KARYOTYPE OF A SEI WHALE

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The chromosomes of the Mammalia have been studied for a long period, and many species of mammals except those belonging to the Lemures, Proboscidea, Hyracoidea and Sirenia were analyzed their karyotypes (Matthey, 1949).

In the order Cetacea, Makino (1948) first reported a study on the chromosomes of Dall's porpoise, *Phocoenoides dalli* (True). He concluded that the diploid number of the chromosome is 44 and it has sex chromosomes of XY type. And also he pointed out the resemblance between the karyotype of the Dall's porpoise and that of the Ungulata rather than that of the Carnivola.

Nowosielski-Slepowron and Peacock (1955) reported a study on the chromosomes of the blue, fin and sperm whales. In this report chromosome numbers were not decided and were only suggested the approximate diploid number 48 in the above three species.

I report here a study on the chromosomes of the sei whale based on a testes sample obtained from the North Pacific.

I am much indebted to Dr. M. Sasaki of the Hokkaido University, who gave me valuable suggestions on the cytological technique. Greater thanks are due to Prof. T. Hibiya of the University of Tokyo who kindly discussed the draft.

MATERIAL AND METHOD

The material used in this report was collected from a sei whale captured in the northern part of the North Pacific in the summer season of 1966 by a Japanese whaling factory ship. In the external characters and internal organs of this whale, no abnormality was found.

The animal had been dead about 4 and a half hour when the testicular material was collected. After removal from the animal, the piece of testicular tissue was minced with sharp scissors on a small petri dish without adding any saline. 2 ml of 0.6% hypotonic sodium citrate solution was added to about 0.5 ml of the tissue shred and mixed. Then it was allowed to stand at the room temperature for about 40 minutes. Then, the cells suspending in the hypotonic solution were fixed by adding 6 ml of acetic alcohol (acetic acid 1 : methanol 3) for about 30 minutes. The suspension was kept undisturbed until relatively larger tissue shreds had settled down to the bottom of the tube. Then the upper layer of dispersed cell suspension was separated.

After centrifugation at 3,000 r.p.m. for about 3 minutes, the supernatant was decanted, and 6 ml of fresh fixative was added. The fixative was changed three times at the interval of about 20 minute or more. The cell pellet of the final centri-

fugation was resuspended in 2 ml fresh fixative.

Some preparations for microscope were made on the factory ship after the method of Sasaki (1964). But most of the preparations were made by the same method at The Whales Research Institute, after the cell suspension was stored in a freezer for about 4 months.

For staining the slides, diluted Giemsa solution (1 : 20) was applied for 10 to 20 minutes.

NUMBER OF CHROMOSOMES

Chromosomes in spermatogonia

The material provided fairly numerous spermatogonial nuclei undergoing division. And many of these were relatively free from overlapping of chromosomes, which allowed the counting of number. Most of these nuclei contained one or two of the nucleoli stained pale, which suggested that these cells are not in the stage of metaphase but probably in the late prophase.

Spermatogonial nuclei with diploid chromosomes were easily recognized from those of the spermatocyte by their large chromosome number and the simple shape of each chromosomes.

TABLE 1. NUMBER OF CHROMOSOMES OBSERVED IN THE SPERMATOGONIAL NUCLEI

Number of chromosomes	40	41	42	43	44	45	46	47	48	49	Total
Number of occurrence	2	2	1	1	38	3	4	1	1	1	54
Percentage of occurrence	3.7	3.7	1.9	1.9	70.3	5.4	7.4	1.9	1.9	1.9	100.0

By the treatment of the hypotonic sodium citrate solution, the chromosomes in some spermatoganial nuclei revealed the chromatids and the position of the attachment of fibre. They were used for the morphological investigation mentioned later.

The number of diploid chromosomes was counted after their relative arrangement in a nucleus was sketched. In some nuclei which seemed difficult to count the chromosomes, above procedure was repeated independently two or three times. And the coincided number was taken.

The chromosome number was counted on 54 spermatogonial nuclei by this method. Its results is shown in Table 1. It shows that about 70 percent of the nuclei examined gave counts of 44, though the counts falls relatively wide range, 41 to 49 in diploid number. And it can be concluded that 44 is the diploid number of the chromosomes of the sei whale.

Chromosomes in spermatocytes

The spermatocyte nuclei undergoing division were not common comparing with those of spermatogonia. Four primary spermatocytes were observed with spreaded chromosomes. And only one secondary spermatocyte nucleus was observed with the chromosomes spreading fairly well.

Though, only from these spermotocyte nuclei the final conclusion on the number of chromosomes of the sei whale could not obtained, all of them suggested the diploid number of chromosomes to be 44.

MORPHOLOGY OF THE CHROMOSOMES

Morphological measurements were made on the enlarged photograph of 10 spermatogonial nuclei which showed fine structure of the chromosomes, in parallel with microscopical observation.

After the total length and the length of the shorter arm of each chromosomes were measured, the total length was standardized by the following formula.

$$\text{R.L.} = \frac{\text{Total length of a chromosome}}{\text{Length of (21A's+X)}} \times 1{,}000$$

R.L.: Relative length of a chromosome
A : Autosome
X : X chromosome

TABLE 2. RELATIVE LENGTH, PERCENTAGE OF SHORTER ARM AND RATIO OF ARMS[1]

Serial No.	Relative length		Percentage of shorter arm	Ratio of arms[2]
	range	mean		
1	76–102	86	18	4.5
2	67–83	73	30	2.3
3	62–78	67	23	3.3
4	58–65	60	26	2.8
5	53–58	55	26	2.8
6	49–53	51	23	3.4
7	47–49	49	32	2.1
8	44–48	46	32	2.1
9	43–47	46	44	1.3
10	42–46	44	34	1.8
11	41–46	43	37	1.7
12	39–44	42	46	1.2
X	39–45	42	43	1.4
13	37–42	40	35	1.9
14	34–42	39	32	2.1
15	34–41	38	40	1.5
16	29–39	35	40	1.5
17	29–38	35	32	2.1
18	27–35	32	33	2.0
19	25–34	30	41	1.4
20	24–27	27	44	1.3
21	17–22	20	29	2.4
Y	7–12	9	—	—

1) Mean value obtained from ten spermatogonial nuclei.
2) Calculated from the former column.

The ratio of the two arms, which are devided by a kynetochore, are calculated by dividing the longer arm by the shorter. And the relative position of kynetochore is also shown by the percentage of the length of shorter arm to the total length of a chromosome.

Each spermatogonial chromosomes were placed into the homologous pairs, being based on the relative length, the relative position of kinetochore and the characteristic shape observed under microscope. A representative serial alignment is given in Fig. 1, which is a rearrangement of the chromosomes shown in PLATE 1.

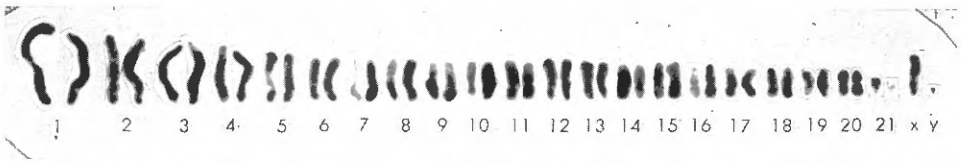

Fig. 1. Serially aligned arrangement of the paired chromosomes of the sei whale.

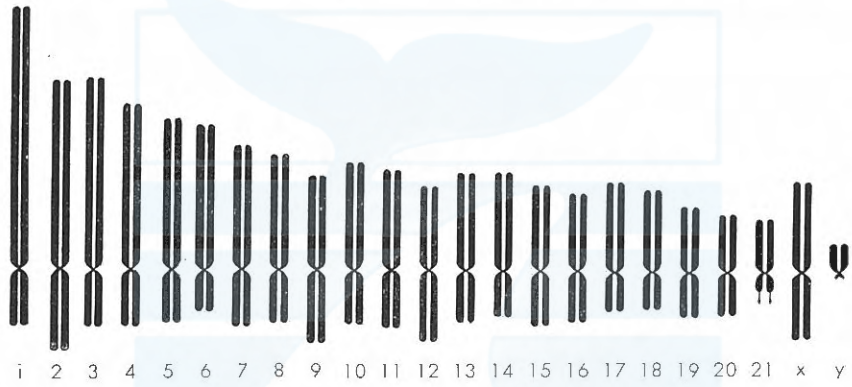

Fig. 2. Schematic figure of the chromosomes of the sei whale drawn from the measurements shown in Table 2.

The mean value of the measurements of chromosomes in 10 spermatogonial nuclei are shown in Table 2, in which the ratios of the two arms are calculated from the second column. Fig. 2 shows a schematic shape of chromosomes drawn from the value in Table 2. Out of 44 diploid complements, 42 elements can be recognized as constituting 21 homologous pairs, but 2 members are remained without mate of same size or shape. These are the sex-chromosomes. The longer one is considered to be X chromosome and the shorter Y chromosome. The length of X chromosome is nearly same with that of 12th autosome but the percentage of the shorter arm is smaller. It shows somewhat peculiir form having a vague constriction on the longer arm and can be distinguished relatively easily.

Y chromosome is the smallest in the 44 diploid chromosomes and its relative length is 9. Though it is difficult to observe the morphology, I suggest it to be a tero- or acrocentric chromosome.

The karyotype of the sei whale is the polymorphic same as that of many mammals, and the lengthes of chromosomes distribute continuously from the longest to

the shortest. It is usually very difficult to classify the chromosomes without taking into consideration the relative length and the position of the kinetochore of the all chromosomes in a nuclei. When they are considered, the chromosomes can be classified into some groups.

As shown in Table 2 and Fig. 2. the chromosomes from 1st to 6th are acrocentric and have relatively larger length. The ratio of arms is 2.3 or more.

The 7th and 8th have medial length and their ratios of arms are 2.1. The ratio of arms of the 9th is 1.3 and kinetochore seems to situate nearly middle of the chromosome. The 10th and 11th chromosomes have the nearly same length and ratio of arms. The 12th and X chromosomes have the same length and both are metacentric chromosomes but the latter have the shorter arms than the former.

From the 13th to the 18th chromosomes are composed with acrocentric and relatively shorter chromosomes, and it is difficult to distinguish each other. Their ratios of arms fall between 1.5 and 2.1.

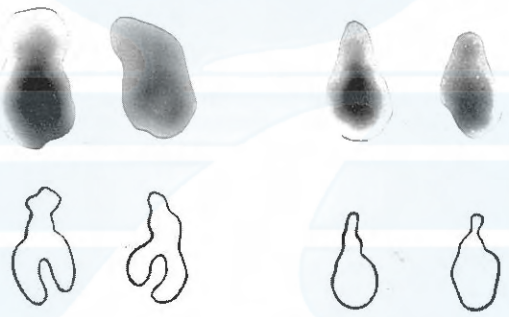

Fig. 3. Satellite on the 21st autosome.

The 19th and 20th are nearly metacentric chromosome, the ratios of the arms are 1.4 and 1.3 respectively. The 21st is an acrocentric chromosome of which ratio of the arms is 2.4. A satellite is observed on the shorter arms of this chromosome, which are shown on Fig. 3. The Y chromosome, which is the shortest chromosome, was already mentioned above.

When the karyotype of the sei whale is compared with that of the Dall's porpoise (Makino, 1948) some resemblances are found, namely the total number, X chromosome with the length corresponding to 10th or 11th (in the case of sei whale 12th), Y chromosomes with shortest length and autosomes composed of metacentric and acrocentric elements. But when compared precicely, some differences are found. In the case of the Dall's porpoise (Makino, 1948) the 5th and 6th elements are metacentric chromosomes and other relatively larger chromosomes are the acrocentric, and he suggest the members of the 9th to the 21st pairs to be acrocentric.

Among relatively larger autosomes of the sei whale, the 9th and 12th are metacentric or V shaped chromosomes. And I think that these differences of karyotypes between the two species are not unreasonable, when it is considered that they belong to the different suborder or Odontoceti and Mysticeti.

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EXPLANATION OF PLATES

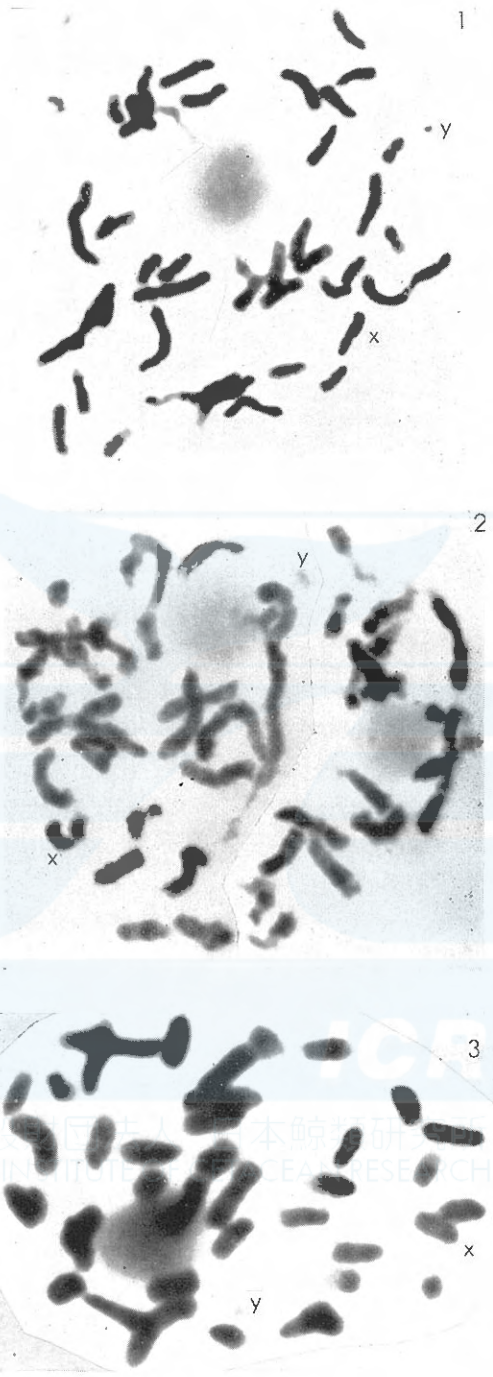
PLATE I

Fig. 1–3. Spermatogonial nuclei of the sei whale at the late prophase of the division.

PLATE II

Fig. 1. Spermatogonial nucleus of the sei whale at the late prophase of the division.

Fig. 2. Spermatocyte nucleus at the second division.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