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Karyotypes and Cellular DNA Contents of Some Sharks in the Order Carcharhiniformes

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Abstract The karyotypes of two sharks and cellular DNA contents of four sharks in the order Carcharhiniformes were studied and determined to be as follows: *Prionace glauca*: 2n=86, M-SM=30, ST-A=56, FN=116, DNA=8.6 pg/cell; *Sphyrna lewini*: 2n=86, M-SM=20, ST-A=66, FN=106; *Carcharhinus obscurus*: 2n=ca. 78, M-SM=ca. 20, ST-A=ca. 58, FN=ca. 98, DNA=6.0 pg/cell; *C. plumbeus*: 2n=ca. 74, M-SM=ca. 18, ST-A=ca. 56, FN=ca. 92, DNA=6.0 pg/cell; *C. galapagensis*: DNA=8.5 pg/cell.

These carcharhinid sharks showed a smaller size and range of chromosomes compared with those of triakidid and scyliorhinid sharks. Smaller sized meta-, submeta- and subtelocentric chromosomes in the carcharhinid sharks may have resulted from pericentric inversions. From karyological features, *Sphyrna lewini* shows close affinities to sharks in the family Carcharhinidae, supporting a recent cladistic analysis of the order Carcharhiniformes in which the Carcharhinidae included the genus *Sphyrna*.

Several karyological studies of the order Carcharhiniformes have been made (Stingo, 1979; Yabu and Ishii, 1984; Schwartz and Maddock, 1986; Asahida et al., 1988; Asahida and Ida, 1989; Maddock and Schwartz, unpubl. data). However, emphasis on the family Carcharhinidae has been very limited (Yabu and Ishii, 1984; Maddock and Schwartz, unpubl. data).

Most workers have placed the hammerhead sharks in a separate family (Sphyrnidae) from other carcharhinoids. Recently, however, Compagno (1988) included them in the Carcharhinidae as a supertribe (Sphyrnini) on the basis of many morphological characteristics. Not with standing, some uncertainties remain in the cladistic relationships of carcharhiniforms, although the group is well studied compared with other shark orders. It is necessary therefore, to compare cytogenetic data among these sharks.

In this study the karyotypes of two carcharhiniform sharks, *Prionace glauca* and *Sphyrna lewini*, and the cellular DNA contents of four carcharhinid sharks, *Carcharhinus plumbeus*, *C. obscurus*, *C. galapagensis* and *P. glauca*, were examined and are described below with comments on the phyletic relationships of the family Carcharhinidae.

Materials and Methods

The materials used in the present study are listed in Table 1. 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 (Nikon Vickers M85a). Blood samples were stained according to the Feulgen 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. Chromosome spreads were obtained from gill tissue.

Chromosome size was measured with a micrometer equipped with a 1000× microscope.

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.

The classification of carcharhiniform sharks used in Table 4 follows Compagno (1988).

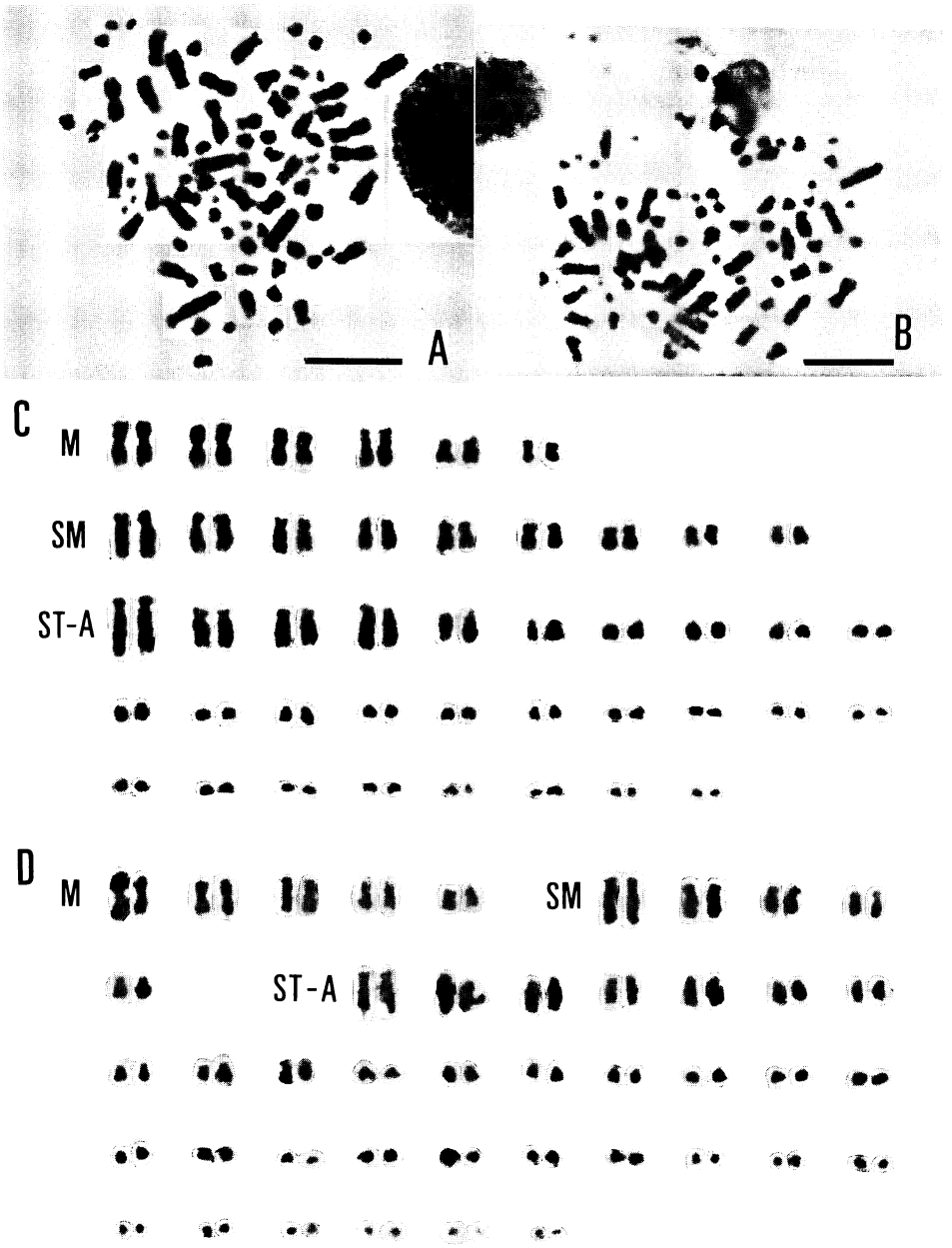

Fig. 1. Photomicrographs of metaphase cells and karyograms of two carcharhinid sharks. A, C) *Prionace glauca*, 2n=86; B, D) *Sphyrna lewini*, 2n=86. Scale indicates 10 μm.

Results

Prionace glauca (Fig. 1A, C).—The diploid chromosome number was determined as 86 (Table 2). The karyotype consisted of 30 meta- or submetacentric (M-SM) chromosomes and 56 subtelocentric or acrocentric (ST-A) chromosomes (Fig. 1A, C). The fundamental number (FN) was 116. Chromosome sizes ranged from 2.4 to 4.8 μm (M), 1.9 to 5.2 μm (SM), 1.4 to 9.1 μm (ST) and 0.9 to 2.1 μm (A). The DNA value was determined to be 8.6 pg/cell (Table 3).

Sphyrna lewini (Fig. 1B, D).—The diploid chromosome number was determined as 86 (Table 2).

The karyotype consisted of 20 M-SM chromosomes and 66 ST-A chromosomes. The FN value was 106. Chromosome sizes ranged from 2.5 to 5.8 μm (M), 2.8 to 5.8 μm (SM), 1.8 to 5.5 μm (ST) and 0.8 to 3.5 μm (A).

Carcharhinus plumbeus, *C. obscurus* and *C. galapagensis*.—Because good chromosome spreads were not obtained, the karyotypes were unable to be determined. The DNA contents were determined to be 6.0 pg/cell, 6.0 pg/cell and 8.5 pg/cell, respectively (Table 3).

Discussion

The karyotype of *Prionace glauca* determined here differs from the results of Yabu and Ishii (1984), who reported the diploid chromosome number to be 78, with the karyotype comprising 2 pairs of metacentric chromosomes, 3 pairs of submetacentrics, 10 pairs of acrocentrics, 16 pairs of chromosomes with intermediate constriction and 8 pairs of spherical chromosomes. The different results may be due to an imcomplete chromosome figure, which seems to lack

Table 1. Specimens used for chromosome (C) and cellular DNA content (D) studies

Species	Date of sampling	Locality	TL (mm)	BL (mm)	BW (g)	Sex	Usage
Sphyrna lewini	July 24, 1991	Ogasawara	800	563	ca. 4000	Female	C
Carcharhinus obscurus	June 21, 1989	Tateyama	1238	960	ca. 6500	Male	C, D
C. plumbeus	Aug. 22, 1988	Ogasawara	686	519	1990	Female	C, D
	Aug. 22, 1988	Ogasawara	650	492	1550	Male	D
C. galapagensis	July 26, 1991	Ogasawara	875	656	ca. 2000	Male	D
Prionace glauca	Dec. 2, 1985	Off Sanriku	1600	1210	8600	Female	C
	Dec. 2, 1985	Off Sanriku	1640	1220	12,600	Female	D
	May 29, 1986	Tateyama	510	372	386	Male	C, D
	Aug. 29, 1990	Sanriku	732	531	1214	Female	C

Table 2. Distribution of chromosome counts obtained in the present study. N indicates number of cells observed

Species	Chromosome count										N
	<76	78	80	82	84	85	86	87	88	90<	
Sphyrna lewini	2	1	0	0	0	1	5	1	1	1	12
Prionace glauca	2	1	3	2	2	1	7	0	1	2	21

Table 3. DNA measurements obtained in the present study

Species	Cells observed	Arbitrary DNA unit	Standard error	Standard deviation	Relative DNA unit	Absolute DNA pg/cell
Carcharhinus obscurus	100	36.17	0.156	1.563	1.75	6.0
*Cyprinus carpio**	100	20.62	0.058	0.578	1.0	3.4
Carcharhinus plumbeus (male)	100	30.74	0.069	0.694	1.72	5.9
*Cyprinus carpio**	100	17.84	0.048	0.484	1.0	3.4
C. plumbeus (female)	100	32.88	0.062	0.621	1.76	6.0
*Cyprinus carpio**	100	18.33	0.050	0.499	1.0	3.4
Carcharhinus galapagensis	50	53.47	0.378	2.657	2.51	8.5
*Cyprinus carpio**	50	21.31	0.096	0.682	1.0	3.4
Prionace glauca (male)	100	38.82	0.086	0.861	2.5	8.5
*Cyprinus carpio**	100	15.55	0.042	0.423	1.0	3.4
P. glauca (famale)	100	39.73	0.118	1.180	2.55	8.7
*Cyprinus carpio**	100	15.60	0.043	0.429	1.0	3.4

* As control.

some small acrocentric chromosomes, in Yabu and Ishii (1984).

As seen in Table 4, *P. glauca* shows a similar karyotype to those of other sharks in the family Carcharhinidae, such as *Carcharhinus acronatus* and *C. limbatus* (Maddock and Schwartz, unpubl. data). Carcharhinid sharks have a large number of diploid chromosomes and a smaller number of meta- or submetacentric chromosomes compared with triakidid and scyliorhinid sharks.

Sphyrna lewini has a larger number of diploid chromosomes and a smaller number of meta- and submetacentric chromosomes than most other sharks belonging to the order Carcharhiniformes, with the exception of the family Carcharhinidae (Table 4). The karyotype of *S. lewini* is similar to that of sharks in the latter, especially *P. glauca*. Karyotypes of *Carcharhinus plumbeus* and *C. obscurus* could not been clarified during the present study, because the spreads appeared to lack some small chromosomes. However, karyotypes based on imcomplete chromosome spreads of these two species suggested similar features to other reported sharks in the family Carcharhinidae. More detailed study is necessary for clarification of the karyotypes of these species.

The chromosome size and range in *S. lewini*, *P. glauca* and other carcharhinid sharks reported are smaller than those of triakidid and scyliorhinid

Table 4. Karyotypes and cellular DNA contents of the order Carcharhiniformes

Species	2n	M-SM	ST-A	FN	DNA (pg/cell)	Reference
Family Scyliorhinidae						
Cephaloscyllium umbratile	64	34	30	98	14.7	Asahida et al., 1988
C. uter	——	——	——	——	15.4	Hinegardner, 1976
C. ventriosum	64	46	18	110	18.1, 13.8[e]	Maddock and Schwartz, unpubl.
Scyliorhinus canicula	62	42	20	104	11.3[c]	Stingo, 1979
S. stellaris	72	50	22	122	12.3[c]	Stingo, 1979
S. torazame	64	26	38	90	13.2	Asahida et al., 1988
Galeus eastmani	——	——	——	——	11.0	Asahida and Ida, 1989
G. melastomus	——	——	——	——	12.3	Stingo et al., 1989
G. nipponensis	——	——	——	——	11.1	Asahida and Ida, 1989
Family Triakididae						
Mustelus asterias	——	——	——	——	8.6	Stingo et al., 1989
M. canis	80	44	36	124	9.6, 9.2[b]	Maddock and Schwartz, unpubl.
M. californicus	——	——	——	——	12.8	Hinegardner, 1976
M. manazo	68	44	24	112	9.3	Asahida and Ida, 1989
M. norrisi	——	——	——	——	9.0	Hinegardner, 1976
M. sp.	——	——	——	——	9.6	Hinegardner, 1976
Triakis scyllia	72	36	36	108	9.8	Asahida and Ida, 1989
T. semifasciata	72	52	20	124	9.6[b]	Schwartz and Maddock, 1986
Family Galeorhinidae						
Galeorhinus galeus	——	——	——	——	17.3	Stingo et al., 1980
Family Carcharhinidae						
Galeocerdo cuvier	86	40	46	126	8.3[b], 13.1[e]	Maddock and Schwartz, unpubl.
Sphyrna lewini	86	20	66	106	7.0[b], 6.6[d], 8.9[e]	present study
S. tiburo	——	——	——	——	7.8	Hinegardner, 1976
Rhizoprionodon porosus	——	——	——	——	7.8	Stingo et al., 1989
R. terraenovae	ca. 90	ca. 32	ca. 58	ca. 124	7.2[b]	Maddock and Schwartz, unpubl.
Carcharhinus acronatus	ca. 86	ca. 35	ca. 51	ca. 122	6.7, 6.8[b]	Maddock and Schwartz, unpubl.
C. limbatus	ca. 86	ca. 33	ca. 53	ca. 120	7.3, 7.4[b], 8.2[e]	Maddock and Schwartz, unpubl.
C. longimanus	——	——	——	——	6.7	Mirsky and Ris, 1951
C. obscurus	ca. 78	ca. 20	ca. 58	ca. 98	6.0, 5.5[a]	present study
C. perezi	——	——	——	——	11.7	Stingo et al., 1989
C. plumbeus	ca. 76	ca. 18	ca. 56	ca. 92	6.0	present study
Negaprion brevirostris	——	——	——	——	7.4	Hinegardner, 1976
Prionace glauca	86	30	56	116	8.6, 8.6[b]	present study

M-SM, meta-submetacentrics; ST-A, subtelo-acrocentrics; FN, fundamental number; [a]Mirsky and Ris, 1951; [b]Hinegardner, 1976; [c]Stingo et al., 1980; [d]Schwartz and Maddock, 1986; [e]Stingo et al., 1989.

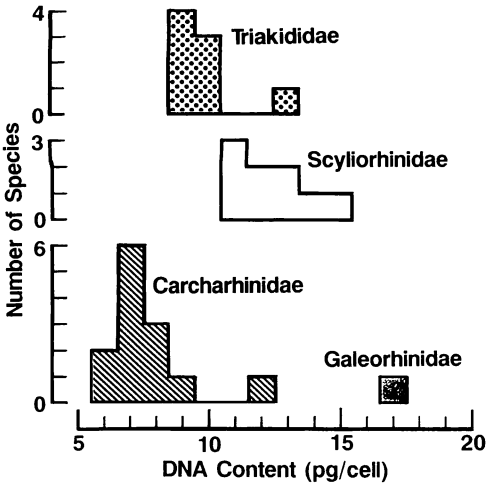

Fig. 2. Frequency histogram showing the distribution of cellular DNA contents of carcharhiniform sharks.

sharks. For example, the chromosome size ranges from 0.7 to 12.0 μm in the scyliorhinid shark, *Cephaloscyllium umbratile* (Asahida et al., 1988), with the larger-sized chromosomes being some 16 to 18 times as large as the smaller-sized ones in that species. Large-sized chromosomes in scyliorhinid sharks seem to have originated from structural modifications such as tandem fusions and tandem gene duplications (Asahida et al., 1988). In contrast to those in scyliorhinid sharks, such as *C. umbratile*, the larger-sized subtelocentric chromosomes in *P. glauca* are about 9 times as large as the smaller acrocentric ones, and the larger-sized submetacentric chromosomes in *S. lewini*, about 7 times as large as the smaller acrocentric ones. These relatively smaller-sized metacentric, submetacentric and subtelocentric chromosomes of carcharhinid sharks and *S. lewini* seem to have originated from a structural modification such as pericentric inversion, judging from their karyological features, including the size distribution of the chromosomes and the relationship between the diploid chromosome number and fundamental number.

The DNA values in the family Carcharhinidae and two *Sphyrna* species range between 6.0 and 8.6 pg/cell (Table 4), being generally lower than values in the Triakididae and Scyliorhinidae (Table 4). Figure 2 shows a frequency histogram of the distribution of the cellular DNA contents of carcharhiniform sharks, the two *Sphyrna* species being included in the Carcharhinidae. All of the carcharhiniform families are characterized by a certain range and value of cellular DNA content, with a tendency for increasing DNA value with increase in chromosome size and range.

The DNA value, karyotype and features of chromosome size and range of *S. lewini* are very similar to those of carcharhinid species, rather than triakidids and scyliorhinids. Also, the karyological features of *S. lewini* seem to be more similar to typical carcharhinid sharks, such as *Carcharhinus limbatus* (Maddock and Schwartz, unpubl. data), *C. obscurus* and *P. glauca*, rather than to other carcharhinids, such as *Galeocerdo cuvier* and *Rhizoprionodon terraenovae* (Maddock and Schwartz, unpubl. data), suggesting a close relationship of *S. lewini* to the family Carcharhinidae.

Most workers have placed the hammerhead sharks (*Sphyrna and Eusphyrna*) in a separate family, usually on the basis of the cephalofoil in the former and the many other associated modifications. However, Compagno (1988) proposed a cladistic classification of the order Carcharhiniformes, in which the family Sphyrnidae was ranked down to a supertribe (Sphyrnini) in the Carcharhinidae on the basis of many morphological characteristics. Recently, Martin et al. (1992) reported phylogenetic relationships among carcharhinoid and lamnoid sharks, based on mitochondrial DNA sequence data, including topologies which showed similar branch lengths among *Carcharhinus*, *Negaprion* and *Sphyrna*. Furthermore, the branch length of *Galeocerdo* was more distant from *Carcharhinus*, than that of *Sphyrna*. Their molecular data suggested that *Sphyrna* was more closely related than *Galeocerdo* to *Carcharhinus* and *Negaprion*. If the hammerhead sharks rightly belong to the Carcharhinidae, carcharhiniform sharks would appear to be rather clearly represented by three families, based on karyotypic features and cellular DNA content, i.e., Scyliorhinidae, Triakididae and Carcharhinidae, plus some families that presently have no karyological information.

The cladistic classification of the family Carcharhinidae proposed by Compagno (1988) seems to be supported by both karyotypic features and cellular DNA content (Table 4), and mitochondrial DNA sequence data of some carcharhinoid and lamnoid sharks (Martin et al., 1992).

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メジロザメ目魚類の核型および核内 DNA 量

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メジロザメ目魚類，ヨシキリザメ *Prionace glauca* とアカシュモクザメ *Sphyrna lewini* の核型を簡易組織培養法を用いて観察し，メジロザメ *Carcharhinus plumbeus*，ドタブカ *C. obscurus*，ガラパゴスザメ *C. galapagensis* およびヨシキリザメ *P. glauca* の核内 DNA 量を顕微分光濃度計を用いて測定した．ヨシキリザメの核型は染色体数 2n＝86，中部・次中部着糸型染色体 (M-SM)＝30，次端部・端部着糸型染色体 (ST-A)＝56，腕数 (FN)＝116 であり，核内 DNA 量は 8.6 pg/cell であった．アカシュモクザメの核型は 2n＝86，M-SM＝20，ST-A＝66，FN＝106 であった．メジロザメ，ドタブカ，ガラパゴスザメの核内 DNA 量はそれぞれ，6.0 pg/cell，6.0 pg/cell，8.5 pg/cell であった．これらの結果を，他のメジロザメ目魚類の核型および核内 DNA 量と比較した結果，シュモクザメ類の核型や核内 DNA 量の特徴はメジロザメ科魚類と同じであり，他科魚類とは明確に異なった．また，メジロザメ科魚類とシュモクザメ類では染色体のサイズとその範囲が比較的小さい．従って，これらの中・小型の中部・次中部着糸型および次端部着糸型染色体は，動原体を含む逆位によって生じたと推定された．以上の類似性は，シュモクザメ類とメジロザメ科魚類の強い近縁性を示唆しており，シュモクザメ類をメジロザメ科に含める分類体系を支持している．

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