NASH, C.E., KUO, C.M. & MCCONNELL, S.C. 1974. Operational procedures for rearing larvae of the grey mullet (*Mugil cephalus* L.). *Aquaculture* 3: 15–24.

NASH, C.E. & KONINGSBERGER, R.M. 1981. Artificial Propagation. In: Aquaculture of Grey Mullets, (ed.) Oren, O.H. International Biological Programme 26. Cambridge University Press, Cambridge.

YASHOUV, A. & BERGER-SAMSONOV, E. 1970. Contribution to the knowledge of eggs and early larval stages of mullets (Mugilidae) along the Israel coast. *Bamidgeh* 22: 72–89.

ZHITENEV, A.N., KALININ, D.S. & ABAYEV, Y.I. 1974. The state of the gonads of the striped mullet (*Mugil cephalus*) and the sharpnose mullet (*Mugil saliens*) leaving estuaries to spawn and their reaction to a pituitary injection. *J. Ichthyol.* 14: 232–239.

# A rapid, non-sacrificial chromosome preparation technique for freshwater teleosts

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The use of fin epithelium from the tilapia, *Oreochromis mossambicus*, and the grass carp, *Ctenopharyngodon idella*, was investigated to provide a rapid, non-sacrificial procedure for determining ploidy. A combination of colchicine, prolonged hypotonic treatment, dissociation of cells followed by Giemsa staining makes it possible to achieve good quality metaphase chromosome spreads using small fish without the use of sterile conditions, centrifuges or sacrificing the specimen. In situations such as the induction of triploidy or tetraploidy, it is necessary to have a quick, reliable method of assessing the results of experimental design. The technique presented in this report provides numerous, well-spread metaphase chromosomes with a tissue handling time of less than 2 h.

Die gebruik van die vinepiteel van die bloukurper, *Oreochromis mossambicus*, en die graskarp, *Ctenopharyngodon idella*, is geëvalueer as 'n vinnige metode om chromosoomgetalle te bepaal sonder dat die vis doodgemaak word. 'n Kombinasie van colchicine, verlengde hipotoniese behandeling en die dissosiasie van selle gevolg deur Giemsakleuring, lewer hoë-kwaliteit metafase-chromosome. Die tegniek leen hom daartoe dat klein vissies gebruik kan word wat nie gedood hoef te word nie en dat geen gesofistikeerde apparaat of steriele toestande benodig word nie. In situasies soos die induksie van triploïede of tetraploïede is dit wenslik om op 'n baie vroeë ouderdom te kan bepaal of die induksie 'n sukses was ten einde die eksperimentele prosedure te evalueer. Die tegniek wat in hierdie studie gebruik is, lewer verskeie goedverspreide metafase-chromosome binne slegs 2 h.

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There are many uses to which the chromosomal information of fish can be put, particularly in the areas of cytotaxonomy, mutagenesis and aquaculture (Kligerman & Bloom 1977). Chromosome numbers have been described for a wide range of fish species (eg. Beamish & Miller 1977; Bertollo, Takahashi & Filno 1983; Beck & Biggers 1980; Blaxhall 1983: Hinegardner 1976; Vervoort 1980).

Roberts (1967) suggested that fish are likely to have more intraspecific chromosomal polymorphism than other vertebrates and that karyotypic differences could be used as racial markers while interspecific karyotypic differences are criteria for separating morphologically similar species (Boothroyd 1959; Fukuoka 1972).

It is possible, however, that some of the observed chromosome variations reflect an inadequacy of the technique used, particularly the squash method which often results in poor morphological detail and overlapping of chromosomes (Blaxhall 1975; Hartley & Horne 1985). Until techniques are refined and 'normal' karyotypes are accurately known, only extreme variations can be detected.

Genetic mechanisms of sex determination have been described for a number of species with the complete range from synchronous hermaphroditism through primitive polygenic sex determination to distinct sex chromosomes being exhibited in various fish species (Atz 1964; Avtalion & Hammerman 1978; Harrington 1963; Uyens & Miller 1971; Ohno 1967). A knowledge of the sex-determining mechanisms has great implications in fish breeding, for example in the monosex culture of the tilapias employing the techniques of sex reversal and hybridization.

In reviews of current fish chromosome techniques (Blaxhall 1975; Hartley & Horne 1985; Ojima 1982) criticism has been made of the consistency of results, the sophistication and length of techniques, the frequent necessity of sacrificing the specimen and the specimen size required. It was the purpose of this study to modify the solid tissue techniques of Denton & Howell (1969) and Kligerman & Bloom (1977), to provide a rapid, technically unsophisticated means of deriving accurate chromosomal information from young freshwater fish without sacrificing the specimen. The value of this technique would be both in field studies and in the laboratory where a quick accurate result is desired but without the use of centrifuges, grinders, digestive enzymes or tissue culture and when the specimen must be kept alive.

The fish used in this study were maintained in 300 l, aerated, glass aquaria kept at 28°C by submersible, thermostatically controlled heaters. The two species investigated were *Oreochromis mossambicus* and *Ctenopharyngodon idella*.

The most suitable method for obtaining a high mitotic index is described below.

Trim the edges of the caudal fin two to three days prior to processing to stimulate regeneration of the epithelium. Place fish in aerated 0,005% colchicine solution at 28°C for 3 h. Remove specimen and rinse under running water. Trim caudal fin margins which will

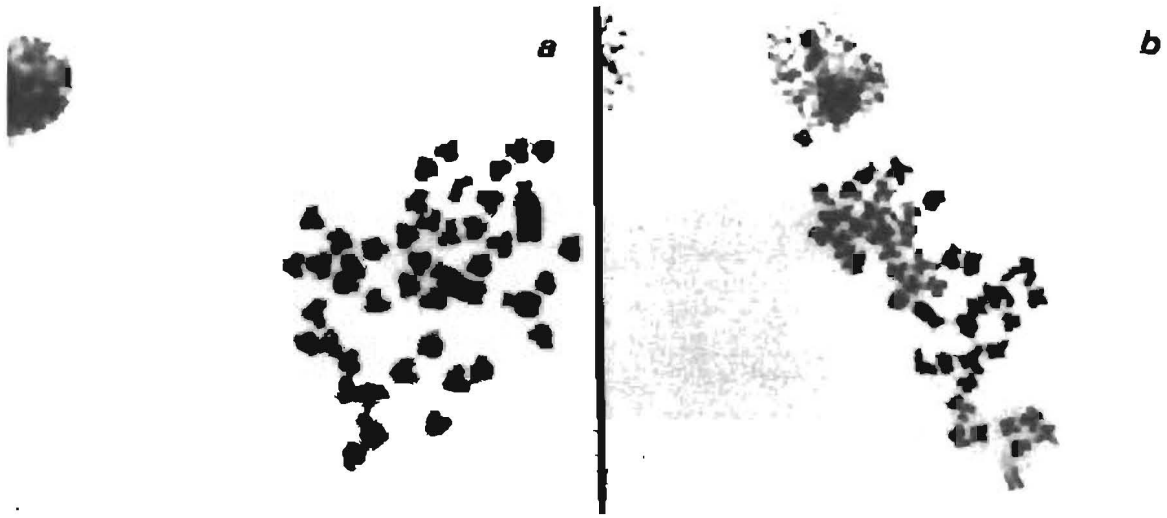


**Figure 1** (a) A metaphase chromosome spread of *O. mossambicus* (× 1000). (b) A metaphase chromosome spread of *Ctenopharyngodon idella* (× 1000).

appear colourless indicating epithelial growth and place trimmings in 0,075 mol $dm^{-3}$ HCl solution for 30–45 min to swell. Add 5% by volume of freshly prepared, chilled methanol-acetic acid (3:1) fixative. After 5 min, aspirate the solution. Add two fresh changes of fixative and place in the well of a depression slide, add 2–3 drops of 50% glacial acetic acid and gently mince the tissue for 1 min.

Using a Pasteur pipette and rubber bulb, withdraw the cell suspension and expel it onto a clean slide heated to 45°C on a slide warmer. Quickly withdraw the suspension back into the pipette leaving a ring of cells approximately 1 cm in diameter. Repeat 2–3 times per slide. Remove slide from slide warmer and allow to air dry.

Stain for 20 min in phosphate buffered fiemsa stain, pH 7,2. Rinse slides in distilled water and air dry. Mount and view under oil.

The range and quality of cells arrested in metaphase for both species was sufficient to permit karyotyping and chromosome counting. (See Figure 1a & 1b).

The stress of handling the fish appeared to influence the mitotic index. This effect was reduced by painting the colchicine aquarium to reduce stress from disturbances and by treating more than one fish simultaneously. Increasing the time in the colchicine solution or increasing the colchicine concentration, while raising the mitotic index, produces chromosomes of poor morphology.

It was found that poor spreading of the chromosomes was improved by additional time in the hypotonic KCl solution allowing the cells to swell more. Conversely, scattering of the chromosomes was found to occur if the cells were exposed to the KCl solution for too long.

Yolked fry may be treated in a similar manner except that it is necessary to sacrifice the specimen. The following alterations are made to the above method:

(a) the first step is omitted.
(b) after removing the fry from the colchine, rinse and dry, then remove the head and yolk sac.

The time and cost associated with many chromosome preparation techniques is a serious constraint to documenting accurate, reproducible genetic blueprints as often special laboratory facilities and skills are required.

The rapid, inexpensive tissue handling technique described in this article overcomes many of these problems and enables several fish to be investigated simultaneously. Also, because of the young age at which fish can be processed, any necessary corrections to experimental design can be made relatively early in the fish's life, thereby reducing fish maintenance costs.

Owing to the simplicity of this technique, without the use of centrifuges, digestive enzymes or aseptic culture conditions, it is ideally suited to application in both the field and the laboratory, whenever a quick, simple method is required.

## References

ATZ, J.W. 1964. Intersexuality in fishes. In: Intersexuality in vertebrates including man. Academic Press, London, New York. 145–232.

AVTALION, R.R. & HAMMERMAN, I.S. 1978. Sex determination in *Sarotherodon* (Tilapia). Introduction to the theory of autosomal influence. *Bamidgeh* 30: 110–115.

BEAMISH, R.J. & MILLER, R.R. 1977. Cytotaxonomic study of gila trout, *Salmo gilae*. *J. Fish. Res. Bd Can.* 28: 1745–1748.

BECK, M.L. & BIGGERS, C.J. 1980. Karyological analysis of *Ctenopharyngodon idella*, *Aristichthys nobilis* and their F1 hybrid. *Trans. Am. Fish. Soc.* 109: 433–438.

BERTOLLO, L.A.C., TAKAHASHI, C.S. & FILNO, O.M. 1983. Multiple sex chromosomes in the genus *Moplias* (Pisces: Erythrinidae). *Cytologia* 48: 1–12.

BLAXHALL, P.C. 1975. Fish chromosome techniques — a review of selected literature. *J. Fish. Biol.* 7: 315–320.

BLAXHALL, P.C. 1983. Chromosome karyotyping of fish using conventional and G-banding methods. *J. Fish. Biol.* 22: 417–422.

BOOTHROYD, E.R. 1959. Chromosome studies on three

Canadian populations of Atlantic salmon. *Can. J. Genet. Cytol.*. 1: 161–172.

DENTON, T.E. & HOWELL, W.M. 1969. A technique for obtaining chromosomes from the scale epithelium of teleost fishes. *Copeia*. 392–393.

FUKUOKA, H. 1972. Chromosome number variations in the rainbow trout. *Jap. J. Genet.* 47: 455–458.

HARRINGTON, R.W. 1963. Environmentally controlled induction of primary male gonochorists from eggs off the self-fertilising hermaphroditic fish, *Rivulus marmoratus*. *Biol. Bull.* 132: 174–199.

HARTLEY, S.E. & HORNE, M.T. 1985. Cytogenic techniques in fish genetics. *J. Fish. Biol.* 26: 575–582.

HINEGARDNER, R. 1976. The cellular DNA content of sharks, rays and some other fishes. *Comp. Biochem. Phys.*. 55B, 3: 367–370.

KLIGERMAN, A.D. & BLOOM, S.E. 1977. Rapid chromosome preparations from solid tissues of fishes. *J. Fish. Res. Bd Can.*. 34: 266–269.

OJIMA, Y. 1982. Methods in fish cytogenetics. *The Nucleus*. 25(12): 107.

OHNO, S. 1967. Sex chromosomes and sex linked genes. Springer Berlin, Heidelberg, New York.

ROBERTS, E.L. 1967. Chromosome cytology of the Osteichthyes. *Progve. Fish Cult.* 29: 75–83.

UYENS, T. & MILLER, R.R. 1971. Multiple sex chromosomes in a Mexican cyprinodontid fish. *Nature, London* 23: 452–453.

VERVOORT, A. 1980. The karyotypes of seven species of *Tilapia* (Teleostei: Cichlidae). *Cytologia* 45: 651–656.