Functional Organization of Polytene X-chromosome in Two X-chromosome Inversion Carrying Larvae of *Drosophila melanogaster* Reared at 24°C or at 10°C

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Larvae of *D. melanogaster* carrying either the In(1)B^{M1} or In(1)B^{M2} inversion have been reared at 24° or at 10°C to study the morphology, transcription and replication of the X-chromosome in the salivary gland polytene nuclei. These two inversions share a similar left-hand breakpoint in euchromatin (16A2-5 region in polytene X-chromosome) but have different right-hand breakpoints in the proximal heterochromatin. In 10°C reared B^{M1} male larvae, the polytene X appears somewhat more diffused than in 24°C reared larvae. On an average, in 36% of the polytene nuclei of 10°C reared B^{M2} male larvae the single X-chromosome appears considerably shortened in length, enlarged in width and has very indistinct bands, while the other nuclei show "normal-looking" X as in B^{M1} larvae. This highly disorganized form of X-chromosome, referred to as "pompon-like" X, is never seen in cold-reared female or in warm-reared female as well as male B^{M2} or B^{M1} larvae. Hoechst 33258 fluorescence reveals that the "pompon-like" morphology of the X is due to loose packing of chromatin in different band regions. It is suggested that the "pompon-like" morphology is due to position effect variegation associated with the particular heterochromatin breakpoint in the B^{M2} inversion. ^{3}H-uridine and ^{3}H-thymidine labelling and autoradiography of polytene nuclei from cold-reared male and female larvae of the two genotypes shows that the "pompon-like" or the "normal-looking" X in male nuclei continues its hyperactive transcription and faster replication as in 24°C reared wild-type larvae. It appears that the hyperactive organization of the hemizygous X in larval male polytene nuclei predisposes its pattern of chromatin condensation to be specifically affected by a variety of genetic, chemical and physical factors. However, the type of chromatin dispersion seen in "pompon-like" X in cold-reared B^{M2} male larvae does not seem to affect the basic functional (hyperactive) organization of the hemizygous X in male polytene nuclei.

Contrary to the earlier general concept of the genetic inertness of the heterochromatin in *Drosophila* genome[1], it is now well known that DNA sequences localized in the heterochromatin regions of *D. melanogaster* are transcribed[2,3] and that functional genes can also be localized, although in a low density, in heterochromatin regions by genetic methods[4]. It has also been known for a long time that a chromosomal rearrangement involving heterochromation often results in position effect variegation which involves alteration in the stuctural and functional organization of the euchromatic region which is brought nearer to the heterochromatin region[5–8]. However, not all break-points in the heterochromatin have the same effect[7] which implies that different regions of the heterochromatin have different properties. In this context, we have undertaken studies to examine the effects, if any, of chromosomal rearrangements involving heterochromatin, on the functional organization of the X-chromosome in polytene nuclei of *D. melanogaster*. Earlier studies[9–12] have demonstrated differences in the structural and functional organization of the polytene X-chromosome in male and female cells in relation to the phenomenon of dosage compensation. Thus, we have specifically looked for any changes brought about by the rearrangement in this aspect of X-chromosome

organization. In this initial study we present the results of studies on two X-chromosomal inversions of *D. melanogaster*, viz. In(1)B^{M1} and In(1)B^{M2} (for more details see Results and Ref. 13) in relation to their effects on the functional organization of the polytene X-chromosome. These two inversions share similar euchromatin breakpoint but have different heterochromatin breakpoints, and thus provide an opportunity to examine the influence, if any, of the neighbourhood of different heterochromatin segments.

Materials and Methods

Two X-chromosome inversion carrying stocks of *D. melanogaster*, namely In(1)B^{M1} and In(1)B^{M2} have been studied. The stocks are marked as follows (for details of genetic markers, see ref. 13):

1. In(1)B^{M1}, *B*^{M1} (tan-like)
2. In(1)B^{M2}, *r*^{v} *B*^{M2}

The stocks, obtained from *Drosophila* stock center at CALTECH, Pasadena, have been maintained under standard laboratory conditions. The flies of In(1)B^{M1} and In(1)B^{M2} stocks were raised at 24° ± 1°C and eggs collected from healthy flies on agar-cornmeal-brown sugar-yeast food at intervals of 1 hr. After hatching at 24°C, the larvae were reared either at 24° or at 10°C (see ref. 14 for details). The two inversion stocks will be referred to as B^{M1} and B^{M2}, respectively.

For studying the salivary gland polytene chromosomes of these 2 inversion strains, the salivary glands from 24° or 10°C grown mature third instar larvae of B^{M1} and B^{M2} were excised in Poels' salt solution[15], fixed with 1:3 acetomethanol, stained with aceto-orcein-carmine stain, and squashed in 50% acetic acid. These temporary squash preparations were observed under phase contrast optics. For analyzing the fluorescence patterns of salivary gland chromosomes in the two inversion strains, the salivary glands from mature third instar larvae of B^{M1} and B^{M2} grown at 24° or at 10°C, were fixed and squashed (without staining) as mentioned above. After removing the cover glasses the chromosomes were stained with an aqueous solution of Hoechst 33258 (5 μg/ml) for 5 min and examined as described earlier[16].

For studying the replication patterns, the salivary glands from mature third instar larvae of B^{M1} and B^{M2} reared at 10°C, were pulse labelled with ^{3}H-thymidine (Activity 250 μCi/ml; Sp. Act. 15.8 Ci/mM, BARC, Bombay) for 10 min, fixed, squashed and processed for autoradiography as described earlier[17]. To study the transcription patterns, the salivary glands from 10°C reared mature third instar larvae of B^{M1} and B^{M2} were excised and pulse labelled for 10 min and processed for autoradiography. The autoradiographic exposure time was 9 and 7 days, respectively, for ^{3}H-thymidine and ^{3}H-uridine labelled preparations.

Results

X-chromosome morphology at low developmental temperature—In the B^{M1} and B^{M2} inversions, the region 16A2-5 to 20B and 16A2-5 to 20E, respectively, of the normal sequence of the X-chromosome is inverted[13]. In aceto orcein-carmine stained squash preparations of salivary glands from 24°C reared B^{M1} and B^{M2} larvae, the inverted region (16A2-5 to 20B in B^{M1} and 16A2-5 to 20E in B^{M2}) normally appears like a ring with both ends attached to the chromocenter; occasionally, one end remains free while in some nuclei this segment appears away from the chromocenter, either in the form of a closed ring or with the ends remaining free. When detached from the chromocenter, the inverted region or the ring is usually found to carry a small block of β-heterochromatin. The remaining non-inverted part, i.e. region 1A-15F, projects out free from the chromocentre. The inverted and the non-inverted regions of the X-chromosome of B^{M2} female and B^{M1} male larvae (24°C reared) are shown in Figs. 1a, b, respectively. The salivary gland polytene chromosomes from cold-reared larvae appear much thicker[14,18]. Unlike the female X-chromosome (Fig. 1c), the male X-chromosome of cold grown B^{M1} and B^{M2} larvae exhibits varying degrees of alterations

in morphology. In 10°C reared B^{M1} male larvae the inverted as well as the non-inverted regions of the X-chromosome appear somewhat diffused (Fig. 1d) throughout their length. In the case of 10°C reared B^{M2}, although the organization of the X-chromosome in female is similar to that observed in cold-reared B^{M1}, the X-chromosome in male exhibits marked differences in its morphology. In addition to the "normal-looking" X-chromosome comparable to that seen in cold-reared B^{M1}, the male X-chromosome in B^{M2} shows another structural form (see Fig. 1e-f) which is referred to as the "highly diffused" or "pompon-like" X-chromosome[19]. The "pompon-like" X-chromosome is considerably shortened in length but enlarged in width while no bands can be easily identified except for the very prominent landmarks at 3C and 10F. In such nuclei the inverted region is also comparably disorganized in which none of the bands of the region 16A-20 can be identified. It is significant that the autosomes in these nuclei do not show any difference from those in male nuclei with a "normal-looking" X-chromosome (Fig. 1e, f). Nuclei with the "normal-looking" and with the "pompon-like" X-chromosome are seen in same salivary gland. The "pompon-like" forms of X-chromosome are seen in high as well as low level polytene nuclei. The frequency of the "pompon-like" form is, however, lower than that of the "normal-looking" Xs, since out of 751 nuclei observed from salivary glands of 8 male 10°C reared B^{M2} larvae, 270 (36.0%) nuclei had "pompon-like" X-chromosome, while the remaining 481 (64.0%) nuclei showed the "normal-looking" X. Comparable "pompon-like" forms are never seen in the cold-reared larvae of B^{M1} male or in 24°C reared B^{M2} larvae.

Examination of the Hoechst 33258 (H) fluorescence patterns of salivary gland polytene chromosome preparations from male and female larvae of B^{M1} and B^{M2} reared at 24°C or at 10°C reveals that as in wild-type *D. melanogaster*[16], in B^{M1} and B^{M2} polytene nuclei also two to three more H-bright regions are seen in the generally brightly fluorescing chromocentric heterochromatin (see Fig. 2a, b). However, neither the inverted nor the non-inverted region of the X-chromosome has been seen to carry any of these more H-bright regions (Fig. 2c). A comparison of the H-fluorescence of the various forms of male X-chromosome in salivary gland polytene nuclei of 10°C reared B^{M1} and B^{M2} larvae (Fig. 2d, e) shows that as the degree of band disorganization increases, the characteristic differences in the fluorescence of the different band regions seen in a "normal-looking" X-chromosome (Fig. 2d) are obliterated in the "pompon" form, and even the relatively more bright bands in 3C, 10F-11A, 14AB regions also become feebly fluorescing (Fig. 2e).
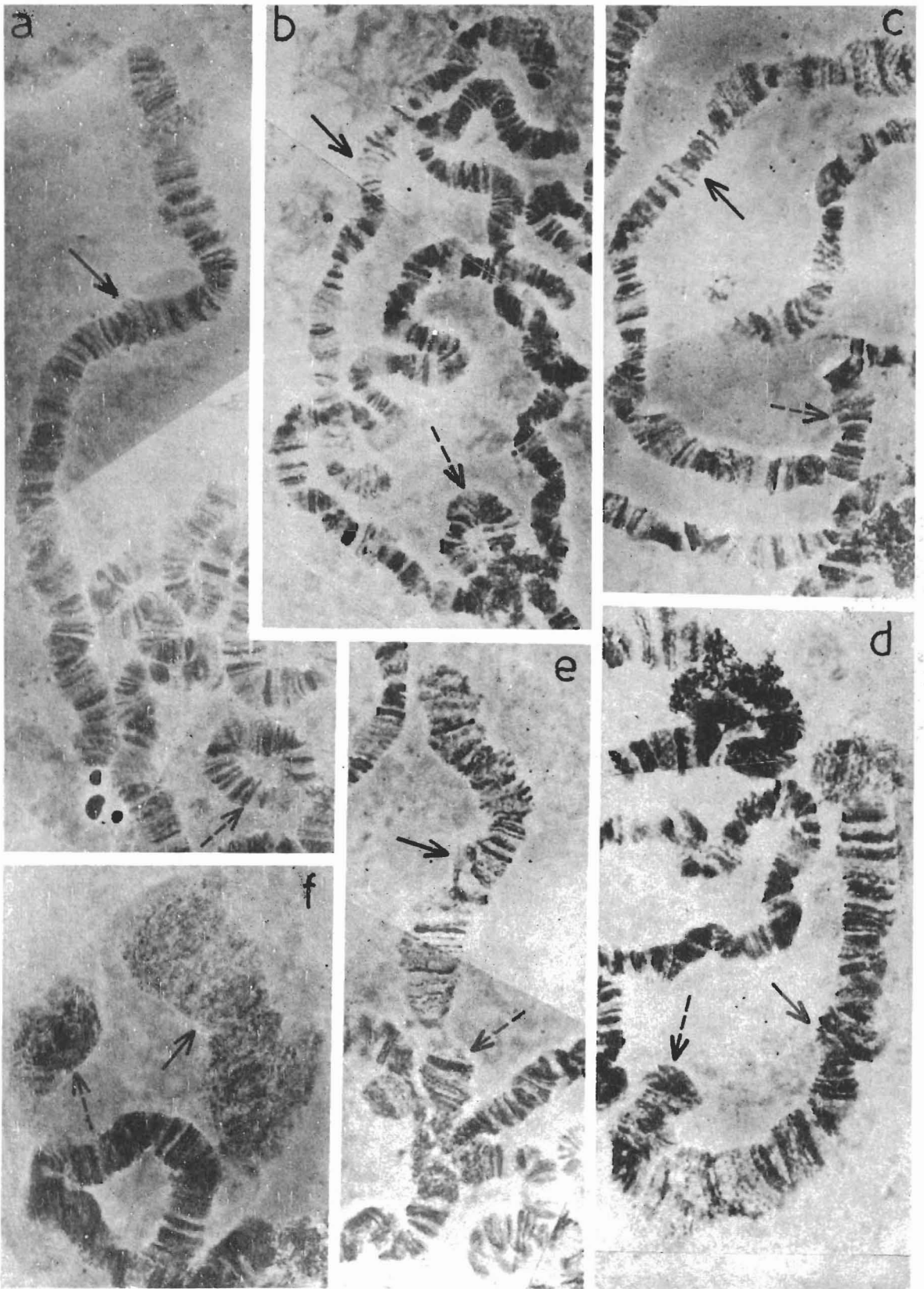
Fig. 1—The non-inverted (solid arrow) and the inverted (broken arrow) segments of X-chromosome from salivary gland polytene nuclei of 24°C or 10°C grown larvae of In(1)B^{M2} (a, c, e and f) or In(1)B^{M1} (b, d). The X-chromosome in cold-reared B^{M2} female larvae (c) appears similar to that at 24°C (a), but in cold-reared B^{M2} males, the X-chromosome assumes a "normal-looking" (e) or "pompon-like" (f) form. In warm (b) as well as cold-reared (d) B^{M1} males, only normal-looking Xs are seen. Magnification, X 1100

Rate of RNA synthesis by the X-chromosome in male and female salivary gland nuclei of B^{M1} and B^{M2} larvae reared at $10°C$—In view of the above noted alterations in the organization of the X-chromosome of the cold-reared larvae of B^{M1} and B^{M2}, the relative transcriptional activity of the X-chromosome in male and female salivary gland nuclei from 10 C reared larvae has been examined after ^{3}H-uridine labelling. The chromosomal segments chosen for this analysis

Fig. 2 Hoechst 33258 fluorescence of the chromocenter (**a, b**) and the Xs of 24 C and 10 C grown In (1)B^{M1} (**c, d**) and In(1)B^{M2} (**e**) larvae. Magnification, X 1060

are 1A-3C and 1A-10F on the X-chromosome, and 58A-60F and 56A-60F on the 2R chromosome. The numbers of silver grains present on these shorter and longer segments of X and 2R in a nucleus have been counted and the mean X/2R grain ratios for the shorter and longer segments calculated. The data are presented in Table 1. It is seen that in both the inversions, the relative rates of X-chromosome transcription remain comparable between male and female polytene nuclei since the X/2R grain ratios for the shorter as well as the longer segments are not significantly different between the two sexes (see Table 1). In the case of B^{M2} male larvae, nuclei with "normal-looking" and "pompon-like" forms of X-chromosome have been analysed separately (Table 1) and the relative rates of X-chromosome transcription in the two forms have also been found to be similar (also see Figs. 3a-c).

Replicative organization of the X-chromosome in B^{M1} and B^{M2} larvae reared at $10°C$—The general features of polytene replication in cold-reared B^{M1} and B^{M2} larvae are seen to be similar to those observed earlier[14] in cold-reared wild type *D. melanogaster* larvae. All the seven categories of the chromosomal labelling patterns seen after a brief pulse of ^{3}H-thymidine to freshly dissected larval salivary glands, observed in earlier studies[14,20,21], could be seen in these two cases also. In the present study, the replication of the X-chromosome in the cold-reared B^{M1} and B^{M2} has been specifically examined. The details of the replicative organization of the individual replicating units in the cold- and warm-reared B^{M1} and B^{M2} larvae will be presented elsewhere; here only the general features of X-chromosome replication are presented.

As seen in polytene nuclei from warm-reared wild-type larvae[11,22,23], in the cold-reared B^{M1} and B^{M2} larvae, the replication phase of the male X-chromosome in the salivary gland polytene nuclei has been found to be always a step ahead of the autosomes. Thus, in most of the cold-reared male nuclei of the two genotypes showing the mid-interband (MIB) type of labelling pattern on autosomes, the X-chromosome exhibits heavy interband (HIB) type of ^{3}H-thymidine incorporation; in a few MIB nuclei, the male X-chromosome also shows MIB type of labelling, but the intensity of labelling on X is seen to be higher than that on the autosomes in the nucleus. In nuclei with Continuous or late Discontinuous types of autosomal labelling also, the male X-chromosome appears in a characteristically advanced stage of replication as in the case of warm-reared larvae[11].

The "pompon-like" X-chromosome in male polytene nuclei in cold-reared B^{M2} larvae is also seen to
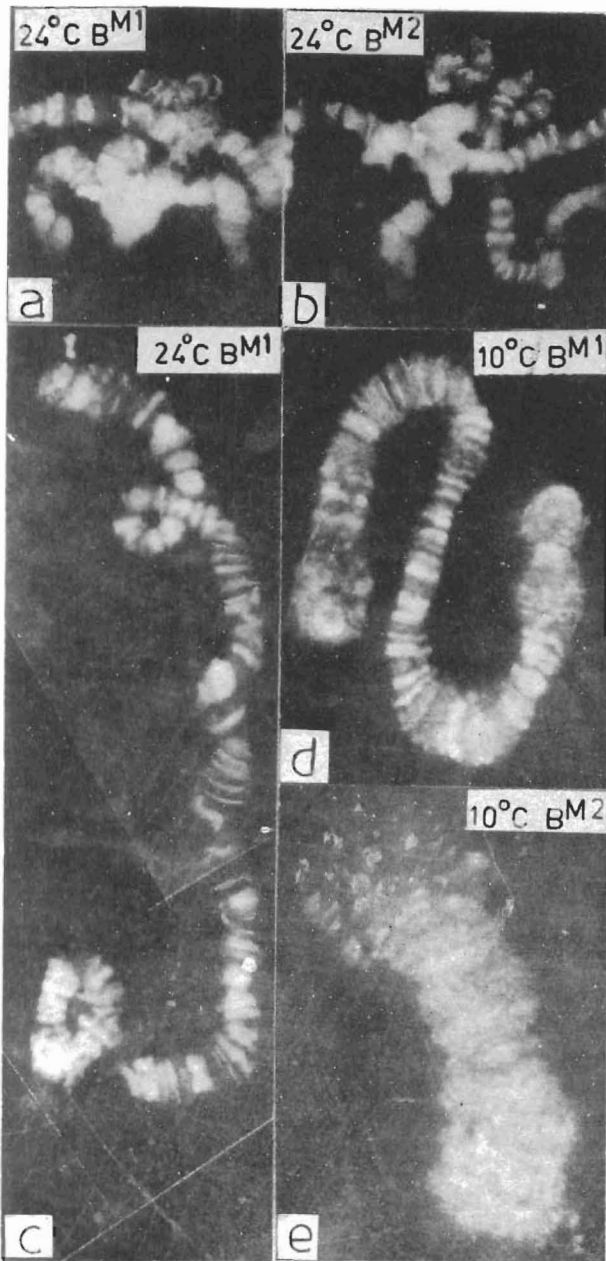
replicate as fast as the "normal-looking" X in B^{M1} male larvae. Two examples are shown in Fig. 4. In Fig. 4a, the autosomes show HIB type labelling while the "pompon-like" X-chromosome shows 2C-3C type; in Fig. 4B, the labelling of autosomes is of 3C type while the "pompon-like" X-chromosome shows 3D-2D type labelling. A comparison of the discretely labelled sites on the "normal-looking" and the "pompon-like" X-chromosomes in 2D or 1D type labelled B^{M2} nuclei also reveals a close similarity between the programme of their replication. As can be seen from Fig. 5, in spite of the indistinct banding pattern in the "pompon-like" X, the discretely labelled sites in the two forms of X-chromosome in 2D type nuclei are comparable.

Following the method of Lakhotia and Mukherjee[11], the numbers of discretely "late" labelled sites on the 2R (56A-60F) and X(1A-15F) segments in different 1D type nuclei from male and female cold-reared B^{M2} larvae have been scored. In the case of preparations from male larvae, nuclei with "normal-looking" and "pompon-like" X-chromosomes were scored separately. The data in Fig. 6 show that in male nuclei with similar number of labelled sites on the 2R segments, the number of discretely labelled sites on the "pompon" and the "normal" Xs are comparable. Furthermore, as is known for warm-reared wildtype larvae[11], both the forms of the male X-chromosomes show fewer number of labelled sites than the Xs in female nuclei with comparable 2R labelling (see Fig. 6).
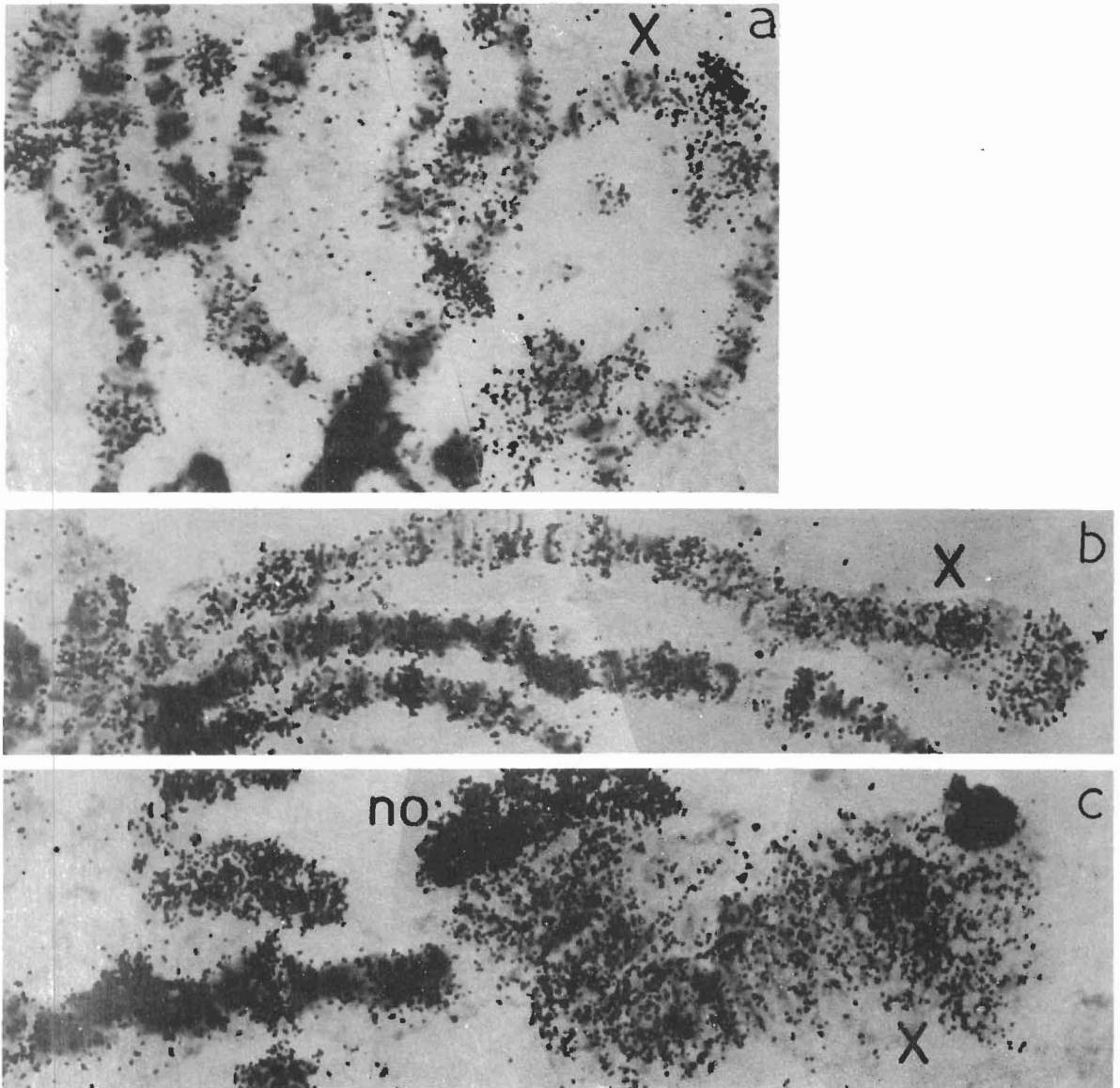

Fig.3 Autoradiograms showing ^{3}H-uridine labelling of X-chromosomes and autosomes in female (a) and male (b,c) polytene nuclei from 10°C reared B^{M2} larvae. The "normal-looking" (b) and the "pompon-like" (c) forms of male X-chromosomes show similar degree of labelling relative to that of autosomes. Magnification, X 1200

A comparison of the rate of ^{3}H-thymidine incorporation in the two forms of X-chromosomes in cold-reared B^{M2} male larvae also shows that the replicative organization of the X-chromosome is not modified due to its altered morphology. Data on the mean numbers of silver grains scored on 1A-15F segment on X-chromosome and on 56A-60F segment of 2R in male and female late 1D type labelled nuclei (showing labelling only on 4 to 6 discrete late replicating sites of 2R, viz., 56AB, 56F, 57AB, 58A, 59CD, 60F) are presented in Table 2. Comparison of the mean grain counts and the 2R/X grain ratios in

male nuclei having "pompon" or "normal" X-chromosome have shown no difference between the two. These grain count data also show that the mean numbers of grains of the 2R segment in these samples of male and female nuclei (Table 2) are comparable, while in the same samples the mean grain count on the Xs of female nuclei is much more than twice that on the single X of male nuclei. This is also reflected in the much higher mean 2R/X grain ratio for male as compared to that for female (Table 2). These differences in male and female B^{M2} larvae are generally similar to those reported earlier for wild-type D. melanogaster[11].
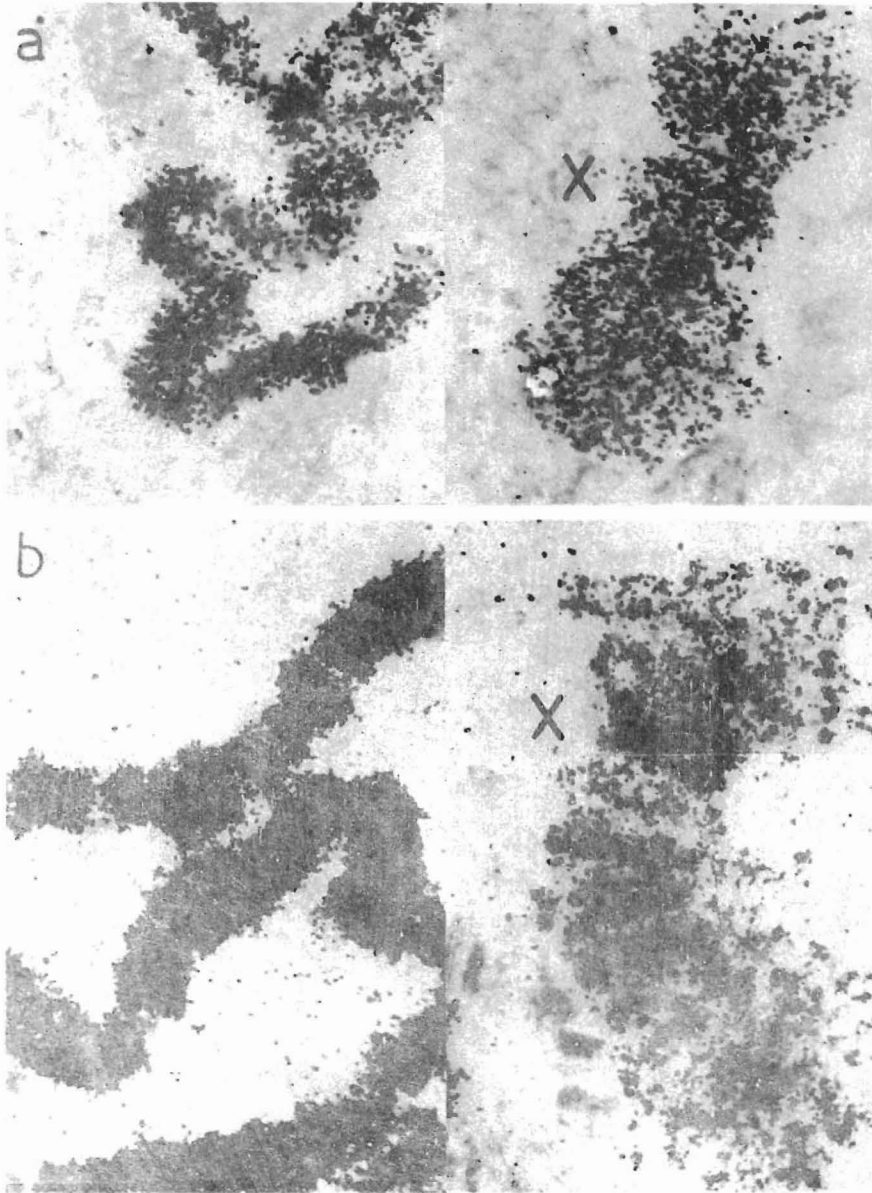

Fig. 4 Autoradiograms of part of two nuclei showing ^{3}H-thymidine labelling patterns of the "pompon-like" X in cold-reared B^{M2} male larvae. In (a) the autosomes are of H1B type, the X is of 2C type; in (b) the autosomes are of 3C type, the X is of 3D-2D type. Magnification, X 1600

Table 1 — ^{3}H-Uridine Labelling of Segments of X- and Right Arm of the Second Chromosome (2R) in Salivary Gland Polytene Nuclei from In(1)B^{M1} and In(1)B^{M2} Larvae Reared at 10°C

Genotype	Sex	Mean ($\pm$SE) No. of grains on				Mean ($\pm$SE) X/2R ratio	
		X		2R		1A-3C/58A-60F	1A-10F/56A-60F
		1A-3C	1A-10F	58A-60F	56A-60F		
B^{M1}	Male	106.07 ± 9.14 (30)	273.17 ± 18.96 (12)	94.40 ± 1.77	157.67 ± 10.29	1.13 ± 0.04	1.75 ± 0.08
	Female	97.86 ± 5.29 (44)	311.63 ± 16.72 (27)	93.20 ± 4.16	186.22 ± 9.60	1.06 ± 0.04	1.69 ± 0.06
B^{M2}	Male (N)	133.04 ± 2.18 (28)	401.46 ± 5.56 (13)	120.61 ± 2.08	258.54 ± 12.24	1.12 ± 0.04	1.57 ± 0.06
	Male (P)	122.53 ± 13.55 (15)	348.58 ± 32.08 (12)	110.47 ± 9.89	220.00 ± 25.26	1.12 ± 0.06	1.64 ± 0.05
	Female	168.35 ± 10.25 (23)	396.17 ± 57.76 (6)	159.83 ± 10.15	241.17 ± 36.92	1.07 ± 0.04	1.70 ± 0.14

The numbers in parentheses indicate the number of nuclei examined.

In B^{M2} male, N and P indicate nuclei with "normal-looking" and "pompon-like" X-chromosome, respectively.

On student's 't' test analysis, no significant difference in the mean X/2R ratio is seen between the two sexes, in both In(1)B^{M1} as well as B^{M2} larvae grown at 10 C

Discussion

The highly disorganized and "pompon-like" morphology of the polytene X-chromosome in 10°C reared B^{M2} male larvae is most interesting. It appears that the "pompon-like" morphology of the X in many nuclei of cold-reared B^{M2} male larvae is an extreme manifestation of the tendency towards greater diffusion of polytene X-chromosome seen in 10 C reared wild type (unpublished observations) or in B^{M1} male larvae. It is remarkable that this drastic disorganization of banding pattern in the male X-chromosome occurs only in B^{M2} but not in B^{M1} larvae, and that too only when they are reared at low temperature. This difference in the response of B^{M1} and B^{M2} inversions is significant in view of their similar euchromatic breakpoints[13] in 16A2-5 region of X-chromosome. Since the two inversions differ in their right hand breakpoints in the basal X heterochromatin[13], it appears likely that the particular heterochromatin breakpoint in B^{M2} inversion is involved in bringing about this alteration in X-chromosome packing in cold-reared male larvae, since earlier cytogenetic studies have adequately demonstrated different effects of different blocks of X-heterochromatin[4,7]. It may, however, be pointed out that although at cytological level the B^{M1} and B^{M2} inversions appear to have similar euchromatin breakpoints in 16A2-5 region, the precise breakpoints within this region in the two inversions may still differ and this difference in euchromatin breakpoint could also contribute to the "pompon-like" morphology in cold-reared B^{M2} male larvae. However, since all nuclei in cold-reared B^{M2} male larvae do not show "pompon-

Table 2 — ^{3}H-Thymidine Incorporation on Segments of X (1A-15F) and 2R (56A-60F) in Late 1D Type Polytene Nuclei of In(1)B^{M2} Larvae Reared at 10°C

Sex	No. of labelled sites on 2R	Mean (+SE) no. of grains on		Mean (±SE) 2R/X grain ratio
		2R	X	
		(56A-60F)	(1A-15F)	
Female	4-6	116.89 ± 8.67 (29)	255.28 ± 21.96	0.48 ± 0.02
Male (Normal)	-do-	99.53 ± 12.05 (15)	61.53 ± 9.24	1.81 ± 0.15
Male (Pompon)	-do-	102.22 ± 19.75 (9)	45.00 ± 7.67	2.19 ± 0.14

Figures in parentheses indicate the number of nuclei examined in each case

On student's 't' test analysis, no significant difference in the rate of replication was noted between the "normal" and the "pompon" forms of X-chromosome

like" morphology, and since this is also temperature dependent, it appears more likely that this particular morphology is related to the heterochromatin breakpoint in B^{M2} inversion. In fact, the "pompon-like" morphology of the male X-chromosome in cold-reared B^{M2} larvae qualifies well to be considered as an example of position effect variegation in which the "phenotype" being modified is the packing of chromatin in the hemizygous X-chromosome. In this context, it is interesting to note that in a related reinversion mosaic stock of In(1)B^{M2}, Majumdar and Mukherjee[24] have also reported comparable mor-
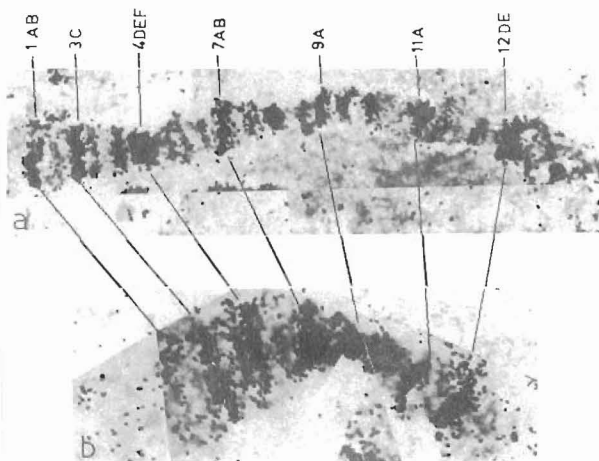
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Fig. 5—Autoradiograms showing "normal" (a) and "pompon-like" (b) X-chromosomes with 2D type labelling from cold-reared B^{M2} male larvae. Note the similarity of discretely labelled sites in the two forms. Magnification, X 1600
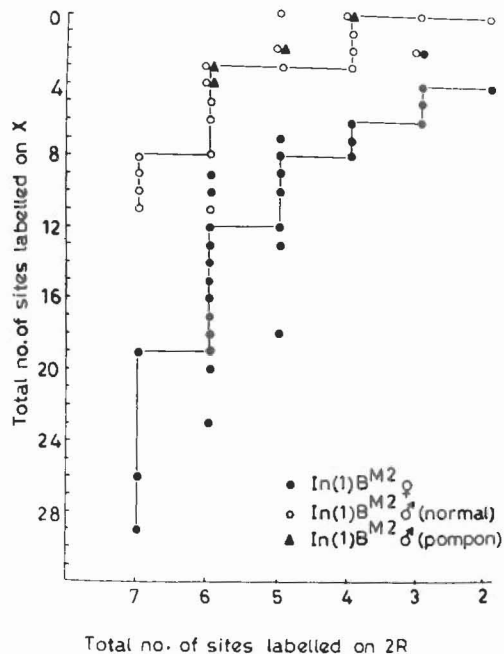

Fig. 6—Graphical representation of the number of labelled sites on the X relative to that on the 2R segment in 1D type labelled nuclei from male and female B^{M2} larvae reared at 10 C

phological variations in the X-chromosome in polytene nuclei of 24 C reared male larvae. Although, in their case, the morphological alterations in male X-chromosome occur under different conditions than the present, the basic cause in both appears to be related to the particular breakpoints in the B^{M2} inversion. The Hoechst 33258 fluorescence analysis reveals that the "pompon-like" morphology of the X-chromosome in some of the cold-reared B^{M2} male nuclei is due to a loose chromatin packing since the intense fluorescence of several compact bands seen normally is not present in the "pompon" form. Apparently the duller fluorescence of these bands is due to differences in packing density of chromatin since Hoechst 33258 fluorescence is modifiable by chromatin condensation[25,26].

The cytogenetic nature of the section 20 in X-chromosome of *D. melanogaster* has been debated[27-30]. Irrespective of whether this region is considered to be heterochromatic, or euchromatic or quasi-euchromatic in cytological terms, this region does contain functional sequences. Our observations on B^{M2} suggest that one of the functions of this region may be to regulate the chromatin packing in the euchromatin part of X-chromosome, particularly in male. The B^{M2} inversion thus provides an unique example of position effect variegation in which chromatin packing along the entire X-chromosome or in specific regions[24] is variably modified.

The "pompon-like" morphology of the X-chromosome in cold-reared B^{M2} male nuclei is comparable in some respects to the changes seen in $l(3)tl$ larvae[19,31-33]. However, in the case of $l(3)tl$ larvae, even autosomes have been seen to assume

similar forms although the male X is the first to get modified. The functional organization of the hemizygous X-chromosome in polytene nuclei of male *Drosophila* is known to be normally different from that of the two Xs in female nuclei and this difference is related to hyperactivity of the male X in relation to dosage compensation[9,10,12,34]. It appears that the selective alteration in the morphology of the hemizygous X in cold-reared male larvae is a consequence of the above basic differences in the organization of the X in male and female polytene nuclei. In several earlier studies also the male X has been found to be more susceptible to alterations in its morphology under different physical, chemical or genetic conditions[19,24,33,35-37]. Probably the less compact chromatin organization of normal male X, required for its hyperactivity[10,38], preconditions this chromosome to respond selectively to different conditions.

The more diffused and "puffy" appearance of the X-chromosome in cold-reared male larvae raises the possibility that the male X in these cases may become still more hyperactive in transcription than at 24°C since the degree of puffing or the enlarged appearance of polytene X in male *Drosophila* has generally been correlated with higher transcription rates[9,10,35,39-41]. In this context, the similar X/2R ^{3}H-uridine grain ratios between male and female polytene nuclei from

cold-reared B^{M1} and B^{M2} larvae are rather unexpected. Likewise the "pompon-like" and the "normal-looking" X in cold-reared male B^{M2} larvae have been seen to transcribe at comparable rates. Thus, in these cases, in spite of the more diffused appearance, the relative rates of transcription are not correspondingly increased. The present results are in agreement with the earlier studies on X-chromosome transcription in $l(3)tl$ larvae of *D. melanogaster*[19] or in *Phryne*[42], where morphological changes in the polytene X-chromosome organization were not associated with corresponding changes in transcription. Apparently the pattern of chromatin dispersion which occurs during puffing or for the normal hyperactivity of the male X, is of a different nature than the chromosomal diffusion seen in the cold-reared male X, although as considered above, the hyperactive organisation of the male X may pre-dispose it to further diffusion under low temperature growth conditions.

Analysis of ^{3}H-thymidine labelled autoradiograms revealed the typical pattern of faster replication of the male X-chromosome in polytene nuclei of the two strains reared at $10°C$ as is observed in $24°C$ grown wild type larvae[11,12]. The "pompon-like" X of B^{M2} did not differ in any way from that of the "normal-looking" X: this is clearly evidenced from the comparable numbers of discretely labelled sites in the "pompon-like" and the "normal-looking" Xs during the late stage of polytene replication cycle and by the comparable rates of DNA synthesis in these two structural forms of the male X-chromosome in cold-reared B^{M2} larvae (see table 2). In an earlier study[23], it was proposed that the polytene X-chromosome in normal male larvae is faster replicating because the lesser number of chromatids and their loose packing facilitates quicker penetration of the enzymes and precursors needed for DNA synthesis. However, the present results show that the comparatively more loose packing of the chromatin material in the highly diffused "pompon-like" X-chromosome does not further accelerate the rate of ^{3}H-thymidine incorporation. In a more recent study, Hägele and Kalisch[43] have also seen that the puffing of a site does not modify the basic programme of replication of the involved site.

Acknowledgement

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