Preparation of Fish Chromosomes by *in Vitro* Colchicine Treatment

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Cytogenetic studies of fish progressed markedly in the last decade. The objectives of such studies range from taxonomy or systematics to genetics with a variety of methods of chromosomal preparation. In most methods, material fish was treated with colchicine in advance and gills, kidney or other tissues were used for chromosome preparation (McPhail and Jones, 1966; Arai, 1973; Gold, 1974; Kligerman and Bloom, 1977). More than five hundred fish species have been examined for karyotype. The results were reviewed by Park (1974) and Ojima et al. (1975).

Various tissue culture methods using leukocyte (Labat et al., 1967; Heckman and Brubaker, 1970; Ojima, 1970), the scale (Denton and Howell, 1969; Ojima et al., 1972; Kobayasi, 1975), and other tissues (Roberts, 1964; Chen, 1970; Yamamoto and Ojima, 1973) have been reported. Fish chromosomes have also been analyzed by use of banding techniques (Howell and Bloom, 1973; Abe and Muramoto, 1974). Most techniques of these workers, however, seem not to be applicable to large or migratory species, hard to transport to or to keep alive in a laboratory. With a hope of overcoming such difficulties, a simple and rapid technique was developed.

Materials and methods

Materials were sampled from fish caught by two separate set-nets located along the coast of Okkirai Peninsula (39°06′N, 141°53′E) and another of Ryori Peninsula (30°03′N, 141°53′E), Iwate Prefecture, Japan. These include the three species, *Thunnus thynnus* (Linnaeus), *Scomber tapeinocephalus* Bleeker, and *Seriola quinqueradiata* Temminck et Schlegel, taken between autumn of 1976 and spring of 1977, and ranging 55 to 100 cm, 26 to 35 cm, and 26 to 26.5 cm of fork length, respectively (Table 1).

Classification of chromosomes is adopted from Levan et al. (1964).

Medium solution for incubating the material tissue was prepared and tissues of gills were processed by the following procedures.

1) An isotonic incubating medium (Ringer solution or sea water one-half diluted with distilled water), containing 0.003 to 0.005% colchicine, was saturated with pure oxygen gas or oxygen with 5% carbondioxide at room temperature, immediately before use.

2) Remove the gill from animal by a pair of scissors immediately after being sacrificed. Pieces (ca. 0.3 to 0.5 g, ca. 1 cm long) of tissues of the gill washed by sea water were soaked in incubating medium and shaken gently for 3 to 10 hours continuously, being longer for larger or less active fish.

3) Place tissue pieces in one of the three hypotonic solutions, 0.005 N potassium chloride, 0.005 N sodium citrate or distilled water, at room temperature for 30 to 60 minutes.

4) Fix tissues in Carnoy solution, containing methanol and acetic acid (2: 1) at least for one hour.

5) Pick tissue pieces up from Carnoy solution by a forceps, and tap to spread them on a cooled slide glass (ca. 5°C) for a proper density of cells.

6) Dry the slide glass by a alcohol lamp and leave it at room temperature for a few hours. The staining is performed by Giemsa solution. Mount the preparation with a cover glass, if necessary.

Table 1. List of fishes examined.

Species	Date sampled	Number of fish	Fork length range (cm)
Thunnus thynnus	October, November 1976 and May 1977	5	55.0～100.0
Scomber tapeinocephalus	November 1976	3	25.0～ 35.0
Seriola quinqueradiata	November 1976	2	26.0～ 26.5

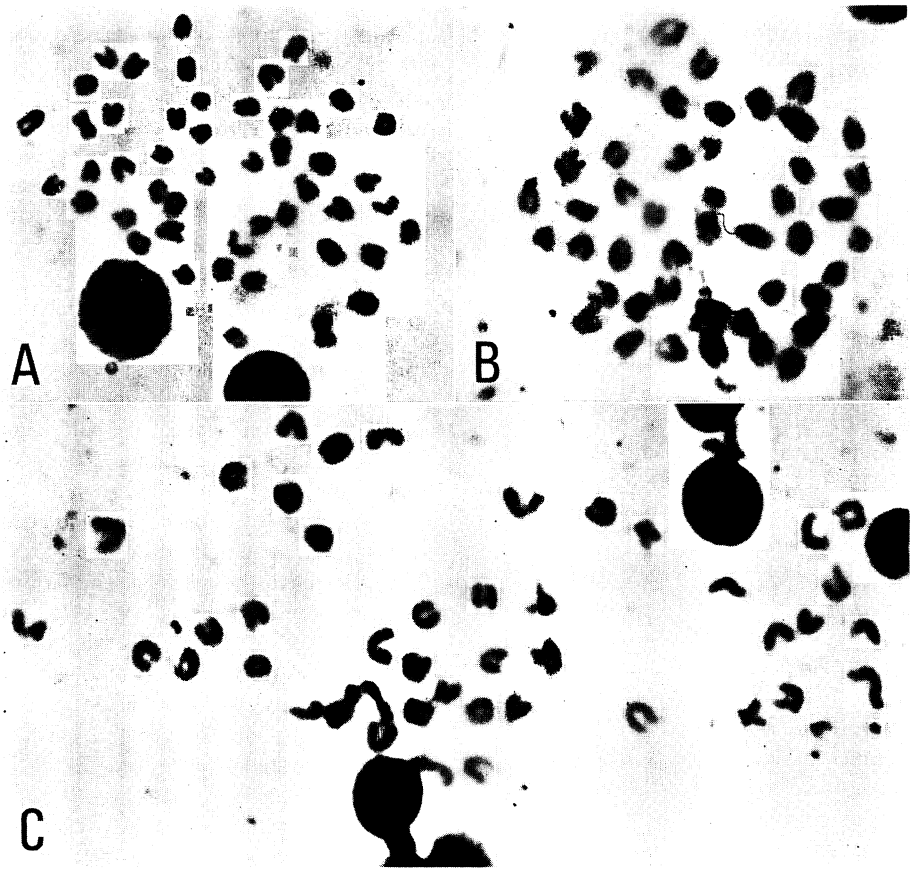

Fig. 1. A: Mitotic metaphase chromosomes from *Thunnus thynnus*, ×1600. B: *Scomber tapeinocephalus*, ×1340. C: *Seriola quinqueradiata*, ×1490.

Table 2. Karyotypes of two scombrid and a carangid fishes.

Species	Cells examined	Karyotypes				2n	Number of arms
		M	SM	ST	A		
Thunnus thynnus	27	2	0	2	44	48	50
Scomber tapeinocephalus	26	0	0	2	46	48	48
Seriola quinqueradiata	36	0	2	2	44	48	50

Results and discussion

Microscopic photographs of mitotic metaphases from gill cells in the three species prepared through the present techniques are shown in Figs. 1 and 2. Among three hypotonic solutions used, potassium chloride gave the best chromosome figures. Their karyotypes are listed in Table 2, indicating that the diploid chromosome number is 48 in all species. The karyotype comprises one pair of metacentrics (M), two pairs of subtelocentrics (ST), and twenty-two pairs of acrocentrics (A) in *Thunnus thynnus*, one pair of subtelocentrics and twenty-three pairs of acrocentrics in *Scomber tapeinocephalus*, and one pair of submetacentrics (SM), one pair of subtelocentrics, and twenty-two pairs of acrocentrics in *Seriola quinqueradiata*.

Significance of the present techniques can be stated as follows:

1) Applicable to fish species, which are hard to handle for colchicine treatment due to their

Fig. 2. A: Karyotype of *Thunnus thynnus*, from Fig. A, 2n=48 (M: 2, ST: 2, A: 44). ×1400, B: *Scomber tapeinocephalus*, from Fig. B, 2n=48 (ST: 2, A: 46), ×1170. C: *Seriola quinqueradiata*, from Fig. C, 2n=48 (SM: 2, ST: 2, A: 44), ×1300.

large size or high speed.

2) Saving for colchicine. Our experiments indicated that only 1.5 to 2.5 mg colchicine suffice for tuna 100 cm long or ca. 30 kg by weight, whereas 750 to 1,200 mg colchicine will be necessary with the conventional *in vivo* treatment to the same sized material, e. g., 100~400 mg colchicine for a large paddlefish (Dingerkus and Howell, 1976).

3) Easy to operate. Procedures up to soaking tissues in incubating medium can be performed even under a rough condition like on shipboard. Hours of incubation will make it possible to bring the tissue pieces back to the laboratory. The entire processes do not require any expensive instruments nor expertized techniques.

4) Increased opportunity for sampling fish of high commercial value, because a piece of the gill filaments (ca. 1 cm long) is sufficient with the present method.

The entire processes can be completed within 24 hours. Although having been unsuccessful in obtaining mitotic metaphase chromosomes for demersal species such as flatfish or rockfish, the present technique with an aid of tissue culture could be applied to deep sea fish, which can be taken alive by conventional fishing gears.

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鰓振盪培養による魚類の染色体の簡易観察法

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中・大型の魚類の染色体観察は従来の方法では生体の輸送，収容の設備，およびコルヒチン処理量の経済性など多くの問題点がある．これらの点を解決すべくインビトロの手法の開発を試みた．

コルヒチン濃度 0.003～0.005% の等張液（リンゲル液又は 2 倍稀釈海水）50 m*l* と酸素とを充填した容器に鰓切片 0.3～0.5 g を漬け，数時間室温で振盪することでマグロ，ゴマサバ，ブリの染色体像を得ることが出来た．染色体数はいずれも 2n＝48 で核型はマグロ中部着糸染色体 (M)＝2，次端部着糸染色体 (ST)＝2，端部着糸染色体 (A)＝44；ゴマサバ ST＝2，A＝46；ブリ　次中部着糸染色体 (SM)＝2，ST＝2，A＝44 であった．本方法は，高速で泳ぐ中・大型魚に応用出来る，コルヒチン処理量が少ない，処理が簡単である，可食部を汚染しないなどの利点がある．

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