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Cytogenetic analysis of the *echinoid* (*ed*), *dumpy* (*dp*) and *clot* (*cl*) region in *Drosophila melanogaster*

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Summary

The chromosomal region surrounding the *ed*, *dp* and *cl* genes has been studied cytogenetically (24–26 on 2L chromosome). It contains three *Minutes* and a haplo-sterile function. For isolation of deficiencies and mutations these haplo-insufficient functions were covered by an insertional translocation of 24D4-25F2 into the X chromosome, or by tandem duplications. 112 lethal and visible mutations induced by EMS and X-rays have been localized by deficiency mapping to 20 subregions. They specify 42 loci in a 48 band interval consistent with the notion that most of the bands encode a single lethal function. The *dp*, *DTS*, *tkv* and suppressor/enhancer loci for position-effect variegation were studied in detail. A dominant suppressor function was localized within the structural part of the *dp* complex. New non-conditional lethals have been isolated for the *DTS* locus. Complementation analysis with the previously identified dominant heat-sensitive alleles places the site for heat sensitivity in the middle of the locus. Two haplo-abnormal enhancers of position-effect variegation were localized in the region 25F2–26A1. A triplo-abnormal suppressor function maps to 26B2–5; 26B9. The dose-dependent functions of these loci were studied by the use of deficiencies and duplications.

1. Introduction

The vicinity of the *dp* locus (the cytological region from 24 to 26 on chromosome 2L) contains several interesting genetic functions which are poorly characterized. A locus for which dominant cold- and heat-sensitive mutations were isolated has been localized between the *dp* and *cl* genes (Suzuki & Procunier, 1969; Rosenbluth, Ezzel & Suzuki, 1972). Such mutations can be isolated only for a few loci and no definite information about their genetic and functional properties is available. Furthermore, two loci proved to be modifiers of position-effect variegation (Reuter & Szidonya, 1983). One expresses a haplo-abnormal suppressor effect in genotypes carrying *dp* deletions and the white variegating w^{m4h} position-effect rearrangement. The other was identified by the use of tandem duplications as a triplo-abnormal suppressor. Since in heterochromatic position-effect a change in chromatin structure is a main aspect of gene inactivation, these mutations have already proved useful for genetic dissection of chromatin functions (Dorn *et al.* 1986). Cytogenetic studies of these loci are a prerequisite for their detailed genetic and molecular characterization.

The region of interest contains three *Minutes* and a haplo-sterile function. For haplo-insufficient genes even small deficiencies result in a significantly reduced viability and fertility (Lindsley *et al.* 1972). They are therefore not easily amenable to genetic fine structure studies. Attempts to isolate deficiencies utilizing the pseudodominance of recessive visible mutations or the reversion of the dominant temperature-sensitive (*DTS*) mutations localized in this region failed (Velissariou & Ashburner, 1980; Reuter & Szidonya, 1983). Such deficiencies will be lost not only because of the three strong *Minutes* but also because of the presence of a haplo-sterile function. For cytogenetic fine structure studies several specific genetic tools are necessary, most importantly insertional and tandem duplications to cover these functions.

In an earlier study we isolated duplications for this region. With the help of some of these rearrangements we have now recovered 26 new deletions out of 85394 X-ray-treated second chromosomes. From 10148 X-ray and EMS mutagenized chromosomes we recovered 103 lethals, 6 visible and 3 *Minute* mutations. These mutants were assigned to complementation groups,

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Table 1. *Mutations and chromosomes used*

Mutations or chromosomes	Breakpoint/origin	Reference[a]
Df(2L)2802	25F2–3; 25F4–26A1	3
Df(2L)50075a	25F2–3; 25F4–26A1	3
Df(2L)50078a	25F2–3; 25F4–26A1	3
$Df(2L)cl^{1}$	25D2–4; 25F2–4	2, 3
$Df(2L)cl^{2}$	25D2–4; 25F2–4	2, 3
$Df(2L)cl^{7}$	25D5–6; 26A7–8	2, 3
$Df(2L)cl^{h1}$	25D2–4; 25F1–2	5, 6
$Df(2L)cl^{h2}$	25D5–6; 25F4–5	5, 6
$Df(2L)cl^{h3}$	25D2–4; 26B2–5	6
$Df(2L)cl^{h4}$	25D6–E1; 25E5–F1	5, 6
$Df(2L)dp^{h19}$	24E5–F1; 24F7–25A1	5, 6
$Df(2L)dp^{h24}$	Not visible	6
$Df(2L)dp^{h25}$	24E2–4; 25B2–5	5, 6
$Df(2L)dp^{h28}$	24D7–8; 24F7–25A1	5, 6
$Df(2L)dp\text{-}cl^{h1}$	24F7–25A1; 26A2–3	6
$Df(2L)dp\text{-}cl^{h2}$	24E4–F1; 26B9–C1	6
$Df(2L)dp\text{-}cl^{h3}$	24F7–25A1; 25E2–4	6
$Df(2L)ed^{Sz\text{-}1}$	24A3–4; 24D3–4	4
$Df(2L)ed\text{-}dp^{h1}$	24C3–5; 25A2–3	5, 6
Df(2L)M11	24D3–4; 25A2–3	4, 5
$Df(2L)M\text{-}z^{B}$	24D8–E1; 24F7–25A1	1, 5
$Df(2L)sc^{19\text{-}1}$	24D4–5; 25C8–9	5, 6
$Df(2L)sc^{19\text{-}3}$	24E2–3; 25A7–B1	5, 6
$Df(2L)sc^{19\text{-}4}$	25A4–5; 25E5–F1	5, 6
$Df(2L)sc^{19\text{-}5}$	25A4–5; 25D5–7	5, 6
$Df(2L)sc^{19\text{-}6}$	24F1–2; 25C3–5	5, 6
$Df(2L)sc^{19\text{-}7}$	24D2–4; 25C2–3	6
$Df(2L)sc^{19\text{-}8}$	24C2–8; 25C8–9	6
$Df(2L)sc^{19\text{-}9}$	24D4–5; 25F4–26A1	6
$Df(2L)sc^{19\text{-}10}$	25A4–5; 25B9–C1	6
$Df(2L)sc^{19\text{-}11}$	24D2–4; 25B2–4	6
$Df(2L)sc^{19\text{-}12}$	25A4–5; 26A6–B1	6
$Df(2L)sc^{19\text{-}13}$	24E2–4; 25B2–5	6
$Df(2L)tkv^{Sz\text{-}2}$	25D2–4; 25D6–E1	4
$Df(2L)tkv^{Sz\text{-}3}$	25A2–3; 25D5–E1	4
Dp(2;1)B19	9B/25F2; 24D4/9C	4, 5
Dp(2;2)B3	23E2–3; 26E2–F1	4
Dp(2;2)B17	23A3–B1;25C3–8	4
$In(1)w^{m4h}$	3C2–3; 20	1
$T(Y;2)dp^{h14}$	—	6
$T(Y;2)dp^{h15}+Df(2L)ed^{-}\ dp^{-}$	—	6
$T(2L;3R)dp^{h27}$	91D/24F4–7; 32B2/91E	5, 6
dp^{hx}	X-ray induced *dp* mutations	Isolated over *Dp(2;2)B3*
a-x *b-x* *sz-x*	EMS induced lethal and visible mutations	Isolated over *Dp(2;1)B19*
h-x	X-ray induced lethal and visible mutations	Isolated over *Dp(2;1)B19*

[a] 1, Lindsley & Grell (1968); 2, Velissariou & Ashburner (1980); 3, Kotarski, Pickert & MacIntyre (1983); 4, Reuter & Szidonya (1983); 5, Semeshin & Szidonya (1985); 6, described herein.

which were mapped onto the polytene chromosome regions using the newly recovered deletions. Mutants for *dp*, *DTS*, and *tkv* as well as modifiers of position-effect variegation are discussed in detail.

2. Materials and methods

(i) *Culture conditions and stocks*

Drosophila cultures and stocks were maintained on a standard medium of cornmeal, yeast, sucrose and agar. The mutant and balancer stocks not described in Lindsley & Grell (1968) are listed in Table 1. All the crosses were carried out at 25 °C unless otherwise indicated.

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(ii) *Deficiency isolation*

Deficiencies for the *ed*, *dp* and *cl* genes were induced by 4000 R X-ray treatment (150 kV, 0·5 mm Al filter, 1000 R/min) of 2- to 4-day-old Oregon R males. These flies were crossed to *Dp(2;2)B3, ed dp^{o2} cl/ In(2L)Cy+In(2R)Cy, Cy cn^2 sp^2* virgins. The newly induced deficiencies were selected using the visible markers *ed*, *dp* and *cl*. The exceptions were crossed to *Dp(2;2)B3, ed dp^{o2} cl Sco/In(2L)Cy^{L}t^R+In(2R)Cy, Cy Roi cn^2 sp^2(=InCyRoi)* for phenotypical proof as well as to cover haplo-insufficient functions. The *Dp(2;2)B3, ed dp^{o2} cl Sco/'+'* progeny were crossed to *In(2L)Cy+In(2R)Cy, Cy cn^2 sp^2/Dp(2;2)B3, ed dp^{o2} cl* flies and the mutations and deficiencies were balanced as heterozygotes over the second chromosomal balancers *In(2L)Cy+In(2R)Cy, InCyRoi* or over *Dp(2;2)B3*. This series of mutations has been symbolized by the superscript '*h*' followed by an Arabic number.

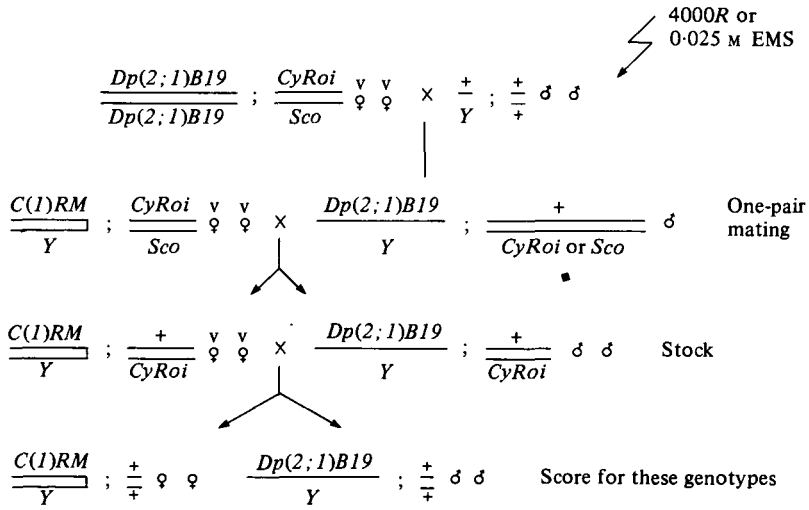
Dp(1;2)sc^{19} inserting *y$^+$* into 25A and *Dp(2;1)B19, ed dp^{o2} cl* were used in another deficiency isolation scheme. Virgins *y Dp(2;1)B19/y Dp(2;1)B19; Dp(1;2)sc^{19}/InCyRoi* were crossed to irradiated *y Dp(2;1)B19/Y; Dp(1;2)sc^{19}/InCyRoi* males (4000R). A deletion of *y$^+$* function of translocation *T(1;2)sc^{19}* will result in yellow phenotype in flies carrying a female-derived *InCyRoi* chromosome. These exceptions were crossed to *y Dp(2;1)B19; Dp(1;2)sc^{19}/InCyRoi* flies and *y Dp(2;1)B19; Df/InCyRoi* stocks were constructed. This set of deletions is symbolized as *Df(2L)sc$^{19\text{-}x}$*.

(iii) *Isolation of visible and lethal mutations*

Recessive lethal and visible mutations were isolated for the region covered by *Dp(2;1)B19, ed dp^{o2} cl* (24D4–25F2) after EMS (0·025 M) treatment according to Lewis & Bacher (1968) or X-ray (4000R) mutagenesis as described above. The isolation scheme is shown in Fig. 1. The mutations were balanced in *C(1)RM, y^2 su(w^a) w^a bb/Y; lethal/InCyRoi* × *Dp(2;1)B19/Y; lethal/InCyRoi* as well as in duplication free stocks.

(iv) *Deletion mapping and complementation analysis*

The recessive lethal and visible mutations were crossed to the deletion-bearing stocks with breakpoints in the region covered by *Dp(2;1)B19*, and the presence or absence of *Df/mutant* heterozygotes were recorded. In this way, the mutations were assigned to different



Viable	Viable	No new lethal induced on the treated chromosome
Lethal	Lethal	New lethal induced, not covered by duplication
Lethal	Viable	New lethal induced in the region covered by duplication
Mutant phenotype	Mutant phenotype	New visible mutation, not covered by duplication
Mutant phenotype	Wild type	New visible mutant covered by duplication other than *ed*, *dp* or *cl*
ed, dp or cl	ed, dp or cl	New *ed*, *dp* or *cl* mutant induced

Fig. 1. Isolation scheme of recessive lethal and visible mutations in the region covered *Dp(2;1)B19, ed dp^{o2} cl* (24D4–25F2).

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subregions. Those falling into the same subregion were crossed to each other and to the previously known mutants. The number of complementation groups was determined within each subregion and designated by the letters *jf* followed by Arabic numbers.

(v) *Cytology*

For the determination of breakpoints of chromosomal aberrations, third instar larvae grown on enriched yeast-glucose medium at 18 °C were dissected in 45% acetic acid solution and their salivary glands were stained with orcein acetic acid and squashed in lactic acetic acid. Breakpoints were determined according to the revised chromosome map of Bridges (1942) and the electron microscopic analysis of Saura (1980). For some of the deletions, the breakpoints were determined by EM analysis (Semeshin & Szidonya, 1985).

(vi) *White position-effect variegation in* In(1)w^{m4h}

On the w^{m4h} background the suppressor and enhancer effect on white variegation can be visually observed. This effect was quantified by measurements of the relative content of red eye pigments (Reuter & Wolff, 1981) and expressed as a percent of that found in the wild-type strain Canton-S.

The strongly dominant suppressor *Su-var(2)1*^{o1} (Reuter, Dorn & Hoffmann, 1982) was used to demonstrate the haplo-abnormal enhancer functions covered by some of the *clot* deletions. All the rearrangements used for the localization of the triplo-abnormal suppressor function are cl^-. Because of the lack of the appropriate eye pigment content their effect could only be visually examined.

3. Results

(i) *Cytology*

The banding pattern within the cytological extent of the duplication *Dp(2;1)B19* as compared from different sources, is shown in Figure 2. According to the Bridges (1942) map the region from 24D4 to 25F2 contains 61 bands. In an EM study Saura (1980) additionally described a few new bands and counting some doublets as single bands estimated the number of bands between 60–63. However Semeshin *et al.* (1985) and Semeshin & Szidonya (1985) using different fixation procedures showed that most of the 'doublets' have to be counted as single bands reducing the number of bands to 48 (cf. Fig. 2).

Although the breakpoints of the deletions were determined in larvae heterozygous for *Dp(2;1)B19* to cover the *Minutes*, difficulties arose especially from puffing and band morphology. If some discrepancies were found between the genetical data and the earlier

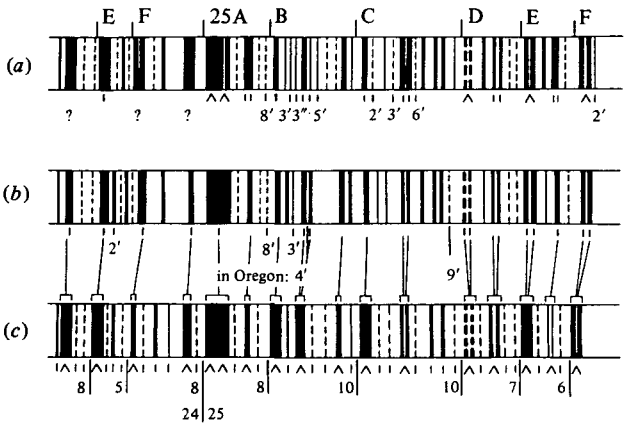

Fig. 2. Salivary gland chromosome banding pattern derived from different sources in region 24D4–25F2. (*a*) Saura (1980); (*b*) Semeshin *et al.* (1985) and Semeshin & Szidonya (1985); (*c*) Bridges (1942). ? = not decided whether singlet or doublet.

cytological studies (Semeshin & Szidonya, 1985), the breakpoints have been reexamined. The breakpoints of all deficiencies and other rearrangements used are listed in Table 1.

(ii) *Deficiency isolation and their genetic extent*

In the first set of experiments *Dp(2;2)B3, ed dp*^{o2} *cl* was used to cover the *Minute* and the haplo-sterile functions in the region. Out of 43422 treated chromosomes, 10 *ed*, 56 *dp*, *16 cl*, *2 ed dp* and 5 *dp cl* exceptions were found. Stocks could be established from 2 *ed dp*, *29 dp*, *3 dp cl* and 4 *cl* mutations. The cytological analysis proved the existence of deletions in 13 out of 38 mutations isolated (2 *ed-dp*, *3 dp*, *3 dp-cl* and 4 *cl*). A fourth *dp* mutation was cytologically normal, but subsequent genetic analysis established that it, too, was a deletion. The mutations dp^{h14} and dp^{h15} are Y; 2 translocations and dp^{h27} is an insertional translocation of the cytological region 24–32 into 3R (Table 1). The long *dp–cl*^{h} deletions are only viable over duplications and could not be used for complementation analysis.

In the second set of experiments the insertional duplication *Dp(2;1)B19* was used to cover the haplo-insufficient functions of the region while we screened for an X-ray induced loss of the y^+ function inserted by the translocation *T(1;2)sc*^{19} into region 25A. From 83944 *CyRoi* flies scored only half carried the irradiated paternal translocation. Altogether 24 yellow exceptions were detected and 13 of them were successfully established as stocks. Of these 12 proved to represent deletions. Five delete all the 3 *Minutes* of the region: *M(2)LS2*, *M(2)z* and *M(2)S1*, 1 deletes *M(2)LS2* and *M(2)z*, 1 *M(2)z* and *M(2)S1*, 2 only *M(2)z* and 2 only *M(2)S1*. All the deficiencies which include *M(2)S1* are sterile. Only 1 deficiency, *Df(2L)sc*$^{19-10}$, is M$^+$ and it is fertile.

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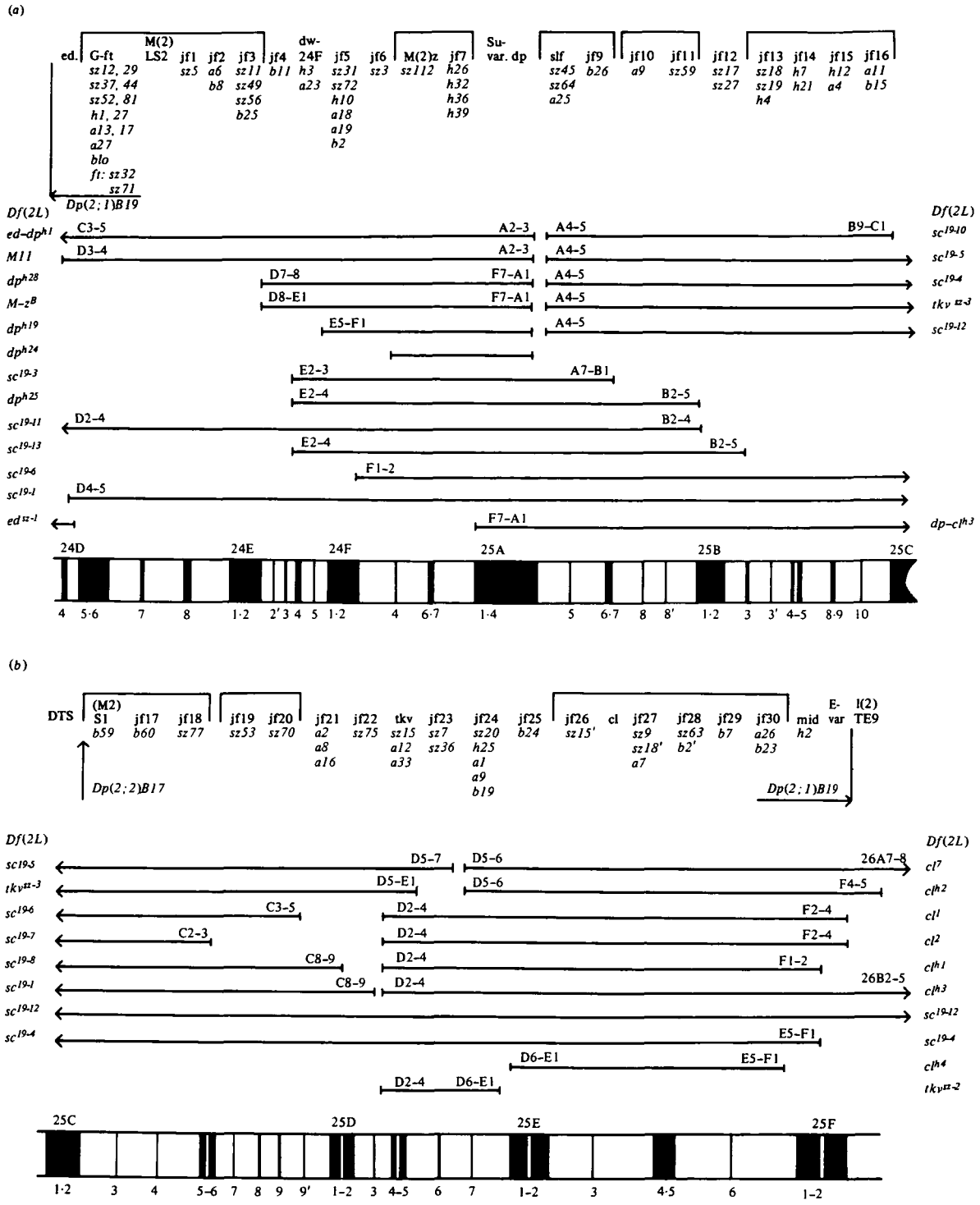

Fig. 3. Complementation map of the mutations isolated over *Dp(2;1)B19*. Deficiencies are placed according to their complementation behavior with respect to the point mutations. The polytene chromosome bands have been placed to fit as many deficiencies as possible. The *dp* and *DTS* mutations are listed in Table 2 and Fig. 4 respectively.

(iii) *Isolation of mutations within 24D4 and 25F2*

Because most of the deletions are *Minute* and show reduced viability and fertility, the insertional duplication *Dp(2;1)B19* was used to isolate lethal and visible mutations for this region. In the isolation scheme only those mutations can be recovered whose mutant function is covered by the duplication and, therefore, chromosomes with recessive second site lethal mutations outside the region are excluded (cf. Fig. 1).

We recovered 91 mutants from 7496 EMS and 20 from 2652 X-ray treated chromosomes. Of these 103 were lethals, 6 visibles and 3 *Minutes*. The frequency of mutant recovery for the region therefore is 1·22% for EMS and 0·75% for X-ray mutagenesis.

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Table 2. *Phenotypic distribution of the* dp *mutations*

Phenotype	Isolated in experiment		Mutagene origin
	Dp(2;2)B3	*Dp(2;1)B19*	
olv, Su-var	$Df(2L)dp^{h19,24,25,28}$	—	X-ray
	$dp^{h7,17,22,26}$	—	X-ray
olv	$dp^{h2,3,4,5,11}$	—	X-ray
	$dp^{h14,18,27,29}$	—	X-ray
	—	*b-12, sz-28*	EMS
o^{D}(l)v	—	*a-22*	EMS
ol, Su-var	$dp^{h6,8,9,10}$	—	X-ray
	$dp^{h12,20,21}$	—	X-ray
	—	*sz-24*	EMS
(o)l	—	*h-20*	X-ray
o(l)	—	*b-27, b-42*	EMS
lv	—	*h-37*	X-ray
	—	*b-21*	EMS
l	—	*b-1, sz-31, sz-46*	EMS
	—	*h-40, h-41*	X-ray
o	dp^{h13}	—	X-ray
	—	*sz-66*	EMS
v	—	*sz-4*	EMS

Two *olv* and two *ol* mutations which were lost from the dp^{h} series are not listed here.

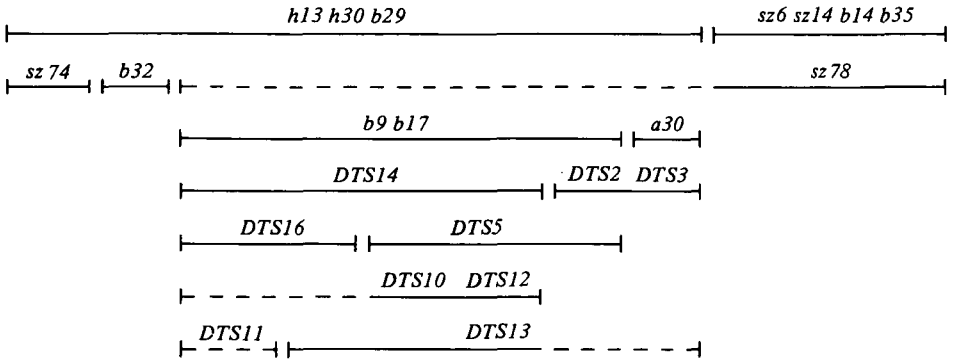

Fig. 4. Complementation map of the *DTS* alleles. Dashed line means partial complementation (less that 25% of the complete complementation).

(iv) *Deletion mapping and complementation analysis*

The mutations were first tested against a set of overlapping deficiencies $Df(2L)ed\text{-}dp^{h1}$, $Df(2L)sc^{19-3}$, $Df(2L)tkv^{Sz-3}$ and $Df(2L)cl^{h3}$ (Table 1, Fig. 3), followed by finer localization with the remaining deficiencies. In this way the mutations were each localized to 1 of the 20 subregions. Mutations within a subregion were crossed *inter se* to determine the number of complementation groups and their cytogenetic localization. Of the 42 complementation groups 17 are represented by a single mutant allele, 9 by 2 and 12 by 3 or more alleles (Fig. 3). Three loci showed a high mutability: the *Gull-fat* locus with 2 new visibles and 12 lethals, the *dp* locus with 1 *vortex*, 1 *oblique* and 14 recessive lethals and the *DTS* locus with 13 recessive lethals. In addition, for the *M(2)z* and *M(2)S1* functions EMS-induced mutants were isolated. None were detected for *M(2)LS2, ed* and *cl*.

The 112 mutations were found on 108 chromosomes, as four chromosomes carried double mutations covered by *Dp(2;1)B19*. The *sz-18* chromosome does not complement with *l(2)jf13* and *l(2)jf27* mutations while *sz-15* shows tkv phenotype in heterozygotes with *tkv* mutations and becomes lethal over $Df(2L)cl^{h4}$. Chromosome *b-2* is lethal over *l(2)jf5* and *l(2)jf28* mutations while *sz-31* carries *dp* lethal and does not complement the *l(2)jf5* mutations. These chromosomes are designated by a comma in superscript (see Fig. 3). None of the mutations of the *Gull-fat* locus complement *inter se* and they all show a lethal or visible mutant phenotype in heteroallelic combination.

The *dumpy* mutations were grouped according to their phenotypic effects in homozygotes, in *inter se* combinations, and in heterozygotes to the following alleles: *v2, lv, o2, olS* and *lvI*. From the isolation experiment using $Dp(2;2)B3, ed\ dp^{o2}\ cl$, altogether 19 *olv*, 9 *ol* and 1 *o* mutation were found. From the experiment designed to isolate recessive lethal and

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Table 3. *Complementation analysis of the tkv mutations*

Genotypes	*tkv*	*sz-15 (tkv)*	*a-12*	*a-33*	*Df(2L)tkv^{Sz-2}*	*l(2)str*
Df(2L)tkv^{Sz-3}; T(2;3)tkv^{Sz-3}	tkv	Strong tkv	Strong tkv	—	—	Strong tkv
tkv	tkv	tkv	Weak tkv	Weak tkv	tkv	tkv
sz-15 (tkv)		Recessive second site lethal	—	—	Strong tkv	—
a-12			—	—	—	—
a-33				—	—	—

tkv, thick wing vein phenotype; —, heterozygotes are lethal.

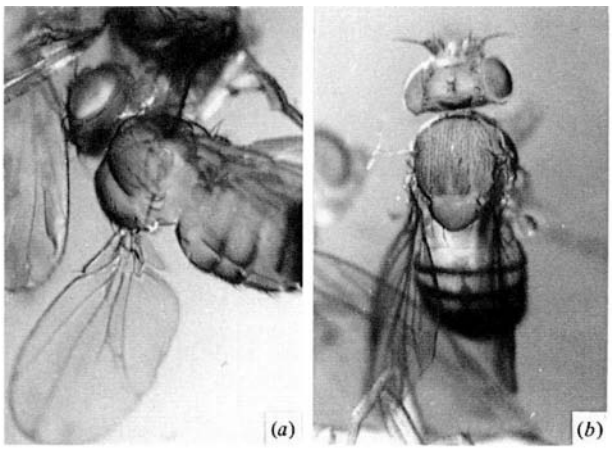

Fig. 5 (*a*)/ Developmental defects of *a-12/Df(2L)tkv^{Sz-3}+ T(2;3)tkv^{Sz-3}/+* flies. (*b*)/ Defects in bristle development of *b-24/Df(2L)cl^{h4}* flies.

visible mutations covered by *Dp(2;1)B19* only 3 *olv* were obtained, 1 showing a dominant oblique phenotype, as well as 4 *ol, 2 lv, 1 v, 1 o* and 5 lethal *dumpy* mutations (Table 2). In the case of chromosome *a-22* with dominant oblique phenotype of 100% penetrance no abnormalities were observed cytologically. Some of the homozygous *a-22* flies survive with a very strong ov phenotype. The *sz-4* mutation is a weak hypomorphic allele of the *dp* complex. Flies homozygous for this chromosome are viable with a very slight vortex phenotype and blistering wings but in combination with the majority of the *olv*, *ol*, *lv* or *l* chromosomes they are lethal or semi-lethal.

The deficiencies *Df(2L)M-z^{B}*, *Df(2L)dp^{h19}* and *Df(2L)dp^{h28}* which do not complement with any of the *dp* mutations, have no cytological effect on the 25A1·4 band (Semeshin & Szidonya, 1985). The smallest deficiency *Df(2L)dp^{h24}* which does not complement three complementation groups (Fig. 3), does not show any visible deletion on the salivary gland chromosome (Semeshin & Szidonya, unpublished result).

Another complex locus in the region is the *DTS* cluster. For this gene 13 new mutations were isolated. The *sz* series of mutations were isolated at 22 °C, and the others at 25 °C. In the complementation analysis we included the mutations isolated by Suzuki & Procunier (1969). Lethal interaction at the permissive temperature (18–22 °C) was determined in the offspring of *InCyRoi/l(2)X^{DTS}* × *InCyRoi/l(2)Y^{DTS}* crosses. (The *CyRoi* balancer was utilized to avoid errors of *Cy* classification.) The complementation map is represented in Fig. 4. Altogether 14 complementation groups and 8 complementation units (complon) could be identified. From our isolations only chromosome *a-30* showed a strong dominant temperature sensitive lethal effect, while *b-9* and *b-17* alleles are moderate *DTS* mutations.

Three new mutations represented alleles of the *tkv* locus. *Inter se* and crosses to *tkv* deficiencies revealed a complex complementation pattern (Table 3). Two types of mutations could be identified, one resulting in the thick wing vein phenotype and the other in a recessive lethal effect. The embryonic lethal mutation *str* isolated by Nüsslein-Volhard, Wieschaus & Kluding (1984) does not complement with both types of mutations.

(v) *Modifiers of position-effect variegation*

All the deficiencies and the X-ray and EMS induced *dp* mutations were tested for a dominant effect on position-effect variegation in *In(1)w^{m4h}*, since our earlier studies demonstrated the existence of such a function in the region covered by deficiency *Df(2L)M-z^{B}* (Reuter & Szidonya, 1983). Among the X-ray induced *dp* mutations, all the deletions and 11 of the *dp^{h}* mutations showed a suppressor effect. (Table 2). The differences in the eye pigment content of *w^{m4h}* flies due to the suppressor effect are shown for representative mutations in Table 4. None of the neighbouring complementation groups showed any suppressor effect. The salivary gland chromosomes of three *dp* mutations showing a suppressor effect (*dp^{h7}*, *dp^{h21}* and *dp^{h22}*) were examined by EM thin sectioning and none of them proved to be a deficiency of the 25A1·4 band or the surrounding region (Semeshin & Szidonya, unpublished).

Of the *cl* and *dp-cl* deficiencies all except *Df(2L)cl^{h1}*

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Table 4. *Dominant suppressor effect of some of the* dp *mutations on* w^{m4h} *position-effect variegation*

Mutation studied	Pigment values of w^{m4h}/Y males[a]		R[b]	Suppressor effect	Phenotype	Origin
	$dp^{hz}/+$	$CyRoi/+$				
dp^{h6}	33·8 ± 2·2	8·3 ± 2·7	4·1	Yes	ol	X-ray
dp^{h7}	37·6 ± 1·3	9·6 ± 2·2	3·9	Yes	olv	X-ray
dp^{h9}	23·6 ± 4·9	9·2 ± 2·0	2·6	Yes	ol	X-ray
dp^{h19}	31·0 ± 6·2	10·9 ± 2·7	2·8	Yes	olv	X-ray
dp^{h27}	8·5 ± 0·7	7·9 ± 1·3	1·1	No	olv	X-ray
sz-24	9·9 ± 0·5	3·1 ± 0·8	3·2	Yes	ol	EMS
a-22	7·0 ± 1·8	5·2 ± 1·3	1·3	No	o^{D}(l)v	EMS
b-1	2·6 ± 0·4	2·6 ± 0·9	1·0	No	l	EMS
b-12	1·5 ± 0·4	1·6 ± 0·7	1·0	No	olv	EMS
b-27	6·6 ± 2·6	3·9 ± 1·3	1·7	No	o(l)	EMS
b-42	3·1 ± 0·9	2·2 ± 0·8	1·4	No	o(l)	EMS

[a] Result of crosses of w^{m4h}/w^{m4h}; +/+ females with $+/Y$; $CyRoi/dp^{hz}$ males.
[b] Ratio of the relative pigment content of $dp^{hz}/+$ to $CyRoi/+$.

Table 5. *Effect of deficiencies for region 25–26 on* w^{m4h} *position-effect variegation*

Genotype[a] and pigment values[b]/Phenotype[c]				R[d]
$Df(2L)^{h1}/Su$	41·0 ± 5·3	$CyRoi/Su$	51·5 ± 5·8	0·8
$Df(2L)cl^{h2}/Su$	20·9 ± 2·7	$CyRoi/Su$	94·1 ± 8·4	0·2
$Df(2L)cl^{1}/Su$	18·0 ± 1·9	$CyRoi/Su$	67·8 ± 5·7	0·27
$Df(2L)cl^{2}/Su$	22·6 ± 1·7	$CyRoi/Su$	68·4 ± 8·6	0·33
$Df(2L)cl^{h3}/Su$	1·2 ± 0·3	$CyRoi/Su$	63·7 ± 3·5	0·01
$Df(2L)cl^{7}/Su$	1·7 ± 0·3	$CyRoi/Su$	65·0 ± 5·4	0·03
$Df(2L)cl^{h4}/Su$	55·2 ± 4·2	$CyRoi/Su$	69·8 ± 7·6	0·8
Df(2L)2802/Su	56·5 ± 2·9	$CyRoi/Su$	54·3 ± 3·2	1·04
Df(2L)70075a/Su	23·5 ± 1·2	$CyRoi/Su$	56·1 ± 2·9	0·42
Df(2L)70078a/Su	15·7 ± 0·9	$CyRoi/Su$	56·1 ± 3·1	0·28
$Df(2L)dp\text{-}cl^{h1}/DpB3$	Suppressor effect			
$Df(2L)dp\text{-}cl^{h2}/DpB3$	Strong mottling			
$Df(2L)dp\text{-}cl^{h3}/DpB3$	Suppressor effect			
$Df(2L)cl^{h3}/DpB3$	Suppressor effect			
$Df(2L)cl^{7}/DpB3$	Suppressor effect			

[a] Females of the genotype w^{m4h}; $CyRoi/Su\text{-}var(2)1^{o1}Sco$ and w^{m4h}; $CyRoi/Dp(2;2)B3$, $ed\ dp^{o2}\ cl$ were crossed to $+/Y$; $Dfcl^{z}/CyRoi$ and w^{m4h}/Y; $Dfdp\text{-}cl^{z}/Dp(2;2)B3$ males, respectively.
[b] Relative content of red eye pigment given as a percent of that found in the wild type stock, Canton-S.
[c] Genotypes $Dfcl^{z}/Dp(2;2)B3$, $ed\ dp^{o2}\ cl$ are *clot* and express the mutant eye color phenotype. White variegation therefore was studied in the eye phenotype only.
[d] Ratio of the relative pigment content of $Dfcl^{z}/Su\text{-}var(2)1^{o1}$ to $CyRoi/Su\text{-}var(2)1^{o1}$.

and $Df(2L)cl^{h4}$ express a strongly dominant enhancer effect. These deletions result in an almost white-eyed phenotype of w^{m4h} flies. In order to quantify this effect by pigment measurements the strongly dominant suppressor mutation $Su\text{-}var(2)^{o1}$ (Reuter, Dorn & Hoffmann, 1982) was used. As a result of the pigment measurements the deficiencies can be divided into two groups (Table 5). The two deficiencies $Df(2L)cl^{h3}$ and $Df(2L)cl^{7}$ express a significantly stronger enhancer effect than the others, indicating two different haplo-abnormal enhancers in the given region. One can be localized between the proximal breakpoints of $Df(2L)cl^{h1}$ and $Df(2L)cl^{h2}$ still covered by $Dp(2;1)B19$ (cf. Fig. 3) while the other maps between the proximal breakpoints of $Df(2L)cl^{h2}$ and $Df(2L)cl^{7}$ in region 25F2–3 to 26A2–5. For a further proof of the existence of the latter enhancer the independently isolated deficiencies *Df(2L)2802*, *Df(2L)50075a* and *Df(2L)50078a* (Kotarski, Pickert & MacIntyre, 1983) were tested. Both *Df(2L)50075a* and *Df(2L)50078a* express the enhancer effect whereas deficiency *Df(2L)2802* genetically with the leftmost proximal breakpoint is without any effect (Table 5). Deficiencies *Df(2L)50075a* and *Df(2L)50078a* do complement with $Df(2L)cl^{h1}$ and $Df(2L)cl^{h2}$.

In an earlier study using tandem duplications a triplo-abnormal suppressor function was localized into region 25F4-26B9. In this case three doses of a wild type gene resulted in a strong suppressor effect suggesting that a deletion over a duplication will

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restore the normal extent of mottling. Only *Df(2L)dp-cl*^{h2} shows such an effect (Table 5). Therefore, the triplo-abnormal suppressor of position-effect variegation has to be localized between 26B2–5 and 26A9.

4. Discussion

(i) *Genetic dissection of a haplo-insufficient region*

Many regions of the *Drosophila* genome contain haplo-insufficient functions (Lindsley *et al.* 1972) causing difficulties for cytogenetic analysis. The region 24–25 contains 3 *Minute* loci and 1 haplo-sterile function (Velissariou & Ashburner, 1980). To avoid these difficulties we previously suggested the isolation of deletions and mutations with the help of duplications covering these insufficient functions (Reuter & Szidonya, 1983). For this purpose the tandem duplication *Dp(2;2)B3, ed dp*^{o2} *cl* and the insertional duplication *Dp(2;1)B19, ed dp*^{o2} *cl* were used. Using the tandem duplication we isolated deficiencies based on the pseudodominance of their recessive markers. The insertional duplication was successfully applied to schemes for the selection both of deficiencies by screening for a loss of the y^+ function of *T(1;2)sc*^{19}, and of recessive lethals, by the rescue of mutant homozygotes in *Dp(2;1)B19* flies.

Over duplication *Dp(2;2)B3* 12 new deletions and 4 translocations were isolated from 43422 treated chromosomes while the experiment with *Dp(2;1)B19* yielded 12 new deletions among 41972 irradiated chromosomes. By comparison Gausz, Awad & Gyurkovics (1980) from 63000 X-ray-treated chromosomes recovered 18 new deletions for the *kar* locus, the surrounding of which do not contain haplo-insufficient functions. These numbers demonstrate that the effectiveness of the isolation schemes designed to cover these functions is similar to that of earlier investigations. Most of the deletions isolated are relatively large, especially those isolated using *T(1;2)sc*^{19}. This phenomenon also seems to be consistent with other studies. Velissariou & Ashburner (1980) using only this translocation to recover deletions for the region 25AB found nine yellow progeny from 3500 treated chromosomes. All were *Minutes* and sterile. Since the loss of *M(2)z* gene does not result in sterility, this factor can be be located to the vicinity of 25C1·2 because *Df(2L)sc*$^{19\text{-}10}$ is fertile. This implies that all these flies from that experiment were probably large deficiencies.

In our experiment the average mutation rate per locus (Barrett, 1980) is 0·029% for EMS and 0·018% for X-ray mutagenesis, but this does not include those chromosomes lost due to a second site lethal outside the region covered by *Dp(2;1)B19*. The limit of this rate varies between 0·03–0·05% (Lim & Snyder, 1974; Gausz *et al.* 1979) in different saturation experiments. Nüsslein-Volhard *et al.* (1984) after EMS mutagenesis tested 5756 second chromosomes for lethality. From these they found 4217 lines which carried one or more lethals corresponding to a total of 7600 lethal hits which gives 1·3 lethal hits/chromosome. Therefore, the average mutation rate per locus is calculated as 0·038% for EMS mutagenesis (0·023% for X-ray) which is similar to the rate for other experiments.

(ii) *Genetic organization of the region from 24D4 to 25F2*

The region studied comprises 48 bands. Altogether 42 complementation groups were identified. In general, this is in good accordance with previous reports that most band/interbands of the salivary gland giant chromosomes encode single genetic units (Judd, Shen & Kaufman, 1972; Hilliker, Clark & Chovnick, 1980; Gausz *et al.* 1979, 1981; Robert *et al.* 1985). However, recent molecular and cytogenetic fine structure studies (Wadworth, Craig & McCarthy, 1980; Zhimulev *et al.* 1981; Hall, Mason & Spierer, 1983) suggest that a single band may include more than one gene, of which one is a lethal function and the others may cause visible, behavioural, etc. phenotypes. The opposite situation is also observed. For example overlapping deletions, deficient for two bands in the *Adh* region, yield viable complementary progeny (Woodruff & Ashburner, 1979). The 70000 Da heat-shock protein genes in region 87A–C are repeated, so a complete loss of the 87C1·2 band does not decrease viability (Gausz *et al.* 1979). Since in the present study we did not search for behavioural or fertility mutants and the recovery of visibles was restricted by the presence of *y* and w^a markers, we cannot argue for or against either of these hypotheses. However, in the region covered by deficiency *Df(2L)cl*^{h4} seven independent complementation groups were found in a four band interval, at least four complementation groups can be localized in the single 25C1·2 band, and in the two bands interval of 24D5,6-7 there are five complementation groups. In the five-band interval between the proximal breakpoints of the *Df(2L)sc*$^{19\text{-}6}$ and *Df(2L)sc*$^{19\text{-}8}$ deficiencies only one complementation group could be placed and in the three band interval between the proximal breakpoint of *Df(2L)sc*$^{19\text{-}1}$ and the distal breakpoint of *Df(2L)tkv*$^{Sz\text{-}2}$ no genes could be identified. In the other subregions the number of the bands corresponds well with the number of complementation groups.

(iii) *The* dumpy *complex locus*

Previous studies localized the *dp* and *M(2)z* genes into bands 25A1-4 (Velissariou & Ashburner, 1980; Broderick & Roberts, 1982). With the new deficiencies more precise cytogenetic mapping could be performed. Most of the *dp* deficiencies coming from the left fail to remove any part of band 25A1·4, as revealed by EM studies (Semeshin & Szidonya, 1985). Therefore, we

suggest that the *dp* locus is adjacent or at the lefthand end of 25A1·4. *M(2)z* can be localized to the left of *dp* by deficiency $Df(2L)dp\text{-}cl^{h3}$ which has the distal breakpoint between *dp* and *M(2)z* because $Df(2L)dp\text{-}cl^{h3}/Dp(2;3)tkv^{Sz\text{-}3}$ is M^+. Two deletions, $Df(2L)ed\text{-}dp^{h1}$ and *Df(2L)M11* include 25A1·2 and no additional complementation group to the right of *dp* in 25A1·2 could be identified. It is very likely that the whole 25A1·4 region is devoid of any essential genetic function because between the proximal breakpoints of $Df(2L)dp^{h28}$ and $Df(2L)M\text{-}z^B$ and the distal breakpoints of deficiencies $Df(2L)sc^{19\text{-}4}$ and $Df(2L)sc^{19\text{-}5}$ (25A4–5) no complementation groups could be mapped. Furthermore, $Df(2L)ed\text{-}dp^{h1}$ and $Df(2L)tkv^{Sz\text{-}3}$, both with a breakpoint in 25A2-3, survive as heterozygotes.

The phenotypic distribution of the new *dp* mutations is different depending on which isolation scheme was used (Table 2). The use of the oblique phenotype of $Dp(2;2)B3, ed\ dp^{o2}cl$ resulted in recovery of mutants expressing an oblique phenotype independently of being vortex or lethal as well. There were twice as many *olv* mutations as *ol* but only one *o* allele (Table 2), indicating that mutations in an *o-olv* subsite usually result in an olv phenotype. The recovery of fewer *ol* mutants corresponds to the finding of Grace (1980) that the *dumpy* locus contains at least three *o-olv* subsites but only one which includes *ol* alleles. All the *ol* mutations isolated also express a dominant suppressor effect whereas only about half of the *olv* alleles show such an effect on position-effect variegation (Table 2). In the experiment with *Dp(2;1)B19* both lethal and visible *dp* mutations could be found. Out of 16 mutations 13 were connected with lethal effect (Table 2). One third of them showed only a lethal phenotype, demonstrating that in a random sample of mutations the lethal (*l*) sublocus (Grace, 1980) is as mutable as the other subloci connected with lethality (*olv*, *ol* and *lv*).

(iv) *The* DTS *complex*

Suzuki (1970) placed the *DTS* gene in 25CD. The deletion mapping places this locus between the proximal breakpoints of $Df(2L)sc^{19\text{-}10}$ and $Df(2L)sc^{19\text{-}7}$ localizing the gene in the 25B9–25C1·2 bands. In this interval at least three more complementation groups are situated. One of them is the *M(2)S1* function. The haplo-sterility also maps here but the *M(2)S1* mutation isolated does not show reduced fertility. However, this does not exclude the possibility that a *M(2)S1* deficiency results in haplo-sterility. The near vicinity of the *DTS* locus to the haplo-sterile function could explain our failure to isolate deficiencies by screening for the loss of the DTS mutant phenotype (Reuter & Szidonya, 1983), although the duplication *Dp(2;2)B17* separates the two genes placing the *DTS* proximal to *M(2)S1*.

The *sz* series of mutations were isolated at 22 °C and the rest at 25 °C. None of the mutations from the *sz* series showed any dominant heat sensitivity and none of the mutations isolated at 25 °C showed any dominant cold sensitivity (except of chromosome *a-30* which is heat sensitive) suggesting that the DTS phenotype is not the result of an amorphic or hypomorphic mutation. *Inter se* complementation analysis of the recessive lethals isolated in the current work, and most of the *DTS* alleles isolated by Suzuki & Procunier (1969), was performed at 22 and 18 °C. The 22 mutations studied fall into 14 complementation groups which define 8 complementation units at 22 °C (Fig. 4). There is only a slight interaction between the left and the right parts of the complementation map. Chromosome *sz-78* is completely lethal with the right side alleles but only semilthals with the alleles in the middle of the map, where the dominant heat sensitive mutations are localized. This suggests that only a limited part of the gene product is responsible for the heat sensitivity which would explain the low frequency of occurence of *DTS* mutations (Rosenbluth, Ezzel & Suzuki, 1972). There were no differences between the complementation maps determined at 18 and 22 °C.

(v) *The* thick vein *locus*

Kotarski, Pickert & MacIntyre (1983) already pointed out that the *tkv* locus might also be associated with a recessive lethal function. Our results clearly show that the *tkv* locus expresses both a visible and recessive lethal phenotype. We have already shown that $Df(2L)tkv^{Sz\text{-}3}$ has a breakpoint in the *tkv* gene itself (Reuter & Szidonya, 1983). As demonstrated in Table 3, lethal *a-12* is a *tkv* lethal allele showing a strong visible phenotype when heterozyous over $Df(2L)tkv^{Sz\text{-}3}+Dp(2;3)tkv^{Sz-3}$ whereas the *a-33* chromosome is lethal in this constitution. These heterozygotes not only express thick wing vein phenotype but are also characterized by a very strong effect on the thorax development: the scutellum is very much shortened and a deep furrow can be seen along the midline of dorsal thorax. Tergites along the dorsal midline of the abdomen are not properly fused (Fig. 5*a*). The *tkv* lethals are allelic to the *str* embryonic lethals isolated by Nüsslein-Volhard *et al.* (1984). The $str/Df(2L)tkv^{Sz\text{-}3}$ flies show this adult phenotype, indicating that the *str-tkv* gene exerts its effect not only in embryonic development. The proximal breakpoint of $Df(2L)tkv^{Sz\text{-}3}$ and the distal breakpoint of four *clot* deletions overlap only in the *tkv* gene, placing it in 25D4·5 band.

(vi) *Modifiers of position-effect variegation*

In the region from 24A3–4 to 26C1–2 one haplo-abnormal and one triplo-abnormal suppressor were identified as well as two enhancer functions. The haplo-abnormal suppressor function is closely linked to the *dp* gene. The *dp* mutations expressing a

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dominant suppressor effect are of the *ol* and *olv* type occurring independently of the mutagen used (Tables 2 and 3). It was not possible to identify any separate lethal complementation group distally from the *dp* locus with a suppressor function. According to the suggestion of Grace (1980) that the *ol* subsite represents the structural part of the *dp* gene, we may conclude that the suppressor effect is also a function of this part of the *dp* locus.

A haplo-abnormal enhancer effect can be placed between the proximal breakpoints of $Df(2L)cl^{h1}$ and $Df(2L)cl^{h2}$ in 25F2–4 (Table 5). Duplication *Dp(2;1)B19* also includes this locus but only shows a weak suppressor effect indicating that a duplication for this locus is not connected with the observed strong triplo-abnormal suppressor effect. In the interval between the proximal breakpoints of $Df(2L)cl^{h1}$ and cl^{h2} no recessive lethal complementation group was identified. Since both deficiencies $Df(2L)cl^{h3}$ and $Df(2L)cl^{7}$ express a significantly stronger enhancer effect than the above mentioned *cl* deficiencies as shown in $Su\text{-}var(2)1^{01}$ background (Table 5), the existence of another haplo-abnormal enhancer which is not covered by *Dp(2;1)B19* can be assumed. With the independently isolated deficiencies *Df(2L)2802, 50075a* and *50078a* this gene can be localized between 25F4 and 26A1 (Table 5).

None of these deletions, however, influence the strong triplo-abnormal suppressor effect displayed by duplication *Dp(2;2)B3*. The only deficiency showing a normal mottling phenotype when heterozygous over *Dp(2;2)B3* is $Df(2L)dp\text{-}cl^{h2}$ (Table 5). Therefore, the gene connected with the strong triplo-abnormal suppressor effect is located between the proximal breakpoints of $Df(2L)cl^{h3}$ and $Df(2L)dp\text{-}cl^{h2}$ in the region between 26B2–5 and 26B9. From these data we cannot decide whether this gene, if deleted shows a haplo-abnormal enchancer function, because the appropriate deficiencies are not yet available.

In a region comprising, according to the Bridges (1942) map, altogether about 100 bands, four position-effect variegation modifying loci were identified leading to an estimate of about 160 such loci for the whole *Drosophila* complement. This is in good accordance with the results received for a third chromosomal region (86C–88C) which suggested 120 such genes (Reuter *et al.* 1987).

(vii) *Miscellaneous loci*

The *Gull-fat* complex is placed between the distal breakpoints of $Df(2L)sc^{19\text{-}1}$ and $Df(2L)dp^{h28}$ in region 24D5–7 together wtih four more complementation groups. The *dw-24F* locus represented by two new lethal alleles. Heterozygous combinations with *dw-24F* show dwarf phenotype. This gene can be placed in the bands 24E4–5 (Fig. 3).

Complementation group *l(2)jf24* is represented by five lethal mutations which show wings up phenotype in *inter se* crosses. The heterozygous flies are very weak and not able to recover after even a slight CO_2 treatment. This complementation group occupies the distal site of $Df(2L)cl^{h2}$ and cl^{7} which overlaps $Df(2L)tkv^{Sz\text{-}2}$, placing this gene in 25D6. The next complementation group to the right, *l(2)jf25* is represented by one mutation which expresses bristle phenotype when heterozygous over $Df(2L)cl^{h2}$ or $Df(2L)cl^{h4}$ but is lethal with other *clot* deletions. The bristle phenotype means a nearly complete loss or strong Minute-like reduction of all the bristles while hairs are not effected (Figure 5*b*). This locus is placed at band 25D7.

The complementation group *l(2)jf27* , which is located within the shortest *cl* deletion $Df(2L)cl^{h4}$, is represented by two lethals and one semi-lethal expressing in heteroallelic combination a small rough-like eye phenotype. This phenotype complements with *pi* which is probably located proximal from the region we studied. The lethal function of the chromosome carrying the *TE 9* (w^{a} rst^{+}) transposon of Ising & Block (1981) in 25D maps in 25F2–3 between the proximal breakpoint of $Df(2L)cl^{1}$ and cl^{h2}, and is not covered by *Dp(2;1)B19*.

New alleles were found for two other zygotic loci (*mid* and *slf*) described by Nüsslein-Volhard, Wieschaus & Kluding (1984) and they were mapped in the region covered by duplication *Dp(2;1)B19* (Fig. 3). Another zygotic gene (*slp*) is placed in the overlapping part of $Df(2L)ed^{Sz-1}$ and $Df(2L)ed\text{-}dp^{h1}$ between the 24C4–D3 bands. We also tried to localize the lethal phenotype of the *Streak* (*Sk*) mutation but none of the deletions cover this function.

With the help of various genetic means we have been able to dissect a relatively large haplo-insufficient chromosomal region of the *Drosophila* genome. The mutations and deletions isolated in this study will provide the opportunity for further developmental and molecular analysis of the genetic loci described.

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