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Source: Folia Zoologica, 60(2) : 115-121

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/fozo.v60.i2.a5.2011>

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Karyotype diversity of the offspring resulting from reproduction experiment between diploid male and triploid female of silver Prussian carp, *Carassius gibelio* (Cyprinidae, Actinopterygii)

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Received 8 June 2010; Accepted 25 November 2010

Abstract. Populations of silver Prussian carp (*Carassius gibelio*) are known to exhibit different ploidy levels among their individuals. No consistent information is available regarding chromosome number of triploid biotype. Generally diploids have 100 chromosomes while triploids have 150–160 chromosomes. The karyotype of the *C. gibelio* triploid biotype is characterized by a variable number of small chromosomal elements called supernumerary chromosomes. Here we report the results of a reproduction experiment between a diploid male and triploid female with respect to chromosome numbers of the parents and their offspring. Thirty metaphases of both parents and fifteen individuals of the offspring were investigated. We found variability in chromosome numbers among analysed offspring with a fluctuation from 150 to 159. In comparison, the chromosome numbers of male and female individuals were found to be 100 and 159 respectively. Our results show a high chromosomal plasticity of the *Carassius gibelio* triploid biotype.

Key words: cytogenetic, chromosome number, ploidy level, hybridization

Introduction

Silver Prussian carp (*Carassius gibelio*, Bloch, 1782) occurs in a vast territory of Eurasia in two main biotypes: diploid – evolutionary tetraploid with 100 chromosomes and triploid – evolutionary hexaploid with approximately 150 chromosomes (Kottelat & Freyhof 2007). The triploid biotype is also known for its gynogenetic form of reproduction – sperm dependent parthenogenesis (Golovinskaya et al. 1965, Peňáz et al. 1979). Some authors use the term allogynogenesis to describe a specific form of reproduction of silver Prussian carp wherein the male sperm partially contributes to the genome of offspring (Yi et al. 2003, Zhao et al. 2004).

The original distribution of silver Prussian carp throughout Europe is unclear due to a number of introductions, confusion with feral goldfish (*Carassius auratus*), as well as misinterpretation of the taxonomical status of *C. gibelio* in older literature (Kottelat 1997, Kalous et al. 2004). Although there are many ambiguities of the origin; the expansion of the triploid biotype of *C. gibelio* in Central Europe is well documented (Holčík & Žitňan 1978). Lusk et al. (1977) described the first occurrence of an all female population of *C. gibelio* in a lower stretch of the River Dyje in the territory of the Czech Republic. The study of Peňáz et al. (1979) and Lusk & Baruš (1978) revealed that the fish were all triploids and female,

and therefore should reproduce only gynogenetically. The carp aquaculture in the Czech Republic was then responsible for the expansion of the triploid biotype of *C. gibelio* to two other major European basins of the Elbe and Oder. This was caused by a number of reasons, e.g. the accidental introduction of silver Prussian carp into common carp stock or its use as baitfish by anglers, as well as its occasional escapes during the draining of ponds or due to floods (Lusk et al. 1980, Kubečka 1989, Slavík & Bartoš 2004). Surprisingly, at the beginning of the 1990's, males and diploids started to appear within the population of *C. gibelio* in the River Dyje alluvium (Halačka et al. 2003, Lusková et al. 2004). In a relatively short time, the once all female triploid population transformed to a diploid-polyploid complex with various percentages of males reaching 43 % (Vetešník 2005). Similar

and Hydrobiology in Vodňany, Czech Republic. Prior to any handling, the fish were anaesthetized with 0.6 ml.l⁻¹ 2-phenoxyethanol (Merck Co., Darmstadt, Germany). Hormonal stimulation and gamete collection followed the methodology of Linhart et al. (2003), while fertilization and egg incubation in experimental trays were carried out according to Linhart et al. (2006). Blood was sampled according to Svobodová et al. (1991). Ploidy levels of both specimens were determined as a relative DNA content in erythrocytes by means of flow cytometry (Partec CCA I; Partec GmbH, EU) using 4', 6-diamidino-2-phenylindol (DAPI). Samples were processed according to Flajšhans et al. (2008). Erythrocytes of a diploid male gave a relative DNA content of 2n as the diploid standard. A number of the hatched offspring (approximately

Table 1. Chromosome numbers of *Carassius gibelio* reported from Europe.

Locality	Numbers of chromosomes	Reference
Belarus	94, 141	Cherfās (1966)
Former Yugoslavia	160	Vujosevic et al. (1983)
Czech Republic	160 (166)	Peňáz et al. (1979)
Romania	98	Raicu et al. (1981)
Former Yugoslavia	158	Fister & Soldatovic (1989)
Poland	100, 150	Boroń (1994)
Hungary	100, 148–156	Tóth et al. (2005)

complexes were also recorded from other places in Europe (Černý & Sommer 1994, Abramenko et al. 1998, Tóth et al. 2000). Few cytogenetic studies exist on the European population of *C. gibelio* and the results are quite variable especially in the triploid biotype – see Table 1. In any case, cytogenetics can be considered a crucial approach in explaining mechanisms of mysterious phenomena within former all female populations of *C. gibelio* such as a sudden appearance of males and a ploidy level reduction from 3n to 2n in a short period (Ráb et al. 2007). Here we present the results of a reproduction experiment between diploid male and triploid female originating from the locality where the population of silver Prussian carp was originally established within the Czech Republic.

Material and Methods

Parental fish were captured during the spring of 2006 in alluvium of the River Dyje close to its confluence with the River Morava, in South Moravia – Czech Republic. Fish were transported to the University of South Bohemia, Research Institute of Fish Culture

100 fish larvae) and both parental specimens were subsequently kept in aquaria until chromosome preparation was carried out. Parental male and female were investigated using a standard direct procedure for chromosome preparation from the kidneys according to Ráb & Roth (1988). Both male and female nuclei suspensions were dropped on slides and air-dried. Fifteen specimens of offspring were investigated from 2008 to 2010 using a non-destructive method of chromosome preparation from regenerated tissue of caudal fin; a slightly modified protocol of Völker & Kullmann (2006) was used. The result of a single preparation was, in most cases, composed of two slides with three nuclei rings per specimen. Staining was processed in a buffered 5 % solution of Giemsa-Romanowski for 10 minutes, and 30 best metaphase spreads per individual were examined each time using a system composed of a Microscope Olympus BX41TF (magnification 1000 ×), an Olympus SP-350 digital camera and a computer with QuickPHOTO MICRO version 2.3 software (PROMICRA, s.r.o., Praha, Czech Republic) running on Microsoft® Windows® XP.

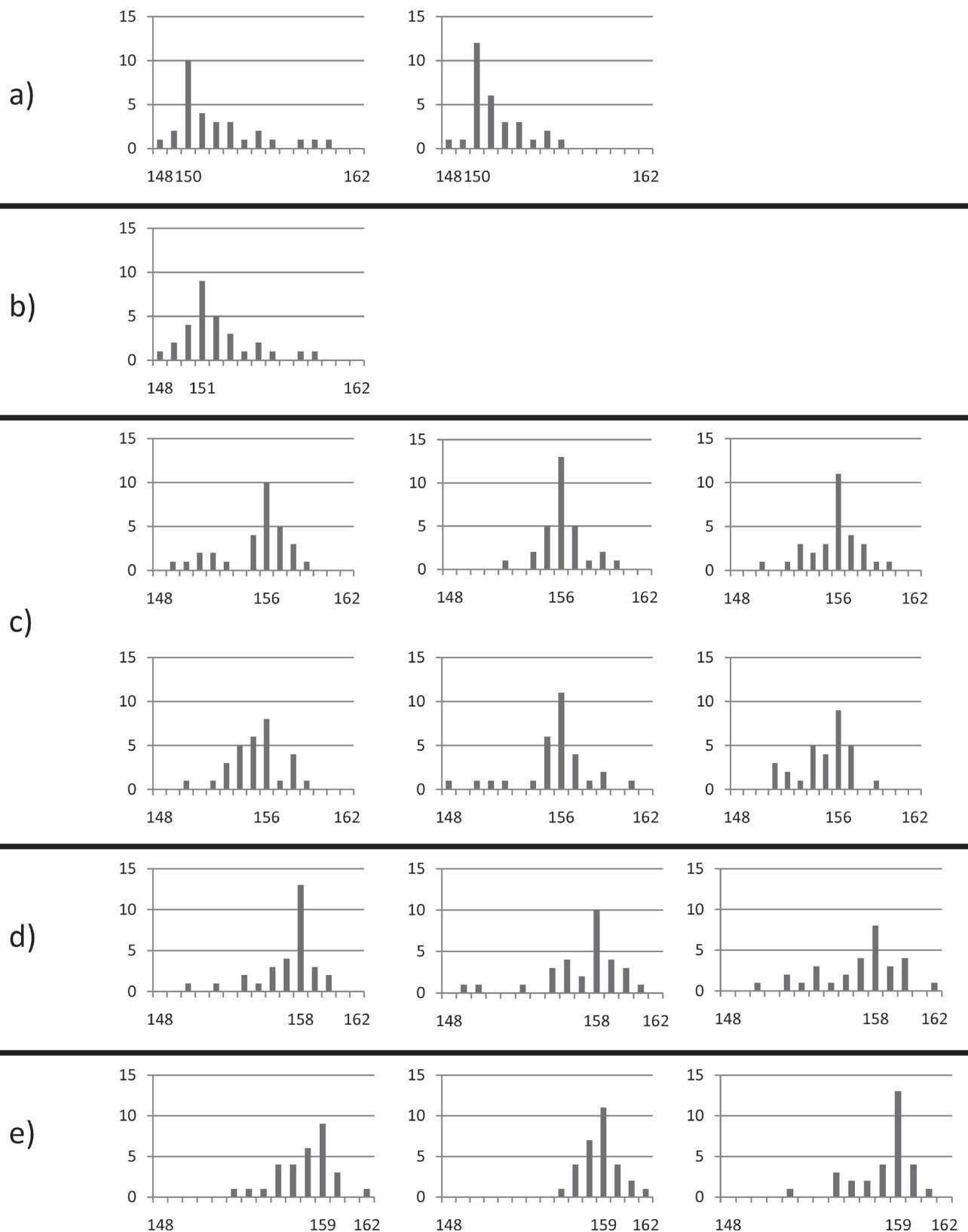


Fig. 1. Frequency distribution of chromosome numbers of individuals of offspring resulting from reproduction experiment. a) two individuals with modal number of chromosomes 150, b) one individual with modal number of chromosomes 151, c) six individuals with modal number of chromosomes 156, d) three individuals with modal number of chromosomes 158, e) three individuals with modal number of chromosomes 159.

Chromosome counting was carried out on a PC through the use of QuickPHOTO-MICRO version 2.3 software with a “Counting Points” function. We counted all chromosomes and chromosomal structures, and we did not separate microchromosomes due to their difficult definition and unclear size limits with respect to others chromosomes.

Results

Male and female were identified by flowing cytometry as diploid and triploid respectively. The modal chromosome number of diploid parental male was 100 (70.0 % of investigated metaphases) and the modal chromosome number of triploid female was 159 (46.6 % of investigated metaphases). Fifteen analysed individuals of offspring were divided into five groups with different chromosome numbers – see Fig. 1.

Discussion

Several decades after the appearance of the *C. gibelio* triploid biotype in the territory of the Czech Republic, a fascinating change of ploidy and the male female ratio within the population was observed. A cytogenetic study, conducted shortly after the appearance of all female triploid biotype of *C. gibelio* in South Moravia, recorded 160 chromosomes and six microchromosomes (Peňáz et al. 1979). Unfortunately, no other karyological data from the Czech Republic have been available since 1979.

Our analysis of *C. gibelio* caught in the same locality after almost 30 years revealed different chromosome numbers for male and female, 100 (Fig. 2) and 159 (Fig. 3) respectively. A reproduction experiment of the $2n$ male and $3n$ female shows a variability of chromosome numbers in 15 specimens of the offspring (sex undetermined). It is clear that the *C. gibelio* triploid biotype does not bear a defined number of chromosomes, although it does oscillate above 150. This is in agreement with Zhou & Gui (2002) and supports the hypothesis of close relations between East Asian and European populations of triploid biotypes of silver Prussian carp (Kalous & Šlechtová 2004, Kalous et al. 2007) in terms of karyology. The presented findings can also explain inequality in older literature regarding chromosome numbers of *C. gibelio* in Europe (see Table 1). In previous times, authors commonly investigated only few specimens from one locality caught at the same time, and therefore they came to the conclusion that the triploid biotype of *C. gibelio* possesses only one specific number of chromosomes. There is also always a high probability of error when the chromosomes in

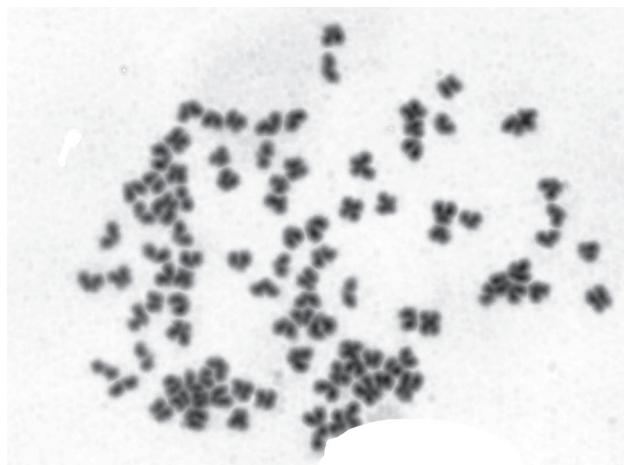


Fig. 2. Metaphase of diploid male parent of silver Prussian carp ($2n = 100$) ($\times 1000$).



Fig. 3. Metaphase of triploid female parent of silver Prussian carp ($3n = 159$) ($\times 1000$).

metaphases are counted; this is primarily due to their high numbers, small size, and the presence of small chromosomal elements called microchromosomes or supernumerary chromosomes (Boroń 1994). Although many authors published karyotypes of *C. gibelio*, they are generally in disagreement. Without additional staining or *in situ* hybridisation it is very complicated to be oriented in karyological structure. We therefore present only chromosome numbers gained from a sizable dataset. Fifteen analysed individuals of the offspring were divided into five groups according to

their modal chromosome numbers: 150, 151, 156, 158 and 159. This variability supports a process of interaction between male and female gametes after the fertilization of eggs from 3n individual with the sperm of 2n male. In any case, this interaction leads to a production of triploid offspring. Since a genetic analysis of progeny was not performed, we can not be sure whether these are recombinant offspring, or that

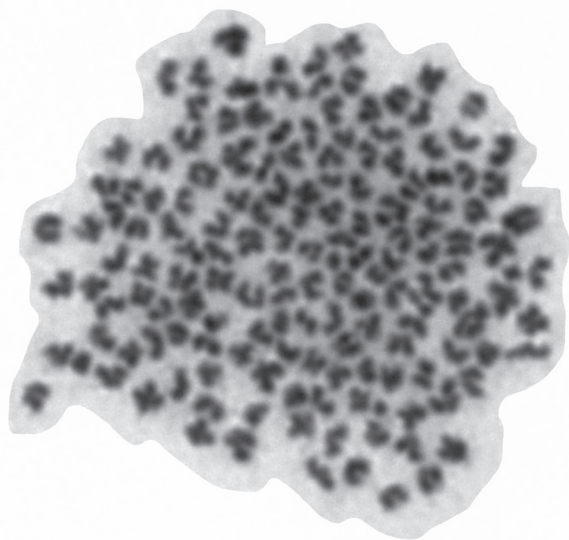


Fig. 4. Metaphase of triploid offspring specimens of silver Prussian carp with most common chromosome number ($3n = 156$) ($\times 1000$).

some/all of the individuals are of gynogenetic origin. The most common modal chromosome number of offspring was 156 (Fig. 4), which is also often recorded from other studies (Yi et al. 2003, Zhao et al. 2004). Tóth et al. (2005) found 148-156 chromosomes within the offspring of diploid male and triploid female. Unfortunately, the authors grouped together the chromosome numbers of analysed specimens resulting from the aforementioned reproduction experiment, and it is not clear if the variability was observed among the offspring or just within the metaphases.

The dual reproduction modes, including gynogenesis and sexual reproduction, have been demonstrated to coexist in the triploid silver Prussian carp (Gui & Zhou 2010). In other words, when the eggs are inseminated by heterologous sperm from other species, they produce a clonal lineage of all females by gynogenesis. However, when homologous sperm of triploid (evolutionary

hexaploid) male inseminate eggs of triploid (evolutionary hexaploid) female, the responding development mode is sexual reproduction, which produces recombinant offspring. This reproduction strategy requires production of sperm with a “haploid” chromosome number (in triploids this means a 1.5 ploidy level). The discovery of haploid sperm production of diploid *C. gibelio* males and aneuploid sperm production (close to 1.5n) in triploid *C. gibelio* males was proven by Flajšhans et al. (2008). The theoretical fertilization of eggs of 3n female by homologous sperm (1n) of diploid male should result in offspring with approximately 125 chromosomes. Our results do not support this hypothesis because all of the analysed specimens of the offspring were identified to be triploids with at least 151 chromosomes. It seems that silver Prussian carp with 125 chromosomes are rare, or the number of chromosomes is not in accordance with the viability of fertilized egg or even cannot be formed in ova.

The variability in karyological structure was found in the gynogenetic fish *Poecilia formosa*, which is of hybrid origin (Lamatsch et al. 2004). Hybridisation is considered a main evolutionary factor in the occurrence of gynogenetic polyploid animals (Dawley 1989, Vrijenhoek et al. 1989). In the case of triploid biotype of *C. gibelio*, it is not clear which species could have been the parental ones. However, recent findings of a much higher genetic variability of the genus *Carassius* in Eurasia presented by Takada et al. (2010) and Rylková et al. (2010) open new perspectives in finding a potential parental species.

Acknowledgements

We thank Martin Flajšhans and his colleagues from FROV, JČU in Vodňany for the reproduction of fish, flow cytometry analyses, and his valuable advices regarding the problems associated with ploidy levels of silver Prussian carp. We also thank Šárka Pelikánová and Jörg Bohlen for their meticulous care of the fish. We appreciate proof reading of the manuscript made by Brian Kavalir and finally, we are grateful to two anonymous referees for their comments and recommendations. This study was financially supported by grants: GACR 206/05/2159, IRP FAPPZ, CZU No. MSMT 604670901 and IRP IAPG No. AV0Z50450515.

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