Japan. J. Ichthyol.
40(3): 317-322, 1993

魚類学雑誌
40(3): 317-322, 1993

The Karyotype and Cellular DNA Content of a Ray, *Mobula japonica*

Takashi Asahida,[1] Hitoshi Ida,[2] Hiroaki Terashima[3] and Hui-Yun Chang[4]

[1] Tohoku National Fisheries Research Institute, 3-27-5 Shinhama, Shiogama, Miyagi 985, Japan
[2] School of Fisheries Sciences, Kitasato University, Sanriku-cho, Kesen-gun, Iwate 022-01, Japan
[3] Hanshin Rinkai Sokuryo, 5-57-6 Honkomagome, Bunkyo, Tokyo 113, Japan
[4] College of Fisheries Science, National Taiwan Ocean University, Pei-ning Road, Keelung 20224, Taiwan, Republics of China

(*Received June 2, 1992; in revised form February 17, 1993; accepted August 19, 1993*)

Abstract The karyotypes of an adult female and male foetus of a ray, *Mobula japonica*, were observed following short-term tissue culture, and the cellular DNA content measured. The karyotype and cellular DNA content of *Mobula japonica* were determined as follows: 2n=66, M=26, SM=12 (female) and 11 (male), ST-A=28 (female) and 29 (male), FN=104 (female) and 103 (male), NAN=98, DNA=9.5 pg/cell. A small difference recognized between the male and female karyotypes, in the shape and size of the one of the middle-sized chromosome pairs, may be related to sex determination in this species. The phyletic relationships of the order Myliobatiformes is discussed from karyological viewpoint.

Karyological studies have been carried out on about 60 species of elasmobranchs. In spite of their conspicuous sexual dimorphism, there is very limited information on elasmobranch sex chromosomes (Donahue, 1974; Kikuno and Ojima, 1987). Male heterogamety has been reported for a stingray, *Dasyatis sabina*, and a guitarfish, *Rhinobatos hynnicephalus*. In addition, there are a few reports dealing with cellular DNA content of elasmobranchs (Hinegardner, 1976; Stingo et al., 1980; Ida et al., 1986; Schwartz and Maddock, 1986; Stingo and Capriglione, 1986; Asahida et al., 1987, 1988; Asahida and Ida, 1989, 1990).

The results of an examination of cellular DNA content and karyotypes of a female and a male foetus of *Mobula japonica*, collected by set-net in Sanriku, Iwate, Japan, are described below with some comments on the phyletic relationships of rays.

Materials and Methods

The material used is a female (ca. 150 cm in disc length [DL], ca. 200 cm disc width [DW], ca. 120 kg body weight [BW]) and a male foetus (53 cm DL, 69 cm DW, 8.45 kg BW) of *Mobula japonica*. The cellular DNA content, expressed as the DNA value of the red blood cells relative to that of the common carp, *Cyprinus carpio*, was measured using a scanning microdensitometer. Blood samples were stained according to Feulgen's technique (Macgregor and Varjley, 1983).

A short-term tissue culture method (Asahida and Ida, 1990) was adopted for preparation of metaphase chromosome spreads, followed by routine air-drying and Giemsa staining.

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

Elasmobranch classification follows Compagno (1973).

Results

Chromosome spreads were obtained from gill tissue. The diploid chromosome number was determined as 66 (Table 1). The female karyotype consisted of 26 metacentric chromosomes (M), 12 submetacentric chromosomes (SM), and 28 subtelocentric or acrocentric (ST-A) chromosomes. The male karyotype consisted of 26 M, 11 SM and 29 ST-A (Fig. 1). Chromosome sizes ranged from 12.4 to 3.2 μm (M), 8.9 to 3.2 μm (SM) and 4.9 to 2.0 μm (ST-A). In the karyotypes, a small difference was

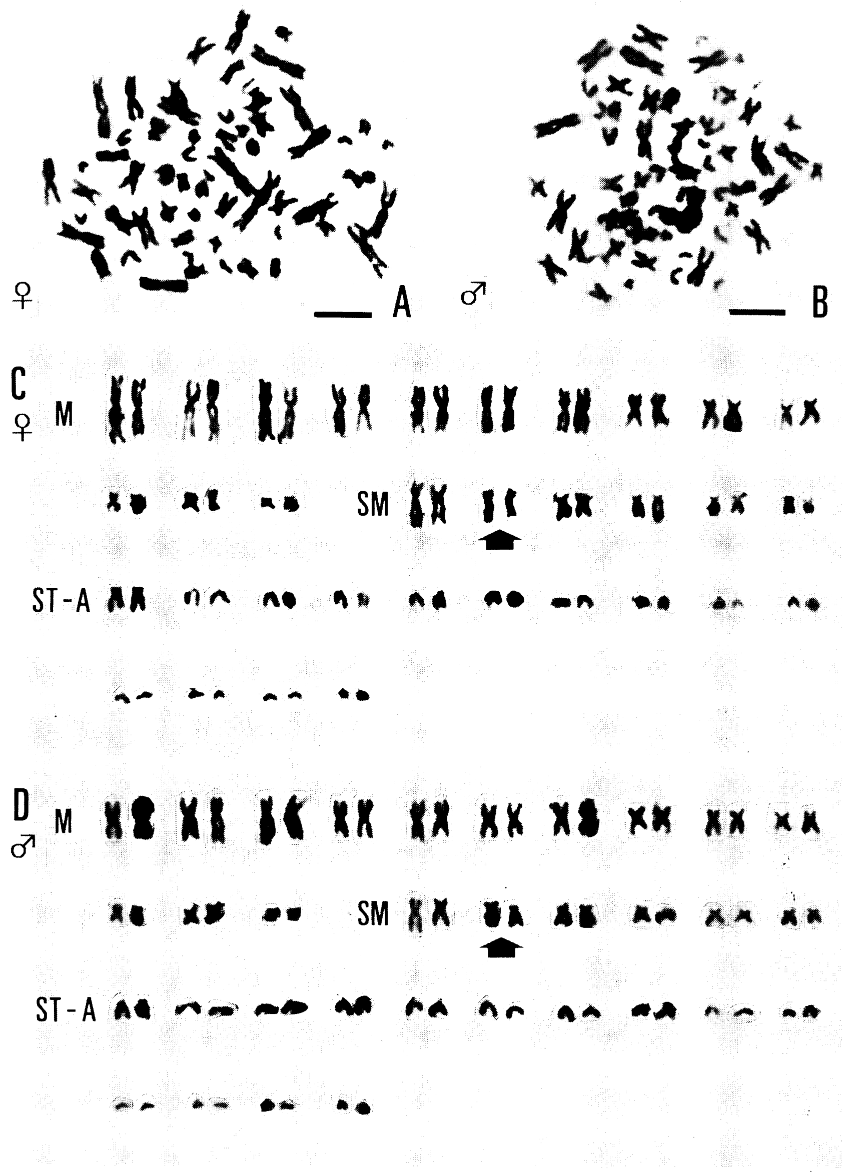

Fig. 1. Photomicrographs of metaphase cells and karyograms of a male and female *Mobula japonica*. A, C) female; B, D) male. 2n=66. Scale indicates 10μm. Arrow indicates chromosome pair wherein a difference was recognized between the male and the female in chromosome shape and size.

Table 1. Distribution of chromosome counts for *Mobula japonica*

Sex	Chromosome count									N*
	<60	62	64	65	66	67	68	70	72<	
Female	6	1	2	2	8	0	1	0	1	21
Male	3	0	2	1	4	0	1	0	0	11

* Number of cells observed.

found between the male and female in the shape and size of the one of the middle-sized chromosome pairs (Fig. 1, indicated by arrows). In the female, the two elements of the pair were identical in shape (arm ratio is 2.5) and size, but in the male, one of the pair was smaller than the other (arm ratio is 2.5), being

Table 2. DNA measurements of *Mobula japonica*

Species	Cells observed	Arbitrary DNA unit	Standard error	Standard deviation	Relative DNA unit	Absolute DNA pg/cell
Mobula japonica (♂)	50	35.42	0.188	1.331	2.77	9.4
*Cyprinus carpio**	50	12.80	0.042	0.294	1.0	3.4
Mobula japonica (♀)	50	37.22	0.177	1.255	2.84	9.7
*Cyprinus carpio**	50	13.09	0.035	0.248	1.0	3.4
Mobula japonica (♀)	50	37.36	0.140	0.988	2.73	9.3
*Cyprinus carpio**	50	13.71	0.033	0.230	1.0	3.4

* Control.

Table 3. Karyotypes and cellular DNA contents of the order Myliobatiformes

Species	2n	M-SM	ST-A	FN	Sex-C	NAN	DNA (pg/cell)	Reference
Order Myliobatiformes								
Family Urolophidae								
Urolophus aurantiacus	52	44	8	96	U	96?	13.1	Asahida et al., 1987
U. halleri	72	22	50	94	U	90?	13.0[a]	Maddock and Schwartz, unpublished
Family Dasyatididae								
Dasyatis akajei	72	34	38	106	U	96?	8.3	Asahida et al., 1987
D. americana	78	9	69	87	U	83?	9.3	Maddock and Schwartz, unpublished
D. kuhlii	ca. 64				U	U		Asahida and Ida, unpublished
D. matsubarai	64	40	24	104	U	98	9.5	Asahida and Ida, 1990
D. sabina	68	28	40	96	X–Y	96?	8.7[c]	Donahue, 1974
D. sayi	68	34	34	102	X–Y?	98?	9.4[b] (9.2[c])	Donahue, 1974
D. violacea	58	ca. 30	ca. 28	ca. 88	U		13.7[a]	Stingo and Capriglione, 1986
	58	ca. 20	ca. 38	ca. 78	U	98	9.6	Asahida and Ida, 1990
Family Gymnuridae								
Gymnura micura	56	44	12	100	U	98?	11.4[d] (16.2[a])	Maddock and Schwartz, unpublished
G. japonica	56	32	24	88	X–Y?	88?		Asahida and Ida, unpublished
Family Mobulidae								
Mobula japonica	66	38	28	104	X–Y?	98	9.5	Present study
Family Myliobatididae								
Myliobatis californica	52	50	2	102	U	94?	10.4[d] (9.8[b])	Maddock and Schwartz, unpublished
M. equila							10.8	Stingo et al., 1980
M. freminvillei	52	50	2	102	U	94?	10.6[d] (9.8[b])	Maddock and Schwartz, unpublished
M. tobijei	54	40	14	94	U	90?	8.7	Asahida et al., 1987
Family Rhinopteridae								
Rhinoptera bonasus	64	42	22	106	U	92?	10.0[d]	Maddock and Schwartz, unpublished
R. quadriloba							10.4	Hinegardner, 1976

Sex-C, sex-chromosome type; NAN, new arm number (Arai and Nagaiwa, 1976); U, unknown; X–Y, X–Y type. X–Y?, suggests existence of sex-chromosomes. NAN?, count determined from reported figure, but inexact. a, Stingo et al., 1980; b, Hinegardner, 1976; c, Maddock and Schwartz (unpublished); d, Ohno et al., 1969.

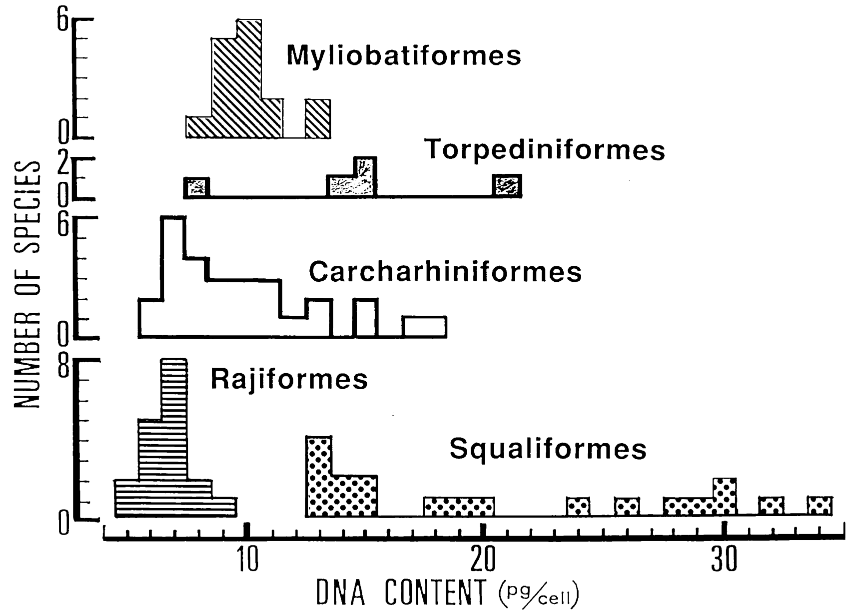

Fig. 2. Frequency histograms showing the distribution of cellular DNA contents of myliobatiform rays and some orders of elasmobranchs.

identified as a subtelocentric chromosome (arm ratio is 3.0). The fundamental numbers were 104 (female) and 103 (male). The DNA value was determined as 9.5 pg/cell (Table 2).

Discussion

Table 3 shows the karyotypes and cellular DNA contents of fishes belonging to the order Myliobatiformes. Amongst them, the cellular DNA content of *Mobula japonica* was typical. Figure 2 shows the range of DNA values of some elasmobranch orders. The DNA values of myliobatiform fishes showed a smaller variation compared with other elasmobranchs. The karyotype of *Mobula japonica* was similar to other myliobatiform rays, especially *Dasyatis matsubarai*, the karyotypes of these species being characterized by a large proportion of meta- or submetacentric chromosomes. Also, the fundamental number of *Mobula japonica* is about 100, being close to other myliobatiform rays, such as *Dasyatis*. Judging from their similar DNA values and fundamental numbers, the smaller number of diploid chromosomes and larger size of meta- or submetacentric chromosomes in *Mobula japonica* compared with other myliobatiform rays, suggests a centric fusion origin of the large-sized chromosomes. Recent studies have recognized that the large proportion of meta- or submetacentric chromosomes in elasmobranch fishes is a specialized state (Stingo et al., 1980; Ida et al., 1986; Schwartz and Maddock, 1986).

There are very few reports dealing with the karyotypes of both sexes in elasmobranchs. In myliobatiform rays, the only sex chromosome type so far reported is male heterogamety in the stingray, *Dasyatis sabina*, which has heterochromosomes in a submetacentric chromosome pair (Donahue, 1974). The difference in shape and size of the sex chromosomes between male and female *D. sabina* seems very small. In *M. japonica*, it appeared that the male had differently shaped chromosomes comprising the 2nd pair of the middle-sized group (Fig. 1), suggesting that these chromosomes are related to sex determination in the species. Further chromosomal analyses, such as G and C-banding and observation of meiosis in reproductive cells, are necessary for a more detailed understanding of the role of these chromosomes. It is interesting to note that *M. japonica* is similar to *D. sabina* in the condition of these submetacentric chromosomes. In previous reports, sex chromosomes have been observed only in batoid species amongst elasmobranchs (Donahue, 1974;

Kikuno and Ojima, 1987), suggesting that in this feature, batoids are more specialized than the other elasmobranchs.

The fundamental numbers of myliobatiform rays show rather small variation, from 78 to 106, such apparently resulting mainly from pericentric inversions. Conversion of the FNs to NAN (Arai and Nagaiwa, 1976), gives values ranging from 83 to 98, with most species being 98. This may be further evidence for the monophyly of the myliobatiform rays.

Mobula japonica is more closely related to the Dasyatididae and Myliobatididae in having similar NAN and DNA values than to the Urolophidae and Gymnuridae.

Acknowledgments

We would like to express our thanks to Mr. Chikara Nakajima, master fisherman and other staff of Kokabe set-net, Iwate Prefecture, for their kind offer of the study materials. We are also indebted to Dr. Michael B. Maddock and Dr. Frank J. Schwartz (Institute of Marine Science, The University of North Carolina at Chapel Hill) for permission to quote their results. Part of this work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan, to T. Asahida.

Literature Cited

Arai, R. and K. Nagaiwa. 1976. Chromosomes of tetraodontiform fishes from Japan. Bull. Natn. Sci. Mus., Ser. A (Zool), 2: 59–72.

Asahida, T. and H. Ida. 1989. Karyological notes on four sharks in the order Carcharhiniformes. Japan. J. Ichthyol., 36: 275–280.

Asahida, T. and H. Ida. 1990. Karyotypes of two rays, *Torpedo tokionis* and *Dasyatis matsubarai*, and their systematic relationships. Japan. J. Ichthyol., 37: 71–75.

Asahida, T., H. Ida and S. Inoue. 1987. Karyotypes of three rays in the order Myliobatiformes. Japan. J. Ichthyol., 33: 426–430.

Asahida, T., H. Ida and T. Inoue. 1988. Karyotypes and cellular DNA contents of two sharks in the family Scyliorhinidae. Japan. J. Ichthyol., 35: 215–219.

Compagno, L. J. V. 1973. Interrelationships of living elasmobranchs. Pages 15–61 *in* P. H. Greenwood, R. S. Miles and C. Patterson, eds. Interrelationships of fishes. Zool. J. Linnean Soc., Vol. 53, Supp. 1.

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

Hinegardner, R. 1976. The cellular DNA content of sharks and rays and some other fishes. Comp. Biochem. Physiol., 55B: 367–370.

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. of Japan, Tokyo.

Kikuno, T. and Y. Ojima. 1987. A karyotypic studies of a guitarfish, *Rhinobatus hynnicephalus* Richardson (Pisces, Rajiformes). La Kromosomo, 2(47–48): 1538–1544.

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 & Sons, New York.

Ohno, S., J. Muramoto, C. Stenius, L. Christian, W. A. Kittrell and N. B. Atkin. 1969. Microchromosomes in holocephalian, chondrostean and holostean fishes. Chromosoma, 26: 35–40.

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. of Japan, Tokyo.

Stingo, V., M. D. Buit and G. Odierna. 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. of Japan, Tokyo.

イトマキエイの核型および核内 DNA 量

朝日田　卓・井田　齊・寺島裕晃・張　慧雲

イトマキエイ（雌）とその胎仔（雄）の核型を簡易組織培養法を用いて分析し，核内 DNA 量を顕微分光濃度計を用いて測定した．本種の染色体数は 2n＝66 で，核型は雌雄でやや異なり，雌では中部着糸型染色体 (M)＝26, 次中部着糸型染色体 (SM)＝12, 次端部—端部着糸型染色体 (ST-A)＝28, 腕数 (FN)＝104, 雄では M＝26, SM＝11, ST-A＝29, FN＝103 であった．核内 DNA 量は 9.5 pg/cell であった．雌の第 2 SM ペアに対応する雄の染色体は SM と ST から成る異形対で，これらはその形態と大きさか

ら，性染色体である可能性が示唆された．核型と核内 DNA 量の検討より，トビエイ目魚類の系統類縁関係，特に各科の近縁性や単系統性について論じた．

（朝日田：〒985 宮城県塩釜市新浜町 3-27-5 東北区水産研究所；井田：〒022-01 岩手県気仙郡三陸町越喜来　北里大学水産学部；寺島：〒113 東京都文京区本駒込 5-57-6　阪神臨海測量（株）；張：中華民国　国立台湾海洋大学水産学院）