Cytotaxonomy of Trox Fabr.
(Troginae: Scarabaeidae: Coleoptera)

In several instances, analysis of morphology of karyotype led the way to a new and better and sometimes to novel understanding of the systematic relationships within a major biological group. Based on karyotype morphology Smith\textsuperscript{1,2} solved the taxonomic puzzle in three North American and two Finnish species of Hyllobius. Trox provides another good example of complete agreement between classical taxonomy and relationships based on the structure and behaviour of chromosomes.

Vaurie\textsuperscript{3,4} placed North American species of Trox into species subgroups on the basis of male genitalia and certain other morphological characters. Trox foveicollis and T. spinulosus dentibius are the more primitive species belonging to terrestris subgroups. Trox scabrous is the name species of the scabrous complex. Trox punctatus, Trox sectellaris and Trox monachus belong to the suberosus subgroup, which is believed to contain more specialized forms. The South American subgroup brevicollis, to which Trox ariceus belongs, is regarded to be intermediate between the suberosus group and other Trox. The Indian Trox omancambus can be best fitted in the North American suberosus group.\textsuperscript{5}

Cytologically, all the above mentioned species of Trox possess 9 + Xyp karyotype. However, they differ in morphological details of the chromosomal complement. All the chromosomes are metacentric in the complement of Trox foveicollis; Trox scabrous and Trox spinulosus dentibius whereas the chromosomes are acrocentric in Trox punctatus, Trox sectellaris, Trox monachus and Trox omancambus. Since scarabaeid chromosomes are typically metacentric Virkki\textsuperscript{7} considered the latter karyotype as derivative. Comparatively high chiasma frequency and hence a high recombination index in the Indian Trox omancambus also lends support to the derived nature of the acrocentric karyotype. Virkki\textsuperscript{7} assumed a complete series of pericentric inversions to be responsible for the evolution of karyotype from a totally metacentric to a totally acrocentric one during which the "fundamental number" of chromosome arms is also reduced to one half. Since it is hard to imagine a sudden change the inversions must have had a selective value and got accumulated during a long evolutionary history. Consequently, there should be intermediate forms between the two extremes in Trox foveicollis type and Trox punctatus type of karyotypes. This gap is filled by Trox ariceus which has in its karyotype mostly metacentric chromosomes, but at least one pair of chromosomes is acrocentric.

The foregoing account shows how beautifully and exactly the cytological picture; Trox foveicollis, Trox spinulosus dentibius, Trox scabrous → Trox ariceus → Trox punctatus, Trox sectellaris, Trox monachus and Trox omancambus fits into the one drawn on the basis of morphotaxonomy; terrestris, scabrous → brevicollis → suberosus, in that phylogenetic sequence.

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Giemsa-Banding in Fish Chromosomes

Differential banding patterns obtained by various techniques are currently in vogue for identification of animal chromosomes, particularly the human chromosomes (cf. Sharma and Taksdhar). These bands (obtained by the use of quinacrine fluorescentes) appear as high and low intensity regions under the fluorescent microscope; if produced by other methods, these are visible as differentially stained segments under the light microscope. Of methods tried on the fish chromomeres, the following technique, which involves a modified procedure, gave very satisfactory results.

The fish chromosome slides were prepared by the usual colchicine-citrate-acetic alcohol-air drying method described elsewhere. For obtaining the G-bands, 5-6 days old slides were dipped in a 10\% solution of hydrogen peroxide for 5 min, washed in distilled water and treated for 10 minutes with 0.027 solution of trypsin kept at 4°C (± 1°C). After washing the slides with 5-10 changes of distilled water, they were stained in dilute Giemsa stain (pH neutral). The slides were differentiated in distilled water, dried at 37°C for 6 hours and mounted in DPX after cleaning in xylene.
The most important step in the present method is the slowing down of the action of trypsin by low temperature treatment. In this way, it is easy to arrest the action of the enzyme at the optimum stage. It is known that the action of trypsin occurs in two stages. It initially denatures the protein substrate and then the protein becomes more sensitive to the hydrolytic action of the enzyme. The denaturation activity brings about suitable changes in the protein molecules associated with the chromosomes. It is only in this phase that the differentially staining bands can be obtained. Thus, it is very necessary that this phase is prolonged by slowing down the action of the enzyme (done presently by low temperature treatment) so that the action is easily controlled before the next phase of digestion sets in. Such a slowing down can also be attempted by versene or by ethylenediaminetetraacetic acid.

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The Figs. 1 and 2 show somatic metaphases obtained from the kidney cells of male and female Channa punctatus (Bloch) (2n = 32) belonging to the family Ophiocephalidae. The elements show clear G-bands which are more discrete on the longer chromosomes. Since the lighter regions on the elements are the ones that have been altered by the enzyme to become unselectable with Giemsa, these are the real G-bands. The application of banding pattern studies to the fish chromosomes can be very useful due to the fact that the fishes usually have a larger number of elements not distinguishable by normal morphometric criteria.

The author is grateful to Professor A. K. Datta-Gupta, Head of his department, for providing the research facilities.

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**CYTOCHEMISTRY AND ULTRASTRUCTURE OF THE YOLK NUCLEUS IN OOECYES OF THE LOACH, MISGURNUS ANGUILLICAUDATUS (CANTOR)**

No structure of the cell intrigues the cytologist as much as the yolk nucleus of fish oocytes. It is generally assumed that the yolk nucleus takes part in the synthesis of certain substances that are subsequently incorporated in the yolk. On the contrary, Kudo believed that the yolk nucleus is concerned with the formation of cortical alveoli. None of these authors has however shown any physical continuity between the yolk nucleus and the formation of yolk. The present cytochemical and ultrastructural study is related to the yolk nucleus in the oocytes of *M. anguillicaudatus*.

Adult loaches were obtained from fresh water streams of Gunma prefecture, Japan. Fresh pieces of the ovaries were fixed in Carnoy, Bouin, 10% neutral formalin and formal-calcium fixatives. Methyl green/pyronin, Trichloroacetic acid and Ribonuclease digestion, Feulgen reaction, Hg-BBP, Trypsin digestion/Hg-BBP, Fast green FCF, Blocking of fuchsin/fast green, Millon's reagent, Performic acid-alcan blue and PAS tests were employed for cytochemical analysis. The technique of electron microscopy is similar to that described by Dutt and Inoue.

The yolk nucleus which is juxtanuclear in position in the cytoplasm of immature oocytes appears as a spherical body surrounded by a clear space. Cytochemically it is rich in RNA, acidic proteins and basic proteins containing arginine. These findings in the loach corroborate the earlier reports on the occurrence of RNA and protein in the yolk nucleus in other fish oocytes. The yolk nucleus is however devoid of any detectable amounts of DNA, tyrosine, cystine and...