Karyological Notes on Four Sharks in the Order Carcharhiniformes

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The order Carcharhiniformes is one of the most prosperous groups in sharks and their way of life and distribution show rather wide variation among the species. This large group contains about 55% of all the shark species, and is divided here into 8 families. Some authors reported systematic relationships in their studies on morphological and ecological features. Adding to these studies, some cytotaxonomical studies have been published in reference to their phyletic relationships (Stingo, 1979; Schwartz and Maddock, 1986). In the present paper, the results of two karyotypes and four cellular DNA contents of carcharhiniform sharks are reported for comparison among three families in the order Carcharhiniformes. Their systematic relationships are discussed from these karyotypes and cellular DNA contents.

Materials and methods

Materials used in the present study are listed in Table 1. The cellular DNA content was measured as the relative DNA values of red blood cells of objective species in comparison with the value of the common carp, *Cyprinus carpio* (3.4 pg/cell: Hinegardner and Rosen, 1972) 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 method or in-vitro method (Ida et al., 1978) was used.

Details of the preparation of chromosomes are as follows.

Colchicine treatment: a) In vivo.—The 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 the gill, kindey and intestine were removed. b) In vitro.—After removing the tissues from the body, they were washed with sea or isotonic water and soaked in isotonic incubating or minimum essential medium with colchicine at concentrations of 1 to 3 μg/ml 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 0.075 M KCl solution or distilled water and then fixed with Carnoy's fixative for at least 60 minutes.

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

Classification of the chromosomes followed

Table 1. List of the materials for chromosome study (C) and cellular DNA content analysis (D).

Species	Date	Locality	Sex	T. L. (mm)	B. W. (g)	Usage
Mustelus manazo	29, Jun.'83	Tateyama	female	600	600	C
	31, May'86	Tateyama	female	450	250	D
Triakis scyllia	9, Jun.'80	Misaki	male	483	300	C
	29, May'86	Tateyama	female	1,288	12,000	D
Galeus eastmani	8, Apr.'86	Suruga Bay	female	405	210	D
Galeus nipponensis	5, Feb.'86	Suruga Bay	female	490	280	D

Table 2. Frequency distribution of chromosome counts in the material fishes.

Species	Chromosome count									Number of cells observed
	<63	64	65	66	67	68	70	72	74<	
Mustelus manazo (intestine and kidney)	11	2	3	0	0	9	0	3	1	29
Triakis scyllia (kidney)	7	1	0	2	1	3	2	7	3	26

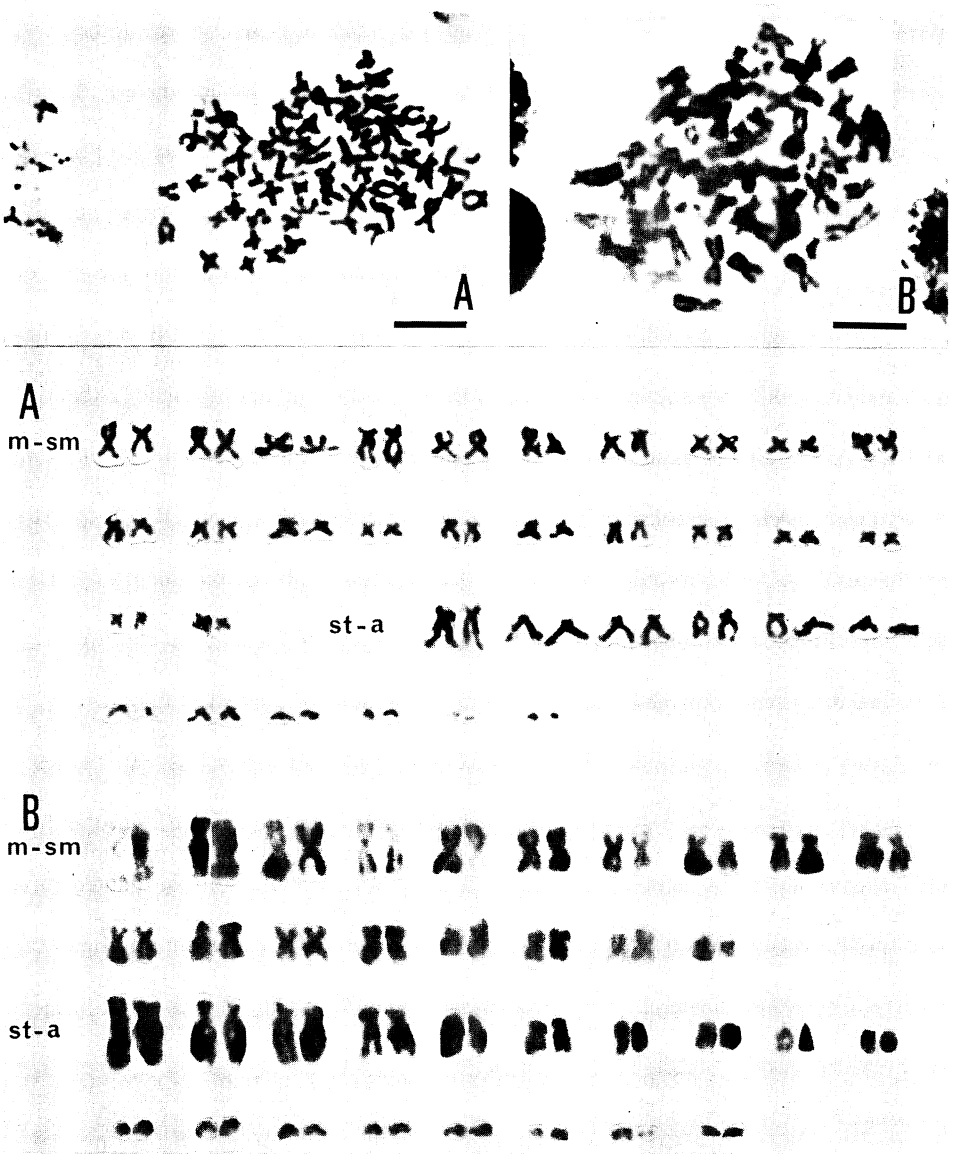

Fig. 1. A: A photomicrograph of metaphase and a karyogram of *Mustelus manazo*, 2n=68. The karyotype comprises 44 meta- or submetacentric (M, SM) and 24 subtelocentric or acrocentric (ST, A) chromosomes. Scale indicates 10 μm. B: A photomicrograph of metaphase and a karyogram of *Triakis scyllia*, 2n=72. The karyotype comprises 36 meta- or submetacentric (M, SM) and 36 subtelocentric or acrocentric (ST, A) chromosomes. Scale indicates 10 μm.

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

Results

Mustelus manazo: Two specimens were available for chromosome observations. Good chromosome spreads were obtained from the tissues of the kidney and intestine. The diploid chromo-

Table 3. DNA measurements for the material fish. * As control.

Species	Cells observed	Arbitrary DNA unit	Standard error	Standard deviation	Relative DNA unit	Absolute DNA pg/cell
Mustelus manazo	96	67.19	0.180	1.766	2.759	9.4
*Cyprinus carpio**	100	24.35	0.063	0.627	1.0	3.4
Mustelus manazo	100	75.07	0.260	2.600	2.682	9.1
Triakis scyllia	74	80.50	0.374	3.218	2.876	9.8
*Cyprinus carpio**	100	27.99	0.109	1.088	1.0	3.4
Galeus eastmani	100	63.69	0.102	1.015	3.127	10.6
*Cyprinus carpio**	100	20.37	0.039	0.386	1.0	3.4
Galeus eastmani	98	81.83	0.149	1.484	3.361	11.4
*Cyprinus carpio**	100	24.35	0.063	0.627	1.0	3.4
Galeus nipponensis	111	13.30	0.052	0.550	3.364	11.4
*Cyprinus carpio**	104	3.952	0.028	0.288	1.0	3.4
Galeus nipponensis	107	13.86	0.134	1.390	3.190	10.9
*Cyprinus carpio**	61	4.380	0.024	0.190	1.0	3.4

Table 4. Karyotypes and cellular DNA contents in three families in the order Carcharhiniformes. [a] Stingo et al. (1980); [b] Hinegardner (1976). MC, microchromosomes. As defined by Dingerkus (1979), microchromosomes are small, usually less than 0.5 μ in the greatest dimension, and without discernible centromere or chromosome arms. Because of its size, the precise counts cannot be determined.

Species	2n	M-SM	ST-A	FN	MC	DNA (pg/cell)	Reference
Family Scyliorhinidae							
Cephaloscyllium umbratile	64	34	30	98	2	14.7	Asahida et al., 1988
C. uter						15.4	Hinegardner, 1976
C. ventriosum	64	46	18	110	2	18.1	Schwartz and Maddock, 1986
Scyliorhinus torazame	64	26	38	90	4	13.2	Asahida et al., 1988
S. canicula	62	42	20	104	2	11.3[a]	Stingo, 1979
S. stellaris	72	50	22	122	4	12.3[a]	Stingo, 1979
Galeus eastmani						11.0	present study
G. nipponensis						11.1	present study
Family Triakididae							
Galeorhinus galeus						17.3	Stingo et al., 1980
Mustelus manazo	68	44	24	112	0	9.3	present study
M. canis						9.2	Hinegardner, 1976
M. californicus						12.8	Hinegardner, 1976
M. norrisi						9.0	Hinegardner, 1976
M. sp.						9.6	Hinegardner, 1976
Triakis scyllia	72	36	36	108	0	9.8	present study
T. semifasciata	70–72	52	18–20	122–124	0	9.6[b]	Schwartz and Maddock, 1986
Family Carcharhinidae							
Carcharhinus longimanus						6.7	Mirsky and Ris, 1951
C. obscurus						5.5	Mirsky and Ris, 1951
C. limbatus	ca. 80	ca. 30	ca. 50	ca. 110	0	7.8(7.4[b])	Schwartz and Maddock, 1986
C. acronatus	84	32	52	116	0	7.3(6.8[b])	Schwartz and Maddock, 1986
Galeocerdo cuvier	86	38	48	124	0	8.3	Schwartz and Maddock, 1986
Rhizoprionodon terraenovae	ca. 80	ca. 44	ca. 36	ca. 124	0	7.2	Schwartz and Maddock, 1986
Prionace glauca	78	ca. 28	ca. 50	ca. 106	0	8.6(8.6[b])	Asahida et al., unpublished
Negaprion brevirostris						7.4	Hinegardner, 1976

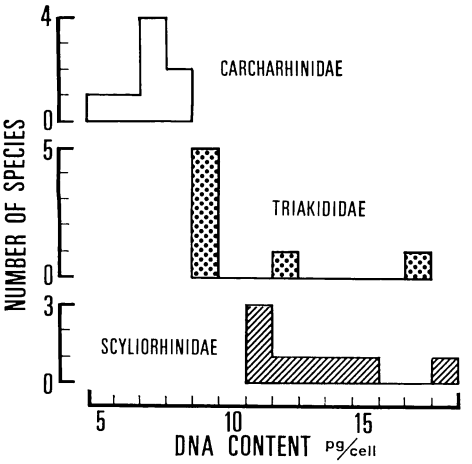

Fig. 2. Frequency diagrams showing the distribution of DNA contents of three families of carcharhiniform sharks so far reported. Value of *Mustelus* sp. (Hinegardner, 1976) is not included.

some number was determined as 68 (Table 2). The karyotype consisted of 44 meta- or submetacentric (M-SM) chromosomes and 24 subtelocentric or acrocentric (ST-A) chromosomes (Fig. 1A). The fundamental number was 112. The DNA values ranged from 9.1 to 9.4 pg/cell (Table 3), and the value for this species was determined as 9.3 pg/cell (Table 4).

Triakis scyllia: Two specimens were available for chromosome observations. Good chromosome spreads were obtained from the tissue of the kidney. The diploid chromosome number was determined as 72 (Table 2). The karyotype consisted of 36 meta- or submetacentric chromosomes and 36 subtelocentric or acrocentric chromosomes (Fig. 1B). The fundamental number was 108. The DNA value was determined as 9.8 pg/cell (Table 3).

Galeus eastmani: DNA measurements were slightly different between the two smear samples, 10.6 and 11.4 pg/cell (Table 3). The DNA value was thus determined as 11.0 pg/cell.

Galeus nipponensis: Simillar DNA values of 10.9 and 11.4 pg/cell were obtained (Tabl 3). The DNA value was thus determined as 11.1 pg/cell.

Discussion

Karyotypes and cellular DNA contents of the family Triakididae and other carcharhiniform sharks so far reported are summarized in Table 4. This Table shows few results from triakidid sharks. The DNA value ranges between 9.3 and 17.3 pg/cell in the family Triakididae, but most species have values of around 9.5 pg/cell. These values are situated in about the middle portion of the range of the whole carcharhiniform sharks. For example, the family Carcharhinidae show lower DNA values ranging between 5.5 and 8.6 pg/cell, and higher DNA values are shown by the family Scyliorhinidae, ranging from 11.0 to 18.1 pg/cell (Table 4). Adding to this point, the family Triakididae show different karyotypes in comparison with those of the family Scyliorhinidae and Carcharhinidae (Table 4). Particularly, the family Triakididae have large numbers of meta- or submetacentric chromosomes (50 to 70% of 2n) and have no microchromosomes. Difference in size among chromosomes is small in the family Triakididae in comparison with that in the family Scyliorhinidae, and this feature seems to be expressed as "symmetrical" (Morescalchi, 1977; karyotypes consist of chromosomes similar in shape and size). The larger-sized chromosomes are about 4 to 5 times the smallest chromosome in *Mustelus manazo* and about 5 to 6 times that in *Triakis scyllia*.

Asahida et al. (1988) stated that scyliorhinid sharks showing primitive or generalized features in morphology have much difference in the size of chromosomes, which is expressed as "asymmetrical" (Morescalchi, 1977; the karyotypes consist of chromosomes with much difference in size and shape). Their karyotypes are accompanied with microchromosomes and they have a higher DNA content. If "symmetrical" karyotypes are derived from "asymmetrical" ones (Morescalchi, 1977), the family Triakididae seems to be a more advanced group than the family Scyliorhinidae.

In terms of the range of DNA value, the three families of carcharhiniform sharks (Scyliorhinidae: 11.0–18.1 pg/cell; Triakididae: 9.0–17.3 pg/cell; and Carcharhinidae: 5.5–8.6 pg/cell) are rather clearly separated into three groups except for two species of the family Triakididae (Fig. 2). These three families are arranged in the declining order of Scyliorhinidae, Triakididae and Carcharhinidae with regard to their amounts of DNA. Hinegardner (1976) stated that high DNA contents are often found in species that represent lineages which have evolved slowly over long periods of

time, and these are considered to be generalized or primitive organisms. If this explanation is fitted for carcharhiniform sharks, these three families are arranged in the order of Scyliorhinidae, Triakididae and Carcharhinidae with regard to their generalized features. Ohno (1970: 125–129) stated as follows: "Possession of the minimum-sized genome does not indicate so-called "primitiveness." None of the more ancient holocephalian, chondrostean or holostean fish are endowed with such a small genome. Hagfish and lampreys, which represent the most ancient jawless state of vertebrate evolution, are also possessors of rather large genomes.... The trend of more ancient and presumably primitive fish having larger genomes than those which are modern and highly specialized is evident in report of Hinegardner (1968).... It appears probable that as a result of extensive experiments with gene duplication which began as early as Devonian times, various lineages of ancient fish acquired rather large genomes; perhaps between a 40 to 100% value of that of placental mammals. So far as those which depended exclusively upon tandem duplication were concerned, subsequent modernization and progressive specialization have been accompanied by progressive reduction in the degree of genetic redundancy.... The trend for a diminished degree of genetic redundancy with progressive modernization has also been noted in angiosperm species of plants belonging to the genus *Lathyrus*. All 18 species of *Lathyrus* have nearly identical diploid chromosome complements made of 14 metacentric chromosomes, but the genomes of more ancient species contained twice the DNA of that of more modern species (Rees and Hazarika, 1969)." Difference in DNA value among the three genera in the order Carcharhiniformes treated here seems to fit his explanation. Whether large amounts of DNA, such as scyliorhinid sharks have, were accomplished exclusively by tandem duplication or by a combination of tandem duplication and tetraploidy cannot be resolved at the moment.

Nakaya (1975) proposed a scheme of relationships of Scyliorhinid-Carcharhinid line on the basis of some morphological characters (e.g., vertebral calcification, orbital processes, pectoral and dorsal fin, etc.) together with the characters of the reproductive system. According to his scheme, the family scyliorhinidae is situated as most primitive in the order Carcharhiniformes, and the genera *Mustelus* and *Triakis*, both belonging to the family Triakididae, are situated as intermediate between the families Scyliorhinidae and Carcharhinidae. He also stated that the genus *Triakis* is slightly advanced than *Mustelus*, and that the genus *Galeus* may occupy an intermediate position between *Scyliorhinus* and *Halaelurus*.

The order of specialization of these groups proposed by Nakaya (1975) seems to be almost identical with that of karyotypes and cellular DNA contents of these groups in the present study. For the mechanism of accumulation of large amounts of DNA value and the polarity of DNA value and karyotypes in elasmobranchs, a more detailed study by biochemical methods seems to be needed.

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日本産メジロザメ目 4 種の核型および DNA 量

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日本産ドチザメ科魚類 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．また，トラザメ科 2 種の DNA 量も同様に測定した．ホシザメ *Mustelus manazo* の核型は 2n＝68，中部-次中部着糸型染色体 (M-SM)＝44，次端部-端部着糸型染色体 (ST-A)＝24，腕数 (FN)＝112，DNA 量＝9.3 pg/cell であり，ドチザメ *Triakis scyllia* では，2n＝72，M-SM＝36，ST-A＝36，FN＝108，DNA 量＝9.8 pg/cell であった．ヤモリザメ *Galeus eastmani* およびニホンヤモリザメ *Galeus nipponensis* の DNA 量はそれぞれ 11.0，11.1 pg/cell であった．核型と DNA 量の検討の結果，ドチザメ科魚類は，トラザメ科魚類より特化したグループであると判断された．

(022–01 岩手県気仙郡三陸町　北里大学水産学部)