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Position Effect Variegation in *Drosophila melanogaster*: Relationship Between Suppression Effect and the Amount of *Y* Chromosome

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ABSTRACT

Position effect variegation results from chromosome rearrangements which translocate euchromatic genes close to the heterochromatin. The euchromatin-heterochromatin association is responsible for the inactivation of these genes in some cell clones. In *Drosophila melanogaster* the *Y* chromosome, which is entirely heterochromatic, is known to suppress variegation of euchromatic genes. In the present work we have investigated the genetic nature of the variegation suppressing property of the *D. melanogaster Y* chromosome. We have determined the extent to which different cytologically characterized *Y* chromosome deficiencies and *Y* fragments suppress three V-type position effects: the *Y*-suppressed lethality, the *white mottled* and the *brown dominant* variegated phenotypes. We find that: (1) chromosomes which are cytologically different and yet retain similar amounts of heterochromatin are equally effective suppressors, and (2) suppression effect is positively related to the size of the *Y* chromosome deficiencies and fragments that we tested. It increases with increasing amounts of *Y* heterochromatin up to 60–80% of the entire *Y*, after which the effect reaches a plateau. These findings suggest suppression is a function of the amount of *Y* heterochromatin present in the genome and is not attributable to any discrete *Y* region.

VARIEGATION due to the chromosomal position of a gene was first described in *Drosophila melanogaster* by MULLER (1930) and has since been observed in several species. The phenomenon is the consequence of the inactivation of euchromatic genes which are placed into a disrupted block of heterochromatin by a chromosome rearrangement. The heterochromatin-directed *cis*-inactivation only occurs in a proportion of cells during development. Thus the individual carrying the chromosome rearrangement is phenotypically a mosaic of mutant and wild-type clones. (For review, see SPOFFORD 1976.)

Cytogenetic analysis of *D. melanogaster* polytene chromosomes has shown that segments containing variegating genes lose their typical banded morphology and show irregular or diffuse banding (SHULTZ and CASPERSON 1934; PROKOFYEVA-BELGOVSKAYA 1939; HARTMAN-GOLDSTEIN 1967; KORNER and KAUFFMAN 1986). This suggests that the adjacent heterochromatin causes some modification(s) in the chromatin structure of the variegating gene, which possibly results in the inhibition of its normal expression. A recent molecular analysis of the *rosy* locus has shown in fact that the rosy-variegated phenotype is related to a decrease in transcription (RUSHLOW, BENDER and CHOVNICK 1984).

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Although the molecular basis of position effect variegation remains as yet unknown, different sets of data suggest that histone proteins play an important role in this phenomenon. In fact both reduction of histone gene multiplicity (MOORE *et al.* 1979; MOORE, SINCLAIR and GRIGLIATTI 1983) and histone protein modifications result in the suppression of position effect variegation (MOTTUS, REEVES and GRIGLIATTI 1980). Furthermore, the dominant mutation *Su-var(2)1^{01}*, which suppresses position effect variegation and shows a lethal interaction with the *Y* chromosome, correlates with histone H4 hyperacetylation and with a significantly increased chromatin accessibility to endogenous nucleases (DORN *et al.* 1986). Altogether these findings indicate that changes in chromatin structure and organization play an important role in modifying variegating gene expression.

In the last few years nonhistone chromosomal proteins (NHC proteins) were isolated which bind predominantly to the heterochromatic sequences of *D. melanogaster* and may be involved in the acquisition and/or the maintenance of a compact heterochromatin structure (HSIEH and BRUTLAG 1979; LEVINGER and VARSHAVSKY 1982; JAMES and ELGIN 1986) and thus in position effect variegation.

In *D. melanogaster* position effect variegation can be enhanced or suppressed by a variety of physical, chemical and genetic factors (reviewed by SPOFFORD 1976). In particular, addition of heterochromatin to the ge-

nome suppresses, while subtraction enhances, typical variegation. The *Y* chromosome, which is entirely heterochromatic, suppresses position effect variegation (GOWEN and GAY 1934) in a cell autonomous way (BECKER and JANNING 1977). Attempts were made to identify specific *Y* region(s) involved in suppression of position effect variegation. Early studies on the white mottled position effect indicated that the amount of *Y* chromosome added to the genome was unrelated to the efficiency of suppression (BAKER and SPOFFORD 1959). In a further analysis, the B^S variegated phenotype was investigated and two suppressing regions, one close to the *kl-2* fertility factor and another proximal to *ks-1*, were identified (BROSSEAU 1964).

It has recently been suggested that the *Y* chromosome suppresses position effect variegation by competing for free histone or NHC protein(s) responsible for the "heterochromatinization" of the variegating gene (ZUCKERKANDL 1974; MOORE *et al.*, 1979). That would result in a general dilution of these proteins at the variegating sites, thus reducing the probability that these sites become inactive (MOTTUS, REEVES and GRIGLIATTI 1980).

In the last few years the *Y* functions have been analyzed cytogenetically. The *Y* chromosome heterochromatin has been subdivided into 25 differentially stained regions by combining Quinacrine, Hoechst 33258 and N-band staining techniques. Genetic complementation tests coupled with the cytological analysis of several rearranged *Y* chromosomes have led to mapping of the *Y* functions to these cytological regions (GATTI and PIMPINELLI 1983). The loci defined in these studies are physically very large. The *kl-5*, *kl-3* and *ks-1* fertility factors are 3 to 4 Mb long, for example. Different types of genetic organization are evident among the component of the *Y* chromosome: (1) the six fertility factors require structural integrity for functioning; (2) the collochore (COOPER 1964) and the *Cry* locus (HARDY and KENNISON 1980; HARDY *et al.* 1984), like the *bb* locus (reviewed by RITOSSA 1976), are inactivated only by deletions (rather than by breakpoints) and therefore possibly consist of a large number of repeated subunits (reviewed by PIMPINELLI *et al.* 1986); and (3) the *ABO* factors, which map to specific heterochromatic sites of different chromosomes and may correspond to another kind of repeated genetic element (PIMPINELLI *et al.* 1985). Furthermore, the *Y* chromosome heterochromatin includes several classes of highly-repeated and middle repetitive DNAs (PEACOCK *et al.* 1977; SPRADLING and RUBIN 1981) which may be functional parts of the genetic loci (GATTI and PIMPINELLI 1983; LIVAK 1984).

In the present work we have asked whether the suppression of position effect variegation is a general feature of the heterochromatic *Y*-DNA or whether it is due to specific *Y* regions. To this end we have tested the effectiveness of nine different *Y* chromosome deficiencies and five *Y* fragments in suppressing three different V-type position effects: the *Y*-suppressed lethality, the *white mottled* and the *brown dominant* phenotypes, associated with the *In(1)lv231*, $In(1)w^{m4L}$ w^{m51bR} and $In(2R)bw^{VDe2}$ rearrangements, respectively. We have found that the suppression of all three variegated phenotypes depends on the amount of the *Y* chromosome present in the genome and is not attributable to any discrete *Y* region.

MATERIALS AND METHODS

For the description of *FM7* and $In(1)w^{m4L}$ w^{m51bR} rearrangements see APPELS and HILLIKER (1982). Other chromosomes and genetic markers used in this work are described in LINDSLEY and GRELL (1968).

Culture conditions: Flies were maintained on a standard *Drosophila* medium containing cornmeal, yeast, sucrose agar with nipagin added as a mold inhibitor. All crosses were grown at 25°.

Male-sterile *Y* chromosomes: The genetics and cytology of the y^+Y deficiencies have been previously described (GATTI and PIMPINELLI 1983) except for *Df(Y)G22*, B^Sy^+, which was characterized genetically by KENNISON (1981) and cytologically by C. PISANO, S. BONACCORSI and M. GATTI (personal communication).

***Y* chromosome fragments:** The *Y* fragments employed in this work are *Y* distal-*X* proximal elements ($X^P Y^D$) derived from five different fertile reciprocal *X-Y* translocations (*V36*, *V24*, *W19*, *P7* and *W28*) that involve a *y w f X* chromosome and the $B^SY y^+$ chromosome (see Figure 2). The *X-Y* translocations were induced and genetically characterized by KENNISON (1981). The *X* chromosome breakpoints are located in the heterochromatin (*Xh*) proximal to the *bb* locus. (For the cytological map of the *X* heterochromatin, see PIMPINELLI *et al.* 1985.) The $B^SY y^+$ chromosome breakpoint of *V24*, *P7* and *W28* is located in the long arm (YL), while the *Y* chromosome breakpoint of *W19* and *V36* in the short arm (YS). Therefore, besides the different portions of *Y* chromosome heterochromatin, the $X^P Y^D$ elements carry either y^+Xh ($X^P YS^D$ *V36*, $X^P YS^D$ *W19*) or B^sXh ($X^P YL^D$ *V24*, $X^P YL^D$ *P7* and X^P-YL^D *W28*) blocks. Both y^+Xh and B^SXh blocks are derived from the proximal *Xh* region and are comparable in size. (For the nomenclature of the heterochromatic blocks of y^+Y and B^SYy^+, see GATTI and PIMPINELLI 1983.) These elements X^PY^D were isolated from the parental translocations by crossing females homozygous for *YSX.YL*, *In(1)EN*, *y* (=*XY*, *y*) to *T(X;Y) y w f* $B^S y^+$ males and by recovering the $XYy/X^P Y^D$, B^S or y^+ F_1 males. Except for *P7*, cytologically described in the paper by HARDY *et al.* (1984), the cytological analysis using Hoechst and N-banding techniques of the *X-Y* translocations was carried out by S. BONACCORSI (personal communication).

Calculation of the *Y* deficiency and fragment sizes: The cytological size of *Y* deficiencies and $X^P Y^D$ elements, including the y^+ or B^S *Xh* blocks (Figures 1 and 2) is expressed in percent according to the cytological map of the standard fertile y^+Y (Figure 1) elaborated by GATTI and PIMPINELLI (1983).

Eye pigment measurement. Heads were collected 2 days after esclosion by freezing the adults in Eppendorf tubes and vortexing for a few seconds. The red pigment was extracted according to EPHRUSSI and HEROLD (1944). Spec-

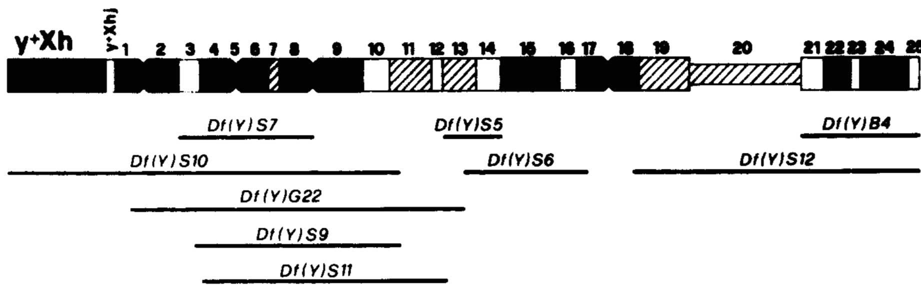

FIGURE 1.—Hoechst staining banding pattern of the standard y^+Y chromosome according to GATTI and PIMPINELLI (1983). The dark areas correspond to bright regions; the hatched areas, to dull regions and the open areas, to nonfluorescent regions. The bars below indicate the physical size and the chromosome location of the deficiencies employed in our analysis.

trophotometric analysis was performed at 480 nm. For each chromosome samples consisting of five heads were analyzed.

RESULTS

Suppression of three different variegated position effect was analyzed: (1) the *Y*-suppressed lethality (LINDSLEY, EDINGTON and VON HALLE 1960), (2) the white mottled (w^m), and (3) the brown variegated phenotypes (bw^V), which are respectively associated with the following rearrangements: (1) *In(1)v231* an inversion with breakpoints 1B/C; 20F on the cytogenetic map, (2) $In(1)w^{m4L}\ w^{m51bR}$, and (3) $In(2R)bw^{VDe2}$ (41A-B; 59 D6-E1). In both *In(1)v231* and $In(1)w^{m4}\ w^{m51bR}$ the variegation-inducing regions are derived from the *X* chromosome heterochromatin, whereas the heterochromatin of the right arm of the second chromosome induces the bw^V phenotype. All three rearrangements induce a strong variegated position effect, which can be efficiently suppressed by the addition of a *Y* chromosome to the genome.

Y chromosome deletions (Figure 1) and $X^P\ Y^D$ elements (Figure 2) recovered from fertile *T(X;Y)s* were tested for their ability to suppress these V-type position effects. It is worth noting here that the *Df(Y)s* are from the same parent *Y*. As can be seen in Figures 1 and 2, the rearrangements used in this work altogether cover the entire *Y* length. Thus, no *Y* regions were left genetically unexplored in our experiments.

The *Y* suppression effect on *l(1)v 231*: In a first set of experiments, the suppression of the lethal phenotype of *l(1)v231*, associated with *In(1)l v231*, was analyzed.

$YL \cdot X \cdot YS/Y^*$ males (where Y^* are either *Y* deficiencies or X^PY^D elements marked with y^+ or B^S) were crossed with *FM7, y* w^a *v B/l(1)v231 y* females. We have suppressed the absolute viability of the F_1 males as the ratio of surviving *l(1)v231* males to the total female offspring whose viability was assumed to be constant. The ratio of $l(1)v231/Y^*$ male absolute viability to that of $l(1)v231/y^+Y$ males, which carry a cytologically normal and genetically fertile y^+Y, gave a relative viability which measured the extent of suppression exerted by each rearranged *Y* chromosome relative to that caused by y^+Y.

Table 1 shows the absolute and relative viability values calculated for different male genotypes bearing the *Y* chromosome deficiencies assayed in this experiment. In Figure 3 the absolute viability of each class of *l231* males bearing different *Y* chromosomes is plotted *vs.* their physical size.

Deficiency mapping shows that chromosomes still retaining 60 to 95% of the total heterochromatin do not drastically differ from the control y^+Y in their effectiveness of suppression. In particular the suppression effectiveness is the same for *Df(Y)s S10, S11, S7, S6* and *S5*. Since the deficiencies used altogether cover the entire *Y* chromosome length except for the centromeric region (Figure 1), these results suggest that the suppression effect does not map to specific *Y* region.

The possibility might still remain that the *Y* centromere or its neighboring regions, which are retained in all deficiencies, are responsible for the observed suppression. This possibility was tested using five different fragments (Figure 2). These were recovered as half translocations ($X^P\ Y^D$ elements) from the fertile *Xh-Y* translocations *V36, V24, W19, P7* and *W28* (KENNISON 1981), which lack the *Y* centromere. The X^PY^D elements retain, in addition to the y^+ or B^S *Xh* blocks present in the $B^S\ Y\ y^+$ chromosome from which they originated, different amounts of the *Xh* proximal to the *bb* locus. In particular, both *V36* and *V24* contain roughly the whole of the proximal *Xh* region, while *P7, W28* and *W19* retain only smaller *Xh* portions next to the centromere. The heterochromatic content of $X^P\ Y^D$ elements, including y^+ or B^S *Xh* blocks, is expressed as a percent of the cytological size of the control y^+Y. In this calculation the *Xh* proximal to the *bb* locus is not included, since its cytological content does vary among the elements. Furthermore, since the y^+Y chromosome heterochromatin retained in the $X^P\ Y^D$ elements represent 12–55% of the control y^+Y size, that also enabled us to investigate the pattern of suppression obtained with *Y* portions representing less than 60% of the y^+Y. The results are shown in Table 1 and are graphically expressed in Figure 3.

The maximum background of suppression attributable to the proximal *Xh* present in the $X^P\ Y^D$ elements does not go beyond 26%, observed with *V36* which carries the largest *Xh* amount (*i.e.*, the *Xh* region proximal to *bb* plus the y^+Xh block derived from the original B^SYy^+). Therefore the *Yh* present in *V24, W19,*

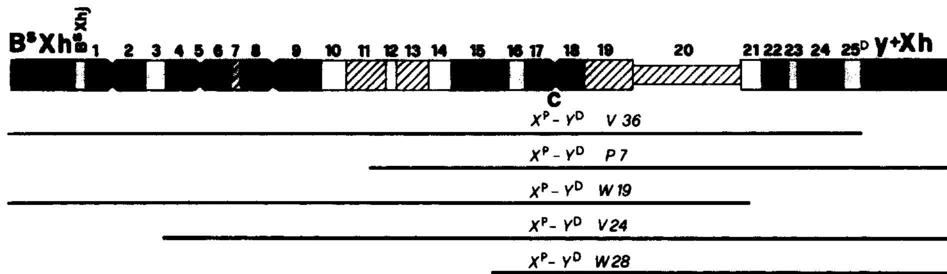

FIGURE 2.—Hoechst staining banding pattern of the standard B^SYy^+ chromosome according to GATTI and PIMPINELLI (1983). The bars below indicate the physical size and the chromosome location of five different deficiencies recovered as $X^P Y^D$ elements from reciprocal $T(X;Y)$s (see MATERIALS AND METHODS).

TABLE 1

Suppression effect of different *Y* chromosomes on the *l(1)v231* phenotype

Chromosome	Progeny: *l(1)v231* Y^*	Progeny: Total females (*yB* and yB^+)	a.m.v.[a]	± SE	r.m.v.[b] (%)
0	101	2663	0.038	0.001	7.3
V36	231	1409	0.164	0.004	31.5
V24	386	1223	0.31	0.008	59.6
W19	910	2820	0.32	0.005	61.5
P7	370	957	0.386	0.01	74
W28	443	1020	0.43	0.01	82.7
S10	589	1151	0.51	0.013	98
S12	929	1879	0.494	0.009	95
G22	660	1349	0.49	0.01	94.2
S11	596	1164	0.51	0.012	98
S7	1190	2261	0.526	0.009	101
S6	940	1780	0.528	0.01	101
B4	637	1291	0.493	0.011	95
S5	469	899	0.521	0.014	100
y^+Y	1449	2769	0.52	0.009	100

[a] The absolute male viability is expressed by the ratio of vital *l(1)v231* males to the total of females.

[b] Relative male viability (%) $= \dfrac{l(1)v231/Y^*\ \text{(a.m.v.)}}{l(1)v231/y^+Y\ \text{(a.m.v.)}} \times 100.$

[c] Y^* = *Y* chromosome deficiencies and fragments.

P7 and *W28* fragments is indeed responsible for the increased suppression exerted by those $X^P Y^D$ fragments in comparison to *V36*.

It is apparent from the plot in Figure 3 that suppression increases as a function of the amount of the *Y* added to the genome. Small amounts of *Y* chromosome are able to induce a relevant suppression effect. In fact, it appears that the *Yh* present in *V24* and *W19* (about 10% and 15% of the control y^+Y, respectively) is responsible at least for the 30% suppression difference observed between these elements and *V36*. These data show that the *Y* centromere does not play a specific effect in suppressing *l(1)v231*, since the tested $X^P Y^D$ elements lack the *Y* centromere and all of them show a suppression effect related to the physical size. Furthermore the similarly sized *V24* and *W19* show similar effects, although they contain opposite ends of the *Y* chromosome (see Figure 2).

Taken together, these findings indicate that suppression of *l(1)v231* is a quantitative phenomenon, independent from the genetic constitution of the *Y* chromosome heterochromatin present in the genome.

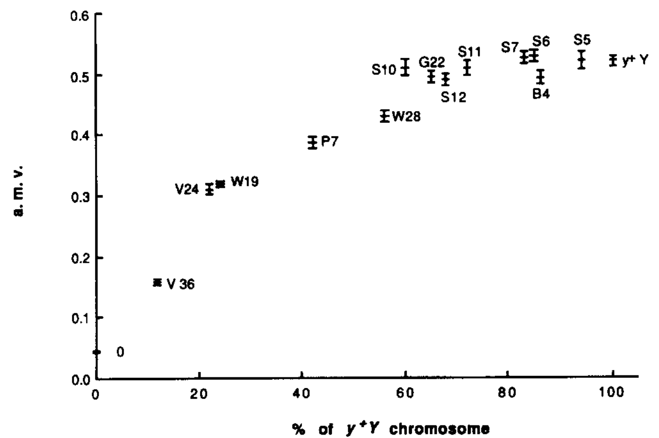

FIGURE 3.—The suppression-effect exerted by different *Y* chromosome amounts on the lethality associated to the *In l(1)v231*. The absolute viability of each class of *l(1)v231* males bearing different *Y* chromosomes is plotted *vs.* their physical size. The physical size of *Y* deficiencies and X^D-Y^D elements, including the y^+ or B^S *Xh* blocks, is expressed as a percent of the cytological size of the control y^+Y, according to the cytological map of the standard fertile y^+Y stained with Hoechst 33258 elaborated by GATTI and PIMPINELLI (1983). In that calculation the *Xh* proximal to the *bb* locus present in the $X^P Y^D$ elements is not included.

A critical threshold (60% of the y^+Y) is apparent, at which the maximum suppression occurs. Therefore the possibility that any specific *Y* region is responsible for the suppression of that position effect variegation can be ruled out.

The white mottled phenotype: The deletions used in the test with *l(1)231* were also used to test w^m and bw^V position effect variegation. Only the y^+-marked and *X-Y* rearrangements could be used since the B^S marker in the others drastically reduces the number of ommatidia.

The effect exerted by the *Y* chromosome in suppressing $In(1)w^{m4}$ $w^{m51bR}(w^m)$ was tested by crossing single w^m/Y males by $y\ w\ f/y\ w\ f/Y^*$ females (where Y^* were either *Y* chromosome deficiencies or $X^P Y^D$ elements from $T(X;Y)$ marked with y^+). In each cross, the red pigment levels were measured in both the $y\ w\ f/y\ w^m$ and the $y\ w\ f/y\ w^m/Y^*$ F_1 female offspring taken as control and experimental values, respectively. The results are summarized in Table 2 and the suppression values are shown in Figure 4.

Suppression values obtained with *W19* (in which the 21 to 25 *Yh* regions represent in size 15% of the y^+Y) element confirms that small fragments of y^+Y hetero-

TABLE 2

Suppression effect of different *Y* chromosome amount on the white mottled phenotype

Chromosome	No. of observations[a] C	E	$\overline{OD}$ C	E	$\Delta\overline{OD}$[b]	± SE	Percent of suppression
V36	11	11	0.036	0.087	0.051	0.005	26.7
W19	7	10	0.046	0.131	0.085	0.0047	44.5
S10	13	18	0.035	0.198	0.163	0.004	85.3
S12	15	18	0.039	0.197	0.158	0.0045	82.7
S11	9	12	0.047	0.208	0.161	0.004	84.4
S9	9	9	0.036	0.201	0.165	0.006	84.8
S7	10	8	0.035	0.220	0.185	0.005	97.4
S6	13	21	0.039	0.220	0.181	0.003	94.7
B4	7	11	0.047	0.222	0.175	0.005	92
S5	7	10	0.043	0.219	0.176	0.005	92.1
y^+Y	18	14	0.041	0.232	0.191	0.005	100

Optical density (OD_{480nm}) levels were measured in $ywf/yw^m/Y^*$(E) and ywf/yw^m(C) siblings females (see text).

[a] Each observation based on a pigment extracted from five heads.

[b] $\Delta\overline{OD} = \overline{OD}(E) - \overline{OD}(C)$.

[c] Percent of suppression = $(\Delta\overline{OD})Y^*/(\Delta\overline{OD})y^+Y$.

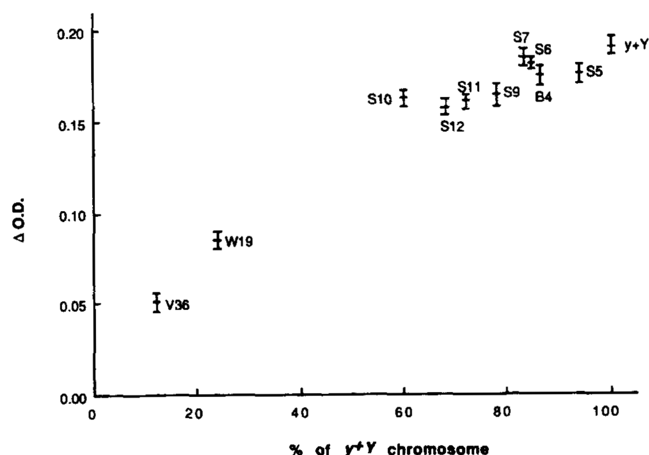

FIGURE 4.—The suppression effect exerted by different *Y* chromosome amounts on the *In(1)w*m4l w^{m51BR} variegated phenotype. The percent of the *Y* chromosome added to the genome is plotted *vs.* the $\Delta\overline{OD}(\overline{OD}(E)\overline{OD}(C))$ values. The physical size of *Y* deficiencies and $X^P Y^D$ elements, including the y^+ or B^S *Xh* blocks, is expressed as a percent of the cytological size of the control y^+Y, according to the cytological map of the standard fertile y^+Y stained with Hoechst 33258 elaborated by GATTI and PIMPINELLI (1983). In that calculation the *Xh* proximal to the *bb* locus present in the $X^P Y^D$ elements is not included.

chromatin are highly effective in suppressing the variegated phenotype. The presence of the y^+Xh block retained in both *V36* and *W19* is not crucial *per se*, as can be seen by comparing *Df(Y)S10* (deleted in the y^+Xh block) with *Df(Y)S12* which is of a comparable size and yet retains the y^+Xh block (Figure 4). Both *S10* and *S12*, which remove the opposite ends of the *Y*, leave about 60% of the chromosome and both show a suppression effect approaching 85% of the y^+Y control. Suppression remains constant for deficiencies retaining 60 to 80% of heterochromatin, after which the maximum suppression is reached.

Taken together these results show that suppression of the w^m phenotype, as previously found for *l(1)v231*, is a function of the amount of *Y* chromosome present in the genome.

The brown dominant variegated phenotype: The effect of the *Y* chromosome heterochromatin on bw^{VDe2} expression was tested by crossing single *y/Y*; $bw^{VDe2}/+$ males to *ywf/ywf/Y** females. The amount of red pigment was measured in the F_1 female offspring. The F_1 females from each cross are either *y w f/y*; $bw^{VDe2}/+$ (control group) or *y w f/y/Y**; $bw^{VDe2}/+$ (experimental group). The results from these experiments are summarized in Table 3 and plotted in the graph in Figure 5.

The *V36* suppression effect on *bw* variegation is similar to that observed on both previously tested phenotypes. The *W19* fragment is more effective in bw^V suppression (70%) than in either *l(1)v231* or w^m suppression. The *W19* and *V36* fragments differ in that *W19* carries the 21 to 25 regions of the *Y* short arm, which represent 15% of the y^+Y in size and appear to be responsible at least for the 50% difference between the suppression values of those fragments. That suggests either that small amounts of *Y* heterochromatin—regardless of genetic content—are sufficient for effective suppression of bw^V, or that *W19* fragment includes a specific bw^V suppressor located in the h21-h25 regions of the *Y*. The effect of these regions present in *W19* can be evaluated from *Df(Y)B4* which is deleted for the same regions (Figure 1). Suppression efficiency observed for *Df(Y)B4* is identical to that shown by the control y^+Y chromosome. Thus the effect of the *W19* element seems to be only attributable to its quantitative rather than to its qualitative heterochromatic content. The bw^V suppression with heterochromatic portions representing 60% or more of the y^+Y is very similar to that observed for suppression of the w^m phenotype (Figures 4 and 5). However, this is an exception to the otherwise generally observed quantitative correlation. *Df(Y)S6* is similar in size to *S7* and *B4* chromosomes, but its suppression value is closer to that observed for *S10, S12, S11* and *S9* than to the control. We are unable to analyze in any further detail the region removed in *Df(Y)S6* (*kl-1*$^-$), which appears to be responsible for a 9% decrease in suppression compared to the y^+Y control. Thus *S6* chromosome may identify a cytological region relatively more efficient than the rest of the *Y* in suppressing the bw^V phenotype.

Besides the weak suppressor region identified by the *S6* chromosome, it is apparent from the graph that, on the whole, bw^V suppression does not substantially differ from that reported for either *l(1)231* or w^m in that no discrete region of the *Y* chromosome is responsible for a strong suppression effect.

TABLE 3

Suppression effect of different *Y* chromosome amount on the brown variegated dominant phenotype

Chromosome	No. of observations[a] C	E	$\overline{OD}$ C	E	$\Delta\overline{OD}$[b]	± SE	Percent of suppression
V36	9	8	0.018	0.064	0.046	0.012	17.4
W19	9	12	0.022	0.206	0.184	0.005	69.7
S10	11	10	0.022	0.264	0.242	0.007	91.7
S12	22	14	0.021	0.268	0.247	0.0045	93.5
S11	15	14	0.019	0.257	0.238	0.006	90
S9	9	8	0.018	0.256	0.242	0.007	91.7
S7	14	16	0.021	0.282	0.261	0.0035	98.8
S6	9	12	0.033	0.272	0.239	0.0076	90.5
B4	6	6	0.019	0.285	0.266	0.0084	100.7
S5	9	4	0.029	0.293	0.264	0.0085	100
y^+Y	11	11	0.014	0.278	0.264	0.007	100

Optical density (OD_{480nm}) levels were measured in *ywf/y/Y**; bw^{VDe2}/+ (E) and *ywf/y*;bw^{VDe2}/+ (C) siblings females (see text).

[a] Each observation based on a pigment extracted from five heads.

[b] $\Delta\overline{OD} = \overline{OD}$ (E) − $\overline{OD}$ (C).

[c] Percent of suppression = ($\Delta\overline{OD}$) Y^*/($\Delta\overline{OD}$) y^+Y.

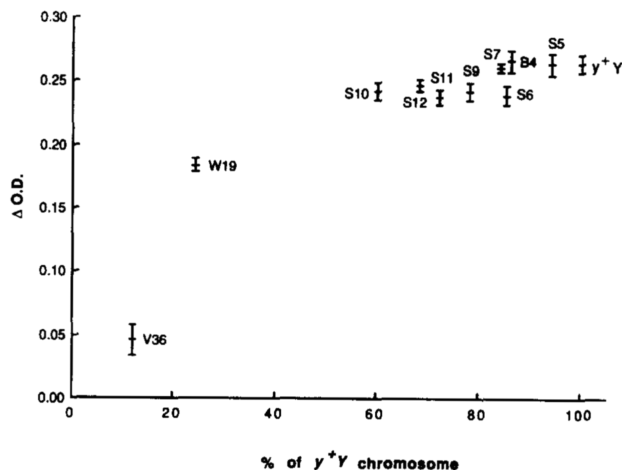

FIGURE 5.—The suppression effect exerted by different *Y* chromosome amounts on the $In(2R)bw^{VDe2}$ variegated phenotype. The percent of the *Y* chromosome added to the genome is plotted versus the $\Delta\overline{OD}(\overline{OD}(E)-\overline{OD}(C))$ values. The cytological size of *Y* deficiencies and X^PY^D elements, including the y^+ or B^SXh blocks, is expressed as a percent of the cytological size of the control y^+Y, according to the cytological map of the standard fertile y^+Y stained with Hoechst 33258 elaborated by GATTI and PIMPINELLI (1983). In that calculation the *Xh* proximal to the *bb* locus present in the X^PY^D elements is not included.

DISCUSSION

The *Y* chromosome has been known for a long time to be an efficient suppressor of position effect variegation (GOWEN and GAY 1934). Early investigations favored the view that discrete suppressor regions could be mapped to specific *Y* sites (BAKER and SPOFFORD 1959; BROSSEAU 1964). Chromosome banding techniques were not available at that time. Therefore, both the qualitative and quantitative content of the *Y* chromosomes assayed in those studies were poorly resolved. Some of the *Y* fragments used in those studies may have in fact included euchromatin from *X* or autosomes that was not identified as euchromatic, as well as heterochromatin from other sources that could have the same generalized effect as the *Y* heterochromatin.

In the present work, we have found that suppression of the variegating state is a function of the quantity of *Y* heterochromatin present in the genome at least for three different V-type position effects: the *Y*-suppressed lethality, the *white mottled* and the *brown variegated* phenotypes. We wish to point out the consistency with which this phenomenon was observed. In fact similar suppression patterns were found in males (*l(1)v231*) and females (w^m and bw^V) using unrelated analytical methods: recovery of viable male offspring and eye pigment measurement.

Our studies were based on a detailed cytological analysis of the *Y* chromosome deficiencies and fragments that were employed. Since the different deletions and fragments together cover the entire length of the *Y*, we are confident that in our tests no *Y* regions were left genetically unexplored.

Several points are worth stressing. Suppression is unrelated to the cytogenetic content of the *Y* chromosome rearrangements that were tested. In fact *Df(Y)S10* and *Df(Y)S12* chromosomes, which are cytogenetically different and yet retain similar amounts of heterochromatin are equally effective suppressors of all three variegated phenotypes. The same applies to *V24* and *W19* X^PY^D elements as suppressors of the lethal variegated phenotype.

The suppression effect appears to be related to the size of the *Y* chromosome deficiencies and fragments. Suppression increases with increasing amounts of *Y* heterochromatin up to 60–80% of the entire *Y*, after which the effect reaches a plateau. In particular, for *l(1)v231* a critical threshold (60% of the y^+Y) is apparent, above which the maximum suppression occurs.

Small *Y* chromosome fragments (*V24* and *W19* elements) were found to be already highly effective in suppressing the variegated phenotypes. This effect reflect again the quantitative feature of the suppression phenomenon, suggesting that in the absence of a *Y* chromosome, the repressed chromosomal state of the variegating genes is particularly sensitive to the addition of small amounts of *Y* heterochromatin. Since the variegation-inducing heterochromatic regions are different for the three position effects examined in this study (*Xh* and *2Rh*), it appears that the suppression effect exerted by the *Y* is also largely independent from the genetic constitution of the inducer sites, being only related to their common heterochromatic organization.

These results indicate that the variegation-suppression property is a general feature of the *Y* heterochromatin, in that it is homogeneously spread along the

entire length of this chromosome rather than being associated with a specific mappable element. That may be explained postulating that proteins involved in the "heterochromatinization" of the variegating genes are actually structural components of all the heterochromatic regions of the *Y* chromosome and are present in limiting amount in the cell. According to this model, the greater the amount of *Y* chromosome heterochromatin added to a variegating genome, the better the chance that heterochromatic proteins (histones or NHC proteins), would be "sequestered" by the *Y* DNA. Because these proteins are present in limiting amount, the addition of *Y* material would result in a progressive increase of the suppression effect on a variegating gene until a threshold is reached. Such a mechanism may indeed apply to other heterochromatic regions which proved effective in modifying position effect variegation.

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