Puffing patterns in polytene chromosomes of different tissues of *Melanagromyza obtusa* during development

ABSTRACT: A completely new and improved system for the study of comparative puffing and banding patterns in different tissues of *Meianagromyza obtusa* during development is reported. A comparative study of puffing in polytene chromosomes of salivary and midgut epithelial cells at the third instar larval and prepupal stages was made. Based on the behavior of these puffs, three categories of puffs were observed—common puffs, tissue-specific puffs, and stage-specific puffs. The functional significance of these puffs in relation to the cellular activity during development is discussed. In addition, the data on banding patterns between the homologous chromosomes in different tissues has further strengthened the concept of the constancy of banding patterns.

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PUFFING is common in cells with polytene chromosomes, but it was not until 1952 that puffing was clearly understood through the detailed study of its behavior during the development of two dipteran insects—*Chironomus*[5] and *Rhynchosciara*[11,12]. Since these initial studies were published a voluminous literature has accumulated on the subject. Recently, there have been numerous reviews on puffing in polytene chromosomes of different insects[2,4,10,13].

Despite the extensive studies on puffing, data on comparative puffing patterns in different tissues of the same organism at different stages of development are still meager and fragmentary. Since polytene chromosomes from the salivary glands attain the highest degree of polyteny of all tissues, it is the salivaries that have been the target of the most studies of puffing. Apart from a few abstracts[14,16,19,21,22] a detailed comparison of puffing is that of Berendes[8,9] with *D. hydei*.

Melanagromyza obtusa is a dipteran pest of the family Agromyzidae, causing substantial damage to an important pulse crop (*Cajanus indicus*) in oriental regions of the world. While checking its larval salivary chromosomes for naturally occurring chromosomal polymorphism it was noticed that larval midgut epithelial cells also possess adequately large polytene chromosomes. This characteristic prompted a detailed comparative study of the banding as well as puffing patterns in different tissues during the development of this insect.

Material and Methods

The material for the present study was collected from the natural breeding sites of this insect. Although partial rearing could be done satisfactorily by supplying its natural food (*Cajanus* seed), it has not been possible to maintain its culture in the laboratory through successive generations. Thus, infested pods of *Cajanus* containing larvae of different age groups were brought to the laboratory and the larvae were allowed to continue their development inside the seed. In order to stage larvae and prepupae the number of pores of the spiracles and the appearance of a yellowish hard covering around pupating larvae were used as important markers. In addition, the time interval (approximately 8 ± 1 hours) between two successive stages also was a consideration. Several larvae and prepupae of similar age were examined in order to generalize the pattern of puffing for a particular

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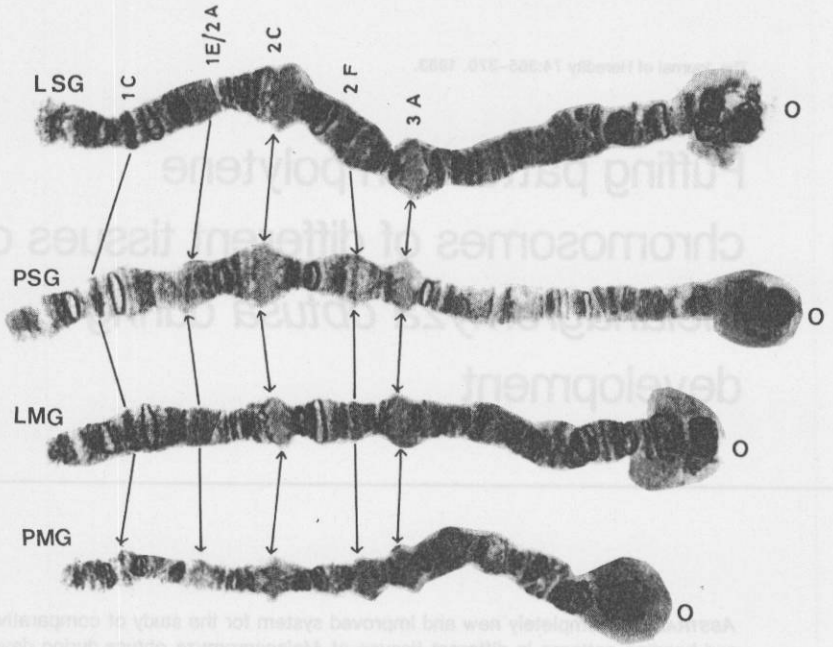

FIGURE 1 Comparative puffing activity in the salivary gland and midgut cells during third instar larval and prepupal stages of development in the X chromosome. Circles at the right represent the centromeric positions of the chromosome. Abbreviations used: LSG—larval salivary gland; PSG—prepupal salivary gland; LMG—larval midgut; PMG—prepupal midgut.

tissue at a certain stage of development. The use of a particular portion of a tissue was made so as to avoid any possibility of variation in puffing due to different regions of the organ.

Slides of polytene chromosomes were made by the usual squash method. All preparations were observed with high power and oil immersion objectives. The puffed sites were located with the help of the reference map of Singh and Gupta[23]. The activity of these puffs were estimated following the method of Ashburner[3]. To generalize the pattern of puffing and level of activity, nuclei from 20 individuals of each type were examined.

Results

A comparison of the banding sequence in polytene chromosomes of salivary gland cells and midgut cells during third instar larval and prepupal stages of *M. obtusa* revealed an apparently identical banding sequence in both tissues. However, a striking difference was observed in their puffing patterns. Altogether 49 loci were examined that formed observable puffs. Based on the behavior of these puffs during development, three different categories could be identified (Table I; Figures 1–5), e.g.,

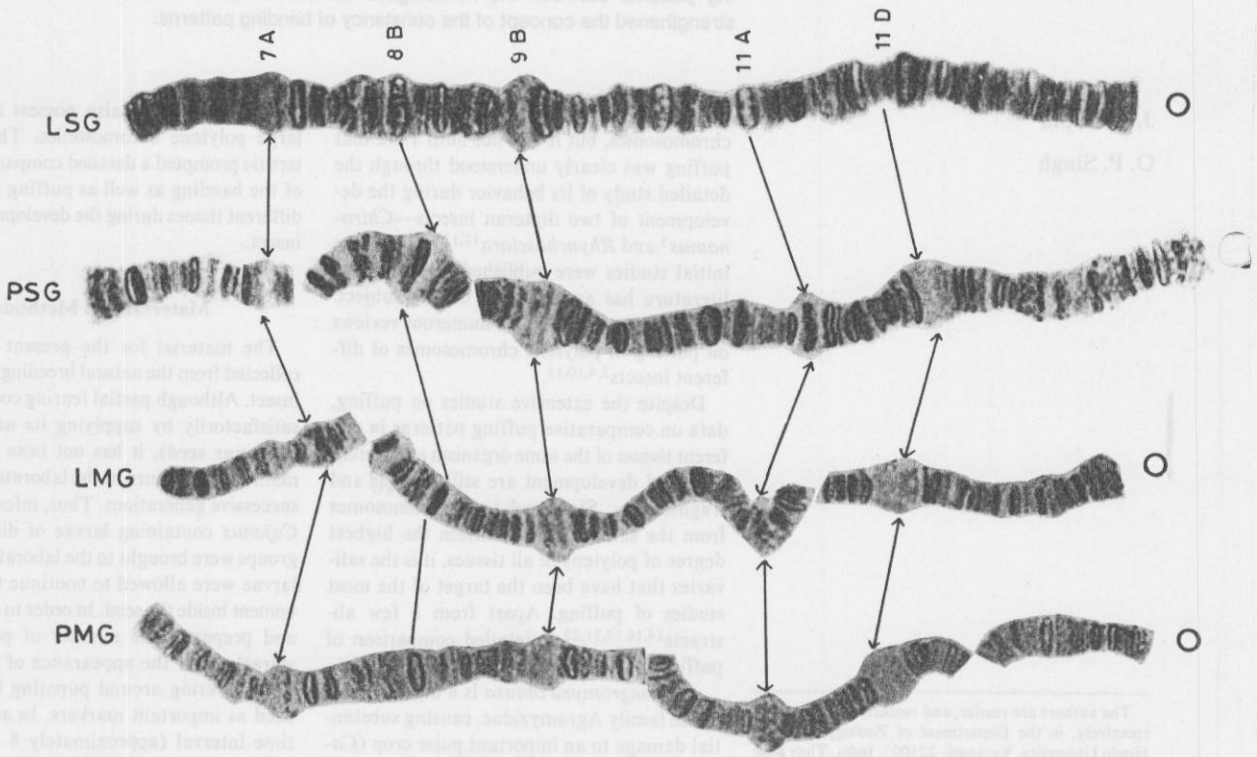

FIGURE 2 Puffing activity in salivary gland and midgut cells of chromosome A (see Figure 1 for description and abbreviations).

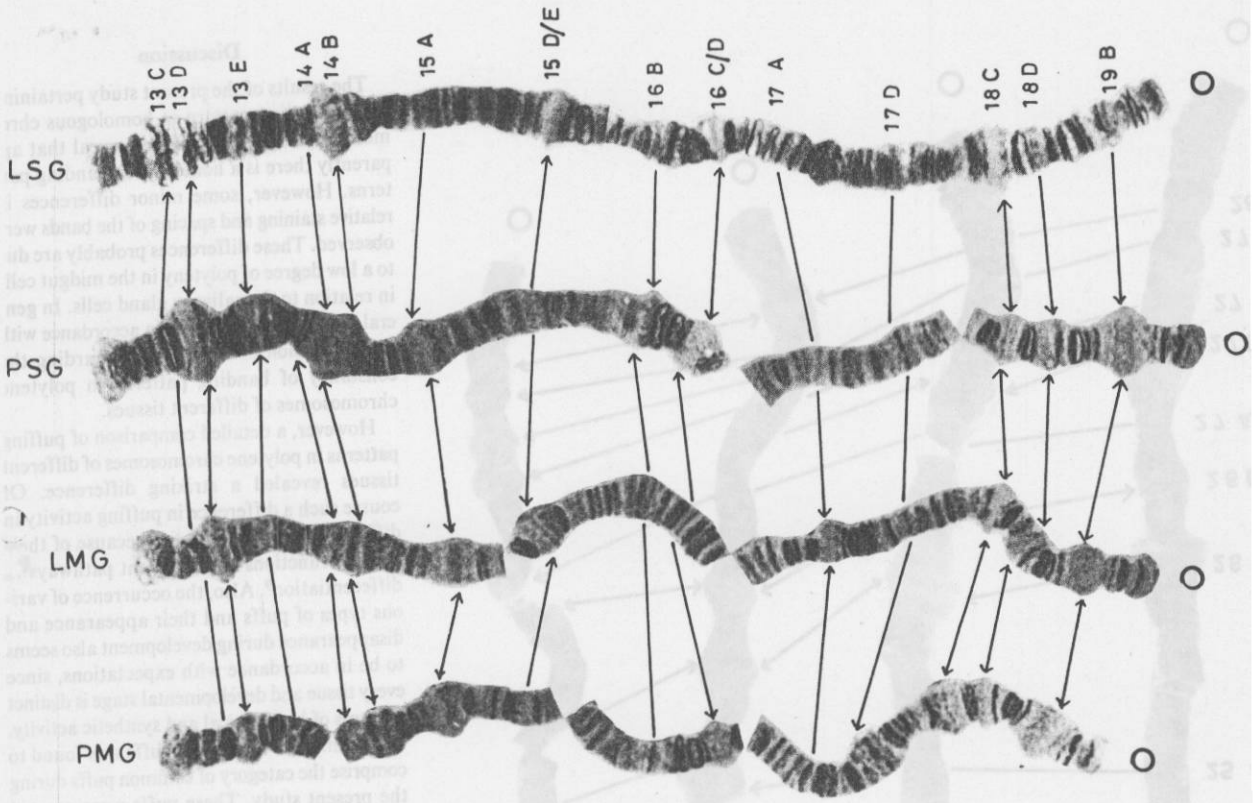

FIGURE 3 Puffing activity in salivary gland and midgut cells of chromosome B (see Figure 1).

the first category contained puffs that were common to both tissues and stages of development. A total of 12 such puffs was detected involving loci 2C, 3A, 7A, 9B, 13D, 14B, 18C, 26B/C, 26D, 29B, 36B, and 39B.

The second category of puffs included those that were associated with specific tissue only. During the present study only 5 puffs specific to the salivary gland, and 12 puffs specific to the midgut tissue were detected. The salivary specific puffs were located at 8B, 13C, 13E, 16B, 21C, and the midgut puffs were exclusively associated with 1C, 17A, 17D, 20B, 22D, 23A, 24B/C, 25A, 27A, 27D, 27E, and 28A.

The third category contained the stage-specific puffs that are always found to be associated with a particular stage of development, e.g., three exclusively larval puffs (13C, 15D/E, 29A) and 19 exclusively prepupal puffs (1E/2A, 2F, 8B, 11A, 11D, 13E, 14A, 15A, 16B, 18D, 19B, 22A, 22B/C, 30C, 32C, 35A, 37B, 38D, 39A) were recognized in the salivary gland cells. In addition, six puffs associated with the larval stage (15D/E, 17A, 20B, 22B/C, 22D, 25A) and 12 puffs attached to the prepupal stage (1C, 1E/2A, 2F, 16C/D, 17D, 22A, 27A, 27B/C, 27D, 27E, 32C, 35A) were observed in the larval and prepupal midgut cells, respectively.

Apart from the occurrence of different types of puffs, differences in the number and the level of activity of these puffs also were observed.

Table I. Details of various types of puffs in salivary and midgut tissues during third instar larval and prepupal stages of *Melanagromyza obtusa*

Types of puffs		No. puffs	Details of loci forming puffs
Common		12	2C, 3A, 7A, 9B, 13D, 14B, 18C, 26B/C, 26D, 29B, 36B, 39B
Tissue-specific			
salivary gland		5	8B, 13C, 13E, 16B, 21C
midgut		12	1C, 17A, 17D, 20B, 22D, 23A, 24B/C, 25A, 27A, 27D, 27E, 28A
Stage-specific			
salivary gland:	larval stage-specific	3	13C, 15D/E, 29A
	prepupal stage-specific	19	1E/2A, 2F, 8B, 11A, 11D, 13E, 14A, 15A, 16B, 18D, 19B, 22A, 22B/C, 30C, 32C, 35A, 37B, 38D, 39A
midgut:	larval stage specific	6	15D/E, 17A, 20B, 22B/C, 22D, 25A
	prepupal stage specific	12	1C, 1E/2A, 2F, 16C/D, 17D, 22A, 27A, 27B/C, 27D, 27E, 32C, 35A

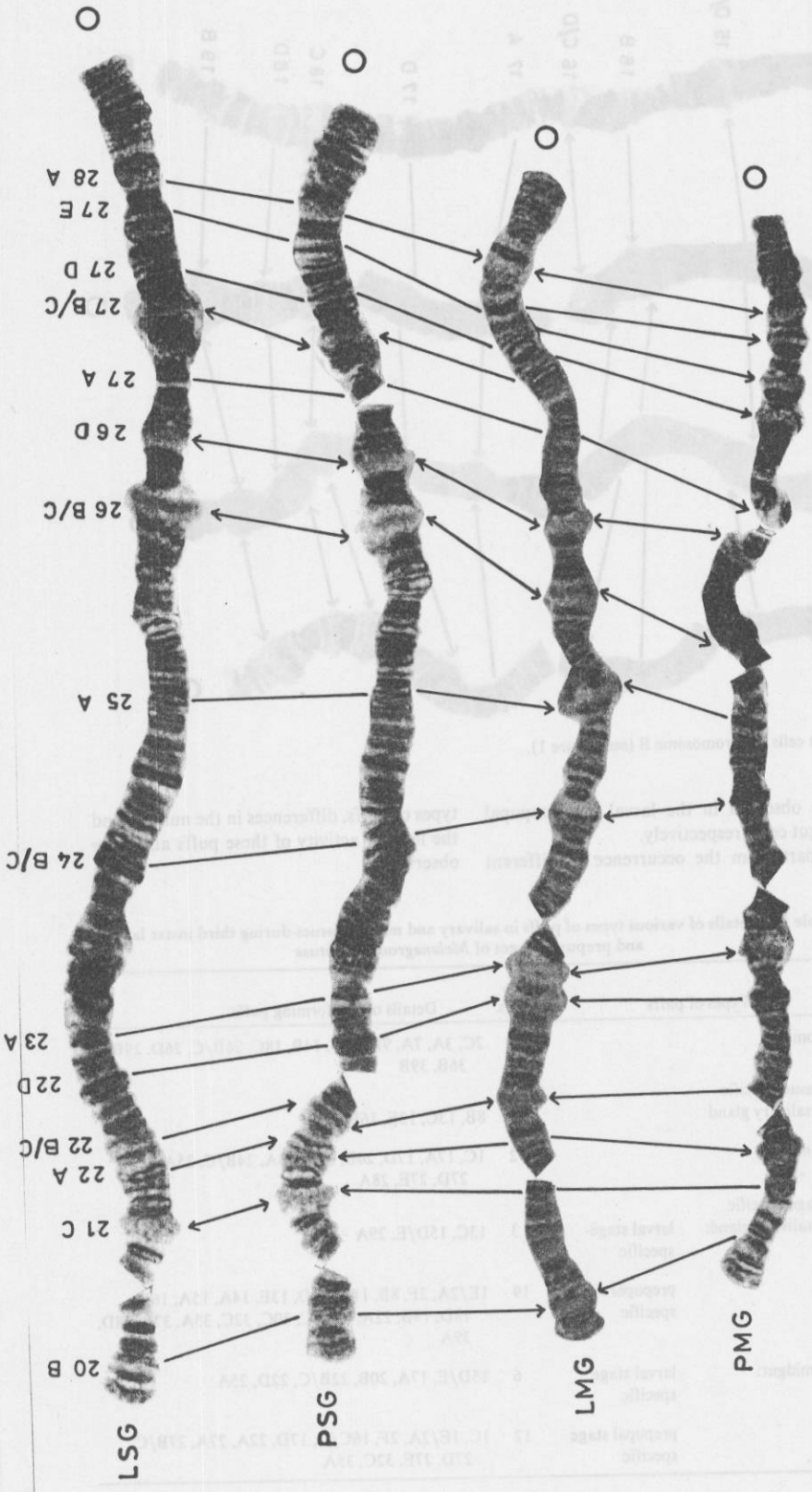

FIGURE 4 Puffing activity in salivary gland and midgut cells of chromosome C (see Figure 1).

Discussion

The results of the present study pertainir to the banding pattern in homologous chr mosomes of different tissues reveal that ap parently there is a homology in banding pa terns. However, some minor differences i relative staining and spacing of the bands wer observed. These differences probably are du to a low degree of polyteny in the midgut cell in relation to the salivary gland cells. In gen eral, the present results are in accordance witl the conclusion of Beermann[7] regarding th constancy of banding patterns in polyten chromosomes of different tissues.

However, a detailed comparison of puffing patterns in polytene chromosomes of different tissues revealed a striking difference. Of course such a difference in puffing activity in different tissues is expected because of their specific functions and different pathways of differentiation[9]. Also, the occurrence of various types of puffs and their appearance and disappearance during development also seems to be in accordance with expectations, since every tissue and developmental stage is distinct in terms of biochemical and synthetic activity. For example, a total of 12 puffs was found to comprise the category of common puffs during the present study. These puffs were present irrespective of the tissue and the stage of development, indicating that the products of these puffs probably are essential for the general maintenance of the cell metabolism during the development.

In contrast to the above category of puffs, the stage-specific puffs are always associated with a particular stage of development. Altogether 3 larval and 19 prepupal puffs were detected in the salivary gland cells, and 6 larval and 12 prepupal puffs were observed in the midgut cells. Unfortunately, very little is understood about the function of these puffs except that they are always associated with a particular stage of development. Ashburner[1] has suggested that these puffs may code for proteins of little significance to the tissue itself, i.e., proteins exported from the tissue are perhaps utilized by the imaginal tissues during histogenesis.

The third category of puffs observed were tissue-specific puffs. Altogether five puffs specific to the salivary gland and 12 puffs specific to midgut tissue were identified. From the functional point of view these puffs may be related to functions specific to a particular cell type, e.g., the secretion of glue substance by larval and prepupal salivary glands or the secretion of the digestive enzymes by the midgut cell may be assigned to these puffs.

Apart from the occurrence of different types of puffs, differences in the level of ac-

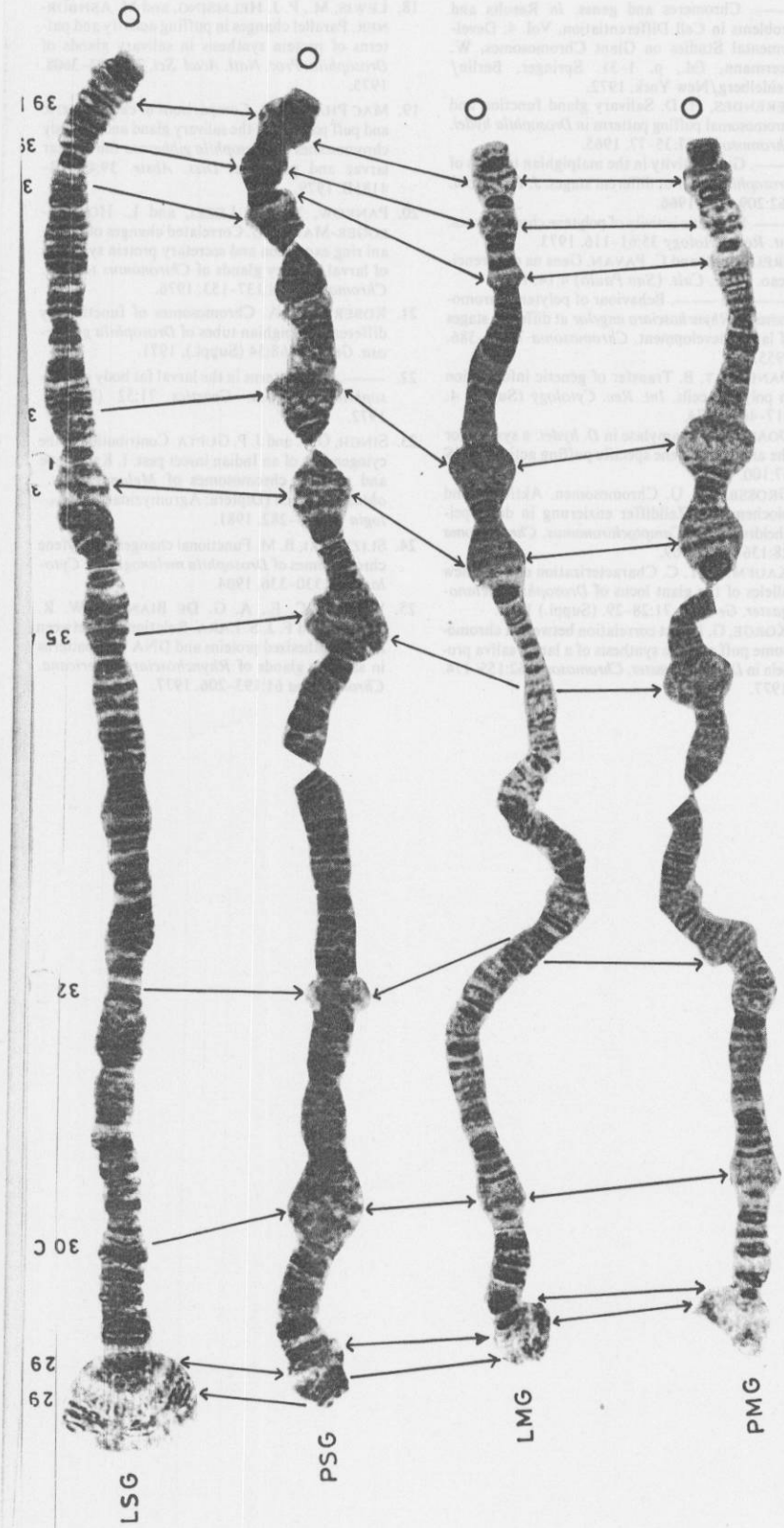

FIGURE 5 Puffing activity in salivary gland and midgut cells of chromosome D (see Figure 1).

tivity of these puffs also was observed. The activity of an individual puff was estimated by taking the ratio between the maximum diameter of the puff region and the diameter of an adjacent band not involved in puffing activity[3]. The number of puffs and their total activity in different cell types is shown by a histogram in Figure 6. Such differences in the level of activity are considered as the special feature of both stage and tissue specificities. The results of further studies on the level of activity of individual puffs in different cell types of this insect will be published elsewhere.

Another interesting observation made during this study is that differences in puffing at one particular time are more striking than indicated by the number of tissue-specific puffs. However, Berendes[9] has argued for such differences by assuming that cell metabolism of different tissues and of different cells within the same tissue is regulated by asynchronous changes in gene activity. It should, however, be possible to relate a particular puff with a specific cellular function. In fact, there have been a few good correlations between the appearance of specific puffs and the appearance of specific cell products[6,15,17,18,20,24,25].

The demonstration of puffing patterns in *M. obtusa* provides further support to the concept

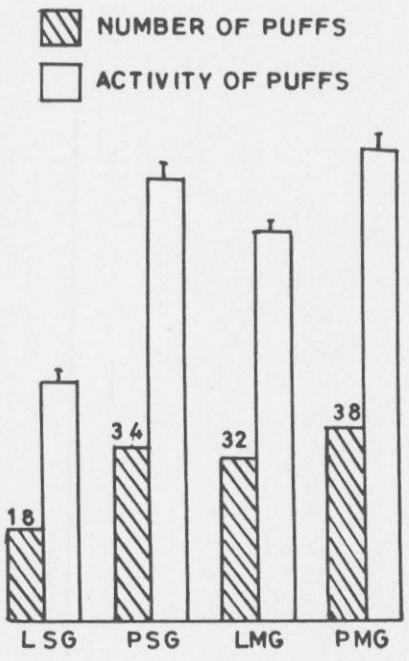

FIGURE 6 Histogram showing number of puffs and their total activity in different cell types of *Melanagromyza obtusa*.

of differential gene activity. Moreover, the extremely favorable polyteny found in tissues other than salivary glands in *M. obtusa* provides the background for a detailed investigation into the mechanisms of puffing.

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