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Chromosome preparations from freshly dead fish

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Abstract Chromosome preparations were obtained from kidney tissues of freshly dead fish *Labeo rohita* and *Cirrhinus mrigala*, using *in vitro* colchicine treatment. The chromosomal spreads were of good quality, suitable for karyotyping and chromosome banding studies. The present technique is highly suitable for cytogenetic studies in wild stock of fish species for which there is difficulty in getting live fish and also in those species which die immediately in captivity. This is the first report on use of tissues from dead fish for studying metaphase chromosomes.

(**Keywords :** chromosome/*Labeo rohita/cirrhinus mrigala*)

Chromosome spreadsin fish have been commonly obtained from tissues such as gill, kidney, intestine and blood by using techniques such as 1) *in vivo* colchicine pretreatment and sacrificing fish specimens[1-3]. 2) cell culture techniques[4-6]. 3) *in vitro* colchicinization technique[7,8]. All these techniques require live fish for chromosome preparations which sometimes can be very difficult especially under field conditions. So far no report is available in literature on use of tissues from dead fish for obtaining chromosomes and it is widely believed that dead fish can not be used for chromosomal studies. The present investigation was therefore, undertaken to explore the possibility of using tissues from freshly dead fish for karyomorphological studies.

For the present study, five specimens of two species i.e., *Labeo rohita* and *Cirrhinus mrigala*, approximately 0.5 to 1 kg in weight were procured from the local market in the month of December. The kidney tissues were dissected out from dead specimens, approximatley 2 hr. after death. The cephalic kidney was taken and homogenized to prepare cell suspension in 8ml of RPMI 1640 culture medium. The cell suspension was taken in a petridish and to this cell suspension 50 µl of 0.05% of colchicine was added. The cell suspension was incubated at 37°C for 30 minutes and then the contents in petridish were centrifuged at 1200 rpm for 10 minutes. The cell pellet was suspended in 8 ml of freshly prepared 0.56% potassium chloride solution for 22 minutes at room temperature. The hypotonic treatment was terminated by adding 1ml of freshly prepared, chilled Carnoy's fixative (Methanol 3 parts: acetic acid 1 part) to the centrifuge tube. After thoroughly mixing the contents the tubes were again centrifuged at 1200 rpm for 10 minutes. The supernatant was discarded and the cell

pellet was resuspended in 7 ml of chilled fixative. The process of washing of the cell pellet with fixative was repeated thrice to get clear whitish pellet. The slides were prepared by the flame drying technique and stained with Giemsa in phosphate buffer (pH 6.8).

More than 25 good quality metaphase spreads were observed in each slide from all dead specimens. The diploid chromosome number in both the species was 2n= 50 (Figs. 1&2), which was in agreement with the chromosome number, reported earlier[9-10]. The quality of metaphase spreads obtained was suitable for karyotyping and chromosome banding studies. The number of metaphase spreads obtained in the present study was generally sufficient for cytogenetic characterization in these species.

For cytogenetic studies of fishes, the *in vivo* techniques are commonly used and optimized in different species to get a large number of metaphase spreads. This method has, however, a disadvantage that specimens have to be kept alive at least for one hour after injecting colchicine. In field conditions it is very difficult to get and maintain live specimens. In some species like *Tenuolosa ilisha* the fishes die immediatley in captivity[11]. Similarly setting up of cell cultures in these species would be very difficult. At present cell culture techniques have been reported in some carps and trout only[4,6,12]. The difficulty in getting live fishes may be one of the reasons for lack of karyological information in many fish species especially wild species.

Thus, with the technique discussed above, cytogenetic investigation could be undertaken in wild stocks of freshly dead fishes under field conditions obtained from routine catches at landing centers. This technique has an additional advantage that cytogenetic studies can be undertaken along with biochemical and molecular genetics work as an integrated study leading to better interpretation of results. This is the first report on chromosomal preparations from tissues of freshly dead fishes.

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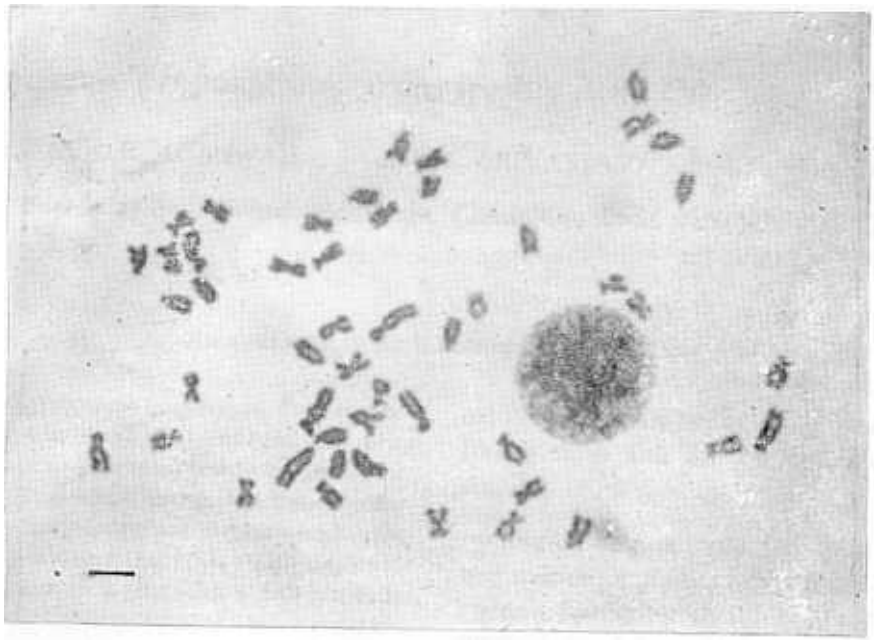

Fig. 1 Metaphase spread of *L. rohita*

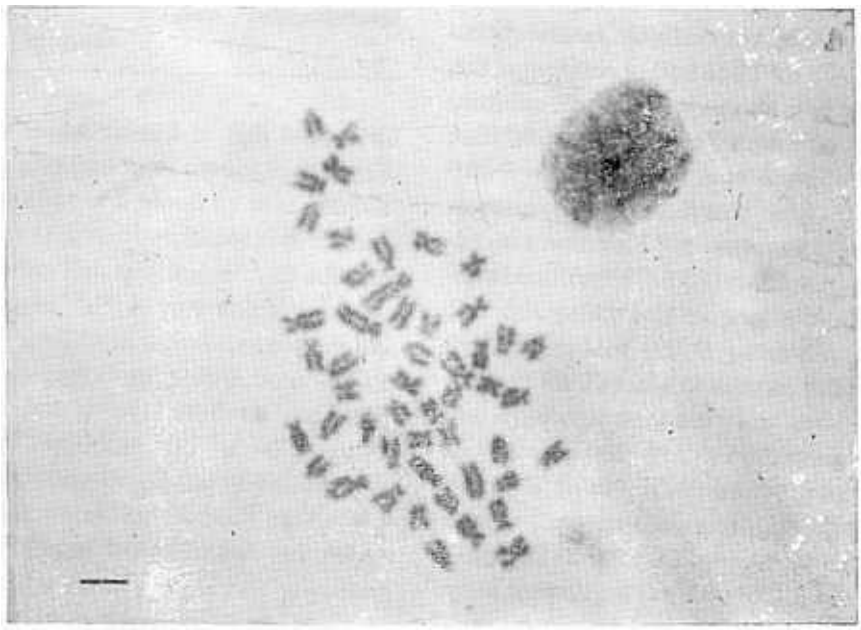

Fig. 2 Metaphase spread of *C. mrigala*
Bar represents 10μm