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Original Article

Developmental incompatibility between cell nucleus and cytoplasm as revealed by nuclear transplantation experiments in teleost of different families and orders

YAN SHAOYI*[1], TU MIAO[1], YANG HUIYI[1], MAO ZHONGRUNG[1], ZHAO ZIYONG[1], FU LIJUAN[1], LI GUANSHAN[1], HUANG GUOPING[1], LI SHUHONG[1], JIN GUANQUIN[2], MA LI[2], LIN LIDONG[3], XIA SHILING[3] and YU LEINING[4]

[1]*Institute of Developmental Biology, Academia Sinica, Beijing,* [2]*Guangxi Fisheries Research Institute, Nanning,* [3] *Pearl River Fisheries Research Institute, The Chinese Fisheries Academy, Guangzhou* and [4]*Chang Jiang Fisheries Research Institute, The Chinese Fisheries Academy, Shashi, China*

ABSTRACT Teleosts from different families and orders were used as materials for nuclear transplantation experiments. (1) The nuclei of goldfish (*Carassius auratus*, family Cyprinidae, order Cypriniformes) were transplanted into the enucleated egg cytoplasm of loach (*Paramisgurnus dabryanus*, family Cobitidae, order Cypriniformes) and vice-versa. (2) The nuclei of Tilapia (*Oreochromis nilotica*, order Perciformes) were transplanted into the enucleated egg cytoplasm of goldfish (*Carassius auratus*, order Cypriniformes). The chromosome number of the nucleus donor fish is different from that of the cytoplasmic recipient fish in each of the two combinations. In the first case, only a few early nucleo-cytoplasmic hybrid (NCH) larval fish were obtained in each combination. In second case, even though a high percentage of NCH blastulas were also obtained, the majority of them died at the same developmental stage, except a few which survived until early gastrula stage. The examination of the metaphase chromosome figures of the NCH blastulas or embryos obtained in all three combinations indicated that they were of nucleus-donor type. The developmental rates of all the NCH eggs were similar to those of cytplasmic-recipient type. Scanning electronmicroscopy examination showed that the morphology of NCH blastula cells, which were obtained from the combination of Tilapia nucleus and goldfish cytoplasm, manifested obviously abnormal features and the cells were arrested at different stages of cell disintegration. Two-dimension polyacrylamide gel electrophoretograms of the homogenates of Tilapia, goldfish and their NCH blastula cells showed that the protein synthetic pattern of NCH blastula was similar to that of Tilapia nucleus type. The results of experiments which failed to obtain NCH adult fish in all three combinations can be explained as a result of developmental incompatibility between the donor nucleus and the enucleated recipient egg cytoplasm, which were from distantly related fish species. And the chromosome numbers of all the component fish of the three combinations which were examined in the experiment and shown to be quite different from each other in the tested fish, should not be overlooked as one of the essential factors causing the developmental incompatibility in NCH fish in this experiment.

KEY WORDS: *developmental incompatibility, nuclear transplantation, teleosts*

Introduction

Our previous reports showed that several kinds of nucleo-cytoplasmic hybrid (NCH) fish were obtained by using the technique of nuclear transplantation. NCH fish obtained from the combination of nucleus and cytoplasm belonging to different varieties of fish (the nucleus of crucian carp, *Carassius auratus* wild type, was transplanted into the enucleated egg cytoplasm of goldfish, *Carassius auratus* domestic type) (Tung and Yan, 1985); NCH fish obtained

Abbreviations used in this paper: NCH, nucleo-cytoplasmic hybrid

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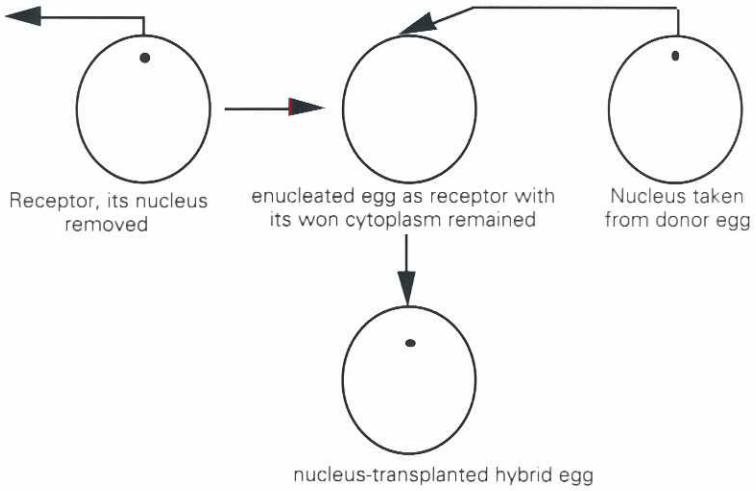


NUCLEUS RECEPTOR	COMBINATION BETWEEN	NUCLEUS DONOR
1. *Carassius auratus*, domestic type	Species & Species (1985)	*Carassius auratus*, wild type
2. *Carassius auratus*, wild type	Genera & Genera (1980, 1986)	*Cyprinus carpio*
3. *Cyptinus carpio*	Genera & Genera (1984)	*Carassius auratus*, wild type
4. *Megalobrama amblycephala*, subfamily Abramidinae	Subfamily & Subfamily (1985)	*Ctenopharyngodon idellus*, subfamily Leucinae
5. *Paramisgurnus dabryanus*, family Cobitidiae	Family & Family (1990)	*Carassius auratus*, domestic type, family Cyprinidae
6. *Carassius auratus*, domestic type	Family & Family (1990)	*Paramisgurnus dabryanus*, family Cobitidae
7. *Carassius auratus*, order Cypriniformes	Order & Order (1990)	*Oreochromis nilotica*, order Perciformes

from the combination of nucleus and cytoplasm belonging to different genera of fish (the nucleus of common carp, *Cyprinus carpio*, genus Cyprinus Linnaeus was transplanted into the enucleated egg cytoplasm of crucian carp, *Carassius auratus* wild type, genus Carassius Jarocki) (Tung *et al.*, 1963; Yan *et al.*, 1986) and their reciprocal nucleo-cytoplasmic transplantation combination, in which the nucleus of crucian carp was transplanted into the enucleated egg cytoplasm of common carp (Yan *et al.*, 1984); NCH fish obtained from the combination of nucleus and cytoplasm belonging to different subfamilies of fish (the nucleus of grass carp, *Ctenopharyngodon idellus*, subfamily Leucinae was transplanted into the enucleated egg cytoplasm of blunt-snout bream, *Megalobrama amblycephala*, subfamily Abramidinae) (Yan *et al.*, 1985).

The common characteristics of all these NCH fish are:

1. All four kinds of NCH eggs could develop into adult fish. The survival rates of these kinds of NCH fish were between 0.9%-3.2%.
2. All these kinds of NCH fish were fertile or unilateral sex fertile. For example: (a) the female NCH fish obtained from the combination of the nucleus of crucian carp and the egg cytoplasm of goldfish was fertile (Tung and Yan, 1985) while no male NCH fish of this combination (inter-varieties) has yet been found; (b) both male and female NCH fish obtained from the combination of the nucleus of carp and the egg cytoplasm of crucian carp (inter-genus), were fertile (Tung 1980; Yan *et al.*, 1986). They could produce normal sperm and eggs and lasted for 4 generations, and their offspring were obtained via self-crossing (Yan, unpublished data). (c) NCH fish from the combination of the nucleus of crucian carp and the egg cytoplasm of common carp were obtained. Among them at least two fish were identified as a male and a female. They survived until spawning season (Yan *et al.*, 1984). (d) Several NCH fish were obtained from the combination of the nucleus of grass carp and the egg cytoplasm of blunt-snout bream and were identified as male. They were able to release normal sperm to fertilize the eggs of grass carp. However, no mature female NCH fish were found during the experiment (Yan *et al.*, 1985).
3. Routine chromosome examination of the four kinds of combination for producing NCH fish revealed that the metaphase chromosome figures of the nucleus donor and the cytoplasm recipient might or might not show distinguishable differences. However, the nucleus donor and the cytoplasm recipient in each combination had the same chromosome number. For example, the chromosome number of both crucian carp and goldfish is 2n= 100; the chromosome number of both common carp and crucian carp, 2n= 100; the chromosome number of both grass carp and blunt-snout bream, 2n= 48.
4. In each kind of combination for producing NCH fish, the nucleus-donor fish and the cytoplasm-recipient fish were also used for sexual hybridization and different percentages of sexual hybrid fish were obtained. The results indicated that in closely related species, such as the crucian carp and goldfish, sexual hybridization between them gave rise to very high percentages of hybrid offspring which were fertile in both males and females. On the other hand, when rather distantly related species, such as the common carp and crucian carp, were hybridized sexually, some sexual hybrid fish were

TABLE 1

SURVIVAL PERCENTAGES OF DEVELOPMENTAL STAGES OF: NUCLEUS TRANSPLANTED EGGS OBTAINED FROM THE COMBINATION OF GOLDFISH NUCLEUS AND LOACH CYTOPLASM, AND THE COMBINATION OF LOACH NUCLEUS AND GOLDFISH CYTOPLASM (BOTH OF THESE COMBINATIONS ARE OF INTER-FAMILY HYBRID) AND THE NUCLEUS TRANSPLANTED EGGS OBTAINED FROM THE COMBINATION OF GOLDFISH NUCLEUS AND GOLDFISH CYTOPLASM, THE COMBINATION OF LOACH NUCLEUS AND LOACH CYTOPLASM (BOTH OF THESE COMBINATIONS ARE OF INTRA-SPECIES NONHYBRID)

	Developmental stages of transplanted eggs									
Combinations nucleus and cytoplasm	No. of transplanted eggs (%)	No. of early cleavage stage (%)	No. of blastula stage (%)	No. of gastrula stage (%)	No. of neurula stage (%)	No. of eye cup stage (%)	No. of heart-beat stage (%)	No. of blood stage (%)	No. of larva circulation (%)	No. of adult fish (%)
Nucleus of goldfish + Cytoplasm of loach	825 (100%)	615 (74.5%)	513 (62.1%)	124 (15%)	30 (3.6%)	24 (2.9%)	7 (0.8%)	5 (0.6%)	5 (0.6%)	0 (0%)
Nucleus of loach + Cytoplasm of goldfish	620 (100%)	386 (62%)	354 (57%)	114 (18.3%)	10 (1.6%)	5 (0.8%)	4 (0.6%)	4 (0.6%)	4 (0.6%)	0 (0%)
Nucleus of goldfish + Cytoplasm of goldfish	127 (100%)	96 (76%)	51 (40%)	29 (23%)	12 (9%)	5 (4%)	4 (3%)	2 (1.6%)	2 (1.6%)	2 (1.6%)
Nucleus of loach + Cytoplasm of loach	279 (100%)	198 (71%)	173 (62%)	111 (40%)	26 (9%)	20 (7%)	14 (5%)	10 (3.5%)	8 (2.8%)	8 (2.8%)

obtained but the males were sterile; the sexual hybridization between grass carp and blunt-snout bream produced a few sexual hybrid offspring but in this case as well, their gonads did not differentiate at all (Yan, unpublished data).

5. All the NCH fish obtained from these combinations were basically similar to the nucleus-donor type fish in their morphology, physiology and biochemistry but with some modifications (Yan, 1989).

In this experiment, fish of more distantly related species were

TABLE 2

COMPARISON OF THE NORMAL TABLE OF THE EGGS OF GOLDFISH, LOACH, GOLDFISH (♀) x LOACH (♂) AND LOACH (♀) x GOLDFISH (♂)

WATER TEMPERATURE

NORMAL TABLE OF FERTILIZED EGGS (MIN)

Developmental stages	Goldfish	Goldfish (♀) x Loach (♂)	Loach (♀) x Goldfish (♂)	Loach	Remarks
Fertilization	0	0	0	0	
1 cell	10±1.5	10±1.5	10±1.5	10±1.5	First period, the
2 cell	25±2	25±2	25±1.5	25±1	developmental rates
4 cell	50±2	50±2.5	50±2	50±2	for 4 kinds of eggs
8 cell	64±3	64±2	64±2	64±2	were the same
16 cell	86±2	86±2	85±2	84±2	
32 cell	105±3	106±3	104±2	103±2	
64 cell	124±3	125±3	122±2	120±2	
High blastula	174±3	175±3	170±3	168±2	Second period, the development
Middle blastula	202±3	203±3	198±3	195±3	for 4 kinds of egg still kept up
Late blastula	260±3	262+3	255±3	250±3	close rate. The development of
Early gastrula	315±4	318±4	301±3	296±3	loach became faster than that of goldfish
Middle gastrula	405±4	408±4	345±3	340±3	Third period, the developmental
Late gastrula	507±4	512±4	438±3	430±3	rates for the eggs of goldfish and loach
Neurula (blasto-pore closed)	630±4	655±4	538±4	520±4	became obviously different from each other. The development of loach became much
Eye cup	810±5	835±5	680±5	655±5	faster than that of goldfish. The developmental
Tail bud	1050±5	1080±6	970±5	910±5	rates of 2 kinds of hybrid
hatching	2125±9	2175±9	1470±8	1400±8	were of maternal type.

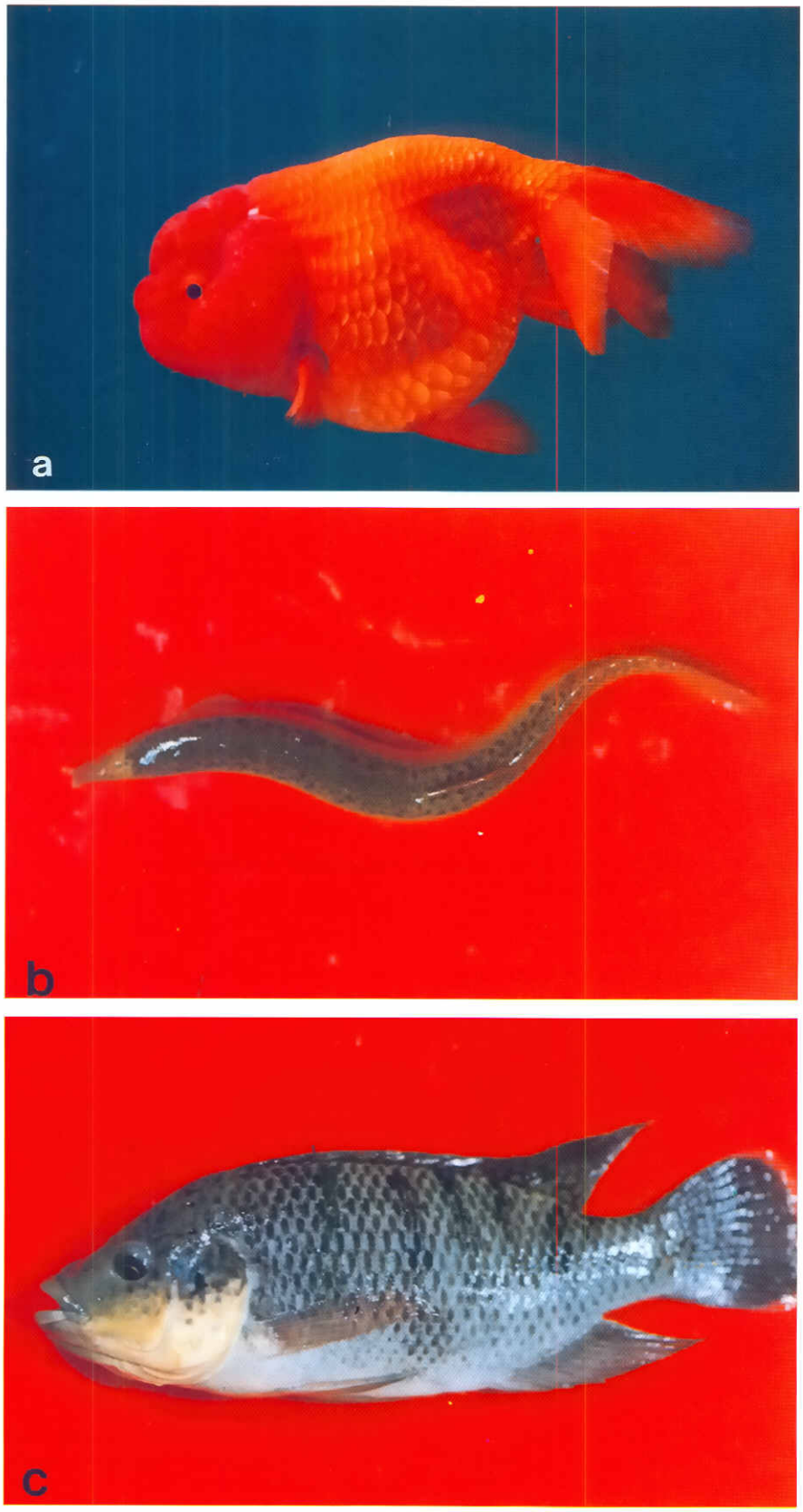


Fig. 2. Photograph shows the different fish species used in both nuclear transplantation and sexual hybridization experiments. (a) *Goldfish* (Carassius auratus, *family Cyprinidae, order Cypriniformes). **(b)** Loach* (Paramisgurnus dabryanus, *family Cobitidae, order Cypriniformes). **(c)** Tilapia* (Oreochromis nilotica, *family Cichlidae, order Perciformes*).

used as materials. (a) The nucleus of goldfish (*Carassius auratus*, family Cyprinidae, order Cypriniformes) (Fig. 2a) was transplanted into enucleated egg cytoplasm of loach (*Paramisgurnus dabryanus*, family Cobitidae, order Cypriniformes) (Fig. 2b) and vice versa (both are inter-families); (b) the nucleus of Tilapia (*Oreochromis nilotica*, order Perciformes) (Fig. 2c) was transplanted into the enucleated egg cytoplasm of goldfish (*Carassius auratus*, order Cyprinifomes) (inter-orders). These three kinds of nucleus transplantation experiments are illustrated in Fig. 1. The aim of this experiment was to investigate the developmental compatibility of NCH eggs in more distantly related fish species and the factors that may influence compatibility.

Results

Nuclear transplantation experiments in teleosts of different families

(1) The nuclei of goldfish were transplanted into the enucleated eggs of loach. It can be seen in Table 1 that from 825 nucleus-transplanted eggs, 513 developed into blastula stage (survival rate 62.1%), 124 into gastrula stage (survival rate 15%) and 5 into larval fish stage (survival rate 0.6%). Fig. 3a shows one of the NCH larval fish from this combination. The metaphase chromosome figure of the NCH embryo is of goldfish type (nucleus-donor type, see Fig. 3b)

(2) The nuclei of loach were transplanted into the enucleated eggs of goldfish (and vice-versa). It can also be seen in Table 1 that of 620 nucleus-transplanted eggs, 354 developed into blastula stage (*i.e.* survival rate 57%), 114 into gastrula stage (survival rate 18.3%) and 4 into larval fish stage (survival rate 0.6%). Fig. 3c shows one of the NCH larval fish from this combination. The metaphase chromosome figure of the NCH embryo is of loach type (nucleus-donor type, see Fig. 3d).

As is shown in Table 3, no differences in the developmental rate among two kinds of NCH eggs and their two kinds of recipient eggs in early embryonic stage (up to mid-gastrula stage) were observed. However, the time taken by NCH eggs to develop into larval fish stages was similar to that of each recipient egg type and to the maternal fish of each sexual hybrid between the same two fish species.

Since the NCH eggs were obtained by individual micro-operation, no detailed statistics are available in comparison with those of sexual hybrid eggs.

The larval fish of both kinds, which were obtained from the above two kinds in nuclear transplantation experiments, finally died of extravasated blood or enlargement of the cardiac sac. Both cases showed the common lethal syndrome as well as syndromes occurring either in other nuclear transplantation experiments or in the sexual hybridization experiments when distantly related teleosts were used as materials.

According to these results, it can be concluded that the nuclei of the NCH embryos in both experiments were derived from the diploid donor-nuclei instead of from the residual haploid nuclei of the recipient eggs. Thus they were true NCH embryos or larval fish.

In order to check whether the failure to obtain complete development of the above two kinds of NCH eggs resulted from the mechanic injuries of micro-operation, the following two kinds of intra-species nuclear transplantation experiments were carried out as controls and the results are shown in Table 1, indicating that:
(1)When the nuclei of goldfish were transplanted into the enucleated eggs of goldfish, from 127 nucleus-transplanted eggs, 2 adult fish were obtained (survival rate 1.6%).
(2)When the nuclei of loach were transplanted into the enucleated eggs of loach, from 279 nucleus-transplanted eggs, 8 adult fish were obtained (survival rate 2.8%).

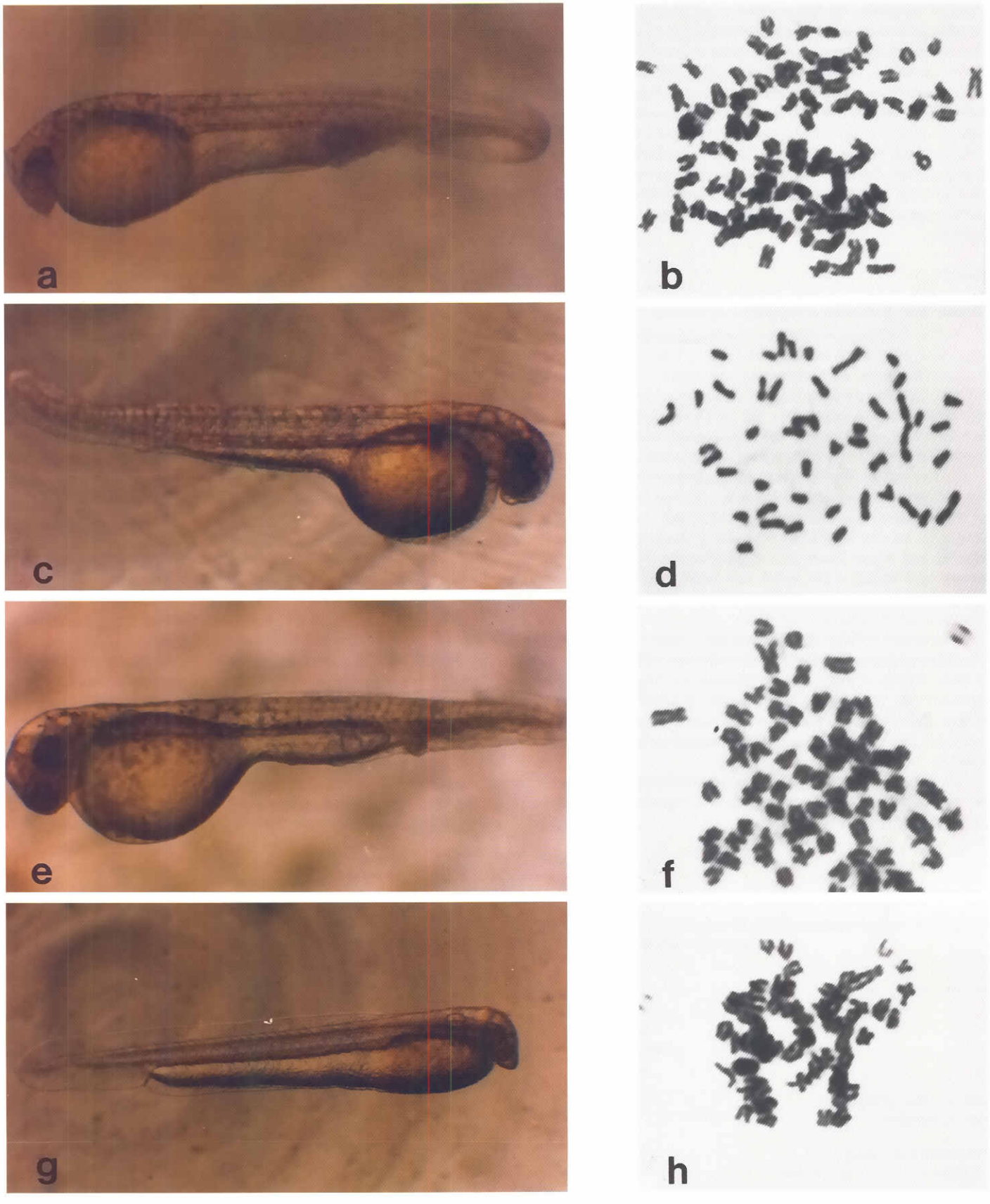
Since intra-family nucleus-transplanted adult fish were obtained in both experiments, this indicates that the operation injury of the nucleus-transplanted egg obviously was not a main factor influencing whether the NCH eggs developed into adult fish when the nucleus and cytoplasm, which belong to different families of fish, were combined together. On the other hand, the survival rates of adult fish were also very low in both kinds of intra-species nucleus-transplanted eggs, and the possibility of operation injuries influencing the process of the development of the nucleus-transplanted eggs should not be excluded.

The sexual hybridization experiments between goldfish and loach were also carried out in order to examine whether sexual hybrid fish could be obtained from those two distantly related species of fish. The results showed that when goldfish eggs were fertilized with loach sperm, of 450 fertilized eggs, 210 developed into normal heart-beating stage (survival rate 46.6%). But all of them became abnormal and died at different larval fish stages (Fig. 3e). The metaphase chromosome figure is of heteroploid type (Fig. 3f). When loach eggs were fertilized with goldfish sperm, the same

TABLE 3

THE SURVIVAL PERCENTAGES OF DEVELOPMENTAL STAGES OF NUCLEUS TRANSPLANTED EGGS, WHICH WERE OBTAINED FROM THE COMBINATION OF TILAPIA NULEUS AND GOLDFISH CYTOPLASM

	Developmental stages of nucleus transplanted eggs				
Combination of nucleus and cytoplasm	**No. of transplanted eggs (%)**	**No. of early cleavage stage (%)**	**No. of early blastula stage (%)**	**No. of late blastula stage (%)**	**No. of gastrula stage (%)**
The nucleus of Tilapia was transplanted into the enucleated eggs of goldfish	3907 (100%)	2907 (74.4%)	2809 (71.8%)	2111 (53.7%)	6 (0.15%)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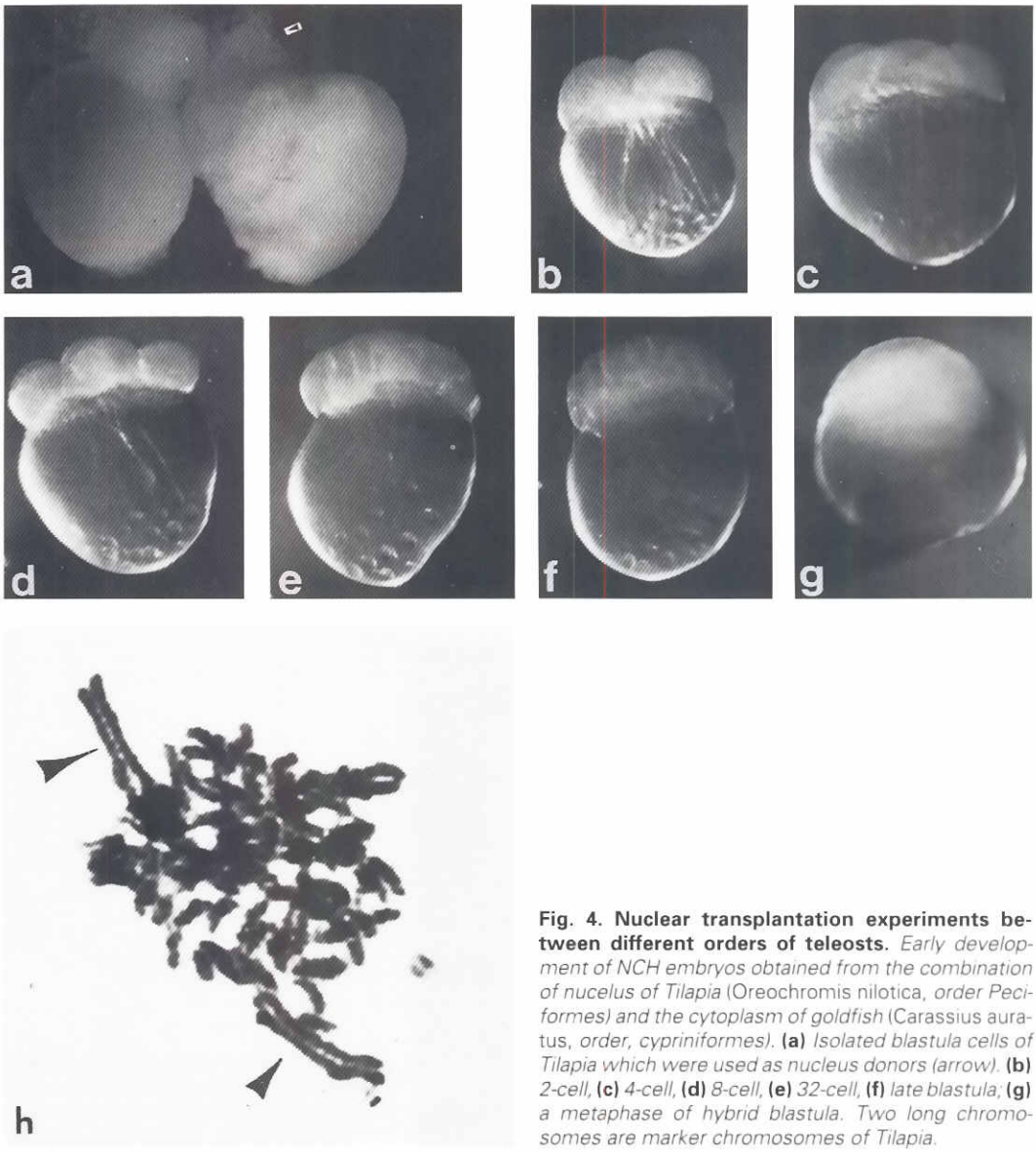

Fig. 4. Nuclear transplantation experiments between different orders of teleosts. *Early development of NCH embryos obtained from the combination of nucelus of Tilapia* (Oreochromis nilotica, *order Peciformes) and the cytoplasm of goldfish* (Carassius auratus, *order, cypriniformes).* **(a)** *Isolated blastula cells of Tilapia which were used as nucleus donors (arrow).* **(b)** *2-cell,* **(c)** *4-cell,* **(d)** *8-cell,* **(e)** *32-cell,* **(f)** *late blastula;* **(g)** *a metaphase of hybrid blastula. Two long chromosomes are marker chromosomes of Tilapia.*

results were obtained, *i.e.* of 340 fertilized eggs, 140 developed into normal heart beating stage (survival rate 46.6%). Then, all of them gradually became abnormal and died at different larval fish stages at length (Fig. 3f). Its metaphase chromosome figure is of heteroploid type (Fig. 3g). Therefore, no sexual hybrid fish were obtained from the two inter-family sexual hybridization experiments.

Table 2 shows the comparison of the developmental rates of goldfish and loach and sexual hybrids between them. It can be seen that the developmental rates of goldfish and loach in early embryonic stages are similar to each other, but they have differences after mid-gastrula stage until larval fish stage. It also shows that the developmental rates of both sexual hybrids, which developed after late gastrula stage, were of maternal type.

Nuclear transplantation experiment in teleosts of different orders

In this experiment the nuclei of Tilapia were transplanted into the enucleated eggs of goldfish. As shown in Table 3, of 3907 nucleus-transplanted eggs, 2111 developed into blastula stage (Fig. 4b-g). The survival rate of the NCH embryos at this stage (53.7%) is close to those obtained in above two inter-family nuclear transplantation

Fig. 3. The nucleocytoplasmic hybrid and sexual hybrid larval fish and their metaphase chromosome figures. (a) *NCH larval fish (43.5 h, 26°C) obtained from the combination of goldfish nucleus and loach cytoplasm.* **(b)** *Metaphase chromosome figure MCF of NCH of the combination of 3a (goldfish type, diploid).* **(c)** *NCH larval fish (45 h, 26°C) obtained from the combination of loach nucleus and goldfish cytoplasm.* **(d)** *MCF of NCH of the combination of 3c (loach type, diploid).* **(e)** *Goldfish (♀) x Loach (♂), larval fish (55 h, 26°C).* **(f)** *MCF of sexual hybrid of Goldfish (♀) x Loach (♂) (heteroploid).* **(g)** *Loach (♀) x Goldfish (♂), larval fish (61h, 26°C).* **(h)** *MCF of sexual hybrid of loach (♀) x goldfish (♂) (heteroploid).*

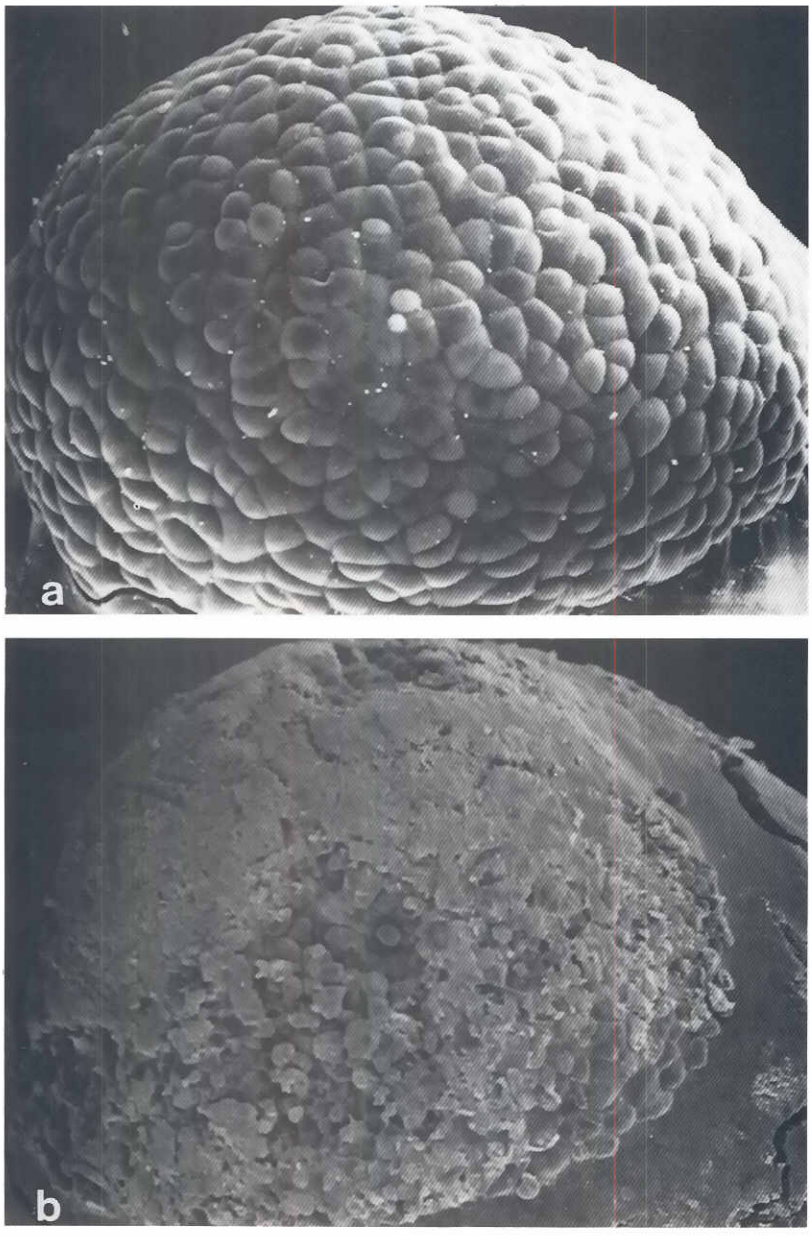

Fig. 5. The scanning electron microscopic pictures of normal goldfish *(Carassius auratus)* blastula and NCH blastula *obtained from the combination of Tilapia* (Oreochromis nilotica) *nucleus and goldfish cytoplasm.* **(a)** *Normal goldfish blastula (x200). Showed regular blastula cells.* **(b)** *NCH blastula (x200). Wilt blastula cells were released from the egg.*

experiments (compared with the data in Table 1). However, the majority of the NCH blastula obtained in this experiment did not develop furtherly except 6 of them which were able to develop into the beginning of gastrula stage and died soon after.

The comparison of the developmental rates of early embryonic stages among Tilapia, goldfish and the NCH embryos indicated that the developmental rate of NCH embryo was more closely similar to that of goldfish (cytoplasmic-recipient type) rather than to that of Tilapia (nucleus-donor type). For example, after nuclear transplantation, the duration between 1-cell to 2-cell stage in NCH egg took about 50 mins, the same duration in goldfish egg took about 45 mins, while in Tilapia egg it took about 120 mins; another example is that the duration between 1-cell to late blastula stage in NCH egg took about 260 mins, the same duration in goldfish took about 240 mins while in Tilapia egg it took about 660 mins. These three kinds of eggs were cultured in Holtfreter's solution at 26°C.

The examination of the metaphase chromosome figure of the NCH blastula showed that it was of Tilapia type (nucleus-donor type, Fig. 4h, 2n= 44, with 2 pairs of long-armed chromosomes).

Since most of the NCH embryos died at late blastula stage, we examined the morphology of their blastula cells by means of scanning electronmicroscopy and found that when the NCH eggs developed into blastula stage, their cells became obviously abnormal and arrested at different stages of cell disintegration (Fig. 5a, b).

Two-dimension polyacrylamide gel electrophoretograms of the

homogenates of Tilapia, goldfish and their NCH blastula cells showed that the protein synthetic pattern of NCH blastula was basically similar to that of Tilapia nucleus type except that 2 polypeptides dots appeared in the pattern of Tilapia blastula which were not found in that of NCH blastula and 4 polypeptide dots in the pattern of NCH blastula were not found in that of Tilapia blastula (Fig. 6a, b, c). The nature of those polypeptides dots has not been accurately identified. This means that the expression activities of Tilapia nucleus genome with minor modifications were observed in the NCH egg cytoplasm as early as in the blastula stage instead of in the gastrula stage.

The reciprocal nuclear transplantation experiment between these two fish, *i.e.* the combination of goldfish nucelus and Tilapia cytoplasm, has not been done because the Tilapia egg is too fragile and it is not suitable for doing nuclear transfer operations.

The inter-order sexual hybridization experiments between Tilapia and goldfish were also done in order to investigate whether any sexual hybrid fish could be obtained between such distantly related fish species. The results showed that no early embryos of sexual hybrid or adult fish developed when goldfish eggs were fertilized with Tilapia sperm or Tilapia eggs were fertilized with goldfish sperm. In both cases, only 1 or 2 regular or irregular cell divisions of the eggs were observed in the very beginning of their development. Then these blastomeres fused together and the whole egg soon died.

In summary, neither inter-family nor inter-order nuclear-cytoplasmic hybrid fish could be obtained from these experiments and the incompatibility between nucleus and cytoplasm seemed to be a key factor which may influence the normal development of those NCH eggs in comparison with the results obtained in the inter-variety, inter-genus and inter-subfamily nuclear transplantation experiments.

Discussion

According to our previous experimental results, the conclusion could be drawn that: (1) developmental compatibility existed under the interaction of the nucleus and cytoplasm which were derived from either the diversity between different varieties, different genera or different subfamilies of fish; (2) the genetic characteristics of NCH fish in all those combinations might be modified by the interaction of nucleus and cytoplasm (Yan, 1989); (3) the possiblity for obtaining NCH fish is almost comparable to the possibility of obtaining sexual hybridized fish in all those combinations (unpublished data). The only difference between the NCH fish and sexual hybrid fish is that the NCH fish are fertile in general while the sexual hybrid fish of the same combination is almost infertile or infertile in unilateral sex, even though the fish species used for those experiments have the same chromosome number (Yan, 1989). This indicated that the developmental compatibility between nucleus and cytoplasm was greater in nucleo-cytoplasmic hybrid fish than that in sexual hybridized fish.

Generally speaking, the interaction of nucleus and cytoplasm in NCH fish did not seem to seriously influence the normal development of their gonads. But in sexual hybridization, the developmental compatibility between diploid nuclei of sperm and that of egg as well as the compatibility between the hybrid zygot nucleus and egg cytoplasm in distantly related species will play the role of influencing the normal development of their hybrid's gonads. Therefore, it seemed that the normal development of gonads in sexual hybrid fish was expected to be determined more essentially by the diversity between male and female diploid nuclei, rather than by the diversity between nucleus and cytoplasm if compared with the same distantly related species used in sexual hybridization.

The results of this experiment showed that it was very difficult to obtain NCH fish between very distantly related species, *i.e.* inter-family and inter-order by means of nuclear transplantation. However, some differences in developmental compatibility of NCH fish obtained from the combination of nucleus and cytoplasm in the fish belonging to different families and different orders were observed. For example, the NCH egg obtained from the combination of goldfish nucleus and enucleated loach egg cytoplasm develop into larval fish stage (inter-family), the NCH egg obtained from the combination of loach nucleus and enucleated goldfish egg cytoplasm developed into larval fish stage too (inter-family) while the NCH egg obtained from the combination of Tilapia nucleus and enucleated goldfish egg cytoplasm could only develope into late blastula stage, except a few which reached very early gastrula stage (inter-order).

These results indicate that when the nucleus and cytoplasm from the fish of different families or orders are combined together in a NCH egg, the developmental compatibility between the nucleus and cytoplasm is highly restricted. The more distantly related the species used in combination, the more serious the developmental incompatibilities revealed.

On the other hand, the results from the sexual hybridization experiments showed that it was vey difficult to obtain any normal sexual hybrid fish among the aforesaid fish species. When goldfish and loach were crossed, some sexual hybrid embryos were able to continue developing until larval fish stage (inter-family) and no normal embryos were obtained when Tilapia and goldfish was sexually crossed (inter-order). So, the developmental incompatibility in both NCH and sexual hybrid eggs seemed to be comparable. In addition, the common lethal syndromes which caused the death of NCH as well as sexual hybrid larval fish would also confirm this point.

It is worth noting that the chromosome numbers of all those fish species used as materials in this experiment are different from each other. For example, the chromosome number of goldfish is 2n= 100, loach 2n= 48 and Tilapia, 2n= 44. Therefore, the contradiction between the nucleus-donor with different chromosome numbers and the recipient egg cytoplasm in nuclear transplantation experiments, and the inharmonious chromosome numbers in diploid sperm and egg nucleus, as well as the contradiction between the heteroploid chromosome numbers of the hybrid zygot nucleus and its surrounding cytoplasm in sexual hybridization, seemed to be the essential factors influencing the developmental compatibility of NCH eggs and of the sexual hybrid eggs at different levels.

Tung *et al.* (1973) reported that when the nucleus of goldfish (domestic *Carassius auratus*, subfamily Cyprininae, 2n= 100) was transplanted into the enucleated egg cytoplasm of the bittering (*Rhodus sinensis*, subfamily Acheilognathinae, 2n= 46) or sexual hybridization of these two kinds of fish (inter-subfamily) was carried out, neither NCH nor sexual hybrid fish could be obtained, with the exception of the NCH or the sexual hybrid egg which only develop into larval fish stage. As both NCH and sexual hybrid fish could be obtained when grass carp and blunt-snout bream were used as materials (also inter-subfamily) and both of them had the same chromosome numbers, it can be assumed that the difference in chromosome numbers between goldfish and bittering may also be

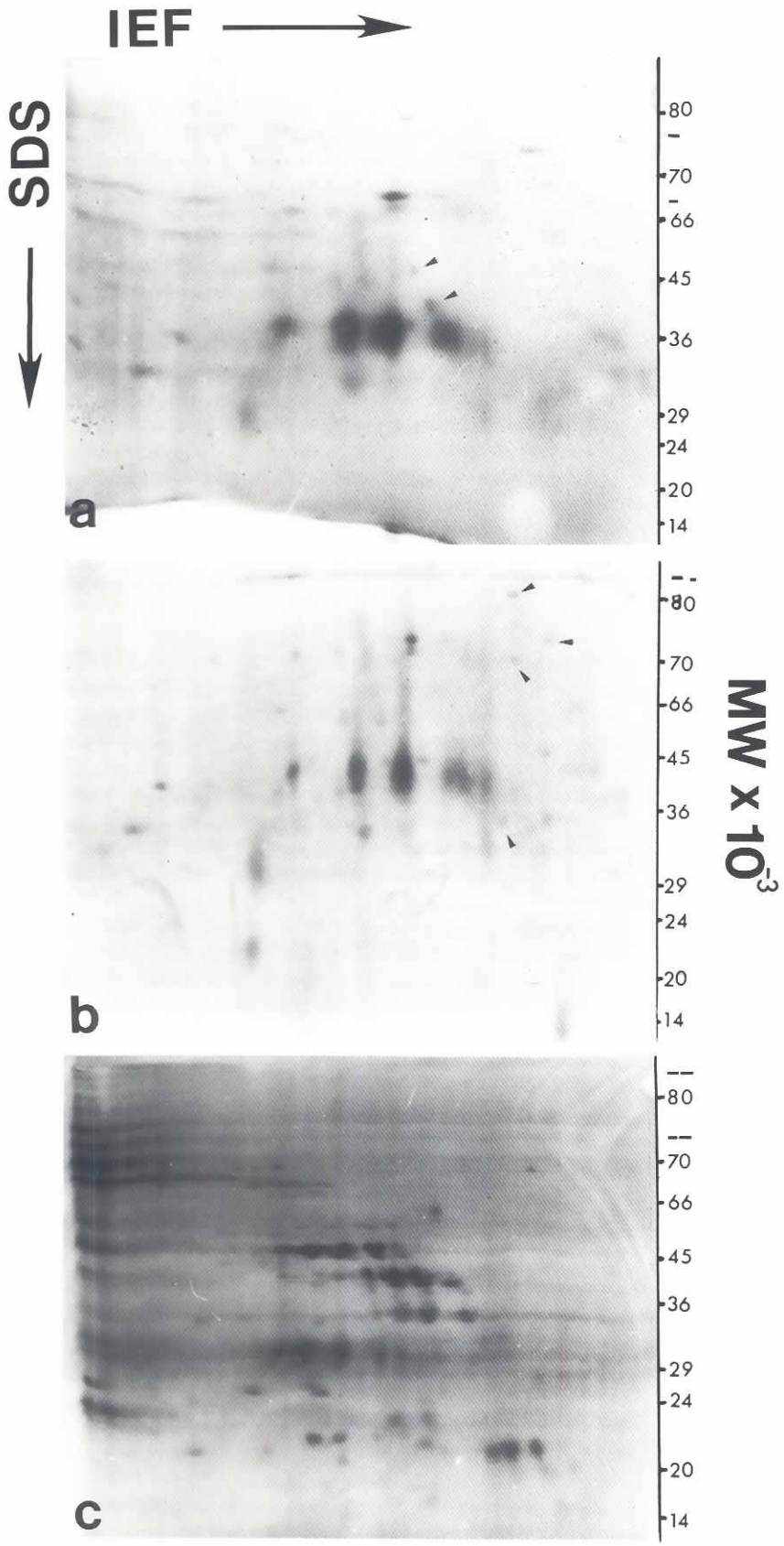

Fig. 6. Two-dimensional electrophoretograms of polypeptides extracted from blastula of (a) *Tilapia* (Tilapia nilotica). **(b)** *Hybrid blastula obtained from the combination of the nucleus of Tilapia and the cytoplasm of goldfish.* **(c)** *Goldfish* (Carassius auratus). *Arrows show the differences of polypeptides dots between (a) and (b).*

considered as one of the essential factor influencing the developmental compatibility of their NCH and sexual hybrid fish.

Wu *et al.* (1980) reported that when different varieties of goldfish were used for nuclear transplantation experiments, the cytoplasmic effects were strengthened through serial transplantations. They proposed that during serial nuclear transplantations, the accumulative effect of the cytoplasm of several recipient eggs of the nucleus becomes strong enough to modify the original function of the donor nucleus. If this were true, then the possibility would existe to enhance the developmental compatibility of NCH egg by using serial nuclear transplantation. We tried serial nuclear transplantation in the aforesaid fish combinations too. Unfortunately, improvement in the developmental compatibility of the NCH eggs has not yet been observed. Probably the developmental incompatibility of the NCH eggs in this experiment surpassed their threshold for further development as compared with the NCH eggs obtained from the combination of fish species in different varietes, genera and subfamilies.

It is important to notice that as the nucleus of Tilapia was transplanted into the enucleated egg cytoplasm of goldfish, a high percentage of NCH blastula were obtained. This indicated that the potential developmental compatibility between Tilapia nucleus and goldfish cytoplasm seemed to occur at the very early developmental stages. However, it disappeared very quickly as soon as the late blastula stage was reached. Scanning electronmicroscopy examinations showed that the embryonic cells of these NCH blastulas became abnormal and their development was arrested at different scales of cell disintegration which made the egg impossible to develop further.

Two-dimensional polyacrylamide gel electrophoretograms of the homogenates of Tilapia, goldfish and their NCH blastula cells showed that the protein synthetic pattern of NCH blastula was basically similar to that of Tilapia nucleus type except that some minor modifications could be identified. This meant that the expression of the genomic activities of Tilapia nucleus occurred in the recipient egg cytoplasm of goldfish, prior to gastrula stage, while in normal state, the genomic activities would be expressed when gastrulation starts, since the maternal mRNAs, which are stored in the egg cytoplasm, are responsible for early development up to blastula stage (Browder, 1980). These results indicated that the reprogram of genomic activities could happen when different nucleus and cytoplasm from diverse species, were combined together in a new developing system (Yan, 1989).

Nowadays, it was generally believed that when a foreign gene is transferred into a fertilized egg, a transgenic animal may result because the foreign gene can automatically be integrated into the egg genome, reprogramming its genomic activities in spite of the existence of the protective mechanisms of gene regulation of the egg itself, which remained species-specific during evolution. As a matter of fact there was also mch experimental evidence, indicating that many factors (proteins) exist in the cell cytoplasm may have the regulatory functions of gene activities. On this basis, to assume the possibility of the existence of some sorts of reprogramming mechanisms of genomic activities in a NCH egg or embryo would be a reasonable explanation.

In addition, the developmental rates of NCH and sexual hybrid fish in all three combinations showed that they are similar to that of the recipient egg cytoplasm type. This also confirmed that the maternal cytoplasmic influences have changed the cell division cycles of donor nucleus in both cases (Satoh, 1989).

Comparison of these results which were obtained in our previous experiments and those of these experiments indicated that the developmental rates of goldfish and crucian carp, common carp and crucian carp, grass carp and blunt-snout bream are all similar to each other (Yan *et al.*, unpublished data) while those of goldfish and loach are different in late embryonic stages; and those of Tilapia and goldfish are obviously different from each other as early as in the cleavage stages. It can be proposed that the developmental incongruities between the nucleus and cytoplasm derived from distantly related fish species (inter-family and inter-order) and combined in a new NCH egg, may also influence developmental compatibility.

These experimental results have raised some important questions for further investigating, such as (1) what about the molecular basis that may control the developmental incompatibility between nucleus and cytoplasm in NCH fish as well as in sexual hybrid fish obtained from distantly related fish species? (2) what is the difference in the cytoplasmic mechanisms influencing the developmental incompatibility of NCH and sexual hybrid eggs? (3) does the possibility exist of overcoming the developmental incompatibility in NCH and sexual hybrid fish in some other way? This is the target we will be aiming towards in the near future.

Materials and Methods

Fish stocks and technique for nuclear transplantation

Freshwater teleosts, the goldfish, loach and Tilapia were provided by the Aquaculture facility of the Institute of Developmental Biology, Academia Sinica, Beijing, China. The method for taking eggs and blastula cells from the above fish and the nuclear transplantation technique were reported in previous papers (Tung *et al.*, 1963; Yan, 1989).

Chromosome examination

The technique for obtaining chromosome preparations in fish was according to Yamazaki's method (1981).

Technique for scanning electronmicroscopy

Laboratory routine procedure for scanning electron microscopy were followed: the specimen were fixed with 2.5% glutaraldehyde (Sigma) in 0.1 M cacodylate buffer, pH 7.2 for several hours, then rinsed with the same buffer and dehydrated with ethanol. After absolute alcohol was substituted by iso-amyl acetate (Sigma), the specimen underwent critical-point drying in carbon dioxide, was coated with gold, and examined with JSM-T200 scanning electron microscope.

Technique for two-dimension polyacrylamide gel electrophoresis

Sample preparation

Blastulas from each kind of fish were transferred into a 50% Holtfreter's solution and the blastoderm of each blastula was cut from the yolk part by using a glass microneedle under a dissection microscope, then rinsed three times with boiled water. The blastoderms were lysed in lysis buffer (0.5% NP-40, 150 mM NaCl, 50 mM Tris-HCl at pH 7.4, 5µl/blastoderm) with several times of cryolysis, then mixed and stirred vigorously on a vertex and centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was taken and stored at -80°C until used (Hamaguchi *et al.*, 1982).

Two-dimensional gel electrophoresis

IEF- the first dimension. Measured sample volumes (5µl lysis buffer per blastula) were loaded onto the tops of first dimension IEF tube gels (4% acrbis, 9 M Urea, 2% NP-40 (Fluka), 6% Ampholine (LKB) pH 5-7, 1.2% Ampholine pH 5-7, 0.2% Ampholine pH 3-10). The focusing gel was in diameter of 1.0 mm and length of 90 mm, focusing between 0.1 M H_3PO_4 anode buffer and 0.2 M NaOH cathode buffer overnight for 6,000 V. Hr.

(constant voltage). SDS (Serva).

PAGE- the second dimension. The focusing gel was extruded from its tube and applied directly to the top of slab gel (4% stacking gel and 10% running gel). Then the tube gel was sealed to the slab with a minimum amount to gelling agarose (Pharmacia) (1% LMP agarose [BRL], 1% SDS, 1 mM MTT, 50 mM Trils-HCl). Electrophoresis in the second dimension was 5 hours at constant current of 15 mA. (O'Farrell, 1975; Austerberg, 1982).

Siver-stain

The gel slab was soaked in formaldehyde-ethanol solution (7% formaldehyde, 4% ethanol) for 30 min. Then the gel slab was washed in 10% ethanol for 10 min three times and in distilled water 10 min three times. The water was drained off andfreshly made ammonical silver solution was added, followed by soaking for 12 min. The gel was then washed for 5 min in distilled water and developed in 1% acetic acid for 3 min, then transferred to glass distilled water for taking the picture (Oakley, 1980; Austerberg, 1982).

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