CHAPTER 01
“Cytogenetics”, is the scientific study, which deals with the study of structure and function of chromosomes. Chromosomes were first observed in plant cells by Swiss botanist Karl Wilhelm Von Nägeli and in animal cells of Ascaris worms by Belgian scientist Edouard Van Beneden (1842). The role of chromosomes was described in detail by a German anatomist Walther Flemming (1882) in Salamander cells during mitosis.

John Gregor Mendel’s experiments with the Peas, established that the traits from parents pass as unmodified “units”, (which we now call “genes”) to the successive generations according to set ratios. Individuals possess two sets of factors, one of each derived from either one of the parents. It makes no difference whether a particular character is inherited from the mother or the father; both the parents contribute in the same way. Furthermore these factors are sometimes expressed and sometimes concealed but never lost. Studies on heredity illustrated that the phenomenon described by Mendel’s laws corresponds completely to the observations made of the division of the nucleus and the fusion of the nuclei of the chromosomes derived from two parents. In 1904 W. S. Sutton and T. Boveri combined these ideas to postulate the chromosome theory of inheritance and convinced that the chromosomes behave just like the Mendelian units (soon to be called genes) and that each chromosome must carry many of them. In 1910, Morgan by his observations made on the common fruit fly, proved that the Mendelian factors were nothing but genes which were situated in the chromosomes and later it was established that the chromosomes were in fact complexes of nucleic acids and proteins. Their condensed state is due to the binding of nucleic acids to basic proteins (Benjamin, 2000). Each species has a specific number of chromosomes called its diploid chromosome number, which is maintained through generations by two types of cell divisions in eukaryotes called mitosis (somatic) and meiosis.
During mitosis prophase stages of the cell division, the duplicated elongated chromosomes start condensing to the maximum degree, into short structures by metaphase stage. The structural details of the chromosomes can be best studied during this period of the cell division, with the aid of a transmitted light microscope. The duplicated chromosomes at this stage are held together at their centromeres on the equatorial plane. In metaphase stage the kinetochore which is a protein structure, assembles on the centromere and serve as the point of attachment for microtubules (spindle fibers) assembled from the centriole. The ability of the microtubules to assemble and disassemble is utilized by the cytogenetists to arrest highly condensed chromosomes at the metaphase, where the chromosome structure can be illustrated.

1.1 Chemical constituents of chromosomes

Chromosomes are nucleo-protein complex structures located in the cell nucleus. They are composed of DNA, histone and non-histone proteins, RNA, and polysaccharides. DNA is the essential component of the chromosomes, which may be regarded as a device for carrying genetic information from one generation to the next generation. All other components serve as the supporting elements to accomplish this purpose and also to maintain the integrity of the genetic material. DNA normally occurs as a double stranded polymer, the two strands held together by hydrogen bonding between complementary bases. The common form of the double helix, not only in aqueous solution, but probably also in the cells (Hamilton et al. 1959; Hanlon et al. 1972) is right handed helix, generally B-form DNA. The double stranded DNA can be denatured upon exposure to variety of agents such as heat, acid or alkali treatments. However, on return of the favourable conditions, the separated strands reanneal to form the duplex structure.

Proteins are the second important constituents of the chromosome structure. Primarily two types of
proteins are found in the chromosome structures, that is histone chromosomal proteins (HCP) and non-histone Chromosomal proteins (NHCP). The major proteins being histone proteins, the histones are a group of small, basic proteins that are highly conserved throughout the evolution and are confined to nuclei and chromosomes. These proteins are present in a quantity roughly equal to that of DNA (Sumner, 1990). Five types of histones are normally present in the eukaryotic chromosomes: H1, which is lysine rich; H2A and H2B, slightly lysine rich; and H3 and H4, which are arginine rich. H1 differs from the other histones in having a larger molecule, and consisting of several different but closely related fractions. Histones are principally structural proteins. All the histones are subject to modification by methylation, acetylation, phosphorylation or ADP-ribosylation, and in addition H2A and H2B may also combine with the polypeptide ubiquitin (Wu et al. 1986). It has been suggested that such modifications might be involved in functions such as transcription or chromosome condensation, but conclusive evidence is lacking as yet. All chromosomal proteins other than histones tend to be lumped together as non-histone chromosomal proteins (NHCP). There are at least several hundred different NHCPs (Peterson, 1976) although only about 15-20 major ones (Elgin, 1975). Together these make up the bulk of mitotic chromosomes, being up to three or four times as abundant as DNA (Bostock and Sumner, 1978). The other important constituent of the chromosomes is the RNA. Metaphase chromosomes have a higher ratio of RNA to DNA than interphase chromatin (Bostock, 1980), but the values obtained for this ratio are highly variable, and there is no doubt that a substantial amount of non-chromosomal RNA can become attached to chromosomes during isolation procedure (Mendelsohn, 1974).

Number of divalent cations is also associated with the chromosomes. Potassium and chloride are most abundant ions in chromosomes; there is moderate amount of sodium and relatively small quantities of magnesium and calcium (Cameron et al. 1979; Warley et al. 1983). Extraction of divalent cations, from chromosomes causes them to swell, and this swelling can be reversed if the appropriate ions are restored (Hadlaczky et al. 1981; Zelenin et al. 1982). At finer level, the presence or absence of divalent cations affects the appearance of chromatin fibers (Ris, 1975), thus there is good evidence that certain
inorganic ions have an important structural function in chromosomes. It is also believed that the chromosome structure also involves trace amounts of carbohydrates (Daskal et al. 1978).

1.1.2. Assembly of Chromosomal Constituents into chromosome

The major problem in the construction of chromosomes is how to pack such an enormous length of DNA into the Cell nucleus of about just a few micrometers. In order to overcome this problem cells have evolved a sophisticated mechanism of condensation. A variety of evidence has shown that the first level of packaging of DNA is into bodies called nucleosomes (Pederson et al. 1986; Van Holde, 1989). These consists of 146 base pair (bp) of DNA wrapped round an octomeric histone bead which contains two molecules each of histones H2A, H2B, H3 and H4. A short length of linker DNA connects these beads to each other, this linker DNA is wrapped around a linker histone molecule i.e. H1 and certain HMG proteins may also be bound to the linker (Einck and Bustin, 1985).

Chromosomes have been characterized in many species belonging to various phyla, still there are many species belonging to different phyla are not yet cytogenetically characterized. In the present study the chromosomes of *Perna viridis* are analysed in detail. *Perna viridis* apart from being an important bivalve which is used as food in the coastal regions of India, is unique to possess diploid chromosome complement number as 30, where as all the other species of the genus *Perna* have 28 as the diploid chromosome number.

1.2 Biology of the *Perna viridis* (Linnaeus, 1758).

In the present thesis molecular cytogenetic studies has been carried out on the commercially important bivalve mollusca *Perna viridis*, commonly known as “The Green Mussel” The species forms an important fishery along the coasts of India and an esteem delicious item of marine food.
The genus *Perna* consists of three species, *Perna canaliculus*, *Perna perna* and *Perna viridis*. Along the coast of India, two species of the genus *Perna* are distributed i.e. *Perna viridis* and *Perna perna*. *Perna viridis*, the green mussel are among the commonest of marine molluscs and constitute an important element in the ecology of coastal waters. *Perna viridis* inhabit inter-tidal and sub-tidal coastal waters up to the depth of 15 meters. The mussel’s being sessile, prefer rocky open coasts and are always found attached to rocks and other hard substratum by means of byssus threads secreted by them. These mussels measure 80–100 mm in length, occasionally reaching 165 mm. The shell has a smooth surface covered with a periostracum that is vivid green to dark brownish near the outer edge and olive green near the attachment point. The ventral margin of the shell is straight or slightly concave. The interior of the shell valves is shiny and pale bluish green. The life span of the mussels is 2–3 years. They have a wide tolerance of salinity and temperature. They are suspension or filter feeders, feeding mainly on the small zooplanktons and phytoplanktons. During the process they accumulate toxic substances in to their soft tissues and are thus excellent biomonitoring organisms of coastal water quality.

In similarity with all other members of pelecypoda, *Perna viridis* lacks a head, pharynx, tentacle, radulla and are completely enclosed in a bivalve shells of which the valves are lateral, hinged together middorsally, with correspondingly bi-lobed mantle. These are untorted bilaterally symmetrical mollusks. Absence of anterior adductor muscle, two retractor scars, 10–18 primary lateral teeth, usually two dysodont teeth, sometimes one, pitted resilial ridge and smooth shell set apart genus *Perna* from other three closely related genera of the family Mytilidae i.e. *Mytilus*, *Aulacomya* and *Choromytilus*.

### 1.2.1 Taxonomic Position of *Perna viridis*

Molecular Cytogenetic Studies in *Perna viridis* (Bivalvia:Mollusca) from Goa, West Coast of India.
Phylum : Mollusca (Linnaeus 1758, Cuvier 1795)
Class : Bivalvia (Linnaeus 1758)
SubClass : Pteriomorphia
Superorder : Isofilibranchia
Order : Mytiloida (Ferussc1872)
Superfamily : Mytilacea
Family : Mytilidae (Rafinesque 1815)
Subfamily : Mytilinae (Rafinesque 1815)
Species : *viridis*

1.2.2. Distribution

*Perna viridis* is broadly distributed in the Northern Indian Ocean, where it ranges west from the Persian Gulf and east to New Guinea and Japan and New Guinea for north and south ranges respectively. The native range of the green mussel is along the Indian coast and throughout the Indo-Pacific region. It is also believed that this mussel has the potential to increase its geographical distribution by stepwise larval dispersal or “Island hopping” (Siddal, 1988).

*Perna viridis* occurs naturally and is widely distributed along the inter-tidal coasts of India (Jones and Alagarswami, 1973). In India it is distributed along the east as well as along the west coasts of India. Along the east coast, the green mussel is found on small scanty beds along Chilka Lake, Visakhapatnam, Kakinada, Madras, Pondicherry, Cuddalore and Port Novo and Port Blair. Where as these mussels are abundant with extensive beds along the west coast of India and found all along the Quilon, Allepepy, Cochin, Calicut, Kasargod, Mangalore, Karwar, Goa, Bhatia Creek, Malvan, Ratnagiri and Gulf of
Animal collection stations
Dona - Paula

Odexel

Siolim
Perna viridis, Green mussel

Male

Female
Kutch (CMFRI, 1980). Along the coast of Goa, green mussel, *Perna viridis* is found throughout its length.

### 1.2.3. Reproduction and Growth

Sexes are separate, spawning normally occurs during February to March and September to November (Rao, *et al.* 1975). However green mussels located in the Johore straits (Tham *et al.* 1973; Choo, 1974) and Quozon Philippines (Walter, 1982) exhibit continuous breeding throughout the year. Spawning is initiated by either sex with each releasing its gametes into the water, where the fertilization occurs (Stephen and Shetty, 1981). The zygote develops into the motile trochophore larvae in 7 – 8 hours of fertilization after 16 – 19 hours it is transformed into the D-shaped veliger larvae. The larvae completely metamorphose into the adult in 8-12 days (Tan, 1975).

### 1.3. Need of the present thesis

#### 1.3.1. Aquaculture Practices

With global population expansion and shrinking resources of the food production. Number of modern technologies have been successfully applied to increase the agriculture production, still is not sufficient to sustain the continuously increasing population pressure. Thus the demand for high-quality proteins, especially from aquatic sources is rising enormously. There is a paradigm shift in demand for supply of sea-food. Increased aquaculture production is obviously essential to meet this demand in the third millennium.

Aquaculture has been in existence for the centuries as a traditional farming practice in Europe and Asian countries mainly for the edible oysters, fresh water finfishes and brakishwater shrimps. Global aquaculture production has increased from 26.6 million metric tons in 1996 to 45.46 million metric tons in 2004, recording fast growth rate all through these years (CMFRI, 2006).
India with a coastline of about 7000 Km and an exclusive economic zone (EEZ) of two million square kilometers reflects its full potential in the aquaculture industry. India’s annual marine fish catch is only about 2.4 million tonnes, which includes variety of finfishes and shellfishes. Most of the fish catch is supplied to the local markets. By adopting better aquacultural practices and the application of modern technologies such as genetic improvement programs can tremendously increase the supply of marine finfishes and shellfishes. Among molluscs, oysters (4.6 million metric tons) top the production followed by mussels (1.86 million metric tons) and scallops (1.16 million metric tons) in 2004 (CMFRI, 2006). The world mussel production by culture during 2004 was around 1.86 million tons. The largest producer of the mussel is China contributing 7,17,368 tons followed by Spain, France and Netherlands.

Molluscs, especially edible bivalves, are considered as very suitable for culture, as they have a high growth rate, high yield per total weight, high nutritional value, gives highest conversion of primary producers (Phytoplankton) to human food and moreover have a good export potential apart from easiness in culture practices.

Mussels, oysters and clams are an important source of nutrition in the coastal states of India. Goa state in India with a coastline of about 106 kms has vast resources of the edible bivalves. Among the bivalves, mussels are consumed in large quantities. Apart from their natural occurrence, mussels can be cultivated in the coastal waters on the raft cultures (Qasim, et al. 1977; Rivonker, 1991). The green mussel, *Perna viridis* and brown mussel *Perna perna* are the two species occurring along the Indian coasts. The green mussel enjoys a wider distribution along the east and west coasts of India including Andaman Islands, whereas the brown mussel is restricted to the southwest coast of India.

01. General Introduction

*Perna viridis*, being the most common bivalve species along the west coast, has a primary importance in the aquaculture industry. In these mussels, spawning can be induced throughout the year by using some physical and chemical treatments (Coeroli *et al.* 1984). These mussels are easy to breed and culture in the laboratory conditions, *Perna viridis* has very high growth rate (Shafee, 1979; Chatterji *et al.* 1984; Rajagopal *et al.* 1998). As an aquaculture industry it is not yet developed to its potential. Only few raft cultures operate along the southwest coast of India. Most of the fishing is done in the natural beds all along the west coast of India but scope for increasing natural production from existing beds is rather limited (CMFRI, 2006). Intervention of modern techniques in the aquaculture industry will be helpful not just in overcoming difficulties in obtaining better yields but also to increase the quality and quantity of the meat. Better quality and total yield will in turn be helpful in obtaining better market values.

Genetic improvement programmes can play vital role in setting better goals in the aquaculture industry. Two important genetic improvement programmes, which can be applied to the species of aquacultural importance, especially to the marine animal models like the green mussel, are chromosome set alteration (Polyploidy) and hybridization.

Technology of experimental alteration of the chromosome set is one important area, which has the potential to meet the demand for the high quality meat. Chromosome set manipulation permits the production of monosex and sterile individuals, which exhibit better growth rate, since they do not contribute their energy in the gametogenesis and absence of gametes in turn increases the quality of the meat. However for any genetic improvement programme, involving chromosome set manipulation, it is essential to have the knowledge of the normal chromosome complements. Even in the successful hybridization experiments, knowledge of karyotype may not be absolutely essential but obligatory, since similar chromosomes number indicates a better chance of hybridization. As a general principle, smaller the number of chromosomes, the more important it is for the hybridizing species to have equal number.
of chromosomes (Bye, 1983).

*Perna viridis* being a species of aquacultural importance and is a potential candidate for the genetic improvement programmes in India. Elsewhere in related species belonging to genus *Mytilus*, the genetic improvement techniques have shown promising results (Gosling, 1989)

### 1.3.2. Chromosomes and Cytotaxonomy

The genetic constitution of a population may change through time and space, consequently leading to evolutionary divergence. It is also evident that the genetic constitution of a species varies to a greater or lesser degree from one population to another and from generation to generation. Therefore, different populations of a single species would be expected to be at different evolutionary stages of divergence mainly due to intrinsic genetic adaptation to their slightly different microenvironments and degrees of extrinsic isolation. Such genetic differentiation in different populations of a species may lead to speciation. Thus the process of speciation essentially involves a divergence of genetic constitution, which must be sufficient to increase genetic incompatibility to various degrees between the newly evolved sibling species. It has generally been appreciated, that the chromosome number can shed light on the phylogenetic relationships of organisms (Nakamura, 1986).

Karyotype evolution is mainly due to chromosome segment rearrangements and differentiation / accumulation of heterochromatin and provides a useful tool for cytotaxonomy of many groups of animals (Baimai, 1998). There are numerous reports on inter- and intraspecific heterochromatin variation in relation to karyotypic evolution in both plants and animals (Pathak *et al.* 1973; Baverstock *et al.* 1983; Patton and Sherwood 1982). Furthermore, detectable differences in constitutive heterochromatin in mitotic chromosomes provide a useful criterion for separation of cryptic (isomorphic) species and of closely related species.

Chromosome morphology, as revealed by the standard metaphase preparations and banding techniques, has been used to distinguish species, hybrids within the species or even strains of a species and populations. In mollusca, cytotaxonomic work is confined only to limpet-form snails (Nakamura, 1986)
The general significance of karyotype evolution is obscure, in spite of our understanding to some extent the mechanism of change and their consequences. The diploid number of chromosomes, \(2n\), varies from two in the nematode *Parascaris* to 254 in the hermit crab *Eupagurus*, and over 500 in some ferns. In some taxa, the number is relatively uniform and conserved: for example \(2n=26\) in most of the dragonflies, and \(2n=36\) in many snakes. In some groups, acrocentric and metacentric chromosomes can be distinguished; the number of chromosome arms may then be more constant than the number of chromosomes. There can however be big differences between the numbers of chromosomes in some species. For example in the Assam sub-species of the Indian muntjac, *Muntiacus muntjac vaginalis*, male with \(2n=7\) and female \(2n=6\) and the Chinese muntjac, *M. reevesi* with \(2n=46\) (Wurster and Benirschke, 1970; Wilkinson *et al.* 1988; 1989; Lin *et al.* 1991; Brinkley *et al.* 1984). Chromosome number in fishes is highly conserved but few species belonging to the Scorpaenidae show high chromosome number diversity (Cataudella and Capanna, 1973; Sola *et al.* 1978; Thode *et al.* 1983; Amores *et al.* 1990; Yokoyama *et al.* 1992; Caputo *et al.* 1996; 1997; Corrêa and Galetti, 1997), especially in genus Scorpaena, where closely related species have different chromosome number, for example *S. notata* has \(2n = 34\), *S. isthimensis* has \(2n = 40\), *S. porcus* has 42 whereas *S. brasiliensis* possess diploid number as 46. Chromosome number variation in the closely related species have also been reported from order veneroida of the subclass pelecypoda, all species so far cytogenetically characterized show \(2n=38\) (Menzel 1968a; Ieyama, 1980; Rasotto *et al.* 1981; Gerard, 1978; Nishikawa and Hisatomi, 1959) except in *Tapes philippinarum* which has \(2n=28\) (Nishikawa and Hisatomi 1969). The green mussel, *Perna viridis* is unique, to have diploid number of chromosomes as 30 (Ahmed, 1974; Goswami and Fernandes, 1993), where as all other members of the genus *Perna* and *Mytilus* have \(2n=28\) (Ahmed *et al.* 1970; Ieyama, 1983; 1984 Moynihan and Mahon, 1983; Thiriot-Quievreux, 1984a; Dixon and Flavel, 1986; Pasantes *et al.* 1990; Insua and Thiriot-Quievreux, 1994). This suggests the need for the detailed cytogenetic investigation to establish the evolutionary and phylogenetic
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**1.3.3. Cytogenetics and Environmental Impact assessment**

Industrial and agricultural effluents are discharged into the ocean through the rivers and streams. These effluents have a wide range of potential toxic chemicals, some of which show genotoxic effects apart from being toxic at physiological levels. Genotoxic effects at molecular level range from base substitutions in the DNA to the breakages at chromosomal level. Such effects at chromosomes are manifested by chromosome breakages, rearrangements and nondisjuncture during meiotic and mitotic divisions. Normal chromosome structure studies, chromosome aberrations (CA) and sister chromatid exchange Assays (SCE) are important biomarkers for assessing impact of various effluents, discharged into the water bodies. Detailed chromosomal investigations are very much indispensable to use important species as the biomarkers in ecological studies. In recent past, the increased concern about the marine pollution has led to many investigations of the cytological effects of pollutants like surfactants (Abel and Skidmore, 1975; Schmid and Mann, 1961) and metals on fish gills (Skidmore and Tovell, 1972). In recent years the scientific attention has turned from fish to bivalves, particularly mussels, clams and oysters and their associated changes induced by environmental stressors. Bivalve animal models have several advantages over fish models in toxicology studies. Foremost advantage of the bivalves is cost and easy maintenance, when compared to fishes and secondly bivalves are less prone to secondary stressors such as infection by fungi or bacteria whereas fishes are very susceptible to such infections especially in the culture conditions (Bayne, *et al.* 1985). Bivalves are sessile, plentiful, inexpensive and relatively easy to maintain in the laboratory conditions and these qualities propose them as the best animal model candidate for scientific investigations and for biomarker studies.

Mussels, clams and oysters can concentrate a variety of chlorinated hydrocarbons (Risebrough *et al.* 1976), aromatic hydrocarbons (Di Salvo *et al.* 1975; Fossato and Canzonier, 1976) and metal ions (Friedrich and Filice, 1976; Philips, 1976; 1977a; 1977b; 1978; 1979a; 1979b; 1980; Simpson, 1979;
Lowe and Moore, 1979) within their soft tissues and therefore represent good indicators of xenobiotic bioaccumulation. Goldberg (1975) proposed that mussels should be used on a worldwide basis as indicators of environmental pollution. Bayne (1976) elaborated this proposal and suggested that mussels could also be used to measure the biological effects of observed pollution load, thus reflecting the ecological consequences of environmental contamination.

It has been observed and reported that the *Perna viridis* can adapt to harsh environmental conditions (Morton, 1987) and has wide salinity tolerance. Realization of the fact that this bivalve can bioaccumulate number of pollutants (Philips, 1980), inspires the need to investigate the clastogenic efficiency of some of the common pollutants.

### 1.3.4. Gene mapping

In recent years the molecular techniques have been applied to the cytogenetic studies. The most commonly used molecular cytogenetic technique is fluorescence in situ hybridization (FISH). FISH is usually applied to standard cytogenetic preparations on microscope slides, but it can be used on slides of formalin-fixed tissue, blood or bone marrow smears, and directly fixed cells or other nuclear isolates. The basic principle of the method is that single-stranded DNA will bind or anneal to its complementary DNA sequence. Thus, a DNA probe for a specific chromosomal region will recognize and hybridize to its complementary sequence on a metaphase chromosome or within an interphase nucleus. Both have to be in single-strand conformation, therefore the DNA probe and the target DNA must be denatured, usually by heating them in a formamide-containing solution.

The probe is hybridized to the target DNA under conditions that allow the DNA to reanneal in double-strand form. Added to the hybridization mixture is an excess of repetitive sequence DNA to block non-specific binding of the probe to the target. After hybridization, the slides are washed to remove excess or non-specifically bound probe. To detect the location of the probe on the target DNA, the probe
DNA can be directly labeled with a fluorescent tag. It can also be chemically modified by the addition of hapten molecules (biotin, digoxigenin or fluorescein) that can then be indirectly fluorescently labeled with immunocytochemical techniques. The target DNA is counterstained with another fluorochrome of a complementary color.

The probe DNA can be observed on its target by using a fluorescent microscope with filters specific for the fluorochrome label and the counterstain. Special filters have been developed to allow simultaneous visualization of several fluorochromes. Digital cameras designed to detect low light level emissions and computer imaging is used to increase the sensitivity of probe detection. Because fluorescent dyes are subject to photobleaching, the preparations are not permanent and must be stored away from light. Use of an antifade solution (phenylenediamine or DABCO) has improved the capacity to observe and document fluorescently labeled samples.

Microdeletion are small and often difficult to detect by conventional methods but can be detected precisely in a short time by applying in situ hybridization techniques. Combinations of FISH probes and standard cytogenetics can characterize structural rearrangements and the technique is helpful to identify the chromosome structural abnormalities.

The autosomal and sex chromosomal aneuploidies are responsible for most of the chromosomal disorders found in many animals including human conceptuses. Chromosome-specific cosmids or alpha satellite probes can be used in FISH studies to document chromosome gain or loss in cells that are not dividing. Interphase cytogenetics using FISH is a rapidly growing field. One can enumerate the number of fluorescent signals present in interphase nuclei.

FISH is used to map genes to specific chromosomes and chromosomal regions. Labeling the probe with different fluorochromes and detecting their hybridization color pattern can establish the order of genes or gene sequences within a chromosome. In a new technique called fiber FISH, chromosome specific chromatin fibers can be spread on a slide and then hybridized with locus-specific probe allow fine resolution mapping of DNA sequences.
1.4. Review of literature

1.4.1 Cytogenetic studies in Marine animals

Introduction of squash method coupled with colchicines treatment to arrest the chromosomes at the
metaphase stage (Roberts, 1964) and flame air-drying method (Denton and Howell, 1969) for the
preparation of the good metaphase plate has revolutionized the cytogenetic research. Study of cyto-
genetics became prominent after the analysis of the human chromosomes (Tjio and Levan, 1956) and also
recognition of some pathological conditions associated with the chromosomes, this led to the technical
advances in the field of human cytogenetics. Initially scientific investigations were mainly concentrated
on the mammalian cytogenetics in general and human cytogenetics in particular. Later cytogenetic
techniques were extended to analyze the chromosome structure and morphology of number of animals
and plants.

Cytogenetic investigations of the marine fishes have increased in the recent times; about 13000 marine
fish species exist (Nelson, 1994). Of these only 2% are cytogenetically characterized (Brum, 1996).
Fishes show little divergence in the chromosome number.

Most marine fishes studied had diploid complement of 48 acrocentric chromosomes (Sola et al. 1981;
Klinkhardt et al. 1995; Brum, 1996) with few exceptions, some species of Nototheniidae with 2n
between 22 to 26 (Ozouf-Costaz et al. 1997), an Antarctic fish group, has 2n=240–260 and in some
anadromous Acipenseridae, which show several micro chromosomes (Fontana et al. 1997). However,
some species belonging to Blenniidae, Gobiidae and Scorpaenidae, for instance, exhibit a remarkable
change in the chromosome number (Cataudella and Capanna, 1973; Sola et al. 1978; Thode et al.
1983; Amores et al. 1990; Yokoyama et al. 1992; Caputo et al. 1996; 1997; Corrêa and Galetti,
1997).

Along the coast of India, around 150 marine species have been cytogenetically characterized which
includes mainly commercially important fishes belonging to the groups teleost, cat fishes, cyprined fishes (Manna and Prasad, 1968; Mann, 74; Manna and Khuda-Bukhsh, 1977b; Lakra and Rishi, 1991)

1.4.2 Cytogenetic Studies in Phylum Mollusca

The living molluscs are estimated to include some 107,000 species divided in to seven classes (Nicol, 1969; Dolph et al. 1970). The class Gastropod makes up about 84% of all the species in this phylum. The pelecypods represent about 14% of the mollusks and remaining five classes polyplacophora, aplacophora, monoplacophora, cephalopoda and scaphopoda make up the remaining 2% of the phylum mollusca and includes about 2000 living species (Dolph and Humphery, 1970).

The first studies in the field of Molluscan cytology dates back to the nineteenth century, numbers of attempts were made to observe the sub cellular organelles. Because of inferior optical equipments and methods, however, many of the earlier reports were shown to be inaccurate (Patterson and Burch, 1978). Chromosome numbers of only 32 species were listed in Patterson’s review (Patterson 1970) and in the later years reports on the chromosome number in this group increased (Nakamura, 1986). These early works provide only fragmentary information since they generally aimed at the establishment of chromosome counts, often using meiotic preparations with sectioning method, and therefore chromosomes were poorly characterized.

The literature on karyotype analysis on mollusca is not abundant, only a few investigators have succeeded in the more detailed analysis on the karyotypes. This state of information on molluscan chromosomes has been said to be mainly due to the small size of the chromosomes and to technical difficulties (Ramammorthy, 1958; Burch, 1968.). Owing to this, it is difficult to obtain mitotic fields with enough quality to carry out proper chromosomal studies. However, in the gastropods and pelecypods some investigations to obtain the diploid number, have been completed (Burch, 1968; Menzel, 1968a; Ahmed and Sparks, 1970; Patterson, 1973; Ieyama and Inaba, 1974). Other karyotype characteristics have

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not been reported in most cases. In the last four decades especially since 1960, a considerable amount of information has been accumulated on variable taxa of mollusca. These advances were mainly in consequence to the interest in the cytogenetics of Homo sapiens. In Harvey’s list (1920) chromosome numbers of only 44 molluscan species has been recorded and later in Makino’s list (1950) those of only 127 species and subspecies. Then just two decades after Makino’s work, the chromosome numbers of 622 species and subspecies were reported (Patterson, 1970). Chromosome numbers are now known from members belonging to seven molluscan classes, i.e. cephalopoda, gastropoda, bivalvia, Scaphopoda, monoplacophora, polyplacophora, and aplacophora. Most of the cytogenetic work is concentrated on commercially important species of the classes such as gastropod and bivalvia (pelecypoda). Summaries of these investigations of about 1951 species along with other works on the class have been widely reported (Makino, 1950; Burch, 1965; Inaba, 1959).

In gastropods, two Pulmonate orders, Basommatophola and Stylommatophola are well surveyed cytogenetically. Recently, some members of the families Oxynoidae, Patellaceae and Littorinidae of class gastropoda have been cytogenetically characterized (Vitturi et al. 2000b; Diupotex et al. 2003; Libertini et al. 2004) Cytogenetic studies have been carried out in some of the Indian species of class Gastropoda belonging to genus Cryptozona, Oncidium and also estuarine basommatophorous snail (Natarajan, 1958a; 1958b; 1959)

In the Extant cephalopods, chromosome studies have been carried out in both the sub classes i.e. Coleoidea and Nautiloidea, In subclass Coleoidea cytogenetics studies are mainly confined to order sepioidea and octopoda. In order sepioidea diploid number of chromosomes is not clear (Vitturi et al. 1982) whereas Octopus variabilis and O. vulgaris have diploid number as 56 (Inaba, 1959; Vitturi et al. 1982). Important references in the subclass Nautiloidea includes in the species Nautilus macromphalus and N. pompilius, which have diploid number of chromosomes as 52 (Nakamura, 1985; Vitturi et al. 1990)

Molecular Cytogenetic Studies in Perna viridis (Bivalvia:Mollusca) from Goa, West Coast of India.
Though Archeogastropoda, a prosobranch order, is thought to be the stem of the gastropod phylogenetic tree, and other orders are conceived as more advanced and probably evolved from this primitive order, cytogenetic information on this group is comparatively scarce except some reports on the *Haliotis discus hannai* and *Haliotis discus discus* $2n=36$ and *Haliotis tuberculata* $2n=28$ (Arai and Noel, 1986).

Chromosome studies in class Polyplacophora is limited to some genera of suborder Ischnochitonina, *Lepidozona, Middendorffia caprearam*, (Nishikawa and Ishida, 1969) of the family Ischnochitonina, *Chiton olivaceus, Liolophura japonica* and *Onithochiton hirasei* of the family Chitonidae (Vitturi *et al.* 1982; Nishikawa and Ishida, 1969; Kawai, 1976). Species in these two families of the suborder Ischnochitonina, have a diploid chromosome number as 24 except *Chiton olivaceus* which has diploid number 26 (Vitturi *et al.* 1982). But in the suborder Achanthochitonina, diploid chromosome number varies from 16 in *Achanthochiton defilippi* (Nishikawa and Ishida, 1969; Kawai, 1976), *Achanthochiton crinitus* has 18 (Colombera and Tagliaferri, 1983) to *Achanthochiton communis* having $2n=24$ (Vitturi *et al.* 1982).

1.5. Aims and Objectives of the Thesis

This thesis is aimed at detailed chromosome study of the commercially important bivalve mollusk Green Mussel, *Perna viridis*. Some key areas of study embodied in the thesis are as follows:

1.5.1. Mitotic chromosome analysis

Mitotic index is calculated in the gill and gonadal tissues. The percent mitotic index is estimated in both the tissues in both male and female sexes of the *Perna viridis*. Detailed chromosomal morphological analysis of the green mussel has been carried out. Based on the morphometric analysis of the chromosomes, karyogram and ideogram was prepared.
1.5.2 Constitutive heterochromatin Banding

Chromosomes produce characteristic banding pattern upon treatment with certain chemicals and dyes. Banding studies help in understanding the euchromatic and heterochromatic regions on the chromosomes. Some classes of Constitutive heterochromatic regions have been banded by means by BSG – banding technique. Constitutive heterochromatic banding will be utilized for the discussion on the diploid chromosome number 30 in *Perna viridis* as against 2n=28 in all the other members of the genus *Perna* and a very closely related *Mytilus*.

1.5.3 Localization of Ag-NOR Regions

Argiophilic Nucleolus organizer regions reflect the presence of rDNA genes on the chromosomes (Sumner, 1990). In this study Ag-NOR’s are localized on the chromosomes. Frequency of Ag-NOR activity in the larval and adult stages collected from different locations has been compared.

1.5.4. Chromosome Engineering by induction of triploidy

*Perna viridis* is the most important species of aquacultural importance among the class bivalvia (Qasim *et al.* 1977; Rivonkar, 1991). So far no attempt has been made to increase the yield of the green mussels by utilizing the genetic techniques. In this study chromosome set alteration has been carried out to induce triploidy by using some physical and an aneugenic chemical. Cumulative effect of aneugenic agents compared to increase the percentage of triploid animals.

1.5.5. Chromosome aberration Studies

Intertidal habitat of this animal exposes them to number of water pollutants, which are discharged in to the seas by river discharges. It has also been established that these mussels accumulate number of pollutants and heavy metals (Chipman, 1972; Roberts, 1976; Grimas, 1976; Risebrough *et al.* 1976; Di Salvo *et al.* 1975; Fossato and Canzonier, 1976; Friedrich and Filice, 1976; Philips, 1976; 1977a;
1977b; 1978; 1979a; 1979b; 1980; Simpson, 1979; Lowe and Moore, 1979). Many of the common pollutants have been suspected to alter the integrity of the chromosomes, in this study ability of two substances suspected to be mutagenic (insecticide and an herbicide) to induce chromosome aberrations were studied in adult and in larval stage.

1.5.6. Gene probes and Mapping

In recent years several molecular techniques like fluorescent in situ hybridization (FISH), fiber FISH, Comparative genomic hybridization (CGH) have been applied to localize and map the genes on the chromosomes in a variety of animal species In the present study a 18 S ribosomal gene was amplified, characterized and the gene probe was developed by using fluorescent haptane molecule. The position the gene on the interphase and metaphase chromosomes was localized by in situ hybridization technique.