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A KARYOTYPIC STUDY OF THREE SPECIES OF RAJIFORMES (CHONDRICHTHYES, PISCES)

WILLIAM H. DONAHUE, C.S.C.
Department of Biology
King's College, Wilkes-Barre, Pa. 18702

A karyotypic study has been made of three species of Chondrichthyes of the order Rajiformes: the lesser electric ray (*Narcine brasiliensis*) of the Torpedinidae, and two stingrays, the Atlantic stingray (*Dasyatis sabina*) and the bluntnose stingray (*Dasyatis sayi*) of the Dasyatidae. The karyotypes were derived by a squash technique from gill epithelial cells after specimen injection with colchicine. The lesser electric ray has a diploid number of 28 (18 m, 4 sm, and 6 st), the smallest diploid count yet discovered in the Chondrichthyes. Both stingrays have a diploid count of 68. The Atlantic stingray has 28 m-sm and 40 st-t,; the bluntnose stingray has 34 m-sm and 34 st-t. The suggestion is made that these species may show a relationship to skates previously described in that they possess a similar number of chromosome arms. A preliminary note is made on the chromosome number of the Atlantic guitarfish.

Introduction

At the present time, our knowledge of the karyotypes of the sharks, skates and rays is extremely small (Table I). No karyotype of a shark has yet been published, although chromosomal numbers (with some morphology in a few cases) are available for four species (Makino, 1937; Matthey, 1947; Nogusa, 1960 and Nygren *et al.*, 1971). Karyotypes for three species of skates have recently been published (Nygren *et al.*, 1971). Chromosome numbers with some morphological information are available for a stingray (Nogusa, 1960) and for a skate (Makino, 1937). Any studies earlier than those cited, especially those that mention chromosome numbers in conjunction with other cytological studies, would have to be verified before their chromosome data could be accepted.

The present study was undertaken to determine the karyotypes of the lesser electric ray, *Narcine brasiliensis* (Olfers) and of two species of stingrays, the Atlantic stingray, *Dasyatis sabina* (Lesueur), and the bluntnose stingray, *Dasyatis sayi* (Lesueur). A preliminary observation of the chromosomes of the Atlantic guitarfish, *Rhinobatus lentiginosus* (Garman) was also made. The research was conducted at the Marine Institute of the University of Texas in Port Aransas during the summer of 1972.

Materials and Methods

The specimens were collected by trawling in the waters of the Gulf of Mexico adjacent to Port Aransas, Texas. Ten specimens (six male and four female) of the lesser electric ray, 10 specimens (7 male and 3 female) of the Atlantic stingray, two specimens of the bluntnose stingray, and a single female specimen of the Atlantic guitarfish were studied. The fish were maintained in large, concrete holding tanks for periods ranging from a day to several weeks. When a choice was possible, smaller specimens were chosen for study.

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Each specimen was injected in the dorsal musculature with 0.2 to 0.4 ml. (depending on size) of 0.5% colchicine in distilled water. Injected specimens were maintained in well aerated sea water for approximately 15 to 16 hr (overnight). Shorter periods were attempted but were found to be not as satisfactory. Chromosome spreads were prepared from gill epithelial cells according to the method of McPhail and Jones (1966). Four to six slides were prepared from each specimen. The slides were studied under phase-contrast, and those spreads suitable for karyotyping were photographed on Kodak Plus-X 35 mm film and developed in Kodak DK-50. Karyotypes were prepared with the chromosomes arranged according to the classification of Levan *et al.* (1965). The terminology proposed by these authors is used throughout this paper with m referring to a chromosome with the centromere in the median region; sm, submedian region; st, substerminal region; and t, terminal region. The fish names and classification correspond to Bailey *et al.* (1970).

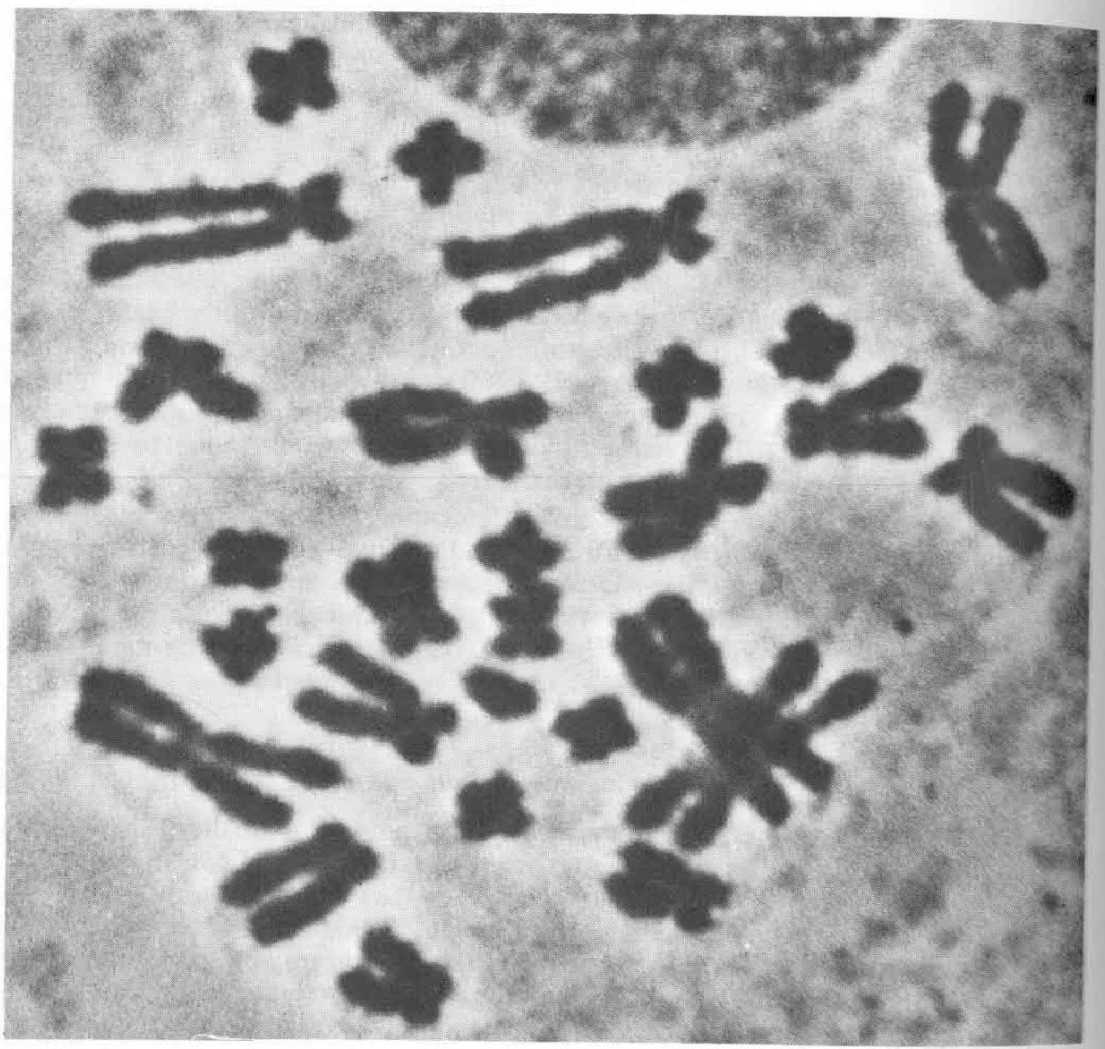

Fig. 1. Mitotic chromosomes of *Narcine brasiliensis.* A squash preparation of gill epithelial cells from a colchicine-injected male specimen. Phase-contrast photograph.

Results

Excellent chromosome spreads were obtained from the gill epithelial cells of the lesser electric ray (Fig. 1). The 10 specimens studied resulted in 263 spreads that were countable. Of these, 263, 210 gave counts of 28 chromosomes. The other 53 spreads gave counts ranging from 7 to 35. The diploid chromosomal number of *N. brasiliensis* is clearly 28.

The morphology of the chromosomes was so delightfully evident in many of the spreads that karyotypes were easily prepared (Fig. 2). There were 9 m chromosomes, 2 sm and 3 st. There were no chromosomes with the centromeres in the terminal region. Three of the nine pairs of m chromosomes are large and sufficiently distinct that the homologs are easily paired. The other six pairs are small and remarkably similar in form so that the pairing of the chromosomes is largely arbitrary although a slight gradation in size of these small chromosomes is evident. The four sm chromosomes are rather similar and are paired somewhat arbitrarily. The largest pair of the st chromosomes is brilliantly evident while the two smaller pairs are quite similar. No heterogamety was found in the electric ray.

Work with both species of stingrays was as difficult and frustrating as work with the electric ray was easy and rewarding. A large number of chromosomes with many of them being very small made counting difficult and uncertain. However, the ten specimens of *Dasyatis sabina* yielded 140 countable spreads of which 107 gave counts of 68 chromosomes. The other 33 spreads gave counts ranging from 34 to 71. The diploid count of *D. sabina* is therefore 68. Two very young males born in the holding tanks were most productive in the number of cells in division.

Karyotypes of both male and female specimens are presented in Fig. 3. The suggestion of Levan *et al.* (1965) of grouping the chromosomes of a newly described species into two groups, m-sm and st-t, was followed. The m-sm group contains 28 chromosomes. Of these, 20 chromosomes are large, while 8 are notably smaller. Of the 40 chromosomes falling in the st-t classification, only the largest pair was clearly distinguishable as homologous. Male heterogamety is suggested by pair 10 of the m-sm group (Fig. 3). However, the size distinction is not always as evident as it is in this karyotype selected for illustration.

Although slides were prepared from two male and two female specimens of *Dasyatis sayi,* only the two females resulted in countable chromosome spreads. Counts of 68 were obtained from 23 cells. A few of these spreads, such as the one used in the karyotype (Fig. 4) showed chromosome morphology very clearly. However, counts in general were very difficult to make, and frequently were doubtful. Such doubtful counts were made of 28 spreads and these resulted in numbers between 61 to 70. However, the diploid count of *D. sayi* can be placed with confidence at 68.

The karyotype of *D. sayi* (Fig. 4) shows 34 chromosomes in the m-sm grouping with 20 of these being notably larger than the other 14. The arrangement of chromosomes into homologous pairs is again arbitrary, although the larger chromosomes are clearly distinguishable from the smaller ones. There are also 34 chromosomes in the st-t group with the largest pair of st chromosomes clearly homologous.

Only a preliminary note on the chromosome number and morphology of the Atlantic guitarfish is valid here. While two specimens were obtained, one died after injection with colchicine probably as a result of temperature change in the water in which it was kept. The other, a female specimen, yielded only four spreads on which counts could even be attempted. These four spreads reveal that

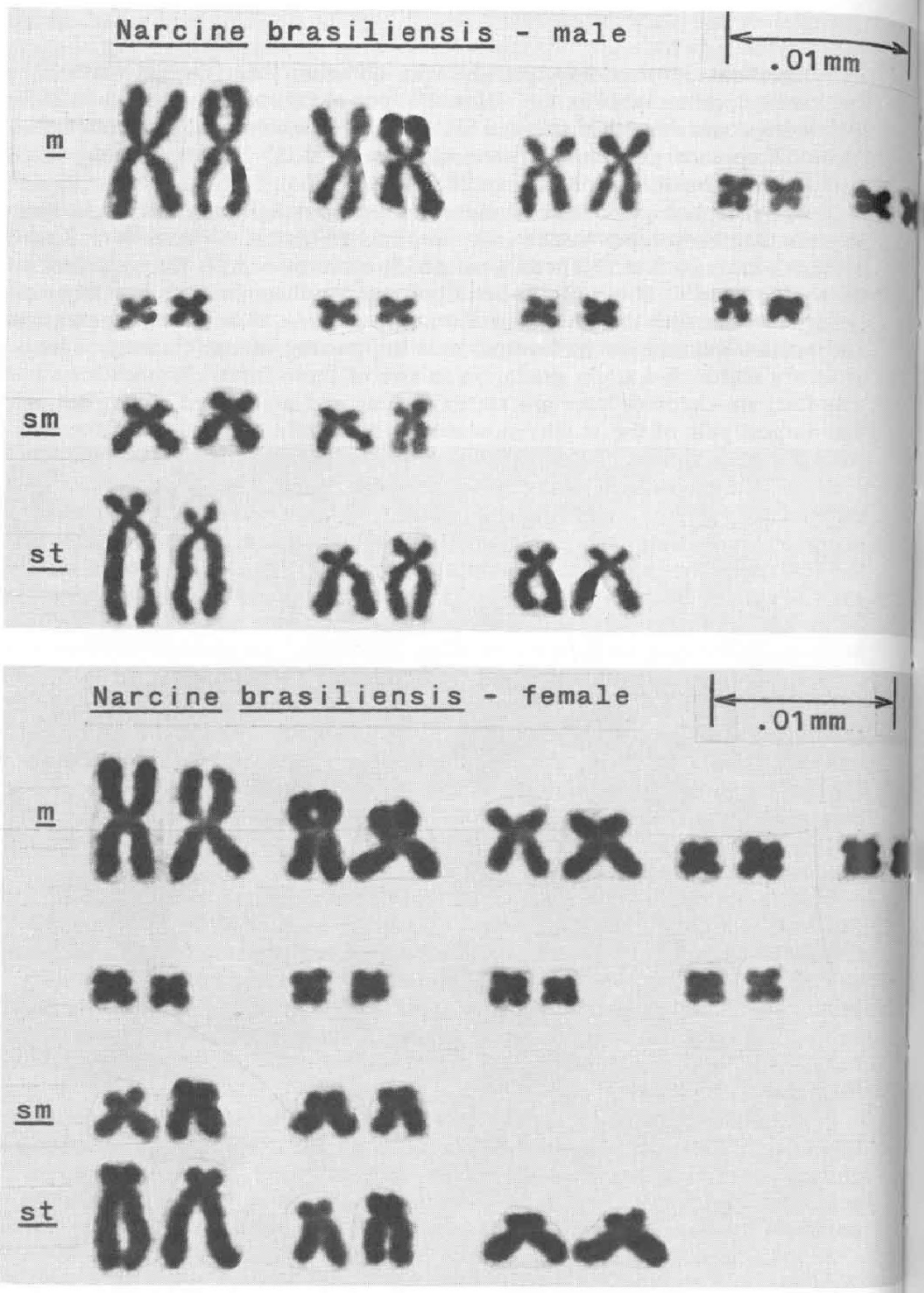

Fig. 2. Karyotypes of male (upper) and female (lower) *Narcine brasiliensis*.

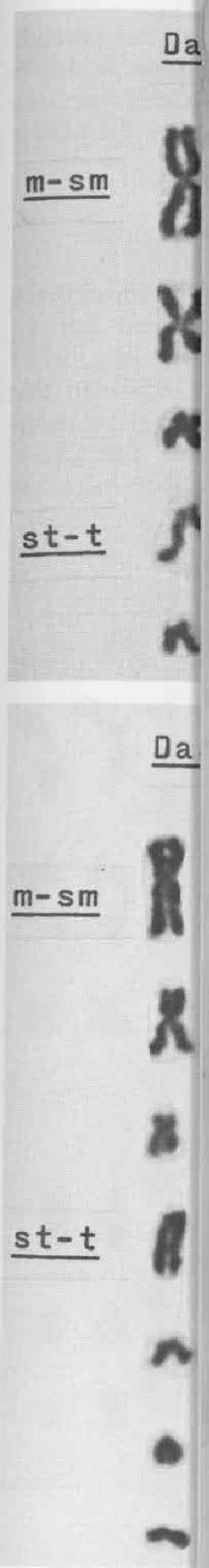

Fig. 3. K

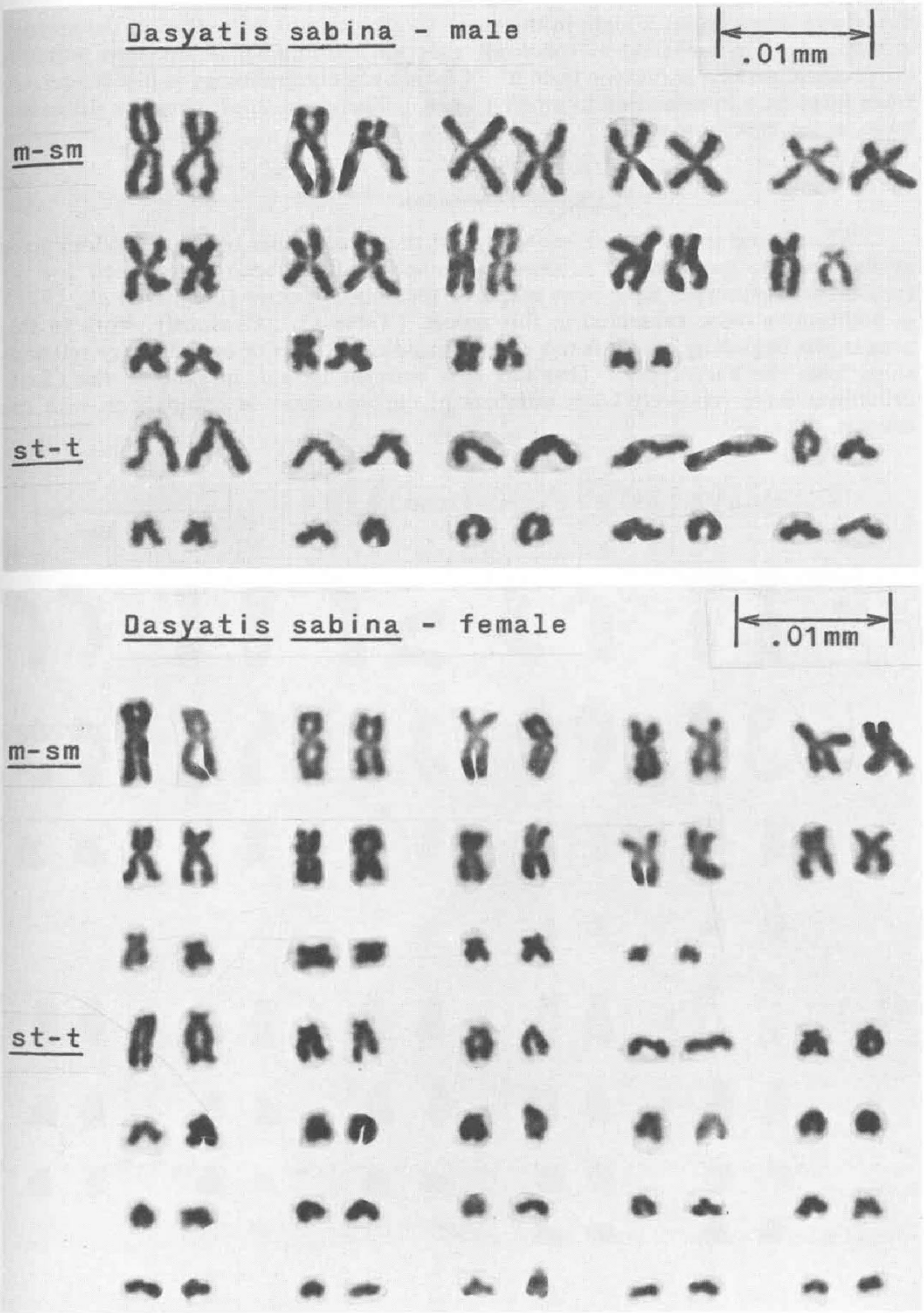

Fig. 3. Karyotypes of male (upper) and female (lower) *Dasyatis sabina*.

the chromosome count is high, in the range of 80, perhaps 84. One of the spreads is rather good in revealing morphology, although the number of overlaps prohibits the preparation of a karyotype from it. Chromosome morphology is highly variable from large m chromosomes to small t ones. There are approximately 30 rather large m-sm chromosomes.

Discussion

What is the status of our knowledge of the cytogenetics of the Chondrichthyes at the present moment? Chromosome numbers have been determined for 12 species, yet karyotypes have been prepared for only 3 species (Nygren *et al.*, 1971) in addition to those presented in this report. (Table I). Obviously, work in this area is just beginning, and it is too early to predict patterns of evolutionary relationships from the karyotypes. One fact that emerges is that, in general, the Chondrichthyes have relatively large numbers of chromosomes in comparison with the teleosts.

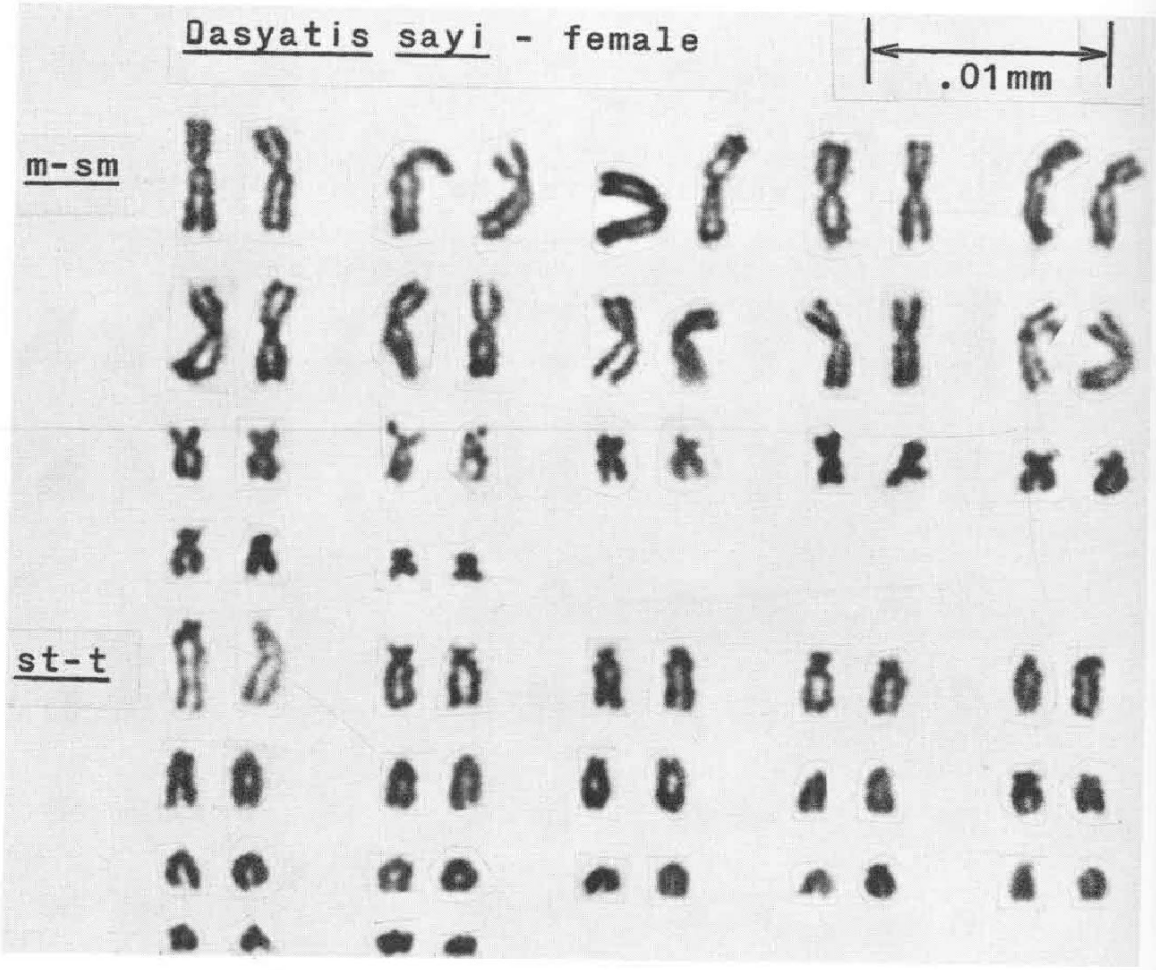

Fig. 4. Karyotype of a female *Dasyatis sayi*.

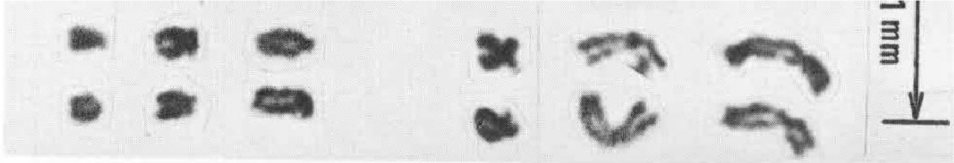

TABLE I

A summary of current information on chromosome numbers and chromosome morphology of the Chondrichthyes

Family	Species	Chromosome number	Chromosome morphology	Authority
Triakidae	*Mustelus manazo*	$2n = 72$	12 or more with median or submedian centromeres 60 telomitic	Nogusa, 1960
Squalidae	*Squalus suckleyi*	$2n = 62$	20 atelomitic, remaining rod-shaped	Makino, 1937
	Scylliorhinus catula	$n = 31$		Matthey, 1947
	Squalus acanthius	$n = 31$		Nygren *et al.* 1971
Dasyatidae	*Dasyatis akajei*	$2n = 84$	approximately 20 metacentric or submetacentric, remaining telocentric	Nogusa, 1960
	Dasyatis sabina	$2n = 68$	28 m-sm, 40 st-t	present study
	Dasyatis sayi	$2n = 68$	34 m-sm, 34 st-t	present study
Rajidae	*Raja meerdervoortii*	$2n = 104$	telomitic	Makino, 1937
	Raja batis	$2n = 98$	metacentrics not more than 3 pairs	Nygren *et al.* 1971
	Raja clavata	$2n = 98$	metacentrics not more than 3 pairs	Nygren *et al.* 1971
	Raja radiata	$2n = 98$	metacentrics not more than 3 pairs	Nygren *et al.* 1971
Torpedinidae	*Narcine brasiliensis*	$2n = 28$	18 m, 4 sm, 6 st	present study

In view of the unusually high chromosome numbers in the Chondrichthyes, the diploid number of 28 in *N. braciliensis* is quite unexpected. When compared with other published information, the karyotype of this species stands in a class of its own. It will be most interesting to see whether future studies reveal similar karyotypes for other members of the Torpedinidae or for any of the other supposedly closely related families of Chondrichthyes. Until it is verified by modern techniques, the report of Kastschenko (1890) of about 50 chromosomes for *Torpedo ocellata* must be seriously questioned.

The relative ease of karyotype preparation, the small number of chromosomes, many with distinctive morphology, should make the lesser electric ray a desirable experimental animal for cytogenetical studies of fish, or more specifically, of the Chondrichthyes.

The only karyotypic study of the Dasyatidae previous to the present study was that of Nogusa (1960) on *Dasyatis akajei*. He gave the diploid number as 84, and stated that "Approximately 20 elements in a rough calculation are of metacentric or submetacentric structure and occupy the peripheral zone of the equatorial plate, surrounding the remaining telocentric ones which are scattered in the central region, though it was difficult to determine with certainty the shape of individual chromosomes." From this description, it seems that the notable difference between the count of 84 for *D. akajei* and of 68 for *D. sabina* and *D. sayi* lies in a greater number of smaller chromosomes in *D. akajei*. The 20 elements which he singles out as different from the rest might well correspond to the 20 larger m-sm chromosomes in *D. sabina* and *D. sayi*. While the colchicine and squash technique of the present study greatly disrupts chromosome arrangements, some spreads were observed that did suggest that the larger chromosomes occupy the periphery, and the smaller ones occupy the central region of the equatorial plate as observed by Nogusa in paraffin sections.

Can any relationship be suggested between the karyotypes of the Dasyatidae of this study and the Rajidae of the studies by Makino (1937) and Nygren *et al.* (1971)? Makino determined that *Raja meerdevoortii* had a diploid count of 104 telomitic chromosomes while Nygren *et al.* published karyotypes of *Raja batis, R. clavata* and *R. radiata* with diploid counts of 98. Although the illustrations are not perfectly clear on the point, these authors state: "In all three species, the number of metacentric chromosomes does not seem to exceed three pairs. However, accurate counts have been very difficult to carry through. This means that the N.F. value for all three species is probably 104." In this way, they imply a relationship between their 98 counts and the 104 of Makino's earlier study. When one compares the stingray karyotypes of the present study with the skate karyotypes of Nygren *et al.*, the absence of large m-sm chromosomes in the skates and their relative abundance in the stingrays is remarkable. If one counts the number of arms in the stingray chromosomes, the number is certainly close to 104. This suggests that the number 68 may be related to the 104 by centric fusion in many of the chromosomes of the stingrays.

Can *Dasyatis akajei* ($2n = 84$) described by Nogusa (1960) fit into this scheme? Recall that approximately 20 of the 84 chromosomes were regarded as metacentric. This again results in an N.F. value of approximately 104!

It will be most interesting to see whether future studies will confirm this chromosomal relationship between the skates and stingrays. That such a relation-

ship probably does not exist among all the families of the Rajiformes is strongly indicated by the 28 chromosomes of the lesser electric ray of the family Torpedinidae.

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