A field-trip microtechnique for studying fish leukocyte chromosomes

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An ideal technique for field cytotaxonomy of fishes should be rapid, efficient, and time- and distance-independent. It should work on small as well as big specimens, and it should be amenable to air-drying. A technique meeting these requirements is as follows. A few drops of whole blood are collected from the specimens during field trips and kept in culture medium. In the laboratory, the medium is changed, Phytohemagglutinin is added, and the cells are cultivated at room temperature for about 3 days, after which air-drying fixation is performed. For very small specimens, the blood from several individuals may be pooled in one culture vessel.

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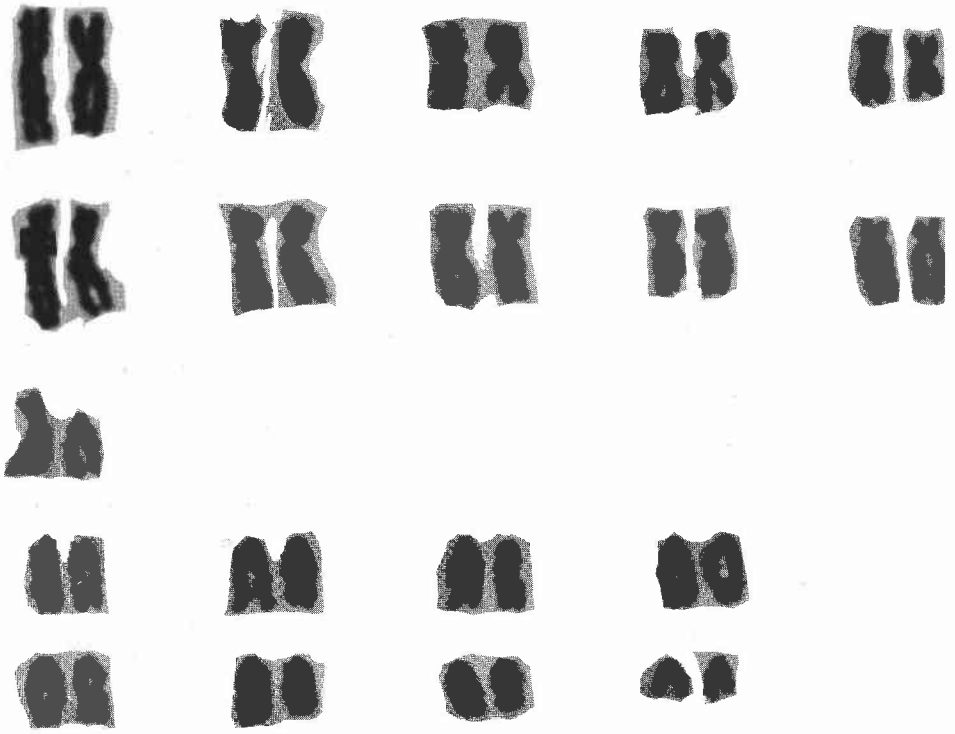
Une technique optimale de caryologie des poissons appliquable sur le terrain devrait posséder les qualités suivantes: elle devrait être rapide, produire un grand nombre de bonnes métaphases et être indépendante de la distance et du temps de retour au laboratoire; elle devrait être appliquable à de petits spécimens aussi bien qu'à des gros, et permettre de plus le séchage à l'air. La technique suivante satisfait à ces conditions. Sur le terrain, on prélève sur les spécimens quelques gouttes de sang que l'on garde dans un milieu de culture. De retour au laboratoire, on change le milieu de culture et on lui ajoute de la Phytohémagglutinine. On cultive les cellules dans ce milieu pour environ 3 jours à la température de la pièce, après quoi l'on fixe les cellules selon la technique du séchage à l'air. Pour les spécimens très petits, on peut mettre dans la même bouteille de culture le sang prélevé sue plusieurs individus.

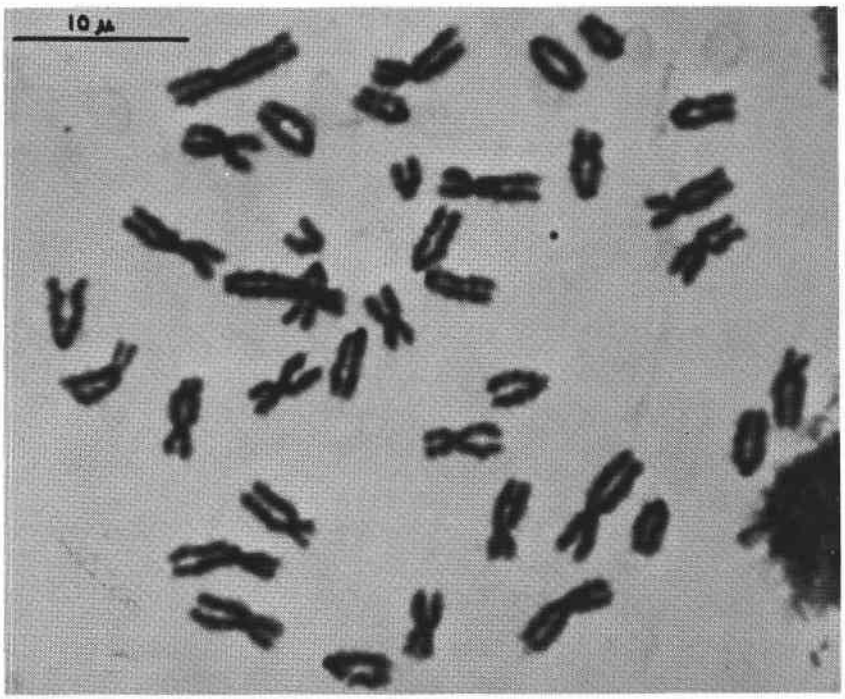
Introduction

Fish chromosomes have been considered difficult to study ever since cytologists became interested in them about 40 years ago. Workers have experimented with various techniques in the laboratory, which have been well summarized by Ivanov (1972), but most of them have shortcomings. The main techniques are (1) the classical embryo squash, for which it is difficult to get material from wild fish populations; (2) the direct preparation of somatic cells (McPhail and Jones 1966; Denton and Howell 1969; etc.), which has a relatively low yield of good metaphase plates, and is not directly amenable to air-drying; (3) long-term tissue culture (for instance in Gravell and Malsberger 1965), which requires a very long culture time and has a low rate of success; (4) primary culture of hard body tissue (Roberts 1964), which works well and could be used in the same way as the technique suggested in the present paper, which is based upon (5) short-term culture of peripheral blood: this method has been proposed by some workers (Labat *et al.* 1967; Ojima *et al.* 1970; Heckman and Brubaker 1970), who adapted to fish material the method currently in use in human cytogenetics, which was initiated by Moorhead *et al.* (1960).

We have come to the conclusion that an optimal method for field cytotaxonomy of fishes should have the following six qualities: (1) it should be rapid; (2) it should have a high yield of good-quality metaphase plates; (3) it should be usable on small as well as big specimens (this requirement was added in view of cyprinid taxonomy); (4) it should not require the transport of live specimens to the laboratory, as limited equipment in field work often restricts such transportation; (5) it should be relatively time-independent, especially if one wants to sample far away from the laboratory; (6) the cells should be amenable to air-drying if chromosome banding staining, or scanning electron microscopy (Webb 1974) are to be used.

The technique presented hereafter fulfills these requirements. Furthermore, the experience required for its successful completion is minimal. The basis is Moorhead's (1960) mammalian





peripheral blood culture technique, modified to make it a micromethod, to adapt it to fish, and to include an option for field work.

Materials and Methods

(a) Anesthetic—Bath of 0.01% MS-222 (Sandoz Pharmaceuticals) or bath of 1% urethane.

(b) Culture medium—Parker 199 medium supplemented with 30% foetal bovine serum, 1% penicillin–streptomycin solution (5000 U penicillin and 5000 µg streptomycin per millilitre), and 2% Phytohemagglutinin-M (Difco).

(c) Spindle-inactivating pretreatment—Addition to the culture medium of 0.002 to 0.01 µg/ml (depending upon the species, for the best results in terms of metaphase yield and degree of contraction) of Vinblastine Sulfate (*Velbe*, Eli Lilly & Co.).

(d) Hypotonic solution—Two to 3 parts (depending on the species) of distilled water added to 1 part of culture medium.

(e) Fixative—Methanol : glacial acetic acid, 3:1. Fresh fixative is mixed before every fixation.

(f) Strain—Five minutes in Giemsa (Gurr) 1:25 in phosphate buffer, pH between 6.8 and 7.2, or 20 min in 1:100 Giemsa.

Procedure

Whole blood is obtained from a fish by cutting the tail off at the level of the anal fin, after sterilization of the surrounding skin with 95% ethanol. It may be necessary to anesthetize the fish for this operation. Blood is drawn in a sterile Pasteur pipette wetted with heparin. The specimen can then be preserved for identification. If the blood is obtained away from the laboratory, it can be kept in culture medium free from Phytohemagglutinin, and away from strong heat. Three or 4 drops of whole blood are then added to 5 ml of culture medium in a sterile vial or tube. Blood can be kept this way for a few days.

Upon arrival at the laboratory, the cell suspension is centrifuged, and the cells are aseptically transferred to fresh culture medium containing Phytohemagglutinin. The vials are incubated *at room temperature* in the dark for about 3 days. Mitoses have been obtained between 2½ and 4½ days of incubation. The cells are treated with the spindle-inactivating agent for 1 h.

For the hypotonic treatment, the cell suspension is transferred to a test tube and centrifuged for 5 min at 500–1000 rpm. Most of the medium is removed, distilled water is added to the remaining medium to obtain the desired concentration (see *d* above), and the cells are suspended for 15 min in this solution, which is kept close to 20 °C. The suspension is again centrifuged at 500–1000 rpm. All but 0.2 ml of the hypotonic solution is removed and 2 ml of fresh fixative is added drop by drop; the cells are resuspended very often. The suspension should be drawn slowly through a fine disposable syringe a couple of times to reduce the clumps. It is allowed to stand for 25 min.

After centrifugation, the liquid phase is removed and the test tube inverted for a few seconds on a filter paper. Then 1 to 2 ml of fresh fixative is added and the cells are resuspended. The suspension should again be drawn a couple of times through the fine disposable syringe. It is allowed to stand for 10 min. The solution is centrifuged and the fixative removed. Fresh fixative is added until a weakly opalescent suspension is obtained. One or 2 drops of the cell suspension are put on an alcohol-clean slide wetted in distilled water; it is blown on a little, then left to dry completely in vertical position. It should not be flamed or heated if chromosome banding is to be attempted. The slides are dehydrated very well by running them through steps of alcohol, or by placing them for 1 or 2 h in vacuum, or by storing them for at least 3 days in sealed boxes containing a desiccant.

The slides are stained when needed, washed with tap water, and air-dried. Stain contrast can be controlled by examining the slide without a cover slip under the microscope. Slides are immersed in xylol and mounted with Permount medium.

Remarks

When the specimens are too small, the blood from several individuals may be pooled to obtain enough cells for a fixation. Fishes as small as 29 mm in total length were studied in this way, in which case the blood from three to five individuals had to be pooled. Fishes from 60 mm in total length and up are big enough in general to provide the 3 or 4 drops of heparinized blood needed for one culture vessel.

A drawback of this method is that, although it works very well with most species, with some others it does not work *at all*. We have never succeeded, for instance, in stimulating the blood of *Lota lota* and of *Phoxinus phoxinus*, and transforming their mononuclear lymphocytes into dividing cells. The reason for this is unknown to us: it may be due to some deficiency of the culture medium, which could perhaps be accounted for by the use of fish serum instead of bovine serum, or it may be related to the action of Phytohemagglutinin, whose action is still not fully understood. In these cases, a piece of body tissue could be aseptically removed from the fish and brought to the lab in a vial of culture medium, and Roberts's (1964) primary culture method could be used instead of blood culture.

FIG. 1. Chromosomes of a female *Anguilla anguilla* from Kävlingeån River in the province of Scania, Sweden (13°13′35″ E, 55°46′40″ N). Collected in October 1971. Giemsa.

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DENTON, T. E., and W. M. HOWELL. 1969. A technique for obtaining chromosomes from the scale epithelium of teleost fishes. Copeia, 1969(2): 392–393.

GRAVELL, M., and R. G. MALSBERGER. 1965. A permanent cell line from the fathead minnow (*Pimephales promelas*). Ann. N.Y. Acad. Sci. **126**: 555–565.

HECKMAN, J. R., and P. E. BRUBAKER. 1970. Chromosome preparation from fish blood leukocytes. Prog. Fish-Cult. **32**(4): 206–208.

IVANOV, V. N. 1972. Techniques for fish chromosome preparation. English edition of Vopr. Ikhtiol. **12**(3): 389–396.

LABAT, R., R. LARROUY, and L. MALASPINA. 1967. Technique de culture des leucocytes de *Cyprinus carpio* L. C.R. Acad. Sci. Ser. D, **264**: 2473–2475.

MCPHAIL, J. D., and R. L. JONES. 1966. A simple technique for obtaining chromosomes from teleost fishes. J. Fish. Res. Board Can. **23**(5): 767–769.

MOORHEAD, P. S., P. C. NOWELL, W. J. MELLMAN, D. M. BATTIPS, and D. A. HUNGERFORD. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res. **20**: 613–616.

OJIMA, Y., S. HITOTSUMACHI, and M. HAYASHI. 1970. Jpn. J. Genet. **45**(2): 161–162.

ROBERTS, F. L. 1964. A chromosome study of twenty species of *Centrarchidae*. J. Morphol. **115**(3): 401–417.

WEBB, C. J. 1974. Fish chromosomes: a display by scanning electron microscopy. J. Fish Biol. **6**: 99–100.