THE KARYOTYPE OF THE FIN WHALE

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INTRODUCTION

THE chromosomes of a whale were studied for the first time by MAKINO (1948) in Dall's porpoise (*Phocoenoides dallii* TRUE). He determined the chromosome number to 2n=44 and described 22 bivalents in male meiosis, including one heteromorphic pair. A second report on whale chromosomes appeared in 1955, when NOWOSIELSKI-SLEPOWRON and PEACOCK published their observations in three species, the blue whale (*Balaenoptera musculus* RAFINESQUE), the fin whale (*Balaenoptera physalus* L) and the sperm whale (*Physeter catodon* L), from material collected during the antarctic whaling season of 1946—47. Since the chromosome analyses of these authors were all made on prophase stages of spermatogonial cells and spermatocytes, their counts were highly approximate. They estimated the haploid numbers of all three species to 24 but did not find the corresponding diploid number of 48, the highest diploid number counted by them being 39.

The first authors to utilize modern tissue culture technique for chromosome analysis in whales were WALEN and MADIN (1965), who described the karyotypes of two whale species, the bottlenosed dolphin (*Tursiops truncatus* MONT.) and the pilot whale (*Globicephala scammonii* COPE) from cultured kidney cells. Both species had 2n=44 and the karyotypes showed great similarities. In 1966, the karyotype of the sei whale (*Balaenoptera borealis* LESS.) was reported by KASUYA, and the chromosome number of 2n=44 was found in direct preparations of spermatogonial cells.

Of the species of whales so far studied, the blue, fin and sei whales belong to the whalebone whales (Mysticeti), the sperm, pilot, Pacific killer whale, Dall's porpoise and the bottlenosed dolphin belong to the toothed whales (Odontoceti).

The purpose of the present paper is to describe the somatic chromosomes of the fin whale, determine the chromosome number and con-

struct an idiogram from measurements of the chromosomes of a number of mitotic metaphases, utilizing the tissue culture technique.

Material and methods

The material was collected at a whaling station in Hvalfjördur located about 50 miles from Reykjavik, Iceland. Three whale species are brought to this station, viz. the fin, sei and sperm whales. The whales are caught 150 to 200 miles offshore and are brought into the station usually some 20 to 30 hours after death.

Tissue samples were taken of lung, heart, kidney and spleen from adults and fetuses of the fin whale. In addition, lung tissue of the sperm whale was taken. In both species, the confluent cell method was used, and in the sperm whale also the plasma clot method. Cell growth was recorded in 3 out of 70 milk dilution bottles set up of the fin whale. These samples were all from lung tissue, two were from the same male individual, and the third was from a female. No growth was recorded in any of the cultures from the sperm whale. The low proportion of successful cultures may be due to the fact that temperature does not go down in the killed animals during their transport to the station, and this may cause unsuitable conditions for the cells. The two animals from which viable cultures were obtained had been dead in 21 to 22 hours. The female was 65 feet in length, the male 61. The sperm whales have always been dead a longer period of time than the fin whales, when brought into the station.

Initially Hank's-Eagles medium was used, but after passage 3 of the male cells it was substituted by Parker 199; both media were used with 20 % calf serum and with penicillin and streptomycin. The change to Parker seemed to better the conditions of the cultures. After 7 months and 18 passages the male cells still showed an unchanged karyotype. The female cells died after little more than one month in culture; they were then in passage 6.

For chromosome preparations, rapidly growing cultures were treated for one hour with colcemid (Ciba) in the concentration 0.2 μg/ml medium. After trypsinization and hypotonic treatment in medium diluted 3:1 with distilled water, the cells were fixed in 9 parts of 60 % acetic acid and 1 part of 1 N hydrochloric acid. After 20 minutes the fixative was discarded, the cells resuspended in 2 % orcein in 60 % acetic acid and squashed under microscopic control.

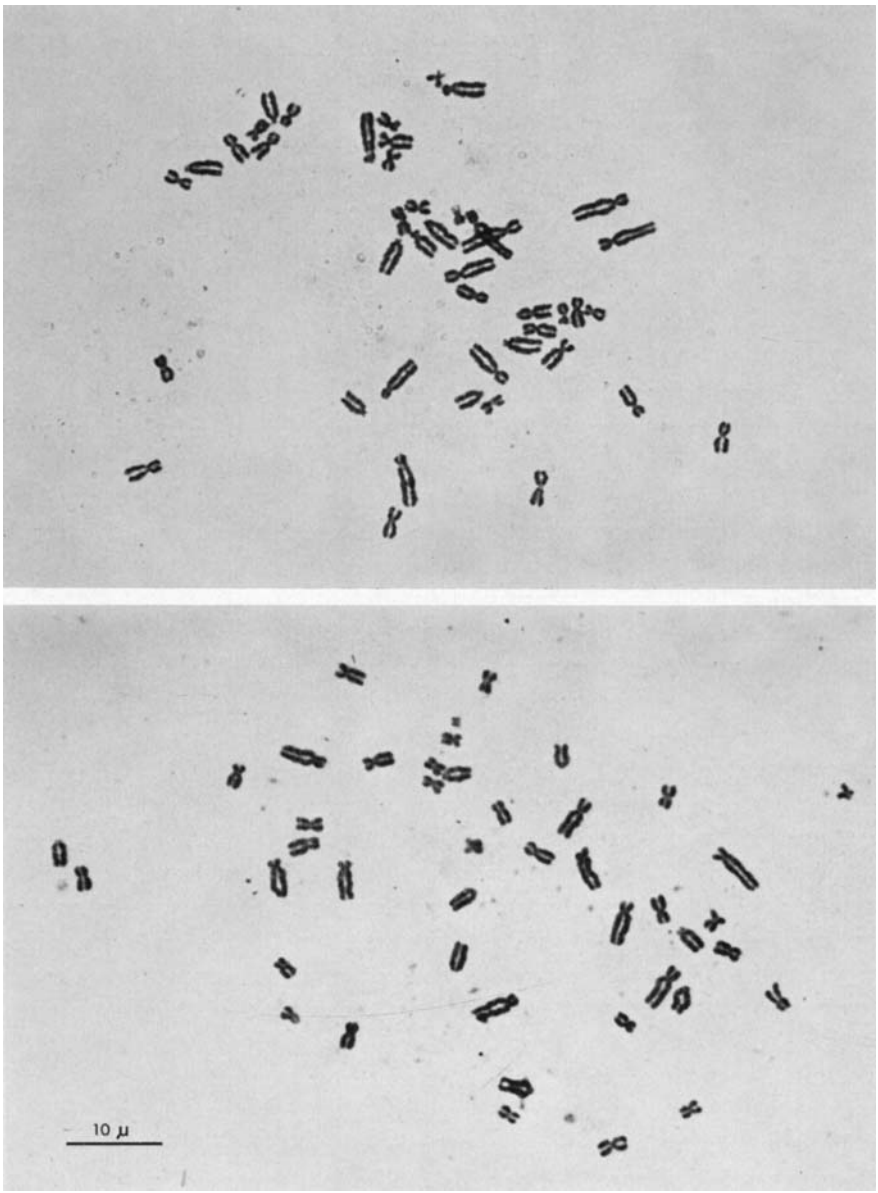

Fig. 1. Metaphase chromosomes of *Balaenoptera physalus*. Female cell above, male cell below.

TABLE 1. *Grouping of the whale chromosomes according to arm ratio.*

Group	Arm ratio	Number of autosome pairs	Sex chromosomes
m	1.0—1.7	8	Y
sm	1.7—3.0	6	
st	3.0—7.0	3	X
t	7.0—∞	4	

OBSERVATIONS

The chromosome observations of the present investigation were all made in cells from passage 3. Chromosome numbers were determined in the only 2 female cells analyzable and in 50 male cells. The 2 female cells and 48 of the male cells had 2n=44, 1 male cell had 43 and 1 had 45. The general appearance of the metaphase chromosomes is seen in Figure 1, in which one female and one male cell at metaphase are presented with their chromosomes *in situ*.

Six male cells and one female cell were analyzed and the chromosomes measured by the present author, and five additional male cells were analyzed and measured by Dr. A. LEVAN. The chromosomes were divided into 4 groups (Table 1) according to the location of their centromeres, as suggested by LEVAN and collaborators (1964). Within each group, the chromosomes were numbered after falling length, except X and Y which were not given numbers.

The results of the measurements of the 12 metaphases are summarized in Table 2, in which average lengths, variation extremes and arm indices are given for each chromosome type. The length measurements are in microns. The two sex chromosomes represent the extreme length limits of the karyotype, X being the longest chromosome with an average length of 8.7 μ, and Y the shortest, 1.1 μ. This amplitude in size between the X and the Y is among the greatest so far found in mammals. It is a characteristic feature that the longest chromosomes of the whale karyotype are assymmetric, whereas the m group chromosomes are all medium-sized or small. The total average length of the whale karyotype is 102 μ, which is roughly the same total length as in man. In Figure 2, the chromosomes are arranged into a female and a male karyotype. The arrangement is the same as in Table 2, except that the X and Y chromosomes have been placed separate from the other chromosomes of their groups.

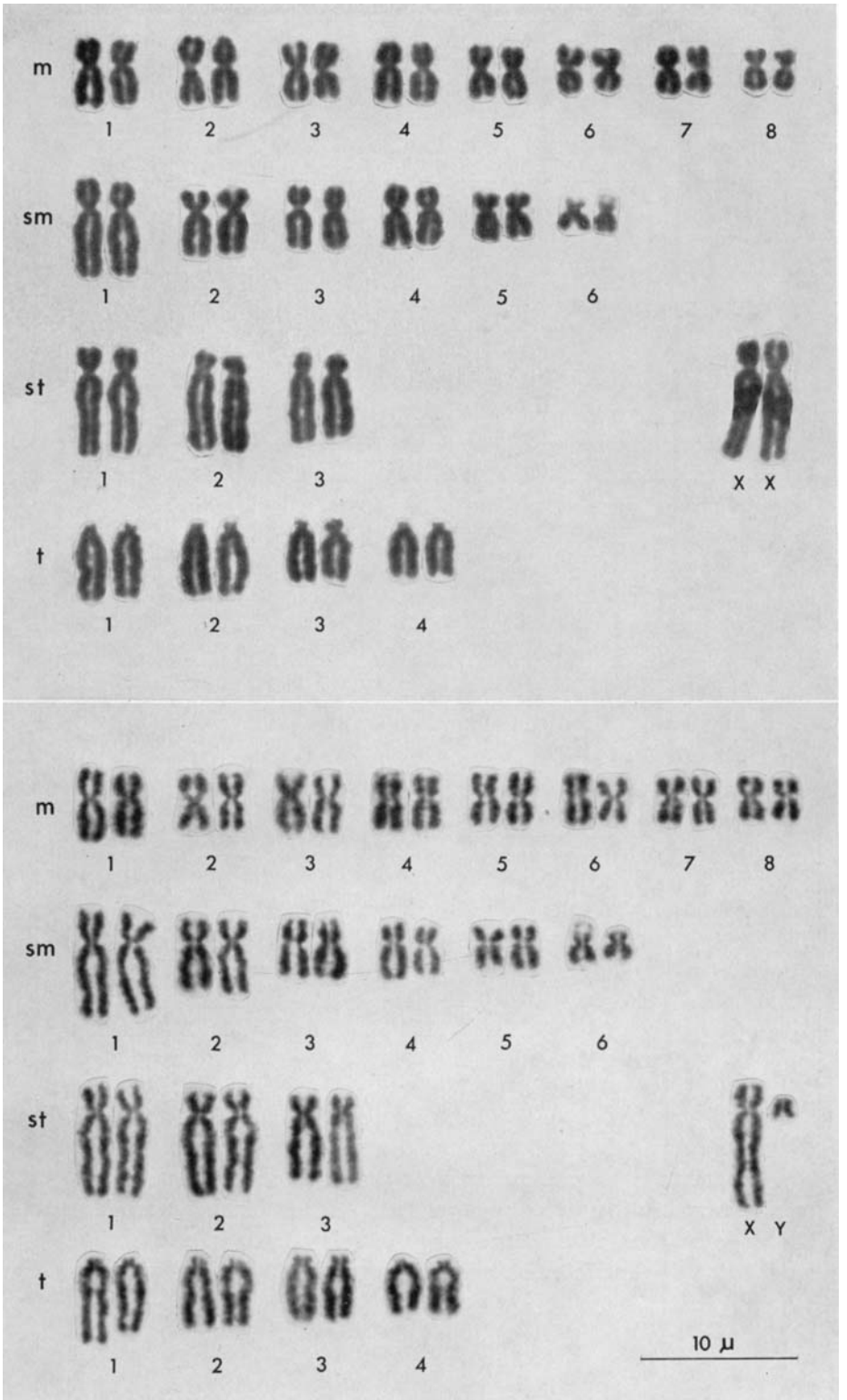

Fig. 2. The karyotype of *Balaenoptera physalus*.

TABLE 2. *Absolute measurements and arm ratio of chromosomes of twelve cells.*

Chromosome group	Number in group	Average length, μ	Variation exstremes, μ	Average arm ratio
m	1	4.6	3.5—6.1	1.61
	2	4.4	3.3—5.4	1.11
	3	4.2	2.9—5.8	1.45
	4	3.8	2.7—5.2	1.40
	5	3.4	2.7—4.3	1.11
	6	3.2	2.4—3.9	1.24
	7	3.0	2.4—3.8	1.14
	8	2.8	2.1—3.8	1.26
	Y	1.1	0.7—1.6	1.20
	Total	30.5	0.7—6.1	
sm	1	7.2	5.0—9.6	2.43
	2	4.8	3.8—6.4	2.18
	3	4.2	3.0—5.9	1.96
	4	3.7	2.5—5.1	1.89
	5	3.1	2.4—4.1	1.86
	6	1.9	1.2—2.4	2.52
	Total	24.9	1.2—9.6	
st	X	8.7	6.9—10.6	3.72
	1	7.6	5.7—10.4	3.43
	2	7.1	5.3— 9.5	3.79
	3	6.1	4.3— 7.7	4.36
	Total	29.5	4.3—10.6	
t	1	5.4	4.0—7.8	20.2
	2	4.8	3.4—6.2	21.4
	3	4.4	3.3—5.8	8.4
	4	3.8	2.8—5.0	16.3
	Total	18.4	2.8—7.8	
	Grand total	103.3	0.7—10.6	

Chromosome pair sm 6 had small satellites on the short arms. Usually the satellites were clearly visible only in one member of the pair, but occasionally they were seen in both. Association between the satellited ends of these chromosomes was often noticed. In one instance, in one of the two female cells, a satellite was observed proximally in one

TABLE 3. *Relative length and arm ratio. Comparison between measurements by the author (U A) and by Albert Levan (A L).*

Chromosome group	Number in group	Relative chromosome length			Arm ratio		
		U A	A L	t	U A	A L	t
m	1	45.6±0.41	43.1±1.46	1.649	1.53±0.042	1.73±0.095	0.608
	2	43.2±0.69	42.1±1.02	0.854	1.14±0.035	1.06±0.024	1.905
	3	40.6±0.46	41.5±0.83	0.926	1.40±0.078	1.53±0.026	1.585
	4	37.9±0.59	35.3±1.50	2.009	1.43±0.063	1.36±0.021	1.061
	5	34.5±0.46	31.5±0.55	4.156**	1.11±0.017	1.10±0.014	0.455
	6	31.7±0.47	29.8±0.46	2.866*	1.20±0.044	1.30±0.093	1.961
	7	30.8±0.52	28.4±0.34	3.850**	1.17±0.038	1.11±0.026	1.304
	8	28.0±0.31	26.5±0.78	1.740	1.24±0.035	1.29±0.078	0.588
	Y	12.0±0.74	7.9±0.46	4.775**	1.19±0.049	1.22±0.056	0.400
sm	1	68.0±0.85	72.6±0.99	3.540**	2.47±0.026	2.38±0.138	0.647
	2	47.5±0.72	48.7±0.66	1.302	2.10±0.038	2.29±0.124	1.462
	3	40.8±0.78	44.0±0.64	3.168*	1.87±0.045	2.09±0.064	2.716*
	4	36.2±0.91	37.1±0.87	0.719	1.80±0.052	2.02±0.138	1.528
	5	30.4±0.61	30.3±1.43	0.570	1.73±0.019	2.04±0.080	3.780**
	6	19.4±0.61	17.7±0.85	1.614	2.39±0.138	2.71±0.483	1.576
st	X	83.9±1.18	86.2±2.10	0.922	3.69±0.099	3.77±0.162	0.133
	1	72.7±1.11	75.6±2.41	1.093	3.34±0.100	3.55±0.065	1.765
	2	68.8±1.19	70.1±0.76	0.899	3.77±0.127	3.81±0.203	0.167
	3	59.2±0.92	59.3±1.12	0.035	4.17±0.128	4.62±0.093	2.848
t	1	52.8±0.80	53.6±0.58	0.801	16.91±0.593	24.72±1.330	5.379***
	2	46.8±0.73	46.9±0.90	1.036	20.64±3.980	22.39±1.361	0.416
	3	43.4±0.62	42.8±0.57	0.773	7.85±0.422	9.13±0.405	2.188
	4	37.8±0.43	36.8±0.55	1.445	16.52±2.451	15.97±1.763	0.182

of the t chromosomes. In a few cases, secondary constrictions were seen in almost all of the t chromosomes. They were located near the middle of the long arm. Interphase nuclei contained clearly visible nucleoli and heterochromatic bodies. A sex chromatin body was visible in most female cells, even though the material had been exposed to hypotonic treatment.

The absolute lengths of the chromosomes were converted into relative values, viz. fractions of the total chromosome length of the haploid X containing female genome, this being made equal to 1000 units. Average values of the relative length measurements and the arm ratios,

together with their standard errors are given in Table 3. As seen, the material has been divided into the two sets of measurements made by the author (UA) and by Dr. LEVAN (AL), respectively. This has been done, because the techniques employed in the two cases were different, the UA set being measured on photographs at a magnification of 4500 times, the AL set on camera lucida drawings at 7000 times. The agreement between the two sets is good and the differences are within reasonable limits of personal and optical errors (cf. HSU and ZENZES, 1964). It was attempted to minimize the optical error in both series, in the UA series by utilizing several negatives from each cell and only measuring chromosomes near the optical axis of the picture, in the AL series by moving each chromosome into the center of the view field before drawing.

In the table, the t values and the statistical significances for the differences between the two sets of measurements (UA and AL) have been calculated both for lengths and arm ratios. The high t values for the measurements of chromosomes m 5, m 6 and m 7 may be accounted for in the following way. The length differences between these three pairs are small and their arm ratios similar. Thus, the chromosomes could not be identified individually but were placed in length order, the two longest as pair 5 and the two shortest as pair 7. In doing so, automatically differences between the chromosomes within each pair are minimized and thus result in lower standard errors with consequent higher t values between the two sets of measurements. The relatively high t values in these pairs are therefore an artificial effect of the impossibility to distinguish these pairs from each other. In the case of the Y chromosome, in which the two sets of measurements also show a rather great difference, it should be remembered that only half the number of measurements are available compared with the autosomes, which will give more leeway for factors of coincidence.

On close analysis of the length values of Table 3, a small but consistent disagreement becomes apparent between the two sets of measurements. If all chromosomes are arranged in order of length this disagreement becomes evident (Table 4). The differences UA minus AL become negative in the 8 longest chromosomes, and all positive in the 8 shortest, whereas in the 7 chromosomes of middle length, 4 differences are positive and 3 negative. In other words, the UA measurements are consistently shorter than the AL measurements in long chromosomes, but longer in short chromosomes. This can only mean that there is a constant discrepancy between the two methods of

TABLE 4. *Differences between average length measurements U A—A L.*

Number in order of length	Number in karyotype	Difference between averages of U A and A L
1	X	−2.3
2	st 1	−2.9
3	st 2	−1.3
4	sm 1	−4.6
5	st 3	−0.1
6	t 1	−0.8
7	sm 2	−1.2
8	t 2	−0.1
9	m 1	+2.5
10	t 3	+0.6
11	m 2	+1.1
12	sm 3	−3.2
13	m 3	−0.9
14	t 4	+0.1
15	sm 4	−0.9
16	m 4	+2.6
17	m 5	+3.0
18	m 6	+1.9
19	sm 5	+0.1
20	m 7	+2.4
21	m 8	+1.5
22	sm 6	+1.7
23	Y	+4.1

measurement, probably in the decision in each chromosome, where the outer boundary of the end is. It seems that a little more of the halo, always present in the microscopic picture, has been included in the UA measurements than in the AL measurements. This would tend to make the smaller chromosomes relatively larger and the large chromosomes relatively smaller. This would also influence the arm ratios of asymmetric chromosomes making them lower in the UA than in the AL series. That this is actually so is seen in Table 3, in which all of the 14 arm ratios of sm, st and t chromosomes, except two (sml and t4), agree with this supposition. That the present discrepancy between measurements on photographs and drawings is perhaps dependent on the two techniques employed rather than on personal idiosyncrasies of the two workers responsible for the present measurements is indicated by similar results obtained earlier in the Cancer chromosome laboratory, when similar sets of measurements have been compared.

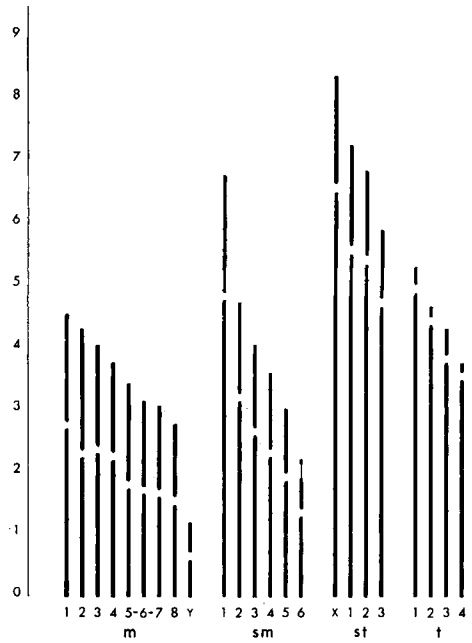

Fig. 3. The idiogram of *Balaenoptera physalus*. Chromosomes m 5-6-7 cannot be safely identified.

The idiogram of Figure 3 is based on the values of Table 3, and the chromosome lengths are given as fractions of the haploid female set. Chromosomes that cannot be identified safely have their numbers joined with hyphens. Although chromosomes st 1 and st 2 are similar on the idiogram, they are distinguishable by the aid of their general appearance.

COMPARISON BETWEEN THE WHALE SPECIES

Various authors have discussed the phylogeny of the whales on the basis of their anatomy and paleontology without reaching agreement as to whether the phylogenetic development of the whales should be considered as mono- or diphylogenetic (for references *see* VALEN, 1968). In this connection, it may be of interest to make a comparison karyologically between those species in which the chromosomes are known. As pointed out above, the chromosomes have been analyzed with techniques suitable for measurements only in four whale species, the fin

TABLE 5. *Comparison between four whale species.*

Whale species	Autosomes[1]				Sex chromosomes			
	m	sm	st	t	m	sm	st	t
Fin whale	8	6	3	4	Y	—	X	—
Sei whale	6	12	3	0	X	—	—	Y
Pilot whale	8	6	3	4	Y	X	—	—
Bottlenosed dolphin	9	7	2	4	—	—	—	—

[1] In the bottlenosed dolphin the identity of X and Y is not known; they are therefore included among the autosomal counts.

whale, the sei whale, the bottlenosed dolphin and the pilot whale, and therefore the comparison has to be limited to these species. All of them have 44 chromosomes somatically, just as the Dall's porpoise studied by MAKINO.

In Table 5, the chromosomes of the four species are arranged into groups according to their arm ratios. In the pilot whale and the bottlenosed dolphin, this arrangement is based on measurements on the photographs in WALEN and MADIN (1965), and thus only tentative, but should still give a fairly good indication of the relations between the chromosomes of these species. It appears that there is great similarity between the karyotypes of three of the species, viz. the fin whale, pilot whale and bottlenosed dolphin, whereas the sei whale shows some differences. These, however, may be due to the fact that different methods were employed in the preparation of the chromosome slides of the sei whale. The similarity between the karyotypes of the fin whale, a whalebone species, and the two tooth whales undoubtedly supports the idea of a monophylogenetic origin of the whales.

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SUMMARY

The somatic chromosomes of the fin whale (*Balaenoptera physalus* L.) were studied and measured in preparations from cultures of lung tissue and an idiogram was made from measurements of all chromosomes of seven cells. The chromosome number of the fin whale is 2n=44 with an unusually long X chromosome. A tentative comparison between the karyotype of the fin whale and three other species of whales yielded some viewpoints on the phylogeny of the whales.

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