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Studies on the Karyotypes and Comparative DNA Values in Several Korean Cyprinid Fishes

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한국산 담수어류(잉어과) 몇종의 핵형과 DNA 상대량에 관한 연구*

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적 요

한국산 담수어류 4종 (잉어과)에 대한 염색체 및 DNA 상대량을 조사하여 아래와 같은 결과를 얻어 이들의 세포유전학적 특징을 계통학적인 면에서 고찰하였다.

1) 참마자 (*Hemibarbus longirostris*) 및 누치 (*H. labeo*)의 염색체수는 2n=50으로써 그중 참마자는 7쌍의 metacentric (A 그룹), 14쌍의 submeta와 subtelocentric (B 그룹) 그리고 acro 내지 telocentric 염색체 (C 그룹) 4쌍으로 구성되며 arm수 (AN)는 92였다. 누치의 핵형은 A, B 및 C 그룹이 각각 9,11 그리고 5쌍으로써 AN은 90이 된다.

2) 버들개 (*Moroco lagowskii*) 및 버들치 (*M. oxycephalus*)의 염색체수는 역시 2n=50이었으며, 이들의 핵형도 다같이 A그룹이 6쌍, B그룹이 14쌍 그리고 5쌍의 C그룹으로 이루어져 있으며, AN은 90이었다.

3) 4종의 DNA 상대량은 모두 붕어의 약 60%에 해당하였다.

INTRODUCTION

Karyological characteristics have been used as a valuable aid to taxonomical and evolutional studies in many groups of plant and animal. But a little is known about the fish karyology because chromosomes of fishes are small and available techniques have often yielded questionable counts and minimal morphology.

Since 1967 improvements in methodology could be avoided technical problems that plagued earlier works, and has facilitated precise and reliable analyses of chromosomes in fish species. In view of the above facts cytogenetical study in Korean ichthyological fauna, which contains more than 100 fresh-water species,

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would be of interest.

The family *Cyprinidae* of Korea consists of 31 genera included 59 species. Nogusa (1960) reported the chromosomes of 9 species involved in 6 genera with Japanese population by classic gonad-section method. More recently, five species which are common in Korea and Japan were also investigated karyologically and cytophotometrically (Ojima *et al.*, 1972). It is a purpose of this paper to describe the somatic chromosomes and DNA contents of four species of Korean *Cyprinidae* with special concern to phylogenetic significance of cytogenetical characters.

MATERIALS AND METHODS

Species and sex of specimens studied along with their collection locality are shown in Table 1.

Table 1. Species and their localities

Species	No. of sample	Sex	Localities
Hemibarbus longirostris	11	♀ : 8, ♂ : 3	Han River (Chuncheon)
H. labeo	6	♀ : 1, ♂ : 4, ? : 1	〃 (〃)
Moroco oxycephalus	4	♀ : 2, ♂ : 2	Ui-dong
M. lagowskii	10	♀ : 5, ♂ : 5	Stream of Mt. Sorak

Chromosome preparations were made following the technique of Ojima and Hitotsumachi (1967). Anterior part of kidney tissue was isolated, minced into fine fragments, and suspended in BSS for fish. Colchicine was added at a final concentration of about 0.2~0.5μg/ml and kept at room temperature for 30 minutes. Following cell collection by means of centrifugation, a pelet of cell was resuspended in 0.075 M hypotonic KCl solution for 15~30 minutes at room temperature, and fixed in 1 : 3 acetic alcohol. Chromosome slides were made following the routine air-drying method with Giemsa stain. At least four clean metaphases were selected in each species for karyotype analysis.

DNA content were obtaind from liver nuclei stained with Feulgen reagent. Liver tissues were simultaneously fixed in acetic alcohol at 4°C for 30 minutes. Admixture of raw rubber (Tissuemat, Fisher Co., Chicago) was used for paraffin embedding. Sections were made 7~8 micra thick, hydrolyzed in 5N HCl at room temperature for 2 hours, and stained in Feulgen for 2 hours. Slide was then

washed 3 times in SO_2 water, dehydrated and mounted in Cargille oil. Liver tissues of gold fish (*Carassius auratus*) were used as the control.

The DNA contents in individual nuclei were checked using two-wavelength method according to Mendelsohn (1958 a,b) using a microspectrophotometer (Olympus MSP-A-IV). Instrument was tested previously by measuring nucleated erythrocytes of domestic fowl according to the advice of Ruthmann (1970).

RESULTS

1. Chromosomes

Chromosome counts obtained for each species are shown in Table 2. Although counts ranged from 45 to 52, the strong modal diploid chromosome numbers of 50 were appeared in all species. There was no interindividual chromosome polymorphism among the kidney cells. Counts of less than 2n=50 may be attributed primarily to chromosome loss through cell breakage.

Table 2. Distribution of diploid chromosome number in four species of family *Cyprinidae*

Species	Frequency of chromosome No. of								No. of cells scored	2n No.
	45	46	47	48	49	50	51	52		
H. longirostris	1	0	2	9	10	102	6	1	131	**50**
H. labeo	2	4	4	7	15	229	1	0	262	**50**
M. oxycephalus	0	0	1	1	1	48	2	3	56	**50**
M. lagowskii	0	1	2	7	24	389	8	1	432	**50**

According to the position of centromere and chromosome size, diploid compliments were tentatively categorized into three groups, A, B and C. Group A includes nearly metacentric chromosome, group B submeta- and subtelocentric chromosomes, and group C telo-or acrocentric ones. The karyotype analysis was done on 50 well-delineated metaphasic chromosomes in which elements showed minimum overlapping (Figs. 1~4). Matching of homologous pairs was rather subjective since many elements were morphologically similar.

Representative karyotypes of two species of *Hemibarbus* are presented in Figs. 5 and 6. It is clear from these figures that the two species are different in respect of their karyological characteristics. *H. longirostris* has 7 pairs of A group chro-

mosomes, 14 pairs of B, and 4 pairs of C, whereas *H. labeo* has 9, 11 and 5 pairs respectively. These results will give the total arm numbers of 92 and 90.

The karyotypes analyzed in two species of genus *Moroco* are shown in Figs. 6 and 7. Both species posses 6 pairs of chromosome of group A, 14 pairs of B, and 5 pairs of C, since these two species have an equal arm number of 90. A comparison of karyotypes of above two species failed to reveal any visible variation of individual chromosomes either numerically or morphologically.

No. karyotypic sexual dimorphism that may reflect sex chromosomes was observed not only in *H. longirostris* and *H. labeo* but also in *M. oxycephalus* and *M. lagowskii*. Summary of chromoscme constitution of each species is outlined in Table 3.

Table 3. Karyotype constitution of four species

Species	2n No.	Group (Pair)			Arm No.
		A	B	C	
H. longirostris	50	7	14	4	92
H. labeo	50	9	11	5	90
M. oxycephalus	50	6	14	5	90
M. lagowskii	50	6	14	5	90

2. *Relative DNA values*

Relative DNA values, number of nuclei measured and arm number are summarized in Table 4. In this analysis the DNA value of gold fish was used as control, and these of other species were discussed in comparison with control value.

The four species have DNA values of about 60 % of gold fish, and there was no significant difference among four species observed.

Table 4. Relaitve amounts of DNA/cell for members of *Cyprinidae*

Species	No. of cells measured	Relative amount of DNA ±S.E.	Arm No.
H. longirostris	50	0.618±0.068	92
H. labeo	50	0.602±0.065	90
M. oxycephalus	50	0.632±0.072	90
M. lagowskii	50	0.582±0.060	90
Carassius auratus	50	1.000±0.143	148*

* Ojima and Hitotsumachi (1967)

DISCUSSION

Cytogenetic analysis of family *Cyprinidae* (Ord. *Ostariophysi*) in Old World species have been carried out by Ohno *et al.*(1967) and Ojima *et al.*(1972). For the New World member Lieppman and Hubbs (1969) reported two species included two genera.

The chromosome number obtained from kidney tissue for *H. longirostris* was identical with that found from gonad tissue by Nogusa (1960). In the present study karyotype of same species was found to be different from that reported by Nogusa(1960) who indicated the occurrence of 50 acrocentric- or telocentric chromosomes. The difference may be attributable to the method used for study, since his study was made by classic sectioning method. *H. labeo*, *M. oxycephalus* and *M. lagowskii* have not been studied in previous chromosome works as far as we know.

In the family *Cyprinidae* it was not surprising that some species of inter— or intragenus had identical karyotype with each other. For example gold fish (*Carassius auratus*) and carp (*Cyprinus carpio*) or *Zacco temminckii* and *Z. platypus* etc. were identical in karyotype (Ojima & Hitotsumachi, 1967; Ojima *et al.*, 1972). Two species of genus *Moroco* in this study also had exactly identical chromosome constitution with each other (See Figs. 7 & 8). So, karyotype may not be useful in elucidation of the phylogenetic relationship between these two species.

Cytogenetically every organism is characterized by its own specific karyotype, in number and morphology. Recently, reports have appeared showing that karyotypic variation through Robertsonian type translocation occurs indifferent individuals of same species of the *Centrachidae* and *Salmonidae* (Becak *et al.*, 1966; Fukuoka, 1972). Aside from above two families, similar polymorphism has been reported especially in *Moroco jouyi* of Japan which is closely related to *M. oxycephalus* belonging to the family *Cyprinidae* (Ojima, 1973). Individuals with diploid chromosome number of 50, 51 and 52 coexisted in 17 specimens of the same species. The three modal types were related to each other by the Robertsonian principle. But in the present species such interindividual chromosome polymorphism was not observed.

In the present study, no distinction was observed in the karyotype produced from female and male of each species. Therefore, it was difficult to indicate that there was chromosomal heteromorphism between both sexes in all species that may reflect sex chromosomes such as those found for some teleostean fishes by Ebeling and Chen (1970).

Ohno *et al.*(1968), in a comprehensive examination of vertebrates from fish to

mammals, demonstrated the occurrence of several manners of karyotype diversification in the class of Fishes, such as unequal exchanges between the chromatids of the same chromosome, unequal crossing over between homologous chromosomes during meiosis, duplication of DNA molecule within chromosome segments, and polyploidization. In this way, a change in the original amount of DNA per cell, which corresponds to about 20% of the DNA in placental mammals, took place, leading to the existence of groups of fishes with DNA amounts of 30% (Ord. *Percomorphi*), 40% (Subord. *Clupeoidea*), 50% (Ord. *Ostariophysi*), 80~90% (Some *Salmonids*), etc. as compared with mammals.

Fish, the lowest vertebrate from an evolutionary point of view, demonstrated a reduced morphologic and genetic differentiation of the sex chromosome. Polyploidization seems to be occurred frequently and playes a relatively important role in evolution, e.g., the salmonid fishes have a DNA content of 80% of that of placental mammals, and a diploid compliment consisting of about 100 chromosome arms, while *Clupeoidea* have a DNA content of 40% and diploid compliment consisting of 48 acrocentrics (Ohno, 1969 ; 1970).

A similar situation is found among cyprinid fishes. The two species of genus *Barbus*, *B. tetrazona* and *B. fasciatus*, had the diploid chromosome number ranging around 50 and the DNA value of 20~22 percent that of placental mammals, while the gold fish and the carp had the diploid chromosome number of about 100 and the DNA value of 50~52% that of placental mammals (Ohno *te al.*, 1967). According to this data it is generally agreed that the carp and the gold fish are tetraploid forms with respect to two species of *Barbus*. Furthermore, recent extensive works of Ojima *et al.*(1972) proposed that this type of relationship might also be considered to exist between gold fish and *Acheilognathus* and *Rhodeus*.

In consideration of the above facts, DNA content and chromosome number of present species, the suggestion that these species are also diploid forms with respect to gold fish in tetraploid state seems to have some positive support (See Table 4).

SUMMARY

Karyological characteristics and relative DNA values were investigated in four species of the family *Cyprinidae* of Korean fresh-water. The results obtained were as follows ;

1) The two *Hemibarbus* species, *H. longirostris* and *H. labeo*, had the diploid chromosome number of 50. The compliment of former consisted of 7 metacentric, 14 submeta- and subtelocentric and 4 telo- or acrocentric pairs, whereas latter had 9, 11 and 5 pairs respectively, Total arm numbers (AN) were 92

and 90.

2) In the case of two species of genus *Moroco*, *M. oxycephalus* and *M. lagowskii*, their diploid number and karyotype showed an identical pattern for each other (2n=50): 6 pairs of metacentrics, 14 pairs of submeta- and subtelocentrics and 5 pairs of telo- or acrocentrics. The AN of both species were 90.
3) Relative DNA values of all species were measured as about 60% that of gold fish.

From the above observation, the cytogenetical character was discussed with special concern to the phylogenetic significance.

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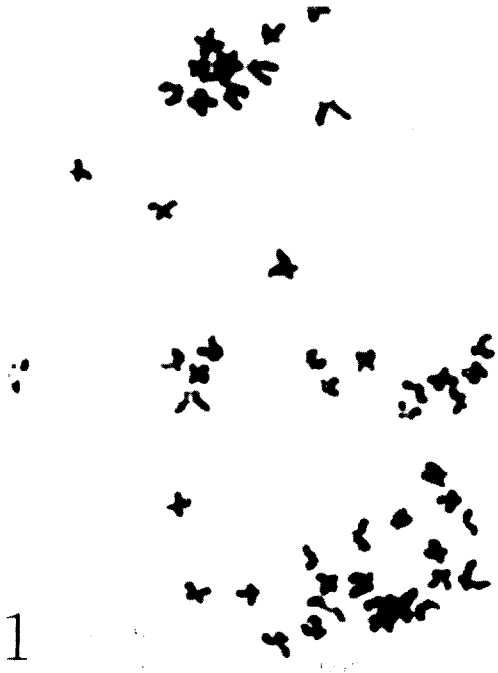
EXPLANATION OF PLATES

Figs. 1—4. Metaphase spreads of *H. longirostris* (Fig. 1), *H. labeo* (Fig. 2), *M. lagowskii* (Fig. 3) and *M. oxycephalus* (Fig. 4). Diploid number of all species is 50.

Figs. 5 and 6. Karyotypes of *H. longirostris* (Fig. 5) and *H. labeo* (Fig. 6). A; Metacentric group, B; submeta-and subtelocentric group, C; acro- or telocentric group.

Figs. 7 and 8. Karyotypes of *M. lagowskii* (Fig. 7) and *M. oxycephalus* (Fig.8). A; Metacentric group, B; submeta- and subtelocentric group, C; acro- or telocentric group.



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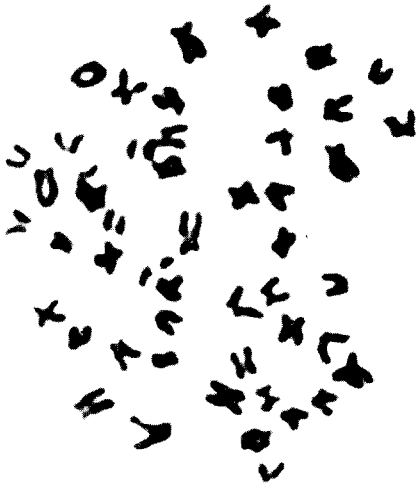

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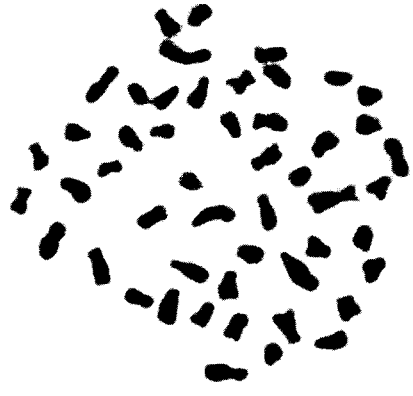

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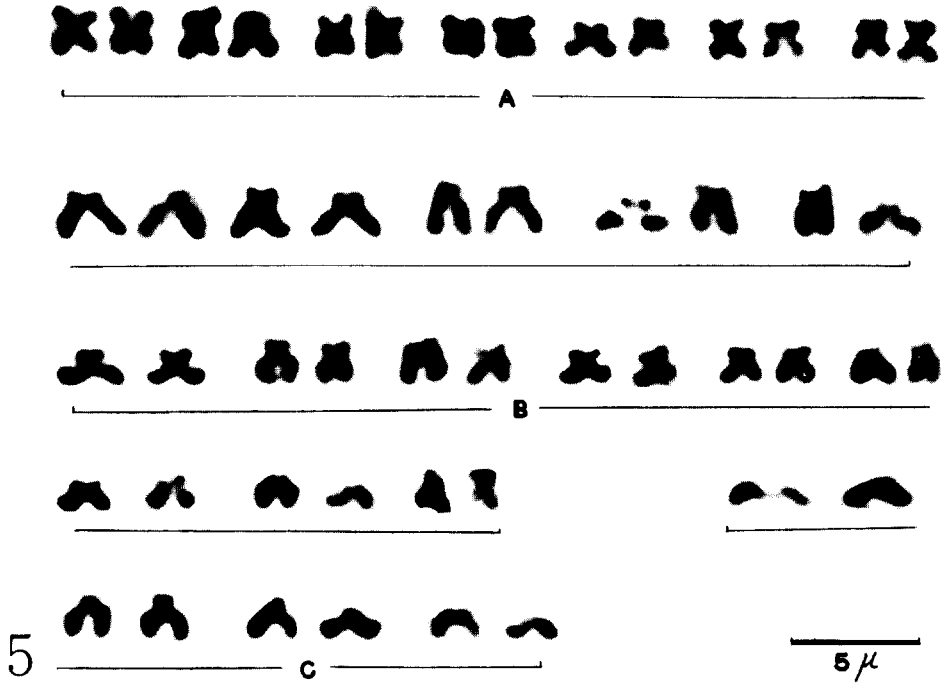

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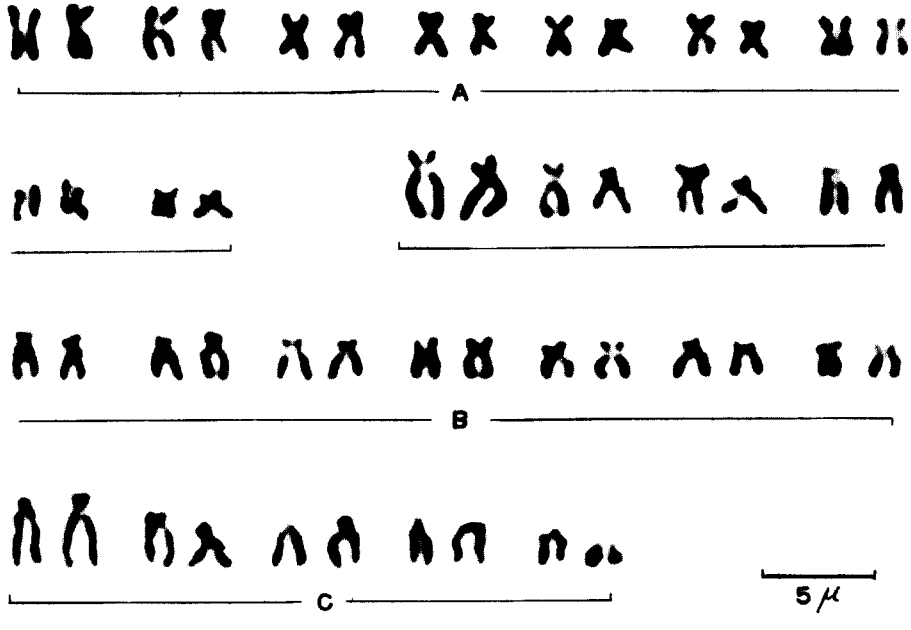

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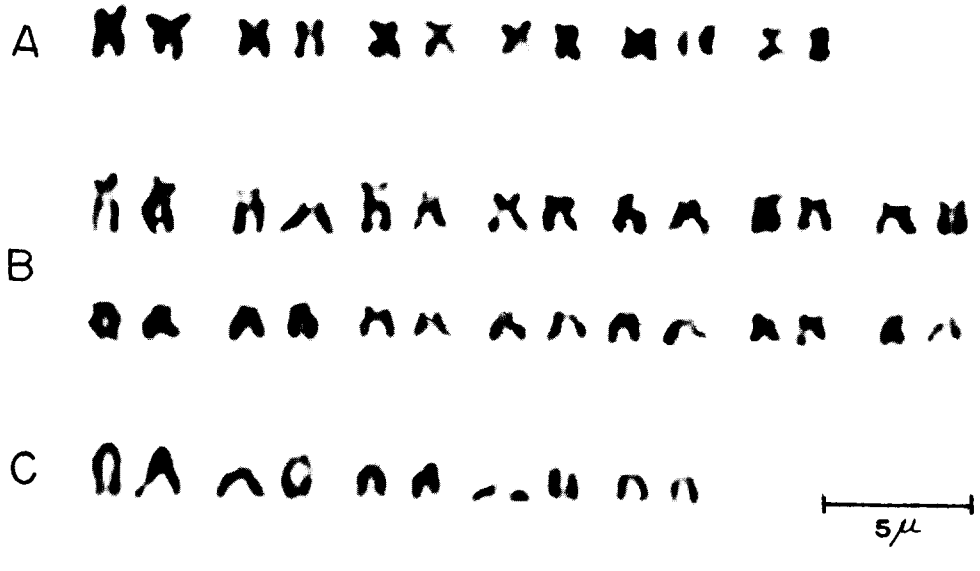

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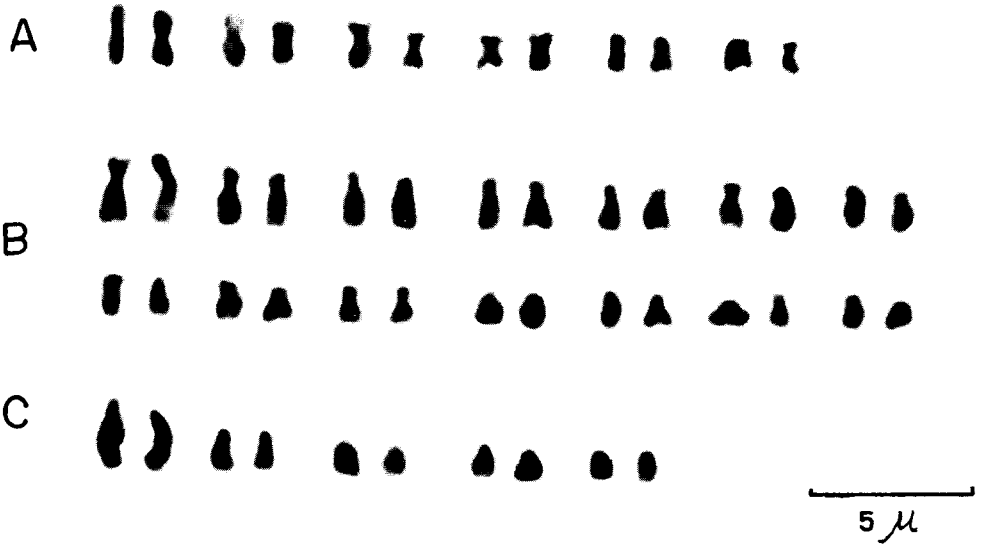

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