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Cytogenetical Study of Fishes from Coastal Area in Jo Island, Busan, Korea I. *Parapercis sexfasciata* (Temminck et Schlegel), *Sebastiscus marmoratus* (Cuvier) and *Pleuronectes yokohamae* (Günther)

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ABSTRACT

Karyotype analysis and DNA content determination of three species of saddled weever, *Parapercis sexfasciata* (Temminck et Schlegel), marbled rockfish, *Sebastiscus marmoratus* (Cuvier) and marbled sole, *Pleuronectes yokohamae* (Günther) from coastal area in Jo island, Busan, Korea were performed. The karyotype of *P. sexfasciata* has a diploid number of 2N = 26 and fundamental number of FN = 50 with a chromosome formula of 22M+2A+2T. The karyotype of *S. marmoratus* has a diploid number of 2N = 48 and fundamental number of FN = 50 with a chromosome formula of 2M+46T. The karyotype of *P. yokohamae* has a diploid chromosome number of 2N = 48 and fundamental number of FN = 48 with a chromosome formula of 24T. The variation in their DNA values (*P. sexfasciata* = 1.50 pg/nucleus; *S. marmoratus* = 1.31 pg/nucleus; *P. yokohamae* = 1.03 pg/nucleus) is positively related to the variation in chromosome fundamental number in material fish examined.

Key words: DNA content, Jo island, Karyotype, *Parapercis sexfasciata, Pleuronectes yokohamae, Sebastiscus marmoratus.*

INTRODUCTION

Fish cytotaxonomy refers to the study of phenetic and/or phylogenetic relationships among species, based on comparisons of chromosome number and morphology, genome size, or the amount of DNA per nucleus (Gold, 1979; Kim and Park, 1990; Park, 1992; Park et al., 1999). Particularly, regarding the chromosome numbers and genome sizes among diploid teleost fishes there is a highly significant, positive correlation between chromosome number and genome size (Perdersen, 1971; Hinegardner and Rosen, 1972).

The saddled weever, *Parapercis sexfasciata* (Temminck et Schlegel) of the family Pinguipedidae is widely distributed in the South Sea and around Jeju

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Island of Korea, however, at these locations also exist many fishes from parts of Japan and Taiwan (Choi et al., 2002). The marbled rockfish, *Sebastiscus marmoratus* (Cuvier) of the family Scorpaenidae and the marbled sole, *Pleuronectes yokohamae* (Günther) of the family Pleuronectidae are usually found near the coastal areas from the East Sea to the East China Sea (Choi et al., 2002).

The present study aims to clarify the cytogenetical aspects of *P. sexfasciata*, *S. marmoratus* and *P. yokohamae* which were collected off the coast of Jo island, Busan Korea. Details of their karyological features and flowcytometry are described below.

MATERIALS AND METHODS

Sampling and species identification

The saddled weever, *Parapercis sexfasciata* (Temmick et Schlegel), the marbled rockfish, *Sebastiscus marmoratus* (Cuvier) and the marbled sole, *Pleuronectes yokohamae* (Günther) were collected by using traps and netting at the shore around Jo island, Korea Maritime University (KMU), Busan, Korea, at which this experiment was performed from August to September, 2004. The fish were transported to Aquarium of Fishery Genetics and Breeding Laboratory, KMU, where they remained alive until they were analyzed.

The current classifications of three material fish examined were based on Choi et al. (2002). After anaesthesis with 200 ppm lidocaine-HCl/1,000 ppm $NaHCO_3$ at 22°C, total length was measured by vernier caliper to 0.1 cm and external morphology was taken by digital camera using photocopy stand.

Chromosome analysis

Twenty specimens of each species (10 females and 10 males) were subjected to karyological analyses. Each specimen was given colchicine (1-10 μg per g body weight) by intraperitoneal injections. After 3 hours the specimens were sacrificed and kidney tissues were used for chromosome preparations. The kidney cells were suspended in hypotonic 0.075 mM KCl solution, fixed with 1 : 3 acetic alcohol, and chromosome sliders were made by means of conventional air-drying technique. Detailed procedures for preparation may be referred to Im et al. (2001) and Kim et al. (2001).

At least 20 countable metaphases from each specimen were observed for chromosome counts and karyotypes. Well spread chromosomes at metaphases were selected and photographed. Fundamental number of each chromosomes were calculated and nomenclature of the chromosomes is based on the calssification made by Levan et al. (1964).

Flowcytometry

Flowcytometric analysis was performed to estimate average cellular DNA content of 20 individuals from each species. After anaesthetizing the fish with 200 ppm lidocaine-HCl/1,000 ppm $NaHCO_3$, a 0.5 mL of whole blood was collected in 1 mL acid citrate dextrose (ACD) solution (0.48 g citric acid, 1.32 g sodium citrate, 1.47 g dextrose in 100 mL of distilled water), washed once with PBS by centrifugation (200 g for 10 mins), and then fixed in 50% ethanol or 1% parafromaldehyde for one hour. One million cells were taken and stained using propidium iodide (100 μg/mL) at 4°C for one hour.

Samples stained were analyzed on a Partec PA-II flowcytometer (Partec GmbH, Münster, Germany) to determine relative DNA content. The red blood cells (2.36 pg DNA/nucleus) of cyprinid loach *Misgurnus arguillicaudatus* were used as a standard reference (Park, 1992; Kim et al., 1993).

RESULTS

Figure 1 shows the external morphology of fish used in this experiment. The mean total body length in saddled weever, *Parapercis sexfasciata* (Temminck et Schlegel) was 19.2 ± 1.1 cm, marbled rockfish, *Sebastiscus marmoratus* (Cuvier) was 17.1 ± 0.9 cm and marbled sole, *Pleuronectes yokohamae* (Günther) was 23.1 ± 1.3 cm.

The modal chromosome numbers of *P. sexfasciata* was 2n = 26 with the mode of chromosome distribution frequency of 88% (Table 1), consisting of 11 pairs of metacentrics, 1 pair of acrocentrics and 1 pair of telocentrics. The fundamental number of saddle weever was 50 (Table 1, Fig. 2A). Interestingly, there was chromosome breakage at long arm of one acrocentrics (Fig. 2A, arrow).

The modal chromosome numbers of *S. marmoratus* and *P. yokohamae* were identical as 2n=48 with the mode of chromosome distribution frequency of 84% in *S. marmoratus* and the mode of chromosome distribution frequency of 82% in *P. yokohamae* (Table 1). An idiogram of the *S. marmoratus* was consisted of 1 pair of metacentric and 23 pairs of telocentrics, and the

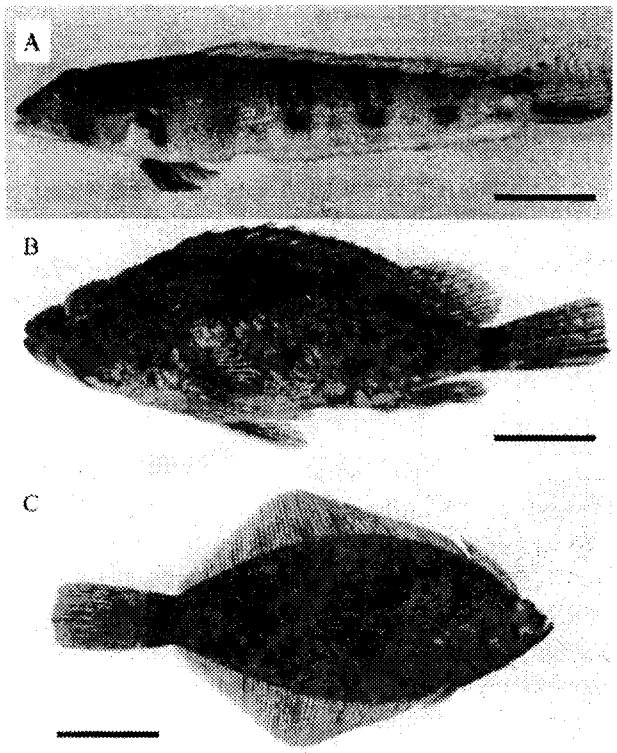

Figure 1. External morphology of fish used in this experiment. (A) *Parapercis sexfasciata*, (B) *Sebastiscus marmoratus* and (C) *Pleuronectes yokohamae*. Bars are 3 cm.

fundamental number of these fish was 50 (Table 1, Fig. 2B). An idiogram of the *P. yokohamae* was consisted of 24 pairs of telocentris, and the fundamental number of these fish was 48 (Table 1, Fig. 2C). There was no evidence of polymorphism including aneuploidy or sex-related heteromorphic chromosome for all species examined.

Table 1. Karyotypes and frequency distribution (%) of diploid chromosome numbers of material fish examined. M, metacentrics; A, acrocentrics; T, telocentrics.

Species	Karyotype			FN	Frequency distribution (%)				Total cell count
	M	A	T						
Parapercis sexfasciata	22	2	2	50	24 (2)	25 (4)	26 (88)	27 (6)	260
Sebastiscus marmoratus	2	0	46	50	46 (2)	47 (11)	48 (84)	49 (3)	220
Pleuronectes yokohamae	0	0	48	48	46 (2)	47 (10)	48 (82)	49 (6)	200

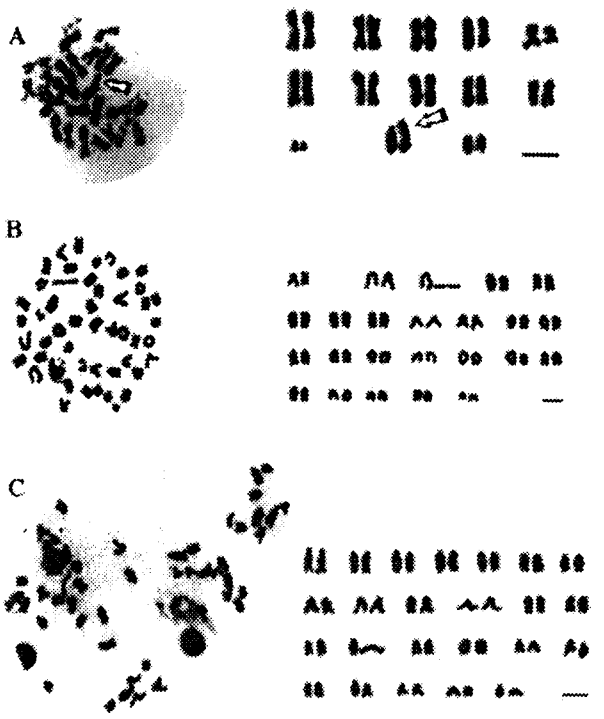

Figure 2. Metaphases and idiograms of (A) *Parapercis sexfasciata*, (B) *Sebastiscus marmoratus* and (C) *Pleuronectes yokohamae*. Arrow indicates broken acrocentric chromosome. Bars are 10 μm.

Table 2. Nuclear DNA content of material fish in this experiment.

Species	DNA content (pg/nucleus)*
Parapercis sexfasciata	1.50 ± 0.04
Sebastiscus marmoratus	1.31 ± 0.02
Pleuronetes yokohamae	1.03 ± 0.05
Misgurnus anguillicaudatus standard**	2.36 ± 0.05

*Values are means ± SE.
**From Park, 1992 and Kim et al., 1993.

The mean values of DNA contents (2C) of each species examined using cyprinid loach red blood cell as a standard reference are shown in Table 2. The mean value of DNA content of the saddle weever was 1.50 pg/nucleus. The mean values of DNA contents of marbled rockfish and marbled sole were 1.31 pg/nucleus and 1.03 pg/nucleus, respectively.

DISCUSSION

Chromosome numbers and variabilities in chromosome number distinguish certain major taxonomic groupings of fishes (Gold, 1979; Park et al., 1995, 1997, 1999; Park and Lee, 1996). To the best of our knowledge this is the first report on the karyotype of *Parapercis sexfasciata* (Temminck et Schlegel). The observation in the current study of a chromosome breakage at the long arm of one acrocentric in *P. sexfasciata* phenomenon worthy of further study.

The diploid chromosome number and kayotype of *Sebastiscus marmoratus* in the present study were in accordance with findings of Nishikawa et al. (1977). As Fukuoka and Niiyama (1970) have already pointed out, the diploid chromosome number of *Pleuronectes yokohamae* (Günther) is 2n = 48 and its karyotype consists of all telocentrics with the fundamental number of 48.

Evidence has been largely provided stating the DNA nuclear content is statistically constant in all cells exhibiting identical ploidy in various tissues from the same animal or from animals of the same species, whereas it is significantly divergent in different species (Chiarelli and Capanna, 1973). According to this knowledge of the nuclear DNA amount and the karyotype, in a given individual, the correlation logically ensures namely the number and shape of the chromosome characteristics of the species (Lovett et al., 1980; Park et al., 1999; Park et al., 2000; Park et al., 2003).

Considering the present study of the karyotype and DNA content per nucleus of the tested fishes showed 1.50 pg/nucleus in *P. sexfasciata*, 1.31 pg/nucleus in *S. marmoratus* and 1.03 pg/nucleus in *P. yokohamae*, it is concluded 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. These mechanisms likely occurred during the evolution of the karyotypes of *P. sexfasciata*.

For future reference, the application of chromosome banding techniques to *P. sexfassiata* including *P. multifasciata* (Döderlein) of the family Pinguipedidae, is a possible solution to the phenomenon of chromosome breakage, in *P. sexfassiata*. Further evaluation based on a classification and evolutionary approach in the family Pinguipedidae should also be considered.

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