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Study of Chromosome Morphology of Venerid Clam Paphia Textile

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Abstract

In recent years, the number of cytogenetic studies on bivalves has remarkably increased. Karyotypes of mollusc species have been studied by banding techniques and fluorescence in situ hybridization (FISH), although most of them are limited to a few families, such as Mytilidae, Ostreidae, Pectinidae, Cardiidae and Veneridae. Chromosome spreads of bivalves were also used using different tissues: gonad, embryos, and adult gills. During the present study, chromosome spreads of venerid clam, P. textile were used to study the karyotypes of this clam. The study indicated that the species had 38 chromosomes with 7 metacentric, 5 submetacentric, 3 subtelocentric and 4 telocentric pairs. The Absolute length of the chromosomes ranged from 1.1 to 2.8. The Relative length ranged from 3.23 to 8.24. The Centromeric index ranged from 0 to 48.14. The Arm ratio showed a variation between 1.08 to ". Fundamental arm number for P. textile was 64. During the current study, the percentage of euchromatin observed in both the long and short arms of the chromosomes of the clam species was more compared to the heterochromatin, indicating active regions of replication and transcription. This also indicated that the phenotypic expression of genes and also the variations observed in the species were due to the euchromatin.

Keywords : P.textile, Veneridae, karyotypes, euchromatin, heterochromatin

Introduction

Class Bivalvia (formally named Pelecypoda - axe foot or Lamellibranchia – leaf like gills) is a class of molluscs that first appeared in the Lower Cambrian period. Bivalves underwent a period of limited diversity during the Palaeozoic and then boomed during the Mesozoic so that they are now the second largest molluscan class. There are roughly 10,000-15,000 species globally found today, present in both freshwater and marine environments. One of the Subclass of this class, Heterodonta of the marine bivalve molluscs includes family Veneridae which has over 400 living species such as the edible clams, the cockles etc. The word 'clam' is popularly used while referring to the members of several bivalve families which burrow into the substratum with the help of usually well developed foot. Among the exploited bivalve resources of India, clams are by far the most widely distributed and abundant. Clam fishery in Maharashtra is mainly dependent on M. meretrix, K. opima and Paphia laterisules (Ranade, 1964). Good clam fishery is reported from the Ratnagiri coast of Maharashtra. It is represented by clams belonging to genus Paphia, Meretrix and Katelysia. These clams support an artisanal fishery along the major

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estuaries i.e. Kalabadevi and Kajali estuaries along Ratnagiri coast, where Paphia textile (Chinese clam) has great demand for its taste. Paphia textile, commonly known as 'Chinese clam', has very glossy shell with v-shaped markings. Shell is elongate, moderately inflated and elliptical-ovate in outline; anterior and posterior margins rounded; lunule is lanceolate, delicately outlined by a finely impressed line; outer shell surface smooth, glossy, pale yellowish-white in colour and marked with pale purplish grey inverted V-shaped markings; hinge narrow, concentrated under the umbo of each valve, with 3 radiating cardinal teeth but without lateral teeth (Barash and Danin, 1973).

Though the morphological characteristics based on the shell structure, shape, depth, concentric lines, ridges, and the cardinal teeth which can be used as taxonomical tools in the identification of these bivalves, variations in the colour patterns and markings are observed. Under stressful or unfavourable conditions, the shell morphology tends to change and may pose a problem in identifying the species. Chromosomes, being species specific, can prove an appropriate tool for the identification and comparison of the genetic relationship among the genus belonging to one family and species belonging to one genus.

Material and Methods

Samples of *P. Textile* collected monthly from the estuarine areas along the Ratnagiri coast using drag nets and by hand picking, were transported in polythene bags to the laboratory and stored in tubs with filtered sea water. They were cleaned by a wire brush to remove adhered sand, mud and other organisms. Then they were kept into well aerated individual beakers having filtered sea water at 20-25°C for acclimatization for 24 hours before experimentation. The clams that had open valves after the acclimatization period were discarded. Species identification was done following the diagnostic characters described by Anon (1974).

The acclimatized healthy clams were kept in colchicine solution (0.005 %) for 6–8 hrs to arrest the chromosomes in Metaphase. Gills and gonads were dissected from each treated clam and treated twice with 0.56% KCl solution for 15 min at room temperature. They were fixed by freshly prepared Carnoy's solution (ethanol: glacial acetic acid, 1:3 by volume) kept at 2- 4^{0}C. The solution was changed 3-4 times every 15 min and then kept at 4^0 C till chromosome preparation (Martínez et al., 2002; Ferna´ndez-Tajes et. al., 2003).

For karyotyping, photographs of metaphase chromosomes spreads were taken with the help of a Leica DM 500 microscope and enlarged pictures were obtained. Individual chromosomes were cut out from the spread, arranged by their decreasing sizes and classified according to the centromeric index, following the nomenclature of Levan, et al., (1964). The chromosomes were then matched up using size and centromere position as guides. Following calculations would be done from the observations.

$$\text{The relative length} = \frac{\text{Chromosome length}}{\text{Total length of haploid set}} \times 100$$

$$\text{Centromeric index} = \frac{\text{Length of shorter arm of a chromosome}}{\text{Total chromosome length}} \times 100$$

$$\text{Arm ratio} = \frac{\text{Length of the long arm of the chromosome}}{\text{Length of the short arm}}$$

The chromosomes were classified into metacentric, submetacenteric, telocentric as per Ganai and Yousuf (2013). Chromosome measurements were also made using the computer application MicroMeasure version 3.2 (Reeves and Tear, 1999).

Result and Discussion

P. textile has medium sized solid, equivalve, inequilateral shell. The umbones are well in the anterior part of shell. Outline of the shell is elongate, almost twice longer than high; dorsal margin sloping to a narrowed posterior margin; anterior rounded slightly acute. Hinge is without lateral teeth. The well defined lunule is slightly depressed. Surface of the shell is shinny. Sculpture is smooth with growth lines only. Pallial sinus is round pointing to the beaks reaching half the shell's height. Internal margin is also smooth. The chromosome morphology and ideograms of P. textile (Plate 1 and 2, Table 1, Fig. 1) indicated that the species had 38

chromosomes with 7 metacentric (Nos. 1 to 7), 5 submetacentric (Nos. 8 to 12), 3 subtelocentric (Nos. 13 to 15) and 4 telocentric (Nos. 16 to 19) pairs. The Absolute length of the chromosomes ranged from 1.1 to 2.8. The Relative length ranged from 3.23 to 8.24. The Centromeric index ranged from 0 to 48.14. The Arm ratio showed a variation between 1.08 to ". Fundamental arm number for *P. textile* was 64.

Measurements of the chromosomes were taken by using vernier calipers as well as by the MicroMeasure 3.3 software, during the current study. The marked chromosomes are represented by Plate 3. Point-like features along several chromatids have been marked: centromeres are marked with large circles and the segments are marked by green lines. The excel data generated by the software is represented in Table 2. The data generated by the software showed the length measurements of long and short arms, the total length of the chromosomes, arm ration and Centromeric index. The amount of euchromatin and heterochromatin in each arm were also calculated by the software MicroMeasure 3.3.

In recent years, the number of cytogenetic studies on bivalves has remarkably increased. Since 1992, karyotypes of about 65 species have been studied by banding techniques and fluorescence in situ hybridization (FISH), although most of them are limited to a few families, such as Mytilidae, Ostreidae, Pectinidae, Cardiidae and Veneridae (Thiriot-Quievreux, 2002). Lin et. al (2008) reported that chromosome spreads of bivalves were usually prepared with three methods using different tissues: gonad, embryos, and adult gills. The authors did comparative experiments on chromosome preparation from the three tissue-specific methods and showed that gonad chromosome preparation, especially with mature eggs, didn't produce clear metaphase plates, and chromosome arms were tangled probably because of meiotic pairing. There were few well-spread chromosomes, so it was difficult to do karyotypic analysis. They got more mitotic metaphase plates from adult gill chromosome preparation in M. mercenaria, than other species, such as M. meretrix (Lu et al., 2003) and Tegillarca granosa (Zheng et al., 1996), and better chromosomal morphologies. But all chromosomes were too small to be measured accurately for karyotypic analysis. During the present study, chromosome spreads were prepared by using gonad and adult gill tissues of the selected clams. Better results were obtained from the adult gill tissues.

The chromosome number reported in the Venerid clams during the present study was reported to be 2n = 38. Within the bivalve class this is the most frequent chromosome number (Thiriot-Quievreux, 2002). Their karyotypes are very different and could be used for identification of one species from another. This also suggests the rearrangement of the chromosomes during the divergence of the species.

Present study used MicroMeasure, a new computer application written for the collection and analysis of cytogenetic data. Measurements of the chromosomes were taken by using vernier calipers as well as by the MicroMeasure software, during the current study. With MicroMeasure, the user creates an on-screen tracing of chromosomes and other features present in an image. Measurements are then calculated from this tracing. Green et al., (1980, 1984) and McGurk and Rivlin (1983) developed methods for computer-aided measurements. A set of programs by Oud et al., (1987) allowed researchers to measure chromosomes and perform limited statistical analyses on these measurements. In this study, the researchers chose not to include routines for automated chromosome identification. This software allowed marking the chromosomes and related features had been carried out, by using length measurements including total length, arm length, arm ratio, and centromere index and exported to Microsoft Excel spreadsheets.The chromosome segments or the chromatids as well as the centromeres were marked and lengths were measured by the software.

Table 1. Karyomorphometric analysis of somatic metaphase complement of Paphia textile

Chromosome pair No.	Length of long arm (L) (μm)	Length of short arm (S)(μm)	Absolute length of chromosome (L+S)(μm)	Centromeric index = Short arm/Total length*100	Arm ratio =Long arm /Short arm (L / S)	Relative length = Chromosome length/Total haploid set*100	Chromo-some class
1	1.5	1.3	2.8	46.42	1.15	8.24	m
2	1.4	1.3	2.7	48.14	1.08	7.94	m
3	1.1	0.9	2.0	45.00	1.22	5.88	m
4	1.2	0.8	2.0	40.00	1.5	5.88	m
5	1.1	0.7	1.8	38.39	1.57	5.29	m
6	1.3	1.1	2.4	45.84	1.18	7.05	m
7	1.3	1.2	2.5	48.00	1.08	7.35	m
8	1.3	0.7	2.2	31.81	1.85	6.47	sm
9	1.2	0.7	1.9	36.84	1.71	5.58	sm
10	1.0	0.6	1.6	37.35	1.7	4.70	sm
11	1.2	0.7	1.9	36.84	1.71	5.58	sm
12	1.1	0.6	1.7	35.29	1.83	5.00	sm
13	0.9	0.3	1.2	25.00	3.0	3.52	st
14	1.0	0.3	1.3	23.07	3.33	3.82	st
15	0.9	0.3	1.2	25.00	3.0	3.52	st
16	1.3	-	1.3	00	∞	3.82	t
17	1.3	-	1.3	00	∞	3.82	t
18	1.1	-	1.1	00	∞	3.23	t
19	1.1	-	1.1	00	∞	3.23	t
Total	**22.3**	**11.5**	**34.0**	**564.64**	**26.9**	**99.92**	

Diploid chromosomes number **= 38**

Chromosomes formula **= 7m + 5sm + 3st +4t**

Fundamental arm number **= 64**

Total complement length **=34.0**

Table.2. Excel data generated by MicroMeasure 3.3 software for P. textile

Set ID: PT		
Mag:	600	
Image resolution:	37.79	pixels per cm

Marking order	Rank	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Eu. in long arm	Het. in long arm	Eu. in short arm	Het. in short arm
2	1	12.28	6.69	5.59	1.20	0.46	0.00	6.69	0.00	5.59
6	2	9.09	5.98	3.10	1.93	0.34	0.00	5.98	0.00	3.10
15	3	9.07	5.74	3.33	1.72	0.37	5.74	0.00	3.33	0.00
5	4	8.78	4.73	4.04	1.17	0.46	4.73	0.00	4.04	0.00
13	5	8.37	5.15	3.23	1.59	0.39	0.00	5.15	0.00	3.23
12	6	7.60	4.68	2.92	1.60	0.38	4.68	0.00	2.92	0.00
1	7	7.46	4.49	2.97	1.52	0.40	0.00	4.49	3.53	0.00
7	8	7.25	3.82	3.43	1.11	0.47	3.82	0.00	0.00	3.43
4	9	6.89	5.29	1.60	3.30	0.23	0.00	5.29	0.00	2.97
3	10	6.61	3.44	3.17	1.09	0.48	3.44	0.00	3.17	0.00
26	11	6.53	3.49	3.03	1.15	0.46	3.49	0.00	3.03	0.00
28	12	6.42	3.59	2.83	1.27	0.44	3.59	0.00	2.83	0.00
10	13	6.32	3.43	2.89	1.19	0.46	3.43	0.00	0.00	2.89
23	14	5.02	3.83	1.19	3.2	0.24	3.83	0.00	2.93	0.00
19	15	5.00	3.75	1.25	3.00	0.0.25	3.75	0.00	2.70	0.00
9	16	3.23	3.23	0.00	∞	0.00	0.00	4.13	0.00	1.86
20	17	3.17	3.17	0.00	∞	0.00	3.17	0.00	0.00	0.00
22	18	2.83	2.83	0.00	∞	0.00	2.83	0.00	0.00	0.00
11	19	2.83	2.83	0.00	∞	0.00	2.83	0.00	0.00	0.00

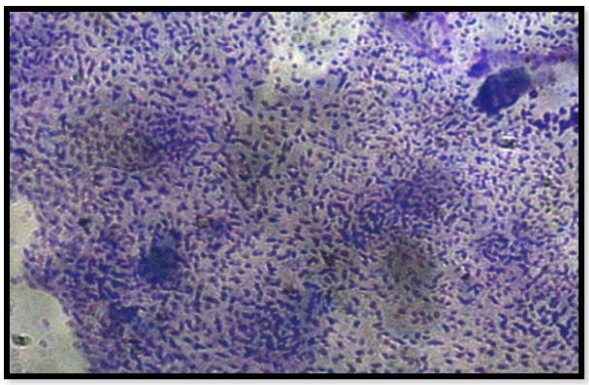

Plate.1. Giemsa-stained metaphase chromosomes of *P. textile*

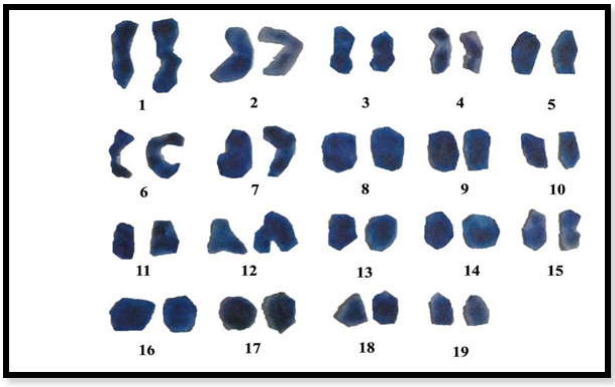

Plate.2. Karyotype of *P. textile*

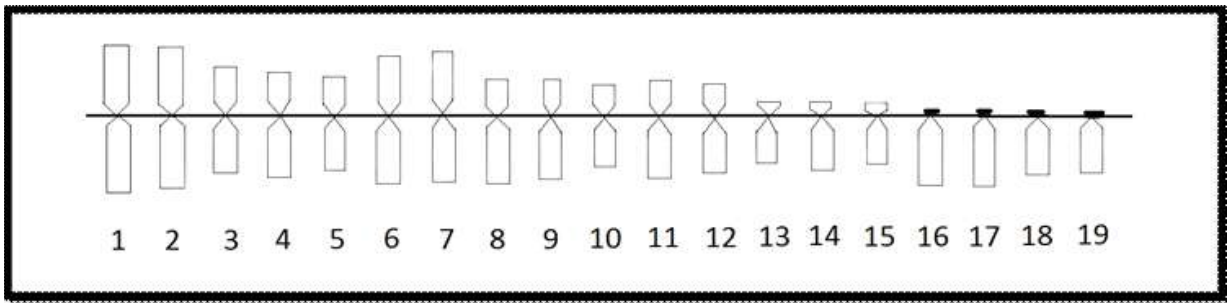

Fig.1. Ideogram of *P. textile*

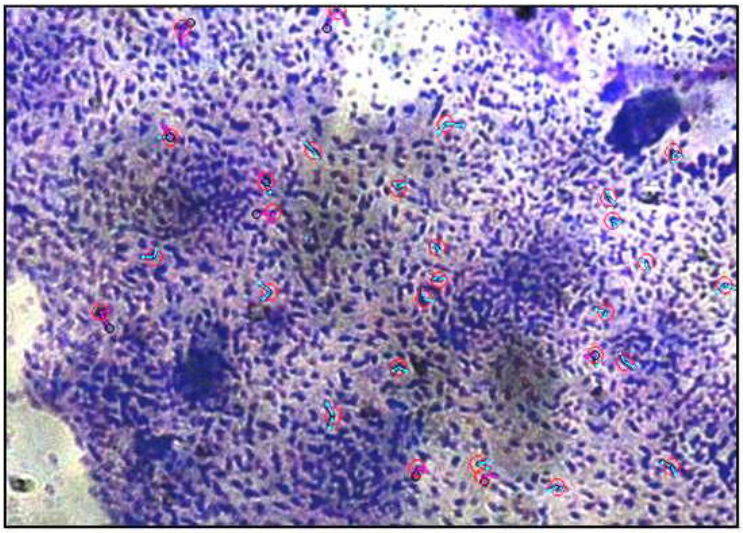

Plate.3. Chromosome measurements done by MicroMeasure 3.3 for *P. textile*

Chromatin is the basic material of chromosome. Chromatin can be of two types depending on staining properties. Euchromatin is the portion of chromosome with lightly packed DNA, partially condensed and stain lightly. DNA is packed in 3 to 8nm fibers. Genes situated on this portion are structural genes, these genes undergo replication and participates in the active transcription. Euchromatin comprises the genetically most active portion of the genome, have role in phenotypic expression of genes. Most of this chromatin disperses after completion of mitosis. Heterochromatin is the portion of chromosome with tightly packed DNA that always remains in the condensed state and stain dark. DNA is tightly packed in the 30nm fibers. Heterochromatin shows late replication and limited transcription. It also has high content of repetitive DNA sequence and it contains very few structural genes. During the current study, the percentage of euchromatin observed in both the long and short arms of the chromosomes of the clam species was more compared to the heterochromatin, indicating active regions of replication and transcription. This also indicated that the phenotypic expression of genes and also the variations observed in the species were due to the euchromatin. The morphological and structural variations within the species could be well attributed to the amount of euchromatin present in the chromatids. No satellite chromosomes were observed during the present investigations.

Ming-y et. al., (2012) studied the karyotype of P. undulate and reported that the length and the arm ratio of chromosomes were measured and calculated with Micromeasure 3.3 software. The results showed that the chromosome number of P. undulate was 38 and the karyotype formula was summarized as 2n = 38. The formula derived was 14m+12sm+4st+8t.The number of chromosome arm was 64. No satellite chromosome had been found in the chromosome set of P. undulate. The length of chromosome was about 1.5-4.0 ìm.

Chromosome numbers of Veneridae were reported as of three types, i.e, 2n = 28, 30, 38 (Zheng et al., 2000; Que et al., 1999; Borsa and Thiriot-Quiévreux, 1990). The most common type is 2 n = 38. Wang et. al., (1999) believed that 2 n = 38 seem to be the initial diploid type of bivalve mollusc, and the number of diploid evolves in two directions in the course of evolution, one is increase and the other is decrease.

The chromosomal changes accompanying bivalve evolution are an area about which few reports have been published. In the similar work, Pérez-García et. al., (2014 a and b) reported about the chromosomes of five species of venerid clams (Venerupis corrugata, Ruditapes philippinarum, Ruditapes decussatus, Dosinia exoleta, and Venus verrucosa). Both the chromosome numbers and the karyotypes determined in this work for Ruditapes philippinarum, R. decussatus, Venerupis corrugata, Venus verrucosa, and Dosinia exoleta were in agreement with previous results (Borsa and Thiriot-Quiévreux, 1990; Insua and Thiriot-Quiévreux, 1992; Ebied & Aly, 2004 and Hurtado & Pasantes, 2005) and further confirm that, unlike other families within the order Veneroida in which interspecific differences in chromosome numbers have been detected, all Veneridae species have the same chromosome number, 2n = 38. The high variation in karyotype composition detected in these species indicated that speciation in Veneridae was accompanied by some chromosome rearrangements. Present work confirms the findings, where the selected clam species, *P. textile* showed diploid chromosome number as 38.

Conslusion

The study of Venerid clam, *P. textile* indicated that the species had 38 chromosomes with 7 metacentric, 5 submetacentric, 3 subtelocentric and 4 telocentric pairs. The Absolute length of the chromosomes ranged from 1.1 to 2.8, while the Relative length ranged from 3.23 to 8.24. The Centromeric index ranged from 0 to 48.14. The Arm ratio showed a variation between 1.08 to Fundamental arm number for *P. textile* was 64. During the current study, the percentage of euchromatin observed in both the long and short arms of the chromosomes of the clam species was more compared to the heterochromatin, which could be attributed to the phenotypic expression of genes that may result in the variations observed in the shell markings.

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