1.8. Silver Crucian Carp (Carassius auratus gibelio Bloch, XXX) in the Danube river basin

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1.8.1. Introdution

The silver crucian carp can be originated from East-Asia. It is not really clear, that this species are indigenous or not in the Danube river basin. Natural migration could be done after the ice-age, when melting ice filled areas, that could make a connection between the Danube and the Volga river basin. Taking into the consideration the really simple environmental demands of the s.c.c., it is possible, that some individuals was able to survive in astatic waters. It is a fact, that the s.c.c. was introduced to Hungary at 1954 by fish culturists. The main target of the settlement was to fill the ecological niche beside the carp (Cyprinus carpio). It was also videly regarded, that s.c.c. are has a dropsy resistance. From the Danube river J. Tóth detected this species for the first time in 1975. According to Holcík (1980) in the Fig. 1 the s.c.c. distribution can be seen between 1970 and 1980.

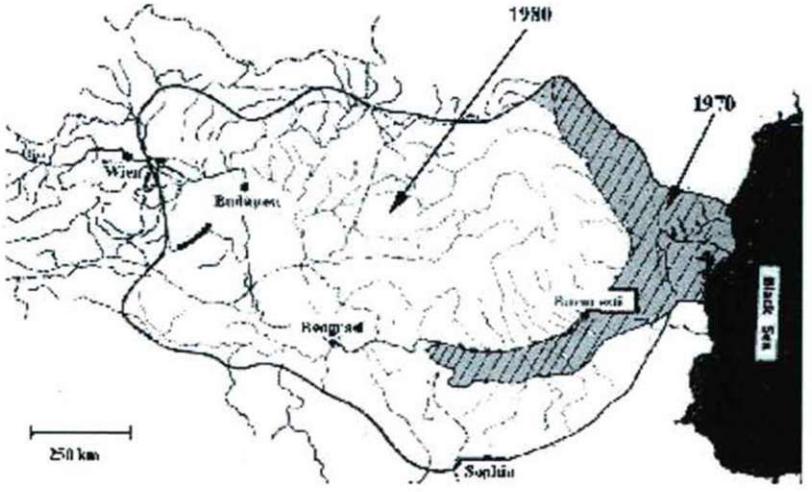

Fig. 1. Distribution of the Silver crucian carp in 1970 and 1980 (Holcík 1980)

This is not known if the settlement influenced this spreading process or not, but anyway we have realized, it was not a good business. The s.c.c. has a wide adaptability, and a very successful reproductive mechanism called gynogenesis. In the other hand there were changes in water habitats, – because of the water management – that mostly turned the stream, and river habitats into a lake habitat, which meant favourable conditions for the s.c.c. populations. Nowadays the s.c.c. are widely spreaded fish pushing out other valuable

rare species as tench (Tinca tinca), and crucian carp (Carassius carassius). 10-15 years ago triploid unisex (female) populations were recorded only, but few years ago male individuals – which were not studied genetically – were also detected in natural waters, (Pénzes et al.) and those were spreaded quickly in our waters. To summarise the written above nowadays there are two forms of the s.c.c. in Hungary:

Type of triploid unisex populations with chromosome number of 150 and a diploid type containing male and female members too, that has 100 chromosomes and reproduce traditionally.

The triploid females reproduce by gynogenesis. Development of the gynogenetic eggs are induced by sperm of other fish species (carp or other cyprinids), but the genetic staff of the sperm does not play role in the development of the offsprings. As the literature on the subject showes, in this process the duplication of the chromosomes can be observed, but the first meiotic division doesn't occur. During the second meiotic division the second polar body eliminates, which resulst triploid eggs. (Horváth and Orbán, 1995).

In our study we try to find the genetic background of the special adaptability of s.c.c. We want to examine the relations between the two genetic types from the wiew of reproduction and genetics too. We also would like to get closer to find a method, wich can help us to make a ballance in fish populations. For the first step we separated diploid and triploid individuals. Morpho-meristic estimations, erythrocyte nucleus diameters, and chromosome number had been examined.

1.8.2. MATERIALS AND METHODS

After fishes were taken from the Isaszegi-pound, they were transfered into the laboratory in 10 litre tanks. In the laboratory the individuals were kept in 50 litre tanks at 28 C for 1 month. Morpho-meristic data were detected using a simple calliper and weight was measured by scale.

The erythrocyte nucleus diameters were detected by using Burker-chamber. The result was appraised according to Katsutoshi et al. (1990)

Chromosome preparations were made from fin fibroblast cultures. Fish-fins were clipped with sterile scissors and washed with ethanol (70%). They were put into Petri-dishes with trypsin-PBS solution (0.025%) and cut into small pieces, about 2x2 mm. The pieces of fins were soaked in trypsin for 10 minutes, and then transferred to sterile tissue culture flasks without any solution and allowed to stick for 1 or 2 h. After this procedure, the flask was filled up with sterile TC-199 medium containing 15% FCS (fetal calf serum, Sigma F 7524). After four weeks when the fibroblast monolayer was almost confluent in the flasks, one drop of 0.05% colchicine (Sigma C 9754) was added. After two hours the medium was removed from the flask into a centrifuge-tube, replaced with 0.5 - 1 ml trypsin (0.025%) and the fibroblasts incubated for 7-10 min. at 28°C. The cell suspension was added to the culture medium and after 7 min centrifugation (1500g) the supernatant was removed and the cells resuspended in 0.35% KCl. Hypotonic treatment took 10 minutes at room temperature and was followed by 3 changes of methanol/acetic acid (3:1) fixative. The cell suspension was spread on slides, dried at room temperature, and stained with 5% fresh Giemsa (in phosphate buffer pH 7.0) for 7-8 min. Five slides from each individual were prepared.and at least 20 metaphase spreads per individual examined.

1.8.3. RESULTS

Comparing the different sexes we have found that the body weights of females are higher than male's. The differences of relative size of head (body lenght/head lenght) between females and males shows that females are probably more successful in competition, living in unfavourable conditions. The triploid and the diploid form cannot be separated by morphologically, even we have found some diploid individuals with morphological default, that was detected only in goldfish till this time.

The amount of the genetic staff was found to be different between females and males. The average erythrocyte nucleus diameter of males 6.28 mm, and females 7.69 mm. (Fig. 2). Those studies was verified with determination of chromosome number. Almost all of the males are diploid, but we have found some male individuals showig chromosome mosaicity with a chromosome number of 100, 125, 134, 156, 174 and 186, usually between 100 and 190.

As it known the s.c.c. females produce triploid eggs, that induced by males of other species. We have done an artificial fertilisation using different males and both triploid and diploid females.

In case of artificial spawning we have found the next results:

Triploid female

male	hatching ratio
S. crucian carp (Carassius auratus gibelio)	98%
Goldfish (Carassius auratus auratus)	95%
C. carp (Cyprinus carpio)	95%
Roach (Rutilus rulitus)	90%
Rosybarb (Barbus conchonius)	75%
Pearch (PercaJluviatilis)	0%
Ruffe (Gymnocephalus cernuus)	0%

Diploid female

Silver crucian carp (Carassius auratus gibelio)	95%
Goldfish (Carassius auratus auratus)	90%
Rosybarb (Barbus conchonius)	3%

1.8.4. DISCUSSION

It is clear, that Silver crucian carp has an ability to survive extraordinary ecological conditions. We have been trying to find the reasons for the wide tolerance interval of this species, and we also would like to find a method, for regulating the growth of their population. We have done a study, that showed the diploid and triploid populations of s.c.c. live together the same natural water area. Some of the diploid individuals have morphological default, but the two forms can not be separated simply by measuring morphological parameters. Genetic measurements showed that there are differences between the amount of genetic staff in the males. We have found a phenomenon of mosaicity. Mosaicity was found in related species Carassius carassius by Lingenfelser et al. (1997) in Ukraine near Chernobil.

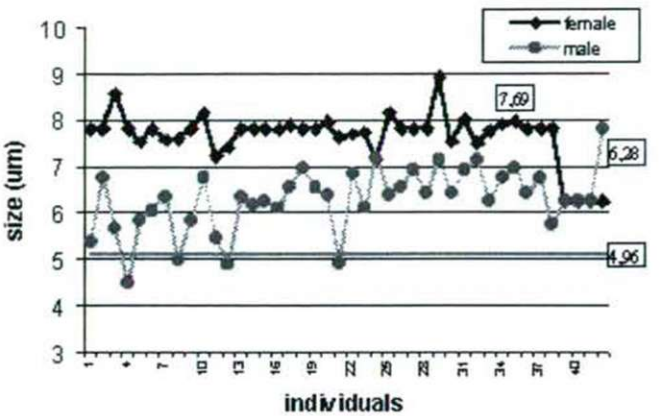

Fig. 2. Erithrocyte nucleus diameter of female and male silver crucian carps

This study raised some questions. Where the diploid form and the mosaic males came from? Our hypothesis is that mosaic males can be offsprings from the situation, when the two fomrs spawn together. This idea suppose, that the diploid males spawn with the triploid females, and the genetic staff of the sperm stays in the eggs after inducing the development of the offsprings. Later some of these "strange chromosomes" eliminate. A related study was done by Taniguchi and Dong (1997), that proved, that the heat shock during the fertilization of gengoroubuna (Carassius auratus cuvieri) eggs - treated with common carp sperm - led to the apperance of the carp's fragments in the offsprings genome.

Further studies have to be carried out to find if there is any difference between the two form's ecological demands. The females are more successful on competition in bad ecological circumstances. We have to examine if there is a connection between the ploidy level and ecological demands.

As it is known there was a spontaneous polyploidisation in the cyprinids about 1 million years ago, so some of the cyprinids including the Carassius genus has nearly 100 chromosomes instead of 50. Furthermore the silver crucian carp had gone through a process called triploidization, so the chromosome number increased to 150. This way the s.c.c. is hexaploid behaving triploid gynogenetic unisex populations. Our hyphotesis is the following: The hexaploidy may provide a lot of possibilities to produce different kind of proteins depending the ecological circumstances. To point out, are there any reality of the hypothesis RAPD analysis and protein polymorphism analysis were carried out, but before any conclusion it has to be completed with allelspecific examinations.

1.8.5. Summary

The number of the silver crucian carp has been increasing in the last 10-15 years in the hungarian section of the Danube river basin. This process leads to the loss of biodiversity in natural waters, and causes damages in fish farms. This huge population growth can be explained by several reasons as the changes of the water habitats, and the special characteristic of this species includig a very successful reproductive mechanism, and the ecological simplicity. In our study we try to find the reasons of it's wide

adaptability and also try to get closer to find a method, which can help us to make a ballance between the populations of s.c.c. and other species. We tried to separate diploid and triploid individuals. Morpho-meristic estimations, erythrocyte nucleus diameters, and chromosome number had been examined. In this study we also wanted to point out some of the causes of the huge flexibility of the s.c.c. using RAPD analysis and protein polymorphism.

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