Chromosome studies on the marine shrimp *Penaeus (Fenneropenaeus) merguiensis* from the Persian Gulf

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Progress in Penaeid shrimp genetics, inbreeding and biotechnology has been slower than fishes; perhaps one of the reasons is the lack of knowledge on fundamental aspects of their biology (Xiang et al., 1991). A group of basic information for such studies is karyological information and their use for better understanding of the genealogy, taxonomy, chromosome manipulation, inbreeding experiments, identified genetically abnormal shrimps or polyploid individuals (Chavez Justo et al., 1991).

Research on chromosome numbers, structure and composition in penaeidae has been relatively difficult, mainly due to the relatively small size and large number of chromosomes. Another reason is karyological techniques limitation.

Shrimp culture is the most important beneficial trade sector in Southeast Asia; it started in Iran in 1994 and has rapidly expanded during the last two decades (Kakoolaki et al., 2010). *Penaeus (Fenneropenaeus) merguiensis* L., 1975 is one of the most important species from the viewpoint of catching in the Persian Gulf for Iranian fishermen. However, several studies have been carried out on specimens of this species from different locations around the world (Xiang et al., 1996; Petsiri, 1997). There are several reports based on existing geographical differences in chromosome numbers of Penaeidae shrimps (Campos-Ramos, 1997). For understanding these differences, we ventilate this investigation on *P. merguiensis* from the Persian Gulf.

For obtaining the best chromosome spread, adult specimens, 21 wild mature shrimps (13 females and 8 males) were used. The mature shrimps were brought alive by placing them in fresh, aerated seawater, transferring them to the Hatchery and placing them in concrete tanks; they were fed with Melalis and Clams. Adult male and female specimens were 110-120 mm TL, 30.0-46.0g BW and 130-140 mm TL, 34.0-70.0g BW, respectively. Mature shrimps were obtained by trawling in fishing zones between eastern Qeshm Island in the Persian Gulf and western Jask in the Oman Sea.

For chromosome spread preparations from adult tissues, shrimps were injected intraperitoneally (between

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mouth and first periopod) or intramuscularly (first or the second abdominal segment) with 2µg of Colchicine solutions (0.5% per gram of body weight) (Chavez Jesto et al., 1991, Tan et al., 2004) and kept alive in aerated sea water at 30 ± 2^{0}C for periods of 3.30, 4.30, 5, 6 or 7 hr, after which they were sacrificed. Tissue from the gills, hepatopancreas, ovaries and testes tissue was dissected out and cut into small pieces of approximately 2mm. The tissues pieces were immersed in 0.075 M KCl or 0.9% Sodium Citrate in distilled water for hypotonization and stayed in the hypotonic solution for 20, 25, 30, 35, 50 or 60 min at room temperature (approximately 25^{0}C). The samples were then fixed in a fresh chilled solution of methanol and acetic acid (3:1) for 30, 40 or 60 min with several changes.

Two methods, warm and cold were used for chromosome preparations. In the cold method, the pieces of tissue were squashed on a chilled (4^{0}C) glass slide by stamping the tissue using forceps. The warm method was done according to the method of Campos-Ramos (1997) with some modifications. In this procedure, tissue pieces were squashed on the Petri dish by using a 7 cm plastic rod in the fixative solution until a suspension was obtained. 1ml of the suspension was dropped from a height of 5-20 cm on a hot (45^{0}C) glass slide, or the suspension was put into Eppendorf tubes and then was centrifuged at 1500 rpm for 3 min; the supernatant was decanted and the cells were re-suspended in fresh fixative. 1 ml of suspension was dropped on a hot glass (40-50^{0}C) slide and then the slides were allowed to dry up.

The slides were kept at room temperature to dry and then they were stained with 10% Giemsa (Merck, Germany) diluted with Sorensons Phosphate buffer (pH 6.8) for 10, 15 or 20 min; then rinsed gently with distilled water and allowed to air dry for at least 24h.

Mitotic and meiotic chromosomes were observed at 40 × magnifications and photographed at a magnification of 100× using an Olympus photomicroscope with digital camera (Olympus C-35DX, Japan) and Fuji colour films (ISO 400/27^0). The chromosome counts were made on the photographs of each metaphase plate. Three of the best mitotic metaphases were chosen for karyotyping. The morphometric measurements of chromosome were conducted using the photographic software Photoshop 6.0 (Adobe systems). Each chromosome was tagged with a reference number. Chromosomes were arranged in homologous pairs, classified by their relative arm-length ratio and then arranged by decreasing size.

Suitable metaphase plates were not obtained from Gill, hepatopancreas and ovary tissues. The fat tissue in hepatopancreas and the yolk in ovary, especially in advanced stages of sex maturation were hampering the preparation of suitable metaphase chromosomes. Suitable metaphase chromosomes were obtained from testes; the testicular tissue due to containing both mitotic cells (spermatogonias) and meiotic cells (spermatocytes); both diploid and haploid chromosome numbers were obtained. The evident difference was observed in the

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chromosome condensation using different Colchicine treatment incubation.

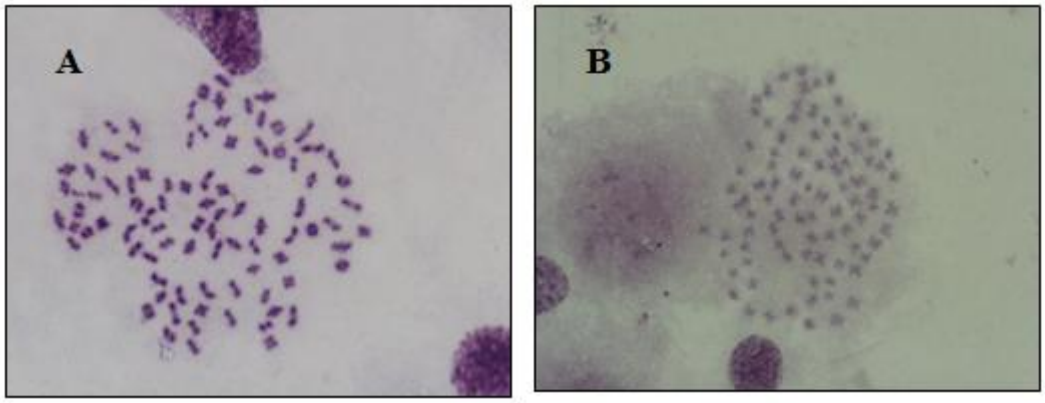

Figure 1: Different degrees of condensation on mitotic metaphase chromosomes obtained from testes. A, 2n = 88, Colchicine treatment = 5hr, 400X. B, 2n = 88, Colchicine treatment = 3hr and 30 min, 400X.

However, longer incubation led to a greater chromosome condensation. In this study, the most condensed chromosomes were obtained by the 7hr treatment (Fig. 2C). The most effective colchicine and hypotonic treatment for suitable metaphase plate was for 5-6 hr and 45 min, respectively (Figs. 1A, 2C) and the optimum method for chromosome preparation was the Squash method. The best Giemsa concentration & time for staining was 10 -12% and 15-20 min, respectively.

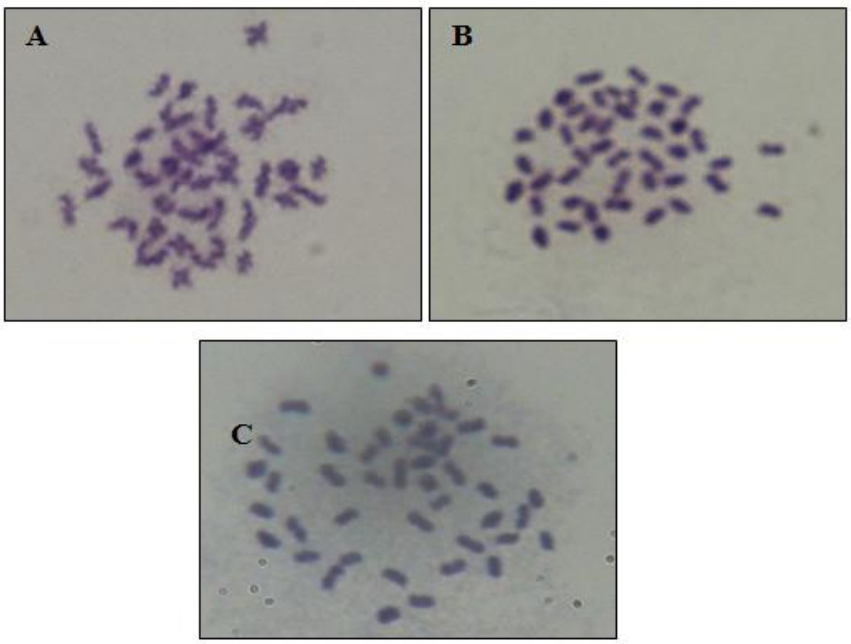

Figure 2: Different degrees of condensation on miotic metaphase chromosomes obtained from testes. A, n = 44, Colchicine treatment = 5hr, 1000X. B, n = 44, Colchicine treatment = 3hr and 30 min, 1000X. C, n = 44, Colchicine treatment = 7hr, 1000X

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From the metaphase chromosome counts obtained from testes, the diploid chromosome numbers were considered to be 2N=88. The percentage of the modal diploid cells was 26.5% of 64 cells. The chromosome number ranged from 79-90 (Table 1). The diploid chromosome number was confirmed by obtaining the haploid chromosome number of N = 44 from testes tissue. The chromosome number varied from 38- 47 (Table 2). The modal haploid cells were 54.7% of 84 cells.

Table 1: Diploid chromosome numbers of the *P. merguiensis*

	79	80	81	82	83	84	85	86	87	88*	89	90	Total
Cells	3	2	3	3	4	5	4	5	6	17	7	8	64
Percentage	4.7	3.2	4.7	4.7	6.2	7.8	6.2	7.8	9.4	26.5	11.0	7.8	100

* Modal diploid chromosome number (2N = 88).

Table 2: Haploid chromosome numbers of the *P. merguiensis*

	38	39	40	41	42	43	44*	45	46	47	Total
Cell	3	7	6	4	9	4	46	1	3	1	84
Percentage	3.6	8.3	7.1	4.8	10.7	4.8	54.7	1.2	3.6	1.2	100

* Modal haploid chromosome number (N = 44).

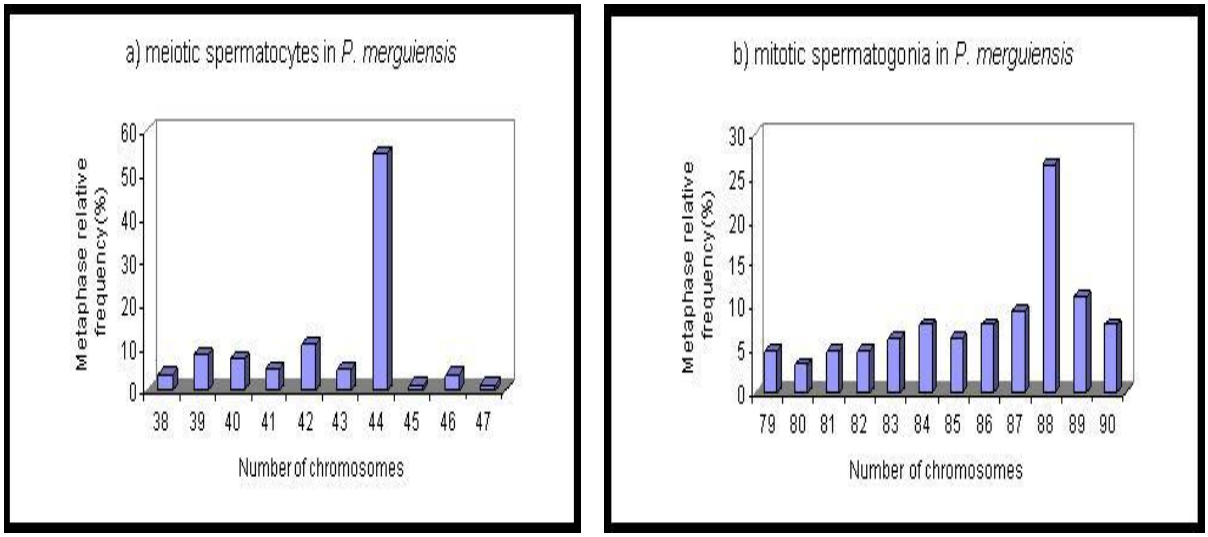

Figure 3: Frequency distribution for haploid (a) and diploid (b) chromosome counts in *P. merguiensis*

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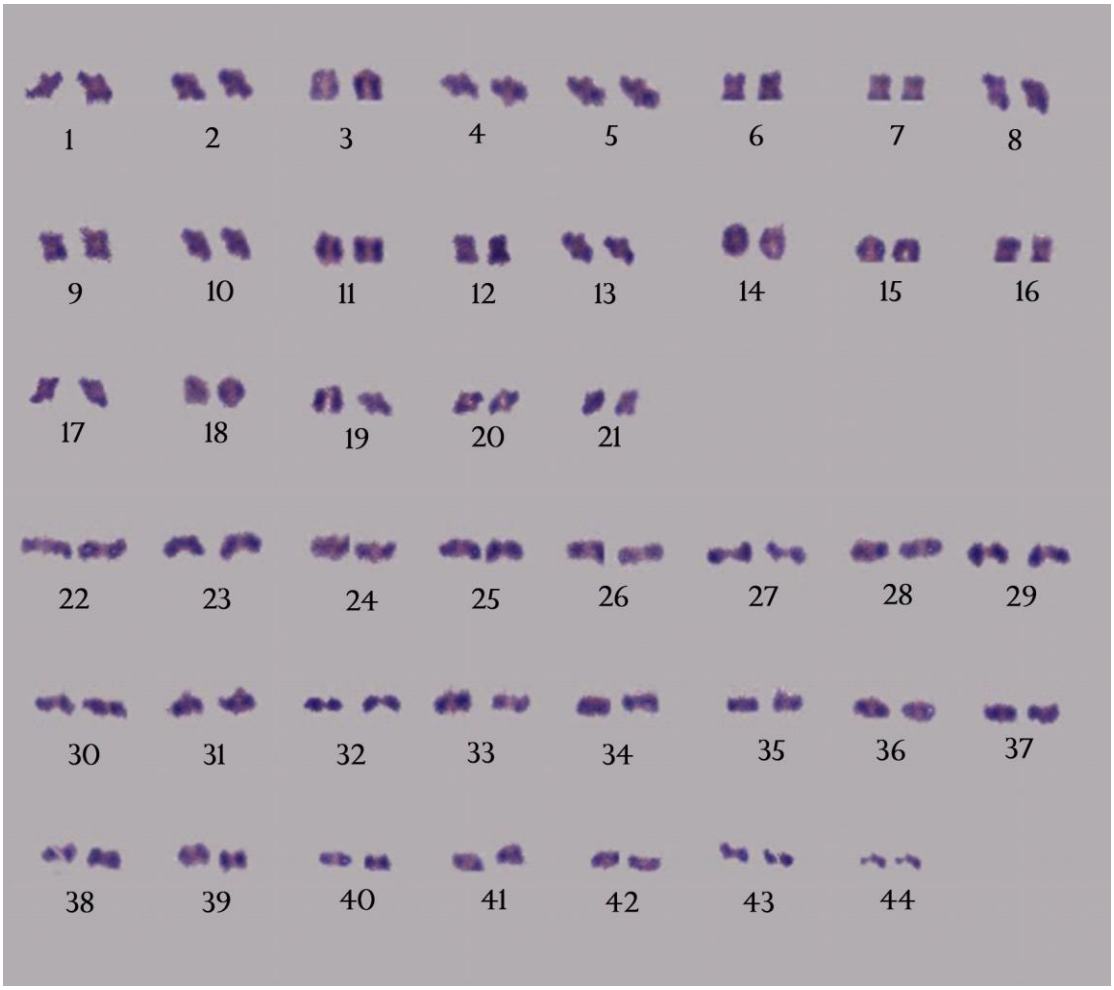

Figure 4: Karyotype of *P. merguiensis*. 21 pairs are metacentric and submetacentric (numbers 1-21); the remaining 23 pairs are considered as acrocentric and telocentric

Rapidly growing tissues are most suitable for obtaining numerous chromosome spreads in metaphase for cytogenetic studies (Tan et al., 2004). In previous studies, the testis was proven to be an excellent tissue for karyotype analysis since it is not only a source of meiotic metaphases but also a source of mitotic metaphases (Chow et al., 1990; Xiang et al., 1994), which was also granted by our results.

The Colchicine concentrations of 1-2 µg /g BW with a 4-6 hr maintenance period have effectively arrested dividing cells in metaphase in various temperate crustacean species (Hasegawa, 1981, Murofushi and Deguchi, 1983). In this study, we administrated colchicine at a dose of 2µg /g BW by injecting it into the muscle of the first or second abdominal segment of the male and successfully obtained both mitotic and meiotic metaphases (3-7 hr maintenance period at 30 ± 2^0C, see Figs. 1, 2).

In another study, Xiang et al., 1996 only used males for chromosome preparation. They injected 0.1-0.3 ml/g BW of 0.2% (w: w) colchicine solution to *P. merguiensis* and *P. esculentus* males for 4-5 hr and successfully obtained meiotic and mitotic chromosomes in testis tissue.

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During karyotype analysis the extent of chromosome condensation may differ greatly in different cells or during different mitotic stages, it can also be attributed to different treatments (Jixun et al., 1989). In this study, different colchicine incubation periods were examined. Colchicine treatment with incubation period of 7hr resulted in ample metaphase chromosomes (from testis tissue) but chromosomes were condensed (Fig. 2C). This implies that the duration of incubation in colchicines strongly affects the chromosome quality where clearness is decreased. In colchicine treatment with 3hr and 30 min incubation, the chromosomes were not clear, therefore it can be deduced, if the duration of colchicine incubation is short, the quality of chromosomes will decrease as well (Figs. 1B, 2B).

The existence of microchromosomes, the large number and small size of chromosomes with progressively decreasing length in penaeidae shrimps make it difficult to identify them individually (Campos-Ramos, 1997). Most of the chromosomes in penaeid karyotypes are subtelocentric or acrocentric (Goswami, 1985).

In the present study, 21 pairs were metacentric and submetacentric (numbers 1-21) and 23 pairs were acrocentric and telocentric (numbers 22-44). It was difficult to distinguish between acrocentric and telocentric chromosomes due to the small size of chromosomes. The karyotype formula proposed for *P. merguiensis* from the Persian Gulf was: 2n = 88 = 21(m, sm) + 23(t, a) (Fig. 3). However, Petsiri (1997) observed 28(m, sm) + 16(T,t) and Xiang et al. (1996) have not conducted karyotype due to the small size of individual chromosomes.

The number of diploid chromosomes of *P. merguiensis* from Australia and Thailand have been recorded, 2n = 88, by Xiang et al. (1996) and Petsiri (1997). The present study demonstrated that *P. merguiensis* from the Persian Gulf has numbers of chromosomes which are similar to those found in other members of the species in Thailand and Australia. Therefore, further research will be necessary to verify geographic difference in chromosome numbers among species of Penaeus.

Karyological studies are not only useful to the taxonomy but also essential to genetic and inbreeding studies and chromosome manipulation. Up to now, polyploidy manipulation has successfully been applied to Oysters and Salmonid fishes at commercial scales (Nell et al., 1994; Benfey, 2001) and in shrimp *Litopenaeus vannamei* it has only been conducted at experimental scales (Chen et al., 1997; Dumas and Ramos, 1999; Li et al., 2003) but in *P. merguiensis* it has not yet been studied. However, our study adds knowledge on the chromosome preparation but it also foresees the possible difficulty for polyploidy manipulation in this species duo to the large number and small size of chromosomes.

Eight of 26 species in Decapoda and one of 6 species in the Isopoda have heteromorphic pairs of sex chromosome (Niiyama, 1959; Jixun et al. 1989). No heterotrophic pairs of sex chromosomes were recorded in all of the species in the Penaeus studied up to now, therefore it can be reasoned that the sex in Penaeus may be

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determined by other mechanisms and not by sex chromosomes. In the present study we did not observe heteromorphic pairs of chromosomes.

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