Chromosoma (Berl.) 81, 281–306 (1980)

CHROMOSOMA

Cytogenetic Analysis of the 2B3-4–2B11 Region of the X-Chromosome of *Drosophila melanogaster*

I. Cytology of the Region and Mutant Complementation Groups

E.S. Belyaeva[1], M.G. Aizenzon[1], V.F. Semeshin[1], I.I. Kiss[2], K. Koczka[2], E.M. Baritcheva[1], T.D. Gorelova[1], and I.F. Zhimulev[1]

[1] Institute of Cytology and Genetics of the Academy of Sciences of U.S.S.R., Siberian Branch, Novosibirsk, 630090, U.S.S.R.; [2] Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Abstract. There are, at least, 11 distinct single bands and one real doublet in the region 2B1-2–2C1-2 of the X-chromosome of *Drosophila melanogaster*. This figure coincides with that in Bridges' revised map with most of the "doublet" bands being artifacts. Three puffs appear in the region. The early ecdysone-specific puff 2B5-6, small at PS(puffing stage) 1, increases sharply at PS 2. The late ecdysone-specific puff 2C1-2 appears at PS 4–5. At PS 9–10, when 2B5-6 disappears completely and 2C1-2 decreases, a third puff at 2B11 appears. None of these puffs is active at PS 11. Morphological analysis of puff appearance and autoradiographic study of ^{3}H-uridine incorporation into chromosomes carrying rearrangements within the 2B region suggest that the early ecdysone-specific puff derives from bands 2B5 and may be 2B6, while the neighbouring bands 2B1-4, and 2B7-10 do not show appreciable transcription at the investigated stages.

There are 42 mutations affecting viability in the region where the 2B5-6 puff is located. The mutations belong to 6 complementation groups; two of the groups *dor* and *swi,* are independent while the rest are overlapped by several lethal mutations (overlapping complex.) Mutants of the different groups have series of similar characteristics: temperature sensitivity, dose sensitivity, larva-pupal lethality and similar morphological abnormalities. It can be assumed that there is a functionally linked cluster of genes within the region 2B. Complementation groups *br, rbp, l*(1)*pp*-1, *l*(1)*pp*-2 (overlapping complex) have been located by rearrangements in very narrow cytological limit 2B3-4–2B5 that is in the area of developing puff. Two other loci *dor* and *swi* are situated some to the right of 2B5.

Introduction

Beermann (1952, 1962) first concluded that puffs on the polytene chromosomes develop through decondensation of single bands. These observations and assumption that one band represents one genetic function led to the idea that

0009-5915/80/0081/0281/$05.40

one puff is morphologically equivalent to one gene (Clever, 1964; Pelling, 1972). However, the "one band – one gene" hypothesis has not yet received a firm factual foundation, and its general extension to all the bands remains questionable (Lefevre and Wiedenheft, 1974; Belyaeva et al., 1976; Judd, 1976; Jarry, 1979). The problem of the informational content of the puffs is further complicated by the observations that, n certain cases, decondensation also extends into a series of neighbouring bands (Mechelke, 1961; Panitz, 1964; Kiknadze, 1972; Berendes et al., 1974) and, following a pulse of ^{3}H-uridine, the label simultaneously appears over all the bands (Berendes et al., 1974; Belyaeva and Zhimulev, 1976).

Firm evidence has already been presented in several cases for a direct relationship between puffing activity and synthesis of specific proteins: puffs 3C11-12 and 68BC in *D. melanogaster* were shown to contain genes controlling the synthesis of some of the salivary glue proteins (Korge, 1977; Akam et al., 1978).

"Heat shock" puffs were shown to contain genes coding for heat-induced proteins (Ashburner and Bonner, 1979, review). However, relating the puffs to the synthesis of specific proteins does not entirely solve the problem of their genetic organization; the possibility remains that they also contain other genes not expressed in the protein spectrum. The above problems can be elucidated only by genetic analysis.

In the present study, we attempted to unravel the genetic content of the 2B3-4–2B11 region on the X chromosome of *D. melanogaster* by saturating it with lethal mutations. The region holds the 2B5-6 puff, which belongs to the "early ecdysone-specific" group induced by the rising ecdysone concentration just before puparium formation (Becker, 1959; Ashburner, 1975).

Materials and Methods

Drosophila Cultures. They were maintained on a standard yeast-cornmeal-agar medium at 25° C.

List of the Stocks Used. This is shown in Table 1. For the genetic symbols, see Lindsley and Grell (1968). Cytological information is given in Results.

Chromosome Preparations for Light Microscopy. There were made from the salivary glands of larvae and prepupae of different ages, as described earlier (Zhimulev and Belyaeva, 1974).

Fixation for Electron Microscopy (EM). Either a mixture of ethanol: acetic acid (3:1) or 45% acetic acid (Kerkis et al., 1975) was used for fixation. A description of the puffing activity was based on the puff stages (PS) after Ashburner (1975). Chromosomal regions were identified according to the revised map of Bridges (Lindsley and Grell, 1968).

Labelling of the RNA Synthesis. This was done by incubating freshly dissected salivary glands for 5 min in a solution of ^{3}H-uridine (100 μCi/ml, 44 Ci/mM, Amersham) followed by autoradiography (Zhimulev and Belyaeva, 1974)

Mutations in the 2B Region. These were isolated in two ways. The first scheme was suggested by M.M. Green (Fig. 1a).

Females carrying attached X-chromosomes and a Y-chromosome with the duplication Dp(1)y^2Y67g were crossed to y/Y males that had been treated for 18 h with ethyl methanesulphonate (EMS) (0.025 M EMS in 1% sucrose solution; Lewis and Bacher, 1968). In the F_1 generation,

Table 1. List of the stocks used

Stock	Source	Reference
giant ($gt\ w^a/gt^{13z}$)	T. Kaufman	Kaufman, 1972
Df(1)*pn*7b/FM-6, $y^{31d}sc^8dm\ B$	Obtained in our laboratory	
T(1;3)*sta*/FM-6, $y^{31d}sc^8dm\ B$	I.I. Oster	Lindsley and Grell, 1968
Df(1)A94/FM-6, $y^{31d}sc^8dm\ B$	G. Lefevre	
Df(1)RA19 v^{74k}/FM-6, $y^{31d}sc^8dm\ B$	J.K. Lim (from G. Lefevre originally)	
Df(1)S39/FM-6, $y^{31d}sc^8dm\ B$	L. Craymer (from G. Lefevre originally)	
Dp(1)y^2Y67*g*24.2/$\overline{XX}$	M.M. Green	
Dp(1)y^{59b}Y (2)/$\overline{XX}$	M.M. Green	
Dp(1)y^2Y67g/$\overline{XX}$	J.K. Lim (from M.M. Green originally)	
Dp(1;f)101	I.I. Oster	Lindsley and Grell, 1968
Dp(1)y^2Y21T	Obtained by T. Gorelova	
Dp(1)y^2Y53T	Obtained by T. Gorelova	
Dp(1)$y^2$40T	Obtained by T. Gorelova	
T(1;3)2B7; 84A5/sc^{S1}In(1)dl49	H.J. Becker	
br Pgd$^-$ *pn*	V.A. Gvozdev	Lindsley and Grell, 1968
$br^3dx^{st}ed\ su(dx)^2$	L. Craymer	Lindsley and Grell, 1968
$br^4 = br^{59j}$	M.J. Fahmy	Lindsley and Grell, 1968
dor/ClB	Bowling Green Stock Center	Lindsley and Grell, 1968
y dor^1/FM-6 l^{69j}	V.A. Gvozdev	
dor^{66g}/C(1)RM	J.C. Lucchesi (from G. Lefevre originally)	
$dor^{l69F}/y^2w^av\ B$	J.C. Lucchesi	
l(1)*d.norm.*-1^a, *y w sn*^{3}/Binsn	J.W. Fristrom	Stewart et al., 1972
sc ec cv pt v g^3		Lindsley and Grell, 1968

only those males survived that had no lethal mutation on the X-chromosome or that had one within the region covered by the duplication (Fig. 1aI). All surviving males were pair mated to females heterozygous for a deficiency for 1E5–2B11 (Fig. 1aII). If the EMS-treated X-chromosome carried a lethal mutation within this region *yl*/Df(1)RA19 females, having normal (B^+) eyes, do not appear among the offspring.

In the second scheme, the EMS-treated *y*/Y males were crossed to homozygous *Basc*/*Basc* females (Fig. 1bI). The F_1 females were pair mated to males with an X-chromosome deficient for the 1E5–2B11 region (Fig. 1bII). If the female had any lethal mutation within the region of the deficiency, no females with normal (B^+) eyes appeared in the offspring.

Mutations *l*(1)*npr*-1 (non-pupariating), *l*(1)*npr*-2 and *l*(1)*d.norm.*-24 were also induced with EMS (Kiss et al., 1976a).

The mutations were balanced over the FM-6 l^{69j} or the Binsn balancer chromosomes.

Cytological Localization of the Mutations. *yl*/FM-6 l^{69j} heterozygous mutant females were crossed to males carrying different deletions or duplications (Fig. 10). If the mutation was located within the region covered by the appropriate deletion or duplication, females with normal (B^+) eyes or males were absent or present in the offspring, respectively.

Complementation Tests. Females heterozygous for a lethal mutation were crossed to males carrying another lethal mutation and a Y-chromosome with Dp(1)y^2Y67g.

Each cross was made at 18° C, 25° C and 29° C. In the case of the *dor* and *swi* groups,

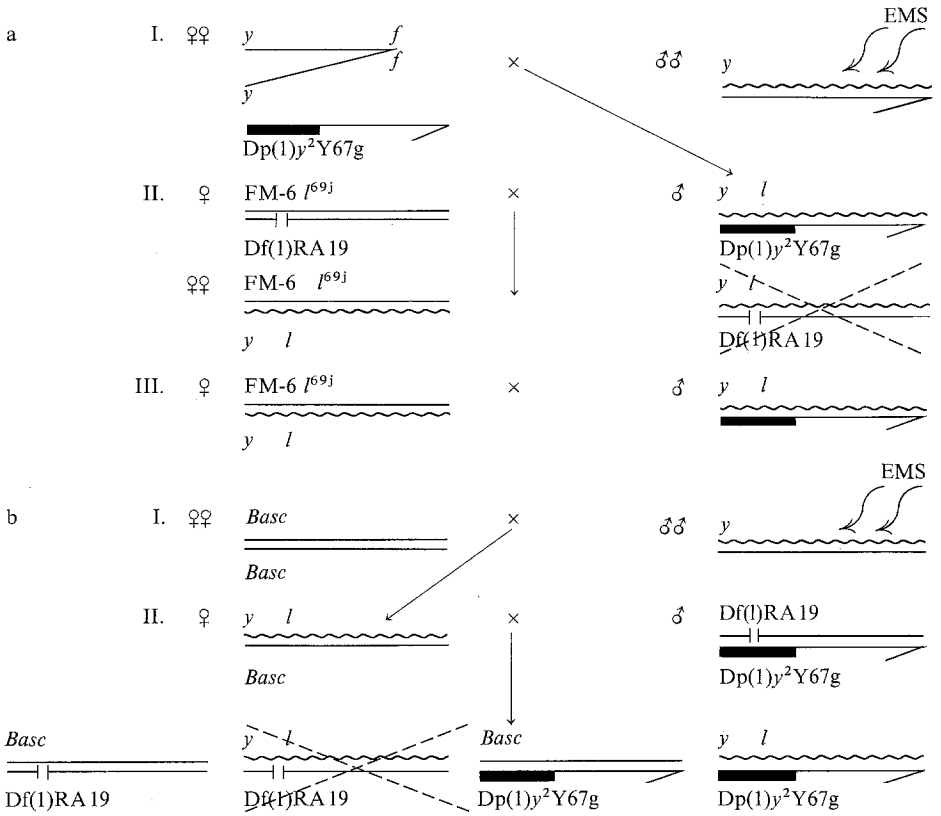


Fig. 1. Induction and recovery of mutations in the 1E5–2B11 region

the crosses were made only in one direction, while the other four groups were tested both in direct and in reciprocal crosses.

Genetic mapping of *l*(1)*npr*-2 was accomplished by crossing *y sc npr*-2 *w*/+++ heterozygous females of wild-type males. The number of males of each genotype was counted and used to calculate the recombination frequencies.

Results

Number and Morphology of the Bands in the 2B1-2 to 2C1-2 Interval

According to the map of Bridges (see Lindsley and Grell, 1968), this region contains 20 bands, 7 of which are doublets. In an EM study, Sorsa (as quoted by King, 1975), found 11 single and one double (2C1-2) bands. Berendes (1970) also studied the region, but did not determine the exact number of the bands. Ananiev and Barsky (1978), using chromosomes stretched with the aid of a micromanipulator, counted only 8 single bands in the region.

Our EM results confirmed the validity of the map of Bridges, with the exception of the double bands. The doublet appearance of certain bands as observed by Bridges was probably a consequence of the long exposure to acetic acid during fixation (Semeshin et al., 1979). All the thick bands of the region, except 2C1-2, seemed to be homogenous (i.e., single) when fixed in the alcohol: acetic acid (3:1) mixture, but appeared to be a doublet after fixation in acetic

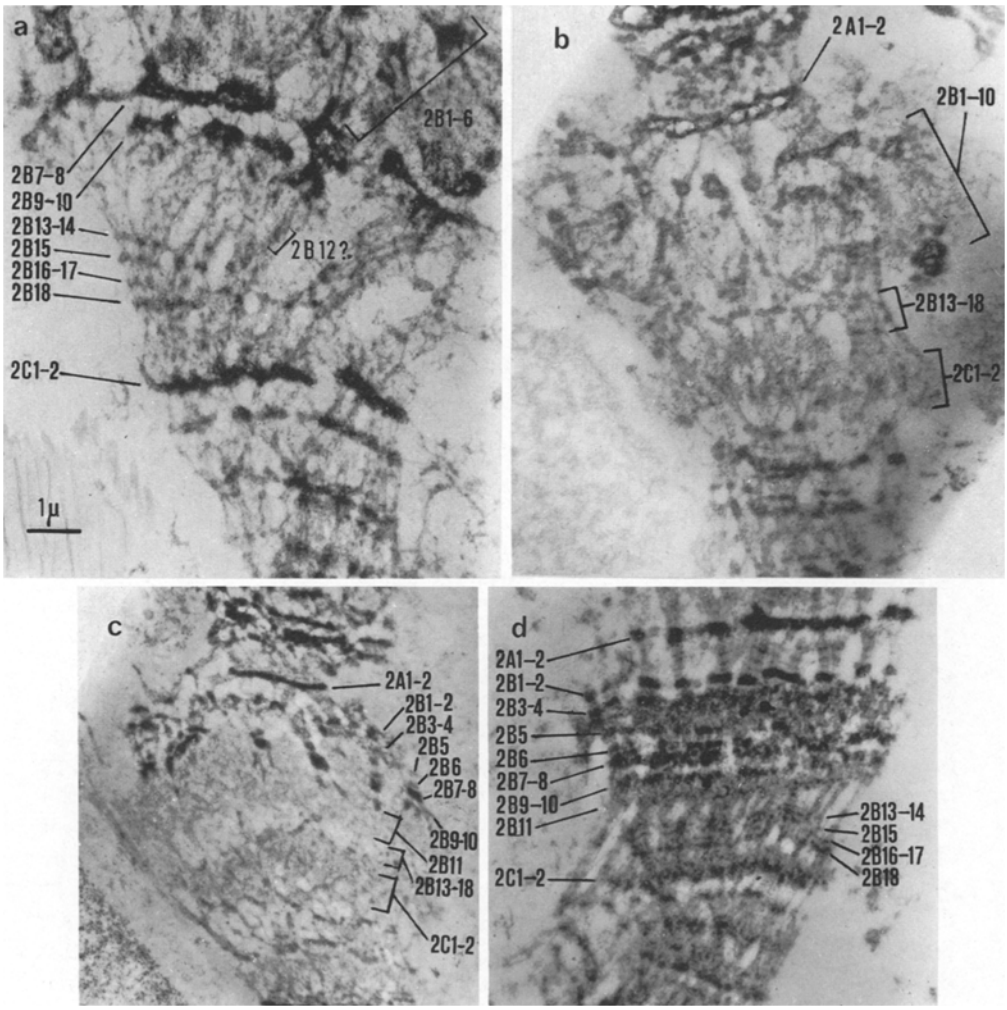


Fig. 2a–d. Morphology of the 2B1-2–2C1-2 region at EM level. **a** PS1; **b** PS4-5; **c** PS9-10; **d** PS11; **b** fixation in 45% acetic acid; **a, c, d** fixation in ethanol: acetic acid (3:1)

acid (Fig. 2). As for the 2B1-2–2B9-10 region, only 6 bands were clearly visible: the thick ones of 2B1-2, 2B3-4, 2B7-8 and 2B9-10 as well as the thinner ones of 2B5 and 2B6. The latter can be clearly seen only at puffing stage (PS) 10–11 when there is no puffing in the region (Fig. 2d). We did not find the distinct thin 2B12 band. Sometimes separate small pieces can be seen in its place (Fig. 2a). Possibly, the continuous puffing takes place here and it is difficult to count the exact number of bands. Therefore the interval from 2B11 to 2B13 we call conditionally 2B12. In this way, 13 distinct bands were found in the 2B1-2–2C1-2 region. To facilitate comparisons between our results and formerly published ones, we have not changed the original nomenclature used by Bridges (2B1-2, 2B3-4, etc).

It is an interesting characteristic of the 2B1-2–2B9-10 region that the bands

show a mirror image symmetry in their arrangement and appearance, suggesting the presence of a "reverse repeat" (Bridges, 1935; Offermann, 1936). The different bands often engage in ectopic contacts with one another, resulting in the formation of anastomoses and interfering with the exact identification of the bands, especially during puffing (Fig. 2a, b; 3c, d). The participation of the bands of the 2B1-2–2B9-10 region in ectopic pairing with other regions, many of which have been described as heterochromatic (Prokofyeva-Belgovskaya, 1939; Zhimulev and Kulichkov, 1977), as well as the intensive hybridization of labelled "bulk" RNA and cRNA to the region (Gvozdev et al., 1980) suggests the presence of repeats in it.

Puffing Activity in the 2B1-2–2C1-2 Region

Puffing activity was studied in both female larvae and prepupae heterozygous for two alleles of giant ($gt\ w^a/gt^{13z}$) having large polytene chromosomes, and in homozygous y/y larvae. The puffing pattern of 2B–2C region in these stocks is very similar to, if not identical with, that in wild-type strains (Becker, 1959; Ashburner, 1975). Starting at the 96th–100th hour after egg laying when the salivary gland polytene chromosomes become analyzable (PS1), one small puff can be found in the region; it is seen in the place of the bands 2B5 and 2B6 (Fig. 3a). Following a pulse-incorporation of ^{3}H-uridine, the whole 2B1-2–2B9-10 region is uniformly labelled (Fig. 3b). After PS2, when the first ecdysone-specific puffs appear, the decondensation of the region reaches its maximum. In the maximally developed puff the distance between the pairs of the large bands becomes somewhat greater, the bands loosen up, but the anastomoses between them remain intact; in most cases, condensed and decondensed material can be seen mixed in the puff (Figs. 2b; 3c–d). Following incorporation of ^{3}H-uridine, the label is found uniformly distributed through the whole zone of 2B1-2–2B9-10 (Fig. 3e). After PS4, when puffing in the region is still at the maximum, the 2C1-2 band also decondenses and forms another large puff. Both puffs remain active throughout PS6-8 (Fig. 3d–e) and then start to regress. By PS9-10, which coincides about with the short period of non-moving larvae having the salivary gland lumen filled with the secretion product, the puff in the 2B1-2–2B9-10 region disappears and the other puff at 2C1-2 decreases, although the latter one remains somewhat decondensed and still incorporates labelled uridine. At this time a new puff appears in the region of 2B11, which becomes especially evident in EM pictures (Figs. 2c; 3f, g, h). Decondensed material of this puff seems to penetrate the 2B7-8 and 2B9-10 bands, the latter one losing its clearcut boundaries during the process. By the time of puparium formation (PS11), the three puffs regress (Figs. 2d, 3i) and, in respect to incorporation of ^{3}H-uridine, the region does not differ from other chromosomal parts containing no observable puffs (Zhimulev and Belyaeva, 1974). The second peak of puffing activity in this region occurs during PS18-20, 8–12 h after puparium formation (Fig. 3j).

In this way, our observations on the behaviour of the puff in the 2B1-2–2B9-10 region coincide with data of other authors (Becker, 1959; Ashburner, 1975). As for the region to the right of this puff, two other distinct active sites were

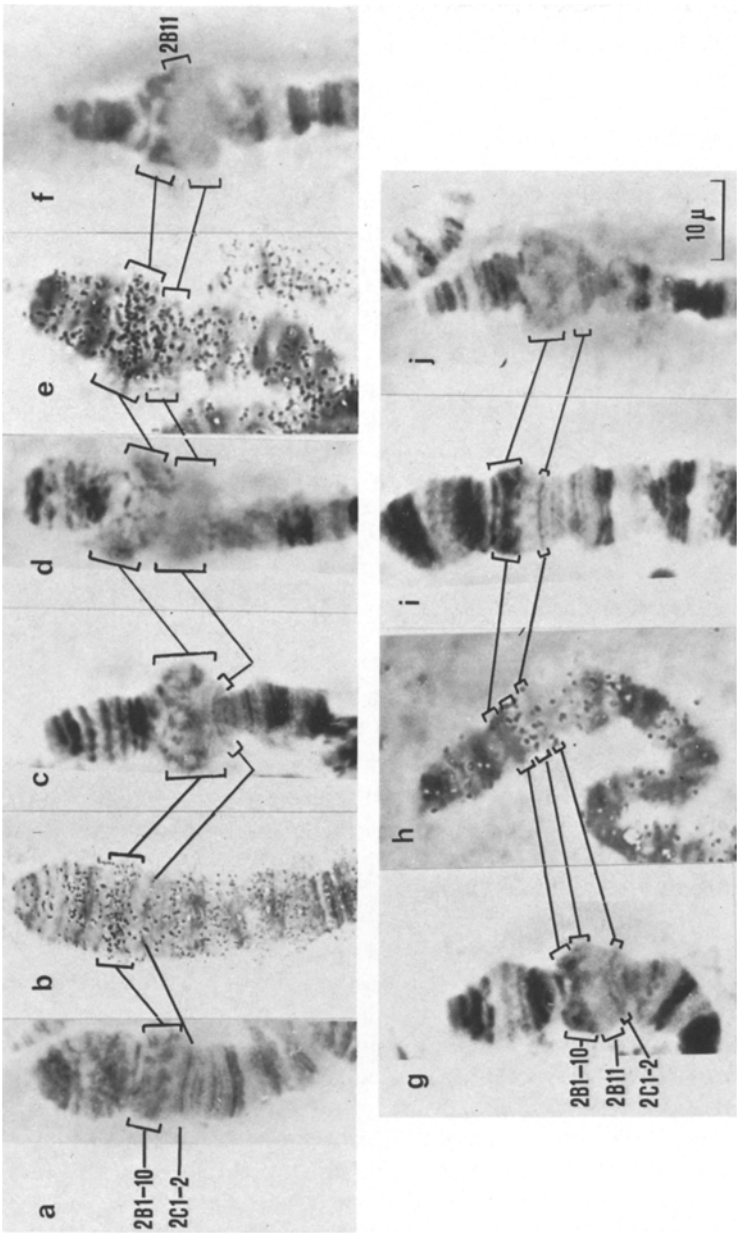


Fig. 3a–j. Puffing in the 2B1-2–2C1-2 region in the course of ontogenesis in homozygous *y/y*. **a**, **b** PS1; **c** PS3–4; **d**, **e** PS7; **f** PS9; **g**, **h** PS10; **i** PS11; **j** PS18

found (2B11 and 2C1-2), which in most of the earlier studies were regarded as one under the designation 2B13-17 (Becker, 1959; Kiknadze, 1972; Ashburner, 1975).

The puff in the 2B1-2–2B9-10 region appears to be early ecdysone-specific in the sense that its activity rises suddenly during the first hour following

the hormone application (Ashburner, 1972). The other two puffs (2C1-2 and 2B11) belong to the late ecdysone-induced series.

Cytology of Chromosome Rearrangements

Lefevre has found that Df(1)RA19 removes bands from 1E3-4± to 2B12± (personal communication). Our EM data confirm this localization since we have not detected any material in the interval between 1E3-4 and 2B12 in the chromosome with deletion (Fig. 4). The absence of 2B11 puff is clearly seen at PS10 in the chromosome with the deletion (Fig. 4b, c).

Df(1)A94 has the same limits (Fig. 4d).

Df(1)*sta* has been described as loss of bands from 1D3–1E1 to 2B (Lindsley and Grell, 1968). We have found that in this case all bands in the region 1D are present and that the part of 1E1-2 is likely to be present also, the latter one pairing with the homologous band of the normal partner (Fig. 5a). It is followed by bands that are identified as 2B5, 2B6, 2B7-8, 2B9-10 (Fig. 5e). We suggest that breaks occurred within 1E1-2 and 2B3-4, resulting in their fusion. In any case the material of 2B1-2 is absent in a chromosome with the deletion, but is seen well in duplication Dp(1:3)*sta* as well as the material of 1E1-2 (Fig. 5c, d).

Df(1)*pn*7b has similar limits (Fig. 6) with the exception that band identified as the part of 1E1-2 according to its pairing with the respective band of the normal partner is thinner than that in Df(1)*sta* (Fig. 6a). In this case the material of the right part of 2B3-4 is less likely to remain and to fuse with 1E1-2.

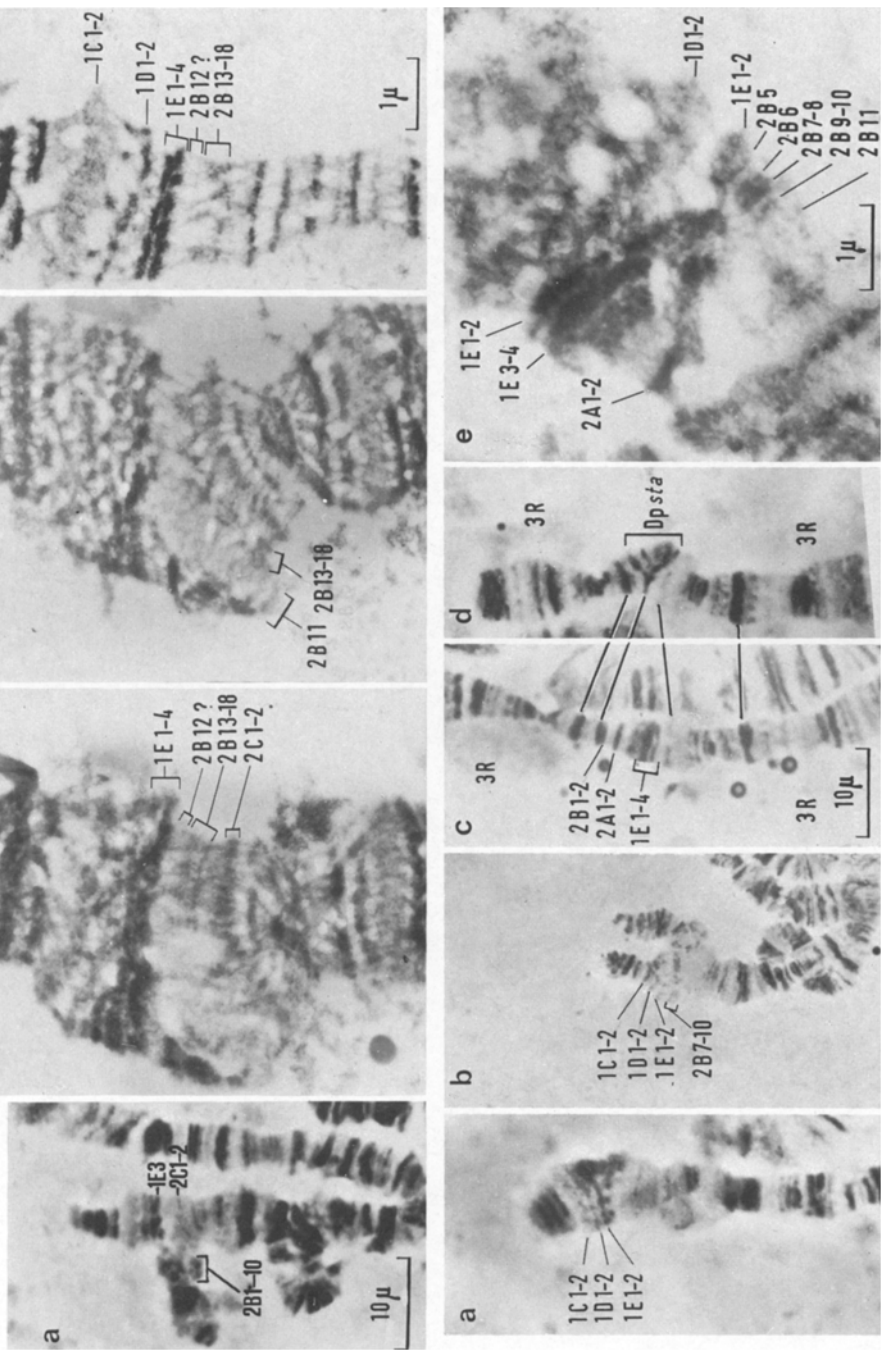
Df(1)S39, according to Lefevre, is deficient from 1E4± to 2B10± (personal communication). In chromosomes with Df(1)S39 light microscopic observations show all the bands of the 1D region, further a very thin band that has no morphologically identical partner in the normal homologue (Fig. 7a), and two bands frequently paired with proximal part of 2B5-6 puff of the normal partner. However, with the EM four bands between 1D3-4 and 2B11 can be seen well (Fig. 7b, c). Three bands adjacent to 2B11 are easily identified as 2B9-10, 2B7-8, 2B6 by their typical morphology and since they pair with the homologous region in the heterozygote. Thus the thick band which appeared single under the light microscope really consists of the two bands 2B6 and 2B7-8. Therefore it can not be related to 1E3-4. The thin band to the left could represent either 2B5 or the rest of 1E1-2. We prefer the second version for the following reasons:

1. As usual, the 2B5 band can not be seen under light microscope since it either puffs or is fused with adjacent bands;

2. in the chromosome with Df(1)S39 the puff normal by arising from 2B5-6 does not form, implying that 2B5 is absent.

3. in the heterozygote DF(1)S39/Df(1)*pn*7b the band under question reveals distinctive homology with the rest of 1E1-2 in the chromosome carrying Df(1)*pn*7b (Fig. 7d).

Thus it is possible to believe that Df(1)S39 is a result of breaks in 1E1-2 and either in the interband after 2B5 or in the right- most part of 2B5, the rests of which have fused with 1E1-2. Thus, to the right Df(1)S39 extends



Figs. 4 and 5. The 2B region. **Fig. 4a–d**. The 2B region in chromosomes with Df(1)RA19 and Df(1)A94. **a–c** Df(1)RA19/+; **a** PS11, **b, c** PS10; **d** homologue with Df(1)A94, PS11. **Fig. 5a–e.** The 2B region in chromosomes with Df(1)*sta* and Dp(1;3)*sta*. **a, b, c** Df(1)*sta*/+, **a, e** PS11, **b** PS5; **c, d** Dp(1;3)*sta*; **c** non-paired 3R homologue with duplication; **d** Dp(1;3)*sta*/+

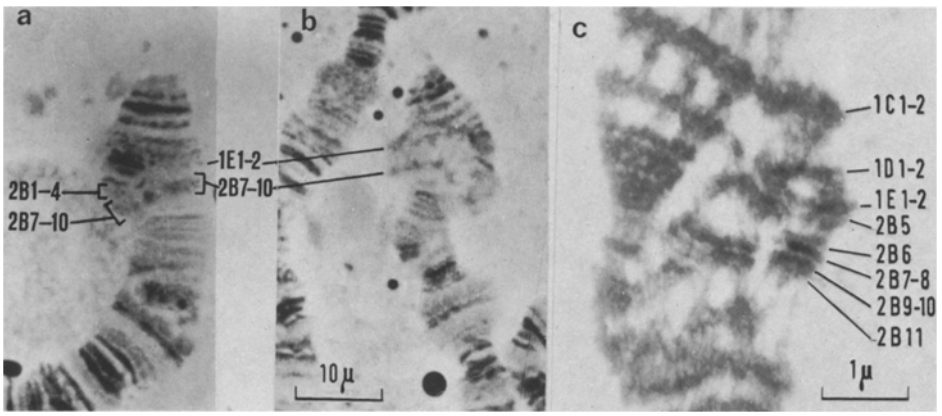


Fig. 6a–c. The 2B region in chromosome Df(1)*pn*7b/+. **a** PS1; **b** PS2; **c** PS11

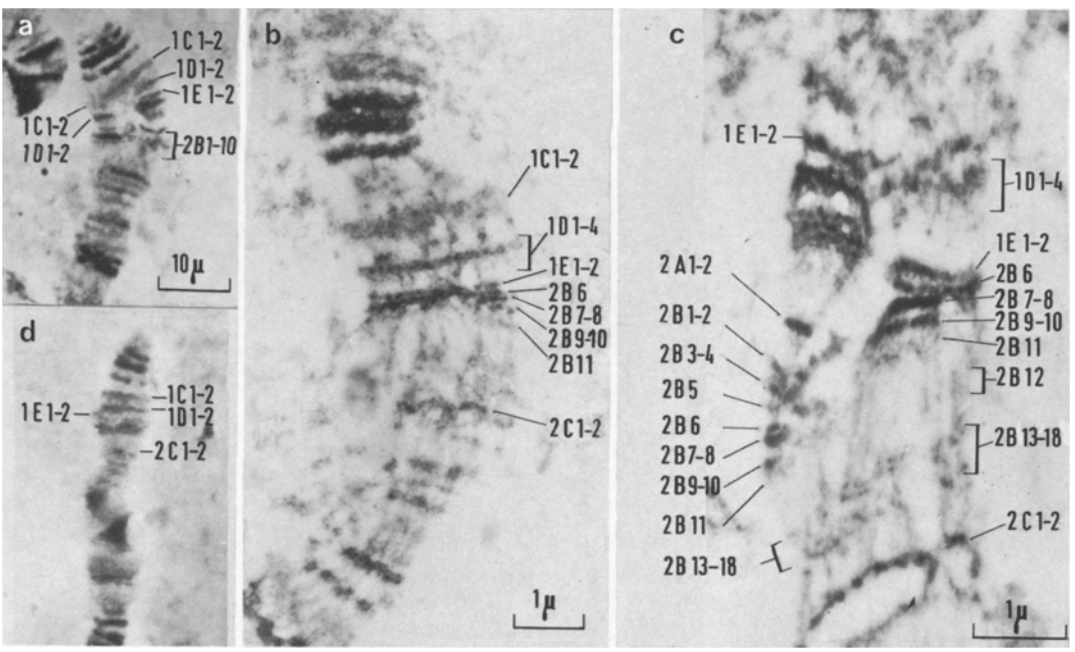


Fig. 7a–d. The 2B region in chromosomes with Df(1)S39 **a, c** Df(1)S39/+, PS11; **b** homologue with Df(1)S39, PS11; **d** heterozygote Df(1)S39/Df(1)*pn*7b, PS11

one band further than Df(1)*pn*7b. When in the heterozygote homologues with these deletions appear absolutely identical at the stage when the 2B5-6 puff is absent (Fig. 7d).

All the duplications used in the experiments except Dp(1:*f*)101 are different parts of the X-chromosome distal end, that are translocated to the Y-chromo-

some with unidentified parts of heterochromatin (19–20 region). (Left breakpoints in the X-chromosome are given in Fig. 10.) The most important for the purposes of our investigation are three cytologically identical duplications- Dp(1)y^2Y67g24.2, Dp(1)y^2Y53T, Dp(1)y^2Y21T. Under the light microscope it is possible in all three duplications to recognize both 2B1-2 and 2B3-4 followed by the light zone corresponding to the 2B5-6 puff and connected with the chromocenter (Fig. 8a–d). We have not succeeded in identifying any parts of 2B7-8 and 2B9-10. Under the EM, 2B5 and 2B6 can be seen in light zone adjacent to heterochromatin (Fig. 8h, i). The whole zone 2B1-2–2B9-10 is clearly seen in Dp(1)y^2Y4OT (Fig. 8f).

Translocation T(1:3)2B7 has well identifiable breakpoints in 2B7-8 and 84A4-6 (Fig. 9a, b) (resulting combination 81A–84A4-6–2B9-10–19A–99A–84A6–2B7-8–1A).

It must be emphasized that the 2B1-10 region is rather difficult for cytological and EM mapping due to constant ectopic contacts the bands in the region. All the bands at the same time are clearly seen only on rare sections or even parts of sections. From one to two hundred of chromosomes with each rearrangement were analysed under the EM to map their breakpoints.

Puffing on Chromosomes Carrying Rearrangements

Analysis of stocks with rearrangements having breakpoints within the 2B region revealed the structural independence of the puffs in the region: they develop normally despite being separated in space. On chromosomes with the translocation T(1:3)2B7, which separates the puffs at 2B7-8, all the 3 puffs develop normally (Fig. 9). Total absence of the 2B1-2–2B1-region in the case of Df(1)RA19 and Df(1)A94 does not alter the activity of the 2C1-2 puff. On duplication Dp(1)y^2Y67g24.2 the puff between 2B1-2 and 2B9-10 has the usual size, although the region with the 2B11 and 2C1-2 puffs is missing (Fig. 8a). In all the cases mentioned above, the behaviour of the puffs does not depend on conjugation between the rearangement-carrying and the normal homologues.

Participation of distinct bands in the formation of the puff studied was determined with the help of rearrangements. As mentioned above, although decondensation was maximal in region 2B5–2B6 during puff-formation, the neighbouring thick bands also loosened up partially, and the silver grains after autoradiography were spread equally above the territory of 2B1-2–2B9-10. The same phenomenon was especially well demonstrated on stretched chromosomes by Ananiev and Barsky (1978). On this basis, it can be supposed that all the bands of the given region are active in transcription. It was a surprise that deletion Df(1)*pn*7b lacking the 2B1-2 band and the greater part – may be the whole – of 2B3-4, did not cause any apparent reduction of the puff size (Fig. 6b). To investigate this further, we determined the effect of the same deletion on the incorporation of ^{3}H-uridine into the active puff. In order to eliminate mistakes caused by unequal incorporation of the label into different nuclei, we used Df(1)*pn*7b/FM-6 heterozygous larvae, comparing the two X-chromosomes in the same nucleus: silver grains were counted in the puffs

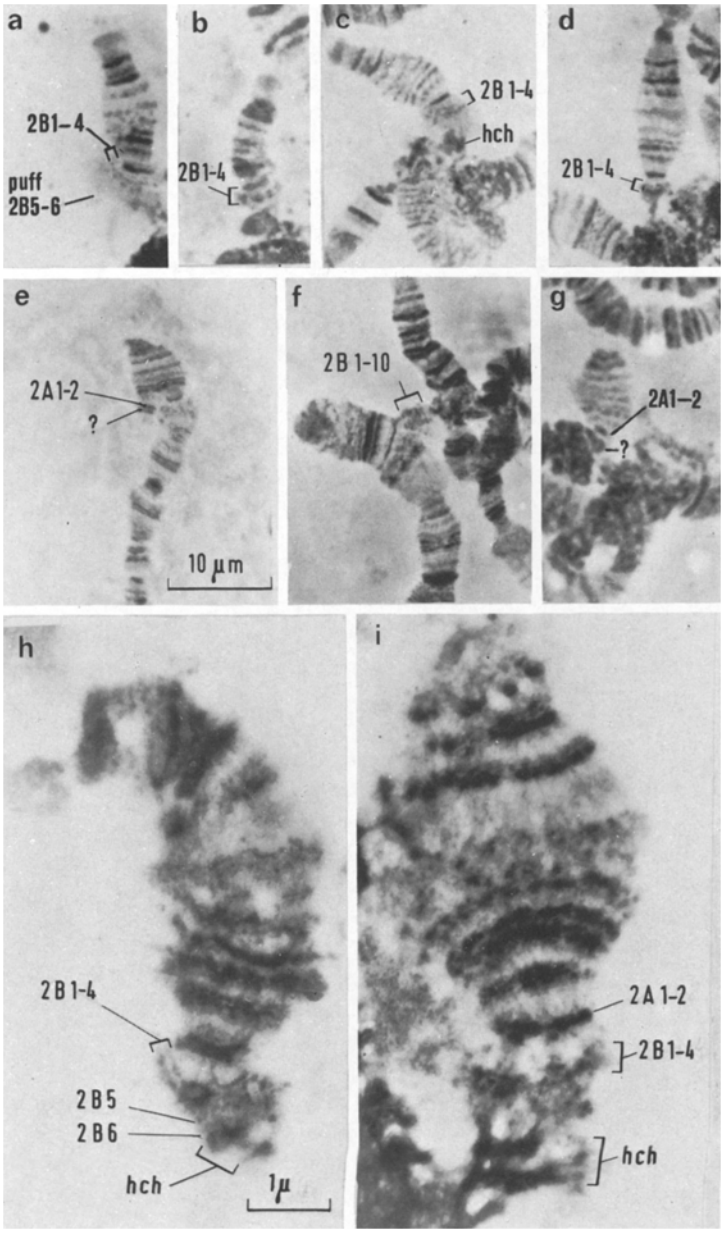


Fig. 8a–i. Duplications of the X-chromosome **a, b, h, i** Dp(1)y^2Y67g24.2, **a** PS5, **b, h, i** PS11; **c** Dp(1)y^2Y53T. PS11; **d** Dp(1)y^2Y21T, PS11; **e** Dp(1;f)101, PS1; **f** Dp(1)y^2Y40T, PS5; **g** Dp(1)y^{59b}Y2, PS1; *hch* heterochromatin

Table 2. Grain numbers over the 2B5-6 puff in normal and rearrangement-bearing 2B regions

No. of larvae	No. of nuclei	Df(1)*pn*7b/FM-6		No. of larvae	No. of nuclei	Dp(1)y^2Y67g24.2, X		No. of larvae	No. of nuclei	T(1;3)2B7/In(1)d*l*49	
		Homologue with Df	FM-6			Duplication	Normal X-chromosome			Homologue with translocation	In(l)d*l*49
1	1	15	15	1	1	12	13	1	1	19	16
	2	18	19		2	15	14		2	20	22
	3	22	21						3	21	23
				2	1	11	11		4	23	26
2	1	12	15		2	7	6				
	2	9	5		3	6	8	2	1	17	17
					4	9	8				
3	1	7	8		5	8	10	3	1	12	15
	2	13	12		6	15	16		2	20	20
					7	14	15	4	1	10	11
4	1	14	12		8	11	13		2	6	6
	2	8	9		9	8	8				
	3	10	12		10	4	5	5	1	27	26
					11	9	12		2	28	29
5	1	18	19		12	8	11		3	29	30
	2	18	17		13	8	8		4	27	25
									5	36	36
6	1	12	15	3	1	14	16		6	16	14
	2	11	12		2	7	9		7	18	20
	3	11	12		3	34	36		8	35	36
									9	28	30
7	1	8	10						10	18	19
	2	9	10						11	20	21
	3	17	17								
	4	13	13								
	5	15	14								
	6	7	8								
8	1	12	8								
	2	17	17								

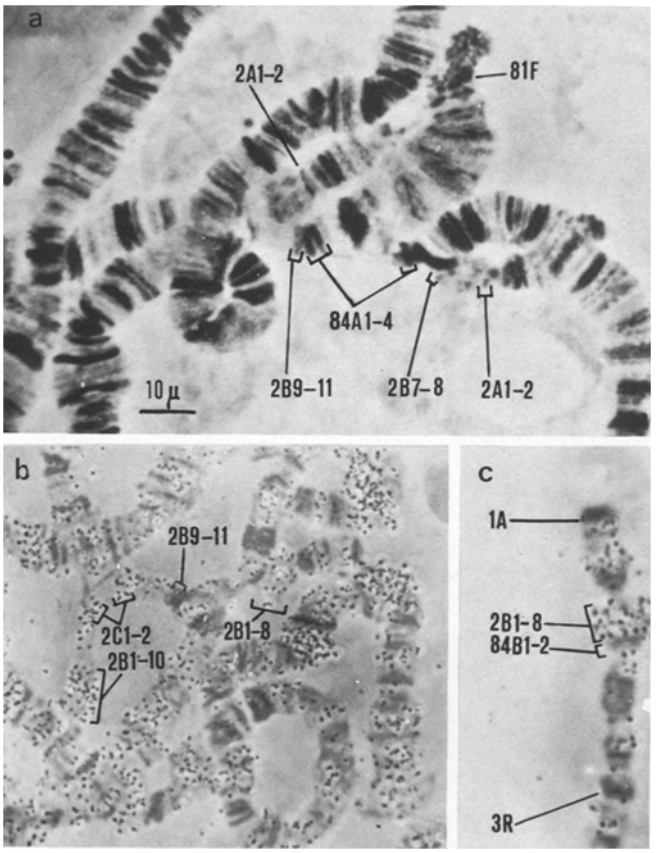


Fig. 9a–c. The 2B1-2–2C1-2 region in the T(1;3)2B7/In(1)dl49 stock. **a** PS9; **b, c** ^{3}H-uridine incorporation, 5 min, PS7

of non-conjugating homologues, one with and one without the deletion. The autoradiographic analysis confirmed the morphological observations showing that the deletion had no detectable effect on the incorporation of ^{3}H-uridine (Table 2).

Analogous results were obtained in stocks where bands to the right of 2B5 and 6 were missing. Here, the size of the puff and the incorporation into it of ^{3}H-uridine were the same on duplication Dp(1)y^{2}Y67g24.2 and on the normal X-chromosome, both in the same nucleus (Table 2).

Analysis of translocation T(1;3)2B7 corroborates these findings. This translocation separates band 2B9-10 and probably part of 2B7-8 from the puff (Fig. 9), but this has no effect on the puff size and the incorporation of label into it (Fig. 9b, c; Table 2). In addition, bands 2B9-11, translocated to the 3R chromosome, do not contain any significant amount of label during the period of maximum puffing at PS7 (Fig. 9b). These facts persuade us to believe that bands 2B1-4 and 2B7-10 do not contribute significantly to either puffing or

transcription. Thus the limits of origination of the puff have been narrowed to 2B5-6. Both bands are not identified in the developed puff. However, the chromosome with Df(1)S39 contains the band 2B6 with the adjacent left interband, but has no features of puff (Fig. 7a). Hence, 2B6 either does not take part in puffing, or cannot puff without 2B5. It is impossible to choose between these versions until we obtain a deletion removing 2B6, but leaving 2B5 intact. At present we are to assume that 2B5-6 is the puff-forming zone ("Entstehungsort", Beermann, 1962). This confirms the original nomenclature of the 2B5-6 puff introduced by Becker (1959). Participation in the puff of the right part of 2B3-4, as well as of the left part of band 2B7-8, which are directly connected to the zone of intense decondensation, remains questionable.

Results of the present work raise the question of caution in the interpretation of light microscopic autoradiography; they show that mere localization of the silver grains above some structures may not always be taken as an evidence for their activity. The cause of this phenomenon is not entirely understood. It can not be explained by the spreading of the β-particles of tritium. First, no gradient of label is formed in the region of the puff. Second, in T(1;3)2B7, bands within the 84B region, which are in a direct contact with the puffing region occupying the former place of 2B9-11, do *not* contain any label (Fig. 9b, c). It is more than likely that in this case transcriptionally active and inactive parts are mixed within the region of the 2B5-6 puff. This situation can be caused by the firm anastomoses between the 2B3-4 and 2B7-10 bands retained during the development of the puff. They can prevent the puff from pushing aside the blocks of inactive material, that, partly decondensed, is mixed with the zone of active RNA synthesis. Such an interpretation is supported by the morphology (Figs. 2b, c and 3c, d).

Recovery and Characterization of Mutants

In six independent experiments 17, 120 mutagenized X-chromosomes were tested and 98 mutations were isolated. All of these mutations are uncovered by the deficiency Df(1)RA19 so that they would be localized between 1E3-4 and 2B12 (Fig. 10); 34 of them were non-allelic with Df(1)*sta*, i.e., they were located between the right breakpoints of the latter deletion and Df(1)RA19 in the interval 2B3-4–2B11. Alleles of some previously known genes (*br* and *dor*) are also situated in this region. The rest of the mutants localized in the region uncovered by both deficiencies, i.e., in the interval 1E5–2B3-4 (Fig. 10), were not studied further in the present work.

Mutants in the region 2B3-4–2B11 can be divided into two basic classes: 35 mutations express the lethality in homo- or hemizygous (male) condition, and another 7 that in homozygous or hemizygous condition have good viability, but in heterozygotes with deficiencies are nearly inviable or even completely lethal. On this basis we consider these mutations as hypomorphs. In the latter group, there are alleles showing temperature sensitivity, mostly expressing an enhanced mutant phenotype or lethality at 29° C, while being normal or at

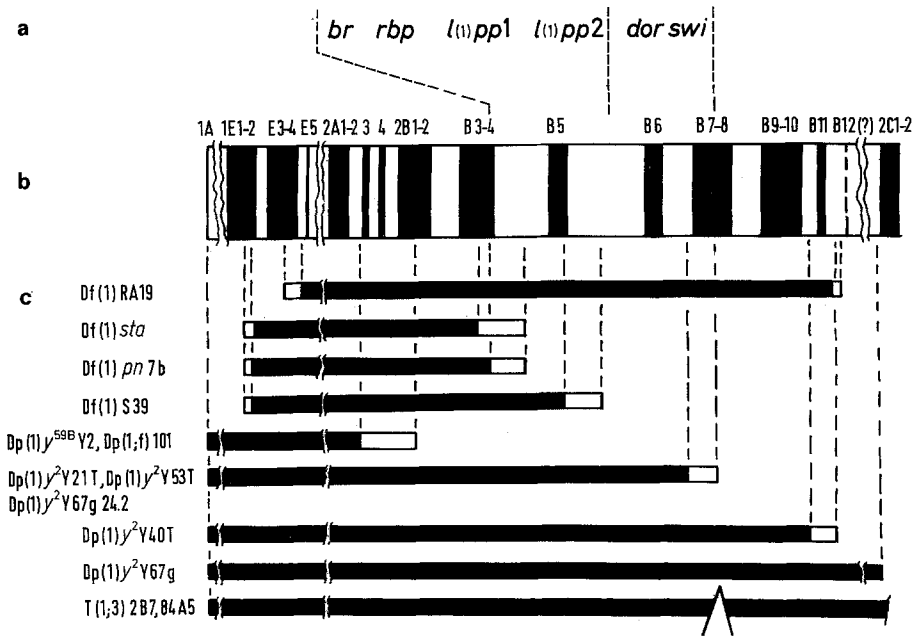


Fig. 10a–c. Cytological map of the 2B1-2–2B9-10 region. **a** Complementation groups; **b** banding pattern in the region 1D–2C1-2 according to the revised map of Bridges; **c** localization of the chromosome rearrangements. Unshaded zones correspond to regions of uncertainty in the determination of the breakpoints

least viable at 25 or 18° C. The *br* and br^3 alleles, however, show the maximum lethal effect at 25 and 18° C when heterozygous with Df(1)RA19 (Table 3).

Many individuals that are viable in both homo- or hemizygous and haplo-heterozygous combinations, show morphological abnormalities (Table 3). The deviations belong to several basic types, as follows: "short, broad wings" (*br* phenotype): this feature is expressed clearly at low temperature. In addition, the wings frequently have a rugged, blistered surface, and in many cases one wing is shorter than the other. "Singed wings": this feature does not have a full penetrance and expressivity. Some of the mutations cause a reduction of bristle number on the palpus, others have an abdomen of "faded" or "swollen" appearance. A detailed description of the phenotypic characteristics of the different mutants has been published earlier (Aizenzon et al., 1980).

Complementation Analysis

The complementation map of the mutants at 25° C is shown in Table 5. Six complementation groups are evident. The *dor* (deep orange) group includes the hypomorphic *dor* and its lethal alleles (dor^l, dor^{lt81}, etc.). All the members of this group are fully complementing with mutants belonging to other complementation groups at all the temperatures tested. There is no complementation

Table 3. Influence of hypomorphic mutations on the viability in homo-, hemi- and heterozygous [over Df(1)RA19] conditions

Mutant	Homozygous[a]	Hemizygous[b]	Heterozygous[c]
y (control)	289/359	80/67	8/50
	443/450	224/211	50/122
	71/101	135/139	20/71
y t132	$194/77^{I, III, IV}$	$115/100^{I}$	113/0
	$567/553^{I}$	$325/334^{I}$	$203/3^{I}$
	$53/66^{I}$	$121/111^{I, III}$	161/0
y t219	$197/16^{III-V}$	$162/13^{III, V}$	114/0
	$424/267^{III-V}$	$329/175^{III, V}$	$182/20^{III-V}$
	$73/64^{III-V}$	$112/135^{III, V}$	$98/74^{III-V}$
y t251	$213/47^{III, IV}$	48/36	129/0
	$485/364^{III, IV}$	272/298	$187/53^{III, IV}$
	$86/57^{IV}$	179/194	$93/78^{III, IV}$
y t252	51/51	65/34	184/0
	50/59	103/50	$287/20^{IV}$
	74/94	118/97	$69/18^{I, III, IV}$
$y^{+}dor$	255/0	108/0	142/0
	$233/182^{VI}$	$203/131^{VI}$	$100/13^{VI}$
	$87/92^{VI}$	$159/178^{VI}$	$47/43^{VI}$
$y^{+}br$	70/62	81/64	$46/8^{II, III}$
	122/131	276/329	118/0
	$103/104^{II}$	$153/159^{II}$	91/0
$y^{+}br^{3}$	120/131	65/55	$77/53^{II, III}$
	148/143	235/244	$147/6^{II}$
	105/116	113/104	$87/1^{II}$

[a] Ratio of ♀♀ *B*: ♀♀ B^{+} from the cross $\frac{\text{FM-6 } l^{69j}}{y\ l} \times \frac{y\ l}{\text{Dp(1)}y^{2}\text{Y67g}}$

[b] Ratio of ♀♀ *B*:♂♂ as under[1]

[c] Ratio of ♀♀ *B*:♀♀ B^{+} from the cross $\frac{\text{FM-6 } l^{69j}}{y\ l} \times \frac{\text{Df(1)RA19}}{\text{Dp(1)}y^{2}\text{Y67g}}$

The 3 lines for each mutation show results at 30° C, 29° C and 18° C, respectively.

I – reduction of bristle number on palpus
II – short broad wings
III – faded wings
IV – swollen abdomen
V – singed wings
VI – deep orange eyes

within the *dor* group: heterozygotes of any two lethal alleles die. It is interesting that heterozygotes carrying *dor* and a lethal allele are lethal at 29° C, partially viable at 25° C and fully viable at 18° C. The surviving individuals show the *dor*-phenotype.

The *swi* (singed wings) group includes 4 lethal and 2 hypomorphic (t219, t251) mutations. Rare survivors show the characteristic abnormalities of the wings. Mutations of this group are fully complementing with mutations of

any other group. There is no full complementation among the alleles of *swi* group as the surviving heterozygotes show morphological abnormalities of the wing and can have a "swollen" abdomen. In some of the larvae homozygous for mutation t467, puparium formation occurs only on the anterior half of the body. As this phenomenon was characteristc of the mutant *hfw* (halfway), localized to the same region between 2B4 and 2B8 (Rayle, 1967; Rayle and Hoar, 1969), *hfw* probably belongs to the *swi* group.

The *rbp* (reduced bristle number on palpus) group includes the hypomorph rbp^{t132} which, when homozygous, leads to the reduction of the palpal bristles, as well as 4 other lethals. All of them are fully complementing with mutations of any other group, except *br* (see below). Heterozygotes of the different *rbp* lethal alleles remain lethal. Heterozygotes containing rbp^{t132} and a lethal allele are completely viable at 18° C, but at higher temperatures most of them die and the survivors show a mutant phenotype (reduced bristles on the palpus, shortened bristles on the scutellum, shrivelled or swollen abdomen, shrivelled wings, eyes with a crumpled surface).

The *br* (broad) group contains several hypomorphs (*br*, br^3) and four lethals. Mutants of this group complement with mutants of all the other groups except *rbp* (see below). There is also no complementation within the *br* group itself, i.e., heterozygotes of the different lethals or of br^4 and a lethal allele do not survive. A few animals of the *l*t336/*l*t103 genotype survive with short, broad wings. Heterozygotes containing a lethal allele and *br* or br^3 survive and show the *br* phenotype, especially at 18° C; br^3/lt366 and br/br^3 heterozygotes, as well as br^3/br^3 homozygotes, have a normal phenotype, however.

As it has been mentioned earlier, the complementation between alleles of the *rbp* and *br* groups is not always complete. Beside such heterozygotes as lt103/t132 or lt366 with other alleles of the *rbp* group (except t99)., which are fully viable and have a normal phenotype, there is a series of combinations where the surviving heterozygotes carry mutant characteristics of both groups separately or in combination. For example, *l*t35 or *br*, when heterozygous with any allele of the *rbp* group, have a decreased number of bristles on the palpus; the *br*/t132, *l*t103/t144, *l*t103/t358 combinations, in addition to the reduced bristle number on the palpus, have short, broad wings. Another feature of the *br* and *rbp* groups is that heterozygotes carrying an allele of these groups and the t4 or t126 mutations, which rarely survive at 18° C or 25° C, show the characteristics of both groups. In the case of combinations t4/*br*, t126/*l*t103 and several others, short, broad wings are accompanied by a reduced number of bristles on the palpus.

The *l*(1)*pp*-1 group consists of 4 lethal alleles $l(1)pp\text{-}1^{t110}$, etc. that are fully complementing with representatives of any other groups, but lethal in combination with each other.

The *l*(1)*pp*-2 group contains only one hypomorph $l(1)pp\text{-}2^{t252}$. When heterozygous with any of the rest of the mutations, excepting long "polar" one, it shows the normal phenotype and viability. However, when heterozygous with Df(1)RA19 or t324, this mutation causes a series of abnormalities (see Table 3).

Groups *rbp*, *br*, *l*(1)*pp*-1 and *l*(1)*pp*-2 are overlapped by 5 lethal mutations: *l*(1)t324, *l*(1)t435, *l*(1)*npr*-1, *l*(1)*npr*-2 and $l(1)d.norm.\text{-}1^a$. All of them are lethal

in homo- and hemizygotes, as well as in heterozygotes with each other or with representatives of the overlapped groups. Some of their other characteristics were published earlier (Kiss et al., 1976a, b; Kiss et al., 1978). The rare surviving heterozygotes are phenotypically similar to the mutants participating in the cross: t132/t324, t132/t435 and t376/t324 escapers show a reduction of the bristle number on the palpus; *br*/t324, *br*/t435, br^3/t324 and br^3/t435 have short, broad wings; t252/t435 show the normal phenotype, which is characteristic also for t252/t252 homozygote. Interestingly, rare surviving individuals of t4/t324 had short, broad wings, a typical feature of mutants in the *br* group.

In addition, there is another, "short" group of overlapping mutants *l*(1)d.norm.-24, *l*(1)t143, *l*(1)t126, *l*(1)t4 that do not complement only with the *rbp* and *br* groups.

The complementation map remained essentially constant at all the temperature tested, except that at 29° C a partial overlap was found between the "short overlapping" mutations and *l*(1)*pp*-1 alleles: t197/t4 and t149/t4 heterozygotes showed a strongly reduced viability.

It should be mentioned that the position relative to each other of the complementing groups is given in accordance with the results of genetic mapping (Aizenzon, not published).

Cytological Localization of the Mutations

All the mutations were overlapped by Dp(1)y^2Y67g24.2, Dp(1)y^2Y53T, Dp(1)y^2Y21T, Dp(1)y^2Y40T, but none of them was covered by Dp(1)y^{59b}Y(2) and Dp(1;f)101. It has to be mentioned that *dor* is not covered by Dp(1;f)101, as it was reported earlier (see Lindlsey and Grell, 1968). The mutation dor^l is the only exception. It is lethal in males with Dp(1)y^2Y67g24.2, Dp(1)y^2Y53T, Dp(1)y^2Y21T. This effect seems to be due to presence of another mutation that is located to the right of 2B7-8 insofar as homozygotes and hemizygotes dor^l die at larval third instar and males having dor^l and one of duplications enumerated above die at late pupa. This lethal factor acting at late pupa is overlapped by Dp(1)y^2Y67g.

A special "complementation" can be observed between Df(1)pn7b and the mutations studied. Heterozygotes carrying this deficiency and mutations of groups *l*(1)pp-1, *l*(1)pp-2, *dor* or *swi* are fully viable and do not show any deviation from the wild-type phenotype. However, heterozygotes containing the overlapping mutations or certain alleles of the *br* group over Df(1)*pn*7b are either lethal or have a reduced viability. The rest of the alleles in group *br* as well as alleles of the *rbp* group are completely viable when heterozygous with Df(1)*pn*7b, but show a mutant phenotype (Table 4). The interaction between Df(1)*pn*7b and the alleles of the *br* and *rbp* groups might suggest that there is some kind of position effect in the *br* locus resulting from rearrangement. Overlapping between the locus *br* and the deletion seems to be absent since the affected *br* alleles behave in relation to Df(1)*pn*7b not as combined with a deletion but with a recessive mutation of lt103 type, Another possibility is that, although the deficiency does not overlap the locus *br*, an independent

Table 4. Interaction of mutations with Df(l)pn7b. Viability and phenotype of the deletion-carrying individuals

Complete lethality	Reduced viability	Normal viability	
	br wings	*br* wings	reduced bristle number on palpus (*rbp*)
t4	*l*t103	br	t99
*l*t35	t126	br^3	t132
t143	*l*t336		t144
t324	*l*t366		t358
	t435		t376

br mutation exists on the deficient chromosome. All of the *dor* and *swi* alleles have normal viability in heterozygote with Df(1)S39 unlike the groups overlapped by noncomplementing mutations that die with Df(1)S39.

Genetic Mapping of l(1)npr-2

The ratio of recombinants between *y* and *l*(1)*npr*-2 as well as *sc* and *l*(1)*npr*-2 was 15/3518 = 0.0043 for both cases. The frequency for recombination between *l*(1)*npr*-2 and *w* and *ec* was found to be 36/3518 = 0.0102 and 159/3518 = 0.0452, respectively. Double recombinants were not found. Accordingly, *l*(1)*npr*-2 is located between *y* and *w* at the map position 0.43.

Discussion

There is a series of lethal mutations in the region between 2B3-4 and 2B7-8 of the X-chromosome. With the help of complementation analysis, the 42 mutants were divided into 6 complementation groups. There were 9 alleles, however, that did not complement with more than one complementation group (Table 5): 4 of them overlapped the *rbp* and *br* groups while the other 5 did not complement with *rbp*, *br*, *l*(1)*pp*-1 and *l*(1)*pp*-2 groups, as well as with the former 4 "overlapping" alleles.

In addition to the lethal mutants isolated by us, the *dor* group includes previously known alleles *dor*, dor^l, dor^{66g} and dor^{169F}. All the viable transheterozygotic combinations show the characteristic orange eye colour.

The previously known mutant *hfw*, which inhibits development at puparium formation, probably belongs to our *swi* complementation group. The characteristic feature of this mutation – pupariation of the anterior half of the larva only – was observed in some of the homozygotes of swi^{t467}.

Mutants of the *dor* and *swi* groups always complement with each other and with mutants of the other groups. On this basis we can suppose that there are, at the minimum, 3 independent genetic loci in the region studied: *dor*, *swi*, and the "overlapping" complex. This assumption is supported by

Table 5. Complementation map of the mutant alleles:

l(1)t435				*l*t257	t467
l(1)t324				*l*t187	t251
l(1)*npr*-1				*l*t148	t219
l(1)*npr*-2				*l*t141	t200
l(1)*d.norm.*-1[a]				*l*t128	t63
l(1)*d.norm.*-24		t197	t252	*l*t81	t32
l(1)t143		t149		66g	
l(1)t126		t76		*l*69F	
l(1)t4		t10		*dor*[1]	
*l*t366	t376			*dor*	
*l*t336	t358				
*l*t103	t144				
*l*t35	t132				
br[4]	t99				
br[3]					
br					
br	***rbp***	***l*(1)*pp*-1**	***l*(1)*pp*-2**	***dor***	***swi* (*hfw?*)**

the observation on separation of these loci by crossing-over (Rayle and Hoar, 1969; Aizenzon, in preparation). Furthermore, there is enough basis to suppose that *dor* and its lethal alleles belong to the same cistron (Bischoff, 1973).

The situation is not so clear with the analysis of those groups showing overlaps on the complementation map. Complicated interactions were found between alleles of the *br* and *rbp* groups. All the individuals heterozygous for *br* and *rbp* alleles were viable (full complementation for lethality) but some of them had an abnormal phenotype showing the characteristics of both groups (the complementation is partial in the respect of morphological characteristics). It is also important that the phenotype of the heterozygote changes at different temperatures: *l*t35/t99, *l*t35/t132 and *l*t35/t358 heterozygous females have a normal phenotype at 18 and 25° C, but show a reduced number of bristles on the palpus at 29° C.

The effect of temperature on the complementation pattern is regarded as a typical characteristic of interallelic complementation (Fincham, 1959; Partridge, 1960; Garen and Garen, 1963). In the case of Drosophila, interallelic

complementation is known for the mal (Chovnick et al., 1969; Duck and Chovnick, 1975) and ss^a (Mglinetz, 1977) loci, although in the latter case the existence of two cistrons can not be excluded. In our case, either the *br* and *rbp* groups or the whole complex could represent one single locus and the "group" would be produced by allelic complementation.

Another explanation for the complex complementation map could be that an operon-like system exists in the region. An example for such genetic organization in *D. melanogaster* is represented by the complex rudimentary (*r*) locus, which shows a similarly complicated complementation pattern and codes for the first three enzymic activities in the biosynthetic pyrimidine pathway (Nørby, 1973; Fausto-Sterling, 1977; Jarry, 1979). In our case the overlapping alleles would represent either deletions or mutations with polar effects. As we did not find any visible aberrations in the 2B region of the chromosomes carrying the overlapping alleles, we favour the second possibility. However, no unambiguous interpretation of the "overlapping" effect can be given on the basis of the genetic data presented.

Apparently some loci in the region have not been revealed and can be brought out with other mutagens or under other selection background. According to Lefevre (personal communication), the interval between the right ends of Df(1)*sta* and Df(1)RA19 includes 9 complementation groups. We have found that Dp(1)y^2Y67g24.2 does not cover completely the chromosome region removed by deficiencies RA19 and A94. Males having any of these deficiencies and the duplication are rather inviable at 29° C. At 25° C they can live up to 7 days, but remain sterile. Therefore, to the right of band 2B7-8 there may be at least one more essential locus. Besides there can be repeated genes in the region that are not revealed with standard genetic methods.

As mutations in the different complementation groups show a whole series of common characteristics, we may suppose that all these genes are linked to each other functionally. All the groups except *l*(1)*pp*-1 show temperature sensitivity. Some of the mutants are viable in heterozygotic combinations only at lower temperatures. It is also known that *hfw* forms normal prepupae at 18° C in the hemizygous males (Rayle, 1967). In the case of *dor* and dor^l, two temperature-sensitive periods were found: one during early embryogenesis and the other from the 3rd larval instar until the prepupal period (Pucket et al., 1977). On the contrary, the viable *br* alleles showed the most conspicuous abnormalities in wing shape at low temperature (Table 3).

The second characteristic that is common for all the groups except *l*(1)*pp*-1 is dose-sensitivity. The *D. melanogaster* genome is known to have a number of regions that in one dose, i.e. in heterozygous deficiencies, can not maintain normal functioning (Lindsley et al., 1972, Stewart and Merriam, 1973; Lefevre and Johnson, 1973). In our case we are dealing with loci whose normal alleles in one dose provide viability both in males and in females heterozygous for the deletion. However, some mutant alleles of these loci (groups *br, dor, rbp,* l(1)*pp*-2), severely reduce viability of females with heterozygous deletions. At the same time in males one dose of the mutant allele is sufficient for survival as a result of dose compensation. Females homozygous with the mutation are fully viable also. A similar situation has been described for some mutations

of *pur*-1 and *fa* loci (Johnson et al., 1979, Welshons, 1971). In agreement with these authors we classify such mutations as hypomorphs.

Finally, we have to mention some common morphological features of mutants belonging to the different groups. Similarity of the morphological anomalies can not only be found among groups overlapped by the noncomplementing mutations, but among independent complementation units, too. Such parallels suggest related functions of the genes involved. As mentioned above, the 2B region, according to its morpholgy, might represent a reverse repeat, so that the functional similarity of the genetic loci would stem from their originally common ancestry.

The majority of the mutants in the different complementation groups express developmental arrest and lethality at the end of the larval period or in the prepupal-pupal development. In addition, analysis of the puffing patterns in mutant larvae and prepupae belonging to different groups showed similar abnormalities of the ecdysone-induced puffs (a detailed report on these results will be published elsewhere).

Nonpupating mutations from the group of "long" overlapping alleles have normal ring glands which, when being transplanted into some other nonpupating mutants restores their development. Experiments with transplantations and genetic mosaics have shown, that tissues homozygous for *l*(1)*npr*-1, *l*(1)*d.norm.*-1^a can not undergo postlarvae development under normal ecdysone concentration (Kiss et al., 1978).

Taken together, these facts persuade us to speculate that within the studied region a cluster of functionally linked genes exists which control one of the early steps of metamorphosis and are involved, directly or indirectly, with the mechanism of action of ecdysone. The connection of the activity of these genes with puffing at the sites involved is of considerable interest. The solution of this question depends on the precision of the genetic and cytological localization of the mutations. A few of the mutants were mapped genetically earlier: *dor* and *br* to 0.3 and 0.6, respectively (see Lindsley and Grell, 1968); *l*(1)*d.norm.*-1^a to 0.0 (Stewart et al., 1972); *l*(1)*npr*-1 to 0.2, *l*(1)*d.norm.*-24 to 0.3 (Kiss et al., 1976); *hfw*, which is possibly allelic to the *swi* group, is known to be located to the right of *dor* (Rayle and Hoar, 1969). Although in the studies quoted above relatively small numbers of flies were scored, the results indicate that the mutations mentioned are located within a short segment close to the distal end of the X-chromosome. The cytological data also support this conclusion.

The *br* mutation is localized between 1C5–2C10 on the cytological map (see Lindsley and Grell, 1968). Data on the localization of *dor* are contradictory. It was reported that Dp(1;f)101, with its right breakpoint between 2A3-4 and 2B1-2 contains the wild-type allele of *dor* (see Lindsley and Grell, 1968). However, using series of duplications, *dor* and *hfw* were localized to the 2B4-8 region (Rayle and Hoar, 1969); it has also been suggested that *dor* is situated in 2B11-12 (Lefevre, 1976).

With the rearrangements used by us, all of the obtained mutations could be located within the region limited by the right part of the 2B3-4 band and by the left part of 2B7-8 band. The left margin of the region is limited by the right ends of deletions Df(1)*sta* and Df(1)*pn*7b located either in 2B3-4 or in

the interband between 2B3-4 and 2B5. At the moment the right margin of the region can be detected only with the help of duplications Dp(1)y^2Y67g24.2, Dp(1)y^2Y53T and Dp(1)y^2Y21T that cover all of the loci involved, but morphologically do not contain euchromatic material beyond 2B5-6. Certainly, mapping with the help of duplications adjacent to heterochromatin is not ideal. It was suggested that Dp(1)y^2Y67g24.2 itself is rearranged and contains more of 2B in the chromocenter, (Lefevre, personal communication). In a special effort to solve this point we could not find any features of position effect for our loci in the duplications used which is not in agreement with the suggestion of heterochromatinization of bands carrying these loci. Besides, duplications of this kind arise rather frequently (we have obtained 2 of 6 in one experiment) which is hardly in accordance with their derivation from multiple breaks in X-chromosome material translocated into the Y-chromosome. All of our data, taken together with Rayle and Hoar's findings (1969) lead us to conclude that the right limit of the *dor* and *swi* (*hfw?*) loci mutations is not further to the right the left part of 2B7-8. The problem may be solved more exactly with the use of other deletions. The relationship of the *dor* and *swi* (*hfw?*) loci to the puff is still uncertain inasmuch as they are situated outside of the area of deletion Df(1)S39 removing 2B5 and the puff. If 2B6 would take part in the puff formation in normal salivary gland chromosomes, it is to be assumed that either the *dor* and *swi* loci in a salivary gland are not active at the developmental stages studied or they are located in interbands.

The location of the "overlapping" mutation complex is clearer: since it falls into the deletion region of Df(1)S39, its limits coincide with the puff limits and are the following: 2B5 and possibly the right part of 2B3-4. The activity of the complex is very likely connected to the puff development. However, to discuss the question of the informational content of 2B5-6 puff in more detail would be untimely because of the lack of data on participation of the 2B6 band in formation of this puff and data genetic organization of the "overlapping complex".

Acknowledgements. The authors are indebted to G. Lefevre for critical reading of the manuscript, to II.I. Becker, V.A. Gvozdev, M.M. Green, L. Craymer, T. Kaufman, J.K. Lim, J.C. Lucchesi, I.I. Oster, M.J. Fahmi and J.W. Fristrom for kindly providing Drosophila stocks as well as to V.E. Grafodatskaya and V.A. Melnikov for the technical assistance.

References

Aizenzon, M.G., Belyaeva, E.S., Kiss, I., Koczka, K., Zhimulev, I.F.: Cytogenetic analysis of the 2Bl-2–2B9-10 region of the X-chromosome of Drosophila melanogaster. II. Complementation groups. Genetika (U.S.S.R.) **16**, 251–269 (1980)

Akam, M.E., Roberts, D.B., Richards, G.R., Ashburner, M.: *Drosophila:* the genetics of two major larval proteins. Cell **13**, 215–225 (1978)

Ananiev, E.V., Barsky, V.E.: Localization of RNA synthesis sites in the 1B-3C region of the Drosophila melanogaster X-chromosome. Chromosoma (Berl.) **65**, 359–371 (1978)

Ashburner, M.: Patterns of puffing activity in the salivary gland chromosomes of Drosophila. VI. Induction by ecdysone in salivary glands of D. melanogaster cultured in vitro. Chromosoma (Berl.) **38**, 255–281 (1972)

Ashburner, M.: The puffing activities of the salivary gland chromosomes. In: Handbook of genetics (R.C. King, ed.), vol. 3. p. 793–811 New York-London: Plenum Press 1975

Ashburner, M., Bonner, J.J.: The induction of gene activity in Drosophila by heat shock. Cell **17**, 241–254 (1979)

Becker, H.J.: Die Puffs der Speicheldrüsenchromosomen von Drosophila melanogaster. I. Mitteilung. Beobachtungen zum Verhalten des Puffmusters im Normalstamm und bei zwei Mutanten, giant und lethal-giant-larvae. Chromosoma (Berl.) **10**, 654–678 (1959)

Beermann, W.: Chromomerenkonstanz und Spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von Chironomus tentans. Chromosoma (Berl.) **5**, 139–198 (1952)

Beermann, W.: Riesenchromosomen. Protoplasmatologia, Bd. 6/D. Wien: Springer 1962

Belyaeva, E.S., Zhimulev, I.F.: RNA synthesis in the Drosophila melanogaster puffs. Cell Different. **4**, 415–427 (1976)

Belyaeva, E.S., Pokholkova, G.V., Zhimulev, I.F.: Cytogenetic analysis of the complementation groups in the 10A 1–2 band of the X-chromosome of Drosophila melanogaster. Dokl. Akad. Nauk. U.S.S.R. **228**, 1208–1211 (1976)

Berendes, H.D.: Polytene chromosome structure at the submicroscopic level. I. A map of region X, 1–4E of Drosophila melanogaster. Chromosoma (Berl.) **29**, 118–130 (1970)

Berendes, H.D., Alonso, C., Helmsing, P.J., Leenders, H.J., Derksen, J.: Structure and function in the genome of Drosophila hydei. Cold Spr. Harb. Symp. quant. Biol. **38**, 645–654 (1974)

Bischoff, W.L.: Fine structure mapping of three lethal mutants within the deep orange gene of Drosophila melanogaster. Dros. Inf. Serv. **50**, 172 (1973)

Bridges, C.B.: Salivary chromosome maps. J. Heredity **26**, 60–64 (1935)

Chovnick, A., Finnerty, V., Schalet, A., Duck, P.: Studies of genetic organization in higher organisms. I. Analysis of a complex gene in Drosophila melanogaster. Genetics **62**, 145–160 (1969)

Clever, U.: Puffing in giant chromosomes of Diptera and the mechanism of its control. In: The Nucleohistones (J. Bonner and P. Ts'o, eds.) p. 317–334. San Francisco: Holden Day 1964

Duck, P., Chovnick, A.: Resolution of an equivocal genetic element in Drosophila melanogaster: organization of the maroon-like locus. Genetics **79**, 459–466 (1975)

Fausto-Sterling, A.: Studies on the female sterile mutant rudimentary of Drosophila melanogaster. II. An analysis of aspartate transcarbamylase and dihydroorotase activities in wild-type and rudimentary strains. Biochem. Genet. **15**, 803–815 (1977)

Fincham, J.R.S.: On the nature of the glutamic dehydrogenase produced by inter-allele complementation at the am locus of Neurospora crassa. J. gen. Microbiol. **21**, 600–611 (1959)

Garen, A., Garen, S.: Complementation in vivo between structure mutants of alkaline phosphatase from E. coli. J. molec. Biol. **7**, 13–22 (1963)

Gvozdev, V.A., Ananiev, E.V., Kotelyanskaya, A.E., Zhimulev, I.F.: Transcription of intercalary heterochromatin regions in Drosophila melanogaster cell culture. Chromosoma (Berl.) **80**, 177–190 (1980)

Jarry, B.P.: Genetical and cytological location of the structural parts coding for the first three steps of pyrimidine biosynthesis in Drosophila melanogaster. Molec. gen. Genet. **172**, 199–202 (1979)

Johnson, M.M., Woloshyn, E.P., Nash, D.: Cytogenetical localization of the purine 1 and guanosine 1 loci of Drosophila melanogaster; the purine 1 locus specifies a vital function. Molec. gen. Genet. **174**, 287–292 (1979)

Judd, B.H.: Genetic units of Drosophila-complex loci. In: The genetics and biology of Drosophila (M. Ashburner and E. Novitsky, eds.), vol. 1, p. 767–799, London, New York, San Francisco: Academic Press 1976

Kaufman, T.C.: Characterization of three new alleles of the giant locus of Drosophila melanogaster. Genetics **71**, Suppl., p. 328–329 (1972)

Kerkis, A.Yu., Zhimulev, I.F., Belyaeva, E.S.: Electron-microscopic autoradiography method to study the structural and functional organization of polytene chromosomes. Tsitologia (U.S.S.R.) **17**, 1330–1331 (1975)

Kiknadze, I.I.: Functional organization of chromosomes. Nauka, p. **33** (1972)

King, R.C.: Drosophila melanogaster: An introduction. In: Handbook of genetics, (R.C. King, ed.), vol. **3**, p. 625. New York, London: Plenum Press 1975

Kiss, I., Bencze, G., Fekete, E., Fodor, A., Gausz, J., Maróy, P., Szabad, J., Szidonya, J.: Isolation and characterization of X-linked lethal mutants affecting differentiation of the imaginal discs in Drosophila melanogaster. Theoret. Appl. Genet. **48**, 217–226. (1976a)

Kiss, I., Bencze, G., Fodor, A., Szabad, J., Fristrom, J.W.: Prepupal-larval mosaics in Drosophila melanogaster. Nature (Lond.) **262**, 136–138 (1976b)

Kiss, I., Major, J. Szabad, J.: Genetic and developmental analysis of puparium formation in Drosophila melanogaster. Molec. gen. Genet. **164**, 77–83 (1978)

Korge, G.: Direct correlation between a chromosome puff and the synthesis of a larval saliva protein in Drosophila melanogaster. Chromosoma (Berl.) **62**, 155–174 (1977)

Lefevre, G., Jr.: A photographic representation and interpretation of the polytene chromosomes of Drosophila melanogaster salivary glands. In: The genetics and biology of Drosophila (M. Ashburner and E. Novitsky, eds.), vol. 1a, p. 31–66, London, New York, San Francisco: Academic Press 1976

Lefevre, G. , Jr., Johnson, T.K.: Evidence for a sex-linked haplo-inviable locus in the cut-singed region of Drosophila melanogaster. Genetics **74**, 633–645 (1973)

Lefevre, G., Jr., Wiedenheft, K.B.: Two genes in one band? Dros. Inf. Serv. **51**, 83–84 (1974)

Lewis, E.B., Bacher, F.: Method of feeding ethyl methanesulfonate (EMS) to Drosophila males. Dros. Inf. Serv. **43**, 193 (1969)

Lindsley, D.L., Grell, E.H.: Genetic variations of Drosophila melanogaster. Carnegie Inst. Wash. Publ. **627**, 1968

Lindsley, L., Sandler, L., Baker, B.S., Carpenter, A.T.C., Denell, R.E., Hall, J.C., Jacobs, P.A., Miklos, G.L.G., Davis, B.K., Gethman, R.C., Hardy, R.W., Hessler, A., Miller, S.M., Nozawa, M., Parry, D.M., Gould-Somero, M.: Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics **71**, 151–184 (1972)

Mechelke, F.: Das Wanders des Aktivitätsmaximums im BR_4-Locus von Acricotopus lucidus als Model für die Wirkungsweise eines komplexen Locus. Naturwissenschaften **48**, 29 (1961)

Mglinetz, V.A.: Interallelic interaction in spineless-aristapedia locus of Drosophila melanogaster. Genetika (U.S.S.R.) **13**, 1073–1078 (1977)

Nørby, S.: The biochemical genetics of rudimentary mutants of Drosophila melanogaster. I. Aspartate carbamoyltransferase levels in complementing and non-complementing strains. Hereditas (Lund) **73**, 11–16 (1973)

Offermann, C.A.: Branched chromosomes as symmetrical duplications. J. Genet., **32**, 103–116 (1936)

Panitz, R.: Hormonkontrollierte Genaktivitäten in den Riesenchromosomen von Acricotopus lucidus. Biol. Zentrbl., **83**, 197–230 (1964)

Partridge, C.W.H.: Altered properties of the enzyme adenylosuccinase, produced by inter-allelic complementation at the Ad-4 locus in Neurospora crassa. Biochem. biophys. Res. Comm. **3**, 613–619 (1960)

Pelling, C.: Transcription in giant chromosomal puffs. In: Results and problems in cell differentiation (W. Beermann, ed.) v. 4 p. 87–99, Springer Verlag, Berlin, Heidelberg, New York (1972)

Prokofyeva-Belgovskaya, A.A.: Inert regions of the inner parts of the X-chromosome. Izv. Akad. Nauk. U.S.S.R., Ser. Biol., No 3, 362–370 (1939)

Pucket, L., Mackay, L., Petty, K., Tabery, E., Mueller, D.: Temperature sensitivity of the mutant deep orange of Drosophila melanogaster. Genetics **86**, 549 (1977)

Rayle, R.E.: A new mutant in Drosophila melanogaster causing an ecdysone-curable interruption of the prepupal molt. Genetics **56**, 583 (1967)

Rayle, R.E., Hoar, D.I.: Gene order and cytological localization of several X-linked mutants of Drosophila melanogaster. Dros. Inf. Serv. **44**, 94 (1969)

Semeshin, V.F., Zhimulev, I.F. Belyaeva, E.S.; Cytogenetic study of the region 9E–10A of Drosophila melanogaster X chromosome. Genetika (U.S.S.R.), **15**, 1784–1792 (1979)

Stewart, B., Merriam, J.R.: Segmental aneuploidy of the X-chromosome. Dros. Inf. Serv. **50**, 167–170 (1973)

Stewart, M., Murphy, C., Fristrom, W.: The recovery and preliminary characterization of X-chromosome mutants affecting imaginal discs of Drosophila melanogaster. Develop. Biol. **27**, 71–83 (1972)

Welshons, W.J.: Genetic basis for two types of recessive lethality at the Notch locus of Drosophila. Genetics, **68**, 259–268 (1971)

Zhimulev, I.F., Belyaeva, E.S.: Incorporation of ^{3}H-uridine into the chromosomes of the larval salivary gland of Drosophila melanogaster. Genetika (U.S.S.R.) **10**, 71–80 (1974)

Zhimulev, I.F., Kulichkov, V.A.: Break sites in polytene chromosomes of Drosophila melanogaster: their location and replication features. Genetika (U.S.S.R.) **13**, 85–94 (1977)

Received August 13, 1979 – May 13, 1980 / Accepted May 19, 1980 by W. Beermann
Ready for press May 26, 1980