A Preliminary Study of the Polytene Chromosomes in Malpighian Tubules and Rectum Cells of Chironomus sp.

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Although the polytene chromsomes of Chironomus were discovered by Balbiani as early as 1881, little is known of the genetics of Chironomus and few researches were on cytogenetics of Chironomus. In recent years, the cytogenetics of Chironomus aroused the interest of genetists because all the somatic cells, which make up the digestive organs of Chironomus, have chromosomes that are metabolically active during the interphase. This fact indicates that the polytene chromsomes persist throughout the whole life cycle of Chironomus. These characteristics combined with its low number and varied chromosomes make the insects good material for studying chromosome differentiation and its function in development. Our present research tries to explore the relationships between the chromosome and cellular differentiation and their function by studying reversible changes in chromosomes structures of Chironomus.

Materials and Methods

The life cylce of Chironomus includes five stages of egg, embryo, larva, pupa and adult. Our study began from larva and ended at the eclosion, i.e. the last three stages of the life cycle. The digestive system of larvae is comprised of mouth, oesophagus, cardiac chamber of stomach, proventriculus, midgut, malpighian tubules, dilated chamber, small intestine and rectum. Among which the salivary gland contains salivary gland cells, cavern cells and tube cells. Malpighian tubules have four long tubes, the wall of each being a layer of cells that can be divided into three types:

(1) α cells, about 28, big and shuttle-like make the main part of malpighian tubules. (2) β cells, about 10, small and Y shape, located among α cells. There are 2-3 α cells for every β cell. The β cells are probably a type of cell that makes α cells to be tubules. (3) δ cells, about 15, located at the center of malpighian tubules, link between the Malpighian tubules and the digestive tract. There are about 200 big cells in the rectum. When

Chironomus develops from larva to adult, the changes of the digestive system are tremendous, especially in the foregut. For instance, the salivary gland disappears. On the contrary, little change takes place in the hindgut, such as malpighian tubules and rectum. Malpighian tubules and rectum persisting from larva to adult is advantageous to developmental genetics.

The samples of Chironomus were collected from the campus of Beijing University and were classified into form A and form B. We dissected the digestive organs of different stages and observed them under low magnification light microscopy using morphological and histochemical methods. Most of the samples were stained by the aceto-orcein method; a few samples by Feulgen and Unna reactions to observe the chromosomes histochemically.

The chromsomes were mapped with a micro-trace device.

Result of Observation

1. The basic patterns of the polytene chromsome set.

There are 8 chromosomes in the meiocytes of Chironomus but only 4 chromosomes in the cells of digestive system because of chromosome fusion in somatic cells. We observed that all the chromosomes in the cells of the digestive system are interphase and endopolyploid. Among which, salivary gland, malpighian tubules and rectum cells have giant chromosomes with high degrees of polytenization. The chromosomes of these three tissues have well spread, more distinct bands, fine structures and have individual characteristics. Therefore, we will use the 4th chromosome of malpighian tubules and rectums of adult to demonstrate the basic structures of polytene chromsome pattern.

The 4 chromosomes are named the 1st, 2nd, 3rd and 4th chromosomes according to their length and shape (see plate II, a). The 1st chromosome is the longest one with a calabash-like head and a heterochromatin-rich tail; more than 400 bands can be distinguished. The 2nd chromosome is the second longest one having a pair of eye-like structure in the centromere;

also more than 400 bands can be distinguished. The 3rd chromosome is shorter than the 2nd one with flat ends; about 300 bands can be distinguished. The 4th chromosome is the shortest one with big ends and thin middle part. There are 63 bands and a giant nucleolus at its head.

The chromosomes can also be divided into several regions. For example, the 4th chromosome consists seven (A-G) regions. There is a nucleolar organizer in region A, a constriction in the region C, three wide bands in region D and a wide band in region E. The region G is an expanded part. Generally puffs appear in regions A, D, E and G, in A (A10-A11), D (D1-D2), D and E (D3-E1), F and G (F5-G1), respectively. (See plate I, 4).

Histochemical study indicated that most of DNA is located in the bands and only a small amount of DNA is distributed in interbands; most of the RNA is located in the nucleolus and chromosome puffs. On the slides prepared by Feulgen reaction, sometimes we could see clearly the decondensed parts of chromatin, showing that chromosomes contain an enormous number of DNA fibres.

During all the developmental stages of the digestive system of Chironomus, the chromosomes from different cells such as salivary gland cells, α cells of malpighian tubules and rectum cells have the same number and similar structure (see plate I, 1, 2 and 3, showing the 4th chromosomes of a salivary gland cell, α cell and rectum cell). These facts indicate that all the somatic cells have a similar genetic background. We can identify the chromosomes of Chironomus on the basis of structural stability.

2. Changes of polytene chromosomes during individual development.

Although the chromosomes of different tissues present similarity and stability because of the same genetic basis, it is impossible for these vigorously metabolic chromosomes not to change their morphological characters to carry out physiological and biochemical functions.

These changes are most noticeable in the activities of puffs. From the 4th chromosomes of Malpighian tubules and rectum which we mapped from larva until adult, it was inferred that the puffs were developed from bands by the processes of formation, swelling and regression.

The larval development of Chironomus includes three stages: the length of stage I is 1.3 cm, stage II 1.7 cm, stage III 2.4 cm. Both the prepupa and adult consist of early and late stages.

Observation indicates Malpighian tubules only form puffs in A, D and G regions with different degrees of swelling : the biggest puff in A, a moderate puff in G and the smallest puff in D. All of them appear during the stage I of the larva, gradually swell, and begin to regress in the pupal stage. At the last stage of adult all bands are restored except the puff in D, in which a trace remains. All the A, D, E and G regions of the rectum form puffs, but always one stage later than in the Malpighian tubules i.e. they begin to form puffs in stage II of the larva. Puffs in A and G swell early, about in stage III of larva. The former continues for a long time and up to the late stage of adult, traces still can be seen; the latter regress at the early stage of the adult and banding is restored at the late stage of adult. Puffs in both D and E are in band state at the stage I of the larva and begin to puff with less swelling at stage II of the larva. But their swelling continues until the early stage of the adult. They are similar to puff G at the late stage. The difference in the formation of puffs between Malpighian tubules and rectum is determined by their different structures and functions.

In addition we found that the 1st chromosome of Malpighian tubules puffed most actively in the adult stage. Some regions of the chromosome form a lot of puffs to link together.

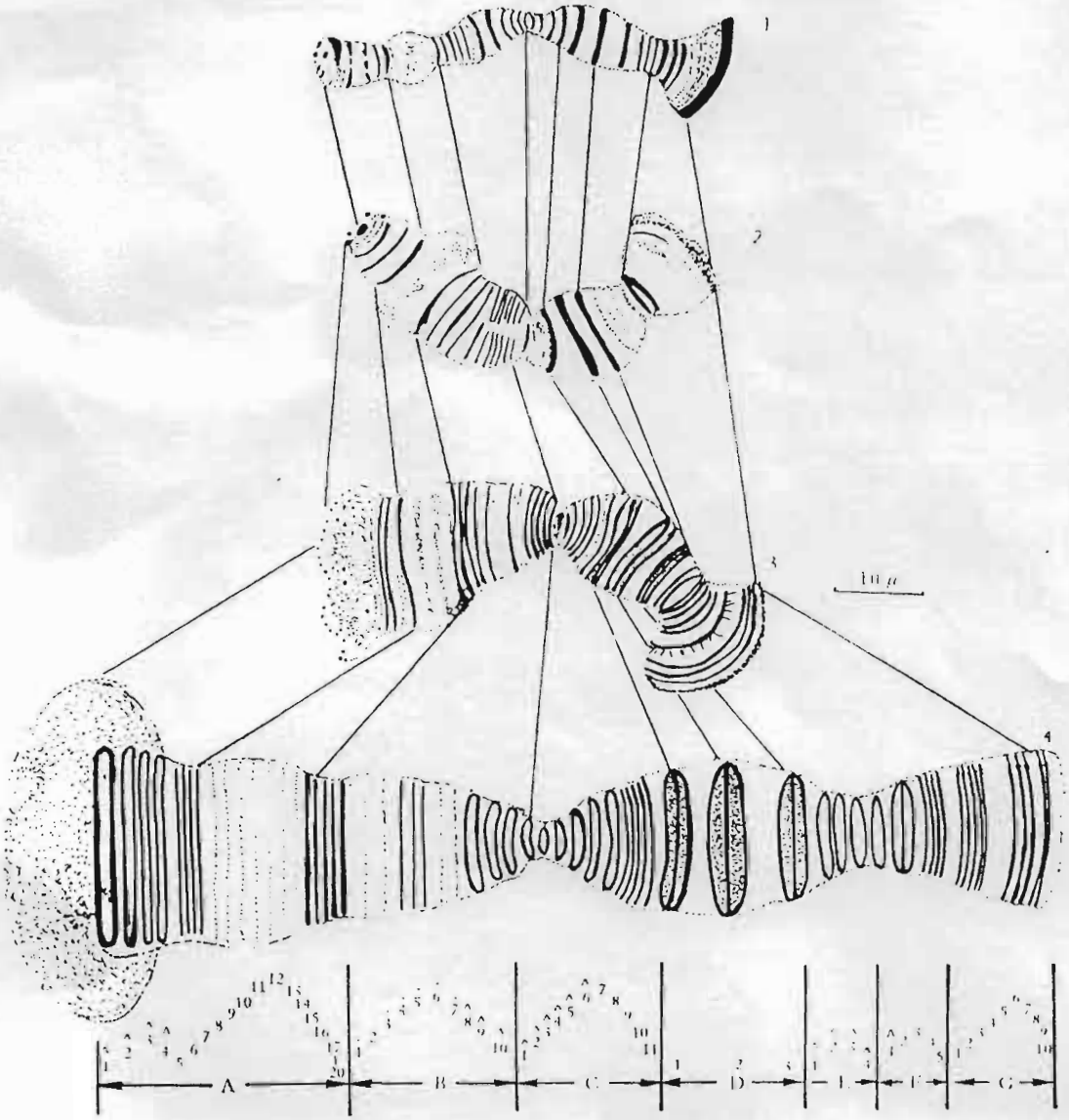
It has been noted that in some areas of Malpighian tubules and rectum chromosomes, the coiled heterochromatic DNA was cast out of the chromosome, particularly in the head of the 4th chromosome (see plate II, b). Other workers also found in other insects that the DNA of puff increased rapidly. We will discuss below the significance of activities of DNA puffs or heterochromatin in the regulation of developmental genes.

Another important change of chromosomes is the differentiation of chromosome polytenization. The different degrees of polytenization give chromosomes of different tissues or cells variety in length, width, size, degree of extension and decondensation, number of distinct bands and properties of staining and consequently result in the differentiation in the size or even in the functions of the cells. For example, in the tissues of Malpighian tubules, the high degree of polytenization of the chromosomes of the α cells enlarges the cell and enhances its secretory function. On the other hand, β cells owing to the low degree of polytenization, are small and probably have no function of secretion. It appears that the differentiation of chromosome polytenization is the cause of differentiation of cells. At the same time, the puffs increasing simultaneously along with polytenization have also been recorded.

Discussion

Recently, many researches dealing with the changes of function of chromosome during development aimed at understanding the cellular differentiation from the viewpoint of nucleo-cytoplasmic interaction. Most of the studies concentrated on puffs and rings of chromosomes. Beermann [5], Sorsa [14] and Ashburner [1,2] pointed out that the bands which form puffs vary in different tissues of Chironomus larvae, so the patterns of puff-formation are tissue specific. The puffs are the DNA fibers that are decondensed and spread from a band and adjacent interbands. Beermann [4] and Pelling [12] also indicated that puffs are the sites of RNA synthesis : the different forms of puffs reflect their different forms of RNA. RNA accumulates and forms into puffs. The regression of puffs was the result of restoration of the bands and transfer of the RNA. The formation and regression of the puffs are suggested to correspond to the switching on and off of transcription of specific gene (or genes). An important theory that the different genes cause different bands to form puffs was put forward. This conclusion was confirmed by the recent work done by Daneholt [6,7], Lambert [11] and Serfling [13] on the 4th chromosome of C. tentans in which Balbiani ring II produced 72s RNA, and the work done by Beermann [3] and Grossbach [9,10] on the anterior salivary gland lobe cells from heterozygotes of C. pallidivittatus and C. tentans. The results we obtained and of other author's work indicate the cellular differentiations are

reflected in functional differentiation of chromosomes. This topic deserves intensive study.



不同组织细胞第IV染色体。1.直肠细胞；2.马氏管α细胞；3.唾腺细胞；4.模式图。

The chromosome IV of the different tissues. 1. Cells of rectum; 2. α cells of Malpighian tubules; 3. Cells of the Salivary gland; 4. The arbitrary regional divisions of the chromosome IV.

a

b

a. 摇蚊成虫期直肠染色体组，染色体 I, II, III, IV　染色体 I 膨泡区（箭头指示）。
The giant chromosomes of the rectum in the adult of *Chironomus* sp.. Chromosome I, II, III, IV. The puffing regions of the chromosome I (Arrows).

b. 摇蚊马氏管、直肠染色体 IV 异染色质的积累和排出（箭头指示）。
The accumulation and discarding of heterochromatin of chromosome IV in the rectum and Malpighian tubules.