147. Triploidy appeared in the Back-Cross Offspring from Funa-Carp Crossing

By Yoshio OJIMA,^{*)} Makoto HAYASHI,^{*)} and Koichi UENO^{**)}

(Comm. by Sajiyo MAKINO, M. J. A., Oct. 13, 1975)

Although a considerable work has so far been done on fish hybrids by a number of ichthyologists in both scientific and practical interest, knowledge has remained meagre on the cytogenetic problems. Analyses of genetic systems by means of experimental hybridization have proved important for the understanding of cytotaxonomic difference of related forms. On the other hand, significant co-relationships between the geographical distribution and chromosomal polymorphism or polyploidism have been announced in fishes. Importance of experimental hybridization in fishes has been increasing for the critical consideration of species differentiation.

The genus *Carassius* known by a common name of the Funa is a member of the Cyprinidae distributing in Japan, and subdivided into 5 subspecies according to Nakamura (1969): *Carassius auratus* subsp.; *C. a. buergeri*; *C. a. grandoculis*; *C. a. langsdorfi*; and *C. a. cuvieri*. Two types of reproduction have been suggested in these subspecies: bisexual and gynogenesis. Kobayashi (1970, 1972, 1973) reported three chromosomal polymorphisms characterized by diploid, triploid and tetraploid forms in *C. a. langsdorfi*. Diploid and triploid forms have been noted in *C. a. buergeri* (Kobayashi 1975). It has been shown that in gynogenetic forms, triploid eggs are activated by sperm from bisexual species, and that the paternal genome does not participate in the fertilization. Then the offspring possess the maternal genome only.

Implicated in speciation, polyploidism and reproduction of the genus *Carassius*, the present authors have been dealing with some cytogenetic investigations in intergeneric hybrids between the Funa (*Carassius auratus cuvieri*) and the Carp (*Cyprinus carpio*) produced by experimental way.

Materials and methods. When kept under a confined condition, the Funa (♀) and the Carp (♂) mated and produced hybrids (F₁). Reciprocal crosses were also possible with similar results. The hy-

^{*)} Department of Biology, Kwansei Gakuin University, Nishinomiya.

^{**)} Department of Fishery, Kinki University, Higashi-Osaka.

Reprint request: Dr. Y. Ojima, Dept. of Biology, Faculty of Science, Kwansei Gakuin University, Nishinomiya, 662 Japan.

brids grew rather rapidly showing no observable abnormalities. The egg-cleavage proceeded without mitotic disturbance. The breeding experiments by Ojima since 1945 resulted in that the hybrid males were completely sterile. It has recently been shown by Ojima that F_1 females laid eggs in back-crossing with the Carp, and that they developed into adults without any visible abnormality. The present chromosome study was made on the Funa (*C. a. cuvieri*), the Carp (*Cyprinus carpio*), F_1 (Funa ♀ × Carp ♂), and back-cross offspring (F_1 ♀ × Carp ♂).

Kidney tissues were removed, minced with scissors and suspended in fish-BSS. They were treated with colchicine ($0.5 \mu\text{g/ml}$) for 30 minutes at room temperature. Following KCl-hypotonization (0.075 M) for 15–20 minutes, and Carnoy-fixation as usual, slides were air-dried and Giemsa-stained.

Results and remarks. Details of the chromosome morphology of the Funa (*Carassius auratus cuvieri*) and the Carp (*Cyprinus carpio*) were given in the former papers (Ojima *et al.* 1966, 1967). It was shown that the Carp and the Funa had 100 chromosomes in the diploid complement. Morphologically the chromosomes showed

Figs. 1-6. Metaphase chromosomes of the Funa, Carp, F_1 hybrids and back-cross offspring. Fig. 1: The Funa. $2n$, 100. Fig. 2: The Carp. $2n$, 100. Fig. 3: The F_1 . $2n$, 100. Fig. 4: Diploid back-cross individual. $2n$, 100. Fig. 5: Triploid-range chromosomes of a triploid back-cross individual. $3n$, 156. Fig. 6: The same of a back-cross individual. $3n$, 155.

Fig. 7. Karyotype of the F_1 with 100 chromosomes. Arrow shows the largest submetacentric chromosome originated from the Funa.

Fig. 8. Karyotype of a $3n$ -range cell from a triploid back-cross specimen. $3n$, 156.

no appreciable difference between the two forms, except the largest submetacentric pair (Figs. 1-2). It is natural to speculate that the F_1 offspring between the Funa (♀) and the Carp (♂) have 100

chromosomes in $2n$. Then, the chromosome counting of the F_1 offspring showed 100 chromosomes in diploid (Fig. 3). Karyotypically the F_1 chromosomes did not differ from those of the parent species. The diploid complement was characterized by 6 pairs of relatively metacentrics, 18 pairs of mostly submetacentrics and 26 pairs of acrocentric or telocentrics, except the largest submetacentric chromosome which originated from the Funa (Fig. 7, by arrow). Evidently the F_1 chromosomes, 100 in number, are a total sum of the haploid set of the parent species.

The author's experiments (Ojima *et al.* 1961) resulted in that the F_1 males were completely sterile, while the females were capable of laying eggs. Theoretically, the back-cross individuals between the F_1 female and the male Carp should have 100 chromosomes in diploid. Opposed to this assumption, the back-cross offspring having a $3n$ -range chromosome complement appeared in one half of the progenies. The chromosome number of the diploid back-cross offspring was 100 (Fig. 4) which consisted of 6 pairs of metacentrics, 21 pairs of submetacentrics, and 23 pairs of telo- and acrocentrics.

The chromosomes of the triploid back-cross offspring were 145 to 160 in number. The karyotype of the 156-chromosome cells provided an evidence of the trisomic condition for the meta- and submetacentric chromosomes which were morphologically well-defined, though no informative feature was obtained for the telo- and acrocentric chromosomes on account of the paucity of characteristic configuration for analysis (Fig. 8). Noticeable is the occurrence of minute elements, or microchromosomes, in some of the $3n$ -range metaphases of the back-cross individuals.

The significance of triploidy has remained unclear in the course of evolution of the Funa. The occurrence of $3n$ -range back-cross specimens seems to throw some light on the differentiation of species in the Funa. Further, interesting are the facts that the most individuals showing the $3n$ -range chromosomes were females, and that they showed an inter-chromosomal variation similar to the findings in *Carassius a. langsdorffi* by Muramoto (1975). Further information and discussion on the significance of triploidy will be available in the near future, since crossing experiments have been in progress.

Summary. A chromosome study is made on the Funa, the Carp, F_1 , and back-cross individuals ($F_1 \text{ } \text{♀} \times \text{Carp } \text{♂}$). The occurrence of triploidy in the female back-cross offspring is noted. The significance of triploidy is discussed in relation to the differentiation of species in the Funa.

Acknowledgments. The authors are grateful to Dr. S. Makino, M. J. A., Professor Emeritus, Chromosome Research Unit, Hokkaido

University, for going over the manuscript and for invaluable advices. The author's thanks are also due to Dr. A. Hamaguchi and Mr. S. Shibata, Hyogo Fisheries Research Station, for the facility of breeding fishes used.

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