15. A Karyotypical Study of the Conger Eel (Conger myriaster) in in vitro Cells, with Special Regard to the Identification of the Sex Chromosome\textsuperscript{*1}

By Yoshio OJIMA and Hirotsugu UEDA
Department of Biology, Faculty of Science, Kwansei Gakuin University, Nishinomiya
(Communicated by Sajiro MAKINO, M. J. A., March 12, 1981)

Most studies of chromosomes in Pisces have been made by using conventional staining methods. Recent literature refers to several studies carried out with the application of the banding techniques (Kligerman and Bloom 1977; Ojima and Ueda 1978; Ueda and Ojima 1978; Uwa et al. 1981). On account of small size and high number of chromosomes, there are considerable difficulties in chromosome investigations of fishes in general. Cultured cells, however, have proved advantageous due to a high mitotic index and fine quality for banding analysis of the chromosomes.

This article deals with a chromosome analysis and DNA replication in in vitro cells cultured from the Conger eel (Conger myriaster) with the application of the staining method of constitutive heterochromatin and the terminal BrdU pulse method.

Materials and methods. Conger myriaster, collected at Tarumi, Kobe City, Hyogo Prefecture, was used as a tissue donor. Freshly killed and anesthetized with MS-222 (methane sulfonic acid, Sigma) fishes, were sterilized by rinsing with 0.2% benzalkonium chloride followed 70% ethanol. A heart was aseptically removed and minced with scissors. The tissue fragments thus obtained were rinsed twice with calcium and magnesium-free saline which contained 0.206 M NaCl. The fragments were placed onto a tissue culture flask (Nunclon, 25 cm\textsuperscript{2}) with a small amount of FCS (fetal calf serum). They were cultivated at 20\textdegree C in the closed screw-cap bottles. After half an hour, 5 ml of a growth medium was added gently. The growth medium consisted of Hanks' BME containing 0.206 M NaCl (Clem et al. 1961), 20 mM of HEPES, 100 units/ml penicillin and 100 \mu g/ml streptomycin being supplemented with 15% of FCS. About two weeks after cultivation, fibroblast-like cells outgrew from the tissue. The medium was refreshed at one day interval. To obtain the primary mono-sheet of cells, the trypsinization was employed very gently. As

\textsuperscript{*1} This work was supported by a grant-in-aid (No. 540381) for the scientific research from the Ministry of Education, Science and Culture of Japan.
the culture cells of this fish apt to be damaged, trypsinization (also contained 0.206 M NaCl) was applied at 4°C, and the duration of treatment was reduced to a minimum. The subsequent subcultivations were performed by the same procedures. Following the treatments of cultured cells with colchicine, and KCl-hypotonization, they were fixed with Carnoy's solution as usual, air-dried and Giemsa-stained. Following the Giemsa C-banding technique (Sumner 1972), the chromosomes were analysed. The late DNA replication pattern of chromosomes was detected according to a modification of the terminal BrdU pulse method (Latt 1973). After subcultivation of the cells, BrdU (20 μM/ml) was added to the bottle, when the cells showed a subconfluent state, and incubated in the dark for 6- to 14-hs before fixation. Colchicine was added to the culture for 1-h. Air-dried preparations were stained with 2% 4Na-EDTA and 3% Giemsa for 4 min at 25°C (after the technique modified by Takayama, personal communication).

Results and remarks. Two females and six males were used for chromosome investigations. The diploid chromosome number was 38, and the arm number was 56. The karyotype consisted of 4 pairs of metacentric, 5 pairs of submetacentric, and 10 pairs of acrocentric chromosomes (Fig. 1). The C-bands were found located on the centric regions of all chromosome pairs, except a pair of the smallest acrocentrics, and the pericentric regions of some chromosome pairs. Most probably, meta- and submetacentrics may be induced by pericentric inversion of acrocentrics. A remarkable sexual difference of chromosomes was detected in a submetacentric pair. The female had a structurally heteromorphic pair characterized by short arms different in length, whereas in the male all pairs were homomorphic. This situation will indicate the ZZ/ZW type of the sex differentiation in this species.

The heteromorphic chromosomes as found in the female showed no difference in length. The W-chromosome had a C-band as a deeply stained band in its pericentric region. In the light of the above findings, it is apparent that the morphological differentiation of the Z and W chromosomes might be resulted from pericentric inversion (Fig. 2). On the basis of the late replication pattern by means of 6-hs terminal BrdU pulse incorporation, the W-chromosome was distinguishable from the Z-chromosome (Fig. 3). Generally the late replication pattern corresponded to the G-band pattern. Especially, the part of DNA-BrdU incorporation in chromatids showed a strong affinity to the combination staining with 4Na-EDTA and Giemsa (Takayama et al. 1981), though the characteristic and reproducible G-band patterns did not appear in the same chromosome in this study.
Finding a similar feature in the chromosomes of Amphibia, Schempp and Schmid (1981) have reported that the reason for the apparent lack of multiple banding patterns in the euchromatin of amphibian chromosomes is partly explained by the high degree of condensation of the amphibian chromosomes in the metaphase. Probably, the difficulty of G-band demonstration in fish chromosomes would be attribute.

Figs. 1-3. 1: C-banding karyotype patterns of male (a) and female (b) in Conger myriaster. Certain meta- or submetacentric elements may probably be induced by pericentric inversion. The sexspecific chromosomes are shown as ZZ in male andZW in female. 2: Diagram and C-band chromosomes illustrating the differentiation of Z and W chromosomes through pericentric inversion. 3: Late DNA replication pattern in the female chromosomes according to 6-hs terminal BrdU pulse method.
to the differences of chromosomal proteins in comparison with that of mammals.

Park and Kang (1979) reported recently that the karyotype of *Conger myriaster* was composed of 6 pairs of metacentrics, 4 pairs of submetacentrics, and 9 pairs of acrocentric and telocentric chromosomes with a heteromorphic pair consisting of the largest and the smallest metacentrics. In the present study, the relation of the increase of acrocentric pair to the decrease of submetacentric pair was definitely detected in the same species together with the heteromorphic chromosomes. The most reasonable explanation for the above feature is that there exist two different forms in *Conger myriaster* in relation to pericentric inversion.

On the basis of a consensus of estimates through the findings and views on fish chromosomes, it seems that the fish group is now in the course of evolution, and that their sexual differentiation is still in an unstable and primitive condition. The progressive cell-culture and banding techniques will make it possible to identify various kinds of sex chromosomes in the future.

References