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STUDY ABOUT THE ORIGIN AND THE CELLULAR GENETIC MECHANISMS RESPONSIBLE FOR THE CHROMOSOME POLYMORPHISM IN SOME INTERSPECIFIC HYBRIDS IN FISH

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Key words: cellular genetic mechanisms,chromosome polymorphism intespecific hybrids, fish

Abstract: Crossings between the common carp females and *Ictiobus cyprinelus, Ctenopharyngodon idella,* or *Hypophthalmychtys molitrix* males led to offsprings with polymorph chromosome numbers and different natures, according to the cellular genetic mechanisms which we analyzed. These mechanisms involved a pre- or postmeiotic diploidization of the maternal genome, amphimixis of the two pronuclei or with the second polar body, somatic cell multiplications or reductions, additions or loses of chromosomes from one or both genitors; mechanisms which led usually to aneuploidy. The factors which regulate this mechanisms are unknown and they had a random manifestation, different during ontogenesis, and different in each cell line.

INTRODUCTION

The intespecific hybrids inherit the chromosome sets from their two genitors. A recognition between these chromosome sets occurs, which leads to the establishing of the future fate of each chromosome. When the mated species are distant related, the homology of their chromosomes is small and great disorders occur in the genome of their hybrid offsprings. In this situation, some mechanisms interfere, trying to balance the function of the new created genome, so that the carrying individual will survive (CUELLAR 1974).

The mechanisms, implicated in the restoring of the disturbed balance, induce important (large) genome mutations, materialised in a polymorph state of the chromosome number. The origin of the chromosomes in the hybrid genome is different and stronglz related with the genetic mechanism which led to the restoring of this balance (BARSHIENE 1980).

The common carp (*Cyprinus carpio* L.) was often used in hybridisation experiments with chinese carp species (BAKOS and all. 1978, 1995) or with the crucian carp (CHERFAS and all. 1993).

In this study we analyzed the cellular genetic mechanisms which generate some interspecific hybrids and their chromosome polymorphism. We analyzed also the origin of the chromosomes in the genome of these experimentally obtained interspecific hybrids.

MATERIAL AND METHODS

The interspecific hybrids were obtained in the Fish Culture Research Station in Nucet – Dambovița County, using common carp (*Cyprinus carpio* L.) eggs, stripped from Ineu breed females (homozygous in the recessively inherited scattered scale pattern) (20=100 chromosomes) (RAICU and all. 1972) and milt from *Ictiobus cyprinellus* (2n=100 chromosomes), *Ctenopharyngodon idella* (2n=48 chromosomes) (MARIAN and KRASZNAI 1978), or *Hypophthalmichtys molitrix* (2n=48 chromosomes) (MARIAN and KRASZNAI 1978) males.

We analyzed cytogenetically some individuals from each experimental hybridisation batch in order to establish their chromosome number (partial performed in V.R.I.B. Pasteur-Bukarest) (NICOLESCU and all. 1990). The origin of the offsprings was analysed according to their morphological phenotype and their scale cover patterns.The cellular genetic mechanisms, which induce the chromosome polymorphism, were analyzed according to the chromosome sets counted in the cells from different tissues, which were analyzed in different stages of their ontogeny.

RESULTS AND DISCUSSIONS

The origin and the cellular genetic mechanisms which occur in *Cyprinus carpio* x *Ictiobus cyprinellus* hybrids

In the offsprings populations, obtained in this experimental hybridisation, we found 78% of the individuals with a diploid chromosome set (2n=100 chromosomes), with common carp morphologic phenotype and with a scattered scale cover (maternal inherited pattern). These individuals are for certain ***gynogenetic diploids***. They did not have polymorphic chromosome numbers. In this case, the diploidisation of their maternal genetic material occurred spontaneously and was not experimentally induced.

The other 22% from this population had a common carp morphologic phenotype, scattered scale cover, but a very large chromosome polymorphism, with 120-150 chromosomes in the sets. These offspings could be considered ***gynogenetic triploids*** (***Table 1*** and ***Table 2***).

Gynogenesis occurs in many fish species as a fertility restoring mechanism of the hybrid, in principal when the mated species are distant related (PURDOM and LINCOLN 1974, CHOURROUT 1985, NA-NAKORN and all. 1993). TURNER and all. 1980 demonstrated that the unisexual gynogenetically reproducing species *Poecilia formosa* is a hybrid.

The possible mechanisms, which induced the counted polymorph states, are presented in ***Fig. 1***. The origin of the chromosomes of the gynogenetic offsprings is easy to establish. These offsprings have only two maternal chromosome sets, one of them from the egg's pronucleus and the other one from the second polar body, which was retained in the egg cell when this egg was fertilised with a heterogen sperm. The mechanism, which determines the sperm to induce this retention is unknown. Probably there are involved some factors which prevent the forming of the division spindle or it's function, when it appeares. The male pronucleus does not participate in amphimixis because of the incompatibility of its chromosomes (they come from distant related species), which have a small homology. This genetic material remains inactive in the cell and it is probably lost in the future zygotic mitoses. It was not found in adult cells. Because of the presence in the cell of the homologue genetic material of the female pronucleus and the second polar body, the amphimixis takes place between these two cell formations.

Another diploidisation mechanism occurring in the gynogenetic offspring, is possibly induced by the sperm cell. It could have a postmeiotic manifestation. In this case, the egg finishes it's second meiotic division and extrudes the second polar body, after it was penetrated by a sperm cell. Then, because of the non homology of the two chromosome sets, induced after the recognition mechanism of the chromosomes, or because of other causes, the sperm cell would induces an endomitosis of the female pronucleus and a diploid zygote would be formed. It continues a normal division. This mechanism probably occurred as a necessity to restore the diploid state, which permits a good function of all genes. The factors involved in the manifestation of this mechanism probably occurred during evolution as a necessity to restore the viability and the fertility of the interspecific hybrids.

Triploidy occurred often in interspecific fish hybrids (CHEVASUS and all. 1983, GRAY and all. 1993). The interspecific hybrids of carp are often triploids (VASILYEV and all. 1975, REDDY and all. 1990). The three chromosome sets belong usually to only one genitor (mostly the mother) and extremely rare to both genitors. In order to balance these genomes, one can observe many intermediate states with interindividual and intraindividual polymorphism in chromosome numbers, determined by very complicated mechanisms, until a diploidisation of the triploid will occur and generate a new species (in their phylogeny).

Table 1 **- Types of interspecific hybrids obtained by fertilising common carp eggs with sperms from males which belonged to different pecies**

male genitor	scale cover pattern	nature of offsprings	morphologic phenotype	code
Ictiobus cyprinellus	scattered	gynogenetic diploids	common carp	IC-GD
	scattered	gynogenetic hiperdiploids	common carp	IC-GHD
Ctenopharyngodon idella	scaled	aneuploid diploid hybrids	intermediate	CI-DH
	scaled	aneuploid diploid hybrids	intermediate	CI-DH
	scaled	aneuploid diploid hybrids	intermediate	CI-DH
	scaled	real hybrids	intermediate	CI-H
	scaled	anuoploid triploid hybrids	intermediate	CI-TH
	scaled	anuoploid triploid hybrids	intermediate	CI-TH
	scaled	anuoploid triploid hybrids	intermediate	CI-TH
	scaled	anuoploid triploid hybrids	intermediate	CI-TH
	scattered	gynogenetic diploids	common carp	CI-GD
	scattered	gynogenetic diploids	common carp	CI-GD
	scattered	gynogenetic diploids	common carp	CI-GD
Hypophthalmichthys molitrix	larvae	real hybrids	-	HM-H

A possible mechanism in the triploids could be the total or partial retention of the father's chromosomes. In this hybrid, this hypothesis is infirmed by the scale cover of the offsprings. The cytogenetically performed analyzes did not identified the origin of the chromosomes in the obtained smears. If the paternal chromosomes were retained (THORGAARD and all. 1985), they did not contain genes which determine the scale cover or other morphological patterns.

More probably, the triploids inherited only mother genomes, which randomly lost some chromosomes during the cell division, in order to balance the function of these genomes in the triploid cell.

It is not excluded, but less possible, that after the second polar body extrusion, there occurred a normal amphimisis between the pronuclei of the two genitors. Subsequently, the mother chromosomes doubled by endomitosis and the father chromosomes were gradually eliminated during embriogenesis.

The aneuploidy with increased the chromosome number was observed mostly in one-summer-old individuals. The two years old fish had mostly normal diploid chromosome sets, state that shows that the triploidy is only a transitive state. Finally the father chromosomes will be totally eliminated and the offspring will have balanced diploid gynogenetic chromosome sets.

The origin and the cellular genetic mechanisms which occur in *Cyprinus carpio* x *Ctenopharyngodon idella* hybrids

According to the chromosome sets of the genitors, a real hybrid between this two species would have 2n=74 chromosomes,.

24% of the hybrid offsprings obtained by this crossing, were individuals with common carp morphologic pattern, scattered scale cover (mother inherited pattern) and chromosome sets with 98-100 chromosomes (a normal state for a diploid common carp, according to RAICU and all. 1972). We considered them ***gynogenetic diploids***. CERFAS and all. 1993, 1994 described such gynogenetic diploid hybrids, obtained by crossing the crucian carp with the common carp and STANLEY 1976 reported androgenetic and gynogenetic hybrids obtained between *Ctenopharyngodon* and other *Cyprinidae.*

The other 76% of the hybrid offsprings had normal scale cover and the morphologic pattern intermediate between the two genitor species. We considered them ***hybrids***. 48% of them were ***triploid aneuploid hybrids*** with 2n=100-150 chromosomes, 4% ***real hybrids*** with 63% of their cells with 70-74 chromosomes and 24% of them ***diploid aneuploid hybrids*** with 2n=98-101 chromosomes ***(Table 1*** and ***Table 2).***

In the individuals with scattered scale cover, the gynogenetic mechanism determined the retention, in the offspring´s genome, only of the maternal chromosomes, which performed spontaneously a diploidisation. The cobserved variation in the chromosome number was in normal limits for a diploid individual. The normal scaled individuals were generated by many complex cellular genetic mechanisms which induced the counted interindividual and intraindividual aneuploidy.

The individuals appointed as triploid-aneuploid hybrids could have different origin. A possible mechanism, which explain the appearance of these individuals, determined an addition in the hybrid genome of two maternal chromosome sets (by retention of the second polar body, or by an endomitosis of the female pronucleus) and a paternal chromosome set and the generation of triploid hybrid cells. Then, during ontogeny, the paternal chromosomes, which were not homologue with the double female chromosomes, were gradually eliminated. Because the paternal chromosomes were eliminated during the whole life of the individual, they could have some active genes in the earlier stages, and induce phenotypic patterns (FERRIS and all. 1977 a, b). This mechanism is sustained by the intermediate morphological patterns and the normal scale cover of this hybrid. Even in the morphological pattern there was a decisive influence of the maternal inheritance. When we agree this theory we can consider also the normal scaled offsprings to be aneuploid triploids with an advanced chromosome elimination and not aneuploid diploid hybrids. Because the analysed individuals had all rather the same age and dimensions, we could not prove this assumption for certain. The so formed triploid cells, could perform a reductional division, which generates cell clones with 60 chromosomes.

In these cells there will subsequently occur random chromosome reductions and additions. Another possible mechanism that led to offsprings with normal scale cover and 100-122 chromosomes, could be the aneuploid hybrid origin. According to this hypothesis, in this case, the two pronuclei performed an amphimixis which led to a real hybrid zygote. It subsequently performed a diploidisation in order to balance the gene function and became a normal diploid.

Then, due to the aleatory manifestation of the genetic regulation in different cells, a random elimination of heterochromatic chromosomes occurred, chromosomes which could belong to one of the genitors (probably the father), but also to both genitors. More, some of these diploid hybrid cells with 140 chromosomes could perform a similar reductional division as in triploid hybrid cells and generate cell clones with 70 chromosomes. This genome can perform then random chromosome reductions or multiplications. In this way, the same chromosome number as in a simple real hybrid could occur in this case, but here were involved major chromosome restructurings, most of them at the functional level. The so formed chromosome set is probably a mosaic of maternal and paternal chromosomes, randomly chosen in different cells. If this hybrid is fertile, during generations it could lead to the stabilisation of this new ploidy level and form a new species.

The cell clones with 60 chromosomes probably appeared after a random reductional division of the triploid aneuploid cells and the cells with 70 chromosomes after a reductional division of some diploid hybrid cells with 140 chromosomes, or after an advanced chromosome elimination process.

Table 1 **– Number of chromosomes of the interspecific hybrids obtained by fertilising common carp eggs with sperms from males which belonged to different species**

code	nr. anal. indv.	% of analysed cells																							
		number of chromosomes																							
		60	69	70	72	74	75	80	84	86	90	92	98	100	101	104	110	120	122	125	130	132	140	142	150
IC-GD	10	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-
C-GHD	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	⇐	48	⇒	⇐10⇒		⇐21⇒		21
CI-DH	3	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-
CI-DH	2	-	10	-	-	-	-	-	-	-	-	-	-	90	-	-	-	-	-	-	-	-	-	-	-
CI-DH	1	-	-	-	-	-	-	-	-	-	-	-	⇐	100	⇒	-	-	-	-	-	-	-	-	-	-
CI-H	1	-	-	⇐	63	⇒	-	⇐	5	⇒	-	-	⇐	27	⇒	-	-	-	-	-	-	-	-	-	-
CI-TH	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-
CI-TH	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	⇐				100				⇒
CI-TH	3	-	-	-	-	-	-	-	-	-	-	-	-	⇐		100		⇒	-	-	-	-	-	-	-
CI-TH	1	16	-	17	-	-	-	-	-	-	-	-	-	17	-	-	-	50	-	-	-	-	-	-	-
CI-GD	1	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-
CI-GD	2	-	-	-	-	-	-	-	-	-	-	-	-	⇐	100	⇒	-	-	-	-	-	-	-	-	-
CI-GD	3	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-		
HM-H	6	-	⇐		100			⇒	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

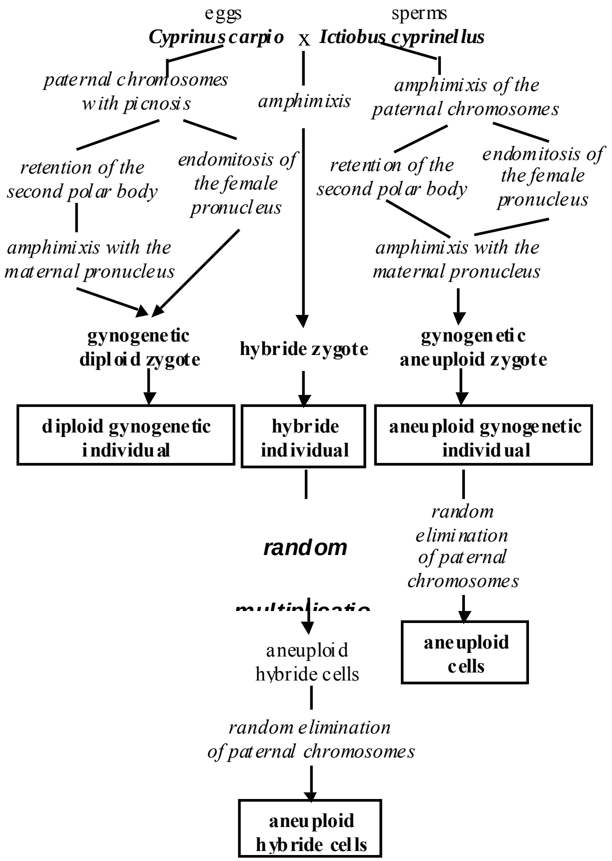

Fig. 1 – **Cellular genetic mechanisms possible to be involved in the generation of the *Cyprinus carpio* x *Ictiobus cyprinellus* hybrids.**

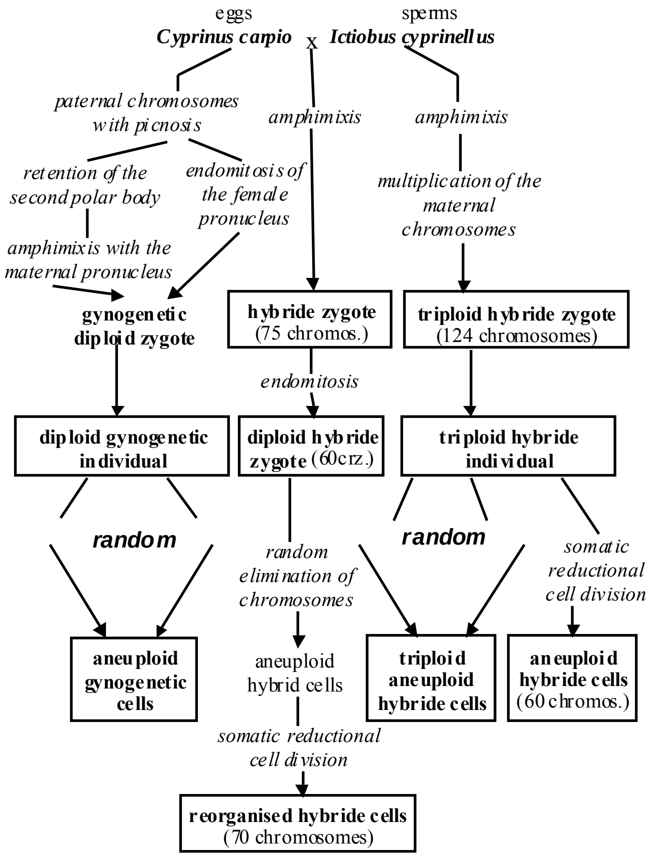

Fig. 2 – **Cellular genetic mechanisms possible to be involved in the generation of the *Cyprinus carpio* x *Ctenopharyngodon idella* hybrids.**

3,4% of the normal scaled population had chromosome sets with 70 chromosomes in the most cells. The other cells had more chromosomes until 100 ones. This is the most eloquent expression of a possible real hybridisation process. In this case the cells with encreased chromosome numbers were determined by postzygotic chromosome additions. More plausible is the hypothesis that there was a haploidisation process of the tiploids or of the diploid hybrids; that did not exclude some chromosome losses or duplications, in some triploid cell clones.

The offspring is a real hybrid if it had in the majority 70-74 chromosomes. They come from the retention of the two haploid genomes of the genitor species (50 chromosomes from the carp and 24 from *Ctenopharyngodon*). The cells with different chromosome number could disappear during ontogeny until the mature age of the individual.

The individual with diploid normal chromosome sets and with normal scale cover, in their majority, are probably gynogenetic diploids, which added paternal functional genes.

It is difficult to make an interpretation upon which of the described mechanisms was involved. It is possible that each individual or even each cell line followed it's owen way of development, so that we counted all that cases in different ontogenetic stages and in different tissues.

We could not establish the factor that determined the gynogenetic or hybrid development of the offspring. The sperm penetrated certainly the egg, but the later evolution of its genetic material could be different, probably determined by many cellular regulatory factors from the egg cell.

The intraindividual heterogeneity of the hybrid genome, in the first month of development is a trace (sign) of the possible chromosome segregation in the hybrid cells, analysed both "in vivo" and in cell cultures. In the over one years old hybrids the balanced ploidy level was high.

The origin and the cellular genetic mechanisms which occur in *Cyprinus carpio* x *Hypophthalmichtys molitrix* hybrids

We analysed cytogenetically larvae from this offsprings, which did not survived until the fingerling age, so that we could not analyze their morphologic phenotype. These larvae had constantly 67-80 chromosomes in their sets ***(Table 1*** and ***Table 2)***, so that we considered them ***real hybrids***. The variation of the chromosome number indicated an aneuploid state, determined by random extrusions and multiplications of some chromosomes ***(Fig 3).***

The mortality of the individuals took place because the two parental genomes were not balanced, so that the chromosomes reductions and additions could be performed. In this way the function of the cells was strongly affected, or some lethal genes became expressed. The mortality of the individual, in their first year of life, was determined by the expression of some lethal genes until this age or by some fitness deficiency induced by genomic unbalances.

CONCLUSIONS

- By crossing *Cyprinus carpio* x *Ictiobus cyprinellus*, we obtained 78% gynogenetic diploids and 22% aneuploid gynogenetic triploids with polymorphism of the chromosome number,
- By crossing *Cyprinus carpio* x *Ctenopharyngodon idella*, we obtained 24% gynogenetic diploids, 24% aneuploid diploid hybrids, 48% aneuploid triploid hybrids and 4% real hybrids
- By crossing *Cyprinus carpio* x *Hypophthalmychtys molitrix,* we obtained real interspecific hybrids showing polymorphism of the chromosomes. The offspring did not survive the one summer age because of the expression of some lethal genes, because their genomes were not balanced so that the genetic regulation mechanisms were disturbed, or because of a lose in their fitness.
- The gynogenetic diploid offspring were obtained by a premeiotic or postmetiotic diploidisation of the maternal genome.
- The aneuploid diploid or triploid hybrids were obtained by random additions and eliminations of some chromosomes from one or both genitors.
- The real hybrids were obtained by the amphimixis of the parental genomes.
- The polymorph chromosome numbers are a result of the following processes :
  - the partial or total amphimixis of the two pronulei,
  - the partial or total random elimination of some chromosomes which belong to a diploid or triploid gynogenome, obtained by the multiplication of the matrnal genome,
  - the random duplication of some chromosomes or the whole sets of gynogenomes,
  - somatic reductional divisions or aleatory mitosis in the cells of the gynogenetic offspring,
  - the multiplication of the maternal genome and its amphimixis with the paternal one, leading to triploid hybrids. Subsequently the aneuploidy appeared by the extrusion or duplication of some chromosomes.
  - the multiplication of the hybrid chromosomes by amphimixis of the two heterogene pronuclei, which led to diploid hybrid cells, which subsequently could suffer reductional divisions or abnormal mitosis and generate aneuploid diploid hybrid cells.
- In different cells of the same organism there acted different cellular genetic mechanisms.
- The cellular genetic mechanisms become functional in different stages of ontogeny.
- In each individual, the cellular genetic mechanisms evolve in different ways, because the hybrid state generates different unbalances of the genetic regulation.

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