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Cytogenetic Studies of Two Species of Porpoise

THIS investigation was undertaken to compare the chromosomes of two species of porpoise, the Atlantic bottlenose (*Tursiops truncatus*) and the Pacific white-striped porpoise (*Lagenorynchus obliquidens*), both small odontacete cetaceans of the family Delphinidae. The chromosome number and karyotypes were established for a male and female *T. truncatus* and for two males and one female *L. obliquidens*.

The animals used in this study are members of the cetacean research colony, maintained at the Marine Biology Facility, U.S. Naval Missile Center, in California. The two species differ markedly in external appearance. *T. truncatus* dwells mostly in coastal or inshore waters, often swimming into bays and rivers[1]. They are common in the Atlantic Ocean along the East and Gulf coasts of the United States, as well as in the Mediterranean Sea and several other areas of the world. *L. obliquidens* ranges from Mexico to Alaska in the deeper offshore waters of the eastern Pacific ocean.

Blood samples were obtained by venipuncture of small vessels which run laterally down the centre of each side of the fluke. About 10 ml. of heparinized blood were withdrawn and refrigerated or kept at room temperature for periods not exceeding 2 h before a culture was set up. The microtechnique for culturing leucocytes from whole blood described by Arakaki and Sparkes[2] was modified slightly for use with porpoise blood. The type of medium used, duration of incubation of the cultures and the amount of phytohaemagglutinin (PHA) were varied to determine the optimal conditions for preparation of metaphase chromosomes. The samples from *L. obliquidens* were the easiest to obtain and to culture; almost every modification produced satisfactory preparations, while those from *T. truncatus* were of limited success. Table 1 summarizes these results. In general the chromosome preparations were inferior to those obtained from human blood, but were satisfactory for counting chromosomes and for establishing karyotypes for these mammals.

Metaphases were accumulated by exposure to colchicine for 4 h. After incubation the cells were subjected to hypotonic treatment with 0·95 per cent sodium citrate, washed in methyl alcohol and glacial acetic acid fixative (3 : 1) mounted on slides by the air-dry method and stained with aceto-orcein. To establish a karyotype which could be regarded as representative, the chromosomes of several cells from each porpoise were counted, photographed and karyotyped. These results are summarized in Table 2 and the karyotypes are illustrated in Figs. 1 and 2.

The modal chromosome number is constant at forty-four for both species, male and female, and the chromo-

Table 1. SUMMARY OF RESULTS WITH VARIED TISSUE CULTURE CONDITIONS

Culture media	Culture results	
	L. obliquidens	*T. truncatus*
(1) Eagle's MEM (2 ml.) with: 20 per cent foetal calf serum, 0·2 ml. of whole blood and 0·01 ml. of PHA-P*	Good	Poor
(2) TC 199 (5 ml.) with: 20 per cent foetal calf serum, 0·7 ml. of leucocyte rich plasma and 0·2 ml. of PHA-M*	Good	Fair
(3) Eagle's MEM Spinner (5 ml.) with: 20 per cent foetal calf serum, 0·7 ml. of leucocyte rich plasma and 0·2 ml. of PHA-M*	Good	Good metaphases but few in number
(4) NCTC 109 (5 ml.) with: 20 per cent foetal calf serum, 0·7 ml. of leucocyte rich plasma and 0·2 ml. of PHA-M*	Very good	Poor

Incubation time: 3–4 days—acceptable results.
More than 4 days—clots formed and few metaphases seen.
Optimum conditions: *L. obliquidens:* NCTC 109 incubated for 3 days.
T. truncatus: Eagle's MEM Spinner incubated for 3 days.

* 'Difco', as reconstituted.

somes can be readily paired. There seemed to be sufficient diversity in size and morphology of the chromosomes to warrant an attempt to establish provisional karyotypes for these animals. Comparison of karyotypes between individuals of the same and different species supported the establishment of a similar karyotype for both species.

The chromosomes are sub-classified into groups given designations of *A*, *B*, *C*, *D*, and the sex chromosomes (*X*, *Y*). Within the chromosome groups most of the individual pairs of chromosomes can be recognized and identified. Group *A* contains the first five pairs of large sub-metacentrics: the centromere of *A*-1 is more medial than *A*-2 although both pairs are about the same size; the centromere of *A*-3 is less medial than *A*-4 and is slightly larger; *A*-5 is the smallest of this group. The long arms of one *A*-2 chromosome seemed to be consistently longer than those of its paired chromosome. Group *B* comprises the smaller sub-metacentrics arranged in order of decreasing size. The centromere position is also characteristic in that it is: sub-median in *B*-6; sub-terminal in *B*-7; sub-terminal in *B*-8; sub-median in *B*-9; sub-terminal in *B*-10; sub-median in *B*-11; and sub-median in *B*-12.

Group *C* consists of four metacentric or very nearly metacentric pairs of chromosomes. *C*-13 is distinctly larger than the other three. *C*-14 to *C*-16 become progressively smaller; *C*-16 is slightly smaller than the last sub-metacentric, *B*-12. Group *D* is composed of acrocentric pairs; *D*-17 is considerably larger than any of the other pairs in group *D*. *D*-21 is the smallest of the autosomes. Because of the size of this pair in our preparations it was not possible to determine accurately whether

Table 2. SUMMARY OF METAPHASE ANALYSES

Animal studied	No. of cells in which chromosomes were counted	No. of chromosomes/cell	No. of karyotypes
Tursiops truncatus, male	5	44	4
Tursiops truncatus, female	5	44	5
Lagenorynchus obliquidens, male (*A*)	5	44	4
Lagenorynchus obliquidens, male (*B*)	4	44	3
Lagenorynchus obliquidens, female	8	44	4

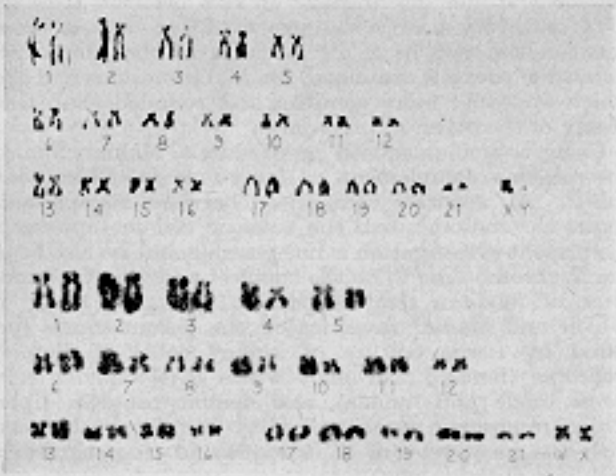

Fig. 1. Representative karyotypes of male (top) and female (bottom) of porpoise species *T. truncatus*. The pairs of chromosomes have been numbered and grouped according to scheme outlined in text. The male and female seem to differ only in their sex chromosome make-up. The very small *Y* chromosome is paired with the *X* in the male.

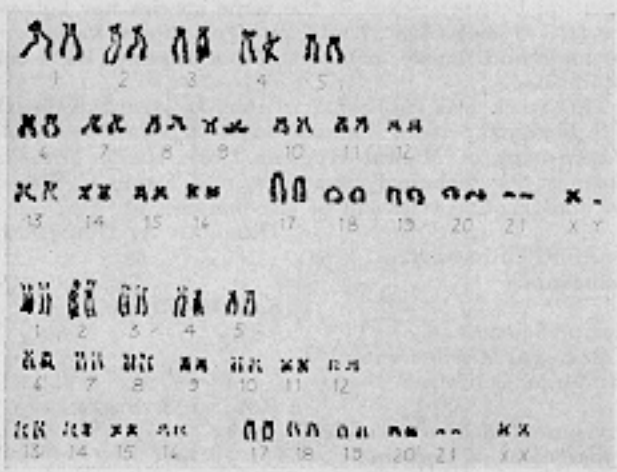

Fig. 2. Representative karyotypes of male (top) and female (bottom) of porpoise species *L. obliquidens*. Comparison with the karyotypes of species *T. truncatus* in Fig. 1 shows no obvious gross differences.

it was best grouped with the acrocentrics or sub-metacentrics, but as the smallest it was given the last of the autosomal designations.

The sex chromosomes are of the *X*, *Y* type, characteristic of mammals. The males have one *X* chromosome and one *Y*, the females two *X* chromosomes. The *Y* chromosome is the smallest of all the chromosomes. In two preparations it appeared to be sub-metacentric but generally the centromere was indistinct. The *X* chromosome is

metacentric and intermediate in size between *C*-14 and *C*-15 and very nearly the size of *B*-11; the difference between the two is in the position of the centromere. In most of the cells examined, the *X* chromosome had arms which appeared more compact and rounded than those of any of the other chromosomes.

Using testicular squash preparations, Makino found a chromosome complement of forty-four in *Phocoenoides dalliii*[3]. An accurate comparison between his spermatogonial chromosomes and the somatic cell metaphases of the present investigation is not possible, but he also found the *Y* chromosome to be the smallest and the *X* chromosome of medium size comparable with pair 10 or 11. Walen and Madin[4] investigated the chromosomes (prepared by tissue culture of kidney cells) of *Tursiops truncatus* (female) and pilot whale (*Globicephala scammonii*, male and female), and demonstrated a diploid ($2n$) chromosome number of forty-four for both species with the karyotype of *T. truncatus* in close agreement with our observations.

A diploid ($2n$) chromosome number of forty-four has been found for two species of porpoise, *Tursiops truncatus* and *Lagenorynchus obliquidens*. Except for differences in sex chromosomes between male and female, there appear to be no obvious gross differences in chromosomal constitution between sexes in the same species nor between the two species (Figs. 1 and 2). Provisional karyotypes for male and female members of both species have been established.

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