Japanese Journal of Ichthyology
Vol. 33, No. 4 1987

魚類学雑誌
33巻4号 1987年

Karyotypes of Three Rays in the Order Myliobatiformes

Takashi Asahida, Hitoshi Ida
and Shu Inoue

(Received April 15, 1986)

A large number of karyological studies have been carried out for the class Osteichthyes. On the contrary, only about thirty species of the class Chondrichthyes have been studied (Nygren *et al.*, 1971; Nygren and Jahnke, 1972; Donahue, 1974; Stingo, 1979; Ida, 1984; Ida *et al.*, 1985). Twelve species of rays mostly Torpediniformes and Rajiformes were analyzed on their karyotype. At present, both inter- and intraordinal relationships of rays are not well defined. The reported karyotypes together with their genome sizes are variable according to the species. Thus karyological analysis seems to be useful for the further understandings of phyletic relationships of the groups. We examined karyotypes and DNA content of three myliobatiform species, viz. *Dasyatis akajei*, *Urolophus aurantiacus*, and *Myliobatis tobijei*. The results are described in relations with some informations on their morphology.

Materials and methods

Materials used in the present study are shown in Table 1. Counts for the vertebrae and fin supporting elements were based on X-ray photographs. Vertebrae of elasmobranchs were classified into two types, monospondylous and diplospondylous. In the present case, the two types were not well defined, so vertebrae anterior to the anus were regarded as precaudal ones and those posterior to the anus were regarded as caudal. Thirty-six samples, 11 specimens of *Dasyatis akajei*, 12 *Urolophus aurantiacus*, 10 *Myliobatis tobijei* and 3 *Gymnura japonica* (other than those used for chromosome study) were collected from Shimokita, Sanriku, Tateyama, Izu, and Miyake, and were used for meristic comparison. For the preparation of chromosomes, the routine air-drying method or *in vitro* method (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 and intestine were removed. (b) *In vitro*: After removing the tissues from the body, they were washed with sea water, soaked in isotonic incubating medium with colchicine at the concentration of 1 to 3 μg/ml and shaken gently for 12 to 24 hours continuously.

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 with Carnoy's fixative for at least 60 minutes.

Preparation and Staining. The cell suspension was expanded over the entire slide by dropping Carnoy's fixative on the slide. Then the preparation was stained with Giemsa solution diluted to 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. The nuclear DNA content was measured on blood smears using scanning microspectrophotometer. Blood samples were stained according to Feulgen's technique (Macgregor and Varjley, 1983).

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

Species	Date	Locality	TL (mm)	DL (mm)	DW (mm)	BW (g)	Sex	Usage
Dasyatis akajei	83-6-8	Tateyama	373	188	220	405	F	C
	85-10-23	Shimokita	334	116	135	80	M	G
Urolophus aurantiacus	79-5-10	Miyake I.	325	209	226	409	F	C
	85-9-23	Tateyama	263	155	167	200	F	G
Myliobatis tobijei	80-6-11	Misaki	530	220	366	770	M	C
	85-10-23	Shimokita	597	182	303	550	F	G

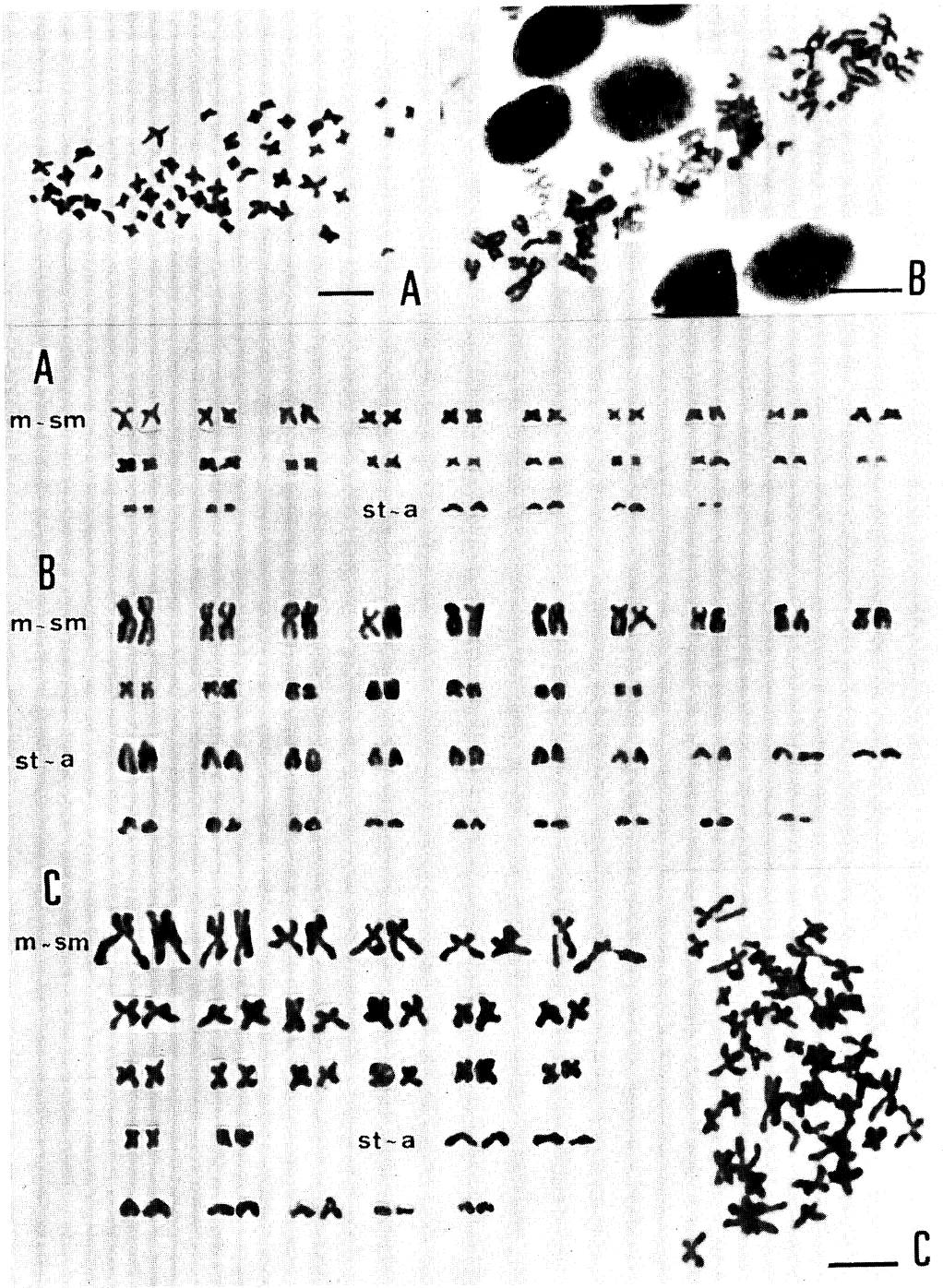

Fig. 1. Chromosome spreads and karyograms of three myliobatiform fishes. A, *Urolophus aurantiacus*; B, *Dasyatis akajei*; C, *Myliobatis tobijei*. Bars indicate 10 μm.

Table 2. Distribution of chromosome counts for three species of the Myliobatiformes.

Species	Chromosome count									Number of cells observed
	58	66	68	70	71	72	73	74	78	
Dasyatis akajei	1	1	1	1		7		1	1	13
	<42	44	46	48	50	51	52	54	56	
Urolophus aurantiacus	4	1	1	2	3		5		1	17
	<48	50	52	53	54	55	56	58	60	
Myliobatis tobijei	2		1		6					9

Table 3. Genome sizes of three species of the Myliobatiformes.

Species	Cells observed	Arbitrary DNA unit	Standard deviation	Standard error	Relative DNA unit	Absolute DNA pg/cell
Dasyatis akajei	72	9.213	0.148	0.017	2.442	8.30
Myliobatis tobijei	68	9.595	0.154	0.019	2.543	8.65
*Cyprinus carpio**	62	3.773	0.030	0.004	1.000	3.40
Urolophus aurantiacus	94	15.88	0.786	0.081	3.839	13.1
*Cyprinus carpio**	58	4.136	0.036	0.005	1.000	3.40

* As control.

Table 4. Some selected meristic characters of four species of the Myliobatiformes. Figures show the modal counts and those in parentheses show the ranges.

Species	Vertebrae			Fin radials		Pectoral pterygiophore*	Number of specimens observed
	Precaudal	Caudal	Total	P_1	P_2		
Dasyatis akajei	39 (38–46)	97 (82–110)	136 (106–149)	109 (104–114)	24 (20–29)	7 (7–8)	11
Urolophus aurantiacus	31 (29–35)	109 (103–114)	140 (133–150)	91 (87–95)	21 (19–26)	8 (7–11)	12
Gymnura japonica	34	118 (115–121)	152 (149–155)	116	18	6	3
Myliobatis tobijei	38 (35–42)	100+**	140+**	95 (64–99)	23 (19–27)	6	10

* Pterygiophore numbers of the first supporting elements of pectoral fin.

** Due to their smaller size and fusion, the actual numbers could not be counted.

Table 5. Karyotypes and genome sizes of the Myliobatiformes.

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	30?	28?	88?	13.7*	Stingo and Capriglione, 1986
D. akajei	72	34	38	106	8.3	present study
Urolophus halleri	72	20	52	92	13.0*	Schwartz and Maddock, 1986
U. aurantiacus	52	44	8	96	13.1	present study
Gymnura micrura	56	44	12	100	11.4 (16.2*)	Schwartz and Maddock, 1986
Myliobatis freminvillei	52	50	2	102	10.6 (9.8*)	Schwatrz and Maddock, 1986
M. californicus	52	50	2	102	10.4 (9.8*)	Schwartz and Maddock, 1986
M. aquila					10.8	Stingo, 1980
M. tobijei	54	40	14	94	8.7	present study

* Stingo, 1980.

Results

Dasyatis akajei: Three specimens were available for chromosome observations but a female specimen gave good chromosome spreads for analysis. The diploid chromosome number was 72 (Table 2). The karyotype consisted of 34 meta- or submetacentric and 38 subtelocentric or acrocentric chromosomes (Fig. 1B). The fundamental number was 106. The genome size was 8.30 pg/cell (Table 3).

Urolophus aurantiacus: Two specimens were available for chromosome observations but only one female specimen gave good chromosome spreads for analysis. The diploid chromosome number was 52 (Table 2). The karyotype consisted of 44 meta- or submetacentric and 8 subtelocentric or acrocentric chromosomes (Fig. 1A). The fundamental number was 96. The genome size was 13.1 pg/cell (Table 3).

Myliobatis tobijei: Four specimens were available for chromosome observations but only one for male specimen gave good chromosome spreads analysis. The diploid chromosome number was 54 (Table 2). The karyotype consisted of 40 meta- or submetacentric and 14 subtelocentric or acrocentric chromosomes (Fig. 1C). The fundamental number was 94. The genome size was 8.65 pg/cell (Table 3).

The vertebral composition and the number of supporting elements of fins were selected as the meristic characters (Table 4). Most characters showed large variation, especially in the number of caudal vertebrae of *Dasyatis* and *Myliobatis*. In *Urolophus*, the number of precaudal vertebrae was rather small but the range of the count was the largest among the three species.

Discussion

The karyotype and DNA content of the myliobatid fishes so far reported are summarized in Table 5. The genome size ranges between 8.3 and 16.2 pg/cell in myliobatiform species. Except for the genus *Gymnura*, most genera have a genome size of about 9 to 14 pg/cell and no apparent ploidy can be detected from the table. The genome size range of the genus *Dasyatis* is large, about 8 to 14 pg/cell, in comparison with that of genera *Urolophus* (about 13 pg/cell) and *Myliobatis* (about 9 to 11 pg/cell). The genome size range of the genera mentioned above seem to reflect the numerous species in each genus. The karyological diversification of the genus *Dasyatis* is most conspicuous. On the other hand, the karyotypes and genome sizes of the genus *Myliobatis* are less diversified in comparison with that of other two genera. In *Urolophus*, the karyotypes of the two species are markedly different (Table 5). Recent studies are in accordance with the recognition of primitiveness or generalized state of large proportion of acrocentric or telocentric chromosomes in elasmobranch fishes (Ida *et al.*, 1986; Schwartz and Maddock 1986; Stingo and Capriglione, 1986). It may be said that the karyotype of *Urolophus helleri* is more generalized in having numerous acrocentric chromsomes than that of *aurantiacus*. The equal genome size, nearly equal fundamental number, smaller number of diploid chromosome number and larger sizes of meta- or submetacentric chromosomes in *Urolophus aurantiacus* in comparison with those of *U. halleri* may suggest centric fusion origin of the large-sized chromosomes. It seems that a more detailed comparison on the morphology of the genus *Urolophus* is needed for the further understandings of the large difference in karyotype within a genus in the order Myliobatiformes.

In conventional systematics, these groups are classified into two families. Dasyatidae, including *Dasyatis* and *Urolophus*, and Myliobatidae (Garman, 1913; White, 1937; Matsubara, 1955). Compagno (1973) ranked up these families to superfamilies, Dasyatoidea and Myliobatoidea, and subdivided Dasyatoidea into the family Urolophidae and Dasyatidae on the basis of skeletal features. Nishida (1985) analyzed more detailed osteology of the myliobatids and emphasized the difference between *Dasyatis* and *Urolophus*.

We examined some meristic characters of *Urolophus aurantiacus*, *Dasyatis akajei* and *Myliobatis tobijei* for a comparison of these species. *Urolophus aurantiacus* has 29–35 precaudal vertebrae, as compared to 38–46 in *Dasyatis akajei* and 35–42 in *Myliobatis tobijei*. The actual numbers of caudal vertebrae in *Dasyatis akajei* and *Myliobatis tobijei* could hardly be counted, because of their smaller size and fusion of the elements. *Urolophus aurantiacus* is different from the other two genera in having fewest precaudal vertebrae and pectoral fin radials (Table 4).

Adding to these meristic features, *Urolophus aurantiacus* has caudal fin, showing the most generalized state in this character, as compared to specialized whip-like tail of *Dasyatis akajei* and *Myliobatis tobijei*. The morphological and karyological features suggest that *Urolophus aurantiacus* has closer affinity to *Dasyatis akajei* than to *Myliobatis tobijei*. A more detailed study on the morphology in relation with karyology of myliobatiform species seems to be needed.

Acknowledgments

We would like to express our thanks to Mr. Kohjiroh Matsuhashi, manager of Mutsu Sekine set net of Aomori Prefecture, Mr. Hitoshi Ebihara, master fisherman of Banda set net of Chiba Prefecture, and the Staff of Ohgusu set net of Kanagawa Prefecture, and of Igaya set net of Miyake Island, for their kind offer of the materials. We are also indebted to Mr. Yasuyuki Koike, Tokyo University of Fisheries, who offered the facilities of the study.

Literature cited

Compagno, L. J. V. 1973. Interrelationships of living elasmobranchs. *In* Interrelationships of fishes. Zool. J. Linnean Soc., Suppl. 1, 53: 15–62.

Donahue, W. H. 1974. A karyotypic study of three species of Rajiformes (Condrichthyes, Pisces). Can. J. Genet. Cytol., 16: 203–211.

Garman, S. 1913. The Plagiostomia. Mem. Mus. Comp. Zool., Harv. Coll., 36: 1–515, pls. 1–75.

Ida, H., M. Murofushi, S. Fujiwara and K. Fujino. 1978. Preparation of fish chromosomes by *in vitro* colchicine treatment. Japan. J. Ichthyol., 24(2): 281–248.

Ida, H. 1984. Karyological relationships of sharks and rays. *In* Sharks and rays as the fishery resources. Fishery Science Ser. 49, Koseisha-koseikaku, Tokyo, 136 pp. (In Japanese.)

Ida, H., I. Sato and N. Miyawaki. 1985. Karyotypes of two species in the order Torpediniformes. Japan. J. Ichthycl., 32(1): 107–111.

Ida, H., T. Asahida, K. Yano and S. Tanaka. 1986. Karyotypes of two sharks, *Chlamydoselachus anguineus* and *Heterodontus japonicus*, and their systematic implications. Pages 158–163 *in* T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds. Indo-Pacific fish biology: Proceedings of the Second International Conference on Indo-Pacific Fishes. Ichthyol. Soc. Japan, Tokyo.

Levan, A., K. Fredga and A. A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201–220.

Macgregor, H. C. and J. M. Varjley. 1983. Measuring nuclear or chromosomal DNA. Pages 227–239 *in* Working with animal chromosomes. John Wiley and Sons, New York.

Matsubara, K. 1955. Fish morphology and hierarchy. I. Ishizaki Shoten, Tokyo, xi+789 pp. (In Japanese.)

Nishida, K. 1985. Comparative skeletal anatomy of six myliobatoid fishes from Japan. Rep. Japan. Group Elasmobranch Studies, (19): 21–42. (In Japanese.)

Nygren, A., B. Nilsson and M. Jahnke. 1971. Cytological studies in Hypotremata and Pleurotremata (Pisces). Hereditas, 67: 275–282.

Nygren, A. and M. Jahnke. 1972. Microchromosomes in primitive fishes. Swedish J. Agric. Res. 2: 229–238.

Schwartz, F. J. and M. B. Maddock. 1986. Comparisons of karyotypes and cellular DNA contents within and between major lines of elasmobranchs. Pages 148–157 *in* T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds. Indo-Pacific fish biology: Proceedings of the Second International Conference on Indo-Pacific Fishes. Ichthyol. Soc. Japan, Tokyo.

Stingo, V. 1979. New developments in vertebrate cytotaxonomy. II. The chromosomes of the cartilaginous fishes. Genetica, 50(3): 227–239.

Stingo, V. 1980. Genome size of some selachian fishes. Bull. Zool., 47: 129–137.

Stingo, V. and T. Capriglione. 1986. DNA and chromosomal evolution in cartilaginous fish. Pages 140–147 *in* T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds. Indo-Pacific fish biology: Proceedings of the Second International Conference on Indo-Pacific Fishes. Ichthyol. Soc. Japan, Tokyo.

White, E. G. 1937. Interrelationships of the elasmobranchs with a key to the order Galea. Bull. Am. Mus. Nat. Hist., 74(3): 25–138, pls. 1–51.

(School of Fishery Sciences, Kitasato University, Sanriku-cho, Kesen-gun, Iwate Pref. 022–01, Japan)

日本産トビエイ目3種の核型

朝日田　卓・井田　斉・井上　衆

日本産トビエイ目3種の核型を air-drying 法により分析し，それぞれのゲノム量を顕微分光濃度計を用いて測定した．アカエイの核型は 2n=72，中部-次中部着糸型染色体 (MS-M)=34，次端部-端部着糸型染色体 (ST-A)=38，腕数 (FN)=106，ゲノム量=8.3 pg/cell であり，ヒラタエイでは 2n=52，M-SM=44，ST-A=8，FN=96，ゲノム量=13.1 pg/cell；トビエイでは 2n=54，M-SM=40，ST-A=14，FN=94，ゲノム量 8.7 pg/cell であった．核型及びゲノム量などの検討の結果，ヒラタエイとアカエイの近縁性は，ヒラタエイとトビエイの近縁性以上のものではないと判断された．

(022–01 岩手県気仙郡三陸町　北里大学水産学部)