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Conservation of the 93D Puff of *Drosophila melanogaster* in Different Species of *Drosophila*

S.C. Lakhotia and Ajit Kumar Singh
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India

Abstract. Temperature shock (TS) results in activation of a specific set of puffs in polytene nuclei of *D. melanogaster*. Earlier studies in this species from several laboratories revealed certain unique features of the major TS puff at 93D locus, which is also specifically induced by benzamide (BM) and by incubation of glands in heat shocked glands' homogenate (HSGH). We have now extended studies on TS response to several other species of *Drosophila* to ascertain whether loci homologous to 93D puff of *D. melanogaster* are present in other species. In polytene nuclei of two closely related (*D. ananassae, D. kikkawai*) and in two distantly related species (*D. hydei, D. nasuta*), six to nine puffs are induced by TS. Interestingly, in each species one of the major TS puffs, viz., 2L-2C in *D. ananassae,* E-11BC in *D. kikkawai,* 2R-48A in *D. nasuta* and 2–48C in D. hydei, is also specifically induced by BM, autologous species' HSGH and vitamine-B_6 (vit-B_6) treatment. HSGH of a different species fails to induce these puffs. These puffs thus resemble the 93D locus of *D. melanogaster,* although the 93D puff does not respond to vit-B_6. These observations are discussed in relation to the conservation of 93D puff locus in different species of *Drosophila.*

Introduction

The temperature shock (TS) response in different tissues and species of *Drosophila* has been found to be similar (Ashburner and Bonner, 1979). Among the 9 puff sites induced by TS in polytene nuclei of *D. melanogaster,* the 93D puff locus is distinctive (Bonner and Pardue, 1976; Spradling et al., 1977; Ashburner and Bonner, 1979; Mukherjee and Lakhotia, 1979, 1982; Lengyel et al., 1980). Earlier studies in our laboratory have shown that the 93D puff can be selectively induced by benzamide (Lakhotia and Mukherjee, 1980) or by incubation of fresh glands in a homogenate of heat shocked glands (Mukherjee and Lakhotia, 1981). Moreover, our studies also suggest that the 93D transcription products are probably not translated

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(Lakhotia and Mukherjee, 1982). These features make the 93D locus very interesting. Equally interesting is the heat shock induced 2–48C puff in *D. hydei* since this puff can be selectively induced by vitamine-B_6, can regress independent of the other TS puffs and has transcription products with certain unusual features (Leenders and Beckers, 1972; Leenders et al., 1973; Derksen, 1975; Lubsen et al., 1978). These unique features of the 2–48C puff of *D. hydei* led us to suspect that this puff may be similar to the 93D locus of *D. melanogaster*. However, the 93D puff of *D. melanogaster* is not sensitive to vit-B_6 (Derksen, 1975) and also *D. melanogaster* heat shock nuclear RNA does not hybridize to 2–48C puff of *D. hydei;* thus the two sites may not appear to be similar (Peters et al., 1980). Nevertheless, to further explore the possible homology of the 93D puff site of *D. melanogaster* in other species of *Drosophila,* we undertook the present study where the responses of polytene nuclei of four species of *Drosophila,* viz. *D. ananassae, D. kikkawai, D. nasuta* and *D. hydei* to heat shock, benzamide, vitamine-B_6 and homogenate of heat shocked glands have been analyzed. Our observations show that like the 93D puff of *D. melanogaster,* in each of the four species examined, one of the major heat shock puff sites is selectively induced by benzamide and vitamine-B_6 as well as by homogenate of heat shocked glands (same species). These puffs in different species including the 2–48C of *D. hydei,* thus appear functionally homologous to the 93D of *D. melanogaster.*

Materials and Methods

Late third instar larvae of wild strains of four species of *Drosophila,* viz. *D. ananassae, D. kikkawai, D. nasuta* and *D. hydei* have been used. Eggs were collected at hourly intervals in food-filled plastic Petri dishes. Adults and larvae were grown on standard agar-corn meal-brown sugar-yeast food at 24±1° C. Larval cultures were provided with additional yeast suspension for healthy growth. Freshly excised salivary glands from actively migrating late third instar larvae were subjected to the following treatments (also see Table 1). The medium used for dissection and incubation of glands for the treatments included only the inorganic salt constituents of Poels' tissue culture medium (see Lakhotia and Mukherjee, 1980), except in the case of treatment with Pyridoxal-5-phosphate (vit-B_6, see below):

Temperature Shock (TS). Freshly excised sister salivary glands from larvae of each species were separated and incubated either at 37° C or at 24° C for 20 min. Subsequently, the treated (37° C) and control (24° C) glands were labelled with ^{3}H-uridine (300 μCi/ml, sp. act. 10.9 Ci/mM, BARC, Bombay) for 10 min at 37° C or at 24° C.

Benzamide (BM) Treatment. Salivary glands from larvae of four species were dissected. The sister glands were separated and incubated either in medium freshly mixed with benzamide (1 mg/ml) or in benzamide-free medium (control) at 24° C for 10 min. After 10 min the treated and control glands were labelled with ^{3}H-uridine as above. The treated glands were labelled in the presence of BM while control glands were labelled in BM-free medium.

Heat Shocked Glands' Homogenate (HSGH) Incubation. Salivary glands of *D. hydei* or *D. nasuta* were heat shocked at 37° C for 45 min and homogenised as described earlier (Mukherjee and Lakhotia, 1981). Freshly dissected sister salivary glands of *D. hydei* or *D. nasuta* were separated and incubated either in auto- or heterologous species' HSGH at 24° C for 45 min or were incubated in a homogenate of corresponding species' non-heat shocked glands

Table 1. Summary of the treatments applied and the methods of analysis in different species

Treatments	*Drosophila* species			
	D. annassae	*D. kikkawai*	*D. nasuta*	*D. hydei*
Temperature shock (TS)	ARG	ARG	ARG	ARG
Benzamide (BM)	ARG	ARG	ARG	ARG
Heat shocked glands' homogenate (HSGH)				
a) *D. hydei*	–	–	–	ARG
b) *D. nasuta*	–	–	ARG	ARG
Pyridoxal-5-phosphate (Vit-B_6)				
a) Vit-B_6	PS	PS	PS	–
b) Vit-B_6 + TS	PS	–	PS	–
c) Vit-B_6 + BM	PS	–	–	–
d) Vit-B_6 + TS + BM	–	–	PS	–

ARG = Autoradiography; PS = Puff size measurement; – = Experiment not done

Table 2. Summary of the puff sites induced by different treatments in the different species

Treatments	Puff(s) induced				
	D. melano-gaster[a]	*D. ananassae*	*D. kikkawai*	*D. nasuta*	*D. hydei*[b]
TS	2L-33B	2L-*2C*, 5A, 8B, 16A	D-5AB, 9E, *17A*, *20B*	2L-38C	2-*48C*, *32A*, *36A*
	3L-*63BC*, 64F, *67B*, 70A	2R-*4D*, 7C, *11C*	E-*5DE*, *11BC*, *15BC*	2R-*59B*, *48A*	3-31C
	3R-*87A*, *87C*, *93D*, *95D*	3L-6C		3-*62A*, *63AB*, *69A*	4-*81B*, *85B*
		XL-8B			
BM	3R-93D	2L-2C	E-11BC	2R-48A	2-48C
Vit-B_6	No puff induced	2L-2C	E-11BC	2R-48A	2-48C
HSGH					
a) *D. melano-gaster*	3R-93D	–	–	–	–
b) *D. hydei*	No puff induced	–	–	–	2-48C
c) *D. nasuta*	No puff induced	–	–	2R-48A	No puff induced

[a] Information for *D. melanogaster* from Mukherjee and Lakhotia (1979, 1981), Lakhotia and Mukherjee (1980) and Derksen (1975)

[b] Information for TS and Vit-B_6 induced puff sites in *D. hydei* from Berendes et al. (1965) and Leenders et al. (1973). The major TS puff sites in italics

(control gland homogenate, CGH). All sets of glands were subsequently labelled with ^{3}H-uridine as earlier.

After ^{3}H-uridine labelling, the control and treated glands of different experiments were fixed, stained, squashed and processed for autoradiography with Ilford L4 or K5 nuclear emulsions in the usual manner. The autoradiograms were exposed for 4–5 days in dark at

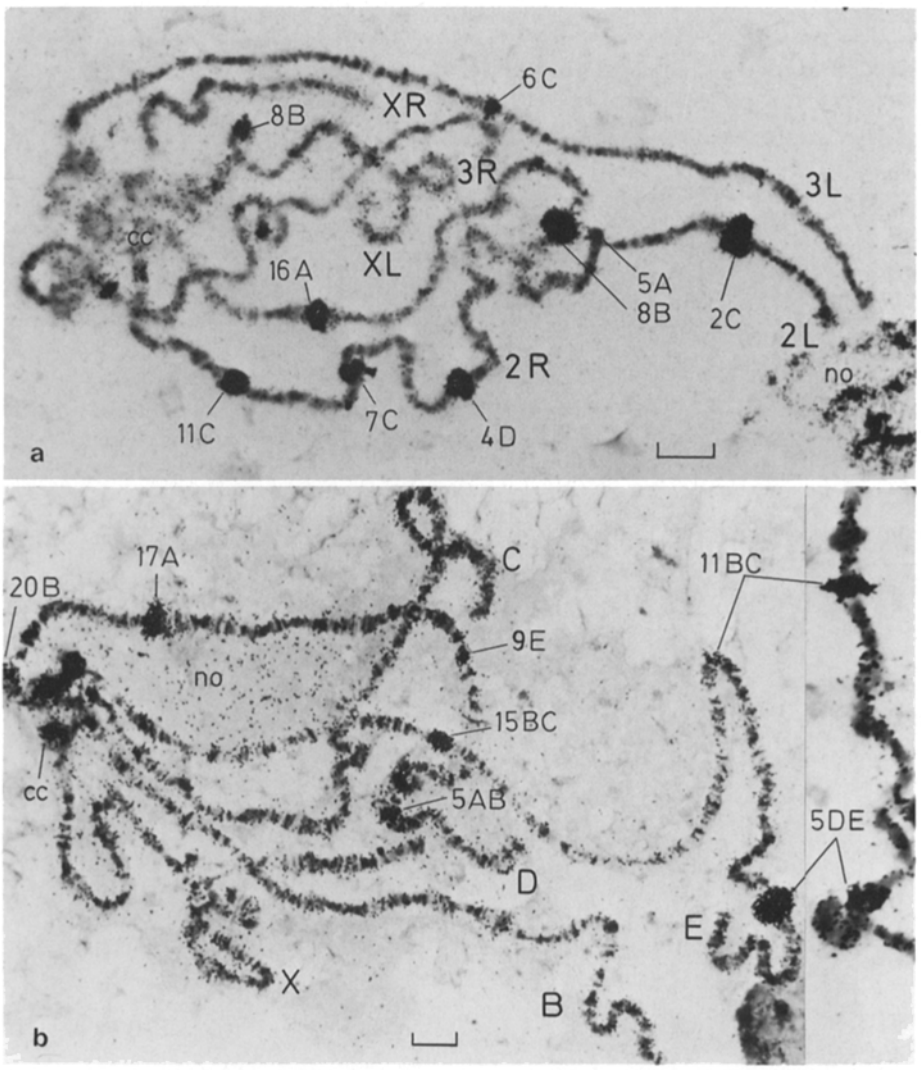

Fig. 1 a–d. ^{3}H-uridine labelled autoradiographs of heat shocked polytene nuclei of **a** *D. ananassae,* **b** *D. kikkawai,* **c** *D. nasuta* and **d** *D. hydei.* The heat shock induced puffs and the chromosome arms in each case are indicated. In **b**, the inset shows a segment of arm *E* from another nucleus in which the 11BC puff is highly induced. *cc* chromocentre, *no* nucleolus. The bar in these and all other figures indicates 10 μm

4–6° C. Slides were developed using D-19b developer and fixed in acid fixer, following which the slides were washed, stained with Giemsa and mounted with D.P.X.

Vitamine-B_6 (vit-B_6) Treatment. Pyridoxal-5-phosphate (vit-B_6, Sigma) was dissolved (2.5×10^{-2} M) in a modified Poels' inorganic salt medium (devoid of KCl and NaCl, see Derksen, 1975). Sister salivary glands from larvae of *D. ananassae, D. kikkawai* and *D. nasuta* were incubated either in vit-B_6 medium (treated) or in medium without vit-B_6 (control) for 30 min at 24° C. After incubation the glands were fixed, stained with 2% acetocarmine and

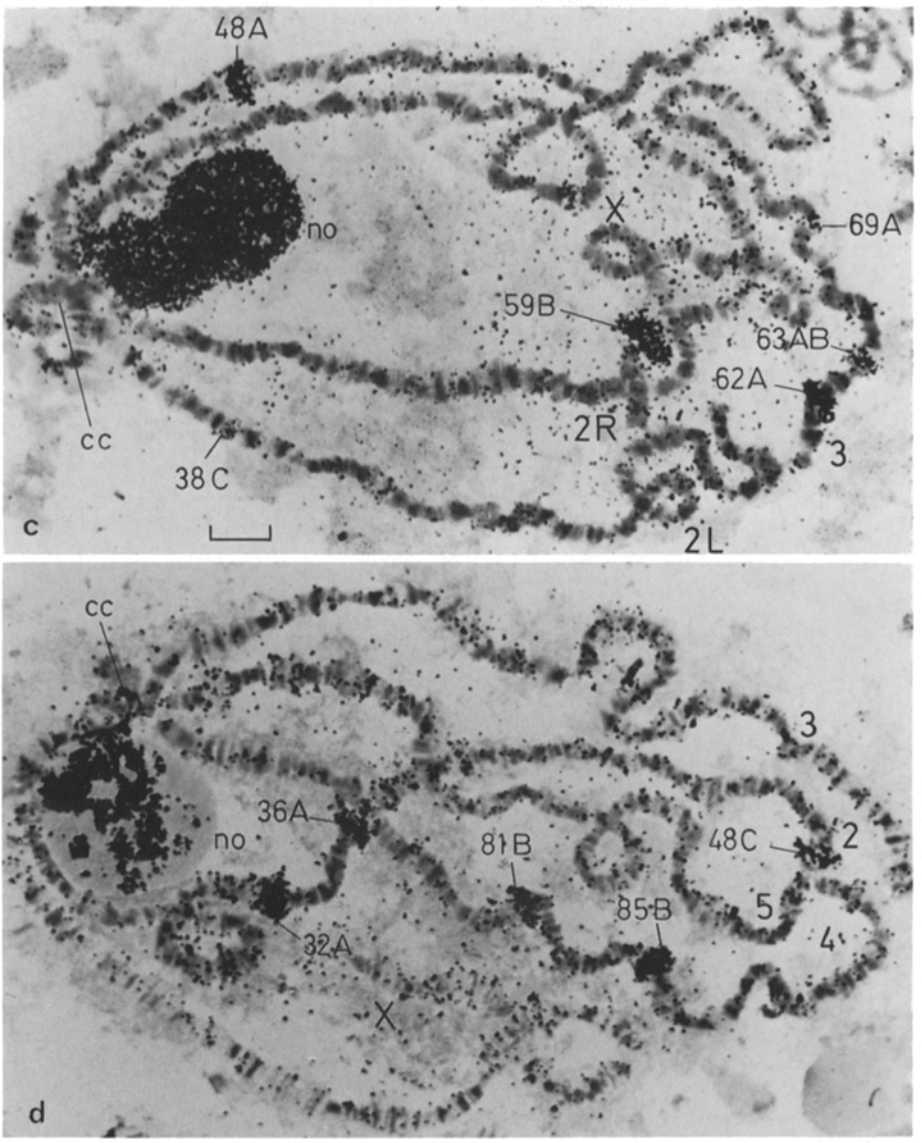

Fig. 1 c, d

squashed in 50% acetic acid. The cover glasses were sealed with nail polish and the preparations were examined under phase contrast optics for measurement of the sizes of induced puff/s.

Vit-B_6, BM and TS Combination Treatments. Larval salivary glands of *D. ananassae* and *D. nasuta* were treated with vit-B_6 (2.5×10^{-2} M) at 37° C (vit-B_6 + TS treatment) for 30 min and squashed for measurement of puff sizes. Salivary glands of *D. ananassae* larvae were also treated with vit-B_6 in the presence of BM (vit-B_6 + BM) for 30 min and the squash preparations were examined for the induced puff/s. In another set, salivary glands of *D. nasuta* were incubated at 37° C for 30 min in a medium containing BM as well as vit-B_6 (vit-B_6 + BM + TS treatment). The puffs induced in this case were also examined in squash preparations.

Table 1 gives a summary of the different treatments applied and the method (autoradiography or puff size measurement) used to assess activity of the induced puff/s.

Observations

The cytological sites affected by the different treatments in the four species have been identified on the basis of the polytene chromosome maps of the four species described earlier by Berendes (1963) for *D. hydei,* Raychaudhuri and Jha (1964) for *D. ananassae* and Roy and Lakhotia (1979, 1981) for *D. kikkawai* and *D. nasuta,* respectively. The cytological locations of the puffs induced by the different treatments in these four species and also in *D. melanogaster* (for comparison) are listed in Table 2. The data on mean ^{3}H-uridine grain counts on the TS puff sites in these species after TS, BM or HSGH treatments are presented in Figure 5. The induced activity of these puff sites after TS (Figs. 1, 2) or benzamide (Fig. 3) or HSGH incubation (Fig. 4) is illustrated in Figures 1–4. Since TS induced puffing activity in *D. hydei* has been described earlier (Berendes et al., 1965), data

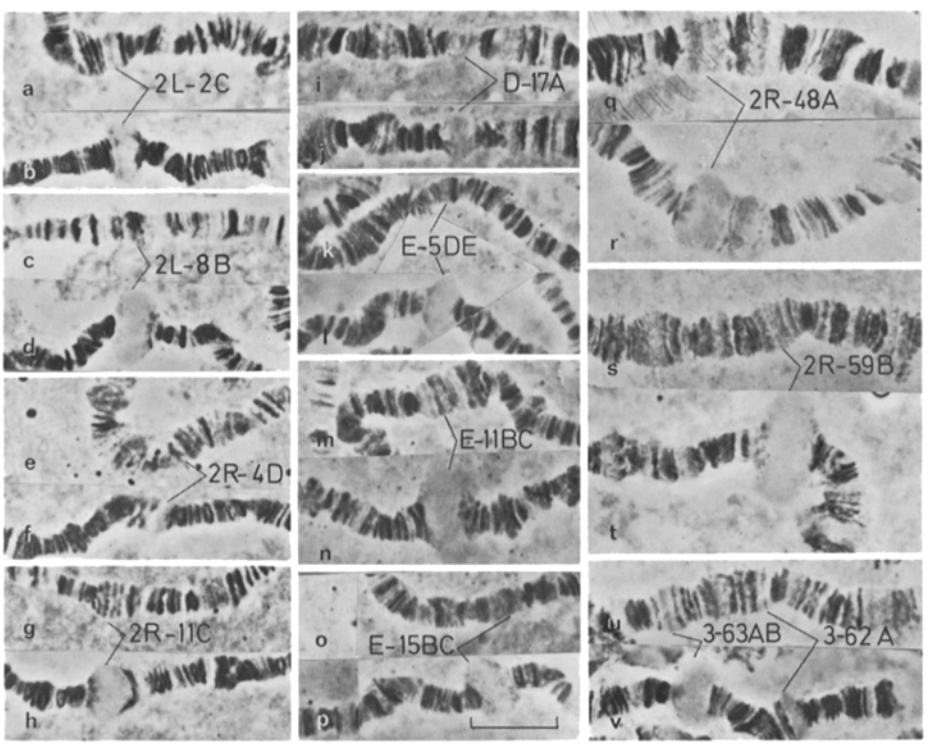

Fig. 2a–v. Phase-contrast photomicrographs of polytene chromosome segments from control and heat shocked glands showing the major TS puff sites in **a–h** *D. ananassae,* **i–p** *D. kikkawai* and **q–v** *D. nasuta.* For each chromosome segment, the upper cut-out shows the morphology in control while the lower shows the TS-induced puff

on TS induced activity in *D. hydei* are not presented here. Our present observations are in agreement with the earlier studies.

We have identified 9 TS puff sites in *D. ananassae,* 7 in *D. kikkawai* and 6 in *D. nasuta,* while 9 and 6 TS puff sites have been described earlier in *D. melanogaster* and *D. hydei* (see Table 2 and Fig. 1). In each species, 4 to 5 sites, located on 2 chromosome arms, form major TS puffs as evidenced by their size (Fig. 2) and ^{3}H-uridine incorporation (Table 2, Figs. 1, 2 and 5). In all species, the TS puffs are restricted to three chromosome arms and only in *D. ananassae,* a small TS puff is seen on the X-chromosome (see Figs. 1 and 5). As in *D. melanogaster,* in these species also TS causes inhibition of chromocentre and general chromosomal RNA synthesis while the nucleolar transcription is not much affected (Fig. 1). However, the inhibition of general chromosomal RNA synthesis in heat shocked glands of *D. kikkawai* is less marked than in the other three species (data not presented).

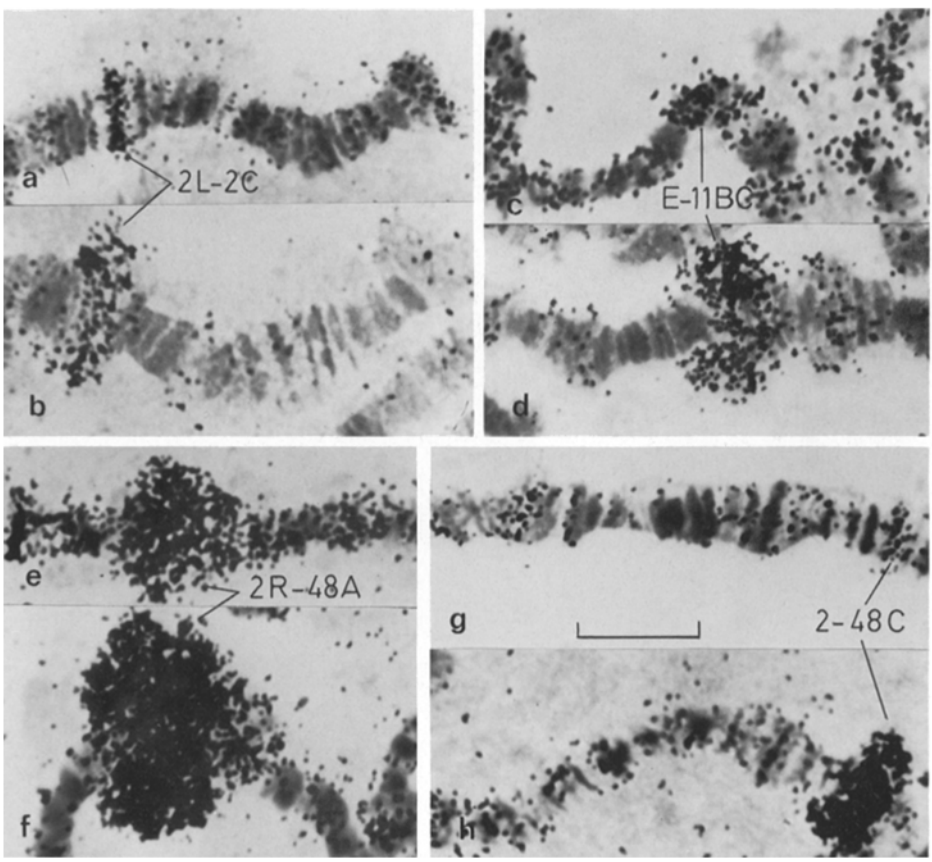

Fig. 3a–h. ^{3}H-uridine labelling of the benzamide-inducible TS puff site in control (**a, c, e** and **g**) and BM-treated (**b, d, f** and **h**) salivary glands of **a–b** *D. ananassae,* **c–d** *D. kikkawai,* **e–f** *D. nasuta* and **g–h** *D. hydei*

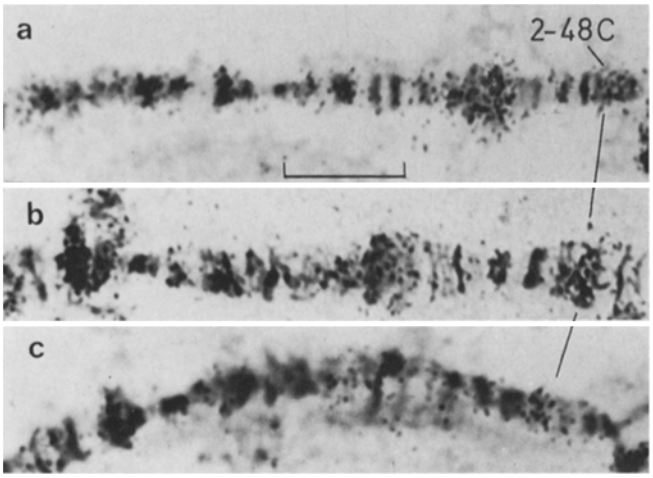

Fig. 4a–c. ^{3}H-uridine labelling of the 2-48C puff site in salivary glands. **a** Incubated in *D. hydei* CGH, **b** incubated in *D. hydei* HSGH, **c** incubated in *D. nasuta* HSGH. Note the increased labelling of 2-48C puff in **b** but not in **c**

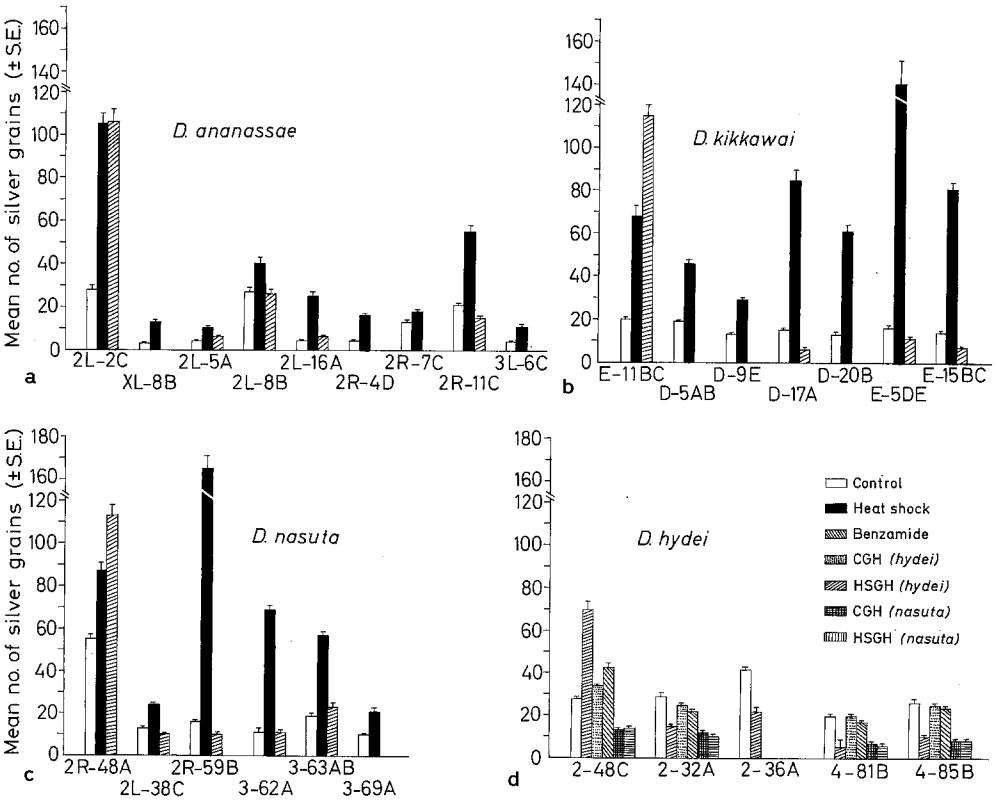

Fig. 5a–d. ^{3}H-uridine incorporation (Mean silver grain counts ± S.E.) on different TS puff sites in control and variously treated salivary glands of **a** *D. ananassae*, **b** *D. kikkawai*, **c** *D. nasuta* and **d** *D. hydei* larvae. In case of BM or HSGH (*D. hydei*) treated glands, data for some of the TS puff sites have not been collected. Each data-point is mean of grain counts scored in 25 to 45 polytene nuclei

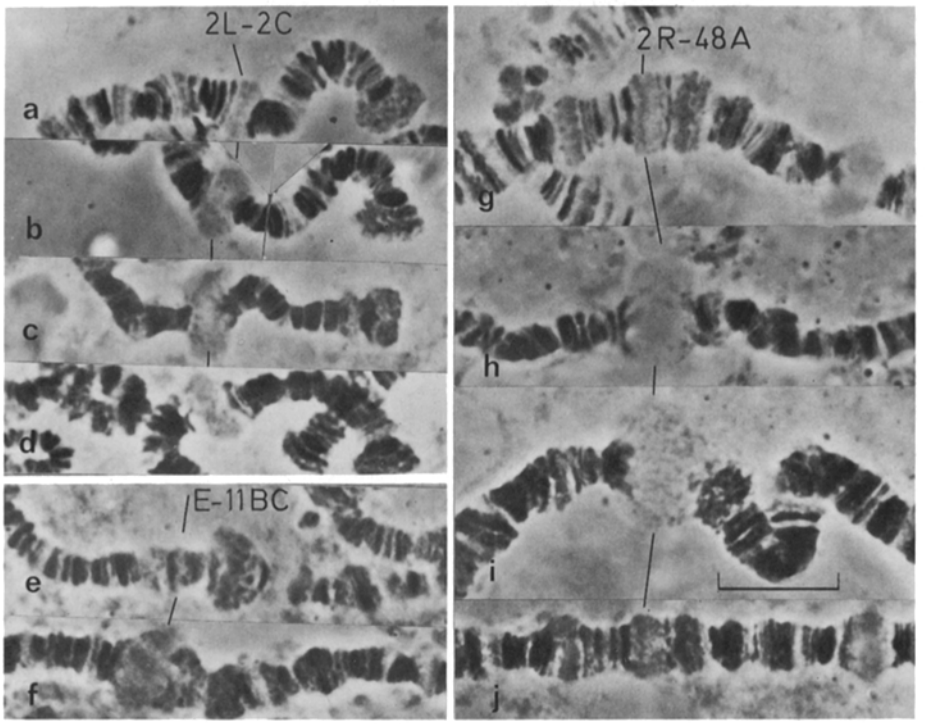

Fig. 6a–j. Phase-contrast photomicrographs showing the effect of vit-B_6 and other combination treatments on the BM-inducible TS puff site in different species. **a–d** *D. ananassae:* Control (**a**), vit-B_6 (**b**), vit-B_6 + TS (**c**) and vit-B_6 + BM (**d**) treated. **e–f** *D. kikkawai:* Control (**e**) and vit-B_6 treated (**f**). **g–j** *D. nasuta:* Control (**g**), vit-B_6 (**h**), vit-B_6 + TS (**i**) and vit-B_6 + TS + BM (**j**) treated. For details of treatments, see text

As in the case of *D. melanogaster* (Lakhotia and Mukherjee, 1980), ^{3}H-uridine incorporation in general chromosomal regions in the BM treated glands of the four species is significantly inhibited while the nucleolar uptake is not affected (detailed data not presented here). However, it is very interesting that in each of the four species one of the major TS puff, viz., 2L-2C in *D. ananassae,* E-11BC in *D. kikkawai,* 2-48C in *D. hydei* and 2R-48A in *D. nasuta* is significantly and singularly induced by BM: while the ^{3}H-uridine grain counts on all other TS loci are reduced in the BM treated glands, the grains on these puffs are increased several fold (Figs. 3 and 5). No other site in any species shows increased activity after BM treatment although a few of the normally active puffs in some cases are not much inhibited e.g. X-7C in *D. nasuta,* 10B and 13B on 3R in *D. ananassae,* and E-11E in *D. kikkawai* (data not presented).

The 2-48C puff of *D. hydei* incorporates significantly more ^{3}H-uridine in glands incubated in *D. hydei* HSGH than in glands incubated in *D. hydei* CGH (Figs. 4 and 5d). None of the other TS puff sites are affected by this treatment (Fig. 5d). *D. nasuta* HSGH fails to induce the 2-48C or any other TS puff site in *D. hydei* (see Figs. 4 and 5d) although *D. nasuta* HSGH induces 2R-48A puff in *D. nasuta* glands (data not presented).

Table 3. Effect of Pyridoxal-5-phosphate (Vit-B_6) and its different combinations with BM and TS on activity of the BM-inducible puff in different species of *Drosophila*

Species and their BM inducible puff	Mean (±S.E.) puff size of the BM-inducible puff after different treatments				
	Control	Vit-B_6	Vit-B_6+TS	Vit-B_6+BM	Vit-B_6+BM+TS
D. ananassae					
2L-2C	2.16±0.07 (39)	3.27±0.13 (35)	3.32±0.12 (24)	2.21±0.11 (23)	–
D. nasuta					
2R-48A	1.44±0.04 (27)	2.01±0.09 (24)	2.25±0.10 (24)	–	1.79±0.08 (22)
D. kikkawai					
E-11BC	1.42±0.06 (25)	2.27±0.10 (29)	–	–	–

(Puff sizes, measured with an ocular disc, represent the ratio of maximum diameter of the puff to that of a specific reference band in each species. The puff sizes in all treated samples except in Vit-B_6+BM experiment are significantly different ($P<0.05$) from corresponding control values)

Measurement of puff sizes in vit-B_6 treated glands reveals that in all the four species, the BM-inducible TS puff site is also selectively induced by the vit-B_6 treatment (see Table 3 and Figs. 5, 6). Other TS puffs are not affected by vit-B_6 (data not presented). It is already known that in *D. hydei,* the 2-48C puff is significantly induced by vit-B_6 treatment (Leenders et al., 1973).

In *D. ananassae* as well as in *D. nasuta,* vit-B_6 treatment at 37° C induces the 2L-2C and 2R-48A puff, respectively, to the same extent as at 24° C since the mean sizes of these puffs are found to be same in the two treatment conditions (Table 3, Fig. 6). However, when vit-B_6 treatment is given to glands of *D. anassae* at 24° C in the presence of BM, the 2L-2C puff remains uninduced as indicated by its size (Table 3, Fig. 6). Interestingly, when vit-B_6, TS and BM treatments are applied simultaneously to glands of *D. nasuta,* the mean size of 2R-48A puff is found to be intermediate between that in control and vit-B_6 treated glands (Table 3, Fig. 6).

Discussion

As expected the different species of *Drosophila* tested here, show comparable changes in the pattern of ^{3}H-uridine incorporation in polytene nuclei when exposed to 37° C. The small but similar numbers of gene loci which are specifically activated at the elevated temperature and their restriction to certain chromosome arms indicate their evolutionary homology. In other species of *Drosophila* also TS puffs have been found on evolutionarily homologous chromosomal arms (Berendes, 1965; van Breugel et al., 1968; Ashburner and Bonner, 1979; Pierce and Lucchesi, 1980). In this context,

the presence of a small TS puff site on the X-chromosome of *D. ananassae* is interesting. In *D. pseudoobscura,* Pierce and Lucchesi (1980) have found TS puffs on one arm of the metacentric X and this is traceable to translocation of an original autosomal arm to the X-chromosome of *D. pseudoobscura* (Sturtevant et al., 1937). However, in the evolutionary history of *D. ananassae* there has been no evidence so far of translocation of any autosomal material onto the X-chromosome, (Patterson and Stone, 1954). A further molecular characterization of the XL-8B puff of *D. ananassae* and its product will help to trace its evolutionary origin. It also remains to be seen if this heat shock locus is dosage compensated like the X-linked TS puffs in *D. pseudoobscura* (Pierce and Lucchesi, 1980).

In order to study the evolutionary conservation of the 93D puff of *D. melanogaster,* we have examined species which are its close or distant relatives. *D. ananassae, D. kikkawai* and *D. melanogaster* belong to the same *melanogaster* species group under the sub-genus *Sophophora* while *D. nasuta* and *D. hydei* are very distant relatives belonging to the sub-genus *Drosophila; D. hydei* and *D. nasuta* are not very closely related to each other also since they belong to *repleta* and *immigrans* species groups, respectively (Patterson and Stone, 1954). The most interesting finding of the present investigation is that irrespective of the phylogenetic relationships, one and the same major TS puff site in each species is inducible by BM, autologous HSGH and vit-B_6 treatments. During earlier studies in our laboratory (Lakhotia and Mukherjee, 1980; Mukherjee and Lakhotia, 1981) it has been seen that the major TS puff locus at 93D in polytene nuclei of *D. melanogaster* is also specifically induced by BM or HSGH treatments. In view of these common inducible features of the major TS puff at 2L-2C in *D. ananassae,* E-11BC in *D. kikkawai,* 2R-48A in *D. nasuta* and 2-48C in *D. hydei,* we believe that these puffs in the respective species are homologous to the 93D puff site of *D. melanogaster.* Results of the combined vit-B_6, TS and BM treatments in *D. ananassae* and *D. nasuta* further reveal similarity of 2L-2C of *D. ananassae* and 2R-48A of *D. nasuta* with the 93D site of *D. melanogaster.* In earlier studies in *D. melanogaster* (Lakhotia and Mukherjee, 1980; Mukherjee and Lakhotia, 1982) it was seen that when two treatments which individually induce the 93D puff are applied simultaneously, there is no additive effect on 93D induction, rather the 93D puff is actually regressed in most cases. In parallel with these we find that after simultaneous vit-B_6 and BM treatments, the 2L-2C puff of *D. ananassae* fails to be induced at all, while combined vit-B_6 and TS treatment does not cause the 2L-2C puff to be induced to a greater degree than after any one of the treatments alone. Similarly in *D. nasuta* glands exposed to vit-B_6 and BM at 37° C, the 2R-48A puff is induced to a lesser extent than after any one of the treatments alone. As in the case of the 93D puff of *D. melanogaster,* the BM-inducible puff sites in each species are also developmental puffs as evidenced by ^{3}H-uridine uptake at these loci in the control glands (Fig. 5). Again like the independent response of the 93D puff to TS (Mukherjee and Lakhotia, 1979), these particular puff sites in the respective species show a variable degree of activity in different heat

shocked nuclei (data not presented, but see Fig. 1b). In view of the above similarities the absence of any effect of vit-B_6 on the 93D or any other puff site of *D. melanogaster* (Derksen, 1975 and our own observation) is curious, more so since two of its close relatives, *D. ananassae* and *D. kikkawai,* respond to vit-B_6 in the same manner as the more distant relatives. Therefore, the insensitivity of the 93D puff of *D. melanogaster* to vit-B_6 seems to be an exceptional feature.

The absence of vit-B_6 induced puffing activity in *D. melanogaster* and the failure of the heat shock induced *D. melanogaster* nuclear RNA to hybridize in situ with 2-48C or any other site in polytene nuclei of *D. hydei* led Peters et al. (1980) to conclude that a site homologous to 2-48C does not exist in *D. melanogaster*. Nevertheless, our present observations and the other available information on the transcription products of these two puffs (Bisseling et al., 1976; Spradling et al., 1977; Lubsen et al., 1978; Peters et al., 1980; Lengyel et al., 1980), strongly suggest homology of the 93D puff of *D. melanogaster* and the 2-48C puff of *D. hydei.* The heat shock induced transcription products of both these loci share similar unusual features in being mainly intranuclear and poly A^- (Bisseling et al., 1976; Spradling et al., 1977; Lubsen et al., 1978; Lengyel et al., 1980; Peters et al., 1980). Indirect considerations also suggest that at both these loci, there may be sequence repetition and also more than one kind of transcriptionally active sequences are located at 2-48C or 93D loci (Lubsen et al., 1978; Peters et al., 1980; Lengyel et al., 1980). In addition to these similarities in the nature of their products, the *ebony* locus is localized in the 93D region in *D. melanogaster* and in 2-48C region of *D. hydei* (Scalenghe and Ritossa 1977; Peters et al., 1980). In spite of the apparent cytogenetic homology of these loci in the two species, it appears that their DNA sequences have rapidly diversified so that RNA transcripts of one do not hybridize with the other (Peters et al., 1980). In this context, it is also noteworthy that while Brady and Belew (1981) and Belew and Brady (1981) have obtained evidence that vit-B_6 induced activity of 2-48C puff of *D. hydei* results in increased synthesis of a 40 KD protein (tyrosine aminotransferase), in *D. melanogaster* no novel polypeptide/s could be identified after BM or HSGH induced activity of the 93D puff (Lakhotia and Mukherjee, 1982). In view of all these, it is possible that the different agents (TS, BM, HSGH and vit-B_6) that induce puffing at the same cytological site, actually elicit transcription of different sequences which for some reason are always clustered at one site in all these species. As suggested by Peters et al. (1980) these sequences, which for most part are probably not translated, may undergo rapid diversification in evolution. Our results show that in spite of the possible sequence diversification, they continue to exhibit similar response to given inducing agents. In this context, our observations on the inducibility of the BM-puff site by auto- or heterologous species' HSGH are interesting. We have presented data to show that the 2-48C puff of *D. hydei* is inducible by *D. hydei* HSGH but not by *D. nasuta* HSGH. We have also seen (data not presented) that the 2R-48A puff of *D. nasuta* is inducible by *D. nasuta* HSGH but not by *D. hydei* HSGH. Likewise,

the 93D puff of *D. melanogaster* is not inducible by *D. nasuta* or *D. hydei* HSGH (data not presented). Obviously, whatever factor in the HSGH leads to induction of the BM specific puff, it is species specific. This species specificity of the HSGH effect may be related to rapid sequence diversification that is presumed to occur at these loci (Peters et al., 1980). At this stage we do not know if the insensitivity of the 93D puff of *D. melanogaster* is due to absence at 93D site of sequences coding for the 40 KD polypeptide (Brady and Belew, 1981) or is due to absence of other sequences which mediate the vit-B_6 effect. We are trying to analyse this aspect.

The function/s of the 93D puff of *D. melanogaster* are not known but our present studies have revealed that each of the other closely on distantly related species of *Drosophila* examined, has one locus whose response to inducing agents are similar to those of the 93D locus. If the sequences at these loci diverge as rapidly as suggested by the results of Peters et al. (1980), the functional significance of such "conserved" loci becomes more mysterious and challenging for further studies.

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Note Added in Proof

The 93D puff of *D. melanogaster* has been found (T. Mukherjee and S.C. Lakhotia, unpublished) to be specifically induced by colchicine or colcemid and thus appears to be homologous to the 20CD heat shock puff of *D. virilis* since this puff is also inducible by colchicine as well as by vit-B_6 (see Ashburner and Bonner, 1979).