

EFFECTS OF UV-IRRADIATION ON EMBRYONIC DEVELOPMENT OF INBRED STRAINS OF THE TELEOST, *ORYZIAS LATIPES*.

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In order to make inbred strains of the fish, *Oryzias latipes*, sister-brother matings for successive generations have been carried out since 1974. Three families (B-1, B-2 and B-3) from different single-pair matings were produced from the offspring of wild-type fish. Three families (0-1, 0-4, 0-5) were also produced from random single-pair matings from the stock of the orange-red variety kept at the National Institute of Radiological Sciences. During the course of breeding, the fish were kept in plastic aquaria (17×29×15cm) at 25°C on a 16th light/8h dark cycle. The adults were fed once daily on Tetra-min flakes. Newly hatched fry and the young were given *Daphnia* and powdered Tetra-min. Under these conditions, fry developed to sexual maturity within 2-3 months, and five generations were produced in one year. During inbreeding, the reproductive ability of many pedigrees was reduced. However, two pedigrees (HO4 and HO5) of the orange-red variety of *Oryzias latipes* and three pedigrees (HB11, HB12 and HB32) of wild-type (brown) fish have been inbred by full sister-brother mating for 22 generations during a period of five years from 1974 to 1979. Three substrains, HO4A, and HO4B, HO4C, have been separated from HO4 after 5th, 6th, and 14th generations and inbred with 17th, 16th, and 8th generations, respectively. These inbred strains have now been further inbred by brother-sister matings for more than 26 generations. They have become sufficiently homozygous to accept intrastain scale grafts permanently.

As part of the evaluation of inbred strains of this fish as test animals for the detection of mutagenic and carcinogenic factors, an experiment was designed to study effects of UV-irradiation on embryos of different inbred strains. Photoreactivation (PR) of UV damage

in the cells of embryos of these strains has also been studied. Embryos of four different inbred strains (HO4C, HB11, HB12 and HB32C) and two substrains (HO4C1 and HO4C3) were irradiated with UV (250 to 3000erg/mm²) at the morula stage. The animal poles of the eggs were locally irradiated, using a Petri dish without any water. To effect PR, the glass vessels containing irradiated eggs in water were exposed to fluorescent light. Observations of the embryos were made every day up to hatching in order to check on the surviving embryos and to remove dead ones. Critical embryonic death occurred within 2 days after UV-irradiation, embryos being arrested before the formation of optic buds (at stage 19). A second peak of embryonic death was observed around 10 days after fertiliza-

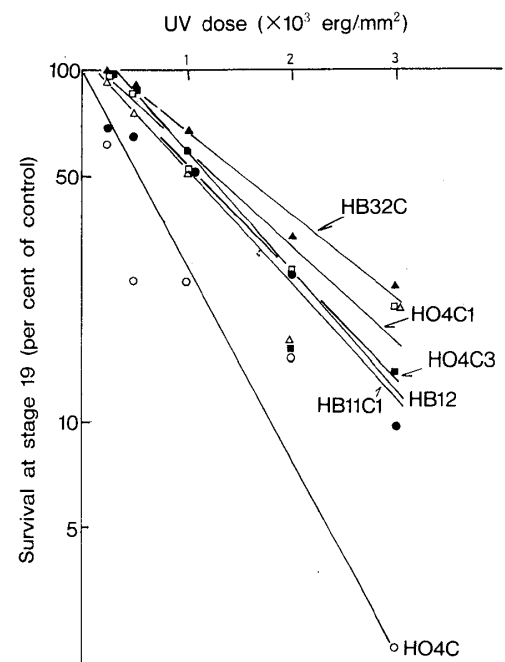


Fig. 1. Survival fractions of the UV-irradiated embryos of different inbred strains of *Oryzias latipes*. Fertilized embryos at the morula stage were locally irradiated, subsequently kept in the dark for 12 hours after UV-irradiation and in a lighted room thereafter.

tion i.e., the time of hatching of these embryos. A dose-dependent decrease in the survival rates at developmental stage 19 was observed in eggs of each strain (Fig. 1) and the PR phenomenon was also found in all strains. Survival fractions in figure 1 showed that the most sensitive strain to UV was HO4C ($D_0 = 800 \text{ erg/mm}^2$) whereas the highest D_0 value (resistant strain) was found in HB32C ($D_0 = 1800 \text{ erg/mm}^2$). The survival rates in embryos of all strains were increased by illumination with visible light, the maximum photoreactivable fraction being 0.40 to 0.72. The rates of survival of embryos at hatching in each

strain were lower than those examined at stage 19. However, the higher sensitivity of HO4C embryos to UV was clearly demonstrated.

These results suggest that (1) as criteria for biological effects on fish eggs, their mortality at early developmental stages is as useful as their hatchability, and (2) visible light illumination after UV-irradiation produces a marked increase in the survival rate. Moreover, these investigations show that inbred strains of this fish provide a valuable test system for studying the effect of environmental factors.