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Replication in *Drosophila* Chromosomes: Part IV—Patterns of Chromosomal Replication in Salivary Gland Polytene Nuclei of *Drosophila nasuta*

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Replication in polytene nuclei of late third instar larvae and early prepupae of *D. nasuta* has been studied by autoradiography of squash preparations of salivary glands following a 10 min *in vitro* pulse of ^{3}H-thymidine. A reference photomap of polytene chromosomes of *D. nasuta* is also presented. As described earlier in *D. kikkawai* [Roy and Lakhotia, *Indian J exp Biol*, 17 (1979) 231], in *D. nasuta* also 3 categories of early-S ^{3}H-thymidine labelling patterns (low, mid, and heavy, interband type) are seen in high frequency in late larval stages but not in early prepupae when the conventional discontinuous (2D and 1D) labelling patterns are predominant. In some of the low interband type labelled nuclei, only one puff site, 48A on 2R, is seen to be labelled and this particular puff also remains labelled with a much higher silver grain density in comparison with 4 other puff sites on 2R, in all labelling patterns. Most of the other puffs are unlabelled in late-S patterns. It is suggested that in polytene nuclei of late third instar larvae of *D. nasuta*, a polytene S-period is initiated by DNA synthesis only at the 48A puff, the other disperse regions (puffs and interbands) start replicating later in a sequential manner followed by initiation of replication of band regions. The 48A puff seems to have an unusually extended period of DNA synthesis and shows striking similarity with the E-11E puff of *D. kikkawai* (Roy and Lakhotia, 1979). The patterns of replication of X-chromosome in male and female polytene nuclei of *D. nasuta* in the early stages of polytene-S period have also been examined. The observations suggest that in *D. nasuta* the replication of different sites on male X is initiated slightly later than some sites on autosomes or on female X's. However after this initial delay the male X becomes faster replicating from the heavy interband labelling stage onwards and as in other species, completes its replication cycle in late-S (1D type pattern) before many autosomal or female X chromosomal sites.

Polytene nuclei of larval salivary glands of *Drosophila* undergo a definite sequence of cyclic replication in which a synthetic phase (polytene S-period) alternates with an intersynthetic phase[1,2]. However the absence of cell cycle stage dependent differences in morphology of polytene nuclei has hindered the analysis of the temporal sequence of replication of different chromosome regions and the determination of the duration of a polytene S-period. Nevertheless several indirect considerations have led to the general belief that the generally observed 3 categories of autoradiographic labelling patterns of ^{3}H-thymidine pulse lablelled polytene chromosomes of *Drosophila*, *initious* (labeling of all chromosome regions) and *discontinuous* patterns (with predominant labelling of dense band regions), occur in that order in a polytene S-period in late third instar larval salivary glands[3-5]. The initial stage of the polytene S-period, represented by the interband (1B) labelling patterns (also termed 'disperse discontinuous' or DD patterns by Chatterjee and Mukherjee[3]), has been found to be rather brief in the *Drosophila* species analysed in detail[3,4]. However we have recently presented[5] our observations in larval salivary glands of *D. kikkawai* in which the initial patterns or the 1B phase of polytene S-period was found to be more extended. In this paper we present our observations on polytene replication in salivary glands of *D. nasuta*. In this species also we find that the interband labelling phase is very extended. Furthermore, as reported earlier in *D. kikkawai*[5], one puff in *D. nasuta* polytene chromosomes also shows ^{3}H-thymidine incorporation in all types of labelled nuclei including the late-S (discontinuous labelled) nuclei. Taking advantage of the extended initial phase of the S-period in polytene nuclei of *D. nasuta*, we have also examined the replication of the X-chromosome in male and female nuclei since while a detailed analysis been made earlier, relatively little is known about the initial stages.

Materials and Methods

A wild strain of *D. nasuta* (Varanasi) has been used for this study. Flies and larvae were reared on standard agar-cornmeal-brown sugar-yeast food at 24° ± 1°C. To obtain healthy and synchronized larvae, eggs were collected in food filled petri dishes for 30-60 min. In the larvae of *D. nasuta* the colour of the anterior and posterior spiracles changes during third instar and this colour change was taken as the external marker to obtain a more synchronously growing population of

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larvae. About 30-34 hr prior to anterior spiracle eversion, the posterior pair of spiracles develop a black pigment stripe at their base and this stage is recognized as 0 hr black spiracle stage. The stage of anterior spiracle eversion is referred to as 0 hr prepupae. Salivary glands from late third instar larvae (between 0 hr and 24 hr black spiracle stage) and from 0 hr to 4 hr old prepupae were dissected out in *Drosophila* Ringer (*p*H 7.2). The first group of larvae was dissected between 0 and 8 hr, the second group between 10 and 16 hr and the third group between 18 and 24 hr after the black spiracle stage. In each case the excised salivary glands were labelled for 10 min with ^{3}H-thymidine in Ringer (250 μCi/ml; sp. act. 10.4 Ci/mM; BARC, Trombay). Squash preparations of labelled glands were processed for autoradiography as described earlier[5]. To facilitate analysis of the autoradiograms and to serve as a standard reference, a photomap (Fig. 1) of the polytene chromosomes of *D. nasuta* has also been prepared from phase-contrast photomicrographs of aceto-orcein-carmine stained temporary squash preparations of late third instar larval salivary glands.

Results

Polytene chromosomes of *D. nasuta*:

The metaphase karyotype of *D. nasuta* consists of acrocentric X, metacentric 2nd, acrocentric 3rd and dot like 4th chromosomes[7]. Correspondingly, salivary gland polytene chromosome complement of *D. nasuta* consists of 4 long arms and a short arm. The 4 long arms correspond, respectively, to X, 2L, 2R and 3rd chromosomes while the short arm represents the 4th chromosomes. The chromocentre is very small made up largely of a compact rounded alpha heterochromatic mass with little beta heterochromatic regions at the bases of different arms[8]. Among the long arms of *D. nasuta* polytene nuclei, 2L is the shortest while chromosome 3 is the longest. The X is slightly longer than 2L, but is shorter than 2R.

In photomap (Fig. 1) of *D. nasuta* polytene chromosomes the X, 2L and 2R have been divided into 20 divisions each while the long third chromosome is divided into 40 divisions. X-chromosome has divisions 1 to 20 from the free tip to centromeric end; 2L has divisions 21-40 from tip to centromeric end; the divisions (41-60) on 2R are numbered from centromeric end to the free tip while on chromosome 3, the divisions are numbered from 61-100 from tip to basal region. Chromosome 4 is very small and only one division is recognised on this arm. Each division is further sub-divided into 3 sections (A, B and C) with each section starting from an interband (Fig. 1). Each section is further divisible into several bands (details not given here). It may also be noted that many larvae of the strain used in this study carry an inversion on 2L between 25C and 38B.

Patterns of ^{3}H-thymidine incorporation in polytene chromosomes:

General patterns of autoradiographic labelling and their frequencies in larval and prepupal glands—The general patterns of autoradiographic labelling of different polytene nuclei in *D. nasuta* following a pulse of ^{3}H-thymidine are essentially similar to those described earlier[5] in *D. kikkawai*, and as in that species, we have classified the ^{3}H-thymidine labelled polytene nuclei of *D. nasuta* also into the following 8 categories: (i) low interband (LIB); (ii) mid interband (MIB); (iii) heavy interband (HIB); (iv) medium continuous (2C); (v) heavy continuous (3C); (vi) heavy discontinuous (3D); (vii) medium discontinuous (2D); and (viii) late or low discontinuous (1D) type of labelling patterns. We presume that (see discussion) as in *D. kikkawai*[5], in late third instar larval polytene nuclei of *D. nasuta* also the labelling patterns (i) to (viii) occur in that sequence during the progression of a polytene S-period from initiation to termination; in other words, the LIB type of nuclei are presumed to be in the very early and 1D type nuclei in the late phase of a given polytene S-period. Representative examples of these labelling patterns are illustrated in Fig. 2a-g to show the general distribution of autoradiographic labelling in these categories. As can be seen from the examples in Fig. 2a-g, the LIB nuclei have a low labelling of only one or two puffs and none or a few interbands while in MIB and HIB types, progressively more puffs and interbands and light bands appear labelled. In different HIB type nuclei the labelling of the interbands and the puffs varies from moderate to heavy (Figs 2c, 4c and 4d), but most of the dark bands remain unlabelled. In 3D to 1D patterns, the interband and puff regions become progressively unlabelled and also the intensity of labelling and the number of labelled bands decreases progressively as originally described by Rodman[9]. The chromocentre in polytene nuclei of *D. nasuta* is largely made up of alpha heterochromatin[8]. In all the nuclei labelled with ^{3}H-thymidine, irrespective of their labelling patterns, the alpha heterochromatic mass was always seen to be distinctly unlabelled (Fig. 3a-g).

Preparations from larvae (male and female) and prepupae of different age groups (see material and methods) were scored for the frequencies of different labelling patterns. As can be seen from the data in Tables 1 and 2, frequencies of the different labelling patterns vary in a characteristic manner with larval and prepupal age. It is seen that in both male and female larvae the frequency of unlabelled nuclei increases with increasing larval age. However, in 10-16 hr black spiracle stage larvae, the frequency of

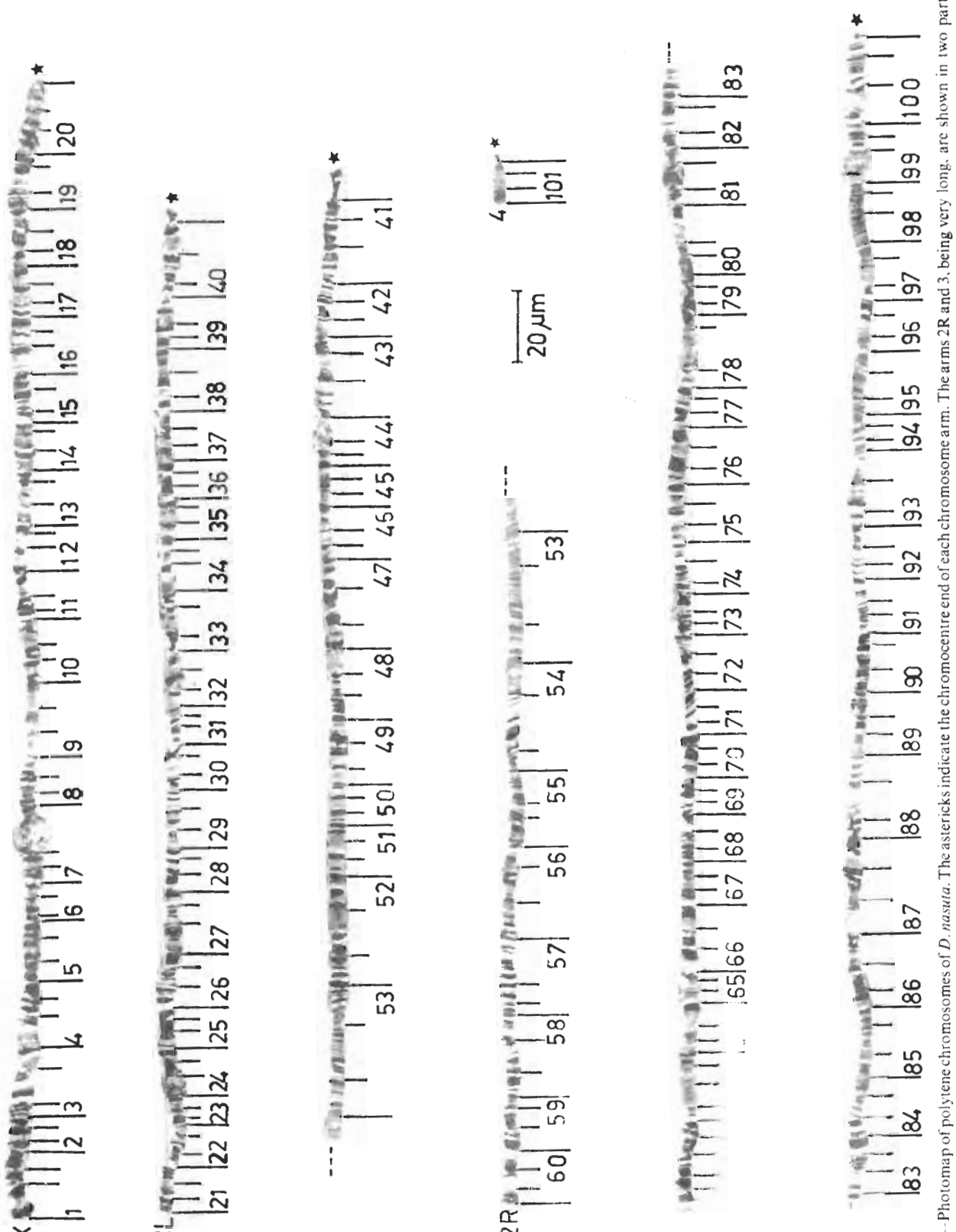

Fig. 1 — Photomap of polytene chromosomes of *D. nasuta*. The astericks indicate the chromocentre end of each chromosome arm. The arms 2R and 3, being very long, are shown in two parts each

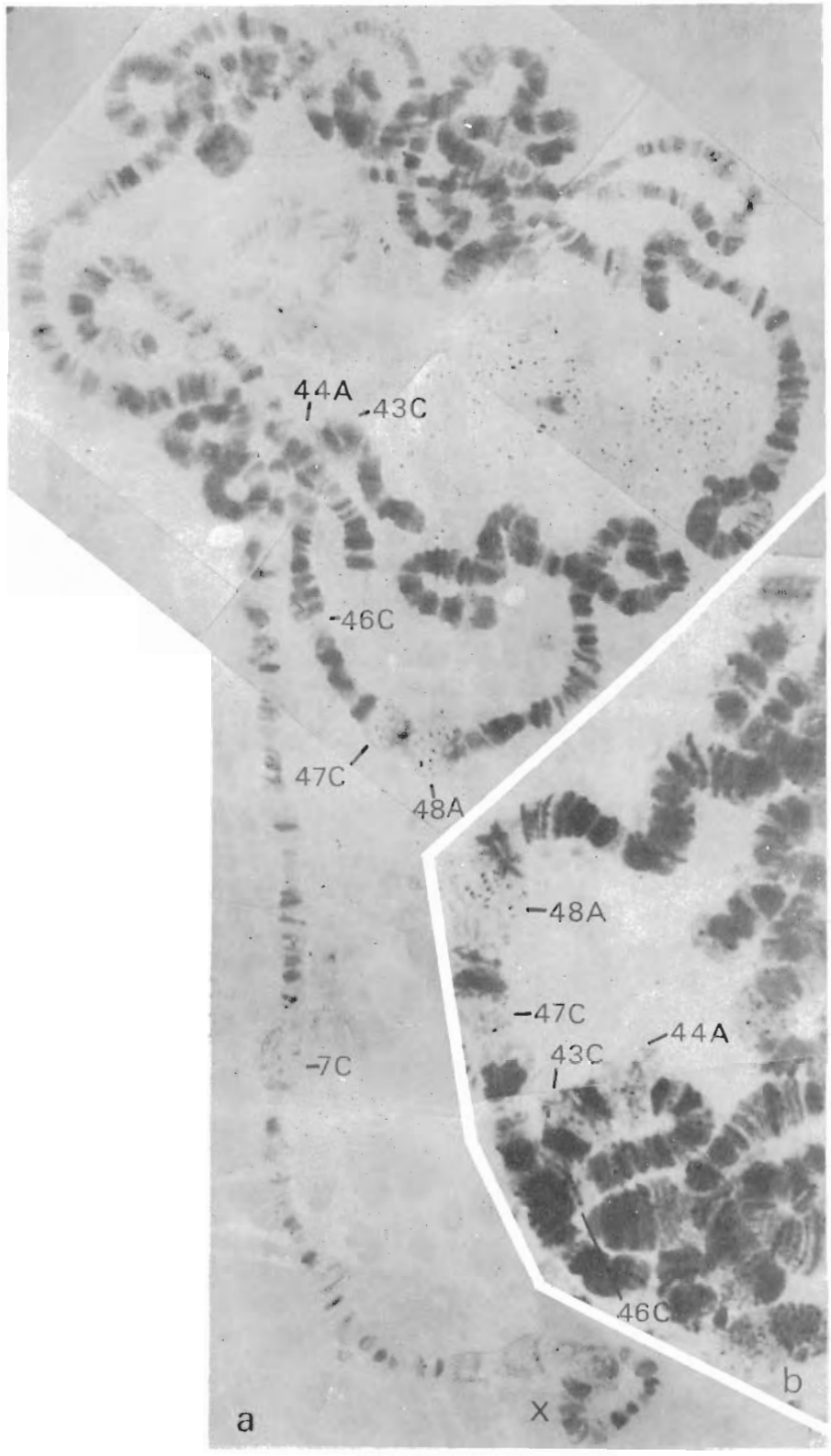

Fig. 2– Autoradiographs of ^{3}H-thymidine pulse- labelled polytene chromosomes of late third instar larvae of *D. nasuta* to show the general features of the 8 categories of chromosomal labelling patterns identified in this species. Some of the puff sites on 2R and X, seen in the different montages, are indicated; × 1200; a, Montage of a complete male nucleus showing low interband (L1B) type labelling with only puff (48A on 2R) being labelled. All other regions including the X-chromosome are completely unlabelled. The intranucleolar DNA is also labelled (for details of intranucleolar labelling, see ref. 19; b, Part of a mid interband (M1B) type labelled nucleus. Note the labelling of several interbands and puffs in addition to 48A puff.

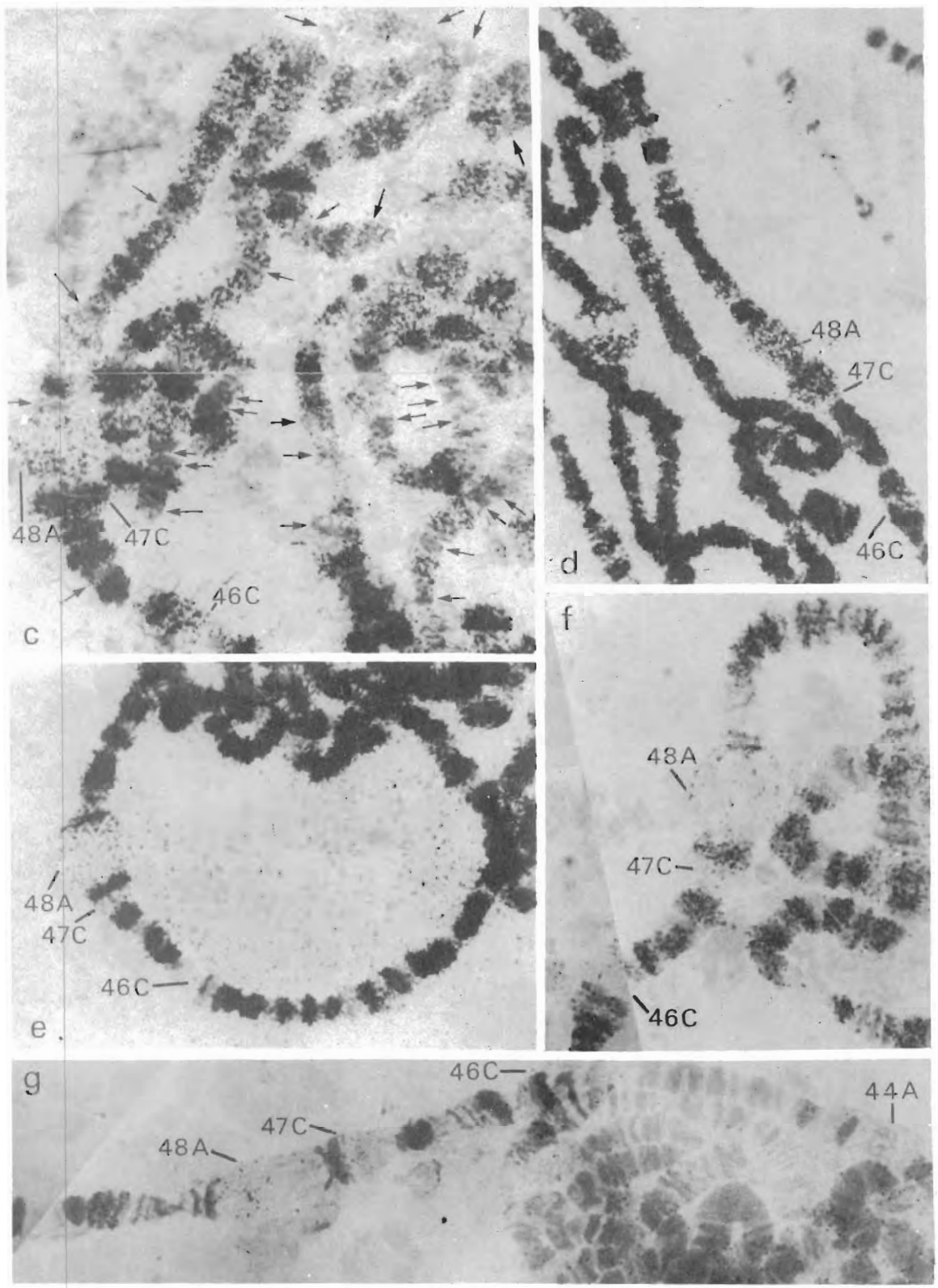

Fig. 2— c, Part of a heavy interband (HIB) type labelled nucleus showing a heavy labelling of most puffs and interbands. Many bands are distinctly unlabelled (arrows); d, Part of a heavy continuous (3C) type labelled nucleus; e, Part of a heavy discontinuous (3D) type labelled nucleus with some puff and interband sites being unlabelled; f,g, Parts of medium (2D) and low (1D) discontinuously labelled nuclei, respectively. Note the progressively reduced labelling of band regions. While most puffs and interbands are unlabelled in these patterns, the 48A puff is well labelled.

unlabelled nuclei in female larvae is much higher (46.03%) than in male larvae (17.97%) while in other 2 age groups the frequency of unlabelled nuclei is nearly similar in male and female larvae. Among the labelled nuclei it is seen that the frequencies of interband patterns (particularly LIB and MIB types) increase with larval age while the continuous (3C) and discontinuous (2D and 1D) patterns show a decline. The very high frequency of LIB and MIB patterns in larvae of either sexes (particularly in the 18-24 hr black spiracle stage) is very interesting.

Analysis of frequencies of different labelling patterns during the first 4 hr after anterior spiracle eversion shows that in these salivary glands, the labelled nuclei are fewer, and among these, the interband patterns are totally absent (Table 2). While in 0 hr prepupal glands, the 3C type patterns are seen in slightly more than 25% of labelled nuclei, in the later stages, these patterns are no more present. Likewise, the 3D and 2D patterns also become fewer in older prepupae, and in 4 hr prepupae only 2D (11.90%) and 1D (88.09%) types of nuclei are seen (Table 2).

^{3}H-thymidine labelling of the 48A puff—The most important feature of the polytene chromosomes of *D. nasuta* is the patterns of ^{3}H-thymidine incorporation in the puff 48A on 2R. It is seen to incorporate ^{3}H-thymidine in all labelled nuclei and in many of the LIB type of nuclei, this is the only site in the nucleus (except the nucleolus), which is labelled (Fig. 2a). In most of the 1D type nuclei also this puff shows a significant ^{3}H-

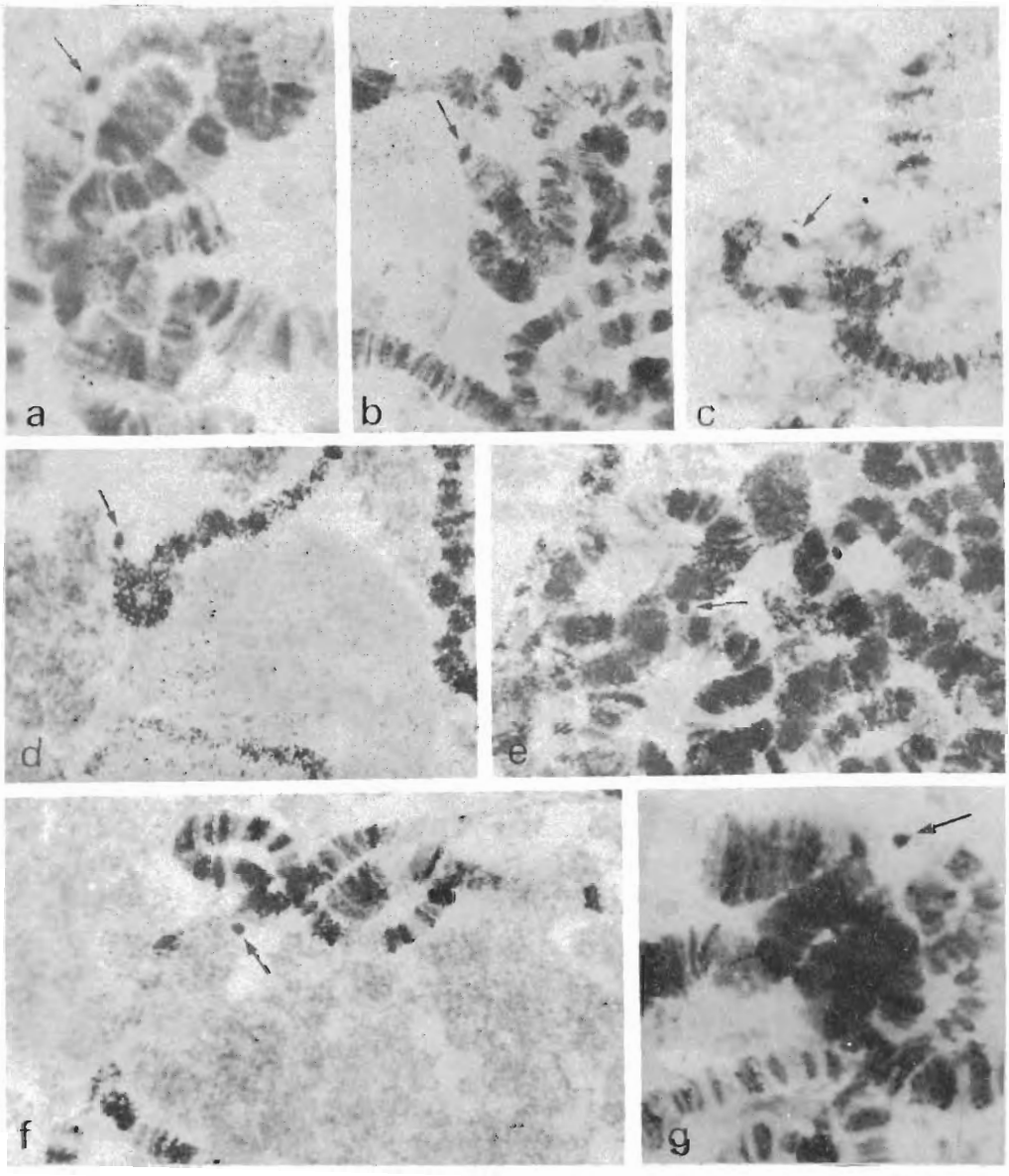

Fig. 3a-g—Parts of autoradiographs of polytene nuclei of *D. nasuta* to show lack of incorporation of ^{3}H-thymidine in alpha-heterochromatin (arrow) in LIB (a), MIB (b), HIB (c), 2C-3C (d), 3D (e), 2D (f) and 1D (g) type labelled nuclei; × 1200

thymidine incorporation (Fig. 2g). To obtain a more detailed information on the replicative organization of this puff, we have counted the number of silver grains on 48A and 4 other (47C, 46C, 44A and 43C) puffs on 2R in nuclei showing different types of ^{3}H-thymidine labelling patterns in preparations of larval salivary glands (Table 3). In this analysis the data of HIB, 2C and 3C types of nuclei could not be obtained because of very low number of scorable HIB and 2C nuclei while in 3C type nuclei, a very heavy labelling of all chromosome regions does not permit a precise grain count on the puff regions. Likewise for the 3D type nuclei, only very few nuclei could be scored since in others the grain density was too high. Nevertheless the

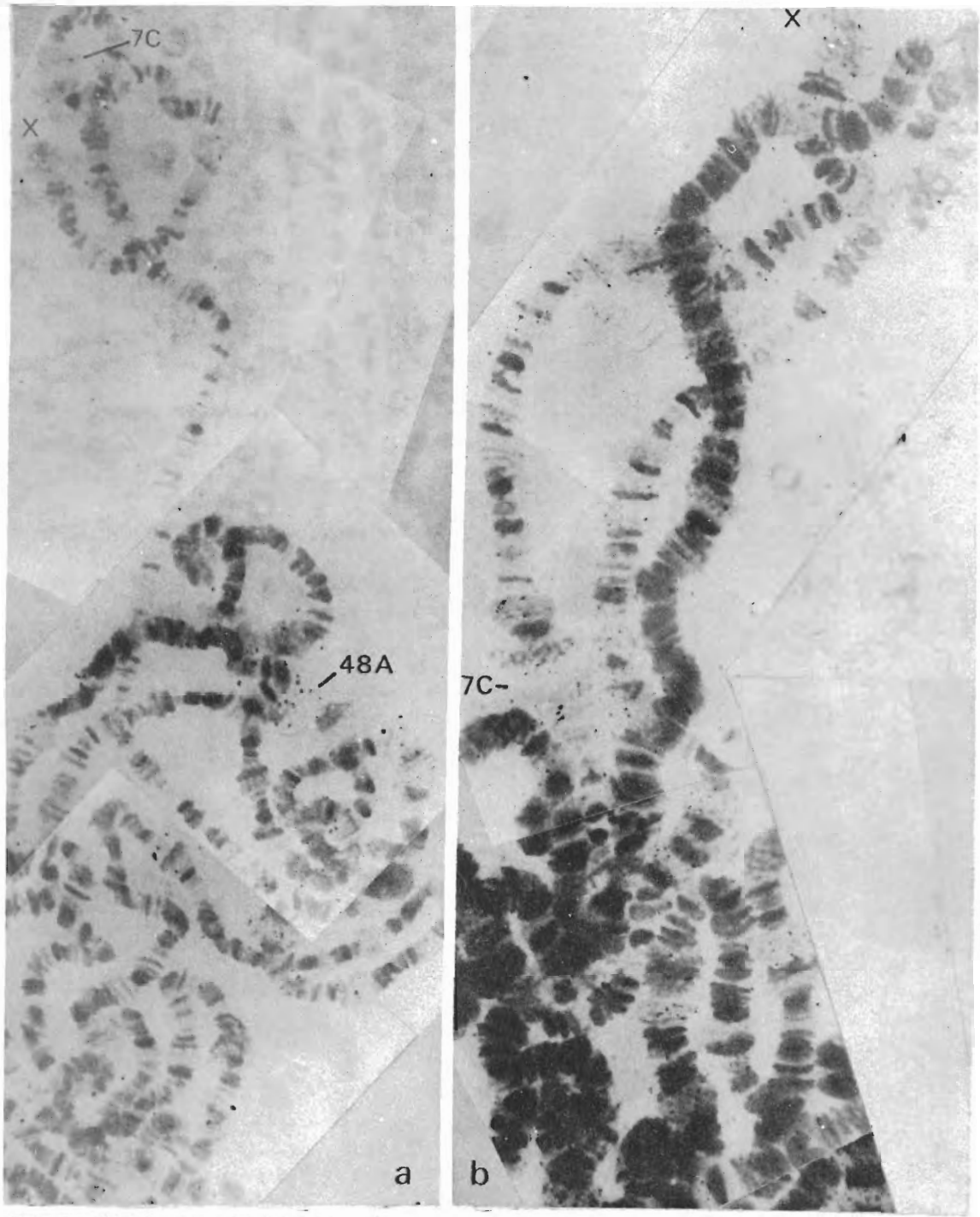

Fig. 4—Examples of interband type labelled polytene chromosomes of male *D. nasuta* to show differential replication of X-chromosome; × 1200; a, Autosomes LIB type with a few puffs low labelled; the lightly stained male X is nearly unlabelled, except for 2-3 grains on a few sites like 7C puff; b, Autosomes MIB type with several puffs and interbands labelled; the male X shows a lesser degree of labelling

data in Table 3 and other observations, (Fig. 2a-g) very clearly reveal interesting differences in the replicative organization of 48A and other puffs. All the 5 puff sites show maximum grain density in 3D (and in 3C) patterns. But only the 48A puff shows a pronounced labelling (with 10 or more silver grains on average) in LIB as well as 1D type nuclei. The other puff sites are mostly unlabelled or very low labelled in these two types of labelled nuclei, particularly in 1D type where the very low labelling in these puffs is seen in only a few nuclei and that too nearer the adjoining band region. On the other hand, in 48A puff, the silver grains appear more generally distributed over the puff area (see Fig. 2a and g), indicating that the 48A puff DNA is replicating even in the late S (see Discussion). Another significant difference between the 48A and other puffs is the several times higher mean grain density on 48A puff compared to the other sites in all types of labelled nuclei (Table 3).

Labelling patterns of X-chromosome in male and female polytene nuclei of D. nasuta—As in other species of *Drosophila*[6,10], ^{3}H-thymidine incorporation

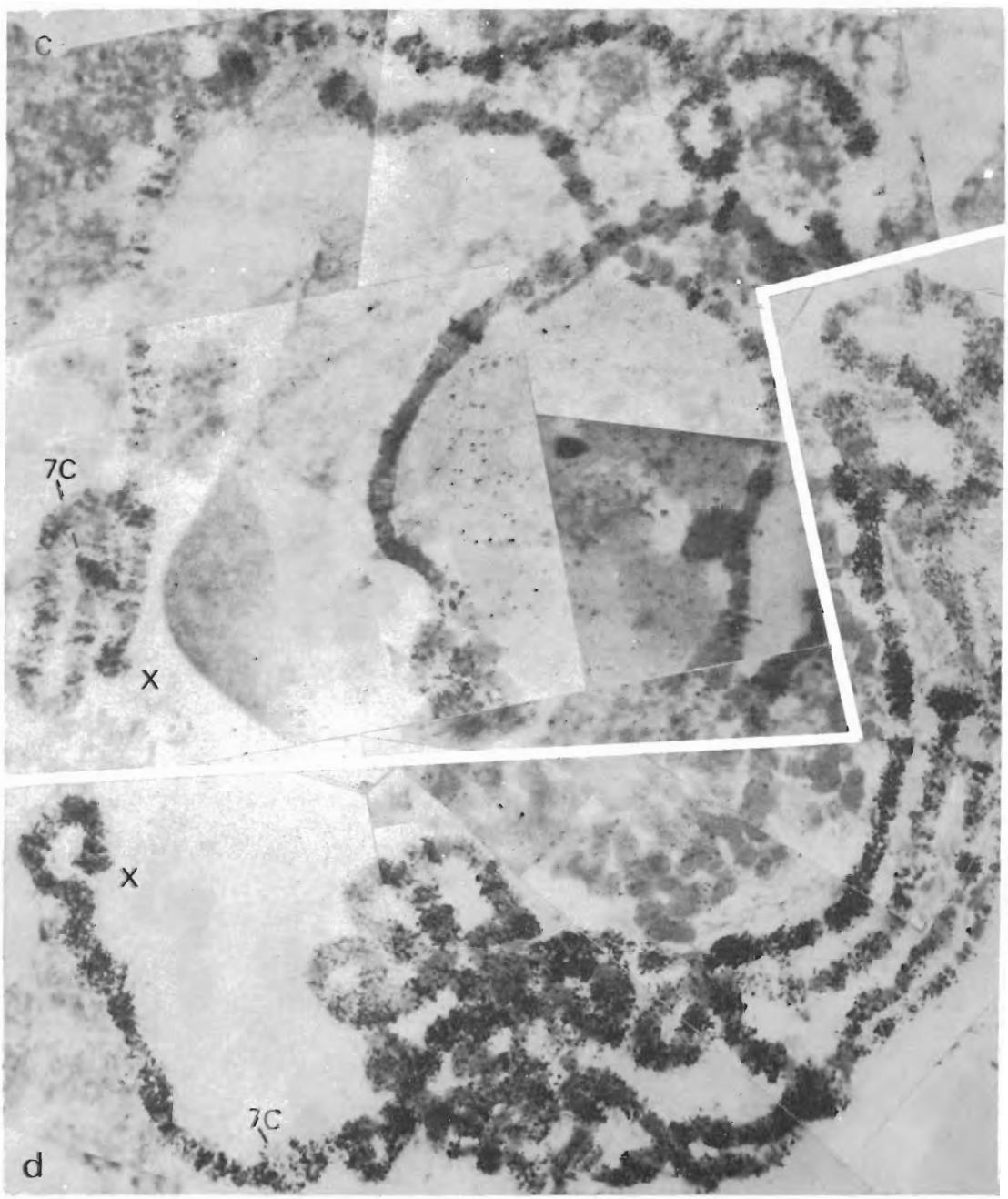

Fig. 4—c, Autosomes with labelling intermediate between MIB and HIB types. The male X shows nearly similar labelling intensity as autosomes; d, Autosomes with typical HIB type labelling; the male X shows a much heavier labelling, approaching the 3C level

in X-chromosome in male polytene nuclei of *D. nasuta* is asynchronous with autosomes. Thus in *D. nasuta* also, male nuclei with continuous and discontinuous type patterns show lesser labelling on the X-chromosomes than the autosomes whereas in female continuous and discontinuous type labelled nuclei the autosomal and X-chromosomal labelling is comparable. The patterns of ^{3}H-thymidine labelling of X-chromosomes in male nuclei showing IB type labelling are, however, different (Fig. 4a-d). The first site in X-chromosome to get labelled in male as well as in female LIB type nuclei is the big puff in 7C section. In an analysis of labelling patterns of small segments of X (6A to 8C) and of 2R (47A to 47C) in male and female nuclei, we have noticed that in LIB type female nuclei, the 7C puff on X can be seen to be labelled when only the 48A puff on the 2R segment of the nucleus is labelled. On the other hand in male LIB nuclei, the 7C puff on X is seen to be labelled only in slightly more advanced LIB stage when on the 2R segment in addition to the 48A puff, the 47C and/or the interband region in the beginning of 47A section is also labelled. Similarly in further advanced stages of LIB and MIB type nuclei, while the relative number of labelled sites on X and autosomes is comparable in preparations from female larvae, those from male nuclei display a distinctly less number of labelled sites on X in comparison to autosomes (Fig. 4a, b). In HIB type nuclei, however, the male X is no longer less labelled than the autosomes. Male nuclei with labelling pattern intermediate between MIB and HIB types, show nearly similar degree of labelling of the X and

Table 1—Frequencies of Different ^{3}H-thymidine Labelling Patterns in Salivary Gland Polytene Nuclei in Different Age Groups of Male and Female Larvae of *D. nasuta*

Larval age (hr)	Sex	Total nuclei*	Unlabelled**	Labelled**	Frequency among labelled nuclei**							
					LIB	MIB	HIB	2C	3C	3D	2D	1D
0-8	M	988(14)	92 (9.31)	896 (90.68)	75 (8.37)	3 (0.33)	15 (1.67)	22 (2.45)	176 (19.64)	15 (1.67)	208 (23.21)	382 (42.63)
	F	1048(16)	87 (8.30)	961 (91.69)	49 (5.09)	13 (1.35)	19 (1.97)	22 (2.28)	249 (25.91)	21 (2.18)	218 (22.68)	370 (38.50)
10-16	M	946(17)	170 (17.97)	776 (82.02)	146 (18.18)	38 (4.89)	0 (0.00)	1 (0.12)	111 (14.30)	18 (2.31)	139 (17.91)	323 (41.62)
	F	693(11)	319 (46.03)	374 (53.96)	105 (28.07)	1 (0.26)	0 (0.00)	6 (1.60)	57 (15.24)	14 (3.74)	59 (15.77)	132 (35.29)
18-24	M	1097(17)	408 (37.19)	689 (62.80)	267 (38.75)	118 (17.12)	42 (6.09)	12 (1.74)	29 (4.20)	15 (2.17)	64 (9.28)	142 (20.60)
	F	1133(14)	632 (55.78)	501 (44.21)	257 (51.29)	77 (15.36)	19 (3.79)	7 (1.39)	24 (4.79)	18 (3.59)	31 (6.18)	68 (13.57)
Total	M	3031(48)	670 (22.10)	2361 (77.89)	488 (20.66)	159 (6.73)	57 (2.41)	35 (1.48)	316 (13.38)	48 (2.03)	411 (17.40)	847 (35.87)
	F	2874(41)	1038 (36.11)	1836 (63.88)	411 (22.38)	91 (4.95)	38 (2.06)	35 (1.90)	330 (17.97)	53 (2.88)	308 (16.77)	570 (31.04)

Figures in parentheses indicate (*) number of salivary gland pairs examined, and (**) per cent value in each group.

Table 2—Frequencies of Different ^{3}H-thymidine Labelling Patterns in Salivary Gland Polytene Nuclei of Prepupae (after anterior spiracle eversion stage) in *D. nasuta*

Age (hr)	Total nuclei	Unlabelled*	Labelled*	Frequency of different labelling patterns among labelled nuclei**							
				LIB	MIB	HIB	2C	3C	3D	2D	1D
0	356	304 (85.39)	52 (14.61)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	14 (26.92)	4 (7.69)	16 (30.77)	18 (34.61)
2	529	455 (86.01)	74 (13.99)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.35)	18 (24.32)	55 (74.32)
4	657	615 (93.61)	42 (6.39)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	5 (11.90)	37 (88.09)
Total	1542	1374 (89.10)	168 (10.89)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	14 (8.33)	5 (2.97)	39 (23.21)	110 (65.48)

*Figures in parentheses indicate the percent value.

Table 3—Mean Grain Number on Five Puff Sites on Arm 2R with Respect to Different ^{3}H-thymidine Labelling Patterns

[Values are mean ± SE. Figures in parentheses indicate the number of nuclei scored in each group]

Labelling pattern	Number of Silver grains on puff sites				
	48A	47C	46C	44A	43C
LIB	9.23 ± 0.37 (148)	2.45 ± 0.14 (148)	1.21 ± 0.17 (143)	0.79 ± 0.11 (134)	2.15 ± 0.19 (134)
MIB	18.46 ± 1.59 (62)	2.71 ± 0.38 (62)	2.59 ± 0.19 (59)	2.64 ± 0.19 (50)	3.72 ± 0.59 (50)
3D	61.33 ± 15.72 (3)	10.00 ± 3.78 (3)	8.33 ± 1.45 (3)	25.00 (1)	10.00 (1)
2D	28.18 ± 1.77 (33)	2.72 ± 0.61 (33)	2.66 ± 0.51 (30)	4.81 ± 1.00 (19)	8.42 ± 1.13 (19)
1D	10.42 ± 0.86 (59)	1.88 ± 0.25 (59)	1.84 ± 0.31 (58)	1.46 ± 0.31 (45)	3.86 ± 0.42 (45)

autosomes (Fig. 4C) whereas in typical HIB type nuclei, the male X is distinctly heavier labelled than the autosomes (Fig. 4d). In polytene nuclei of female *D. nasuta*, in HIB as well as in other types of patterns, the degree of labelling of X and autosomes in a nucleus is similar.

Discussion

The present autoradiographic studies on ^{3}H-thymidine pulse labelled polytene nuclei of *D. nasuta* reveal several interesting features of polytene replication in this species. The high frequency and the variety of interband type of ^{3}H-thymidine labelling patterns in late third instar larval polytene nuclei of *D. nasuta* are striking. A comparable replicative organization of polytene chromosomes has so far been described only in larval salivary glands of *D. kikkawai*[5]. As described in that paper for *D. kikkawai* (also see ref. 3, 4 and 11 for other species of *Drosophila*), it appears that in late third instar larval polytene cells of *D. nasuta* also, a given polytene S-period starts with initiation of replication at a few specific puff sites, with other puffs and interbands becoming active sequentially during MIB and HIB phases and finally followed by replication of different bands. In this context the nuclei in which only the puff 48A is labelled are very interesting. We have reported earlier[3] that in some ^{3}H-thymidine pulse labelled polytene nuclei of *D. kikkawai* also only the puff site 11E on arm E appears labelled and we had suggested that the single puff labelled nuclei represent the very initial stage of a polytene S-period. The same may be suggested for the specific labelling of 48A puff in some LIB type polytene nuclei of *D. nasuta* observed in this study. It is significant that in prepupal glands we did not see any single puff labelled or even more advanced IB (MIB or HIB) type labelled nuclei. This further confirms our belief that the IB patterns represent the initial stages of polytene S-period since it has been shown by Rodman[9,12] (also see ref 1) that in prepupal salivary glands, new initiations of polytene S-period do not occur. The 2D and 1D type patterns, which become progressively more common among labelled nuclei with increasing prepupal age, obviously represent the later stages of a polytene S-period.

The data on the frequencies of different ^{3}H-thymidine labelling patterns in polytene nuclei from different age-groups of larvae show that the LIB type and to a lesser extent the MIB type labelling patterns are very commonly seen, particularly in 18-24 hr black spiracle age-group. Since, as discussed above, the LIB type nuclei represent the initial stage, this would imply that there is a synchronous initiation of a new replication cycle in many nuclei of salivary glands at this stage. This apparent synchrony of initiation of a new replication cycle in groups of polytene nuclei in *D. nasuta* permits us to analyse if the synchronously initiated nuclei with varying polyteny levels proceed synchronously through the S or they have a varying duration of the S-period and a corresponding variation in the duration of different phases of an S-period, as has been suggested earlier[1,2]. This aspect is now being analysed in our laboratory.

The replicative organization of 48A puff in *D. nasuta* is interesting. This puff appears to incorporate ^{3}H-thymidine throughout a polytene S-period as evidenced by its labelled appearance in all types of labelled nuclei from LIB to 1D. Except in *D. kikkawai*[5], in most other species of *Drosophila*[3,4,13], the early replicating puff regions have been seen to complete their replication cycle before the 2D or 1D stages. The replicative organization of the 48A puff in *D. nasuta* is obviously different. In view of the several times higher grain density at 48A (in comparison to other puff sites, Table 3) in all labelled nuclei, and its extended period of ^{3}H-thymidine incorporation, it may be envisaged that the 48A puff is involved in "extra" DNA synthesis. However this possibility may

be negated by two observations. Firstly the pattern of quantitative variation in ^{3}H-thymidine incorporation during the progression of a polytene S-period at 48A site is similar to that of other sites, being low in early S (LIB and MIB type nuclei), very high in mid-S (HIB-2C and 3C-3D type nuclei) and again declining in late-S (2D and 1D type nuclei) phase. Secondly it has also been observed[14] that the 48A puff size remains the same irrespective of low or high degree of ^{3}H-thymidine incorporation at this locus in different labelling patterns. Nevertheless, further analysis of the molecular and cytological organization of replicating sequences at 48A puft of *D. nasuta* is needed to understand the significance of the unusual replicative of this puff site.

The lack of ^{3}H-thymidine incorporation in the alpha heterochromatin in polytene nuclei of *D. nasuta* is as expected since from the other studies[15,16] it is known that the alpha heterochromatin does not polytenize in polytene nuclei of *Drosophila*. Furthermore, using fluorescence techniques, it has been shown earlier[8] that in polytene nuclei of *D. nasuta*, the bulk of chromocentre region is made of non-replicative alpha heterochromatin. The large size and the distinctness of the alpha heterochromatin in polytene nuclei of *D. nasuta* permits an easy analysis of its replicative organization by autoradiographic techniques also.

The faster completion of replication of X-chromosome in polytene nuclei of different species in male *Drosophila* has been studied in detail in several earlier studies[6,10,11]. Similar detailed studies of X-chromosome in male and female polytene nuclei during the early-S period are fewer. Hägele and Kalisch[13] compared the ^{3}H-thymidine labelling of X-chromosome in initial (interband) labelling patterns in male and female polytene nuclei of *D. melanogaster*, and suggested that "in the initial stage of replication the male X-chromosome is in a later replication phase than the X-chromosome in female or autosomes". However in another study, a heavier labelling of male X-chromosome in interband type of labelled nuclei of *D. melanogaster* has been stated to be absent[17]. On the other hand Mukherjee and Chatterjee[11] have reported a heavier labelling of male X in polytene nuclei of *D. pseudoobscura* showing HIB (late DD pattern of Mukherjee and Chatterjee[11]) and 2C type of autosomal labelling. In a study of replication in polytene nuclei of F_1 hybrids of *D. azteca* and *D. athabasca*, Meer[18] has reported that the X-chromosome in male may start its replication earlier than the autosomes. In this context the present observations in *D. nasuta* are interesting, particularly since in this species it appears that the initial phase of replication in polytene nuclei is more extended. Our observations suggest that in *D. nasuta*, the initiation of replication on X-chromosome sites, both in male and female, occurs after some autosomal sites (48A puff on 2R) have already been initiated to replicate. However, relative to female X s, the initiation at specific sites in X-chromosome of male polytene nuclei of *D. nasuta* seems to occur slightly later since more sites on autosomes of male nuclei are labelled when the labelling on the male X-chromosome is first (puff 7C) detectable. In *D. nasuta*, the X-chromosome in male nuclei appears to be more advanced in replication only from HIB stage onwards. It may be noted that the early patterns described in previous studies in *D. melanogaster*[13] or in *D. pseudoobscura*[11] appear to be comparable to the HIB patterns of *D. nasuta*. Apparently, a replication stage comparable to LIB and MIB patterns of *D. nasuta* either does not exist in these two species or is too rare to be recorded. Thus, unlike the condition reported by Meer[18] in the hybrid larvae, in *D. nasuta*, the male X does not start replicating earlier than the autosomes. Rather, the observations indicate that the initiation of male X may, in fact, be slightly delayed compared to the female X s. But after this brief initial delay, the male X-chromosome soon starts replicating faster in comparison to the autosomes or the X-chromosome in female.

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