister-brother mating since 1980 and 1982, respectively, and finally two inbred strains of medaka, designated Hd-rR and HNI, were established in 1988 and in 1989, respectively (Hyodo-Taguchi and Sakaizumi, 1993). In addition, from two other stocks of the University of Tokyo (SOK) and of Nagoya University (d-rr), two more inbred strains were established in 1995 (Hyodo-Taguchi, unpublished). Besides NIRS, a few inbred strains of medaka have been established, e.g., a tester medaka for the specific-locus method has been established at the University of Tokyo and the tester medaka is now in the 24th inbreeding generation (Shima, 1996).

Laboratory stocks of inbred strains

Several different inbred strains of the medaka are maintained in our laboratory at NIRS. The following is the list of the stocks available.

Wide Type (brown or black) Strains

HB32D: Derived from offspring of fish obtained from a stock at Chiba University, which had been collected near Chiba City around 1970;

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inbred for 63 generations. Substrain HB32C was separated from HB32D at the 16th generation and the 69th generation of this strain is now in very low fecundity. Substrain HB32F has been lost by low fecundity in the 62nd generation.

HB12A: Derived from a different pair in the same stock from which HB32D was derived, and inbred for 67 generations.

HB11A: Derived from a pair of the offspring of the original pair from which HB12A was derived and inbred for 66 generations. The eggs are characterized by an unusual pattern of oil globule fusion after fertilization. This trait is under single locus control. The symbol for the gene *of* (oil globule fusion delay) has been proposed, and the gene is recessive and autosomal (Hyodo-Taguchi, 1979). Substrain HB11C was separated from HB11A at the 16th generation and has been inbred for 69 generations.

HNI-II: Derived from the offspring of fish obtained from the stock (NI) at the University of Tokyo, which had been collected from Niigata City in 1980 (Sakaizumi *et al.*, 1980; 1983); inbred for 38 generations.

HNI-I: Derived from a different pair in the same stock from which HNI-II was derived and inbred for 35 generations. HNI has alleles different from those of HO4, HB32 and HB12 at many protein loci.

HSOK: Derived from the offspring of fish from one of the Korean medakas which were collected in Sokcho City (Sakaizumi and Jeon, 1987); inbred for 25 generations and newly described in this report.

Orange-Red Strains

HO4C: Derived from a stock in NIRS which was obtained originally from a dealer in Chiba Prefecture; inbred for 73 generations. Inbreeding of substrains HO4A, HO4B and HO4C3 did not succeed because of low fecundity around 50-60 generations.

HO5: Derived from a different pair in the same stock from which HO4C derived and inbred for 70 generations.

White and Orange-Red Strains

Hd-rR: Derived from the offspring of fish obtained from a stock (d-rR) at Nagoya University. The females are white (*bb*, X^rX^r) and males orange-red strain (*bb*, X^rY^R); inbred for 45 generations. The d-rR strain has been used in experiments on sex differentiation (Yamamoto, 1953; 1975).

Hd-rr: Derived from the offspring of fish obtained from a stock (d-rr) at Nagoya University. The females and males are white, genotype *bb*, X^rX^r and *bb*, X^rY^r and newly reported in this paper.

In 1990 and 1992, we began to inbreed other stocks of medaka, the albino strains which were given by Dr. Tomita at Nagoya University (*i3* and *i*) and derived from a cultivated stock in North Carolina (NCMH). They are now inbreeding around the 14th generation.

Polymorphic variation in the inbred strains and its related studies

Polymorphism in several protein loci has been detected among the inbred strains of medaka, as revealed by electrophoresis (Hyodo-Taguchi and Sakaizumi, 1993). The polymorphic variants in the inbred strains are useful for studies of genetics and embryology.

In the inbred strain HNI which derived from the Northern population of medaka in Japan, protein polymorphism quite differs from those of the other inbred strains of medaka, belonging to the Southern population of Japan (Sakaizumi, 1986). Kurihara *et al.* (1992) detected RFLPs between HNI and other inbred strains by the RFLPs analysis of genomic DNA fragments with various restriction endonucleases. This large genetic difference is advantageous in genome mapping studies. Wada *et al.* (1995) have constructed a genetic linkage map by applying the arbitrarily primed polymerase chain reaction (AP-PCR) technique to three inbred strains of the medaka using many genetic markers. They have shown that the map consists of 28 linkage groups and spans about 2480 contiguous centimorgans (cM) with an average of 323 kilobase pairs (kb)/cM.

Even among the inbred strains of medaka belonging to the Southern population, polymorphism of a few enzymes was observed. Three types of LDH isozymes (type I, II, and III) were found in the muscle of adult medaka from a commercial stock (outbred medaka). The LDH patterns of type I and type III were found to have been fixed in HO4C and HB12, respectively. This difference was useful for studies of the regulatory mechanisms of expression of LDH subunits of isozymes in ontogeny (Ohyama *et al.*, 1986) and for purification and characterization of these subunits (Sasaki *et al.*, 1989).

Establishment of the new inbred strain HSOK belonging to the East-Korean population, which

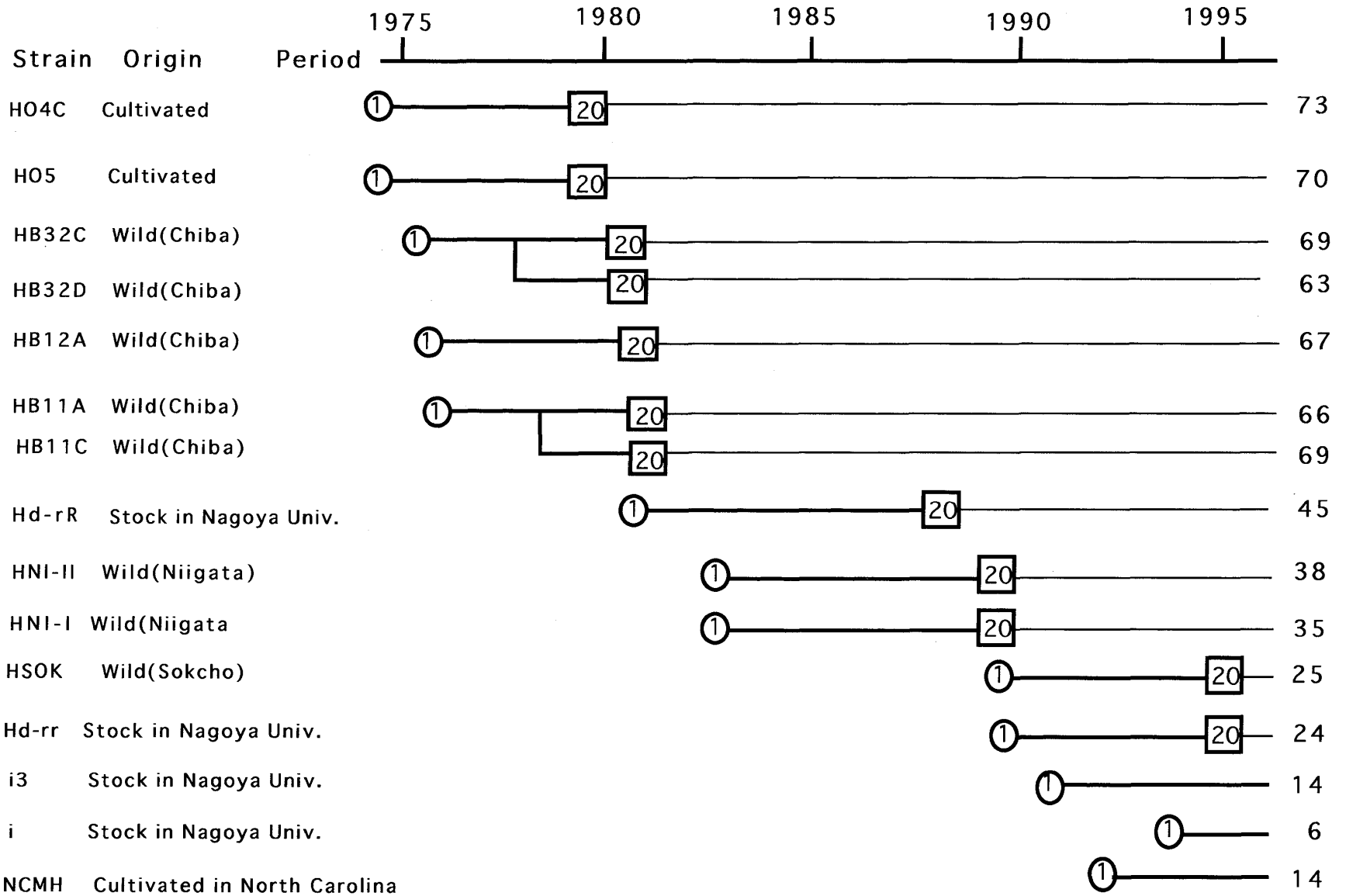

Fig. 1. A chronological table of inbreeding the medaka. Numbers in figure indicate generation of inbreeding by sister-brother mating. Symbol ①— indicates the 1st generation of inbreeding and symbol —20— indicates the time when fish have been inbred by full sister-brother mating for 20 generations. Thick and thin lines indicate period of inbreeding and that after establishment of inbred strains, respectively.

differs in region-specific genetic variation from those of the Southern and Northern populations, will contribute to further analysis of genomic linkage groups in medaka. Moreover, the large genetic difference between the inbred strains of medaka, such as polymorphisms of protein loci and DNA fingerprints, is useful for genetic monitoring to prevent unexpected genetic contamination. It is also advantageous for embryonic engineering experiments and establishment of transgenic and chimeric strains, and may be used as markers for confirmation of technical problems.

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