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An Improved Technique for Chromosome Karyotyping from Embryonic and Soft Tissues of Tilapia and Salmonids

M.G. HUSSAIN* and B.J. McANDREW

Institute of Aquaculture
University of Stirling
Stirling FK9 4LA, Scotland, UK

Abstract - In the present technique, we modified partly or completely, many conventional steps of chromosome methodologies for teleost fishes which have been reported (Kligerman and Bloom 1977; Chourrout and Itskovich 1983; Chourrout and Happe 1986; Don and Avtalion 1986). The modified technique resulted in superior and high quality chromosome spreads on the periphery of cell rings obtained from samples of embryonic and soft tissues of Nile tilapia, *Oreochromis niloticus*, and two salmonids, *Salmo salar* and *S. trutta*.

This improved karyological technique is readily and widely available for tilapia and salmonids, as well as for other finfish species suitable for aquaculture.

The main fish specimen used in this study were *Oreochromis niloticus* col-lected from an electrophoretically tested pure stock of Nile tilapia originally obtained from a wild population of Lake Manzala, Egypt, in 1979 (McAndrew and Majumdar 1983) and presently available at the Institute of Aquaculture, University of Stirling, Scotland. The triploid progenies of the fish were produced by pressure, heat and cold shocks according to Hussain et al. (1991); the dip-loids were collected from the control group of 3n sibs and haploids were made available by *in vitro* fertilization of eggs with UV-irradiated sperm (UV dose: 300- 310 mW/cm^2, duration: 2 minutes).

The triploid fry of two salmonids were collected from the Howeitoun Fish Farm, Stirling, Scotland, where these progenies were induced by applying heat shock to recently fertilized eggs of the Atlantic salmon, *Salmo salar* (shock: 30°C x 10 minutes, 20 minutes after fertilization; Johnstone 1987) and the brown trout, *S. trutta* (shock: 28°C x 8 minutes, 15 minutes after fertilization; Infante 1990).

For karyotyping, embryonic tissues were collected from newly hatched or 1 day-old larvae of haploid, diploid and triploid groups of *O. niloticus* offsprings. For each group (ca 100) 15-20% embryos were placed in a small petri dish containing 8-10 ml of 0.002-0.005% colchicine solution (freshly prepared or stored at 4°C for 4-6 hours at 28°C). Tissues were obtained from the embryos in chilled 0.7% saline solution under a dissecting microscope by removing their heads and yolk sacs and putting these in distilled water (hypotonic solution) for

*Present address: Fisheries Research Institute, Mymensingh 2201, Bangladesh.

8-12 minutes. The tissues were then immersed in a fixative of 4:1 methanol-acetic acid at 4°C. After two changes the tissues were stored in the fixative for 30-90 days. To prepare the slides, the tissues were removed from the fixative and, after blotting out the excess fixative, placed in the cavity of a perspex slide with two to three drops 60% glacial acetic acid (Analar Grade), and minced for 1 minute with a glass rod to allow sufficient dissociation of epithelial cells. After 15-20 minutes, three to four drops of cell suspension were dropped from a height of 30-40 cm onto a clean glass slide on a warmed hot plate (44-48°C) and withdrawn within 8-12 seconds leaving a fine and clean ring of cells using a single micro-hematocrit dropper. Slides were air dried and stained with freshly prepared 10% Giemsa stain (prepared in 0.01M phosphate buffer pH 7.0) for 15-20 minutes. The slides were rinsed in distilled water, air dried and mounted with DPX after 10 minutes of Xylene wash.

Soft tissues like gill epithelia and the soft edges of the caudal fin were collected from 30- and 70-day old (after hatching) fry of Nile tilapia and two salmonids (*Salmo salar* and *S. trutta*), respectively. The fry were placed overnight (10-12 hours) in a plastic container with aerated 0.01-0.02% colchicine solution. The temperature of the colchicine solution was maintained at 28°C for tilapia fry and 10°C for salmonid fry. Tissues were collected with fine scissors and forceps then transferred immediately to distilled water for 15-20 minutes before being fixed in 4:1 methanol-acetic acid (two changes) and stored at 4°C up to 30 days. Slides were prepared according to the same technique described for chromosome karyotyping from embryonic tissues.

Metaphase spreads of chromosomes were checked and chromosome number noted by observing the slides under x400 and x1000 (oil immersion) magnifications, respectively, with an Olympus compound microscope. The karyological examination was carried out by counting the chromosomes of as many karyotypes as possible per slide.

Table 1 presents the results of our recently improved technique for chromosome karyotyping from embryonic and soft tissues of representative fish, Nile tilapia and two salmonids. Slides prepared from embryonic tissues involving a large number of haploid, diploid and triploid progenies confirmed a high percentage (95-100%) of performance with increased number of metaphase spreads. Slide preparations of soft tissues like gill epithelia and soft edges of caudal fins of all 3n progenies was found positively effective (having around 70% slides with metaphases), more or less in both tilapia and salmonids.

Inadequate colchicine and hypotonic treatment led to many unburst nuclei containing uncountable and overlapping chromosomes in the preparation. A 4:1 preparation of the fixative, methanol-acetic acid was found effective in keeping the tissues in good condition before preparing the chromosome slides for 30-90 days (after fixing). Any difference in the proportion of mixing these chemicals led to adverse results. The rate of dissociation of epithelial cells in acetic acid was observed to be rather critical; a concentration of 50% acetic acid (Kligerman and Bloom 1977) did not help to disperse the tissues in the cell suspension, while 70% quickly disintegrated the cells. Mincing the tissues and immersing them in 60% acetic acid for 15-20 minutes was determined to be the standard. In the present method, dropping the cell suspension from a greater

Table 1. Percentage of slides with chromosome spreads and yield of maximum and minimum countable metaphases per slide prepared from embryonic and soft tissues of representative slides.

Tissues/species	Progenies (number)	Age (days a.h.)	Slides with metaphase (%)	Countable metaphase/slide	
				Max.	Min.
Embryonic tissues					
**Oreochromis niloticus*					
Haploid (n)	120	0-1	100	<15	<5
Diploid (2n)	300	0-1	100	<20	<3
Triploid (3n)	1,200	0-1	95-100	<20	<3
Other soft tissues					
O. niloticus (3n)					
Gill epithelia	40	30	80-100	<15	<2
Caudal fins	40	30	40-60	<15	<2
**Salmo salar* (3n)					
Gill epithelia	40	70	60-80	<5	<3
Caudal fins	40	70	80-90	<7	<3
**S. trutta* (3n)					
Gill epithelia	40	70	60-70	<5	<3
Caudal fins	40	70	70-80	<7	<3

*Chromosome number: *O. niloticus* (n=22; 2n=44; 3n=66); *S. salar* (3n=87); *S. trutta* (3n=120).

height (30-40 cm) on a warmed slide resulted in a better spread of metaphase chromosomes. However, the method of simply expelling the cell suspension on the slide as suggested by Kligerman and Bloom (1977) did not work well in our investigation. We also found that the temperature of the prewarmed slide was a most sensitive parameter for improving the quality of slides. Temperatures of 50-55°C and 36-42°C showed poor and scattered chromosome morphology. The best temperature for high quality metaphase spreads was 44-48°C (Hussain et al. 1991). Staining the slides with 4% Giemsa (Kligerman and Bloom 1977; Chourrout and Itskovich 1983; Chourrout and Happe 1986) did not improve the visibility and brightness of the chromosomes in the metaphase; while 20% Giemsa (Don and Avtalion 1986) stained the metaphases too deep, making it difficult to score the chromosomes. In this technique, using a 10% concentration of freshly prepared Giemsa was found most adequate.

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