

STUDY ABOUT THE PARTHENOGENETIC DEVELOPMENT POSSIBILITIES OF SOME CIPRINIDS

CARMEN NICOLESCU

Keywords: parthenogenesis, haploidy, diploidy, ciprinids, temperature,

Abstract: The segmentation and early ontogenetic development of the unfertilised common carp, goldfish and grass-carp eggs were investigated in different temperature conditions. A normal development of the eggs until the morula or disco-blastula stage was observed in all analysed fish. After these stages almost all eggs died. Only very few goldfish or common cap eggs performed a parthenogenetic development. These individuals were more then sure haploid because they didn't survive the yolk sac resorbtion stage. The parthenogenetic events were stimulated by the extreme temperature conditions.

INTRODUCTION

Parthenogenesis is a reproductive modality that supposes the development of an individual from an unfertilised egg-cell. The new individual can be haploid or diploid. To develop a normal individual it must became diploid either through nuclear fusion or through a restriction division. Mostly cleavage products of a haploid egg may undergo fusion, producing a diploid embryo.

Phenomenon includes non-gametic forms of automixis in animals is a common case of male haploidy.

Parthenogenetic development seams to have ecological and evolutionary implications in animals. The absence of males enables rapid production of offspring without food consumption from males. Sometimes there was observed a combination of thelytoky (absence of males) and bisexual fertilisation.

Some instances of thelytoky (automictic or meiotic thelytoky) involve meiotic egg production, and two of the four meiotic products sometimes fuse to restore diploidy.

In other cases (apomictic or ameiotic thelytoky) mitosis produces the egg-cells, or diploidy may be restored by endomitosis after meiosis.

The development of unfertilised eggs using one or other of the above-described events can occur, in rare occasions, in many animal species. It can be also induced artificially. It is still surprisingly rare, especially since it avoids the cost of meiosis. Thelytokous forms seem to be liable to early extinction compared with their bisexual relatives, probably through progressive homozygosity.

The fish egg is at ovulation blocked in the metaphase of the second meiotic division. It contains a large amount of RNA and proteins, able to sustain the fist development stages of the embryo. The second meiosis is continued only if the egg is entered by a sperm-cell. In this case the chromosomes are gathered in the haploid female pro-nucleus and in the haploid second polar body, which is extruded in the environment. The female pro-nucleus passes the fertilisation events by gathering its chromosomes with those from the male pro-nucleus, brought by the sperm-cell's head. After these, the zygote is formed and it begins segmentation. During segmentation the genetic programme encoded in the egg's molecules is performed.

The genetic events are accompanied by some cellular events like hydration of the egg-cell. During hydration water enters in the egg which swells so that the mycropile is closed and no sperm-cell can enter anymore.

In fish, like in other animals, there can sometimes occur parthenogenetic developing events.

Which of the described mechanism is implicated and the conditions inducing this phenomenon is to be investigated. In common carp this process is rare - 0,1-3% (Golovinskaja, 1968) , 0,1 - 1% (Cherfas, 1975), 0,0005% (Nicolescu, 1994). Because it happens, it may have a biological significance.

PURPOSE OF THE INVESTIGATION

In this study there was investigated if parthenogenesis happens in common carp, goldfish and grass-carp and if extreme environmental conditions like extreme temperature values concur to stimulate a parthenogenetic development of the egg-cells.

This investigation has a theoretic importance because it tries to give an answer on the adaptive and evolutionary meaning of parthenogenesis. Because there were analysed a large number of unfertilised eggs, incubated in artificial conditions, it was possible to surprise a very rare phenomenon like parthenogenesis. The experimental conditions avoid the interference of gynogenesis induced by a heterogene sperm-cell. Also it was possible to analyse the if extreme temperature values stimulate parthenogenesis.

If this question will become a positive answer, parthenogenesis can be a useful tool in obtaining haploid fish or diploid totally homozygous ones in practice.

MATERIALS AND METHODS

Common carp (*Cyprinus carpio*), goldfish (*Carassius auratus gibelio*) and grass-carp (*Ctenopharyngodon idella*) eggs were striped after hormonal treatment from mature females.

During 5 years more than 10kg eggs were incubated without fertilisation using the specific incubation technology. The common carp eggs and those from the goldfish were incubated stuck after activation on suspended nyal nets. The grass-carp eggs were incubated suspended in the water-flow.

The incubation temperature was used as variation parameter. The temperature influence was tested at 8°C, 12°C, 18°C, 20°C, 22°C, 23°C, 25°C and 28°C.

Each experiment was compared with a control batch in which normal fertilised eggs, from the same female fish, were incubated in the same temperature conditions.

The other environmental conditions like oxygen content, chemical water quality were maintained constant.

During incubation there were made macroscopic and microscopic observation on the development of the unfertilised eggs.

The survival rate was calculated, as a relative value in accordance with the survival in the control batch, at hydration, at the morula, blastula and gastrula stage.

RESULTS AND DISCUSSIONS

Almost all unfertilised eggs began hydration after their contact with the water (Fig.1a). Hydration is a physic event not depending on fertilisation.

The survival of the fertilized egg-cells in different early ontogenetic stages is presented in Table 1.

The hydration was accomplished in 99,99% of the incubated eggs by a normal segmentation like in the control ones. This high survival rate was observed in all analysed species not depending on the environmental condition until the 16-cell stage. After this stage they began a differentiation.

In the morula and disco-blastula stage the control batch and also the experimental batches had 80-90% alive, normal developed eggs, depending on the environmental incubation conditions.

The beginning of each cytological and genetic event during segmentation was influenced by the temperature. The lower the temperature was the slower the events' succession. At a normal temperature of 22°C the morula stage occurred after 10 hour of incubation. At higher or lower temperatures the segmentation led sometimes to unequal blastomeres.

At the morula stage (Fig.1b) began the massive mortality of the eggs, in accordance with the extreme environmental conditions. No egg treated at 28°C survived this stage. The dead eggs could be distinguished by their opacity (Fig.1c). At lower temperatures, a few grass-carp eggs survived this stage, more common carp and goldfish eggs.

Table 1 – The survival of the fertilized egg-cells in different early ontogenetic stages

temperature [°C]	survival rate of the unfertilized eggs [%]							
	8	12	18	20	22	23	25	28
<i>Cyprinus carpio</i>								
hydration	99,2	99,25	100	99,99	99,99	99,98	99,65	98,9
morula	82,5	85,3	87,8	89,3	98,9	85,2	85,7	81,2
disco-blastula	12,5	9,5	6,2	5,2	4,8	4,2	5,3	0
gastrula	0,003	0,002	0	0,0009	0,0007	0,001	0,002	0
<i>Carrassius auratus gibelio</i>								
hydration	99,78	99,83	100	99,87	99,99	99,98	99,65	98,9
morula	87,23	88,25	90,1	90,2	89,23	87,2	82,5	81,2
disco-blastula	11,7	5,3	6,8	8,7	4,2	8,2	9,4	12,3
gastrula	0,005	0,002	0	0,0009	0,0009	0,0008	0,003	0,21
<i>Ctenoparyngodon idella</i>								
hydration	99,23	99,12	99	98,99	99,01	99,12	99,89	99,8 7
morula	80,1	83,4	85,5	85,7	81,2	81,5	87,3	85,7
disco-blastula	0,0001	0,001	0	0,0002	0,0001	0,0001	0,001	0
gastrula	0	0	0	0	0	0	0	0

After 14 hours the disco-blastula became visible (Fig.1d). In this stage almost all eggs died.

The fish egg-cell has RNA and proteins that allow the normal development until the gastrula stage (Kunz 1978). This capacity is sustained by the existence in the egg-cell of all necessary elements and regulating systems encoded in the egg-RNA and egg-proteins. The death caused by stopping the segmentation occurred at different times according with the individual specificity (eggs from different females). This indicates an individual specificity of the matrocline development of the egg until a specific ontogenetic stage when the regulating genes are inactive or lake. These genes belong to the cytoplasmatic inheritance of the egg. The quantities of RNA in the egg-cell, but also the environment, induce the moment of death. These hypothesis was confirmed by the development of the eggs from different females which stopped their development some of them in the morula stage, other in the disco-blastula stage, even in the same environmental conditions. The same behaviour was observed at all three analysed species.

The goldfish eggs had a higher survival so that some eggs passed this stage. At extreme temperature an increase of the survival was also observed. All grass-carp eggs died at the disco-blastula stage stage.

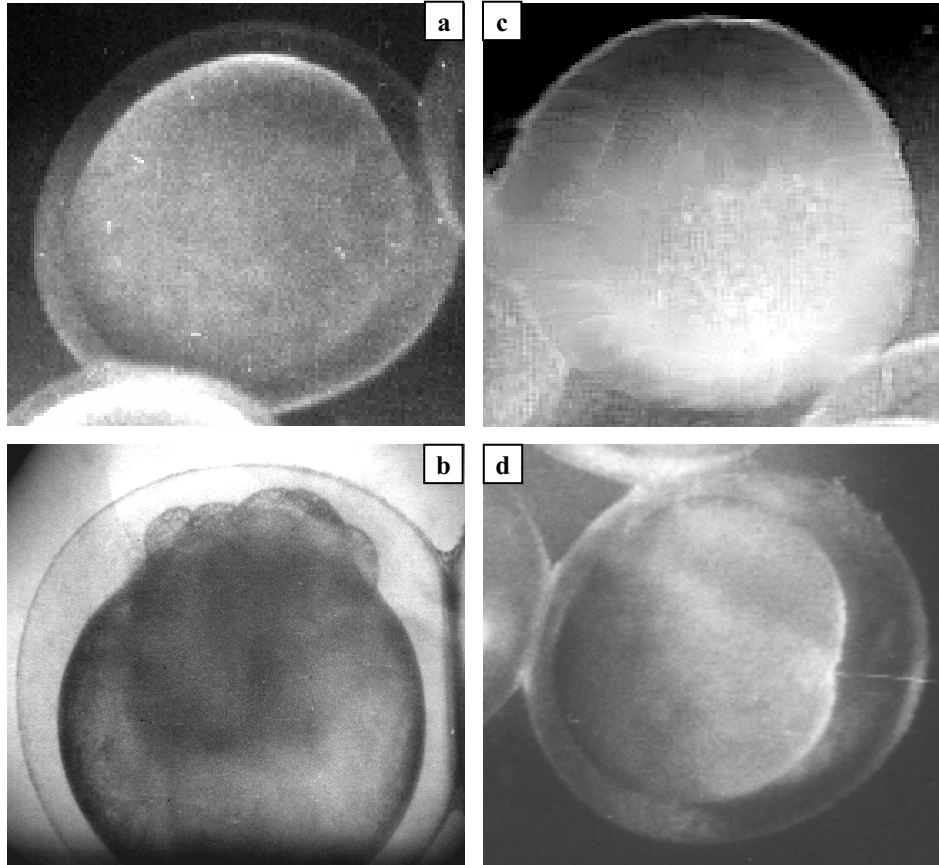


Fig.1 – The segmentation of the unfertilised common carp egg-cell
 a) – hydration, b) - dead cell, c) - morula, d) – disco/blastula

At the gastrula stage a few eggs survived, more in the goldfish batch and more those incubated at extreme temperature conditions. There were found only a very few common carp eggs alive and no grass-carp eggs.

The goldfish and common carp eggs, which survived this stage, had a normal future development and a normal survival rate, like that counted in the control-batch. Some of them hatched, but no one survived the yolk sack resorption stage.

The extreme temperature lowered the normal viability of the eggs so that most of them died before their biologic potential was exhausted. Only an extremely few number survived so that a parthenogenetic development can be diagnosed.

It seems that a further development of the egg needs a sperm-cell not only to balance the genes but also to deliver some cytoplasmic constituents like the centrosome (Busniță et al. mentioned by Lieder, 1959).

The parthenogenetic diagnosed common carp and goldfish eggs can be haploid or diploid ones. They were not cytogenetic investigated to count their chromosome number.

The common carp and the goldfish are tetraploids of an ancient ancestor, so that in some conditions they can survive in a haploid form that can easily generate triploids with adaptive and evolutionary advantages. Haploids can survive only until the larval stage (Nicolescu, 2002), so that the hatched individuals can be considered haploids and not diploids. Diploids can survive even if they are totally homozygous like the mitotic gynogenetic ones until adulthood (Nicolescu, 2002).

The increasing survival in accordance with the restrictive environmental conditions can sustain a spontaneous diploidisation induced by these environmental conditions. These events can have also an evolutionary significance - in a restrictive environment, when survival of the population is low, there became active some reproductive mechanisms to maintain survival of the population.

In the grass-carp no surviving egg was found after the disco-blastula stage, maybe because its higher sensibility or maybe because its genome can not be balanced in a haploid form, and no diploidisation events happened.

CONCLUSION

- At all analysed ciprinids the egg-cell can begin segmentation until the morula, disco-blastula or gastrula stage regulated by cytoplasmic factors of the egg-cell.
- The matrocline segmentation has an intra-individual variation, dependent on the RNA content of the egg-cell, which is the only regulating system active until the gastrula stage.
- Parthenogenesis is possible in the goldfish and common carp, but not in the grass-carp.
- There was observed a haploid parthenogenetic development in the common carp and goldfish.
- The extreme temperature did not induce a spontaneous diploidisation of the egg-cell, but seems to be favourable to increase the frequency of a parthenogenetic development.

REFERENCES

Cherfas, Nina B., 1975. *Investigation of radiation-induced gynogenesis in the carp, *Cyprinus carpio*. I. Experiments on obtaining the diploid gynogenetic progeny in mass quantities.* Genetika, 11, 78-86.

Golovinskaja, K. A., 1968, *Genetics and selection of fish and artificial gynogenesis of the carp, *Cyprinus carpio*.* In : T.V.R. PILLAY (Ed.) *Proceed. FAO Symp. warm-water pond fish cult.* FAO Fish Rep. 44 (4), 215-222.

Kunz, W., Schafer, U., 1978, *Oogenese und spermatogenese.* - VEB Gustav Fischer Verlag Jena pp.32-43.

Lieder, U., 1959, *Über die Entwicklung bei Mannchenlosen Stammen der Silberkarausche, *Carrassius auratus gibelio* (Block) (Vertebrata, Pisces).* Biol. Zentralblatt 78, (2), 284-291.

Nicolescu, Carmen, 1994, *Das Studium der genetischen Eigenheiten der parthenogenetischen Entwicklung der Karpfenrogen.* Analele Universității "Valachia" Târgoviște, anul II, fascicola I, pg. 112-117.

Nicolescu, Carmen, 2002, Types of gynogenetic carps obtained using genetic manipulated gametes. Analele științifice ale Universității "A.I.Cuza" Iași, (seria nouă), secțiunea II a.Genetică și biologie moleculară, tomul III, Ed. Universității A.I.Cuza Iași, pg.68-77.

*"Valahia" University of Târgoviște, B-dul Unirii 1, 0200 Târgoviște, România