Chromomeric patterns and photo-maps of the polytene chromosomes of *Melanagromyza obtusa* in three larval tissues

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Abstract

Melanagromyza obtusa is a dipteran pest and possesses reproducible polytene chromosomes not only in the larval salivary glands, but also in the midgut and malpighian tubules. Thus, it provides an opportunity for comparative studies on chromomeric and puffing patterns in different tissues. The present study revealed, in general, in a comparison of band-interband patterns of polytene chromosomes of the salivary glands, midgut and malpighian tubules, similar chromomeric patterns in the homologous chromosomes of these tissues. This suggests similar genic control of chromomeric differentiation in different tissues. Subsequently, some variations of different types (*i.e.* variations in presence/absence of some bands, their number, thickness, compactness, and spacing) were also noticed, as the result of variations in the level of polyteny and puffing activity.

Introduction

Despite the fact that polytene chromosomes (PCs) are found in a wide variety of larval tissues of Diptera, they have been studied in detail only in salivary glands (SG). This is mainly because of the occurrence of low polyteny in tissues other than SG. As a consequence, only a limited number of comparative studies have been completed on their structure and behaviour in different tissues. Although, such investigations were attempted as early as during the forties, detailed accounts can only be found in a few cases (Beermann, 1952; Berendes, 1966; Ribbert, 1979; Redfern, 1981; Gupta and Singh, 1983; Roberts, 1988).

The suitability of *Melanagromyza obtusa* (Diptera: Agromyzidae) for such studies was recently realized, because of the presence of PCs with a well reproducible banding pattern, not only in larval SG, but also in the midgut (MG) and malpighian tubules (Gupta and Singh, 1983; Singh, 1988a). As a result, some aspects of the structure and behaviour of the polytenized genome have already been studied in different tissues (Singh and Gupta, 1981, 1985; Gupta and Singh, 1983; Singh, 1988b). During the present study, the linear arrangement of bands and interbands, their appearance and total number have been examined in relation to the level of polyteny, chromosome lengths and puffing activity, in the homologous chromosomes of SG, MG and malpighian tubules (MT), employing the complete genome. Photo-maps of the chromosomes of the three tissues are included as reference maps to be used in future studies.

Materials and methods

SG, proximal portions of MG and distal portions of MT of third instar larvae of *M. obtusa* were dissected in insect Ringer solution and treated in 0.1 N hydrochloric acid for about 1—2 min before fixation in aceto-methanol (1:3) for 5

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min. Fixed tissues were stained in lacto-aceto-orcein for 5—10 min and then squashed in a fresh drop of stain. The edges of the coverglass of the squash preparations were sealed with DEPEX and slides were kept for 6—12 h in a refrigerator in order to obtain homogenous bright staining throughout the chromosomes. Observations were recorded from fifty nuclei and twenty-three individuals to generalize the findings.

Microphotographs of the homologous chromosomes from three tissues were arranged side-by-side for the comparison of chromomeric patterns. In order to prepare photo-maps, different chromosomes were named, divided into sections and subsections and labelled following the pattern of previous camera-lucida maps (Singh and Gupta, 1981).

Results

General observations on the PC of three tissues (Figures 1 to 5)

The present study revealed that homologous PC of *M. obtusa* could be identified and homologized in different tissues with the help of their general morphology and characteristic landmarks present in each chromosome irrespective of their origin. Except for some minor modifications most of the landmarks noted in the PC of the SG usually appeared to be more or less similar in other tissues. For instance, the absence of a common chromocentre, the presence of a heterochromatic mass at the base of the X-chromosome, the larger size and broader tip of the D-chromosome, a conspicuous constriction at region 26D/27A on the C-chromosome and prominent bands (*e.g.* a group of bands in regions 2D and 13A—F; dark bands in regions 6B, 10A and 19A; a wide disc-like band in 36A *etc*) were observed in the PC of all tissues. A few regions (1A, 12E, 29A, 36C *etc*) appeared differently in different tissues, but observation of other prominent landmarks and tracing the homology from one end of the chromosome, left no doubt. Among the three tissues studied of *M. obtusa*, the SG nuclei were found to obtain the maximum level of polyteny and possessed the largest chromosomes, similar to other dipteran species. MG nuclei were comparatively less polytenized, but more than the MT and as good as the salivary chromosomes of many *Drosophila* species. Measurements of optimally stretched PCs from different tissues indicated variations in the total length of the chromosomes among the tissues. Salivary chromosomes were found to be slightly larger than the MG and MT chromosomes.

Chromomeric patterns in the PC of three tissues (Figures 1 to 5)

Comparison of the sequential arrangement of the chromomeres (band-interband) between homologous PC of different tissues revealed a more or less similar pattern in the three tissues of *M. obtusa*: the majority of the chromomeres were homologized. However, some bands and regions showed slight variations in their morphology concerning their thickness, spacing, curvature, and compactness, but without altering the basic chromomeric pattern. The variations noticed in different tissues are summarized in Table 1. The variations which appeared due to preparative artifacts are omitted.

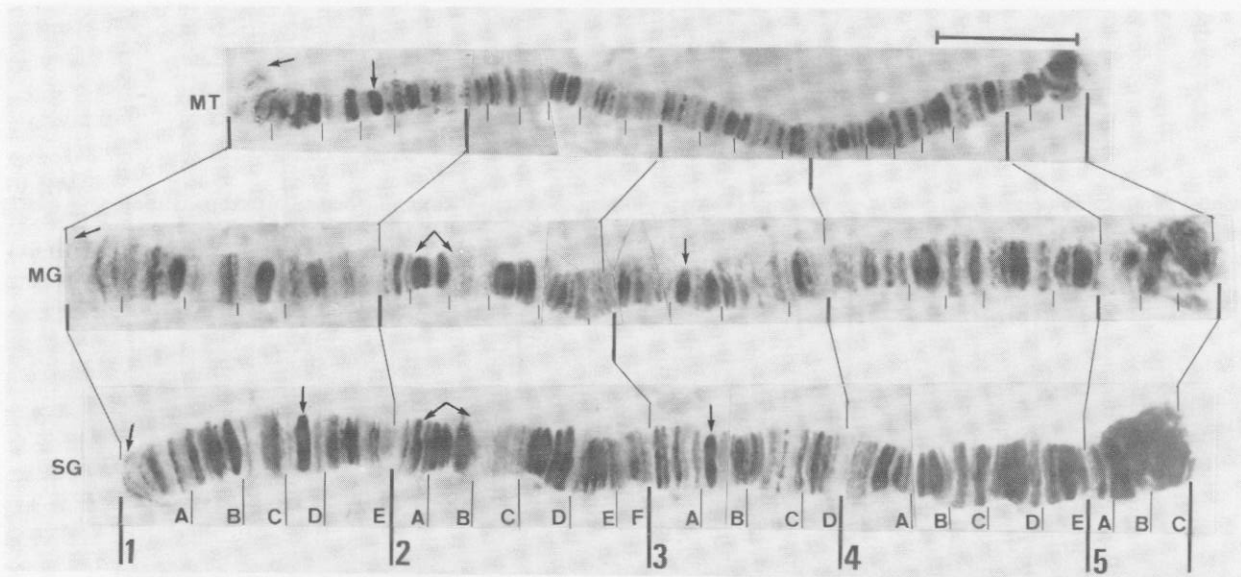

Figure 1 Chromomeric patterns and photo-maps of the X-chromosome of *M. obtusa* in larval SG, MG and MT. Some of the loci where differences in chromomeres between tissues are clearly visible are marked with arrows; details presented in Table 1 are based on the observations of a large number of preparations (see Materials and methods). Right ends of the chromosomes represent centromeres. Bar scale represents 10 μm.

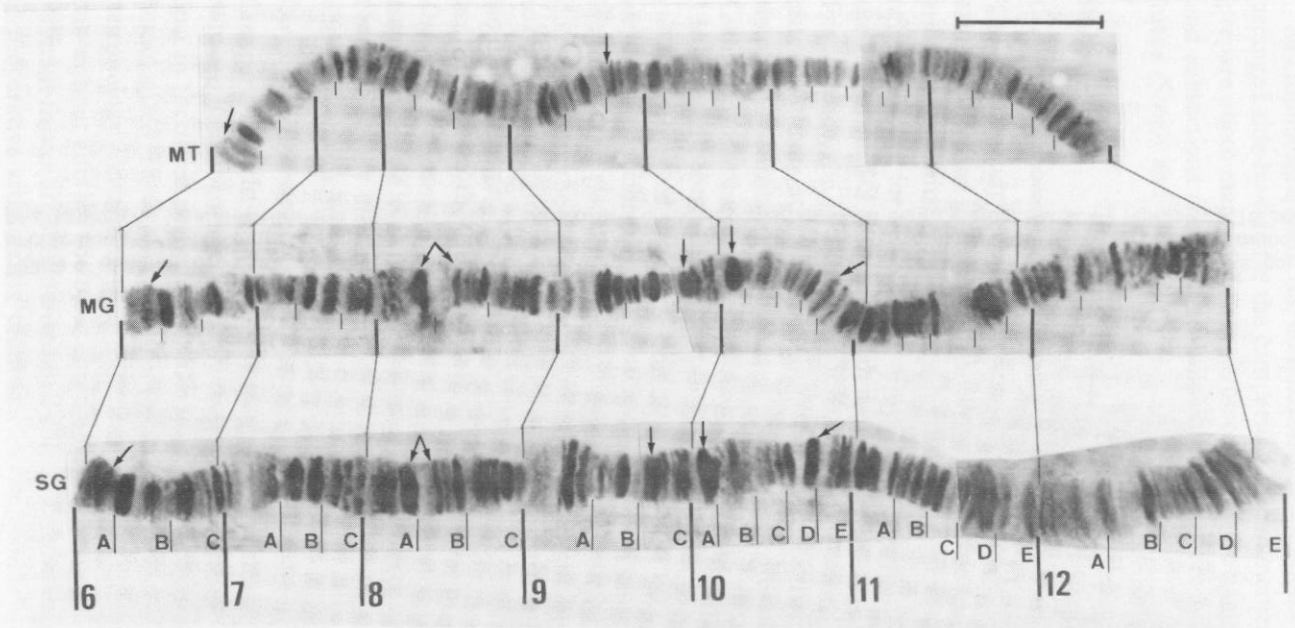

Figure 2 Chromomeric patterns and photo-maps of A-chromosome in three tissues of *M. obtusa* (for details see legend of Figure 1). Bar scale represents 10 μm.

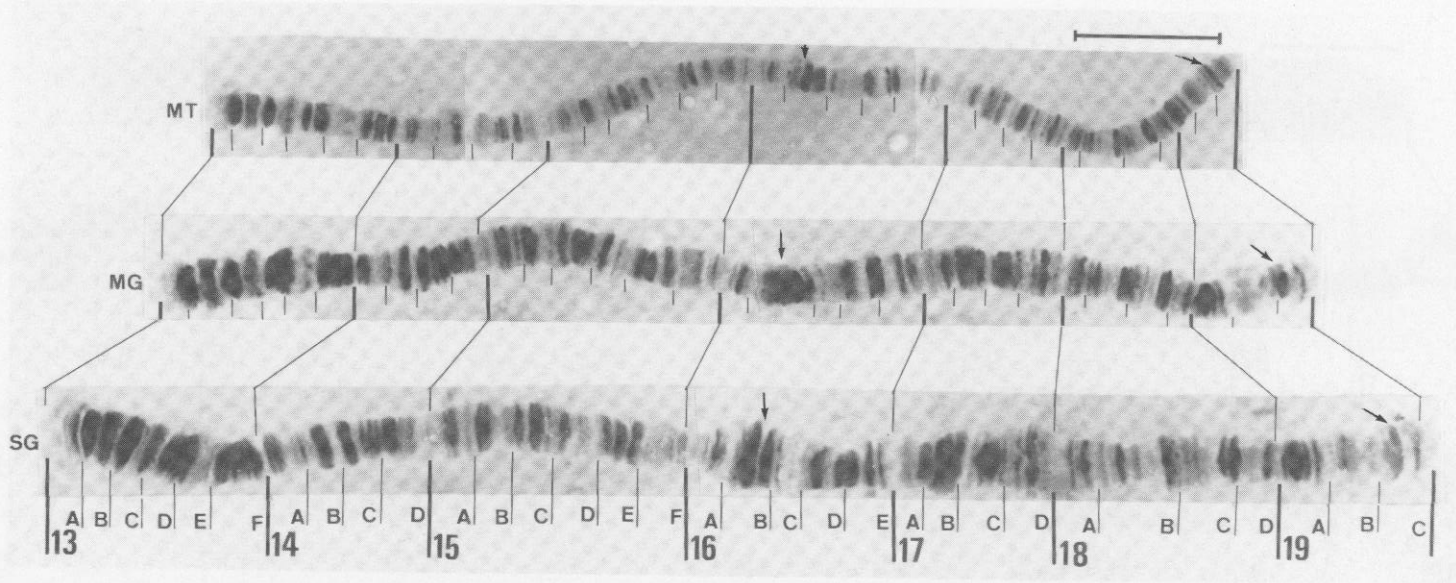

Figure 3 Chromomeric patterns and photo-maps of B-chromosome in three tissues of *M. obtusa* (for details see legend of Figure 1). Bar scale represents 10 μm.

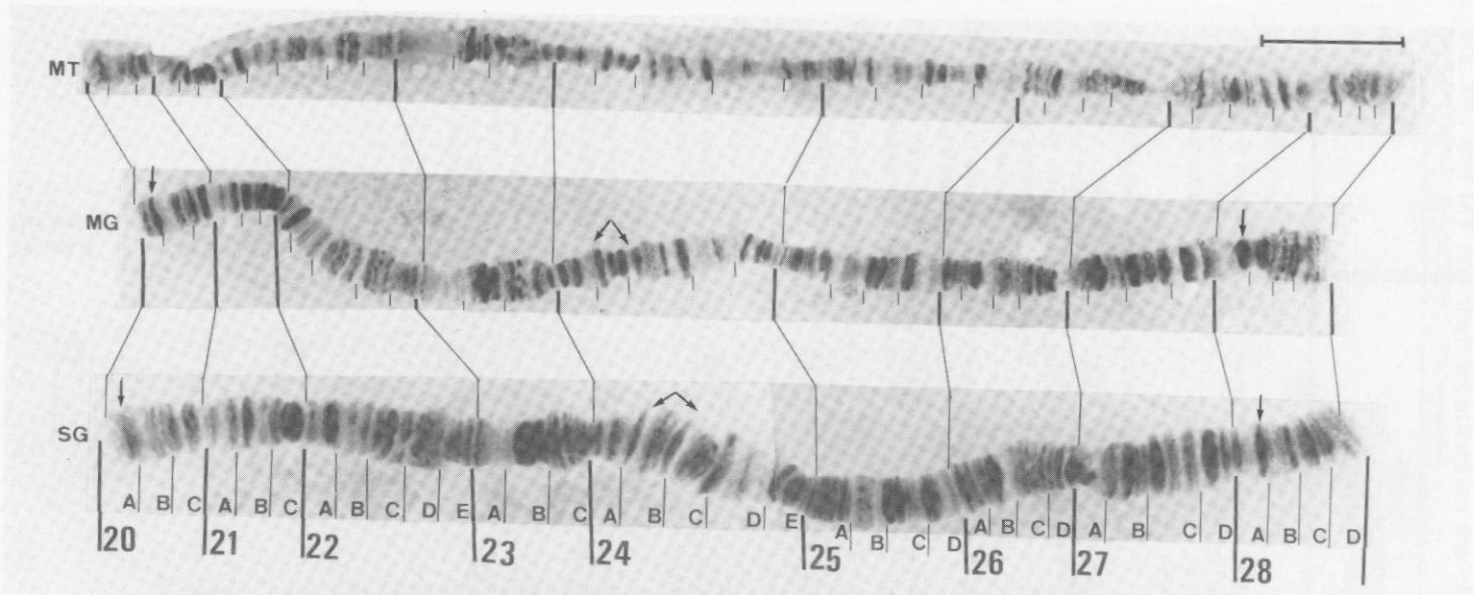

Figure 4 Chromomeric patterns and photo-maps of C-chromosome in three tissues of *M. obtusa* (for details see legend of Figure 1). Bar scale represents 10 μm.

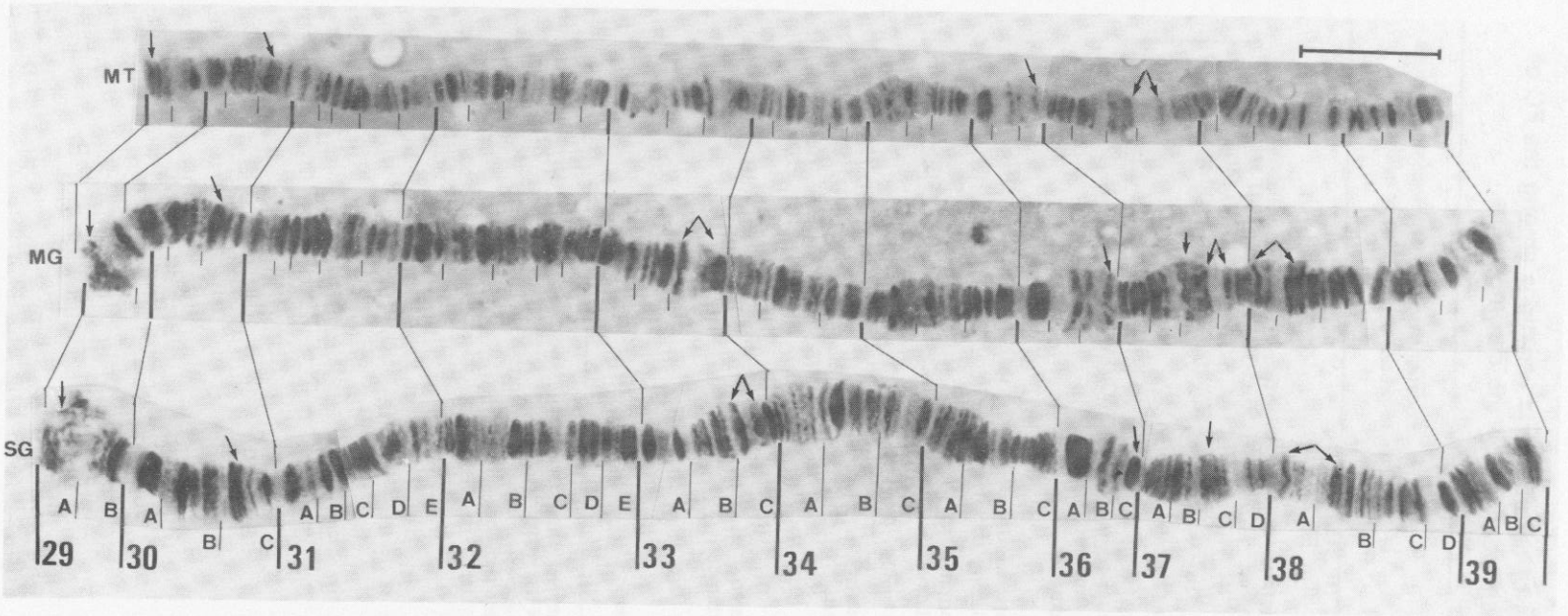

Figure 5 Chromomeric patterns and photo-maps of D-chromosome in three tissues of *M. obtusa* (for details see legend of Figure 1). Bar scale represents 10 μm.

Table 1 Different types of chromomeric variations among the PC of SG, MG and MT and their locations on the chromosomes.

Nature of variations	Locations on the chromosomes (tissues in brackets were clearly evident)
1 Constrictions	
Bands in one/two tissue(s), were short and thick and appeared as constrictions	1D (SG-MT), 2B (SG-MG), 3B (SG-MG), 9C (SG/MT-MG), 10A (SG-MG), 16B (SG-MG), 28A (SG-MG), 36C (SG-MG/MT)
2 Variations in compactness of bands	
(a) Bands in one/two tissue(s) were compact and thick whereas same bands in other tissue(s) appeared loosely packed, elongated and lightly stained	10D (SG-MG/MT), 24A-C (SG-MG/MT) 30C (SG-MG-MT)
(b) Bands in one/two tissue(s) were found diffused due to puffing activity	1A (SG-MG/MT), 29A (SG-MG-MT),
(c) Bands were found discontinuous and broken in one/two tissue(s)	22C-D (SG-MG), 23C (SG-MG/MT) 28C-D (SG-MG), 37C (SG-MG)
3 Variations in number of bands	
More differentiated bands were found in one/two tissue(s)	2B-C (SG-MG-MT), 3C-D (SG-MG-MT) 6C (SG-MG/MT), 12C-E (SG-MG-MT) 13D (SG-MG/MT), 34A (SG-MG/MT)
4 Variation in spacing	
Bands were well separated thus forming wide interbands	8B (SG/MT-MG), 33C (SG-MG/MT), 38A-B (SG-MG)
5 Variation in staining*	
Staining intensity was variable between tissues	6A (SG-MG-MT), 20A (SG/MT-MG), 31C (SG-MG/MT)

* Chromosomes were stained with orcein but no quantitative staining was done. However, loci mentioned here were recorded in most of the preparations.
Variations are based on all preparations so far observed (see Materials and methods), and all such variations cannot be seen in one preparation.

Table 2a Comparison of number of bands (N) and length of the PC (L) in three tissues (lengths are given in μm)

Chromosome arms*	Number of bands and length of chromosomes in different tissues: SG: (N)	(L)	MG: (N)	(L)	MT: (N)	(L)
X	162	80	148	79	134	64
A	140	86	137	78	124	72
B	161	93	150	81	151	74
C	174	94	186	83	172	82
D	227	112	211	104	198	95
Total	864	465	832	425	779	387

*E-chromosome of *M. obtusa*, being heterochromatic and represented by a few diffused bands (Singh and Gupta 1981), is not taken into account during the present study.
Calculations are based on data recorded from fifty nuclei of twenty-three individuals.

Variations of the puffing activity among the three tissues have not been studied in detail, but it should be mentioned, that a considerable amount of puffing variations (both in number of loci and total activity) in the three tissues was noticed. The observed differences between SG and MG have already been summarized (Gupta and Singh 1983), whereas a detailed comparison with the MT, is still awaited.

Total number of bands in the PC of three tissues (Table 2)

Although homologization of chromomeres revealed similar patterns in the three tissues, remarkable differences were noticed when the total number of bands present in the complete genome were counted and compared. The total number of visible bands present in individual chromosomes or in the complete genome of the three tissues revealed surprisingly more bands in the SG chromosomes (maximally polytenized) than in those of the MG and MT (less polytenized).

Table 2b Differences in number of bands and lengths (μm) of the PC in the complete genome

Differences	Number of bands	Length of the PC
Between SG and MG	32 ± 7	40 ± 14
Between MG and MT	53 ± 17	38 ± 8
Between SG and MT	85 ± 17	78 ± 23

Calculations are based on data recorded from fifty nuclei of twenty-three individuals.

Photo-maps of the PC of three tissues (Figures 1 to 5)

The five euchromatic arms designated as X, A, B, C and D, were divided into thirty-nine sections (X, 1 to 5; A, 6 to 12; B, 13 to 19; C, 20 to 28; D, 29 to 39) and each section into subsections following the pattern of the already existing camera-lucida map (Singh and Gupta, 1981). The description of the camera-lucida map for the different chromosomes and the important landmarks is also applicable to the present photo-maps, therefore no further description is given here. Each moderately sized chromosome has a distinct morphology and land marks and can be identified easily by direct comparison with the photo-maps. The E-chromosome (section 40 of camera lucida map), being a small heterochromatic fragment, is not included in present study.

Discussion

One of the important aspects of comparative studies is the pattern of the linear arrangement of chromomeres (band-interband) on homologous polytene chromosomes of different tissues, which reflects light not only on the architecture of the genome, but also on its tissue specific behaviour. The results of earlier studies revealed two different opinions regarding the chromomeric pattern. Most of the researchers found, on the one hand, a more or less similar banding pattern in different tissues and supported the concept of the 'constancy of the banding pattern' (Berendes, 1966; Holden and Ashburner, 1978; Richards, 1980; Redfern, 1981; Tusscher and Derksen, 1982; Sinha *et al.*, 1987; Roberts, 1988; Heino, 1989). On the other hand, in some cases it was difficult to homologize the banding pattern of homologous chromosomes (Ribbert, 1979; King *et al.*, 1981).

The present detailed study, employing the complete genome of *M. obtusa*, revealed a similar banding pattern in the homologous PC of larval SG, MG and MT. Individual chromosomes, based on the banding pattern, were homologized without difficulty in three tissues. No evidence of differential condensation of the chromomeres was found due to variation in the physiological conditions of the tissues. Thus, a similar sequential arrangement of the chromomeric pattern in the three larval tissues of *M. obtusa*, could be concluded. This gives further support to the theory of a constant banding pattern in different tissues and suggests similar genic control of chromomeric differentiation in various tissues.

Minor differences in the morphology of some bands and interbands are reviewed in Table 1. Some of the variations presumably originated as the result of differential puffing activity, which leads to the decondensation and sometimes scrapping of some bands resulting in wide interbands. A few variations have also been realized as a consequence of tissue specific band-interband modifications caused by constant activity/inactivity of some tissue-specific loci. Moreover, some differences are believed to have been caused by variation in the level of polyteny in different tissues, resolution limitations of light microscopy and preparative artifacts. Thus, most of the differences originating as the result of the above factors are of little importance to the basic chromomeric arrangement.

Variations in the number of bands found among the three tissues seem to be mainly due to the level of polyteny and differential puffing activity. For instance, the maximum number of bands could be counted in the SG (the maximally polytenized nuclei among the three tissues) and the minimum in MT (the least polytenized). In fact, many possibilities may be responsible for such differences in the number of bands. The first such possibility is the variation in the level of polyteny. As a matter of fact, very thin bands of SG chromosomes possessing a low amount of chromatin are probably not differentiated into visible bands in the MG and MT due to the extremely low content of chromatin. Therefore, fewer bands were found in the MG and even less in the MT. The second possibility may be the variation in the level of puffing activity. Generally the MG and MT chromosomes possessed comparatively more loci involved in puffing than the SG at the same time (for comparision between SG and MG, see Gupta and Singh, 1983). As a result of considerable puffing activity, some bands in the MG and MT were not resolved due to their diffused and decondensed nature. The third possibility is also of more importance, *i.e.* there may be splitting of some bands in the SG due to 'mini-puffs' within a thick band locus previously condensed in other tissues. This would have given rise to more bands in one tissue. The possibility of fusion of some bands in MG and MT due to their short length, can not be ignored, as some of the regions in the SG possessed more fine bands than at the same place in the MG and MT. Thus, differences in the number of bands could not be considered as a tissue-specific basic architectural variation. It is possible that, if a comparison were made among the PC of the three tissues possessing an almost equal level of polyteny and puffing activity, then such differences in the number of bands might not be detected.

The final lengths of PC in squash preparations are totally dependent on the degree of stretching caused by the pressure applied while squashing. Therefore, it is difficult to comment on the basis of the small differences observed during the present study (Table 2). If differences reflect any tissue-specific variation then it may be due to variations in the level of polyteny.

On the basis of these observations, it is suggested that although the PC of a particular tissue and the developmental stage reached showed some minor differences in banding, the overall pattern of the sequential arrangement of the chromomeres was similar. Differences in the level of polyteny and puffing activity were important reasons for minor differences in the chromomeric patterns of the three tissues. However, technical limitations of the squash method and light microscopy in such studies cannot be ignored. Therefore, further studies employing recent technical developments of chromosome preparation, such as the squash-thin-sectioning method of Sorsa and Sorsa (1967), and the surface-spread polytene chromosome preparation technique of Kalisch and Whitmore (1986) using electron microscopy, are required to generalize the conclusions.

A preliminary study using new techniques has already been attempted by Saura (1986) with the PC of the fat body and SG of *D. melanogaster*, employing the squash-thin-sectioning method. However, due to excessive stretching of the fat body chromosomes during preparation, as a consequence of the low level of

polyteny, it became difficult to identify the chromosomes reliably and only a few major bands could be localized on small sections of chromosomes. Thus, a detailed conclusive study on the chromomeric pattern between tissues employing EM techniques is yet to be done, for which, *M. obtusa* would certainly be a suitable material where PC are sufficiently polytenized in more than one tissue.

References

BEERMANN W. 1952. Chromosomenkonstanz and spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von *Chironomus tentans*. *Chromosoma* **5** 139–98.

BERENDES H. B. 1966. Gene activities in the malpighian tubules of *Drosophila hydei* at different developmental stages. *J. exp. Zool.* **162** 209–17.

GUPTA J. P. and Singh O. P. 1983. Puffing patterns in polytene chromosomes of different tissues of *Melanagromyza obtusa* during development. *J. Hered.* **74** 365–70.

HEINO T. I. 1989. Polytene chromosomes from ovarian pseudonurse cells of the *Drosophila melanogaster otu*-mutant. I. Photographic map of chromosome 3. *Chromosoma* **97** 363–73.

HOLDEN J. J. and Ashburner M. 1978. Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. IX. The salivary and prothoracic gland chromosomes of a dominant temperature sensitive lethal mutant of *Drosophila melanogaster*. *Chromosoma* **68** 205–27.

KALISCH W. -E. and Whitmore T. 1986. The SSP chromosome technique as applied for *Drosophila melanogaster*. *Dros. Inf. Ser.* **63** 142–6.

KING R. C., Riley S. F., Cassidy J. D. and White P. E. 1981. Giant polytene chromosomes from the ovaries of a *Drosophila* mutant. *Science* **212** 441–3.

REDFERN C. P. F. 1981. Homologous banding pattern in polytene chromosomes from larval salivary gland and ovarian nurse cells of *Anopheles stephensi* Liston (Culicidae). *Chromosoma* **83** 221–40.

RIBBERT D. 1979. Chromomeres and puffing in experimentally induced polytene chromosomes of *Calliphora erythrocephala*. *Chromosoma* **74** 269–98.

RICHARDS G. 1980. The polytene chromosomes in the fat body nuclei of *Drosophila melanogaster*. *Chromosoma* **79** 241–50.

ROBERTS P. A. 1988. Developmental changes in midgut chromosomes of *Drosophila gibberosa*. *Chromosoma* **97** 254–60.

SAURA A. O. 1986. Electron microscopic mapping of the second polytene chromosome of *Drosophila melanogaster*. PhD thesis, University of Helsinki, Finland, pp 38–41.

SINGH O. P. 1988a. *Melanagromyza obtusa* - a suitable system for the study of polytene chromosomes. *Curr. Sci.* **57** 48–9.

SINGH O. P. 1988b. Absence of 93D-like locus of *Drosophila melanogaster* in *Melanagromyza obtusa*. Indian J. exp. Biol. **26** 558–9.

SINGH O. P. and Gupta J. P. 1981. Contribution to the cytogenetics of an Indian insect pest. I. Karyotype and salivary chromosomes of *Melanagromyza obtusa* Malloch (Diptera: Agromyzidae). *Caryologia* **34** 275–82.

SINGH O. P. and Gupta J. P. 1985. Differential induction of chromosome puffs in two cell types of *Melanagromyza obtusa*. *Chromosoma* **91** 359–62.

SINHA P., Mishra A. and Lakhotia S. C. 1987. Chromosomal organization of *Drosophila* tumours. I. Polytene chromosome organization and DNA synthesis in ovarian pseudonurse cells in *otu* mutants of *D. melanogaster*. *Chromosoma* **95** 108–16.

SORSA M. and Sorsa V. 1967. The squash technique in electron microscopic studies on the structure of polytene chromosomes. *J. Ultrastruct. Res.* **20** 302.

TUSSCHER B. ten and Derksen J. 1982. The fourth chromosome of *Chironomus tentans* malpighian tubules. An ultrastructural study. *Chromosoma* **85** 643–58.

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