NUCLEAR TRANSPLANTATION IN TELEOSTS* **

— HYBRID FISH FROM THE NUCLEUS OF CRUCIAN AND THE CYTOPLASM OF CARP***

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ABSTRACT

Hybrid fish are obtained from the combination of nucleus and cytoplasm from two genera of fresh-water teleosts using the technique of nuclear transplantation (i.e. the combination of the nucleus of crucian (Carassius auratus) and the cytoplasm of carp (Cyprinus carpio)).

Morphological characteristics of these hybrid fish that have been examined so far indicate that some features such as barbs, pharyngeal teeth, the number of scales along the lateral line, and the number of vertebrae are similar to those of crucian. Some of the hybrid fish grow to normal adult fish.

It has been reported in our previous paper in 1980 that normal hybrid adult fish can be obtained from the combination of nucleus and cytoplasm from two genera of fresh-water teleosts using the technique of nuclear transplantation (i.e. the hybrid fish from the combination of the carp nucleus and the crucian cytoplasm) (CyCa hybrid fish). Morphological characteristics of those hybrid fish showed that some features were inherited from the nucleus donor fish, such as barbs and pharyngeal teeth. Some features seemed to come from the cytoplasm host fish, such as the number of vertebrae. Some features were intermediate, such as the number of scales along the.

* This paper is written in memory of our beloved advisers, Profs. T. C. Tung and Y. Y. F. Tung, who had encouraged us in many ways and had joined us in carrying out this research project before they passed away in 1979 and 1976 respectively.

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*** Abbreviations: CyCa Hybrid fish—Hybrid fish from the combination of the carp nucleus and crucian cytoplasm. CaCy Hybrid fish—Hybrid fish from the combination of the crucian nucleus and carp cytoplasm.
lateral line. The results thus obtained lead us to conclude that both nucleus and cytoplasm can influence the expression of genetic information on this kind of hybrid fish. In addition, it is also a first example which indicates that the mature hybrid fish can be obtained by using the nuclear transplantation technique instead of sexual hybridization[13]. The inbred offsprings of those hybrid fish have been obtained and the results will be reported elsewhere[13].

In this paper we report the experiments on nuclear transplantation between the same two genera of fresh-water teleosts by transplanting the nucleus of crucian into the enucleated carp eggs. This kind of nucleo-cytoplasm combination is the reverse of the previous one (i.e. the hybrid fish from the combination of the crucian nucleus and the carp cytoplasm) (CaCy hybrid fish) reported[13].

I. MATERIALS AND METHODS

Crucian (Carassius auratus Linnaeus) and Carp (Cyprinus carpio Linnaeus) were used for these experiments. Both of them belong to the same family (Cyprinidae) but to different genera. Crucian belongs to the genus Carassius Jarocki and carp belongs to the genus Cyprinus Linnaeus. There are two varieties of crucian: black and red crucian. Both of them are from the local river and ponds in Guangxi Zhuang Autonomous Region. There are also two varieties of carp: black and red carp. The black carp is a local sexual hybrid fish between the common carp and the red carp. The red carp is transferred from Jiangxi Province by the Guangxi Fisheries Research Institute. There are no visual morphological differences between the two varieties of crucian, nor between the two varieties of carp, except that the black pigment cells are present in the black varieties of both crucian and carp.

Some preliminary experiments were carried out at the Institute of Hydrobiology, Wuhan, Hubei Province in the spring of 1976 and few young hybrid fish were obtained. But most of these experiments were continued and carried out at the Guangxi Fisheries Research Institute in Nanning, Guangxi Zhuang Autonomous Region. The weather in Nanning was warmer in general and the water temperature was 18—28°C during the spawning season of both crucian and carp (March to April) when these experiments were begun in 1979.

In order to prevent the higher temperature from damaging the eggs, the nuclear transplantation operations were carried out in a 20°C air-conditioned laboratory. The nuclear transplanted eggs and the hybrid embryos were cultivated in the same conditions and not removed to room temperature until the larval fish stages were reached. The cleaning sand filtered fresh water was used for cultivation of the eggs, embryos and larval fish and changed daily. The technique for nuclear transplantation has been described before[13].

II. RESULTS

In these experiments, the nuclei of crucian blastula cells were transplanted into the enucleated carp mature eggs. According to our own experience, it is more difficult to obtain suitable unfertilized mature eggs (which are used as the host cytoplasm)
from carp than those from crucian by pituitary injection.

The results from these experiments of 1979 were: Among 918 nuclear transplanted eggs, only 9 (0.9%) developed to adult hybrid fish, while 232 were arrested at blastula stage (25.27%), 122 at gastrula stage (13.29%) and 30 at larval fish stage (3.26%) respectively (see Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>The No. of Nuclear Transplanted Eggs and Their Developing Stages</th>
<th>Number of Transplanted Eggs</th>
<th>Arrested at Blastula Stage</th>
<th>Arrested at Gastrula Stage</th>
<th>Arrested at Larval Fish Stage</th>
<th>No. of Adult Hybrid Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival percentages</td>
<td>100%</td>
<td>25.27%</td>
<td>13.29%</td>
<td>3.26%</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

As indicated above, the CaCy hybrid fish can be obtained using the technique of nuclear transplantation.

According to the taxonomic criteria described in our previous paper of 1980(1), some main morphological characteristics of crucian, carp and the...nine...adult...CaCy hybrid fish are shown in Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Type of Fish</th>
<th>Morphological Characteristics</th>
<th>No. of scales on lateral line</th>
<th>No. of vertebrae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pair of barbs</td>
<td>Pharyngeal teeth</td>
<td></td>
</tr>
<tr>
<td>Crucian</td>
<td>None</td>
<td>Wedge-shaped 4 teeth in one row</td>
<td>25–29 Majority 28</td>
</tr>
<tr>
<td>Carp</td>
<td>2</td>
<td>Molar-shaped 5 teeth in 3 rows, 3–1–1</td>
<td>34–36 Majority 35</td>
</tr>
<tr>
<td>CaCy hybrid fish</td>
<td>None</td>
<td>Wedge-shaped 4 teeth in one row</td>
<td>24–28 Majority 25</td>
</tr>
</tbody>
</table>

It can be seen in Table 2: (i) That the crucian have no barbs; their pharyngeal teeth are wedge-shaped, 4 on each side and arranged in one line. The number of scales along the lateral line are 26 to 29, with the majority being 28 and the number of their vertebrae are 4+25 to 4+28, (ii) The carp have two pairs of barbs on each side of the mouth, their pharyngeal teeth are molar-shaped, with 5 on each side, and arranged in 3 rows in order of 3-1-1; The number of scales along the lateral line is 34 to 36, with the majority being 35 and the number of their vertebrae are 4+30 to
4+31; (iii) The CaCy hybrid fish have no barbs, their pharyngeal teeth are wedge-shaped, with 4 on each side and arranged in one line (Plate I, 1c. Since two hybrid fish out of the total nine were lost, only the pharyngeal teeth of seven dead hybrid fish have been examined); the number of their scales along the lateral line are 26 to 29, with the majority being 28 and the number of their vertebrae are 4+25 to 4+28.

Evidently, from the morphological point of view, the CaCy hybrid fish are somewhat like the donor nucleus fish, crucian.

Most likely, the cultivating conditions were not good enough for those hybrid fish as seven of them died in 1980 before they could grow to mature adults. All of the dead fish were fixed in formalin and kept as specimens in our laboratory.

The remains of two adult hybrid fish (Plate I, 1a, b, c, d.) were kept in cultivation in a special cement fish tank with good care. But they didn’t mature in 1981. Not until March, 1982 did we find that both the hybrid fish were almost matured. They grew well and could be distinguished (i.e. the male fish had many small white horny tubercles on the inner margin surface of its pectoral fin and the female had a large belly). We expected that those two hybrid fish could be grown to maturity and be used to breed their offsprings within one or two months later. We removed them into a large wild fish pond in order to improve their living conditions and wait for them to grow to maturity. Unfortunately, these two hybrid fish disappeared in April, 1982 before we could start to work on them. Therefore, the further investigations on those hybrid fish (such as analysis of their chromosome patterns, biochemical properties etc.) had to be suspended until the new hybrid fish of this combination could be obtained in the future.

III. DISCUSSION AND CONCLUSIONS

1. From the results of these experiments, we come to the conclusion that the nuclear transplantation between teleosts of different genera, but from the same family, Cyprinidae, can be obtained not only for the CyCa hybrid fish but also for the hybrid fish from the reverse combination, i.e. CaCy hybrid fish. In our previous paper we reported that the survival rate of nuclear transplanted CyCa hybrid fish was 3.2%[1], whereas that of the nuclear transplanted CaCy hybrid fish was 0.9%.

It seems that the difference of the survival rate between these two kinds of hybrid fish is due to the fact that, (i) It is more difficult to obtain suitable mature eggs (which can be used as host enucleated eggs) from carp than from crucian, because the crucian has a rather small body size and they are much easier to breed in the small tank during their spawning season for the pituitary injection to induce their ovulation. In contrast, carp is much bigger than the crucian and they can only be grown to maturity in large wild fish ponds. Therefore, it seems very difficult to prepare many pairs of carp and to get enough batches of suitable mature eggs from them as we can do with crucian. In addition, the host carp eggs always become over-matured during the nuclear transplantation manipulation operation. In this case, nuclear transplanted eggs would be arrested in early embryonic stages or develop abnormally. On the other hand, if we use the nuclei of carp blastula cells as donor
nuclei for nuclear transplantation, it will be much easier. This is because we only need one pair of male and female carp to produce a lot of carp blastula cells and use their nuclei as donor to be transplanted into many batches of crucian enucleated eggs. This is just the reason why we get the CyCa hybrid fish at first during the past years. (ii) It also might result from inadequate manipulation during the operation that damaged the eggs as we have discussed in the previous paper

(iii) There is another possibility that some natural factors concerning the contradiction between donor nucleus and host cytoplasm in both combinations are somewhat different, which will influence the survival rates of both kinds of hybrid fish.

However, if we compare the survival rate of the CaCy hybrid fish (0.9%) with those reported by Nishioka on different species of frogs (0.08—0.12%)\textsuperscript{14}, it shows that the former is still much higher than the latter. Obviously, the teleosts are much better than the frogs to be used as materials for nuclear transplantation experiments and the success of obtaining hybrid fish from far-related species will throw some new light upon cultivating the new breeds of fish with important economic value.

2. Since all of the hybrid fish obtained in these experiments were dead or lost, there was no opportunity to let us make further investigation on them or even analyse their chromosome patterns and their blood contents as well. It has been shown in our previous paper that the chromosome number of crucian and carp are the same (i.e. $2n = 100$) and none of the marker chromosome can be found in those two species\textsuperscript{14}. But it can be confirmed that the crucian nuclei had been transplanted into enucleated carp eggs, because the hybrid fish obtained in these experiments had no barbs at all. Therefore, it will not be difficult to identify these hybrid fish as the CaCy hybrid fish without analysing their chromosome patterns.

3. According to the taxonomy, it has been shown that the CyCa hybrid fish have some features inherited from the nucleus donor fish, some from the cytoplasm host fish and some being intermediate. However, the results from these experiments indicated that some main morphological characteristics of the CaCy hybrid fish are similar to those of crucian. Since so far there is no additional data available to be used for further analysis of the other characteristics of CaCy hybrid fish, it seems that to give an appropriate explanation for this inconsistency in CyCa and CaCy hybrid fish is difficult. Probably, it is due to the different interactions between the nucleus and the cytoplasm in both kinds of nucleo-cytoplasm combinations.

It seems very interesting to repeat these experiments and make further investigations on the CaCy hybrid fish in the near future.

**References**

1a. *CaCy* hybrid fish (Photograph, female). Number of scales along its lateral line is 26.

1b. *CaCy* hybrid fish (Photograph, male). Number of scales along its lateral line is 28.

1c. X-ray photograph of the fish shown in 1a. The number of its vertebrae is 4+25.

1d. X-ray photograph of the fish shown in 1b. The number of its vertebrae is 4+26.

1e. Teeth pattern of the *CaCy* hybrid fish. Wedge-shaped, with 4 on each side and arranged in one line.