Obervations on the Induction of Position Effect Variegation of Euchromatic Genes in *Drosophila melanogaster*

G. V. Pokholkova, I. V. Makunin, E. S. Belyaeva and I. F. Zhimulev

Institute of Cytology and Genetics, Siberian Division, Russian Academy of Sciences, Novosibirsk 630090, Russia

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ABSTRACT

In the *T(1;2)dor*^{var7} translocation, the 1A-2B7-8 segment of the *X* chromosome is brought to the vicinity of 2R-chromosome heterochromatin resulting in position effect variegation of *dor*, *BR-C* and more distal genes, as well as compaction of chromatin in this segment. By irradiation of *T(1;2)dor*^{var7}, nine reversions (rev) to a normal phenotype were recovered. In two cases (*rev27*, *rev226*), the 1A-2B7-8 section is relocated to the 19A region of the *X* chromosome, forming free duplications (1A-2B7-8/19A-20F-*X*-het). Modifiers of position effect do not change the normal expression of the *BR-C* and *dor* genes in these duplications. In five reversions (*rev3*, *rev40*, *rev60*, *rev167*, *rev175*), free duplications have formed from the 1A-2B7-8 fragment and *X* chromosome heterochromatin. In these rearrangements, modifiers of position effect (low temperature, removal of *Y* and 2R-chromosome heterochromatin and a genetic enhancer (*E-var(3)201*) induce position-effect again. Two reversions (*rev45* and *rev110*) are associated with additional inversions in the original *dor*^{var7} chromosomes. The inversions relocate part of the heterochromatin adjacent to the 1A-2B7-8 section into new positions. In *T(1;2)dor*^{rev45}, position-effect is seen in the 2B7-8-7A element as compaction spreading from 2B7-8 proximally in some cases as far as the 5D region. Thus, in *rev45* the pattern of euchromatin compaction is reciprocal to that of the initial *dor*^{var7} strain. Apparently, it is due to the same variegation-evoking center near the 2R centromere in both cases. In all nine revertants, weakening or complete disappearance of the position-effect is observed despite retention of the 20- kb heterochromatic segment adjacent to the 1A-2B7-8 region. Thus, a 20-kb heterochromatic sequence does not inactivate euchromatin joined to it.

POSITION effect variegation (PEV) in Drosophila results from inactivation of euchromatic genes placed next to pericentric heterochromatin by chromosome rearrangements (reviews: LEWIS 1950; BAKER 1968; SPOFFORD 1976; HENIKOFF 1990; TARTOF and BREMER 1990; ZHIMULEV 1993). The inactivation is associated with a heterochromatin-like compaction of the adjacent euchromatic segment (SCHULTZ 1965; HARTMANN-GOLDSTEIN 1967; ZHIMULEV *et al.* 1986, 1988, 1989; BELYAEVA and ZHIMULEV 1991). At present, the data favor the hypothesis that heterochromatization of the euchromatic loci under position effect is caused by heterochromatin-specific proteins. One of the proteins (HP1), encoded by *Su-var(3)205* (EISSENBERG *et al.* 1990) appears in nuclei during early embryogenesis (JAMES *et al.* 1989) just at the time when the heterochromatin is forming, as revealed by C-staining (VLASSOVA *et al.* 1991). In position effect, compaction of euchromatin also occurs during the first hours of embryogenesis, as evidenced by temperature sensitivity studies (HARTMANN-GOLDSTEIN 1967; ZHIMULEV *et al.* 1988). Recently, the presence of HP1 has been demonstrated in compact euchromatic regions inactivated by position effect (BELYAEVA *et al.* 1993).

It is well known that not all heterochromatic rearrangements induce PEV (DEMEREC 1941; SPOFFORD 1976; ZHIMULEV 1993). In experiments with revertants of PEV, it has been shown that a 3-kb heterochromatic sequence proximal to the euchromatin-heterochromatin junction does not result in inactivation of euchromatin. These data suggest that special compaction domains exist in heterochromatin with centers of inactivation located some distance from the euchromatin-heterochromatin junction (TARTOF, HOBBS and JONES 1984). However, question remains concerning the distance between the eu-heterochromatin junction and heterochromatic sequences inducing inactivation (*i.e.*, what is the size of the compaction domains?). Moreover, the specificity of the heterochromatic sequences inducing compaction is unknown.

In the present study we obtain new information about the heterochromatic regions affecting gene inactivation by analysis of revertants of the position effect for genes in the 2B region associated with *T(1;2)dor*^{var7}. *T(1;2)dor*^{var7} is a complex chromosome rearrangement containing many breaks, one of which occurs in the 2B7-8 band (Figure 1) joining the 2B7–8 region with the most proximal section of 2R-heterochromatin. DNA from the 2B3-5–2B7-8 bands has

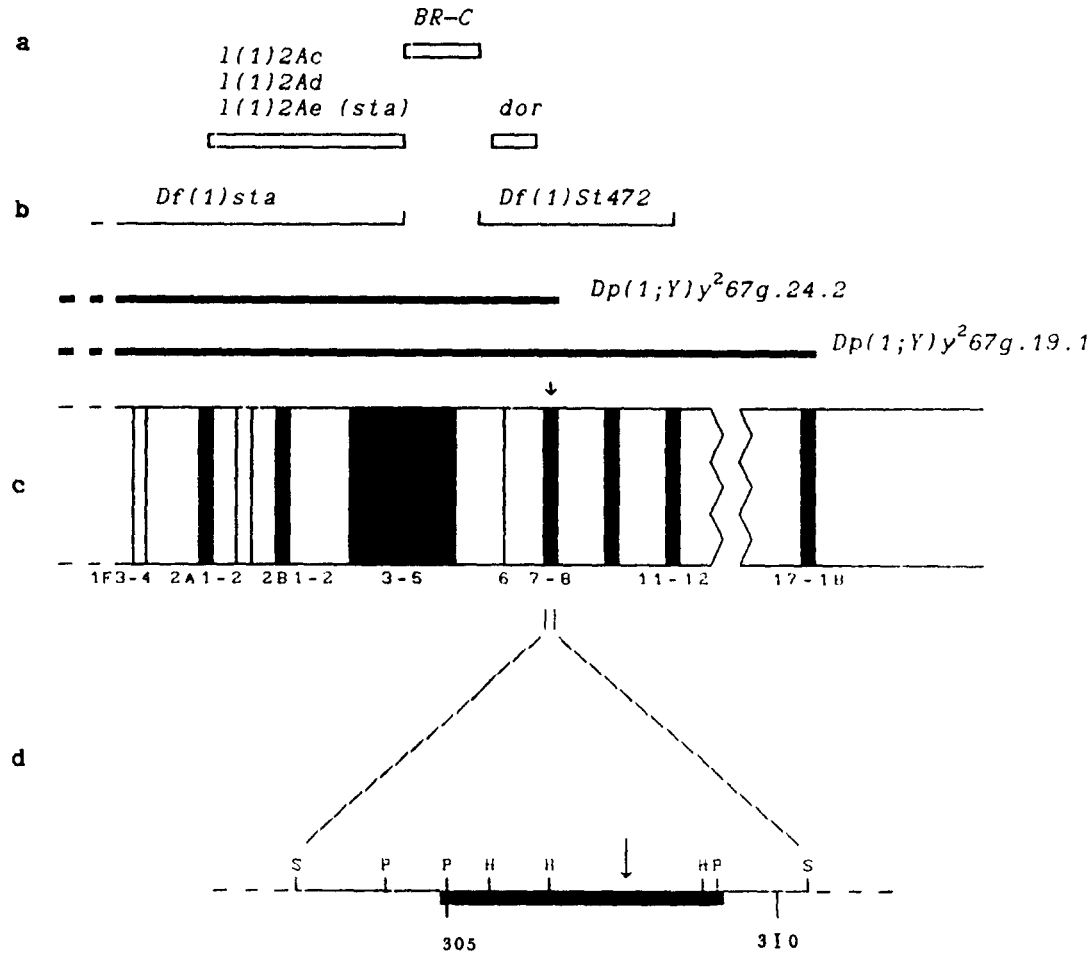

FIGURE 1.—Cytogenetic and MOLECULAR MAP OF THE 1F3-4-2B17-18 REGION OF THE *X* chromosome. (a) Location of the known genes. Cytological limits are shown by open rectangles. (b) Cytological limits of deficiencies (thin lines) and duplications (solid lines). (c) EM map of the region. (d) Partial restriction map of the 2B7-8 band containing the breakpoint of *T(1;2)dor*^{var7}, indicated by arrow (c) and (d). Solid line indicates the probe used for blot hybridizations. S, P, H, restriction sites of *Sal*I, *Pst*I and *Hin*dIII, respectively; 305 and 310 identify kilobase positions on the map of cloned DNA (see BELYAEVA *et al.* 1987; PROTOPOPOV *et al.* 1991).

been cloned and the translocation breakpoint has been mapped on the physical map (Figure 1).

MATERIALS AND METHODS

Stocks: *T(1;2)dor*^{var7} is a complex rearrangement as follows: 1A-2B7-8/2Rh-35B/45A-41A/2B7-8–7A/60C-45A/35A-21A and 60F-60C/7A-*X*-het. The 1A-2B7-8 region is transposed to the proximal part of the pericentric heterochromatin of chromosome 2R. Segment 2B7-8-7A is transposed to the distal section of the same heterochromatic block. Compaction and gene inactivation spread in the distal direction toward 1A and never toward 7A. The *dor* and *BR-C* genes, located in 2B3-6 adjacent to the breakpoint are strongly affected [ZHIMULEV *et al.* (1986) and the present data]. The *nprl*^{8} mutation (synonym *l(1)t435*) is an EMS-induced lesion of the *BR-C* locus, lethal in *nprl*^{8}/*Y* males and *nprl*^{8}/*T(1;2)dor*^{var7} females.

The stocks used to map chromosome rearrangements in region 19-20 were obtained from the Umeå Drosophila Stock Center.

Two stocks have been used as PEV enhancers: *Df(2R)MS2*^{10} deletes the pericentric heterochromatin of the 2R-chromosome and *E-var(3)201*, a semilethal, EMS-induced mutation provided by G. REUTER (REUTER *et al.* 1987).

To compare position effects in *XY* and *XO* males, *C(1)RM/O* and *C(1)RM/Y* stocks were used that are partly isogenic for the autosomes.

Information about all other mutations and rearrangements may be found in LINDSLEY and ZIMM (1992).

Mutagenesis and isolation of revertants: The *T(1;2)dor*^{var7} males were treated with x-rays (4000 r), and mated to *y nprl*^{8} *w sn/FM6, l* females (Figure 2). Among the progeny, the *y nprl*^{8}*w sn/Y* males die due to lethality of *nprl*^{8}, while the *y nprl*^{8}*w sn/T(1;2) dor*^{var7} females die due to PEV inactivation of *nprl*$^{+}$ in the translocation chromosome. The surviving y^{+} males, and females with normal phenotype are selected for study (Figure 2). Phenotypic classes may result from: (1) transposition of the 1A-2B7-8 region to another region of the translocation complex (Figure 2d); (2) gross deletion of the chromosome element carrying the 1A-2B7-8 region to produce a free duplication or minichromosome (Figure 2, c and f); (3) insertion of the 1A-2B7-8 region into an autosome or *Y* chromosome (Figure 2, a, b and e). The selected males (Figure 2, a–c) were mated to the *C(1)DX, y w f* females. If both y and y^{+} females appeared in the progeny of the tested male, then an autosomal or free duplication is

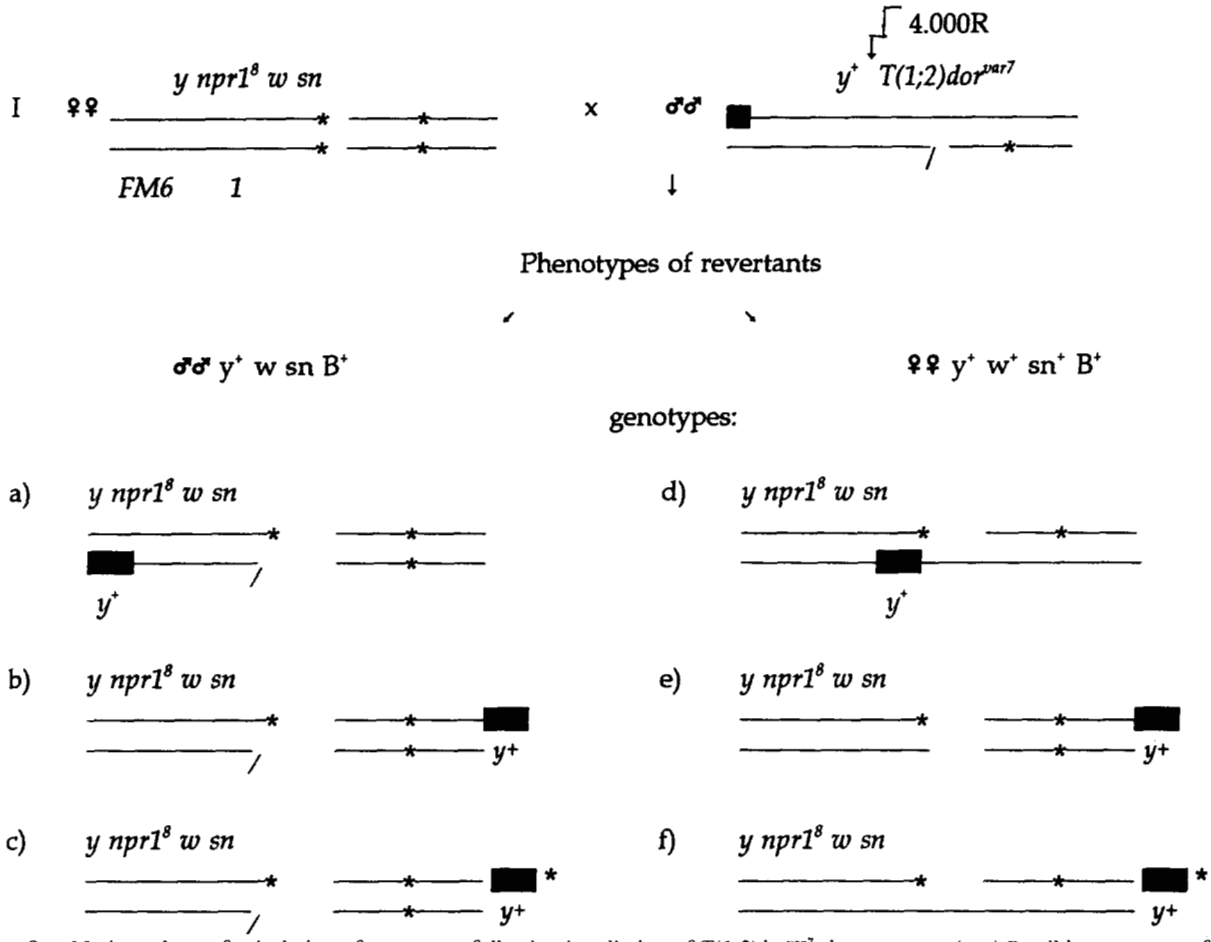

FIGURE 2.—Mating scheme for isolation of revertants following irradiation of *T(1;2)dor*^{var7} chromosome. (a–c) Possible genotypes of males showing y$^+$ w sn B$^+$ phenotype; (d–f) Possible genotypes of females showing y$^+$ w$^+$ sn$^+$ B$^+$ phenotype. Asterisk marks centromere; section 1A-2B7-8 is represented by black rectangle.

inferred. If all the females were y$^+$, then rearrangement to the *Y* chromosome is inferred. Surviving females (Figure 2, d-f) were mated to *FM7*, *y*^{31d} *w*^{a}*B* males. Appearance in the progeny of y$^+$ w^a B females confirms the presence of a free or autosomal duplication. The *C(1)DX, y w f* stock was used to balance the free duplications, and *FM6* was used for other rearrangements.

The rearrangements obtained were designated by adding *rev* (revertant) and a designation number to the standard symbol.

Experimental crosses: All the revertants were checked for PEV under several conditions: temperature (14°, 18°, 25°), removal of *Y* chromosome or pericentric 2R-heterochromatin by the *Df(2R)MS2*^{10} and introduction into the genome of a strong PEV enhancer, *E-var(3)201*. For those cases where the 1A-2B7-8 region is associated with a free duplication, *XY, Dp*^{rev} and *XO, Dp*^{rev} males were examined. These males were recovered by the simultaneous crosses: males *XY, Dp*^{rev} × females *C(1)RM, y v/Y* and *C(1)RM, y v/0*. In these crosses, the paternal *X* chromosome possesses a mutation in the 2B region or a deletion uncovering the *T(1;2)dor*^{var7} breakpoint (Figure 1). If there is no inactivation in the 1A-2B7-8 region of the free duplication, the number of males should comprise 1/3 of the total offspring.

For those cases of reversion associated with transposition of the 1A-2B7-8 region into a new location in *T(1;2)dor*^{var7}, PEV was analyzed in females of B$^+$ phenotype recovered from crossing females *T(1;2)dor*^{rev}/FM6 × males *X/Y/Dp(1)y*^{2}*Y67g19.1*. The paternal-*X* chromosome in this cross carries a mutation or chromosome rearrangement in the 2B region. If PEV caused inactivation of genes in 2B of the revertant chromosomes, then the absence of the B$^+$ phenotypic class is expected. The number of flies exhibiting PEV for the *dor* gene is estimated in analogous crosses.

Junction points between the 1-2B7-8 fragment and the 19-20 region in free duplications were determined by standard complementation tests using a set of mutations and deficiencies located in this region (Table 1).

Cytological preparation: Salivary gland polytene chromosomes were stained with aceto-orcein, squashed in lactic acid and examined in the phase-contrast microscope. Mitotic metaphase chromosome preparations were made from larval neuroganglia. C-banding preparations followed procedures of SUMNER (1972). For *in situ* hybridization, the preparations were denaturated for 1 min in 70% formamide in 2 × SSC at 70°. Following hybridization with a labeled probe for 12–16 h at 37°, the preparations were washed twice in 50% formamide in 2 × SSC and four times in 2 × SSC. Heterochromatic DNA probes (LOHE and ROBERTS 1988) were provided by A. LOHE.

Molecular biology: Genomic DNA was isolated from the imago by phenol deproteinization (HIDD, HOCKETT and YOUNG 1983). Following treatment with *Pst*I endonuclease, genomic DNA fragments were separated by electrophoresis. Southern procedures followed MANIATIS, FRITSCH and SAMBROOK (1982). The 4.0-kb fragment of the Dm 1501 clone

TABLE 1

Complementation tests of the indicated revertant duplications in *X/Y/rev* males against a set of mutations and deficiencies in the base of the *X* chromosome

Revertants	Deficiencies, mutations, and their polytene localizations								Sites of junction of 1A-2B7-8 to *X* chromosome
	Df(1)mal-3 19A1-2;20EF	*Df(1)B57* 19E1-2;19F1	*Df(1)Q54* 19F1-2;20BD	*Df(1)539* 19E6;19F6	*Df(1)16-3-22* 19F6;20A2	*Df(1)GA22* 20A3-het	*Df(1)R-8A* 20BC;20F	*su(f)* 20E	
rev3	−	−	−	−	−	−	−	−	Proximal to *su(f)*
rev27	+	+	+	+	+	+	+	+	19A1-2
rev40	−	−	−	−	−	−	−	−	Proximal to *su(f)*
rev60	−	−	−	−	−	−	+	+	20A3-20BC
rev167	−	−	−	−	−	−	−	−	Proximal to *su(f)*
rev175	−	−	−	−	−	−	−	−	Proximal to *su(f)*
rev226	+	+	+	+	+	+	+	+	19A1-2

(+) = complementation; (−) = failure of complementation.

(PROTOPOPOV *et al.* 1991) serves to probe a breakpoint of the $T(1;2)dor^{var7}$.

RESULTS

Isolation of revertants: From six independent x-ray mutagenesis experiments (MATERIALS AND METHODS), 9 revertants were recovered from a total of 7500 progeny examined: (*rev3*, *rev27*, *rev40*, *rev 45*, *rev60*, *rev110*, *rev167*, *rev175*, *rev226*). Seven exhibited segregation indicating either transposition of the 1A-2B7-8 region to an autosome or formation of a free duplication (Figure 2, e and f). Distinction between these alternatives was accomplished by establishing the linkage for the y^+ gene marking the 1A-2B7-8 region of $T(1;2)dor^{var7}$. For this purpose, crosses were carried out to *y,Cy/Pm,Sb/D*. For all seven of these revertants, the appearance of y^+ *Cy/Pm,Sb/D* among the F_2 progeny allowed one to conclude that the 1A-2B7-8 region was associated with a free duplication.

Two revertants, $T(1;2)dor^{rev45}$ and $T(1;2)dor^{rev110}$, proved to be complex chromosome rearrangements arising due to additional aberrations in the original $T(1;2)dor^{var7}$.

Cytology of the revertants: C-banded metaphase preparations of the revertants are shown in Figure 3. In the initial strain, $T(1;2)dor^{var7}$, one can see two chromosome elements: short (SE) and long (LE). SE corresponds to the fragment of 60F-60C/7A-20/*X*-heterochromatin. LE includes two heterochromatic blocks (Figure 3, b and c), the larger one being characterized by the maintenance of chromatid apposition to late metaphase (Figure 3c). In some metaphases (Figure 3d) a small euchromatic fragment (1A-2B7-8) can be seen distal to the large block, permitting localization of the junction of the 2B7-8 region with the 2R-chromosome heterochromatin. The 1A-2B7-8 region is more likely to be joined to the proximal part of the 2R pericentric heterochromatin rather than to 2L as was believed earlier (ZHIMULEV *et al.* 1986). One euchromatic breakpoint was also more precisely defined: In LE, the 60C region is adjacent to 7A rather than to 6F (Figure 4).

Further rearrangement of $T(1;2)dor^{var7}$ was associated with both $T(1;2)dor^{rev45}$ and $T(1;2)dor^{rev110}$. In $T(1;2)dor^{rev45}$, in contrast to $T(1;2)dor^{var7}$, the heterochromatic block in LE is divided into three fragments, and the centromere is located in the center of the LE (Figure 3, l and m). In the $T(1;2)dor^{rev110}$, the only visible change is the central location of the centromere (Figure 3, n and o) due to an additional inversion. Further mapping of the breakpoints has been carried out by analysis of salivary gland chromosomes. $T(1;2)dor^{rev45}$ retains the breakpoints of the original $T(1;2)dor^{var7}$: 60C, 7A, 2B7-8 (Figure 5a). One additional break occurs in the 49E region joining it with the large heterochromatic blocks and the 1A-2B7-8 fragment. Taking into account the data on mitotic chromosomes, one may conclude that two additional inversions resulted in the following order: 1A-2B7-8/2Rhet/49E-60C/7A-2B7-8/2Rhet-2Lhet-35B/45-2Rhet/49E-45A/35A-21A and 60F-60C/7A-*X*-het. $T(1;2)dor^{rev110}$ arose from an additional inversion between 2Rhet and the 5D region, producing the following order: 1A-2B7-8/2Rhet/5D-2B7-8/2Rhet-45A/35B-2Lhet-2Rhet/5D-7A/60C-45A/35B-21A and 60F-60C/7A-*X*-het (Figures 4 and 5, e and g).

In the metaphases of the seven revertants carrying free duplications $Dp(1;f)dor^{rev3}$, $Dp(1;f)dor^{rev27}$, $Dp(1;f)dor^{rev40}$, $Dp(1;f)dor^{rev60}$, $Dp(1;f)dor^{rev167}$, $Dp(1;f)dor^{rev175}$, and $Dp(1;f)dor^{rev226}$ heterochromatic minichromosomes have been found (Figure 3, e–k). To clarify the origin of heterochromatin in the duplications, we hybridized DNA clones of different *D. melanogaster* satellites with metaphases. While using clone aDm23-24 (LOHE and ROBERT 1988), we found that in all strains with duplications, the label was associated only with the heterochromatin of the normal *X* chromsome and with the duplication (Figure 6). Thus, in these revertants, the duplications are formed by joining the 1A-2B7-8 region to the *X* chromosome heterochromatin and centromere. The 1A-2B7-8 fragment is present in salivary gland preparations of all strains with duplications (Figure 5). In

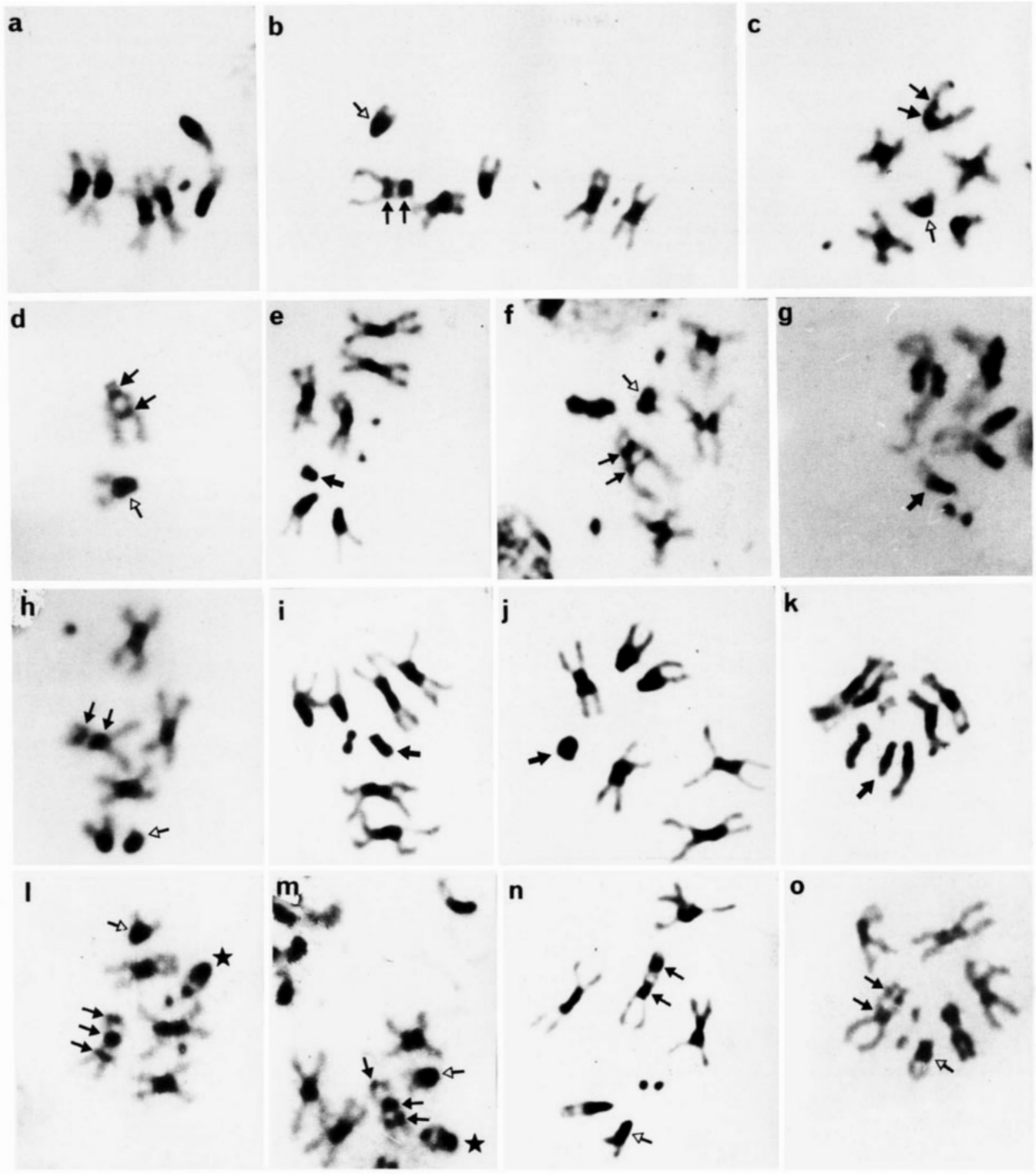

FIGURE 3.—C-banded mitotic chromosomes of the following strains: (a) *yellow*; (b–d) *T(1;2)dor*^{var7}/*y*; (e) *y/y/Dp(1;f)dor*^{rev3}; (f) *T(1;2)dor*^{rev27}/*Y*; (g) *y/y/Dp(1;f)dor*^{rev40}; (h) *T(1;2)dor*^{rev60}/*y*; (i) *y/y/Dp(1;f)dor*^{rev167}; (j) *y/y/Dp(1;f)dor*^{rev175}; (k) *y/Y/Dp(1;f)dor*^{rev226}; (l and m) *T(1;2)dor*^{rev45}/FM6; (n and o) *y/T(1;2)dor*^{rev110}. (b–d) Different stages of mitotic chromosome compaction. Free duplications are indicated by thick arrows; thin arrows point to heterochromatic blocks in the long element; open arrows represent short element rearrangements; asterisk in (m) identifies the *FM6* chromosome. In (f) and (h) long elements are shown as well as free duplications.

rev3, *rev40*, *rev167* and *rev175*, it is adjacent to a chromocenter; in *rev60* one band is observed between 2B7-8 and the chromocenter (Figure 5h); in *rev27* and *rev226*, the 2B7-7-8 region is connected with the 19A–20 region between it and the pericentric heterochromatin (Figure 5f).

Genetic mapping of the duplications (Table 1) shows that *rev27* and *rev226* include all marker loci situated in 19A-20E; *rev60* complements *Df(1)R-8A* and the *su(f)* locus (*i.e.*, the breakpoint in this case is located in the 20A3-20BC region). Four duplications (*rev3*, *rev40*, *rev167* and *rev175*) complement none of the deletions used nor do they complement the *su(f)* mutation; their 1A-2B7-8 region contacts directly with the *X*-heterochromatin.

Modification of position effect variegation in revertants: To assess the extent to which normal function is restored to position-affected genes of

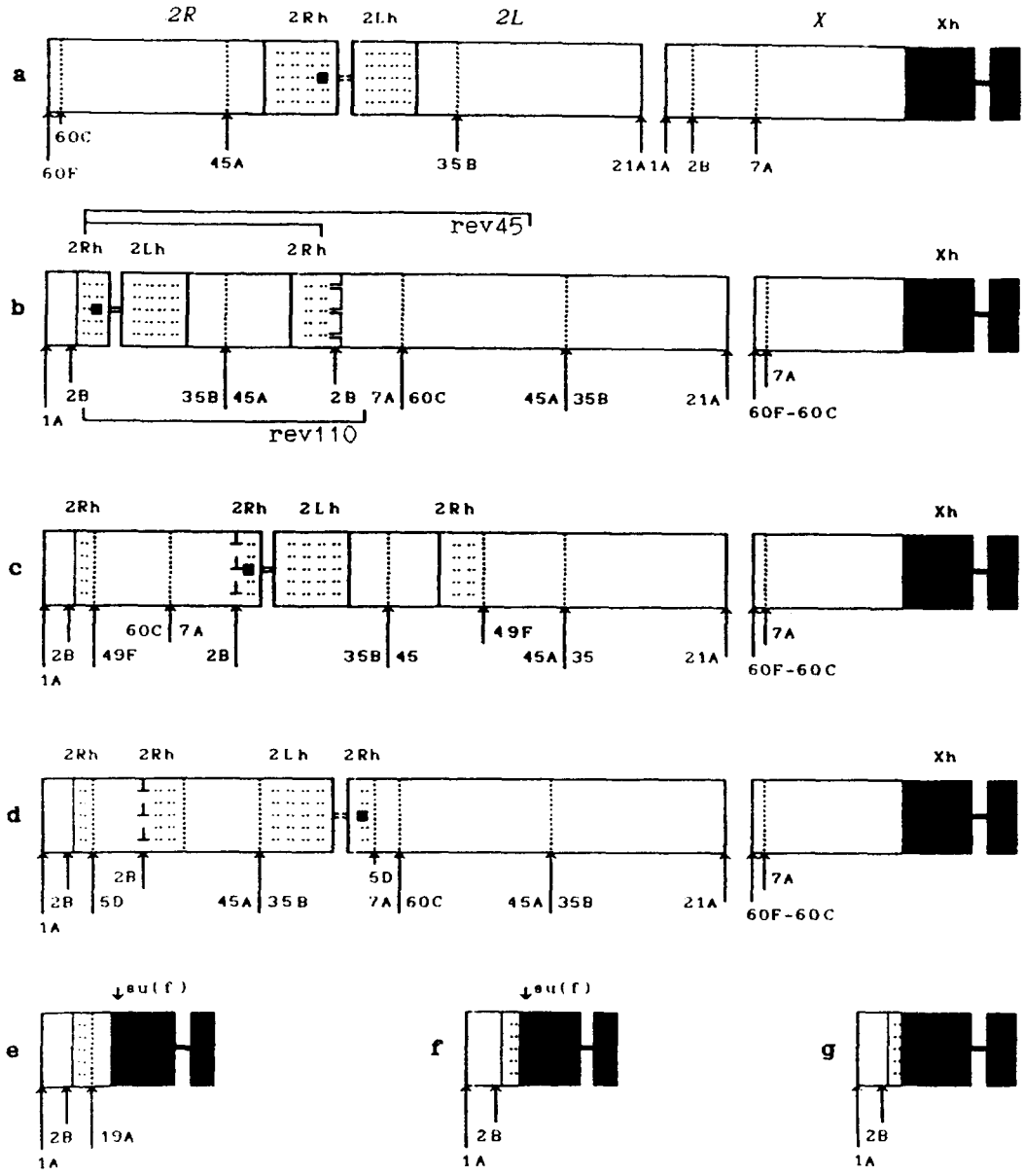

FIGURE 4.—Scheme for the localization of heterochromatin and breakpoints in (a) normal chromsome 2 and *X*; (b) *T(1;2)dor*^{var7}; and in the revertants (c) *rev45*; (d) *rev110*; (e) *rev27* and *226*; (f) *rev60*; (g) *rev3*, *rev40*, *rev167* and *rev175*. Second chromosome heterochromatin is represented by dotted areas while *X*-heterochromatin is represented by solid black areas. Inversions associated with *rev45* and *rev110* are identified by lines above and below the *T(1;2)dor*^{var7} chromosome (b). Dotted areas adjoining euchromatin in *T(1;2)dor*^{var} chromosome represent the 20- and 10-kb heterochromatic segments. Solid black square within the dotted heterochromatin represents the proposed center of heterochromatic compaction.

T(1;2)dor^{var7}, PEV enhancers have been used. With *T(1;2)dor*^{rev45} and *T(1;2)dor*^{rev110}, inactivation of loci in the 1A-2B7-8 region has not been seen, even at low temperature (14° and 18°) (Table 2). Removal of the 2R-heterochromatin or adding a genetic enhancer also do not induce genetic inactivation or variegation (data not shown). The *rev45/Y* males are not viable and *rev110* males are not viable without a *Y* chromosome, even in the presence of *Dp(1;Y)y*$^{+}$*67g.19.1* covering the 1A-2B7-8 region (Figure 1). This may be explained by possible gene inactivation due to PEV in another region of the *X* chromosome. It should be noted that some *rev110/Y* males show reduced rough eyes and thick aristas. These are seen as well in the presence of duplications covering the 2B region. Hence, they are not connected with the genes of 1A-2B7-8 (data not shown).

In *Dp(1;f)dor*^{rev3}, *Dp(1;f)dor*^{rev27}, *Dp(1;f)dor*^{rev40}, *Dp(1;f)dor*^{rev60}, *Dp(1;f)dor*^{rev167}, *Dp(1;f)dor*^{rev175}, *Dp(1;f)dor*^{rev226}, *the display of position-effect was affected by temperature, quantity of Y-* and 2R-heterochromatin and by *E-var(3)201* (Tables 3–5).

In the free duplications *rev3*, *rev40*, *rev60*, *rev167*, *rev175*, PEV for *BR-C* and *dor* may be restored by low temperature and by removing *Y* chromosome heterochromatin (Tables 3 and 4). In the presence of *E-*

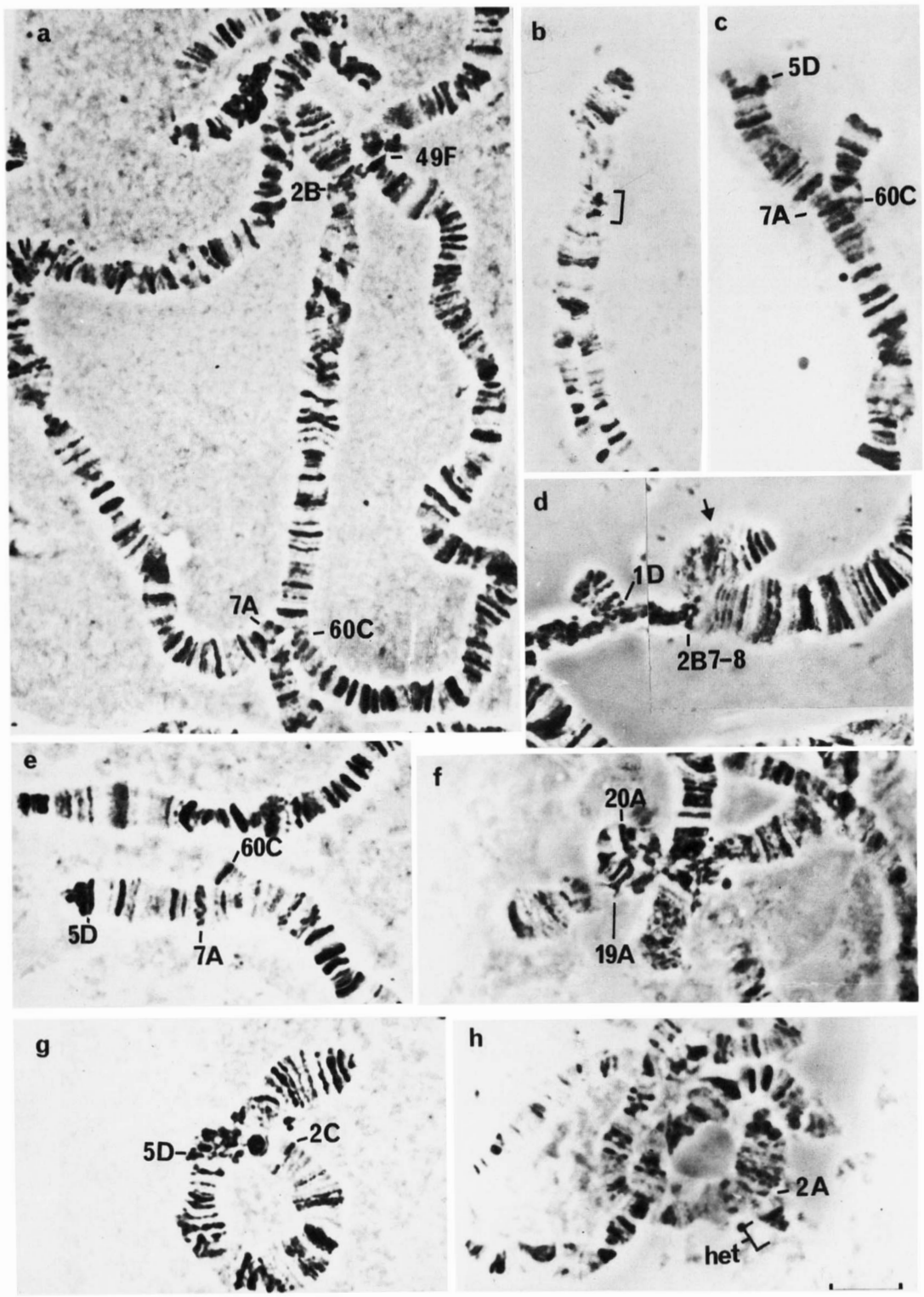

FIGURE 5.—Salivary gland chromsomes of (a–c) *rev45*; (d) *T(1;2)dor*^{var7}; (e and g) *rev110*; (f) *rev27*; and (h) *rev60*. (a) general view of rearrangement *rev45*/+; (b) compaction of the 2B7-8–2D1-2 region bracketed; (c) compaction and underreplication of the 2B7-8–5D section; (d) compaction of the 1D-2B7-8 region in *T(1;2)dor*^{var7}/+. Arrow indicates normal homolog. (e–h) Breakpoints for revertants are indicated. Scale represents 2 μm.

var(3)201 the normal allele of *BR-C* in these duplications is sufficiently inactivated to result in death of *nprl*^{8}/Y/Dp males. However, the enhancer did not increase the variegation of *dor* (Table 5). Removing the 2R-heterochromatin also has no effect on *dor* variegation (data not shown).

In *rev27* and *rev226*, the position effect on *BR-C* and *dor* was not seen with any of enhancers used.

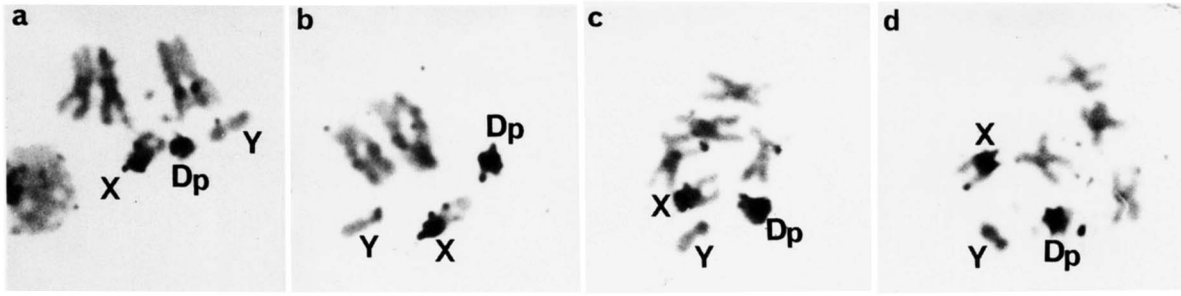

FIGURE 6.—*In situ* hybridization of a Dm23-24 DNA clone comprising 359 bp of *D. melanogaster* satellite 1. 688 with mitotic chromosomes of revertants in X/Y/Dp males: (a) *rev3*, (b) *rev40*, (c) *rev60*, (d) *rev167*. X, Y, Dp-X, Y chromosomes and duplications are noted.

TABLE 2

Complementation of *rev45* and *rev110* with mutations and chromosomal rearrangements (*M*) localized in the 2B region

Mutations or chromosome rearrangements	Temperature	Ratio of B:B$^+$ females	
		T(1;2)dor^{rev45}	*T(1;2)dor^{rev110}*
Df(1)St472	18°	267:319	326:332
		176:180	271:269
dor	14°	243:221[a]	301:287[a]
		262:260[a]	292:306[a]
Df(1)sta	18°	308:299	328:319
		341:316	304:296
nprl^8	18°	329:341	362:367

Ratio of B:B$^+$ daughters of the cross: *rev/FM6* females × *M/Y* males carried out at the indicated temperatures.

[a] Variegation for the eye color is not seen.

TABLE 3

Influence of *Y* chromosome upon variegation for the *dor$^+$* gene in *y,dor/Y/rev,y$^+$* males (upper line) and *y,dor/O/rev,y$^+$* (lower line), resulting from crosses of *y,dor/Y/rev,y$^+$* males to *C(1)RM/Y* or *C(1)RM/O* females

Revertants	Percent of dor$^+$ y$^+$ males with dor variegated eyes (no. of y$^+$ males examined)		
	25°	18°	14°
rev3	0(513); 0(418)	0.7(283); 0(204)	2(156); 1(204)
	56(430); 50(356)	95(210); 98(235)	96(190); 97(220)
rev27	0(236); 0(248)	0(204); 0(268)	0(190); 0(174)
	0(290); 0(268)	0(242); 0(230)	0(166); 0(204)
rev40	0(334); 0(442)	3(214); 3(234)	3.5(284); 1.5(500)
	0(330); 0(448)	40(103); 38(151)	30(145); 25(120)
rev60	0(240); 0(336)	0(412); 0(410)	2(192); 3(210)
	0(380); 0(530)	4(134); 4(162)	6(167); 1(94)
rev167	0(264); 0(320)	0.4(219); 0.5(200)	5(344); 6(328)
	0(426); 0(350)	13(187); 16(166)	21(160); 21(94)
rev175	0(512); 0(440)	0.5(181); 1.2(330)	10(203); 8(217)
	0(368); 0(380)	15(138); 17(253)	16(225); 14(260)
rev226	0(319); 0(208)	0(296); 0(186)	0(234); 0(256)
	0(240); 0(190)	0(420); 0(230)	0(280); 0(298)

The rows present the results of independent experiments.

Change of compaction pattern in chromosome regions of the *T(1;2)dor^{rev45}* revertant: In *T(1;2)dor^{var7}*, the position effect is observed only distally from 2B7-8. There is neither genetic nor cytological indication of inactivation in the region proximal to the breakpoint in 2B7-8 (ZHIMULEV *et al.* 1986). Both *rev110* and *rev45* have been analyzed in order to determine whether compaction exists in other regions translocated to heterochromatin. In *T(1;2)dor^{rev45}*, compaction was seen in the region proximal to 2B7-8. Figure 7 presents the data for the rearrangement under conditions of enhancement of position-effect with *Df(2R)MS2^{10}*. In this case compaction in all nuclei includes the 2B7-8–2B13-18 region and often spreads further, up to section 3C. Length compaction is associated with DNA underreplication. Thus, inactivated regions are not seen in polytene chromsomes, and 2B7-8-7A is almost always separate from the chromocenter (Figure 5, b and c). At 14° and 16° in females carrying *T(1;2)dor^{rev45}* and *Df(2R)MS2^{10}* the underreplication and heterochromatization extend to the 5D region in single nuclei (Figure 5c). Thus, due to translocation of the *T(1;2)dor^{var7}* element to a new heterochromatic site the genetic position-effect in *T(1;2)dor^{rev45}* disappears in 1A-2B7-8 and a cytological position effect appears

TABLE 4

Influence of *Y* chromosome and temperature upon position effect variegation of the *BR-C* gene in male progeny, *nprl^8/Y/rev* (upper line) and *nprl^8/O/rev* (lower line) from crossing of *nprl^8/Y/rev* males × *C(1)RM/Y* and *C(1)RM/O* females

Revertants	Percent of surviving males (total progeny)		
	25°	18°	14°
rev3	30(415); 29(529)	29(353); 31(347)	32(235); 28(518)
	0(700); 0(875)	0(670); 0(540)	0(690); 0(340)
rev27	35(506); 31(480)	29(672); 31(594)	32(462); 30(480)
	33(701); 29(620)	28(684); 34(603)	29(513); 33(561)
rev40	33(409); 29(533)	29(449); 28(458)	27(446); 28(472)
	16(421); 15(381)	12(488); 17(452)	15(580); 20(605)
rev60	38(629); 35(395)	29(544); 27(722)	30(432); 29(609)
	26(758); 24(753)	23(1227); 22(438)	45(258); 20(937)
rev167	29(402); 27(410)	28(807); 29(563)	32(346); 30(609)
	24(670); 25(751)	13(444); 15(472)	18(361); 16(403)
rev175	31(463); 34(510)	34(434); 27(869)	34(417); 30(458)
	21(929); 20(483)	21(618); 24(456)	20(503); 14(489)
rev226	34(504); 32(621)	29(480); 30(520)	31(603); 29(574)
	30(490); 31(520)	34(522); 31(492)	29(496); 33(805)

Paired values represent the results of independent experiments.

TABLE 5

Effect of the *E-var(3)201* enhancer upon inactivation of the *dor* and *BR-C* genes in the progeny of the cross: *y dor/y dor/rev* or *y nprl^{8}/y nprl^{8}/rev* females × *+/Y;E-var/TM3* males carried out at the indicated temperatures

PEV scored	Temperature	Revertants tested						
		rev3	*rev27*	*rev40*	*rev60*	*rev167*	*rev175*	*rev226*
nprl^{8}	25	248:0[a]	321:290	301:0	360:0	272:0	350:0	289:303
dor	25	0(377)[b]	0(328)	0(412)	0(386)	0(368)	0(413)	0(392)
dor	18	2(396)	0(425)	4(382)	0(404)	0(320)	0(378)	0(407)

[a] Ratio of *y nprl^{8}/Y/rev; TM3/+* to *y nprl^{8}/Y/rev; E-var/+*.
[b] Percent of dor variegated males (total *y dor/Y/rev; E-var/+* males).

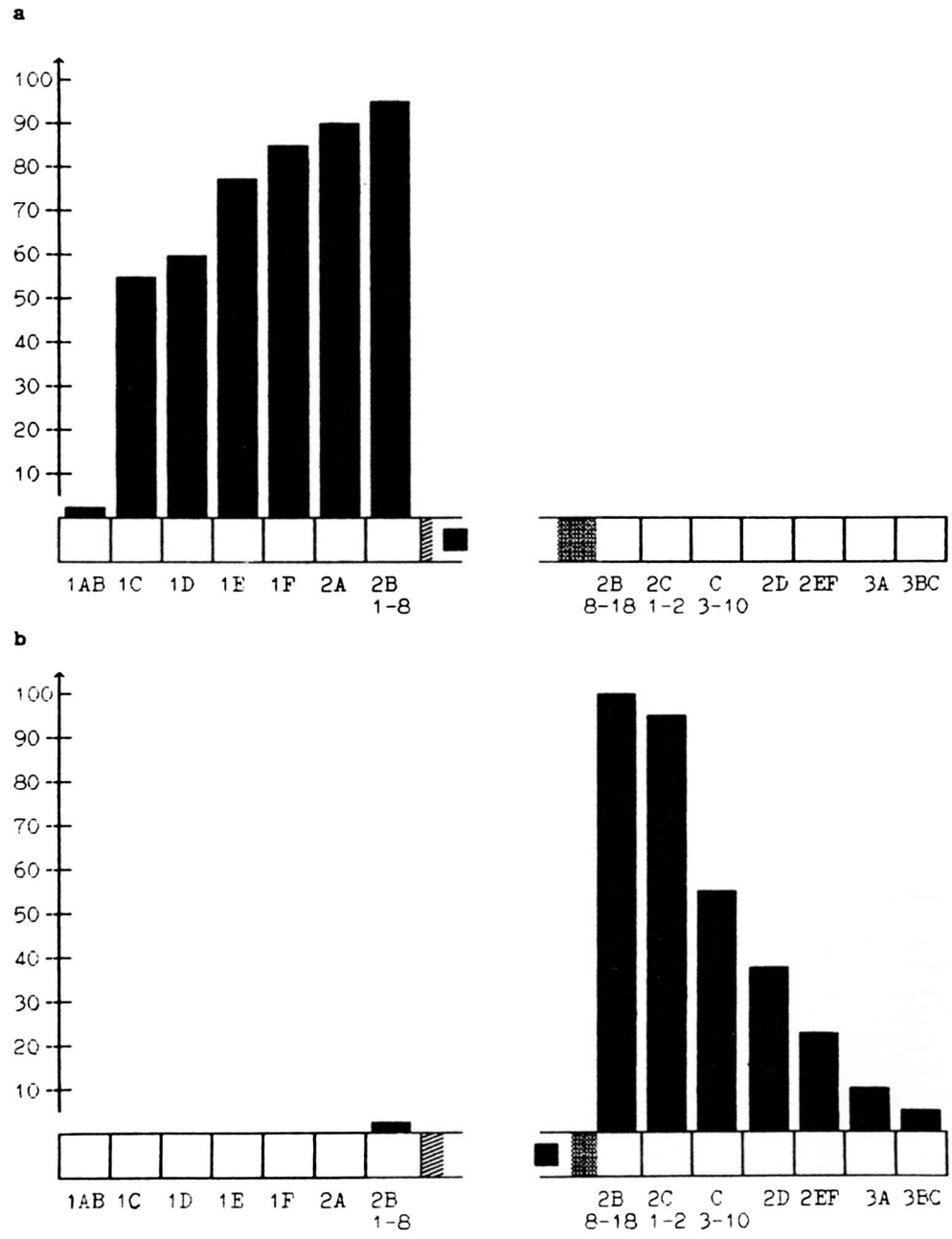

FIGURE 7.—Frequencies of compaction of the euchromatic regions brought into the vicinity of heterochromatin in (a) *T(1;2)dor^{var7}* and in (b) *rev45*. (a) *T(1;2)dor^{var7}/+; Df(2R)MS2^{10}/+*; 14°, (b) *T(1;2)dor^{rev45}/+; Df(2R)MS2^{10}/+*; 25°. Black square represents the proposed heterochromatic compaction center. Shaded regions common to both rearrangements: 20- (left) and 10- (right) kb heterochromatic fragments adjacent to euchromatin. Ordinate: percent of chromosomes examined showing compaction in indicated region. Number of chromosomes examined: (a) 116 and (b) 111.

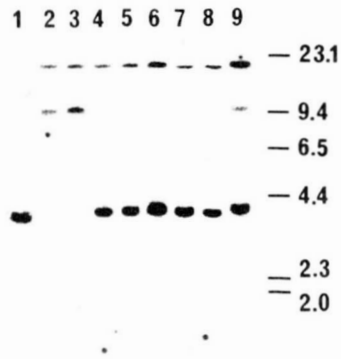

FIGURE 8.—Southern blot hybridization of 4.0-kb *Pst*I fragment (Figure 1) to genomic DNA from the following strains: (lane 1) Batumi-L wild-type female; (lane 2) $T(1;2)dor^{var7}/Y$; (lane 3) $T(1;2)dor^{rev110}/Y$; (lanes 4–8) y/Y, $Dp(1;f)dor^{rev3}$; y/Y, $Dp(1;f)dor^{rev27}$; y/Y, $Dp(1;f)dor^{rev40}$; y/Y, $Dp(1;f)dor^{rev60}$; y/Y, $Dp(1;f)dor^{rev175}$; (lane 9) $T(1;2)dor^{rev45}/FM6$. Molecular weight markers are indicated.

in 2B7-8–7A, *i.e.*, in $T(1;2)dor^{rev45}$, the situation may be reciprocal to that in $T(1;2)dor^{var7}$ (Figure 7).

The length of heterochromatic region involved in translocation: Genomic DNA from revertants was digested with *Pst*I, run in agarose gel and transferred to Hybond-N. Then, it was hybridized with the 4.0-kb *Pst*I fragment of the *DmT*1501 clone from the normal strain (Oregon-R), a fragment broken by $T(1;2)dor^{var7}$ (Figure 1). In the control lane (1), one band can be clearly seen which corresponds to the whole *Pst*I fragment of 4.0 kb (Figure 8). In $T(1;2)dor^{var7}$, this fragment is replaced by two new ones of 20 and 10 kb, representing attachment of its parts to the heterochromatic regions. By hybridization of $T(1;2)dor^{var7}$ DNA with the 0.8-kb *Hin*dIII fragment located distal (Figure 1) to the breakpoint of $T(1;2)dor^{var7}$, only the 20-kb fragment is revealed (data not shown). This identifies the 20-kb fragment as including the heterochromatin adjacent to the 1A-2B7-8 in $T(1;2)dor^{var7}$.

Fragments seen in $T(1;2)dor^{rev110}$ males are identical to those in $T(1;2)dor^{var7}$ (Figure 8). In the revertants carrying free duplications ($X/Y/Dp(1;1)dor^{rev}$), two bands of hybridization were detected with 20- and 4.0-kb fragments. The lower band (4.0 kb) coincides with the fragment in the Oregon-R and Batumi-L strains, and is explained by the presence of a normal *X* chromosome. The 20-kb band indicates that the heterochromatic junction was translocated along with the 1A-2B7-8 region into a new position. DNA from $T(1;2)dor^{rev45}$/FM6 females gives three distinct bands of hybridization with the probe (20, 10 and 4.0 kb). The two upper bands correspond to fragments from $T(1;2)dor^{var7}$ and the low band is a fragment from the *FM*6 balancer chromosome, where the *Pst*I-*Pst*I region is intact. Thus, in all revertants, the same heterochromatic sequence is adjacent to the 1A-2B7-8 region as in the $T(1;2)dor^{var7}$ strain.

DISCUSSION

Position-effect variegation for the *dor* and *BR-C* genes in the $T(1;2)dor^{var7}$ strain is caused by the location of the 2B7-8 region of the *X* chromosome adjacent to a proximal section of 2R-heterochromatin. Heterozygotes for a lethal allele of the *BR-C* gene ($nprl^8$) and the $T(1;2)dor^{var7}$ translocation are completely inviable due to inactivation of the normal allele of the *BR-C* gene in the rearranged chromosome (ZHIMULEV *et al.* 1986). In this study, revertants of position-effect were isolated as viable $nprl^8/rev$ heterozygotes; therefore, one may conclude that in all nine cases, a significant decrease of the position-effect occurred.

Analysis of cytological preparations has shown that the reversions are associated with changes in chromosomal position of the 1A-2B7-8 region. The following types of changes can be detected: (1) The 1A-2B7-8 region has no contact with heterochromatin and is translocated into the euchromatic 19A region of the *X* chromosome (*rev27* and *rev226*; see Figure 4e). In this case of free duplications, a stable reversion to the normal phenotype occurs and none of the tested enhancers of PEV causes genetic inactivation of dor^+ and $BR\text{-}C^+$. (2) In five free duplications (*rev3*, *rev40*, *rev60*, *rev167*, *rev175*) the 1A-2B7-8 region is located near pericentric *X* heterochromatin. In $Dp(1;f)dor^{rev60}$, the new junction is distal to the *su(f)* locus which is itself located in the region of the eu-heterochromatic junction (according to YAMAMOTO *et al.* 1990). In this rearrangement, all or most of the *X*-heterochromatin appears to be retained. For the other revertants associated with free duplications, C-banding of mitotic chromosomes has revealed that a significant part of the *X*-heterochromatin is retained as well (Figure 3). The quantity of heterochromatin in the *X* chromosome is calculated to be approximately equal to that of the second chromosome while the quantity of known repeated and satellite DNA is nearly twice as much (PEACOCK *et al.* 1978). Therefore, it is evident that reversion to normal expression of the *dor* and *BR-C* genes in these five rearrangements occurs simultaneously with a significant increase in the quantity of heterochromatin adjacent to the 1A-2B7-8 fragment. However, reversion is not complete since PEV enhancers restore variegation for the *BR-C* and *dor* genes. Thus, in these experiments carried out to discover whether variegation can be induced in the free duplication revertants, modifiers did restore PEV in *rev3*, *rev40*, *rev60*, *rev167* and *rev175*. (3) In two rearrangements, *rev45* and *rev110*, complete reversion to the normal state is correlated with a significant decrease in the quantity of heterochromatin adjacent to the 2B7-8 region. We failed to see genetic evidence of PEV for *dor* and *BR-C* in these rearrangements, even with the help of enhancers.

In all reversions, at least 20 kb of heterochromatic DNA adjacent to the 1A-2B7-8 fragment in *T(1;2)dor*^{var7} was relocated to a new position. Thus, the presence of flanking DNA encompassing the heterochromatic junction adjacent to the *dor* and *BR-C* loci is not sufficient to induce a position-effect on these loci.

TARTOF, HOBBS and JONES (1984) obtained three w^+ revertants of the *In(1)w*^{m4} inversion, in which a minimum of 3 kb of heterochromatic DNA had accompanied the w^+ gene into a new position. REUTER, WOLFF and FRIEDE (1985) analyzed frequencies of formation of revertants for the same inversion and concluded that the euchromatic-heterochromatic junction itself is responsible for the variegation of the w^+ gene. In the present study, we show that the heterochromatic DNA sequence responsible for position-effect variegation in *T(1;2)dor*^{var7} is not situated in the heterochromatic junction, but is located at least 20 kb from the breakpoint.

The data show that juxtaposition of a euchromatic region to heterochromatin is not sufficient in itself for inactivation of euchromatic genes. It has also long been known that not every eu-heterochromatic rearrangement leads to PEV. Different heterochromatic regions may differentially induce PEV.

TARTOF, HOBBS and JONES (1984) suggested a model in which heterochromatin contains domains having sites responsible for initiation and termination of compaction. In the case of a break separating two sites, genes in the euchromatin translocated near the compaction initiation site are inactivated.

The hypothesis concerning initiation sites for compaction explains the known heterogeneity of heterochromatin for inducing position-effect. However, there is no direct evidence for the existence of such sites. From this point of view, *rev45* isolated in this study is of interest. It reveals a pattern of position-effect and compaction reciprocal to that of the original *var7* strain. The 1A-2B7-8 element (inactivated in *var7*) is translocated to the 49E region together with the adjacent 20 kb of heterochromatin, and consequently loses the position-effect. One may speculate that this results from separation of 1A-2B7-8 from a position-effect initiation site. A heterochromatic section containing this supposed site is relocated to the distal end of the 2B7-8-7A fragment in *rev45*, and compaction occurs spreading from the 2B7-8 region to 5D (*i.e.*, about 170 bands on Bridges' map). If this PEV correlated criterion is used, the position-effect in *rev45* is significantly stronger than in *var7*. This may be due to location of the proposed initiation site of compaction in *rev45* closer to the euchromatic-heterochromatic junction than in *var7*. This is the simplest interpretation of the observed change of compaction in *rev45*.

An initiation site may be necessary for starting the process of joining the chromatin-compacting proteins with DNA. The rest of the heterochromatin is capable of continuing the compaction process, spreading it further. Euchromatin in a rearranged chromosome also binds compaction proteins but not so effectively. Euchromatic regions should be more easily compacted when they contain repetitive sequences homologous to DNA from heterochromatin. For example, in the 2B region, repeated sequences homologous to heterochromatin have been found (E. B. KOKOZA, personal communication). This region is most susceptible to compaction under the influence of heterochromatin (BELYAEVA and ZHIMULEV 1991).

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