An observation of the Chromosomes in the Embryonic Cells of a Goby, *Chaenogobius urotaenia* (Hilgendorf)

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Karyological studies of the testes cells of teleost fishes are sometimes difficult owing to the smallness and relatively high number of the chromosomes in those cells. Recent advanced techniques have made it possible to observe chromosomes in cells of various tissues. Among these, aceto-orcein staining on squashed tissues after colchicine treatment (Roberts, 1964) is simple and easy. The embryonic cells in fishes are assumed to be suitable for chromosome study since they are large in size and show frequent mitotic figures. On the basis of this assumption, the aceto-orcein squashing technique was used on the embryos of a goby. The results are reported in this paper.

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Material and Method

Clusters of the eggs of a goby (Ukigori), *Chaenogobius urotaenia* (Hilgendorf), in early development stages, were collected from a brooklet near our laboratory. They were kept in petri-dishes until the embryos had reached desired stages. They were then decapsuled and immersed in several concentrations of colchicine for one to five hours keeping them alive. It was decided that the immersion in 0.02% colchicine for four to five hours was suitable to obtain good metaphasic figures. After that, the embyros were transferred into 0.9% sodium citrate for ten minutes, meanwhile, as much of the yolk was removed as possible. Each of the yolk-free embryos was then placed on a glass slide and covered with a drop of 2% orcein in 50% acetic acid. After being left fifteen minutes in aceto-orcein solution preventing evaporation, the embryos were squashed under coverslips with thumb pressure. Developmental stages of these embryos were from the blastula to the beginning of the heart-beating.

Results

In early developmental stages up to the late blastula, the chromosomes in the large nuclei of blastomeres were long and filamentous even in metaphase, and therefore, it was difficult to make accurate counts of them. In the head fold stage, the embryonic cells still retained large sizes and their chromosomes appeared to be forty-two in number (Figs. 1 and 2). Most of the chromosomes showed to be rod-

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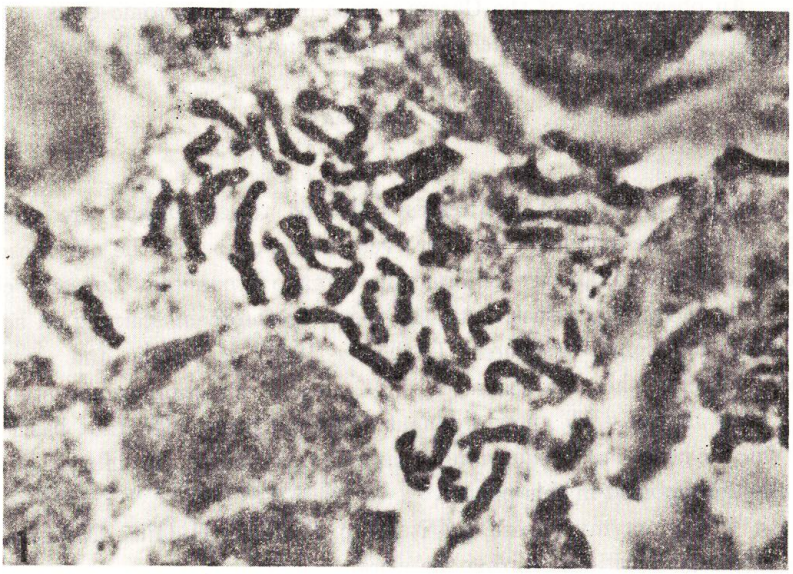


Fig. 1. Metaphase in an embryonic cell from an embryo in the head fold stage × 2300

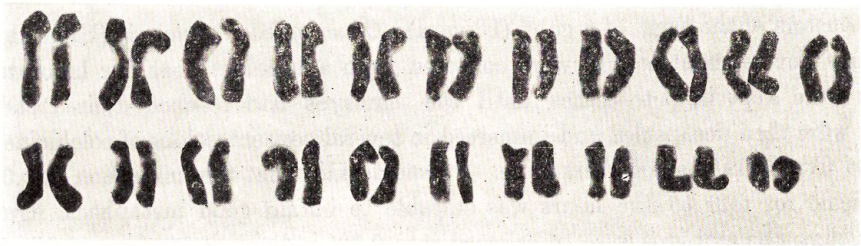
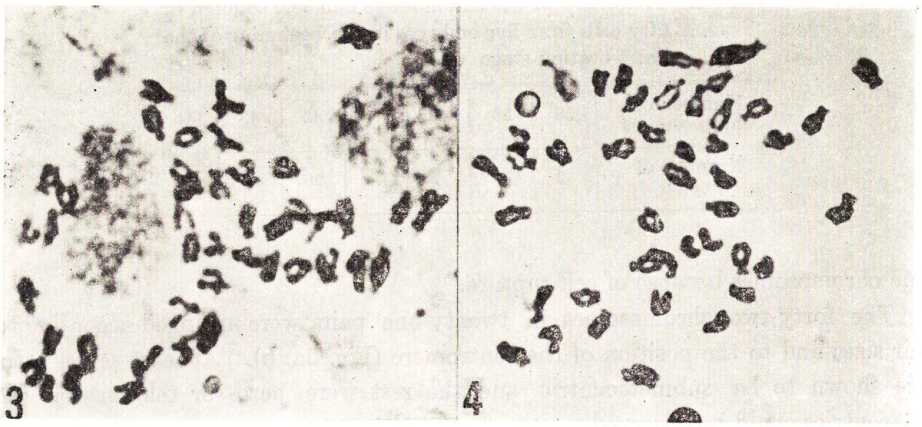


Fig. 2. Chromosome pairs from the cell in Fig. 1, arranged according to their sizes × 2300

shaped and some J- or L-shaped. With regard to their shapes, however, it seemed probable that they were deformed easily by squashing since they still had long and slender forms in this stage.

The most reliable metaphasic figures both for counting the number of chromosomes and for identifying the position of the centromere were obtained from the embryos in the beginning of the heart-beating stage (Figs. 3 and 4). The embryonic cells were smaller as compared with those in the head fold stage, but those having the large metaphasic plate sufficient to observe the chromosomes were frequently encountered in the endodermal region. The numbers of chromosomes counted in fifty selected cells from five specimens are tabulated in Table 1. The mode exists at forty-two which is assumed to indicate the true number of this species. The reason for such a range of numbers could be missing or admixing of



Figs. 3 and 4. Metaphases in two endodermal cells from embryos at the beginning of the heart-beating stage × 2300

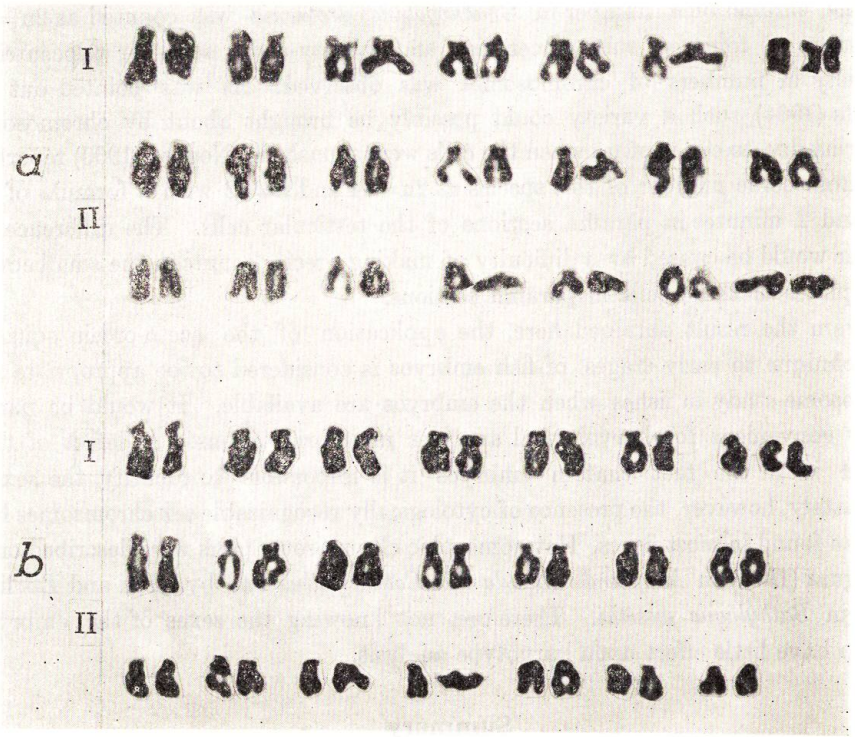


Fig. 5. Karyotype analysis from the cells shown in Figs. 3 and 4. a: from Fig. 3; b: from Fig. 4. The chromosomes were grouped into two; seven pairs of submetacentrics (I) and fourteen pairs of acro- or telocentrics (II). × 3200

Table 1. Frequency of the chromosome numbers counted in fifty cells from five embryos in the beginning of the heart-beating stage

Number of chromosomes	38	39	40	41	42	43	44
Number of cells	2	—	7	—	28	6	7

some chromosomes because of cell rupture.

The forty-two chromosomes in twenty-one pairs were arranged according to their sizes and to the position of the centromere (Fig. 5a, b). At least seven pairs were shown to be submetacentric and the rest were acro- or telocentric. No metacentrics could be observed.

Discussion

The chromosome number in *Chaenogobius urotaenia* was counted as 2n=42 consisting of fourteen submetacentrics and twenty-eight acro- or telocentrics. A variety in numbers of chromosomes was observed. As was pointed out by Roberts (1964), such a variety could possibly be brought about by chromosome scattering due to cell rupture when the cells were squashed. Nogusa (1960) reported the chromosome number of this species as 2n=44 and n=22 with a formula of 42 rods and 2 minutes in paraffin sections of the testicular cells. The difference in number would be caused by a difficulty of making precise counts in the small equatorial plates of those cells in paraffin sections.

From the result obtained here, the application of the aceto-orcein squashing technique to early stages of fish embryos is considered to be appropriate for chromosome study in fishes when the embryos are available. It would be particularly convenient for karyological analyses in hybridizations. A defect of this method is in the fact that in embryos it is impossible to identify the sexes. Fortunately, however, the presence of cytologically recognizable sex chromsomes has not been found in most fishes. Heteromorphic chromosome pairs were described only by Nogusa (1960) in *Mogrunda obscula* and *Cottus pollux* and by Chen and Ebeling (1966) in *Bathylagus wesethi*. Therefore, not knowing the sexes of the embryos seem to have little effect upon karyotype analysis.

Summary

The chromosomes in the embryonic cells of a goby (Ukigori), *Chaenogobius urotaenia* (Hilgendorf) were observed in squashed preparations by aceto-orcein staining. The pre-treatment of the embryos, in the beginning of the heart-beating stage, with 0.02% colchicine for four to five hours turned out to be good for

the purpose. The chromosome number of this species was counted as 2n=42 consisting of 14 submetacentrics and 28 acro- or telocentrics. Advantages of the application of this technique to fish embryos were discussed.

References

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