CHAPTER - I

INTRODUCTION
I. INTRODUCTION

1. General

Balbiani (1881) was the first to describe the giant chromosomes in the salivary gland nuclei of the genus *Chironomus*. These structures were also reported by Koraschelt (1884) and Carnoy (1884), but they mistakenly termed these as 'spireme'. The clear picture and genetic importance of polytene chromosomes in the salivary gland nuclei of Dipteran species was resolved by the studies of Painter (1933) and Heitz and Bauer (1933), in *Drosophila* species; as well as in several other Dipteran groups, like *Sciara, Simulium* and *Cecidomyia* including Chironomids (Alfert 1954, White 1954, Da Cunha 1955). A major portion of our current knowledge on chromosome organisation, function and their evolutionary changes within a taxon has come from the study of Dipteran polytene chromosomes, especially of *Drosophila*, but to a lesser extent of *Chironomus*.

Polytene chromosomes are used as a handy model for studying structural and functional organisation of eukaryotic chromosomes. Great efforts have been made to understand chromosome organisation, correspondence between genes and chromomeres, chromosome replication and transcription related chromosome decondensation or puffing (Beermann 1972). New molecular techniques of gene analysis and *in situ* hybridization offers new ways of experimental approach.
The relationship between the salivary gland cells and the normal mitotic elements was taken up by Bridges (1935a) and Koltzov (1934), who independently put forward the view that the salivaries were similar in structure to prophase chromosomes, but that they had uncoiled and replicated many times without separating from the resulting strands. In other words, they provide us, as a special case of a much more widespread phenomenon of 'Endopolyplody'. The darkly staining bands in the polytene chromosomes clearly correspond to the chromosomes seen at various stages of mitosis and meiosis. Polytene chromosomes have been extremely important in cytogenetics for two reasons: 1) the studies of their detailed morphology, especially of the DNA replication cycle and puffing phenomenon have led to new insights on fundamental problems such as the nature and mode of action of genes, and b) comparison of banding sequences of different individuals, populations and species have been of greatest significance in analysis of the evolutionary cytogenetic problems.

Soon after the first publication of Beermann (1952), that demonstrated the probable nature of structural and functional significance of puffing patterns and the scope for its experimental control of puffing activity in the Dipteran salivary gland nuclei and especially of *Chironomus tentans*, had given an impetus to a series of research activities which altogether had changed the realm of molecular biology, in particular of eukaryotic systems.

Polytene chromosomes demonstrate specific puffing patterns in response to various developmental stimuli. Puffs are the chromosomal sites actively engaged in synthesis of RNA, hence they represent cytological manifestations of
differential gene expression (Ashuburner and Berendes 1978). Therefore, they have been used largely in the studies of the regulation of gene expression by developmental hormones. The role of ecdysone in the regulatory behaviour of the patterns of puffing activity in Drosophila and in Chironomus has been extensively studied (Ashburner and Berendes 1978). Some of the studies induced by hormones were proven well suited for the study of structure and function of genes during developmental phases in Drosophila (Crosby and Meyerowitz 1986) and in Chironomus (Hertner et al. 1986). The application of temperature shocks and other chemical or physical treatments to Drosophila or Chironomus larvae or tissues result in profound changes in pattern of gene activity at the puffing and protein synthesis levels (Ashburner and Bonner 1979).

Balbiani Rings (BR) (giant puff-like structures) that occur in Chironomids are unique in many respects. Their special emphasis was that BRs also respond to external stress. Beermann (1973) in his extensive studies, based on the descriptive and experimental data on the larval salivaries of Chironomus tentans and Chironomus pallidivitatus revealed interesting regulatory relationships among BR-1, BR-2 and BR-6 in response to various sugar treatments. It has been well studied that these BRs under normal conditions synthesize large amounts of a very few specialized proteins of known functions (Grossbach 1969, Daneholt 1975, Edstrom et. al. 1978, Lamb and Daneholt 1979, Hertner et. al. 1980). Several external agents are known to influence dramatically BR activity and thus help in understanding salivary gland functions.
With the use of salivary polytene banding sequences as a parameter, a great majority of the studies of chromosomal evolution and species differentiation have been carried out based on the incidence of chromosome structural rearrangements and on the numerical variations. Such studies were evidently carried out on several members of Dipteran insects (especially the genera, *Drosophila, Sciara, Chironomus, Simulium, Anopheles*). Some species of *Drosophila*, especially with the use of paracentric inversions as a mode of speciation processes has dramatically demonstrated the species relationships from taxa to generic level. Thus, the ‘champion’ species from this point of view were undoubtedly *Drosophila willistoni, Drosophila subobscura, Drosophila paulistorum* to name a few species complexes (White 1973, 1978).

Detailed studies of the polytene chromosomes banding sequences of numerous species of Chironomids (mainly of European species) have been carried out by earlier workers, viz., Bauer (1945), Acton (1957 a, b, c) and Keyl (1957, 1960 a, b, 1961 a, b, 1962, 1963, 1964, 1965 a, b). A brief review of cytology of the Nearctic species of *Chironomus* was published by Wulker et. al., (1968), and by Blaylock (1969) and for Australian species by Martin (1971). Newmann (1977) demonstrated the chromosomal evolution for a number of species inhabiting Hawaiian Islands in which paracentric inversions and centric fusions appears to have implied to pose as an important tool in the establishment of speciation processes. Inversion polymorphisms in chromosomal phylogeny of *Chironomus* have been studied extensively in Northern Europe, Britain, Canada and North America (Basrur 1957, Acton 1962, Topping and Acton 1976).
Most of these reports seem to validate that the occurrence of these chromosomal rearrangements are subject to natural selection.

In recent years, the use of differential chromosome staining techniques has provided new information about the base composition and sequence organization of the DNA in Chironomus thummi sub-species and several other species, in metaphase and polytene chromosomes (Hagele 1977a, b, 1980, Schmidt et al. 1980). Considerable progress has been made in attempting to identify and localize the sex-determining region in an otherwise morphologically homomorphic sex-determining loci in Chironomids (Martin 1981, Martin and Lee 1984, Hagele 1985).

**Labial Teeth (Mentum) – a larval morphology**

Atchley and Martin (1971) used several morphological characteristics of head capsule to discriminate between male and female sex, using morphometric variance of five Australian Chironomidae which closely paralleled with chromosomal polymorphism. The greater significance of this study indicates that the right kind of morphological material could be used as a criterion for study, for example, morphology of labial teeth. This is because the material of choice actually constituted scleratised material that could not ordinarily be influenced on morphology by either external factors or examination. As is well-known that fourth instar larval morphology is not amenable for distinguishing sexes externally at that stage of development and the choice of individual larva for each
kind could then offer a good indicator of external morphological features in
distinguishing individual species. Thus, in the present case, the labial teeth
morphology was also considered as a good structural parameter to distinguish
each species.
2. Review of Literature

a. Cytogenetics and phylogeny of Chironomidae

The banding patterns of polytene chromosome provide useful information on chromosomal rearrangements which may have involved in solving speciation problems pertaining to many siblings of the family Chironomidae (Keyl 1962, Michailova 1989, Martin 1979, Kiknadze et. al. 1981). In some cases, no detectable cytological differences may exist between some closely related species since cytological changes does not appear to be an absolute requirement for speciation. Species which show no cytological differences between species are referred to as homosequential species (White 1973, 1977). In the genus *Chironomus*, the species pairs *Chironomus riparius* and *Chironomus piger* are considered as homosequential, since they have similar polytene banding sequences. However, cytological differences exist in the form of change of DNA content of homologous bands (Keyl 1957, 1965 a, b).

The mechanisms of chromosomal evolution in the Chironomidae seem to be broadly similar to those of Simulidae (Rothfels 1956, Martin 1979). Thus it seemed necessary to concentrate on three cytological features that are essential in unraveling aspects of cytogenetics of chironomids. They are, i) fixed polytene banding differences between the taxa, ii) diverse spectra of floating inversion, and iii) differential sex determining mechanisms, if exists.

Several chromosome groupings have been recognised in Chironomidae (Keyl 1962, Martin et. al. 1974). This grouping was done based on the
combination of whole arm translocations within the chromosomal complement and it is possible to categorise them into seven groups depending on the arm combinations representing four chromosomal segments in the complement. *Thummi* group is characterised by arm combinations AB, CD, EF and G; *Pseudothummi* by AE, BF, CD and G; *Parathummi* with AC, BF, DE and G; *Lacunarius* – AE, BF, CD and G; *Maturus*, AF, EB, CD and G; *Carus* – AC, EB, FD, and G; and *Calligraphus* – AG, BF, CD, and E (Michailova 1985 b). Fusion of arms leads to a reduction in the basic chromosome number. In the majority of recorded cases in Chironomidae, it appears obvious that the involvement of a tandem fusion in which an acrocentric chromosome loses its centromere and attaches to the distal end of a chromosome arm of either another acrocentric or a sub / or metacentric chromosome (Keyl 1962, Martin et. al. 1974, Newmann 1977, Michailova 1989). In the two cases, two species in the *Cryptochironomus defectus* group wherein three chromosome species has led into 2 chromosome species (Bauer 1945), and in the North American *Chironomus* species which possesses only two pairs of chromosomes instead of four (Martin et. al. 1974) may have resulted from centric fusions, although it is also possible by means of other chromosomal rearrangements.

Paracentric inversions which lead to reversal of banding patterns, thereby altering placement of polytene chromosome sequences thus bringing about differences in karyotype. Bauer (1936) was the first to emphasize on the role of polytene chromosome banding differences in the study of karyotype differentiation. Later, Keyl and Strenzke (1956) have combined experiments to

The implicit understanding is that phylogenetic studies of the family Chironomidae has revealed that many of the cytotaxonomic differences noted indicate that the fixation of species differentiation depend mainly on the homozygous reciprocal whole arm translocations and involvement of paracentric inversions. In addition, some species in the species complexes and groups do not differ in their external morphology at the larval stage, while in imaginal stage great variability is observed, in which one species may have the characteristics of other. In such cases, cytogenetic analysis is essential. In this extent, some of the cytogenetic analyses made by earlier workers have provided modes and methods of chromosomal change involved in our current understanding of karyotype systematics of the family Chironomidae. In the following, some of the highlights of the outstanding cytogenetic contributions made earlier in the field are briefly reviewed for the current purpose.
Detailed cytogenetic investigations on European Chironomids have been carried out by Bauer (1945) and by Keyl (1957, 1960, 1961, 1962, 1963, 1964, 1965). Keyl (1962) constructed a comprehensive phylogenetic tree from over twenty species of *Chironomus* of European origin, on the basis of homozygous inversions and whole arm translocations, while analogies were made for species of the other continents. It is generally been possible to homologise chromosome arms throughout the genus by observing the banding patterns and specific markers in the polytene chromosomes. But this is not so simple in many cases. For example, North American *Chironomus attenuatus* and European *Chironomus salinarius* in which banding patterns have been so much altered it becomes extremely difficult to homologise.

*Chironomus decorus*, an American species with wider ecological adaptability has been found abundantly in lakes, ponds and streams throughout the continent, but was found to consist of six different karyotype although Townes (1945) described this as a single species. But, subsequent work by Blaylock (1963, 1965) and by Rothfels and Fairlie (1957) have contributed to the understanding that this highly decorated species complex actually consists of fifteen species under the name of *decorus* complex (Wulker *et. al.* 1968, Wulker and Martin 1974, Sublette and Sublette 1974).

Cytogenetic analysis of *Chironomus plumosus* and further analysis combined with the hybridization tests with allied taxa showed that the populations from North America and those from the Asian part of the USSR appear to point towards the implications that the species is exhibiting ample plasticity in its
genomic content. Some of these taxa have highly differentiated from the original *Chironomus plumosus* genotype amply exhibiting the role of paracentric homozygous inversions and also in demonstrating variability in the amount and extent of heterochromatin (Michailova and Fischer 1984, Michailova 1987), probably associated with speciating processes. Interspecific hybridization with *Chironomus muratensis* did not produce viable progeny but with *vancouveri*, although reciprocal crossings were attempted. Investigations on some European populations of *Chironomus plumosus* showed that the species should not be considered polytype but highly polymorphic (Michailova and Fischer 1986).

A far widely distributed Australian Chironomid, *Chironomus* (Kiefferulus) *interstictus* earlier considered morphologically as a single species, has been distinguished as two independent species, viz. *Chironomus* (Kiefferulus) *interstinctus* and *Chironomus* (Kiefferulus) *parastinctus* on the basis of banding patterns of salivary chromosomes (Martin 1963, 1964).

Two cytologically distinguishable species have been found in the material of *Chironomus australis* (Martin 1971). These species have been called *Chironomus australis* and *Chironomus duplex*. Both belong to the *pseudothummi*-cytological grouping based on AB, CD, EF and G arm combinations. *Chironomus duplex* shows a modified arm pattern due to a tandem fusion of arm G to arm E. Thus, *Chironomus australis* has four chromosome species (2n=8) while *Chironomus duplex* with 3 chromosomes (2n=6) having AEG, BF and CD. The banding patterns of the polytene
chromosomes of two species are comparable and also to the Australian species, *Chironomus oppositus* *Chironomus australis* is very closer chromosomally to *Chironomus oppositus*, while *Chironomus duplex*, which is considered a derived species because of the tandem fusion, shows a number of inversion differences from the morphologically similar *australis*. *Chironomus duplex* is polymorphic for six inversions, two of which are complex, while *australis* appears monomorphic.

In his taxonomic revision of Australian Chironomidae, Freeman (1961) noted that the enlarged male hypopygium of *Chironomus tepperi* and suggested relationship to *Camptochironomus*, although he felt that the species was best left in the genus *Chironomus sensu stricto*. An analysis of the banding patterns of salivary chromosomes, besides providing essential background information for a laboratory colonization, might also be expected to indicate, whether relationships of this species are there to other Australian species, such as *Chironomus oppositus* (Martin 1979) or to the *Camptochironomus* species of Europe and North America (Beermann 1955, Acton 1959). The banding pattern of its salivary chromosome suggests that *Chironomus tepperi* is nearly related to *Chironomus oppositus* Martin, 1974. Arms B, F, E and A appears, identical to sequences present in *Chironomus oppositus*; arm G differ by a simple inversion while arm D and C shows greatest deviations, requiring several inversion steps for their derivation.

Although colonizing species adopt a variety of genetic strategies, it is common to find that they have reduced chromosomal polymorphism compared to
related species. The variability made available by recombination is also considered to be important in the adaptation of colonizing species to new or variable environments. The absence of inversion polymorphism in *Chironomus tepperi* and relatively high chiasma frequency are probably both related to this high colonizing habit.

*Polypedilum nubifer* has been recorded from North Africa, Iraq, Srilanka and Formosa as well as from Australia (Freeman 1961, Edward 1964). Porter and Martin (1977) described the polytene chromosomes for the Australian specimen in which 29 inversions were demonstrated. Phylogenetic considerations have been mainly considered on the basis of recurrent alterations with regard to C, E and G arms. *Polypedilum nubifer* has shown to be female heterogametic in sex determination and heterochromatized differential segment was observed on arm G (41cY-dY).

A large number of paracentric inversions have been recorded during the phylogenetic studies of the many European Chironomids. Some of them have reached fixation and now serve to distinguish species or higher categories, while others are still present in a floating polymorphic condition in the populations or in many species. Keyl (1962) recorded thirtynine inversional changes in the, phylogenies of the twenty European species but it is certain that this number greatly exceed the results obtained from some American and Australian groups of species. For example, the subgenus *Kiefferulus* includes three quite distinct groups of species, whose polytene chromosomes show little resemblance in banding pattern (Martin 1963, 1964). All show considerable inversion
polymorphism; Chironomus interstinctus has demonstrated sixteen well known inversions, parastinctus and martini each eight. Martin (1969, 1979) constructed a comprehensive phylogenetic tree based on arm F, with species drawn from North America, Japan, Africa and Australia in addition to the European species, a total of some 52 species. In this connection it is interesting to note that no directional arrows are assigned since some more could probably be included, particularly at the outer ends of the branches.

In the Bulgarian fauna, Chironomus valkonovi is cosmopolitan in distribution with 2n = 8, but several taxa were found with 2n = 6, that are structurally closer to Chironomus salinarius which is characterised by ABG, CD and EF (Michailova and Chubareva 1977). In the genus Endochironomus, several detailed cytological studies have been carried out (Chironomus albipennis, Chironomus impartendiens) in which a very high variability was found in the basic chromosome number (6 to 8) (Michailova and Gercheva 1982). Interesting is the situation in which placement of NOR was found either on chromosome AB or G, depending on the group orientation, and such kind of loci in the complement has helped in the identification of many karyotypes of species belonging to Endochironomus.

Genus Cricotopus belonging to the subfamily Orthocladinae presents considerable amount of cytogenetic informations especially in the two species groups, viz., fuscus (2n=6) and sylvestris (2n=4) groups in which presence of chromocenter characterises the extent and the presence of constitutive heterochromatin in the polytene nuclei.
Karyosystematic studies pertaining to the species belonging to the genus *Glyptotendipes* has demonstrated a basic chromosome number of $2n=8$, rather conservative compared to other genera in *Chironomidae* in spite of its far more variable geographical distribution, and in fact, it is intercontinental in its distribution. Variable nature of the genome in the case of *Chironomus paripes*, *Chironomus barbipes*, *Chironomus mancunianus* is evident with recorded cases of highly polymorphic taxa in each case. Closer examination of their banding sequences revealed their easy adaptability to variable ecological niches which has had led to clear identification of Palearctic species. Salt water forms have been found to adapt to more concrete conditions and suitably evolved in their karyotypes when compared to fresh water species (Grinchuk and Michailova 1979).

The genus *Glyptotendipes* which has drawn considerable body of research in recent years, has provided another resourceful material of research, as a laboratory system in which hybridization and other parameters could easily be tested in contemplation with cytology. As is well known that this genus is characterised by AB; CD, EF and G arm combination ($2n=8$) and thus has enabled in assessing probable modes of adaptation to variable ecological niches thereby demonstrating variable nature of karyotypes. Michailova and her group (Michailova 1989, Kiknadze et. al. 1981, Michailova et. al. 1994) contends that this genus is highly cosmopolitan and in fact, lead to several speciating species, as it was observed in samples of some watershed locations of Spanish land. Sympatric species of *Glyptotendipes* viz., *Glyptotendipes pallens* and
**Glyptotendipes glaucus**, established at the level of 3% naturally occurring siblings, have been found to be cytological hybrids. Introgressive hybridization has been cited to account for their variable recombination processes. Despite hybridization, natural selection appears to maintain the essential identity of each species as a separate genotypic entity (Michailova 1995, 1996, Michailova and Contreas – Lichtenberg 1995). C-banding analysis has disclosed that *Glyptotendipes pallens* has a telocentric G chromosome, while in *Glyptotendipes glaucus* it is acrocentric (Michailova 1998) in its placement.

Michailova and her collaborators (Michailova and Todorova 1998, Michailova et. al. 1997) have been successful in attempting to colonize the two species viz., *Glyptotendipes riparins* and *Glyptotendipes pallens* at the laboratory level. This has enabled them to conduct number of cytogenetic tests including mutagenic capability of each species, as such providing a material of strength to genetic monitoring in which several environmental factors were tested. Cytotaxonomic features of *Chironomus clarus* demonstrated karyologically more resemblances to *Chironomus obtucidens* species group (wherein *Chironomus clarus* and *Chironomus obtucidens* are considered by some as homosequential species), which is cytologically more closer to *Chironomus thorummi* complex (Michailova and Hirvenoja 1995). *Chironomus brevidentalus* (Hirvenoja and Michailova 1998) demonstrated *salinarivus* type of karyotype although some samples were collected from Finland during the course of investigation. The standard karyotypic study was made for *Chironomus jonmartini* (Hirvenoja and Michailova 1997) and the same was found to correlate
well with other species which were earlier identified on the basis of ecologically as a member species of aberratus group as Chironomus neglectus (Wulker 1991).

A study of the cytogenetics of the Hawaiian Telmatogoton (Chironomidae) is of special interest (n=7 to n=3/4), since it permits examination of chromosomal changes which occur in the evolutionary movement of a species from a marine environment to fresh water (Newmann 1977). The fixation of paracentric inversion and the centric fusion of chromosomes was found to be widespread in the evolution of the freshwater Telmatogoton. A population of T. torrenticola occupies a central position in the proposed phylogeny. Most species and other populations may have been derived from the standard sequence by paracentric inversion. T. abnormis (n=4) has a simple XY system and while T. hirtus (n=3/4) has a complex XY1Y2 system. The unique sequences of band differences in staining intensity of puffs and bands and an inversion form the basis for the differentiation of the various Y-chromosomes in these species.

While considerable progress has been made on the cytogenetics of species of European, American, few Oriental and Australian origin, Indian specimen have received scant attention inspite of fairly widespread faunal range. However, in recent years, some beginning has been made on the Indian scene with some progress. Cytogenetic informations are now available for Nilodorum biroi (Saxena et. al. 1985, Venkatachalaiah et. al. 1998, 1999), Chironomus circumdatus (Kumar and Gupta 1990, Tiwari and Chauhan 1994), Chironomus
striatipennis (Gupta and Kumar 1991), Chironomus niger (now redesignated as incertipennis), (De and Gupta 1994), Chironomus javanus (Tiwari and Chauhan 1994) and Chironomus ramosus (Nath and Godbole 1997) based on description of mitotic and polytene chromosomes and to a certain extent on the nature of their sporadic distribution of inversions encountered. Saxena et. al. (1985) made cursorial comparison of polytene sequences between Indian and Australian fauna and there were no compatibility in respect of inversion polymorphism.

Marine Species

Considerable amount of cytological investigation in Chironomidae has been done on species belonging to the sub family Chironominae and less is known of the cytology of the remaining subfamilies which make up the bulk of the family. A cytological study of Anatopynia dyari a member of the cytologically little known subfamily Tanypodinae has been reported (Bedo 1974). Most species of Tanypodinae are considered to have poor polytene chromosomes due to small size of the salivary glands (Bauer 1945) and detailed cytological (Polytene chromosome) maps are available for only a few species, especially marine species. Very little cytogenetic research has been done on the species dwelling in marine and submarine environments. Thus, it appears obvious that the available cytogenetic data on these groups of chironomids are very inadequate and is in a bad shape for critical analysis. Yet they have been meaningfully exploited to resolve problems relating to speciation, phylogenetic relationships and karyotype evolution (Newmann 1977, Michailova 1979).
b. Inversion polymorphism

Besides, a greater number of studies aimed at the polytene chromosomal polymorphism in natural populations of Drosophilid species (Dobzhansky 1970, White 1973), there are several members of other Dipteran families (e.g., Simulidae, Culicidae, Sciaridae and Chironomidae) wherein polytene chromosome polymorphism have been recorded. Various *Chironomus* species have been studied by earlier authors for example with respect to inversion polymorphism. (Acton 1957a, 1959, Basrur 1957, Hsu and Liu 1948, Martin and Wulker 1971, Hilburn and Atchley 1976, Topping and Acton 1976, Pedersen 1978, Martin 1979).

Many of these studies have demonstrated the existence of adaptive and balanced polymorphism, but investigations have shown that certain conditions in the environments were responsible for the differences. Especially, Dobzhansky and his associates have contributed with information on chromosomal polymorphism with contraselective factors in their pioneer work on *Drosophila pseudobscura* (Dobzhansky 1970). Studies on Chironomids have also shown natural selection to be the cause of differences in the inversion frequencies in different populations (Acton 1957, Topping and Acton 1976).

Inversion polymorphism in chromosomes of *Chironomus tentans* has been studied extensively in northern Europe, U.K., Canada and Alaska (Acton 1957, 1959, 1962, Acton and Scudder 1971). Topping and Acton (1976) studied the populations of larvae of *Chironomus tentans* living in a series of 12 saline lakes...
with variable conductivities to assess the adaptive significance of chromosomal inversions. An experiment was also conducted to demonstrate directly in nature that the inversions are subject to natural selection. The lakes which have been characteristically different in limnological sense, differed primarily in chemical composition and concentration, and thus larvae responded to both biotic and abiotic factors. In their experiment, they also introduced brook Trout into the previously ‘fish-less’ lake. The perturbation of the frequencies during such a short period of time indicates the magnitude of selective forces that must be rather large. Blaylock (1965) assessed inversion polymorphism in natural populations exposed to chronic low-level radiations in the vicinity of Oak Ridge, Tennessee, USA, in which three paracentric inversions were found at a relatively high frequency in three different populations with one exception. The frequency of these endemic inversions showed no temporal fluctuations or significant difference in frequencies between populations.

The three most common inversions in the populations of Australian Chironomus (Kiefferulus) interstinctus at Ocean Groove, Victoria, are tied up in association with one sort of it or the other (Martin 1962, 1965). The detailed analyses of the complexity of inversion systems in this complex taxa revealed non-random association of inversions on second chromosome, i.e. Lonsdale inversion with alternative sequences St$^{Lo}$ and Lo, and the Barwon inversion with alternative sequences St$^{Ba}$ and Ba,. This association has been studied at large for a number of years in the Ocean - Groove population and also in other widely separated populations to learn more about linked inversions. The results from
the Ocean Groove populations indicate that the Lonsdale and Barwon inversions, like the Corio inversion, are maintained by associations rather than by simple heterosis. It appears that selection is favouring the presence of St\textsubscript{Lo} with St\textsubscript{Ba}, and also of Lo with Ba, in the same individual, while it is acting against individuals with St\textsubscript{Lo} and St\textsubscript{Ba} Lo with St\textsubscript{Ba}. This has resulted in favouring double heterozygotes. These double heterozygotes also vary between populations, only showing a significant excess at Ocean Groove. These effects are most likely attributable to the different karyotypes having different adaptive values in the various populations.

Unlike the investigations in *Drosophila* where adults are mated to flies of known karyotype, giving the inversion order on each chromosome, this type of investigation with *Chironomus*, examining larval chromosomes makes it possible the determination of chromosome order in double heterozygotes difficult. The underlying basis of the association may be in withstanding higher water temperatures in summer 'Lo' and 'Ba' together are at an advantage in such conditions, while St\textsubscript{Lo} and St\textsubscript{Ba} together are an advantage in higher rainfall areas.

During the karyosystematic analysis of *Chironomus staegeri*, it became obvious that the population dynamics of the inversion polymorphism of the species was quite complex and showed a number of unusual features (Martin and Wulker 1971). The unusual features include a deficiency or even absence of some heterozygous classes, marked associations of inversions within and between chromosomes and a very strong relationship between some inversion sequences and the ecological habitat. An understanding of the importance of the
inversion associations may help shed some light on the deviations from Hardy-Weinberg principle, particularly as to whether larval niche preference is of any importance. A system of inversion associations which increases the viability of certain homozygous classes, perhaps by adapting them to a particular niche, would be expected in a species as polymorphic as *Chironomus staegeri* because it would increase the fitness of the population as a whole compared to the situation where all inversions were independent and only the multiple heterozygotes had a high adaptive value (White 1958). Obviously, far larger samples will be needed to indicate which associations have real effects.

Pedersen (1978) made comparative analyses of inversion polymorphism in three Danish lake populations on *Chironomus plumosus* for a period of over three years. The populations exhibited differences in the composition of chromosomal polymorphism and in the level of heterozygosity. The polytene inversion frequencies in particular of the longest chromosome showed remarkable differences between the three populations, with reference to Hardy-Weinberg distributions. The differences in the composition and frequencies of inversion heterozygotes in the three populations are presumably due to adaptation to different environments, suggesting the marginal populations of the lakes have lower degree of variation than central population. This study support the idea that adaptedness has incurred at the expenses of adaptive flexibility based on genetic recombination. Populations with a lower degree of polymorphism occupy fewer ecological niches but maintain a higher adaptive flexibility due to fewer genetic recombination.
c. **Chromosome banding techniques**

Differential staining of mitotic chromosomes with different basic dyes after the special treatment or without it has proven to be an important tool for chromosome investigation and has constituted a new approach to the problem of individual chromosome identification as well as in the study of different types of heterochromatin. However, the application of these methods in the study of polytene chromosomes has proven to be of less successful.

Giemsa differential staining techniques, which mostly are useful in differentiating individual chromosomes in metaphase complements of most higher animal systems are helpful in differentiating each complement by means of their typical G, Q, R and C banding sequences, although each staining has its own specific staining property.

The fluorescence study of *Chironomus thummi* chromosomes stained with quinacrine mustared and other related stains was found to be poor (Badaev *et al.* 1973), for the most part the chromosome fluoresce bright green.

Fluorescence of *Chironomus thummi* chromosomes stained by acridine orange, revealed variable effect with respect to mild and wild acid treatment prior to staining. Thus, low level of treatment as well as control slides demonstrated green fluorescence, whereas longer treatment revealed red fluorescence, indicating perhaps of differential functional status of chromosomal packing.
Bedo (1974) described chromosomal complement of *Anatopynia dyari* (belonging to subfamily Tanypodinae) consisting of haploid number of 6 chromosomes (2n=12) characterised by the presence of heterochromatin. Quinacrine staining produced bright fluorescence of the centromeric region stained chromosome IV and some ectopically paired regions of the chromocentre, basal bands and telomeres of some chromosomes. The extra thickness of the IV chromosome suggests of accumulation of extrachromosomal segments due to extra round of replication. The study also highlighted the presence of heterozygosity of the salivary III chromosome in certain populations.

Hagele (1977 a) studied the effect of C-staining and RB-banding profiles of polytene chromosomes of some Chironomid species. Application of C-banding (Arrighi and Hsu 1971) technique on Chironomid polytene chromosomes has changed the chromosome morphology considerably. Chromosome bands as far as they are identifiable are stained pale with the exception of the centromere region and in some cases the telomere region. By Reddish-Blue (RB) method, the centromere bands are stained bright blue, whereas the remainder stain red to red violet. Thus, *Chironomus thummi thummi* chromosomes gave interesting results. C-region staining was prominent in the hybrids of *Chironomus thummi thummi* x *Chironomus thummi piger*; only those interstitial *thummi* bands which are known to have a greater DNA content than their homologous *piger* bands which are C-band positive and blue stained by RB method. These results have obvious impression that the specific staining property at specific regions of
polytene chromosomes show that DNA content disparity along the bands and thereby identifies clearly these two subspecies of *Chironomus thummi*.

The amount and location of constitutive heterochromatin (C-band) in the polytene chromosomes has been considered as an interesting testing ground for establishing phylogenetic relationships of several important Chironomid species group. Thus the distribution of heterochromatin, its amount in homologous sections of the chromosomes is of prime importance for the divergence of the species (Lentzois et. al. 1980, Michailova 1987).

Lentzois et. al. (1980) studied C-banding effect on the polytene chromosome of a number of group of related Australian *Chironomus* species in which C-heterochromatin component appeared quite smaller. C+ regions were usually observed to correlate with single to thick bands at contromeres, telomeres, nucleolic and a few interistial regions. In some species, certain of the above regions were C-band negative, like in most regions of *Chironomus duplex*, while in *Chironomus australis* three C+-bands including at C region corresponds to the X-chromosome (Martin 1979). The greatest amount was seen in chromosomes of *Chironomus nepeanensis*. The low amount of C+ heterochromain in cells of high degrees of polyteny such as those of salivary glands and Malpighian tubules, explains why no bends could be observed in interphase, mitotic and meiotic cells of most species.

The N-banding method (Matsui and Sasaki 1973) is known to extract DNA, RNA and histones from the chromosomes and stain specific intensely
stained region of the chromosome. Funaki (1975) was of the opinion that these N-bands are the regions wherein nonhistone proteins are linked to the nucleolar organizer. The results obtained by Hagele (1977b) are of exceptional quality as far as the polytene chromosomes of *Chironomus thummi* species are concerned. A detailed analysis of the N-banding patterns showed that they correspond to the puffing patterns of the polytene chromosomes. In most of the cases, the size of each N-band and the corresponding puff size coincide. In *Chironomus thummi* the nucleolous organizer region found located in chromosome 4 as revealed by N-banding was more profound. In the case of *Chironomus melanotus*, wherein centromeric heterochromatin was evidently seen even in C-staining and the N-bandings were not at all obvious, whereas N-positive bands were seen in puffs.

The N-banding technique allows to discriminate between two different types of heterochromatin. In *Drosophila*, the alpha-heterochromatin, in contrast to the beta-heterochromatin is N-banding positive, as already reported by Matsui (1974). In the *Chironomus* species studied, heterochromatin is also present as was demonstrated by C-banding methods. This heterochromatin remains unstained by the N-banding technique. The *Chironomus* heterochromatin, like beta-heterochromatin in *Drosophila* is not underreplicated in polytene cells (Walter 1973). These findings and the negative N-banding of both the *Chironomus* heterochromatin and *Drosophila* beta-heterochromatin suggests that the *Chironomus* heterochromatin belongs to beta-heterochromatin type of *Drosophila*. 
The NOR localizing studies by silver nitrate staining in the polytene chromosomes of several European and North American species of *Camptochironomus* complex have shown intra- and inter-specific differences in number, size and chromosomal localization and during developmental stages (Pelling and Beermann 1965, Stockert and Colman 1975). Observations of nucleolus in number of a group of Australian Chironomids in the *Pseudothummi* complex have indicated wide numerical variations between species. Intra-specific polymorphisms for certain nucleoli have also been reported in some species (Martin 1974). The basic chromosomal relationship in this group established by Martin (1966), provide an excellent frame work for studying cytogenetic changes in nucleoli in the evolution of members of the group. An alternative silver nitrate staining technique of Goodpasture and Bloom (1975) and Howell *et al.* (1975) provides data to visualize nucleolar cistrons on chromosomes. Making use of these staining techniques, Lentzois and Stockert (1979) have attempted to establish nucleolar relationships in some Australian *Chironomus* species. Differences in heterochromatin amount have also been observed at different NORs. A scheme for the evolution of nucleolar producing regions in the *Chironomus* group in terms of those and other known chromosomal changes was evidently shown. This investigation has indicated a much wider variation in position and number of nucleoli among closely related Australian *Chironomus* species than that described for European and North American species (Acton 1962, Pelling and Beerman 1965). The most typical number of nucleoli within this group of Australian species is 1 to 2. This is also
typical for European and North American species (Keyl 1957, Pelling and Beerman 1965). Therefore, the most derived species, viz., *Chironomus nepeanensis* is with 8 nucleoli and the species in this group with high number of nuclei are all large with longer periods of larval development. Based on several other parameters, Lentzois and Stockert (1979) have presented a comprehensive scheme for evolution of r-RNA cistrons in this group of Australian *Chironomus* essentially supporting Martin (1966) phylogenetic considerations.

d. Sex-determining mechanisms

As is generally known among Dipterans, Chironomids do not have morphologically identifiable sex chromosomes (either ZZ, ZW, or XX, XY). However, in the genus *Chironomus*, the sex-linkage of certain inversions in *Chironomus tentans*, and *Chironomus pallidivitatus*, as well as eye colour mutants in *Chironomus pallidivitatus*, have been implicated to represent male sex determination and thus designated as male heterogametic sex (Beermann 1955). Extensive survey of the literature pertaining to sex determining mechanism in the genus *Chironomus*, revealed that sex determining region need not be in the same chromosome or chromosome arms (Beermann 1955, Keyl 1961, Rosin and Fischer 1972) and that in some species, (for example, *Chironomus tentans, Chironomus plumosus* and *Chironomus nuditarsis,* there may be more than one location. Beermann (1955) based on his data obtained from several species surveyed for European species, demonstrated Y-chromosomal polymorphism.
In many *Chironomus* species, even though they may be highly polymorphic in expression there is no indication that sex-linkage of inversions occur in natural populations. In fact, this would only be expected when sex-determining loci were actually included in the inverted region. If the sex determiners are linked to the inverted region and crossing over can occur in the chromosome segment between them, the sex determining regions would be randomly distributed in natural populations unless selection or some other factors which could be of recent origin that should lead to linkage disequilibrium (Griringer 1948).

However, in a small number of *Chironomus* species where sex linked inversions occur, it is possible to localize the sex-determining regions to specific chromosomes or chromosome arms (Martin 1981). In most of these studies of *Chironomus*, the males represent heteromorphic sex and the sex-determining region is in different members of the complement. European populations of *Chironomus tentans* and *Chironomus pallidivittatus*, it was found to ascribe to a common sex-determining region, although with differences in the complexity of the inversions involved (Beermann 1955). A group of related Australian species, viz., *Chironomus oppositus*, *Chironomus australis* and *Chironomus duplex*, it was shown to have the sex-determining loci in different chromosomes. Martin (1981), based on the frequency with which the location changed between related species suggested, that the changes were adaptive and integral to the process of speciation and not just coincidence. It is uncertain at present whether there were only a limited number of sites on the chromosomes at which the sex determiner
can occur (Martin et al. 1980, Martin 1981), or whether the sex determiner is on a transposable element (Green 1980), in which case it could insert almost anywhere in the genome.

In the case of *Pseudothummi* group of *Chironomus* species that are found in Australia (viz., *Chironomus oppositus, whitei, australis, alternatus* and *alternatus C*), the sex determiner tend to be located near the centromere of the CD chromosome. This is different from the most common location, arm F of the *thummi* complex in Europe and North America. There is also a group comprising Australian species (*Chironomus oppositus Chironomus f, tyleri, Chironomus cloacalis, Chironomus alternatus b, Chironomus nepeaneusis*) in which sex determiner is found in C-arm, while in other species, distribution was found in either A or B or C. It is not yet clear whether these represent an unstable polymorphism or indicate the existence of cryptic subgrouping within the chromosome complement. This uncertainty means that it is impossible at present, to differentiate and to assimilate the possibility of localizing a translocatable sex determiner as an explanation for variability in its expression to localize sex determiner region which is not convincing at present (Martin 1981).

Now, it appears obvious that most *Chironomus* species have no sex-linked inversion and therefore, it is difficult to predict on the probable position of sex-determiner in the genome (Martin 1981, Martin and Lee 1984, Hagele 1984). Therefore, Hagele (1985) attempted to tackle this problem in a different way, that is by involving reciprocal crosses to evolve some clues with regard to
pairing behaviour of polytene bands, as it was done in the case of *Chironomus thummi* sibling species, *Chironomus thummi thummi* × *Chironomus thummi piger* hybrids and their back cross progeny. This postulated idea was tested on the sex-specific pairing behaviour and it was claimed to have been found in region D3 d-g at the chromosome end of arm F of chromosome III. From these results, it is possible to ascribe that the selectively stained polytene band (D3e) represent the male sex determiner (M) of *Chironomus thummi thummi* stock and that male is the heterogametic sex, with the reservation that the same study did not provide any positive answer as to why *piger* genome lack this locus although both are phylogenetically closer. One possibility is that in *thummi*, the number of chromosome bands within and near the centromere regions have a greater DNA content than the homologus band in *piger* (Keyl 1965), and also has high amounts of repetitive AT-rich DNA families (Schmidt 1981, Vistorin and Schmidt 1983). Thus, one can conclude that, even though they are closely linked to M, this heterochromatic band is not essential for the genetical determination of the male sex. The situation observed in *Chironomus thummi thummi* is very similar to the case that observed in *Calliphora erythrocephala* (Ribbert 1967) in which male is heterogametic in sex determination (Ullerich 1963).

The foregoings in the *Chironomus* seem to suggest that sex-determination mechanism is much simpler than that of the situation in *Drosophila*, thereby providing the probable chance to unravel the molecular aspects of this primitive form of sex determination. Schmidt and his associates (Schmidt 1981, 1984, Schafer and Schmidt 1981) have attempted to isolate and analyse male specific
chromosomal regions which are considered to possess highly repetitive, clustered and interspersed DNA sequences (by mini satellite tagging) called ‘Cla’ elements. These sequences were found in the genome of *Chironomus thummi thummi* approximating 70,000 copies in the genome and are found to be localized at more than 200 different chromosomal sites. In their clustered and interspersed chromosomal distribution pattern, the ‘Cla’ elements are very similar to mini satellite DNA sequences found in humans and many other species. These ‘Cla’ elements seem to provide as molecular probes for sequencing genomic DNA libraries in which clones originating from sex-determining regions which impart sex-specific differences. The type of sequences that were found to differ between male and female, suggests, however, that most of them are not directly involved in the mechanisms of sex-determination. There is good evidence to show that the observed male/female differences are due to an accumulation of noncoding sequences that were typical of heterochromatin within the male sex-determining region. This ‘heterochromatinization’ occurred rather recently in evolution and may be the result of efficient suppression of recombination within the sex-determining region.

Quite recently, Kraemer and Schmidt (1993) have demonstrated the dominant male sex determiner in chromosome III of *Chironomus thummi thummi*. Chromosome III displays a hemizygous cluster of Cla elements in males but not in females. The chromosomal location of this hemizygous Cla element cluster is found in the region of the male determiner designated as M was localized by cytogenetic analysis using Cla elements as hybridization probe.
Molecular analysis of the DNA of males and females in this region displayed a number of differences between the two sexes. One striking difference is that transposable element is associated with the male sex determining region alone, but not in the other chromosome. The sex determining region also contains several other tandem repetitive DNA elements, in addition to the Cla elements.

e. Special features of Chironomids

In *Prodiamesa olivacea* (Diamesinae – subfamily) salivary gland nuclei, a chromocenter connects long arms of chromosome 1 and 2 and the tiny third chromosome with a peculiar concentric banding that never sticks to the chromocenter, although mitotic karyotype (2n=6) reveals 6 chromosomes and spermatogenesis takes place with n=3 chiasmatic bivalents (Zacharias 1981). This study has elucidated the level and degree of allocycly and underreplication in the third polytene chromoosome by cytological and cytophotometric comparison of polytene, polyneme and mitotic chromosomes. In another study by Zacharias (1984) for another Diamesine Chironomid viz., *Pseudodiamesa branickii* which has revealed 4 pairs of mitotic chromosomes, but demonstrated three polytene salivary elements. The microphotometric determinations of DNA contents in mitotic and salivary nuclei yielded a 2C value of 0.23pq DNA and 12 rounds of endoreplicatives. In both salivary gland and Malpighian tubules, chromosome 4 appears as a non-banded network of chromatin in Feulgen preparations. Indirect immunofluorescent staining using antibodies specific for RNA-DNA hybrid, indicated that this structure was transcriptionally active. Its
reaction to heavy metal staining suggested that it included the nucleolar organiser and this was conclusively demonstrated by in situ hybridization with $^{125}$I-rRNA. Chromosome pair 4 comprises about 11.4% of the total DNA in metaphase, but only 3.7% in the highly polytenic salivary nuclei, suggesting about 70% of the chromosome 4 pair is precluded from polytenization.

The Orthocladiinae, another subfamily of the Chironomidae, is characterized by the presence of chromosomes limited to the germ line (K-chromosomes: K-derived from "Keimbahn") (Bauer 1970). These chromosomes, in contrast to the somatic chromosomes (5-chromosomes) pass through a complete chromosome cycle. One speciality of this chromosome cycle which proceeds in both sexes in the same manner, is something to do with the last gonial mitosis, termed differential mitosis. In contrast to Ss (which behave as in a normal mitosis) all Ks move undivided to one cell pole that leads to the duplication of the Ks prior to meiosis, compensating for the elimination of about half the Ks during the first division of the primary germ cells. The cells containing the Ks differentiate into functional spermatocytes and oocytes, whereas the cells with the somatic set develop only into nurse cells. The elimination of Ks from the prospective soma cells takes place during the early cleavage division. Staiber (1987) reported using successful G-banding of Ks in gonial metaphase for Acricotopus lucidus that helped in the identification of nine different K type gonial plates. Various combinations of K chromosomes were found in the complements of different individuals and cells. No two animals were completely alike in the chromosomal complements (Staiber 1989). Usually
the homologous form bivalents, but frequently quadravalents and hexavalents also have been encountered. In some cases, multivalents composed of different limited chromosomes occur, probably non-homologous chromosomes were involved in pairing and crossing over processes.

Noteworthy in this respect is the observation of Ortiz (see Beerman 1955) who reported translocations of K chromosome to the S chromosomes in *Metriocnemus hygropetricus* (Orthocladinae). Polytene fragments of the K-chromosomes were formed in some translocations of this kind. Their banding pattern had no homology with that of the S-chromosomes. The other section of the S-chromosomes were completely heterochromatic. In contrast, a similar kind of translocation generated in *Acricotopus lucidus*; nine fragments of the K chromosomes had a banding pattern identical to that shown by the corresponding regions of the S chromosomes (32% of bands of the S set) (Staiber 1991).

Bauer (1970) observed two cases of germ-line based heterochromatin-insertion into somatic chromosomes in *Smittia parthenogenetica* (belonging to subfamily Orthocladinae of family Chironomidae) and designated as ‘K’ chromosomes. Polytene chromosome preparations showed that this insertion had occurred with II chromosome. Hagele (1980) has characterized this unusual segment and described consisting of C-band positive, late-replicating, inactive RNA synthesis and fluoresces brightly with quinacrine and is polytenic. Although N-banding is negative for the most part, the central portion is N-band positive. He had compared the property of this heterochromatin with that of
heterochromatin found in the cells in *Chironomus melanotus* and *Drosophila melanogaster* and this particular component indeed is undereplicated during polytenization.

Interesting situation arose with a combination study of mode of polytene ultrastructural and cytogenetic analyses which virtually helped in localizing genes in bands more precisely and to determine how the number of bands and genes are related in polytene chromosomes. The method of ultrathin sectioning had enabled to use unsquashed salivary gland polytene chromosomes of *Chironomus thummi*, which could be used for ultrastructural mapping studies (Kiknadze *et. al.* 1976). There was a good agreement between electron micrographs and Hagele’s light microscopic map (Hagele 1970). The three dimensional ultrastructure of isolated salivary gland chromosomes of *Chironomus stigmaterus* for scanning electron microscopy revealed a series of chromatin strands, more extended in interband regions and more lightly coiled or folded in the banded regions (Brady *et. al.* 1977). The nucleolus is observed to be a dense disc, while the Balbiani Rings appear as diffuse regions consisting of both fibrillar and granular elements. Simple and elegant microspreading technique for electron microscopy was described by Kalisech (1982) which avoids ultrathin sectioning of the fixed material, wherein the lateral and longitudinal enlargements or bands and interbands are clearly demonstrated. A slight improvement in the preparation was found ideal for the scanning electron microscopy (Kalisech and Jacob 1983a, b) revealing even photographic depiction of microstructure of polytene chromosomes.
f. Centromeric heterochromatin in polytene chromosome

In polytene chromosomes, centromeric heterochromatin has been accurately mapped in some species of *Drosophila* and in some representative examples of the other Diptera. In many other species including Chironomids, although the chromocentre does form, centromeric blocks of heterochromatin differences in respect of tighter compaction, nevertheless, are frequently connected by long loose strands of ectopic contacts. Such kind of heterochromatic knobs were found in *Chironomus crassimanus* (Keyl 1961), *Chironomus cucini* (Martin 1979), *Chironomus plumosus* (Michailova 1989) and in some anopheline species (Coluzzi and Cancrini 1971). Occasionally, these contacts are extremely in frequent, as in *Chironomus thummi* (Badaev et al. 1973).

Centromeric heterochromatin is often observed to be such bulky blocks that the heterochromatic nature of the material is not in doubt; for example, in *Glyptotendipes barbipes* (Bauer 1936b, Basrur 1957, Michailova 1995), *Orthocladius bipunctellus* (Michailova 1981, Michailova and Belcheva 1982) and *Demeijerea rufipes* (Belyanina 1983).

A small band, somewhat more compact than the others or positively staining for C-heterochromatin, quite frequently is mistaken for centromeric heterochromatin (Hagele 1977 a, Belyanina and Sigareva 1978, Michailova 1987 a) in Chironomids. In many cases, the chromosomes contain no particular bands to which the role of centromeric heterochromatin may be ascribed. Thus,
cytological studies carried out by Keyl (1960) and Michailova (1989) did not reveal typical constitutive heterochromatin in the polytene chromosomes of the 13 Chironomid species investigated.

A stable chromocentre of alpha – heterochromatin type has been observed in *Chironomus melanotus* (Hagele 1977b, Sass 1980b) and in some Yakutian Chironomidae (Kiknadze *et. al.* 1991). The DNA included in the chromocentre of *Chironomus melanotus* is presumably completely polytenized (Steinmann 1978). In their EM sections, alpha-heterochromatin of this species is represented by tightly packed and heavily stained chromatin in which 20 – 25 nm fibrils are distinguished (Sass 1980b), although the degree of compaction make it little doubtful.

In other species, centromeric heterochromatin is not included in the chromocenters as of alpha – heterochromatin type (eg. In *Glyptotendipes*, *Orthocladius* and certain midges, (Chubereva 1979).

Dark knobs are seen in each chromosome of *Orthocladius bipunctatus*. These knobs are blocks of strongly staining compacted vacuolar heterochromatin that lie in the middle or at the ends of the chromosomes. The blocks showed other features of heterochromatin besides morphological ones. For example, ectopic contacts are frequently observed between the ends of the chromosomes where they are located. When stained with quinacrine they are brightly fluorescing and they stain positively for constitutive heterochromatin (Michailova and Belcheva 1982, Michailova 1989).
A block of heavily staining material is connected with the nucleolus at one of the ends of the fourth chromosome of *Cryptochironomous fridmanae*. It contains \( Q^+ \) and \( C^+ \) material (Michailova 1989). Accumulation of heavily staining material presumably of alpha – heterochromatin was identified in *Chironomus nuditorsis* (Fischer and Tichy 1980).

Bridges (1935, 1936) noted that the contacts of various chromosome regions giving rise to loops termed ectopic pairing, are present predominantly in Drosophilid species. The length of the ectopic strands can reach several terms of microns and they are readily identified under the light microscope (Ashburner 1980, Scouras 1984). In unquashed nuclei of the salivary glands of *Acricotopus lucidus* and *Chironomus* sps., the ectopic fibres are as long as 45 \( \mu \)m and are 0.2-0.5 \( \mu \)m thick (Quick 1980). In spite of thorough karyological studies in numerous representatives of Chironomidae, ectopic contacts were rarely seen, if ever identified. Some remarkable ectopic association are the association of nucleoli, or their fragments with chromosome regions, with nucleolar material contacting the chromosome side by side, hanging on long fibres and entering into chromosome fibres produced by breakages (Michailova 1985, Petrova and Michailova 1986, Michailova 1989).

The mechanism of the formation of ectopic contact appears to be the most debatable issue in the whole problem of intercalary heterochromatin. In his early studies, Bridges (1936) suggested that ectopic contacts may be established within the so called cytological repeats, that is, in the chromosome regions showing the...
same banding pattern (Zhimulev 1996). It follows from his suggestion that homologous region pair.

g. B-Chromosomes

Most studies of B chromosomes imply that they are composed mainly of heterochromatin. If such is the case, the study of interphase polytene nucleus of insects offers the possibility of analysing in detail the organization of heterochromatin of another type.

It is characteristic that nuclei with polytene chromosomes in the supernumerary chromosome tend to vary greatly in morphology. These can be structureless clumps of loose or compact chromatin resembling B-heterochromatin (Valkanov and Michailova 1974, Michailova 1976). Although in some cases, for example, in Phyrnecincta (Wolf 1950) it is rounded, but elongated in Chironomus aberratus (Belyanina 1989), strongly heteropycnotic bodies resembling alpha – heterochromatin. In Chironomus behningi, two types of B chromosomes are most frequently encountered. Supernumeraries showing banding patterns have been described in many papers (Chubareva and Petrova 1969, Chubareva 1974, Michailova 1985, 1987 a, b). The number of bands can be large: 11 bands in the left and 15 bands in the right arm of Glyptotendipes paripes (Miseiko et. al. 1971). In Chironomus plumosus size, shape or extent of heterochromatinization varies very drastically from individual to individual larva (Keyl and Hagele 1971). B chromosomes incorporate ^3H Uridine in this species (Illyinskaya et. al. 1991).
Numerous instances are known in which supernumerary chromosomes pair with single regions of the chromosomes of regular set. Thus, the B chromosomes of *Chironomus plumosus* frequently pair with the centromeric region of the four chromosome (Keyl and Hagele 1971, Petrova 1980). It was in *Orthocladiuas bipunctellus*, the B chromosome in the polytene nucleoli pair ectopically with first or second chromosome (Michailova 1981), and also in *Chironomus decatensis* (Procunier 1975), while in *Chironomus heterodantatus* with one of the long arms in the complement (Belyanina 1975).

Keyl and Hagele (1971) reported in the population of ‘Herrenmihle’ of *Chironomus plumosus*, 11% of the individuals contain one supernumerary B-chromosome. This B-chromosome is present both in germ line and somatic cells. In the ‘Falkan’ population, these chromosomes were not found in germ cells. The polytene B-chromosomes of *Chironomus plumosus* exhibits a particular type of banding pattern in the salivary nuclei; further it was shown to form an additional nucleolus in the nuclei of the Malpighian tubules.

**h. Cla-elements**

In the genomes of most *Chironomus* species, a short tandem repetitive DNA element is present, which is characterised by a Cla-restriction site and is therefore called the Cla element or the Cla-DNA family (Schmidt 1981). The genomic concentration of the Cla-family is very different in closely related (sub) species of *Chironomus* (Schaefer and Schmidt 1981). In the case of subspecies *Chironomus thummi thummi* and *Chironomus thummi piger*, the concentration
of Cla elements is clearly correlated to the different genome size, i.e. the subspecies with the larger genome (*Chironomus thummi thummi*) contains about six times more Cla elements than to the smaller genome (*Chironomus thummi piger*) (Schmidt 1981, Vistorin and Schmidt 1983). The different DNA content per genome is the result of small duplications which have occurred in a number of chromosomal bands (Keyl 1965). Apart from the different thickness and the different DNA content of a number of bands, the two subspecies are homosequential and they can be crossed to yield fertile offspring. The subspecies with the smaller genome is thought to be the phylogenetically older genotype according to the cytological analysis (Keyl 1965) and thus the higher concentration of Cla elements in the larger genome must be the consequence of an evolutionary amplification process. However, the difference between the genomes of *Chironomus thummi thummi* and *Chironomus thummi piger* with respect to the Cla-family is not only quantitative, but the distribution of the Cla-elements is also very different, in the chromosomes of the two subspecies as revealed by *in situ* hybridization. The Cla-elements in the chromosomes of *Chironomus thummi piger* are clearly restricted to the heterochromatic centromere region (Schmidt 1981), they are found in more than 200 euchromatic sites in the chromosomes of *Chironomus thummi thummi* in addition to the larger amount present in the centromeric regions.

It has been shown that the Cla-elements have also entered the non-transcribed spacers (NTS) of DNA of *Chironomus thummi thummi*, while *Chironomus thummi piger* is free of Cla-elements (Schmidt et. al. 1982). The
analysis of the ‘empty’ and ‘filled’ Cla-element integration sites represented by NTS of *Chironomus thummi thummi* and *Chironomus thummi piger* respectively, led to the conclusion that the Cla-elements are transposable elements although they might be transferred together with flanking sequences. The Cla-elements however, display another interesting characteristic which might have something to do with their ability to change their position in the chromosomes rapidly when multimeric forms of Cla-elements are run on high percentage polyacrylamide gels, the electrophoretic mobility of Cla-elements is much lower than expected from the real molecular weight. The discrepancy between the real and apparent electrophoretic mobilities is even greater than that reported case of the kinetoplast minicircular DNA. The mobility shift might be due either to ‘bending’ or extensive secondary structures (Israelewski 1983). In either case, the unusual conformation of the Cla-element DNA is determined by the primary base sequences.

Schmidt (1984) was able to sequence clustered and interspersed repetitive DNA sequence family for 36 Cla-element in the genomic DNAs of *Chironomus thummi thummi* and *Chironomus thummi piger* and *Chironomus pallidivitatus*. Many location sites of ‘Cla’ repeats stain positively for C-heterochromatin (Hagele 1977) and replicate late. ‘Cla’ repeats are weakly represented in *Chironomus dorsalis*. In *Chironomus halophilus* the DNA hybridize with the Southern blot analysis and varies in size from more than 25 to only 2 kb which indicates a specific restriction site has been lost (Hankeln 1990).
i. Developmental genetics

Hybridization between different strains of a species or sub-species can create profound genic changes and also sterility in the offspring, if proceeded in a non-reciprocal crossing set up. This unusual genetic phenomenon is thought to be the result of interactions between paternally derived chromosomal and maternally derived cytoplasmic factors. These interactions have gained widespread attention since this type of phenomena was first observed in *Drosophila melanogaster* involving different interaction system, resulting in hybrid dysgenesis to which specific classes of genetic mobile elements are involved to have been ascribed (Breghano and Kidwell 1983, Engels 1983).

Large number of abnormalities were observed in the experimental set up in the case of *Chironomus thummi* subspecies which explicitly expressed in one of the two reciprocal crosses only (Hagele 1984, 1987). Among *Chironomus thummi* hybrids resulting from the cross between *Chironomus thummi thummi* male x *Chironomus thummi piger* female, a high percentage of sterile individuals occur. The sterility is due to the rudimentary development of gonads and affects both sexes (Hagele and Speier 1985). Its appearance depended on the temperature at which embryogenesis and early larval growth of the hybrids involved (Hagele 1987). This type of sterility is induced if specific paternal *piger* chromosomes meet with the specific chromosomal and cytoplasmic *thummi* status of the egg. In a particular programme in which male hybrids of the cross *Chironomus thummi thummi* (stock III) x *Chironomus
*thummi piger*, rudimentary testes were recovered with the following abnormalities (Hagele and Oschmann 1987). Approximately 60% of the hybrid males show allocyclic chromosome behaviour in spermatogonia and I-spermatocyte nuclei. Within these nuclei, two groups of four chromosomes were found which differ from one another in their state of condensation. In cases where allocycly is more pronounced, the chromosomes of both groups disintegrate into numerous unequally sized fragments at meiotic prometaphase I and as such gametes were not produced. In individuals in which the allocycly is less pronounced or absent, the nuclear divisions appear to be normal but chromosomes and chromatid aberrations were quite frequent and number of viable sperms was reduced. In these males, chiasma was reduced several fold in comparison to that of the reciprocal cross products.

Although these two sub species are homosequential in terms of polytene banding sequences, the application of C-banding data on metaphase chromosomes (Hagele and Speier 1988) revealed that *thummi thummi* contain approximately 17% more pericentric C-band heterochromatin. The proportions of heterochromatin in metaphase chromomere in *thummi* is larger than polytene chromosomes. This discrepancy is implied as being due to the specific chromosome organisation rather than the result of under replication of heterochromatin during polytenization.

It was realized that the *piger* chromosome I and III seem to influence significantly on the inducibility of gonadal dysgenesis, but not on chromosome II and IV. Based on the crossing results, Hagele and Oschmann (1989) were of the
opinion that the factors determining rudimentary development of the gonads were located distally to those pericentric chromosome regions of piger I and III polytene chromosomes. However, it was not clear as to whether these effects were due to an act as an epistatic form of interaction or by the activity of transposable elements.

A portion with a sub unit MW of about 148 K Dalton was detected in the fat body of females of the reciprocal hybrids of Chironomus thummi thummi and Chironomus thummi piger and the same was not present in males. Hagele (1990) suggested this protein could be due to the presence of vitellogenin and was found in both hybrids during the late fourth instar larvae.

3. Objectives of the present study

Since, there are no systematic studies available on the cytogenetics of Chironomids, pertaining to tropical regions either at intra or inter-specific level, it is proposed to make a systematic survey of chromosomal analysis of some South Indian Chironomids. Hence, the present study is an attempt to bridge the existing gap for some South Indian fauna. This study was undertaken to gather data on the following areas of research:

1. Chromosome organization of the type-species pertaining to mitotic, meiotic and salivary gland polytene chromosomal karyotype and to indicate their karyological relationships pertaining to Indian fauna.
2. A gross comparison of polytene chromosome banding sequences with that of meiotic pachytene chromosome organization.

3. A report on the nature and kind of endophenotypic variation observed in some natural populations, and

4. A brief description of larval morphological features of labial teeth (Mentum).