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Nuclear DNA Content Variation in Fishes

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Feulgen microspectrophotometry, for the quantitative determination of DNA *in situ*, has been widely employed in animals (Szarski 1974), plants (Price 1976). These studies have shown that variation in nuclear DNA content often provides useful evidence which helps to assess the relationships among related species particularly. Nuclear DNA content variation in fish has been also widely investegated (Hinegardner 1972, Gold 1985, 1987). Here is the report on the nuclear DNA contents of fortytwo species of Chinese freshwater fishes.

Materials and methods

All the specimens used were derived from Wuhan, except *A. chankaensis*, *X. davidi*, *M. chinensis*, *M. aculeatus* from Shashi of Hubei, *P. extremus*, *D. Ptychobarbus dipogon*, *S. Schizothorax oconnori*, *T. Triplophysa siluroides* from Lasa of Tibet.

Relative DNA contents of individual fish were determined microspectrophotometrically using Feulgen-stained erythrocyte nuclei. Blood of single fish is smeared near the frosted end of slides; on the far end of each slide a smear of chichen blood served as the internal standard. The chichen blood was obtained from a strain (XISAISI). The slides were then fixed for 10 min in 90% EtOH, and 30 min in 3: 1 methanol-formaldehyde, rinsed in 70% EtOH (2 min), 50% EtOH (2 min), 30% (2 min) and distilled water, after hydrolysed for 15 min in 5N HCl at 38°C, rinsed briefly in distilled water, and stained one hour in Schiffs reagent. Following staining, rinsing twice (10 min each) in SO_2 water and once (20 min) in distilled water, air dried in the dark, cleared in EtOH and Xylene, mounted in DPX.

The microspectrophotometric apparatus used was a UNIVAR scanning microscope. For each individnal, 50 nuclei were measured and standardized as a percent of the mean absorbancy of chicken erythrocyte nuclei on the same slide. For conversion to picograms of DNA, the standardized, coded data were multiplied by 2.8 pg, the generally accepted DNA content of diploid chichen erythrocyte nuclei (Dhillow 1977).

Results and discussion

Descriptive statistics from the distribution of DNA contents of individual intraspecies are shown in Table 1. The variation over all species ranges from 1.24 pg (*M. albus*) to 19.28 pg (*D. Ptychobarbus dipogon*). One confind that there is close correlation between nuclear chromosome number and nuclear DNA content in fishes. The DNA contents of the species with $2n=50\pm$range from 1.80 pg to 3.10 pg in general, those of the more specialized or evolutionaryily advanced species range from 1.49 pg to 1.70 pg. The DNA contents of the species with $2n=150$ is about 6.0 pg, while in some polyploid forms of Schizothoracine fishes the DNA contents might be very high, which is 19.28 pg. The species that DNA content ranges from 1.8 pg to 2.6 pg account for 64%, which might be the major cases of Chinese fishes that possess a chromosome number of $2n=48$ (58%). However, some species such as Siluriformes, Beloniformes, Perciformes, with $2n=40$–58, their DNA contents are from 1.49 pg to 2.98 pg (10 species in 13 species), the nuclear DNA contents is only 1.24 pg in *M. allous* ($2n=24$). These

Table 1. Nuclear DNA content in the fishes studied

Species	No. of specimens	2n	DNA content	
			Average (AU) (X±ES)	Average (pg)
Cyprinformes				
Cyprinidae				
Leuciscinae				
Mylopharyngodon piceus	4	48	0.756±0.032	2.12±0.09
Ctenopharyngondon idellus	1	48	0.777±0.042	2.18±0.12
Ochetobius elongatus	1	48	0.758±0.044	2.12±0.12
Culterinae				
Pseudolaubuca sinensis	1	48	0.963±0.082	2.70±0.23
Erythroculter ilishaeformis	2	48	0.649±0.040	1.82±0.11
E. mongolicus mongolicus	1	48	0.801±0.089	2.24±0.25
Hemiculter leucisculus	1	48	0.888±0.052	2.43±0.15
Culter erythropterus	1	48	0.844±0.055	2.36±0.15
Parabramis pekinesis	1	48	0.698±0.028	1.95±0.08
Megalobrama amblycephala	1	48	0.801±0.077	2.24±0.22
Xenocyprinae				
Xenocypris davidi	2	48	1.016±0.087	2.84±0.24
X. microlepis	1	48	0.737±0.038	2.06±0.13
Schizothoracinae				
Platypharodon extremus	3	90	1.059±0.116	2.97±0.32
Diptychus dipogon	3	446	6.877±1.344	19.28±3.95
Schizothorax oconnori	3	92	1.090±0.065	3.05±0.18
Acheilognathinae				
Acheilognathus chankaensis	4	44	0.712±0.046	1.99±0.13
Gobioniae				
Abbottina rivularis	2	50	1.098±0.054	3.07±0.15
Hemibarbus labeo	2	50	0.833±0.042	2.33±0.12
H. maculatus	1	50	0.823±0.040	2.30±0.11
Pseudorasbora parva	2	50	1.032±0.043	2.89±0.12
Sarcocheilichthy nigripinnis nigripinnis	1	50	1.100±0.081	3.08±0.23
Cyprininae				
Cyprinus carpio	2	100	1.150±0.080	3.22±0.22
Garassius auratus gibelio	5	150	2.182±0.003	6.11±0.01
Hypophthalmichthyinae				
Aristichthys nobilis	5	48	0.717±0.007	2.01±0.02
Hypophthalmichthys molitrix	5	48	0.728±0.058	2.04±0.16
Cobitidae				
Noemacheilinae				
Triplophysa siluroides	1	48	0.787±0.051	2.20±0.14
Cobitinae				
Paramisgurnus dabryanus	5	48	0.783±0.075	2.19±0.21
Misgurnus anguillicaudatus	2	100	1.611±0.090	4.51±0.27
Siluriformes				
Siluridae				
Silurus asotus	5	58	0.602±0.018	1.69±0.05
Bagridae				
Pelteobagrus fulvidraco	5	52	0.752±0.072	2.11±0.20
Clariidae				
Clarias batrachus	2	56	0.654±0.057	1.83±0.16

Table 1. (continued)

Species	No. of specimens	2n	DNA content	
			Average (AU) (X±ES)	Average (pg)
Cyprinodontiformes				
Poeciliidae				
Gambusia affinis	2		0.553±0.058	1.55±0.16
Beloniformes				
Hemiramphidae				
Hemiramphus kurumeus	3	40	0.533±0.043	1.49±0.12
Synbranchiformes				
Synbranchidae				
Monopterus albus	5	24	0.442±0.039	1.24±0.11
Perciformes				
Serranidae				
Siniperca chuatsi	5	48	0.569±0.046	1.59±0.13
Eleotridae				
Odontobutis obscurus	3	44	0.906±0.072	2.54±0.20
Tilapia				
Tilapia mossambicus	1	44	0.574±0.065	1.61±0.16
Tilapia nilotious	2	44	0.708±0.057	1.98±0.16
Belontiidae				
Macropodus chinensis	3	46	0.906±0.072	2.54±0.20
Channidae				
Channa argus	5	48	0.549±0.017	1.54±0.05
C. asiatica	3	44	0.573±0.047	1.61±0.13
Mastacembelidae				
Mastacembelus aculeatus	2	48	0.575±0.088	1.61±0.25

results suggested that the more specialized or evolutionary species have less DNA content. This is in agreement with previous report by Hinegardner (1972).

The describution of DNA content of eight subfamilies in Chinese Cyprinidae is shown in Table 2. The DNA content in Chinese Cyprinid species studied ranges from 1.82 pg to 19.28 pg, this variation is much higher than that in North America Cyprinidae (Gold 1985, 1987) and than those reported by Hinegardner *et al.* (1972) respectively. The DNA content in the species of six subfamilies, including Leuciscinae, Culterinae, Xenocyprinae, Acheilognathinae, Gobioninae, Hypophthalmich thyinae, is ca. 2.0 pg. There are some differences in the mean DNA content among species of Culterinae, Leuciscinae, Xeuocyprinae, Hypophthalmich-thyinae. These variation might be caused by some change in chromosome size (Rothfels 1966). Unlike these subfamilies the variations in Acheilognathinae and Gobioninae result from the change in chromosome number. The fishes of these six subfamilies belong to a common evolutionary branch, in which Robertsonian translocation might be major modes of evolution, and some kinds of structural change, such as deletions and duplications, might also occured.

Our work shows there is obvious correlation between chromosome ploidy and nuclear DNA content. The DNA content in Schizothoracinae and Cyprininae is approximately times highier than 2.0 pg. Polyploidization might be characteristic for the second branch of evolution in Cyprinid fish. The fishes of the subfamily Cyprininae are thought to be polyploids (4n or 6n), the measurement of cellular DNA content showes that *C. Cyprinus carpio* is tetraploid form (the same as *M. anguillicandatus* in Cobitidae), similarly a hexaploid form is found in *C. auratus gibelio*. The DNA contents of *P. extremus* and *S. Schizothorax oconnori*

are 2.79 pg and 3.05 pg (2n=90 and 92) respectively, which indicate that both species are tetraploid forms. The DNA content of *D. dipogon* is 19.28 pg, except Protopterus which is the highest so far (Fasman 1975), our result is in agreement with the speculation that *D. dipogon* probably be a 16-ploid or 20-ploid arising through multi-polyploidization from a primitive Schizothoracine (Yu 1990).

Previous worh has shown that some natural polyploids have DNA contents that are lower than expected by comparison with their putative diploid ancestors (Verma and Ress 1974, Samuel 1985). A similar decrease has been detected in this study. This decrease was explained by underestimation due to increased DNA condensation and gene redundancy in established polyploids (Verma and Ress 1974); DNA loss (Grant 1976) and selection for smaller component genomes at higher ploidy levels. A decrease in genome size may also accompany environmental stress in nature (Cavalier-Smith 1978). The study on Cyprinidae indicated that a relative decrease in DNA content has been achieved by combining with smaller chromosome. This suggest that Robertsonian rearrangements and some structural changes, such as deletions and duplication, are also typical modes of evolution in polyploidization.

It have been shown that there is DNA sequences polymorphism and chromosome polymorphisms (Gold 1985, Shen Zhujia 1983). The variation of DNA content within population of the species was also detected in this work, which suggests that there also might be DNA comtent polymorphisms in fishes.

Table 2. Nuclear DNA content variation in Chinese Cyprinid fishes

Subfamily	Range (pg)	Subfamily	Range (pg)
Leuciscinae	2.12–2.18	Gobioninae	2.30 –3.08
Culterinae	1.82–2.70	Schizothoracinae	2.97–19.28
Xeuocyprinae	2.06–2.84	Cyprininae	3.22– 6.11
Acheilognathinne	1.99	Hypophthalmich thyinae	2.01– 2.04

Summary

Forty-two species of Chinese freshwater fishes were examined for their cellular DNA content. The result showed that there is a close correlations between nuclear chromosome number or ploids and DNA content in fish. These observations suggest that the more specialized or evolutionarily advanced species, the less nuclear DNA content the fishes possess. The polyploid evolution, the systematic relationship of the fishes and the variations within species are discussed.

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