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Karyotypes and Cellular DNA Contents of Three Species of the Subfamily Clupeinae

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Abstract Karyotypes of three species of the subfamily Clupeinae collected from northern Japan were analyzed by in vitro methods and their cellular DNA contents were measured using an integrating microdensitometer. *Sardinella zunasi* and *Sardinops melanostictus* show very similar karyotypes: 2n = 48, consisting of acrocentric or subtelocentric chromosomes with a gradual decrease in chromosome size, but with differences in cellular DNA of 2.32 and 2.69 pg/cell respectively. *Clupea pallasii* differs from the aforementioned species in karyotype: 2n = 52, consisting of 6 metacentric or submetacentric chromosomes and 46 acrocentric or subtelocentric chromosomes, with a cellular DNA content of 1.96 pg/cell. The results showed two different modes in karyological evolution within the subfamily Clupeinae, i.e. an increase of cellular DNA content without apparent change in karyotype, as shown by *Sardinella zunasi* and *Sardinops melanostictus*, and less change in cellular DNA content but with marked change in karyotype, as shown by *Clupea pallasii.*

The family Clupeidae comprises about 180 species which are divided into five subfamilies. Information on the karyotype and DNA content of the family has been reported for no more than 19 species of three subfamilies (Ohno and Atkin, 1966; Roberts, 1966; Ohno et al., 1968; Mayers and Roberts, 1969; Hinegardner and Rosen, 1972; Rishi, 1973; Skvortsova, 1975; Krysanov, 1978; Khuda-Bukhsh, 1979; Vasil'yev, 1980; Fitzsimons and Doucette, 1981; Doucette and Fitzsimons, 1988). However, most of these studies are descriptions of the karyotypes, and the evolutionary pattern estimated from karyological modification of the family has yet to be studied.

We analyzed karyotypes and DNA contents of three species of the subfamily Clupeinae, i.e., *Sardinella zunasi*, *Sardinops melanostictus* and *Clupea pallasii.* The former two species are studied for the first time and the latter species is reported for the first time from Japanese waters. Details of their karyological aspects are described below and evolutionary patterns in the subfamily are estimated from the data of karyotypes and DNA contents so far reported.

Materials and methods

Fish specimens used for this study were collected from Tokyo Bay, Tokyo and Okirai Bay, Sanriku-cho, Iwate Prefecture. *Clupea pallasii* were one year old offspring from parents collected off Ibaraki Prefecture. Details of the material are shown in Table 1.

For chromosomal study, a short term tissue culture method was adopted. Gill filaments removed from the right side gill arch of live fish were incubated in minimum essential medium (MEM) solution with 0.1–0.2 mcg/ml colcemid, for 2–6 hours at 15–20℃. After incubation, the gill tissues were treated with hypotonic solution (0.075 M KCl) for about 1 hour and then fixed in Carnoy's fixative for at least 1 hour. Chromosome spreads were prepared according to the method described by Ida et al. (1982). Karyotypic data were obtained from photographic negatives. Negatives of chromosome spreads were projected on a NIKON PROFILE PROJECTOR V-12 and the arm lengths of chromosomes were measured to the nearest 0.1 μm by a NIKON DIGITAL COUNTER DP-851. The classification of chromosomes followed Levan et al. (1964). Fundamental number (FN) was established by assigning a value of one to all subtelocentric and acrocentric chromosomes, and a value of two to all metacentric and submetacentric chromosomes. New Arm Number (NAN) was determined following Arai and Nagaiwa (1976). Calculation of the percentage of the length of each chromosome of the total complement length (%TCL) followed Doucette and Fitzsimons (1988) who modified LeGrande's (1975) definition of %TCL. Species identification followed Whitehead (1985).

For the measurement of DNA content, red blood cells obtained from each specimen were stained according to the Feulgen technique (Macgregor and Varjley, 1983), and were measured by an integrating microdensitometer, NIKON VICKERS M-85A. The cellular DNA content was measured relative to that of red blood cells of the common carp, *Cyprinus carpio.*

Results

The distribution of chromosome counts obtained for the three species is given in Table 2.

There was no difference in karyotype between males and females in all species. Chromosomal counts below the modal numbers are probably attributable to a loss of chromosomes during preparation.

The details of karyotype for each species are described below.

Sardinella zunasi (Fig. 1A): The sharp modal count of 2N=48 indicated the diploid number for this species. The karyotype was composed of 48 acrocentric or subtelocentric chromosomes, and both the fundamental number and new arm number were 48 (Table 3). The size of chromosomes decreased gradually. The %TCL ranged from 1.3 to 3.0, with a standard deviation of 0.37 (Table 3). The distribution of %TCL is shown in Fig. 2A. The DNA content of this species was 2.32 pg/cell (Table 3), with a standard deviation of 0.43.

Sardinops melanostictus (Fig. 1B): The sharp modal count of 2N=48 indicated the diploid number for this species. The karyotype was composed of 48 acrocentric or subtelocentric chromosomes, and both the fundamental number and new arm number were 48 (Table 3). The size of chromosomes decreased gradually. The %TCL ranged from 1.3 to 3.0, with a standard deviation of 0.34 (Table 3). The distribution of %TCL is shown in Fig. 2B, showing

Table 1. List of specimens used for studies on chromosome and DNA content of three species of the subfamily Clupeinae.

Species	Date	Locality	Method	Sex	No. of specimens	SL (mm)
Sardinella zunasi	1988-10-15	Tokyo Bay	Angling	male	5	94–128
				female	5	
Sardinops melanostictus	1988- 8-31	Okirai Bay, Sanriku-cho	Set-net	male	1	136–157
				female	4	
Clupea pallasii	1988-11-24	Japan Sea-farming Association Miyako Station	Cultured (Off Ibaraki)	male	3	91–123
				female	3	

Table 2. Frequency distributions of diploid chromosome numbers of three species of the subfamily Clupeinae.

Species	2n																									Total number of cells observed
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	
Sardinella zunasi							1		1			1	2					1	1		1	1			5					14
Sardinops melanostictus																	1						1		7					9
Clupea pallasii		1		1								1					2					1			2		5	1	13	27

Table 3. Karyotypes, DNA content and ranges of %TCL of three species of the subfamily Clupeinae.

Species	2n	M-SM	ST-A	FN	NAN	%TCL range	Standard deviation of %TCL	DNA (pg/cell)
Sardinella zunasi	48	0	48	48	48	1.3–3.0	0.37	2.32
Sardinops melanostictus	48	0	48	48	48	1.3–3.0	0.34	2.69
Clupea pallasii	52	6	46	58	48–50	0.5–2.8	0.42	1.92

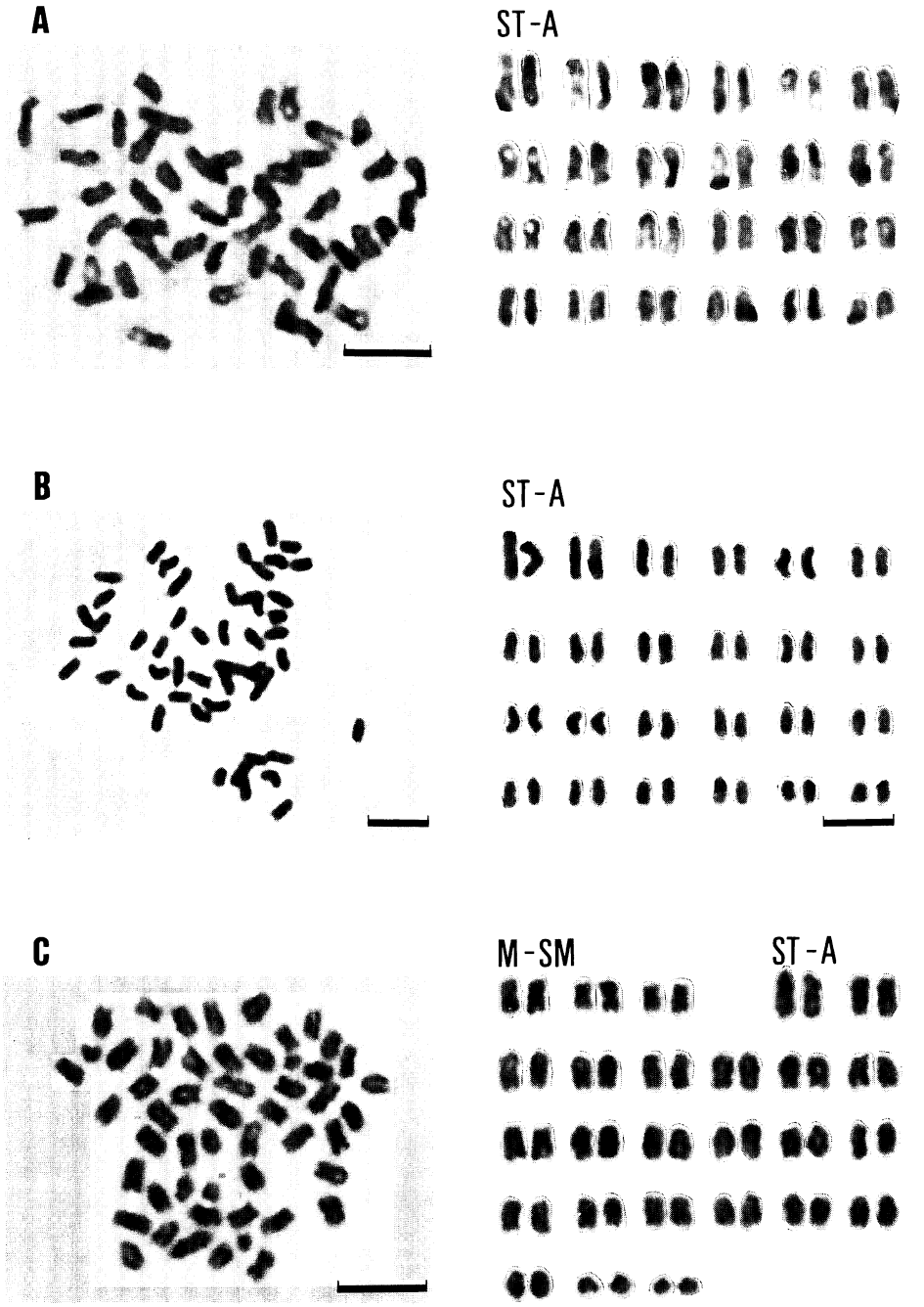

Fig. 1. Photographs of mitotic metaphase chromosomes and their karyotypes. A: *Sardinella zunasi*, 2n=48. B: *Sardinops melanostictus*, 2n=48. C: *Clupea pallasii*, 2n=52. Each scale indicates 10 μm.

a closely similar patern to *Sardinella zunasi*. The DNA content of this species was 2.69 pg/cell (Table 3), with a standard deviation of 0.44.

Clupea pallasii (Fig. 1C): The sharp modal count of 2N=52 indicated the diploid number for this species. The karyotype was composed of six metacentric or submetacentric chromosomes and 46 acrocentric or subtelocentric chromosomes. There were

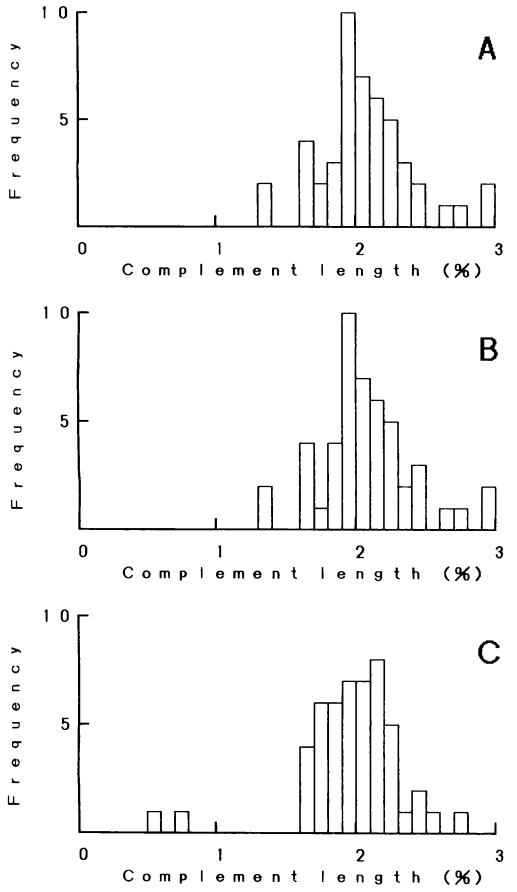

Fig. 2. Frequency diagrams showing the distribution of %TCL in the chromosomes of three species; A: *Sardinella zunasi*, B: *Sardinops melanostictus* and C: *Clupea pallasii*. %TCL is the percent of each chromosome contributed to the total length of the complement (Doucette and Fitzsimons, 1988).

two pairs of acrocentric chromosomes that were smaller than the other chromosomes. The fundamental number was 58 and the new arm number 48–50 (Table 3). The %TCL ranged from 0.5–2.8, with a standard deviation of 0.42 (Table 3). The distribution of %TCL, shown in Fig. 2C, differed from the former two species. The DNA content of this species was 1.92 pg/cell (Table 3), with a standard deviation of 0.23.

Discussion

In the family Clupeidae, 13 species have been analyzed karyologically (Table 4). Of these, six species belong to the subfamily Alosinae (Mayers and Roberts, 1969; Khuda-Bukhsh, 1979; Vasil'yev, 1980; Doucette and Fitzsimons, 1988), two species to the subfamily Dorosomatinae (Fitzsmons and Doucette, 1981) and five species to the subfamily Clupeinae (Roberts, 1966; Rishi, 1973; Skvortsova, 1975; Krysanov, 1978). We added the karyotypes of three species in the subfamily Clupeinae, i.e. *Sardinella zunasi*, *Sardinops melanostictus* and *Clupea pallasii*.

According to Doucette and Fitzsimons (1988), it has been supposed that the ancestral chromosome form of the family Clupeidae had 48 acrocentric chromosomes.

The karyotypes of *Sardinella zunasi* and *Sardinops melanostictus* consisted of 48 acrocentric or subtelocentric chromosomes and there were no peculiar chromosomes, either in size or shape. The grouping of these chromosomes is virtually impossible because of their smooth gradation in size. The ranges of %TCL in the two species were both 1.3–3.0, and distribution patterns of %TCL were very similar (Fig. 2A, B). It seems that these two species have retained their ancestral chromosomal structure.

C. pallasii exhibited a derived feature in having 52 rather than 48 chromosomes. The result of this study is in accordance with Krysanov (1975). The karyotype contained six metacentric or submetacentric, and 46 subtelocentric or acrocentric chromosomes. The six metacentric or submetacentric chromosomes probably resulted from pericentric inversion in each chromosome, because their sizes were about equal to the average size. Judging from their marked differences in size, the four small, acrocentric or subtelocentric chromosomes seem to have resulted from centric fission of one pair of small-sized metacentric or two pairs of submetacentric or subtelocentric chromosomes, because the resultant chromosome sizes are smaller than half of the average chromosome size (Fig. 2C). Based on these assumptions, the ancestral chromosome number of *C. pallasii* was estimated to be 2n=48 or 50.

With regard to cellular DNA content analyses of the family Clupeidae, there are two reports dealing with four species (three species of the genus *Alosa* and *Clupea harengus pallasii* (Table 4)). The DNA content of these species ranged from 1.54 to 2.8 pg/cell.

Among the three species treated here, *Sardinella zunasi* has a DNA content value of 2.32, *Sardinops*

melanostictus 2.69 and *C. pallasii* 1.92 pg/cell. Ohno and Atkin (1966) reported the haploid value for the last-mentioned species as 0.77 pg (=1.54 pg/cell). The reason for the difference between Ohno and Atkin (1966) and the present study is unknown.

Sardinella zunasi and *Sardinops melanostictus* do not seem to vary in karyotype from the ancestral form, but have an apparently larger DNA content than *C. pallasii.* Whereas in *C. pallasii*, considerable changes seem to have occurred in the karyotype, compared with the ancestral form, but there is less variation in the DNA content. The difference in DNA content between *Sardinella zunasi* and *Sardinops melanostictus* does not seem small. Ohno (1970) suggested that DNA molecules increase either by means of unequal exchange, unequal crossing-over or regional redundant duplication. Of these three possibilities, only regional redundant duplication increases DNA content without a change in karyotype. As mentioned earlier, the karyotypes of *Sardinella zunasi* and *Sardinops melanostictus* are very similar. Thus, differences in DNA content between *Sardinella zunasi* and *Sardinops melanostictus* may be caused by regional redundant duplication on all chromosomes.

It may be concluded that there are at least two different patterns of karyological evolution among fishes of the subfamily Clupeinae, i.e. (1) marked change in chromosomal shape and number without marked increase of DNA content, which is shown by *C. pallasii*, and (2) less change in the relative chromosomal shape with marked increase in DNA amount, which is shown by *Sardinella zunasi* and *Sardinops melanostictus.*

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Table 4. Karyological data of the family Clupeidae.

Taxon	2n	FN	NAN	%TCL range	DNA (pg/cell)	Reference
Clupeinae						
Sardinella melanura	44	52				Rishi, 1973
S. zunasi	48	48	48	1.3–3.0	2.32	Present study
Sardinops melanostictus	48	48	48	1.3–3.0	2.69	Present study
Clupea harengus pallasii	52	60			1.54	Krysanov, 1978; Ohno et al., 1968;
	52	58	48–50	0.5–2.8	1.92	Present study
C. harengus pallasii n. *maris-albi*	52	60				Skvortsova, 1975
C. harengus harengus	52–54	66?				Roberts, 1966; Skvortsova, 1975
C. harengus harengus n. *membras*	54	66–70				Skvortsova, 1975
Harengula clupeola	28	52		1.6–5.1		Doucette and Fitzsimons, 1988
Alosinae						
Alosa pseudoharengus	48	48	48			Mayers and Roberts, 1969;
					2.8	Hinegardner and Rosen, 1972
A. kessleri	48	48	48			Vasil'yev, 1980
A. chrysochloris					2.2	Hinegardner and Rosen, 1972
A. sapidissima					2.6	Hinegardner and Rosen, 1972
Brevoortia patronus	46	50	48	1.6–5.4		Doucette and Fitzsimons, 1988
B. smithi	46	50	48	1.5–4.8		Doucette and Fitzsimons, 1988
B. tyrannus	46	50	48	1.4–5.7		Doucette and Fitzsimons, 1988
Gudusia chapra	46	46				Khuda-Bukhsh, 1979
Dorosomatinae						
Dorosoma cepedianum	48	50	48			Fitzsimons and Doucette, 1981
D. pelenense	48	50	48			Fitzsimons and Doucette, 1981

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ニシン亜科3種の核型

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ニシン亜科3種の染色体を air-drying 法により分析し，DNA 量を顕微分光濃度計を用いて測定した．サッパとマイワシの核型は 2n＝48，24 対の次端部—端部着糸型染色体 (ST-A) のみから構成され，ニシンの核型は 2n＝52，6 対の中部—次中部着糸型染色体 (M-SM) と 46 対の ST-A から構成されていた．DNA 量はサッパ 2.32 pg/cell，マイワシ 2.69 pg/cell，ニシン 1.92 pg/cell であった．これら 3 種の核型及び DNA 量より，祖先形から推測される変異において，DNA 量の変化を持つ種(サッパ，マイワシ)と染色体の数と形態の変化を持つ種(ニシン)があった．以上のことから，ニシン亜科内において少なくとも 2 つの進化の方向性があることが示唆された．

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