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Improved Techniques of Chromosome Preparation from Shrimp and Prawns

W.S. LAKRA, P. KUMAR, M. DAS AND U. GOSWAMI¹

*Fish Genetics and Breeding Division
Central Institute of Fisheries Education
Versova, Mumbai 400 061, India*

¹

*National Institute of Oceanography
Dona Paula, Goa 403 004, India*

Abstract

This paper reports simple techniques for obtaining high quality somatic and meiotic chromosome spreads from larval and adult stages of shrimp and prawns. These techniques were successfully applied to study the cytogenetic profile of *Penaeus monodon* ($2n = 88$), *Metapenaeus affinis* ($2n = 88$) and *Macrobrachium rosenbergii* ($2n = 118$). The cephalothorax of postlarvae, and hepatopancreas, regenerated blastema and testicular tissues of adult specimens provided the best chromosomal plates. The improved techniques will be helpful in cytogenetic characterization and improvement of commercially important shrimp and prawn species.

Introduction

The chromosomes of decapod crustaceans were first observed by classical gonadal sectioning or squashing methods as reviewed by Murofushi et al. (1984) and Murofushi and Deguchi (1990). Despite the development of the air-drying method of chromosome preparation, progress in chromosome research in decapod crustaceans has been very slow, mainly due to the small size and large number of chromosome complements in most crustacean species. However, some workers have made notable contributions in crustacean cytogenetics (Mittal and Dhall 1971; Milligan 1976; Hughes 1982; Goswami 1985; Hayashi and Fujiwara 1988; Nakamura et al. 1988; Murofushi and Deguchi 1989; Chow et al. 1990; Justo Chavez et al. 1991; Murofushi et al. 1991; Xiang et al. 1994; Kumar and Lakra 1996). In this paper, we describe improved techniques for obtaining good quality metaphase and meiotic chromosomes from a variety of shrimp and prawn tissues.

Materials and Methods

Postlarvae of *Penaeus monodon* (2-3 cm TL) and *Metapenaeus affinis* (2.5-3 cm TL), and adult specimens of *Macrobrachium rosenbergii* (8.1-12.4 cm TL) were collected live from Kakinada (Andhra Pradesh), Versova (Maharashtra) and Naigaon (Maharashtra), respectively. These animals were brought to Bombay and maintained in the wet laboratory of the Central Institute of Fisheries Education. The larvae were fed with *Moina* and *Artemia nauplii*, while the adult specimens were given clam, beef liver, carrot, potato, etc.

The postlarvae were treated with 0.0065% colchicine for 10 h during the night, then the cephalothorax region was used for chromosome preparation after removing the rostrum, antennae, eye stalk, legs and abdomen. Minced cephalothorax tissue was kept for hypotonic treatment in 0.9% sodium citrate for 1 h at room temperature. It was then transferred to Carnoy's fixative with three changes at an interval of 15 minutes. Metaphase chromosome slides were prepared from the fixed tissue following Kligerman and Bloom (1977). The slides were stained in 10% Giemsa in Sorenson's phosphate buffer for 25 minutes and differentiated in distilled water. The well-spread metaphase plates were photomicrographed with an Olympus BHS-313 research microscope.

Superior quality metaphase spreads were obtained from regenerated blastema. In this technique, the first pair of periopods were cut, and the animals kept in a well aerated tank with proper feeding. After 8-10 d, regenerated blastema had grown to 2-4 mm in size. It was then removed and treated with 0.025% colchicine solution for 1 h. The hypotonic treatment, fixation and slide preparation were done in the same manner as described above.

The adult specimens of *M. rosenbergii* were injected intraperitoneally with 0.01-0.1% colchicine at the rate of $1 \text{ ml} \cdot 100 \text{ g}^{-1}$ body weight. The colchicine solution was prepared in 50% filtered seawater. The injected specimens were allowed to swim in well aerated aquaria for 3-4 h, after which they were sacrificed and their hepatopancreas and gonadal tissues dissected out. The rest of the protocol was the same as described earlier.

The meiotic chromosome preparation required male gonadal tissue, i.e., testes. A cell suspension of testicular tissue was made in hypotonic solution (0.9% sodium citrate) by gently shaking in a test tube for 20 minutes. It was then centrifuged at 1,200 rpm for 7 minutes. The supernatant was poured off and the cells re-suspended in fresh fixative. Further processing of the tissue was the same as described above for postlarvae.

Results and Discussion

Each step in the preparation of tissues and slides for cytogenetic analysis is important in attaining a large number of well-spread metaphases. The first step in the procedure is the treatment of the organisms with colchicine, which

arrests cell division at metaphase. The optimum concentration for postlarvae was determined for the first time as 0.0065% solution for 10 h.

The 0.01-0.1% colchicine treatment (injection) to *M. rosenbergii* adults for 3-4 h was found effective for obtaining both mitotic and meiotic chromosomes. However, Justo Chavez et al. (1991) reported that for tissues of antennal gland and testis in *M. rosenbergii*, the most effective treatment was colchicine injection at 1-2 $\mu\text{g}\cdot\text{g}^{-1}$ body weight, (maintenance for 8-12 h at 28°C). Chow et al. (1990) even obtained meiotic chromosomes in *P. aztecus*, *P. duorarum* and *P. setiferus* without colchicine treatment.

The blastema tissue was treated with 0.025% colchicine for 1 h. This provided superior quality metaphase plates in *P. monodon*. This concentration is higher than that used by Hayashi and Fujiwara (1988) on *P. japonicus* where they placed the tissue in a physiological saline solution with 0.01% colchicine at room temperature for 30-60 minutes. The hypotonic solution used in the present study was 0.9% sodium citrate which gave satisfactory results when the treatment was carried out for 30-60 minutes at room temperature. Hayashi and Fujiwara (1988) and Justo Chavez et al. (1991) used 0.075 M and 0.01 M KCl for 30 minutes and 11-12 minutes in *P. japonicus* and *M. rosenbergii*, respectively. Chow et al. (1990) obtained good results by using only distilled water as a hypotonic solution for 10-15 minutes in a chromosomal study on *P. aztecus*, *P. duorarum* and *P. setiferus*.

The fixative treatment was found to be as important as the hypotonic and colchicine treatments in obtaining well-spread metaphases. A series of three short exposures (15-20 minutes) at room temperature, with fresh fixative added to each change, followed by an overnight exposure at 4°C, proved to be the most effective method of fixing.

The method of slide preparation was crucial in obtaining slides suitable for cytogenetic analysis. Good slides for cytogenetic analysis were obtained by the following technique: the tissue was gently macerated with fine-tipped forceps in 50% acetic acid. The cell suspension was dropped from a height of 15-20 cm, onto ethanol-cleaned hot slides (40-60°C). The slides were allowed to dry for 2-3 d, then stained with 10% Giemsa solution for 25 minutes.

The techniques described above resulted in slides with good chromosomal plates in *P. monodon* ($2n = 88$), *M. affinis* ($2n = 88$) and *M. rosenbergii* ($2n = 118$) (Figs. 1-3). These techniques are easy to use for cytogenetic analysis in field conditions.

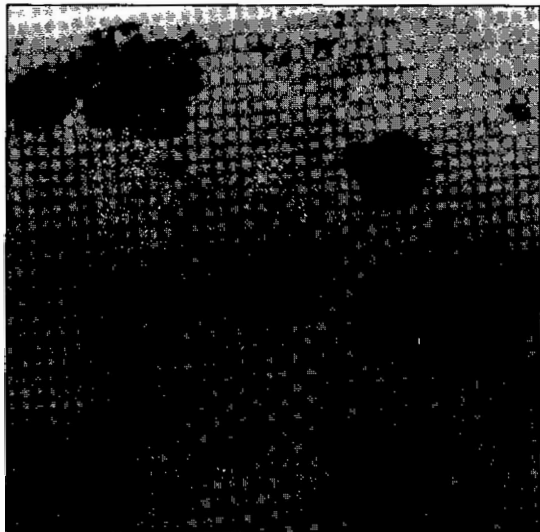


Fig. 1. Mitotic metaphase chromosomes (multiple spreads) from cephalothorax tissue of *Penaeus monodon* ($2n = 88$) x 350

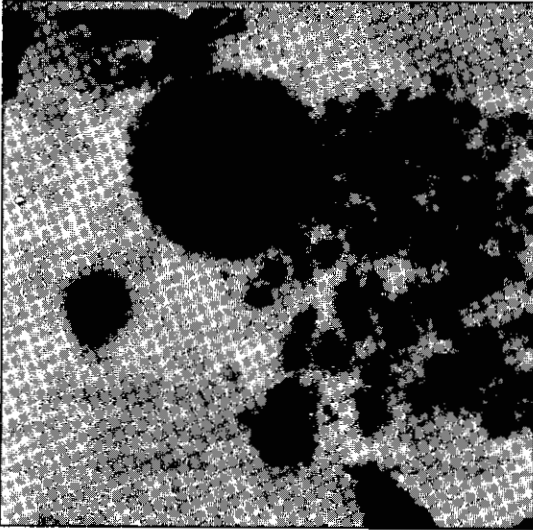


Fig. 2. Mitotic metaphase chromosomes from blastema tissue of *Penaeus monodon* ($2n = 88$) x 750.

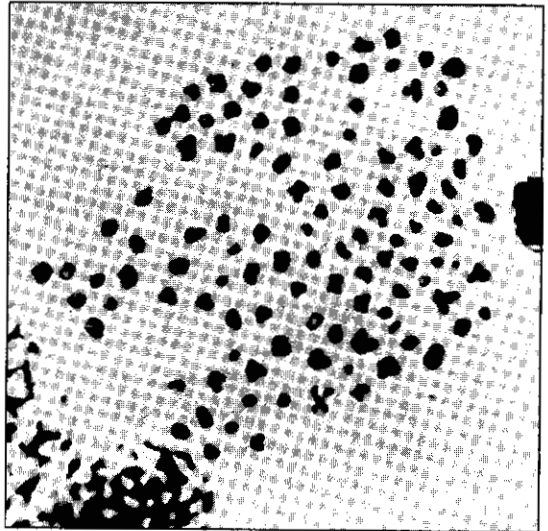


Fig. 3. Mitotic metaphase chromosomes from hepatopancreas of *Macrobrachium rosenbergii* ($2n = 118$) x 750.

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