Replication in *Drosophila* chromosomes
XIII. Comparison of late replicating sites in two polytene cell types in *D. hydei*

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Abstract

We have compared the temporal order of completion of replication of specific sites of X and 2nd chromosomes in two polytene cell types of *D. hydei* by examining the patterns of autoradiographic labelling in ^{3}H-thymidine pulse (10 min) labelled salivary glands and gastric ceaca of mid 3rd instar larvae. Present results are in agreement with our earlier finding in *D. nasuta* (Lakhotia & Tiwari, 1984, Chromosoma, 89: 212–217 that in spite of a general similarity in the cytological identity of independently replicating sites in the two polytene cell types, their temporal programme of replication varies in different tissues. This may be related to differential gene activity patterns and polytene organization in the different cell types.

Introduction

The late replicating sites in polytene chromosomes of *Drosophila* (Rudkin, 1972) have certain special attributes like being frequently involved in or associated with ectopic pairing, chromosome breaks, tandem repeat sequences etc. and thus they are equated with intercalary heterochromatin (Evgen'ev *et al.*, 1977; Zhimulev *et al.*, 1982). In a recent study (Lakhotia & Tiwari, 1984) we found significant differences in the temporal order of completion of replication of specific chromosome regions in two polytene cell types of *D. nasuta*. Since the active replicons associated with late replicating sites in polytene nuclei have been suggested to be differently organized than those active in early S (Lakhotia & Sinha, 1983) and since the late replicating intercalary heterochromatin sites may possibly be under-replicated in a tissue- and/or sex-specific manner (Zhimulev *et al.*, 1982; also see Laird, 1980), differences in the temporal order of replication of these sites in different polytene cell types (Lakhotia & Tiwari, 1984) may have functional implications. However, in an earlier study with mosquito, Redfern (1981) did not find any difference in the terminal replication patterns in larval salivary-gland and ovarian nurse-cell polytene nuclei. Therefore, it will be useful to have more data on the temporal programme of replication of specific late-replicating sites in different polytene cell types to ascertain, in the first place, whether the kind of differences noted by us in *D. nasuta* (Lakhotia & Tiwari, 1984) are more widespread or exceptional. In the present study, therefore, we have compared the temporal programme of replication of certain late labelling sites in polytene nuclei in salivary glands and gastric caeca of mid 3rd instar larvae of *D. hydei*.

Material and methods

A wildtype stock of *D. hydei*, obtained from Prof. A. S. Mukherjee (Calcutta), has been used. Flies and larvae were reared on the standard food medium under uncrowded conditions at 20 °C ± 1 °C. Under these conditions of rearing, pupation occurs on the 13th day after oviposition.

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Gastric caeca and salivary glands from mid 3rd-instar female larvae (216–220 h after oviposition) were dissected out in modified Poels' medium (Sinha & Lakhotia, 1980) and pulse-labelled with ^{3}H-thymidine (100 μci/ml; Sp. Act. 46 Ci/mM, Amersham) for 10 min. After the pule, the tissues were washed, squashed and processed for autoradiography as described earlier (Lakhotia & Tiwari, 1984).

Observations and discussion

As found earlier in *D. nasuta* (Lakhotia & Tiwari, 1984), the polytene chromosomes in gastric caeca of *D. hydei* are thinner than in salivary glands. The polytene banding pattern in the two polytene cell types is similar in general aspect although, as noted by earlier workers (Beermann, 1962; Richards, 1980; Zhimulev *et al.*, 1982), differences in relative staining and/or thickness of certain band/interband regions in the two cell types are obvious (Fig. 1).

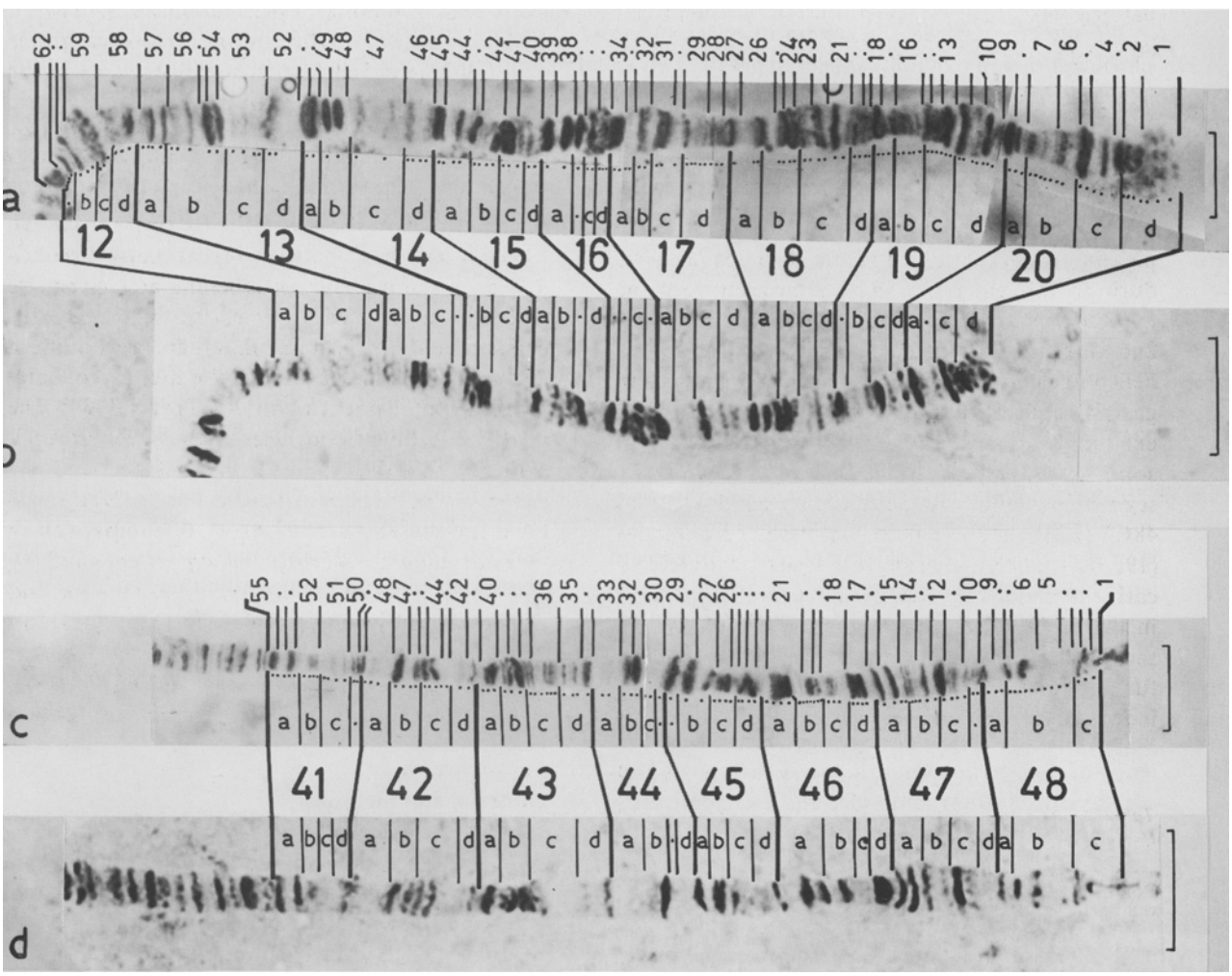

Fig. 1. Cytology of the segments of X (*a* and *b*) and 2nd chromosome (*c* and *d*) from salivary gland (*a* and *c*) and gastric caecum (*b* and *d*) polytene nuclei analysed for ^{3}H-thymidine labelling. The cytological map positions (after Berendes, 1963) for the two segments are indicated in the middle panels, while the independently labelling sites identified in the study are shown in the upper panels of the X and 2nd chromosome segments. Dots along the length of the chromosome segments (X and 2nd) refer to distinctly identifiable bands in each subdivision. Bars represent 10 μm.

Patterns and frequencies of chromosomal labelling in salivary gland and gastric caecum polytene cells

Fewer nuclei are labelled with ^{3}H-thymidine in gastric caeca than in salivary glands. The chromosomal patterns of ^{3}H-thymidine incorporation in the labelled gastric caecum polytene nuclei are, however, generally similar to those seen in salivary glands and have been accordingly designated as interband (IB), continuous (2C, 3C) or discontinuous (3D, 2D and 1D) types, respectively (for details of these patterns see Rodman, 1968; Roy & Lakhotia, 1981). The relative frequencies of these different patterns vary to some extent in the two cell types (see Table 1).

Temporal order of termination of replication of specific sites in polytene nuclei from salivary glands and gastric caeca

For comparison of the temporal order of completion of replication of specific chromosome sites during a polytene S-period in the two cell types, the autoradiographic labelling on segments of X and 2nd chromosomes in 2D and 1D type (late-S) labelled nuclei in preparations of salivary glands and gastric caeca of the same set of larvae have been examined. The analysed segment of the X chromosome extends from band 12A1 to 20D3, while the segment of chromosome 2 covers band 41A1 to 48C3 (Fig. 1). The cytological maps of Berendes (1963) have been generally followed for the identification of major subdivisions of the banding pattern in the X and 2nd chromosome segments; within these subdivisions, the identifiable bands have been further numbered serially (Fig. 1). On the basis of independent labelling properties (Rudkin, 1972), the different band and interband regions of the X and 2nd chromosome segments have been subgrouped into independent replicating sites identifiable at chromosomal level; 62 replicating or labelling sites on the X and 55 on the 2nd chromosome segments have thus been marked (Fig. 1). Within the limits of resolution offered, the different independent replicating sites are cytologically comparable in the two polytene cell types.

A given replicating site has been considered labelled only when 3 or more silver grains are seen within its cytological limits in the autoradiograms. Following the usual procedure (Rudkin, 1972; Mishra & Lakhotia, 1982; Lakhotia & Tiwari, 1984), the observed distributions of the silver grains over the X and 2nd chromosome segments in the different 2D and 1D type salivary gland and gastric caecum polytene nuclei have been arranged in matrices (Fig. 2) to define the most likely temporal order in which the different labelling sites complete their replication (Rudkin, 1972; Lakhotia & Tiwari, 1984). Since these matrices reveal many discontinuities in the presence of labelling at a given site (Fig. 2), the labelled chromosome segments have been ordered in such a way that there are minimum discontinuities in the case of the more frequently labelled site (also see Lakhotia & Tiwari, 1984). The relative labelling frequencies of the different replicating sites (in the labelled nuclei) on the X and 2nd chromosome segments in the two polytene cell types are presented in Figure 3. A comparison of the ordered arrays of the labelling patterns and the labelling frequencies of different sites of X and 2nd chromosome segments in two cell types (Fig. 2 and 3) reveals differences which, as discussed earlier (Lakhotia & Tiwari, 1984) are attributable to altered temporal order of completion of replication

Table 1. Frequencies of different ^{3}H-thymidine labelling patterns in polytene nuclei from salivary glands and gastric caeca of mid 3rd instar larvae of *D. hydei*.

Polytene cell type	Frequency (%) of different patterns							Total No. of nuclei
	Interband	Continuous		Discontinuous			Unlabelled	
	(IB)	2C	3C	3D	2D	1D		
Salivary gland	0.08	7.89	8.51	27.55	16.25	19.66	20.05	1292
Gastric caecum	–	–	0.15	21.44	7.25	18.21	53.24	1296

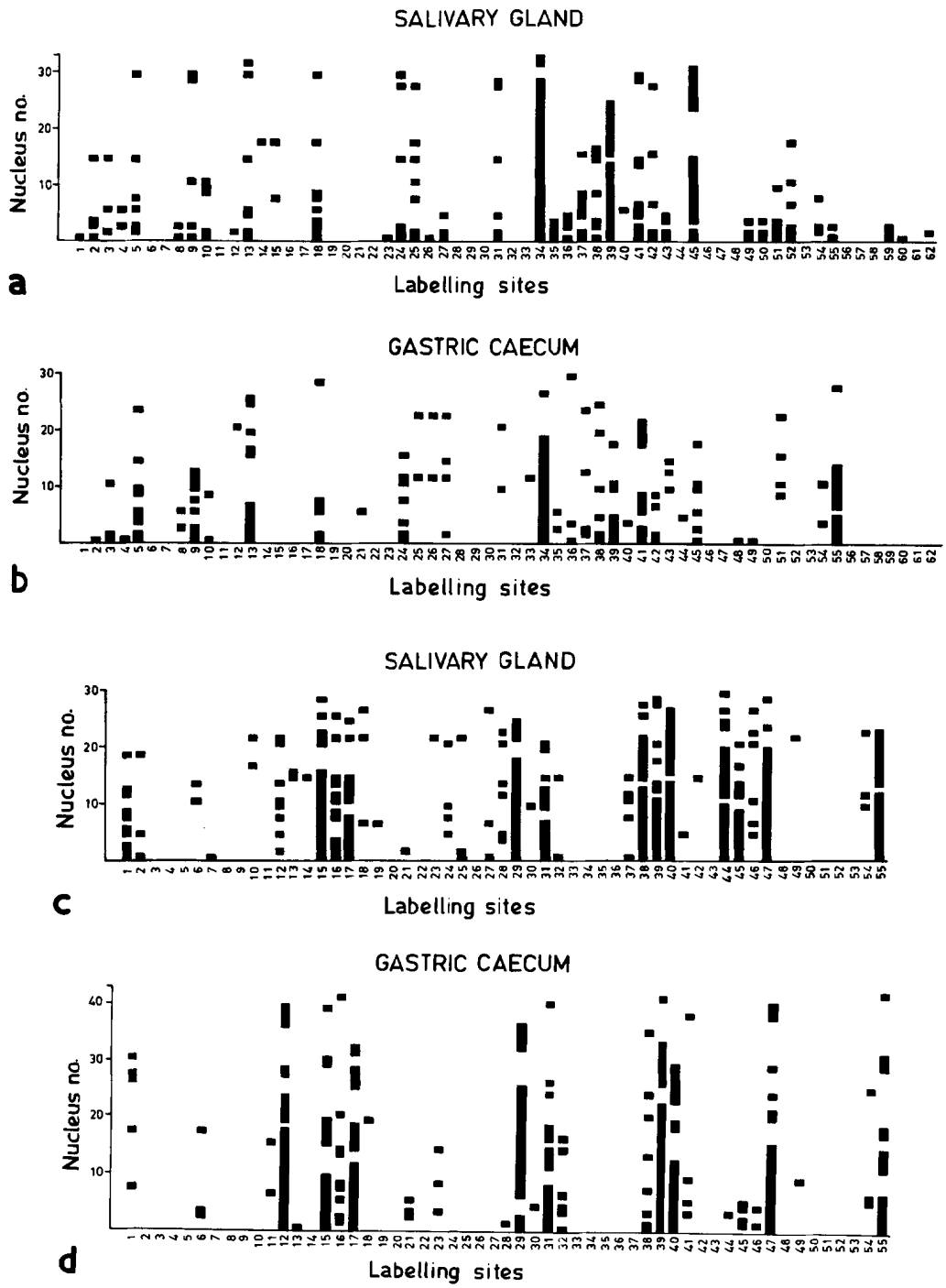

Fig. 2. Graphical representation of the ordered arrays of ^{3}H-thymidine labelling patterns of the different sites on the X (*a*, *b*) and on the 2nd chromosome (*c*, *d*) in different 1D type nuclei from salivary glands (*a* and *c*) and gastric caeca (*b* and *d*). Black bars indicate the presence of labelling at a site. The labelling pattern seen in one nucleus is represented along one horizontal line.

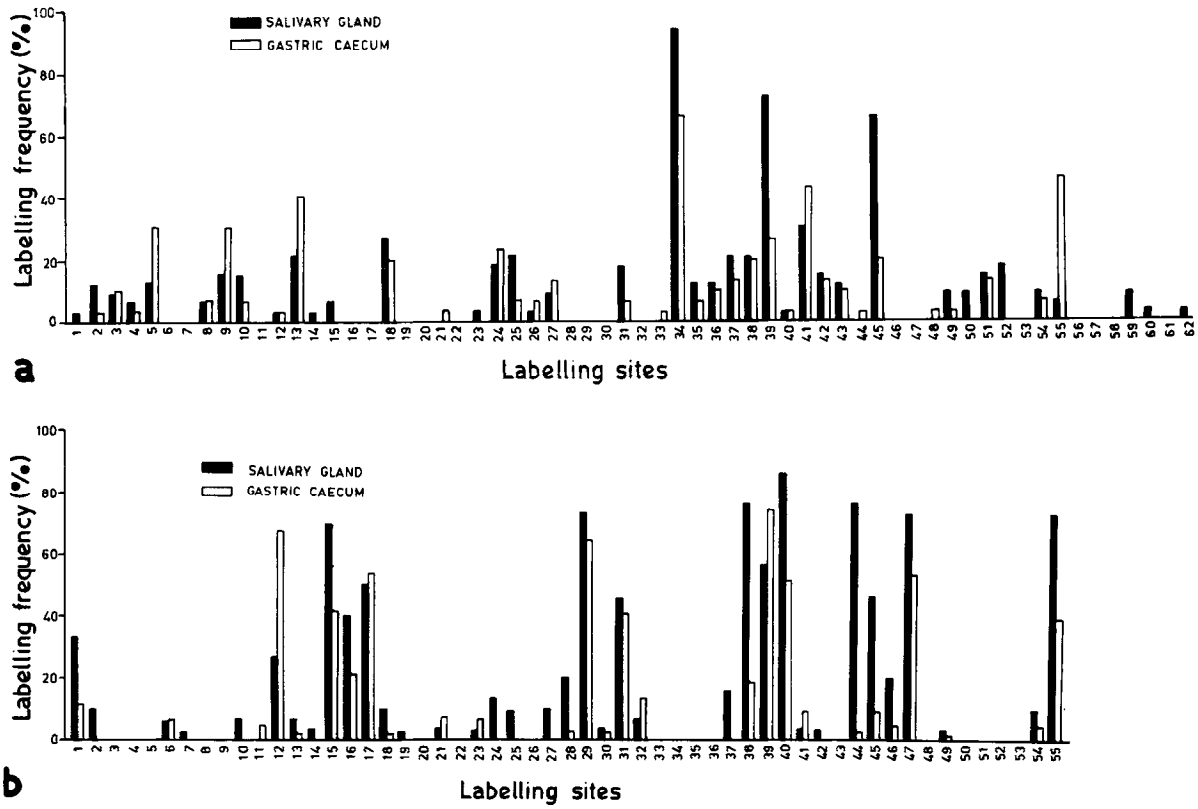

Fig. 3. Comparison of the labelling frequencies of different sites on the X (*a*) and 2nd chromosome (*b*) segments in 1D type labelled polytene nuclei from salivary glands and gastric caeca. Data taken from Figure 2.

of certain regions (like labelling site nos. 5, 9, 13, 34, 39, 41, 45 and 55 on the X and site nos. 1, 12, 15, 16, 38, 39, 40, 44, 45, 47, and 55 on the 2nd chromosome segment) in the two tissues. A χ^2-test of the labelling frequencies (Redfern, 1981; Lakhotia & Tiwari, 1984) of the 55 sites of the 2nd chromosome segment in salivary gland and gastric caecum nuclei reveals the difference to be highly significant ($P < 0.005$), although the χ^2-value for the 62 sites on the X chromosome segment is non-significant ($P > 0.05$). However, since the X chromosome segment has relatively fewer late replicating sites (i.e., with high labelling frequencies, see Fig. 3), it is possible that differences in the labelling frequencies of the late replicating sites are overshadowed by the apparently similar labelling frequencies of the other large number of early completing sites. To check this, the labelling frequencies of those X chromosomal sites which show labelling frequencies higher than 20% in any one or both tissues have also been compared; the χ^2-value for this comparison reveals the labelling frequencies of the late replicating sites of X chromosome segment to be significantly ($P < 0.001$) different between the two cell types. Thus, we conclude that the temporal order of completion of replication of certain sites of X and 2nd chromosome segments is different in salivary gland and gastric caecum polytene nuclei of *D. hydei* larvae (for further discussion see Lakhotia & Tiwari, 1984). Some examples of these differences in the two tissues are illustrated in Figure 4.

Since the result of the present comparison of the temporal order of completion of replication of different independently labelling sites in polytene nuclei from salivary glands and gastric caeca of *D. hydei* larvae is in complete agreement with the result of a similar analysis in *D. nasuta* larvae (Lakhotia & Tiwari, 1984), it appears that differences in the temporal programme of replication of specific chromosomal sites in different polytene cell types of

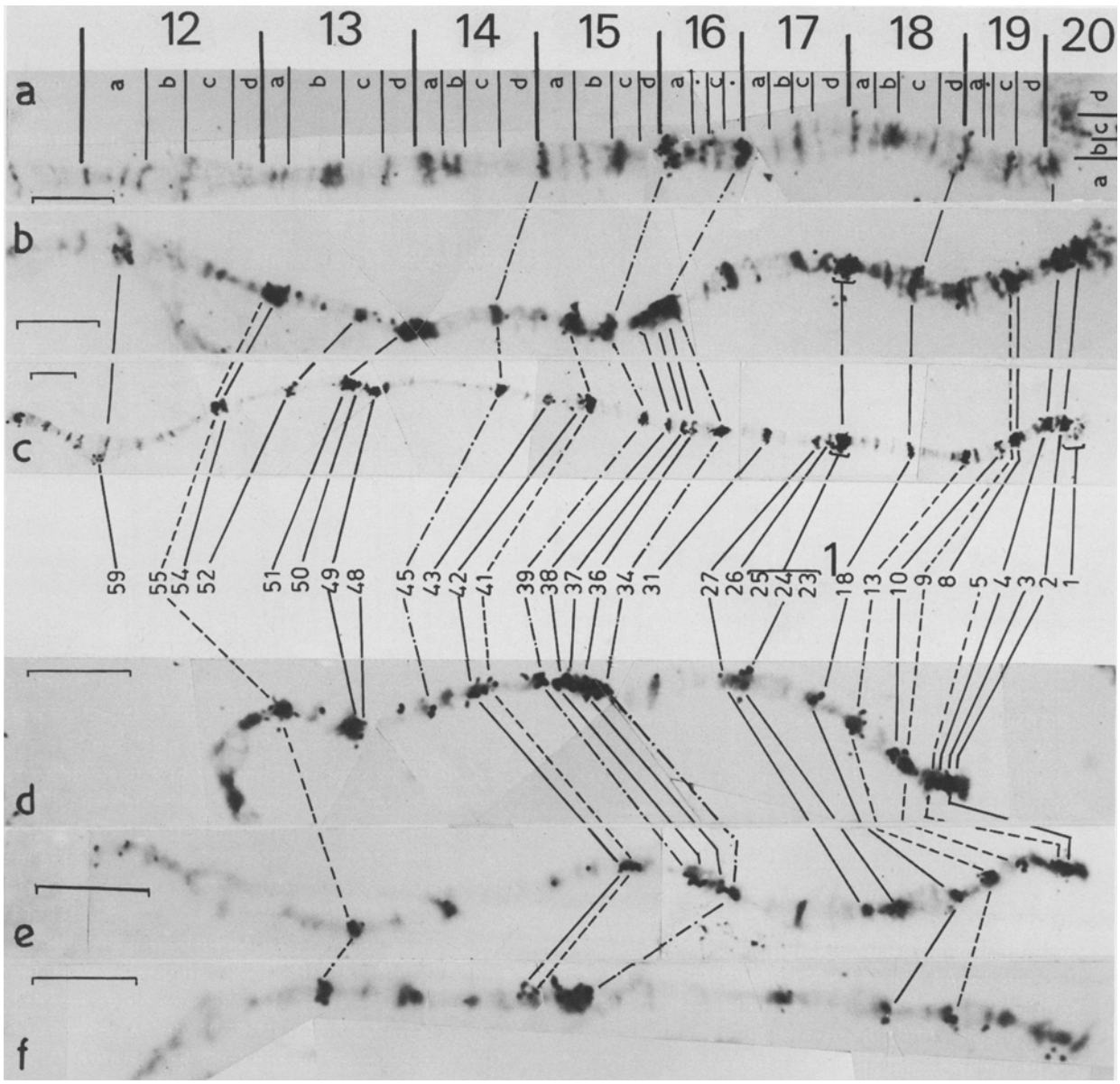

Fig. 4. Examples illustrating differences in the terminal labelling patterns of some late replicating sites on the X (*a–f*) and 2nd chromosome (*g–l*) segments in salivary glands (*a–c* and *g–i*) and in gastric caeca (*d–f* and *j–l*). (*a*) and (*g*) from salivary glands and (*f*) and (*l*) from gastric caeca are from very late 1D nuclei. The labelled sites are indicated in the middle panels: —— sites with comparable labelling frequencies in the two cell types, – · – sites with higher labelling frequency in salivary glands and – – – sites with higher labelling frequency in gastric caeca. Bars represent 10 μm.

larvae is widespread. The functional significance and consequence of these differences in the replication programme are not known but as suggested earlier, these differences become significant in the context of possible variations in levels of polyteny along the length of a chromosome (Laird, 1980) and the late replicating regions being also the sites of intercalary heterochromatin (Zhimulev *et al.*, 1982). It will, therefore, be interesting to compare the distribution of intercalary heterochromatin

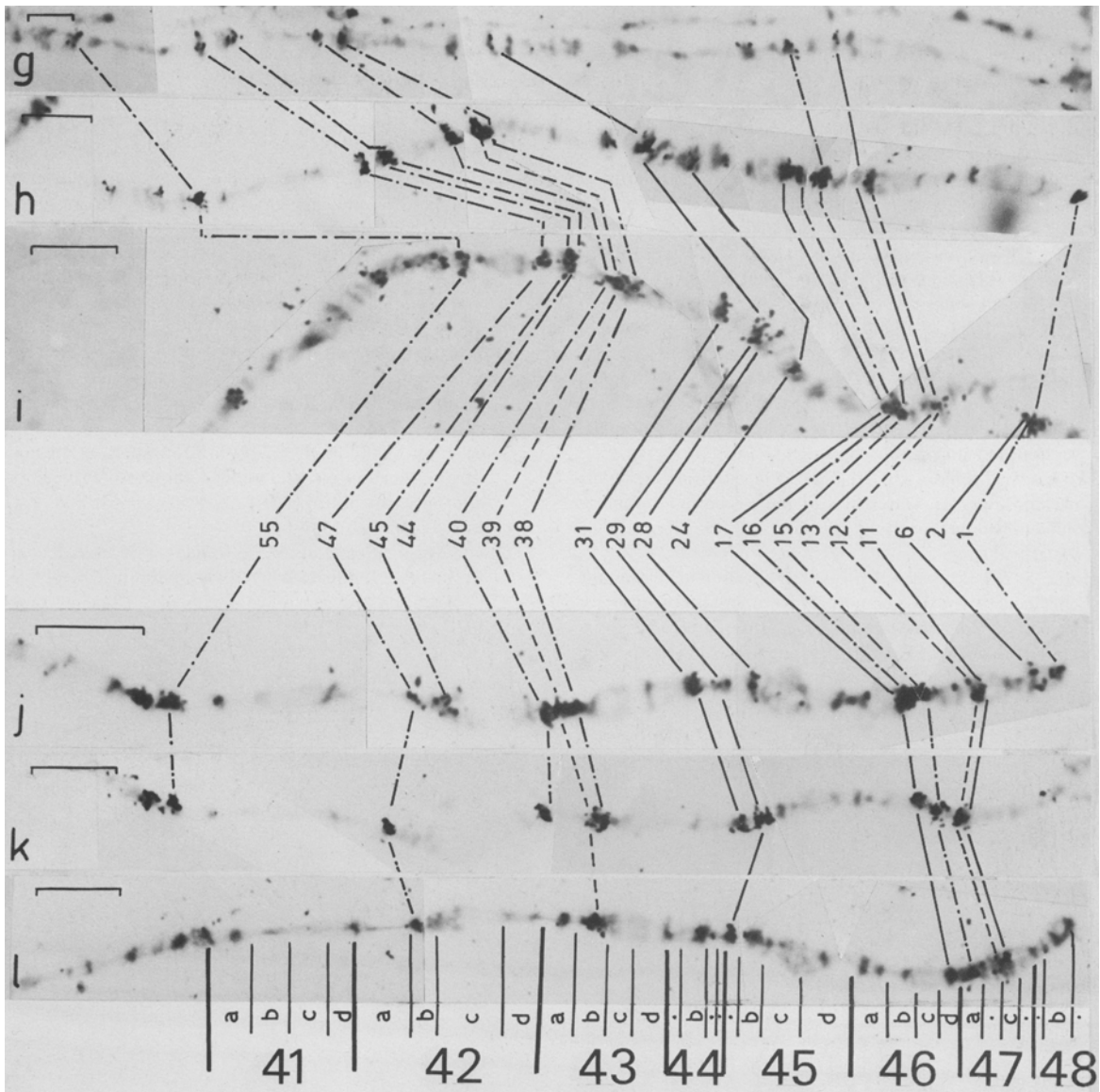

Fig. 4. (Continued).

(with reference to ectopic pairing, 'weak-points' etc.) in the two polytene cell types at those sites which show differences in replication behaviour. These studies are in progress but it may be noted here that while in salivary gland nuclei, the telomeric region of chromosome 2 shows ectopic pairing with terminal regions of other chromosomes in about 90% of nuclei (see Berendes & Meyer, 1968), fewer nuclei in gastric caeca have been seen to have such contacts. Significantly, the present data have shown that this region (48C3) in salivary glands is more late replicating than in gastric caeca.

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References

Beermann, W., 1962. Riesenchromosomen. Protoplasmatologia Handbuch der Protoplasmaforschung. Band IVd (Springer-Verlag, Vienna).

Berendes, H. D., The salivary gland chromosome of Drosophila hydei Sturtevant. Chromosoma 14: 195–206.

Berendes, H. D. & Meyer, G. F., 1968. A specific chromosome element, the telomere of Drosophila polytene chromosomes. Chromosoma 25: 184–197.

Evgen'ev, M., Levin, A. & Gubenko, I., 1977. Are late replicating regions in polytene chromosomes of Drosophila enriched by repeated nucleotide sequences? Nature 268: 766–767.

Laird, C. D., 1980. Structural paradox of polytene chromosomes. Cell 22: 869–874.

Lakhotia, S. C. & Sinha, P., 1983. Replication in Drosophila chromosomes X. Two kinds of active replicons in salivary gland polytene nuclei and their relation to chromosomal replication patterns. Chromosoma 88: 265–276.

Lakhotia, S. C. & Tiwari, P. K., 1984. Replication in Drosophila chromosomes XI. Differences in temporal order of replications in two polytene cell types in D. nasuta. Chromosoma 89: 212–217.

Mishra, A. & Lakhotia, S. C., 1982. Replication in Drosophila chromosomes. VIII. Temporal order of replication of specific sites in polytene chromosomes from 24 °C and 10 °C reared larvae of Drosophila melanogaster. Ind. J. exp. Biol. 20: 652–659.

Redfern, C. P. F., 1981. DNA replication in polytene chromosomes: similarity of termination patterns in somatic and germ-line derived polytene chromosomes of Anopheles stephensi Liston (Diptera:Culicidae). Chromosoma 84: 33–47.

Richards, G., 1980. The polytene chromosomes in fat body nuclei of Drosophila melanogaster. Chromosoma 79: 241–250.

Rodman, T. C., 1968. Relationship of developmental stage to initiation of replication in polytene nuclei. Chromosoma 23: 271–287.

Roy, S. & Lakhotia, S. C., 1981. Replication in Drosophila chromosomes. Part IV. Patterns of chromosomal replication in salivary gland polytene nuclei of Drosophila nasuta. Ind. J. exp. Biol. 19: 797–807.

Rudkin, G. T., 1972. Replication in polytene chromosomes. In: Developmental studies in giant chromosomes (W. Beermann, ed.) Berlin, Heidelberg, New York - Springer: pp. 59–85.

Sinha, P. & Lakhotia, S. C., 1980. Replication in Drosophila chromosomes. Part V. Polytene chromosome replication after in vitro culture of larval salivary glands. Ind. J. exp. Biol. 18: 1059–1065.

Zhimulev, I. F., Semeshin, V. F., Kulichkov, V. A. & Belyaeva, E. S., 1982. Intercalary heterochromatin in Drosophila. I. Localization and general characteristics. Chromosoma 87: 197–228.

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