The Desoxyribose Nucleic Acid (DNA) Content in Liver Cells of the Carp-Funa Hybrids[1)]

By
Yoshio Ojima

(Kwansei Gakuin University)

(*With 2 Text-figures*)

A series of studies on sterility of hybrids between the Carp (*Cyprinus carpio*) and Funa (*Carassius carassius*) have been carried out rather extensively : Makino, Ojima and Matsui (1955) cytologically investigated male sterility in the hybrid. They reported that the Carp and the Funa mate easily and can produce hybrids, and that reciprocal crosses are also possible with similar results. The hybrids are just intermediate between the parent species in several characters. The hybrid males are completely sterile; meiosis in hybrid testes is highly disturbed, being arrested at early stages of the meiotic prophase. Most of the germ cells undergo pycnotic degeneration through the period from leptotene to pachytene, and no spermatozoa are produced.

It is expected that cytochemical analysis will serve to solve many problems dealing with hybrid sterility. At the suggestion of Dr. Schrader and Dr. Moore, Columbia University, the author has undertaken the cytochemical study of hybrid sterility. The author wishes to report in this paper the results of a micro-spectrophotometric study of the Desoxyribose nucleic acid (DNA) and the total protein content in cells of liver tissues of the Carp and Funa, and their hybrids. It would be resonable to expect from the results of the cytological study that the DNA and the total protein content of hybrid cells must be intermediate between the parent species.

The greater part of this study was carried out at Columbia University, through the expert direction of Dr. Pollister, Dr. Moore and Dr. Schrader, to whom the author is greatly indebted for their valuable suggesions and for the use of the laboratory. Thanks must be extended to Dr. Leuchtenberger, Professor of Western Reserve University, for her generosity, and also to Professor Makino, Hokkaido University, for going over the manuscript for publication.

The following study was done on the liver cells of the Carp (*Cyprinus carpio*), the Funa (*Carassius carassius*) and their hybrids. They were fixed in Carnoy's fluid (Alcohol-acetic acid 3 : 1), embedded in paraffin, and cut at different thicknes-

1) This paper is dedicated to Professor Tohru Uchida in commemoration of his sixtieth birthday.

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ses required for microspectrophotometry.

The DNA content in individual cells was determined through microspectrophotometric analysis of the Feulgen reaction according to Stowell 1945. For the photometric microscopic measurements of total protein (tyrosin and tryptophane), the sections were stained with Millon reagent (Pollister and Ris 1947).

The photometric microscopic method after Pollister and Ris (1947) was adopted since it allows the estimation of relative amounts of colored precipitates within individual nuclei of fixed and stained cells.

The morphological appearance of liver cells of the Carp, the Funa and their hybrids are shown in the figures (Fig. 1, a-c). The hybrid nuclei closely resembled the Carp nuclei in morphological aspects. On the other hand, the Funa nuclei were somewhat oval shaped and larger in size than the Carp nuclei. A survey from a morphological study of Feulgen preparations of the Funa nuclei, showed strikingly larger amounts of DNA in comparison with the results from the Carp or the hybrids.

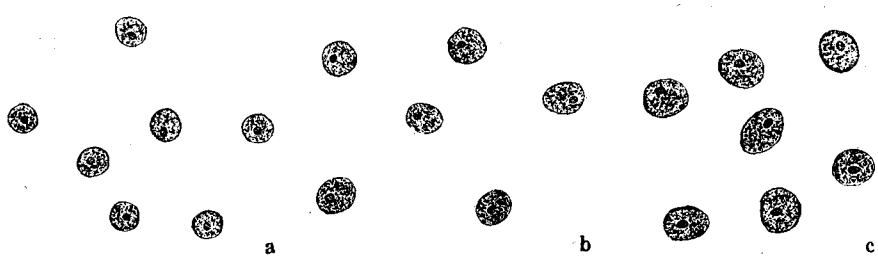

Fig. 1. Morphological comparison of nuclei. (10×100).
a, Carp. b, F_1. c, Funa.

In Table 1 are presented the results from the comparison of the nuclear volume in liver cells of three specimens. It is evident from this table that the nuclear volume of the hybrid specimen is just intermediate between the Carp and the Funa.

Table 1. Comparison of the nuclear volume in liver cells of three specimens

	Carp	F_1	Funa
Mean volume of liver nuclei in cubic microns	57.6±17.9	71.8± 6.79	87.9±18.1
No. of nuclei measured	60	60	60

The results of the DNA measurments in individual nuclei of liver cells from

three specimens are presented in Text-Fig. 2. On the basis of the DNA data shown here, it can be seen that there is a definite difference between the Carp and the Funa. The Funa has DNA values significantly higher than those of the Carp. It can be noted also that the value of the DNA of hybrids are just intermediate between the parents.

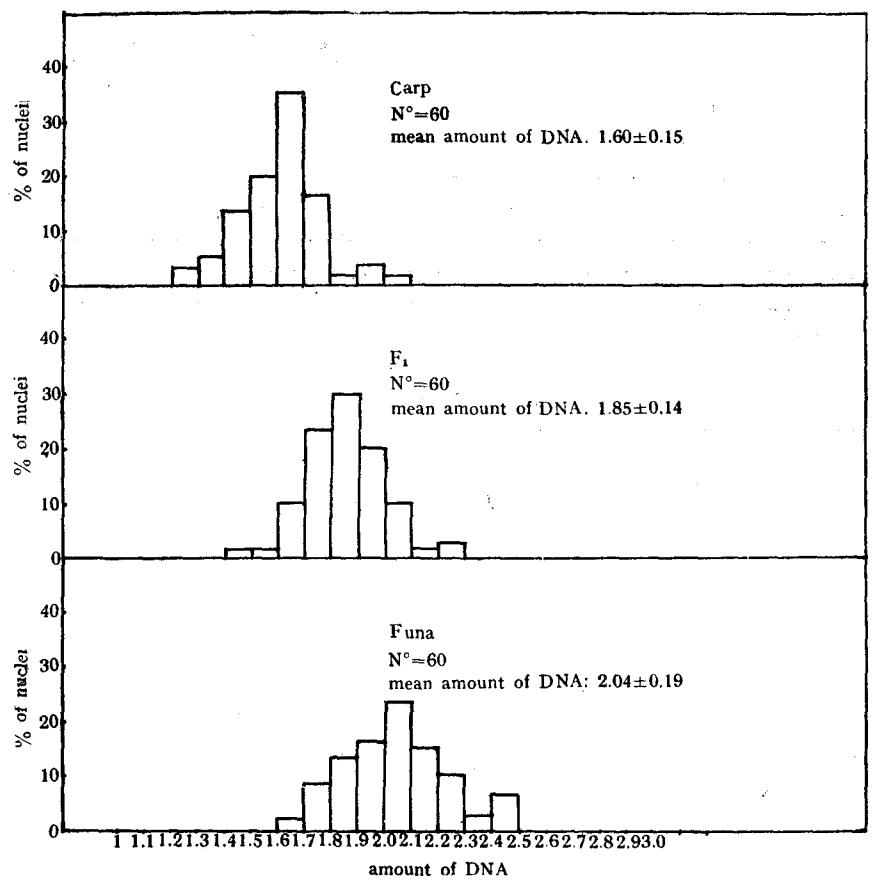

Fig. 2. Comparison of the amount of DNA (microspectrophotometry) in individual nuclei of the Carp, the Funa and their hybrid. N°=number of nuclei measured.

The results of absorption measurements of total protein of liver nuclei through the Millon reaction in three specimens are shown in Table 2. The actual extinction is higher in Carp nuclei than in Funa nuclei. If the values are converted into the total amount of protein for the nuclear volume, it is evident that the Funa

has much higher amount of protein than the Carp. Of course, the hybrid protein are just intermediate between the Carp and the Funa. Evidently there is a parallel relationship between the total protein and the DNA.

Table 2. Comparison of the actual extinction through the Millon reaction in three specimens

	Carp	F_1	Funa
Mean extinction	0.114± 0.018	0.093± 0.020	0.087± 0.013
No. of nuclei measured	20	20	20

The present investigation primarily shows the results of the fundamental measurements of the biochemical characters of hybrids. From these results it seems difficult to solve the confused problems dealing with hybrid sterility. But, it may not be uncalled for to record such fragmental data, since the accumulation of the data seems to contribute something forward the final solution of the problems in the future.

References

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