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Review Article

REVIEW OF POLYTENE CHROMOSOME

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ABSTRACT

In the recent years it has been strongly emphasized that cytogenetic studied involving chromosomal polymorphism in the natural populations of mosquito vectors of malaria should form an integral part of research programmes on vector cytogenetics. On keeping in mind present review has been written.

INTRODUCTION

In general, the chromosomes of most of the organisms are too numerous to be considered a good subjects for cytological investigations. Therefore, for a long time, cytogenticists had been in search of an experimental organism, in which the chromosomes are large enough to facilitate the observations of the qualitative difference along their length, corresponding to the genes. The answer was found in the classic genetic studies, carried out on certain traditional materials like *Chironomous* and *Drosophila* (Lemonier, 1973; Zhimulev, 1974; Gurzdev, 1975; Zhimuleve and Belyaeva, 1975' Kiknadze *et al*., 1976 .These insects were considered important for genetic studies, not only from the point of view of their lower diploid chromosome number (2n=8) and short life cycle, but also for the special type of giant sized chromosomes in their salivary gland nuclei. They are well suited for understanding the various problems related to the behaviour of genes, puffing activity and DNA regulatory mechanism.

Bridges in 1935 made extensive and detailed investigations of the salivary gland chromosomes of *Drosophila melnogaster*. He published a detailed salivary gland chromosome map of *Drosophila melanogaster* and described the relation of the bands to the genes. This was a significant discovery which opened up new fields in cytogenetical research.

The banding patterns of the polytene chromosomes have been of particulars value in genetic studies. Cytological and genetic maps of all the chromosomes of *Drosophila melanogaster* have been prepared and it is now possible to identify the sites of genetic activity in chromosome from the banding pattern. Radiation studies have substantiated these findings. For example, the absence of the specific band or a set of bands removed from a chromosome by induced breakage of the chromosome, can be correlated with the changes in the genetic behaviour of an organism. Other types of abnormalities in configuration of the chromosome can also bee mined in this way.

Most of the work, on the chromosomal polymorphism has been done on dipteron insects in which the genera *Drosophila* (Goldschmidt, 1956; Richmond and Dobzhansky, 1976), (Casron, 1946) and *Chrionomous* (Action 1956, Beerman, 1956, Martin and Walker, 1971, Brady *et al* 1977, Martin, 1979) have been investigated more extensively than any other specues. Dobzhansky (1944, 1947b, 1948, 1950, 1957, 1970), Ranganath and Krishnamurthy (1981) and Clyde (1982) have done extensive work on the evolution of various species groups of the genus *Dropsophila* i. e. *D. pseudoobscura* group, *D. paulistorum* group, *D. willistoni* group *D. replete* group and *D. nasuta* group by studying the salivary chromosome morphology in different populations. Changes in banding pattern during different seasonal cycles were also studied in *D.funebris, D. robusta, D melangoster* and

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Chironomous plumoscus. Epaling and Lower (1957) and Strickberger and Willis (1946) observed monthly frequency of inversion in the 3rd chromosome of *Dropophila sp.* While Mettler *et al* (1977) studied 20 different populations of *D. melanogaster* and classified inversions on the basis of their frequency and geographical distribution. Structural variability in chromosomes of urban and rural populations of *D. fineries* was noticed by Dubinin *et al* (1987) and Dubinin and Tiniakov (1946).

For a long time, the mosquito workers had been taking keen interest in studies on the various aspects of mosquito behaviour and morphology because of the role played by these insects in disease transmission and human economy all over the world. During the last three decades, the work on culicine cytogentics has grown to an international level an the scientists from the various research centers of the world have contributed significantly towards our understanding of their basic biology, evolution, speciation and insecticide resistance, linked with the hereditary process (Davidson, 1956, 1957, 1959, 1979; Mosna *et al*. 1958; Brown, 1958, 1959, 1961, 1967; Mason and Bron, 1963; Mariani *et al*., 1964' Singh and Mohan, 1965; WHO 1970; Coluzzi and Kitzmiler, 1975).

In mosquitoes, work of similar nature was carried out in several species of the family Culicidae in which details of standing banding pattern of polytene chromosomes have been taken into account in solving the problems of speciation and taxonomy in the genus *Anopheles* (Firzzi,1947 a, b,c, 1949-5a, b 1954, 1958 a, b, c Firzzi and De Carli, 1954, Firzzi and Holstein, 1956, Frizzi and Kitzmiller, 1959, Baker and kitzmiler, 1963 a, b kitzmiller, 1966, Coluzzi and Sabatini, 1967, Coluzi *et al*, 1970 wattal andkalra, 1967, Puri 1960, Rabanni and Kitzmiller, 1974 Chowdiah *et al*, 1971, Chaudhary 1971, 1973, 1975, 1979, 1985, Kreutzer *et al*, 1970, Belcheva and Mikhailona, 1970, 1972, Brown and Pal, 1971, Kabanova et, al, 1973, Green, 1972a, 1982, 1983, Green and Miles, 1980, Green *et al*, 1985, Smithson, 1972, Mehmood and Sakai 1984, 1985, Coetzee, 1983, 1984).

Study of the salivary chromosome morphology is an intera part of investigations on various aspects of cytogenetics of insects likes mosquitoes which are malaria and filaria carries and human population. Study of the gene arrangement or bands on various chromosomes is essential due to the facts that these bands are specific for the species. The occurrence of chromosomal polymorphism originating from Inversions and translocations is an important source in insect evolution and speciation (White, 1957, 1973, Baker and Kitzmiller 1961, 1963b, Coluzzi & Sabtaini 1967, Coluzzi Di Deco and Cancrini, 1970, Dobzhansky, 1970, Kreutzer 1972a, b, 1973, Kreutzer and Kitzmiler 1971a, Narang *et.* 1973, Kretuzer and Kitzmiler 1971a, Narang *et al,* 1973, Kitzmille *et al* 1973, a,b, 1976, Green 1982, Sharma et, al, 1968b, 1976, 1982, Sharma & Chaudhary, 1972, 1973, 1979.

Members of the family culicidae have a unique problem of forming species complexes of one or more subspecies and verities. Such complexes of sibling species have indistinct or poorly defined morphotaxonomic characters but very clear difference sin their chromosomal banding pattern. In other words, they chromosomal binding pattern. In other words, they are morphologically similar but chromosomally different. *Anopheles gambiae* complex and *A. maculipennis.* Such complexes, which were detected on the basis of chromosomal banding pattern comparison in their subspecies. Accordingly, *A. gambiae* of the African subcontinent was recognized as a complex of 6 subspecies which were detected on the basis of chromosomal banding pattern comparisons in their subspecies. Accordingly, *A. gambia*, of the African subcontinent was recognized as a complex of 6 subspecies which are morphologically indistinguishable. These are *A. arabiensis, A. melas, A. merus. A. gaudrimaculatus, A. bwambae and A. arabiensis, A. arabiensis,A. melas, A. merus, A. gaudrimaculatus, A. bwambae and A. gambiae* (Who, 1984, 1989) Their recognition was made by applying concepts and techniques of evolutionary systematic and genetics. Recent studies have shown further complexities involving chromosomal polymorphism and incipient speciation process (Robert et al 1989. Similarly, *A. maculiennis* was also identified as a group of species over Europe, Asia and North Africa with the species, namely, *A. labranchiae, A. atroparus, A. message, A. sacharovari, A. maculipennis, A; melanoon, A. subbalpinis*,. In North America, a closely related group of species constitute an important part of the anopheline fauna.

In addition to these two species complexes, the following have also been indemnified as group of species forming complex of sibling species. Many of these have lately been elevated to the level of species.

Anopheles stephensi. It has two geographical races viz. type form, which is an urban inhabitant and an active vector of malaria, and variety mysorensis, a poor vector of malaria,. The two forms were identified on the basis of the number of the ridges on the egg. Examination of several *A. stephensi* strains in the laboratory revealed that there were three variants instead of two, with reference to ridge numbers i.e. type form, variety mysorensis and an intermediate form.

Amopheles culcificies. The genetic and cytogenesis studies carried out so far on this species have reveled that of is also a complex of at least 4 sibling species (a, B,C & D) with the possibility of a few more, Preliminary. Experiments suggest that these sibling species also differ in their seasonal prevalence, geographic location and response to insecticides (Subbbarao & Sharma, 1984, Green and Miles, 1980.

Anopheles hyrcans. To date, there are 14 known taxa in the oriental region which possibility which possibility belong to this complex (Kanda and Oguma, 1972, Takai and Kand, 1986) of these only 10are prevalent in the Indian region, out of which only *A. hycanus* (type form) *A. nigerrimus*, *A. sinusis and A. hycanus (type form), A. nigerrimus, A. sinusis and A. peditaeniatus* are common in India (Harrison,1972,). The cytogenetic information is available only for *A. nigerrimus* (seetharam & Chowdiah, 1971). *A sinensis* (Sharma, 1971) and *A. peditaeniatus* (Chaudhary, 1979).

Anopheles barbirostris. As early as 1962, Reidhad identified *A. barbinstoris* to be a group species to which further information was added by Chowdiah *et al*,(1970) about the species represented in India.

Anopheles barratries. As. Early as 1962, Reid had identified. Brainstorms to be a group of species to which further information was added by Chowdiah *et al*, (1970) about the

species represented in India.. This consists of 5 species A,B,C,D and E (Baimai *et al* 1988). Limited cytological information about its karyotype has been presented by Wibawo *et al*. (1984), Baimai et, al. (1984) and Kanda *et al* (1980)

In a monograph of WHO (1989) there are three more complexes, listed below:-*Anopheles maculates:* It consist of nearly 7 sibling species, out of which only the type form, *A. maculates* has been cytologically worked out by Narang et. al. (1973 b), whereas its variety willl has been elevated to the rank of a species (Green *et al*, 1985).

Anopheles punctuates. In this, at least 5 species are prevalent in the Australasian zone which have been indentified as, *A. farauti* No.1, No2, and No.3, *A. koliensis* and *A. punctuates*. *Anopheles balabacensis.* Within the *A. leucosphyrus* group*, balabacensis* complex forms a subset of its own forms, while another set comprising A. dirus in it forms a subset of at least 5 species.

A. funestus. In this complex there are 6 members comprising *A. funestus arwni*, *A. parinsis, A. confuses, A. leesoni, A. rivulorun and A. brucii.* Out of these six, only the type form A. funestus is a recognized vector of Malaria next to *gambiae* in Afro-tropical region.

Koryakov á D.E. *et al*. (1998) studied region 20 of the polytene *X* chromosome of *Drosophila melanogaster* was studied in salivary glands (SG) and pseudonurse cells (PNC) of *otu* mutants. In SG chromosomes the morphology of the region strongly depends on two modifiers of position effect variegation: temperature and amount of heterochromatin. It is banded in *XYY* males at 25°C and *β*-heterochromatic in *X0* males at 14°C, i.e. *su(f)* in section 20C, the nucleolar organizer and 359-bp satellite in 20F. The 359-bp satellite, which has been considered to be specific for heterochromatin of the mitotic *X* chromosome, was found at two additional sites on chromosome 3L, proximally to 80C. The right arm of the *X* chromosome in SG chromosomes was localized in the inversion *in (1LR)pn2b*: the telomeric *HeT-A* DNA and AAGAG satellite from the right arm are polytenized, having been relocated from heterochromatin to euchromatin.

Vatolina T.Y. *et al.* 2011reported that the Salivary gland polytene chromosomes demonstrate banding pattern, genetic meaning of which is an enigma for decades. Till now it is not known how to mark the band/interband borders on physical map of DNA and structures of polytene chromosomes are not characterized in molecular and genetic terms. It is not known either similar banding pattern exists in chromosomes of regular diploid mitotically dividing non polytene cells. Using the newly developed approach permitting to identify the interband material and localization data of interband-specific proteins from modencode and other genome-wide projects, we identify physical limits of bands and interbands in small cytological region 9F13-10B3 of the X chromosome in *D. melanogaster*, as well as characterize their general molecular features. results suggests that the polytene and interphase cell line chromosomes have practically the same patterns of bands and interbands reflecting, probably, the basic principle of interphase chromosome organization. Two types of bands have been described in chromosomes, early and late-replicating, which differ in many aspects of their protein and genetic content. As appeared, origin recognition complexes are located almost totally in the interbands of chromosomes.. Gilmour, D. S. and Lis J.T. (1985), examined the in vivo distribution of RNA polymerase II on the hsp70 heat shock gene in *Drosophila melanogaster* Schneider line 2 cells. In heat shock-induced cells, a high level of RNA polymerase II was detected on the entire gene, while in non induced cells, the RNA polymerase II was confined to the 5' end of the hsp70 gene, predominantly between nucleotides -12 and +65 relative to the start of transcription. This association of RNA polymerase II was apparent whether the cross-linking was performed by a 10-min UV irradiation of chilled cells with mercury vapor lamps or by a 40-microsecond irradiation of cells with a high-energy xenon flash lamp. We hypothesize that RNA polymerase II has access to, and a high affinity for, the promoter region of this gene before induction, and this poised RNA polymerase II may be critical in the mechanism of transcription activation.

Sutton, E. (1940), In a study of euchromatic bands transferred to heterochromatic regions, and vice versa, evidence of change in structure was found in a single case, where bands from 2B transposed to the chromocenter of X sometimes appear darker than the homologous bands in the normal X. Bands from the white-Notch region of X (3C) do not appear to be lost or changed when they are adjacent to heterochromatin, nor do bands from some other euchromatic regions of the chromosomes. Heterochromatic regions inserted between euchromatic bands retain their characteristic structure. It is concluded that these studies provide no evidence of visible change or loss of bands due to an interaction between euchromatin and heterochromatin.

Prokofyeva-Belgovskaya (1937) states that “a transfer of any chromosome section to the chromocentral region modifies the structure of that section into a chromocentral structure. Conversely, the removal of chromosome sections of the inert region from the chromocenter (by an inclusion into the active region) brings about a change in the cytological structure of the translocated sections; they become indistinguishable from the active part of the chromosome.”

Lefevre, G. JR. `1971 A cytogenetic analysis of a series of recessive sex-linked lethal mutants located in the ras-U-m-fw region indicates that the frequency of recombination observed between neighboring loci is directly correlated with the salivary chromosome banding pattern. Regions containing dark-staining heavy bands, such as 1 OA1-2 with which the U locus is associated, exhibit more crossing over than do regions populated by equal numbers of thin, faint bands.

Rudkin (1965) on the relative DNA content of successive intervals along the X chromosome can be used to calculate the length of DNA, in nucleotide pairs, that is associated with specific recombination frequencies in the long interval from w to f, which is free from interference by the telomere and centromere. Further, the nucleotide length of an "average" band can be calculated, together with the 512 G. LEFEVRE, JR. amount of crossing over that can be attributed to it. These correlations make possible predictions of the cytological locations of genes from linkage data, and vice uersa-Even though bands vary in size from those barely resolvable and containing 5 x 10”. or fewer, nucleotide pairs (per haploid strand) to those like 1 OA1-2, containing nearly 2.5 x lo5 nucleotide pairs, there appars to be a oneto-one relationship

between genes and bands. Thus, larger bands contain a great excess of "quiet" DNA whose significance is not immediately evident. A smaller band like 3C, with which the locus is associated, appears to have mutant sites distributed throughout its substance, but even such a band might have a significant amount of "quiet" DNA intercalated between mutant sites. Only the thinnest bands. such as those between 10A1-2 and 10B1-2, may be totally composed of information-containing DNA associated with specific loci.

Vatolina T.Y. *et al*. 1967 Salivary gland polytene chromosomes demonstrate banding pattern, genetic meaning of which is an enigma for decades. Till now it is not known how to mark the band/interband borders on physical map of DNA and structures of polytene chromosomes are not characterized in molecular and genetic terms. It is not known either similar banding pattern exists in chromosomes of regular diploid mitotically dividing nonpolytene cells. Using the newly developed approach permitting to identify the interband material and localization data of interband-specific proteins from modencode and other genome-wide projects, we identify physical limits of bands and interbands in small cytological region 9F13-10B3 of the X chromosome in *D. melanogaster*, as well as characterize their general molecular features. Our results suggests that the polytene and interphase cell line chromosomes have practically the same patterns of bands and interbands reflecting, probably, the basic principle of interphase chromosome organization. Two types of bands have been described in chromosomes, early and late-replicating, which differ in many aspects of their protein and genetic content. As appeared, origin recognition complexes are located almost totally in the interbands of chromosomes.

Michael and Judd 1974 from earlier work, there appears to be an underlying one-to-one correspondence of polytene chromosome bands and complementation groups within a sizeable, continuous X-chromosome segment, 3A1-3C7). However, most of the data supporting this one-to-one relation of bands and genes were gathered from mutants that upset vital functional units, thus leading to lethality. Among this series of mutants, only four loci, zeste, white, roughest and verticals, have no known lethal alleles. If phenotypic changes less drastic than lethality result from the loss of other chromosomal segments, they probably would not have been recognized in the earlier studies.-We report here some chromosomal sequences localized in 3A, 3B, and 3C whose loss effects no lethal change in the development of the animal. A portion of the 3A3-3A4 region can be disrupted in a nonlethal fashion, yet this sequence does not seem to be a part of either the zeste locus or l(Z) zwZ, which are known to be located in these bands. Two more complementation groups have been discovered that have no lethal alleles and map to 3B4-3B6; a third falls within 3B1-2. The loss of a sequence in 3C2-3 is tolerated without any genetically observable effect. Between 3C7 and the boundary of 3D there is at least one more sequence that behaves in this manner.-The discovery of these units, which are not allelic to any of the loci previously known, makes it clear that division 3B contains more genes (i.e., complementation groups) than polytene chromosome bands, while portions of 3A and 3C seem to have no functional significance. Accordingly many polytene chromosome bands may be composites of several complementing functional units. This investigation also indicates that there are chromosomal segments that are seemingly dispensible and thus function in a manner that is difficult 01' impossible to define with available methods.

Madueno, E. *et al*. 1995 assembling contiguous arrays of cosmids that were selected by screening a library with DNA isolated from microamplified chromosomal divisions. This map, consisting of 893 cosmids, covers -64% of the euchromatic part of the chromosome. In addition, 568 sequence tagged sites (STS), in aggregate representing 120 kb of sequenced DNA, were derived from selected cosmids. Most of these STSs, spaced at an average distance of -35 kb along the euchromatic region of the chromosome, represent DNA tags that can be used as entry points to the fruitfly genome. Furthermore, 42 genes have been placed on the physical map, either through the hybridization of specific probes to the cosmids or through the fact that they were represented among the STSs. These provide a link between the physical and the genetic maps of *D. melanogaster*. Nine novel genes have been tentatively identified in *Drosophila* on the basis of matches between STS sequences and sequences from other species.

Sesman *et al.* (2005) In this study the effects of AFB1, a mold metabolite, on third instar larval salivary gland polytene chromosomes of *Drosophila melanogaster* were investigated. The various chromosomal abnormalities (= aberrations) as fragment loss from teminal, semibreak, asynapsis, regional shrinking, ectopic pairing on arms of polytene chromosome, were observed in polytene chromosome slides from third instar larvae which have developed in the medium containing AFB1. It was found that the difference between control and test groups from the point of view of the chromosomal abnormalities is statistically significant ($P < 0.05$). The possible mechanism of the all chromosomal abnormalities caused by AFB1 is discussed. *Drosophila ananassae* exhibits a high degree of chromosomal polymorphism. A total of 70 paracentric and 17 pericentric inversions and 13 translocations have been described in *D. ananassae*. However, only three paracentric inversions, namely, AL in 2L, DE in 3L and ET in 3R, are coextensive with the species. Chromosomal polymorphism has also been studied in Indian populations of *D. ananassae*, and there is evidence for geographic differentiation of inversion polymorphism.

Zhimulev F. *et al*. 2009 Using the polytene chromosomes, numerous biological phenomena were discovered. First the polytene chromosomes served as a model of the interphase chromosomes in general. In polytene chromosomes, condensed (bands), decondensed (interbands), genetically active (puffs), and silent (pericentric and intercalary heterochromatin as well as regions subject to position effect variegation) regions were found and their features were described in detail. Analysis of the general organization of replication and transcription at the cytological level has become possible using polytene chromosomes. In studies of sequential puff formation it was found for the first time that the steroid hormone (ecdysone) exerts its action through gene activation, and that the process of gene activation upon ecdysone proceeds as a cascade. Namely on the polytene chromosomes a new phenomenon of cellular stress response (heat shock) was discovered. Subsequently chromatin boundaries (insulators) were discovered to flank the heat shock puffs. Major progress in solving the problems of dosage compensation and position effect variegation phenomena was mainly related to studies on polytene

chromosomes. This review summarizes the current status of studies of polytene chromosomes and of various phenomena described using this successful model.

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