Cytogenetic Analysis of Bagrid Catfish, *Pseudobagrus fulvidraco* (Teleostomi : Siluriformes)

In - Seok Park and Chung - Lyul Lee*

Department of Marine Living Resources, Kunsan National University,
Kunsan 573 - 360, Korea
*Department of Biology, Kunsan National University, Kunsan 573 - 360, Korea

The chromosome numbers of bagrid catfish, *Pseudobagrus fulvidraco* was 52, nine pairs (No. 1 to 9) were metacentrics with the range of relative length 2.89~6.22 and arm ratio 1.09~1.58 ; thirteen pairs (No. 10 to 22) were submetacentrics with the range of relative length 2.88~5.88 and arm ratio 1.80~3.65 ; and all other pairs (No. 23 to 26) were acrocentrics with the range of relative length 2.63~3.30 and arm ratio 9.01~10.67, and fundamental number was 104. Heteromorphic sex chromosomes were not found. There was not exist significant difference in resultant erythrocyte measurements and parameters between female and male ($p<0.05$). The mean sizes of cell and nucleus, were $11.03\times9.67\mu m$ and $4.18\times3.66\mu m$ respectively. The number of erythrocytes of both females and males were $6\sim7\times10^5/ml$. Gill tissues from diploid individuals had cells with one or two nucleoli.

Introduction

The bagrid catfish (*Pseudobagrus fulvidraco*) of the family Bagridae has been widely distributed in Western - Southern part of Korea and is fast growing and highly esteemed food fish in Korea (Chyung, 1977 ; Lee and Kim, 1990). Unfortunately, such highly esteemed food fish has so far remained cytologically the least investigated.

At sexual maturation, the growth rate of *P. fulvidraco* becomes rapidly decreases, because of their high gonadosomatic index (unpublished data). These problems can be greatly reduce by inducing triploid. Generally triploid fish showed gonadic sterility (Kim *et al.*, 1994 ; Park *et al.*, 1994 ; Lincoln and Scott, 1984) and the manipulation of chromosomes appears to be a valuable technique that normally leads to an increase of economic value in this species.

It is known that polyploid shows phenomena of their increasing, compare to their diploid counterpart, in number of chromosome and nucleolus, and size of cell and nucleus of erythrocyte. Although size increase in cell and nucleus of polyploid, cell number of polyploid was contrary to their diploid counterpart (Ueno, 1984 ; Sezaki *et al.*, 1988 ; Aliah *et al.*,1991 ; Park and Park, 1994). In this respect, measurement of the chromosome number, nucleolus number, nuclear and/or cellular sizes, and erythrocyte

number can be used to determine the level of ploidy in this species (Thorgaard, 1986 ; Kim *et al.*, 1994 ; Park and Kim, 1994).

In this study we have performed cytogenetic analyses including chromosome number, karyotype, nucleolus number, nuclear and cellular sizes, and erythrocyte number of *P. fulvidraco* in order to obtain baseline information for future chromosome manipulation.

Materials and Methods

The analyses were based on preparations obtained from 22 specimens (10 females and 12 males weighting 400~500g) of *Pseudobagrus fulvidraco* collected from the Kum River (the city of Kunsan, Korea). All specimens were sexed by gonad examination.

Chromosome preparations were obtained using the standard kidney procedure described in Kim *et al.* (1982). An intraperitoneal injection of colchicine was given to each specimen (1~10 μg/g body weigth) three to four hours before sacrificing. Kidney tissue was minced in 0.075M KCl. The suspensions were centrifuged, supernatants discarded and cell sediments fixed in two successive changes of fresh methanol - acetic acid solution (3 : 1). Slide were prepared by routine air - drying method (Kim *et al.*, 1982) and stained with Giemsa solution. At least 20 countable metaphases from each specimen were observed for the determination of chromosome number and for the analysis of karyotype. Well spread chromosomes at metaphases were selected and photographed. Relative length (%) and arm ratio (long arm/short arm) of each chromosomes were calculated, respectively and karyotypes were analyzed based on the method of Levan *et al.* (1964).

Erythrocytes were used for the measurement of nuclear and cellular sizes. Heparinized peripheral blood was collected from the caudal artery of each specimen. Blood smears were prepared by the conventional method (Park and Kim, 1994) and stained with Giemsa or May - Grünbaldt - Giemsa solution. Major and minor axes of a minimum of 120 erythrocytes and of their nuclei were measured by a micrometer under 1,000 fold magnification. Then the mean values of major (a) and minor (b) axes characteristic of each subject were calculated. From the two means a further three additional parameters were determined : major axis/minor axis (a/b) ; surface area (S)=abπ/4 (Sezaki and Kobayashi, 1978) ; Volume (V)=4π(a/2)(b/2)^2/3 (Lemoine and Smith, 1980). Significant difference from measurements and prameters between sexes was tested using student t - test. The number of erythrocytes was counted by the standard method using a Thoma Zeiss's haemocytometer.

Small pieces of gill tissue were cut off and placed in fixative. After fixing overnight or longer, slide were prepared using the method of Kligerman and Bloom (1977) and stained to reveal nucleoli by using the method of Gold (1984). For each individual, two slides were silver stained and the number of nucleoli/cell recorded for 50 cells/slide.

Results

The diploid chromosome number 52 was determined and regardless of sex, all specimens had 104 as the fundamental number (FN) (Table 1). The chromosomes of both females and males were arranged into 26 pairs and the heteromorphic sex chromosomes were not found in this species (Table 1 and Fig. 1). Nine pairs (No. 1 to 9) were metacentrics with the range of relative length 2.89~6.22 and arm ratio 1.09~1.58 ; thirteen pairs (N0. 10 to 22) were submetacentrics with the range of relative length 2.88~

Table 1. Results of karyotypic analysis of *Pseudobagrus fulvidraco*

Number and sex of specimens	Cells examined	Karyotypes*			2n	Fundamental number(FN)
		M	SM	A		
10(♀)	216	18	26	8	52	104
12(♂)	247	18	26	8	52	104

*M=metacentric, SM=submetacentric, A=acrocentric.

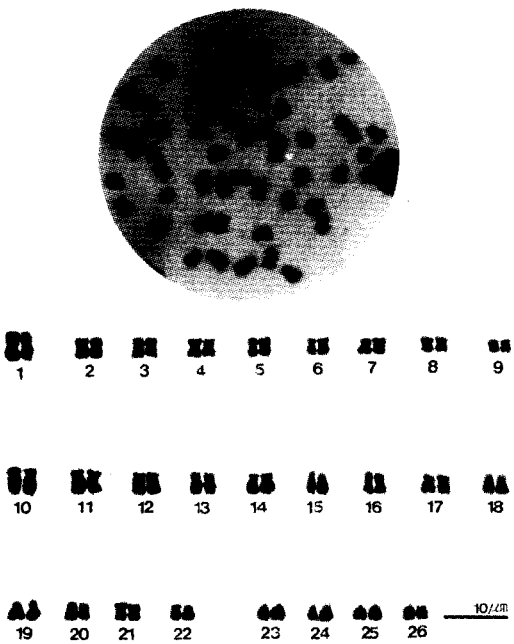

Fig. 1. Metaphase and idiogram of *Pseudobagrus fulvidraco*.

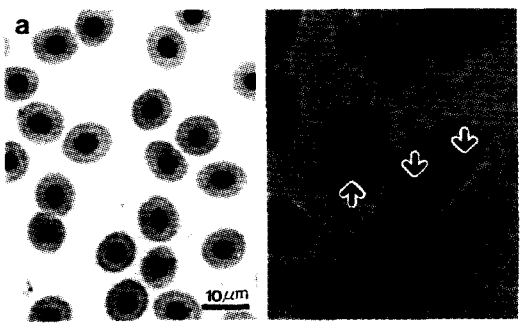

Fig. 2. External morphology of erythrocyte (a) and silver stained gill tissue (b) of *Pseudobagrus fulvidraco*. Arrows indicate nucleoli silver stained gill tissue.

Table 2. Chromosome characteristics of karyotypes of *Pseudobagrus fulvidrac*

Chromosome pair No.	Relative length(%)		Arm ratio*		Type**
	Mean	SD	Mean	SD	
1	6.22	0.53	1.09	0.09	M
2	4.15	0.31	1.58	0.12	M
3	3.75	0.31	1.25	0.11	M
4	3.59	0.29	1.23	0.09	M
5	3.53	0.26	1.19	0.10	M
6	3.38	0.25	1.19	0.10	M
7	3.29	0.24	1.20	0.11	M
8	3.12	0.23	1.18	0.08	M
9	2.89	0.12	1.16	0.10	M
10	5.88	0.49	2.54	0.21	SM
11	5.27	0.42	2.37	0.22	SM
12	4.69	0.31	1.94	0.17	SM
13	4.52	0.36	1.93	0.11	SM
14	4.38	0.30	1.86	0.12	SM
15	4.32	0.29	1.98	0.13	SM
16	4.10	0.32	1.97	0.10	SM
17	4.05	0.31	2.03	0.20	SM
18	3.92	0.27	1.99	0.16	SM
19	3.56	0.25	1.99	0.17	SM
20	3.41	0.22	2.78	0.21	SM
21	3.08	0.20	1.80	0.13	SM
22	2.88	0.21	3.65	0.25	SM
23	3.30	0.23	9.67	0.82	A
24	3.18	0.20	9.33	0.83	A
25	2.91	0.18	9.01	0.72	A
26	2.63	0.17	10.67	0.66	A

* Long arm/short arm

** M=Metacentric, SM=Submetacentric, A=Acrocentric.

5.88 and arm ratio 1.80~3.65 ; and all other pairs (No. 23 to 26) were acrocentrics with the range of relative length 2.63~3.30 and arm ratio 9.01 ~10.67 (Fig. 1 and Table 2).

Fig. 2 - a shows spherical and elliptic form of erythrocyte. There was not exist significant difference in resultant erythrocyte measurements and parameters between female and male ($p<0.05$). The mean sizes of cell and nucleus, were

$11.03 \times 9.67\mu m$ and $4.18 \times 3.66\mu m$ respectively and consecutively the ratios of major axis/minor axis in cell and nucleus were 1.14 and 1.20 respectively. The mean surfaces area and volumes of cell and nucleus were $83.82\mu m^2$, $542.79\mu m^3$ and $12.58\mu m^2$, $28.96\mu m^3$ respectively.

The number of erythrocytes of both females and males were $6 \sim 7 \times 10^5/ml$. As shown in Fig. 2 – b, gill tissues had one or two nucleoli.

Discussion

The diploid chromosome number and karyotype of *P. fulvidraco* were similar to those reported by Lee (1988). Of particular interest, large metacentric chromosome with the high range of relative length 6.22 of one pair (No. 1) chromosome in this species, hereafter, should be useful marker (Thorgaard and Allen, 1987 ; Park and Kim, 1994) to detect the level of ploidy. Most catfish species except North American catfish, *Noturus taylori* studied previously have undifferentiated sex chromosomes (LeGrande, 1981 ; Kim *et al.*, 1982, 1988). Absence of heteromorphic sex chromosomes in *P. fulvidraco* also reported by Kim *et al.* (1982) and these facts indicate that their sex chromosomes are not morphologically differentiated.

However the polyploid cell and nucleus are larger in size than diploid ones, extensive variations in cellular and nuclear size within individual fish of known ploidy and among individuals of a given ploidy have been reported (Thorgaard, 1986). Considering this point of view to estimate polyploid further study such as DNA content measurement by flowcytometry will be necessary in addition to nuclear or cellular size measurement. Our result of erythrocyte number $6 \sim 7 \times 10^5/ml$ is a reliable estimation of polyploidy because of triploid organisms have lower erythrocyte numbers than diploid organisms (Ueno, 1984 ; Kim *et al.*, 1988 ; Sezaki *et al.*, 1988 ; Park and Park, 1994).

Counting nucleoli method, involves silver staining cells and determining the maximum number of nucleoli per cell, is a simple inexpensive alternative to flowcytometry which may be applicable to a variety of fish species (Phillips *et al.*, 1986). In some species including mudminnow, carp, walleye pike, and various Gymnotiformes (Kligerman and Bloom, 1977 ; Foresti *et al.*, 1981 ; Takai and Ojima, 1982 ; Gold, 1984 ; Phillips *et al.*, 1986) exist with one chromosome pair with nucleolar organizer regions (NORs), each nucleolar organizer region usually forms a separate nucleolus in rapidly dividing tissuse, but the chromosomes with NORs often form a single nucleolus in tisstue with a lower mitotic index (Phillips *et al.*, 1986). The present results show that *P. fulvidraco* is species such as those described in other papers which have only one chromosome pair with NORs per diploid genome.

Consider results from this study, we conclude the cellular and nuclear sizes measurement, erythrocyte number count, nucleoli count, and karyological analysis of diploid *P. fulvidraco* should be used as a valuable estimation of future induced polyploidy in this species.

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동자개, *Pseudobagrus fulvidraco* (Teleostomi : Siluriformes)의 세포유전학적 연구

박 인 석 · 이 충 렬*
군산대학교 해양자원육성학과 · 군산대학교 생물학과*

동자개, *Pseudobagrus fulvidraco*의 염색체 수는 2n=52(1~9번 중부염색체: 상대길이 2.89~6.22, 완비 1.09~1.58 ; 10~22번 차중부염색체: 상대길이 2.88~5.88, 완비 1.80~3.65 ; 23~26번 단부염색체 : 상대길이 2.63~3.30, 완비 9.01~10.67)였으며 fundamental number는 104이였다. 이형의 성염색체는 발견되지 않았다. 적혈구를 대상으로한 각 측정, 계측 항목에서 암 · 수 간 유의한 차이는 없었으며($p<0.05$), 적혈구 세포와 핵의 크기는 각각 11.03×9.67μm와 4.18×3.66μm이였다. 암 · 수에서 적혈구 수는 6~7×105/*ml*이였다. 아가미 조직에서 인 계수시 1개 혹은 2개의 인형성부위가 나타나 2배체의 특성을 나타내었다.