

A MODEL ON THE STRUCTURE AND ORGANIZATION OF THE POLYTENE CHROMOSOME BASED ON THE STUDY ON *CHIRONOMUS STRIATIPENNIS* KIEFFER (DIPTERA: CHIRONOMIDAE)

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ABSTRACT

A study on the polytene chromosomes obtained from the salivary gland cells of *Chironomus striatipennis* Kieffer indicated that a polytene chromosome is not a typical ribbon like body formed of a number of thread like chromatin elements aligned side by side having condensed chromatin thread at locations forming bands, simple linear threads of chromatin as interbands and highly extended chromatin threads forming Balbiani rings and puffs at places as conventionally thought of, instead a chromosome appears to be a cylindrical body with thin and thick circular bands as elevated regional differentiation of the chromatin threads having interbands in between as depressed regions over a chromosome. The polytene chromosomes prepared from the Chironomid larvae showed the characteristic configurations to support the organization of polytene chromosomes as conjectured. The variable dimensions of bands appeared due to variable amount of condensed chromatin material and intervening interbands also varied in their magnitude of extension. Besides some regions of excessive constriction also appeared as waists along a chromosome arm and such differentiation could be possible due to presence of some quasifluid substance making the core of the cylindrical chromosome. The multiple chromatin threads in the polytene chromosome appeared to be arranged on the surface line of the long chromosome with coiled or kinky orientation of the thread at identical locations in all the chromatin threads. The chromatin threads were so arranged that the surface of the chromosome appeared smooth laterally except some swelled protrusions at places. The swelled protrusions were the bands and the depressed regions between them were the interbands. The inner core of the cylindrical chromosome appeared to be filled up with a material of quasi-fluid consistency.

INTRODUCTION

Polytene chromosomes represent a special type of chromosomal configuration at interphase stage of cell cycle in several types of organisms including dipterous insects (Zhimulev, 1999). In comparison to their metaphase counterparts these chromosomes are greatly enlarged both in length and breadth and therefore, they are called giant chromosomes (Koltzoff, 1934). Not only that the homologous counterparts of the polytene chromosomes in the somatic cell during polytene chromosome formation come in pairing and the chromosomes in paired condition undergo multiple rounds of replication without the separation of the chromatids (Ashburner, 1970; Keyl, 1962; Sarkar *et al.*, 2011). Hence, the chromosomes in paired state appear as long ribbon like structures. Such multiple round of replication without chromatid separation is called endoreduplication. Not only that all the chromatids of a polytene chromosome at particular points show chromatin condensation forming highly pyknotic chromatin zones called bands and therefore, along the whole length of a chromosome many band structures appear with mostly unstained interband regions within two adjacent bands. Occasionally a band on the chromosome may show hyperactivity with laterally extended threads engaged in RNA synthesis (Beermann, 1952; Keyl, 1957; Hagele, 1975). Such extended puffy structure at a specific point on the polytene

chromosome is called Balbiani ring. Though polytene chromosomes were first noticed in some somatic cells of the larvae of *Chironomus plumosus*, but the chromosomes were also observed in the interphase nuclei of the somatic cells of many other members of dipterous insects, Collembolans, infusorians and mammals (Zhimulev, 1999). Because of their unique organization showing functional activities at cytological level the polytene chromosomes have achieved attraction of Cytologists, Geneticists and Molecular Biologists as they give clue to many mysteries of the science of Genetics (Michailova, 1989). Based on the study on the polytene chromosomes of *Chironomus striatipennis* Kieffer an attempt has been given in the present investigation to formulate a model on organization of the polytene chromosomes in eukaryotes.

MATERIALS AND METHODS

Fourth instar larvae of *Chironomus striatipennis* Kieffer collected from laboratory culture were dissected to obtain their salivary glands and the glands were fixed in 3: 1 alcohol-acetic acid by standard method. The gland tissues were then stained with 2% aceto-orcein for 10 minutes and squashed on clean glass slide with the help of a cover glass. The squashed materials were then observed under the microscope to study the configurations and organization of the polytene

Table 1: Polytene chromosomes of *C. striatipennis* and their measurements at specific regions

Chromosome	Centromeric position	Measure of maximum wide region (μm)	Measure of maximum narrowed region (μm)	When the arm is cylindrical its circumference at the broadest region (μm)
I	Middle	3.46	1.15	10.87
II	Middle	2.55	1.24	8.01
III	Middle	2.99	1.44	9.39
IV	Subterminal	5.92	0.95	18.60

chromosomes. Besides the conventional study of the chromosome in bright fields, the chromosomes were also studied under phase contrast objectives.

Identification of the polytene chromosomes was done following the conventional methods as they were numbered according to their decreasing order of lengths. The largest chromosome was marked as chromosome I and the smallest one as chromosome IV. The length and breadth of each chromosome and the dimensions of the major bands were also recorded (Table 1). The largest and smallest interbands were also measured along with the dimension of a puff or Balbiani ring over the chromosomes. Other characteristic features related to the polytene chromosome organization were also noted.

Metaphase chromosomes from the neural cells of brain of the larva were also prepared after treatment with 0.02% Colchicine for 2 ½ hours in the culture medium for studying the structure and number of the metaphase chromosomes in a cell of this fly. For metaphase chromosome preparation the standard technique was followed (Ayala and Serdar, 2009). The metaphase chromosomes prepared from the neural cells were stained with 2% aceto orcein for their study under the microscope.

RESULTS

The salivary gland cells in squash preparations exhibited four well spread polytene chromosomes in a cell and each of the chromosomes displayed distinct bands and inter-bands (Fig. 1). Occasionally at some points the chromosomes exhibited the presence of swelled regions called the puffs. Besides, at

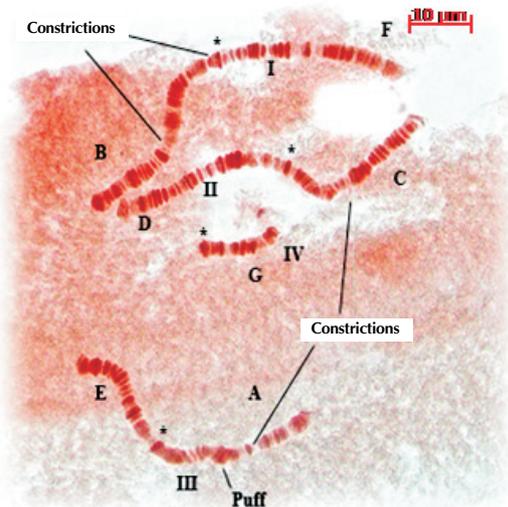


Figure 1: Under bright field the polytene chromosomes of *C. striatipennis*. Centromeric positions are shown by star marks and I, II, III and IV are the four polytene chromosomes of variable size. Two constrictions and a puff have been shown by line tool

several points highly extended Balbiani rings were also noticed. The four polytene chromosomes were the representatives of the eight chromosomes of a cell in diploid state (Fig. 2). Because of pairing of the homologous chromosomes at interphase stage of the cell cycle and multiple round of replication of the chromatids in a chromosome showing highly extended condition each chromosome appeared as very long ribbons like component with bands, interbands and puffs (Fig. 1). Among the four polytene chromosomes three chromosomes were metacentric and one chromosome appeared acrocentric. As per conventional procedure they were marked as I, II, III and IV according to their decreasing order of length (Fig. 1).

A polytene chromosome along its whole length showed presence of constrictions of variable thickness in which due to the presence of a constriction some points appeared extremely narrow whereas some regions were with broader constrictions. In this consideration the chromosomes were irregular along their longitudinal display. The centromeric region appeared as thick band taking more staining in comparison to the other bands. The telomeric part of some of the chromosomes showed puffy conformation. The fourth acrocentric chromosome mostly contained an oval nucleolus attached to the distal end. The bands along a chromosome arm varied in dimension when some appeared to be broad and some appeared as extremely thin (Table 1). The interbands between two adjacent bands were also differing in dimension and mostly taking no stain during processing. A measure of the most broad and constricted site over the polytene chromosomes may be shown (Table 1).

The phase contrast picture indicated that each of the polytene chromosomes was like an elongated cylinder remained engraved in the matrix spread of a cell (Fig. 3, 4 & 5). The

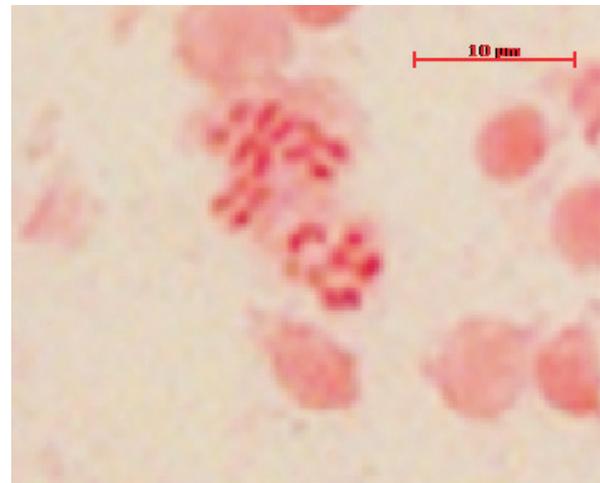


Figure 2: Metaphase chromosomes from neural cell of brain from *C. striatipennis*. Each cell exhibited presence of eight small rod shaped chromosomes



Figure 3: 3D Image of four polytene chromosomes of *C. striatipennis*. Star marks represent the centromeric positions and I, II, III and IV are the different chromosomes

bands along a chromosome arm appeared as swelled structure of different dimensions with interband as depressed grooves varying in dimension (Fig. 4). However, the elevated band and grooves along the chromosome arms always showed a smooth surface under phase contrast objectives. The aberrant structure like inversion (heterozygous) in a chromosome appeared to be non-linear structure (Fig. 5). The idea of the existence of a chromosome core having fluidic consistency was supported by observation of the chromosomal configurations as indicated in the following figures (Fig. 6 & 7). Occasionally a polytene chromosome under pressure during its preparation showed exit of inner contents from the core (Fig. 6). Sometimes due to such liberation of the inner contents of the core region a polytene chromosome was found to be flattened with depressed walls (Fig. 7). Further a cylindrical conformation with a chromatin free core region is logically acceptable because the circumference of the cylindrical body appears capable of providing space for accommodation of multiple chromatin threads as they may be produced during endoreduplication

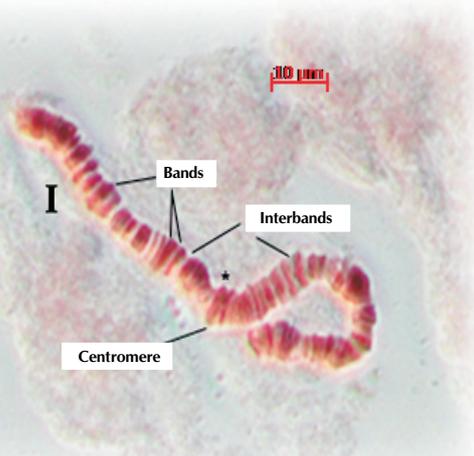


Figure 4: 3D image of polytene chromosome I of *C. striatipennis* (under phase contrast objective) within the cellular matrix. The star mark represents the centromere of the chromosome and the bands and interbands are shown by line tools. In the figure bands appear as elevated structures and interbands as depressed regions. Dimensions of bands and interbands also vary as observed in the figure

DISCUSSION

Polytene chromosomes in dipterous insects because of their highly extended configuration with display of bands,

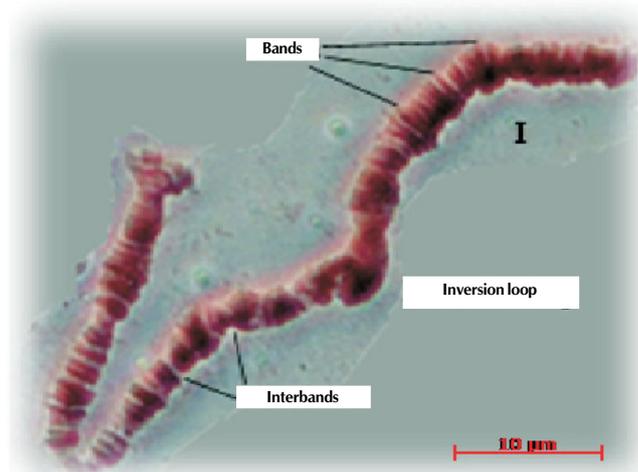


Figure 5: Chromosome I with an inversion loop showing a characteristic contour

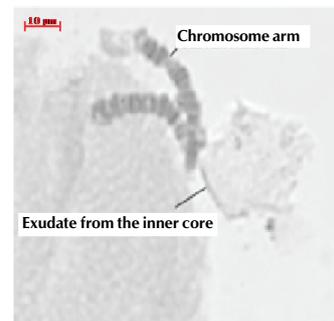


Figure 6: Polytene chromosomes folded from the middle when the inner content came out as fluidic matrix through the distorted segment of the chromosome

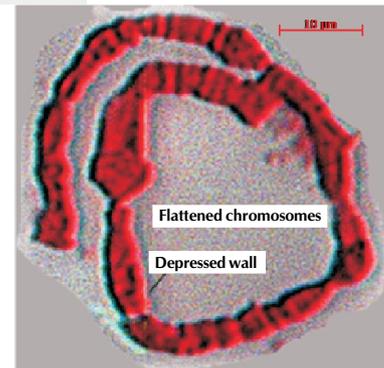


Figure 7: A preparation of polytene chromosome in which due to liberation of inner fluidic contents the chromosome took a flattened shape under phase contrast microscope

interbands and puffs appear to give a new dimension to the structure of the eukaryotic chromosomes. On the basis of light microscopic observation the polytene chromosomes were compared with cylindrical cables with a distinct banding pattern (Zhimulev, 1999) containing multiple chromatin threads (Koltzoff, 1934). Taniguchi and Takayama (1987) suggested that the polytene chromosomes appeared to be formed of dense mass of kinky fibers and the interbands were formed of 30 nm chromatin fibres. On the other hand, less extended polytene chromosomes with indistinct interbands and bands appeared to be formed of 150-200 nm chromatin fibers. However, most of the models advocated the presence of multiple chromatin fibres arranged within the polytene chromosome in compact fashion as electric wires in the electrical cable. Taniguchi and Takayama (1987) suggested coiled organization of the chromatin fibers so that they may

be visible on staining under the light microscope. The model suggested according to the present study claims an orientation of the unit fibers of ~10 nm dimension along the interband zones and kinky or coiled orientation of the unit fibers along the band region making them visible on staining. Instead of coiled arrangement of the fibers, a kinky organization of the same at the bands appears more logical because a band DNA during functional activity may be extended at ease without developing any local strain at any particular point. The dimension of the chromatin fibres and their higher order organization is presumed to be consistent with the model as suggested by Woodcock (2005). The inner contents of the central core of the polytene chromosome might be semifluid by consistency and probably gelatinous in nature and the material acts as the glue for holding the chromatids together side by side. Further, variation in arm thickness of a polytene chromosome at specific points as well as chromosomes at different stages suggests that these glue materials of the chromosome core are at least partly contributed by the chromosome itself. Based on such analysis a model to explain the structural organization of a polytene chromosome may be depicted in the following diagrams (Fig. 8 and 9).

The model on the organization of a polytene chromosome may be elaborated as under.

1. The polytene chromosome appears as a cylindrical pipe in which chromatin threads are peripherally arranged.
2. The cylindrical pipe structure is having unequal thickness along the whole regions. A region may be extremely constricted and the other may be highly expanded appearing as puff.
3. The constricted and expanded regions are set with number of bands and interbands of which some are broad and thick, whereas some are narrow and thin.

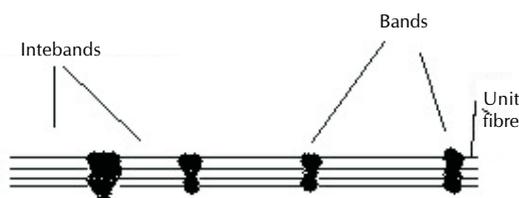


Figure 8: A model of the polytene chromosome segment for bands and interbands



Figure 9: A model proposed to explain the structure of a typical segment of a polytene chromosome showing outer chromatin fibres encircling the inner core filled with probably a substance of fluidic consistency. The elevated bands and depressed interbands as well as the structure of a puff region may be shown as above

4. The band regions are stainophilic and the interband regions are stainophobic.
5. The stainless interband zones appear as depressed whereas stained band regions appear as elevated zones along the

chromosome arms.

6. The cylindrical chromosome may be divisible into two distinct zones: the central chromatin free zone formed of contents of fluidic consistency and the peripheral fibrous zone of chromatin.
7. The fibrous peripheral layer forms a lamina over the central cylinder and the peripheral layer is formed of closely set chromatin fibers probably of ~10 nm dimension

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