Chromosomes of Three Flatfishes (Pleuronectiformes)

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The chromosomes of the pleuronectiform fishes have been reported in 23 species from five families within eight families (Nogusa, 1960; Ohno and Atkin, 1966; Ivanov, 1969; Fukuoka and Niiyama, 1970; Barker, 1972; LeGrande, 1975). In the present paper, the karyotypes of a paralichthyid, *Paralichthys olivaceus*, and two cynoglossids, *Paraplagusia japonica* and *Cynoglossus interruptus* are described and compared with other members of this order. *P. olivaceus* has been karyotyped by Nogusa (1960) using the gonad section method, and the other species represent the second and third records on the karyotypes in the family Cynoglossidae respectively.

Material and methods

Paralichthys olivaceus, three specimens, HUMZ 78599, female, 256.7 mm in total length, HUMZ 78600, sex unknown, 214.5 mm in total length, HUMZ 78601, female, 237.5 mm in total length; *Paraplagusia japonica*, one specimen, HUMZ 78598, female, 361.0 mm in total length. These specimens were captured by gill net at Futami, Yamaguchi Prefecture, on April 10, 1978. *Cynoglossus interruptus*, two specimens, HUMZ 87209, female, 145.2 mm in total length, May 16, 1979, HUMZ 87210, female, 120.0 mm in total length, July 10, 1979. These specimens were captured by small trawl net at Yanai Bay, Yamaguchi Prefecture, Japan.

The fishes were kept in aquaria for a short period. They were given an intraperitoneal injection of colchicine (1 μg/g body weight) 4 to 5 hours before sacrificing. Pieces of kidney were removed, minced with a pair of scissors in a shalet and suspended in 0.075 M KCl hypotonic solution for 30～40 minutes at room temperature. The dispersed cells were collected in a centrifuge tube and fixed with 3 : 1 methanol-acetic acid for 40 minutes. Slides were prepared according to routine air-drying method and stained with a Giemsa solution.

Classification of chromosomes is after Levan et al. (1964). The arm number (NF) was established by assigning a value of one to all acrocentric chromosomes, and a value of two to all metacentric, submetacentric and subtelocentric chromosomes.

Specimens used in the present study are deposited in the Laboratory of Marine Zoology, Faculty of Fisheries, Hokkaido University (HUMZ).

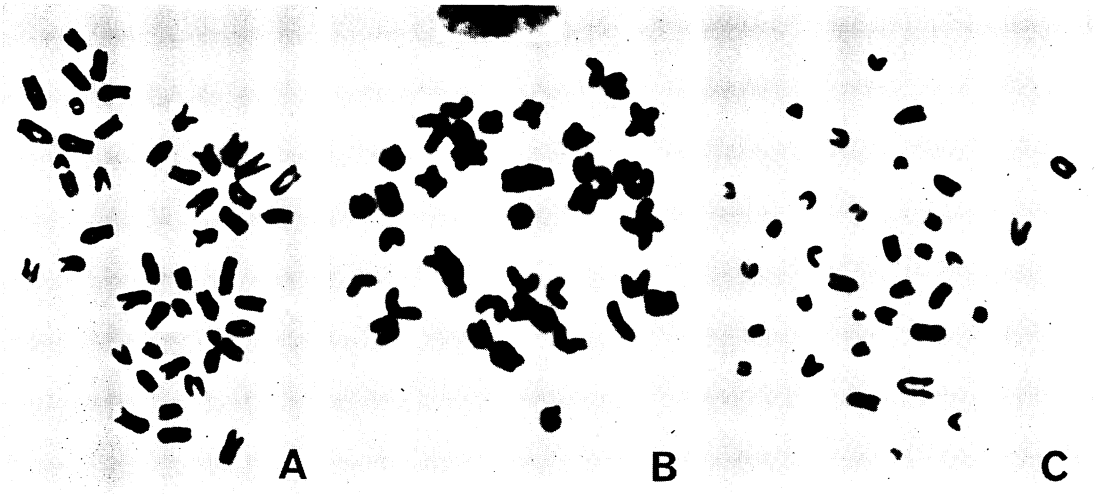

Fig. 1. Photomicrographs of mitotic metaphase chromosomes. A: *Paralichthys olivaceus*. ×1750. B: *Paraplagusia japonica*. ×2150. C: *Cynoglossus interruptus*. ×2950.

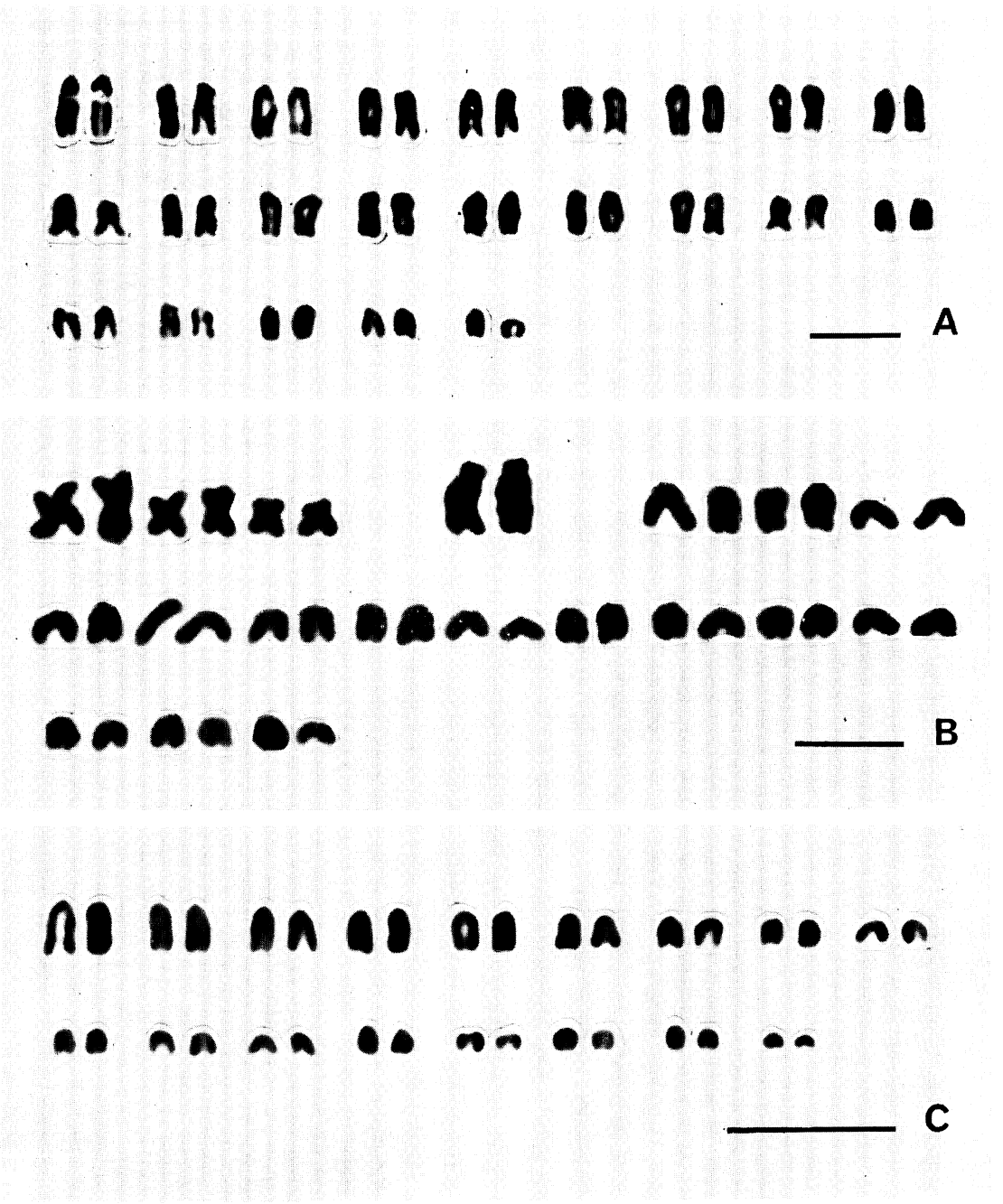

Fig. 2. Karyotypes of three flatfishes. A: *Paralichthys olivaceus*, from Fig. 1A. ×2700. B: *Paraplagusia japonica*, from Fig. 1B. ×3080. C: *Cynoglossus interruptus*, from Fig. 1C. ×4950. Scales indicate 5 μm.

Results

Paralichthys olivaceus (Temminck et Schlegel); Japanese name: Hirame (Figs. 1A, 2A): The diploid chromosome number is 46 (Table 1). The karyotype consists of 46 acrocentric chromosomes, gradually declining in size. The arm number is 46. Secondary constrictions were observed at the first pair of acrocentric chromosomes.

Remarks: These results agree in number and elements with those of Nogusa (1960) using the gonad section method, but disagree in that secondary constrictions are observed at the first pair of acrocentrics in the present specimens. Secondary constrictions characterize the karyotype of this species among those members of the family Paralichthyidae

Table 1. Frequency distribution of diploid chromosome numbers in three flatfishes.

Species	Number of specimens	Number of diploid chromosomes													Total cell count
		32	33	34	35	36	37	38	39	44	45	46	47	48	
Paralichthys olivaceus	3									6	4	41	5	9	65
Paraplagusia japonica	1					12	8	49	1						70
Cynoglossus interruptus	2	3		24	1										28

Table 2. Diploid and fundamental chromosome numbers in flatfishes. According to Park (1974) and Ojima et al. (1976), the data taken by the gonad section method are excluded here.

Species	2n	NF	References
Scophthalmidae			
Scophthalmus maeoticus	40	≥60	Ivanov (1969)
Paralichthyidae			
Paralichthys lethostigma	48	68	LeGrande (1975)
P. olivaceus	46	46	Present study
Xystreurys liolepis	48	48	Ohno and Atkin (1966)
Etropus crossotus	38	74	LeGrande (1975)
Citharichthys spilopterus	28	50	LeGrande (1975)
Pleuronectidae			
Cleisthenes pinetorum herzensteini	44	48	Fukuoka and Niiyama (1970)
Verasper moseri	46	48	Fukuoka and Niiyama (1970)
Limanda punctatissima	48	48	Fukuoka and Niiyama (1970)
L. herzensteini	48	48	Fukuoka and Niiyama (1970)
L. schrenki	48	48	Fukuoka and Niiyama (1970)
L. yokohamae	48	48	Fukuoka and Niiyama (1970)
Platichthys stellatus	48	48	Fukuoka and Niiyama (1970)
Kareius bicoloratus	48	48	Fukuoka and Niiyama (1970)
Microstomus achne	48	48	Fukuoka and Niiyama (1970)
Pleuronectes platessa	48	48	Barker (1972)
Pleuronichthys verticalis	48	48	Ohno and Atkin (1966)
Soleidae			
Trinectes maculatus	40	50	LeGrande (1975)
Achirus lineatus	40	64	LeGrande (1975)
Solea lascaris nasuta	42	≥50	Vasilyev (1978)
S. solea	42	—	Barker (1972)
Zebrias zebra	46	46	Fukuoka and Niiyama (1970)
Cynoglossidae			
Paraplagusia japonica	38	46	Present study
Symphurus plagiusa	45, 46 (♂)(♀)	70, 72 (♂)(♀)	LeGrande (1975)
Cynoglossus interruptus	34	34	Present study

so far examined. In the pleuronectiform karyotypes, such constrictions have been reported only in a pleuronectid, *Microstomus achne* (Fukuoka and Niiyama, 1970), and a cynoglossid, *Cynoglossus interruptus*, in the present study.

Paraplagusia japonica (Temminck et Schlegel); Japanese name: Kuro-ushinoshita (Figs. 1B, 2B): The diploid chromosome number is 38 (Table 1). The karyotype comprises 6 metacentric, 2 subtelocentric and 30 acrocentric chromosomes. The arm number is 46.

Cynoglossus interruptus (Günther); Japanese name: Genko (Figs. 1C, 2C): The diploid chromosome number is 34 (Table 1). The karyotype consists of 34 acrocentric chromosomes, gradually declining in size. The arm number is 34.

Remarks: Secondary constrictions are observed at the largest pair of acrocentrics in other mitotic metaphase chromosomes, although they are unclear in Fig. 2. The karyotype of this species is simple in comparison with those of *Symphurus plagiusa* and *Paraplagusia japonica*, which have been examined in the Cynoglossidae. Furthermore, the chromosomal constitution of this fish is peculiar in having the smallest number of arms within the Pleuronectiformes so far studied.

Discussion

In the Pleuronectiformes, the diploid chromosome number widely ranges from 28 to 48, and the arm number from 34 to 70 (Table 2).

LeGrande (1975), judging from the prevalence of 48 as the modal diploid and modal fundamental chromosome numbers so far studied, considered that the primitive karyotype for the pleuronectiforms probably consisted of 48 subtelocentric to acrocentric chromosomes. From this assumption, he induced that the decrease in diploid number below 48 and the increase in the fundamental number above 48 probably resulted from two main mechanisms of chromosomal change, centric fusion and pericentric inversion, in the course of the evolution of karyotypes in this group.

However, the present study indicates that karyotypes having fundamental numbers below 48 (NF=48) clearly exist in the pleuronectiforms. In addition, the fundamental number of a soleid, *Zebrias zebra*, is 46 (Fukuoka and Niiyama, 1970). These chromosomal constitutions can not be explained only by mechanisms of chromosomal change mentioned above.

Acknowledgments

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ヒラメ，クロウシノシタおよびゲンコの染色体

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カレイ目魚類3種，ヒラメ科のヒラメおよびウシノシタ科のクロウシノシタとゲンコの体細胞染色体を通常の air-drying 法により観察した．染色体数はヒラメ 2n=46，クロウシノシタ 2n=38，ゲンコ 2n=34 である．ヒラメの核型は 23 対の端部着糸染色体から構成され，NF=46 である。最も大きい端部着糸染色体対にこれまでヒラメ科魚類の核型では報告されていない二次くびれが観察された．クロウシノシタの核型は 3 対の中部着糸染色体，1 対の次端部着糸染色体および 15 対の端部着糸染色体からなり，NF=46 である．ゲンコの核型は 17 対の端部着糸染色体から構成され，NF=34 である．

これら3種の核型はいずれも腕数が少なく，カレイ目魚類の中では特異的である．

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