

研究报告

NUCLEAR TRANSPLANTATION IN TELEOSTS\* \*\* \*\*\*

IVA. NUCLEAR TRANSPLANTATION BETWEEN DIFFERENT  
SUBFAMILIES-HYBRID FISH FROM THE NUCLEUS OF GRASS  
CARP (*CTENOPHARYNGODEN IDELLAS*) AND THE  
CYTOPLASM OF BLUNT-SNOUT BREAM (*MEGALOBrama  
AMBLYCEPHALA*)\* \*\* \*

Yan Shaoyi, Lu Deyu, Du Miao,

Li Guangsan, Han Zhou, Yang Huiyi, Wu Zhengan

(Institute of Developmental Biology, Academia Sinica, Beijing)

Yang Yongquan, Xia Dequan,

Liu Aizhu, Zhang Xingzhong

(Chang Jiang Fisheries Research Institute, The China State Fisheries Institution, Shashi)

ABSTRACT

Nucleo-cytoplasmic hybrid fish are obtained from the combination of nucleus and cytoplasm from two subfamilies of fresh-water teleosts using the technique of nuclear transplantation, i.e. the combination of the nucleus of grass carp (*Ctenopharyngoden idellas*) and the cytoplasm of blunt-snout bream (*Megalobrama amblycephala*). The average survival rate of the hybrid fish is 3.6%.

Some morphological characteristics of those nucleo-cytoplasmic hybrid fish (head in body length, depth in body length, width in body length, dorsal spine, abdominal keel, anal rays, gillraker, pharyngeal teeth, lateral line scales and number of vertebrae) are similar to those of grass carp. It indicates that the expression of genetic information related to those characteristics of nucleo-cytoplasmic hybrid fish were influenced by the donor nucleus of grass carp.

Analysis of the chromosome patterns of grass carp, blunt-snout bream

\* 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.

\*\* Contribution No. 8501 of the Institute of Developmental Biology, Academia Sinica.

\*\*\* This work is partially supported by Rockefeller Foundation Grant 79008.

\*\*\*\* Abbreviations: *Ct* fish—Grass carp; *Me* fish—Blunt-snout bream; *CtMe* fish—nucleo-cytoplasmic hybrid fish from the combination of Grass Carp nucleus and Blunt-snout bream cytoplasm.

and the nucleo-cytoplasmic hybrid fish shows that their chromosome numbers are all the same ( $2n=48$ ) and most chromosomes in above three kinds of fish are difficult to be distinguished morphologically. However, preliminary examination shows that the eleventh pair of subtelocentric chromosomes are somewhat different in grass carp and blunt-snout bream which we used in our experiments: the arm-ratio of the eleventh pair of chromosomes in grass carp is  $>3$ , and that in blunt-snout bream is 2. Examination also confirms that the arm-ratio of the eleventh pair of chromosomes of nucleo-cytoplasmic hybrid fish is  $>3$ . Evidently, the nucleus of nucleo-cytoplasmic hybrid fish is obtained from grass carp cell by nuclear transplantation.

Preliminary observation shows that the growth rate of the nucleo-cytoplasmic hybrid fish is much faster than that of blunt-snout bream and also a little faster than that of grass carp. None of the nucleo-cytoplasmic hybrid fish was found to be dead of infective disease which is always observed in grass carp.

So far, one of the nucleo-cytoplasmic hybrid male fish has matured and produce normal sperms which could be used to fertilize the grass carp eggs. In view of the fact that in sexual hybridization between remote species of fish such as that in grass carp and blunt-snout bream, their hybrids are always abnormal, lethal at early embryonic stages or sterile (due to the poor development of their gonads) even few of them survived, it is clear that the method of nuclear transplantation as an useful biotechnique can be used for obtaining the nucleo-cytoplasmic hybrid fish clones. As well as to provide a possibility in forming new breeds of edible fish. The further study of this nucleo-cytoplasmic hybrid fish will be reported in separate paper elsewhere.

## INTRODUCTION

In our previous paper we reported that two kinds of normal nucleo-cytoplasmic hybrid fish were obtained from same family but two genera of fresh-water teleosts using the technique of nuclear transplantation: (1) Both male and female nucleo-cytoplasmic hybrid fish were obtained from the combination of carp (*Cyprinus carpio* Linnaeus) nucleus and crucian (*Carassius auratus* Linnaeus) cytoplasm. Its survival rate is 3.2%. Morphological characteristics of those nucleo-cytoplasmic hybrid fish showed that some features were inherited from the nucleus donor fish. Some features seemed to come from the cytoplasm host fish and some were intermediate. It indicated that both nucleus and cytoplasm can influence the expression of genetic

information on this kind of hybrid fish<sup>[1]</sup>. (2) Nucleo-cytoplasmic hybrid fish were also obtained from the combination of crucian nucleus and carp cytoplasm. Its survival rate is 0.9%. Morphological examination showed that some main characteristics of this kind of nucleo-cytoplasmic hybrid fish are similar to those of nucleus donor fish, crucian. Some of them could also be grown to maturity<sup>[2]</sup>. According to the principle of taxonomy, above two kinds of nucleo-cytoplasmic hybrid fish are obtained from two combinations of two remote species of fish. However, the question related to the possibility of cultivating them as new breeds of fish with better economic value are still remained to be studied.

In addition, before we reported above two kinds of nucleo-cytoplasmic hybrid fish, Tung et al also reported the experiments of nuclear transplantation in different subfamilies of fresh-water fish<sup>[3]</sup>. They used gold fish (*Carassius auratus*) which belongs to the subfamily Cyprininae and one kind of Chinese bitterling (*Rhodeus Sinensis*), which belongs to the subfamily Accheilognathinae as materials to the nuclear transplantation experiments. The results showed that either in the combination of blastula nucleus of Chinese bitterling and the gold fish enucleated egg cytoplasm or in the combination of blastula nucleus of gold fish and the enucleated egg cytoplasm of Chinese bitterling, more than 60% of the nuclear transplanted eggs can be developed into blastula stage. Among them, about 1% of the embryos can continue to develop into various/embryonic stages later than gastrula stage and up to larval stages. The majority of the nucleo-cytoplasmic hybrid embryos were dead within five days of cultivation and only few of them can live for one week. Unfortunately, those experiments did not indicate that nucleo-cytoplasmic adult hybrid fish could be obtained from the combinations of nucleus and cytoplasm from two different subfamilies of fresh-water fish using the technique of nuclear transplantation.

In this paper, we also report the experiments on nuclear transplantation between two subfamilies of fresh-water fish by transplanting the nuclei of grass carp into the enucleated blunt-snout bream eggs in order to explore: (1) the possibility of producing adult nucleo-cytoplasmic hybrid fish from the combination of nucleus and cytoplasm in different subfamilies of fish; (2) the morphological characteristics of the nucleo-cytoplasmic hybrid fish; and (3) the possibility of the nucleo-cytoplasmic hybrid fish to be cultivated as a new breed of fish with some economic advantages.

## MATERIAL AND METHODS

Grass carp (*Ctenopharyngodon idellus*) (*Ct*) and blunt-snout bream (*Megalobrama amblycephala*) (*Me*) were used for these experiments. Both of them belong to the same family (*Cyprininae*) but to different subfamilies and genera. Grass carp belongs to the subfamily *Leucinae* and blunt-snout bream belongs to the subfamily *Abramidinae*. Both *Ct* fish and *Me* fish are important edible fresh-water fish in China. *Ct* fish is a herbivorous one, growing faster and bigger, but its survival rate is very low owing to its poor diseases resistance. This is the main problem dealing with the fish farming of grass carp. *Me* fish is also a herbivorous one. Although it has a small body but also grows faster. It tastes delicate and delicious. Its diseases resistance is stronger than that of *Ct* fish. Both *Ct* and *Me* fish are very much liked by the people for fish dishes in China. To select these two kinds of fish for the experiments of nuclear transplantation is not only for the basic study of interrelationship between nucleus and cytoplasm, but also for the purpose of searching for a way to produce an improved breed of fish with both advantages of *Ct* and *Me* fish. Experiments were carried out at Chang Jiang Fisheries Research Institute, Shashi, Hubei province. *Ct* fish and *Me* fish were cultivated in the ponds of the institute. The spawning season of *Ct* and *Me* fish in Hubei province is from the end of April to the beginning of June. The best spawning period is the first and second ten days in May. Nuclear transplantation operations were carried out within this period. The weather temperature of this period was 18—26°C. Other characteristics of those hybrid fish were examined on routine basis without any seasonal limitation.

In these experiments, the sperms of *Ct* fish were obtained by naturally matured male fish. Egg of *Ct* fish were obtained from those fish induced by LRH injection (60–100 µg/kg body weight). Eggs of *Me* fish were obtained from those fish induced by pituitary injection (6–8 mg carp pituitary body/kg body weight) or LRH and pituitary combined injection (5 µg LRH + 3 mg carp pituitary body/kg body weight).

After artificial insemination, the fertilized eggs of *Ct* fish were cultivated in room temperature ( $\pm 24^\circ\text{C}$ ) until middle blastula stage for providing suitable donor cell nucleus. The technique for nuclear transplantation has been described before<sup>[4]</sup>.

The lymphocyte cultural method described by Wu et al.<sup>[6]</sup> was used for analysing the chromosome patterns of fish.

## RESULTS

### (1) The development of nucleo-cytoplasmic hybrid fish

In these experiments, the normally developed middle blastula of *Ct* fish were selected and their nuclei were transplanted into the enucleated eggs of *Me* fish. The quality of the recipient enucleated eggs is very important for their further development after nuclear transplantation. The main criterion of the egg quality is its maturity situation, which can be identified by fertilizing some eggs taken from the experimental batches with normal *Ct* fish sperms to see whether their fertilization ratio are high or not as we reported in previous paper<sup>[1]</sup>. After nuclear transplantation, if the quality of eggs are poor, their early segmentation will be irregular and no normal embryos could be developed. Most of them will die at abnormal blastula or early gastrula stages. If the quality of eggs are good, normal hybrid fish in different ratio could be obtained in various batches. In Table I, the results obtained from seven nuclear transplantation experiments during the years of 1977, 1978 and 1979 were listed.

As indicated in Table I, the different numbers of adult nucleo-cytoplasmic hybrid fish were obtained in different batches of nuclear-transplanted eggs. Among them, 2 adult hybrid fish (2.5%) were obtained in first batch, 1 (0.07%) in second batch, no adult hybrid fish in third batch (0.0%), 1 in fourth batch (0.08%), 5 in fifth batch (4.8%), 3 in sixth batch (7.5%) and 12 in seventh batch (14.1%). Among 659 nuclear transplanted eggs in seven batches, 24 developed to adult nucleo-cytoplasmic hybrid fish, while 474 arrested at blastula stage (71.9%), 214 at gastrula stage (32.5%) and 42 at larval fish stages (6.2%). The average survival percentage of *CtMe* adult fish is 3.6%. Obviously, although the numbers of adult hybrid fish obtained from different batches of nuclear transplanted eggs are different. However the *CtMe* adult fish could be definitely obtained using the technique of nuclear transplantation.

### (2) Morphological characteristics of nuclear-transplanted hybrid fish

According to the taxonomy, ten main different morphological characteristics between 13 *CtMe* fish, 30 *Ct* fish and 14 *Me* fish were observed and comparison of them is listed in Table II.

As indicated in Table II, ten main morphological characteristics, i.e. head in body length, depth in body length, width in body length, dorsal spine, abdominal keel, anal rays, gillraker, pharyngeal teeth, lateral line scales, and number of vertebrae were examined in these kinds of fish. It showed

Table I The survival percentage of embryonic stages and adult nucleo-cytoplasmic hybrid fish from the nuclear transplantation experiments between grass carp nucleus and blunt-snout bream cytoplasm(CtMe)fish during the year of 1977,1978 and 1979.

| Batches of Experiments and Date of Experiments | No. of Transplanted Eggs | Arrested at Blastula Stage | Arrested at Gastrual Stage | Arrested at Larval Fish Stage | No. of Adult nucleo-cytoplasmic hybrid fish(CtMe fish) |
|--|--------------------------|----------------------------|----------------------------|-------------------------------|--|
| 1. 1977,5,19                                   | 80(100%)                 | 65(81.2%)                  | 20(25%)                    | 4 (5%)                        | 2 (2.5%)   |
| 2. 1977,5,21                                   | 135(100%)                | 120(88.8%)                 | 30(22.2%)                  | 3 (2.2%)                      | 1 (0.07%)  |
| 3. 1977,5,25                                   | 94(100%)                 | 72(76.6%)                  | 23(24.5%)                  | 2 (2.1%)                      | 0 (0.0%)   |
| 4. 1978,5,30                                   | 120(100%)                | 40(33.3%)                  | 26(21.6%)                  | 4 (3.3%)                      | 1 (0.08%)  |
| 5. 1979,5,12                                   | 105(100%)                | 75(71.4%)                  | 59(56.2%)                  | 8 (7.6%)                      | 5 (4.8%)   |
| 6. 1979,5,12                                   | 40(100%)                 | 30(75%)                    | 20(50%)                    | 5 (12.5%)                     | 3 (7.5%)   |
| 7. 1979,5,12                                   | 85(100%)                 | 72(84.7%)                  | 36(42.3%)                  | 16(18.8%)                     | 12(14.1%)  |
| 8. Total                                       | 659(100%)                | 474(71.9%)                 | 214(32.5%)                 | 42(6.4%)                      | 24(3.6%)   |

that the morphological characteristics of CtMe fish are basically similar to those of donor nucleus fish(grass carp).Plate Ia,Ib,andIc show the different morphological shapes of Ct fish, Me fish and CtMe fish. Plate IIa, IIb, and IIc show the X-ray photographs of Ct fish,Me fish and CtMe fish and their vertebral numbers,Plate IId,Ile and IIe show the pharyngeal teeth pattern of Ct fish,Me fish and CtMe fish.

### (3) Analysis of chromosome patterns

The calculation of chromosome numbers and analysis of chromosome patterns in Ct fish, Me fish and CtMe fish indicate that: the diploid chromosome number of Ct fish and Me fish are the same( $2n=48$ ), including ten pairs of metacentric and submetacentric chromosome and other fourteen pairs of telocentric and subtelocentric chromosomes. It is very difficult to distinguish the difference of every pair of chromosome in Ct fish and Me fish morphologically. However it was found that, the largest pair of chromosomes, i. e. the eleventh pair of chromosomes both in Ct fish and Me fish which were used as our experimental materials at Chang Jiang Fisheries Research Institute are different with their arm-ratio; the arm-ratio of the eleventh pair of chromosomes in Ct is  $>3$  (plate Id and Id') and that of in Me fish is equal to 2 (plate Ie and Ie'). The chromosome number of CtMe fish was also found to be equal to those of Ct fish and Me fish( $2n=48$ ). They can also identified as 10 pairs of metacentric and submetacentric chromosomes and 14 pairs of telocentric and subtelocentric chromosomes. Among them,

Table I Comparison of some morphological characteristics of grass carp(Ct),CtMe fish and blunt-snout bream(Me)

| Morphological Characteristics | Type of fish      |                   |                       |
|-------------------------------|-------------------|-------------------|-----------------------|
|                               | Grass carp (Ct)   | CtMe Fish         | Blunt-snout bream(Me) |
| Head in body length           | 4.16<br>(average) | 4.17<br>(average) | 4.82<br>(average)     |
| Depth in body length          | 4.10<br>(average) | 4.35<br>(average) | 2.10<br>(average)     |
| Width in body length          | 6.34<br>(average) | 6.25<br>(average) | 8.36<br>(average)     |
| Dorsal spine                  | —                 | —                 | +                     |
| Abdominal keel                | —                 | —                 | +                     |
| Anal rays                     | 3.8               | 3.7—9             | 3.27—30               |
| Gillraker                     | 16—18             | 18—20             | 13—17                 |
| Pharyngeal teeth*             | 2 line            | 2 line            | 3 line                |
| Lateral line scales           | 37—43             | 31—43             | 50—60                 |
| Vertebrate number             | 39—42             | 39—40             | 42—43                 |

\* Only 7 dead CtMe fish were examined.

the arm-ratio of the largest eleventh pair of subtelocentric chromosomes is also  $>3$ , (plate If and If') which is similar to that pair of chromosomes in Ct fish. In this sense, it indicate that the CtMe fish were grown from the combination of Ct nucleus and Me cytoplasm.

#### (4) The growth and reproductive capability of CtMe fish

According to the observation of 10 CtMe fish obtained from different batches of experiments which were cultivated in the ponds before 1981, it shows that their growth rate are: the average body length of one year hybrid fish is 36.5cm long, while that of 2 years is 62.3cm long, and 4 years is 87cm long. Since those CtMe fish were obtained individually from different batches of experiments during the last several years, and were few in numbers, so that it is very difficult to carry out the growth rate comparison experiments in same durations and same conditions with the Ct fish and Me fish as the control. However, according to the comparison of the data of the growth rates of Ct fish and Me fish reported in other references (the average body length of one year Ct fish is 34.5cm long, while 2 years is 60cm long, 4 years is 75.7cm long, the average body length of one year Me fish is 16.4cm long, while 2 years is 30.7cm long and 4 years is 41.8cm long)<sup>(1)</sup>, it indirectly shows that the growth rate of CtMe fish is much

faster than that of *Me* fish and a little faster than that of *Ct* fish. Beyond that, none of infective diseases like that always observe in *Ct* fish cultivation has been observed.

So far, one of *CtMe* male fish had matured in 1981 and produced normal sperms. The back sexual cross hybrid fish were obtained by using those sperms to fertilize the *Ct* fish eggs (the results will be reported elsewhere). It indicates that the gonads of this male *CtMe* fish grow well and the fish itself is fertile. Unfortunately, there is no mature female *CtMe* fish available for carrying out inbreeding experiments with that mature male *CtMe* fish, because the durations of maturity of different individuals are different.

## CONCLUSIONS AND DISCUSSION

From the results of these experiments, we come to the following conclusions:

(1) We have continuously reported that nucleo-cytoplasmic hybrid fish were obtained between different genera of fresh-water teleosts using the technique of nuclear transplantation. The survival rate of the hybrid fish obtained from the combination of carp nucleus and crucian cytoplasm is 3.2%<sup>[1]</sup>. The survival rate of the nucleo-cytoplasmic hybrid fish obtained from the combination of crucian nucleus and carp cytoplasm is 0.9%<sup>[2]</sup>.

In addition, in 1973 Tung et al.<sup>[3]</sup> reported that when different fresh-water teleosts of subfamilies (goldfish and one kind of bitterling) were used as material in the nuclear transplantation experiments, they only obtained some nucleo-cytoplasmic hybrid embryos rather than any adult hybrid fish. However, in these experiments, the adult nucleo-cytoplasmic hybrid fish were obtained between *Ct* fish and *Me* fish using the technique of nuclear transplantation.

The average survival percentage of *CtMe* fish which was obtained from seven experiments (see Table I) is 3.6%. It is obviously higher than that of the hybrid fish obtained from the combination of crucian nucleus and carp cytoplasm and also a little higher than that of the hybrid fish from carp nucleus and crucian cytoplasm. As indicated in Table I, the survival rates of the hybrid fish in seven different batches of experiments are of great difference. In the worst batch (third batch), no adult hybrid fish were obtained. In the best batch (seventh batch), the survival percentage of adult hybrid fish is as high as 14.1%. The comparison of these results with those obtained from the nuclear transplantation experiments between different genera and subfamilies of fresh-water teleosts, it is not difficult to observe



that difference in subfamily is not an insurmountable barrier for obtaining adult hybrid fish using the technique of nuclear transplantation. The main factor which influenced the success for obtaining hybrid fish believed to be the quality of the recipient enucleated eggs. Of course, how to keep the transplanted nucleus without damage during operation, the time used for nuclear transplantation (the faster the better) and the suitable location of the transplanted nucleus in the enucleated eggs are all the technical factors which can influence the success of nuclear transplantation. These results are in keeping with what we obtained in our previous experiments of nuclear transplantation in fish.

As for the reason why no adult nucleocytoplasmic hybrid fish could be obtained from the fish of different subfamilies using the technique of nuclear transplantation when goldfish and Chinese bitterling were used as materials is probably due to their different developmental patterns between those two kinds of fish. The egg of Chinese bitterling has much egg yolk, and a special kind of embryonic movement (blastokinesis) was observed during its early development stages when the blastoderm shield was completed, i.e., the blastoderm shield will rotate 180° of its location on the egg yolk. In addition, during its early developmental stages, two horn-like embryonic projections consist of egg yolk inside and epithelium outside appeared in both side of the embryonic head. It is a very special developmental pattern which was ever seen in the other fish of the family *Cyprinidae*. The developmental speed of Chinese bitterling is also much lower than that of goldfish. Therefore, the inconsistent of the early developmental patterns between goldfish and Chinese bitterling seems to be a natural barrier to make their nucleocytoplasmic hybrid embryos arrested at most at larval stages. On the other hand, the early developmental patterns and speeds between *Ct* fish and *Me* fish are rather similar, and it seems that this point can be used as one of the reasons to explain why the adult nucleocytoplasmic hybrid fish were easier to be obtained between *Ct* and *Me* fish, even they also belong to different subfamilies.

(2) The three kinds of chromosome patterns obtained in these experiments indicate that although *Ct* and *Me* fish have same number of chromosomes ( $2n=48$ ) and most of their chromosomes were difficult to be distinguished in both fish morphologically. However, according to the preliminary examination of our fish materials at Chang Jiang Fisheries Research Institute, it shows that the arm-ratio of the largest eleven pair of chromosomes in *Ct* fish is  $>3$ , that of in *Me* fish is equal to 2, and that of in *CtMe* fish

is also  $>3$ , so that it could be identified that the nucleus of *Ct* fish had been transplanted into the enucleated eggs of *Me* fish and thus the *CtMe* fish were developed from the nuclear transplanted eggs. Further more, the morphological characteristics of *CtMe* fish were basically identified to be similar to those of *Ct* fish. Obviously, it also confirms that the obtained nucleo-cytoplasmic hybrid fish must be the combination of *Ct* nucleus and *Me* cytoplasm.

(3) According to the taxonomic criteria of the nucleo-cytoplasmic hybrid fish described in our previous paper of 1980<sup>[1]</sup> and 1984<sup>[2]</sup>, the results are different in different combinations of nucleus and cytoplasm. Morphological characteristics of those hybrid fish obtained from the combination of carp nucleus and crucian cytoplasm showed that some features were inherited from the nucleus donor fish, some features seemed to come from the cytoplasm host fish and some features were intermediate. It indicates that both nucleus and cytoplasm can influence the expression of genetic information on this kind of hybrid fish. Morphological characteristics of those hybrid fish obtained from the combination of crucian nucleus and carp cytoplasm showed that their main features are similar to those nucleus donor fish, crucian. It seems that the genetic influences from the donor nucleus fish were obtained on this kind of nucleo-cytoplasmic hybrid fish. Some morphological characteristics of *CtMe* fish obtained in these experiments, such as head in body length, depth in body length, width in body length, dorsal spine, abdominal keel, anal rays, gillrakers, pharyngeal teeth, lateral line scales and number of vertebrae were basically similar to those of donor nucleus *Ct* fish. It also indicated that the main morphological characteristics were genetically influenced by donor nucleus on this kind of hybrid fish. These results are coincident with those obtained from the nucleo-cytoplasmic hybrid fish of crucian nucleus and carp cytoplasm and different from those obtained from the nucleo-cytoplasmic hybrid fish of carp nucleus and crucian cytoplasm.

(4) So far one male *CtMe* fish had been obtained. It shows that the sexual gonads of that fish developed well and normal sperms can be produced by that fish to fertilize *Ct* eggs, and normal fish were developed from those fertilized eggs. It means that this kind of nucleo-cytoplasmic hybrid fish was fertile as we found in the nucleo-cytoplasmic hybrid fish obtained from the combination of carp nucleus and crucian cytoplasm.

(5) Although there are no experimental data available now to let us make a complete evaluation of the growth rate, nutrition value, diseases resistance and the quality of their offsprings of *CtMe* fish. But, according to pre-

eliminary observation of their growth rate and compared it with those reported in other references<sup>(6)</sup> about the growth rates of both *Ct* and *Me* fish in general, it seems that *CtMe* fish is better than its donor nucleus and host cytoplasm fish. In addition, since none of the *CtMe* fish was observed to be dead of disease infection as normal *Ct* fish always do. Therefore it can be proposed that this kind of experimental approach in the creation of new breeds between remote species in fish using the technique of nuclear transplantation has certain advantages which will provide an attractive future for improving the economic quality of the fish.

The further analysis of some characteristics of *CtMe* fish at biochemical level which showed some evidence of cytoplasmic influence on gene expression of *CtMe* fish has been briefly reported in a separate congress abstract<sup>(7)</sup> and will be published in details elsewhere.

## REFERENCES

- [1] Tung, T. C. et al., *Scientia Sinica*, 23:517—523, 1980.
- [2] Yan Shaoyi et al., *Scientia Sinica*, 27:1029—1033, 1984.
- [3] Tung, T. C. et al., *Acta Zoologica Sinica*, 19:201—212, 1973.
- [4] Tung, T. C. et al., *Scientia Sinica*, 14:1244—1245, 1963.
- [5] Wu Zhengan et al., *Acta Zoologica Sinica* 26:18—23, 1980.
- [6] 湖北省水生生物研究所, 鱼类研究室, 《长江鱼类》, 科学出版社, 1976, pp98, pp108.
- [7] Yan Shaoyi et al., JEEM Special Issue, Journal of Embryology and Experimental Morphology, European Developmental Biology Congress, Abstracts, Southampton, UK, 2—7, September, 1984, 105.

## Key words

Nuclear transplantation; fish(teleosts)

## 硬骨鱼类的细胞核移植

### IV a. 不同亚科间的细胞核移植——由草鱼

#### 细胞核和团头鲂细胞质配合而成的核质杂种鱼

严绍颐 陆德裕 杜 森 李光三 韩 胄 杨慧一 吴政安

(中国科学院发育生物学研究所, 北京)

杨永铨 夏德全 刘爱珠 张兴忠

(中国水产科学院长江水产研究所, 沙市)

本文报道了, 用细胞核移植的方法, 获得亲缘关系更远的, 不同亚科鱼类之间的核质杂种鱼, 即由鲤科 (Cyprinidae) 中, 雅罗鱼亚科 (Leucinae) 的草鱼 (*Ctenopharyngodon idellus*) 的胚胎细胞核和鲂亚科 (Abramidinae) 的团头鲂 (*Megalobrama amblycephala*) 的卵细胞质配合而成的核质杂种鱼。被移植的卵子共659个, 得杂种成鱼24尾, 成活率为3.6%。

这些核质杂种鱼的若干形态特征, 如体长/头长, 体长/体高, 体长/体宽, 背鳍硬刺, 腹棱、臀鳍条数, 鳃耙数, 咽喉齿, 侧线鳞片数和脊椎骨数等, 与草鱼者相似, 这表明此核质杂种鱼的上述特征受草鱼细胞核的影响。

检查草鱼, 团头鲂和由草鱼细胞核和团头鲂细胞质配合而成核质杂种鱼的染色体组成表明, 它们的染色体数目均为  $2n = 48$ 。但这三种鱼的绝大多数染色体在形态上很难彼此区别。不过, 根据我们初步观察发现, 在我们于长江水产研究所选用的草鱼和团头鲂染色体组型中, 它们的第十一对亚端着丝点染色体可以区别: 草鱼中, 该对染色体的臂比指数  $> 3$  (见图 1d、1d'), 而团头鲂中该对染色体的臂比指数为 2 (见图 1e、1e')。经检查也发现核质杂种鱼染色体组型中的第十一对染色体的臂比指数也  $> 3$  (见图 1f、1f')。由此可见, 核质杂种鱼的细胞核系来源于被移植的草鱼细胞核, 与核质杂种鱼所表现的若干形态特征所表明的结果相一致。

初步观察还表明, 核质杂种鱼的生长速度明显比团头鲂快, 也稍优于一般报道的草鱼的生长速度。没有发现在这些杂种鱼中, 有象草鱼那样往往因感染疾病而死亡的例子。

目前, 已有一尾雄性核质杂种鱼成熟并能产生正常的精子使草鱼卵受精。鉴于在这远缘鱼类之间进行有性杂交, 例如草鱼和团头鲂之间进行有性杂交, 它们的杂交胚胎往往不正常, 或在不同发育阶段致死, 或即使能获得少数成鱼, 它们也因性腺发育不良而不能繁殖后代形成杂种体系。而用细胞核移植的方法则能在远缘鱼类之间获得核质杂种鱼, 它们因能繁殖而有可能发展为新的鱼类克隆。为用生物工程的方法培育鱼类新品种探索一条新途径。有关此核质杂种鱼的生化等性状分析, 将另文报道。

**关键词** 细胞核移植; 鱼类 (硬骨鱼)