5.

MATERIAL AND TECHNIQUE

For the purpose of the present studies, the fishes were collected from the Saugor lake and from the rivers and seasonal streams of Saugor. The fishes which were kept in the aquariums in the laboratory for some days failed to yield favourable material as in almost all cases the nuclei in the cells of the germinal tissue were always found to withdraw in the resting stage. It was thus extremely difficult to obtain suitable material for the study of the chromosomes in the laboratory kept specimens and therefore a large number of fishes had to be collected from their natural environments directly at different seasons and dissected on the spot for the fixation of the gonads. It was, however, found that the collections made from April to August were mostly suitable in most of the cases.

TECHNIQUE

The entire study of the chromosome survey is based on squash preparations (Aceto-orcein used after the improved formulae of De Tomassi 1936). The dye used for
these studies was obtained from Messrs. G.T. Gürr of United Kingdom, which proved very satisfactory. The studies on squash preparations were supplemented by a study of sections. Before fixation, the testes were always treated with a 0.33% hypotonic salt solution for a period of 5 to 10 minutes, and then processed according to their hardness. This was done by fixing small pieces of testes in 1.1% Aceto-orcein and kept at a temperature of 38°C to 40°C in a constant temperature bath in 45% Acetic acid, until the material became suitably softened for squash preparations. The time for keeping the material in the bath at the above mentioned temperature varied from half an hour to one and a half hour, according to its hardness.

Temporary squashes were mounted in Aceto-glycerine (45% Acetic acid + 55% glycerine). Permanent preparations were made by removing the coverslips in Aceto-alcohol (1:3) and dehydrating both the slide and coverslip through ascending grades of alcohol; they were cleared in Xylol and finally mounted separately in neutral Canada balsam. During the process if the stain was found to be insufficient, the material was re-stained in 1.1% Aceto-orcein
before dehydration and mounting.

**FIXATIVES AND STAINS USED**

1. Fixative for squashes : Aceto-orcein 1.1% in 45% Acetic acid.

2. Stain for squashes : Aceto-orcein and Feulgen (Modified).

3. Fixative for sections : Champy's fluid, Alcoholic Bouin solution and Aceto-alcohol (1:3).

4. Stain for sections : Feulgen (Modified), Heidenhains Iron Haematoxylin and light green.

For staining the squash preparations, the Aceto-orcein and Feulgen method as modified by the present author was found to be most satisfactory. For this modification the author used an aqueous solution of 0.5% Fuchsin Basic after the usual hydrolysis for 6 minutes at 60° C instead of staining by leuco-basic fuchsin as is done in the normal Feulgen method. The material was stained in 0.5% basic
Fuchsin from half an hour to one and a half hours and subsequently washed in 80₂ water thrice for 20 minutes each.

The figures in the present work were drawn with the aid of Camera-lucida and were further magnified. Due to the extremely small size of the chromosomes, it was not possible to get good photomicrographs of the chromosome patterns (Karyotypes) in one plane after using oil-immersion objective and high power plane eye-pieces.

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