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Karyotypes and Cellular DNA Contents of Two Sharks in the Family Scyliorhinidae

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Recently, some cytotaxonomic and immunological studies of sharks, skates and rays have been reported (Stingo, 1979; Stingo and Capriglione, 1986; Schwartz and Maddock, 1986; Tomonaga et al., 1984, 1985; Davies et al., 1986), adding some clues for clarification of the phylogenetic relationships of elasmobranchs. Out of these, scyliorhinid sharks were dealt with in two papers, Stingo (1979) and Davies et al. (1986). Stingo (1979) reported the karyotypes and genome sizes of two sharks, *Scyliorhinus canicula* and *S. stellaris*. *S. canicula* was analyzed by Davies et al. (1986) from the immunological aspects.

Adding to these reports, some revisional studies on the morphology of elasmobranchs have been published, offering better understandings on the classification of the group. But studies on scyliorhinid sharks are very few. For the Japanese scyliorhinid sharks, or the cat sharks, Nakaya (1975) discussed the phyletic relationships of the group based on comparative anatomy and reproductive system.

We examined karyotypes and cellular DNA contents of the Japanese swell shark and cat shark in order to get information for the further understandings of the phyletic relationships of the group.

Materials and methods

Materials used in the present study are shown in Table 1. The cellular DNA content was measured as the relative DNA values determined in comparison with red blood cells of the common carp, *Cyprinus carpio*, using a scanning microspectrophotometer. Blood samples were stained according to Feulgen's technique (Macgregor and Varjley, 1983). For the preparation of chromosomes, the routine air-drying or in-vitro methods (Ida et al., 1978) were used.

Details of the preparation of chromosomes are as follows.

Colchicine treatment. a) In vivo: Samples were injected with colchicine at a concentration of 15 to 30 μg/g body weight. About 12 to 24 hours after the injection, the specimens were sacrificed and the tissues of gill, kidney, intestine, spleen and gonad were removed. b) In vitro: After removing the tissues from the body, they were washed with sea water or isotonic water, soaked in isotonic incubating medium or minimum essential medium with colchicine at concentrations of 1 to 3 μg/m*l* for 12 to 24 hours at 15°C to 20°C.

Hypotonic treatment and fixation. The tissues were treated for 60 to 120 minutes with hypotonic 0.075 M KCl solution or distilled water and then fixed in Carnoy's fixative for at least 60 minutes.

Preparation and staining. The cell suspension with Carnoy's fixative was dropped and spread over the entire slide. Then the preparation was stained with Giemsa solution diluted 20 times by a phosphate buffer (pH 6.8).

Classification of chromosomes followed Levan et al. (1964). Meta- and submetacentrics are described as two-arm chromosomes, and subtelocentrics and acrocentrics as one-arm chromosomes.

Counts for the vertebrae were based on X-ray photographs. Twenty samples, 13 specimens of *Cephaloscyllium umbratile* collected from Suruga Bay and 7 specimens of *Scyliorhinus torazame* from Shimokita, were used for meristic comparison.

Results

Cephaloscyllium umbratile: Four specimens were available for chromosome observations. Good chromosome spreads were obtained from the gill tissues. The diploid chromosome number

Table 1. List of the materials for chromosome (C) and genome size (G) study.

Species	Date	Locality	TL (mm)	BL (mm)	BW (g)	Sex	Usage
Cephaloscyllium umbratile	86-2-5	Suruga Bay	264	191	49	male	G
	86-5-7	Suruga Bay	194	150	28	male	C
Scyliorhinus torazame	86-11-12	Shimokita	431	341	327	female	G
	86-11-28	Shimokita	436	334	322	male	C

was 64 (Table 2). The karyotype consisted of 34 meta- or submetacentric and 30 subtelocentric or acrocentric chromosomes (Fig. 1A). The fundamental number was 98. Similar DNA values of 14.5 and 15.0 pg/cell were obtained for the swell shark (Table 3). The genome size was thus determined as 14.7 pg/cell. Mode of monospondylous vertebrae number was 50 ranging between 48 and 51. Percentage of monospondylous vertebrae in comparison with that of the total was 41.7% (Table 4).

Scyliorhinus torazame: Two specimens were

Table 2. Frequency distribution of chromosome counts for two sharks of the family Scyliorhinidae.

Species (tissues)	Chromosome count												Number of cells observed
	<30	31	32	33	34	<60	61	62	63	64	65	66	
Cephaloscyllium umbratile													
(gills and shell gland)						5	0	0	1	6	1	1	14
Scyliorhinus torazame													
(testis)	9	1	7	1	2								20
(spleen)						4	0	0	1	3	1	1	10

Table 3. Cellular DNA content of two sharks of the family Scyliorhinidae.

Species	Cells observed	Arbitrary DNA unit	Standard error	Standard deviation	Relative DNA unit	Absolute DNA pg/cell
Cephaloscyllium umbratile	50	115.5	0.180	2.979	4.251	14.5
Cyprinus carpio	45	27.17	0.158	1.061	1.0	3.4
Cephaloscyllium umbratile	100	89.65	0.196	1.959	4.401	15.0
Cyprinus carpio	100	20.37	0.039	0.386	1.0	3.4
Scyliorhinus torazame	100	130.5	0.417	4.170	4.044	13.7
Cyprinus carpio	100	32.27	0.115	1.147	1.0	3.4
Scyliorhinus torazame	100	133.9	0.413	4.132	3.74	12.7
Cyprinus carpio	100	35.77	0.097	0.966	1.0	3.4

Table 4. Vertebral number of two sharks of the family Scyliorhinidae. Figures show the modal counts and those in parentheses the ranges.

Species	Vertebrae		Total	Percentage of mono-spondylous vertebrae	Number of specimens observed
	Mono-spondylous	Diplo-spondylous			
Cephaloscyllium umbratile	50	70	120	41.7 (%)	13
	(48–51)	(69–72)	(118–122)	(41.0–42.5)	
Scyliorhinus torazame	36	79	115	31.5 (%)	7
	(35–38)	(77–82)	(112–119)	(30.7–32.5)	

Table 5. Karyotypes and cellular DNA contents of scyliorhinid sharks. M-SM, meta-submetacentrics; ST-A, subtelo-acrocentrics; FN, fundamental number. * Stingo et al. (1980).

Species	2n	M-SM	ST-A	FN	DNA (pg/cell)	Reference
Cephaloscyllium umbratile	64	34	30	98	14.7	present study
C. uter					15.4	Hinegardner (1976)
C. ventriosum	64	46	18	100	18.1	Schwartz and Maddock (1987)
Scyliorhinus torazame	64	26	38	90	13.2	present study
S. canicula	62	42	20	104	11.3*	Stingo (1979)
S. stellaris	72	50	22	122	12.3*	Stingo (1979)
Galeus eastmani					11.0	unpublished
G. nipponensis					11.1	unpublished

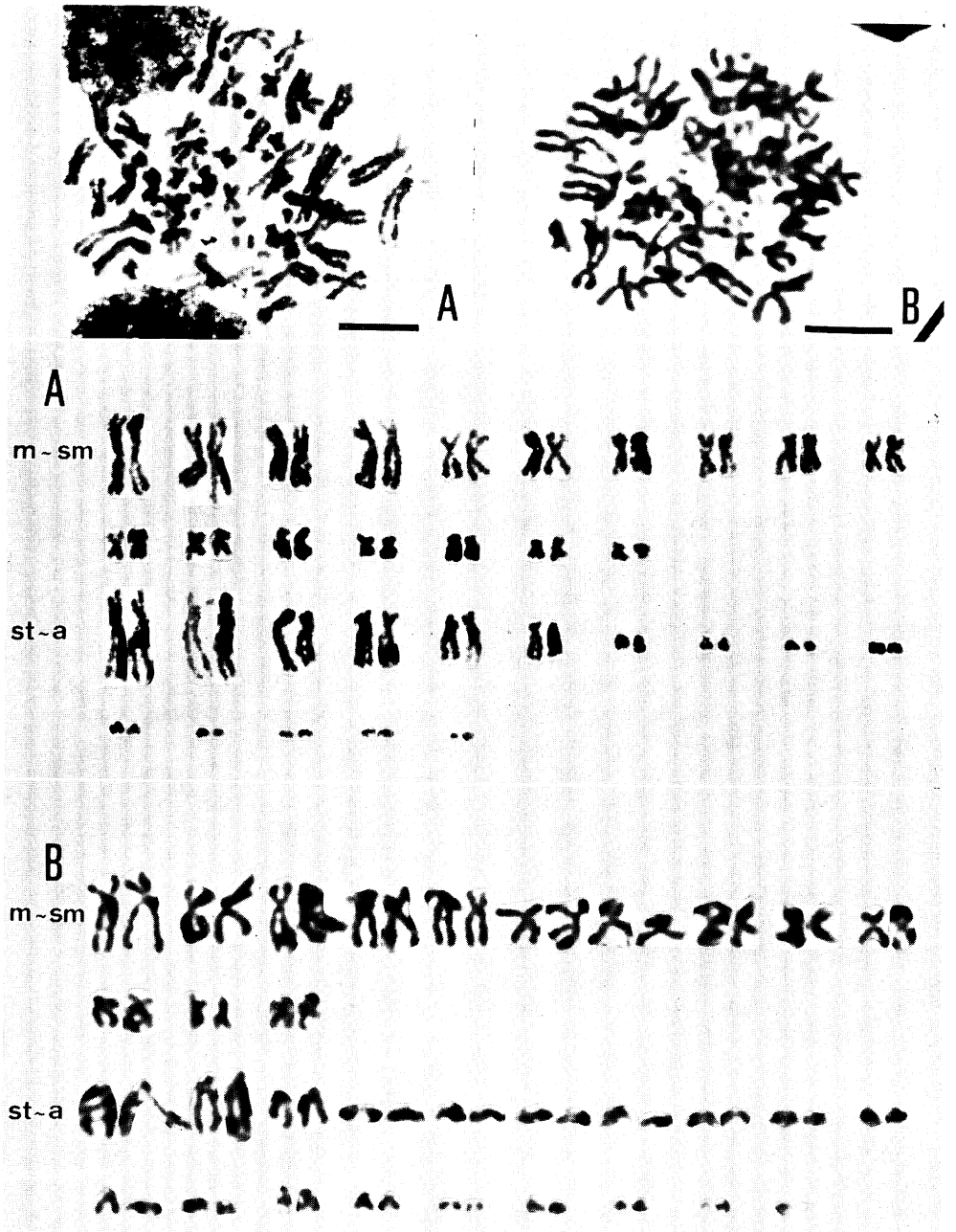

Fig. 1. A photomicrograph of metaphase and its karyogram of two scyliorhinid sharks. A: *Cephaloscyllium umbratile*, 2n=64. The karyotype is composed of 34 meta- or submetacentric (m~sm) and 30 subtelocentric or acrocentric (st~a) chromosomes. B: *Scyliorhinus torazame*, 2n=64. The karyotype is composed of 26 meta- or submetacentric (m~sm) and 38 subtelocentric or acrocentric (st~a) chromosomes. Each scale indicates 10 μm.

available for chromosome observations. Good chromosome spreads were obtained from the tissue of spleen. The modal chromosome counts of the cat shark testis and spleen preparations were 32 and 64, respectively (Table 2). Thus the diploid chromosome number was determined as 64. The karyotype consisted of 26 meta- or submetacentric and 38 subtelocentric or acrocentric chromosomes (Fig. 1B). The fundamental number was 90. DNA measurements for the cat shark are slightly different between the two smear samples, 12.7 and 13.7 pg/cell (Table 3). The genome size was thus determined as 13.2 pg/cell. Mode of monospondylous vertebrae number was 36 with the range between 35 and 38. Percentage of monospondylous vertebrae in comparison with that of the total was 31.5% (Table 4).

Discussion

About 90 species of the scyliorhinid sharks of the world are recognized, but only about 10% were studied on their karyotypes and DNA contents. The results of these studies are summarized in Table 5. The genome size ranges between 11.0 and 18.1 pg/cell in scyliorhinid sharks. These values are high for the members of galeomorph sharks. The genome sizes of species of the genus *Cephaloscyllium* are large, about 15 to 18 pg/cell, in comparison with those of genera *Scyliorhinus* (about 11 to 13 pg/cell) and *Galeus* (about 11 pg/cell, unpublished). Adding to this feature, there exist much difference in chromosome sizes between smaller acrocentrics and larger meta- or submetacentrics in these species. The larger sized chromosomes are about 16 to 18 times and about 8 to 10 times as large as the smaller sized ones in *Cephaloscyllium umbratile* and in *Scyliorhinus torazame*, respectively. Most chromosome sizes in galeomorph sharks vary between about 2 and 8 μm. But in *Cephaloscyllium umbratile*, chromosome size ranges between about 0.7 and 12 μm. Differential intensity of the Giemsa staining along with the arms and larger sizes of metacentric and acrocentric chromosomes in swell shark and cat shark seem to originate from structural modification such as tandem fusions among these chromosomes. Furthermore, *Cephaloscyllium umbratile* and *Scyliorhinus torazame* have some microchromosomes. These karyotypic features show a similar tendency with those of primitive sharks, e.g., Heterodontiformes and Hexanchiformes (Schwartz and Maddock, 1986; Ida et al., 1986). Asahida et al. (1987) reported that the morphological specialization of myliobatid rays seemed to be related with karyological specialization. Numbers of vertebrae of swell shark and cat shark were examined for meristic comparison (Table 4). Though there exists slight difference in the relative composition of vertebrae between the two species (*Cephaloscyllium umbratile* has higher counts of monospondylous but smaller counts of diplospondylous vertebrae than *Scyliorhinus torazame*, vertebrae totaling about 120 in both species), the polarity of variation in vertebral composition could not be determined because of scarce information.

Nakaya (1975) stated the primitiveness of the swell shark and cat shark in scyliorhinid and carcharhinid groups based on comparison with chondrocranium and vertebral calcification. Moreover, Nakaya emphasized that the genus *Cephaloscyllium* is regarded as the most primitive form among Japanese scyliorhinid sharks and the genus *Scyliorhinus* may be a little more advanced group. Supposing that the swell shark and cat shark are the representatives of the primitive galeomorphs, it may be said that scyliorhinid sharks which are primitive or have generalized features in morphology have much differences in chromosome size which is expressed as "asymmetrical" of Morescalchi (1977), higher DNA content and microchromosomes.

Ohno (1970) stated as follows: "Unlimited increase of the genome size exclusively by tandem duplication is a hopeless proposition in that it is the best way to conserve and the worst way to change. Lungfish and salamanders clearly show the tragic consequences of exclusive dependence upon tandem duplication as the means of achieving gene duplication. No matter how many copies of the structural cistron they might have acquired by repeated tandem duplication, as long as tandem duplicates remain under the control of a single regulatory gene locus, they can only serve to produce more of the same gene products as do the multiple copies of the gene for 18S and 28S *ribosomal* RNA which occupy the nucleolar organizing region."

Results of the present study fit his explanation quite well, i.e. larger genome size shows less difference in chromosome number and much difference in chromosome size.

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日本産トラザメ科魚類2種の核型および DNA 量

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日本産トラザメ科魚類2種の核型を 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．ナヌカザメの核型は 2n=64，中部—次中部着糸型染色体 (M–SM)=34，次端部—端部着糸型染色体 (ST–A)=30，腕数 (FN)=98，DNA 量=14.7 pg/cell であり，トラザメでは 2n=64，M–SM=26，ST–A=38，FN=90，DNA 量=13.2 pg/cell であった．この2種の核型及び DNA 量は，より基本的とされる板鰓類（ラブカ，ネコザメなど）に似た特徴を示し，Galeomorphii の中では基本群に含まれると判断された．

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