Karyotypes of Two Rays, *Torpedo tokionis* and *Dasyatis matsubarai*, and Their Systematic Relationships

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Karyological studies have been carried out on about 40 species of elasmobranchs (Stingo, 1979; Ida et al., 1985, 1986; Stingo and Capriglione, 1986; Schwartz and Maddock, 1986; Kikuno and Ojima, 1987; Asahida et al., 1987, 1988). Out of them, about 10 species were electric rays (order Torpediniformes) and sting rays (order Myliobatiformes). Phyletic relationships from karyological features on the order Torpediniformes were discussed by Ida et al. (1985) and on the order Myliobatiformes by Asahida et al. (1987).

We examined karyotypes of *Torpedo tokionis* and *Dasyatis matsubarai*, and cellular DNA contents of *D. matsubarai* and *D. violacea*. The results of the present study are described below with comments on the phyletic relationships within these two groups.

Materials and methods

The materials used in the present study are listed in Table 1. The cellular DNA content was measured as the relative DNA value of the red blood cells of objective species in comparison with that of the common carp *Cyprinus carpio* using a scanning microdensitometer. Blood samples were stained according to Feulgen's technique (Macgregor and Varjley, 1983).

The short-term tissue culture method was adopted to prepare metaphase chromosomes and the routine air-drying method with Giemsa staining was used. Details of the short-term tissue culture method are as follows:

After removed from the body, the tissues were rinsed with the culture medium for elasmobranchs. The medium was prepared from modified Eagle's minimum essential medium (Nissui) by adding 4 to 5 g/*l* NaCl, 20 to 25 g/*l* Urea and 30 to 35 m*l*/*l* of Hepes. Then the tissues were incubated in the culture medium with colcemid at final concentrations of 0.2 to 1.0 μg/m*l* for 6 to 12 hours at 15 to 20℃.

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.

Results

Torpedo tokionis: Chromosome spreads were obtained from the tissue of the kidney. The diploid chromosome number was determined as 86 (Table 2). The karyotype consisted of 86 acrocentric chromosomes (Fig. 1A). The fundamental number was 86.

Dasyatis matsubarai: Chromosome spreads were obtained from the tissues of the kidney and spleen. The diploid chromosome number was determined as

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

Species	Date of sampling	Locality	T. L. (mm)	D. L. (mm)	D. W. (mm)	B. W. (g)	Sex	Usage
Torpedo tokionis	14 Mar. 1985	Suruga-Bay	ca. 900	ca. 480	ca. 550	ca. 15000	male	C
Dasyatis matsubarai	12 Nov. 1986	Shimokita	598	264	315	975	male	C, D
D. violacea	31 Jul. 1986	off Niijima	715	336	460	2450	male	D

Table 2. Distribution of chromosome counts for two rays in the present study.

Species	Chromosome count									Number of cells observed
Torpedo tokionis (kidney)	<80	82	84	86	88	90	92	94	96	5
	1	1	0	3	0	0	0	0	0	
Dasyatis matsubarai (kidney and spleen)	<52	54	56	57	58	60	62	64	66	14
	6	1	0	1	1	0	0	4	1	

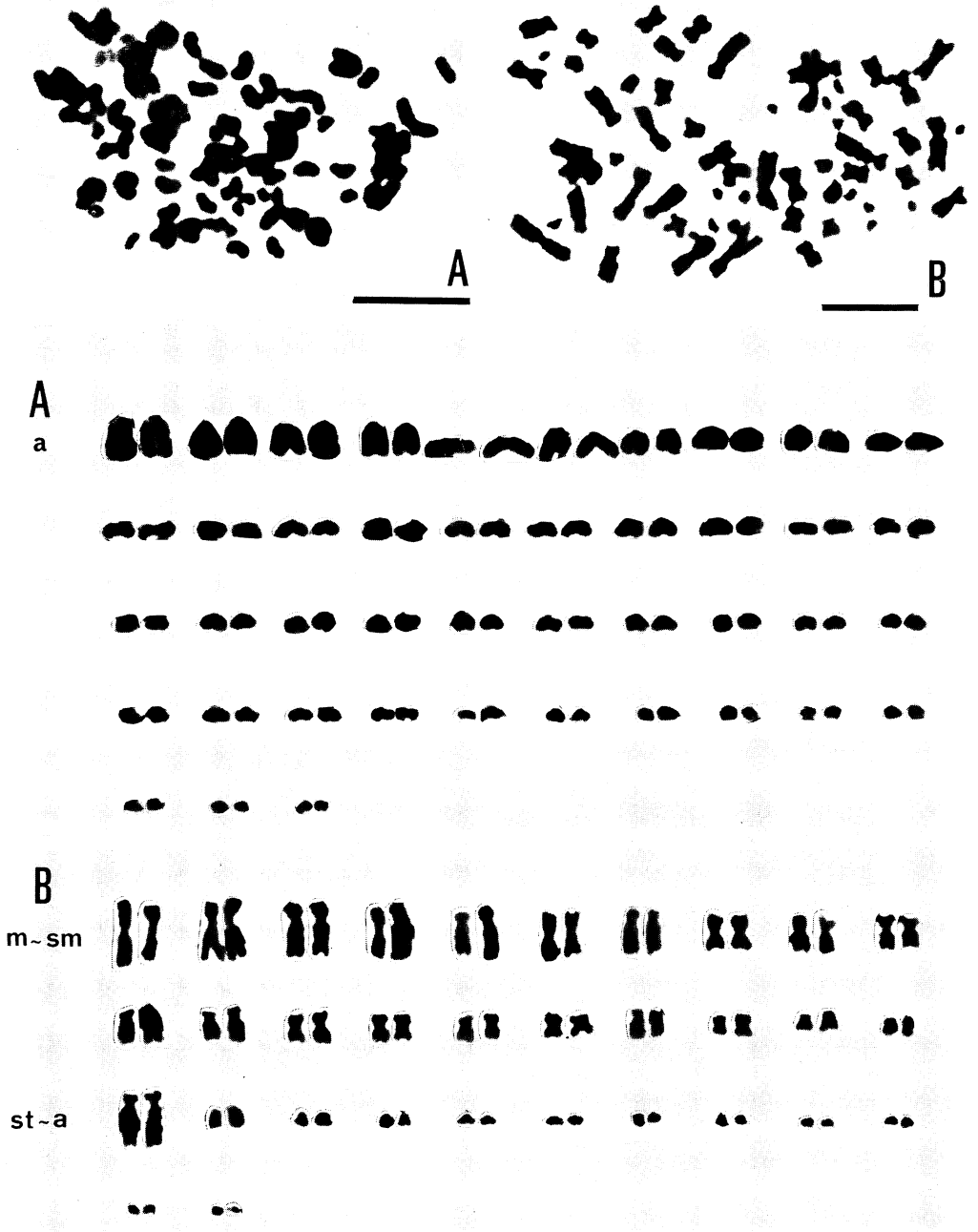

Fig. 1. A photomicrograph of metaphase and a karyogram of torpedinid and dasyatid rays. A: *Torpedo tokionis*, 2n=86. The karyotype consisted of 86 acrocentric chromosomes. B: *Dasyatis matsubarai*, 2n=64. The karyotype consisted of 40 meta- or submetacentric (m~sm) and 24 subtelocentric or acrocentric (st~a) chromosomes. Each scale indicates 10 μm.

64 (Table 2). The karyotype consisted of 40 meta- or submetacentric (m-sm) chromosomes and 24 subtelocentric or acrocentric (st-a) chromosomes (Fig. 1B). The fundamental number was 104. The DNA value was determined as 9.5 pg/cell (Table 3).

Dasyatis violacea: The DNA value was determined as 9.6 pg/cell (Table 3).

Discussion

Table 4 shows the karyotypes and cellular DNA contents of the fishes of the order Torpediniformes so far reported. The karyotype of *Torpedo tokionis* consists of 86 acrocentric chromosomes, and is identical with that of *Torpedo marmorata*. The karyotypes of the fishes of the Torpediniformes show similar fundamental numbers except for *Narcine brasiliensis*. As regards the genus *Torpedo*, the fundamental numbers are 86 in three species. Recent studies are in accordance with the recognition of a large proportion of acrocentric or telocentric chromosomes in elasmobranch fishes as in primitive or generalized state (Ida et al., 1986; Schwartz and Maddock, 1986; Stingo and Capriglione, 1986). If such a tendency is fitted for the order Torpediniformes, the species having less biarmed (metacentric) chromosome numbers may be regarded as primitive. Thus, the karyotypes of *Torpedo tokionis* and *T. marmorata* seem to be close to the ancestral karyotype of the genus *Torpedo*. These karyotypes seem to have undergone a similar structural modification in chromosomes from their ancestor.

The cellular DNA contents of *Narcine* are 8.4 pg/cell, but those of *Torpedo* are 14.0 to 15.0 pg/cell. These values suggest the tetraploid origin of *Torpedo* from an ancestral form having about 8 pg/cell in genome size (Ida et al., 1985). Further, Ida et al. (1985) stated that the Torpedinoidea (including *Torpedo, Narke, Temera*, etc.) seem to have been derived from the Narcinoidea (*Narcine*) or a Narcinoidea-like ancestor by tetraploidization and some modification in karyotype on the basis of comparison

Table 3. Cellular DNA contents of two rays, *Dasyatis matsubarai* and *D. violacea*. *Stingo et al. (1980).

Species	Cells observed	Arbitrary DNA unit	Standard error	Standard deviation	Relative DNA unit	Absolute DNA pg/cell	Reported DNA pg/cell
Dasyatis matsubarai	100	65.97	0.122	1.215	2.81	9.5	
Cyprinus carpio	100	23.51	0.044	0.438	1.00	3.4	
Dasyatis violacea	100	77.45	0.159	1.558	2.82	9.6	13.7*
Cyprinus carpio	100	27.47	0.073	0.732	1.00	3.4	

Table 4. Karyotypes and cellular DNA contents of the order Torpediniformes. *Hinegardner (1976); **Stingo et al. (1980); ***Asahida et al. (unpublished).

Species	2n	m-sm	st-a	FN	DNA (pg/cell)	Reference
Narcine brasiliensis	28	28	0	56	8.4*	Donahue (1974)
Narke japonica	54	28	26	82	21–24?***	Ida et al. (1985)
Torpedo ocellata	66	12	54	78	15.0**	Stingo (1979)
T. californica	82	4	78	86	14.6*	Ida et al. (1985)
T. marmorata	86	0	86	86	14.0**	Stingo (1979)
T. tokionis	86	0	86	86		present study

Table 5. Karyotypes and cellular DNA contents of the genus *Dasyatis*. *Hinegardner (1976); **Stingo et al. (1980); ***Asahida et al. (unpublished).

Species	2n	m–sm	st–a	FN	DNA (pg/cell)	Reference
Dasyatis sabina	68	28	40	96		Donahue (1974)
D. sayi	68	34	34	102	9.4*	Donahue (1974)
D. violacea	58				9.6*** (13.7**)	Stingo and Capriglione (1986)
D. akajei	72	34	38	106	8.3	Asahida et al. (1987)
D. matsubarai	64	40	24	104	9.5	present study

of their DNA values and karyotypes. On the other hand, *Narke japonica* (Torpedinoidea) shows a large amount of DNA content, about 21 to 24 pg/cell (Asahida et al., unpublished). If that value of DNA content is accurate, the karyological evolution in the order Torpediniformes stated by Ida et al. (1985) should be revised as the DNA content of the genus *Narke* might be increased not only by tetraploidization but also by other gene duplication events such as tandem duplication. As regards the mechanism of the accumulation of DNA and karyological modification, a more detailed study seems to be needed.

Table 5 shows the karyotypes and cellular DNA contents of the genus *Dasyatis* rays so far reported. *Dasyatis matsubarai* and *D. violacea* show almost similar value in DNA content, 9.5 and 9.6 pg/cell respectively. Stingo et al. (1980) reported the DNA value of *Dasyatis violacea* as 13.7 pg/cell. In the present study, the DNA value of *Dasyatis violacea* is 9.6 pg/cell. To understand the difference in the values of DNA contents for this species, detailed comparison of the materials from the Pacific and Atlantic oceans is required. The cellular DNA contents of the family Dasyatidae range between 8.3 and 9.6 pg/cell except for the value of *Dasyatis violacea* reported by Stingo et al. (1980). Myliobatiform families have peculiar values of cellular DNA contents, e.g., Urolophidae: 13.0 to 13.1 pg/cell, Myliobatidae: 8.7 to 10.8 pg/cell, Rhinopteridae: 10.0 to 10.4 pg/cell. These features may suggest that a species group in each family has a closer affinity than the groups of other order, e. g., Squaliformes (Asahida et al., unpublished).

The karyotype of *Dasyatis matsubarai* consists of 40 meta- or submetacentric chromosomes and 24 subtelocentric or acrocentric chromosomes (Fig. 1). The fundamental number is close to the other Dasyatid rays. The similar DNA value, similar fundamental number, smaller number of diploid chromosome number, and larger size of meta- or submetacentric chromosomes in *Dasyatis matsubarai* in comparison with those of other *Dasyatis* species may suggest a centric fusion origin of the large-sized chromosomes. It may be said that the karyotype of *Dasyatis matsubarai* is more specialized in having a large proportion of meta- or submetacentric chromosomes than the other species of the genus.

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ヤマトシビレエイとホシエイの核型および系統類縁関係

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日本産エイ類，ヤマトシビレエイ *Torpedo tokionis* とホシエイ *Dasyatis matsubarai* の核型を，簡易組織培養法を用いて分析した．またホシエイとカラスエイ *Dasyatis violacea* の DNA 量を，顕微分光濃度を用いて測定した．ヤマトシビレエイの核型は 2n＝86 で，すべての染色体が端部着糸型であった．ホシエイでは 2n＝64, 中部–次中部着糸型染色体 (M–SM)＝40, 次端部–端部着糸型染色体 (ST–A)＝24, 腕数 (FN)＝104 であり，DNA 量は 9.5 pg/cell であった．またカラスエイの DNA 量は 9.6 pg/cell であった．核型と DNA 量の検討の結果，ヤマトシビレエイの核型は同属の基本核型に近いものと考えられた．シビレエイ類の倍数性進化については，その機構についてより詳しい研究が必要であると判断された．またホシエイはその核型の特徴から，アカエイ科魚類の中では特化した種であると判断された．

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